

### Does the Risk of Childhood Diabetes Mellitus Require Revision of the Guideline Values for Nitrate in Drinking Water?

Jan M.S. van Maanen,<sup>1</sup> Harma J. Albering,<sup>1</sup> Theo M.C.M. de Kok,<sup>1</sup> Simone G.J. van Breda,<sup>1</sup> Danielle M.J. Curfs,<sup>1</sup> Ingrid T.M. Vermeer,<sup>1</sup> Anton W. Ambergen,<sup>2</sup> Bruce H.R. Wolffenbuttel,<sup>3</sup> Jos C.S. Kleinjans,<sup>1</sup> and H. Maarten Reeser<sup>4,5</sup>

<sup>1</sup>Department of Health Risk Analysis and Toxicology, <sup>2</sup>Department of Methodology and Statistics, Faculty of Health Sciences, University of Maastricht, Maastricht, The Netherlands; <sup>3</sup>Department of Endocrinology, University Hospital, Maastricht, The Netherlands; <sup>4</sup>Juliana Children's Hospital, The Hague, The Netherlands; <sup>5</sup>TNO Prevention and Health, Leiden, The Netherlands

In recent years, several studies have addressed a possible relationship between nitrate exposure and childhood type 1 insulin-dependent diabetes mellitus. The present ecologic study describes a possible relation between the incidence of type 1 diabetes and nitrate levels in drinking water in The Netherlands, and evaluates whether the World Health Organization and the European Commission standard for nitrate in drinking water (50 mg/L) is adequate to prevent risk of this disease. During 1993–1995 in The Netherlands, 1,104 cases of type 1 diabetes were diagnosed in children 0–14 years of age. We were able to use 1,064 of these cases in a total of 2,829,020 children in this analysis. We classified mean nitrate levels in drinking water in 3,932 postal code areas in The Netherlands in 1991–1995 into two exposure categories. One category was based on equal numbers of children exposed to different nitrate levels (0.25–2.08, 2.10–6.42, and 6.44–41.19 mg/L nitrate); the other was based on cut-off values of 10 and 25 mg/L nitrate. We determined standardized incidence ratios (SIRs) for type 1 diabetes in subgroups of the 2,829,020 children with respect to both nitrate exposure categories, sex, and age and as compared in univariate analysis using the chi-square test for trend. We compared the incidence rate ratios (IRRs) by multivariate analysis in a Poisson regression model. We found an effect of increasing age of the children on incidence of type 1 diabetes, but we did not find an effect of sex or of nitrate concentration in drinking water using the two exposure categories. For nitrate levels > 25 mg/L, an increased SIR and an increased IRR of 1.46 were observed; however, this increase was not statistically significant, probably because of the small number of cases (15 of 1,064). We concluded that there is no convincing evidence that nitrate in drinking water at current exposure levels is a risk factor for childhood type 1 diabetes mellitus in The Netherlands, although a threshold value > 25 mg/L for the occurrence of this disease can not be excluded. *Key words:* drinking water, guideline values, nitrate, type 1 diabetes. *Environ Health Perspect* 108:457–461 (2000). [Online 30 March 2000]

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Dietary vegetables and drinking water are the two main sources of nitrate intake. Drinking water can be the main, if not the only, source of nitrate, particularly in bottle-fed infants and in individuals on low-vegetable diets. Setting standards for nitrate in drinking water therefore is an important instrument in the control of nitrate-related health risks. Several adverse health effects of nitrate exposure have been identified, and different molecular mechanisms have been studied to explain the method of action in different target tissues. After gastrointestinal resorption and recirculation of nitrate taken up by food and/or drinking water, reduction of nitrate to nitrite occurs in the oral cavity and reingested nitrite enters the bloodstream. After absorption in the blood, nitrite is rapidly oxidized to nitrate. In the bloodstream, nitrite is involved in the oxidation of

hemoglobin to methemoglobin, resulting in the impairment of oxygen transport. Nitrite may also react in the stomach with amines and amides to form *N*-nitroso compounds, which have genotoxic properties (1,2), and may also play a causative role in the initiation of gastric cancer (3). A role of *N*-nitroso compounds in the etiology of cancer of the esophagus and nasopharynx (4) as well as a linkage between the occurrence of non-Hodgkin lymphoma and nitrate exposure have been suggested (5). Furthermore, a relationship was discovered between nitrate exposure and congenital malformations, in central nervous system defects in particular, but the evidence was found to be inconclusive (3).

Even though experimental data raise serious concern about the formation of *N*-nitroso compounds and carcinogenic risk, the epidemiologic evidence for an association

between the intake of nitrate and cancer is regarded to be insufficient. Therefore, the World Health Organization (WHO) guideline value for nitrate in drinking water (50 mg/L nitrate units as nitrate ion) was established solely to prevent methemoglobinemia (6). In the European Union, the maximum admissible nitrate level in drinking water was also set at 50 mg/L (7). In a recent review on the role of drinking water nitrate as a cause of infantile methemoglobinemia (7), it was concluded that the standard of 50 mg/L might be unnecessarily strict and that gastrointestinal infection and inflammation and the ensuing overproduction of nitric oxide may be the cause of infantile methemoglobinemia attributed to drinking water nitrate (8). In our view, however, rather than raising the standard of nitrate to levels > 50 mg/L, other adverse health effects of nitrate exposure should be taken into consideration. We suggest that the present drinking water guideline value for nitrate of 50 mg/L needs to be reconsidered for multiple reasons. Even though the guideline value is aimed at the protection of infants as the highest risk group against methemoglobinemia, cases of methemoglobinemia as a result of exposure to nitrate in drinking water < 50 mg/L have been reported (9). In a previous study (1), we observed increased *hprt* variant frequencies in lymphocyte DNA of subjects who used private well water with nitrate levels below the guideline value of 50 mg/L as drinking water. Also, formation of the carcinogenic *N*-nitrosodimethylamine was observed during uptake of nitrate at the acceptable daily intake level—corresponding with the standard of nitrate in drinking water—in combination with nitrosatable

Address correspondence to J.M.S. van Maanen, Department of Health Risk Analysis and Toxicology, University of Maastricht, PO Box 616, 6200 MD Maastricht, The Netherlands. Telephone: 31 43 3881094/3881097. Fax: 31 43 3884146. E-mail: j.vanmaanen@grat.unimaas.nl

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precursors (2). These studies indicate possible genotoxic and carcinogenic effects of nitrate exposure; the current guideline value of 50 mg/L might not be sufficiently protective against these effects. New targets for nitrate exposure were recently defined, including thyroid and pancreas. Nitrate exposure can lead to hypertrophy of the thyroid due to inhibition of uptake of iodine by the thyroid. We observed this effect at drinking water nitrate concentrations above the guideline value of 50 mg/L (10), whereas a German study demonstrated this effect at nitrate levels as low as 22.5 mg/L (11).

In the health risk evaluation of nitrate in drinking water, the possible relationship between nitrate exposure and childhood insulin-dependent diabetes mellitus has not been taken into account. However, in recent years, several investigators have described such a relationship. Again, the formation of *N*-nitroso compounds may be a causative factor in the etiology of this nitrate-induced type 1 diabetes. *N*-nitroso compounds such as the nitrosamides streptozotocin and *N*-nitrosomethylurea have toxic effects on pancreatic  $\beta$ -cells in animals; streptozotocin is used as a model compound to induce type 1 diabetes in rats (12,13). A nitrosamine-rich diet of smoked and cured meat induced damage to pancreatic  $\beta$ -cells and diabetes in mice (14). In an investigation in humans it was suggested that the nitrosamine content of smoked mutton after consumption by the parents caused diabetes in the progeny (15). In the Swedish Childhood Diabetes Study (a case-control study), a dose-response relationship was observed between the frequency of intake of foods rich in nitrosamines, nitrate, and nitrite, and type 1 diabetes (16,17). A Finnish case-control study indicated that high dietary intake of nitrite in children was related to type 1 diabetes, but there was no effect of intake of nitrate or nitrite via drinking water (18). In a case-control study in Australia, no relationship was found between estimated intake of nitrosamines from food and type 1 diabetes (19). In Yorkshire, England, Parslow et al. (20) reported a positive association between nitrate levels in drinking water > 14.85 mg/L and the incidence of type 1 diabetes. An ecologic analysis in the United States also suggested a positive correlation between low-level nitrate exposure through drinking water and type 1 diabetes at nitrate levels < 10 mg/L (21). It should be emphasized that the possible  $\beta$ -cell destruction due to endogenous formation of *N*-nitroso compounds from nitrate is also dependent on other factors such as the presence of amines as nitrosatable precursors, the pH of the stomach, and the presence of inhibitors of nitrosation, such as ascorbic acid.

In The Netherlands, the incidence of type 1 diabetes mellitus has been increasing for several years (22,23). Of particular interest is that between 1990 and 1995 the incidence of type 1 diabetes in children 0–4 years of age has doubled (24). From 1980 to 1995, the incidence of type 1 diabetes mellitus in children 0–14 years of age increased from 11.1 to 14.6 per 100,000/year, which implies a rise of 32% in 15 years (24). This increase in incidence of type 1 diabetes mellitus has been found in several countries (25). The increase of 32% in 15 years cannot be explained by a change in the genetic pool of the general population; it could be suggested that environmental factors may play a role in this increase. Possible dietary factors that may be related to type 1 diabetes are nitrosamines (16) and zinc as well as nitrate concentration in drinking water (20,26).

The aim of our study was *a*) to investigate the possible association between nitrate in drinking water and the incidence of type 1 diabetes mellitus in children in The Netherlands; *b*) to add knowledge to the limited data in this field of research, in particular to make a comparison with other ecologic studies possible [studies that describe the effects of nitrate in drinking water at levels of 10 mg/L (21) and 15 mg/L (20)]; and *c*) to evaluate whether the WHO and European Commission (EC) standard for nitrate in drinking water (50 mg/L) are adequate to prevent risk of this disease.

## Research Design and Methods

We assessed the geographic differences (based on postal code areas) in incidence of type 1 diabetes mellitus in relation to the nitrate concentration in drinking water in The Netherlands. Postal code areas provide a hierarchic classification of addresses. We identified 3,932 four-digit postal code areas in The Netherlands; these areas contain addresses from small villages to town districts. Most of the areas have diameters of approximately 2–3 km.

**Study population.** In 1993, the Dutch Paediatric Surveillance Unit (DPSU) in Leiden, The Netherlands, started a nationwide registry of childhood diabetes in The Netherlands. The target population for the registry is the total population of The Netherlands. Each month, every pediatrician is asked by mail to report to the DPSU with the number of new cases of type 1 diabetes mellitus (and nine other disease states) diagnosed (if any). DPSU registry inclusion criteria are *a*) < 15 years of age at the date of the first insulin injection; *b*) residency in The Netherlands at the start of insulin treatment; and *c*) permanent insulin dependency. Members of the Dutch Diabetes Association (Amersfoort, The Netherlands) provide a

secondary source of concurrence. The registration covers the child's date of birth, the date of the first insulin injection, the place of residence at the time of the first insulin injection (postal code), and the country of origin of both parents (24). We obtained population data for three groups of children 0–14 years of age: 0–4, > 4–9, and > 9–14 years of age, including male/female distribution data in all postal code areas, from Statistics Netherlands (27). A total of 1,104 children 0–14 years of age were diagnosed with type 1 diabetes mellitus between 1993 and 1995.

**Nitrate in drinking water.** We obtained the 1991–1995 drinking water nitrate levels within each four-digit postal code area in from the National Institute of Public Health and Environmental Protection (RIVM) in Bilthoven, The Netherlands (28), and from 25 water supply companies that supply drinking water for the entire country. We obtained the mean, minimum, and maximum nitrate concentrations in the postal code areas for the years 1991–1995. Regulations under the Water Supply Act (28) require that the water supply companies measure nitrate levels per 10,000 inhabitants twice a year. We translated the RIVM and the water supply company data into data for postal code areas; one water supply company could not provide the data for postal code areas and was omitted from the analysis. We calculated mean values of nitrate concentrations in drinking water based on the samples taken from 1991 to 1995 per postal code area.

We studied two categories of nitrate exposure levels. The first category (nitrate exposure levels) involved three nitrate concentration ranges: < 10, 10–25, and > 25 mg/L. We chose these values because 25 mg/L is the recommended nitrate level in drinking water as defined by the EC Drinking Water Directive (29). The maximally acceptable concentration of nitrate in drinking water as defined by the European Union is 50 mg/L (7). In this study we observed that the mean levels of nitrate in drinking water in the postal code areas do not exceed this limit value of 50 mg/L in The Netherlands. We chose 10 mg/L as the cut-off value because an ecologic study in the United States (21) indicated an effect of a 10-mg/L nitrate exposure level on the incidence of type 1 diabetes, and to make a comparison with the U.S. study possible.

The second category was based on an equal distribution of the population of children 0–14 years of age. The nitrate concentration ranges in this category were 0.25–2.08, 2.10–6.42, and 6.44–41.19 mg/L for the three age groups of 0–4, > 4–9, and > 9–14 years of age, respectively; a distinction was

made between males and females. In the three ranges of drinking water nitrate concentrations with an equal distribution of the population, the population numbers are approximately equal because of the differences in numbers of inhabitants in 3,932 postal code areas—each code with a distinct mean nitrate concentration—which have to be divided over the three ranges of nitrate concentrations.

Data from diabetes incidences diagnosed between 1993 and 1995 were correlated with the mean nitrate concentration in drinking water in the postal code areas in 1991–1995. Of the 1,104 cases of type 1 diabetes, 23 cases could not be used in the analysis because the residence postal code area was unknown. Because of the unavailability of the data of nitrate concentrations in postal code areas of one water supply company, as well as from a small amount of other postal code areas of other water supply companies, 17 additional cases of type 1 diabetes were lost. We used a total of 1,064 type 1 diabetes cases in the period 1993–1995 in the study among a total number of 2,829,020 children 0–14 years of age.

**Statistical analysis.** We performed statistical analyses using SPSS (SPSS Inc., Chicago, IL). Two different analyses were performed: the chi-square test for trend (univariate analysis) and a Poisson regression model (multivariate analysis). This statistical approach is the same as that used in the Yorkshire study on the effect of nitrate in drinking water on the incidence of type 1 diabetes in children (20). The two statistical analyses were performed using two different categorizations of nitrate exposure levels.

We calculated standardized incidence ratios (SIRs) for type 1 diabetes mellitus as the ratio of observed to expected cases  $\times 100$ . We determined SIRs for the subgroups with different nitrate levels, sex, and age, and compared SIRs in univariate analysis using the chi-square test for trend (30). We performed a comparison of incidence rate ratios (IRRs) by multivariate analysis in a Poisson regression model (log-linear model) using nitrate concentrations, sex, and age as variables. In the Poisson regression model, three groups with different nitrate exposure ranges, two groups (one of each sex), and three groups with different ages were formed; thus, 18 subgroups were compared with respect to the proportion of diabetes cases to total subgroup numbers in one model. We evaluated the two categories of nitrate exposure levels in the univariate as well as in the multivariate analysis. Thus, no control population was involved in this ecologic study, but we studied the differences between subgroups of all children 0–14 years of age in The Netherlands by both univariate and multivariate analysis. We also

analyzed the possible interactions between nitrate levels, sex, and age. The relationship between nitrate concentrations and the incidence of type 1 diabetes was also studied in the Poisson regression model using the nitrate concentration in drinking water as a continuous variable.

## Results

The results of the statistical analysis using the univariate analysis are shown in Table 1; the results of the Poisson regression modeling are shown in Tables 2 and 3. The statistical parameters show that there is no effect of increased nitrate concentrations on the incidence of type 1 diabetes mellitus in children 0–14 years of age in The Netherlands. This result is obtained when using the categorization of drinking water nitrate concentrations with cut-off values of 10 and 25 mg/L, as well as the different nitrate exposure levels with three groups of equal populations. The univariate analysis showed an increased SIR of 1.457 for the nitrate concentration range  $> 25$  mg/L (Table 1). However, the number of patients was very small (15 of the 1,064 patients were within this range) and the corresponding 95% confidence interval of the SIR was 0.85–2.07. Thus, the increase in SIR was not statistically significant. The chi-square test for trend showed a  $p$ -value of 0.573, indicating that the increase in SIR was not statistically significant. Using the three ranges of drinking water nitrate concentrations with equal population numbers, we did not find any increased SIRs. The multivariate analysis using the Poisson regression model showed  $p$ -values of 0.385 and 0.599 for the two categories of nitrate exposure levels [cut-off values of 10 and 25 mg/L, respectively (Table 2), and equal population numbers (Table 3)]. Thus, both the univariate and multivariate analyses show no effect of nitrate drinking water levels on the incidence of type 1 diabetes. No effect of sex on the incidence of type 1 diabetes was observed. However, a clear effect of increasing age among children

0–14 years of age on the incidence of type 1 diabetes was observed in both the univariate and multivariate analysis. No effect of possible interaction between nitrate levels, sex, and age was observed. In addition, when nitrate concentrations were studied as a continuous variable, there was no effect on the incidence of type 1 diabetes ( $p = 0.65$ ).

## Discussion

Recently, Parslow et al. (20) found a positive association between nitrate levels  $> 14.85$  mg/L in drinking water and the incidence of type 1 diabetes in Yorkshire, north-ern England. This paper was published after the 1996 revision of the guidelines for drinking water quality (6). Such an association at drinking water levels below the target value of 25 mg/L set by the EC (29) raises the question whether the WHO guideline value of 50 mg/L, which is suitable to prevent methemoglobinemia, should be reevaluated in the context of risk of type 1 diabetes.

Our ecologic study is comparable with the Yorkshire study (20) in the design and statistical approach used. Both studies have certain limitations in terms of interpretation, e.g., we did not gather information on the quantity of water consumed for each individual, information on the length of residence time in a particular postal code area, or information on other risk factors such as family history of type 1 diabetes. However, the strength of both studies lies in the large population studied. In the Yorkshire study, 1,797 cases of diabetes in children 0–16 years of age were diagnosed over a period of 16 years (between 1978 and 1994) in a total population of 696,032 children; the nitrate data were based on samples analyzed between 1990 and 1995. In our study, which describes incidences of type 1 diabetes diagnosed in 1993–1995 for the entire country of The Netherlands, 1,064 cases were observed in 2,829,020 children 0–14 years of age; the nitrate data are based on samples tested between 1991 and 1995.

**Table 1.** Standardized incidence ratios for type 1 diabetes by two categories of mean nitrate levels, sex, and age.

| Variable            | Range      | Population <sup>a</sup> | O   | E   | SIR   | 95% CI    | $p$ -Value <sup>b</sup> |
|---------------------|------------|-------------------------|-----|-----|-------|-----------|-------------------------|
| Mean nitrate (mg/L) | < 10       | 2,318,360               | 869 | 872 | 0.997 | 0.93–1.06 | 0.573                   |
|                     | 10–25      | 483,295                 | 180 | 182 | 0.990 | 0.84–1.14 |                         |
|                     | > 25       | 27,365                  | 15  | 10  | 1.457 | 0.85–2.07 |                         |
| Mean nitrate (mg/L) | 0.25–2.08  | 941,200                 | 363 | 354 | 1.025 | 0.92–1.13 | 0.949                   |
|                     | 2.10–6.42  | 942,085                 | 338 | 354 | 0.954 | 0.85–1.06 |                         |
|                     | 6.44–41.19 | 945,735                 | 363 | 356 | 1.021 | 0.92–1.12 |                         |
| Sex                 | Male       | 1,446,430               | 525 | 544 | 0.965 | 0.88–1.05 | 0.244                   |
|                     | Female     | 1,382,590               | 539 | 520 | 1.037 | 0.95–1.12 |                         |
| Age (years)         | 0–4        | 983,755                 | 305 | 370 | 0.824 | 0.72–0.93 | 0.000                   |
|                     | > 4–9      | 943,125                 | 367 | 355 | 1.035 | 0.93–1.14 |                         |
|                     | > 9–14     | 902,140                 | 392 | 339 | 1.155 | 1.05–1.26 |                         |

Abbreviations: E, expected; O, observed.

<sup>a</sup>Ages 0–14 years. <sup>b</sup>Chi-square test for trend.

**Table 2.** Incidence rate ratios of type 1 diabetes for different mean nitrate levels (based on cut-off values of 10 and 25 mg/L), sex, and age in a Poisson regression model.

| Variable                  | Range  | IRR  | 95% CI    | p-Value |
|---------------------------|--------|------|-----------|---------|
| Mean nitrate level (mg/L) | < 10   | 1    | —         | 0.385   |
|                           | 10–25  | 0.99 | 0.85–1.17 |         |
|                           | > 25   | 1.46 | 0.88–2.43 |         |
| Sex                       | Male   | 1    | —         | 0.244   |
|                           | Female | 1.07 | 0.95–1.21 |         |
| Age (years)               | 0–4    | 1    | —         | 0.00004 |
|                           | > 4–9  | 1.25 | 1.08–1.46 |         |
|                           | > 9–14 | 1.40 | 1.21–1.63 |         |

Thus, our study included approximately 2.8 million children 0–14 years of age, approximately 4 times the number of children included in the Yorkshire study. The number of cases of type 1 diabetes mellitus in Yorkshire was higher than in our study, and cases were gathered over a period of 16 years (approximately 100 cases/year). In our study, we gathered data over a period of 3 years (350 cases/year); also, the periods of collecting data on nitrate levels and diabetes cases are more coincident with our study. The Yorkshire study found higher nitrate drinking water concentrations as compared to our study. In our study, 82% of the children 0–14 years of age were exposed to nitrate concentrations < 10 mg/L; 17% were exposed to nitrate concentrations > 10 mg/L but below the 25 mg/L European Union target value for nitrate (7); approximately 1% were exposed to nitrate concentration between 25 and 41 mg/L (the highest value). In the Yorkshire study, 30% of the drinking water samples exceeded the 25 mg/L level; the nitrate levels in the highest of the three exposure groups fell in the range of 14.85–40.01 mg/L. In our study, the highest range of nitrate levels of the three exposure groups with equal populations was 6.44–41.19 mg/L. It could be suggested that higher nitrate levels in drinking water in Yorkshire (e.g., > 15 mg/L) are responsible for the increased incidence of type 1 diabetes mellitus. Although we observed an increased SIR and IRR for nitrate levels > 25 mg/L, this increase was not statistically significant. However, the number of cases was very small: 15 of 1,064. Thus, in contrast to the Yorkshire study finding of a threshold value for type 1 diabetes > 15 mg/L nitrate in drinking water, the results of our study substantiate the possibility of a threshold value > 25 mg/L. We found an effect of increasing age among children 0–14 years of age on the incidence of type 1 diabetes. However, we did not find any interaction between nitrate levels and age; the possible effect of nitrate on the incidence of type 1 diabetes did not vary across age groups.

**Table 3.** Incidence rate ratios of type 1 diabetes for different mean nitrate levels (based on equal numbers of exposed children), sex, and age in a Poisson regression model.

| Variable                  | Range      | IRR  | 95% CI    | p-Value  |
|---------------------------|------------|------|-----------|----------|
| Mean nitrate level (mg/L) | 0.25–2.08  | 1    | —         | 0.599    |
|                           | 2.10–6.42  | 0.94 | 0.81–1.08 |          |
|                           | 6.44–41.19 | 1.00 | 0.87–1.16 |          |
| Sex                       | Male       | 1    | —         | 0.244    |
|                           | Female     | 1.07 | 0.95–1.21 |          |
| Age (years)               | 0–4        | 1    | —         | 0.000014 |
|                           | > 4–9      | 1.27 | 1.09–1.48 |          |
|                           | > 9–14     | 1.43 | 1.23–1.66 |          |

We cannot confirm the conclusion of the Yorkshire study (20) that nitrate in drinking water may be a precursor of chemicals which are toxic to the pancreas. The results of our study are also in contrast with an ecologic analysis in Colorado. Kostraba et al. (21) suggested a positive correlation between nitrate levels < 10 mg/L and the incidence of type 1 diabetes mellitus. They found an increased risk in the third tertile of water nitrate levels (0.77–8.2 mg/L) as compared to the first tertile (0.0–0.084 mg/L). Lower nitrate levels were observed in the Colorado study than in our study; nitrate levels in the third tertile were even below the range of our third tertile of 6.44–41.19 mg/L. In our study, > 18% of the subjects were exposed to nitrate levels > 10 mg/L; therefore, it was possible to study differences in the effect of nitrate in drinking water on the incidence of type 1 diabetes above and below 10 mg/L. The nitrate level categories, which were based on equal numbers of children (0.25–2.08, 2.10–6.42, and 6.44–41.19 mg/L), made it possible to study an effect already occurring < 10 mg/L. No effect of increased nitrate levels in drinking water on the incidence of type 1 diabetes was observed in either category. This result is in contrast with the result of the Colorado study. The Colorado study used a simple linear regression analysis between nitrate levels and the incidence of type 1 diabetes ( $p$ -value = 0.03), which is less suitable for an ecologic analysis than the models used in Yorkshire and in our study.

The evidence that increased intake of nitrate, nitrite, and *N*-nitroso compounds can lead to increased incidence of type 1 diabetes is conflicting. Case-control studies applying food frequency questionnaires show both negative [an Australian study (19)] and positive [a Swedish study (16)] effects of intake of foods with high nitrosamine content. In the Swedish study (16), intake of food rich in nitrosamines and intake of food rich in nitrate and nitrite were associated with increased incidence of type 1 diabetes, whereas a Finnish study showed an

effect of intake of nitrite—but not of nitrate—in food, but no effect of intake of nitrate and nitrite via drinking water (18). The ecologic studies of the possible correlation between nitrate in drinking water and incidence of type 1 diabetes now comprise a positive study [the Yorkshire study (20)], a less clear-cut positive study [the Colorado study (21)], and our study, which is negative for nitrate in drinking water. The Finnish study (18) is also negative for nitrate in drinking water, but the nitrate levels in domestic water were particularly low (< 5 mg/L).

The mechanism of  $\beta$ -cell damage leading to type 1 diabetes mellitus is probably a T-cell mediated autoimmune process. The concordance of diabetes mellitus type 1 among monozygotic twins is < 50%, which indicates that the disease must be caused, in part, by nongenetic mechanisms, i.e., environmental factors. In recent years, hypotheses involving a multifactorial etiology have been developed that imply the role of fetal virus infections, early exposure to cow's milk, and a high exposure level of nitrosamines as determinants of initiators of  $\beta$ -cell autoimmunity (31,32). Viral-nutritional interactions in the gut, including nitrosamines in particular, have been proposed to be involved (31,32). As an example, a 10-fold increase in risk was observed in the Swedish study (17) when combining a variable measuring infectious disease with the frequency of food consumption containing nitrosamines. However, this role for nitrosamines as nutritional factor still has to be proven. Haverkos (32) mentions that this hypothesis involving nitrosamines is one of several possible combinations of environmental factors associated with type 1 diabetes mellitus.

There is no convincing evidence that the intake of nitrate in The Netherlands at present levels in drinking water leads to increased risk of diabetes mellitus type 1, although our results indicate the possible presence of a threshold value > 25 mg/L. The possible role of nitrate in a multifactorial process cannot be excluded. Environmental factors are still considered to play an important role in the etiology of type 1 diabetes mellitus. In a study of incidence of type 1 diabetes among Moroccan children in The Netherlands (children whose parents both emigrated from Morocco, which is considered a low risk area for type 1 diabetes), the incidence was 1.5 times higher than that of Dutch children (33). Again, environmental factors may play an important role in this phenomenon. The sharp rise (78%) in incidence of type 1 diabetes mellitus between 1990 and 1995 in children 0–4 years of age in The Netherlands and the 32% rise in 15 years between 1980 and 1995 in children 0–14 years of age suggest an important role for

environmental factors that are involved in an early process of  $\beta$ -cell destruction; alternatively, environmental factors may have changed the expression of genetic factors. We conclude that further studies, including case-control studies, are needed to evaluate the possible role of environmental factors in the increased incidence of type 1 diabetes mellitus, and to indicate more accurately a safety level of nitrate in drinking water to avoid increased risk of diabetes. The present WHO and EU standards of 50 mg/L might not be adequate to prevent risk of this disease (6,7).

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