Commentary: Nitrites, nitrates and nitrosation as causes of brain cancer in children: epidemiological challenges

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Very little is known about the causes of brain cancer in childhood although this is the most common solid tumour diagnosed in paediatric populations. Globally, it is estimated that there are over 20,000 new diagnoses and 10,000 deaths from brain cancer in children under 15 years annually. A compelling hypothesis of carcinogenesis for childhood brain cancer, originally based on animal experiments, was formulated over 35 years ago and implicated prenatal exposure to environmental nitrate and nitrite. This is because many commonly found proteins (amines, amides, ureas) can be converted (nitrosated) to their carcinogenic N-nitroso derivatives by reaction with nitrite. Nitrite is occasionally found in the environment but most human exposure occurs through ingested nitrate that can be broken down to nitrite by oral bacteria often found in saliva. Nitrate salts are among the key components within inorganic fertilizers and the increased dependency of farming practices on such fertilizers over several decades has led to increasing levels of human exposure to nitrates. This arises from consumption of the resulting crops and, to a greater extent, from drinking water supplies that have been subject to growing nitrate contamination from agricultural land run-off. Drinking water contamination from agricultural land run-off.

has been subject to legislation providing maximum permitted nitrate and nitrite concentrations for some time.\textsuperscript{8,9}

The nitrosation pathway leading from nitrates to cancer has generated a mass of laboratory research since first formulated\textsuperscript{5,10} but, in general, has failed to find much support from epidemiology. One important reason for this has been the Achilles heel of so much epidemiology: lack of adequate methods of historical exposure assessment. Crude measures of nitrate exposure from food sources have been estimated in case–control\textsuperscript{11} and cross-sectional study designs\textsuperscript{12} using questionnaires but these measures suffer from all the usual difficulties associated with dietary epidemiology compounded with limited data on nitrate concentrations in foods. The considerable variability (both geographic and historic) of nitrate levels in drinking water make this parameter even harder to assess in epidemiological studies although a number of attempts have been made.\textsuperscript{13} Understandably these have been more successful in situations where stable communities have been dependant on single sources of drinking water with constant levels of nitrate such as areas of South America with high natural deposits.\textsuperscript{14} In developed countries with mobile populations consuming both public and bottled water, ascertainment of a reliable exposure measure becomes an extremely difficult undertaking.

A further complicating factor is that the cancers most often studied with regard to nitrate exposure have been those of the upper gastrointestinal tract, notably stomach, where latency periods are lengthy and relevant exposure windows maybe long and extend back decades in the past. The hypothesis that prenatal exposure to nitrates may be aetiologically involved in childhood brain cancer does define a relatively small critical time window that, at least in theory, may make the epidemiological logistics somewhat more straightforward. This was the hope in the international collaborative study of these cancers reported by Mueller and colleagues.\textsuperscript{15} In this study of 836 childhood cases and 1485 matched controls from seven countries, detailed questions were asked regarding the source of water consumed by the mother during the index pregnancy. The objective was then to sample the residential drinking water supply and test for nitrate and nitrite using a rapid dipstick procedure.

This represents just about the best possible attempt at trying to target the relevant exposure information within the context of a retrospective study design. The time interval of relevance can be precisely specified and it is both quite short and relatively close to the time period of information ascertainment (at least compared with other types of cancer). Use of objective and quantitative exposure data from the dipstick determinations should be a significant advantage although these do depend on the assumption that nitrate/nitrite levels have been constant in the time since the pregnancy and, of course, that the dipstick measures are reliable and valid.

Unfortunately the epidemiological practicalities did not keep pace with the design—only 185 and 341 of the case and control mothers, respectively, were able to provide a water sample from the residence during pregnancy (no samples at all could be collected from three of the nine study centres). Additional stratification of the results, by histological subtype of brain cancer, involved a further halving of these numbers. It was these stratified data, based on approximately 15% of the subjects that produced the ‘headline’ result of a statistically significant association between elevated nitrite levels in drinking water and risk of astrocytoma. Mueller and colleagues would have preferred to refine the analysis still further, by excluding cases and controls where mothers reported drinking bottled water during pregnancy, but at this point in the study against declining study numbers was declared lost.

It is important to note that the statistically significant results were for nitrite and not nitrate. There was an increased but non-significant odds ratio for water nitrate above the recommended guideline level of 50 mg/litre but no indication of elevated risk below this level. Nitrite concentrations in drinking water are normally negligible and the presence of detectable levels is an indication of either bacterial contamination or inadequacies in the chloramination purification process. The presence of nitrite in 19% of the water samples in this study may, therefore, be regarded as surprisingly high given that the threshold for dipstick detection was 1 mg/ml and, for example, the European Community Drinking Water Directive has a maximum allowance of 0.5 mg/ml.\textsuperscript{9} The authors do raise concerns about the accuracy of some of the nitrate determinations but not those for nitrite. Well water is more likely to contain nitrite than public water and, in a separate analysis, there was a positive association of risk associated with the observed small number of well water users during pregnancy but only in some study centres.

So what can be concluded from the Mueller study? From a public health perspective, the nitrate results maybe considered reassuring. Nitrate pollution of the environment is a growing concern\textsuperscript{7} but the suggested health risks, especially the cancer risks, have never been confirmed and certainly not quantified (indeed some recent work\textsuperscript{5} has indicated beneficial effects on health). The absence of any observed brain cancer risk with nitrate in drinking water below guideline levels is, therefore, a helpful conclusion. The results for nitrite are certainly of more concern but, assuming the dipstick quantification is correct, water with the concentrations of nitrite associated with the increased risk should not now be publicly supplied. Well water users have been warned for some considerable time about the non-cancer health risks associated with high levels of nitrate and nitrite, especially methaemoglobinemia.\textsuperscript{7}

The foregoing messages have to be tempered, however, with a consideration of the methodological limitations. The study exemplifies just how hard it can be at times to obtain relevant exposure information in epidemiology\textsuperscript{16} especially for diseases, such as cancer, with extensive latency periods. Despite the objective nature of the exposure assessment tool, it is unknown what degree of bias can be introduced when samples are missing from such a large proportion of cases and controls—this is in addition to the bias bought about by failures in the initial recruitment process in this type of study.\textsuperscript{17} The hypothesis of prenatal exposure to N-nitroso compounds should, in principle, have been an area where one may have anticipated molecular epidemiology to have made an impact given the substantive understanding of how these compounds interact with DNA. Indeed nitrosation was a topic that engendered several innovative methodologies within the emerging discipline of molecular epidemiology.\textsuperscript{18–20} These were, however, all techniques that assessed whether nitrosation as a mechanism was associated with cancer—an important biological question but not one that addresses the public health issue of the hazard...
associated with drinking water contaminants. There is currently no biomarker that assesses nitrate or nitrite in drinking water better than direct measurement of these ions in the water supply. Perhaps one way out of this exposure dilemma, at least for exposures such as those considered here, is to conduct studies in populations where historical drinking water monitoring data are relevant, reliable, and available to researchers. Water quality is subject to regular inspection in many countries and is now often sampled on a small area basis employing state-of-the-art analytical technology. Such data, if they can be accessed and extend back long enough, should be an epidemiological goldmine for this type of study. Routine statistics have been a longstanding foundation stone for epidemiology and still have a major role to play in the epidemiology of the future.

References