

EXHIBIT C

“When Does Nitrate Become a Risk for Humans?,” Journal of Environmental Quality
37:291-295 (2008)

ACLC R5-2016-0531 Sweeney Submission of Evidence

University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Agronomy & Horticulture -- Faculty Publications

Agronomy and Horticulture Department

1-1-2008

When Does Nitrate Become a Risk for Humans?

David S. Powlson
Rothamsted Research

Tom M. Addiscott
Rothamsted Research

Nigel Benjamin
Derriford Hospital

Kenneth G. Cassman
University of Nebraska - Lincoln, kcassman1@unl.edu

Theo M. de Kok
University Maastricht

See next page for additional authors

Follow this and additional works at: <http://digitalcommons.unl.edu/agronomyfacpub>

 Part of the [Plant Sciences Commons](#)

Powlson, David S.; Addiscott, Tom M.; Benjamin, Nigel; Cassman, Kenneth G.; de Kok, Theo M.; van Grinsven, Hans; L'hirondel, Jean-Louis; Avery, Alex A.; and Van Kessel, Chris, "When Does Nitrate Become a Risk for Humans?" (2008). *Agronomy & Horticulture -- Faculty Publications*. Paper 102.
<http://digitalcommons.unl.edu/agronomyfacpub/102>

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

David S. Powlson, Tom M. Addiscott, Nigel Benjamin, Kenneth G. Cassman, Theo M. de Kok, Hans van Grinsven, Jean-Louis L'hirondel, Alex A. Avery, and Chris Van Kessel

When Does Nitrate Become a Risk for Humans?

David S. Powlson and Tom M. Addiscott Rothamsted Research

Nigel Benjamin Derriford Hospital

Ken G. Cassman University of Nebraska

Theo M. de Kok University Maastricht

Hans van Grinsven Netherlands Environmental Assessment Agency

Jean-Louis L'hirondel Centre Hospitalier Universitaire de Caen

Alex A. Avery Hudson Institute

Chris van Kessel* University of California–Davis

Is nitrate harmful to humans? Are the current limits for nitrate concentration in drinking water justified by science? There is substantial disagreement among scientists over the interpretation of evidence on the issue. There are two main health issues: the linkage between nitrate and (i) infant methaemoglobinaemia, also known as blue baby syndrome, and (ii) cancers of the digestive tract. The evidence for nitrate as a cause of these serious diseases remains controversial. On one hand there is evidence that shows there is no clear association between nitrate in drinking water and the two main health issues with which it has been linked, and there is even evidence emerging of a possible benefit of nitrate in cardiovascular health. There is also evidence of nitrate intake giving protection against infections such as gastroenteritis. Some scientists suggest that there is sufficient evidence for increasing the permitted concentration of nitrate in drinking water without increasing risks to human health. However, subgroups within a population may be more susceptible than others to the adverse health effects of nitrate. Moreover, individuals with increased rates of endogenous formation of carcinogenic N-nitroso compounds are likely to be susceptible to the development of cancers in the digestive system. Given the lack of consensus, there is an urgent need for a comprehensive, independent study to determine whether the current nitrate limit for drinking water is scientifically justified or whether it could safely be raised.

Is nitrate harmful to humans? Are the current limits for nitrate concentration in drinking water justified by science? These questions were addressed at a symposium on "The Nitrogen Cycle and Human Health" held at the annual meeting of the Soil Science Society of America (SSSA). Although they sound like old questions, it became clear there is still substantial disagreement among scientists over the interpretation of evidence on the issue—disagreement that has lasted for more than 50 years.

This article is based on the discussion at the SSSA meeting and subsequent email exchanges between some of the participants. It does not present a consensus view because some of the authors hold strongly divergent views, drawing different conclusions from the same data. Instead, it is an attempt to summarize, to a wider audience, some of the main published information and to highlight current thinking and the points of contention. The article concludes with some proposals for research and action. Because of the divergent views among the authors, each author does not necessarily agree with every statement in the article.

Present Regulatory Situation

In many countries there are strict limits on the permissible concentration of nitrate in drinking water and in many surface waters. The limit is 50 mg of nitrate L⁻¹ in the EU and 44 mg L⁻¹ in the USA (equivalent to 11.3 and 10 mg of nitrate-N L⁻¹, respectively). These limits are in accord with WHO recommendations established in 1970 and recently reviewed and reconfirmed (WHO, 2004). The limits were originally set on the basis of human health considerations, although environmental concerns, such as nutrient enrichment and eutrophication of surface waters, are now seen as being similarly relevant. It is the health

Copyright © 2008 by the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Published in *J. Environ. Qual.* 37:291–295 (2008).
doi:10.2134/jeq2007.0177

Received 10 Apr. 2007.

*Corresponding author (cvankessel@ucdavis.edu).

© ASA, CSSA, SSSA

677 S. Segoe Rd., Madison, WI 53711 USA

D.S. Powlson and T.M. Addiscott, Soil Science Dep., Rothamsted Research, Harpenden, Herts AL5 2JQ, United Kingdom; N. Benjamin, Derriford Hospital, Brest Rd, Derriford, Plymouth, PL6 5AA, United Kingdom; K.G. Cassman, Dep. of Agronomy and Horticulture, Univ. of Nebraska, Lincoln, NE, 68583 USA; T.M. de Kok, Dep. of Health Risk Analysis and Toxicology, University Maastricht, P.O. Box 616, 6200 MD the Netherlands; H. van Grinsven, Netherlands Environmental Assessment Agency, P.O. Box 303, 3720 AH Bilthoven, the Netherlands; J.-L. L'hirondel, Service de rhumatologie, Centre Hospitalier Universitaire de Caen, 14033 Caen Cedex, France; A.A. Avery, Center for Global Food Issues, Hudson Inst., PO Box 202, Churchville, VA 24421 USA; C. van Kessel, Dep. of Plant Sciences, Univ. of California, Davis, CA, 95616 USA.

issues that are the main cause of disagreement; the contrasting views are set out in the following two sections.

Nitrate and Health

There are two main health issues: the linkage between nitrate and (i) infant methaemoglobinaemia, also known as blue baby syndrome, and (ii) cancers of the digestive tract. The evidence for nitrate as a cause of these serious diseases remains controversial and is considered below.

An Over-States Problem?

The link between nitrate and the occurrence of methaemoglobinaemia was based on studies conducted in the 1940s in the midwest of the USA. In part, these studies related the incidence of methaemoglobinaemia in babies to nitrate concentrations in rural well water used for making up formula milk replacement. Comly (1945), who first investigated what he called "well-water methaemoglobinaemia," found that the wells that provided water for bottle feeding infants contained bacteria as well as nitrate. He also noted that "In every one of the instances in which cyanosis (the clinical symptom of methaemoglobinaemia) developed in infants, the wells were situated near barnyards and pit privies." There was an absence of methaemoglobinaemia when formula milk replacements were made with tap water. Re-evaluation of these original studies indicate that cases of methaemoglobinaemia always occurred when wells were contaminated with human or animal excrement and that the well water contained appreciable numbers of bacteria and high concentrations of nitrate (Avery, 1999). This strongly suggests that methaemoglobinaemia, induced by well water, resulted from the presence of bacteria in the water rather than nitrate *per se*. A recent interpretation of these early studies is that gastroenteritis resulting from bacteria in the well water stimulated nitric oxide production in the gut and that this reacted with oxyhaemoglobin in blood, converting it into methaemoglobin (Addiscott, 2005).

The nearest equivalent to a present-day toxicological test of nitrate on infants was made by Cornblath and Hartmann (1948). These authors administered oral doses of 175 to 700 mg of nitrate per day to infants and older people. None of the doses to infants caused the proportion of haemoglobin converted to methaemoglobin to exceed 7.5%, strongly suggesting that nitrate alone did not cause methaemoglobinaemia. Furthermore, Hegesh and Shiloah (1982) reported another common cause of infant methaemoglobinaemia: an increase in the endogenous production of nitric oxide due to infective enteritis. This strongly suggests that many early cases of infant methaemoglobinaemia attributed at that time to nitrate in well water were in fact caused by gastroenteritis. Many scientists now interpret the available data as evidence that the condition is caused by the presence of bacteria rather than nitrate (Addiscott, 2005; L'hirondel and L'hirondel, 2002). The report of the American Public Health Association (APHA, 1950) formed the main basis of the current recommended 50 mg L⁻¹ nitrate limit, but even the authors of the report

recognized that it was compromised by unsatisfactory data and methodological bias. For example, in many cases, samples of water from wells were only taken for nitrate analysis many months after the occurrence of infant methaemoglobinaemia.

About 50 epidemiological studies have been made since 1973 testing the link between nitrate and stomach cancer incidence and mortality in humans, including Forman et al. (1985) and National Academy of Sciences (1981). The Chief Medical Officer in Britain (Acheson, 1985), the Scientific Committee for Food in Europe (European Union, 1995), and the Subcommittee on Nitrate and Nitrite in Drinking Water in the USA (NRC, 1995) all concluded that no convincing link between nitrate and stomach cancer incidence and mortality had been established.

A study reported by Al-Dabbagh et al. (1986) compared incidence of cancers between workers in a factory manufacturing nitrate fertilizer (and exposed to a high intake of nitrate through dust) and workers in the locality with comparable jobs but without the exposure to nitrate. There was no significant difference in cancer incidence between the two groups.

Based on the above findings showing no clear association between nitrate in drinking water and the two main health issues with which it has been linked, some scientists suggest that there is now sufficient evidence for increasing the permitted concentration of nitrate in drinking water without increasing risks to human health (L'hirondel et al., 2006; Addiscott, 2005).

Space does not permit here to discuss other concerns expressed about dietary nitrate, such as risk to mother and fetus, genotoxicity, congenital malfunction, enlarged thyroid gland, early onset of hypertension, altered neurophysiological function, and increased incidence of diabetes. For differing views of other possible health concerns, see L'hirondel and L'hirondel (2002) and Ward et al. (2006).

Nitrate is made in the human body (Green et al., 1981), the rate of production being influenced by factors such as exercise (Allen et al., 2005). In recent years it has been shown that body cells produce nitric oxide from the amino acid L-arginine and that this production is vital to maintain normal blood circulation (Richardson et al., 2002) and protection from infection (Benjamin, 2000). Nitric oxide is rapidly oxidized to form nitrate, which is conserved by the kidneys and concentrated in the saliva. Nitrate can also be chemically reduced to nitric oxide in the stomach, where it can aid in the destruction of swallowed pathogens that can cause gastroenteritis.

Evidence is emerging of a possible benefit of nitrate in cardiovascular health. For example, the coronaries of rats provided water for 18 mo that contained sodium nitrate became thinner and more dilated than the coronaries of the rats in the control group (Shuval and Gruener, 1977). Nitrate levels in water showed a negative correlation coefficient with the standardized mortality ratio for all cardiovascular diseases (Pocock et al., 1980). In healthy young volunteers, a short-term increase in dietary nitrate reduced diastolic blood pressure (Larsen et al., 2006). Based on these data, one could hypothesize that nitrate might also play a role in the cardiovascular health benefit of vegetable consumption (many vegetables contain high concentrations of nitrate) (Lundberg et al., 2004).

The Need for Caution

Although there is little doubt that normal physiological levels of nitric oxide play a functional role in vascular endothelial function and the defense against infections (Dykhuizen et al., 1996), chronic exposure to nitric oxide as a result of chronic inflammation has also been implicated, though not unequivocally identified, as a critical factor to explain the association between inflammation and cancer (Sawa and Oshima, 2006; Dincer et al., 2007; Kawanishi et al., 2006). Nitric oxide and NO-synthase are known to be involved in cancer-related events (angiogenesis, apoptosis, cell cycle, invasion, and metastasis) and are linked to increased oxidative stress and DNA damage (Ying and Hofseth, 2007). Rather than nitrate, the presence of numerous classes of antioxidants is generally accepted as the explanation for the beneficial health effects of vegetable consumption (Nishino et al., 2005; Potter and Steinmetz, 1996).

A recent review of the literature suggests that certain subgroups within a population may be more susceptible than others to the adverse health effects of nitrate (Ward et al., 2005). Although there is evidence showing the carcinogenicity of N-nitroso compounds in animals, data obtained from studies that were focused on humans are not definitive, with the exception of the tobacco-specific nitrosamines (Grosse et al., 2006). The formation of N-nitroso compounds in the stomach has been connected with drinking water nitrate, and excretion of N-nitroso compounds by humans has been associated with nitrate intake at the acceptable daily intake level through drinking water (Vermeer et al., 1998). The metabolism of nitrate and nitrite, the formation of N-nitroso compounds, and the development of cancers in the digestive system are complex processes mediated by several factors. Individuals with increased rates of endogenous formation of carcinogenic N-nitroso compounds are likely to be susceptible. Known factors altering susceptibility to the development of cancers in the digestive system are inflammatory bowel diseases, high red meat consumption, amine-rich diets, smoking, and dietary intake of inhibitors of endogenous nitrosation (e.g., polyphenols and vitamin C) (de Kok et al., 2005; De Roos et al., 2003; Vermeer et al., 1998). In 1995, when the Subcommittee on Nitrate and Nitrate in Drinking Water reported that the evidence to link nitrate to gastric cancer was rather weak (NRC, 1995), the stomach was still thought to be the most relevant site for endogenous nitrosation. Previous studies, such as those reviewed in the NRC (1995) report, which found no link between nitrate and stomach cancer, concentrated on the formation of nitrosamines in the stomach. Recent work indicates that larger amounts of N-nitroso compounds can be formed in the large intestine (Cross et al., 2003; De Kok et al., 2005).

Some scientists argue that there are plausible explanations for the apparent contradictory absence of adverse health effects of nitrate from dietary sources (Van Grinsven et al., 2006; Ward et al., 2006). Individuals with increased rates of endogenous formation of carcinogenic N-nitroso compounds are more likely to be at risk, and such susceptible subpopulations should be taken into account when trying to make a risk-benefit analysis for the intake of nitrate. In view of these complex dose-response mechanisms, it can be argued that it is not surprising that ecological and cohort

studies (e.g., Van Loon et al., 1998) in general do not provide statistically significant evidence for an association between nitrate intake and gastric, colon, or rectum cancers. The experimental design of most of these studies may not have been adequate to allow for the determination of such a relationship.

Population studies have the problem that factors influencing health tend to be confounded with each other. This necessitates molecular epidemiological studies aimed at improving methods for assessing exposure in susceptible subgroups. This approach requires the development of biomarkers that enable the quantification of individual levels of endogenous nitrosation and N-nitroso compounds exposure and methods for accurate quantification of exposure-mediating factors.

Nitrate, Food Security, and the Environment

It is beyond dispute that levels of nitrate and other N-containing species have increased in many parts of the ecosystem due to increased use of fertilizers and combustion of fossil fuels. At present, 2 to 3% of the population in USA and the EU are potentially exposed to public or private drinking water exceeding the present WHO (and USA and EU) standard for nitrate in drinking water. The proportion of the exposed population in the emerging and developing economies is probably larger and increasing (Van Grinsven et al., 2006).

The environmental impacts of reactive N compounds are serious, and continued research on agricultural systems is essential to devise management practices that decrease losses and improve the utilization efficiency of N throughout the food chain. At the same time, the central role of N in world agriculture must be considered. Agriculture without N fertilizer is not an option if the 6.5 billion people currently in the world and the 9 billion expected by 2050 are to be fed (Cassman et al., 2003). Losses of reactive N compounds to the environment are not restricted to fertilizers: losses from manures and the residues from legumes can also be large (Ad-discott, 2005). Research indicates that simply mandating a reduction in N fertilizer application rates does not automatically reduce N losses because there is typically a poor relationship between the amount of N fertilizer applied by farmers and the N uptake efficiency by the crops (Cassman et al., 2002; Goulding et al., 2000). Instead, an integrated systems management approach is needed to better match the amount and timing of N fertilizer application to the actual crop N demand in time and space. Such an approach would lead to decreased losses of reactive N to the environment without decreasing crop yields. Many of the potential conflicts between the agricultural need for N and the environmental problems caused by too much in the wrong place are being studied within the International Nitrogen Initiative (INI; <http://initrogen.org/>), a networking activity sponsored by several international bodies.

The adverse environmental impact of reactive N species (i.e., all N-containing molecules other than the relatively inert N_2 gas that comprises 78% of the atmosphere) deserves attention. Some of these molecules, such as nitrogen oxides, come from combustion of fossil fuels in automobiles and power plants. Agriculture, however, is the dominant source through the cultivation of N_2 -fixing crops and the manufacture and use of N fertilizers (Turner and Rabalais, 2003). Both have increased greatly over the

last few decades, and the trend is set to continue (Galloway et al., 2003; 2004). The subsequent N enrichment causes changes to terrestrial and aquatic ecosystems and to the environmental services they provide. Examples include nitrate runoff to rivers causing excessive growth of algae and associated anoxia in coastal and estuarine waters (James et al., 2005; Rabalais et al., 2001) and deposition of N-containing species from the atmosphere causing acidification of soils and waters and N enrichment to forests and grassland savannahs (Goulding et al., 1998). All of these impacts can radically change the diversity and numbers of plant and animal species in these ecosystems. Other impacts almost certainly have indirect health effects, such as nitrous oxide production, which contributes to the greenhouse effect and the destruction of the ozone layer, thereby allowing additional UV radiation to penetrate to ground level with the associated implications for the prevalence of skin cancers.

Losses of nitrate to drinking water resources are also associated with leaky sewage systems. Leaky sewage systems need to be improved for general hygiene considerations. This need is especially important in developing countries and poor rural areas that do not have well developed sewage and waste disposal infrastructure.

Returning Question

In considering the management of nitrogen in agriculture and its fate in the wider environment, the debate keeps returning to the original question: "Is nitrate in drinking water really a threat to health?" Interpretations of the evidence remain very different (L'hirondel et al., 2006; Ward et al., 2006). The answer has a significant economic impact. The current limits established for ground and surface waters require considerable changes in practice by water suppliers and farmers in many parts of the world, and these changes have associated costs. If nitrate in drinking water is not a hazard to health, could the current limit be relaxed, perhaps to 100 mg L⁻¹? The relaxation could be restricted to situations where the predominant drainage is to groundwater. Such a change would allow environmental considerations to take precedence in the case of surface waters where eutrophication is the main risk, and N limits could be set to avoid damage to ecosystem structure and function. Phosphate is often the main factor limiting algal growth and eutrophication in rivers and freshwater lakes, so a change in the nitrate limit would focus attention on phosphate and its management—correctly so in the view of many environmental scientists (Sharpley et al., 1994). It is possible that a limitation on phosphate might lead to even lower nitrate limits in some freshwater aquatic environments to restore the diversity of submerged plant life (James et al., 2005). It could be argued that setting different limits, determined by health or environmental considerations as appropriate, is a logical response to the scientific evidence.

Given the criticisms of the scientific foundation of present drinking water standards and the associated cost-benefits of prevention or removal of nitrate in drinking water, we propose the need to consider the following issues in discussing an adjustment of the nitrate standards for drinking water:

- Nitrogen intake by humans has increased via drinking water and eating food such as vegetables.

- There is circumstantial and often indirect evidence of the enhanced risk of cancers of the digestive system after an increase in the concentration of nitrate in drinking water. There is an urgent need to synthesize existing data and understanding, or to carry out additional research if necessary, to reach clear and widely accepted conclusions on the magnitude of the risk. This will require greater collaboration between scientists who hold opposing views over the interpretation of currently available data. The possibility that subgroups within the population respond differently requires quantification and critical examination.
- Nitrogen oxides have a functional role in normal human physiology, but they are also involved in the induction of oxidative stress and DNA damage. The challenge is to quantify and evaluate these risks and benefits of nitric oxide exposure in relation to the intake of nitrate in drinking water. If humans have a mechanism to combat infectious disease with nitric oxide, produced from nitrate consumed in drinking water and food, what are the long-term effects of the nitric oxide benefits compared with the potential negative health effects from higher intake of nitrate?
- If the evaluation of potential adverse health effects from chronic exposure to nitrate levels in drinking water above 50 mg L⁻¹ demonstrates that these adverse effects can be considered minor compared with other issues of health loss associated with air pollution or life style, would the removal of nitrate from drinking water to meet the current allowable concentration standards be cost-efficient relative to other potential investments in health improvement?

Although science may not provide society with unequivocal conclusions about the relationship between drinking water nitrate and health over the short term, there are good reasons to further explore the issue (Ward et al., 2005). Unfortunately, it remains difficult to predict the health risks associated with chronic nitrate consumption from water that exceeds the current WHO drinking water standard. One complication is the endogenous production of nitrate, which makes it more difficult than previously realized to relate health to nitrate intake in water or food.

Practical management strategies to overcome inefficient use of nitrogen by crops and to minimize losses of nitrate and other N-containing compounds to the environment have to be developed for agricultural systems worldwide.

Given the lack of consensus, there is an urgent need for a comprehensive, independent study to determine whether the current nitrate limit for drinking water is scientifically justified or whether it could safely be raised. Meta-analyses are valuable tools for generating conclusions about specific chronic health effects (e.g., stomach cancer, colon cancer, bladder cancer, specific reproductive outcomes). Unfortunately, the number of suitable studies for any particular health effect is likely too small to be detected by meta-analyses (Van Grinsven et al., 2006). Empirical studies focused on susceptible subgroups, development of biomarkers for demonstration of endogenous nitrosation, and methods for

accurate quantification of mediating factors may provide part of the answers. Moreover, there is also a separate need for determining water quality standards for environmental integrity of aquatic ecosystems. It is time to end 50 yr of uncertainty and move forward in a timely fashion toward science-based standards.

References

- Acheson, E.D. 1985. Nitrate in drinking water. HMSO, London, UK.
- Addiscott, T.M. 2005. Nitrate, agriculture, and environment. CABI Publ., Wallingford, Oxfordshire, UK.
- Al-Dabbagh, S., D. Forman, D. Bryson, I. Stratton, and R. Doll. 1986. Mortality of nitrate fertilizer workers. *Brit. J. Industr. Med.* 43:507-515.
- Allen, J.D., F.R. Cobb, and A.J. Gow. 2005. Regional and whole-body markers of nitric oxide production following hyperemic stimuli. *Free Radical Biol. Med.* 38:1164-1169.
- APHA. 1950. Committee on water supply: Nitrate in potable waters and methaemoglobinemia. *Am. Public Health Assoc. Yearb.* 40:110-115.
- Avery, A.A. 1999. Infantile methaemoglobinemia: Reexamining the role of drinking water nitrates. *Environ. Health Perspect.* 107:583-586.
- Benjamin, N. 2000. Nitrates in the human diet—Good or bad? *Ann. Zootechnol.* 49:207-216.
- Cassman, K.G., A.D. Dobermann, and D.T. Walters. 2002. Agroecosystems, N-use efficiency, and N management. *Ambio* 31:132-140.
- Cassman, K.G., A.D. Dobermann, D.T. Walters, and H. Yang. 2003. Meeting cereal demand while protecting natural resources and improving environmental quality. *Ann. Rev. Environ. Resour.* 28:315-358.
- Comly, H.H. 1945. Cyanosis in infants caused by nitrates in well water. *JAMA* 129:112-116.
- Cornblath, M., and A.F. Hartmann. 1948. Methaemoglobinemia in young infants. *J. Pediatr.* 33:421-425.
- Cross, A.J., J.R. Pollock, and S.A. Bingham. 2003. Heme, not protein or inorganic iron, is responsible for endogenous intestinal n-nitrosation arising from red meat. *Cancer Res.* 63:2358-2360.
- de Kok, T.M.C.M., L.G.J.B. Engels, E.J. Moonen, and J.C.S. Kleinjans. 2005. Inflammatory bowel disease stimulates formation of carcinogenic N-nitroso compounds. *Gut* 54:731.
- De Roos, A.J., M.H. Ward, C.F. Lynch, and K.P. Cantor. 2003. Nitrate in public water systems and the risk of colon and rectum cancers. *Epidemiology* 14:640-649.
- Dincer, Y., Y. Erzin, S. Himmetoglu, K. Nur Gunes, K. Bal, and T. Akcay. 2007. Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. *Dig. Dis. Sci.*, DOI 10.1007/s10620-00609386-8.
- Dykhuizen, R.S., A. Fraser, C. Duncan, C.C. Smith, M. Golden, B. Benjamin, and C. Leifert. 1996. Antimicrobial effect of acidified nitrite on gut pathogens: Importance of dietary nitrate in host defense. *Antimicrob. Agents Chemother.* 40:1422-1425.
- European Union. 1995. European Commission Directorate-General III Industry. Scientific Committee for Food. Opinion on Nitrate and Nitrite. Annex 4 to Document III/5611/95.
- Forman, D., A. Al-Dabbagh, and R. Doll. 1985. Nitrate, nitrite, and gastric cancer in Great Britain. *Nature* 313:620-625.
- Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, and B.J. Cosby. 2003. The nitrogen cascade. *Bioscience* 53:1-16.
- Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asier, C. Cleveland, P. Green, E. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A. Townsend, and C. Vorosmary. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70:153-226.
- Goulding, K.W.T., N.J. Bailey, N.J. Bradbury, P. Hargreaves, M. Howe, D.V. Murphy, P.R. Poulton, and T.W. Willison. 1998. Nitrogen deposition and its contribution to nitrogen cycling and associated processes. *New Phytol.* 139:49-58.
- Goulding, K.W.T., P.R. Poulton, C.P. Webster, and M.T. Howe. 2000. Nitrogen leaching from the Broadbalk Wheat Experiment, Rothamsted, UK, as influenced by fertilizer and manure inputs and weather. *Soil Use Manage.* 16:244-250.
- Green, L.C., K. Ruiz de Luzuriaga, D.A. Wagner, W. Rand, N. Isfan, V.R. Young, and S.R. Tannenbaum. 1981. Nitrate biosynthesis in man. *Proc. Natl. Acad. Sci. USA* 78:7764-7768.
- Grosse, Y., R. Baan, K. Straif, B. Secretan, F. El Ghissassi, and V. Cogliano. 2006. Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *Lancet Oncol.* 7:628-629.
- Hegesh, E., and J. Shiloah. 1982. Blood nitrates and infantile methaemoglobinemia. *Clin. Chim. Acta* 125:107-125.
- James, C., J. Fisher, V. Russel, S. Collings, and B. Moss. 2005. Nitrate availability and hydrophyte species richness in shallow lakes. *Freshwater Biol.* 50:1049-1063.
- Kawanishi, S., Y. Hiraku, S. Pinlaos, and N. Ma. 2006. Oxidative and nitrate DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol. Chem.* 387:365-372.
- Larsen, F.J., B. Ekblom, K. Sahlin, J.O. Lundberg, and E. Weitzberg. 2006. Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.* 355:2792-2793.
- L'hirondel, J.-L., A.A. Avery, and T. Addiscott. 2006. Dietary nitrate: Where is the risk? *Environ. Health Perspect.* 114:A458-459.
- L'hirondel, J., and J.L. L'hirondel. 2002. Nitrate and man: Toxic, harmless, or beneficial? CABI Publ., Wallingford, Oxfordshire, UK.
- Lundberg, J.O., E. Weitzberg, J.A. Cole, and N. Benjamin. 2004. Opinion—Nitrate, bacteria and human health. *Nat. Rev. Microbiol.* 2:593-602.
- National Academy of Sciences. 1981. The health effects of nitrate, nitrite and N-nitroso compounds. Committee on Nitrite and Alternative Curing Agents in Food. Part 1. National Academy Press, Washington, DC.
- National Research Council. 1995. Nitrate and nitrite in drinking water. National Research Council. Subcommittee on Nitrate and Nitrite in Drinking Water. National Academy Press, Washington, DC.
- Nishino, H., M. Murakoshi, W.Y. Mou, S. Wada, M. Msuda, Y. Ohsaka, Y. Satomi, and K. Jinno. 2005. Cancer prevention by phytochemicals. *Oncology* 69:38-40 (suppl.).
- Pocock, S.J., A.G. Shaper, D.G. Cook, R.F. Packham, R.F. Lacey, P. Powell, and P.F. Russell. 1980. British regional health study—Geographic variations in cardiovascular mortality, and the role of water quality. *BMJ* 280:1243-1249.
- Potter, J.D., and K. Steinmetz. 1996. Vegetables, fruit, and phytoestrogens as preventive agents. *IARC Sci. Publ.* 139:61-90.
- Rabalais, N.N., R.E. Turner, and W.J. Wiseman. 2001. Hypoxia in the Gulf of Mexico. *J. Environ. Qual.* 30:320-329.
- Richardson, G., S.L. Hicks, S. O'Byrne, M.T. Frost, K. Moore, N. Benjamin, and G.M. McKnight. 2002. The ingestion of inorganic nitrate increases gastric S-nitrosothiol levels and inhibits platelet function in humans. *Nitric Oxide* 7:24-29.
- Sawa, T., and H. Oshima. 2006. Nitrate DNA damage in inflammation and its possible role in carcinogenesis. *Nitric Oxide* 14:91-100.
- Sharpley, A.N., S.C. Shapra, R. Wedepohl, J.T. Sims, T.C. Daniel, and K.R. Reddy. 1994. Managing agricultural phosphorus for protection of surface waters: Issues and options. *J. Environ. Qual.* 23:437-451.
- Shuval, H.I., and N. Gruener. 1977. Health effects of nitrates in water. Report EPA-600/1-77-030. USEPA, Cincinnati, OH.
- Turner, R.E., and N.N. Rabalais. 2003. Linking landscape and water quality in the Mississippi River basin for 200 years. *Bioscience* 53:563-572.
- Van Grinsven, H.J.M., M.H. Ward, N. Benjamin, and T.M.C.M. de Kok. 2006. Does the evidence about health risks associated with nitrate ingestion warrant an increase of the nitrate standard for drinking water? *Environ. Health* 5:26 doi:10.1186/1476-069X-5-26.
- Van Loon, A.J., A.A. Botterweck, R.A. Goldbohm, H.A. Brants, J.D. van Klaveren, and P.A. van den Brandt. 1998. Intake of nitrate and nitrite and the risk of gastric cancer: A prospective cohort study. *British J. Cancer* 78:129-135.
- Vermeer, I.T.M., D.M.F.A. Pachet, J.W. Dallinga, J.C.S. Kleinjans, and J.M.S. van Maanen. 1998. Volatile N-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet. *Environ. Health Perspect.* 106:459-463.
- Ward, M.H., T.M. de Kok, P. Levallois, J. Brender, G. Gulis, B.T. Nolan, and J. VanDerslice. 2005. Workgroup report: Drinking water nitrate and health—Recent findings and research needs. *Environ. Health Perspect.* 113:1607-1614.
- Ward, M.H., T.M. de Kok, P. Levallois, J. Brender, G. Gulis, J. VanDerslice, and B.T. Nolan. 2006. Respond to dietary nitrate: Where is the risk? *Environ. Health Perspect.* 114:A459-A460.
- World Health Organization. 2004. Recommendations: nitrate and nitrite. p. 417-420. *In* Guidelines for drinking-water quality, 3rd ed. WHO, Geneva, Switzerland.
- Ying, L., and L.J. Hofseth. 2007. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res.* 67:1407-1410.

EXHIBIT D

“Saturated Zone Denitrification: Potential for Natural Attenuation of Nitrate Contamination in Shallow Groundwater Under Dairy Operations,” Environmental Science and Technology, 41:759-765 (2007)

ACLC R5-2016-0531 Sweeney Submission of Evidence

Saturated Zone Denitrification: Potential for Natural Attenuation of Nitrate Contamination in Shallow Groundwater Under Dairy Operations

M. J. SINGLETON,*† B. K. ESSER,†
J. E. MORAN,† G. B. HUDSON,†
W. W. MCNAB,‡ AND T. HARTER§

Chemical Sciences Division, Lawrence Livermore National Laboratory, Environmental Restoration Division, Lawrence Livermore National Laboratory, and Department of Land, Air, and Water Resources, University of California at Davis

We present results from field studies at two central California dairies that demonstrate the prevalence of saturated-zone denitrification in shallow groundwater with ^3H / ^3He apparent ages of <35 years. Concentrated animal feeding operations are suspected to be major contributors of nitrate to groundwater, but saturated zone denitrification could mitigate their impact to groundwater quality. Denitrification is identified and quantified using N and O stable isotope compositions of nitrate coupled with measurements of excess N_2 and residual NO_3^- concentrations. Nitrate in dairy groundwater from this study has $\delta^{15}\text{N}$ values (4.3–61‰), and $\delta^{18}\text{O}$ values (–4.5–24.5‰) that plot with $\delta^{18}\text{O}/\delta^{15}\text{N}$ slopes of 0.47–0.66, consistent with denitrification. Noble gas mass spectrometry is used to quantify recharge temperature and excess air content. Dissolved N_2 is found at concentrations well above those expected for equilibrium with air or incorporation of excess air, consistent with reduction of nitrate to N_2 . Fractionation factors for nitrogen and oxygen isotopes in nitrate appear to be highly variable at a dairy site where denitrification is found in a laterally extensive anoxic zone 5 m below the water table, and at a second dairy site where denitrification occurs near the water table and is strongly influenced by localized lagoon seepage.

Introduction

High concentrations of nitrate, a cause of methemoglobinemia in infants (1), are a national problem in the United States (2), and nearly 10% of public drinking water wells in the state of California are polluted with nitrate at concentrations above the maximum contaminant level (MCL) for drinking water set by the U.S. Environmental Protection Agency (3). The federal MCL is 10 mg/L as N, equivalent to the California EPA limit of 45 mg/L as NO_3^- (all nitrate concentrations are hereafter given as NO_3^-). In the agricultural areas of California's Central Valley, it is not uncommon

to have nearly half the active drinking water wells produce groundwater with nitrate concentrations in the range considered to indicate anthropogenic impact (>13–18 mg/L) (2, 4). The major sources of this nitrate are septic discharge, fertilization using natural (e.g., manure) or synthetic nitrogen sources, and concentrated animal feeding operations. Dairies are the largest concentrated animal operations in California, with a total herd size of 1.7 million milking cows (5).

Denitrification is the microbially mediated reduction of nitrate to gaseous N_2 , and can occur in both unsaturated soils and below the water table where the presence of NO_3^- , denitrifying bacteria, low O_2 concentrations, and electron donor availability exist. In the unsaturated zone, denitrification is recognized as an important process in manure and fertilizer management (6). Although a number of field studies have shown the impact of denitrification in the saturated zone (e.g., 7, 8–11), prior to this study it was not known whether saturated zone denitrification could mitigate the impact of nitrate loading at dairy operations. The combined use of tracers of denitrification and groundwater dating allows us to distinguish between nitrate dilution and denitrification, and to detect the presence of pre-modern water at two dairy operations in the Central Valley of California, referred to here as the Kings County Dairy (KCD) and the Merced County Dairy (MCD; Figure 1). Detailed descriptions of the hydrogeologic settings and dairy operations at each site are included as Supporting Information.

Materials and Methods

Concentrations and Nitrate Isotopic Compositions. Samples for nitrate N and O isotopic compositions were filtered in the field to 0.45 μm and stored cold and dark until analysis. Anion and cation concentrations were determined by ion chromatography using a Dionex DX-600. Field measurements of dissolved oxygen and oxidation reduction potential (using Ag/AgCl with 3.33 mol/L KCl as the reference electrode) were carried out using a Horiba U-22 water quality analyzer. The nitrogen and oxygen isotopic compositions ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of nitrate in 23 groundwater samples from KCD and MCD were measured at Lawrence Berkeley National Laboratory's Center for Isotope Geochemistry using a version of the denitrifying bacteria procedure (12) as described in Singleton et al. (13). In addition, the nitrate from 17 samples was extracted by ion exchange procedure of (14) and analyzed for $\delta^{15}\text{N}$ at the University of Waterloo. Analytical uncertainty (1 σ) is 0.3‰ for $\delta^{15}\text{N}$ of nitrate and 0.5‰ for $\delta^{18}\text{O}$ of nitrate. Isotopic compositions of oxygen in water were determined on a VG Prism isotope ratio mass spectrometer at Lawrence Livermore National Laboratory (LLNL) using the CO_2 equilibration method (15), and have an analytical uncertainty of 0.1‰.

Membrane Inlet Mass Spectrometry. Previous studies have used gas chromatography and/or mass spectrometry to measure dissolved N_2 gas in groundwater samples (16–19). Dissolved concentrations of N_2 and Ar for this study were analyzed by membrane inlet mass spectrometry (MIMS), which allows for precise and fast determination of dissolved gas concentrations in water samples without a separate extraction step, as described in Kana et al. (20, 21). The gas abundances are calibrated using water equilibrated with air under known conditions of temperature, altitude, and humidity (typically 18 °C, 183 m, and 100% relative humidity). A small isobaric interference from CO_2 at mass 28 (N_2) is corrected based on calibration with CO_2 -rich waters with known dissolved N_2 , but is negligible for most samples. Samples are collected for MIMS analysis in 40 mL amber

* Corresponding author address: P.O. Box 808, L-231, Livermore, California, 94550; phone: (925) 424-2022; fax: (925) 422-3160; e-mail: singleton20@llnl.gov.

† Chemical Sciences Division, Lawrence Livermore National Laboratory.

‡ Environmental Restoration Division, Lawrence Livermore National Laboratory.

§ University of California at Davis.

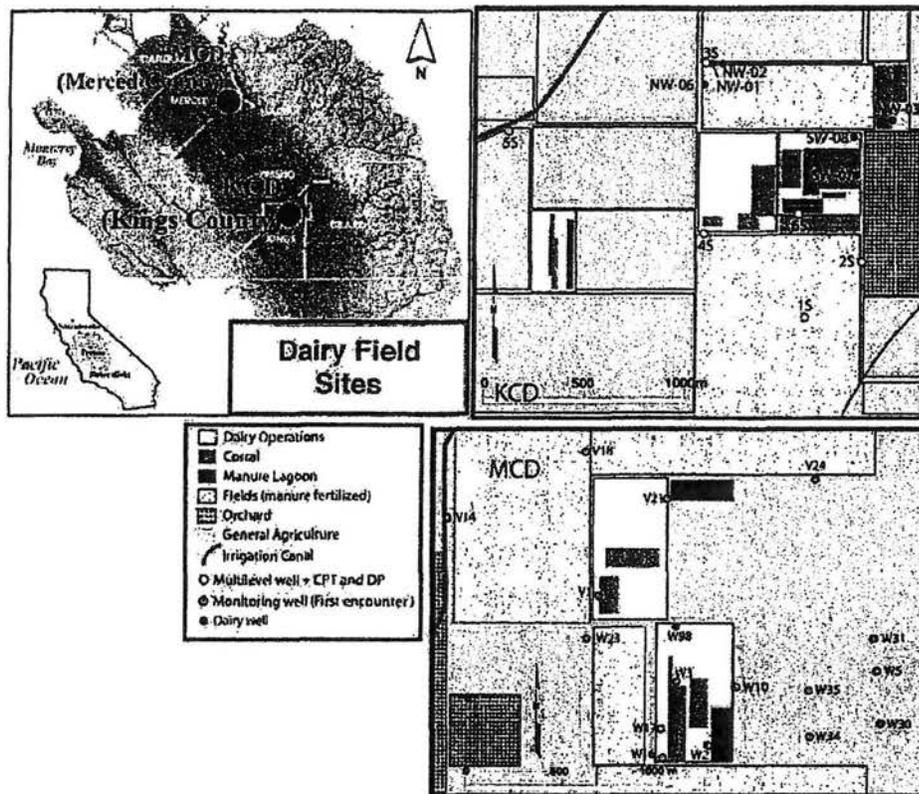


FIGURE 1. Location of dairy study sites, and generalized maps of each dairy showing sample locations relative to lagoons and dairy operations.

glass VOA vials with no headspace that are kept cold during transport, and then analyzed within 24 h.

Noble Gases and $^3\text{H}/^3\text{He}$ Dating. Dissolved noble gas samples are collected in copper tubes, which are filled without bubbles and sealed with a cold weld in the field. Dissolved noble gas concentrations were measured at LLNL after gas extraction on a vacuum manifold and cryogenic separation of the noble gases. Concentrations of He, Ne, Ar, and Xe were measured on a quadrupole mass spectrometer. The ratio of ^3He to ^4He was measured on a VG5400 mass spectrometer. Calculations of excess air and recharge temperature from Ne and Xe measurements are described in detail in Ekwurzel (22), using an approach similar to that of Aeschbach-Hertig et al. (23).

Tritium samples were collected in 1 L glass bottles. Tritium was determined by measuring ^3He accumulation after vacuum degassing each sample and allowing 3–4 weeks accumulation time. After correcting for sources of ^3He not related to ^3H decay (24, 25), the measurement of both tritium and its daughter product ^3He allows calculation of the initial tritium present at the time of recharge, and apparent ages can be determined from the following relationship based on the production of tritogenic helium ($^3\text{He}_{\text{tr}}$):

$$\text{Groundwater Apparent Age (years)} = -17.8 \times \ln(1 + ^3\text{He}_{\text{tr}}/^3\text{H})$$

Groundwater age dating has been applied in several studies of basin-wide flow and transport (25–27). The reported groundwater age is the mean age of the mixed

sample, and furthermore, is only the age of the portion of the water that contains measurable tritium. Average analytical error for the age determinations is ± 1 year, and samples with ^3H that is too low for accurate age determination (< 1 pCi/L) are reported as > 50 years. Significant loss of ^3He from groundwater is not likely in this setting given the relatively short residence times and high infiltration rates from irrigation. Apparent ages give the mean residence time of the fraction of recently recharged water in a sample, and are especially useful for comparing relative ages of water from different locations at each site. The absolute mean age of groundwater may be obscured by mixing along flow paths due to heterogeneity in the sediments (28).

Results and Discussion

Nitrate in Dairy Groundwater. Nitrate concentrations at KCD range from below detection limit (BDL, < 0.07 mg/L) to 274 mg/L. Within the upper aquifer, there is a sharp boundary between high nitrate waters near the surface and deeper, low nitrate waters. Nitrate concentrations are highest between 6 and 13 m below ground surface (BGS) at all multilevel wells (0.5 m screened intervals), with an average concentration of 98 mg/L. Groundwater below 15 m has low nitrate concentrations ranging from BDL to 2.8 mg/L, and also has low or nondetectable ammonium concentrations. The transition from high to low nitrate concentration corresponds to decreases in field-measured oxidation–reduction potential (ORP) and dissolved oxygen (DO) concentration. ORP values are generally above 0 mV and DO concentrations are > 1 mg/L in the upper 12 m of the aquifer, defining a more oxidizing zone (Figure 2). A reducing zone is indicated below

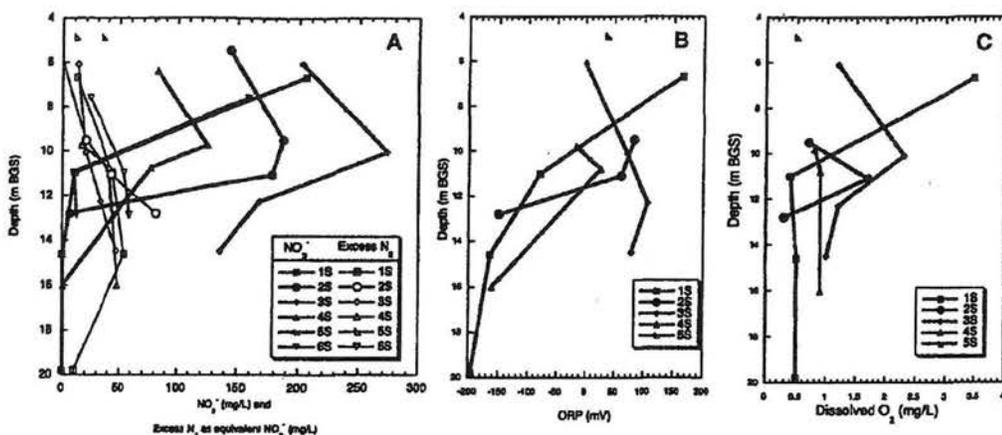


FIGURE 2. (A) Average excess N_2 and nitrate concentrations, (B) oxidation-reduction potential (ORP), and (C) dissolved oxygen in multilevel monitoring wells at the KCD site.

12 m by ORP values as low as -196 mV and DO concentrations <1.2 mg/L. Vertical head varies by less than 10 cm in the upper aquifer multilevel wells.

Nitrate concentrations at MCD monitoring wells sampled for this study range from 2 to 426 mg/L with an average of 230 mg/L. Several wells (W-02, W-16, and W-17) located next to a lagoon and coral have lower nitrate but high ammonium concentrations (Table 1 in Supporting Information). The MCD wells are all screened at the top of the unconfined aquifer except W96, a supply well that is pumped from approximately 57 m BGS. Nitrate concentrations observed for this deeper well are <1 mg/L.

Dissolved Gases. Nitrogen gas, the comparatively conservative product of denitrification, has been used as a natural tracer to detect denitrification in the subsurface (16–18). Groundwater often also contains N_2 beyond equilibrium concentrations due to incorporation of excess air from physical processes at the water table interface (23, 29, 30). In the saturated zone, total dissolved N_2 is a sum of these three sources:

$$(N_2)_{\text{dissolved}} = (N_2)_{\text{equilibrium}} + (N_2)_{\text{excess air}} + (N_2)_{\text{denitrification}}$$

By normalizing the measured dissolved concentrations as N_2/Ar ratios, the amount of excess N_2 from denitrification can be calculated as

$$(N_2)_{\text{denitrification}} = \left(\frac{N_2}{Ar} \right)_{\text{measured}} - \left(\frac{N_2_{\text{equilibrium}} + N_2_{\text{excess air}}}{Ar_{\text{equilibrium}} + Ar_{\text{excess air}}} \right) Ar_{\text{measured}}$$

where the N_2 and Ar terms for equilibrium are calculated from equilibrium concentrations determined by gas solubility. The N_2/Ar ratio is relatively insensitive to recharge temperature, but the incorporation of excess air must be constrained in order to determine whether denitrification has shifted the ratio to higher values (19). Calculations of excess N_2 based on the N_2/Ar ratio assume that any excess air entrapped during recharge has the ratio of N_2/Ar in the atmosphere (83.5). Any partial dissolution of air bubbles would lower the N_2/Ar ratio (30, 31), thus decreasing the apparent amount of excess N_2 .

For this study, Xe and Ne derived recharge temperature and excess air content were determined for 12 of the monitoring wells at KCD and 9 wells at MCD. For these sites, excess N_2 can be calculated directly, accounting for the contribution of excess air and recharge temperature. Site

representative mean values of recharge temperature and excess air concentration are used for samples without noble gas measurements. Mean annual air temperatures at the KCD and MCD sites are 17 and 16 °C, respectively (32), and the Xe-derived average recharge temperatures for the KCD and MCD sites are 19 and 18 °C. Recharge temperatures are most likely higher than mean annual air temperature because most recharge is from excess irrigation during the summer months. The average amount of excess air indicated by Ne concentrations is 2.2×10^{-3} cm³(STP)/g H₂O for KCD and 1.7×10^{-3} cm³(STP)/g H₂O for MCD. From these parameters, we estimate the site representative initial N_2/Ar ratios including excess air to be 41.2 for KCD and 40.6 for MCD. Measured N_2/Ar ratios greater than these values are attributed to production of N_2 by denitrification.

The excess N_2 concentration can be expressed in terms of the equivalent reduced nitrate that it represents in mg/L NO_3^- based on the stoichiometry of denitrification. Considering excess N_2 in terms of equivalent NO_3^- provides a simple test to determine whether there is a mass balance between nitrate concentrations and excess N_2 . From Figure 2, there does not appear to be a balance between nitrate concentrations and excess N_2 in KCD groundwater, since nitrate concentrations in the shallow wells are more than twice that of equivalent excess N_2 concentrations in the anoxic zone. There are multiple possible causes of the discrepancy between NO_3^- concentrations and excess N_2 concentrations including (1) the NO_3^- loading at the surface has increased over time, and denitrification is limited by slow vertical transport into the anoxic zone, (2) mixing with deeper, low initial NO_3^- waters has diluted both the NO_3^- and excess N_2 concentrations, or (3) some dissolved N_2 has been lost from the saturated zone. All three processes may play a role in N cycling at the dairies, but we can shed some light on their relative importance by considering the extent of denitrification and then constraining the time scale of denitrification as discussed in the following sections.

Isotopic Compositions of Nitrate. Large ranges in $\delta^{15}N$ and $\delta^{18}O$ values of nitrate are observed at both dairies (Figure 3). Nitrate from KCD has $\delta^{15}N$ values of 4.3–61.1‰, and $\delta^{18}O$ values of -0.7 –24.5‰. At MCD, nitrate $\delta^{15}N$ values range from 5.3 to 30.2‰, and $\delta^{18}O$ values range from -0.7 to 13.1‰. The extensive monitoring well networks at these sites increase the probability that water containing residual nitrate from denitrification can be sampled.

Nitrate $\delta^{15}N$ and $\delta^{18}O$ values at both dairies are consistent with nitrification of ammonium and mineralized organic N

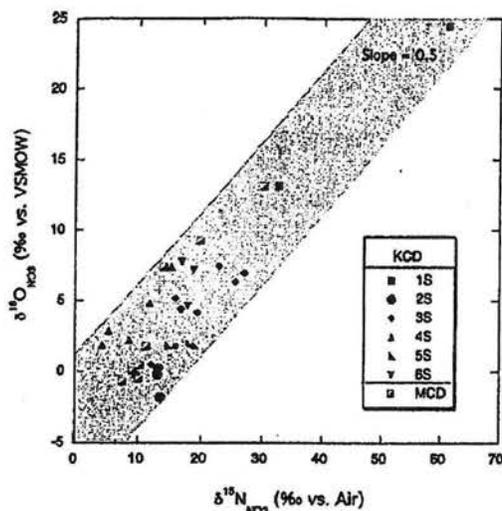


FIGURE 3. Oxygen and nitrogen isotopic composition of nitrate in dairy groundwater from multilevel monitoring wells at KCD and first encounter wells at MCD. The shaded region indicates a slope of 0.5 for a range of starting compositions. Calculated slopes for linear fits to multilevel wells at KCD and first encounter wells at MCD range from 0.47 to 0.60.

compounds from manure-rich wastewater, which is stored and used as a fertilizer at both dairy sites. At some locations, nitrification has been followed by denitrification. Prior to nitrification, cow manure likely starts out with a bulk $\delta^{15}\text{N}$ value close to 5‰, but is enriched in ^{15}N to varying degrees due to volatile loss of ammonia, resulting in $\delta^{15}\text{N}$ values of 10–22‰ in nitrate derived from manure (33, 34). Culture experiments have shown that nitrification reactions typically combine 2 oxygen atoms from the local pore water and one oxygen atom from atmospheric O_2 (35, 36), which has a $\delta^{18}\text{O}$ of 23.5‰ (37). Different ratios of oxygen from water and atmospheric O_2 are possible for very slow nitrification rates and low ammonia concentrations (38), however for dairy wastewater we assume that the 2:1 relation gives a reasonable prediction of the starting $\delta^{18}\text{O}$ values for nitrate at the two dairies based on the average values for $\delta^{18}\text{O}$ of groundwater at each site (–12.6‰ at KCD and –9.9‰ at MCD). Based on this approach, the predicted initial values for $\delta^{18}\text{O}$ in nitrate are –0.7‰ at KCD and 1.1‰ at MCD. Samples with the lowest nitrate $\delta^{15}\text{N}$ values have $\delta^{18}\text{O}$ values in this range, and are consistent with nitrate derived from manure. There is no strong evidence for mixing with nitrate from synthetic nitrogen fertilizers, which are used occasionally at both sites, but typically have low $\delta^{15}\text{N}$ values (0–5‰) and $\delta^{18}\text{O}$ values around 23‰ (39).

Denitrification drives the isotopic composition of the residual nitrate to higher $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values. The stable isotopes of nitrogen are more strongly fractionated during denitrification than those of oxygen, leading to a slope of approximately 0.5 on a $\delta^{18}\text{O}$ vs $\delta^{15}\text{N}$ diagram (34). Nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values at individual KCD multilevel well sites are positively correlated with calculated slopes ranging from 0.47 to 0.60; the slope of first encounter well data at MCD is 0.66 (Figure 3). These nitrate $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values indicate that denitrification is occurring at both sites. Because a wide range of fractionation factors are known to exist for this process (40), it is not possible to determine the extent of denitrification using only the isotopic compositions of nitrate along a denitrification trend, even when the initial value for manure-derived nitrate can be measured or calculated.

Extent of Denitrification. The concentrations of excess N_2 and residual nitrate can be combined with the isotopic composition of nitrate in order to characterize the extent of denitrification. In an ideal system, denitrification leads to a regular decrease in nitrate concentrations, an increase in excess N_2 , and a Rayleigh-type fractionation of N and O isotopes in the residual nitrate (Figure 4). In the Rayleigh fractionation model (41) the isotopic composition of residual nitrate depends on the fraction of initial nitrate remaining in the system ($f = C/C_{\text{initial}}$), the initial $\delta^{15}\text{N}$, and the fractionation factor (α) for denitrification:

$$\delta^{15}\text{N} = (1000 + \delta^{15}\text{N}_{\text{initial}}) f^{(\alpha-1)} - 1000$$

The fractionation factor α is defined from the isotopic ratios of interest ($R = ^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$):

$$\alpha = \frac{(R)_{\text{Product}}}{(R)_{\text{Reactant}}}$$

This fractionation can also be considered as an enrichment factor (ϵ) in ‰ units using the approximation $\epsilon \approx 1000 \ln \alpha$. The extent of denitrification can be calculated as $1 - f$. Rather than relying on an estimate of initial nitrate concentration, the parameter f is determined directly using field measurements of excess N_2 in units of equivalent reduced NO_3^- :

$$f = C_{\text{NO}_3^-} / (C_{\text{NO}_3^-} + C_{\text{excess N}_2})$$

Heterogeneity in groundwater systems can often complicate the interpretation of contaminant degradation using a Rayleigh model (42). Denitrified water retains a proportion of its excess N_2 concentration (and low values of f) during mixing, but the isotopic composition of nitrate may be disturbed by mixing since denitrified waters contain extremely low concentrations of nitrate (<1 mg/L). The sample from 1S with a f value close to zero and a $\delta^{15}\text{N}$ value of 7.6‰ was likely denitrified and is one example of this type of disturbance. However, in general, groundwater samples from the same multilevel well sites at KCD fall along similar Rayleigh fractionation curves, indicating that the starting isotopic composition of nitrate and the fractionation factor of denitrification vary across the site (Figure 4).

Values of $\delta^{15}\text{N}$ and f calculated from nitrate and excess N_2 fall along Rayleigh fractionation curves with enrichment factors (ϵ) ranging from –57‰ to –7‰ for three multilevel well sites at KCD and first encounter wells at MCD. As expected for denitrification, the enrichment factors indicated for oxygen are roughly half of those for nitrogen. The magnitude of these enrichment factors for N in residual nitrate are among the highest reported for denitrification, which typically range from –40‰ to –5‰ (34, 40). Partial gas loss near the water table interface at MCD could potentially increase the value of f , resulting in larger values of ϵ . Gas loss is unlikely to affect fractionation factors at KCD since most excess N_2 is produced well below the water table. Considering the large differences observed for denitrification fractionation factors within and between the two dairy sites, it is not sufficient to estimate fractionation factors for denitrification at dairies based on laboratory-derived values or field-derived values from other sites. The appropriate fractionation factors must be determined for each area, and even then the processes of mixing and gas loss must be considered in the relation between isotopic values and the extent of denitrification. Nevertheless, direct determination of the original amount of nitrate using dissolved N_2 values significantly improves our ability to determine the extent of denitrification in settings where the initial nitrate concentrations are highly variable.

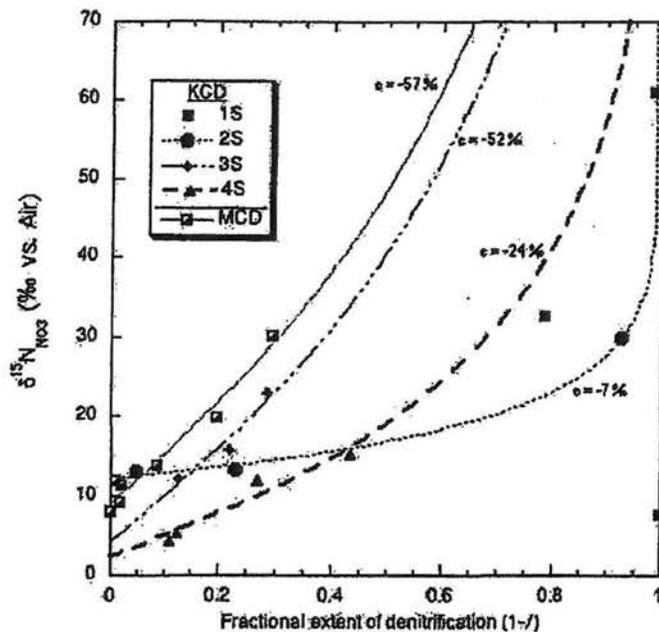


FIGURE 4. Nitrate $\delta^{15}\text{N}$ values plotted against the fractional extent of denitrification ($1 - f$) based on excess N_2 and residual nitrate. Enrichment factors (ϵ) are calculated by fitting the Rayleigh fractionation equation to data from three multilevel well sites at KCD and wells at MCD.

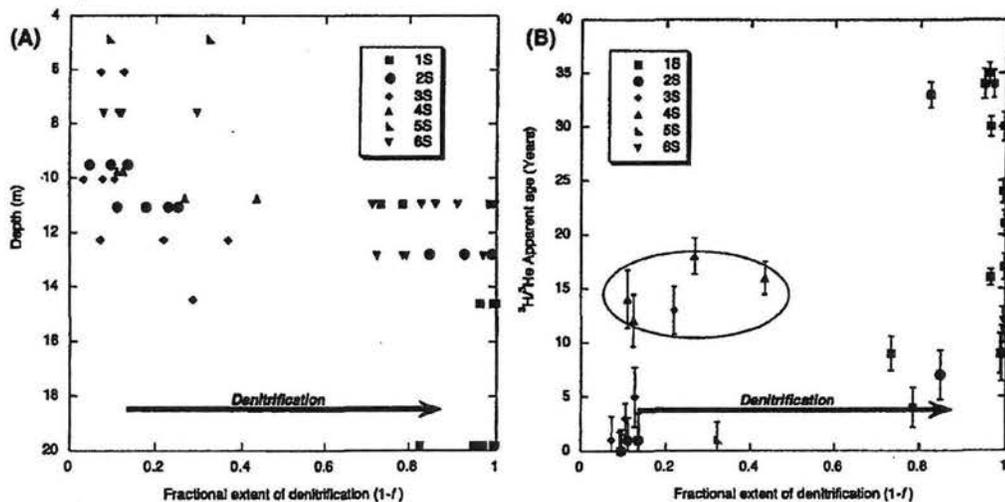


FIGURE 5. Sample depth (A) and $^3\text{H}/^3\text{He}$ apparent age (B) plotted against the fractional extent of denitrification ($1 - f$). Samples at two sites have experienced less denitrification than is typical for samples with $^3\text{H}/^3\text{He}$ apparent age > 8 years (circled, see text).

Time Scale of Denitrification. Modern water (i.e., groundwater containing measurable tritium) is found at all multilevel wells completed in the upper aquifer at KCD, the deepest of which is 20 m BGS. The upper aquifer below KCD has $^3\text{H}/^3\text{He}$ apparent ages of < 35 years. At well 1D1 (54 m BGS), the lower aquifer has no measurable NO_3^- and tritium below 1 pCi/L, indicating a groundwater age of more than 50 years. The sum of nitrate and excess N_2 is highest in the young, shallow dairy waters at KCD. Samples with $^3\text{H}/^3\text{He}$ ages > 29 years were below the MCL for nitrate prior to denitrification. These results are consistent with an increase in nitrate loading

at the surface, which followed the startup of KCD operations in the early 1970s.

The extent of denitrification at KCD is related to both depth and groundwater residence times based on $^3\text{H}/^3\text{He}$ apparent ages (Figure 5). There is a sharp transition from high nitrate waters to denitrified waters between 11 and 13 m depth across the KCD site. This transition is also related to the apparent age of the groundwater, as the high nitrate waters typically have apparent ages of between 0 and 5 years, and most samples with ages greater than 8 years are significantly or completely denitrified. There are five samples

that do not follow this pattern. These outliers are from sites 3S and 4S where the shallow groundwater has much higher $^3\text{H}/^3\text{He}$ apparent ages due to slow movement around clay zones at the screened intervals for these samples. The existence of older water that is not significantly impacted by denitrification indicates that it is the physical transport of water below the transition from oxic to anoxic conditions rather than the residence time that governs denitrification in this system.

At the MCD site, groundwater $^3\text{H}/^3\text{He}$ apparent ages indicate fast transit rates from the water table to the shallow monitoring wells. Most of the first encounter wells have apparent ages of <3 years, consistent with the hydraulic analysis presented by Harter et al. (5). The very fast transit times to the shallow monitoring wells at MCD allow for some constraints on minimum denitrification rates at this site. Based on the comparison of the calculated ages with the initial tritium curve, these shallow wells contain a negligible amount of old, ^3H -decayed water. In shallow wells near lagoons (e.g., W-16 and V-21), the observed excess N_2 (equivalent to 71 and 40 mg/L of reduced NO_3^-) accumulated over a duration of less than 1 year, indicating that denitrification rates may be very high at these sites. Complete denitrification of groundwater collected from well W-98 (excess N_2 equivalent to 51 mg/L NO_3^-) was attained within approximately 31 years, but may have occurred over a short period of time relative to the mean age of the water.

Occurrence of Denitrification at Dairy Sites. The depth at which denitrified waters are encountered is remarkably similar across the KCD site. This transition is not strongly correlated with a change in sediment texture. The denitrified waters at all KCD wells coincide with negative ORP values and generally low dissolved O_2 concentrations. Total organic carbon (TOC) concentration in the shallow groundwaters range from 1.1 to 15.7 mg/L at KCD, with the highest concentrations of TOC found in wells adjacent to lagoons. The highest concentrations of excess N_2 are found in nested well-set 2S, which is located in a field downgradient from the lagoons. However, sites distal to the lagoons (3S and 4S) that are apparently not impacted by lagoon seepage (43) also show evidence of denitrification, suggesting that direct lagoon seepage is not the sole driver for this process.

The chemical stratification observed in multilevel wells at the KCD site demonstrates the importance of characterizing vertical variations within aquifers for nitrate monitoring studies. Groundwater nitrate concentrations are integrated over the high and low nitrate concentration zones by dairy water supply wells, which have long screened intervals from 9 to 18 m BGS. Water quality samples from these supply wells underestimate the actual nitrate concentrations present in the uppermost oxic aquifer. Similarly, first encounter monitoring wells give an overestimate of nitrate concentrations found deep in the aquifer, and thus would miss entirely the impact of saturated zone denitrification in mitigating nitrate transport to the deep aquifer.

Monitoring wells at MCD sample only the top of the aquifer, so the extent of denitrification at depth is unknown, except for the one deep supply well (W98), which has less than 1 mg/L nitrate and an excess N_2 content consistent with reduction of 51 mg/L NO_3^- to N_2 . This supply well would be above the MCL for nitrate without the attenuation of nitrate by denitrification. The presence of ammonium at several of the wells with excess N_2 indicates a component of wastewater seepage in wells located near lagoons, where mixing of oxic waters with anoxic lagoon seepage may induce both nitrification and denitrification. Wells that are located in the surrounding fields have high NO_3^- concentrations, and do not have any detectable excess N_2 , a result consistent with mass-balance models of nitrate loading and groundwater nitrate concentration (5).

While dairy operations seem likely to establish conditions conducive to saturated zone denitrification, the prevalence of the phenomenon is not known. Major uncertainties include the spatial extent of anaerobic conditions, and transport of organic carbon under differing hydrogeologic conditions and differing nutrient management practices. Lagoon seepage may also increase the likelihood of denitrification in dairy aquifers. The extent to which dairy animal and field operations affect saturated zone denitrification is an important consideration in determining the assimilative capacity of underlying groundwater to nitrogen loading associated with dairy operations.

Acknowledgments

This work was performed under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under Contract W-7405-Eng-48. Funding for this project was from the California State Water Resources Control Board Groundwater Ambient Monitoring and Assessment Program and from the LLNL Laboratory Directed Research and Development Program. We thank Mark Conrad and Katharine Woods for use of the LBNL Center for Isotope Geochemistry's stable isotope lab and help with analyses. We are grateful for the efforts of two journal reviewers, who provided helpful critiques of this work.

Supporting Information Available

A table of chemical, isotopic, and dissolved gas results from this study, a plot of apparent age with depth, and detailed descriptions of the study sites. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Literature Cited

- (1) Fan, A. M.; Steinberg, V. E. Health implications of nitrate and nitrite in drinking water - an update on methemoglobinemia occurrence and reproductive and developmental toxicity. *Regulat. Toxicol. Pharmacol.* 1996, 23, 35-43.
- (2) Nolan, B. T.; Hitt, K. J.; Ruddy, B. C. Probability of nitrate contamination of recently recharged groundwaters in the conterminous United States. *Environ. Sci. Technol.* 2002, 36, 2138-2145.
- (3) California Department of Health Services Geotracker Database. State Water Resource Control Board of California: Sacramento, CA, 2003. <http://geotracker.swrcb.ca.gov/>.
- (4) Squillace, P. J.; Scott, J. C.; Moran, M. J.; Nolan, B. T.; Kolpin, D. W. VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States. *Environ. Sci. Technol.* 2002, 36, 1923-1930.
- (5) Harter, T.; Davis, H.; Mathews, M. C.; Meyer, R. D. Shallow groundwater quality on dairy farms with irrigated forage crops. *J. Contam. Hydrol.* 2002, 55, 287-315.
- (6) Cameron, K. C.; Di, H. J.; Reijnen, B. P. A.; Li, Z.; Russell, J. M.; Barnett, J. W. Fate of nitrogen in dairy factory effluent irrigated onto land. *N. Z. J. Agric. Res.* 2002, 45, 217-216.
- (7) Mariotti, A.; Landreau, A.; Simon, B. ^{15}N isotope biogeochemistry and natural denitrification process in groundwater: Application to the chalk aquifer of northern France. *Geochim. Cosmochim. Acta* 1988, 52, 1869-1878.
- (8) Puckett, L. J.; Cowdery, T. K.; Lorenz, D. L.; Stoner, J. D. Estimation of nitrate contamination of an agro-ecosystem outwash aquifer using a nitrogen mass-balance budget. *J. Environ. Qual.* 1999, 28, 2015-2025.
- (9) Puckett, L. J.; Cowdery, T. K. Transport and fate of nitrate in a glacial outwash aquifer in relation to ground water age, land use practices, and redox processes. *J. Environ. Qual.* 2002, 31, 782-796.
- (10) Korom, S. F. Natural denitrification in the saturated zone - a review. *Water Resour. Res.* 1992, 28, 1657-1668.
- (11) DeSimone, L. A.; Howes, B. L. Nitrogen transport and transformations in a shallow aquifer receiving wastewater discharge: A mass balance approach. *Water Resour. Res.* 1998, 34, 271-285.
- (12) Casciotti, K. L.; Sigman, D. M.; Hastings, M. G.; Bohlke, J. K.; Hilbert, A. L. Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Anal. Chem.* 2002, 74, 4905-4912.

- (13) Singleton, M. J.; Woods, K. N.; Conrad, M. E.; Depaolo, D. J.; Dresel, P. E. Tracking sources of unsaturated zone and groundwater nitrate contamination using nitrogen and oxygen stable isotopes at the Hanford Site, Washington. *Environ. Sci. Technol.* 2005, 39, 3563-3570.
- (14) Silva, S. R.; Kendall, C.; Wilkison, D. H.; Ziegler, A. C.; Chang, C. C. Y.; Avanzino, R. J. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. *J. Hydrol.* 2000, 228, 22-36.
- (15) Epstein, S.; Mayeda, T. K. Variation of O-18 content of waters from natural sources. *Geochim. Cosmochim. Acta* 1953, 4, 213-224.
- (16) Bohlke, J. K.; Denver, J. M. Combined use of groundwater dating, chemical, and isotopic analyses to resolve the history and fate of nitrate contamination in two agricultural watersheds, Atlantic Coastal Plain, Maryland. *Water Resour. Res.* 1995, 31, 2319-2339.
- (17) McMahon, P. B.; Bohlke, J. K. Denitrification and mixing in a stream-aquifer system: Effects on nitrate loading to surface water. *J. Hydrol.* 1996, 186, 105-128.
- (18) Vogel, J. C.; Talma, A. S.; Heaton, T. H. E. Gaseous nitrogen as evidence for denitrification in groundwater. *J. Hydrol.* 1981, 50, 191-200.
- (19) Wilson, G. B.; Andrews, J. N.; Bath, A. H. The nitrogen isotope composition of groundwater nitrates from the East Midlands Triassic Sandstone Aquifer, England. *J. Hydrol.* 1994, 157, 35-46.
- (20) Kana, T. M.; Darkangelo, C.; Hunt, M. D.; Oldham, J. B.; Bennett, G. E.; Cornwell, J. C. Membrane inlet mass spectrometer for rapid high precision determination of N_2 , O_2 , and Ar in environmental water samples. *Anal. Chem.* 1994, 66, 4166-4170.
- (21) An, S. M.; Gardner, W. S.; Kana, T. Simultaneous measurement of denitrification and nitrogen fixation using isotope pairing with membrane inlet mass spectrometry analysis. *Appl. Environ. Microbiol.* 2001, 67, 1171-1178.
- (22) Ekwurzel, B. *LLNL Isotope Laboratories Data Manual*; UCRL-TM-203316; Lawrence Livermore National Laboratory: Livermore, CA, 2004; p 133.
- (23) Aeschbach-Hertig, W.; Peeters, F.; Beyerle, U.; Kipfer, R. Palaeotemperature reconstruction from noble gases in ground water taking into account equilibration with entrapped air. *Nature* 2000, 405, 1040-1044.
- (24) Aeschbach-Hertig, W.; Peeters, F.; Beyerle, U.; Kipfer, R. Interpretation of dissolved atmospheric noble gases in natural waters. *Water Resour. Res.* 1999, 35, 2779-2792.
- (25) Ekwurzel, B.; Schlosser, P.; Smethie, W. M.; Plummer, L. N.; Busenberg, E.; Michel, R. L.; Weppernig, R.; Stute, M. Dating of shallow groundwater - comparison of the transient tracers H-3/He-3, chlorofluorocarbons, and Kr-85. *Water Resour. Res.* 1994, 30, 1693-1708.
- (26) Poreda, R. J.; Cerling, T. E.; Solomon, D. K. Tritium and helium isotopes as hydrologic tracers in a shallow unconfined aquifer. *J. Hydrol.* 1988, 103, 1-9.
- (27) Solomon, D. K.; Poreda, R. J.; Schiff, S. L.; Cherry, J. A. Tritium and He-3 as Groundwater Age Tracers in the Borden Aquifer. *Water Resour. Res.* 1992, 28, 741-755.
- (28) Weissmann, G. S.; Zhang, Y.; LaBolle, E. M.; Fogg, G. E. Dispersion of groundwater age in an alluvial aquifer system. *Water Resour. Res.* 2002, 38, art. no.1198.
- (29) Heaton, T. H. E.; Vogel, J. C. Excess air in groundwater. *J. Hydrol.* 1981, 50, 201-216.
- (30) Holocher, J.; Peeters, F.; Aeschbach-Hertig, W.; Hofer, M.; Brennwald, M.; Kinzelbach, W.; Kipfer, R. Experimental investigations on the formation of excess air in quasi-saturated porous media. *Geochim. Cosmochim. Acta* 2002, 66, 4103-4117.
- (31) Holocher, J.; Peeters, F.; Aeschbach-Hertig, W.; Hofer, M.; Kipfer, R. Gas exchange in quasi-saturated porous media: Investigations on the formation of excess air using noble gases (abstr.). *Geochim. Cosmochim. Acta* 2002, 66, A338-A338.
- (32) Peterson, T. C.; Vose, R. S. An overview of the Global Historical Climatology Network temperature database. *Bull. Am. Meteorol. Soc.* 1997, 78, 2837-2849.
- (33) Kreitler, C. W. Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. *J. Hydrol.* 1979, 42, 147-170.
- (34) Kendall, C. Tracing nitrogen sources and cycling in catchments. In *Isotope Tracers in Catchment Hydrology*; Kendall, C., McDonnell, J. J., Eds.; Elsevier: New York, 1998; pp 519-576.
- (35) Andersson, K. K.; Hooper, A. B. O_2 and H_2O are each the source of one O in NO_2^- produced from NH_3 by Nitrosomonas - N15-NMR evidence. *FEBS Lett.* 1983, 164, 236-240.
- (36) Holocher, T. C. Source of the oxygen atoms of nitrate in the oxidation of nitrite by *Nitrobacter agilis* and evidence against a P-O-N anhydride mechanism in oxidative phosphorylation. *Arch. Biochem. Biophys.* 1984, 233, 721-727.
- (37) Kroopnick, P. M.; Craig, H. Atmospheric oxygen: Isotopic composition and solubility fractionation. *Science* 1972, 175, 54-55.
- (38) Mayer, B.; Bollwerk, S. M.; Mansfeldt, T.; Hutter, B.; Veizer, J. The oxygen isotope composition of nitrate generated by nitrification in acid forest floors. *Geochim. Cosmochim. Acta* 2001, 65, 2743-2756.
- (39) Kendall, C.; Aravena, R. Nitrate isotopes in groundwater systems. In *Environmental Tracers in Subsurface Hydrology*; Cook, P. G., Herczeg, A. L., Eds.; Kluwer Academic Publishers: Norwell, MA, 2000; pp 261-297.
- (40) Hübner, H. Isotope effects of nitrogen in the soil and biosphere. In *Handbook of Environmental Isotope Geochemistry: Volume 2b, The Terrestrial Environment*; Fritz, P., Fontes, J. C., Eds.; Elsevier: New York, 1986; pp 361-425.
- (41) Criss, R. E. *Principles of Stable Isotope Distribution*; Oxford University Press: New York, 1999; p 254.
- (42) Abe, Y.; Hunkeler, D. Does the Rayleigh equation apply to evaluate field isotope data in contaminant hydrogeology? *Environ. Sci. Technol.* 2006, 40, 1588-1596.
- (43) McNab, W. W.; Singleton, M. J.; Moran, J. E.; Esser, B. K. Assessing the impact of animal waste lagoon seepage on the geochemistry of an underlying shallow aquifer. *Environ. Sci. Technol.* 2007, 41, 753-758.

Received for review May 25, 2006. Revised manuscript received November 13, 2006. Accepted November 15, 2006.

ES061253G

Supporting Information

Singleton et al, Saturated Zone Denitrification....

Supporting Information for "Saturated Zone Denitrification: Potential for Natural Attenuation of Nitrate Contamination in Shallow Groundwater Under Dairy Operations" by M. J. Singleton^{1*}, B. K. Esser¹, J. E. Moran¹, G. B. Hudson¹, W. W. McNab², and T. Harter³

Contents: 7 Pages, 1 Figure, and 1 Table

Description of Dairy Sites

Study Site 1:

Study Site #1 is located at a dairy operation in Kings County, CA (KCD). Manure management practices employed at KCD, with respect to corral design, runoff capture and lagoon management are typical of practices employed at other dairies in the region. KCD has close to the 1000-cow average for dairies in the area, and operates three clay-lined wastewater lagoons that receive wastewater after solids separation. Wastewater is used for irrigation of 500 acres of forage crops (corn and alfalfa) on the dairy and on neighboring farms; dry manure is exported to neighboring farms.

KCD is located in the Kings River alluvial fan, a sequence of layered sediments transported by the Kings River from the Sierra Nevada to the low lying southern San Joaquin Valley of California (1, 2). The site overlies an unconfined aquifer, which has been split into an upper aquifer from 3m to 24m below ground surface (BGS) and a lower aquifer (>40 m BGS) that are separated by a gap of unsaturated sediments. Both aquifers are predominantly composed of unconsolidated sands with minor clayey sand layers. The lower unsaturated gap was likely caused by intense regional groundwater pumping, and a well completed in this unsaturated zone has very low gas pressures. There are no persistent gradients in water table levels across the KCD site, but in general, regional groundwater flow is from the NW to SE due to topographic flow on the Kings River fan. The water table is located about 5 m BGS. Local recharge is dominated by vertical fluxes from irrigation, and to a lesser extent, leakage from adjacent unlined canals. Transient cones of depression are induced during groundwater pumping from dairy operation wells.

The regional groundwater is highly impacted by agricultural activities and contains elevated concentrations of nitrate and pesticides (3, 4).

KCD was instrumented with five sets of multi-level monitoring wells and one "up-gradient" well near an irrigation canal. These wells were installed in 2002, and sampled between Feb. 2002 and Aug. 2005. The multi-level wells have short (0.5 m) screened intervals in order to detect heterogeneity and stratification in aquifer chemistry. One monitoring well was screened in the lower aquifer, 54m BGS. The remaining monitoring wells are screened in the upper aquifer from 5m to 20m BGS. In addition, there are eight dairy operation wells that were sampled over the course of this study. These production wells have long screens, generally between 9 to 18 meters below ground surface (BGS).

Study Site 2:

The second dairy field site is located in Merced County, CA. The Merced County dairy (MCD) lies within the northern San Joaquin Valley, approximately 160 km NNW from the KCD site. The site is located on the low alluvial fans of the Merced and Tuolumne Rivers, which drain the north-central Sierra Nevada. Soils at the site are sand to loamy sand with rapid infiltration rates. The upper portion of the unconfined alluvial aquifer is comprised of arkosic sand and silty sand, containing mostly quartz and feldspar, with interbedded silt and hardpan layers. Hydraulic conductivities were measured with slug tests and ranged from 1×10^{-4} m/s to 2×10^{-3} m/s with a geometric mean of 5×10^{-4} m/s (5). Regional groundwater flow is towards the valley trough with a

gradient of approximately 0.05% to 0.15%. Depth to groundwater is 2.5 m to 5 m BGS. The climate is Mediterranean with annual precipitation of 0.5 m, but groundwater recharge is on the order of 0.5–0.8 m per year with most of the recharge originating from excess irrigation water (3). Transit times in the unsaturated zone are relatively short due to the shallow depth to groundwater and due to low water holding capacity in the sandy soils. Shallow water tables are managed through tile drainage and groundwater pumping specifically for drainage. The MCD site is instrumented with monitoring wells that are screened from 2-3 m BGS to a depth of 7-9 m BGS. The wells access the upper-most part of the unconfined aquifer, hence, the most recently recharged groundwater (6). Recent investigations showed strongly elevated nitrate levels in this shallow groundwater originating largely from applications of liquid dairy manure to field crops, from corrals, and from manure storage lagoons (6). For this study, a subset of 18 wells was sampled. A deep domestic well was also sampled at MCD. This domestic well is completed to 57 m BGS, and thus samples a deeper part of the aquifer than the monitoring well network.

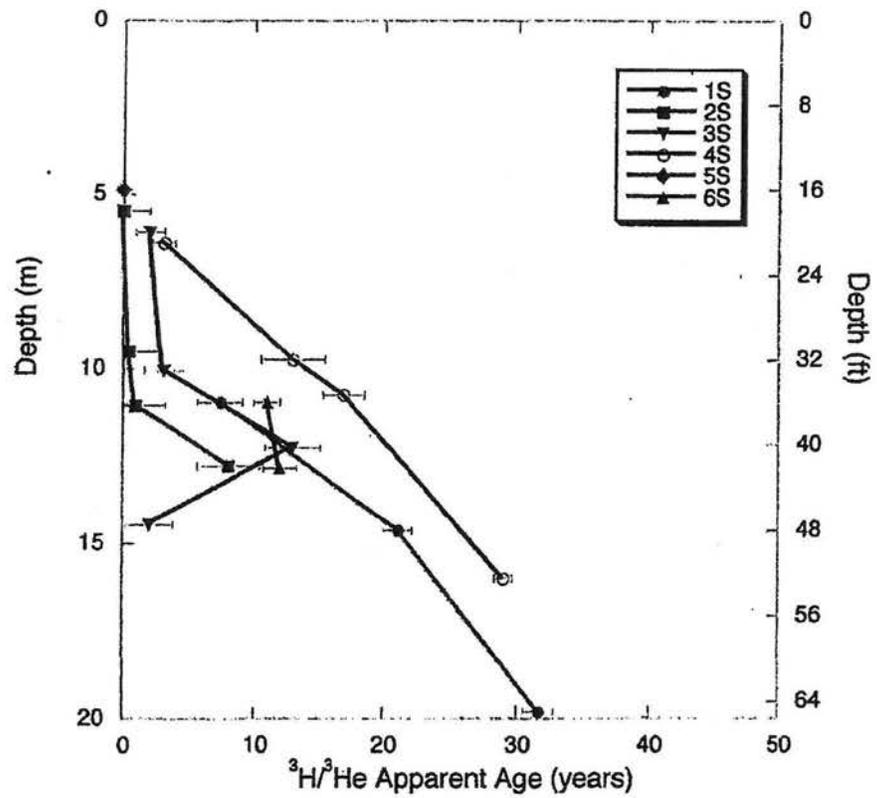


Figure S1. Groundwater $^3\text{H}/^3\text{He}$ apparent ages from multilevel monitoring wells at KCD. Error bars show analytical error.

Table S1. Chemical, dissolved gas, and isotopic compositions for multilevel groundwater monitoring wells and lagoons. Average values are given for wells sampled more than once. Excess N₂ values in bold are fully constrained by noble gas determinations of excess air and recharge temperature.

Site	Depth of multi- level well (m)	Cl ⁻ (mg/L)	NO ₂ ⁻ (mg/L)	NH ₄ ⁺ (mg/L)	ORP	DO (mg/L)	TDC (mg/L)	δ ¹⁸ O H ₂ O (‰ SMOW)	δ ¹⁵ N NO ₃ ⁻ (‰ Air)	δ ¹⁵ O NO ₃ ⁻ (‰ SMOW)	³ H/ ⁴ He age (yr)	+/- (yr)	Excess air determined from Ne (cc STP/g)	Recharge Temp. from Xe (°C)	+/- (°C)	² H pC/L	+/- (pC/L)	N ₂ /Ar
KCD-CANAL-1		1.5	1.2	0.2		10.0		-12.9								13.3	0.6	
KCD-LAGOON-1		304.5	28.6	360.8		0.4	480.0	-10.2										68
KCD-LAGOON-2		265.2	13.9	292.1		0.5	490.0	-10.0										58
KCD-LAGOON-3		212.2	22.4	181.3		0.5	420.0	-9.9										41
KCD-1D1	54.3	1.9	0.2	<0.1	-264	0.2	0.8	-13.7	7.1		>50		3.40E-03	15	1.2	0.5	0.1	41
KCD-1S1	6.7		206.0		166	3.5		-12.7										46
KCD-1S2	11.0	52.5	11.1	0.3	-79	0.4	2.5	-12.8	46.9	18.8	7.3	1.8	<1E-4	16	1.1	32.0	1.2	62
KCD-1S3	14.6	36.0	0.5	1.3	-164	0.5	1.3	-12.9	7.6		21.1	1.1	2.82E-03	14	1.1	31.4	1.2	63
KCD-1S4	19.8	9.8	0.4	2.5	-196	0.5	1.1	-13.3			31.7	1.1	4.02E-03	16	1.1	28.3	1.1	46
KCD-2S1	5.5	107.7	144.5	<0.1			5.0	-12.3			0.0	2.0	1.70E-03	19	1.0	21.9	0.9	39
KCD-2S2	9.5	95.0	187.2	0.6	84	0.7	4.2	-12.2	13.1	-0.2	0.5	2.2	1.78E-03	22	1.1	19.5	0.8	49
KCD-2S3	11.1	101.1	178.2	0.1	62	1.7	3.0	-12.1	13.2	0.2	1.0	2.1	<1E-4	21	1.1	19.3	0.8	62
KCD-2S4	12.8	72.7	7.1	1.0	-149	0.3	1.8	-12.4	29.9		8.0	2.4	<1E-4	23	1.8	19.8	0.8	101
KCD-3S1	6.1	170.4	203.1	0.4	0	1.2	5.3	-11.7	14.5	2.4	2.0	1.0	1.42E-03	19	1.1	17.8	0.7	46
KCD-3S2	10.1	255.6	273.6	<0.1	72	2.3	14.2	-11.2			3.0	1.4	6.35E-04	21	1.1	21.2	0.9	49
KCD-3S3	12.3	162.7	167.8	0.5	107	1.2	9.0	-11.9	15.8	5.2	13.0	2.2	1.30E-03	18	1.0	16.4	0.8	53
KCD-3S4	14.5	194.0	136.4	<0.1	79	1.0	5.6	-11.8	22.9	7.4	2.0	1.7	<1E-4	20	1.0	18.6	0.7	59
KCD-4S1	6.4	127.0	83.3	<0.1					8.6	2.2	3.0	0.8	3.35E-04	20	1.0	35.6	1.4	
KCD-4S2	9.8	32.1	125.4	0.4	-16	0.8	1.1	-11.8	4.7	2.3	13.0	2.5	5.07E-03	18	1.3	20.3	0.8	51
KCD-4S3	10.8	42.3	77.1	0.5	27	0.9	1.1	-12.0	13.5	6.1	17.0	1.6	3.54E-03	19	1.2	22.7	0.9	60
KCD-4S4	16.0	35.0	0.9	1.8	-161	0.9	3.5	-13.0			29.0	0.7		18	1.0	46.5	1.7	61
KCD-5S1	4.9	14.5	35.4	1.3	37	0.5	1.5	-13.4	18.9	1.8	<1		<1E-4	18	1.0	12.5	0.6	46
KCD-6S1	12.9	129.3	12.7	20.4		1.0	15.7	-11.9	12.1		12.0	1.3	<1E-4			29.1	1.1	70
KCD-6S2	11.0	140.6	10.1	3.2		1.2	14.6	-11.8			11.0	1.0	<1E-4			33.3	1.2	67
KCD-6S3	7.6	129.5	159.3	0.9			6.7	-11.6	19.0	7.7			2.13E-04			33.9	1.3	51
KCD-NW-01	9-18	140.8	114.7	1.9		1.9		-12.0	15.0									54
KCD-NW-02	9-18	163.4	75.2	3.4		1.3		-12.0	18.2							17.0	0.9	71
KCD-NW-03	9-18	100.3	67.2	<0.1														
KCD-NW-04	9-18	2.8	2.0	<0.1				-13.7			>50		7.72E-04	12	0.9	0.2	0.2	
KCD-NW-06	9-18	92.8	48.6	2.6				-12.2	17.2							22.9	1.2	61
KCD-SW-02	9-18	52.6	91.0	<0.1				-12.7	23.5							24.8	1.4	
KCD-SW-03	9-18	45.1	29.2	1.9		1.5		-12.4	27.3							30.4	1.3	57
KCD-SW-07	9-18	165.5	25.8	<0.1														
KCD-SW-08	9-18	184.1	116.6	2.3		3.8		-10.9	16.9							19.7	0.8	53
MCD-LAGOON		514.0	<0.1	691.8														62
MCD-V-01	7.0	317.8	425.1	<0.1	111	5.6	12.7	-9.3	13.9	7.4	12.0	1.7	<1E-4	25	1.2	36.0	1.4	61
MCD-V-14	7.6	71.4	316.0	<0.1			5.8		11.2	1.7	2.0	2.9	1.26E-03	18	1.0	12.4	0.5	41
MCD-V-18	6.1	77.2	195.5	1.7	193	3.3	8.1		10.1	-0.5						12.2	0.5	39
MCD-V-21	9.1	145.5	163.1	<0.1	147	1.4	22.6	-9.1	19.9	9.2	<1					15.3	0.6	51
MCD-V-24	9.1	30.2	201.5	<0.1	161	7.0	5.4	-10.5	7.4	-0.7	<1		4.31E-04	20	1.0	13.8	0.6	37
MCD-W-99		73.0	303.2	2.4			12.2		10.3	0.4	1.0	2.1	<1E-4	19	1.0	14.5	0.6	39
MCD-W-02	7.0	226.1	2.0	148.5		0.6	12.7	-9.1								17.9	0.7	121
MCD-W-03	7.0	82.2	341.8	0.7		0.8	14.5	-10.5			3.0	3.1	2.13E-03	17	1.0	13.7	0.6	45
MCD-W-05	7.0	48.3	230.6	<0.1				-10.7	6.8							14.5	0.8	39
MCD-W-10	9.1	55.5	426.1	<0.1			11.7	-10.3	9.1	0.0	3.0	3.4	2.52E-03	19	1.1	13.5	0.6	44
MCD-W-16	9.1	298.9	6.1	113.9	176	0.7	9.1	-8.1			<1	0.7	<1E-4			18.9	0.9	134
MCD-W-17	9.1	136.9	171.7	26.7	208	0.7	9.8	-9.4	30.2	13.1			<1E-4			15.9	0.7	90
MCD-W-23	9.1	80.9	356.1	1.9	121	1.1	10.4	-10.2			2.0	2.8	1.65E-03	20	1.0	13.9	0.5	43
MCD-W-30	9.1	49.1	324.8	<0.1				-9.9	5.3		1.0	2.3	1.23E-03	17	0.8	16.3	0.9	38
MCD-W-31	9.1	46.8	187.9	<0.1				-10.9	8.0		<1		1.82E-03			15.9	0.7	40
MCD-W-34	7.3	63.4	185.6	<0.1				-10.8	7.9		1.0	3.8	2.77E-03	17	0.8	13.7	0.7	41
MCD-W-35	7.3	159.6	304.4	<0.1				-9.7	11.8		<1		1.52E-03	17	0.8	16.3	0.8	41
MCD-W-98	57	69.6	0.4	<0.1			2.1	-10.6			31.0	0.6	1.76E-03	18	1.0	21.8	0.9	64

References

- (1) Weissmann, G. S.; Fogg, G. E., Multi-scale alluvial fan heterogeneity modeled with transition probability geostatistics in a sequence stratigraphic framework. *Journal of Hydrology* 1999, 226, 48-65.
- (2) Weissmann, G. S.; Mount, J. F.; Fogg, G. E., Glacially driven cycles in accumulation space and sequence stratigraphy of a stream-dominated alluvial fan, San Joaquin valley, California, USA. *Journal of Sedimentary Research* 2002, 72, 240-251.
- (3) Burrow, K. R.; Shelton, K. R.; Dubrovsky, N. M. *Occurrence of nitrate and pesticides in ground water beneath three agricultural land-use settings in the eastern San Joaquin Valley, California, 1993-1995*; Water-Resources Investigations Report 97-4284; U.S. Geological Survey: 1998; p 51.
- (4) Burrow, K. R.; Shelton, K. R.; Dubrovsky, N. M. *Occurrence of nitrate and pesticides in ground water beneath three agricultural land-use settings in the eastern San Joaquin Valley, California, 1993-1995*; Water-Resources Investigations Report 97-4284; United States Geological Survey: 1998; p 51.
- (5) Davis, H. H. In *Monitoring and evaluation of water quality under Central Valley dairy sites*, Proceedings of the California Plant and Soil Conference (California Chapter of American Society of Agronomy and California Fertilizer Association, Visalia, California), Visalia, California, 1995; California Chapter of American Society of Agronomy: Visalia, California, 1995; pp 158-164.
- (6) Harter, T.; Davis, H.; Mathews, M. C.; Meyer, R. D., Shallow groundwater quality on dairy farms with irrigated forage crops. *Journal of Contaminant Hydrology* 2002, 55, 287-315.

EXHIBIT E

“Water Quality Regulations for Dairy Operators in California’s Central Valley—Overview and Cost Analysis,” November 2010, prepared by California Department of Food and Agriculture

ACLC R5-2016-0531 Sweeney Submission of Evidence

Water Quality Regulations for Dairy Operators in California's Central Valley – Overview and Compliance Cost Analysis

Casey Walsh Cady and Mike Francesconi¹
November 2010

Table of Contents

1. Executive Summary
2. Introduction and Background
3. Study Scope and Methodology
4. Dairying in California's Central Valley
5. Consultants Addressing the General Order
6. Requirements of the General Order Waste Discharge Requirements
7. Dairy Operators' Time
8. Capital Investment
9. Technical and Financial Assistance
10. Analysis and Conclusions
11. References and Acknowledgements

1. Executive Summary

To protect beneficial uses of surface waters and groundwater, the Central Valley Regional Water Quality Control Board adopted a general Waste Discharge Requirements order for dairies (the General Order) in May 2007. Approximately 1,600 dairies were initially covered under the General Order which established a timeline for operators to develop and implement both a waste management plan (WMP) and a nutrient management plan (NMP). The General Order includes a monitoring and reporting program (MRP) that identifies mandatory sampling and reporting. The General Order also requires that registered professionals perform specified tasks. To comply with the General Order, dairy operators have become much more sophisticated at using the nutrients in manure to match crop needs.

CDFA analyzed the costs of compliance with the General Order by interviewing dairy operators and their consultants. Dairy operators are incurring significant costs to comply with the General Order requirements for a NMP, WMP, and MRP. Future costs related to groundwater monitoring and infrastructure improvement are uncertain at this time but will significantly increase compliance costs in 2011 and beyond. These costs are not offset by the increased efficiency of using manure for crop production, although some financial and technical assistance is available to operators to help them comply with the General Order and offset some of the initial costs of implementation.

Results from the survey show that from 2007 - 2010 total compliance costs for individual dairy operators (not including additional groundwater monitoring) in the Central Valley vary widely from \$11,768 to \$162,804 with an average of \$54,975. One time costs range from \$2,250 to \$34,000 with an average of \$11,575 without additional groundwater monitoring. The average annual estimated costs of compliance is \$14,136.

¹ Casey Walsh Cady is Staff Environmental Scientist, Division of Marketing Services, California Department of Food and Agriculture. Mike Francesconi, is Supervising Auditor, Dairy Marketing Branch, California Department of Food and Agriculture. Corresponding author: ccady@cdfa.ca.gov

The amount spent ranges widely based on dairy size location, number of fields, herd size and other factors. This report was prepared in response to a November, 2009 request from the California Department of Food and Agriculture (CDFA).

2. Introduction and Background

The Central Valley of California is over 500 miles long and extends from the Oregon border to the Tehachapi Mountains south of Bakersfield. The region currently has approximately 1,400 dairies. Herd size (mature cows) for dairies permitted under the General Order vary widely, from 58 to 10,925. Nitrates and salts from dairies can result in contamination of surface water and groundwater, and so dairies are regulated by the Central Valley Regional Water Quality Control Board (RB5). Other sources of nitrate such as irrigated agriculture and septic systems are also regulated by RB5.

Prior to May 2007, most of the approximately 1,600 dairies operating in the Central Valley were not regulated under a formal order issued by RB5. In May 2007, RB5 adopted Order R5-2007-0035 "*Waste Discharge Requirements General Order for Existing Milk Cow Dairies*" (the General Order). The General Order applies to dairies that submitted a complete Report of Waste Discharge (ROWD) by October 17, 2005, have not expanded their herd size by more than fifteen percent since they submitted their ROWD, do not discharge wastes that originate outside the dairy, and do not discharge manure or process water to waters of the State. The purpose of the General Order is to regulate the discharge of wastes from the dairy production area and associated cropland. Such wastes are generated from the storage and use of manure, and may transport nutrients, pathogens, and/or salts that can adversely affect the quality of surface water and groundwater.

The General Order applies to both the dairy production area and land application area. The General Order defines requirements for land application of manure based on nutrient budgets developed in a site-specific Nutrient Management Plan (NMP) and requires dairies to have sufficient storage capacity to contain all wastewater generated at the dairy, including rainfall runoff that has contacted manure or feed, until the wastewater can be applied to cropland pursuant to an NMP or is otherwise properly managed. Wastewater is not allowed to be discharged to waters of the State unless the dairy obtains a National Pollutant Discharge Elimination System (NPDES) permit that allows certain discharges following storms that exceed a 25-year, 24-hour storm event. However, stormwater runoff from cropland where manure was applied pursuant to an NMP may also be allowed if receiving water is not significantly affected. The General Order also prohibits further degradation of groundwater, but does not address the cleanup of groundwater degraded by past dairy operations.

The General Order incorporates a phased compliance schedule that gives operators time to make necessary changes in their facilities and practices, take advantage of opportunities for education, and obtain funding for needed facility improvements. The General Order imposes complex requirements on dairy operators including submission of annual reports; development and implementation of an NMP with annual updates, development and implementation of a WMP; daily, weekly and monthly monitoring; and specific sampling of process wastewater, manure, irrigation water, plant tissue, soils, supply wells, tile drainage, etc.. The General Order requires each dairy to fully implement their NMP and WMP by July 1, 2011. More information on the requirements in the General Order is presented below along with an analysis of the compliance costs.

This report examines the cost of complying with the General Order based on data for some of the approximately 1,400 dairies that are covered by the General Order. The data covers the years when facility assessments, planning, and implementation first began. It is anticipated that for most

dairies these costs will increase as the monitoring program is implemented and infrastructure upgrades are made.

3. Study Scope and Methodology

No two California dairies are exactly alike; dairy operators have different resources and production facilities. Therefore, this report provides a range of compliance costs based on a number of factors including dairy herd size, location, number and size of crop fields, facility wells, age of the dairy, physical layout, lagoon size, options for nutrient export, choice of consultants, soil types, etc. Where appropriate, average compliance costs are presented.

This report evaluates the cost of compliance for dairy operators covered under the General Order. It does not analyze costs for dairies covered under National Pollutant Discharge Elimination System (NPDES) permits or covered under individual Waste Discharge Requirements (WDR) orders (e. g., dairies that did not file a ROWD by October 17, 2005 or those that have expanded their herd size more than fifteen percent after October 17, 2005).

To prepare this report, CDFA staff interviewed personnel from eight consulting firms (one of these firms also provides engineering services), two agricultural laboratories and two engineering firms. These firms work with approximately 77% of the dairy operators in the Central Valley. CDFA also collected information on time spent on compliance and infrastructure costs from 62 dairy operators who participate in CDFA's Cost of Production studies. They represent 4% of Central Valley dairy operators and 5% of Central Valley milking cow population.

4. Dairy Production in California's Central Valley

Milk and associated dairy products (cheese, dry milk powder, butter, ice cream etc.) are California's top grossing agricultural products and California leads the nation in milk production (CDFA, 2010). California produces 21% of the nation's milk supply (CDFA, 2010) and the Central Valley houses an estimated 89% of California's dairy cows. However, in 2009, dairy operators in California were faced with historic low prices for milk and unusually high cost of production, including the cost of compliance with environmental regulations. There was a net loss of 100 dairies across California in 2009, eighty one dairies were located in the Central Valley (CDFA, 2009).

California dairies are complex, advanced operations, especially those facilities with a large herd size. Most all the dairies are family run, and the operators strive for production efficiencies through use of advanced technologies in genetics, nutrition, reproduction, animal housing, and animal welfare. Because the California dairy industry is so large, various entrepreneurs have developed niche markets to provide assistance to dairy operators. So instead of relying on employees, many dairy operators hire consultants who specialize in providing information, services, or trouble shooting. That option doesn't exist in most other states.

5. Consultants Addressing the General Order

The General Order has an intensive monitoring and reporting program. Operators may choose to do none, some, or all of the monitoring on their own, or hire consultants to do it. Components of the WMP such as storage capacity calculations and flood protection must be signed off by a appropriately registered professional. Likewise, only a trained professional can sign off on backflow prevention on well heads. Some components of the NMP such as the Sampling and

Analysis Plan and Nutrient Budget must be signed off by a Professional Soil Scientist, Professional Agronomist, or Crop Advisor certified by the American Society of Agronomy, or by a Technical Service Provider certified in nutrient management in California by the Natural Resources Conservation Service.

Consultants have varied knowledge and understanding of dairy operations. Some consultants have been conducting nutrient management at dairies for years. Other firms are new to nutrient management. Some consulting firms have a long history of service to the dairy industry, including addressing compliance with regulations. Some consultants provide all required services, while others provide only limited services. Some firms serve 300 or more dairies while others may serve fewer than 15 dairies.

This report presents a range of compliance costs that reflect different approaches on structuring services and fees. Some consultants charge a flat fee, while others charge based on herd size. Some focus on a particular aspect of the General Order – such as the record keeping or preparing an NMP or WMP.

6. Requirements of the General Order

The General Order requires that each dairy operation accomplish the following tasks:

- A. Inspection of dairy production area
- B. Annual report (submitted annually, July 1)
- C. Sampling and analysis of wastewater, plant tissue, solid manure, irrigation water, and soil
- D. Sampling and analysis of unauthorized off-site discharges, supply wells, tile drains, some tailwater discharges, and stormwater discharges
- E. Nutrient management plan (completion date July 1, 2009)
- F. Waste management plan (completion date July 1, 2010)
- G. Additional groundwater monitoring (some dairies ordered to begin February 1, 2010)
- H. Implementation of the NMP and WMP by July 1, 2011

In this analysis various compliance costs were examined, including:

- Reporting and documentation required by RB5
- Dairy operators (and staff) time associated with implementing the General Order
- Fees paid to consultants
- Laboratory costs
- Infrastructure | Upgrades to dairy
- Annual fees paid to RB5

A. Monthly Inspections/Service of Samples

The General Order requires a number of inspections of production and land application areas by the dairymen or a consultant, including:

- Inspection of waste storage areas (weekly or monthly depending on the time of year);
- Inspections of storm water containment structures (after significant storm events);
- Pond inspection with photo documentation showing current freeboard (monthly).
- Inspections of land application areas when process wastewater is being applied (daily).

Many of the consultants report that operators do the daily, weekly, and monthly inspections themselves. For the consultants who do this service, the fee is typically bundled with annual reporting and/or an NMP. Also some consultants charge a separate fee to travel and conduct water and soil sampling (see Subsection C below). These costs are termed "servicing of samples". Six consultants provided cost data for monthly inspections. Costs range from \$600 to \$9600 per year with an average annual cost of \$5,148.

B. Annual Report

An annual report (AR) is due by July 1 of each year, and includes a General Section, Groundwater Reporting Section, and a Storm Water Reporting Section. Table 1 provides a comprehensive list of the AR requirements.

Six consultants provided cost data for AR preparation. Costs range from \$150 to \$3,000. Some consultants reported that in general the costs to prepare the annual report increase with an increase in the number of fields utilized by the dairy. Larger dairies tend to have more fields for land application of manure.

Each application of nutrients, water, or soil amendments to each field for each crop must be tracked, recorded and data submitted within the AR. Some consultants report that they have been able to lower the fees for the AR as their staff have increased their proficiency, and some consultants alter their fee structure based on herd size. Consultants report that larger dairies may have more skilled staff who are more proficient at handling the paperwork requirements. Some consultants have raised their fees to address poor record keeping. Consultants with numerous clients generally achieve an organizational structure that permits rapid entry and review of all required data.

Table 1 - Annual Report Requirements

An annual monitoring report is due by 1 July of each year and represents activities from the previous calendar year.

A. General Section:

1. Information on crops harvested
2. An Annual Dairy Facility Assessment (an update to the Preliminary Dairy Facility Assessment)
3. Number and type of animals, whether in open confinement or housed under roof,
4. Estimated amount of total manure and process wastewater generated by the facility,
5. Estimated amount of total manure and process wastewater applied – with calculations of the nitrogen, phosphorus, potassium and total salt content.
6. Estimated amount of total manure and process wastewater transferred to other persons – with calculations of the nitrogen, phosphorus, potassium and total salt content.
7. Total number of acres for all and actual application areas used during the reporting period for application of manure and process wastewater;
8. Summary of all manure, process wastewater discharges from the production area
9. Summary of all storm water discharges from the production area
10. Summary of all discharges from the land application area to surface water
11. A statement regarding NMP update
12. Copies of all manure/process wastewater tracking manifests and written agreements for transfer of process wastewater
13. Copies of laboratory analyses of all discharges
14. Tabulated analytical data for samples of manure, process wastewater, irrigation water, soil, and plant tissue
15. Results of the Record-Keeping Requirements for the production and land application areas

B. Groundwater Reporting Section

Laboratory data for annual results from supply well and subsurface (tile) drainage systems. Additional sampling and reporting is required once groundwater monitoring wells are required and installed. For those dairies that currently have groundwater monitoring results shall be included with the annual reports.

C. Stormwater monitoring results

The report shall include a map showing all sample locations for all land application areas, rationale for all sampling locations, a discussion of how storm water flow measurements were made, the results (including the laboratory analyses, chain of custody forms, and laboratory quality assurance/quality control results) of all samples of storm water, and any modifications made to the facility or sampling plan in response to pollutants detected in storm water.

C. Sampling and Analysis of Wastewater, Manure, Plant Tissue, Soil and Irrigation Water, Supply Well, Storm Water Discharges and Unauthorized Discharges

The General Order calls for a significant amount of sampling and analyses. – including

- Sampling of solid manure
- Process wastewater (liquid manure)
- Irrigation water
- Plant tissue
- Soil
- Domestic and agricultural supply wells
- Subsurface (tile) drainage systems

Discharge Monitoring

- Unauthorized discharges of manure or process wastewater
- Stormwater discharges to surface water from production area
- Stormwater discharges to surface water from land application area
- Tail water discharges to surface water from land application area

For a detailed list of sampling frequency and minimum analyses required, see guidance from the California Dairy Quality Assurance Program (http://www.cdqa.org/docs/1.4_sampling_requirements_crib_sheetv3_9-30-07.pdf).

The General Order identifies sample handling procedures, completion of chain-of-custody documents, and approved analytical methods.

Some dairy operators hire consultants to collect samples and record appropriate information others collect samples and deliver them to the laboratory for analysis. CDFA interviewed two laboratories that conduct sampling. The reported annual costs for sampling and analysis range from \$1,500 per year for a smaller dairy to \$15,000 per year for very large dairies. The reported average annual cost was \$3,350.

One of the primary factors influencing the cost of the sampling is irrigation water source. Those dairies that are served by canal water may use data from irrigation districts (if available). For those dairies with multiple wells, each well must be sampled annually.

D. Nutrient Management Plan

The NMP is a collection of documents detailing how nutrients will be managed to prevent contamination of groundwater or discharges of nutrients to surface water. All dairies under the General Order were required to certify their NMP completed in the AR due 1 July 2009. The NMP is not required to be submitted to RB5; however, operators were required to submit numerous statements of completion during the first 30 months after the adoption of the General Order and to maintain documents and all records at the dairy for at least five years. The NMP must be made available to RB5 staff upon request during an inspection. Updates to the NMP are required when changes are made in manure management practices, including changes to crop rotation.

One of the key objectives of the NMP is to ensure that nitrogen application rates do not exceed 1.4 times the nitrogen removal rates of crops and thus be protective of groundwater quality. According to the General Order:

The purpose of the NMP is to budget and manage the nutrients applied to the land application area(s) considering all sources of nutrients, crop requirements, soil types, climate, and local conditions in order to prevent adverse impacts to surface water and groundwater quality. The NMP must take the site-specific conditions into consideration in identifying steps that will minimize nutrient movement through surface runoff or leaching past the root zone (RB5, 2007).

Required information in the NMP includes:

- a) Land application area map identifying: each field, application of solid manure or process wastewater, infrastructure for irrigation, nearby water conveyances and waterways, etc.,
- b) Written agreements for third parties receiving wastewater (including updates in each annual report),
- c) Sampling and analysis plan that documents protocols for sample collection, identifies material to be sampled and frequency of sampling, and identifies the field and laboratory data required,
- d) Nutrient budgets for each field with planned rates of nutrient applications for each crop. Nutrient budgets include: 1) rate of manure and process wastewater for each crop in each field; 2) application timing, 3) method of application of manure and process wastewater; and 4) review of P and K application rates to avoid build-up of these nutrients in the soil,
- e) Setbacks, buffers and other alternatives to protect surface water,

- f) Field risk assessment to evaluate the effectiveness of management practices used to prevent off site discharges of waste constituents,
- g) Detailed record keeping,
- h) Nutrient management plan review.

The Sampling and Analysis Plan and the Nutrient Budget require signatures of a certified nutrient management specialist.

CDFA interviewed eight consultants who prepare NMPs. Some of the consultants bundled the cost of the NMP with annual reports and monthly monitoring, particularly for the annual NMP updates; while others treat the preparation of an NMP as a separate service. The cost of NMP varies by the size of the dairy and the number of fields that receive manure applications. Reported costs for the NMP range from \$250 to \$7,000 for a dairy with 25 fields. The average cost of an NMP is \$3,295. In addition to the cost to prepare the NMP are costs for sampling and record keeping associated with the NMP.

NMP updates may trigger additional costs. Because the NMP was required in 2009 and updates are only required if changes are made, there is insufficient data at this time to determine those costs. However some consultants estimate that 20% of the NMPs need an update and will charge on a time and material basis. One consultant reports that they have had 5 or 6 dairies update their plans in mid-2010. The costs for these revisions ranged from approximately \$450 on the low side to \$1600 on the high side.

As operators become more adept at implementing their NMP, they may experience some economic benefit from improving manure management. Optimizing the use of manure as a fertilizer may result in less purchase of synthetic fertilizers or more sale of manure to neighboring farms. This report does not consider the economic benefits that may accrue.

E. Waste Management Plan

The General Order also calls for each dairy to submit a WMP. Initially, the WMP was to be submitted in July 2009; however, RB5 allowed an additional year to meet this deliverable.

The Waste Management Plan is a comprehensive document with many components, including:

- a) Facility information summary;
- b) Updated maps of structures, milking parlor, other buildings, corrals, ponds settling basins, etc.;
- c) Documentation of lagoon capacity (requires Registered Professional signature);
- d) Evaluation of flood protection (may require Registered Professional signature);
- e) Evaluation of design and construction of the production area;
- f) Operation and maintenance plan;
- g) Backflow prevention implementation by July 1, 2010 (trained professional signature).

Some engineering firms are partnering with dairy consulting firms for WMP completion. Other engineering firms are contracting directly with operators. Some consultants charge a flat fee for the WMP, while others charge a range. In addition to the costs to prepare the WMP, there will be costs to make any necessary improvements to implement the WMP. For example, if pond capacity is inadequate for storage of process water, there will be design and construction costs for additional storage. Because the General Order requires additional analysis for dairies located in a flood zone, most firms assess an extra fee for such dairies. The costs of implementing the NMP

also vary with the amount of information previously collected and with the number of wells that require backflow certification.

Engineering consultants report that the WMP will be highly site-specific and that the herd size of the dairy is not a significant factor in the cost of the WMP, though the size of the production area is. The following factors will affect the cost of WMP development:

- The amount of data needed to be collected (to save money, some operators may conduct that data collection themselves)
- Flood protection evaluations (Depending on the terrain and creeks in the vicinity of the dairy, this can be a significant cost component. No guidance was provided to consultants regarding the information to be included in the evaluation, so costs are difficult to predict.),
- The need to use more sophisticated modeling software.

Reported costs of the WMP vary widely from \$2,000 for a smaller dairy not in a flood zone up to \$27,000 for a large dairy located in a flood zone.

F. Additional Groundwater Monitoring

The General Order calls for additional groundwater monitoring beyond the monitoring discussed in Section 6(D) above. The purpose of this additional monitoring is to confirm that the facility, including cropland, wastewater retention system and the production area, is in compliance with the groundwater limitations. Operators must install a sufficient number of monitoring wells to characterize:

- Groundwater flow direction and gradient beneath the site;
- Groundwater quality upgradient of the dairy (water that is not affected by the dairy operations, but that may have been affected by upgradient activities);
- Groundwater quality down gradient of the corrals, retention ponds, and land application areas.

This means that a minimum of three wells will be necessary, and perhaps many additional wells will be needed depending on site characteristics. The depth to groundwater is a major factor that can increase costs. If both shallow aquifer and a deeper aquifer must be monitored, costs can increase dramatically.

The General Order calls for phased implementation of additional groundwater monitoring. At this time, based on an evaluation of the dairies' threat to water quality, 100 to 200 dairies per year may be directed by RB5 to submit a monitoring well installation plan, install monitoring wells, and sample those wells.

The first group of dairies ordered to install groundwater monitoring wells were those who did not complete the NMP by 1 July 2009 and had nitrate-nitrogen levels of 10 mg/l or more detected in a well or subsurface drainage system in the vicinity of the dairy.

RB5 will further prioritize groundwater monitoring requirements based on a number of factors including the location of the production area or land application area relative to California Department of Pesticide Groundwater Protection Area; the distance of production area or land application area from an artificial recharge area; the distance from the dairy production area or land application area and the nearest off-property domestic well; the distance from dairy production

area or land application area and the nearest off-property municipal well; the number of crops grown per year per field; and Whole Farm Nitrogen Balance.

A registered engineer or geologist must prepare the monitoring well installation plan and submit it for approval by RB5. Initial estimates for the cost of Individual Groundwater Monitoring developed by Dairy CARES (an association of dairy operators and dairy industry representatives) are \$42,500 for upfront costs (well plan, drilling of at least 3 wells, annual sampling and analysis), and \$5,000 per year for reporting.

Alternative Representative Groundwater Monitoring Program

The General Order also allows for establishing an alternative groundwater monitoring program in lieu of each producer installing monitoring wells and conducting sampling. Representatives of Dairy CARES, Western United Dairymen and other industry associations are actively developing an alternative plan which is subject to approval by the Executive Officer of the RB5.

As of September, 2010, the Alternative Representative Groundwater Monitoring Program has not been approved by RB5. In addition there are some dairies that will not be included in the program.

The current draft of the alternative plan includes establishing a nonprofit organization with a Board of Directors to manage clustered groundwater monitoring program and collect fees from enrolled dairy operators to support the monitoring. This approach would allow operators to enroll in the groundwater monitoring organization and pay a fee. The collected fees will support the installation of groundwater monitoring wells and associated sampling, analyses, and reporting requirements on a select group or groups of dairies.

Table 2 includes estimates for the representative groundwater monitoring network developed by Dairy CARES. The fee estimate is based on the number of dairymen who enroll in the representative monitoring program and this cost range is based on estimates of 60% to 80% of the industry participating. The 5-year total cost for the representative monitoring program could range \$3,320 to \$4,860 including well installation, sampling, analysis, and reporting). Compared to groundwater monitoring by individual dairies, the representative monitoring plan is considerably less expensive – especially given that the monitoring will continue into the future.

The final cost list (Table 3) includes both the representative groundwater program and the individual monitoring since there is uncertainty regarding the final structure of this requirement. If this program is not approved and implemented then costs for individual dairy operators to develop and install wells will increase significantly.

Table 2. Estimated Costs for Representative Monitoring Program

One time Sign Up Fee	\$500
Annual Membership Fee (estimate)	\$664 - \$972
Total 2010	\$1164 - \$1472

Dairy CARES - Jan 2010

7. Dairy Operators' Time

One cost factor that must be evaluated is the dairy operators' time dedicated to fulfilling the General Order requirements. CDFA Dairy Marketing Branch collects cost of production information

from approximately 10 percent of the dairies located in the Central Valley. CDFA surveyed 62 operators to determine how much time an employee or manager spent on the General Order on a monthly basis to maintain records, taking samples, etc. Estimates of the amount of time operators dedicated to complying with the General Order range from 1 to 28 hours per month. Additional time is needed to attend classes, read reports, and review documents.

The average hourly wage for employees working on a dairy in 2009 was \$28.00 (CDFA, 2010). This average wage value and estimates of time spent was used to establish the cost of complying with the General Order. The annual cost ranges from \$336 to \$9,408 with an average of \$3,148.

8. Capital Investment

Capital investment upgrades to dairy facilities and structures are another cost operators have to incur to comply with the General Order. ***At this time we are only noting that these costs are occurring but we have no way of determining a representative cost to apply, so they are not included for this study, however it is likely that these are significant costs.*** Since every dairy facility is designed and operated differently, each facility had a different set of issues they had to deal with for their NMP and WMP. Infrastructure improvements related to NMPs and WMPs in many cases have not yet been implemented and are not required to be completed until 2011. Capital investment for infrastructure may include expanding retention ponds, exporting nutrients offsite, adding equipment to process manure on site for export, installation of irrigation delivery systems and related equipment such as flow meters, and installation of flood/runoff control structures such as berms and tailwater return systems.

Interviews with operators show that some had made no capital improvements while others have invested up to \$350,000 in facility improvements. However, in many cases it is difficult to distinguish between general facility improvements and improvements necessary to comply with the General Order. Facility upgrades that were completed include back flow prevention, raising stand pipes, upgrading irrigation pipes, installing concrete silage pads, installing rain gutters, corral grading, adding a new lagoon, and expanding an existing lagoon.

9. Technical and Financial Assistance

Both technical and financial assistance is available to dairy operators to help them understand and implement the General Order. The CA Dairy Quality Assurance Program (CDQAP) is a partnership among California's dairy industry, federal, state and regional government agencies and the University of California Cooperative Extension. CDQAP provides technical assistance to operators and helps them understand and comply with the regulations. A range of services is provided including educational workshops targeted at consultants to provide detailed information and greater understanding of compliance requirements. Producer workshops have focused on providing updated information and immediate deliverable requirements. The curriculum developed has been reviewed by RB5 staff. When possible, example documents and templates have been created to assist operators and their consultants to comply with the General Order. Lastly, CDQAP also provides a voluntary evaluation program with certification available for facilities and managers meeting local, state and federal environmental requirements.

RB5 also provided funding to Merced County to create and maintain on-line forms tailored to meet annual reporting requirements.

Limited financial assistance is also available for dairy operators for planning and implementation on a cost-share basis. The USDA Natural Resources Conservation Service (NRCS) Farm Bill conservation programs are a key funding source.

From 2008 – 2010, NRCS invested \$32.5 million for 1,064 contracts with California dairy and other livestock farmers to implement conservation practices that will help them comply with regulations, manage and use the manure from their animals to fertilize their crops, and improve water quality. The key farm bill programs are Environmental Quality Incentives Program (EQIP), Cooperative Conservation Partnership Initiative (CCPI), and the Agricultural Water Enhancement Program (AWEP – a partnership program with Western United Dairymen).

These programs provide funds on a cost-share basis. Most operators must provide 50% of the cost in order to receive funds. Some of the common practices are concrete stacking pads which reduce leaching to groundwater; manure transfer pipelines which increase the ability to evenly distribute liquid manure to land; flow meters and other devices so that manure applications can be precisely measured; mechanical separators which reduce solids getting in to ponds and tail-water return systems which capture drainage water and return it to the field. Waste management plans are also a cost-share practice; in 2009, NRCS was able to fund the development of more than 600 waste management plans.

Dairy trade associations have also been awarded funds through Farm Bill programs mentioned above. In addition, the California Dairy Campaign received \$750,000 in NRCS Conservation Innovation Grant funds to provide compliance assistance.

Limited assistance was also available through Proposition 50 grant funds administered by the State Water Resources Control Board. Both Western United Dairymen and the California Dairy Campaign had programs to assist dairy operators obtain grant funding for necessary improvements in manure management.

The amount of financial assistance that an operator receives varies widely. Because funds are limited, screening and ranking criteria for the programs are subject to change each year and not all operators apply for or receive funding; these funds are not included as a potential offset in the total costs table below. However, it is important to know that funds may be available for those who apply, and that funding is critically important.

However even with the significant amount of funds available, supply is insufficient to meet current demand. In 2010, the NRCS EQIP dairy programs were largely over-subscribed with 200 applicants placed on waiting list or placed in the pool for following year's application. From 2008 – 2010 only 50% of funding applications for these programs were approved.

10. Analysis and Conclusions

Table 3 presents a total of all the costs of compliance with the General Order. Again it should be emphasized that these costs are estimates and that they are likely to rise in the 2011 and beyond when groundwater monitoring is fully implemented and dairies invest in capital improvements identified in the WMP's.

The table is divided into one-time costs and annual (reoccurring) costs. One-time costs are those associated with specific deliverables such as the NMP and the WMP. Annual costs occur each year as long as the dairy is in operation and has a permit from RB5.

As discussed above there is uncertainty about the additional groundwater monitoring program. Table 3 below includes estimated for both the representative and individual approaches. If the representative program is approved, we expect a majority of dairy producers to join this program; due to its significantly lower costs.

Not including the costs for additional groundwater monitoring, the average one-time costs for operators range from \$2,750 to \$35,984 with an average of \$12,567. Average annual costs range from \$3,006 to \$42,440 with an average of \$14,136. Groundwater monitoring will add significantly to the cost of the program. Total one-time compliance costs including individual groundwater monitoring will range from \$45,250 to \$77,984 with an estimated average of \$55,067 with annual compliance costs of \$8,006 to \$47,440 with an average cost of \$19,136.

Based on the data in Table 3, and using 2007 as the beginning date when compliance costs began, an "average" dairy of 1,000 cows has spent approximately \$55,000 in compliance costs; while a larger dairy with more crop fields may have spent \$160,000 or more.

In 2007, estimates of the cost of compliance with the General Order were made by Dairy CARES and RB5 as the General Order was being developed. Dairy CARES estimated that the cost of compliance would be \$49,780 for one-time costs and \$33,570 for costs that will occur annually for as long as the dairy is producing.

In 2007, RB5 estimated \$41,700 for up-front costs and \$33,300 reoccurring. While it appears that CDFA's estimates are lower - direct comparisons to Dairy CARES and RB5 are problematic because of differences in study methodology.

While this paper provides compliance costs for water quality concerns, dairy operators are also faced with air quality regulations and associated compliance costs from the San Joaquin Valley Air Pollution Control District. CDFA will examine these regulations and costs in future studies.

Table 3. Range of Cost Estimates for Central Valley Dairy Operators to Comply with WDR.

	ONE-TIME COSTS ¹			ANNUAL COSTS ²		
	LOW	HIGH	AVERAGE	LOW	HIGH	AVERAGE
Existing Conditions Report & Preliminary Dairy Facility Assessment (2007)	\$500	\$1,484	\$992	n/a	n/a	n/a
Waste Management Plan (2010)	\$2,000	\$27,000	\$8,280	n/a	n/a	n/a
Nutrient Management Plan (2009)	\$250	\$7,000	\$3,295	n/a	n/a	n/a
Monitoring and Reporting Program						
Laboratory Sampling and Analysis	n/a	n/a	n/a	\$1,500	\$15,000	\$3,350
Monthly Inspections	n/a	n/a	n/a	\$600	\$9,600	\$5,148
Annual Report	n/a	n/a	n/a	\$150	\$3,000	\$810
RWQCB Annual Discharge Fee ³	n/a	n/a	n/a	\$420	\$5,600	\$1,680
Dairy Labor ⁴	n/a	n/a	n/a	\$336	\$9,240	\$3,148
SUBTOTAL	\$2,750	\$35,484	\$12,567	\$3,006	\$42,440	\$14,136
Representative Groundwater Monitoring Program ⁵	\$500	\$500	\$500	\$664	\$972	\$818
Additional Groundwater Monitoring (Individual) ⁶	\$42,500	\$42,500	\$42,500	\$5,000	\$5,000	\$5,000
TOTAL COMPLIANCE COSTS - Representative Groundwater Monitoring Program	\$3,250	\$35,984	\$13,067	\$3,670	\$43,412	\$14,954
TOTAL COMPLIANCE COSTS - Individual Groundwater Monitoring	\$45,250	\$77,984	\$55,067	\$8,006	\$47,440	\$19,136

¹ One-time costs meet specific deliverables in the General Order.

² Annual costs will re-occur each year.

³ 2009-2010 RWQCB Waste Discharge Fee: http://www.swrcb.ca.gov/resources/fees/docs/confined_animal_facilities_fees.pdf

⁴ Work done on dairy by employee and/or managers taking samples, filling out reports, etc.

⁵ Estimated enrollment and annual fees for Representative Program

⁶ Estimated cost (\$42,500) well plan, drilling of at least 3 wells, annual sampling and analysis, and \$5,000 per year for reporting.

Table 4. Total Cost Estimates of General Order by RB5 and CARES, 2007

Requirement	RB5 Upfront (one-time)	RB5 Annual (reoccurring)	CARES Estimate Upfront (one-time)	CARES Estimate Annual (reoccurring)
Existing Conditions Report	\$2,100	\$0.00	\$2,000	\$0
Waste Management Plan	\$11,400	\$0.00	\$9,400	\$0
Nutrient Management Plan	\$800	\$3,800	\$2,700	\$3,500
Monitoring and Reporting	\$27,400	\$29,500	\$35,680	\$30,070
Total Costs	\$41,700	\$33,300	\$49,780	\$33,570
Cost Range	\$12,000 to \$56,000	\$30,000 to \$36,000		

RB5, 2007 and CARES 2007

11. References

California Department of Food and Agriculture. 2010. California Cost of Milk Production Annual Report for 2009.

Central Valley Regional Water Quality Control Board. 2007. Order No. R5-2007-0035: Waste discharge requirements general order for existing milk cow dairies.

Central Valley Regional Water Quality Control Board. 2007. Staff Presentations at 3 May 2007 Public Hearing, Presentation 1, Part 3, Rancho Cordova, CA.
http://www.waterboards.ca.gov/centralvalley/water_issues/dairies/dairy_program_regs_requirements/index.shtml

USDA NRCS. 2010. "CA NRCS and California Dairies Invest Approximately \$12 Million in Water Quality in 2010". May 24, 2010. Davis, CA. http://www.ca.nrcs.usda.gov/news/releases/2010/dairy_5-21-10.html

Dairy CARES Base Cost Comparison, TGO Cost Analysis 2-23-07.

Acknowledgements

Dairy trade organizations, dairy operators, University of California, Davis scientists and staff from the State Water Resources Control Board and RB5 reviewed preliminary drafts and provided insightful comments. We also thanks those consultants who provided the data.