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Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins

Yann Grosse, Robert Baan, Kurt Straif, Béatrice Secretan, Fatiha El Ghissassi, Vincent Cogliano, on behalf of the WHO International Agency for Research on Cancer Monograph Working Group

In June, 2006, 19 scientists from eight countries met at the International Agency for Research on Cancer (IARC) in Lyon, France, to assess the carcinogenicity of ingested nitrate and nitrite, and the cyanobacterial peptide toxins microcystin-LR and nodularins. These agents are linked environmentally through the runoff of agricultural fertilisers that increase nitrogen concentrations in surface water and groundwater, and that could contribute to cyanobacterial growth in surface water. The assessments will be published as volume 94 of the IARC Monographs.[1]

Nitrate and nitrite are naturally-occurring ions. In the past century, the global nitrogen cycle has been increasingly affected by nitrogen fixation for agricultural activities, which now exceeds the amount that occurs naturally. Both groundwater and surface water can be contaminated by excess nitrate as a result of agricultural activities. Human exposure to nitrate and nitrite is mainly from the ingestion of food. Important sources include vegetables, cereal products, and cured meat. Drinking-water is generally not the main source of nitrate, unless concentrations exceed the WHO guideline of 50 mg/L, which is especially found in contaminated groundwater.

Ingested nitrate (NO_3^-) is excreted in the saliva and reduced to nitrite (NO_2^-) mainly by oral bacteria. Under acidic conditions in the stomach, nitrite then reacts readily with nitrosatable compounds, especially secondary amines and alkyl amides, to generate *N*-nitroso compounds. Several *N*-nitroso compounds are potential human carcinogens.[2] The nitrosation reactions can be inhibited by the presence of vitamin C or other antioxidants. Some epidemiological studies assessed the risk of cancer in people who had high intake of nitrite or nitrate and low intake of vitamin C, a dietary pattern that could result in increased endogenous formation of *N*-nitroso compounds. The Working Group weighted these studies more heavily than studies without this information.

From the epidemiological studies of nitrate in food, no increased risk of cancer was seen. For nitrate in drinking-water, epidemiological studies were few, exposure levels were low, and endogenous nitrosation was not often considered.

For ingested nitrite, the risk for stomach cancer was investigated in seven well-designed case-control studies. Six of these showed consistent, positive associations, four of which were significant. Two studies[3,4] looked at effect modification, and the risk was most pronounced in people who had high nitrite and low vitamin C intake. Neither of the two cohort studies reported a clear positive association. No study accounted for potential confounding or effect modification by *Helicobacter pylori*, an important risk factor for stomach cancer.

For oesophageal cancer, two well-designed case-control studies investigated an association with nitrite intake. Both reported a positive association for nitrite intake overall; for people with high nitrite and low vitamin C intake, these associations were significant.

For brain tumours, two of five case-control studies in children showed positive associations with nitrite intake. In one study, children born to mothers with the highest intake of nitrite from cured meat during pregnancy had a three-fold increased risk for brain tumours.[5] The other study[6] reported an increased risk for astroglial brain tumours in the children of mothers whose drinking-water had high nitrite concentrations. For adult brain cancer, no clear pattern emerged from seven case-control studies.

The Working Group concluded that there is "limited evidence of carcinogenicity" for nitrite in food based on the association with stomach cancer.[1] For nitrate in food and nitrate or nitrite in drinking-water, the studies provide "inadequate evidence of carcinogenicity".[1]

No increased incidence of tumours was recorded in mice and rats if nitrate alone was added to the drinking-water or to the diet, providing inadequate evidence of carcinogenicity. Mice given nitrite in drinking-water showed a significant trend in the incidence of forestomach papillomas and carcinomas combined.[7] Rats exposed to nitrite in utero and throughout life had an increased incidence of lymphoreticular tumours, and mice with similar exposure had raised incidences of lymphoma and lung tumours. These results provide limited evidence of carcinogenicity for nitrite alone. Many studies of mice and rats tested nitrite in combination with specific secondary or tertiary amines or amides, added to the diet or drinking-water, or by gastric intubation. Most combinations resulted in increased incidences of benign and malignant tumours at many organ sites.[8,9] The Working Group concluded that these results provided "sufficient evidence of carcinogenicity" for nitrite in combination with amines or amides.[1]

The combination of positive and negative results from epidemiological

and animal studies is coherent with the mechanism of endogenous formation of *N*-nitroso compounds. The strongest associations were recorded in individuals with high nitrite and low vitamin C intake, a combination that promotes these reactions. The lack of association for nitrate in food might be explained by the fact that vegetables, usually the main source of nitrate intake, are also a source of vitamin C and other antioxidants that inhibit endogenous formation of *N*-nitroso compounds. The lack of association for nitrate in drinking-water might be because of the low exposure in the few studies available; nevertheless, nitrate in drinking-water could result in endogenous nitrosation because water can be consumed without concurrent exposure to nitrosation inhibitors.

Overall, the Working Group concluded that "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (group 2A)".[1] The Working Group refrained from doing a separate overall assessment for nitrate or nitrite, because nitrite is produced endogenously from nitrate and the conditions leading to endogenous formation of *N*-nitroso compounds are often present in a healthy human stomach.

Microcystins and nodularins are cyclic peptide toxins produced by cyanobacteria, a group of organisms found in water and soil. Of these toxins, microcystin-LR is the most common. The toxins are released when the bacteria die or are destroyed (for instance, in the stomach or after water treatment). Eutrophication can cause these toxins to occur at unusually high concentrations. People are exposed to these toxins most frequently through the ingestion of drinking-water or during recreational activities when water is swallowed. Moreover, microcystins and nodularins accumulate in fish, shellfish, and crustaceans. Another potential source of human exposure is through dietary supplements sold as blue-green algae supplements.

In China, several studies of hepatocellular carcinoma[10] and one study of colorectal cancer[11] showed raised incidences in populations that used surface water compared with water from wells. These findings and an apparent correlation with microcystin concentrations in these water sources are noteworthy, but few details were given about microcystin concentrations, other contaminants, and potential confounders, therefore providing inadequate evidence of carcinogenicity.

No 2-year bioassays have been done for these toxins. Microcystin-LR in three experiments and nodularins in one experiment promoted liver preneoplastic lesions in rats.[12]. In a study in mice, microcystins promoted colon preneoplastic foci. A subchronic study with microcystin-LR resulted in persistent neoplastic nodules in mouse liver. Although these findings are indicative of promoting activity, the Working Group regarded them as inadequate evidence.

Strong evidence supported a plausible tumour promoter mechanism for these liver toxins. This mechanism is mediated via the inhibition of protein phosphatases 1 and 2A, an effect shown in rodent liver and hepatocytes.[13] The resulting hyperphosphorylation of intracellular proteins leads to disruption of intermediate filaments forming the cellular scaffold in human and rodent hepatocytes.[12,14] These toxins modulate the expression of oncogenes, early-response genes, and tumour necrosis factor α, and affect cell division, cell survival, and apoptosis.[12,15–17]

After review of the evidence, the Working Group concluded that microcystin-LR is "possibly carcinogenic to humans" (group 2B).[1] For nodularins, fewer studies were available; accordingly, the Working Group regarded nodularins as "not classifiable as to their carcinogenicity" (group 3).[1]

The authors declare no conflicts of interests.

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