N-nitrosamines in food: effects and legislation

Effects and response

The safety of exposure of humans to inorganic nitrite and nitrate received increased scrutiny in 1960s. Cases of infantile methemoglobinemia associated with high nitrate in drinking water were documented. Besides, the formation of N-nitrosamines in certain foods, which had been shown to be carcinogenic, raised awareness regarding potential human health concern.

A plausible biological mechanism which explains carcinogenicity of ingested nitrate and nitrite is endogenous N-nitrosation reactions (Bryan *et al.,* 2012). Normal intakes are not proven to have carcinogenic effects. On the other hand, it is the excessive nitrate or nitrite intake which can generate N-nitroso compounds which are carcinogenic and mutagenic by causing DNA alkylation.

N-nitrosamines are also genotoxic, which interacts with DNA directly or indirectly, inducing permanent genetic changes in cells, and causing cancer. For this group, as there is no dose which does not result in a possible effect of the genotoxic carcinogens, thus a no observable effect level (NOEL) cannot be estimated (Ravnum *et al* ., 2014).

In addition, exposure to nitrosamine affects the immune response strongly. Nitrosamine-induced response towards the immune system is much stronger than nitrosamide-induced response, in a same exposure period. Nitrosamine induces a higher percentage of modulated genes, and involves more pathways. This immunosuppressive effect in turn influences the innate immune response of cells. This plays an important role in the promotion

phase of carcinogenic processes, indicating an additional way for nitrosamines to cancer risk (Hebels *et al* ., 2011).

Animal toxicology research serves as an important area for investigation which provides us with safety data. About 90% of the 300 nitrosamines tested showed carcinogenic effects in laboratory animals and bioassays. Nevertheless, the usage of animal models requires understanding of the difference between human and animal systems. Rodents used for this purposes have fore-stomachs and Hardarian glands, which is not analogous as in humans (Bryan *et al* ., 2012)..

Acute toxic effects of nitrate intake had been encountered only at very high doses. On the other hand, nitrite causes acute toxicity in much smaller doses. In laboratory animals, the LD50 of inorganic nitrite is approximately 2. 6 mmol/kg. Some early studies may have shown methaemoglobinaemia when exposed to lower doses of nitrate due to contamination with nitrite (Gilchrist, Shore and Benjamin, 2010).

A follow-up study of the Swedish Mamography Cohort found that there is a two-fold elevated risk of stomach cancer with intake of dietary nitrosamines (Larsson, Bergkvist and Wolk, 2006). On the other hand, Loh *et al* . (2009) suggested that there is a positive association between N-nitrosamine intake and gastrointestinal cancer, especially rectal cancer (Loh *et al* . 2009).

N-nitrosodiphenylamine has shown carcinogenic effects at levels of 1000 parts per million (ppm) to 4000 ppm in both sexes of rats, and there is induced transitional cell carcninoma of the urinary bladder of male and female mice.

Dimethylamines and diethylamines are two of the most potent carcinogens among nitrosamines. 50 ppm of dimethylamines in the diet was found to produce malignant liver tumours in rats in 26 to 40 weeks. Meanwhile, higher doses were shown to cause kidney tumours. For diethylamines, a lag period between dosing and onset of tumours increases with dosage below 0. 5 mg/kg, with the total tumour yield remaining roughly the same. There is not yet a clear threshold dose for carcinogenicity of nitrosamines in diet established (Shibamoto and Bjeldanes, 2009).

According to the Netherlands Cohort study, nitrate and nitrite exposure based on food intake and drinking water show no significant elevation in stomach cancer occurrences, and shows no apparent trend (Larsson, Bergkvist and Wolk, 2006). On the other hand, there is evidence that long-tern consumption of drinking water which contains more than 4 mg/L nitrosamine has been positively associated with risk of non-Hodgkin’s lymphoma.

Although nitrates are absorbed quickly in mostly excreted within the next few hours, the internal dose or nitrosamine cannot be measured as a 24-hour urinary excretion. A study done by Levallios *et al* ., (2000) showed that there is a stronger correlation between urinary nitrate excretions with dietary nitrate as compared to urinary nitrate excretion with water nitrate intake. Nevertheless, there is no relation found between nitrosamine excretions with nitrate intake. This might be due to low nitrate concentrations in water, thus causing it to be harder to observe for immediate effects. Further studies are required to determine if the use of urinary nitrosamine excretion as a biomarker of exposure is useful (Levallois *et al* ., 2000).

Food laws (limits)

EU legislation allows nitrite and nitrate addition of 150 mg/kg respectively for each additive in meat products. On the other hand, Denmark only permits the use of 60 mg/kg of nitrites in meat preservation for Danish products (Herrmann, Duedahl-Olesen and Granby, 2015). Further studies are required to determine if the addition of 150 mg/kg or 60 mg/kg of nitrite added would cause an increase in average nitrosamine levels. On the other hand, there are no maximum limits established by EU for nitrosamine content in processed meat products. The United States had set a limit of 10 µg/kg of total volatile N-nitrosamine content for cured meat products (Crews, 2010).

The highest amount of a contaminant allowed in drinking water is known as maximum contaminant level (MCL). The US Environmental Protection Agency (EPA) has set a (MCL) for N-nitrosodiphenylamine of 7 µg/L (micrograms/Liter) or 7 ppb (parts per billion) based on a lifetime cancer risk of 1 in 1 000 000 (ATSDR, 2010). The EPA established a cancer risk if 0. 7ng/kg body weight of N-nitrosodimethylamine is consumed daily.

In drinking water, the maximum allowed concentration of N-nitrosodimethylamine in Canada is 40 ng/L and 10 ng/L in Germany (Mestankova *et al* ., 2014). Although the EPA has not established a limit for maximum contaminant level in water, the California Department of Health Services has established 10 ng/L as notification level for action to be taken (Mestankova *et al* ., 2014).

The amounts of N-nitrosodiphenylamine in some commonly eaten foods are 0. 023 µg/100g in buns, muffins and bagels, 0. 149µg/100g in ham, and even 0. 109µg/100g in oysters (Stuff *et al* ., 2009).

Current issues (worldwide)

Apples from America have recently encountered some export issues to other countries due to its toxicity. In America, apples which are to be exported are treated with diphenylamine (DPA), which is a preservative added to prevent the apples from turning brown for as long as a few months. This is to prevent cold injury during cold storage, since apples are usually harvested once a year. By itself, DPA isn’t harmful, but it breaks down into carcinogenic elements, namely nitrosamines.

The European Union has banned the use of DPA in 2012. They set the maximum allowable limit of DPA on apples to 0. 1 parts per million (ppm). Nonetheless, DPA residues with an average reading of 0. 42 ppm have been found on over 80 apple samples imported from America, which is well over the maximum allowed limit. Thus, the EU is banning apples from America, until the readings are found to be in accordance with the regulation (Lunder, 2014).

Although the US EPA and World Health Organisation (WHO) found that long-tern exposure to DPA is unlikely to cause a public health concern, the EU maintains that absence of evidence of harm is not a strong enough indicator. The EU claims that there is insufficient testing regarding DPA to prove that their products as well as chemicals formed are safe to be consumed when broken down. The main source of concern is the presence of nitrosamines.

As DPA is the most common chemical used for apples preservation, the presence of cancer-causing nitrosamines should present a great concern.

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