University of East Anglia

and

Drinking Water Inspectorate

# Review of evidence for relationship between incidence of cardiovascular disease and water hardness

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## Authors

[1] Louise Catling (LC)
[1] Ibrahim Abubakar (IA)
[2] Iain Lake (IL)
[1] Louise Swift (LS)
[1] Paul Hunter (PH)

[1] School of Medicine, Health Policy and Practice
[2] School of Environmental Sciences

University of East Anglia, Norwich, Norfolk, NR4 7TJ.

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**Commonly used abbreviations**

| | |
|---|---|
| AHD | arteriosclerotic heart disease |
| AMI | acute myocardial infarction |
| Ca | calcium |
| Cd | cadmium |
| CHD | coronary heart disease |
| CI | confidence interval |
| CVD | cardiovascular disease |
| HHD | hypertensive heart disease |
| ICD | international classification of disease code |
| IHD | ischaemic heart disease |
| Mg | magnesium |
| OR | odds ratio |
| Pb | lead |
| RR | relative risk |
| SMR | standardised mortality ratio |

# Executive summary

## Summary statement

The literature examining the association between drinking water hardness and cardiovascular disease was identified and reviewed, however a comparatively small proportion of these studies were considered of high quality. This review found evidence to support a protective effect of drinking water hardness against cardiovascular disease from a number of studies. If a causal association is established, the comparatively small reduction in cardiovascular risk seen with higher levels of water hardness, calcium and magnesium concentrations may translate to a substantial public health benefit at the population level. At present there is insufficient evidence to elucidate the nature of this apparent effect, with significant inverse associations seen with both drinking water calcium and magnesium and cardiovascular disease. There is also insufficient evidence to make any substantive conclusions regarding drinking water hardness and non-cardiovascular health outcomes.

## Background and purpose

A large body of literature has amassed over a 40 year period examining the apparent inverse association between drinking water hardness and cardiovascular disease in a number of countries. Cardiovascular disease is the leading cause of death in industrialised nations responsible for approximately 235,000 deaths in the UK in 2004. Furthermore, recent studies have begun to examine more diverse non-cardiovascular health outcomes, specifically cancer. Therefore the possible association between drinking water hardness and health outcomes is of great interest given the widespread exposure of the population and the capacity for modification of drinking water hardness levels. Renewed interest in the possible health effects of drinking water hardness has culminated in a series of recently commissioned reviews by the World Health Organisation (WHO).

This study was commissioned by the Drinking Water Inspectorate to review and critically assess the quality of available studies examining the health effects of soft water, primarily the long standing apparent inverse association between water hardness and cardiovascular disease. This review exceeds that undertaken by the WHO with regard to both the consideration of a wider range of primary data papers, and a greater focus upon a systematic assessment of the quality of each study informing the conclusions of this review.

The scope of this review was restricted to an evaluation of the currently available data reporting upon aspects of drinking water quality, specifically hardness, calcium, magnesium, cadmium and lead, and cardiovascular health and other health outcomes. The purpose of this document is to provide an evaluation of the evidence base for policy makers, but not to comment upon any potential policy implications or to recommend any particular course of action.

**Objectives**

To this aim, the objectives of this study were to:

1. Review and critically assess the merits of available studies concerning the health effects of soft and softened water and to categorise the identified studies based on relevant features such as size, study design, conclusions, etc.
2. Advise on the evidence relating to the incidence of cardiovascular disease, or other adverse health effects to consumption of soft or softened water and to comment on the reliability and strength of any reported associations.
3. Advise whether the studies indicate a "no effect" level in relation to water composition and whether a causal or protective agent relating to incidence of illness is evident.
4. To comment on whether the results indicate the need to consider re-hardening of softened water, or whether supplies of naturally soft water should be hardened and provide an indication of the scale of the health benefits of such action.
5. To comment on the evidence relating to specific postulated causal (lead, cadmium) or protective (calcium, magnesium) elements and their importance with respect to cardiovascular disease

## Methods

A standardised search strategy was devised encompassing drinking water hardness, calcium, magnesium, cadmium and lead together with all aspects of cardiovascular disease and cancer as major search terms. We searched available electronic databases including MedLine, PubMed, EMBASE, CINAHL, Toxline, Web of Science and Cochrane Reviews. We also searched the Grey literature using the SIGLE database and the National Research database. The initial search identified 2096 papers, the abstracts of which were then independently screened for relevance by two reviewers. Papers were retained if they presented primary data of human studies, were directly related to the research question and involved a comparison of populations or individuals at different levels of exposure. Experimental animal studies and human dietary studies were excluded from this review. Overall, 132 studies were identified as primary data papers, of which 17 papers were excluded due to their descriptive but non-analytical content. A total of 115 underwent full article appraisal by two independent reviewers.

Standardised forms for data extraction and study quality assessment were created to extract information including study population characteristics, exposure characterisation, sample size, validity of the health outcome and quality of statistical analysis. Separate forms were constructed and used for assessment by epidemiological study type (ecological, cross-sectional, case control and cohort).

Due to the significant level of clinical and methodological heterogeneity, no attempt was made to assess for evidence of statistical heterogeneity. Data were presented in tabular form with a narrative synthesis of the results presented with no formal meta analysis.

## Results

### *Ecological studies*

Most of the published data relate to the possible association between cardiovascular mortality and water hardness, calcium or magnesium levels. The majority of studies

reviewed were of an ecological study design. Further study quality criteria were applied to categorise the ecological studies by high, medium or low quality. A total of 60 such papers were evaluated, of which 44 met the minimum quality criteria. Of the 12 high quality studies, 9 presented evidence for a significant inverse association between water hardness, calcium and/or magnesium levels and cardiovascular mortality. The remaining 3 studies found no significant association. Of the 32 medium and low quality studies, 22 found a significant inverse association.

*Cross-sectional studies*

Five cross sectional studies were identified, of which only 2 sampled the drinking water quality at the individual level with the remainder using an ecological measure of the drinking water parameters. These papers examined individual level cardiovascular risk factors with an inverse association between drinking water calcium and/or magnesium and blood pressure and serum lipids observed in some, but not all studies.

*Case control studies*

Six case control studies examined both drinking water magnesium and calcium and risk of death from cardiovascular disease. Of these, 4 found a significant inverse association with magnesium concentrations.

*Cohort studies*

Of 3 cohort studies reviewed, 2 were of medium/poor quality and used an ecological measure of drinking water factors and limited or non-existent controlling for possible confounders. The third study was conducted in Great Britain and found no association between drinking water hardness and cardiovascular disease. However this study also suffered from poor exposure characterisation.

*Sudden death*

It has been suggested that the inverse association seen between cardiovascular disease mortality and water hardness may be due to an increase in the proportion of sudden deaths in areas of soft water. Few studies specifically examined sudden death. Our study quality assessment identified only one high quality case control study which demonstrated a protective effect of drinking water magnesium against the risk of death following a heart attack, but not for the risk of a heart attack overall. This is suggestive of a decreased risk of cardiovascular mortality rather than cardiovascular disease incidence overall with increasing drinking water magnesium. To date there is insufficient evidence available upon which to base any conclusion regarding the possible role of sudden death in the inverse association described between drinking water hardness and cardiovascular disease.

There was no evidence to suggest a differential effect of the association between drinking water quality and heart disease or stroke. Whilst evidence has been reviewed supporting a protective role of drinking water quality against atherogenesis, high blood pressure and possibly electrical instability, too few studies have sufficiently examined the possible biological mechanism of association as it may relate to existing major cardiovascular risk factors. The evidence to date is therefore too weak and inconsistent to allow any firm conclusions to be drawn regarding the possible biological mechanism linking drinking water quality and cardiovascular disease. Furthermore, the evidence at present does not support a 'no effect' level.

*Non-cardiovascular health outcomes*

A total of 15 studies examined the association between drinking water hardness, calcium or magnesium and non-cardiovascular health outcomes. Of these, 13 considered cancer as the health outcome of interest and provided evidence for a possible protective effect of drinking water hardness, calcium and magnesium against several malignancies. However, of these 15 studies, 12 were case control studies conducted by a single group in Taiwan. Therefore replication of the results in other settings and centres will be necessary before causal inference can be attempted. As yet, no substantive conclusions can be made in this emerging area of research.

*'Protective' vs 'harmful' effect of drinking water hardness*

A number of biologically plausible hypotheses have been reported attempting to identify a single protective factor (dominantly calcium or magnesium) or harmful factor (dominantly cadmium or lead) associated with varying drinking water hardness to explain the apparent inverse association between hardness and cardiovascular disease. The current evidence based upon the epidemiological studies reviewed here shows a weight of evidence to support the role of a 'protective' factor in drinking water. However it must be acknowledged that the small numbers of studies examining possible 'harmful' factors may be influenced by publication or reporting bias. A significant inverse association between both drinking water calcium and magnesium was reported by a number of studies considered in this review. However, at present there is not enough evidence of a sufficient quality to identify any factor responsible for the apparent cardioprotective effect of hard drinking water.

**Study quality**

This review found that although there was a large volume of literature examining drinking water hardness and cardiovascular disease, only a small proportion of the studies were deemed to be of high quality. Therefore even though the methodologically superior cohort and case control studies have examined the association between drinking water hardness and cardiovascular disease, a common criticism of study quality was poor characterisation of the actual exposure of interest. The majority of individual level studies, including the cohort studies, applied an ecological measure of drinking water quality to the individual level data in the analysis of exposure and outcome. Furthermore, some cohort studies were limited in their examination of potential confounding factors.

Prospective cohort studies are the methodological ideal for the examination of a causal association between an exposure and a health outcome. However, the lack of well designed cohort studies in the literature means that the conclusions of this review must be informed by high quality, but methodologically inferior, studies.

**Future directions**

This assessment highlights that although there is a large body of literature examining the association between drinking water hardness and cardiovascular health, there has been a lack of focus upon examination of key issues such as temporality and investigation of a possible dose-response relationship on scales of health effects. Epidemiological studies to date are therefore deficient in their ability to contribute to an assessment of causality. A major challenge for future studies attempting to establish a causal association between drinking water parameters and cardiovascular health is the accurate quantitative and qualitative characterisation of individual exposure and the biologically relevant dose. More focused research at the individual level is required to address the deficiencies of the studies to date.

Whilst prospective cohort studies are the ideal for examining causal associations, they are also resource intensive and costly. Other possible options for future study include those able to consider the direction of association, such as those examining a change in the hardness of a public drinking water supply and subsequent changes in health outcomes in a defined population.

# 1 Introduction

Interest in a possible association between the drinking water hardness and human health began over forty years ago and continues today. The earliest publication commonly cited as the initial stimulus for this topic was conducted in Japan by Kobayashi (1957). He described an inverse association between death rates from cerebrovascular disease and the acidity of river water used for drinking, with greater mortality associated with a decreasing ratio of carbonates to sulphates. This study was closely followed by Schroeder (1960a) who examined 163 municipalities in the United States and demonstrated that drinking water hardness was significantly and inversely correlated with male and female cardiovascular mortality. Since that time, a large volume of literature has amassed examining drinking water hardness and human health in a number of countries including Great Britain, USA, France, Spain, Sweden and Finland. A new dimension in the continued interest in what has become known as 'the water story' is presented by the increased likelihood of utilising water desalinisation plants for the provision of drinking water in regions where resources are becoming increasingly strained due to population growth. The identification of any population health effect that may be associated with the chemical quality of drinking water may therefore be of paramount importance before such desalinised, and hence demineralised then reblended (WHO 1979), water may be utilised on a large scale.

The possible interactions between environment and human health have become the focus of much research attempting to explain the distinct geographies of disease that have been observed in a number of countries. The renewed interest in the possible effects of drinking water hardness has culminated in a series of recently commissioned reviews by the World Health Organisation (WHO) summarising published evidence for an association between drinking water hardness and cardiovascular diseases (Calderon and Craun 2004 (*draft*), Monarca *et al.* 2004 (*draft*): these are currently in draft form and are available to view on the WHO website). Whilst the majority of studies have primarily focused upon cardiovascular disease as the health outcome of interest, recent investigations have begun to examine more diverse non-cardiovascular health effects of the chemical quality of drinking water. The reviews were commissioned by the WHO with the eventual aim of

establishing minimum concentrations of drinking water constituents that may have favourable results on population health.

### 1.1 Definition of drinking water hardness

The hardness of drinking water is determined by the concentration of a number of dissolved polyvalent mineral cations, but predominantly those of calcium and magnesium. Hard water contains higher concentrations of these dissolved minerals (in varying proportions), mainly in combination with bicarbonate, sulphate and chloride, than soft water. The hardness of drinking water is usually dictated by the source of extraction (whether groundwater or surface water) and the underlying geology of the area. Soft rocks including chalk and limestone impart greater concentrations of minerals thereby contributing to harder water than areas of hard rocks such as granite.

Hardness is generally measured as the total concentration of calcium and magnesium present, and is commonly expressed in terms of calcium carbonate equivalent (mg/l $CaCO_3$). There is no universal classification for water hardness, but in general soft water is defined by a concentration of <100 mg/l $CaCO_3$, with moderately hard water as 100 to 200 mg/l $CaCO_3$, and hard water as >200 mg/l $CaCO_3$ (British Water). The most commonly known effects of hard water are due to deposits of calcium carbonate, or scale, which form after evaporation or heating by electrical elements in washing machines and kettles.

### 1.2 Possible health outcomes associated with drinking water hardness

#### 1.2.1 Cardiovascular disease

Cardiovascular disease is the leading cause of death in most industrialised western nations with approximately 235,000 deaths (39% of all deaths) occurring each year in the United Kingdom (BHF 2004). The two most common forms of cardiovascular disease are ischaemic heart disease (IHD), which encompasses conditions such as angina and acute myocardial infarction (heart attack), and cerebrovascular disease (stroke). Of these, IHD is the most common cause of death, responsible for more than

1 in 3 male, and 1 in 6 female deaths. Alongside these mortality figures there is a substantial morbidity burden with an estimated 2.5 million people living with heart disease. This in turn has a huge burden both in terms of individual suffering and impaired quality of life, but also in economic terms with the cost of healthcare being over 1.7 billion pounds a year (www.heartstats.org). Stroke similarly carries a severe morbidity burden in addition to mortality.

The aetiology of cardiovascular disease is multifactorial with many individual level risk factors having been identified including hypertension (high blood pressure), dyslipidaemia (adverse alterations in the lipid profile and concentrations of cholesterol), smoking, alcohol, obesity, lack of exercise and dietary habits. However, distinct geographical patterns in cardiovascular mortality have been observed to exist both within and between countries and appear to be independent of these risk factors.

A spatial analysis of Eurasia detected a trend of increasing cardiovascular and cerebrovascular mortality to the north and north-west (Peter *et al.* 1996). A distinct north-south gradient of cardiovascular mortality in Great Britain has been well established for a number of years demonstrating increased morbidity and mortality in the north in both males and females (West 1977, Pocock *et al.* 1980, MacPherson and Basco 2000). This gradient is observed for both morbidity and mortality and therefore does not reflect differing case fatality rates. Moreover, the geographical trend is not explained in terms of differential access to health care or treatment (Britton *et al.* 2004, Lawlor *et al.* 2003). Notable geographical trends have also been observed in the US (Schroeder 1960b), Finland (Punsar and Karvonen 1979, Kaipio *et al.* 2004) and Sweden (Nystrom *et al.* 1986, Gyllerup *et al.* 1991).

The observation of such distinct patterns in mortality has have been used to generate hypotheses regarding additional possible risk factors for cardiovascular disease that vary in a similar geographical fashion. Several studies have considered the role of the physical environment and the association between cardiovascular disease and temperature (Näyhä 2002, McGregor 2001), rainfall (Pocock *et al.* 1980), and sunlight (Catling 2004). Another possible explanation for the observed geography is the differential distribution of genetic traits. However whilst cardiovascular disease does

show a degree of familial aggregation, it is not commonly considered as clearly genetically driven (Piispanen 1993).

The geographical distribution of water hardness has been found to be inverse to that of cardiovascular mortality in a number of countries. This inverse distribution, whereby areas of harder water correspond to areas of lower cardiovascular mortality, is illustrated for Great Britain in Figures 1 and 2. Cardiovascular mortality is expressed at the local authority district level as the standardised mortality ratio (SMR), showing mortality standardised by the underlying age and gender structure of the district population. These figures clearly show the trend of increasing cardiovascular mortality from the south-east to the north-west for males (Fig 1a) and females (Fig 1b), and the corresponding inverse geographical trend for drinking water hardness grading from hard water in the south-east to soft water in the north-west (Fig 2)[1]. The contribution of drinking water hardness to geographical variations in cardiovascular disease in Great Britain was recently considered in a prospective cohort study. Water hardness remained inversely associated with cardiovascular disease but was not statistically significant after adjustment for a number of individual level risk factors including blood pressure (Morris *et al.* 2001). However, the statistical power to examine the association was limited given only 24 towns were included in analysis.

[1] Reproduced with permission from Catling (2004)

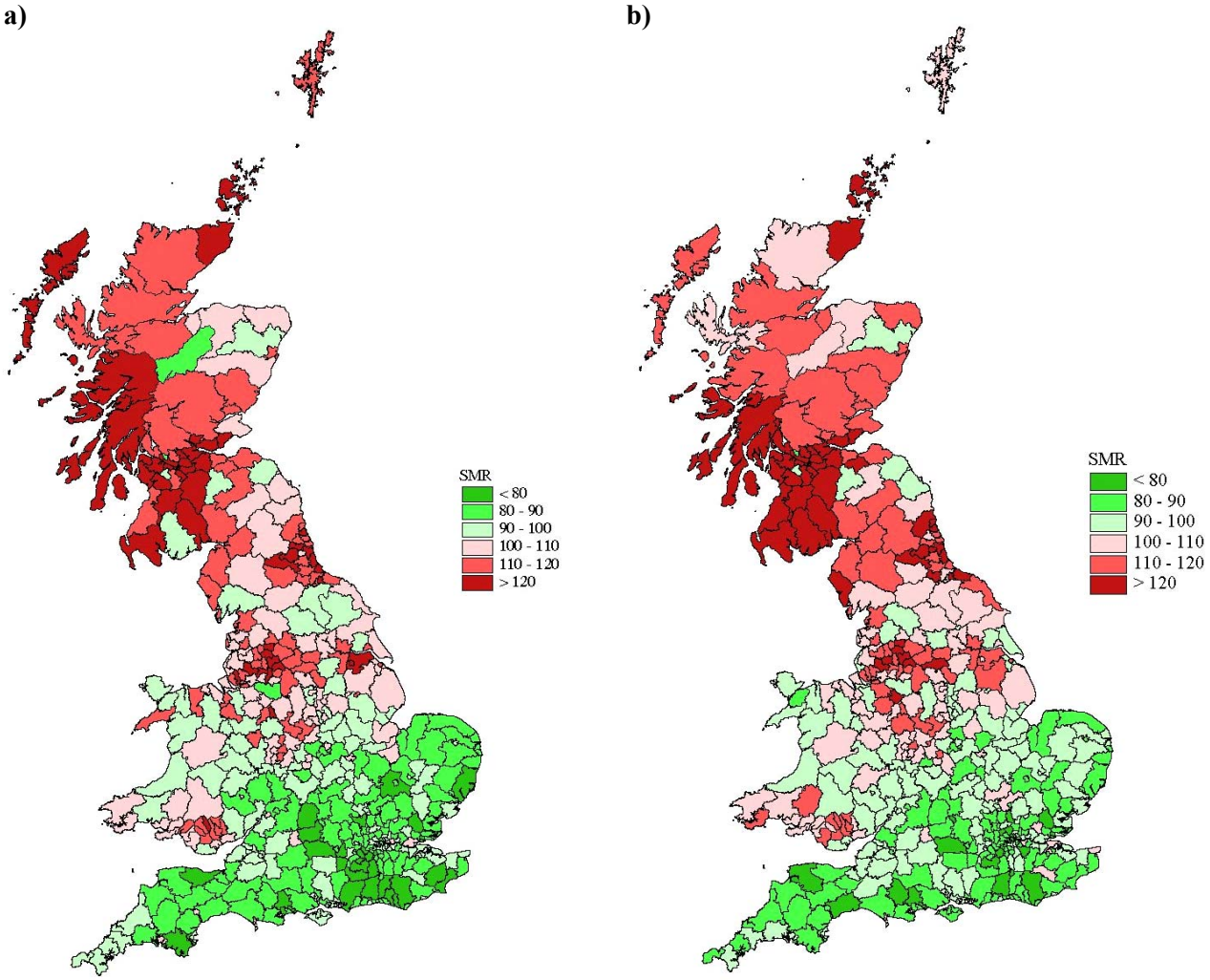


**Figure 1 The geographical distribution of a) male and b) female cardiovascular mortality in Great Britain at the local authority district level. Mortality is presented as the standardised mortality ratio (SMR) for all ages and all codes.**

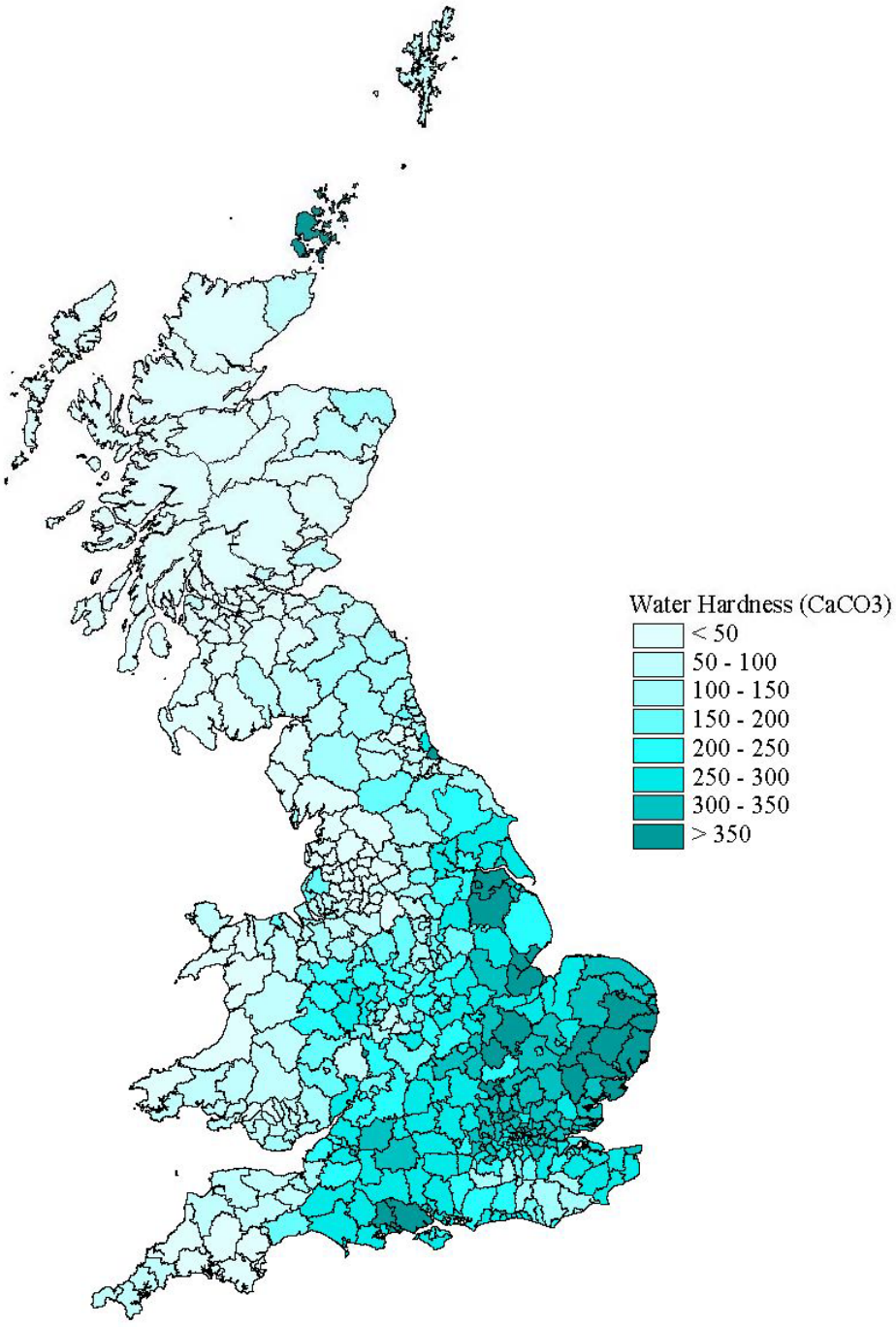


**Figure 2 Drinking water hardness ($CaCO_3$ mg/l) at the local authority district level for Great Britain**

### 1.2.2 Cancer

Possible non-cardiovascular health outcomes have only been considered in more recent studies, therefore the volume of published literature is comparatively smaller. In a recent WHO review, an apparent inverse association between oesophageal cancer, colon cancer and drinking water hardness was suggested (Nam Ong 2004). Colorectal cancer is currently the third most common cancer in Great Britain with approximately 34,500 people diagnosed each year. Oesophageal cancer is a less common form with 7,400 diagnoses made annually (Cancer research UK). These diseases were therefore included as specific search terms for this study to incorporate more recent evidence of an association between drinking water hardness and human health.

### 1.2.3 Other health outcomes

Recent studies have found increasing water hardness to be a risk factor for childhood atopic eczema in England (McNally *et al.* 1998) and Japan (Miyake *et al.* 2004a). Furthermore, drinking water hardness has also been investigated with regard to Type I diabetes (Zhao *et al.* 2001).

However, given the substantially greater morbidity and mortality burden and the economic costs associated with cardiovascular disease and cancer, this review will focus upon studies considering water hardness and adult cardiovascular disease and cancer only.

## 1.3 Protective *vs* harmful drinking water factor

It has been proposed that the apparent lower levels of cardiovascular disease mortality observed in hard water areas, if causal, may be explicable by the presence of a *protective* component in hard drinking water which may prevent disease or modify its severity, or the absence of a *harmful* component. Similarly, the higher levels of mortality observed in soft water areas may be attributable to the possible absence of such a protective factor, or the presence of a harmful factor which may predispose to disease or enhance its severity. These two apparently disparate approaches to the

consideration of a biological mechanism of association have generated studies examining the relationships of individual drinking water components with health outcomes.

Drinking water hardness is chiefly defined by concentrations of the minerals calcium (Ca) and magnesium (Mg). Much research has therefore considered the increased concentration of these minerals in hard drinking water as the hypothesised protective factor. Conversely, soft drinking water has a lower buffering capacity and is therefore more corrosive (Rubenowitz-Lundin and Hiscock 2005). This can result in the increased leaching of heavy metals such as cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) from the water pipes of the drinking water distribution system. The possible increased concentrations of these heavy metals in soft drinking water have therefore been proposed to constitute the harmful factor.

The biological plausibility of these hypothesised associations with both cardiovascular disease and cancer will be briefly considered in the following sections. Supporting evidence is derived from studies such as animal experiments and human intervention studies.

### 1.3.1 Biological plausibility for calcium and magnesium

An increased calcium intake from dietary intervention and supplementation studies has been associated with a general decrease in blood pressure, a major established risk factor for cardiovascular disease (McCarron and Reusser 1999). This observation may, at least partially, explain the reduced significance of the inverse association between water hardness and cardiovascular disease at the individual level in Great Britain following adjustment for blood pressure (Morris *et al.* 2001). Furthermore, there is evidence of a dose-response effect whereby the greatest blood pressure decreases have been observed in individuals with the lowest calcium intakes (Dwyer *et al.* 1998, McCarron and Reusser 1999). A randomised controlled dietary intervention study found the greatest decreases in blood pressure with increased dietary calcium intake to occur in hypertensive individuals (Appel *et al.* 1997).

A similar beneficial role has been suggested for increased magnesium intake (Chakraborti *et al.* 2002). Animal studies have demonstrated magnesium deficiency to be associated with cardiac arrhythmias, which predispose to fatal myocardial infarction (Anderson *et al.* 1975). Magnesium is also essential for regulating vascular tone (Rubenowitz-Lundin and Hiscock 2005). Animal studies have shown that supplemental magnesium delivered via drinking water significantly improved the lipid profile and inhibited atherosclerosis (Sherer *et al.* 1999, Sherer *et al.* 2000, Cohen *et al.* 2002). Furthermore there is also evidence that magnesium deficiency may contribute to insulin resistance (a risk factor for cardiovascular disease) (Ma *et al.* 1995). The strongest evidence of an association between magnesium and heart disease comes from prospective population based cohort studies. Ford (1999) showed that serum magnesium concentrations were significantly and inversely associated with future risk of cardiovascular mortality.

A possible protective effect of calcium against cancer has been illustrated in *in vitro* studies of human cells. Calcium was shown to directly inhibit the proliferation of cells by inducing terminal differentiation (Yang *et al.* 1997a). Magnesium may be central to the cell cycle with a deficient state hypothesised to be an important conditioner in precancerous cell transformation (Tukiendorf and Rybak 2004). Intracellular magnesium may also enhance the fidelity of DNA replication, or may prevent the changes that trigger the carcinogenic process (Yang *et al.* 2000a).

### 1.3.2 Biological plausibility for cadmium and lead

Drinking water is considered an important pathway of exposure to cadmium (Cd) and lead (Pb), which may increase cardiovascular risk (Barry-Ryan *et al.* 2000). Animal studies have demonstrated a hypertensive effect of low level cadmium in drinking water showing increasing severity with chronic exposure (Perry *et al.* 1974). Cadmium accumulates in the kidney and therefore may exert a more chronic effect upon the cardiovascular system (Gyllerup *et al.* 1991). Perry *et al.* (1974) further demonstrated that when the cadmium was delivered via hard water the blood pressure increase associated with the accumulated cadmium was inhibited compared to delivery via distilled water. A degree of interaction between the hypothesised protective and harmful components of drinking water may therefore be present. This

is further supported by animal studies showing the toxic effects of heavy metals were exacerbated in calcium or magnesium deficient animals (Zieghami *et al.* 1990). The evidence of a cardiovascular effect of cadmium in humans is, however, less clear (Sharrett 1979, Nakagawa and Nishijo 1996).

Elevated levels of lead in soft drinking water are significantly associated with increased serum lead levels in individuals (Sparrow *et al.* 1984). Even at low blood levels, lead has long been associated with hypertension and stroke (Perry and Roccella 1998).

However, the consideration of heavy metals as the possible causative factor is complicated by the fact that it is low pH, and not low hardness per se, that increases the release of these metals from the distribution system. Whilst soft waters are generally more acidic and hence contain higher concentrations of Cd and Pb (Yang *et al.* 1999a), Haring and Zoeteman (1980) demonstrated greater metal solvency of hard water in the Netherlands. Here it was found that the softer water generally exhibited a higher pH and lower concentrations of Pb than harder water. Furthermore, early studies of heavy metals in drinking water may have suffered from limited laboratory techniques, therefore the evidence examining Cd and Pd in water will be considered for more recent studies only.

### 1.3.3 Summary

To date, the nature of the apparent cardioprotective effect of hard drinking water remains to be elucidated. Given the evidence supporting both the concepts of a protective factor and a harmful factor in drinking water, both “calcium” and “magnesium” together with “cadmium” and “lead” were included as subject specific search terms in the search strategy.

## 1.4 The purpose of this review and research objectives

This study was commissioned to review and critically assess the quality of available studies examining the health effects of soft water, primarily the long standing apparent association between water quality and cardiovascular disease. This review

exceeds that undertaken by the WHO with regard to both the consideration of a wider range of primary data papers, but also a greater focus upon a systematic assessment of the quality of each study. As such, the conclusions of this review will be drawn from a wider research base but will also be weighted and informed by the methodological quality of the studies.

The objectives are to:

6. Review and critically assess the merits of available studies concerning the health effects of soft and softened water and to categorise the identified studies based on relevant features such as size, study design, conclusions, etc.
7. Advise on the evidence relating to the incidence of cardiovascular disease, or other adverse health effects to consumption of soft or softened water and to comment on the reliability and strength of any reported associations.
8. Advise whether the studies indicate a "no effect" level in relation to water composition and whether a causal or protective agent relating to incidence of illness is evident.
9. To comment on whether the results indicate the need to consider re-hardening of softened water, or whether supplies of naturally soft water should be hardened and provide an indication of the scale of the health benefits of such action.
10. To comment on the evidence relating to specific postulated causal (lead, cadmium) or protective (calcium, magnesium) elements and their importance with respect to cardiovascular disease.

# 2 General quality issues of epidemiological study designs

## 2.1 Potential sources of error and bias

A number of factors will be briefly defined and explained here which are important considerations in the conception and undertaking of any environmental epidemiology study examining exposures and health effects (Morgernstern and Thomas 1993).

The *latency period* refers to the interval between the first exposure to an environmental risk factor, or the start of its causal association, and a health outcome. This may be in the order of years, for example until the manifestation of certain cancers is detected. Failure to adequately consider the latency period in the design of an epidemiological study will reduce the power of the study to detect an association between an exposure and outcome.

Three potential sources of *bias* in the estimation of the association must be assessed to ensure the validity of the findings of any epidemiological study.

1) **Selection bias** – the estimate of the effect of the exposure variable of interest may be distorted by the selection of subjects into the study. This occurs if the process involved in selection yields a non-representative population and is most likely to arise if the base population yielding the study cases is not identified.

2) **Information bias** – this occurs when the method or quality of the measurement or data collection distorts the estimation of association. The primary cause is error in the measurement of variables and misclassification of individuals by exposure or disease status. Important subcategories of this type of bias are:

> *Non-differential misclassification* – where the probabilities of such a misclassification are the same for each group being considered. The consequence of this is a bias of the estimate of effect towards null, meaning the study is less likely to show a significant association between exposure and effect.

*Differential misclassification* – where there is a systematic pattern of misclassification, e.g. a greater probability of higher risk individuals being categorised into a particular exposure group. Consequentially, the effect estimate may become biased in either direction.

3) **Confounding** – the presence of confounding refers to a lack of comparability in the other important characteristics of the different exposure groups (i.e. additional risk factors that may be operating to increase disease risk). A confounder must be associated with both the exposure and the outcome but not lie on the causal pathway. Epidemiologic bias in the estimation of a causal parameter may therefore arise. In order to control for confounding, differences in the distribution of other known risk factors between exposure groups must be considered. The effects must be taken into account either in the study design (e.g. by matching) or in statistical analysis to obtain unbiased estimates.

## 2.2 Definitions of study types

The nature of the epidemiological investigation of environmental exposures and possible health outcomes may take the form of an *experimental* or *observational* study. In experimental studies the exposure of interest is under the control of the investigator with individuals examined for health effects and outcomes. The vast majority of environmental epidemiological studies however are observational in their nature. This means that exposure to the factor of interest is *not* under the control of the investigators. All of the primary data studies examining drinking water components contained within this review were observational studies.

This section will define the four main observational epidemiological study types (ecological, cross-sectional, case control and cohort studies), and are based upon the definitions in the dictionary of epidemiology (Last 2001). The unit of analysis in ecological studies is at the group or area level, whilst the remaining three study designs consider the individual as the unit of analysis. The relative strengths and weaknesses of each study design will also be summarised together with limitations and sources of bias which must be considered in the assessment of the studies.

**Ecological studies**

Ecological studies are defined as studies whereby the unit of analysis is aggregated data, for example populations or groups of individuals. Such studies are also considered as being hypothesis generating as they allow the investigation of hypothesised associations whist being quick, cheap and relatively easy to undertake. A major limitation is the *ecological fallacy* (also known as ecologic bias or aggregation bias). This is a bias that may occur whereby associations found at the aggregate or area level do not necessarily hold at the individual level. The control of covariates and possible confounding factors in these studies is also achieved by the application of area level data. Hence there is the potential for inadequate characterisation of such factors and an overestimation of the relative importance of the exposure factor of interest. The widespread use of ecological studies reflects a fundamental problem of adequate exposure assessment (Morgernstern and Thomas 1993).

**Cross sectional studies**

Cross sectional studies examine the relationship between disease and possible risk factors as they exist in a defined population at a particular moment in time. Disease prevalence rather than incidence is usually measured in cross sectional studies due to the examination of the presence/absence of a disease and the characterisation of the risk factor at the same moment in time. Consequently the cross sectional study design may establish association but not causation, as it is not possible to examine the temporal sequence of exposure and effect. Cross-sectional studies are comparatively cheap to perform and allow the examination of a large number of individuals. However the reliance on retrospective measures increases the likelihood and magnitude of measurement error leading to information bias.

**Case control studies**

Case control studies compare an individual with a disease (*case*) to a suitable individual (or group of individuals) without the disease (*control*). The relationship of a hypothesised risk factor to the disease is then examined by comparing the cases and

controls with regard to the presence, or levels of, the risk factor between the groups. A key methodological issue is in the selection of controls which must be representative of the base population. Case control studies are efficient for examining rarer diseases and those with long latency periods.

**Cohort studies**

The principle of a cohort study is to test for causality, therefore studies employing this epidemiological design yield the strongest evidence for a causal association between a risk factor and a health outcome. A cohort study characterises subsets of a defined population who are, have been, or will be in the future exposed or not exposed (or exposed in different degrees) to a risk factor hypothesised to influence the probability of occurrence of a given health outcome. The main feature of the cohort study is the observation of a large number of individuals over a period of time (usually years) allowing the comparison of incidence rates in groups differing in levels of exposure. Cohort studies are the only observation environmental epidemiological study design able to examine the temporality of association. The other observational study designs described here suffer from temporal ambiguity with regard to the sequence of exposure and effect. However, cohort studies are expensive and with increasing length of follow up may suffer from loss of subjects to follow up, death from other diseases, lack of participation or migration. This can easily yield biased estimates of the association between exposure and health outcome. Furthermore, such studies may require large samples in order to attain sufficient cases upon which estimates may be based. Nevertheless, cohort studies provide the most powerful evidence in the examination of a hypothesised exposure and health outcome.

These definitions of study types were used to classify each paper considered within this review prior to data extraction. Assessment of the study quality of each paper was informed by consideration of the potential sources of error and bias detailed above. The parameters used to evaluate study quality are discussed in greater depth in section 3.5.

## 3 Methods

A standardised search strategy was devised in order to identify a large set of high validity articles regarding water quality and human health. The initial criteria for the identification of such studies covered three broad topics:

- Types of studies: all types of observational epidemiological studies (ecological, cross sectional, case control, cohort).
- Types of participants: populations or individuals exposed to soft or softened water.
- Types of outcome measures: incidence of cardiovascular disease in a population or among cases compared to controls. Incidence of other diseases such as cancer.

Appropriate search terms encompassing these three areas were piloted and it was found that specification of study type resulted in too narrow a return of articles. The search strategy was subsequently modified to increase the sensitivity of the search making it broader and more inclusive. The final search strategy as utilised for the examination of MedLine is presented below.

**MedLine (OVID version rel9.2.0 - 1966 to November week 3 2004)**[2]

| | SearchTerm | Hits |
|---|---|---|
| 1 | Water/ | 51162 |
| 2 | Water.tw | 213506 |
| 3 | 1 or 2 | 233558 |
| 4 | calcium compounds/ or calcium/ | 186192 |
| 5 | Magnesium/ or Magnesium Compounds/ | 51621 |
| 6 | Cadmium/ or Cadmium Compounds/ | 14157 |
| 7 | Lead/ | 16036 |
| 8 | or/4-7 | 243962 |
| 9 | Hardness Tests/ or Hardness/ | 4129 |
| 10 | Hardness.tw. | 3088 |
| 11 | Soft.mp. | 66220 |
| 12 | Water Supply/ or Water Softening/ or Hydrotimetry.mp. | 15168 |
| 13 | Softened.mp. | 360 |
| 14 | or/9-13 | 86754 |
| 28 | 8 or 14 | 328984 |
| 14 | Cardiovascular Diseases/ | 40969 |
| 15 | Coronary Disease/ | 105135 |
| 16 | Coronary Arteriosclerosis/ | 11009 |
| 17 | heart disease.tw. | 66985 |
| 18 | Heart Diseases/ | 35979 |
| 19 | Cerebrovascular Disorders/ | 37105 |
| 20 | Hypertension/ | 128640 |
| 21 | Blood Pressure/ | 177085 |
| 22 | Colonic Neoplasms/ | 40070 |
| 23 | Disease/ | 11977 |
| 24 | Esophageal Neoplasms/ | 22580 |
| 25 | or/14-24 | 566508 |
| 29 | 3 and 28 | 21454 |
| **30** | **25 and 29** | **672** |

The necessary adjustments to the strategy made for the searching of the other electronic databases are detailed in full in Appendix 1.

[2] Example search conducted on: 08/12/2004

**Table 1 Number of papers selected by search strategy by database**

| Electronic database | Number of studies identified |
|---|---|
| MedLine | 672 |
| PubMed | 990 |
| Web of Science | 137 |
| CINAHL | 3 |
| EMBASE | 531 |
| Toxline Core | 297 |
| Special | 563 |
| Cochrane Reviews | 0 |

Table 1 presents the number of studies identified using the search strategy described above (and in Appendix 1). After the removal of duplicates, 2,906 abstracts were identified by the electronic database search. The results of the search were then checked against all papers referenced in the WHO review documents (Calderon and Craun 2004 (*draft*), Monarca *et al.* 2004 (*draft*)) to ensure that these relevant studies were included. Our search strategy captured the 52 papers that had been cited as primary data papers in the WHO review (the remainder of the papers considered in those reviews were intervention or dietary supplementation studies, animal studies, or reviews). A small number of primary data papers were identified from the references of other studies throughout the review process.

### 3.1 Abstract appraisal and study selection

The titles and abstracts of the 2,906 papers were then screened by two reviewers (LC and IA). Papers were retained if they presented primary data of human (not animal) studies, were related directly to the research question and involved a comparison of populations or individuals at different levels of exposure. Due to the broad scope of the search strategy a number of papers identified were subsequently found to be irrelevant to this study (including large number of fisheries papers). A total of 436 were identified and provisionally agreed upon as relevant to this study. Papers studying water hardness and health were reported in wide range of journals encompassing over 56 different journals titles. There are no journals specific to this subject area therefore no handsearching validation of papers was undertaken.

Two reviewers (LC and IA) independently assessed the abstracts of all 436 papers returned by the search and identified those relevant to this review. Of the 436 papers, 57 were non-English language papers. Attempts were made to determine if the papers had been published in English but no such publications were found. Further examination of these papers found that a total of 50 did not present primary data or did not have an abstract. Six of the seven papers identified as possibly relevant were obtained, whilst one paper could not be located. However due to limited resources it was not possible to translate the six foreign language papers for incorporation into this review. A comment on the possible implications of this exclusion is given in section 8. Editorials, letters and reviews were not automatically excluded at this stage, but were assessed for content of new data or references. Experimental studies on animals and those assessing the effects of dietary intake of magnesium and calcium were excluded from this review. Disparities in articles selected were discussed and agreed upon by consensus, resulting in a final list of 379 papers.

Due to both time and resource constraints, it was not possible to conduct a full article appraisal upon the 379 papers identified by their titles and abstracts as possibly relevant. The abstracts were therefore reviewed a second time using more stringent selection criteria. Papers were excluded if they met *all three* of the following conditions: 1) no abstract was available, 2) the paper was not recent (generally pre-1970), and 3) the study was not included in the WHO reviews.

Following this second process, a total of 167 papers remained. Of these, 132 papers were selected as primary data papers and underwent full article appraisal. Seventeen papers were excluded from the final selection due to their descriptive but non-analytical content. An overview of the findings of these papers is given in Appendix 2, and shows an equal proportion of papers yielding positive and negative conclusions. The remainder comprised a number of key review and biological mechanism papers which were also obtained to contribute to a comprehensive discourse on the association between water hardness and human health.

### 3.2 Grey literature and unpublished data

The SIGLE database was examined for relevant grey literature regarding water hardness and health. No information additional to that identified by the electronic database search was found. The National Research Database (http://www.nrr.nhs.uk) was also searched for relevant completed or ongoing studies. Only one possibly relevant study was located examining water consumption as a protective factor against breast cancer (Publication ID N0164087240). The authors were contacted and it was established that no measures of drinking water quality were recorded for this study, therefore it was not necessary to include it in this review. Time and resource constraints negated the possibility of contacting authors identified by the search strategy regarding unpublished results. However, the search strategy returned a large volume of studies yielding a wide variation of results regarding water quality and health. Therefore whilst publication bias remains a possibility it is unlikely to have a major influence upon the conclusions of this review.

### 3.3 Data extraction

Proformas for reviewing study quality and results were created by adapting previously validated forms for observational studies from the Cochrane Collaboration (http://www.medepi.net/meta/forms.html). Separate forms were designed for each study type – ecological/correlational (for ecological and cross sectional studies), case control and cohort studies. The content of the form was explained to each reviewer to ensure consistent interpretation of all aspects of the proforma.

The standard forms were used for data extraction by study type (ecological/correlation, case-control and cohort studies, included in Appendix 3). Each paper was independently reviewed by two members of the working group (LC and one other) using the basic proformas to extract data detailing the patient or population characteristics, location of the study, outcome measures examined (types of illness/health effects considered) and exposure characteristics (levels of drinking water hardness or specific constituents). Information regarding the quality of the study was also recorded including the level of exposure measurement, for example if

proxy measures of drinking water exposure were used or individual level sampling was employed. Data were entered into an MS Excel spreadsheet.

### 3.4 Data Synthesis

Due to the significant level of clinical and methodological heterogeneity, no attempt was made to assess for evidence of statistical heterogeneity. A narrative synthesis of the results is therefore presented with no formal meta analysis. Studies were summarised according to type of exposure (water hardness overall, magnesium and calcium), health effect (cardiovascular, cerebrovascular and other health effects), study quality and similarity of conclusions.

### 3.5 Assessment of study quality

The methodological quality of studies was assessed using components of the Newcastle-Ottawa criteria (Wells *et al.* 2001). Formal scoring for quality criteria was not utilised as suggested in the Cochrane handbook, as these studies are observational. Instead, two reviewers compared the quality of information in the completed proformas and excluded studies that did not meet agreed minimum quality criteria. Disagreements were settled by consensus.

Information to assess the quality of each study was collected using the data extraction forms (Appendix 3). A number of the study quality criteria including examination of sample size and validity of the health outcome being studied, were common to all epidemiological study types considered in this review. Some, however, were specific to the study design employed. Table 2 illustrates the aspects of study quality considered here by study type. The criteria given may be broadly grouped into three themes: exposure characterisation, statistical analysis and population characteristics. This section will define each criterion employed under these headings.

**Table 2 Study quality criteria explicitly evaluated by epidemiological study design**

| | Ecological | Cross-sectional | Case-control | Cohort |
|---|---|---|---|---|
| **Exposure characterisation** | | | | |
| Proxy | ✓ | ✓ | ✓ | ✓ |
| Average | ✓ | ✓ | ✓ | ✓ |
| Bias | ✓ | ✓ | × | × |
| Timing | ✓ | ✓ | × | × |
| Measure | × | × | ✓ | ✓ |
| Exposure | × | × | ✓ | ✓ |
| **Statistical analysis** | | | | |
| Confounding | ✓ | ✓ | ✓ | ✓ |
| Method | ✓ | ✓ | × | × |
| Collinearity | ✓ | ✓ | × | × |
| Migration | ✓ | ✓ | × | × |
| Statistical analysis | ✓ | ✓ | × | × |
| Adequacy of reporting | ✓ | ✓ | × | × |
| **Population characteristics** | | | | |
| Selection | × | × | ✓ | ✓ |
| Adequacy of response rate / follow up | × | × | ✓ | ✓ |
| Adequacy of length of follow up | × | × | × | ✓ |

## Exposure characterisation

*Proxy* – this factor recorded whether an area level proxy for the exposure was applied to population groups, for example the application of a generic label of 'soft' or 'hard' water to an area, rather than a quantitative estimate.

*Average* – this factor was intended to record the specificity of the exposure measurement and was a constant term in all proformas. This recorded whether an individual level measurement was applied for analysis or whether an average, e.g. a value from a water company or individual level measurements averaged over a group, were applied.

*Bias* – the possibility of bias was considered in order to evaluate the quality of the exposure assessment. If exposure to a hypothesised risk factor or self-reported health

status were examined by survey or questionnaire, recall bias may be of importance and may affect the validity of the measures used.

*Timing* – many ecological studies obtained the measure of exposure from water company records or pre-published values. As such, the timing of the measurement may not correspond to the period over which health outcomes were considered. The timing of the water quality measure was recorded as being *before, same* or *after* that of the health outcome of interest.

**Statistical analysis**

The adequacy of the statistical analysis and the reporting of the results were important criteria in the overall assessment of study quality.

*Statistical adequacy* – each study was evaluated to ascertain the appropriateness of the statistical tests used to analyse the data contained therein. The consideration of the method utilised to control for possible confounders was also recorded, i.e. standardisation of rates or simultaneous adjustment in a multivariable model, and contributed to the assessment of statistical adequacy. This criterion not only evaluated the statistical method used, but also the application of the test. A common problem in exploratory descriptive studies is that of *multiple hypothesis testing*. This occurs when an exposure factor such as hardness of drinking water is statistically compared to a number of health outcomes, or when several exposure factors are compared to one outcome. The greater the number of relationships examined, the greater the probability of showing a statistically significant association. In other words, working at the commonly used 5% significance level, one would expect 1 in 20 associations to be statistically significant by chance alone. Thus findings of each study were also evaluated for multiple hypothesis testing and reliability.

*Adequacy of reporting* – this factor was important for the appropriate interpretation of the results presented by each study under consideration. For example, if an estimate of the magnitude of a relationship was presented with no measure of associated uncertainty (e.g. standard errors or confidence intervals) it was therefore not possible to assess the reliability of such an estimate. Similarly, the presentation of the

significance level (p-value) of an association stated as significant in the text was considered important.

*Collinearity* – this is the condition whereby two or more explanatory variables are highly correlated with each other as well as with the dependent variable of interest. The presence of collinearity has implications for the interpretation of the results as it can adversely affect the reliability of the statistical analysis. This was regarded as an important consideration for studies attempting to simultaneously examine both calcium and magnesium for relative importance with the health outcome of interest.

*Migration* – migration bias may arise due to the migration of individuals into or out of the study population group, and as such can distort the assessment of the exposure-outcome association. This can be of particular concern if the health outcome being examined is a chronic disease with long latency. Small levels of differential migration are able to exert a sizeable effect upon the estimation of a relationship within an ecological study.

**Population**

Quality aspects of the study population characteristics were examined for case-control and cohort studies only. A series of multiple choice responses were used to assess the *selection* of both the cases and controls and cohort for representativeness and the possibility of selection bias. Likewise, the determination of the exposure of the individuals constituting the cases, controls and cohort to the drinking water quality parameter of interest was examined. Case control studies were also assessed for differential non-response between groups. Cohort studies were further examined for adequacy of the follow up period and the proportion of individuals lost at follow up.

### 3.5.1 Application of study quality information

Study quality information was used to categorise the ecological studies by high, medium or low quality. The corresponding information for the individual level studies was used throughout this review to inform the assessment of study validity. Full information detailing the quality evaluation of each study is given in Appendix 4.

### 3.5.2 Classification of ecological studies by study quality

Table 3 illustrates the use of the study quality criteria in the categorisation of the ecological studies. Three core study quality criteria were identified as:

- The consideration of confounders
- The quality of the exposure assessment
- The adequacy of the statistical analysis and presentation of results

**Table 3 Use of study quality criteria for the classification of ecological studies**

| | Proxy | Average | Bias | Timing | Method | Confounders | Collinearity | Migration | Statistical analysis | Adequacy |
|---|---|---|---|---|---|---|---|---|---|---|
| **HIGH** | No | ----- | No | ----- | ----- | Age, gender, others | ----- | ----- | Yes | Yes |
| **MEDIUM** | No | ----- | No | ----- | ----- | Age, gender only | ----- | ----- | Yes | Yes |
| **LOW** | Yes/No | ----- | Yes/No | ----- | ----- | None | ----- | ----- | Yes/No | Yes/No |

To be classified as a *high quality study* and therefore have the greatest weight conferred upon the study findings, it was essential that the study in question had accounted for the effects of age and gender together with other major known confounders of the association between drinking water quality and health. Furthermore, high quality studies required the utilisation of quantitative measurements of drinking water parameters together with an appropriate statistical analysis of the association and adequately presented results. *Medium quality studies* were those meeting the criteria for characterisation of the exposure factor and statistical analysis, but accounted for the effects of age and gender only. *Low quality studies* presented a simple and unadjusted analysis of any association and were therefore regarded as weaker studies.

The remaining five criteria were not considered core elements in the categorisation of the ecological studies. Although the method of adjustment for confounders (labelled method in tables) was not considered individually, this criterion informed the assessment of the adequacy of statistical analysis for each study. The criteria examining timing of exposure, collinearity and migration did not influence assignation to a quality criteria however these factors will be addressed in the discussion. The proforma used to assess the ecological studies was also utilised for

cross-sectional studies (i.e. at the individual level). The criterion examining whether an average measure of exposure was used is redundant in the assessment of ecological studies as by their definition, the unit of analysis is grouped data. Each ecological study reviewed was classified according to the criteria in Table 3. A degree of flexibility was required in the categorisation, for example if a study had accounted for age and gender but failed to adequately present the results of analysis, that study would be classified as of low quality.

Aspects of methodological quality for each study design are presented in the narrative synthesis and discussion. Other sources of bias that are not specific to study design such as publication and reporting bias are discussed. Due to the nature of studies included in the review, the data provided was not suitable for the assessment of publication bias using funnel plots.

## 4 Results

The search identified 379 papers. Section 3.1 described how initial review based on the abstract and titles was used to exclude 247 papers. A final list of 115 papers were obtained and the full text was reviewed by two independent reviewers using a standard form (Appendix 3) to extract information on specific aspects of quality and findings of the study. The results are presented in Tables 5 to 10 with studies classified according to study design. These tables summarise the study population examined, the measures of health outcome considered (with ICD code with revision used where given), drinking water parameters investigated (with estimates of mean concentrations and ranges where given), covariates and potential confounders are detailed where they were simultaneously considered in analysis, and finally estimates of the magnitude of association are presented as detailed in each study.

The study quality information extracted for each study and used in the categorisation of ecological studies and the assessment of all epidemiological studies is detailed in Appendix 4.

## 4.1 Ecological studies

The majority of studies examining the potential association between water quality and health outcomes used an ecological design and compared mortality data from registers in administrative units of a country or region. Table 5 presents the results of these studies sorted into categories of decreasing study quality with high, medium and low quality studies respectively denoted by light, medium and dark greyscale shading. Studies are presented in chronological order within these categories.

### 4.1.1 Cardiovascular disease

A total of 60 ecological studies were obtained and the full text reviewed for possible inclusion. Of these 44 were assessed to contain adequate information and satisfied the minimum quality criteria to qualify for inclusion in this review. Ecological studies excluded based on quality criteria are summarised in Appendix 4. Table 5 shows a summary of the studies on the effect of water hardness, calcium or magnesium concentration on cardiovascular disease. For increased clarity, studies finding no significant association have been highlighted in grey.

A total of 12 studies were regarded as high quality ecological studies, followed by 12 of medium quality and 20 of low study quality. It can be seen that, in general, more recent studies were of higher quality than earlier studies (~pre-1980). This reflects improvements in methodological techniques and the recognition of the importance of considering confounding variables. Furthermore, the practical ability to simultaneously adjust for confounding factors has increased over time through increasing computing power leading to more sophisticated statistical models of exposure and outcome. The results of the ecological studies will be assessed, and compared, by these categories.

Table 4 summarises the proportion of ecological studies finding a significant negative or positive association, or no association at all, between drinking water hardness, calcium and/or magnesium and cardiovascular disease. These findings are presented by category of study quality. A number of studies considered more than one water factor as the exposure of interest. The denominator therefore presents the total

number of studies considering that factor (either in isolation or in combination with others) and does not necessarily equal the total number of papers in each study quality category.

**Table 4 Summarised findings of ecological studies examining the association between drinking water hardness, calcium and/or magnesium and cardiovascular disease by category of study quality**

| | Study Quality | | |
|---|---|---|---|
| Total number of studies | High N=12 | Medium N=12 | Low N=20 |
| **Significant inverse association** | | | |
| Hardness | 6 / 8 | 6 / 9 | 9 / 12 |
| Calcium | 2 / 5 | 2 / 7 | 6 / 8 |
| Magnesium | 2 / 5 | 3 / 6 | 6 / 10 |
| **Significant positive association** | | | |
| Hardness | 0 / 8 | 0 / 9 | 0 / 12 |
| Calcium | 1 / 5 | 0 / 7 | 1 / 8 |
| Magnesium | 0 / 5 | 0 / 6 | 1 / 10 |
| **No significant association** | | | |
| Hardness | 2 / 8 | 3 / 9 | 3 / 12 |
| Calcium | 2 / 5 | 5 / 7 | 1 / 8 |
| Magnesium | 3 / 5 | 3 / 6 | 3 / 10 |

Denominator = the number of studies considering each exposure factor and is not necessarily equal to the total as one study may consider more than one factor.

*High quality studies*

Of the 12 high quality studies, 8 examined water hardness as the exposure factor of interest. Of these, 6 studies presented significant results showing an inverse relationship, whilst 2 studies showed no significant association. A smaller number of ecological studies considered both calcium and magnesium as the water quality exposure factors of interest. Of these 5 studies, 2 found a significant inverse association between calcium and/or magnesium and cardiovascular disease. Drinking

water calcium showed a significant positive association in 1 study, with the remainder finding no significant association for either mineral. In summary, of the 12 high quality studies, a total of 9 papers presented evidence for a significant inverse association between drinking water hardness, calcium and/or magnesium and cardiovascular disease mortality, with only 3 studies finding no significant association with any factor examined.

These studies were categorised to be of high quality due to the consideration and incorporation of possible confounding factors in the statistical analysis. All 12 studies accounted for the effects of age, gender and socio-economic factors upon the association between water quality and cardiovascular mortality, with some studies further incorporating climatic factors (Pocock *et al.* 1980, Dudley *et al.* 1969, Catling 2004).

*Medium quality studies*

Of the 12 studies regarded as of medium quality, overall 6 showed no significant association between drinking water hardness, calcium or magnesium and cardiovascular mortality. Of those studies demonstrating a significant relationship, two presented results at the 10% significance level ($p<0.10$; Derry *et al.* 1990, Masironi 1979) as opposed to the commonly used 5% level ($p<0.05$).

Nine studies examined the association between water hardness and cardiovascular mortality, with two thirds demonstrating a significant inverse association. Of those studies considering calcium and/or magnesium, 2 of 7 and 3 of 6 studies respectively showed a significant inverse relationship with some found only in population subgroups (Rylander *et al.* 1991).

Table 5 shows that the medium quality studies considered the possible confounding effects of age and gender only upon the exposure-outcome association of interest. Although Ferrandiz *et al.* (2003) did also simultaneously consider the influence of socioeconomic factors and rural/urban effects, this paper was primarily a methodological paper exploring the construction of spatiotemporal models. The main focus of this paper was therefore a comparison of model functionality with the

findings for the drinking water quality factors only briefly presented. Some authors incorporated additional water constituents in analysis (Kousa *et al.* 2004, Voors 1971).

*Low quality studies*

The majority of ecological studies, a total of 20, were categorised to be of low study quality. We set no restriction by time frame upon our search strategy therefore this reflects the large number of early correlational studies undertaken with no control for confounding. Overall, 16 of the 20 studies illustrated a significant inverse association between water hardness, calcium and/or magnesium and cardiovascular mortality.

Table 4 illustrates a notably consistent proportion of studies demonstrating a significant inverse association between water quality parameters and cardiovascular mortality following study categorisation by quality. A key feature of Table 4 is to demonstrate that classification by study quality criteria did not result in a selection bias of study results for the focus of this review.

The main weaknesses of these ecological studies include the use of average values of drinking water parameters leading to non differential misclassification, lack of evidence for temporality of association and the potential for ecological bias. Cross sectional, case control and cohort studies are less susceptible to these sources of bias and evidence from these studies is summarised below.

**Table 5 Ecological studies examining the association between cardiovascular disease and drinking water hardness, calcium or magnesium**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Kaipio *et al.* (2004) | Finland, 365 rural areas, males and females 35+ years | 1961-1995 | CHD mortality, ICD-7 420, ICD-8 410-414, ICD-9 410-414 | mn, md, rg Calcium (22.70, 17.75 (0.00-259.86) mg/l) Magnesium (7.10, 4.77 (0.00-131.50) mg/l) | Age, gender, mean income, drinking water Ca or Mg, time period | **Calcium (mg/l)** **RR (95%CI)**<br>5.68-14.67 1.00<br>14.68-19.17 1.00 (0.98-1.02)<br>19.18-23.21 1.06 (1.04-1.09)<br>23.22-30.29 1.07 (1.04-1.09)<br>30.30-165.43 1.06 (1.03-1.08)<br><br>**Magnesium (mg/l)** **RR (95%CI)**<br>0.21-3.80 1.00<br>3.81-5.10 0.97 (0.95-0.99)<br>5.11-7.34 0.95 (0.93-0.97)<br>7.35-10.07 0.95 (0.93-0.98)<br>10.08-72.70 0.92 (0.90-0.95) |
| | Ferrandiz *et al.* (2004) | Spain, 538 municipalities, males and females | 1991-1998 | IHD mortality (ICD-9 410-414), cerebrovascular (430-438) | Calcium range 12-480 mg/l), Magnesium (range 1-117mg/l) | Standardised for age, gender and deprivation index | Results presented graphically<br>Significant trend of decreased stroke with increasing Mg, similar but less pronounced for IHD, trends more pronounced for females than males and Mg than Ca |
| | Miyake and Ike (2004b) | Japan, 44 municipalities, males and females | 1995 | Coronary Heart Disease mortality rate (ICD-10 I20-I25) | Water hardness tertiles, <46.5, 46.51.9, >52 mg/l | Age, gender, census average family tax (socioeconomic proxy), number of clinics per 100,000 population (health care status proxy) | **OR (95%CI)**<br>Males Females<br><46.5 1.00 1.00<br>46.5-51.9 0.97 (0.88-1.08) 1.04 (0.94-1.16)<br>>52.0 1.06 (0.91-1.22) 1.00 (0.86-1.16)<br>p for linear trend 0.77 0.74 |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Marque *et al*. (2003) | France, 69 parishes, males and females 65+ years | 1990-1996 | cardiovascular mortality, cerebrovascular mortality (no ICD codes given) | Calcium, magnesium (tertiles) | Age, gender, rurality of parishes | **RR (95%CI)**<br>**Calcium**<br>(highest tertile (94-146mg/l))<br>All cardiovascular 0.90 (0.84-0.96)<br>All excluding stroke 0.90 (0.84-0.97)<br>Stroke 0.86 (0.77-0.96)<br><br>**Magnesium**<br>(highest tertile (11-34mg/l))<br>All cardiovascular 0.93 (0.99-1.04)<br>All excluding stroke 0.96 (0.87-1.05)<br>Stroke 0.92 (0.80-1.06) |
| | Miyake and Ike (2003) | Japan, 44 municipalities, males and females | 1995 | All types of cerebrovascular mortality (no ICD code given) | Water hardness mn±SD (53.2±14.4) rg(35.2-100.0) mg/l | Age, gender, family tax (socioeconomic proxy), total number of health facilities (health care status proxy) | **OR (95%CI)**<br>males+females<br><46.5 (mg/l) 1.00<br>46.5-51.9 0.97 (0.91, 1.03)<br>>51.9 0.93 (0.84, 1.02)<br>*p trend* *0.08* |
| | Maheswaran *et al.* (1999) | England (NW), 305 water supply zones, 21,339 males, 17883 females | 1990-1992 | Acute myocardial infarction mortality ICD-9 410, IHD mortality (410-414) | Calcium (mn±SD 52±43 (rg 5-215)mg/l, md36), magnesium (mn±SD 19±20 (rg 2-111)mg/l, md12) and lead | Age (5 year bands), gender, education level, Carstairs index, drinking water calcium / magnesium / lead northings and eastings of centroids | **RR(95%CI) for quadrupling of concentration:**<br>Acute MI / IHD<br>Log Mg (mg/l) 1.01 (0.96-1.06) / 1.01 (0.96-1.05)<br>Log Ca (mg/l) 0.99 (0.94-1.05) / 0.98 (0.94-1.04)<br>Log Pb (µg/l) 0.94 (0.91-0.98) / 0.99 (0.96-1.03) |
| | Yang *et al.* (1996) | Taiwan, 227 municipalities, males and females | 1981-1990 | coronary heart disease mortality ICD-9 410-414 | Water hardness <75, 75-150, >150 mg/l CaCO3 | Age, gender, urbanisation | r=-0.20, p<0.05, log water r=-0.22, p<0.05<br>**RR (95%CI)**<br>soft 1.096 (1.084-1.108)<br>moderate 1.045 (1.032-1.058)<br>hard 1.00 |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Lovett *et al.* (1986) | England and Wales, 83 county boroughs, males and females, 15-44 years (young adults) | 1969-1973 | Ischaemic heart disease mortality | Water hardness | Age, gender, 'at risk' population, households lacking a car, % households >1 person per room, % persons born in the New Commonwealth | Poisson parameter estimate water hardness: -0.097 (SE 0.020), p<0.05 |
| | Lacey and Shaper (1984) | England, 14 county boroughs, males and females 55-74 years | 1958-1964, 1969-1973 | Change in cardiovascular death rates (ICD-6/7) 330-334, 410-468, ICD-8 393-458 | Water hardness | Age and gender specific death rates, % manual workers, % unemployed | **% change in death rate (standard error)*** <br> Males / Females <br> CVD -8.1 (3.1), p=0.01 / +1.2 (3.8), p=0.70 <br> non-CVD -2.8 (3.4), p=0.40 / +2.1 (3.3), p=0.50 <br> adj CVD -7.5 (not given), p=0.020 <br> *seen with an increase in hardness of 100mg/l in the hardness range <170mg/l |
| | Pocock *et al.* (1980) | Great Britain, 253 towns, males and females, all age and 35-74 years | 1969-1973 | Overall cardiovascular mortality, IHD and stroke mortality | Water hardness + mn±SD 170±109 (rg 10-528)mg/lCaCO3 equiv | Age and gender standardised rates, climatic factors (% days >0.2mm rain, mean daily temp), % manual workers, number of cars per 100 households | Correlation hardness with SMR, r=-0.67 (no p-value given) <br> mg/l $CaCO_3$: ≤170 / >170 <br> water hardness <br> adj standardised reg effect: -7.8 / -0.8 <br> specificity: ≤170 / >170 <br> stroke -6.8 / -3.0 <br> IHD -8.2 / +0.0 <br> non-CVD -0.1 / +0.0 |
| | Dudley *et al.* (1969) | USA, 116 metropolitan areas, white males 45-64 years, age adjusted death rates and proportional mortality | 1949-1951 | Arteriosclerotic heart disease mortality, including coronary heart disease | Water hardness, calcium, magnesium | Age, education, rent, income (city level), climate (comfort index) | Results for CHD mortality only are presented <br> r / p <br> K -0.335 / p<0.001 <br> Na -0.222 / p<0.050 <br> Hardness -0.386 / p<0.001 <br> Ca / n/s <br> Mg / n/s |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Catling (2004) | Great Britain, 457 districts, males and females, 15+ years | 1989-1993 | Mortality from all cardiovascular disease, IHD (ICD-9 410-414), and stroke (ICD-9 430-438) | Water hardness (range 13.8-674.8mg/l) | Age, incident sunlight index, socioeconomic deprivation, ethnicity, alcohol intake | Regression analysis:<br>Regression coefficient (b) per unit increase water hardness (mg/l)<br>All CVD m b=-0.016 (-0.023, -0.008) <0.001<br>All CVD f b=-0.018 (-0.026, -0.010) <0.001<br>IHD m b=-0.015 (-0.023, -0.008) <0.001<br>IHD f b=-0.016 (-0.026, -0.007) 0.001<br>Stroke m b=-0.014 (-0.027, -0.001) 0.035<br>Stroke f b=-0.034 (-0.044, -0.022) <0.001 |
| | Kousa *et al.* (2004) | Finland, 10x10 km grid cells, 18, 946 males 35-74y | AMI pooled for 1983, 1988, 1993 | First attack AMI - hospitalisation attributable to ICD-8/9 410-414 (both fatal and non-fatal) | (mn±SD)<br>Water hardness (3.8±10.0 dH)<br>Calcium (19.9±67.3 mg/l)<br>Magnesium (4.8±5.6 mg/l) | Age, zinc, aluminium, copper, fluoride, iron, nitrate | An increase of 1dH in water hardness was associated with a 1% reduction in risk of AMI (no CI). Fluoride was negatively associated but non-sig. No others sig. Age distribution of population did not affect geographical variation. |
| | Ferrandiz *et al.* (2003) | Spain, 262 municipalities, males and females | 1990-1995 | Cerebrovascular mortality | Calcium, magnesium | Gender, year, socioeconomic factors, urban/rural | Mg (but not calcium) protective against stroke, females found to have lower risk<br>Estimate std.E p-value<br>**Poisson regression results**<br>Ca ($x10^{-4}$) 1.014 2.027 0.6171<br>Mg ($x10^{-3}$) -3.317 0.7909 <0.0001<br>**Negative binomial regression results**<br>Ca ($x10^{-4}$) 5.801 4.479 0.1952<br>Mg ($x10^{-3}$) -3.514 1.500 0.0192 |
| | MacPherson and Basco (2000) | Great Britain, 40 health districts, 4393 males 20+ years | 1991-1995 | CHD mortality | Water hardness and hair calcium concentration | Age, sunshine hours, hair calcium | water hardness (mg Ca/l) b=-0.096 (no CI) adj for hair and sunshine additionally significant with SMR, $r^2$=0.39 (water hardness only)<br>No p-values or CI given |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Rylander *et al.* (1991) | Sweden, 27 municipalities, males and females | 1970-1980 | IHD mortality (410-414), cerebrovascular (430-438) | Water hardness (0.8-20.7 dH), calcium (3.4-131mg/l), magnesium (0.57-15.0mg/l) | Age and gender | **b (95%CI)**<br>**Hardness**<br>IHD M -0.602 (-0.788, -0.315) p=0.001<br>F -0.453 (-0.696, -0.117) p=0.01<br>Cerebro M -0.483 (-0.715, -0.155) p=0.05<br>F -0.367 (-0.638, -0.014) p=0.05<br>**Calcium**<br>IHD M -0.468 (-0.716, -0.115) p=0.01<br>F -0.418 (-0.685, -0.054) p=0.05<br>Cerebro M -0.515 (-0.745, -0.176) p=0.05<br>F -0.323 (-0.622, -0.056) p=0.12<br>**Magnesium**<br>IHD M -0.618 (-0.806, -0.319) p=0.01<br>F -0.452 (-0.706, -0.095) p=0.01<br>Cerebro M -0.165 (-0.507, -0.222) p=0.19<br>F -0.143 (-0.490, -0.243) p>0.40 |
| | Gyllerup *et al.* (1991) | Sweden,<br>259 municipalities,<br>males 40-64y | 1975-1984 | Acute myocardial infarction mortality ICD-9 410 | Water hardness | Age, cold index | Weak non-linear inverse correlation<br>Coefficient of determination (D)<br>Logged water hardness 0.080 |
| | Derry *et al.* (1990) | South Africa, 43 urban sectors, white males and females 25-74 years | 1978-1982 | Cardiovascular disease mortality | Water hardness, calcium, magnesium, potassium | Age and gender | **Spearman's correlation (ρ)**<br>total hardness ρ=-0.26, p<0.10<br>calcium ρ=-0.24, n/s<br>magnesium ρ=-0.24, n/s<br>potasssium ρ=-0.35, p<0.05 |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Leoni *et al.* (1985) | Italy, Abruzzo region communes, 594,323 males and females 45-64 years | 1969-1978 | Cardiovascular disease mortality ICD 390-438, IHD 410-414, cerebrovascular 430-438 | Water hardness range 105.6-443.5 mg/l CaCO3 | Age and gender | Correlation coefficients<br>All CVD / IHD / Stroke<br>m+f: -0.56, p<0.05 / -0.44, p<0.01 / -0.25, p>0.05<br>m: -0.57, p<0.05 / -0.41, p<0.01 / -0.03, p>0.05<br>f: -0.58, p<0.05 / -0.39, p>0.05 / -0.25, p>0.05 |
| | Greathouse and Osborne (1980) | USA, 35 areas, white and non-white males and females 35-74 years | 1968-1972 | Cardiovascular mortality rate | Water hardness (range: 6.5-325 mg/l) | Gender and race specific age-adjusted mortality rates | Hard water areas (300mg/l) experienced an average 10.5% less male deaths from cardiovascular and renal disease than soft water areas (20mg/l), h/e p=0.283. Hardness was n/s with cerebrovascular disease. |
| | Masironi *et al.* 1979) | Europe, 15 towns and cities, males and females 45-64 years | 1974 | AMI attack (rate of occurrence of heart attacks) and incidence rates (rate of occurrence of first attacks) | Water hardness (range 32-354 mg/l CaCO3) | Age and gender adjusted incidence rates | **Correlation (r), p-value**<br>**Attacks**<br>m+f: -0.477, p<0.10<br>m: -0.428, n/s<br>f: -0.468, p<0.10<br>**Incidence**<br>m+f: -0.460, p<0.10<br>m: -0.442, p<0.10<br>f: -0.397, n/s |
| | West (1977) | England and Wales. 15 hospital regions and 115 boroughs | 1969-1971 | IHD mortality (ICD-8 410-414), total mortality | Calcium | Age specific death rates by gender | No association between water calcium and IHD mortality, also calcium not specific to IHD (showed the same association with total mortality). |
| | Voors (1971) | USA, 99 largest US cities, white and non-white males and females | 1959-1961 | Atherosclerotic heart disease mortality rates | Calcium, magnesium | Age adjusted rates by gender and race, drinking water calcium, magnesium, chromium, vanadium, lithium, zinc | Unadjusted negative association between heart disease mortality and drinking water calcium and magnesium. However, no association following adjustment for other elements. |
| | Schroeder (1960b) | USA, states, white males and females 55-64 years | 1949-1951 | Death rates from arteriosclerotic disease, hypertension, liver cirrhosis | Water hardness | Age | arteriosclerotic: M(r=-0.498) F(r=-0.455) (p<0.01),<br>hypertension: M+F (r=-0.567, p<0.01)<br>liver cirrhosis: M+F(r=-0.324, p<0.05) |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Sauvant and Pepin (2000) | France, 52 districts, males and females | 1988-1992 | All cardiovascular ICD-9 390-458, IHD (410-414) and cerebrovascular (430-438) mortality | Water hardness <7.5Fd, 7.5-15.0 Fd, >15.0Fd[3] | Age | **Mortality** **b** **p** **r2**<br>410-414 (m) -0.042, 0.02 0.106<br>410-414 (f) -0.031, n/s 0.031<br>430-438 (m) -0.042, 0.02 0.100<br>430-438 (f) -0.053, 0.01 0.122<br>390-458 (m) -0.048, 0.01 0.119<br>390-458 (f) -0.051, 0.01 0.135 |
| | Sakomoto *et al.* (1997) | Japan, 86 towns and cities | 1975-1979 | Death rates for stomach cancer (ICD-10 C16), cerebrovascular disease (ICD-10 160-169, apoplexy 161+169.9) | Water hardness, calcium, magnesium | None | Total stomach cancer and Mg:Ca ratio r=+0.34, p<0.01<br>cerebrovascular disease and Mg:Ca ratio r=-0.25, p<0.05<br>cerebrovascular disease and Ca r=-0.26, p<0.01 |
| | Piispanen (1993) | Finland, 444 districts, males and females | 1991 | Deaths from cardiovascular disease as a % of all deaths | Water hardness (ºdH: 3.2±1.4 (1-9)<br>Calcium(mg/l)19.3±5.4 (10-40)<br>Magnesium (mg/l) 4.4±2.2 (1-9) | None | **whole Finland (correlation ρ)**<br>females males total<br>hardness 0.002 0.008 -0.005<br>calcium 0.013 0.020 0.045<br>magnesium -0.006 -0.008 -0.039<br>no p-values given – only ρ, states very low |
| | Nerbrand *et al.* (1992) | Sweden, 76 communities, males and females 45-74 years | 1969-1983 | cerebrovascular (ICD-8 432-438) and IHD (410-414) mortality rates | Water hardness range 12.4-35.5 mg/l | None | Negative correlation water hardness and community IHD and stroke mortality for males, females and males and females combined (no r value, p<0.001) |

[3] Fd = French degree whereby 1 Fd = 10 $CaCO_3$ mg/l

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Flaten (1991) | Norway, 97 municipalities, males and females | 1974-1983 | Multiple diseases including cardiovascular and stroke mortality and cancer incidence | Calcium (mean, range 3.09, 0.44-21.7 mg/l) Magnesium (mean, range 0.71, 0.08-2.64 mg/l) | Age specific rates by gender | **Correlations, ρ, p-value**<br>**Calcium** Males Females<br>Heart disease +0.11, n/s +0.22, <0.05<br>Stroke +0.11, n/s +0.18, <0.05<br><br>**Magnesium**<br>Heart disease +0.33, <0.001 +0.23, <0.05<br>Stroke +0.22, <0.05 +0.35, <0.001<br><br>No significant associations or trends were found with cancer incidence |
| | Dzik (1989) | USA (N Dakota), 50 counties, white males and females | 1968-1978 | cerebrovascular disease mortality | Water hardness, magnesium | Age | **correlations** Males Females<br>hardness -0.242, n/s -0.117, n/s<br>Mg -0.180, n/s -0.100, n/s<br>Na +0.066, n/s +0.045, n/s<br>Langeliers -0.171, n/s -0.082, n/s |
| | Smith and Crombie (1987) | Scotland, 56 districts, males 30-64 years | 1979-1983 | Coronary heart disease mortality ICD-? 410-414 | Water hardness | Age | r=-0.17 (water), r=-0.24 (log water)<br>no p-values given |
| | Leary *et al.* (1983) | South Africa, 12 districts, white males | 1978 | IHD mortality | Magnesium (0.04-1.85mmol/l (1-45 mg/l)) | None | Spearman's correlation rho = -0.68, p<0.02 |
| | Crawford *et al.* (1977) | England and Wales, 6 towns hard water, 6 towns soft water, males and females 30-59 years | 1970-1971 | Cardiovascular disease mortality, IHD mortality, sudden death (≤1 hr) | Water hardness, calcium | None - but stated that age, socioeconomic level and season did not affect results | n=697 for sudden deaths, weighted average of all 3 classes. Proportion of sudden deaths in soft water towns: OR=1.327, p=0.071<br>Suggestion of trend of increasing proportion of sudden deaths in soft areas |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Elwood *et al.* (1977) | England and Wales, 61 towns, males and females | 1970-1972 | IHD mortality (410-414), chronic bronchitis (491), all mortality | Calcium (mean 40ppm, range 4.4-118.0), magnesium (mean 6.1ppm, range 1.0-23.6) | Rainfall, temperature, socioeconomic index | Standardised b, t-value<br>IHD **Calcium ppm** **Magnesium ppm**<br>35-44y -0.1714, 1.8 +0.0375, 0.7<br>45-54y -0.1519, 2.2 -0.0016, 0.0<br>55-64y -0.1514, 3.6 -0.0340, 1.4<br>65-74y -0.0549, 1.4 -0.0236, 1.0 |
| | Elwood *et al.* (1974) | Wales, 48 areas, males and females 35-64 years | | Total cardiovascular and IHD mortality | (am v pm, sig for diff)<br>Calcium:<br>25.6±2.34 v 25.2±2.31, p<0.05<br><br>Magnesium:<br>5.2±0.54 v 5.1±0.55, p<0.05<br><br>Lead:<br>0.017±0.0019 v 0.010±0.0011, p<0.05Q | None | **ρ, p-value**<br>**CVD mortality am pm**<br>Mg -0.375, p<0.05 -0.354, p<0.05<br>Ca -0.554, p<0.05 -0.563, p<0.05<br>Pb 0.257, n/s 0.282, p<0.05<br>**Total mortality**<br>Mg -0.354, p<0.05 -0.341, p<0.05<br>Ca -0.665, p<0.05 -0.689, p<0.05<br>Pb 0.292, p<0.05 0.422, p<0.05 |
| | Schroeder and Kraemer (1974) | USA, 94 major cities, white and non-white males and females 45+ years | 1959-1961 | Mortality artertial hypertension (ICD? 330-331, 440-443, 444-447), Mortality atherosclerosis (ICD? 332, 420), Annual death rates 10 leading causes of death (incl cardiovascular) | Water hardness (mn 88, rg 2.4-352), calcium (mn 25, rg 0.3-92 mg/l), magnesium (mn 5.5, rg 0.02-5 mg/l) | Age adjusted death rates by gender | All p<0.05<br>Significant inverse correlation<br>Ca, Mg, Langelier's all ~ same magnitude r=-0.3 (45-64y, slightly higher 65y+)<br>Multiple tables stratified by gender, race and age |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Bierenbaum *et al.* (1973) | Nebraska, USA, London, GB, W-S North Carolina, Glasgow, GB, white and non-white males and females, 30-50 years | Not stated | serum cholesterol, calcium, magnesium, triglycerides | compared soft and hard water communities | None | Serum cholesterol diet related and higher in Omaha and London (hard) than in soft. Triglycerides 14% higher in London than Glasgow. However, lower cardiovascular mortality rates found in hard than soft and were inversely related to serum Ca and Mg. |
| | Neri *et al.* (1972) | Canada, 9 provinces and 516 municipalities, males 35+ years | 1950-1952, 1960-1962 | Mortality ICD-7: 330-334 vascular lesions affecting central nervous system, 420+422 arteriosclerotic and degenerative heart disease, 410-468 other diseases of the circulatory system, non-cardiovascular disease. | Water hardness | Age | Province level (n=9) 1950+1960<br>r<br>All causes -0.87*<br>vascular lesions -0.69*<br>arterio and deg. HD -0.61<br>other circulatory -0.59<br>non-CVD -0.66*<br>*p<0.05<br><br>516 municipalities age st. mortality rates 35-64y: mort diff b. hard (350ppm) and soft (0ppm)<br>(1) (2) (3)<br>All causes 157.5 -16% 100%<br>vascular lesions 15.0 -23% 10%<br>arterio and deg. HD 38.6 -11% 25%<br>other circulatory 11.7 -16% 7%<br>non-CVD 91.9 -18% 58%<br><br>(1) absolute rate per 100,000/yr<br>(2) as % of rate at zero hardness<br>(3) as % of all causes diff. |
| | Roberts and Lloyd (1972) | England and Wales, 50 areas (S Wales), 58 county boroughs (England and Wales), males 35-54 years | 1959-1963 | IHD mortality (ICD-? 420) | Water hardness | Age, rainfall | S.Wales E+W<br>IHD mortality -0.428 -0.462<br>IHD (controlling for rainfall) -0.254, n/s -0.212, n/s |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Anderson and Leriche (1971) | Canada, 34 towns, males and females 35-74 years | 1965-1967 | Indices for sudden cardiovascular death ICD-420: coroner signed certificate, non-institutional death, duration of illness | Water hardness, calcium, magnesium | None – presented sex and age groups but no apparent standardisation | **proportion of sudden deaths**<br>**coroners** **non-institutional** **certificate**<br>**hardness** -0.37* -0.33 -0.33<br>**calcium** -0.42,* -0.31 -0.32<br>**magnesium** -0.31 -0.35* 0.34*<br>*p<0.05 |
| | Crawford *et al.* (1971) | England and Wales, 11 county boroughs, males and females 45-64 years | 1951-1961 | Change in local cardiovascular death rates | Water hardness change of >50 ppm $CaCO_3$ | Age and gender specific death rates | Mean % change in cardiovascular death rates 1951-1961 with a water hardness change (>50ppm)<br>Decreased (n=6) No change (n=72) Increased (n=5)<br>45-64y (m) +20.2 +11.2 +8.5<br>65-74y (m) -1.5 -4.5 -12.5<br>45-64y (f) -9.0 -11.8 -14.9<br>65-74y (f) -9.0 -12.6 -5.4 |
| | Masironi *et al.* (1970) | USA, 42 states, unknown number of communities | 1960-1962 | All cardiovascular deaths, hypertensive heart disease (HHD), arteriosclerotic heart disease (AHD) | Water hardness | Age and gender. Partially adjusted county level: matched for population size. Community level: matched by river basin and population size | Correlation<br>All CVD HHD AHD non-CVD<br>m+f, all ages -0.19 -0.11 -0.44* +0.03<br>m 45-64y -0.17 -0.11 -0.43* +0.21<br>*p<0.05 |
| | Morris *et al.* (1961) | England and Wales, 83 county boroughs, males and females 45-64 and 65-74 years | 1948-1954 | cardiovascular mortality: total and subgroups (n=5) and other mortality, total of 38 causes of death | Water hardness (range <50 to 250+ ppm), calcium (range <10 to 100+ units not stated), magnesium | None | **total hardness** **Ca** **Mg**<br>**45-64y (M)** -0.55** -0.65** -0.04<br>**45-64y (F)** -0.44* -0.58** +0.08<br>**65-74y (M)** -0.53** -0.60** -0.09<br>**65-74y (F)** -0.36* -0.45** +0.05<br>**p<0.001, *p<0.01 |

**Table 5 (continued)**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Schroeder (1960a) | USA, states:males and females, all races; 163 municipalities white males 45-64 years | 1949-1951 | cardiovascular mortality (ICD-? 330-334, 400-468) arteriosclerotic heart disease (420), all cause | Water hardness (state range 17-299 ppm) | Age | total CVD all cause*<br>**State**<br>annual total death rate -0.56, <0.01 +0.02, n/s<br>**Municipality**<br>white males (n=163) -0.58, <0.01 -0.10, n/s<br>Ca (n=163) -0.27, p<0.01 -<br>Mg (n=163) -0.30, p<0.01 -<br>*excluding CVD |

r = pearson's correlation coefficient
ρ = Spearman's rank correlation coefficient
b = regression coefficient
95%CI = 95% confidence interval surrounding point esitmate
SE = standard error of estimate
RR = relative risk
OR = odds ratio

### 4.1.2 Cancers

Only two ecological studies that met our minimum quality criteria for inclusion were identified that examined the potential for an association between water hardness and cancer. These studies are summarised in Table 6. One study (Yang *et al.* 2000a) showed a significant protective effect of both calcium and magnesium on breast cancer mortality after controlling for the potential confounding effect of fertility rates, urbanisation and size of administrative unit. As such this study was classified as of high quality. The other study showed a significant correlation in sub group analysis with evidence of a positive association between drinking water calcium and magnesium levels and colon and rectal cancer in males (Kikuchi *et al.* 1999). However, this study was classified as of low quality. In view of the limited number of studies and the possibility of multiple hypothesis testing in the second study, no causal inference can be inferred from these ecological studies. Several case control studies have been conducted by Yang *et al.* and are reviewed in the next section.

**Table 6 Ecological studies examining the association between drinking water hardness or calcium or magnesium content and cancer**

| | Author | Country, unit of study, population characteristics | Period | Measures of health outcome | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|---|
| | Yang *et al.* (2000a) | Taiwan,<br>252 municipalities | 1982-1991 | breast cancer mortality (ICD-9 174) | Calcium,<br>Magnesium | Fertility rates (females 20-24 years), municipality size, urbanisation, drinking water calcium / magnesium | Calcium mg/l RR<br>≤ 31.6 1.00<br>31.7-42.3 0.84 (0.79-0.90)<br>42.4-81.0 0.87 (0.81-0.93)<br>$\chi 2$ trend=1.011, p=0.498<br><br>Magnesium mg/l RR<br>≤ 8.3 1.00<br>8.4-14.3 0.84 (0.78-0.89)<br>14.4-41.3 0.85 (0.79-0.90)<br>$\chi 2$ trend=1.495, p=0.437 |
| | Kikuchi *et al.* (1999) | Japan, 34 districts, males and females | 1987-1991 | age-adjusted incidence of colorectal cancer by gender | Calcium (mn±SD 8.35±5.22 (rg 0.37-18.29)mg/l), Magnesium (mn±SD 3.83±3.29 (rg 1.00-16.66)mg/l) | Age, gender and 15 other trace elements | st partial regression coefficient<br>Ca r = +0.73, p<0.01 (male colon)<br>Mg r = +1.37, p<0.01 (male rectal)<br>Females all non-significant and not presented |

### 4.1.3 Other diseases

A number of earlier studies investigated the effect of water hardness and association with all cause mortality and several diseases. However the vast majority of the literature in this field has focused upon examining possible associations between components of drinking water and cardiovascular disease and cancer. As such, these health outcomes were the focus of this review (as detailed in the search strategy). Wider reading of the literature undertaken throughout the course of this review did not identify any consistent trend in the evidence from the ecological studies reviewed for an association between water hardness and other specific diseases.

## 4.2 Cross sectional studies

Cross sectional studies were reviewed to assess the effect of water hardness using individual level data (Table 7). A total of 6 studies were identified as cross sectional studies. Of these studies, 3 used an ecological measure of exposure to water hardness based on data obtained from the water supplier (Tukiendorf and Rybak 2004, Zeighami *et al.* 1990, Bierenbaum *et al*. 1975). One study used a proxy for water hardness as it compared hard and soft water towns (Stitt *et al.* 1973). The remaining 2 studies summarised in Table 7 sampled the household drinking water quality at the individual level and examined the association with individual level cardiovascular risk factors (Luoma *et al.* 1973, Nerbrand *et al.* 2003).

**Table 7 Cross-sectional studies examining the association between drinking water hardness or calcium or magnesium and measures of individual health**

| Author | Country, population characteristics | Period | Health parameters | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Tukiendorf and Rybak (2004) | Poland, 219 males and 273 females | 1985-1994 | Liver cancer morbidity | Magnesium | Age | **Logistic regression (Bayesian random effects)**<br>**Slope SD credible 95%CI**<br>Males -0.275 0.127 (-0.527, -0.033)<br>Females -0.196 0.098 (-0.403, -0.017) |
| Nerbrand *et al.* (2003) | Sweden, 207 males and females in 2 communities, 40-59 years | | Serum cholesterol, LDL-cholesterol, triglycerides, systolic and diastolic blood pressure | Household drinking water concentrations of calcium, magnesium | Age and gender | Closer examination of 2 communities identified as extreme (same study as detailed in Table 5)<br><br>**Spearman's correlation, ρ**<br>**Calcium Magnesium**<br>S-cholesterol (m) -0.128, n/s +0.002, n/s<br>S-cholesterol (f) -0.217, p<0.05 -0.158, n/s<br>sysbp (m) 0.195, p<0.05 +0.091, n/s<br>sysbp (f) -0.274, p<0.05 +0.169, n/s<br>diabp (m) -0.020, n/s -0.005, n/s<br>diabp (f) 0.122, n/s 0.066, n/s<br><br>Dietary calcium and magnesium were not significantly correlated with cardiovascular risk factors |
| | | | | | Age, gender, BMI | **Regression:**<br>Water calcium significantly associated with systolic BP after adjustment for age, sex, BMI<br>Water calcium significantly associated with serum cholesterol after adjustment for triglycerides<br>(no results presented) |
| Zeighami *et al.*(1990) | USA, 46 communities, males and females 40-70 years | Not stated | Serum lipids, blood pressure, thyroid hormones | Water hardness ($CaCO_3$):<br>soft water areas rg: 6-107 ppm<br>hard water areas rg: 157-321 ppm | Age, education level, alcohol, smoking, dietary fat, dietary calcium | Water hardness not significantly associated with cholesterol for males or females<br><br>Other parameters not further considered |

| Author | Country, population characteristics | Period | Health parameters | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Bierenbaum *et al.* (1975) | USA, two cities: Kansas City, Kansas and Kansas City, Missouri, 520 males and females | Not stated | Serum cholesterol, triglycerides, blood pressure, serum calcium / magnesium / cadmium | Comparison of hard and soft water towns: Drinking water concentrations (mg/l): (Kansas v Missouri) calcium (57 v 20) magnesium (11 v 4.8) cadmium (0.003 v 0.001) | Matched for age, gender, length of residence. No difference by city in smoking, social class, height or weight | Hard water city (Kansas) had significantly higher cardiovascular mortality rates.<br>No significant difference in serum cholesterol, triglycerides.<br>Systolic and diastolic blood pressures were higher in Kansas than Missouri, together with serum cadmium concentrations an order of magnitude higher than Missouri. |
| Luoma *et al.* (1973) | Finland, 300 males from 4 rural districts | Not stated | Serum cholesterol, triglycerides, self-reported CVD | Household drinking water concentrations of magnesium, fluoride | Age, smoking, diet, drinking water fluoride | Authors stated a trend of decreased cardiovascular disease with increasing Mg and fluoride (no results given)<br>Regression analysis: Mg and F not significantly associated with serum cholesterol or triglycerides) (no results given) |
| Stitt *et al.* (1973) | England and Wales, males 40-65 years in 6 hard water (n=244) and 6 soft water (n=245) towns | Not stated | Heart rate, blood pressure, cholesterol, ECG measurements | Comparison of hard and soft water towns | Age, smoking, occupation | Age, smoking, height and weight were not different between the two groups<br>Mean heart rate and plasma cholesterol were significantly higher in the soft water towns ($p<0.05$). Systolic and diastolic blood pressures were lower in the hard water towns, this difference was more pronounced in the older age group (50-65 years). |

### 4.2.1 Cardiovascular disease

Five studies examined cardiovascular risk factors at the individual level (Luoma *et al.* 1973, Zeighami *et al.* 1990, Nerbrand *et al.* 2003, Bierenbaum *et al.* 1975, Stitt *et al.* 1973). Two of these studies obtained samples of the individual's household drinking water quality. Luoma *et al.* (1973) reported a trend of decreasing self-reported cardiovascular disease with increasing drinking water magnesium, however drinking water magnesium was not associated with serum cholesterol levels. Nerbrand *et al.* (2003) found magnesium concentrations in household water were not associated with any of the cardiovascular risk factors examined. Calcium concentrations were negatively and significantly associated with systolic blood pressure and serum cholesterol after adjustment for age gender and BMI. However when the genders were considered separately, these associations remained significant for females only with a *positive* association seen with male systolic blood pressure. Of the studies using area level measures of drinking water quality, soft water areas exhibited significantly higher serum cholesterol in one study (Stitt *et al.* 1973), but not another (Bierenbaum *et al.* 1975). Drinking water hardness was not associated with serum cholesterol in males or females (Zeighami *et al.* 1990). In comparisons of towns by drinking water hardness, Bierenbaum *et al.* (1975) found a significantly higher blood pressure in the hard water town, whilst Stitt *et al.* (1973) demonstrated a significantly higher heart rate and blood pressure in the soft water towns.

### 4.2.2 Cancers

One cross sectional study was reviewed and reported a significant inverse association between drinking water magnesium and age adjusted morbidity from liver cancer (Tukiendorf and Rybak 2004).

## 4.3 Case Control Studies

In comparison to ecological studies, case control studies are less susceptible to certain sources of bias. The majority of case control studies identified have used an ecological measure of exposure to hard water. This is a valid approach to assessing an

environmental exposure, but studies where hardness was measured in tap water are less susceptible to non differential misclassification.

### 4.3.1 Cardiovascular diseases

Six case control studies examined the association between magnesium and calcium levels in water and the risk of death from cardiovascular disease. Of these, only one study showed a protective effect of drinking water calcium on female mortality from AMI (Rubenowitz *et al.* 1996). Four studies showed a significant protective effect of drinking water magnesium, against mortality from AMI (Rubenowitz *et al.* 2000, Rubenowitz *et al.* 1996), hypertensive disease (Yang *et al*. 1999b) and stroke (Yang *et al.* 1998a) for males and females.

The best evidence for a protective effect of magnesium is provided by the most recent of the Rubenowitz *et al.* (2000) publications which controlled for the possible confounding effects of age, gender and various individual level risk factors for coronary heart disease. This study found evidence of a significant protective effect of drinking water magnesium against death following an AMI, but not for the risk of a myocardial infarct overall. This suggests that the role of the water factor may operate through reducing the risk of cardiovascular mortality rather than affecting the overall risk of cardiovascular disease. The fully adjusted model from this study yielded an OR of 1.39 (95% confidence interval 1.07 to 1.80) demonstrating a significant increase (39%) in the likelihood of survival following an AMI among individuals with intake of magnesium above 13 mg per day in drinking water. This study accounted for dietary intake of calcium and magnesium and measured individual tap water levels of both elements. The main weakness of the study was the inclusion of cancer patients in the control group. Assuming a causal association between a lower drinking water magnesium level and an increased likelihood of various cancers, as suggested by Yang *et al.* (section 4.3.2), the possible effect of this inclusion could be to reduce the magnitude of any observed association.

Only one study considered drinking water hardness as the exposure factor, and found no association with mortality from arteriosclerotic heart disease (Comstock 1971).

**Table 8 Case control studies examining the association between drinking water hardness or calcium or magnesium content and cardiovascular disease**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Rubenowitz *et al.* (2000) | Sweden, 18 municipalities, males and females, 50-74 years | 1994-1996 | 263 cases AMI dead<br>258 controls | Calcium (range 0-235 mg/l), magnesium (range 0-44 mg/l) | Dead: Age, gender, duration at last residence | **OR (95%CI)**<br>**Deaths** **Survivors**<br>**Magnesium***<br>m+f 0.64 (0.42-0.97) 1.16 (0.93-1.45)<br>m 0.69 (0.43-1.09) 1.19 (0.91-1.54)<br>f 0.51 (0.21-1.22) 1.09 (0.70-1.70)<br>**Calcium***<br>m+f 0.89 (0.59-1.33) 0.97 (0.78-1.21)<br>m 1.01 (0.64-1.59) 0.97 (0.75-1.26)<br>f 0.68 (0.29-1.59) 0.90 (0.59-1.38)<br>* upper quartile versus lower three |
| | | | 823 cases AMI alive<br>853 controls | | Alive: Age, gender, BMI, education, occupation, smoking, stress, physical avtivity, fruit and vegetable intake, alcohol, family history of AMI, diabetes, high blood pressure | **Risk of AMI + survival for intake via water:**<br>**Ca >108mg/d** **Mg >13mg/d**<br>**(1)** 0.97 (0.77-1.21) 1.30 (1.04-1.63)<br>**(2)** 0.91 (0.73-1.15) ----------<br>**(3)** 0.89 (0.69-1.15) 1.39 (1.07-1.80)<br>(1) age,gender<br>(2) age,gender,water Mg<br>(3) multivariate |
| Rubenowitz *et al.* (1999) | Sweden, 16 municipalities, females, 50-69 years | 1982-1993 | 378 cases AMI deaths 1368 controls cancer deaths (ICD-9 140-239) | all municipalities (range)<br>Calcium 8-230mg/l<br>Magnesium 1.3-21.5mg/l | Age, drinking water calcium / magnesium | **Calcium mg/l** **OR**<br>≤ 31 1<br>32-45 0.61 (0.39-0.94)<br>46-69 0.71 (0.49-1.02)<br>≥ 70 0.66 (0.47-0.94)<br>**Magnesium mg/l** **OR**<br>≤ 3.4 1<br>3.5-6.7 1.08 (0.78-1.49)<br>6.8-9.8 0.93 (0.64-1.34)<br>≥ 9.9 0.70 (0.50-0.99) |

**Table 8 (continued)**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Yang *et al.* (1999b) | Taiwan, 252 municipalities, males and females, 50-69 years | 1990-1994 | 2,336 cases hypertension deaths 2336 controls all other deaths† | Cases vs controls (mn±SD (mg/l))<br><br>Calcium (32.9±20.3 v 34.8±19.5)<br><br>Magnesium (10.9±7.6 v 11.2±7.5) | Age, gender, urbanisation, drinking water calcium / magnesium | **Calcium mg/l (md) OR**<br>4-11.3 (7.4) 1<br>11.4-30.0 (20.2) 1.23 (0.94-1.62)<br>30.1-37.3 (34.6) 1.32 (0.98-1.78)<br>37.4-53.4 (43.3) 1.12 (0.83-1.51)<br>53.5-81.0 (60.5) 1.26 (0.92-2.02)<br>**Magnesium mg/l (md) OR**<br>1.5-3.8 (3.5) 1<br>3.9-8.2 (7.0) 0.73 (0.57-0.93)<br>8.3-11.1 (9.1) 0.66 (0.50-0.87)<br>11.2-16.3 (13.5) 0.67 (0.50-0.89)<br>16.4-41.3 (19.4) 0.63 (0.47-0.84)<br>$\chi^2$ trend = 29.05, p<0.001 |
| Yang *et al.* (1998a) | Taiwan, 252 municipalities, males and females, 50-69 years | 1989-1993 | 17,133 cases cerebrovascular deaths 17,133 controls all other deaths† | Cases vs controls (mn±SD (mg/l))<br><br>Calcium (34.5±19.5 v 34.8±19.5)<br><br>Magnesium (11.3±7.5 v 11.4±7.5) | Age, gender, urbanisation, drinking water calcium / magnesium | **Calcium mg/l (md) OR**<br>≤ 24.0 (9.2) 1<br>24.4-42.3 (34.6) 1.05 (0.98-1.12)<br>42.4-81.0 (57.0) 0.95 (0.88-1.01)<br>**Magnesium mg/l (md) OR**<br>≤ 7.3 (3.8) 1<br>7.4-13.4 (9.1) 0.75 (0.65-0.85)<br>13.5-41.3 (17.3) 0.60 (0.52-0.70) |

**Table 8 (continued)**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Rubenowitz *et al.* (1996) | Sweden, 17 municipalities, males, 50-69 years | 1982-1989 | 854 cases AMI deaths<br>989 controls cancer deaths (ICD-9 140-239) | all municipalities (range)<br>Calcium 22-225mg/l<br>Magnesium 1.3-20.0mg/l | Age, drinking water calcium / magnesium | **Calcium mg/l (md) OR**<br>≤ 33 (28) 1<br>34-45 (40) 0.88 (0.65-1.19)<br>46-81 (66) 0.84 (0.64-1.10)<br>≥ 82 (82) 1.06 (0.82-1.38)<br>trend: n/s (no value given)<br>**Magnesium mg/l (md) OR**<br>≤ 3.5 (3.5) 1<br>3.6-6.8 (6.7) 0.88 (0.66-1.16)<br>6.9-9.7 (7.8) 0.70 (0.53-0.93)<br>≥ 9.8 (17) 0.65 (0.50-0.84)<br>trend: sig (no value given) |
| Luoma *et al.* (1983) | Finland, males 30-64 years | 1974-1975 | 50 case-control pairs cases first AMI (alive or dead)<br>113 hospital controls (HC)<br>127 population controls (PC) | Calcium, Magnesium | Age, community type (urban/rural) | **RR(95%CI)**<br>**case/HC** **case/PC**<br>Ca ≤ 16mg/l 0.73 (0.22, 1.99) 0.56 (0.25, 1.28)<br>≤ 18mg/l 0.77 (0.30, 1.91) 1.07 (0.48, 2.42)<br>≤ 20mg/l 0.91 (0.35, 2.36) 1.64 (0.73, 3.85)<br>Mg ≤ 1.2mg/l 2.00 (0.69, 6.52) 4.67 (1.30, 25.32)<br>≤ 1.5mg/l 1.11 (0.41, 3.10) 2.29 (0.88, 6.58)<br>≤ 3.0mg/l 1.00 (0.36, 3.08) 1.63 (0.62, 4.52) |
| Comstock (1971) | USA, white males 45-65 years | | 189 cases arteriosclerotic heart disease deaths<br>2 controls per case (other deaths) | Water hardness (range 0-450 ppm CaCO3) | Matched by year of birth | mean hardness RR (age)<br>0-99ppm 0.78<br>150+ppm 1.35<br>*p>0.05 (no CI)* |

† All Yang *et al.* studies excluded from the controls causes of death that have been shown to be possibly associated with the drinking water quality parameters being considered in this review. These exclusions included all cardiovascular and cerebrovascular deaths and deaths from breast, prostate and colorectal cancer.

### 4.3.2 Cancer and other diseases

We identified 12 case control studies examining the association between cancers and measures of water hardness, calcium and magnesium in drinking water (Table 9). All the studies were conducted in Taiwan from the same group and utilised an ecological measure of exposure. Although an a priori hypothesis is stated in all the papers, it is not clear to us how hypotheses were tested by this group. The studies had large sample sizes and excluded controls with history of any condition previously associated with water hardness. All controls were deceased individuals and the studies controlled for the effect of age, sex and urbanisation. The studies did not control for the confounding effect of other factors that may increase the risk of cancers such as smoking.

Evidence of a significant protective effect of hard water was shown for pancreatic cancer (Yang *et al.* 1999c), gastric cancer (Yang *et al.* 1997b), colon cancer (Yang *et al.* 1998b), rectal cancer (Yang *et al.* 1999d) and oesophageal cancer (Yang *et al.* 1999e). Magnesium levels were found to be protective against cancer of the prostate (Yang *et al.* 2000b), oesophagus (Yang *et al.* 2002a), and colon (Yang *et al.* 1997a), but not stomach (Yang *et al.* 1998c) or rectum (Yang *et al.* 1998d). Higher calcium levels in drinking water conferred a protective effect against cancer of the colon, rectum and stomach. The same group also examined drinking water calcium concentrations and two other health outcomes. Increasing calcium levels were significantly associated with an increased likelihood of death from renal failure (Hwang *et al.* 2003), but a slightly decreased incidence of very low birth weight babies (Yang *et al.* 2002b).

The confidence intervals around most of the estimates were narrow with significant evidence of trend for many of the associations observed (Table 9). These studies provide evidence for possible protective effect of calcium and magnesium from several malignancies. However, replication of the results in other settings and centres will be necessary before causal inference can be attempted.

**Table 9 Case control studies examining the association between drinking water hardness or calcium or magnesium content and cancers/other diseases**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Hwang *et al.* (2003) | Taiwan, 252 municipalities, males and females, 50-69 years | 1991-2000 | 2,469 cases renal failure deaths<br>2,469 controls all other deaths† | Calcium | Age, gender, urbanisation, drinking water magnesium | **Calcium (mg/l) (md) OR (95%CI)**<br>≤ 24.4 (14.6) 1<br>25.1-43.0 (34.8) 1.21 (1.03-1.43)<br>43.3-81.0 (57.0) 1.34 (1.12-1.60)<br>$\chi^2$ trend = 7.36, p=0.025 |
| Yang *et al.* (2002a) | Taiwan, 252 municipalities, males and females, 50-69 years | 1991-2000 | 2,204 cases oesophageal cancer deaths<br>2,204 controls all other deaths† | Calcium, magnesium | Age, gender, urbanisation, drinking water calcium / magnesium | **Calcium mg/l (md) OR (95%CI)**<br>≤ 24.0 (9.2) 1<br>24.4-42.6 (34.6) 1.07 (0.88-1.31)<br>43.0-81.0 (57.0) 0.91 (0.74-1.13)<br>**Magnesium mg/l (md) OR**<br>≤ 7.0 (3.6) 1<br>7.3-14.0 (9.1) 0.75 (0.62-0.91)<br>14.3-41.3 (17.6) 0.71 (0.57-0.88)<br>$\chi^2$ trend = 27.75, p<0.05 |
| Yang *et al.* (2002b) | Taiwan, 252 municipalities, first parity babies | 1993-1997 | 1,781 cases females with very low birth weight babies (VLBW <1500g)<br>1,781 controls all other first parity births (>2499g) | Calcium | Sex of baby, month and year of birth, maternal age, marital status, maternal education, urbanisation | **Calcium mg/l (md) OR (95%CI)**<br>≤ 31.6 (14.9) 1<br>32.4-41.9 (34.9) 0.90 (0.75-1.08)<br>≥ 42.2 (57.0) 0.81 (0.69-0.96) |

**Table 9 (continued)**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Yang *et al.* (2000b) | Taiwan, 252 municipalities, males, 60-79 years | 1987-1993 | 682 cases prostate cancer deaths<br>682 controls all other deaths† | Calcium, magnesium | Age, gender, urbanisation, drinking water calcium / magnesium | **Calcium mg/l (md)** **OR (95%CI)**<br>≤ 20.2 (7.5) 1<br>20.3-40.9 (34.6) 1.05 (0.73-1.51)<br>41.0-81.0 (53.8) 1.16 (0.78-1.72)<br>$\chi^2$ trend = 5.29, p<0.05<br>**Magnesium mg/l (md)** **OR (95%CI)**<br>≤ 7.0 (3.6) 1<br>7.1-11.8 (9.1) 0.73 (0.51-1.03)<br>11.9-41.3 (16.9) 0.64 (0.43-0.96)<br>$\chi^2$ trend = 8.07, p<0.01 |
| Yang *et al.* (1999c) | Taiwan, 252 municipalities, males and females 50-69 years | 1990-1994 | 883 cases pancreatic cancer deaths<br>883 controls all other deaths† | Water hardness | Age, gender, urbanisation | **Hardness ($CaCO_3$ mg/l)** **OR (95%CI)**<br>Hard (>150) 1<br>Moderately hard (75-150) 1.09 (0.87-1.37)<br>Soft (<75) 1.39 (1.09-1.76)<br>$\chi^2$ trend = 10.40, p<0.0001 |
| Yang *et al.* (1999d) | Taiwan, 252 municipalities, males and females, 50-69 years | 1990-1994 | 986 cases rectal cancer deaths<br>986 controls all other deaths† | Water hardness | Age, gender, urbanisation | **Hardness ($CaCO_3$ mg/l)** **OR (95%CI)**<br>Hard (>150) 1<br>Moderately hard (75-150) 1.24 (1.01-1.55)<br>Soft (<75) 1.38 (1.10-1.73)<br>$\chi^2$ trend = 13.99, p<0.0001 |
| Yang *et al.* (1999e) | Taiwan, 252 municipalities, males and females, 50-69 years | 1987-1996 | 2,084 cases oesophageal cancer deaths<br>2,084 controls all other deaths† | Water hardness | Age, gender, urbanisation | **Hardness ($CaCO_3$ mg/l)** **OR (95%CI)**<br>Hard (>150) 1<br>Moderately hard (75-150) 0.95 (0.82-1.09)<br>Soft (<75) 1.42 (1.22-1.66)<br>$\chi^2$ trend = 23.43, p<0.0001 |
| Yang *et al.* (1998b) | Taiwan, 252 municipalities, males and females, 50-69 years | 1989-1993 | 1,714 cases colon cancer deaths<br>1,714 controls all other deaths† | Water hardness | Age, gender, urbanisation | **Hardness ($CaCO_3$ mg/l)** **OR (95%CI)**<br>Hard (>150) 1<br>Moderately hard (75-150) 1.22 (1.04-1.43)<br>Soft (<75) 1.46 (1.22-1.75)<br>$\chi^2$ trend = 28.47, p<0.0001 |

**Table 9 (continued)**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Yang *et al.* (1998c) | Taiwan, 252 municipalities, males and females, all ages | 1987-1991 | 6,766 cases gastric cancer mortality<br>6,766 controls all other deaths† | Calcium, magnesium, nitrate | Age, gender, urbanisation, drinking water calcium / magnesium / nitrate | **Calcium mg/l (md)** **OR (95%CI)**<br>≤ 20.2 (7.5) 1<br>22.0-38.7 (32.8) 0.77 (0.69-0.88)<br>39.5-81 (53.4) 0.70 (0.62-0.80)<br>**Magnesium mg/l (md)** **OR (95%CI)**<br>≤ 7.0 (3.6) 1<br>7.3-11.6 (8.9) 1.01 (0.90-1.13)<br>11.8-41.3 (16.3) 0.86 (0.76-0.98)<br>**Nitrate mg/l (md)** **OR (95%CI)**<br>≤ 0.22 (0.04) 1<br>0.23-0.44 (0.37) 1.10 (1.00-1.20)<br>≥ 0.45 (0.67) 1.14 (1.04-1.25) |
| Yang *et al.* (1998d) | Taiwan, 252 municipalities, males and females, 50-69 years | 1990-1994 | 986 cases rectal cancer mortality<br>986 controls all other deaths† | Calcium, magnesium | Age, gender, urbanisation | **Calcium mg/l (md)** **OR (95%CI)**<br>≤ 20.2 (7.5) 1<br>22.0-40.8 (34.6) 0.72 (0.53-0.98)<br>40.9-79.2 (54.8) 0.63 (0.45-0.87)<br>$\chi^2$ trend = 17.73, p<0.0001<br>**Magnesium mg/l (md)** **OR (95%CI)**<br>≤ 7.0 (3.6) 1<br>7.3-11.6 (9.1) 1.12 (0.84-1.49)<br>11.8-41.3 (16.9) 1.11 (0.80-1.54)<br>$\chi^2$ trend = 1.81, p>0.05 |
| Yang *et al.* (1997a) | Taiwan, 252 municipalities, males and females, 50-69 years | 1989-1993 | 1,714 cases colon cancer mortality<br>1,714 controls all other deaths† | Calcium, magnesium | Age, gender, urbanisation, drinking water calcium / magnesium | **Calcium mg/l (md)** **OR (95%CI)**<br>≤ 24.0 (9.2) 1<br>24.4-42.3 (34.6) 0.79 (0.64-0.98)<br>42.4-81.0 (57.0) 0.58 (0.47-0.73)<br>$\chi^2$ trend = 47.47, p<0.0001<br>**Magnesium mg/l (md)** **OR (95%CI)**<br>≤ 7.3 (3.8) 1<br>7.3-13.3 (9.1) 0.72 (0.61-0.84)<br>13.4-41.3 (17.3) 1.06 (0.85-1.32)<br>$\chi^2$ trend = 1.74, p>0.05 |

**Table 9 (continued)**

| Author | Country, population characteristics | Period | Cases and controls | Drinking water parameters | Variables adjusted for | Results | |
|---|---|---|---|---|---|---|---|
| Yang *et al.* (1997b) | Taiwan, 252 municipalities, males and females, all ages | 1987-1991 | 6,766 cases gastric cancer deaths<br>6,766 controls all other deaths† | Water hardness | Age, gender | **Hardness ($CaCO_3$ mg/l)**<br>Hard (>150)<br>Mod hard (75-150)<br>Soft (<75)<br>$\chi^2$ trend = 33.59, p<0.001 | **OR (95%CI)**<br>1<br>1.16 (1.07-1.26)<br>1.65 (1.52-1.79) |

† All Yang *et al.* studies excluded from the controls causes of death that have been shown to be possibly associated with the drinking water quality parameters being considered in this review. These exclusions included all cardiovascular and cerebrovascular deaths and deaths from breast, prostate and colorectal cancer.

## 4.4 Cohort Studies

Three cohort studies were identified by our search strategy (Table 10). Comstock *et al.* reported mortality from stroke (1979) and arteriosclerotic heart disease (1980) in a large cohort of males and females in the US. There was no significant association between water hardness and stroke mortality, and only a weak suggestion that soft water was harmful in females and possibly associated with a slightly greater risk of sudden death. Punsar *et al.* examined coronary heart disease (CHD) incidence and mortality in Finnish males. Neither drinking water calcium or magnesium concentrations were associated with individual level risk factors for CHD (Punsar *et al.* 1975) or mortality (Punsar *et al.* 1975, Punsar and Karvonen 1979). However, the studies conducted by Punsar *et al.* were of poorer quality when compared to Comstock *et al.* with no adjustment for individual level risk factors. Furthermore, although both cohort studies applied an average measure of areal drinking water quality to the individual, greater efforts were made by Comstock *et al.* to increase the likelihood of homogeneity of drinking water exposure by area by aggregating repeated samples at the individual level.

The most recent cohort study reported the findings of a 15 year follow up of the British Regional Heart Study (BRHS) (Morris *et al.* 2001). A total of 7735 males were followed within 24 towns. The occurrence of both fatal and non-fatal CHD was examined at the town level by multilevel modelling of individual level risk factors of males within towns, and town level environmental variables. There was no statistically significant association between water hardness and CHD incidence after controlling for age alone, or all individual level risk factors. Although this is a well designed cohort study, a notable limitation in its ability to examine the water story is the adequacy of the exposure characterisation. An ecological measure of water hardness was utilised at the town level. The towns selected for inclusion in the BRHS had a population of between 50,000 and 100,000. Therefore, a considerable degree of heterogeneity in actual drinking water hardness levels within each town is possible. The application of a town level measure of drinking water hardness may not adequately reflect the actual levels experienced by the individuals. Consequently the large potential for measurement error of the drinking water variable may reduce the ability of this study to detect an association. Furthermore, only 24 towns were

considered in analysis. This small sample size and reduced statistical power may explain the failure of this study to detect the weaker association of water hardness with cardiovascular disease when compared to the individual level risk factors including smoking and blood pressure.

**Table 10 Cohort studies examining the association between drinking water hardness or calcium or magnesium content and cardiovascular disease**

| Author | Country, population characteristics | Period | Health parameters | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Morris *et al*. (2001) | Great Britain, 15 year follow up of 7735 males in 24 towns, aged 40-59 years at baseline | 1978-1996 | Major fatal (ICD-9 410-414) and non-fatal coronary heart disease | Drinking water hardness | Age, smoking, physical activity, BMI, alcohol, systolic blood pressure, cholesterol, social class, height | Multilevel model incorporating individual level risk factor data and town level data (for individuals within towns). Water hardness was considered at the town level (n=24).<br><br>**Water hardness** **OR (95% CI)**<br>Adjusted for age 0.91 (0.83-1.00)<br>Adjusted for all variables 0.96 (0.88-1.05) |
| Comstock *et al*. (1979) | USA, enumeration districts, 12 year follow up of 36.860 males and females adults >25 years | 1963-1975 | Stroke mortality (ICD-7 331, 332, 334) | Categories of drinking water hardness (range 0-389 ppm $CaCO_3$) | Age, gender, housing quality, marital status, education, smoking, mobility, urban/rural | No statistically significant association between water hardness and stroke mortality |
| Comstock *et al*. (1980) | USA, enumeration districts, 12 year follow up of 30,942 white adults >25 years | 1963-1975 | Arteriosclerotic heart disease mortality (ICD-7 420-422) | Categories of drinking water hardness (range 0-389 ppm $CaCO_3$) | Age, gender, duration of residence in household, marital status, education, smoking, urban/rural | Some suggestion that soft water was harmful in females but protective in males. Risk of sudden death is slightly greater for females than males.<br>No evidence for specificity of the association, non-CVD deaths as likely to be associated with soft water. |

**Table 10 (continued)**

| Author | Country, population characteristics | Period | Health parameters | Drinking water parameters | Variables adjusted for | Results |
|---|---|---|---|---|---|---|
| Punsar *et al.* (1975) | Finland, 10 year follow up of 2 rural male cohorts (east, n=622 and west, n=504) | 1970 | Coronary heart disease mortality, survivors classified by heart health status, also examined individual blood pressure, serum cholesterol and ECGs | west v east (mean±SD, rg) mg/l<br>calcium (22.7±3.7 (13.7-27.0) v 11.9±4.5 (3.4-20.6)), magnesium (13.6±6.6 (6.9-27.8) v 3.5±1.5 (0.6-7.3)) | Age groups | Subareas: 10 (west), 33 (east), both areas had soft water. No association between calcium and CHD. Mg concentration was decreased in CHD deaths but was not consistent (no appropriate values to report)<br>Multiple hypothesis testing of 22 trace elements.<br><br>Ca + Mg not correlated with BP or cholesterol<br>Mg did not correlate with clinical severity of CHD. |
| Punsar and Karvonen (1979) | Finland, 15 year follow up of 2 rural cohorts: 888 males (west) and 888 males (east) | 1959-1974 | CHD mortality and sudden death (unexpected and within 1 hour) | Magnesium | None | Significantly higher mortality in the east, water magnesium content for CHD deaths was lower (but n/s). no significant difference for sudden deaths |

## 4.5 Autopsy studies

A number of autopsy studies have investigated the mineral concentrations of the heart muscle (myocardium) by hard and soft drinking water areas and cause of death. Comparisons of areas by drinking water hardness have yielded inconsistent results. Anderson *et al.* (1975) showed myocardial magnesium concentrations in soft water regions to be significantly lower than corresponding hard water areas, whilst Chipperfield *et al.* (1976, 1979) found the opposite to be true. Nevertheless, Chipperfield *et al.* reported that whilst total magnesium concentrations were higher in soft water areas, the authors detected a concurrent *relative* magnesium deficiency. Crawford and Crawford (1967) presented evidence suggesting myocardial disease was more likely to develop in a soft compared to a hard water area (Glasgow *vs* London). These differences in mineral concentration were detected for both cardiovascular and non-cardiovascular deaths. Autopsy studies have also shown a trend of significantly decreased myocardial magnesium concentrations but increased calcium concentrations in heart disease deaths when compared to non-CVD deaths (Anderson *et al.* 1975, Chipperfield *et al.* 1978, Elwood *et al.* 1980). Furthermore, this trend was more pronounced for sudden IHD deaths compared to late IHD deaths in some (Chipperfield *et al.* 1978) but not all studies (Elwood *et al.* 1980). It has been suggested that the elevated calcium may be secondary to the reduced magnesium (Anderson *et al.* 1975). These differences in mineral concentrations were found for the heart muscle only and not other major muscles including the diaphragm and pectoralis major (Anderson *et al.* 1975).

These findings from a small number of autopsy studies are generally compatible with a possible association between increased cardiovascular death rates in soft water areas and a relatively lower magnesium concentration in drinking water. The relative magnesium deficiency may increase the risk of death following myocardial infarction in soft water areas. This hypothesis is also supported by the observation of a greater decrease in myocardial magnesium concentrations in sudden *versus* later IHD deaths. However, the results do show inconsistencies and although controlled for age and gender may be influenced by other factors not considered. Whilst it remains a possibility that magnesium depletion is a consequence of IHD death, the finding of significantly decreased myocardial magnesium concentrations in non-CVD deaths

supports the interpretation of low magnesium as a predisposing cause of sudden death.

Only one study additionally examined myocardial concentrations of cadmium and lead. No significant differences were found between the hard and soft water areas (Anderson *et al.* 1975).

# 5 Discussion of results

## 5.1 Mortality versus incidence

The overwhelming majority of studies examined cardiovascular mortality as the health outcome of interest. Of all the ecological studies, only two medium quality studies examined incidence rather than mortality. Water hardness was significantly associated with a decreased risk of both first AMI (Kousa *et al.* 2004, Masironi *et al.* 1979) and the overall rate of occurrence of heart attacks (Masironi *et al.* 1979). However, these findings were controlled for age and gender effects only and not for other possible confounding factors (hence their classification as medium quality studies). Only 1 of 8 case-control studies of cardiovascular disease reported incidence of first AMI (Rubenowitz *et al.* 2000). Luoma *et al.* (1983) also examined first AMI as the health outcome of interest however the definition encompassed both living and deceased individuals therefore mortality and incidence could not be examined separately. Rubenowitz *et al.* (2000) found high drinking water magnesium levels to be associated with both a reduced likelihood of AMI mortality and an increased likelihood of survival in analyses of deceased and alive individuals, respectively. This study was of high quality and controlled for a number of cardiovascular risk factors at the individual level for survivors of AMI. Of the cohort studies, Morris *et al.*(2001) defined CHD incidence as the occurrence of both fatal and non-fatal events. No cross-sectional studies reported incidence of cardiovascular disease.

There were therefore too few papers examining incidence of cardiovascular disease to allow a comparison of any possible differential effect of drinking water composition

upon incidence or mortality. Hence the conclusions of this review will mainly reflect cardiovascular mortality.

### 5.2 Overview of cardiovascular disease studies

As previously discussed, the classification of mortality from cardiovascular disease encompasses a wide range of conditions as reflected by the large number of ICD codes contained within. Of the 12 high quality ecological studies, 8 examined mortality from IHD, 5 from cerebrovascular disease and 3 from wider definitions of cardiovascular disease. A larger number of studies investigated IHD than cerebrovascular mortality with regard to drinking water parameters. This general trend was consistent between the three study quality groups. The low quality studies were more likely to examine the larger category of all cardiovascular deaths. There was a progressive increase in the proportion of studies failing to report the actual ICD codes examined with decreasing level of study quality. Of the case control studies, 4 examined acute myocardial infarction, a subcategory of IHD (Luoma *et al.* 1983, Rubenowitz *et al.* 1996, 1999, 2000), whilst single studies considered stroke (Yang *et al.* 1998a), hypertensive disease (Yang *et al.* 1999b) and arteriosclerotic heart disease (Comstock 1971). Of the 5 cohort papers, the health outcomes of interest were mortality from IHD (Morris *et al.* 2001, Punsar *et al.* 1975, Punsar and Karvonen 1979, Comstock *et al.* 1980) and stroke (Comstock *et al.* 1979). Overall, a greater number of studies investigated IHD than cerebrovascular disease, with the individual level studies all examining smaller constituent subcategories of these larger groups.

### 5.3 Sudden death versus all cardiovascular deaths

It has been suggested that the inverse association seen in many studies between cardiovascular disease mortality and water hardness is due to an increase in the proportion of sudden deaths in areas of soft water. Few studies specifically examined sudden death as a health outcome of interest. Early ecological studies showed an increased proportion of sudden deaths in soft, compared to hard, water towns (Anderson and Leriche 1971, Crawford *et al.* 1977). However, these were of low study quality and were uncontrolled. Of the cohort studies following individuals for over 10 years, one found a slightly increased risk of sudden death with soft drinking

water in females only (Comstock *et al.* 1980), whilst another found no significant difference between areas of comparatively hard and soft water (Punsar and Karvonen 1979).

Whilst these studies provide no evidence supporting increased sudden death in areas of soft water, the difficulties defining a case as a sudden death and the different definitions used between studies must be acknowledged. The possible role of sudden death in the frequently observed association between water hardness and cardiovascular disease mortality has recently been re-addressed in a well controlled case-control study. Rubenowitz *et al.* (2000) showed that whilst the concentration of drinking water magnesium at the individual level did not influence either the risk or total incidence of AMI, the proportion of survivors was larger at the higher drinking water magnesium concentrations. This association was significant after controlling for major cardiovascular risk factors at the individual level. The authors concluded that increasing magnesium concentrations therefore prevented sudden deaths as opposed to all IHD deaths. A similar, but less consistent, effect was also seen for calcium. It was postulated that the higher magnesium intake from drinking water may contribute to a more rapid correction of electrical instability within the heart, which may otherwise lead to a fatal AMI.

Therefore, to date there is insufficient evidence available upon which to base any form of conclusion regarding the role of sudden death in the water story. However, the evidence presented by Rubenowitz *et al.* (2000) from a well controlled study is supportive of a possible protective role of magnesium in sudden death.

### 5.4 Cerebrovascular disease versus heart disease

As defined in 1.2.1, cardiovascular disease comprises the two broad groupings of heart disease and stroke. Although a number of major risk factors for mortality from these two conditions overlap, including blood pressure (Beevers *et al.* 1995), smoking (Tyroler 2000) and dietary factors (Ness and Powles 1997), the possible roles of water hardness, calcium and/or magnesium as risk factors were examined for differential effects by cardiovascular subgroups.

Of the 12 high quality ecological studies, only 4 studies considered both stroke and IHD mortality. There was no notable difference in the exposure-outcome analysis by stroke or IHD mortality, with the magnitude of the effect estimated to be approximately the same (Pocock *et al.* 1980, Marque *et al.* 2003, Catling 2004) or slightly greater for stroke (Ferrandiz *et al.* 2004). The case-control studies in Table 8 present evidence showing that increasing drinking water magnesium and/or calcium concentrations have been found to be protective against both stroke (Yang *et al.* 1998a) and heart disease (Rubenowitz *et al.* 1996, 1999, 2000). A 12 year follow up of a mixed gender cohort found no significant association between drinking water hardness and mortality from either stroke (Comstock *et al.* 1979) or heart disease (Comstock *et al.* 1980). Two high quality ecological studies examined the wider category of cardiovascular disease mortality. Both the magnitude and significance of the inverse association between mortality and water hardness was found to be comparable for all cardiovascular mortality and stroke (Marque *et al*. 2003) and stroke and IHD (Catling 2004). Therefore, there was no evidence for a differential effect of drinking water hardness, calcium or magnesium and mortality from stroke or heart disease.

### 5.5 Association with major known cardiovascular risk factors

Cardiovascular disease is a disease of complex and multifactorial aetiology. In other words, there are many factors which can contribute to the development and progression of cardiovascular disease. These include atherosclerosis (clogging and hardening of the arteries), blood coaguability (factors which influence the likelihood of the blood to form potentially threatening clots) and electrical factors (affecting the normal rhythm and functioning of the heart). The hypothesised biological mechanisms relating calcium and magnesium to cardiovascular disease have been extensively reviewed elsewhere, and were summarised in the introduction. This section will assess and summarise the evidence presented by primary studies investigating aspects of drinking water quality and cardiovascular disease as it may support any hypothesised biological mechanism of association.

Blood pressure and serum cholesterol concentrations were the only cardiovascular risk factors considered at the individual level by studies of drinking water quality

within this review. Stitt *et al.* (1973) compared individual level cardiovascular risk factors of males residing in hard and soft water towns. Mean heart rate and serum cholesterol concentrations were significantly increased in soft water towns, whilst systolic and diastolic blood pressure were lower in hard water towns. These differences were found after controlling for the effects of age, smoking, occupation and weight. Upon further examination it was shown that the observation of reduced blood pressure was present in the older age group alone (50-65 years), suggesting that any effect may only become evident with increasing age.

Indirect evidence for an association between drinking water quality and blood pressure is given by the finding of an inverse association between magnesium concentrations and the likelihood of mortality from hypertensive disease in males and females (Yang *et al.* 1999b). No such association was demonstrated for drinking water calcium concentrations. However, studies examining individual level measures of drinking water magnesium and blood pressure found no evidence to support this observation (Punsar *et al.* 1975, Nerbrand *et al.* 2003). In contrast, drinking water calcium concentrations were shown to be significantly and inversely related to systolic blood pressure after adjustment for the effects of age, gender and BMI (Nerbrand *et al.* 2003). When the genders were considered separately, this association was retained in females, however a *positive* association was seen with male systolic blood pressure. The gender specific analyses may have been affected by the comparative reduction in the numbers being modelled and therefore a greater sensitivity to random variation. Nevertheless, this difference reduces confidence in the described association between drinking water calcium and decreased blood pressure. There was no evidence of an association between drinking water calcium and blood pressure in Finnish males (Punsar *et al.* 1975).

Drinking water calcium concentrations were shown to be significantly and inversely associated with serum cholesterol levels after adjustment for age, gender and BMI (Nerbrand *et al.* 2003). However, other studies have described no association between male and female serum cholesterol levels and levels of drinking water hardness (Zeighami *et al.* 1990), calcium (Punsar *et al.* 1975) or magnesium (Punsar *et al.* 1975, Luoma *et al.* 1973, Nerbrand *et al.* 2003).

Section 4.3.1 described evidence from a well designed case-control study supporting a protective role of increased drinking water magnesium concentrations in sudden death (Rubenowitz *et al.* 2000). This protective effect was hypothesised to operate via the greater intake of magnesium being able to more rapidly correct any electrical instability in the heart and prevent AMI fatality.

Of the few studies examining individual level factors, an apparent improvement in the cardiovascular risk profile in hard water areas has been described, whilst other studies have identified significant, but inconsistent, associations with only singular risk factors. Section 5.4 described how there was no evidence of a differential effect between stroke and heart disease mortality with regard to drinking water quality indicating the biological mechanism may therefore operate via a risk factor common to both.

Whilst evidence has been presented supporting a protective role of drinking water quality against atherogenesis, high blood pressure and electrical instability, too few studies have sufficiently examined the possible biological mechanism of association as it may relate to existing major cardiovascular risk factors. The evidence to date is therefore too weak and inconsistent to allow any firm conclusions to be drawn regarding the possible biological mechanism linking drinking water quality and cardiovascular disease.

### 5.6 Magnesium versus calcium

As defined in the introduction, total hardness is actually a composite measure of various polyvalent cations present in the drinking water. Of these, calcium and magnesium are the dominant contributors to total hardness, however a single value for hardness may arise from varying proportions of calcium and magnesium. Therefore it is not possible to use the hardness value as a proxy for either calcium or magnesium alone. This section will therefore only consider evidence where calcium and/or magnesium were explicitly studied.

### 5.6.1 Magnesium vs calcium: cardiovascular disease

Of the 12 high quality ecological studies, 5 examined both drinking water calcium and magnesium. Two found no association between IHD mortality and either calcium or magnesium (Maheswaran *et al.* 1999, Dudley *et al.* 1969). Of the remaining 3 studies, increasing magnesium concentrations were significantly associated with decreased mortality from IHD (Kaipio *et al.* 2004) and stroke (Ferrandiz *et al.* 2004). However, Marque *et al.* (2003) found calcium, but not magnesium, to be significantly and inversely associated with total CVD and stroke mortality. A weaker association between calcium and stroke was demonstrated by Ferrandiz *et al.* (2004), with a slight increase in CHD mortality found with increasing calcium concentrations by Kaipio *et al.* (2004).

Section 4.1 presented the proportions of papers examining drinking water calcium and/or magnesium in the high, medium and low quality ecological studies with a summary of findings. A slightly lower proportion of the high quality studies reported a significant inverse association between measures of drinking water magnesium concentrations and cardiovascular mortality than the medium and low quality studies. There was a slightly greater heterogeneity in the proportion of papers reporting a similar association for drinking water calcium by study quality. However, Tables 4 and 5 illustrate that the division of ecological studies by study quality did not disproportionately affect the number of papers considered in this review examining drinking water calcium and magnesium concentrations and cardiovascular disease.

Only two cross-sectional studies examined drinking water calcium and/or magnesium and individual measures of cardiovascular risk factors. Drinking water magnesium was not associated with male or female serum cholesterol (Nerbrand *et al.* 2003, Luoma *et al.* 1973) or blood pressure (Nerbrand *et al.* 2003) in Sweden or Finland respectively. Nerbrand *et al.* (2003) found household concentrations of drinking water calcium to be significantly and inversely associated with female serum cholesterol and systolic blood pressure.

Six of seven case control studies examined drinking water calcium and magnesium and cardiovascular disease. Neither calcium or magnesium were significantly associated with acute myocardial infarction (AMI) in Finland (Luoma *et al.* 1983). Increasing concentrations of drinking water magnesium, but not calcium, were significantly associated with a decreased likelihood of mortality from cerebrovascular disease (Yang *et al.* 1998a) and hypertensive disease (Yang *et al.* 1999b) in Taiwan.

A series of studies conducted by Rubenowitz *et al.* in Sweden examined individual level exposure for drinking water calcium and magnesium and AMI. Increasing magnesium, but not calcium, concentrations were found to be significantly associated with a decreased likelihood of fatal AMI in males (Rubenowitz *et al.* 1996). The opposite pattern was seen for females where increasing calcium, but not magnesium, concentrations were protective (Rubenowitz *et al.*1999). A further case control study examined both males and females and AMI deaths and survivors. This combined study showed no consistent pattern of association for either calcium or magnesium, describing a large but barely statistically significant decrease in the likelihood of AMI deaths with a high magnesium intake.

When individual level drinking water was examined for *survivors* of AMI, only magnesium intake via drinking water was significantly associated with an increased odds of survival. Following adjustment for major cardiovascular risk factors including high blood pressure, smoking and exercise at the individual level (as detailed in Table 8), a magnesium intake of >13mg/day was associated with an increased odds of survival (OR 1.39 (95%CI 1.07-1.80). There was no such association with drinking water calcium.

Only analysis of one cohort examined drinking water magnesium. A lower, but non-significant, drinking water magnesium concentration was described for males dying from CHD during a follow up period of 10 years (Punsar *et al.* 1975) and 15 years (Punsar and Karvonen 1979).

#### 5.6.1.1 Potential problems of statistical analysis

A number of studies simultaneously adjusted for both drinking water calcium and magnesium. Our assessment of study quality showed there to be a high degree of correlation between these two water quality factors in a number of studies. Ferrandiz *et al.* (2004) commented upon the high collinearity introduced to the model with the use of both calcium and magnesium as explanatory factors. The impact of this collinearity is to reduce the significance of the affected variable(s). The two variables are therefore not statistically independent within the model making it impossible to separate the association of calcium and the health outcome from that of magnesium (and vice versa). If a model suffers from collinearity it is therefore not possible to conclude the relative importance of either drinking water calcium or magnesium with the health outcome of interest.

### 5.6.2 Magnesium vs calcium: non-cardiovascular health outcomes

Only one high quality ecological study examined drinking water calcium and magnesium and other health outcomes. Both calcium and magnesium showed a statistically significant protective effect with breast cancer mortality after adjustment for a number of possible confounders (Yang *et al.* 2000a). A cross-sectional study showed drinking water magnesium was associated with a decrease in liver cancer morbidity in males and females, however this study controlled for the age only and did not consider further possible confounders (Tukiendorf and Rybak 2004).

As described in section 4.3.2, a large series of case-control studies were conducted in Taiwan examining drinking water quality and non-cardiovascular health outcomes. Seven studies examined measures of drinking water calcium and/or magnesium. Of these, increasing magnesium, but not calcium, concentrations were significantly associated with a decreased likelihood of mortality from prostate cancer (Yang *et al.* 2000b) and oesophageal cancer (Yang *et al.* 2002a). Conversely calcium, but not magnesium, concentrations were significantly and inversely associated with mortality from cancer of the colon (Yang *et al.* 1997a), rectum (Yang *et al.* 1998d) and stomach (Yang *et al.* 1998c). Furthermore, drinking water calcium concentrations were

significantly associated with an increased likelihood of mortality from renal failure (Hwang *et al.* 2003) but not with the odds of giving birth to very low birth weight babies (Yang *et al.* 2002b).

These case-control studies were controlled for the possible effects of at least age and gender (Yang *et al.* 1998d) with the majority being further controlled for urbanisation and drinking water calcium or magnesium (Yang *et al.* 2000b, 1997a, 2002a, 1998c, Hwang *et al.* 2003). The potential problem of collinearity between drinking water calcium and magnesium (as described in 5.6.1.1) may also affect the results presented here. Yang *et al.* described a high degree of correlation between drinking water calcium and magnesium concentrations (Yang *et al.* 1999b). This may have influenced the results described which consistently illustrated drinking water calcium *or* magnesium as protective against the 5 forms of cancer studied, but with no clear consistent trend as to the relative importance of these drinking water minerals.

A number of studies have therefore shown both drinking water calcium and magnesium to be protective against a number of forms of cancer. However, with the exception of one low quality study (Tukiendorf and Rybak 2004), these results have all been presented by the same study group based in Taiwan. Given this large volume of output, it is not possible to determine how many health outcomes were possibly examined with regard to drinking water quality, i.e. if 'data dredging' had occurred. If this was the case and only significant findings were reported, those findings may be spurious and have occurred through chance alone given the large number of health outcomes that may have been considered.

Although consistent protective effects of increasing drinking water calcium and magnesium have been shown, ecological measures of drinking water parameters were applied in these studies with no consideration of individual level exposure or possible confounding risk factors for the cancers considered. Therefore there is a body of evidence demonstrating a protective effect of both calcium and magnesium upon various forms of cancer, however due regard must be given to the limitations discussed above. Further evidence from different groups in different areas is required before any conclusion can be drawn regarding the possible association between drinking water calcium, magnesium and non-cardiovascular health outcomes.

### 5.6.3 Summary

At present, it is not possible to identify a single nutrient that may be regarded as explaining the water story. This is unsurprising given the complex interrelationships that are known to exist between various nutrients and minerals in physiological functions. It may be more beneficial for future research examining the health effects of specific constituents of drinking water to follow the current trend seen in the consideration of dietary intake and cardiovascular disease, thereby examining combinations of minerals rather than focusing upon singular determinants.

### 5.6.4 Physiological considerations and limitations

It became apparent throughout the course of this review that the physiological justifications for the epidemiological research investigating the water story were based on a small number of repeatedly referenced studies. Wider reading for this topic showed that in the focused field of expertise, many environmental epidemiological studies were considered to have confused epidemiological and pathophysiological data (Durlach 1995).

Serum magnesium concentrations have been used in some studies as an indicator of status, however only severe deficiencies are detectable from this compartment (Rubenowitz *et al.* 1998). Concentrations of drinking water magnesium were not associated with magnesium concentrations in serum (Luoma *et al.* 1973, Nerbrand *et al.* 2003), or muscle or urine (Nerbrand *et al.* 2003). A similar lack of association was also found between drinking water calcium and calcium concentrations within these three compartments (Nerbrand *et al.* 2003).

There is debate regarding the most appropriate compartmental measure of magnesium status as approximately 99% of body magnesium exists as an intracellular ion. Magnesium concentrations of serum and muscle have been investigated with only a weak correlation seen between them (Nerbrand *et al.* 2003). Therefore, to date it is still unclear which measure is most representative of magnesium status. Furthermore a number of epidemiological studies have failed to adequately consider the complex

nature of the biological and homeostatic mechanisms of calcium and magnesium regulation in the body. Serum calcium and magnesium concentrations were used as measures of nutritional status in some studies, however total calcium is a poor measure of status as levels are under tight homeostatic control to maintain concentrations within a narrow limit. Therefore this measure in itself cannot be considered an indicator of calcium status, making comparisons between drinking water calcium concentrations and serum calcium concentrations invalid.

### 5.7 Calcium/magnesium versus trace metals

Although search terms for the trace metals cadmium and lead were included alongside calcium and magnesium, very few studies were returned which examined these trace metals as exposure factors. A total of 5 studies considered cadmium and/or lead, whilst 18 high quality studies specifically examined calcium and/or magnesium.

Two ecological studies considered trace metals as an exposure of interest. Increasing concentrations of lead in drinking water were associated with increased cardiovascular mortality and total mortality in a low quality study (Elwood *et al.* 1974), with no such association found in a high quality study with adjustment for possible confounders (Maheswaran *et al.* 1999).

A case-control study of 135 subjects in Scotland found significantly higher serum lead concentrations in male, but not female, hypertensive individuals compared to age and sex matched normotensives (Beevers *et al.* 1980). This was not explained by differences in weight or smoking habits. Each individual also provided a first run drinking water sample. A significant positive correlation between water lead and serum lead concentrations was found in normal, but not hyptertensive, individuals. A cross-sectional study of 87 males in Birmingham was also conducted by the same study group. No association was detected between blood lead concentrations and blood pressure.

A well controlled cross-sectional study of 246 healthy middle aged males in the US found no significant association between systolic or diastolic blood pressure and individual measures of drinking water exposure to lead, copper or zinc (Sparrow *et al.*

1984). This finding was controlled for age, BMI, educational level and town of residence with no effect seen for smoking or alcohol consumption. A further feature of this study was the collection of two household samples of drinking water: a first use morning sample (representing standing water) and a second sample when the water had run cold (representing mains line water) allowing examination of the possible influence of contact time upon the concentrations of heavy metals. The lack of association remained when the two samples were considered separately and despite the finding that 39% of males had drinking water lead concentrations exceeding the U.S. drinking water limit.

A possible limitation of this analysis is that it was conducted upon a subset of males constituting a larger prospective cohort. The results of this study may be affected by the exclusion of hypertensive males at the formation of the cohort and therefore the possible exclusion of individuals who may be more sensitive to environmental exposures. A more important limiting feature of this study is that the timing of the collection of the water sample did not correspond to that of the blood pressure measurement. Repeated sampling of household drinking water has demonstrated substantial intra-individual variation in exposure to lead. The timing of the sample may therefore be important for adequate exposure assessment (Barry-Ryan *et al.* 2000).

Bierenbaum *et al.* (1975) examined the case of a hard water city with *greater* cardiovascular mortality than a neighbouring city drawing water from the same source but artificially softening it before distribution. This cross-sectional study found a higher concentration of drinking water cadmium in the hard water city in conjunction with significantly increased serum cadmium levels and hypertension. Individuals in this study were matched for age, gender and length of residence, and the findings were not explained by differences in smoking habits (a major potential confounder), social class or weight.

Beevers *et al.* (1980) examined drinking water and serum cadmium concentrations in a similar fashion to that described previously for lead. A total of 70 hypertensive males and females were age and sex matched with 70 normotensives. There was no significant difference in serum cadmium concentrations between these groups and no

correlation between drinking water cadmium (mean 1.28 μg/l (range 0.2-5.9 μg/l)) and blood cadmium concentrations.

Earlier studies attempting to examine drinking water cadmium concentrations may have suffered from analytical limitations in the quantification of concentrations. A recent study found cadmium concentrations to be below detectable limits in 91.9% of samples (Barry-Ryan *et al.* 2000). However as detailed in section 1.3.2, cadmium bioaccumulates within the body therefore chronic exposure to low concentrations may have further health consequences not detected by the epidemiological studies reviewed here.

The current evidence based upon the epidemiological studies reviewed here therefore shows a weight of evidence to support the role of a 'protective' factor in drinking water. However it must be acknowledged that the small numbers of studies examining possible 'harmful' factors may be influenced by publication or reporting bias. Further evidence for the biological effects of cadmium and lead in drinking water comes from other arenas such as animal studies as described in section 1.3.2. Such studies were not within the scope of this review.

### 5.8 Studies of changes in water hardness and health effects

No studies examined the effect of experimental hardening of community drinking water supplies on coronary heart disease. Very few studies have examined changes in cardiovascular mortality subsequent to a permanent change in drinking water hardness. Such instances are also referred to as natural experiments and offer an opportunity to examine, however simplistically, the direction of association of the water story.

Hewitt and Neri (1980) presented a discussion of 2 studies undertaken in Canada to examine domestic water softening and possible health effects. However, the studies and results were not presented in a manner compatible with our study quality assessment as employed for the other epidemiological studies and were therefore not included in the tables of results. Overall, the studies failed to detect any health hazard

or benefit associated with obligate consumption of softened water, however only results for all cause mortality were presented.

The effect of a change in water hardness upon cardiovascular mortality in Great Britain was specifically examined by Crawford *et al*. (1971). County boroughs were selected who experienced a change of at least 50 ppm $CaCO_3$ (50 mg/l $CaCO_3$) in average water hardness over a period of approximately 30 years. Although increases or decreases in water hardness were found in 11 county boroughs, in no area did hardness change from very hard to soft or vice versa. It was found that the significant changes in relative mean death rates between groups was generally consistent with the hypothesis of increasing water hardness being associated with decreased cardiovascular mortality. Death rates for non-cardiovascular mortality showed no trend between groups.

The findings of this study were critically re-assessed and upheld by Lacey and Shaper (1984). Lacey and Shaper conducted a similar study examining comparatively more recent changes in water hardness (1961 to 1971) with a simultaneous consideration of changes in death rates. The authors stated that this approach would therefore be considering possible exposure-outcome responses occurring within one or two years of a change in water hardness and hence not longer time lags. This study utilised the finding of a non-linear association between water hardness and cardiovascular mortality in a previous British study (Pocock *et al.* 1980) to define the extent of change within the water hardness range 0-170 mg/l $CaCO_3$. A total of 14 county boroughs were identified with an effective size of change >20mg/l $CaCO_3$ occurring within this range. Statistical analysis showed an increase of 100 mg/l $CaCO_3$ in the range 0-170 mg/l $CaCO_3$ was associated with a significant decrease of 8.1% in male cardiovascular mortality. No such association was demonstrated for females or non-cardiovascular mortality. This finding remained significant after adjustment for socio-economic factors.

Very few studies have therefore examined the opportunity offered by natural experiments. However, such changes in water hardness over time may be confounded by changes in other cardiovascular risk factors which could be influencing cardiovascular death rates. Natural experiments can not therefore be paralleled to

controlled experiments. However, Lacey and Shaper (1984) found a significant inverse association between changes in water hardness and male cardiovascular mortality after controlling for changes in socio-economic factors. The evidence presented here, though weak, supports the water story.

### 5.9 The relative importance of drinking water: calcium and magnesium intake via water – can it make a difference?

Much of the evidence examining the biological plausibility of an association between calcium and/or magnesium with cardiovascular disease is sourced from animal, experimental and intervention studies. Both calcium and magnesium have been the subjects of a number of recent extensive reviews with regard to cardiovascular disease (Durlach *et al.* 1985, Rylander 1996, Marx and Neutra 1997). Such evidence supporting a biologically plausible role of these minerals will not be repeated here as the purpose of this review was to examine and assess primary studies investigating drinking water quality and health.

Evidence supporting a role of calcium and magnesium in cardiovascular disease therefore comes from a number of epidemiological studies reporting inverse associations between both drinking water calcium and magnesium and cardiovascular disease, together with animal and laboratory studies showing a biologically plausible important role for these minerals in normal heart function. Nevertheless, an important question is whether the amount of calcium and magnesium obtained through drinking water can realistically and critically contribute to body status to an extent that could be reflected in cardiovascular health.

The epidemiological evidence synthesised in this review for assessing the possible relative importance of these two elements in the water story is weak (see section 5.6). Whilst strong evidence from alternative sources has been presented in the literature for a role of both calcium and magnesium with disease, the actual contribution and relevance of the drinking water supply as a route of intake in comparison with other sources (e.g. dietary) remains to be more adequately defined.

The main source of both calcium and magnesium comes from the dietary intake of foods (Nerbrand *et al.* 2003, Rubenowitz-Lundin and Hiscock 2005, Aptel *et al.* 1999). The modern western diet is low in magnesium with modern food processing techniques known to severely decrease magnesium concentrations (Rubenowitz-Lundin and Hiscock 2005). The reference nutrient intake (RNI) defines the amount of a particular nutrient (mineral, vitamin etc) that is at least sufficient for 97% of a population group. RNIs may therefore differ by age and gender. Within the UK, the Department of Health has defined the RNI for magnesium to be 300 and 270 mg/d for males and females respectively. Similarly, the RNI for calcium is 700 mg/d for the genders combined. A wide heterogeneity exists between countries with regard to the definition of RNIs, however the UK values are substantially lower than those specified by other nations including America (calcium >1000 mg/d, magnesium >310 mg/d) and France (calcium 1200 mg/d, magnesium 380 mg/d).

Dietary surveys have shown an insufficient calcium and magnesium intake within both the general population (Galan *et al.* 2002) and the elderly (Aptel *et al.* 1999). A nationally representative dietary survey of British adults (aged 19-64 years) found the mean intake of calcium to be greater than the RNI for males and females at all ages (Ruston *et al.* 2004). However, none of the mean intakes for any of the groups met the US RNI of 1000 mg/d. Dietary magnesium intake in young males and all females did not meet the RNI. Dietary sources of both minerals may therefore be considered to be barely adequate for calcium and inadequate for magnesium provision in the general population. These insufficiencies are accentuated when compared to the higher RNIs applied internationally.

A number of observations support an important role of drinking water in calcium and magnesium intake. The calcium bioavailability of calcium rich waters has been shown to be similar to that of both milk and supplements (Galan *et al.* 2000). Similarly, magnesium sourced from drinking water may be more rapidly and efficiently absorbed than that from the diet (Durlach *et al.* 1985). The form of the mineral in drinking water has been hypothesised to greatly influence the importance of drinking water as a significant source. Hydrated magnesium ions in water may be more bioavailable than the complexed magnesium ions present in food (Durlach *et al.* 1985). An interaction between calcium and magnesium levels in water and those in

food during cooking has also been reported. Haring and Van Delft (1981) showed that the total calcium and magnesium lost from food was lower when cooked in hard water compared to soft water. This implies that a greater proportion of these minerals is leached into soft water during cooking.

A small intervention study demonstrated drinking water as an effective pathway for altering the cardiovascular risk profile of 18 women (Schoppen *et al.* 2004). The study employed a cross-over design with two consecutive periods of two month interventions with consumption of a control water low in minerals (1 litre per day) and a sodium rich carbonated water high in minerals (1 litre per day). This study demonstrated a significant improvement in the atherogenic risk profile with significant decreases in total and LDL cholesterol with increased HDL cholesterol. The authors did not attribute the beneficial effect of consumption of the mineral rich water to any particular constituent but did point out the only small increase in calcium and magnesium compared to the control (calcium 1.7x and magnesium 2.1x control concentrations). Nevertheless, this small intervention study demonstrated that drinking water may be an effective pathway for change in cardiovascular risk.

A four week blinded intervention study was undertaken to examine the effect of water with added magnesium and natural mineral water upon blood pressure (Rylander and Arnaud 2004). A total of 70 males and females (45 to 64 years) identified with borderline hypertension were recruited from an area with a low magnesium concentration in the local drinking water supply. They were allocated to one of three intervention groups to receive water of low magnesium content (*a*), distilled water with supplemented magnesium (*b*), or a highly mineralised water (*c*). Participants consumed at least one litre per day of the provided water for four weeks. Analysis of the whole group showed no significant differences in blood pressure by water intake. Serum and urine measurements of calcium and magnesium over the course of the intervention identified a sub group of individuals with an insufficient body burden of these minerals ($<25^{th}$ percentile). Analysis of this subgroup showed a significant increase in magnesium status following consumption of waters *b* and *c*. There was no effect upon serum magnesium concentrations. Furthermore, this subgroup showed a significant decrease in both systolic and diastolic blood pressure after 2 weeks and 4 weeks of consumption of the highly mineralised water (*c*). This reduction in blood

pressure was detected both in individuals with low initial calcium and those with low initial magnesium. The intervention examining the magnesium supplemented water only (*b*) showed no significant difference in blood pressure. This intervention study therefore confirmed the significant contribution of waterborne minerals to the total body burden. Furthermore, consumption of the highly mineralised drinking water containing high concentrations of calcium (486 mg/l) and magnesium (84 mg/l) (together with other minerals, carbonates and sulphates) was associated with a significant reduction in blood pressure.

The results presented by Rylander and Arnaud (2004) were seen with drinking water concentrations of minerals slightly greater than those seen in public drinking water supplies. However, the possible blood pressure lowering capacity of hard water containing higher mineral concentrations may also contribute to the lack of association between drinking water hardness and coronary heart disease reported by the BRHS cohort study (Morris *et al.* 2001). This study concurrently adjusted for systolic blood pressure when examining town level drinking water hardness and cardiovascular disease. The inclusion of a parameter that may possibly lie on the causal pathway, in addition to the limitations described in section 4.4, would therefore further reduce the ability of the study to detect an association.

A small number of intervention studies have specifically examined the contribution of drinking water magnesium and calcium to total body status. Rubenowitz *et al.* (1998) conducted an oral magnesium loading test to investigate the change in body magnesium status in 12 healthy adults (65-70 years) following a switch from drinking water with a low magnesium content (public supply ~1.6mg/l) to one of higher concentration (25mg/l) for 6 weeks. The higher concentration water was supplied by the study group and was the exclusive water source throughout the study used for all drinking and cooking purposes. The mean magnesium intake via water increased by 1500%, whilst the increase in total magnesium intake (including diet) was 8.4%. Although only a small increase in total intake was seen, measurements of body magnesium status demonstrated a significant increase in status as provided by the enriched drinking water.

A high quality matched case-control study in France examined the contribution of mineral water with differing concentrations of calcium and magnesium to total dietary intake (Galan *et al*. 2002). Four groups of 166 males and females were matched by age, gender, socioprofessional category, education, smoking and geographic area.
They were grouped as regular drinkers (exclusive consumers) of:

1) tap water
2) two low mineralised waters (Ca: 9.9-67.6 mg/l, Mg:1.6-2 mg/l)
3) one moderately mineralised water (Ca: 202 mg/l, Mg: 36 mg/l)
4) one highly mineralised water (Ca: 486 mg/l, Mg: 84 mg/l)

Dietary and mineral water intake were assessed by 24 hour dietary records every two months for one year. Calcium and magnesium intake via food sources did not differ between the four groups. Both the moderately and highly mineralised waters significantly contributed to the total magnesium intake (6-7% and 14-17% for males and females respectively by level of mineralisation). Consumers of the highest mineralised waters had a significantly higher calcium intake than the other 3 groups, with 18-20% of the total intake being derived from the mineral water (compared to 28% from dairy products).

An experimental study in 12 healthy young men found that ingestion of 0.5 litres of water containing 370mg/l of calcium significantly increased calcium status compared with 0.5 litres containing <10mg/l (Guillement *et al.* 2000). Furthermore, the highest absorption efficiency was demonstrated when the total dose ingested was divided up over the day. Therefore a greater absorption of calcium is seen with a frequent intake of small doses rather than one large supplement.

Drinking water at levels detailed by the epidemiological studies reviewed here have therefore been demonstrated to significantly contribute both to the total daily intake and overall body status of calcium and magnesium. This was demonstrated by a well controlled large case-control study (Galan *et al.* 2002) and high quality intervention studies in both healthy elderly (Rubenowitz *et al.* 1998) and young (Guillement *et al.* 2000) subjects. Furthermore, the latter study detailed a detectable biological effect upon calcium status following ingestion of water containing calcium concentrations seen in public drinking water supplies. Two intervention studies also demonstrated

significant improvements in cardiovascular risk factors following consumption of highly mineralised drinking water, with significant blood pressure reductions (Rylander and Arnaud 2004) and beneficial changes in the lipid profile (Schoppen *et al.* 2004). A greater importance of drinking water as a source of calcium and magnesium is supported by the greater absorption efficiency seen with a frequent intake of smaller doses, as would be the case with regular consumption of drinking water throughout the day. Calcium absorption displays marked inter-individual heterogeneity and has been shown to range from 17 to 58% (Wolf *et al.* 2000). Furthermore, calcium absorption capacity decreases with age (Heaney *et al.* 1997). It has therefore been argued that the drinking water route of exposure for calcium and magnesium may be critical for individuals with low dietary intake or absorption capacity but high drinking water calcium and magnesium concentrations.

### 5.9.1 Consumption of tap water in Britain

The intake of calcium and magnesium via drinking water will evidently be dependent upon the concentrations present and the amount of water consumed, with its relative importance being determined by dietary intakes. A survey of tap water consumption in Great Britain was commissioned by The Drinking Water Inspectorate in 1995 (M.E.L Research 1996). A total of 1018 individuals were sampled from 50 local authority areas. Households within these areas were selected to be representative of household type, tenure and ethnicity. This study utilised both questionnaires and a one week diary of liquid consumption to estimate both tap water intake and its fractional importance to the total daily fluid intake. This survey demonstrated that within Great Britain the overall average intake of tap water was 1.4 litres per day. No measure of the variability or range around this figure was given, however the authors discussed a 2.5% sample of the highest consumers as having an average intake of 2.4 litres/d. On average, females had a higher daily intake of tap water than males. A trend of increasing consumption with age was also noted, in conjunction with tap water representing an increasing proportion of total liquid intake. Tap water consumption was highest in the North of Great Britain but there was very little difference by social class. This survey determined that the greatest intake of tap water was via hot drinks, most notably tea (49.2%) and coffee (29.1%), with water straight from the tap constituting only 9.2% of the total intake. Only 1% of households

surveyed used a water softening device. This survey demonstrated an increased summer consumption of tap water therefore representing a seasonality of exposure to drinking water constituents.

Data provided by one of our group (IL) detailed the mean calcium and magnesium concentrations in drinking water by water supply zones covering England and Wales for the year 2003. This allowed us to look at the means and ranges of drinking water concentrations of these minerals. The mean and range of drinking water calcium concentration for England and Wales was 70.2 (0.97-205) mg/l, with the corresponding values for magnesium being 6.5 (0.48-51.1) mg/l.

Using the results of the 1995 survey described above (M.E.L. Research 1996), a crude estimation of the potential contribution of drinking water to the daily RNI of calcium and magnesium is presented in Table 11.

**Table 11 The means and ranges of drinking water calcium and magnesium in England and Wales, with 2 estimates of tap water consumption and potential contribution to the RNI of each mineral**

| | | Mean (mg/l) | Range (mg/l) | As % of RNI | |
|---|---|---|---|---|---|
| **Drinking water calcium** | | 70.2 | 0.97 – 205 | 700mg/d | |
| Average intake | (1.4 l/d) | 98.3 | 1.36 – 287 | 14% | 0.2% - 41% |
| High intake | (2.4 l/d) | 168.5 | 2.33 – 495 | 24% | 0.3% - 71% |
| **Drinking water magnesium** | | 6.5 | 0.48 – 51.1 | 300mg/d* | |
| Average intake | (1.4 l/d) | 9.1 | 0.67 – 71.5 | 3% | 0.2% - 24% |
| High intake | (2.4 l/d) | 15.6 | 1.15 – 122.6 | 5% | 0.4% - 41% |

* using the RNI for males (RNI for females = 270 mg/d)

Table 11 shows a wide potential for drinking water as a source of both calcium and magnesium contributing to the daily intake in England and Wales. However, this table is intended for illustrative purposes only and it is fully acknowledged that a number of other factors contribute to the amount actually absorbed at the individual level.

## 5.10 Potential harm and problems

This review has presented a number of studies supporting an inverse association between water hardness and cardiovascular disease. A natural progression from this finding is the consideration of the hardening of soft water supplies. This section will assess the evidence for potential harm and problems associated with an increase in water hardness as determined by the studies detailed within this review.

Schroeder (1960b) found a significant correlation of increasing death rates from congenital malformations with increasing water hardness. However, this result was unadjusted for age, sex and other factors, and was only one of a number of health outcomes correlated with water hardness, thereby increasing the probability of a spurious association. A more recent study controlling for numerous potential confounding factors found no association between hardness and the occurrence of late adverse pregnancy outcomes in the US (Aschengrau *et al.* 1993).

Enriching water with calcium and magnesium could increase the corrosivity of the supplied water (Durlach *et al.* 1985, see also 1.3.2). Any such change could adversely impact areas with older distribution systems and may increase exposures to heavy metals through leaching. Such problems were considered by Durlach *et al.* (1985) who recommended the 'neutralisation of corrosive water by filtration on calibrated grains of earth-alkaline metals to ensure the highest possible Mg/Ca ratio with the best anticorrosive power.' The feasibility and relevance of this recommendation must be evaluated by experts in such technology with due regard given to the findings of this review.

Non-health effects of the hardening of mains water supplies must also be considered. Increased water hardness leads to an increase in scale deposits upon evaporation and heating. This could have both domestic and industrial implications. Consideration of the economic costs of such hardening programmes and costs associated with scaling would need to be evaluated. Public resistance to such changes in water quality, possibly analogous to that seen for water fluoridation, would also require consideration.

Although the possibility of adverse health outcomes with a programme of water hardening, together with public resistance and the economic costs of hardening and its consequences are essential considerations, the focus of this review was to assess the evidence of a relationship between cardiovascular disease and water hardness. A comparative risk-benefit analysis is therefore beyond the scope of this study.

## 6 Evaluation of findings – the Bradford Hill guide

A commonly employed set of guidelines for the evaluation of epidemiological studies was described by Bradford Hill (Bradford Hill 1965). This comprises nine different factors intended to aid in assessing the probability that an association is *causal* in nature. These factors were applied to the evidence put forward by the papers contained in this review to assess the likelihood that the inverse association between drinking water hardness (and calcium and magnesium) and cardiovascular disease is causal. However, it must be stressed that whilst these criteria are commonly utilised for such purposes of appraisal, they are not definitive.

**Consistency** – This questions whether the findings are replicated by other studies in different populations. Similar results emerging from several studies conducted in different groups reduces the probability that the observed association is coincidental. Sharrett (1981) commented that there was a tendency for studies to have been repeated in the areas where an association had already been found, thereby creating the impression of numerous findings and that the association had been confirmed. However, the assessment of the epidemiological studies by several factors presented in section 5 showed that an inverse association between drinking water quality and cardiovascular disease was found in a number of countries for males and females, and both younger and older population groups. A smaller number of studies failed to detect such an association. The possibility of publication bias cannot be excluded.

**Specificity** – this criterion requires a single cause to produce a single effect, as coincidental associations tend to be non-specific and show associations with many broad disease types. However the rigidity this imposes upon the assessment of environmental exposures and health has been questioned. For example, smoking is a

known major risk factor for lung cancer, however it is also associated with a number of other diseases including cardiovascular disease.

A number of authors examined the specificity of the association between water hardness and health outcome and found a significant association with non-cardiovascular causes of death (Elwood *et al.* 1974, Neri *et al.* 1972). However, these studies investigated possible exposure-effect outcomes for multiple disease categories and hence are weakened due to multiple hypothesis testing. Pocock *et al.* (1980) found a significant inverse association with water hardness to be present for cardiovascular, but not non-cardiovascular, deaths. The percentage change in death rate following a change in drinking water hardness was found to be specific to cardiovascular mortality (Lacey and Shaper 1984). However, Comstock *et al.* (1980) concluded that non-cardiovascular deaths were as likely to be associated with soft water as were arteriosclerotic deaths.

More recent studies as evidenced through the cross-sectional (Table 7) and case-control studies (Tables 8 and 9) have demonstrated an association between water hardness and its compositional characteristics and both cardiovascular disease and cancer of the breast, colon and prostate. These significant non-cardiovascular findings illustrating a possibly wider health protection effect of hard water do not invalidate this criterion of specificity of association. Rather, it may be suggestive of a common underlying homeostatic mineral imbalance manifested in chronic health effects.

**Strength of association** – this considers the fact that weak associations are more likely to be explained by undetected biases, whilst strong associations may be more robust. Of the ecological studies demonstrating a significant association, cardiovascular mortality was estimated to be lower in hard water areas by 10% (Kaipio *et al.* 2004, Marque *et al.* 2003, Yang *et al.* 1996) or up to 15% (Pocock *et al.* 1980) than soft water areas. The case control studies (Table 8) presented odds ratios showing a decreased likelihood of cardiovascular mortality with increasing hardness, calcium and/or magnesium in the order of 35 to 40% (Rubenowitz *et al.* 1996, 1999 and Yang *et al.* 1998a, 1999b). The individual level studies therefore demonstrated a greater strength of association than the high quality ecological studies. However, the

overall strength of association between water quality and cardiovascular disease is weaker than that seen for major cardiovascular risk factors such as smoking. Nevertheless, this criterion does not exclude the possibility that a weak association may be causal.

**Biological gradient** – this criterion assesses the presence of a dose-response relationship whereby greater health effects are seen at higher/lower levels of exposure. Absence of a dose-response effect does not discount causality. Trends of decreasing cardiovascular mortality with increasing drinking water hardness, calcium and/or magnesium have been demonstrated by high quality ecological studies classifying categories by percentiles (Kaipio *et al.* 2004, Yang *et al.* 1996) and case control studies (Rubenowitz *et al.* 1996, 1999 and Yang *et al.* 1998a, 1999b). Conversely, Pocock *et al.* (1980) presented a non-linear association between water hardness and cardiovascular mortality suggestive of a threshold effect.

The categories of drinking water quality used in those studies included percentiles and categories of hardness that have been generated from studies of physical effects such as scaling. Such categorisations are not based upon observations of differential health effects at different concentrations. Therefore these definitions of water hardness may not correspond to those at which health effects may be detected. Such classifications may reduce both the ability of the study to detect an association of a dose-response trend if they cut across a health related threshold.

**Biological plausibility** – this criterion demands a wider assessment of the biological rationale linking an exposure and effect. Biological plausibility is an important criterion for assessment of causality however it is also constrained by the state of current knowledge. Section 1.3 discussed the biological plausibility of the association for water hardness through the possible protective effects of calcium and/or magnesium, or the harmful effects of cadmium or lead. The evidence assessed by this review supports a protective role of calcium and magnesium as described in section 5.5 where significant inverse associations between calcium and magnesium and cardiovascular risk factors were described. However these findings were inconsistent and based on a small number of studies.

Whilst biologically plausible mechanisms of association have been discussed in great depth by a number of authors, constraints of current knowledge with regard to calcium and magnesium and measures of physiological status and effects (5.6.4) must be properly acknowledged.

**Temporality** – this criterion examines the sequence of events, i.e. that exposure must precede effect. The high quality ecological study of change in water hardness and changes in cardiovascular mortality supports a temporal association of cause and effect (Lacey and Shaper 1984). However, as described in section 2.2 only cohort studies are able to examine the temporality of association. Three prospective cohorts were included within this review. No significant association was found between water hardness and cardiovascular mortality (Morris *et al.* 2001, Comstock *et al.* 1979, 1980 and Punsar *et al.* 1975, Punsar and Karvonen 1979). Only Morris *et al.* (2001) and Comstock *et al.* (1979, 1980) controlled for individual level risk factors. However, both studies applied an ecological measure of drinking water hardness with only Comstock *et al.* utilising individual level samples to inform the aggregated exposure measure.

**Experimental evidence** – the strongest support for a causal association is given through experimental evidence whereby the introduction or removal of the hypothesised exposure leads to a detectable change in health outcomes. Whilst this form of evidence may be considered the most powerful in the search for causality, in practice it is often extremely difficult or impossible to obtain in studies of environmental epidemiology. Studies of the health effects hypothesised to be consequent to changes in water hardness have so far been limited to natural experiments. Changes in drinking water hardness at the county borough level in England supported a protective effect of increased water hardness against cardiovascular mortality in studies of both low (Crawford *et al.* 1971) and high quality (Lacey and Shaper 1984).

**Coherence** – this indicates that the association should not conflict with the current state of knowledge regarding the health outcome, i.e. its natural history, biology etc. The hypothesised association between water hardness and cardiovascular disease therefore meets this criterion.

**Analogy** – this utilises previous experience to support causality. An example is the role of sodium chloride in the aetiology of hypertension. It may be possible that other minerals, either in water or diet, may have a role in hypertension or other vascular diseases. Analogy is probably the weakest of the criteria, and can only suggest the possibility of a causal role for an exposure rather than provide proof.

A common example used to illustrate this criterion is the example of thalidomide. Since it is known that this drug induces congenital abnormalities, it can be accepted that other drugs may have similar consequences. This criterion will be more applicable in studies of pharmacological drugs and the chemical structure of environmental contaminants than to the hypothesis being considered here.

**Summary**

This evaluation of the study findings using the Bradford Hill guidelines showed there was sufficient evidence to support the criterion of consistency. However, the evidence, although present, was weak for the other criteria. Nevertheless evidence has been presented throughout this review supporting the possibility of a causal association. However a number of caveats have been discussed both here and within the more detailed section specific evaluations of the evidence. This assessment highlights that although there is a large body of literature examining the association between drinking water and cardiovascular health, there has been a lack of focus upon examination of key issues such as temporality and investigation of a possible dose-response relationship on scales of health effects. Epidemiological studies to date are therefore deficient in their ability to contribute to an assessment of causality. Nevertheless, this large body of evidence has yielded a number of consistent findings (with exceptions), therefore the probability of a non-causal association is reduced. However more focused research evaluating individual measures of exposure and health effects is required.

## 7 Summary of findings by Research Objectives

This section provides a summary of our conclusions by the stated research objectives.

1. *Review and critically assess the merits of available studies concerning the health effects of soft and softened water and to catagorise the identified studies based on relevant features such as size, study design, conclusions, etc.*

This objective was fulfilled throughout sections 4 and 5.

2. *Advise on the evidence relating to the incidence of cardiovascular disease, or other adverse health effects to consumption of soft or softened water and to comment on the reliability and strength of any reported associations.*

Sections 4 and 5 presented a number of high quality studies supporting an inverse association between calcium and magnesium and cardiovascular disease. A number of case-control studies further demonstrated a protective effect of these drinking water parameters against various forms of cancer. This review has discussed in detail the limitations in the interpretation of these studies. Chiefly, very few studies were conducted at the individual level, with even fewer of these examining measures of individual exposure to drinking water factors. Furthermore, evidence of a protective effect of water hardness, calcium and magnesium against a number of cancers is derived from a series of studies conducted by one group in Taiwan. A wider evidence base is therefore required before sound conclusions can be drawn regarding any potential health risk of soft water with respect to cancer. Nevertheless, more recent studies of cardiovascular health and drinking water have yielded evidence to support a cardioprotective effect of hard drinking water.

*3. Advise whether the studies indicate a "no effect" level in relation to water composition and whether a causal or protective agent relating to incidence of illness is evident.*

The evidence presented in this review does not support the concept of a 'no effect' level. Pocock *et al.* (1980) described a non-linear association between water hardness

and cardiovascular mortality in Great Britain whereby the inverse association was no longer observed with increasing water hardness beyond 170 mg/l $CaCO_3$. This non-linearity was not observed in other studies of water hardness and cardiovascular mortality in Britain (Catling 2004). This review found the protective effect of increasing water hardness, calcium and magnesium for cardiovascular disease and cancer to be evident across a range of concentrations.

The small number of studies examining the hypothesised harmful factors, cadmium and lead, did not yield consistent results to support a detrimental effect of soft water. However, this lack of evidence does not exclude the possibility of a causal association between these factors with chronic exposure. The lack of evidence and results in the literature can not be interpreted as evidence of no effect. Nevertheless, the weight of evidence supports a cardioprotective effect of increasing concentrations of water hardness, calcium and magnesium.

*4. To comment on whether the results indicate the need to consider re-hardening of softened water, or whether supplies of naturally soft water should be hardened and provide an indication of the scale of the health benefits of such action.*

Some studies have attempted to quantify predicted improvements in population cardiovascular health outcomes with increases in water hardness. Marier and Neri (1985) synthesised the results of earlier epidemiological studies and estimated an increase in drinking water magnesium concentrations of 6 mg/l would decrease mortality from IHD by ~10%. Rubenowitz *et al.* performed a similar calculation based on the results of her series of well designed case-control studies in Sweden. If all males were to consume drinking water with a magnesium concentration of ≥ 9.8 mg/l, she predicted a decrease in AMI mortality of ~19% (Rubenowitz-Lundin and Hiscock 2005). The corresponding calculation for females showed a 25% decrease in AMI mortality with consumption of ≥ 9.9mg/l.

As yet, no studies have been undertaken to examine the effects of an artificial hardening of drinking water. Assuming a causal association between drinking water hardness and cardiovascular health (which has not yet been proven), this review found no evidence for harm, but a significant potential for benefit with increasing drinking

water hardness. Any changes in drinking water hardness should be implemented within the setting of a carefully designed epidemiological study framework to yield the best possible evidence examining the association between hard water and human health.

*5. To investigate the scale of any proposed interventions by investigating the distribution of water hardness across England and Wales and the number of individuals consuming water at different levels of water hardness.*

Figures 3, 4 and 5 illustrate the drinking water concentrations of water hardness, calcium and magnesium respectively for the cumulative population of England and Wales. They were compiled using water supply zone data for the year 2003. The threshold of effect described by Pocock *et al.* (1980) (170 mg/l $CaCO_3$) is delineated in Figure 3. It can be seen that the drinking water supply of nearly 20 million individuals is below this figure. Rubenowitz *et al.* (2000) found a significant increase in the likelihood of surviving an AMI with consumption of drinking water magnesium exceeding 13 mg/l. This is illustrated in Figure 5 and shows a cumulative total of approximately 47 million individuals are supplied with drinking water below this concentration. Applying the values presented in Research Objective 4, the total population decreases slightly to 40 million. Whilst these figures are purely illustrative and do not consider age or gender distributions, they illustrate that a large proportion of the population of England and Wales may derive a cardioprotective effect from increases in the mineral concentrations in drinking water. Given the substantial morbidity and mortality burden of cardiovascular disease in Great Britain, a 10-20% reduction in mortality attributable to an increase in drinking water hardness translates into a substantial public health effect.

*6. To comment on the evidence relating to specific postulated causal (lead, cadmium) or protective (calcium, magnesium) elements and their importance with respect to cardiovascular disease*

To date, there is still insufficient evidence to assess the relative importance of calcium and magnesium with regard to the water story and cardiovascular disease. Supporting evidence for both minerals has been summarised from a wide range of studies

including supplementation, clinical and autopsies. An important conclusion of this review is that it is not possible to differentiate the effects of calcium from magnesium through the study designs examined within this review.

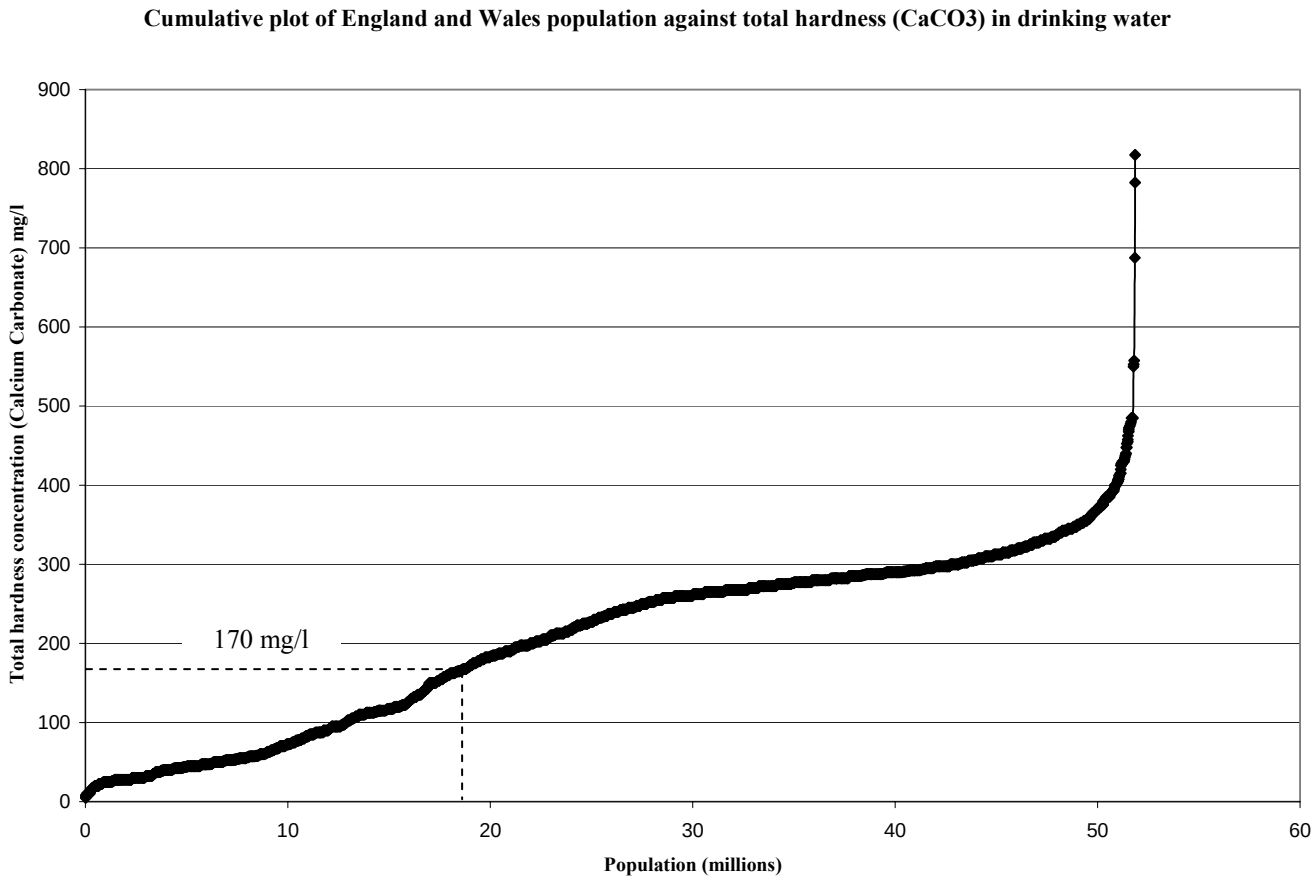


**Figure 3 Cumulative population of England and Wales against drinking water hardness concentrations ($CaCO_3$ mg/l)**

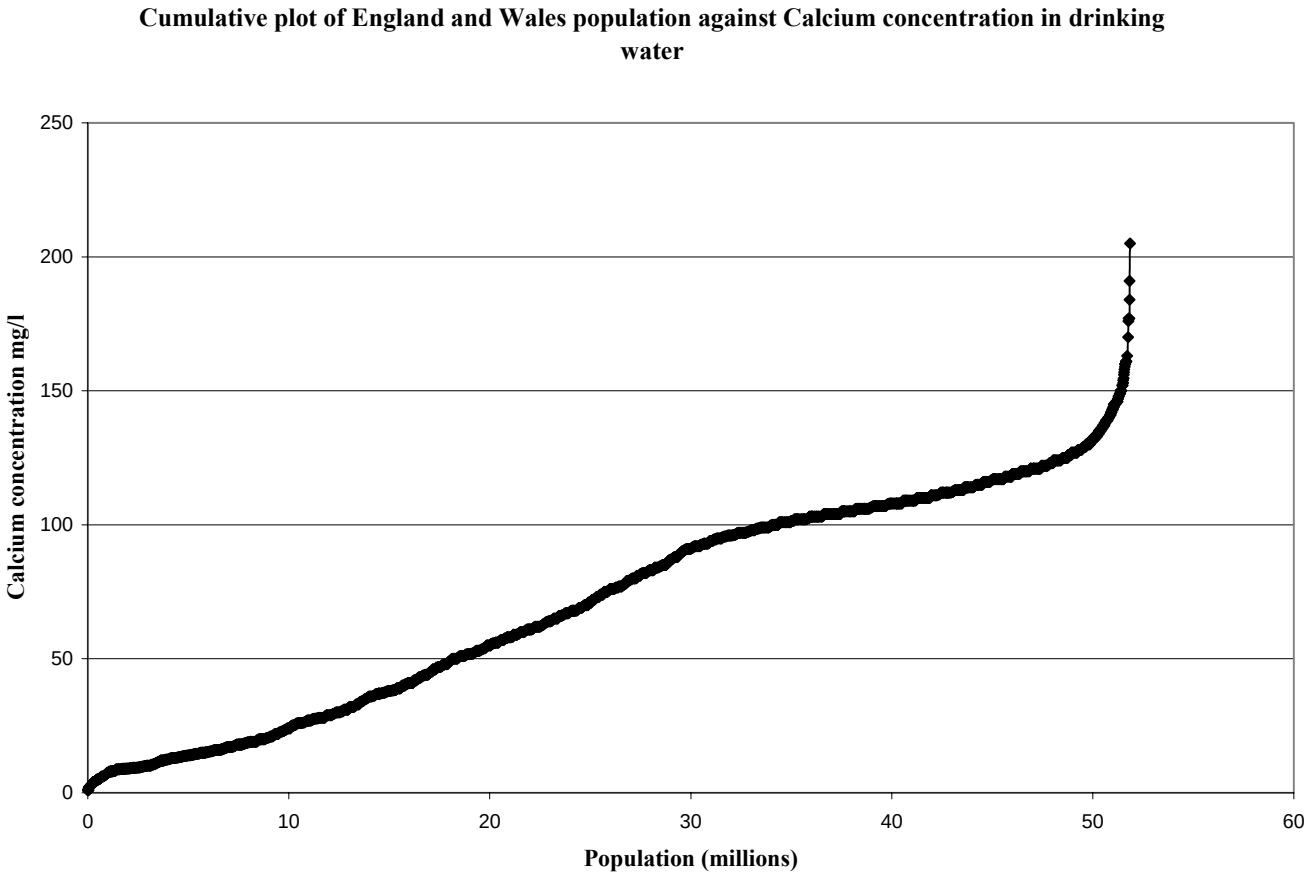


**Figure 4 Cumulative population of England and Wales against drinking water calcium concentrations (mg/l)**

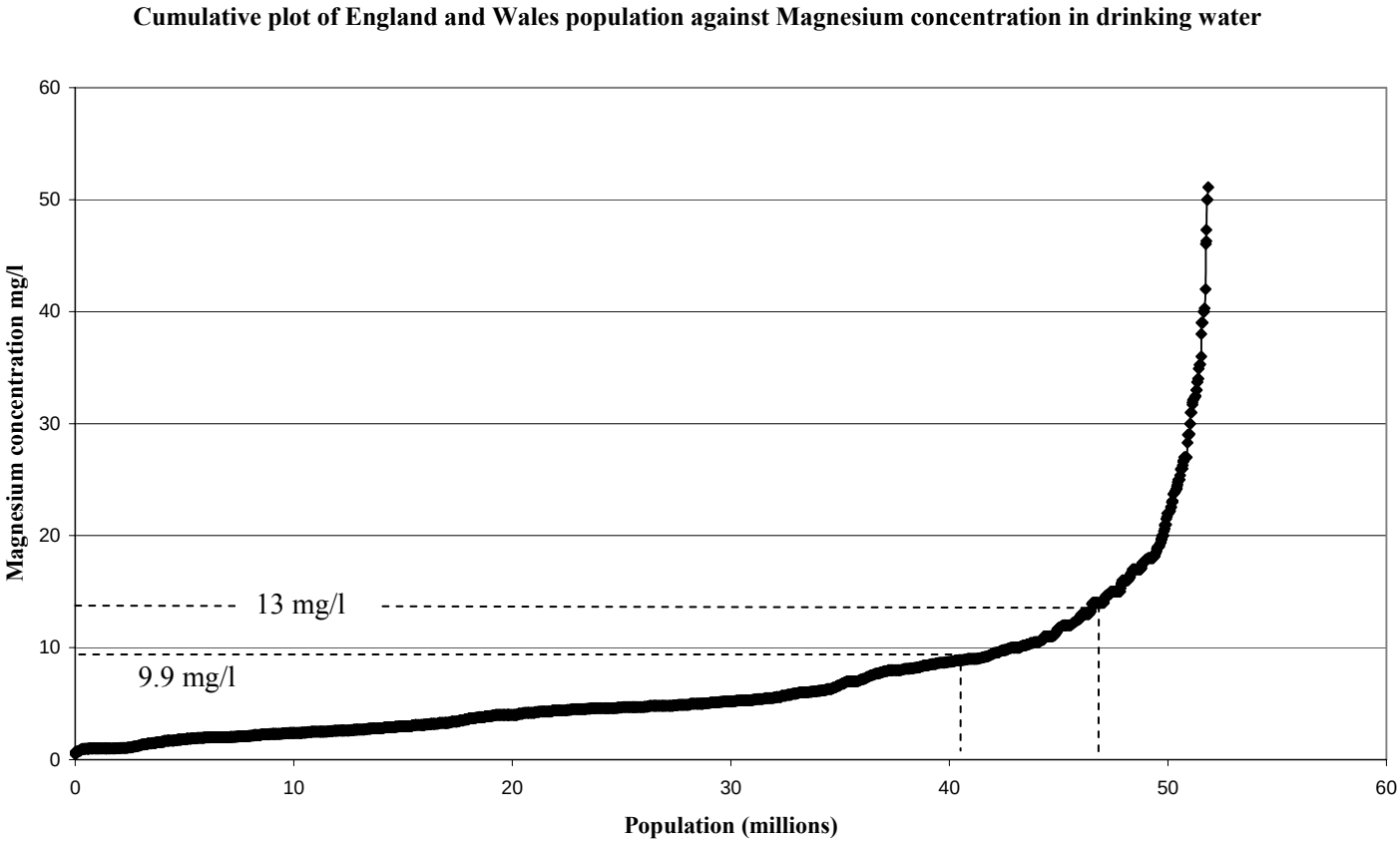


**Figure 5 Cumulative population of England and Wales against drinking water magnesium concentrations (mg/l)**

## 8 Summary of general quality issues and concluding remarks

The limitations of the ecological study design and the ecological fallacy have previously been described. A further limitation is the *modifiable areal unit problem*. This is potential source of error due to the level of aggregation of the data to be analysed. The aggregation of areas will smooth out random variation, however it also increases the heterogeneity of the areal unit making it more difficult to detect an association. A significant association may therefore be found at one level of aggregation, but may not hold at another.

*Migration* may also influence the estimation of effect, as migration into or out of the base population can cause a selection bias if a differential trend occurs. Each ecological study was assessed for the consideration of migration. Only 4 of the 12 high quality studies had recognised and discussed the potential influence of migration upon the estimates derived. Migration may also affect the results of the individual level studies. The latency period of the hypothesised association between water hardness and cardiovascular disease is unknown. Therefore consideration of individuals who may have been exposed to the drinking water parameters for varying lengths of time may affect the estimate of association if they had not been exposed for a length of time sufficient for a health effect to manifest. The majority of individual level studies considered this in their analyses and examined only those who had been resident for a number of years (for example Stitt *et al.* 1973, Punsar and Karvonen 1979, Nerbrand *et al.* 2003).

A number of studies presented only the correlation coefficient to summarise the association between exposure and effect. A major limitation of this measure is that it is dimensionless and therefore gives no indication of the magnitude of the relationship. The correlation coefficient is also affected by sample size and therefore statistically significant associations yielded by a large sample size may not actually be of any notable magnitude or biological relevance.

Section 3.1 described the treatment of foreign language papers for the purposes of this review. The possible implications of the exclusion of the six identified as primary

data studies will be considered here. Four of the six papers were ecological studies, therefore it is not anticipated that their inclusion would substantially affect the conclusions of this review given the large number of English language ecological studies considered. One German study examined drinking water hardness, calcium and magnesium and the incidence of fatal and non-fatal AMI. This study was conducted within the international framework of the WHO myocardial infarction registry network. The contribution of this study may therefore be regarded as considered through the report of Masironi *et al.* (1979). However, the short abstract of an Italian study stated that a greater relative increase in cardiovascular mortality had occurred in a community that had experienced a large reduction in drinking water hardness and calcium and magnesium concentrations (Menotti and Signoretti 1979). This paper was considered to be potentially important given the paucity of studies examining the changes in health outcomes following such changes in drinking water composition. A colleague was able to translate this paper for us. Application of our study quality criteria defined this paper as an ecological study of low quality. Although this study supported a protective role for drinking water hardness against cardiovascular mortality, its inclusion would not have altered any conclusions of this review. This study remained excluded in accordance with the treatment of all other foreign language papers.

A key limitation of all the studies examined within this review is the characterisation of the exposure variable, drinking water hardness, calcium and/or magnesium. The vast majority of individual level studies, including the cohort studies, applied an ecological measure of drinking water quality to the individual level data in the analysis of exposure and outcome. This introduces an ecological bias as the exposure value assigned to the individual does not necessarily reflect that individual's true exposure. A possible consequence of this is a bias towards the null hypothesis, i.e. an increased likelihood of concluding that there is no significant association.

Although this potential bias was avoided in those studies examining both exposure and effect at the individual level, they will still be subject to a degree of measurement error. Gillies and Paulin (1983) specifically examined two common assumptions made by the epidemiological studies reviewed here: 1) people consume approximately the same amount of water, and 2) composition of drinking water at the sampling

points is the same as that actually consumed. The study design attempted to ensure that all samples were obtained on a day when all water consumption was from household supply, and was identical both quantitatively (e.g. sample for each cup of tea consumed) and qualitatively (e.g. if boiled) to amounts actually consumed. They also compared these samples to those taken from the source of supply and straight from the tap. A total of 109 men and women were examined showing a mean intake of 1.25±0.39 litres/day (range 0.26-2.8 litres). Gillies and Paulin demonstrated that the water composition at source can vary markedly from that actually consumed by the household. These differences were observed for both calcium and magnesium. The composition of boiled and non-boiled drinking water was not examined separately, however Gillies and Paulin emphasise that the effect of boiling upon mineral composition is especially important when considering hardness, calcium or magnesium as the exposure factor. A proportion of these parameters will be precipitated out forming insoluble scale upon heating. This comment is especially pertinent in Great Britain as a survey of tap water consumption found that 49.2% of all tap water consumed is in the form of tea, with 29.1 % as coffee. Only 9.2% of all tap water consumption was straight from the tap (M.E.L. Research 1996).

A further source of measurement error is attributable to the wide inter-individual variation in absorption capacity for calcium and magnesium. The measure of drinking water parameters will therefore not equate to the biologically effective dose in each individual.

We have endeavoured to base our assessment on high quality studies thereby aiming to increase the validity of our conclusions. The comparatively small reduction in cardiovascular risk seen with increasing water hardness, calcium and magnesium may translate to a substantial public health benefit at the population level. Furthermore, the accessibility of any drinking water intervention at the population level may bring widespread benefit when compared to individual level resistance at attempts to modify behavioural risk factors such as smoking and obesity. Moreover, risk factors that are easily modifiable at the population level have the potential to yield more substantial benefits with regard to cardiovascular health. A significant finding of the survey of tap water consumption in England and Wales was that intake was greatest in the population groups and areas at highest risk for cardiovascular disease (M.E.L.

Research 1996). The survey showed that tap water intake was higher in the northern region. This corresponds to the areas of softest water (Figure 2) and highest cardiovascular mortality (Figure 1) and therefore represents a greater potential for possible health benefits through drinking water hardness modification. A further observation was that consumption of drinking water increased with age. Both incidence of and mortality from cardiovascular disease increase with age, meaning exposure to any possible cardioprotective effect of drinking water calcium and magnesium could become more pivotal with age.

This review of studies examining drinking water hardness and human health has demonstrated a potential cardiovascular benefit of increasing levels of hardness. The limitations of this evidence have been acknowledged and discussed, however in the absence of harm, the cost effectiveness of any intervention strategy to harden public drinking water supplies becomes an important issue.

A major challenge for future studies attempting to establish a causal association between drinking water parameters and cardiovascular health is the accurate measurement of individual exposure and the biologically relevant dose. The current insufficiency of individual level exposure data in the majority of studies reviewed here has led to probable exposure mismeasurement and bias in the estimation of effect, with the possible loss of precision and power in statistical analysis. Increasingly sophisticated techniques of statistical evaluation and the greater use of GIS for the generation of spatially smoothed exposure variables have not ameliorated the fundamental deficiencies of the ecological design. The study design employed by the BRHS examining geographical variations in cardiovascular disease in Great Britain would form a good basis for future investigations. Utilising this framework but incorporating individual level measures of both drinking water quality and consumption, with a larger sample size integrating a wide range of exposures, would yield strong evidence in the consideration of the water story. More focused research at the individual level is required to address the deficiencies of the studies to date, and to facilitate a more appropriate assessment of a causal association between drinking water quality and cardiovascular disease.

## Appendix 1 – Database specific search strategies

This Appendix details the specific search strategies employed for each electronic database to identify articles relevant to this review. The search strategy used for the MedLine database was detailed in section 2 and formed the basis of those used for the remaining databases. MeSH terms were checked for relevance in each database and were altered accordingly when found to differ. For databases not supporting the MeSH heading format, search words were entered as both text words and key words to maximise coverage. The database specific search strategies are presented in the following tables.

**EMBASE (1980 to 2005 Week 02)**

Scope: The Excerpta Medica database (EMBASE) is a major biomedical and pharmaceutical database indexing over 3,500 international journals. EMBASE is one of the most widely used biomedical and pharmaceutical databases.

| | | Hits |
|---|---|---|
| 1 | Water/ or Deuterium Oxide/ or Drinking Water/ or Fresh Water/ or Ground Water/ or Mineral Water/ or Surface Water/ or Tap Water/ | 63907 |
| 2 | Water Quality/ | 13656 |
| 3 | Water.tw. | 213471 |
| 4 | hardness.tw. | 2200 |
| 5 | soft.mp. | 53229 |
| 6 | softened.mp. | 189 |
| 7 | Water Hardness/ | 488 |
| 8 | hydrotimetry.af. | 0 |
| 9 | Magnesium/ or Magnesium Carbonate/ or Magnesium Hydroxide/ or Magnesium Ion/ or Magnesium Blood Level/ or Magnesium Chloride/ | 29846 |
| 10 | Calcium Chloride/ or Calcium/ or Calcium Carbonate/ or Calcium Hydroxide/ or Calcium Absorption/ or Calcium Blood Level/ or Calcium Ion/ or Calcium Intake/ | 127207 |
| 11 | Lead/ or Lead Blood Level/ | 21583 |
| 12 | Cadmium Chloride/ or Cadmium/ | 22341 |
| 13 | (or/1-3) and (or/4-12) | 13030 |
| 14 | Cardiovascular Disease/ | 40630 |
| 15 | Coronary Artery Disease/ | 34908 |
| 16 | Coronary Artery Atherosclerosis/ | 5105 |
| 17 | Heart Disease/ or Ischemic Heart Disease/ | 60303 |
| 18 | Cerebrovascular Disease/ | 11015 |
| 19 | Hypertension/ or Essential Hypertension/ or Systolic Hypertension/ | 133247 |
| 20 | Blood Pressure/ | 66376 |
| 21 | Colon Cancer/ | 14381 |
| 22 | Esophagus Cancer/ | 7279 |
| 23 | "general aspects of disease"/ or disease association/ or environmental disease/ or pathophysiology/ | 172799 |
| 24 | exp Public Health/ or exp Health/ or exp Health Status/ | 77478 |
| 25 | (or/14-24) | 542221 |
| **26** | **13 and 25** | **531** |

**CINAHL (1982 – December Week 2 2004)**

Scope: The Cumulative Index to Nursing & Allied Health (CINAHL) database provides authoritative coverage of the literature related to nursing and allied health.

| | | **Hits** |
|---|---|---|
| 1 | Water/ | 918 |
| 2 | Water Supply/ or Water/ | 1691 |
| 3 | water.tw. | 3853 |
| 4 | hardness.af. | 137 |
| 5 | hydrotimetry.af. | 0 |
| 6 | soft.mp. | 2771 |
| 7 | softened.mp. | 17 |
| 8 | exp Magnesium/ or exp Magnesium Compounds/ | 651 |
| 9 | exp Calcium/ or exp Calcium Compounds/ | 2321 |
| 10 | Lead/ | 258 |
| 11 | Cadmium/ | 36 |
| 12 | (or/1-3) and (or/4-11) | 112 |
| 13 | Cardiovascular Diseases/ | 4188 |
| 14 | Heart Diseases/ or exp Coronary Disease/ | 14357 |
| 15 | Myocardial Ischemia/ or Arteriosclerosis/ | 2340 |
| 16 | Heart Diseases/ | 2013 |
| 17 | Cerebrovascular Disorders/ | 501 |
| 18 | Hypertension/ | 5730 |
| 19 | Blood Pressure/ | 2497 |
| 20 | Colonic Neoplasms/ | 839 |
| 21 | Esophageal Neoplasms/ | 323 |
| 22 | Disease/ | 1197 |
| 23 | (or/13-22) | 27937 |
| **24** | **12 and 23** | **3** |

**Web of Science** (searched 09/12/2004)

Scope: Incorporating the Science Citation expanded index, the Social Sciences expanded index and the Arts and Humanities expanded index.

| | | Hits |
|---|---|---|
| 1 | TS=(water) or TI=(water) | >100,000 |
| 2 | TS=(hardness) or TI=(hardness) | 29,316 |
| 3 | TS=(soft) or TS=(softened) or TI=(soft) or TI=(softened) | >100,000 |
| 4 | TS=(hydrotimetry) or TI=(hydrotimetry) | 0 |
| 5 | TS=(magnesium) or TI=(magnesium) | 63,937 |
| 6 | TS=(calcium) or TI=(calcium) | >100,000 |
| 7 | TS=(lead) or TI=(lead) | >100,000 |
| 8 | TS=(cadmium) OR TI=(cadmium) | 50,707 |
| 9 | #1 AND (#2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8) | 7,556 |
| 10 | TS=(cardiovascular disease) or TI=(cardiovascular) | 66,821 |
| 11 | TS=(coronary disease) or TI=(coronary) | >100,000 |
| 12 | TS=(coronary arteriosclerosis) OR TI=(arteriosclerosis) OR TI=(atherosclerosis) | 20,289 |
| 13 | TS=(heart disease) or TI=(heart disease) | 83,940 |
| 14 | TS=(cerebrovascular disease) OR TI=(cerebrovascular) | 11,573 |
| 15 | TS=(hypertension) or TI=(hypertension) | >100,000 |
| 16 | TS=(blood pressure) or TI=(blood pressure) | >100,000 |
| 17 | TS=(colonic neoplasm) or TI=(colonic neoplasm) or TI=(colon cancer) | 6,210 |
| 18 | TS=(esophageal neoplasm) or TI=(esophageal neoplasm) or TI=(esophageal cancer) | 2,729 |
| 19 | TS=(disease) or TI=(disease) | >100,000 |
| 20 | #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 | >100,000 |
| **21** | **#9 AND #20** | **137** |

Web of Science field tags:

TS = Topic
TI = Title
AU = Author
SO = Source
AD = Address

**PUBMED** (searched 10/12/2004)

Scope: PubMed provides access to bibliographic information that includes MedLine, OldMedLine, as well as out-of-scope citations from certain MedLine journals, citations that precede the date that a journal was selected for MedLine indexing and some additional life science journals that submit full text to PubMedCentral and receive a qualitative review by the National Library of Medicine.

Note: the individual numbers of hits is not returned for each search term.

"Water" [MeSH Terms] OR Water [Text word] **AND**

| | |
|---|---|
| | ("Hardness" [MeSH Terms] *OR* Hardness (.tw)) |
| OR | Soft (all fields) |
| OR | Hydrotimetry (.tw) |
| OR | Softened (all fields) |
| OR | "Magnesium" (MeSH) |
| OR | "Calcium" (MeSH) |
| OR | "Lead" (Mesh) |
| OR | "Cadmium" (MeSH) |

**AND**

| | |
|---|---|
| | "cardiovascular diseases" [MeSH Terms] |
| OR | "coronary disease" [MeSH Terms] |
| OR | "coronary arteriosclerosis" [MeSH Terms] |
| OR | "heart diseases" [MeSH Terms] |
| OR | "cerebrovascular disorders" [MeSH Terms] |
| OR | "hypertension" [MeSH Terms] |
| OR | "blood pressure" [MeSH Terms] |
| OR | "colonic neoplasms" [MeSH Terms] |
| OR | "esophageal neoplasms" [MeSH Terms] |
| OR | "disease" [MeSH Terms] |

**Hits: 990**

**TOXLINE**

Scope: This database is the National Library of Medicine's bibliographic database for toxicology and comprises two parts: Toxline Core and Toxline Special. Toxline Core incorporates much of the standard journal literature in toxicology and is accessed via PubMed. Toxline Special complements the Core component with references from a range of specialised journals and other data sources including technical reports and research projects.

**Toxline Core –** see PubMed search **Hits: 297**

**Toxline Special –** searched 12/12/2004

| | | | |
|---|---|---|---|
| Water [mh] OR Water [kw] | **AND** | | ( Hardness [mh] *OR* Hardness (kw ) |
| | | OR | Soft [ab] or soft [kw] |
| | | OR | Hydrotimetry [kw] |
| | | OR | Softened [ab] or softened [ti] |
| | | OR | Magnesium [mh] or magnesium compounds [mh] |
| | | OR | Calcium [mh] or calcium compounds [mh] |
| | | OR | Lead [mh] |
| | | OR | Cadmium [mh] or cadmium compounds [mh] |

**AND**

| | |
|---|---|
| | Cardiovascular diseases [mh] |
| OR | Coronary disease [mh] |
| OR | Coronary arteriosclerosis [mh] |
| OR | Heart diseases [mh] |
| OR | Cerebrovascular disorders [mh] |
| OR | Hypertension [mh] |
| OR | Blood pressure [mh] |
| OR | Colonic neoplasms [mh] |
| OR | Esophageal neoplasms [mh] |
| OR | Disease [mh] |

**Hits: 563**

| Author | Country, population | Health outcome | Drinking water parameter | Author's conclusion | Reason for exclusion |
|---|---|---|---|---|---|
| Erb (1997) | USA, counties | Cardiovascular mortality | Area water hardness | Observed an inverse association between water hardness and cardiovascular mortality | Used characteristics of aquifers to generate estimates, poor statistical analysis with incomplete presentation |
| Bernadi *et al.* (1995) | Italy, one region | Small number of sudden cardiac deaths | Area water hardness | Observed elevated sudden deaths in the region and that the water was 'mostly soft' | No statistical analysis |
| Hall and Junger (1993) | Sweden, municipalities | Cardiovascular disease mortality, AMI mortality | Area water hardness | AMI mortality seemed higher in areas of soft water | Water hardness data only available for 7 municipalities therefore weak |
| Van Staden (1990) | South Africa, 276 individuals of the Venda community | Blood pressure and ECG | Area water hardness | Observed CVD rare in the traditional Venda community in a soft water area | No statistical analysis |
| Jeppesen (1987) | Denmark and Greenland | Cardiovascular disease mortality | Area water hardness | No association | No statistical analysis |
| Nystrom *et al.* (1986) | Sweden | Circulatory disease deaths | Area water hardness | Observed higher death rates in the north where water was soft | No statistical analysis |
| MacKinnon and Taylor (1980) | England, 5 towns | Coronary heart disease mortality and sudden death | Area water hardness and calcium concentration | Slight inverse association between calcium and heart disease mortality. No association with sudden deaths | No statistical analysis |

| Author | Country, population | Health outcome | Drinking water parameter | Author's conclusion | Reason for exclusion |
|---|---|---|---|---|---|
| Meyers (1977) | Australia, 6 cities | IHD mortality | Area water hardness and magnesium concentration | No association between water hardness or magnesium and IHD death rate between 6 cities | No statistical analysis |
| Meyers (1975) | Australia, Brisbane and Melbourne | IHD mortality | Area water hardness | A comparison of death rates in two cities showed higher IHD mortality in Brisbane (hard water) | No statistical analysis |
| Hadden (1974) | Northern Ireland | Crude cardiovascular death rates | Water hardness in two towns: one hard, one soft | No difference in unadjusted cardiovascular death rates between the two towns, therefore no association between CVD and water hardness | No statistical analysis |
| Allwright *et al.* (1974) | USA, 3 communities (L.A.) | Cerebrovascular mortality, hypertensive disease mortality | Area water hardness and magnesium concentration | No evident trend between hardness or Mg and community death rates | No statistical analysis |
| Stocks (1973) | England and Wales, 80 boroughs | Cardiovascular mortality, cancer mortality | Water hardness | No association between hardness and coronary heart disease mortality | Poor statistical analysis of proportionate mortality ratio |
| Fodor *et al.* (1973) | Canada, 3 towns | Cardiovascular disease mortality | Area water hardness | Observed a higher death rate in soft water areas | No statistical analysis |
| Morton (1971a) | USA, Colorado, 297 communities | Hypertensive disease mortality | Area water hardness | No observed association between hardness and mortality rate | No statistical analysis |
| Morton (1971b) | USA, Colorado | Hypertension morbidity and mortality | Area water hardness | No association | Not enough information presented to enable quality to be judged |

| Author | Country, population | Health outcome | Drinking water parameter | Author's conclusion | Reason for exclusion |
|---|---|---|---|---|---|
| Anderson *et al.* (1969) | Canada, 55 counties | IHD death rate | Area water hardness | Compared death rates by areas of hardness and found higher IHD mortality in softer water area with higher sudden death | No statistical analysis |
| Morris *et al.* (1962) | England and Scotland, boroughs | Cardiovascular mortality | | | Re-presents results originally described in Morris 1961, no additional information |

## Appendix 3 – Proformas used for data extraction

### Ecological / Correlation Study

| | | |
|---|---|---|
| **Study ID#** | **Author Surname:** | **Location of study (Country)** |
| **Data Extractor**<br>_____ | **Year of Publication**<br>O 200__<br>O 19___ | **Number of subjects included in study**<br>_____ |
| **Language of Publication**<br>O English<br>Other_____ | **Population studied** | **Units of study**<br>O Countries<br>O Regions<br>O Districts<br>O Other…… |

**OUTCOMES MEASURED**

**Type of Illness:**
**Cardiovascular Cerebrovascular**
**Other……..**

***Patient Mortality (Measure of uncertainty)***

***Disease rate (Measure of uncertainty)***

**Outcomes**

| | |
|---|---|
| What are the main outcome measures used in the study? | |
| Main outcome measures accurate (valid and reliable)? | O YES<br>O NO |
| Measured similarly in all areas? | O YES<br>O NO |

**EXPOSURE FACTORS**
Exposure (water hardness and where available specific constituents such as calcium and magnesium)

**Water Hardness**
**Calcium**
**Magnesium**
**Other**_____

**Method used to assess exposure**
Was a proxy or indirect measure of exposure used? YES NO

Was an average measure of exposure used? YES NO
Was recall bias possible with the data collection method used? YES NO

***Study Quality:***

***Timing of measurement of exposure (Temporal ambiguity)***
Before Outcome At the same time After measuring outcome

***Assessment of confounding***
Does the study control for confounders?
YES NO

If YES list confounders controlled
for……..

How was confounding controlled for?
Confounders treated as covariates in a multivariable model
Rates standardised before regression
Other……
……..

**Completeness of exposure and outcome data**
Does exposure data completeness vary geographically? YES NO
Does outcome data completeness vary geographically? YES NO

***Is there evidence of collinearity between variables***
YES NO

***Did the authors discuss/account for the effect of migration across groups?***
YES NO

Comments……
……

**Method of analysis**
Statistically appropriate YES NO
Comment……
…….

Reported adequately with measures of uncertainty and appropriate graphs YES NO

**Result**
(Regression coefficients, Odds Ratios, Risk Ratios etc)

**Conclusion**

## Case Control Study

| Study ID# | Author Surname: | Location of study (Country) |
|---|---|---|
| | | |
| **Data Extractor**<br><br>_____ | **Year of Publication**<br><br>O 200__<br>O 19___ | **Number of subjects included in study**<br><br>_____ |
| **Language of Publication**<br><br>O English<br>Other_____ | **Population studied** | |

**OUTCOMES MEASURED**

**Type of Illness:**
Cardiovascular Cerebrovascular
Other……

Patient Mortality (Measure of uncertainty)

Disease rate (Measure of uncertainty)

**Outcomes**

| | |
|---|---|
| What are the main outcome measures used in the study? | |
| Main outcome measures accurate (valid and reliable)? | O YES<br>O NO |
| Measured similarly between treatment and controls areas? | O YES<br>O NO |

**EXPOSURE FACTORS**
Exposure (water hardness and where available specific constituents such as calcium and magnesium)

**Water Hardness**

**Calcium**

**Magnesium**

**Other**_____

**Method used to assess exposure**
Was a proxy or indirect measure of exposure used? YES NO

Measurement of hardness at

a. household tap b. community level c. other…….

Was an average measure of exposure used? YES NO

***Study Quality:***

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

**Selection**

1) Is the case definition adequate?
a) yes, with independent validation
b) yes, eg record linkage or based on self reports
c) no description

2) Representativeness of the cases
a) consecutive or obviously representative series of cases
b) potential for selection biases or not stated

3) Selection of Controls
a) community controls
b) hospital controls
c) no description

4) Definition of Controls
a) no history of disease (endpoint)
b) no description of source

**Comparability**

1) Comparability of cases and controls on the basis of the design or analysis
a) study controls for _____ _____
_____

**Exposure**

1) Ascertainment of exposure
a) secure record
b) structured interview where blind to case/control status
c) interview not blinded to case/control status
d) written self report or medical record only
e) no description

2) Same method of ascertainment for cases and controls
a) yes
b) no

3) Non-Response rate
a) same rate for both groups
b) non respondents described
c) rate different and no designation

**Result**

**Conclusion**

## Cohort Study

| Study ID# | Author Surname: | Location of study (Country) |
|---|---|---|
| | | |
| **Data Extractor**<br><br>_____ | **Year of Publication**<br><br>O 200__<br>O 19___ | **Number of subjects included in study**<br><br>_____ |
| **Language of Publication**<br><br>O English<br>Other_____ | **Population studied** | |

**OUTCOMES MEASURED**

**Type of Illness:**
Cardiovascular Cerebrovascular
Other……

Patient Mortality (Measure of uncertainty)

Disease rate (Measure of uncertainty)

Outcomes

| | |
|---|---|
| What are the main outcome measures used in the study? | |
| Main outcome measures accurate (valid and reliable)? | O YES<br>O NO |
| Measured similarly between treatment and controls areas? | O YES<br>O NO |

**EXPOSURE FACTORS**
Exposure (water hardness and where available specific constituents such as calcium and magnesium)

**Water Hardness**
**Calcium**
**Magnesium**

**Other**_____

**Method used to assess exposure**
Was a proxy or indirect measure of exposure used? YES NO
Measurement of hardness at
b. household tap b. community level c. other……

Was an average measure of exposure used? YES NO

***Study Quality:***
Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

**Selection**
1) Representativeness of the exposed cohort
a) truly representative of the average exposure to hard water in the community
b) somewhat representative of the average exposure to hard water in the community
c) selected group of users eg nurses, volunteers
d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
a) drawn from the same community as the exposed cohort
b) drawn from a different source
c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
a) secure record
b) structured interview
c) written self report
d) no description

4) Demonstration that outcome of interest was not present at start of study
a) yes
b) no

**Comparability**
1) Comparability of cohorts on the basis of the design or analysis
a) study controls for _____

**Outcome**
1) Assessment of outcome
a) independent blind assessment
b) record linkage
c) self report
d) no description

2) Was follow-up long enough for outcomes to occur
a) yes b) no

3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for
b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an
adequate %) follow up, or description provided of those lost)
c) follow up rate < ____% (select an adequate %) and no description of those lost
d) no statement

**Result**

**Conclusion**

## Appendix 4 – Study quality information

The following tables present the assessment of the quality of each study by the criteria defined in 3.5. Tables 12 to 14 detail the ecological and cross-sectional studies, whilst Tables 15 to 17 present the results for the case-control and cohort studies respectively. All study quality tables are matched to those presented in the results section.

The details of any possible confounding factors that were also considered within the studies were given in the results tables (Tables 5 to 10). The methods used by each ecological and cross-sectional study to control for the effects of potential confounders in the statistical analysis of the data are described in the study quality tables under the heading 'Method'. Here the notation (1) defines that confounders were treated as covariates in a multivariable model, whist (2) defines that rates were standardised before regression.

The presence of collinearity within the study analyses was recorded when discussed by the authors. Where the possibility of collinearity was not addressed in the text by the authors and no information was presented illustrating associations between explanatory variables, this criterion was recorded as 'unknown'. Where collinearity was acknowledged and described, the affected variables are detailed in the study quality tables with correlation coefficients (r) given where available.

Tables 12 to 17 further record the adequacy of the statistical analyses undertaken in each study to examine the association between drinking water quality and health outcome. The statistical methods used are presented together with an assessment of the adequacy of the presentation of results. Where the adequacy of reporting was found to be low (as defined in 3.5), an explanation is given in the study quality tables.

The study quality criteria for case-control studies additionally assessed the selection of cases and controls and the characterisation of exposure. Consideration of the use of a proxy or average measure of exposure was retained, however the level of measurement was recorded for case-control studies, i.e. the application of water quality information at the area *vs* individual level. For ease of presentation, the

notation used in Tables 15 and 16 refer to the classification of each criteria as below (taken from the case-control proforma in Appendix 3):

**Case-control studies**

**Selection**:

1) Is the case definition adequate?
   a) yes, with independent validation
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls
   b) hospital controls
   c) no description

4) Definition of Controls †
   a) no history of disease (endpoint)
   b) no description of source

**Exposure:**

1) Ascertainment of exposure
   a) secure record
   b) structured interview where blind to case/control status
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes
   b) no

3) Non-Response rate
   a) same rate for both groups
   b) non respondents described
   c) rate different and no designation

† throughout the review process, a series of studies were considered where the options for this criterion were too restrictive, they have therefore been identified in the tables as 'source described' and will be described further in the text.

Similarly, Table 17 presents the assessment of study quality for the cohort studies. The notation contained therein refers to the classification of each study by the following criteria (taken from the cohort study proforma in Appendix 3).

**Cohort studies**

**Selection**

1) Representativeness of the exposed cohort
   a) truly representative of the average exposure to hard water in the community
   b) somewhat representative of the average exposure to hard water in the community
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record
   b) structured interview
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes
   b) no

**Outcome**

1) Assessment of outcome
   a) independent blind assessment
   b) record linkage
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ %
   c) follow up rate < ____% and no description of those lost
   d) no statement

**Table 12 Study quality assessment of ecological studies examining the association between cardiovascular disease and drinking water hardness, calcium or magnesium**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Kaipio *et al.* (2004) | No | Yes | No | Before | 1 and 2 | Unknown | Discussed | Yes - negative binomal regression | Yes |
| | Ferrandiz *et al.* (2004) | No | Yes | No | Same | 2 | Yes - (Ca and Mg) | No | Yes - relative risks, covariate analysis of standardised rates | Yes |
| | Miyake and Ike (2004b) | No | Yes | No | After | 1 | No | Discussed | Yes - logistic regression | Yes |
| | Marque *et al.* (2003) | No | Yes | No | Same | 1 | No | No | Yes - model of extra poisson variation regression | Yes |
| | Miyake and Ike (2003) | No | Yes | No | After | 1 | Unknown | Discussed | Yes - multiple logistic regression | Yes |
| | Maheswaran *et al.* (1999) | No | Yes | No | Same | 1 and 2 | Yes - (Ca and Mg) | No | Yes - log linear poisson regression | Yes |
| | Yang *et al.* (1996) | No | Yes | No | Same | 1 | No | No | Yes - correlation and regression | Yes |
| | Lovett *et al.* (1986) | No | Yes | No | Same | 1 | No | No | Yes - compared poisson regression to log normal OLS regression | Yes |
| | Lacey and Shaper (1984) | No | Yes | No | Same | 1 | Unknown | No | Yes - linear regression | Yes |
| | Pocock *et al.* (1980) | No | Yes | No | Same | 1 | Yes | No | Yes - multiple regression | Yes |

**Table 12 (continued)**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Dudley *et al.* (1969) | No | Yes | No | Unknown | 1 | Yes (water factors) | No | Yes - stepwise correlation analysis (large degree of subjectivity) | Yes |
| | Catling (2004) | No | Yes | No | After | 1 | No | Discussed | Yes – multiple regression models | Yes |
| | Kousa *et al.* (2004) | No | Yes | No | Same | 2 | No | No | Yes - bayesian spatial conditional autoregressive model | Yes |
| | Ferrandiz *et al.* (2003) | No | Yes | No | Before | 1 | Yes – authors discuss fully | No | Yes - hierarchical spatiotemporal models | Yes – however many results presented graphically |
| | MacPherson and Basco (2000) | No | Yes | No | Unknown | 1 | Not stated | Yes – resident for at least 4 months | Yes - ANOVA and regression | No – no measure of uncertainty |
| | Rylander *et al.* (1991) | No | Yes | No | After | 2 | hardness and Ca r=+0.93, hardness and Mg r=+0.71, Ca and Mg r=+0.55 | No | Yes - correlation | Yes |
| | Gyllerup *et al.* (1991) | No | Yes | No | Same | 1 | Yes - hardness and cold r=+0.55 | No | Yes - weighted regression analysis | Yes |

**Table 12 (continued)**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Derry *et al.* (1990) | No | Yes | No | Same | 2 | No | No | Yes - Spearman's rank correlation | Yes |
| | Leoni *et al.* (1985) | Yes | Yes | No | Same | 2 | No | Discussed | Yes - correlation coefficient, only 10 data points used | Yes |
| | Greathouse and Osborne (1980) | No | Yes | No | After | 2 | Yes - Ca and hardness r=+0.99, Ca and Na r=+0.53 | No | Yes - regression | No - no measure of uncertainty given |
| | Masironi *et al.* (1979) | No | Yes | No | Same | 2 | No | No | Yes - OLS regression correlation | No - no measure of uncertainty given |
| | West (1977) | No | Yes | No | Same | 2 | No | No | Yes - population weighted correlation coefficients | Yes |
| | Voors (1971) | No | Yes | No | Same | 1 | Yes - between metals, e.g. Ca and Mg r=+0.85 | No | Yes – partial correlation | Yes |
| | Schroeder (1960b) | No | Yes | No | Same | 2 | No | No | Yes - correlation | Yes |

**Table 12 (continued)**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Sauvant and Pepin (2000) | No | Yes | No | Same | 2 | No | No | Yes - correlation and regression | No - no measure of uncertainty given |
| | Sakomoto *et al.* (1997) | No | Yes | No | Unknown | None | No | No | Yes - Pearson correlation and t-tests | Yes |
| | Piispanen (1993) | Yes | Yes | No | Unknown | None | No | No | Yes - Spearman rank correlation, t-tests | Yes |
| | Nerbrand *et al.* (1992) | No | Yes | No | Same (hardness), after (Ca, Mg) | None | No | No | Yes - correlation | No –no measure of uncertainty given |
| | Flaten (1991) | No | Yes | No | Before | 2 | No | Discussed | Yes – Spearman rank correlation but multiple hypothesis testing | Yes |
| | Dzik (1989) | Unknown | Yes | No | Unknown | 2 | Unknown | No | Yes - Pearson correlation | No – no p-values given |
| | Smith and Crombie (1987) | No | Yes | No | Same | 2 | No | No | Yes - correlation | No - no p-value given |
| | Leary *et al.* (1983) | No | Yes | No | After | None | No | No | Yes - Spearman's rank correlation | Yes |
| | Crawford *et al.* (1977) | No | Yes | No | Same | None | No | No | Yes - contingency tables | Yes |
| | Elwood *et al.* (1977) | No | Yes | No | Same | 1 | Yes – rainfall and Ca r=-0.5 | No | Y – multiple linear regression (acknowledged multiple testing) | No – no measure of uncertainty given |

**Table 12 (continued)**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Punsar *et al.* (1975) | No | Yes | No | After | Stratified by age | Yes | No | Yes - correlations | Yes |
| | Elwood *et al.* (1974) | No | Yes | No | After | None | Yes (amongst elements) | No | Yes – Spearman's rank correlation | Yes |
| | Schroeder and Kraemer (1974) | No | Yes | No | After | 2 | No | No | No – large number of unadjusted correlations | No |
| | Bierenbaum *et al.* (1973) | Yes | Yes | No | Unknown | None | Unknown | Worked and lived in area >5y | Yes - ANOVA | Yes |
| | Neri *et al.* (1972) | No | Yes | No | Same | 2 | No | No | Yes - linear correlation and regression analysis | No – no measure of uncertainty given |
| | Roberts and Lloyd (1972) | unknown | Yes | No | Unknown | Partial correlation | Yes - hardness and rainfall (r=-0.48, r=-0.59) | No | Yes - correlation and partial correlation | Yes |
| | Anderson and Leriche (1971) | No | Yes | No | Same | None | No | No | Yes - correlations | Yes |
| | Crawford *et al.* (1971) | No | Yes | No | Before | 2 | No | No | Yes – but subject to multiple hypothesis testing | No – no measures of uncertainty given |

**Table 12 (continued)**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Masironi *et al.* (1970) | No | Yes | No | Same | Matched | α radioactivity and hardness (state r=+0.86, station r=+0.80) | No | Yes - correlations | Yes |
| | Morris *et al.* (1961) | No | Yes | No | After | None | No | No | Yes - correlation | Yes |
| | Schroeder (1960a) | Unknown | Yes | No | Unknown | 2 | No | No | Yes - correlation | Yes |

Ca = calcium
Mg = magnesium
Na = sodium
OLS regression = ordinary least squares regression
ANOVA = analysis of variation

**Table 13 Study quality assessment of ecological studies examining the association between drinking water hardness or calcium or magnesium content and cancer**

| | | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| | Yang *et al.* (2000a) | No | Yes | No | After | 1 | Unknown | Yes | Yes - weighted multivariate linear regression | Yes |
| | Kikuchi *et al.* (1999) | No | Yes | No | Same | 1 | Unknown | No | No - multiple linear regression with 22 elements | No – no measures of uncertainty given |

**Table 14 Study quality assessment of cross-sectional studies examining the association between drinking water hardness or calcium or magnesium and measures of individual health**

| | Exposure factors | | | | | | | Analysis | |
|---|---|---|---|---|---|---|---|---|---|
| **Author** | **Proxy** | **Average** | **Bias** | **Timing** | **Method** | **Collinearity** | **Migration** | **Statistical analysis** | **Adequacy of reporting** |
| Tukiendorf and Rybak (2004) | No | Yes | No | Same | 1 | Unknown | No | Yes – Bayesian logistic regression | Yes |
| Nerbrand *et al.* (2003) | No | No – household tap | No | Same | 1 | Unknown | No | Yes – Spearman's rank correlation | Yes |
| Zeighami *et al.* (1990) | No | Yes | No | Same | 1 | No | No | Yes – ANCOVA | No – p-values only |
| Bierenbaum *et al.* (1975) | No | Yes | No | Same | Matched | No | Yes – lived and worked in area >5yrs | Yes - ANOVA between 2 cities (t-test) | Yes |
| Luoma *et al.* (1973) | No | No – household tap | Yes | Same | 1 | Yes – fluoride and magnesium | No | Yes – correlation and regression (multiple hypothesis testing) | No |
| Stitt *et al.* (1973) | Yes | n/a | n/a | Unknown | Matched | Unknown | Discussed | Yes - t-tests | Yes |

n/a = not applicable
ANOVA = analysis of variance
ANCOVA = analysis of covariance

**Table 15 Study quality assessment of case control studies examining the association between drinking water hardness or calcium or magnesium content and cardiovascular disease**

| | | | | Selection | | | | Exposure | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author** | **Proxy** | **Measured** | **Average** | **1** | **2** | **3** | **4** | **1** | **2** | **3** |
| Comstock (1971) | No | Household | No | b | a | a | a | a | a | a |
| Luoma *et al.* (1983) | No | Household tap | No - individual reading used | a | a | a and b | a | a | a | c |
| Rubenowitz *et al.* (1996) | No | Waterworks | Yes | a | a | a | a | a | a | a |
| Rubenowitz *et al.* (1999) | No | Waterworks | Yes | b | a | a | b | a | a | b |
| Rubenowitz *et al.* (2000) | No | Waterworks and household | Yes | b | a | a | b | a | a | a |
| Yang *et al.* (1998a) | No | Waterworks | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1999b) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |

**Table 16 Study quality assessment of case control studies examining the association between drinking water hardness or calcium or magnesium content and cancers/other diseases**

| | | | | Selection | | | | Exposure | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author** | **Proxy** | **Measured** | **Average** | **1** | **2** | **3** | **4** | **1** | **2** | **3** |
| Yang *et al.* (1999c) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (2000b) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1998b) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1997a) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1999d) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1998d) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1999e) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (2002a) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1998c) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (1997b) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Hwang *et al.* (2003) | No | Municipality | Yes | a | a | a | Source described | a | a | n/a |
| Yang *et al.* (2002b) | No | Municipality | Yes | a | a | a | Source described | a | a | a |

n/a = not applicable

**Table 17 Study quality assessment of cohort studies examining the association between drinking water hardness or calcium or magnesium content and cardiovascular disease**

| | | | | Selection | | | | Outcome | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Author** | **Proxy** | **Measured** | **Average** | **1** | **2** | **3** | **4** | **1** | **2** | **3** |
| Morris *et al.* (2001) | No | Water company | Yes | b | b | a | a | b | a | a |
| Comstock *et al.* (1980) | No | Household sample then aggregated to area level | Yes | a | a | a | a | b | a | d |
| Comstock *et al.* (1979) | No | Household sample then aggregated to area level | Yes | a | a | a | a | b | a | d |
| Punsar and Karvonen (1979) | No | Household sample then aggregated to area level | Yes | a | a | a | a | b | a | d |
| Punsar *et al.* (1975) | No | Household sample then aggregated to area level | Yes | b | a | a | a | b | a | d |

## References


Allwright SP, Coulson A, Detels R, *et al.* (1974) Mortality and water-hardness in three matched communities in Los Angeles. *Lancet* 2(7885):860-864.

Anderson TW, Le Riche WH, MacKay JS. (1969) Sudden death and ischemic heart disease. Correlation with hardness of local water supply. *New England Journal of Medicine* 280(15):805-807.

Anderson TW, Leriche WH. (1971) Sudden death from ischemic heart disease in Ontario and its correlation with water hardness and other factors. *Canadian Medical Association Journal* 105(2):155-160.

Anderson TW, Neri LC, Schreiber GB, *et al.* (1975) Ischemic heart-disease, water hardness and myocardial magnesium. *Canadian Medical Association Journal* 113(3):199-203.

Appel LJ, Moore TJ, Obarzanek E, *et al.* (1997) A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *New England Journal of Medicine* 336(16):1117-1124.

Aptel I, Cance-Rouzaud A, Grandjean H. (1999) Association between calcium ingested from drinking water and femoral bone density in elderly women: evidence from the EPIDOS cohort. *Journal of Bone and Mineral Research* 14(5):829-833.

Aschengrau A, Zierler S, Cohen A. (1993) Quality of community drinking water and the occurrence of late adverse pregnancy outcomes. *Archives of Environmental Health* 48(2):105-113.

Barry-Ryan P, Huet N, MacIntosh DL. (2000) Longitudinal investigation of exposure to arsenic, cadmium and lead in drinking water. *Environmental Health Perspectives* 108:731-735.

Beevers DG, Cruickshank JK, Yeoman WB, *et al.* (1980) Blood-lead and cadmium in human hypertension. *Journal of Environmental Pathology and Toxicology* 4(2-3):251-260.

Beevers G, Lip G, O'Brien E. (1995) *ABC of hypertension*. Fourth ed. London: BMJ Books.

Bernardi D, Dini FL, Azzarelli A, *et al.* (1995) Sudden cardiac death rate in an area characterized by high incidence of coronary artery disease and low hardness of drinking water. *Angiology* 46(2):145-149.

BHF. (2004) British Heart Foundation - mortality statistics: www.heartstats.org.uk.

Bierenbaum ML, Fleischman AI, Dunn JP, *et al.* (1973) Serum parameters in hard and soft water communities. *American Journal of Public Health* 63(2):169-173.

Bierenbaum ML, Fleischman AI, Dunn J, *et al.* (1975) Possible toxic water factor in coronary heart-disease. *Lancet* 1(7914):1008-1010.

Bradford-Hill. (1965) The Environment and Disease: Association or Causation? *Proceedings of the Royal Society of Medicine* 9:295-300.

Britton A, Shipley M, Marmot M, *et al.* (2004) Does access to cardiac investigation and treatment contribute to social and ethnic differences in coronary heart disease? Whitehall II prospective cohort study. *British Medical Journal* 329(7461):318.

Calderon RL, Craun GF. (2004) Water hardness and cardiovascular disease: a review of epidemiological studies 1957-1979 (*Draft*). *World Health Organisation*.

Catling LA (2004) An epidemiological investigation into the influence of solar ultraviolet B radiation on human blood pressure. PhD thesis, University of East Anglia, UK.

Chakraborti S, Chakraborti T, Mandal M, *et al.* (2002) Protective role of magnesium in cardiovascular diseases: a review. *Molecular and Cellular Biochemistry* 238(1-2):163-179.

Chipperfield B, Chipperfield JR, Behr G, *et al.* (1976) Magnesium and potassium content of normal heart muscle in areas of hard and soft water. *Lancet* 1(7951):121-122.

Chipperfield B, Chipperfield JR. (1978) Differences in metal content of the heart muscle in death from ischemic heart disease. *American Heart Journal* 95(6):732-737.

Chipperfield B, Chipperfield JR. (1979) Relation of myocardial metal concentrations to water hardness and death-rates from ischemic heart disease. *Lancet* 2(8145):709-712.

Cohen H, Sherer Y, Shaish A, *et al.* (2002) Atherogenesis inhibition induced by magnesium-chloride fortification of drinking water. *Biological Trace Element Research* 90(1-3):251-259.

Comstock GW. (1971) Fatal arteriosclerotic heart disease, water hardness at home, and socioeconomic characteristics. *American Journal of Epidemiology* 94(1):1-10.

Comstock GW, Cauthen GM, Helsing KJ, *et al.* (1979) Stroke-associated deaths in Washington county, Maryland, with special reference to water hardness. *Stroke* 10(2):199-205.

Comstock GW, Cauthen GM, Helsing KJ. (1980) Water hardness at home and deaths from arteriosclerotic heart disease in Washington County, Maryland. *American Journal of Epidemiology* 112(2):209-216.

Crawford T, Crawford MD. (1967) Prevalence and pathological changes of ischaemic heart-disease in a hard-water and in a soft-water area. *Lancet* 1(7484):229-232.

Crawford MD, Gardner MJ, Morris JN. (1971) Changes in water hardness and local death-rates. *Lancet* 2(7720):327-329.

Crawford MD, Clayton DG, Stanley F*, et al.* (1977) An epidemiological study of sudden death in hard and soft water areas. *Journal of Chronic Diseases* 30(2):69-80.

Derry CW, Bourne DE, Sayed AR. (1990) The relationship between the hardness of treated water and cardiovascular disease mortality in South African urban areas. *South African Medical Journal* 77(10):522-524.

Dudley EF, Beldin RA, Johnson BC. (1969) Climate, water hardness and coronary heart disease. *Journal of Chronic Diseases* 22(1):25-48.

Durlach J, Bara M, Guiet-Bara A. (1985) Magnesium level in drinking water and cardiovascular risk factor: a hypothesis. *Magnesium* 4(1):5-15.

Durlach J. (1995) General considerations on the quality criteria for biomedical papers and some complementary guidelines for the contributors of Magnesium Research. *Magnesium Research* 8(3):191-206.

Dwyer JH, Dwyer KM, Scribner RA*, et al.* (1998) Dietary calcium, calcium supplementation, and blood pressure in African American adolescents. *American Journal of Clinical Nutrition* 68(3):648-655.

Dzik AJ. (1989) Cerebrovascular disease mortality rates and water hardness in North Dakota. *South Dakota Journal of Medicine* 42(4):5-7.

Elwood PC, Abernethy M, Morton M. (1974) Mortality in adults and trace elements in water. *Lancet* 2(7895):1470-1472.

Elwood PC, St Leger AS, Morton M. (1977) Mortality and the concentration of elements in tap water in county boroughs in England and Wales. *British Journal of Preventive and Social Medicine* 31(3):178-182.

Elwood PC, Sweetnam PM, Beasley WH*, et al.* (1980) Magnesium and calcium in the myocardium: Cause of death and area differences. *Lancet* 2(8197):720-722.

Emberson JR, Whincup PH, Morris RW*, et al.* (2003) Re-assessing the contribution of serum total cholesterol, blood pressure and cigarette smoking to the aetiology of coronary heart disease: impact of regression dilution bias. *European Heart Journal* 24(19):1719-1726.

Erb BD, Sr. (1997) Water hardness and cardiovascular death rates in Tennessee. *Tennessee Medicine* 90(8):314-316.

Ferrandiz J, Lopez-Quilez A, Gomez-Rubio V, *et al.* (2003) Statistical relationship between hardness of drinking water and cerebrovascular mortality in Valencia: a comparison of spatiotemporal models. *Environmetrics* 14(5):491-510.

Ferrandiz J, Abellan JJ, Gomez-Rubio V, *et al.* (2004) Spatial analysis of the relationship between mortality from cardiovascular and cerebrovascular disease and drinking water hardness. *Environmental Health Perspectives* 112(9):1037-1044.

Flaten TP. (1991) Geographical associations between drinking water chemistry and the mortality and morbidity of cancer and some other diseases in Norway. *The Science of the Total Environment* 102:75-100.

Fodor JG, Pfeiffer CJ, Papezik VS. (1973) Relationship of drinking water quality (hardness-softness) to cardiovascular mortality in Newfoundland. *Canadian Medical Association Journal* 108(11):1369-1373.

Ford ES. (1999) Serum magnesium and ischaemic heart disease: findings from a national sample of US adults. *International Journal of Epidemiology* 28(4):645-651.

Galan P, Arnaud MJ, Czernichow S, *et al.* (2002) Contribution of mineral waters to dietary calcium and magnesium intake in a French adult population. *Journal of the American Dietetic Association* 102:1658-1662.

Gillies ME, Paulin HV. (1983) Variability of mineral intakes from drinking water: A possible explanation for the controversy over the relationship of water quality to cardiovascular disease. *International Journal of Epidemiology* 12(1):45-50.

Greathouse DG, Osborne RH. (1980) Preliminary report on nationwide study of drinking water and cardiovascular diseases. *Journal of Environmental Pathology and Toxicology* 4(2-3):65-76.

Guillemant J, Le H, Accarie C, *et al.* (2000) Mineral water as a source of dietary calcium: acute effects on parathyroid function and bone resorption in young men. *American Journal of Clinical Nutrition* 71:999-1002.

Gyllerup S, Lanke J, Lindholm LH, *et al.* (1991) Water hardness does not contribute substantially to the high coronary mortality in cold regions of Sweden. *Journal of Internal Medicine* 230(6):487-492.

Hadden DR. (1974) Soft water and ischaemic heart disease: a study of two towns in Northern Ireland. *Ulster Medical Journal* 43(1):45-6.

Hall P, Jungner I. (1993) Hard drinking water and ischemic heart disease: Calcium, bloodlipids, and acute myocardial infarcts. *Journal of Medical Systems* 17(3-4):277-281.

Haring BJ, Zoeteman BC. (1980) Corrosiveness of drinking water and cardiovascular disease mortality. *Bulletin of Environmental Contamination and Toxicology* 25(4):658-662.

Haring BS, Van Delft W. (1981) Changes in the mineral composition of food as a result of cooking in "hard" and "soft" waters. *Archives of Environmental Health* 36(1):33-35.

Heaney R, Barger-Lux M, Dowell M. (1997) Calcium absorptive effects of vitamin D and its major metabolites. *Journal of Clinical Endocrinology and Metabolism* 82(12):4111-4116.

Hewitt D, Neri LC. (1980) Development of the 'Water Story': some recent Canadian studies. *Journal of Environmental Pathology and Toxicology* 4(2-3):51-63.

Hwang SJ, Lai YH, Chiu HF, *et al.* (2003) Association of death from renal failure with calcium levels in drinking water. *Journal of Toxicology and Environmental Health-Part A* 66(24):2327-2335.

Jeppesen BB. (1987) Greenland, a soft-water area with a low incidence of ischemic heart death. *Magnesium* 6(6):307-313.

Kaipio J, Nayha S, Valtonen V. (2004) Fluoride in the drinking water and the geographical variation of coronary heart disease in Finland. *European Journal of Cardiovascular Prevention and Rehabilitation* 11(1):56-62.

Kikuchi H, Iwane S, Munakata A, *et al.* (1999) Trace element levels in drinking water and the incidence of colorectal cancer. *Tohoku Journal of Experimental Medicine* 188(3):217-225.

Kobayashi J. (1957) On geographical relationship between the chemical nature of river water and death rate from apoplexy. *Berichte des Ohara Institutes fur Landurtschaftuche Biologie* 11:12-21.

Kousa A, Moltchanova E, Viik-Kajander M, *et al.* (2004) Geochemistry of ground water and the incidence of acute myocardial infarction in Finland. *Journal of Epidemiology and Community Health.* 58(2):136-139.

Lacey RF, Shaper AG. (1984) Changes in water hardness and cardiovascular death rates. *International Journal of Epidemiology* 13(1):18-24.

Last JM. (2001) *A Dictionary of Epidemiology*. Fourth ed. Oxford: Oxford University Press.

Lawlor DA, Bedford C, Taylor M, *et al.* (2003) Geographical variation in cardiovascular disease, risk factors, and their control in older women: British Women's Heart and Health Study. *Journal of Epidemiology and Community Health.* 57(2):134-140.

Leary WP, Reyes AJ, Lockett CJ, *et al.* (1983) Magnesium and deaths ascribed to ischaemic heart disease in South Africa. A preliminary report. *South African Medical Journal* 64(20):775-776.

Leoni V, Fabiani L, Ticchiarelli L. (1985) Water hardness and cardiovascular mortality rate in Abruzzo, Italy. *Archives of Environmental Health* 40(5):274-278.

Lovett AA, Bentham CG, Flowerdew R. (1986) Analysing geographic variations in mortality using Poisson regression: the example of ischaemic heart disease in England and Wales 1969-1973. *Social Science and Medicine* 23(10):935-943.

Loyke HF. (2002) Effects of elements in human blood pressure control. *Biological Trace Element Research* 85(3):193-209.

Luoma H, Helminen SK, Ranta H, *et al.* (1973) Relationships between the fluoride and magnesium concentrations in drinking water and some components in serum related to cardiovascular diseases in men from four rural districts in Finland. *Scandinavian Journal of Clinical and Laboratory Investigation* 32(3):217-224.

Luoma H, Aromaa A, Helminen S, *et al.* (1983) Risk of myocardial infarction in Finnish men in relation to fluoride, magnesium and calcium concentration in drinking water. *Acta Medica Scandinavica* 213(3):171-176.

Ma J, Folsom AR, Melnick SL, *et al.* (1995) Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin and carotid arterial wall thickness: the ARIC study. *Journal of Clinical Epidemiology* 48(7):927-940.

Mackinnon AU, Taylor SH. (1980) Relationship between 'sudden' coronary deaths and drinking water hardness in five Yorkshire cities and towns. *International Journal of Epidemiology* 9(3):247-250.

MacPherson A, Bacso J. (2000) Relationship of hair calcium concentration to incidence of coronary heart disease. *Science of the Total Environment* 255(1-3):11-19.

Maheswaran R, Morris S, Falconer S, *et al.* (1999) Magnesium in drinking water supplies and mortality from acute myocardial infarction in north west England. *Heart* 82(4):455-460.

Marier JR, Neri LC. (1985) Quantifying the role of magnesium in the interrelationship between human mortality/morbidity and water hardness. *Magnesium* 4(2-3):53-59.

Marque S, Jacqmin-Gadda H, Dartigues JF, *et al.* (2003) Cardiovascular mortality and calcium and magnesium in drinking water: An ecological study in elderly people. *European Journal of Epidemiology* 18(4):305-309.

Marx A, Neutra RR. (1997) Magnesium in drinking water and ischemic heart disease. *Epidemiologic Reviews.* 19(2):258-272.

Masironi R. (1970) Cardiovascular mortality in relation to radioactivity and hardness of local water supplies in the USA. *Bulletin of the World Health Organization* 43(5):687-697.

Masironi R, Pisa Z, Clayton D. (1979) Myocardial infarction and water hardness in the WHO myocardial infarction registry network. *Bulletin of the World Health Organization* 57(2):291-299.

McCarron DA, Reusser ME. (1999) Finding consensus in the dietary calcium-blood pressure debate. *Journal of the American College of Nutrition* 18(5 Suppl):398S-405S.

McGregor GR. (2001) The meteorological sensitivity of ischaemic heart disease mortality events in Birmingham, UK. *International Journal of Biometerology* 45(3):133-142.

McNally NJ, Williams HC, Phillips DR, *et al.* (1998) Atopic eczema and domestic water hardness. *Lancet* 352(9127):527-531.

M.E.L. Research (1996) Tap water consumption in England and Wales: Findings from the 1995 national survey.

Menotti A, Signoretti P (1979) Caratteristiche dell'acqua potabile e cardiopatia coronarica. *Giornale Italiano di Cardiologia* 9:674-677.

Meyers D. (1975) Ischaemic heart disease and the water factor. A variable relationship. *British Journal of Preventive and Social Medicine* 29(2):98-102.

Meyers DH, Williams G. (1977) Mortality from all causes, and from ischaemic heart disease, in the Australian capital cities. *Medical Journal of Australia* 2(15):504-505.

Miyake Y, Iki M. (2003) Ecologic study of water hardness and cerebrovascular mortality in Japan. *Archives of Environmental Health* 58(3):163-166.

Miyake Y, Yokoyama T, Yura A, *et al.* (2004a) Ecological association of water hardness with prevalence of childhood atopic dermatitis in a Japanese urban area. *Environmental Research* 94(1):33-37.

Miyake Y, Iki M. (2004b) Lack of association between water hardness and coronary heart disease mortality in Japan. *International Journal of Cardiology* 96(1):25-28.

Monarca S, Zerbini I, Donato F. (2004) Drinking water hardness and cardiovascular diseases: a review of epidemiological studies 1979-2004 (*Draft*). *World Health Organisation.*

Morgernstern H, Thomas D. (1993) Principles of study design in environmental epidemiology. *Environmental Health Perspectives* 101 (Suppl 4)(54):23-38.

Morris JN, Heady JA, Crawford MD. (1961) Hardness of Local Water-Supplies and Mortality from Cardiovascular Disease in the County Boroughs of England and Wales. *Lancet* 1:860-862.

Morris JN, Crawford MD, Heady JA. (1962) Hardness of Local Water-Supplies and Mortality from Cardiovascular Disease. *Lancet* 2:506-507.

Morris RW, Whincup PH, Lampe FC, *et al.* (2001) Geographic variation in incidence of coronary heart disease in Britain: the contribution of established risk factors. *Heart* 86(3):277-283.

Morton WE. (1971a) Hypertension and drinking water. A pilot statewide ecological study in Colorado. *Journal of Chronic Diseases* 23(8):537-545.

Morton WE. (1971b) Hypertension and drinking water constituents in Colorado. *American Journal of Public Health* 61(7):1371-1378.

Nakagawa H, Nishijo M. (1996) Environmental cadmium exposure, hypertension and cardiovascular risk. *Journal of Cardiovascular Risk* 3(1):11-17.

Nam-Ong C. (2004) Minerals from drinking water: bioavailability for various world populations and health implications (*Draft*). *World Health Organisation*.

NAS-NRC Panel on geochemistry of water in relation to cardiovascular disease. *Geochemistry of water in relation to cardiovascular disease*. Washington: National Academy of Sciences, 1979:1-98.

Näyhä S. (2002) Cold and the risk of cardiovascular diseases. A review. *International Journal of Circumpolar Health* 61(4):373-380.

Nerbrand C, Svardsudd K, Ek J, *et al.* (1992) Cardiovascular mortality and morbidity in seven counties in Sweden in relation to water hardness and geological settings. The project: Myocardial infarction in Mid-Sweden. *European Heart Journal* 13(6):721-727.

Nerbrand C, Agreus L, Lenner RA, *et al.* (2003) The influence of calcium and magnesium in drinking water and diet on cardiovascular risk factors in individuals living in hard and soft water areas with differences in cardiovascular mortality. *BMC Public Health* 3(1):21.

Neri LC, Mandel JS, Hewitt D. (1972) Relation between mortality and water hardness in Canada. *Lancet* 1(7757):931-934.

Ness A, Powles J. (1997) Fruit and vegetables, and cardiovascular disease: a review. *International Journal of Epidemiology* 26(1):1-13.

Nystrom L, Rosen M, Wall S. (1986) Why are diabetes, stomach cancer and circulatory diseases more common in Northern Sweden? *Scandinavian Journal of Primary Health Care* 4(1):5-12.

Perry HM, Jr., Perry EF. (1974) Possible relationships between the physical environment and human hypertension: cadmium and hard water. *Preventive Medicine* 3(3):344-352.

Perry HM, Perry EF, Erlanger MW. (1974) Reversal of cadmium-induced hypertension by selenium or hard water. *Trace Substances and Environmental Health*. (8):51-57.

Perry HM, Roccella EJ. (1998) Conference report on stroke mortality in the southeastern United States. *Hypertension* 31(6):1206-1215.

Peter I, Otremski I, Livshits G. (1996) Geographic variation in vascular mortality in Eurasia: spatial autocorrelation analysis of mortality variables and risk factors. *Annals of Human Biology* 23(6):471-490.

Piispanen R. (1993) Water Hardness and Cardiovascular Mortality in Finland. *Environmental Geochemistry and Health* 15(4):201-208.

Pocock SJ, Shaper AG, Cook DG, *et al.* (1980) British regional heart study: Geographic variations in cardiovascular mortality, and the role of water quality. *British Medical Journal* 280(6226):1243-1249.

Punsar S, Erametsa O, Karvonen MJ, *et al.* (1975) Coronary heart disease and drinking water. A search in two Finnish male cohorts for epidemiologic evidence of a water factor. *Journal of Chronic Diseases* 28(5-6):259-287.

Punsar S, Karvonen M. (1979) Drinking water quality and sudden death: observations from west and east Finland. *Cardiology* 64(1):24-34.

Roberts CJ, Lloyd S. (1972) Association between mortality from ischaemic heart-disease and rainfall in South Wales and in the county boroughs of England and Wales. *Lancet* 1(7760):1091-1093.

Rubenowitz E, Axelsson G, Rylander R. (1996) Magnesium in drinking water and death from acute myocardial infarction. *American Journal of Epidemiology* 143(5):456-462.

Rubenowitz E, Axelsson G, Rylander R. (1998) Magnesium in drinking water and body magnesium status measured using an oral loading test. *Scandinavian Journal of Clinical and Laboratory Investigation* 58(5):423-428.

Rubenowitz E, Axelsson G, Rylander R. (1999) Magnesium and calcium in drinking water and death from acute myocardial infarction in women. *Epidemiology* 10(1):31-6.

Rubenowitz E, Motin I, Axelsson G, *et al.* (2000) Magnesium in drinking water in relation to morbidity and mortality from acute myocardial infarction. *Epidemiology* 11(4):416-421.

Rubenowitz-Lundin E, Hiscock K. Chapter 13 Water hardness and health effects. *Principles of Medical Geology (In print)*, 2005.

Ruston D, Hoare J, Henderson L, *et al.*(2004) The National Diet and Nutrition Survey: adults aged 19 to 64 years.

Rylander R, Bonevik H, Rubenowitz E. (1991) Magnesium and calcium in drinking water and cardiovascular mortality. *Scandinavian Journal of Work, Environment and Health* 17(2):91-94.

Rylander R. (1996) Environmental magnesium deficiency as a cardiovascular risk factor. *Journal of Cardiovascular Risk* 3(1):4-10.

Rylander R., Arnaud MJ (2004) Mineral water intake reduces blood pressure among subjects with low urinary magnesium and calcium levels. BMC Public Health 4:56.

Sakamoto N, Shimizu M, Wakabayashi I, *et al.* (1997) Relationship between mortality rate of stomach cancer and cerebrovascular disease and concentrations of magnesium and calcium in well water in Hyogo prefecture. *Magnesium Research* 10(3):215-223.

Sauvant MP, Pepin D. (2000) Geopraphic variation of the mortality from cardiovascular disease and drinking water in a french small area (Puy de Dome). *Environmental Research* 84(3):219-227.

Sauvant MP, Pepin D. (2002) Drinking water and cardiovascular disease. *Food and Chemical Toxicology* 40(10):1311-1325.

Schoppen S, Perez-Granados AM, Carbajal A, *et al.* (2004) A sodium-rich carbonated mineral water reduces cardiovascular risk in postmenopausal women. *Journal of Nutrition* 134(5):1058-1063.

Schroeder H. (1960a) Relationship between mortality from cardiovascular disease and treated water supplies. Variations in states and 163 largest municipalities of the United States. *Journal of the American Medical Association* 172:1902-1908.

Schroeder H. (1960b) Relations between hardness of water and death rates from certain chronic and degenerative diseases in the United States. *Journal of Chronic Diseases* 12:586-591.

Schroeder HA. (1969) The water factor. *New England Journal of Medicine* 280(15):836-8.

Schroeder HA, Kraemer LA. (1974) Cardiovascular mortality, municipal water, and

corrosion. *Archives of Environmental Health* 28(6):303-311.

Shaper AG, Packham RF, Pocock SJ. (1980) The British Regional Heart Study: Cardiovascular mortality and water quality. *Journal of Environmental Pathology and Toxicology* 4(2-3):89-111.

Shaper AG, Pocock SJ, Packham RF, *et al.* (1983) Softness of drinking water and cardiovascular disease - practical implications of recent research. *Health Trends* 15:22-24.

Shaper AG. (1984) Geographic variations in cardiovascular mortality in Great Britain. *British Medical Bulletin* 40(4):366-373.

Sharrett AR, Feinleib M. (1975) Water constituents and trace elements in relation to cardiovascular diseases. *Preventive Medicine* 4(1):20-36.

Sharrett AR. (1979) The role of chemical constituents of drinking water in cardiovascular diseases. *American Journal of Epidemiology* 110(4):401-419.

Sharrett AR. (1981) Water Hardness and Cardiovascular-Disease. *Circulation* 63(1):A247-A250.

Sherer Y, Shaish A, Levkovitz H, *et al.* (1999) Magnesium fortification of drinking water suppresses atherogenesis in male LDL-receptor-deficient mice. *Pathobiology* 67(4):207-213.

Sherer Y, Shoenfeld Y, Shaish A, *et al.* (2000) Suppression of atherogenesis in female low-density lipoprotein receptor knockout mice following magnesium fortification of drinking water: the importance of diet. *Pathobiology* 68(2):93-98.

Smith WCS, Crombie IK. (1987) Coronary heart disease and water hardness in Scotland - Is there a relationship? *Journal of Epidemiology and Community Health* 41(3):227-228.

Sparrow D, Sharrett AR, Garvey AJ, *et al.* (1984) Trace metals in drinking water: Lack of influence on blood pressure. *Journal of Chronic Diseases* 37(1):59-65.

Stitt FW, Crawford MD, Clayton DG, *et al.* (1973) Clinical and biochemical indicators of cardiovascular disease among men living in hard and soft water areas. *Lancet* 1(7795):122-126.

Stocks P. (1973) Mortality from cancer and cardiovascular diseases in the county boroughs of England and Wales classified according to the sources and hardness of their water supplies, 1958-1967. *Journal of Hygiene* 71(2):237-252.

Tukiendorf A, Rybak Z. (2004) New data on ecological analysis of possible relationship between magnesium in drinking water and liver cancer. *Magnesium Research* 17(1):46-52.

Tyroler H. (2000) Coronary heart disease epidemiology in the 21st century. *Epidemiologic Reviews* 22(1):7-13.

Van Staden DA. (1990) Absence of cardiovascular disease in a rural community using soft water. *South African Medical Journal* 78(4):219.

Voors AW. (1971) Minerals in the municipal water and atherosclerotic heart death. *American Journal of Epidemiology* 93(4):259-66.

Wells G, Shea B, O'Connell D, *et al.* (2001) The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analysis. *http://www.lri.ca/programs/ceu/oxford.htm*.

West RR. (1977) Geographical variation mortality from ischaemic heart disease in England and Wales. *British Journal of Preventive and Social Medicine* 31(4):245-250.

WHO (1979) Health effects of the removal of substances occurring naturally in drinking water, with special reference to demineralized and desalinated water. Report on a Working Group. *EURO Reports and Studies 16*. Copenhagen.

Wolf R, Cauley J, Baker C, *et al.* (2000) Factors associated with calcium absorption efficiency in pre- and perimenopausal women. *American Journal of Clinical Nutrition* 72(2):466-471.

Yang CY, Chin JF, Chiu HF, *et al.* (1996) Relationship between water hardness and coronary mortality in Taiwan. *Journal of Toxicology and Environmental Health* 49(1):1-9.

Yang CY, Chiu HF, Chiu JF, *et al.* (1997a) Calcium and magnesium in drinking water and risk of death from colon cancer. *Japanese Journal of Cancer Research* 88(10):928-933.

Yang CY, Chiu HF, Chiu JF, *et al.* (1997b) Gastric cancer mortality and drinking water qualities in Taiwan. *Archives of Environmental Contamination and Toxicology* 33(3):336-340.

Yang CY. (1998a) Calcium and magnesium in drinking water and risk of death from cerebrovascular disease. *Stroke* 29(2):411-414.

Yang CY, Hung CF. (1998b) Colon cancer mortality and total hardness levels in Taiwan's drinking water. *Archives of Environmental Contamination and Toxicology* 35(1):148-151.

Yang CY, Cheng MF, Tsai SS, *et al.* (1998c) Calcium, magnesium, and nitrate in drinking water and gastric cancer mortality. *Japanese Journal of Cancer*

*Research* 89(2):124-130.

Yang CY, Chu HF. (1998d) Calcium and magnesium in drinking water and risk of death from rectal cancer. *International Journal of Cancer* 77(4):528-532.

Yang CY. (1999a) Relationship between trace metal concentrations and hardness in drinking water in Taiwan. *Bulletin of Environmental Contamination and Toxicology* 63(1):59-64.

Yang CY, Chiu HF. (1999b) Calcium and magnesium in drinking water and the risk of death from hypertension. *American Journal of Hypertension* 12:894-899.

Yang CY, Chiu HF, Cheng MF, *et al.* (1999c) Pancreatic cancer mortality and total hardness levels in Taiwan's drinking water. *Journal of Toxicology and Environmental Health-Part A* 56(5):361-369.

Yang CY, Tsai SS, Lai TC, *et al.* (1999d) Rectal cancer mortality and total hardness levels in Taiwan's drinking water. *Environmental Research* 80(4):311-316.

Yang CY, Chiu HF, Cheng MF, *et al.* (1999e) Esophageal cancer mortality and total hardness levels in Taiwan's drinking water. *Environmental Research* 81(4):302-308.

Yang CY, Chiu HF, Cheng BH, *et al.* (2000a) Calcium and magnesium in drinking water and the risk of death from breast cancer. *Journal of Toxicology and Environmental Health-Part A* 60(4):231-241.

Yang CY, Chiu HF, Tsai SS, *et al.* (2000b) Calcium and magnesium in drinking water and risk of death from prostate cancer. *Journal of Toxicology and Environmental Health-Part A* 60(1):17-26.

Yang CY, Chiu HF, Tsai SS, *et al.* (2002a) Magnesium and calcium in drinking water and the risk of death from esophageal cancer. *Magnesium Research* 15(3-4):215-222.

Yang CY, Chiu HF, Chang CC, *et al.* (2002b) Association of very low birth weight with calcium levels in drinking water. *Environmental Research* 89(3):189-194.

Zeighami EA, Watson AP, Craun GF. (1990) Chlorination, water hardness and serum cholesterol in forty-six Wisconsin communities. *International Journal of Epidemiology* 19(1):49-58.

Zhao HX, Mold MD, Stenhouse EA, *et al.* (2001) Drinking water composition and childhood-onset Type 1 diabetes mellitus in Devon and Cornwall, England. *Diabetic Medicine* 18(9):709-717.

Zielhuis RL, Haring BJ. (1981) Water hardness and mortality in the Netherlands. *Science of the Total Environment* 18:35-45.