

## Nitrate Intake Does Not Influence Bladder Cancer Risk: The Netherlands Cohort Study

Maurice P. Zeegers,<sup>1,2,3</sup> Roel F.M. Selen,<sup>3</sup> Jos C.S. Kleinjans,<sup>4</sup> R. Alexandra Goldbohm,<sup>5</sup> and Piet A. van den Brandt<sup>3</sup>

<sup>1</sup>Department of Public Health and Epidemiology, University of Birmingham, Birmingham, United Kingdom; <sup>2</sup>Comprehensive Cancer Institute Limburg, Department of General Practice, Catholic University of Leuven, Leuven, Belgium; <sup>3</sup>Department of Epidemiology, and <sup>4</sup>Department of Health Risk Analysis and Toxicology, Maastricht University, Maastricht, the Netherlands; <sup>5</sup>Department of Food and Chemical Risk Analysis, TNO Quality of Life, Zeist, the Netherlands

**OBJECTIVES:** *N*-nitroso compounds, endogenously formed from nitrate-derived nitrite, are suspected to be important bladder carcinogens. However, the association between nitrate exposure from food or drinking water and bladder cancer has not been substantially investigated in epidemiologic studies.

**METHODS:** We evaluated the associations between nitrate exposure and bladder cancer in the Netherlands Cohort Study, conducted among 120,852 men and women, 55–69 years of age at entry. Information on nitrate from diet was collected via a food frequency questionnaire in 1986 and a database on nitrate content of foods. Individual nitrate exposures from beverages prepared with tap water were calculated by linking the postal code of individual residence at baseline to water company data. After 9.3 years of follow-up and after excluding subjects with incomplete or inconsistent dietary data, 889 cases and 4,441 subcohort members were available for multivariate analyses. We calculated incidence rate ratios (RR) and corresponding 95% confidence intervals (CIs) using Cox regression analyses. We also evaluated possible effect modification of dietary intake of vitamins C and E (low/high) and cigarette smoking (never/ever).

**RESULTS:** The multivariate RRs for nitrate exposure from food, drinking water, and estimated total nitrate exposure were 1.06 (95% CI, 0.81–1.31), 1.06 (95% CI, 0.82–1.37), and 1.09 (95% CI, 0.84–1.42), respectively, comparing the highest to the lowest quintiles of intake. Dietary intake of vitamins C and E (low/high) and cigarette smoking (never/ever) had no significant impact on these results.

**CONCLUSION:** Although the association between nitrate exposure and bladder cancer risk is biologically plausible, our results in this study do not support an association between nitrate exposure and bladder cancer risk.

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Nitrate is a natural compound of green vegetables, such as lettuce and spinach, and root vegetables, such as beets. Nitrate is also present in drinking water (Gangolli et al. 1994; McKnight et al. 1999; van Loon et al. 1997, 1998). In the late 1990s, the concentration of nitrate in the Netherlands increased in vegetables and drinking water due to cultivation and the use of artificial fertilizers (van Loon et al. 1998), and these nitrate concentrations have remained stable to date (Versteegh et al. 2004). This continued high concentration causes growing concern because of the potential health risks of the metabolites of nitrate and because of their potential relationship with cancer.

There is still a relative deficit of epidemiologic data addressing the association between nitrate exposure and cancer risk. Most of the epidemiologic studies that are available have focused on gastric cancer risk (Boeing et al. 1991; Buiatti et al. 1990; Cantor 1997; Forman 1989; Hansson et al. 1994; Risch et al. 1985; van Loon et al. 1997), but showing little support for the supposed relationship between nitrate and gastric cancer risk. However, an association between nitrate exposure and bladder cancer risk is biologically plausible.

After ingestion, approximately 20% of nitrate is endogenously transformed to nitrite by the bacterial flora of the oral cavity (Weyer et al. 2001). Nitrite can react in the stomach with foodborne secondary amines or amides to form *N*-nitroso compounds (NOCs), depending on the availability of nitrate in the stomach (Mirvish et al. 1987; van Loon et al. 1998; Walker 1990; Weyer et al. 2001). Because approximately 70% of the orally ingested nitrate is excreted in the urine, nitrosation may also occur in the bladder (Gulis et al. 2002; Preston-Martin and Correa 1989; Weyer et al. 2001). Several lifestyle-related factors can influence nitrosation. Food components, such as vitamins C and E, may inhibit the conversion of nitrate into nitrite or block nitrosation (Council of Europe 1995), whereas other factors, such as smoking cigarettes, can promote nitrosation (Ward et al. 2003; Weyer et al. 2001). Smokers appear to have a lower concentration of nitrate in the blood because of the higher thiocyanate concentration, which can be up to four times higher than in nonsmokers (Council of Europe 1995; Mirvish et al. 1987). It is not nitrate per se but its metabolites that are potent rodent carcinogens (Tricker and Preussmann 1991), inducing several types of

cancer including cancer of the stomach, colon, and lymphatic and hematopoietic system, and possibly bladder (Bogovski and Bogovski 1981; Gulis et al. 2002; Mirvish et al. 1987).

Previous epidemiologic studies focused on nitrate exposure and bladder cancer risk are sparse; also, they have addressed nitrate intake only from drinking water and have used ecologic (Gulis et al. 2002; Morales-Suarez-Varela et al. 1995) or case-control (Ward et al. 2003) designs. Only one prospective study on drinking-water nitrate intake and bladder cancer risk has been published so far (Weyer et al. 2001). In that study of the prospective Iowa Women's Health Study, Weyer et al. (2001) found an increased risk of bladder cancer among older women exposed to relatively low drinking-water levels of > 2.46 mg/L/day nitrate, compared with women using < 2.46 mg/L/day [incidence rate ratios (RR), 2.83; 95% confidence interval (CI), 1.11–7.19]. Weyer et al. (2001), however, could not provide data on men (Kantoff 1992).

In the present study, the prospective design, the possibility of investigating nitrate in both food and drinking water, the large study population including both men and women, and the possibility of studying confounding and effect modification by environmental characteristics allowed us to study the association between nitrate intake and bladder cancer incidence in more detail in the Netherlands Cohort Study.

Address correspondence to M.P. Zeegers, Unit of Genetic Epidemiology, Department of Public Health and Epidemiology, University of Birmingham, Public Health Building, Edgbaston, Birmingham, B15 2TT, United Kingdom. Telephone: 44 (121) 414-7878. Fax: 44 (121) 414-7878. E-mail: m.p.zeegers@bham.ac.uk

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