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Health effects of morpholine based coating for fruits and vegetables

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ABSTRACT

Food production and preservation is an important social issue of increasing concern from ancient time onwards. The practice of fruit/vegetable coating was accepted long before their associated chemistries were understood, and are still practiced till date and deserves allocation of more research efforts to investigate the health effects by consumption of coated fruits and vegetables. Morpholine, $O(CH_2CH_2)_2NH$ is a common solvent and emulsifier used in the preparation of wax coatings for fruits and vegetables. Morpholine, by itself, in the doses that are present in fruits and vegetables probably does not constitute a health risk. However, it undergoes nitrosation during the digestion process if there are excess nitrites, formed mainly from naturally occurring nitrate in the diet to form N-nitrosomorpholine (NMOR), a genotoxic carcinogen. Although there is no direct human data on nitrosation rate of morpholine to NMOR, but according to Health Canada Health Hazard Assessment (HHA) 2008 report on Morpholine in wax coatings of apples, safe dose of morpholine in humans is 4.3ng/body weight/day. Sufficient NMOR can be produced in the human gut after ingestion of morpholine-treated fruits/vegetables to pose a health risk raising the need for its effective removal from fruits and vegetables and educating consumers about this risk.

Keywords: Morpholine, Fruits/Vegetables Coating, Emulsifier, NMOR, Carcinogen

INTRODUCTION

Food production and preservation is an important social issue of increasing concern from ancient time onwards and deserves allocation of more research efforts to investigate the preservation of fresh fruits and vegetables. As per National Food Safety Database of Wax Coatings on Fruits and Vegetables, US, 1914 [1], waxing is very common in fruits and vegetables for different roles and applications and summarized in Table 1. Major losses in quality and quantity of fresh fruits which occur between harvest and consumption can be overcome by coating as it can provide an alternative to storage by reducing quality changes and quantity losses through control of internal atmosphere of the individual fruit.

Table 1: Fruits and Vegetables Coating: Roles and Applications

S.No.	Roles	Applications
1	Retain moisture in fruits and vegetables	Maintain wholesomeness and freshness
2	Inhibit mold or bacterial growth	Extended shelf life
3	Enhance appearance	Consumer attraction
4	Prevent physical damage	Prolonged transit and holding periods
5	Protect fruits & vegetables from bruising	Control premature rottening
6	Maintain texture	Delay ripening
7	Reduce weight loss	Slow decaying
8	Minimize discoloration	Avoid aroma loss
9	Inhibit oxygen penetration	Barrier gas exchange with external environment

Along with fruits and vegetables, waxes are also used on candies, pastries and gum extracted from natural sources like plants, food-grade petroleum products, dairy and animal sources or insects (bees wax) and shellac-based wax or resin. Waxing does not improve the quality of any inferior fruit or vegetables; rather, waxing along with proper handling contributes to maintaining a healthful product. Extensive research by governmental and scientific authorities has shown that approved waxes (which must meet the food additive regulations of concern state/ country) are safe to eat and indigestible, which means they go through the body without breaking down or being absorbed. One notable features of wax coating is that, it cannot be removed by regular washing/evaporation due to their higher boiling point and may turn white on the surface of fruits or vegetables, if they have been subjected to excessive heat and/or moisture. This whitening is safe and is similar to that of a candy bar that has been in the freezer for extended period of time.

However, waxes by themselves do not control decay; rather, they are combined with some chemicals to prevent the growth of mold or bacteria. One such chemical is morpholine and its derivatives (MAID) such as 4-(4-aminophenyl) Morpholine [2], 4-(2-Aminophenyl) morpholine [3] and Benzophenone-N-ethyl Morpholine ethers [4]. Mostly, waxes are used only in tiny amounts and each piece of waxed fruit only has a drop or two of wax. Waxes may be mixed with water or other wetting agents like morpholine to ensure that they are applied thinly and evenly. In brief, morpholine reacts readily with fatty acids, forming emulsifying agents, which are used in the formulation of water-resistant waxes/polishes. As the film of polish emulsion gradually dries and morpholine evaporates to form a film highly resistant to water spotting and deterioration. An example of this type of polish is a carnauba wax formulation with the following composition (in parts by weight): Carnauba wax: 11.2, Oleic acid: 2.4, morpholine 2.2, Water 67 [5]. Some more different formulations incorporating a variety of waxes and other substances are listed below (Table 2).

Table 2: Different formulations of wax of commercial origin

S.No	Formulation Components	Concentration (%)
1	Oxidized polyethylene	18.6
	Oleic acid	3.4
	Morpholine	2.8
	Polydimethylsiloxane antifoam	0.01
2	Candelilla wax	18.3
	Oleic acid	2.1
	Morpholine	2.4
	Polydimethylsiloxane Antifoam	0.02
3	Shellac	9.5
	Carnauba wax	8.3
	Morpholine	3.3
	Oleic acid	1.7
	Ammonia	0.17
	Polydimethylsiloxaneantifoam	0.01
4	Shellac	19
	Oleic acid	1.0
	Morpholine	4.4
	Ammonia	0.3
	Polydimethylsiloxane antifoam	0.01
5	Shellac	13.3
	Whey protein isolate	3.0
	Morpholine,	3.1
	Oleic acid,	0.7
	Ammonia,	0.2
	Polydimethylsiloxane antifoam	0.01

MORPHOLINE: INDUSTRIAL POLLUTANT AND FRUITS/VEGETABLES COATING

Morpholine was included in EPA (Environment Protection Agencies, US) master list for toxic substances control act in 1996 (61 Fed. Reg. 65936, 13 Dec 1996) and EPA registered pesticides (EPA inert ingredients list 03) and is designated as a volatile organic compound (VOC) [6]. As per OSHA (Occupational Safety and Health Administration, U.S. Dept of Labor), the permissible exposure limit (PEL) of morpholine is 20ppm [7]. Morpholine is colorless, clear and hygroscopic liquid of maximum water content of 0.3% [8] with an annual production of ~25,000 tons [9, 10]. It has characteristic functional group of amines and ether. Now a days, other than fruits and vegetables coating, morpholine and its derivative are being used in various industries namely, rubber industry (as vulcanization accelerator), iron industry (as corrosion inhibitor), pharmaceuticals industry (as catalysts, antioxidants, bactericides), dyes industry, personal care (hair conditioner, face wash, waxes and polishes) pesticides industry, refinery & power plants (as additive in stream system to adjust the pH levels in water), paper industry (as optical brightener to make paper glossy and preservation of book paper), textile industry (to dissolve cellulose) and as a solvent in various other manufacturing processes. Because of its solubility in water, significant amounts of this chemical compound are released via industrial effluents into the environment where it undergoes chemical or microbiologically nitrosation leading to formation of the carcinogenic compound *N*-nitrosomorpholine (NMOR) (detail in below). It was also reported that morpholine is found in baked ham (0.20 ppm) [11], fishes ($\leq$0.6 ppm in canned tuna) [12], Fruits/Vegetables coating (Orange pulp 5-71.1mg/kg, orange peel 0.3 mg/kg; [13] and cigarettes and cigarette smoke condensate/snuff (0.3 mg/kg) [14, 15] and can enter our body through absorption through the skin, ingestion and other exposure routes, if improperly, handled or consumed directly. The type and severity of symptoms of morpholine toxicity vary depending on the amount of morpholine and the nature of exposure. The link between industrial discharge containing morpholine and direct consumption of coated fruits/vegetables is shown in Figure 1.

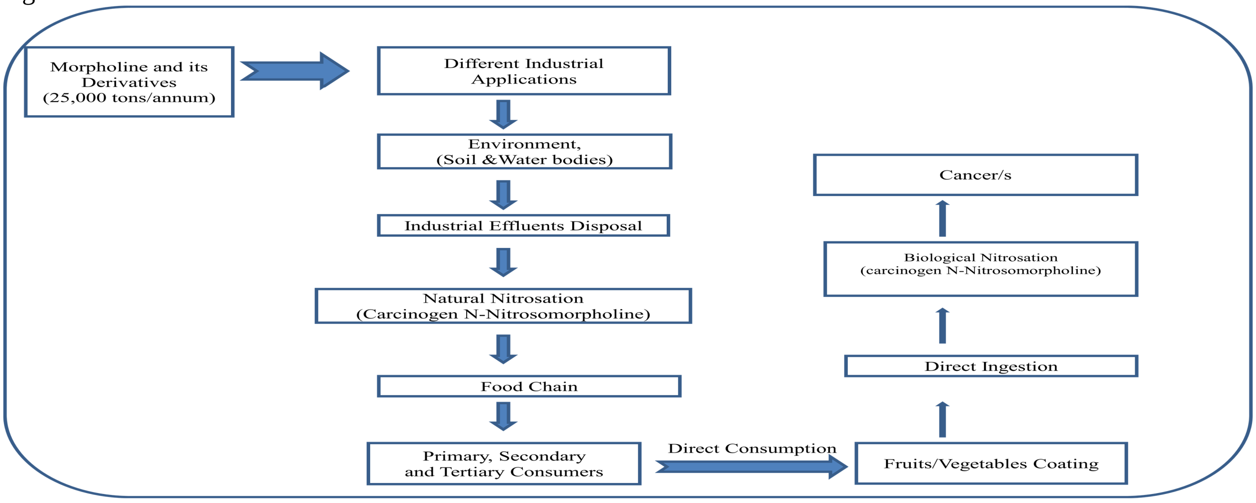

Figure1: Interlink of morpholine in industrial effluents and fruits/vegetables coating

AVAILABILITY OF MORPHOLINE AND ITS NITROSATION (NMOR)

Morpholine and its salts have been used as components of protective coatings applied on fruits and vegetables [16, 17]. There are many reports about the presence of morpholine in different fruits and vegetables. Ohnishi *et al.*, 1983 [13] found morpholine at concentrations of < 71.1 mg/kg in the peel of retail citrus fruits (orange, lemon and mandarin) in Japan while the in the pulp (flesh) of the fruits was much lower, being < 0.7 mg/kg (Table 3). If the fruits were washed in washing-up liquid, morpholine concentrations were reduced, but only by about 25%. Even when the fruit was boiled for 20 minutes, a third to a quarter of the morpholine still remained. Morpholine leached by these processes was detectable in washing and boiling water. Recently, Rupak *et al.*, 2016 [18] also quantified the presence of morpholine in some fruits and vegetables purchased from super market, Hyderabad, India as shown in table 3.

Table 3: Morpholine content in fruits and vegetables

Sample	Part	No. of sample	Morpholine Concentration (mg/kg)	Reference
Orange (Variety a)	Peel	12	ND-57	[13]
	Pulp	03	0.2-0.7	
Orange (Variety b)	Peel	06	5.0-71.1	
	Pulp	01	0.3	
Mandarin	Peel	02	16.1-18.0	
	Pulp	01	ND	
Lemon	Peel	02	ND-5.2	
	Pulp	01	ND	
Grape	Peel	02	2.8-7.0	
	Pulp	01	ND	
Tomato	Peel	02	0.17	[18]
Carrot	Peel	02	1.8	
Capsicum	Peel	02	0.34	

Where a and b variety of Orange, N.D. = not detectable with detection level of 0.2 mg/kg

Sen and Baddoo, 1989 [19] reported the morpholine and NMOR content of waxed and un-waxed apples of Canadian origin obtained either direct from the packers or from retail sources. Apple homogenates and liquid waxes were analyzed for morpholine contents and are shown in table 4. Although the concentration of morpholine found in waxed apples was high, NMOR could not be found in any of the waxed or un-waxed samples. Low levels of morpholine in the un-waxed apples could be due to contamination during packing or transport. Morpholine amounts similar to those found in orange pulp [13], have been found in cigarette tobacco, at a concentration of 0.3 mg/kg, and in snuff and chewing tobacco at concentrations up to 4.0 mg/kg respectively indicating that both smokers and nonsmokers are at an equivalent risk for developing cancer by NMOR. Brunnemann*et al.*, 1982 [15] analyzed 10 popular snuff brands from USA and Sweden for morpholine and NMOR (shown in Table 5). In five USA brands, morpholine and NMOR concentrations was between 1.5-4.0 mg/kg and ~0.7 mg/kg respectively and in Swedish products, these concentrations were between 0.2 and 2.5 mg/kg and 0.044 mg/kg respectively. NMOR formed by nitrosation from morpholine was found in 5/5 of USA and 2/5 of Swedish snuff samples.

Table 4: Concentration of morpholine and NMOR in liquid waxes, waxed and un-waxed apple

Sample	Number of sample	Morpholine Concentration (mg/kg)	NMOR Concentration (mg/Kg)	Reference
Liquid wax	300	27	0.286	[19]
	500	31	0.668	
	400	24	0.138	
	500	38	0.277	
	500	22	0.152	
	300	33	0.585	
Un-waxed apples	300	ND	ND	
	500	0.118	ND	
	400	0.016	ND	
	500	0.041	ND	
	500	ND	ND	
	300	0.018	ND	
Waxed apples	300	4.3	ND	
	500	4.9	ND	
	400	6.3	ND	
	500	7.1	ND	
	500	4.0	ND	
	300	7.7	ND	

Where N.D. = not detected with detection limit is 0.005 mg/kg for morpholine and 0.0005 mg/kg for NMOR

Table 5: Morpholine and NMORconcentration in snuff tobacco/cigarette

	Snuff tobacco										Reference
Concentration (mg/kg)	Snuff brand (USA)					Snuff brand (Sweden)					[15]
	I	II	III	IV	V	I	II	III	IV	V	
Morpholine	2.8	1.5	4.0	3.2	2.2	0.82	0.2	0.78	0.94	2.5	
NMOR	0.024	0.69	0.69	0.63	0.031	0.044	ND	ND	0.01	ND	

Where N.D. = not detectable with detection limit is 0.002 mg/kg

HEALTH HAZARD ASSESSMENT OF COATED FRUITS AND VEGETABLES

As per 2008 summary report of the Health Hazard Assessment (HHA) on “morpholine in wax coatings of apples” by Health Canada [20], fruits and vegetables coating are not free from amine and in the presence of excess nitrite, formed mainly from naturally-occurring nitrate in the diet, morpholine can be chemically nitrosated to form NMOR. Formation of NMOR is well known in aqueous solutions of nitrite or by reaction of gaseous nitrogen oxides, e.g., N_2O_3, N_2O_4, NOx in aqueous solutions even under normal environmental conditions [21-25]. The reaction is summarized as described by Mirvish *et al.,* 1975 and 1988 [21, 22] in Figure 2 shown below:

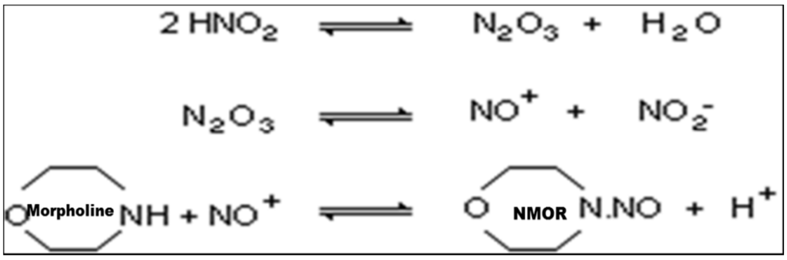

Figure 2: Nitrosation of Morpholine to form NMOR

The unanswered question till date is whether sufficient NMOR can be produced after ingestion in humans, to pose a tangible health risk or not? The possibility that morpholine might be nitrosated by humans to form NMOR during digestion has been investigated indirectly as there is no direct human data on the nitrosation rate of morpholine to NMOR. By direct consumption of coated fruits and vegetables, morpholine and its derivatives pass into gut and the secondary amine functionality inevitably leads to nitrosation and formation of NMOR. Indeed nitrosation of morpholine was reported when morpholine was added to human saliva and NMOR was formed [26]. After oral and parenteral administration or after inhalation, morpholine is well absorbed and is distributed in all tissues and body fluids. Animals exposed to morpholine showed liver and kidney damage [6]. There is direct relationship between morpholine consumption to development of liver or kidney cancer, impaired liver or kidney function in animal model because of the biotransformation and detoxification of foreign substances and hence increased susceptibility to effects of morpholine (Table 6).

Table 6: Adverse health effects of morpholine administration at different doses to animal models

S. No.	Animal Model	Route	Dose	Effects Observed	References
1	Guinea-pigs and rabbit	Dermal application	0.9 g/kg bw	Necrosis of liver & tubules of the kidney	[27]
2	Guinea-pigs and rats	Single oral administration	0.1-10g/kg bw	Hemorrhages in stomach and small intestine	
3	Guinea pigs	Oral, 30 days	0.5 g/kgbw	Necrosis of liver and renal tubules	
4	Rats	Inhalation up to 42 hr	1200 or 1800 ppm (42720 or 6400 mg/m^3)	Liver and kidney necrosis	[28]
5				Blue vision, grey vision or haloes, glaucopsia	
5	Rats	In diet after 270 days	1 g/kg	Fatty degeneration of the liver	[29]
6	Female Sprague-DawleyRats	In diet for 12 weeks	5g morpholine together with 5g nitrite/kg bw/day	6/7 Hepatocellular adenoma, 1/7 carcinomas, 1/7 renal adenoma, 2/7 hemangio-endotheliomas of liver, 1/7 cyst-adenocarcinoma of liver	
		39 weeks	5g morpholine or 5g nitrite alone	No tumors in any tissue	

Where Kg bw: kilogram body weight

Based on HHA conducted chronic oral toxicity study in rat and mice No Observed Adverse Effect Level (NOAEL) is an Acceptable Daily Intake (ADI) of 0.48 mg/kgbw/day. When not considering the potential for nitrosation, morpholine exposure for children and adults is about 8% (0.0384 mg/kg bw/day) and 5% (0.024 mg/kg bw/day) of the ADI respectively, seems to be safe and not a cause for any concern. Although it is assumed that there is some probability of harm at any level of exposure to a genotoxic carcinogen, actual exposure may be so low that the health risk is practically negligible. Regarding the presence of NMOR on apples coated with wax containing morpholine--No NMOR was determined to be present on these apples, and even no NMOR was formed when morpholine and nitrite were combined in experiments conducted in the presence of apple flesh. From the estimated morpholine exposure, the possible endogenously formed NMOR was estimated to be 2.2 and 3.6 ng/kg bw/day for adults and children, which is less than the estimated safe dose of 4.3 ng/kg bw/day. Uncertainties in this estimate of NMOR formation include the physiological differences between humans and rats and actual levels of nitrite consumed. It is unreasonable to assume that these uncertainties would actually increase the estimated NMOR formation in the human gut.

Appropriate literature where rats exposed to morpholine and nitrite showed fore-stomach tumors and other carcinogenic properties has been added, e.g. Mirvish SS *et al.,* 1983 [30] reported the liver and fore-stomach tumors and other fore-stomach lesions in rats treated with morpholine and sodium nitrite. Kitano *et al* 1997 [31] also reported the carcinogenicity of methylurea or morpholine in combination with sodium nitrite in rat multi-organ carcinogenesis bioassay. In a study, after oral treatment with N-nitrosomorpholine, the carcinogenic properties of this substance were studied in rats, mice, hamsters, and various types of fish and as a result, liver and lung cancer were reported in mice, liver and kidney cancer in rats, and liver cancer in hamsters [32]. Zare *et al* 2015 [33] reported that morpholine stress causes changes in blood parameters, increased filtration, decreased reabsorption and absorption, weight loss, inflammation, hyperemia, urinary tract reconstruction and resulted polyuria in NMRI male albino mice. The phenomenon known as blue vision, grey vision or haloes, “glaucopsia” is also well documented effect on eyes of workers exposed to amines, including morpholine and its derivatives, particularly in the foam plastic industry [34, 35]. Corneal oedema with “hazy vision” and halo phenomena around lights have also been described when there was morpholine exposur [36]. Since, no epidemiological studies of morpholine have been conducted and no data were available from human studies on the carcinogenicity of morpholine. The overall IARC (International Agency for Research on Cancer) evaluation was that morpholine was not classifiable for its carcinogenicity to humans [37]. As no direct data about in-situ formation of NMOR as a human carcinogen is available but it was found that prevalence of liver cancer has risen after the introduction of morpholine in UK. Morpholine was introduced in United Kingdom (UK) in late 1970 and gradually it was used intensively in different industries. Exposure of morpholine through various route results adverse health effect which might be one of the reasons (insufficient literature) other than physiological cause of higher incidence of liver cancer as shown in figure 3 as per Cancer Research, UK report, 2010 [38]). Hence in October 2010, UK Food Standards Agency [39] had undertaken an initial risk-assessment of morpholine coated fruits/vegetables and found that morpholine at the levels detected, 0.03-0.3ppm, is likely to be of a low risk to consumer health. Therefore the use of morpholine is prohibited in UK and European Union (EU) member countries. UK Food standards agency banned the import of apple from Chile because it was found to have about 2ppm of morpholine and advised that affected apples should not be on sale in the UK.

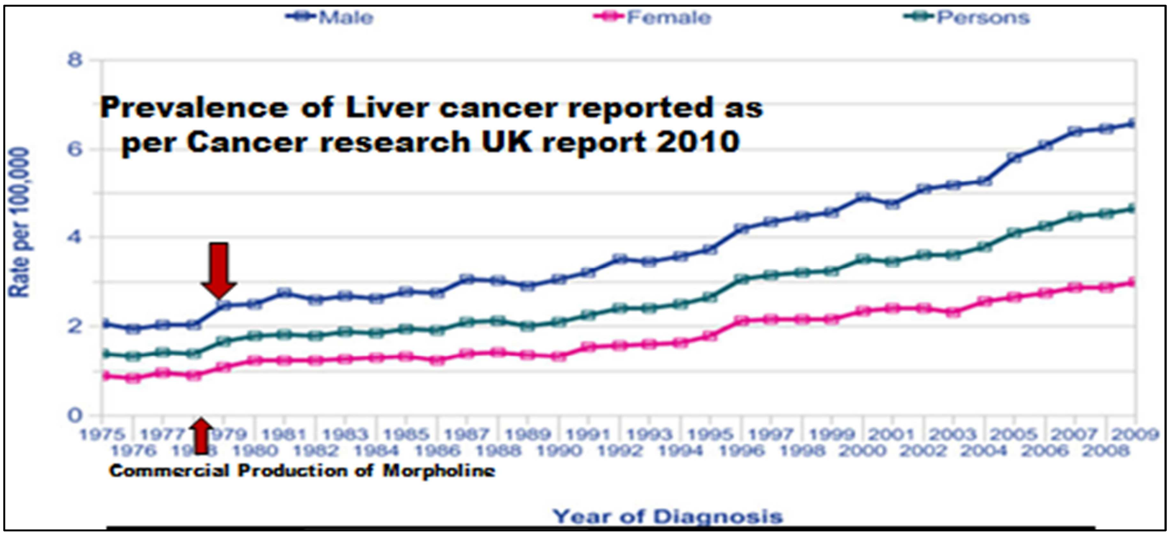

Figure 3: Prevalence of liver cancer as per Cancer Research, UK report, 2010 [38]

CONCLUSION

Even today, fruit and vegetables can be harvested, packed, and stored with the use of waxes and transit to market from remote locations. Since many of fruits and vegetables are typically peeled and washed, only a few common fruits and vegetables present such a problem. Morpholine as a common emulsifying agent in the doses that are present in fruits and vegetables probably does not constitute a health risk. However, during the digestive process, if there are nitrites simultaneously present, morpholine is chemically changed into potential carcinogen NMOR. The estimated safe lower limit for NMOR is 4.3 ng/kg body weight per day. It has been estimated that for adults, consuming waxed apples and a mixed diet, NMOR ingestion can approach (3.6ng/kg body weight) the lower limit of safety [40]. However, these estimates did not actually measure NMOR formation in humans and nitrite ingestion is quite variable in humans. Hence, it is entirely possible that chronic consumption of waxed fruit and vegetables containing morpholine could present a slight risk for cancer in certain individuals. Considering the effects observed in the reported literature, the use of morpholine should be limited or minimal through increasing supervision on its applications. So its monitoring is highly recommended and also educates the consumer about its health effects.

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