Chemoprevention and chemoprotection:
The role of dietary intervention and how to measure its effects

Report of a workshop held on 13–14 June 2000

MRC Institute for Environment and Health

FORA 4

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Preface

This report is based on a workshop which was convened as part of Food Standards Agency-sponsored Food Risk Assessment (FORA) project — a collaborative research programme between the MRC Toxicology Unit and the MRC Institute for Environment and Health in Leicester and the MRC Dunn Human Nutrition Unit in Cambridge. The programme aims to consider and conduct research into fundamental and applied approaches to food risk assessment, most specifically in the context of diet and cancer.

Workshop participants are listed at the end of the report. Many thanks are due to all the workshop participants and especially to the speakers and discussion leaders for their valuable contributions.

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Contents

Executive summary

This report covers the fourth of a series of Food Risk Assessment (FORA) workshops sponsored by the Food Standards Agency and organised by the Institute for Environment and Health. It brings together the various strands of the FORA programme, draws together knowledge about biomarkers related to cancer, and reviews current understanding about how the impact of diet and dietary intervention on cancer, particularly colorectal cancer, can be assessed.

Some of the risk factors for cancer have been proposed or recognised for decades. In particular, many epidemiological studies have linked increased cancer incidences at several sites, especially colorectal cancer, with decreased fruit and vegetable consumption, and vice versa, leading to suggestions for dietary intervention to protect against cancer. However, in contrast to the situation with cardiovascular disease, where many of the risk factors have been established, the scientific basis for chemoprevention of cancer is not well advanced. It is hoped that useful experience may be gained and applied to cancer chemoprevention by comparing the current states of knowledge in the cancer and cardiovascular fields, as is done in this report.

The overall objectives of the workshop on which this report is based were to evaluate the availability and the possible development of biomarkers that are sufficiently predictive of disease outcome (particularly cancer and especially colorectal cancer) and responsive to dietary intervention to be useful tools in measuring the effects of dietary intervention.

To this end, experts in toxicological mechanisms, molecular epidemiology, epidemiology and clinical practice of both cancer and cardiovascular disease were brought together to assess current knowledge about the use of biomarkers, to determine the impact of diet on cancer and to make proposals to improve the measurement of the effectiveness of dietary intervention in cancer chemoprevention and chemoprotection.

The report of the workshop reaches the following conclusions.

- Cancer is perhaps less amenable to intervention and chemoprevention than is cardiovascular disease, as the latter comprises essentially few discrete conditions, whereas there are many forms of cancer, each probably arising via a different pathway (or pathways) and each requiring a different approach to treatment or intervention. Nonetheless, encouragement to continue to search for more effective cancer chemoprevention strategies can be drawn from the fact that, like many current studies on cancer chemoprevention, early studies on intervention to reduce coronary heart disease were also not particularly successful. One possible similarity between the two conditions is that signs of arterial disease, at least to some extent, would be expected to be evident in the whole population, if investigated, and it is possible that the same would be true for early signs of carcinogenesis; for both diseases, strategies to delay disease progression could be as effective as (or more effective than) prevention of disease onset.

- One notable difference between the application of intervention strategies to reduce cardiovascular disease and strategies to modify cancer is the feedback that is available, through the use of biomarkers for the disease, to assess the effectiveness of treatment in the case of cardiovascular disease. Such feedback is important to reinforce intervention strategies and to encourage people to take up and maintain intervention strategies that may require considerable commitment, for example to modify lifestyle, change dietary habits or take medication consistently. Thus it can be expected

that, as has been the case in treatment for cardiovascular disease, cancer intervention will benefit from the development of readily usable biomarkers to monitor the effectiveness of intervention at an individual as well as a population level.

- Currently more is known about the mechanism and process of cardiovascular disease than is known about carcinogenesis. Understanding of the disease mechanism has facilitated the development of biomarkers that are essentially risk factors for cardiovascular disease. Thus understanding mechanisms, both of carcinogenesis and of potential cancer chemoprevention strategies, is of great importance for the development of biomarkers to assess the effectiveness of intervention. Although it has to be recognised that biomarkers for cancer may not necessarily be risk factors for the disease.

- As an initial step in setting up future studies to assess the effectiveness of dietary intervention to protect against colorectal cancer in particular, the development of output markers (i.e. markers associated with the disease process) is likely to be the most useful. Without these the impact of diet can still only be assessed either by waiting for tumours to appear, which takes too long to be of much practical use, or by screening biological samples and seeking to match biomarkers with dietary constituents or types of diet or with eventual tumours in cases.

A number of possible future research directions are proposed.

- Biomarkers for monitoring dietary intervention (either through animal studies or, if possible, through human population studies) and biomarkers of susceptibility (which will usually be genetic markers) should continue to be identified and developed. However, development of such biomarkers requires a long-term research effort. Currently samples taken during the European Prospective Investigation of Cancer (EPIC) studies are being used for this purpose.

- Future research should focus first on identifying and developing reliable output biomarkers for cancer, that is markers of the disease process that are closely associated with the disease endpoint, and preferably markers of early reversible stages of the disease process. For colorectal cancer, studies should focus on the identification of markers of premalignant lesions. The development of output biomarkers will depend, to some extent, on increased understanding of mechanisms. Input or exposure biomarkers, that is markers of, for example, dose or dietary components, should also be developed to monitor how effectively intervention strategies are being adopted. It will be important to establish how input and output markers are linked mechanistically, to be sure that both are measuring different aspects of the same intervention strategy and disease process.

- In the short term, cancer intervention studies should not be undertaken if only input (or exposure) biomarkers are available; suitable output biomarkers should always be used.

- As is the case with cardiovascular disease, markers that could provide immediate objective feedback on the effectiveness of intervention, whether at the general population level or in high-risk individuals, should be developed, especially for clinical applications.

- It is probable that several biomarkers need to be developed and used rather than just one for each outcome; the development of profiles of markers can be envisaged, especially using new genomic techniques, that would be of benefit even at the individual level.

1 General introduction

The Food Standards Agency-sponsored Food Risk Assessment (FORA) project is a collaborative research programme between the MRC Toxicology Unit and the MRC Institute for Environment and Health in Leicester and the MRC Dunn Human Nutrition Unit in Cambridge. The aims of the programme are to consider and conduct research into fundamental and applied approaches to food risk assessment, most specifically in the context of diet and cancer.

This is a report on the fourth of a series of FORA workshops. Reports of three other workshops, on *Probabilistic Approaches to Food Risk Assessment, Energy Metabolism and Carcinogenesis* and *Diet-gene Interactions: Characterisation of Risk,* are published separately (IEH, 2000a,b, 2001).

This report of the final FORA workshop brings together the various strands of the FORA programme and draws together knowledge about biomarkers related to cancer, particularly colorectal cancer, to see what has been achieved and whether it is yet possible to promote advice to the public on risk reduction measures and to suggest further avenues for research.

The overall objectives of the workshop and this report have been to evaluate the availability and the possible development of biomarkers that are sufficiently —

- predictive of disease outcome (particularly cancer and especially colorectal cancer) and
- responsive to dietary intervention

to be useful tools in measuring the effects of dietary intervention.

Discussions at the workshop focused on:

- the current state of knowledge on the role of chemoprevention and dietary intervention to protect against cancer (particularly colorectal cancer) and cardiovascular disease (the latter as an example of a field in which intervention is currently well developed);
- ways to improve the evaluation of dietary intervention;
- possible future research to underpin the development and use of biomarkers to assess dietary intervention; and
- how the use of biomarkers could contribute to decisions about the benefits of dietary intervention to protect against disease, including cancer.

Short summary papers prepared for the workshop by invited speakers are presented in Sections 2 and 3; these were pre-circulated to the workshop participants and provided a basis for discussions. A summary of the discussions at the workshop is given in Section 4, and the conclusions reached together with recommendations for the future are presented in Section 5. A view for the future of the food risk assessment programme is outlined in Section 6.

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IEH (2001) *Diet-gene Interactions: Characterisation of Risk* (FORA 3), Leicester, UK, MRC Institute for Environment and Health

2 Intervention: The successes and the problems

2.1 Chemoprevention and chemoprotection in humans

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The idea that diseases such as cardiovascular disease and cancer can be prevented by taking a simple 'pill' rather than by behavioural change has obvious appeal to many people. Chemoprevention by pharmaceuticals is an established method in the primary and secondary prevention of cardiovascular diseases such as myocardial infarction and stroke. Clinical trials have demonstrated beyond reasonable doubt that both fatal and non-fatal coronary events and strokes can be prevented or the risk of these occurring reduced. Statins are commonly used to lower blood cholesterol concentration, and aspirin is widely used to prevent occlusive vascular diseases. Aspirin and other non-steroidal anti-inflammatory drugs have also shown promise in the chemoprevention of colorectal cancer (IARC, 1997). For two decades or so much observational evidence heralded the use of 'antioxidants' and, notably, β-carotene as a safe, simple panacea for the prevention of coronary heart disease and cancer, only for the latter recently to fail a series of critical tests in human intervention trials (IARC, 1998; Albanes, 1999; Albanes *et al.*, 2000). Recently, disappointing negative results have also been published on the ability of dietary fibre to prevent colorectal adenomas after three to four years of daily intervention (Alberts *et al.*, 2000; Schatzkin *et al.*, 2000). Supplementation with vitamin C, vitamin E, or β-carotene has also not shown any effect in this adenoma recurrence study design (for discussion see Byers, 2000).

Although this is disappointing, lessons have been learned. Both cancer and cardiovascular diseases are difficult to cure, and for a half century their occurrence has been known to vary according to environment and lifestyle. For these reasons one might have expected that both disease groups would to the same extent have generated the inspiration to seek prevention by chemical agents. This is, however, not the case. Risk factors of cardiovascular disease have been known for over 50 years and prevention by pharmaceuticals has been used widely for some ten years. Chemoprevention of cancer is a much more recent phenomenon than chemoprevention of coronary heart disease, even though both diseases are fatal and have a large impact on public health.

Chemoprevention by pharmaceuticals is today an established method for the prevention of myocardial infarction and stroke. It has played an important role in reducing the incidence and mortality of cardiovascular disease world-wide. Chemoprevention of cancer has thus far had only modest achievements. Chemoprevention for some other serious and common health effects, such as osteoporosis, is only now beginning.

Although the chemoprevention of cancer is decades behind that of cardiovascular disease, there is no reason, *a priori*, to believe that progress in cancer chemoprevention will differ substantially from that in cardiovascular disease. Better understanding of the molecular steps critical to carcinogenesis should open new avenues for cancer chemoprevention. Since controlled clinical trials are the cornerstone in developing chemoprevention in practice, an examination of the inherent differences in the preconditions for trials of chemoprevention of cancers and cardiovascular diseases may help to forecast the future of cancer chemoprevention.

The bases of trials for cardiovascular disease are more straightforward than those for cancer, as for the former there are few possible endpoints, mainly myocardial infarct and stroke, whereas there are numerous malignancies. For cancers, real at-risk groups are difficult to find in a population for recruitment into a trial; for heart disease, smoking, arterial blood pressure and serum lipids are relatively easy to study in a population. Any specific cancer is less frequent than myocardial infarction or stroke in most populations. This means that controlled cancer prevention trials must be even larger and more expensive than trials for cardiovascular diseases. In cases of heart disease and stroke, chemoprevention rapidly changes blood pressure or blood lipid concentration, and many good surrogate measures of the clinical disease are available. This helps in selecting the most efficient chemical agents and suitable doses in a series of small studies prior to designing large primary prevention trials of myocardial infarction or stroke. Risk factors and surrogate endpoints of cancers have not been similarly available for measurable chemical modulations in cancer prevention.

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2.2 Cardiovascular risk factors, including dietary risk factors, and the use of biomarkers in measuring risk

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Introduction

Coronary heart disease remains the major cause of death in both men and women in the UK, accounting for about 150 000 deaths per year in England (26% of all deaths). Around one-third of these are of women. Coronary heart disease kills approximately three times more women than breast cancer, although the latter is the major cause of death in pre-menopausal women).

Atheroma is almost ubiquitous in the Western world, affecting virtually all adults to some degree (Adams & Celermajer, 1999). Arterial lesions are present from early childhood, with up to half of children aged 10–14 years having fatty streaks in their coronary arteries. Small atheromatous plaques are found in teenagers and young adults, with more complicated lesions evident by early middle age.

The major risk factors for coronary heart disease are smoking, dyslipidaemia, diabetes and hypertension, each increasing the relative risk of the disease by between three- and fivefold. As far as lipid factors are concerned, the association between cholesterol and coronary heart disease was described almost 100 years ago, and that between the variation of coronary heart disease mortality and dietary fat intake was clearly shown by Ancel and Keys in the Seven Countries Study (Keys, 1970).

There has been too great an emphasis on cholesterol as the most important lipid risk factor for coronary heart disease and on familial hypercholesterolaemia as a major genetic risk factor (the gene frequency for familial hypercholesterolaemia is around 1:500). Although there is a linear relationship between serum cholesterol and coronary heart disease risk, there is almost complete overlap in cholesterol levels in men with and without the disease. Therefore, although serum cholesterol can be used as a marker of risk and in setting treatment targets in patients with established coronary heart disease, its value in screening the population for the disease is limited.

The importance of other, minor, risk factors (homocysteine, fibrinogen, lipoprotein (a), C-reactive protein) is as yet unclear.

In recent years it has become clear that a number of risk factors for coronary heart disease tend to cluster together as part of a syndrome — the Insulin Resistance or Metabolic Syndrome — which, in addition to insulin resistance and carbohydrate intolerance, includes hypertension, a characteristic dyslipidaemia (raised triacylglycerol and low high-density lipoprotein (HDL)-cholesterol but unremarkable total cholesterol), central (abdominal) obesity, hyperuricaemia and a procoagulant state (increased plasminogen activator inhibitor-1 (PAI-1) and factor VII). This syndrome is probably the major risk factor for coronary heart disease in the Western world and, more importantly, in the developing world, where the prevalence of the syndrome may be as high as 50% in some communities (Haffner *et al.,* 1998; Haffner, 2000). It is likely that a number of factors are involved in the pathogenesis of the syndrome. These include physical inactivity, fetal undernutrition and genetic factors (no single gene defect has been identified as a cause of the syndrome; candidate genes include those for β_3-adrenergic receptor, hormone-sensitive lipase, lipoprotein lipase, insulin receptor substrate-1 (IRS-1), glycoprotein PC-1 and skeletal muscle glycogen synthase (Groop, 2000)). In addition, nutritional factors (high levels of saturated fat and, possibly, certain carbohydrates, and low levels of ω-3 polyunsaturated fat) may be involved in the pathogenesis of the syndrome. Recognition of the syndrome is largely based on clinical criteria; screening based on measurement of lipid and haematological parameters, for example, is impractical, but simple markers such as waist:hip ratio (or even simple waist measurement) have been shown to be useful.

Risk factors as biomarkers of coronary heart disease

In view of their power in determining coronary heart disease incidence, risk factors for the disease are widely used as biomarkers. They have the advantage of being sufficiently frequent, easily measurable and quantifiable and, to a large extent, modifiable. However, despite being powerful in predictive terms, they provide little information about the level of activity of the underlying disease process. There has been increasing interest in recent years in markers of endothelial dysfunction and plaque stability — important factors in determining coronary events. These include markers of large vessel function (e.g. flow-mediated dilatation),

measures of arterial structure (using techniques such as ultrasound, electron beam imaging and magnetic resonance imaging (MRI)), and biochemical markers of endothelial damage and inflammation (e.g. adhesion molecules such as ICAM-1; thrombomodulin and endothelin-1; and C-reactive protein). The coronary arteries themselves are, of course, relatively inaccessible, but it has been shown that changes in peripheral large vessels such as the brachial artery correlate closely with (and can be used as surrogates for) measures of endothelial dysfunction and disease severity in the coronary arteries. Such markers are valuable not only in determining risk in individuals with known coronary heart disease, but also in assessing response to treatment with, for example, pharmacological, hormonal and antioxidant agents. In general, however, they have not proved useful in risk assessment in the larger population, although measurement of C-reactive protein, for example, has shown promise in assessing the activity of the inflammatory process, which is now believed to play a major role in both athero- and thrombogenesis (Danesh *et al*., 2000).

Genetic markers of coronary heart disease

A large number of genetic defects associated with coronary heart disease have been identified, and there is an active debate about the usefulness of gene markers in clinical practice. The current consensus is that, although they may be useful in individual cases and in family studies, such biomarkers do not yet have a place in routine clinical practice.

The identification of risk factors has made a major contribution to understanding the pathogenesis of coronary heart disease, has identified public health measures that would reduce the incidence of the disease (stopping smoking, exercise, weight control) and has provided a basis for setting of targets. Risk factors have also proved to be valuable biomarkers of risk, and for monitoring and predicting response to treatment. However, in terms of population screening, no biomarker has yet proved sufficiently precise in identifying individuals with a level of risk that would benefit from drug treatment as primary prevention.

Similarly, screening for genetic factors has not proved useful except in certain high-risk groups. On the other hand, as with drug treatment, it is becoming apparent that certain genotypes may determine an individual's response to nutritional intervention (Minihane *et al.*, 2000). The use of genetic biomarkers in this context is likely to be increasingly important in the future.

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2.3 Clinical applications of the use of biomarkers to measure the effects of dietary intervention

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There is an increasing amount of epidemiological and laboratory data that suggests the potential value of a variety of modifications of dietary intake in reducing the risk of malignancy. Unfortunately most of the epidemiological studies rely on retrospective information and have many potential errors. Laboratory models may not accurately reflect outcomes in humans. The only reliable method of testing the preventative role of dietary modification is a clinical trial in humans.

Cancer preventive intervention trials are associated with many difficulties resulting from the fact that the development of neoplasia is a slow, multistep process that takes many years. In addition the incidence of any particular cancer in the general population is relatively low. As a result, studies which use the frequency of neoplasia as an endpoint are very time-consuming and expensive. An insufficient number of subjects or short follow-up periods significantly decrease the power of these studies, making it impossible to detect real but small protective effects. An alternative trial design involves the use of biomarkers of abnormal cell growth and maturation, which can be measured in subjects entered into a trial so that early indications can be obtained as to whether an intervention is likely to reduce cancer risk. These biomarkers should be the result of actual events occurring in the initiation and promotional phases of tumour development in order to be optimum candidates for modification by the dietary intervention under study. Such intermediate biomarkers can also be tested for their role in identifying populations at increased risk of developing cancer.

Promising intermediate biomarkers of epithelial neoplasms include genomic markers, biochemical markers, proliferation markers, differentiation markers, growth regulatory factors and cellular or structural abnormalities. Examples of genomic markers are nuclear aberrations (e.g. micronuclei), carcinogen–DNA adducts, abnormal DNA content (e.g. polyploidy), oncogene activation, tumour suppressor gene inactivation, and altered DNA methylation. An increasing number of biochemical markers are being developed and include prostaglandin E2, protein tyrosine kinase, tissue inhibitors of metalloproteinase (TIMP), ornithine decarboxylase and polyamines. Proliferation markers can involve measurement of mitotic frequency, thymidine labelling index, ornithine decarboxylase, and polyamine levels. Differentiation markers include levels of keratinisation, specific cytokeratins, *trans* glutamase type I and blood group antigens. Epidermal and transforming growth factor expression are examples of growth-regulatory factors which may be utilised.

The use of these markers may provide a bridge between promising preclinical and epidemiological data and full-scale, long-term clinical trials. Although no biomarker has yet been validated in the context of a clinical cancer intervention study with invasive cancer as the endpoint, there are several biological markers that are specific in the target organs. A good example is aberrant crypt foci, which are precursor lesions for colon cancer and which may be followed during interventions for this disease (Rao *et al.,* 1999).

As the understanding of the process of carcinogenesis in different tissues increases, and as the number of potentially valuable intervention strategies which may reduce cancer risk rises, it is essential that intermediate biomarkers are developed. These must be validated for their accuracy in correlating with cancer development so that their measurement will indicate the potential for an intervention to be clinically valuable. This will reduce the need to recruit large numbers of individuals into a trial which will take years to complete. Ideally such biomarkers should be measurable in readily available clinical samples, such as blood. Unfortunately many markers currently proposed use tissue specimens, which reduces ease of access and patient acceptability and increases cost. Hopefully a variety of easily measured markers which closely correlate with stages in carcinogenesis can be identified and used in a large number of robust clinical trials which include relatively few individuals. It is only with such a development that the many promising new ideas for methods of lowering cancer incidence by modifications of dietary intake can be properly tested.

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3 Biomarkers to measure dietary intervention, especially for colorectal cancer

3.1 Risk factors in the aetiology of colorectal cancer

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Epidemiology

Colorectal cancer is the second most common cancer in many societies, affecting up to 6% of men and women by the age of 75. There is a 15-fold range in age standardised incidence throughout the world; countries with the highest risk include Australia, New Zealand, the USA and parts of Northern Europe, and those with the lowest risk include rural Africa, China and India (IARC, 1997). International and regional studies have shown that there are strong positive associations between fat and meat consumption and large bowel cancer, and strong negative associations with starch and vegetable consumption (Armstrong & Doll, 1975; Bingham, 1996). Up to 80% of colorectal cancer has been attributed to diet (Willet, 1995).

The majority of case–control studies, conducted in widely varying circumstances and populations, including the USA, Japan, Canada, Australia, France and Belgium, have shown that there is an increased risk of colorectal cancer in individuals who report consuming more meat, protein and fat, and for those reporting a decreased consumption of fibre or fibre-containing foods, including vegetables (World Cancer Research Fund, 1997; Department of Health 1998).

Lower rates for cancer would be expected in vegetarians, although in a meta-analysis of five cohorts, non-meat eaters were not at lower risk than meat eaters. However, in the largest of the cohorts studied, meat was associated with increased risk of colorectal cancer (Key *et al.,* 1998; Fraser, 1999).

In general, cohort studies have supported the findings from case–control studies, showing evidence of increased risk of colorectal cancer with 'red' or processed meat, but not with chicken or fish consumption, and with total fat. Vegetables and fibre seem to be protective. However, the magnitudes of the relative risks are low and generally non-significant, in the order of 1–2 for increased risk and 1–0.5 for reduced risk (World Cancer Research Fund, 1997; Department of Health, 1998). This may have arisen from large measurement error introduced into the estimates of dietary exposure, and compares with a relative risk of 15 for smoking and lung cancer.

Intervention studies in colorectal cancer have been confined to testing the effects of supplements of antioxidant vitamins, mostly in order to investigate the ability of these to reduce recurrence of adenomatous polyps, a precursor lesion of colorectal cancer. Those studies that have been reported so far, however, have shown no effect. Supplements of 25 mg β-carotene, 1 g vitamin C or 400 mg of vitamin E, vitamins C and E, or supplements of β-carotene alone in 300 patients studied in Australia did not inhibit polyp recurrence. In Finnish smokers, there was no significant protective effect of β-carotene or vitamin E supplements on the occurrence of colon cancer. Any protective effect of vegetables in colorectal cancer is therefore unlikely to be attributable to antioxidant vitamins (World Cancer Research Fund, 1997; Department of Health, 1998).

Food intervention trials have been limited to supplements of bran, and a low fat diet. In an Australian study, reduction in fat intake was not associated with an overall reduction in polyp numbers, although the combination of a low fat diet and 25 g wheat bran for 4 years did lead to a

significant reduction in the frequency of large adenomas. (World Cancer Research Fund, 1997; Department of Health, 1998). The outcome of further intervention trials with foods (increased vegetables, other polysaccharide supplements) is awaited (Schatzkin *et al.,* 1996).

In men, being overweight is associated with increased risk, and there are reportedly consistent protective effects of increased energy expenditure, assessed from both reported occupational and recreational activity, in case–control and cohort studies (Potter *et al.,* 1994). Although the effect of exercise is usually attributed to reduced transit time of food through the large gut, no such effect has been found in studies in which food intake has been controlled. However, in addition to increased energy intake, exercise is likely to increase consumption of starch and non-starch polysaccharides, which play an important role in colonic function (Bingham, 1996).

Mechanisms

The role of the colonic microbial flora is central to most hypotheses relating diet to the causation of colon cancer. Colonic flora are many and diverse and dependent on residues leaving the small bowel for their nutrition. Carbohydrate, in the form of non-starch polysaccharides, fibre, and some starch and sugars, enters the large bowel and stimulates anaerobic fermentation by the microbial flora. This leads to the production of short chain fatty acids, acetate, propionate, and butyrate, and of gas, and also to an increase in microbial cell mass (biomass). The majority of the carbohydrate that stimulates fermentation is non-starch polysaccharides, but studies in man have shown that a significant amount of starch escapes digestion in the small gut, depending on such factors as the physical form of the food eaten, the granule type, and how it is cooked and processed. This starch, resistant starch, reaches the large bowel where it is also a substrate for fermentation (Bingham, 1996).

Carbohydrate fermentation has a number of implications for protection against large bowel cancer. The stimulation of bacterial growth, together with water binding to residual unfermented non-starch polysaccharide, leads to an increase in stool weight, dilution of colonic contents and faster transit time through the large gut. Long transit time has not been related to large bowel cancer risk, but there is a strong inverse association between high stool weight and colorectal cancer incidence. Low stool weight leads to constipation; this and the use of cathartics are risk factors for colorectal cancer. The association between low stool weight and bowel disease and the linear relationship between non-starch polysaccharide consumption and stool weight, with a 5 g increase for every 1 g of non-starch polysaccharide consumed, is the basis for UK and WHO recommendations for an 18 g population average intake of non-starch polysaccharide, a 50% increase for the UK and most Western populations (World Cancer Research Fund, 1997; Department of Health, 1998).

One of the short chain fatty acids, butyrate, is of particular interest because it is a well recognised anti-proliferative agent, arresting cell growth and inducing differentiation in cell lines. Histone deacetylase is inhibited by butyrate; other short chain fatty acids are much less active in this respect. Changes in chromatin structure lead to alterations in gene expression and accessibility to DNA repair enzymes. Butyrate also induces apoptosis, which may account for its role in reducing proliferation. *In vivo*, rodent studies have shown that luminal butyrate levels are inversely associated with colonic cell proliferation, and positively associated with histone acetylation. However, studies using direct-acting carcinogens (that is, not requiring metabolic activation) in rodents have not shown large amounts of butyrate added to drinking water or food to have a protective effect against carcinogenesis.

The effect of starch on chemical carcinogenesis has not been investigated intensively, and there are few reports in the literature. A large number of studies have shown that bran appears to have a consistently protective effect against chemical carcinogenesis. However, soluble fibres are associated with tumour enhancement (Bingham, 1996).

The association between fat intake and increased bowel cancer risk is generally attributed to the fact that high fat diets increase the concentration of bile acids in the colon. These are converted by the colonic flora to secondary bile acids, one of which, deoxycholic acid, is a known promoter of large bowel cancer, possibly because it is involved in cell signalling via diacylglycerol and protein kinase C. Another reason that diets high in starch and non-starch polysaccharide are thought to be protective is because the production of short chain fatty acids during fermentation leads to a reduction of intestinal pH, when enzymes involved in the formation of secondary bile acids are inhibited. Bile acids are also less soluble and less toxic at low pH. Long chain ω-3 fatty acids, found in fish oils, are, however, thought to be protective, since they are antiproliferative, slowing down the rate of cell division of colonic mucosal cells (Bingham, 1996).

The association between meat consumption and colorectal cancer is usually attributed to the formation of heterocyclic amines in meat when it is cooked. Present estimates of risk from these compounds are rather low when extrapolated from animal carcinogenicity data, but the effect of high fat diets on possible promotion of cancers induced by these compounds and known species differences in levels of adducts formed need to be taken into account (Layton *et al.,* 1995; Turteltaub *et al.,* 1997). Attempts to demonstrate the presence of DNA adducts from these compounds in humans have proved difficult (Schut & Snyderwine, 1999) without recourse to using radiolabelled compounds and detection with accelerator mass spectrometry.

The possibility that meat alters nitrogen metabolism and enhances the production of endogenous promoters and carcinogens within the colon is also attracting increasing attention. Meat increases the amount of nitrogen entering the large bowel, mainly in the form of protein, peptides and amino acids. In humans, these are converted during fermentation to ammonia so that the increase in nitrogen entering the colon as a result of consuming high meat diets increases faecal ammonia concentration. Ammonia is a promoter of carcinogenesis in rodent models, and patients with ureterosigmoidostomies who have very high luminal ammonia concentrations have a greatly increased risk of developing tumours distal to the site of ureteric implantation (Bingham, 1996).

N-nitroso compounds (NOCs) are also found in the colon. They are formed endogenously because the amines and amides produced primarily by bacterial decarboxylation of amino acids can be *N*-nitrosated in the presence of a nitrosating agent. In the 1980s it was thought that faecal samples contain negligible amounts of volatile NOCs, but since that time newer, chemiluminescent, methods of measuring total NOCs have been developed and the presence of NOCs in faeces in animals and man is now well established. An increase in total meat consumption increases faecal NOC levels in a dose-dependent manner, and this effect is not lessened by increasing non-starch polysaccharide or resistant starch intake. The mechanism behind this effect is not certain, but it arises from an increase in nitrosatable material entering the colon in a high protein diet, and possibly an increase in a nitrosating agent, such as nitrous oxide. The precise nature of the NOCs formed has not been determined (Bingham, 1996).

Other dietary factors implicated in protecting against the development of colon cancer include non-nutritive compounds in vegetables, for example the glucosinolates, which are thought to increase levels of enzymes such as glutathione-S-transferase. Vegetables also contain folate, a critical element in methyl group metabolism and thus in thymidine biosynthesis and DNA repair. Free iron is a well accepted catalyst in the Fenton reaction, which yields harmful hydroxyl radicals; it has been proposed that oxidative damage occurs from free iron in the colon, but can be suppressed by the presence of phytic acid, a known chelator of iron. Iron in red meat may also catalyse NOC formation in the colon. Calcium at high intakes (2 g per day) is also proposed to inhibit the toxic effects of free fatty acids entering the large bowel as a consequence of a high fat intake. Results from intervention trials assessing the effects of calcium on proliferation are, however, conflicting.
Vitamin D is classically associated with calcium homeostasis, but a more fundamental role in controlling cell growth and differentiation has more recently emerged, which may be relevant to large bowel cancer (World Cancer Research Fund, 1997; Department of Health, 1998). There are suggestive links between alcohol consumption, particularly beer consumption, and rectal cancer but no conclusive link between alcohol consumption and colon cancer (IARC, 1988).

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3.2 Development of biomarkers to measure the effects of dietary intervention, especially in colorectal cancer

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One of the more challenging areas in food risk assessment is that of measuring effects for which there are no easily accessible indicators of the role of diet. Much of what is known about diet and cancer has been garnered from the use of questionnaires in case–control studies (see Section 3.1). However, many of the complex interactions between hazardous and beneficial food constituents take place within the body and the products of such processes may interact quite differently depending on individual susceptibilities of many kinds. To investigate this, use has been made of biological markers (biomarkers) of processes that are considered to be important in the development of cancer, in particular, the formation of DNA adducts. Two types of DNA damage, one derived from lipid peroxidation and the other from nitrosation of amino acids, were the focus of this study. There are indications that both may be major contributors to damage in DNA.

Malondialdehyde–DNA adducts

Malondialdehyde is the major mutagenic product derived from lipid peroxidation and reacts primarily with DNA to give a guanine adduct (M_1–dG). The details of a sensitive immunoslot-blot (ISB) assay for M_1–dG along with some preliminary results on the effects of diet have been published (Leuratti *et al.,* 1998, 1999). The effects of increasing meat consumption were studied in three volunteers who consumed 0, 60, 240 or 420 g red meat per day as part of an isocaloric diet; carbohydrate was used to substitute for calories from meat. The levels of M_1–dG in blood DNA were increased in all volunteers eating meat but in a non-dose-dependent manner. However, in a subsequent study in our laboratory it was observed that the levels of adducts were greatly influenced by the way in which the meat was prepared. For example, beef eaten as steak and chips resulted in higher levels of M_1–dG than when the same amount was consumed as a lasagne. Volunteers who were maintained on a diet containing a constant amount of meat tended to have stable levels of M_1–dG adducts when followed over a 42-day period.

M_1–dG adducts in colorectal tissue

The Flexi-Scope Trial is a multicentre study carried out in the UK, with the primary aim of investigating whether 'once only' flexisigmoidoscopy (Flexi-Scope) with removal of all detected polyps will prevent or decrease morbidity and mortality from colorectal cancer. Participants are disease-free males and females, aged 55–64. The Norfolk branch of the study is unique in that one-third of the participants also took part in the European Prospective Investigation on Cancer (EPIC) study and therefore their dietary, health and lifestyle data were collected through detailed questionnaires and health checks. During Flexi-Scope screening, colorectal biopsies were collected from EPIC participants with polyps and from matching controls. In the present study biopsy DNA samples were analysed for the presence of M_1–dG adducts using the ISB assay. DNA was extracted from all biopsies received (n = 169). The results suggested a wide inter-individual variation in adduct levels. The total average adduct level was $4.35 \pm 2.99 \times 10^7$ (n = 169), ranging from not detected (13 samples) to 12.2×10^7 adducts. Mean M_1–dG levels of 4.54 ± 2.86 and $4.28 \pm 3.02 \times 10^7$ were detected in females and males, respectively (both cases and controls). Statistical analysis of the data is being carried out.

O^6-Carboxymethyl-dG adducts

Glycine is the simplest α-amino acid and is among the most abundant of the amino acids in dietary proteins. Free glycine occurs in biological fluids at millimolar concentrations (Komorowska *et al.,* 1981); it can be converted to an alkylating agent by nitrosation in the gut. In several studies of DNA damage by nitrosated glycine derivatives the predicted formation of carboxymethyl adducts (N^7-carboxymethylguanine (7CMG), N^3-carboxymethyladenine and O^6-carboxymethylguanine (O^6-CMG) had been observed (Zurlo *et al.,* 1982; Shuker e*t al.,* 1987; Harrison *et al.,* 1997). However, on closer examination it became clear that methylation was also occurring (Shuker & Margison, 1997; Harrison *et al.,* 1999). This latter observation is particularly interesting in view of the number of observations of elevated O^6-methylguanine (O^6-MeG) in DNA from the human gastrointestinal tract being associated with increased risk of cancer (Saffhill *et al.,* 1987; Hall *et al.,* 1991; Jackson *et al.,* 1996). The formation of characteristic carboxymethyl adducts by nitrosated glycine derivatives affords an approach to evaluating their contribution to the overall burden of diet-related N-nitroso compound (NOC) exposures in man. O^6-CMG appears to be

resistant to the action of O^6-alkylguanine transferase (AGT) and would be expected to persist, therefore constituting a good candidate biomarker in DNA. A sensitive ISB method has been developed for O^6-CMG in intact DNA with a detection limit of 15 adducts/10^8 unmodified bases using 1 μg of DNA. In recent studies in our laboratory, examination of human DNA extracted from blood or gastric biopsies from patients attending a gastroenterology clinic revealed the presence of O^6-CMG at levels of up to 67.8 adducts/10^8 unmodified bases.

The presence of specific diet-related DNA damage, as described above, in the human gastrointestinal tract, which can be reliably quantified, provides the basis for the development of biomarkers that can be used for intervention studies. In population studies it would be preferable to use markers that can be detected in blood rather than in biopsy specimens and it is therefore important to establish the extent to which systemic levels of DNA damage reflect the levels in target organs. The role of DNA adducts as markers of risk is still being elucidated but there is little dispute that they provide some information on exposure. In classical dose–response terms a reduction in exposure to a toxic agent would normally be expected to be accompanied by a reduction in adverse effect(s). In carcinogenesis the causal connection between exposure(s) and outcome has often been difficult to establish, primarily because of the long time which elapses between initiation and the appearance of the tumour. The corollary of this is that the effect of reducing exposures (detected directly or through the use of a biomarker) is not likely to be accompanied by a readily detectable health effect in the short term. At the moment there appears to be no easy solution to this dilemma. However, if ongoing prospective studies provide evidence for the role of DNA alkylation in cancer risk then biomarkers of this damage may become valuable tools in dietary intervention studies for colorectal cancer.

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3.3 Use of knowledge about mechanisms in the further development of biomarkers of dietary intervention

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Understanding the mechanisms both of disease and of protection is critical in biomarker design for the future and in determining their utility and relevance for prevention or treatment of disease.

Prevention or treatment

When considering dietary intervention in relation to cancer, it is crucial to make a clear distinction between cancer prevention and cancer treatment. Prevention requires biomarkers related to the critical and multiple early stages in carcinogenesis, whereas treatment will benefit from biomarkers of the tumour phenotype indicative of, for example, invasiveness and growth characteristics. Such phenotypic parameters, including expression of oestrogen receptors, p53, Bcl-2 and HER-2 neu, have been proposed for this purpose in relation to development of carcinoma from pre-malignant breast lesions (Stoll, 1999). Dietary intervention might focus on the limiting step in the disease process so as to delay the onset of disease, or it may concentrate on inhibition of a critical early event. Is the rate limiting step in colon carcinogenesis a late event associated with clonal expansion or an early event of initiation? Effective modes of dietary intervention might be different for different ages. Thus, anti-promotional effects late in life may provide sufficient delay of carcinogenesis, whereas protection against genotoxicity may be more important in the young.

Potential mechanisms

Dietary interventions protecting against chemical carcinogenesis in animal models have been seen to operate at different stages. The mechanisms involved might include inhibition of carcinogen activation, enhanced metabolic detoxication, enhanced DNA repair, and protection against clonal expansion (e.g. reduced proliferative or survival advantage, tumour growth inhibition, enhanced immune surveillance). Biomarkers are required to assess the impact of diet on each of these stages. As an example of the differing impacts of diet, ω-6 polyunsaturated fatty acids can stimulate both oxidative DNA damage and cell proliferation; however, the ω-3 fatty acids can inhibit cell proliferation through interference with signal transduction (Bartsch *et al.,* 1999). There is a particular need for more information on the measurement of dietary factors influencing cell proliferation and apoptosis in target tissues. One study found that mucosal proliferation in rectal biopsy specimens did not have a consistent association with diet (Keku *et al.,* 1998). However, Holt *et al.* (1998) found that an increase of dietary calcium reduced proliferative activity of colonic epithelial cells and restored markers of normal cell differentiation. There is evidence that in some cases of chemical carcinogenesis 'initiated' cells have a selective growth advantage. Assessment of dietary influence on the normal cell population may thus be misleading; understanding differential effects of substances on normal and pre-neoplastic cells becomes important. Consider, for example, a dietary component which elevates apoptosis in cells with abnormally elevated Bcl-2 expression. This may be crucial in chemoprevention but may not be a detectable response in normal cells. It is necessary to identify and isolate cells in preneoplastic stages for comparisons of effects on cell death and survival to be made. In this context, wheat bran (possibly through butyrate production) increased apoptosis and controlled proliferation during colon tumour initiation with azoxymethane in rats (Compher *et al.,* 1999).

Many putative chemopreventative agents have a multitude of potentially beneficial effects. Understanding mechanisms and dose–response relationships will aid in identification of those effects that are relevant to the *in vivo* concentrations achieved. This will be demonstrated in relation to the pleiotropic effects of isoflavonoids (e.g. Harper *et al.,* 1999). Biomarkers must be representative of both beneficial and adverse effects, so that the beneficial window of dose of a chemopreventative agent can be established and nutrition can be optimised rather than dietary components be labelled as 'good' or 'bad' (Milner, 1999).

DNA damage and mutation

Substantial advances have been made in developing biomarkers of DNA damage, such as the sensitive detection of DNA adducts, but relatively noninvasive methods are required for target tissue analyses. Regarding intestinal carcinogenesis, the use of *in vivo* 'mutagen traps' (Bingham *et al.,* 1992), faecal mutagen analyses and mutational analysis (of oncogenes and tumour suppressor genes; Ahlquist *et al.,* 2000) of exfoliated cells show

potential. The analysis of mutational spectra within human tumours and the relationship between specific mutational 'fingerprints' and dietary profiles may also give clues to the identity of causative and protective factors.

Gene–diet interactions

Substantial knowledge has been acquired about genetic polymorphisms (such as in genes encoding detoxification enzymes, DNA repair enzymes or immune system components). Genotyping therefore contributes to an understanding of susceptibility, but many of these proteins and their activities are also influenced by dietary constituents. Consequently susceptibility indicators need to include biomarkers of phenotype so that the combined diet and gene interactions can be considered.

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4 Discussion

4.1 Introduction

The discussion presented herein has been developed, in consultation with several workshop participants, from the discussions taking place at the workshop on *'Chemoprevention and Chemoprotection: The Role of Dietary Intervention and How to Measure its Effects.'* The overall aims of the workshop and this discussion have been to evaluate the availability and the possible development of biomarkers that are sufficiently predictive of disease outcome and responsive to dietary intervention to be useful tools in measuring the effects of dietary intervention to protect against cancer, in particular colorectal cancer. In addressing these aims the workshop participants discussed:

- the current state of knowledge on the role of chemoprevention and dietary intervention to protect against cancer (particularly colorectal cancer) and cardiovascular disease (the latter as an example of a field in which intervention is currently well developed) — discussed in Section 4.2, below;
- ways to improve the evaluation of dietary intervention — discussed in Sections 4.2.3 and 4.2.4;
- possible future research to underpin the development and use of biomarkers to assess dietary intervention — discussed in Section 4.3; and
- how the use of biomarkers could contribute to decisions about the benefits of dietary intervention to protect against disease, including cancer — discussed in Section 4.3.4.

4.2 Intervention

4.2.1 Diet and chemoprotection

As outlined in Sections 2.1 and 2.2, many of the risk factors for some cancers and for cardiovascular disease have been proposed or known for decades. Many epidemiological studies have linked increased cancer incidences at several sites, in particular colorectal cancer, with decreased fruit and vegetable consumption, and vice versa, leading to advice that increasing fruit and vegetable consumption may help protect against cancer.

Chemoprotection strategies based on dietary constituents have been proposed. For example, it was suggested more than 20 years ago that β-carotene in fruit and vegetables might be protective against cancer (Peto *et al.,* 1981), and early observational studies supported a beneficial role for β-carotene (reviewed in IARC, 1998). However, results from the earlier observational studies have not been confirmed in recent intervention trials. The progress of intervention trials on the effectiveness of β-carotene as a chemoprotective agent is particularly informative and provides a salutary lesson about the constraints and requirements for testing natural compounds in chemoprevention studies and the importance of considering the whole diet, not just dietary constituents. As reviewed by the International Agency for Research on Cancer (IARC, 1998), recent intervention trials suggest that high dose supplements of β-carotene may be neither beneficial nor neutral, in terms of human health impact. There is evidence for an increased risk of lung cancer among smokers and asbestos exposed workers given β-carotene supplements at high doses and also for increased cardiovascular death after supplementation. Moreover, there is no clear evidence for a protective effect against cancer at any site, although the studies were not designed to

evaluate whether β-carotene might have any influence on early stages of the disease.

In contrast to the epidemiological studies, results from studies in experimental animals indicate that β-carotene has cancer-preventive activity (IARC, 1998); thus in this case animal models were inadequate to predict human response.

Although studies, such as the β-carotene studies, have investigated the impact of individual components in fruit and vegetables on cancer, it is likely that it is the whole diet, rather than individual chemicals in isolation, that is responsible for any protective effect.

4.2.2 Risk factors for coronary heart disease

A major reason why intervention for cardiovascular disease is better developed and more successful than it is for cancer is that understanding about the disease process and risk factors for the former is so well advanced, as exemplified in Section 2.2 and below.

None of the genetic determinants of atherosclerosis *per se* can account for the vast majority of coronary heart disease. Although there is a clear correlation between serum cholesterol levels and coronary heart disease mortality, cholesterol itself is not a particularly important indicator of risk (see Section 2.2); it is the metabolism of cholesterol, to produce small dense lipoprotein and lipid peroxidation, that leads to the formation of atherogenic agents. When other risk factors are added to the risk associated with elevated cholesterol, the risk of coronary heart disease becomes greater; hypertension and diabetes are effectively risk biomarkers for the disease. This is of great public health importance, as control of these risk factors/biomarkers has a direct effect on the disease outcome. Obesity, as such, is not an especially strong risk factor for coronary heart disease. Gluteo-femoral adiposity (which is more usual in women) is not associated with coronary heart disease. However, central or truncal obesity, which is strongly associated with the Insulin Resistance Syndrome, is associated with the disease (Vague *et al.,* 1956).

Some of the known risk factors for coronary heart disease are powerful biomarkers for the disease. Screening of lipid profiles is used as a biomarker for coronary heart disease, to identify people who would benefit from treatment and to screen high risk groups, including those with clinically diagnosed coronary heart disease, a family history of the disease, hypertension or diabetes.

Dietary risk factors for coronary heart disease are dietary cholesterol, saturated fat, sucrose (possibly), and high calorie intake (owing to its association with obesity). Dietary protective factors are monounsaturated fat (MUFA), ω-3 polyunsaturated fat (PUFA), folate (possibly), and antioxidants (e.g. Durrington, 1995).

As outlined in Section 2.2, a major risk factor for coronary heart disease is the Insulin Resistance Syndrome, with associated dyslipidaemia, and glucose intolerance or diabetes. Although disputed for many years, hypertriglyceridaemia is now recognised as a major risk factor for coronary heart disease (Hokanson & Austin, 1996).

An important metabolic feature of the high-risk Insulin Resistance Syndrome is impaired postprandial clearance of lipids, especially triglycerides. The ability to clear fat after a meal is impaired in patients with coronary heart disease, in people who do not exercise, in people with diabetes and in first-degree relatives of people with coronary heart disease (e.g. Karpe *et al.,* 1994). As described in Section 2.2, genetics, fetal and infant undernutrition, physical inactivity, saturated fat and carbohydrate intake all appear to have some role in the pathogenesis of the Insulin Resistance Syndrome.

4.2.3 Chemoprotective intervention

If chemoprotective intervention is to be undertaken to reduce risks for cancer, the intervention must be easy and have a minimal risk of concomitant toxicity, as benefits to any individual cannot be predicted. Furthermore, a clear understanding of the potential gains and the mechanisms underlying any intervention will increase an individual's motivation to comply with it.

Chemopreventive agents do reduce the risk of some malignancies (either directly or 'accidentally'). For example, taking oral contraceptives for about five years or more reduces the risk of endometrial cancer by around 60% (e.g. Stanford *et al.,* 1993) and of ovarian cancer by around 46% (Hankinson *et al.,* 1992). However, it was only after hundreds of thousands of women had taken oral contraceptives that their protective effect became apparent, which demonstrates how useful an early biomarker of the protective effect would have been.

Similarly the published literature records around 100 000 people who have taken part in randomised trials with carotenoids (IARC, 1998), but only a very few of the 600 carotenoids with potential chemoprotective effects have been investigated. Such a large number of potentially protective

agents cannot be tested in randomised control trials; instead, if appropriate biomarkers were available, well-designed trials using small numbers of people could be used to identify the most effective carotenoids to take forward into larger randomised trials.

As alluded to in Section 2.3, evaluating the impact of cancer chemoprevention has many difficulties. The clinical endpoint (i.e. a particular form of cancer), unlike coronary heart disease, is usually relatively rare; thus to detect statistically significant changes in cancer incidence between those undergoing the intervention and controls, large numbers of people need to be followed up over many years. Such trials will be very costly and will require a substantial infrastructure. Population mobility (which may be an especially important factor where younger people are involved) may further complicate the evaluation. Study design for intervention trials is very complex and has to take into account the need to:

- identify the optimum dose (i.e. how to translate doses used in, for example, 2–3 month trials to interventions over several years);
- isolate the effect of one intervention from that of another;
- monitor toxicity over several years of follow-up; and
- measure and ensure compliance with the intervention protocol.

The availability of appropriate biomarkers could facilitate all these aspects of study design, resulting in the possibility of screening many potentially valuable agents in humans, in smaller studies, with shorter follow-up and thus reduced costs.

4.2.4 Evaluating dietary intervention

Importance of understanding mechanisms underlying dietary intervention

Further research into mechanisms of carcinogenesis and the mode of action of potential chemopreventive dietary factors will greatly facilitate the development of good dietary intervention strategies to protect against cancer. Arguably, dietary intervention trials should not be undertaken in the absence of a mechanistically based hypothesis. If more research were to be directed in this area, trials (such as those with β-carotene) based on assumptions that prove to be less than robust (i.e. that β-carotene was the effective chemoprotective agent in fruit and vegetables and would at worst be neutral in any intervention strategy) could be avoided. For intervention to be successful it should reverse (or halt or delay, as later in life a simple delay in the process may be sufficient) a process on the causal pathways.

A stepwise approach to investigating dietary intervention

As noted above, it is known that diet has an impact on the incidence of some cancers. Fruit and vegetables in the diet, in particular, appear to have a beneficial effect. But it is not yet clear which fruits and vegetables have the most benefit or what it is in the fruit and vegetables that confers the benefit. Indeed, it is not known whether a component of the fruit and vegetables is important or whether eating such foods reduces exposure to (or compensates for) dietary components in other foods that might have an adverse impact on cancer incidence and progression. Although the current consensus is that it is total vegetable consumption that confers the protective effect, there is some suggestion that cruciferous vegetables may be particularly important, and fibre in vegetables is thought to be important for protection against colon cancer (Potter *et al.,* 1994; Bingham, 1996). There is a mechanistic basis, related to faecal weight, for the positive effect of fibre on colon cancer, but there is conflicting evidence even for this (see Section 2.1). Currently there is a paucity of evidence to identify what it is about fruit and vegetable consumption that decreases some cancer risks, although it is expected that results coming out of the European Prospective Investigation of Cancer (EPIC) study will provide valuable information. Thus, starting from the existing knowledge base, a stepwise approach to studying the impact of fruit and vegetable consumption on colorectal cancer could evaluate:

- the beneficial effects of fruit and vegetables in general;
- which fruits and vegetables have most effect; and
- what constituents of fruit and vegetables confer the most benefit.

Such an approach would be substantially enhanced by the development of simple surrogate markers that could be used to assess the effectiveness of intervention at each of the steps. It should be noted that it is most likely that benefit will be provided by a number of interactive effects, rather than just one

food component; ideally any biomarker should be able to accommodate this and be, in practice, a biomarker for the total effect of a mixture of agents or dietary factors.

Studying intervention in high-risk groups

One possibility is that future intervention trials (including dietary trials) should be conducted in known high-risk groups, such as those with precancerous lesions, genetically high risks (for example, mutations in high penetrance genes) or a family history suggesting risk. However, at present it is not easy to identify high-risk groups in the general population. Again, better understanding of disease mechanisms will facilitate the identification of the appropriate high-risk groups to study and also of biomarkers to identify those at high risk and to follow disease progression and the effectiveness of intervention in such groups. However, it should be stressed that it is not clear that any beneficial effect that might be observed in a high-risk group could necessarily be expected to occur to a similar extent or at all in the general population.

Lessons for cancer chemoprevention studies arising from heart disease intervention studies and strategies

One notable difference between chemoprevention for coronary heart disease and chemoprevention for cancer is the feedback. When accepting an intervention that offers protection against coronary heart disease, the individual can see the immediate effects and presumed benefit of the intervention, as assessed by an appropriate biomarker, such as reduced blood pressure or reduced cholesterol levels. There is as yet no immediate marker of the effectiveness of increased fruit and vegetable consumption, for example, in protecting against colorectal (or other) cancer. This indicates the importance of having available appropriate and simple biomarkers to assess the effectiveness of intervention and so motivate people to change their lifestyles, where appropriate.

Although current studies on cancer chemoprevention have not had great success (Section 2.1), this was also true of early studies on chemoprevention for coronary heart disease. Early studies on lipid reduction (both dietary and pharmacological), for example, probably failed to achieve a sufficiently great reduction in lipid levels to see any clinical benefit (Laker *et al.,* 1993). Later secondary prevention trials using more powerful cholesterol-lowering agents (statins) have demonstrated a significant reduction in coronary events and mortality (the Scandinavian Simvastatin Survival Study Group, 1994; Shepherd *et al.,* 1995; Sacks *et al.,* 1996). More recently intervention aimed specifically at patients with low levels of high-density lipoprotein–cholesterol, using a fibrate drug (gemfibrozil), has also been shown to reduce mortality (Rubins *et al.,* 1999). Appreciation of the metabolic interrelationships between triglyceride and high-density lipoprotein–cholesterol has also led to dietary interventions that have improved lipid risk markers for coronary heart disease. However, such interventions (probably because of the limited power of studies when compared with those on pharmacological interventions) have not yet been shown to reduce coronary events (Frost *et al.,* 1999; Williams *et al.,* 1999; Minihane *et al.,* 2000). Again, it is clear that a better understanding of mechanisms has led to improved effectiveness of intervention strategies, both dietary and chemoprotective, for coronary heart disease and it can be expected that the same will be the case for cancer chemoprevention. It is possible that both heart disease and cancer would eventually affect all individuals, even if only to a very slight extent; if so strategies to delay disease progression may be as (or more) effective as strategies to prevent disease onset.

4.3 Biomarkers

4.3.1 The role of biomarkers in establishing the aetiology of colorectal cancer

It has been suggested that about one third of all cancers may be attributed to dietary factors, with the strongest evidence being for colorectal cancer, with possibly 50–80% avoidable by changes in diet (Doll & Peto, 1981; Willet, 1995). Dietary factors appear to have some impact on cancer at most sites, including lung cancer.

Early estimates of the effect of diet on cancer came from cross-sectional comparisons between international cancer incidence rates and data on food consumption. Such ecological studies do not provide strong enough evidence to establish causality, as they cannot determine individual risk or individual risk factors. As noted in Section 3.1, even prospective studies conducted since the 1980s, for example on the association between red and processed meats and colorectal cancer, show only fairly small relative risks (with wide confidence intervals that generally include 1, indicating no statistically significant elevations in risk), which are not large enough to account for 80% of colorectal cancer being attributable to dietary factors. Such small relative risks could easily have arisen through confounding and cannot be taken to imply a causal relationship between the dietary factors examined and colorectal cancer. Thus the available

epidemiological studies are not particularly helpful in evaluating the impact of diet on cancer, hence the increasing interest in the use of biomarkers to study the aetiology of diet-related cancer.

Biomarkers for colorectal cancer for use in epidemiological studies might include:

- biomarkers of metabolic polymorphisms;
- biomarkers to validate ways in which diet is assessed;
- biomarkers to replace usual methods of dietary assessment (although at present there are very few markers that are good enough to replace the best methods of dietary assessment by questionnaire, an exception has been urine markers for aflatoxin exposure, which identified much higher relative risks for liver cancer than was the case using dietary questionnaire data);
- biomarkers of intermediate risk factors, such as serum hormone levels in relation to hormone-related cancers; and
- evidence of somatic damage in tumour tissue relevant to established models for colorectal cancer.

The first type of biomarker listed above is a susceptibility marker; the next two are described, in this report, as 'input' markers (i.e. markers of exposure or dose) and the last two are described as 'output' markers (i.e. markers having some level of surrogacy for the end effect).

4.3.2 Development of biomarkers

In the development of biomarkers for any disease endpoint, including cancer, consideration of the proposal of Salmon (1984) that a genuine causal process is one in which it is possible to follow a mark, which propagates in the course of time, precisely because the causal events are able to induce a structural change that becomes part of the event, is pertinent.

As described above, biomarkers of coronary heart disease (such as plaques and fatty streaks, blood pressure and cholesterol levels) are structurally part of the disease process; they are not surrogate biomarkers. A challenge for the future is to be able to develop biomarkers for cancer that are structurally part of the disease; that is to identify biomarkers that are on the causal pathway to disease. Although the nearer a biomarker is to the disease outcome the better (more reliable) marker of risk it will be, the particular challenge for cancer studies is to identify biomarkers on the casual pathway that are associated with early stages of disease, when intervention can alter the outcome. At present this is easier to do for coronary heart disease, where markers such as cholesterol levels and plaques help identify disease progression, than it is for cancer.

For pathological biomarkers, such as morphological abnormalities (e.g. dysplastic cells), it should be possible to demonstrate that removal of the lesion is associated with reduced risk of cancer. Examples include oral erythroplakia, which is associated with about a 5% risk of developing oropharyngeal cancer, Barrett's oesophagus, which carries a 40-fold elevated risk of adenocarcinoma of the oesophagus, and adenomatous polyps, which are associated with a 10% risk of colorectal cancer. Reversing any of these lesions reduces associated cancer risks, though not necessarily back to background levels of risk (e.g. reversing Barrett's oesophagus does not completely eliminate excess risk). The longer the latency between the detection of the biomarker and the disease the greater is the possibility of effective intervention. Recent trials, in 90 people for one year, identified a selective cox-2 inhibitor that reduced polyp count, compared with a placebo treatment, among individuals with familial adenomatous polyposis (FAP; Steinbach *et al.,* 2000). This, in principle, demonstrates the potential benefits of such a biomarker trial.

If biomarkers are to be developed effectively for use in cancer chemoprevention intervention and epidemiological studies, it will be necessary to:

- know more about *inter*- and *intra*-individual variations in response, in order to develop inexpensive and reliable biomarkers with minimal such variation;
- understand better to what extent changes in the biomarker correlate with reduced risk of cancer, as the level of an ideal biomarker should accurately predict the major expected outcome;
- develop reproducible and rapid assays, which require small samples obtained by minimally invasive means; and
- conduct trials to test correlation(s) between changes in the biomarker(s) and disease outcome.

The latter is particularly relevant, as even in the case of the use of cox-2 inhibitor to reverse polyps

in individuals with FAP, a beneficial effect on colon cancer occurrence has yet to be demonstrated.

Many different markers may be needed to follow different stages of the disease process. Non-invasive methods for biomarkers for colorectal cancer might include *in vivo* mutagen traps (beads with nucleophilic material which can be passed through the gastrointestinal tract to pick up electrophilic agents, Bingham *et al.,* 1992) and isolation of colonocytes from faecal samples, with mutagen fingerprint analysis of exfoliated cells (Ahlquist *et al.,* 2000). More biomarkers that can be determined in blood samples are needed as markers for both early and late changes. The value of markers of genotoxic exposures in cells, including blood and other tissues, that are surrogates for the cancer site (such as those markers described in Section 3.2) should be assessed further. For following late changes in particular, proteomics could be used to look at low levels of specific proteins in the blood as markers for cancer cells in target tissues or organs. Flexi-Scope screening could be extended for biomarker analysis.

4.3.3 The importance of understanding mechanisms for the development of biomarkers

As described in Section 3.3, understanding mechanisms of both disease and protection is crucial to the development of biomarkers. Many dietary components will have effects at (possibly several) different stages of the cancer process. Increased understanding of mechanisms and dose–response relationships will help identify which are the most important interactions and therefore which are the most effective protective strategies and biomarkers to monitor them.

To date much of the emphasis on mechanisms and the identification of biomarkers has been focused on exposure and early molecular events in the disease process; less emphasis has been placed on cellular processes, such as clonal expansion or the balance between apoptosis and cell proliferation. There is a need to find biomarkers to monitor these later processes and assess the impact of diet on them, as well.

Identifying disease mechanisms will certainly help in the identification of the best biomarkers to measure risk, but it is important to recognise the potential complexity of disease mechanisms. For example, as noted in Section 3.3, the influence of diet on gap junction communication (which affects cell proliferation), apoptosis or cell proliferation in preneoplastic cells may not be the same as in a normal cell population, and this should be taken into account in developing biomarkers. Furthermore, intervention to decrease the risk of tumours at one site may not be beneficial across the board. Chemoprevention alters metabolism, which may have a protective effect at one site but cause an increase in tumours at another site.

An important reason for elucidating mechanisms is to link inputs (i.e. exposures such as dietary factors or interventions) and input markers, for a given disease process or intervention, to outputs (i.e. disease endpoints) and output markers, to ensure that both are measuring the same process, albeit at different stages. The ideal biomarker may, therefore, be one that is a measure of both input and output. An example would be serum hormones that are known to be associated with an increased risk of cancer (output marker) but are also affected by dietary intake (input marker).

Certainly, given the number of potential biomarkers for colorectal cancer mentioned above, it would be helpful to establish how closely each potential biomarker, or combination of biomarkers, is associated with the disease endpoint. Biomarkers for cardiovascular disease are directly related to the disease endpoint, but this is not clearly the case for any of the biomarkers for colorectal cancer proposed above. Nonetheless, they may still be useful. In practical terms, for most biomarkers, establishing the strength of the association with disease endpoint will be more important than establishing any mechanistic link between the two. Although it is probably not possible to establish biomarkers that are 100% predictive for a multistage and multifunctional disease such as colorectal cancer, it may be feasible to establish a combination of biomarkers that taken together do achieve a high degree of predictability.

Once such good output biomarkers (i.e. biomarkers strongly associated with disease endpoints) have been established it will then be appropriate to develop the necessary mechanistic studies to work back through the disease pathway to the relevant input markers.

4.3.4 Overview: Use of biomarkers to assess impact of diet and dietary intervention on cancer

What makes a good biomarker of dietary intervention that is practical for use in epidemiological studies or in clinical applications?

In proposing to develop biomarkers of dietary intervention it is first important to recognise what will be a good biomarker for a particular intervention for a particular disease. Furthermore,

what constitutes a good biomarker may depend on whether it is to be used in epidemiological studies or in clinical applications, that is whether it is to be a biomarker for use in the general population (or population subgroups) or a marker that is applicable to monitoring the individual.

Dietary intervention (which can include the use of whole food, food components (including modified or functional foods) or supplementation) may have an effect at any stage through the disease process from exposure onwards, and different biomarkers will be applicable at each stage. The success of intervention at the exposure stage can in principle be measured by modification in an input marker (i.e. a marker of exposure or dose) or possibly in prognostic markers or early markers to detect disease. Efficacy can be assessed by output or risk biomarkers, which have some level of surrogacy for the end effect. In cancer prevention there is a need to develop and use risk biomarkers that can measure the efficacy of intervention at much earlier stages than the onset of clinical disease. Biomarkers may also be used to stratify target populations. For example, biomarkers could be developed and used to identify susceptible groups who may benefit from intervention or to assess whether interventions in a general population have different effects in those with a particular susceptibility marker and those without.

Thus biomarkers can be used in a variety of ways to assess dietary intervention.

- Input: exposure/dose biomarkers — for monitoring — of adherence to intervention
- Output: risk biomarkers — for surveillance — of efficacy of intervention
- Susceptibility markers — for stratification — of susceptible populations

Currently most biomarkers are applicable at the group level rather than at the individual level. The difficulties inherent in extrapolating from groups to individuals are exemplified by studies by Kensler *et al.* (1997) on rats given aflatoxin B1 and treated with the chemopreventive agent oltipraz. Although, in the studies, the occurrence of DNA adducts correlated well with tumour incidence at the group level, the presence of adducts was not predictive of tumour outcome in individual rats. For a biomarker to be applicable at an individual level it would need to have a surrogacy for the end effect of close to 100%. This was not the case, for example, in the aflatoxin B1/oltipraz studies; tumour incidence could be reduced to zero without the elimination of all the biomarker (i.e. aflatoxin B1-related DNA damage; Roebuck *et al.,* 1991).

A good biomarker of dietary intervention can be described as a marker that indicates that the intervention carried out has had an effect. For example, although there is evidence to suggest that eating fruit and vegetables will benefit the health of the population, currently there is no realistic means of measuring the effectiveness of consumption of this in promoting good health. The only course of action at present is to continue to monitor the overall health of the population over a 10–20 year period, in the hope that a reduction in cancer risk might become apparent. A good biomarker could help measure the impact of fruit and vegetable consumption on health, and on cancer risk in particular, on a much shorter timescale. Until such biomarkers are developed it is particularly important to recognise the possibility that a proposed dietary intervention may have unrecognised or unexpected negative impacts as well as or instead of neutral or expected positive impacts.

Biomarkers and risk management

Current advice to the general public is to eat fruit and vegetables and fibre, but it is still not clear how relevant such advice may. As noted above understanding of mechanisms is of particular importance to underpin advice to the public, but apparent associations between dietary components and health outcomes do not necessarily imply causality. For example, it may be that the apparent beneficial effects of β-carotene observed in early studies arose because β-carotene was a marker of a healthy lifestyle rather than a cancer protective agent.

It is important to communicate dietary advice wisely and accurately. For example, current advice is to eat five portions of fruit and vegetables a day, but nothing is said about what effect eating, for example, steak, with these five portions, may have. In fact there is no evidence that fruit and vegetable consumption can counteract the effect of meat consumption on nitrosation, for example. Furthermore, it is possible that high consumption of fruit and vegetables is a marker of some other dietary habit, for example low meat consumption. Where possible, it can be advantageous to identify at risk individuals early and start intervention as soon as possible (as is done for the treatment of diabetes, for example). However, early identification of individuals with high risk for colorectal cancer for example will in most cases (i.e. other than FAP and similar groups) be dependent on the development of suitable biomarkers.

Studies on the effectiveness of medical intervention may appropriately be carried out in identified high-risk groups (e.g. those with FAP or those who already have colorectal cancer). However, this does not seem the best group to study in order to assess the effectiveness of dietary intervention to prevent colorectal cancer. Such studies would be more appropriately undertaken among the general public, as it is hard to see how a dietary intervention might have an impact on a disease process that is already well advanced (i.e. when tumours are already present). It is to be expected that dietary intervention will be more likely to have an impact (whether on disease prevention or delaying disease progression) at early stages of the disease process.

It will be possible to proceed more confidently with dietary interventions for colorectal cancer, for example, when appropriate biomarkers of premalignant lesions become available. Use of such markers would both help identify appropriate subgroups for intervention studies and the effectiveness of intervention. Ideally once such 'output' markers have been developed they should be linked to 'input' biomarkers, that is markers of, for example, dietary exposures; thus variation in input markers for a given intervention (which could be used to measure adherence to the intervention) should be reflected by appropriate variation in output markers of response, which would indicate the effectiveness of the intervention.

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5 Conclusions and recommendations for future work

5.1 Conclusions

This report has compared the current state of knowledge on the role of chemoprevention and dietary intervention to protect against cancer with the parallel knowledge base on cardiovascular disease. The reason for doing this has been to see what lessons may be learned from chemoprevention of cardiovascular disease, where intervention is increasingly well developed and understood, and to see whether any experience gained can be applied to cancer chemoprevention.

Cancer is perhaps less amenable to intervention and chemoprevention than is cardiovascular disease, as the latter comprises essentially few discrete conditions, whereas there are many forms of cancer, each probably arising via a different pathway (or pathways) and each requiring a different approach to treatment or intervention. Nonetheless, encouragement to continue to search for more effective cancer chemoprevention strategies can be drawn from the fact that, like many current studies on cancer chemoprevention, early studies on intervention to reduce heart disease were also not particularly successful. One possible similarity between the two conditions is that signs of arterial disease, at least to some extent, would be expected to be evident in the whole population, if investigated, and it is possible that the same would be true for early signs of carcinogenesis; for both diseases, strategies to delay disease progression could be as effective as (or more effective than) prevention of disease onset.

One notable difference between the application of intervention strategies to reduce cardiovascular disease and strategies to modify cancer is the feedback that is available, through the use of biomarkers for the disease, to assess the effectiveness of treatment in the case of cardiovascular disease. Such feedback is important to reinforce intervention strategies and to encourage people to take up and maintain intervention strategies that may require considerable commitment, for example to modify lifestyle, change dietary habits or take medication consistently. Thus it can be expected that, as has been the case in treatment for cardiovascular disease, cancer intervention will benefit from the development of readily usable biomarkers to monitor the effectiveness of intervention at an individual as well as a population level.

Currently more is known about the mechanism and process of cardiovascular disease than is known about carcinogenesis. Understanding the disease mechanism has facilitated the develop biomarkers that are essentially risk factors (or output markers) for cardiovascular disease. Thus understanding mechanisms, both of carcinogenesis and of potential cancer chemoprevention strategies, is of great importance for the development of biomarkers to assess the effectiveness of intervention. Although it has to be recognised that biomarkers for cancer may not necessarily be risk factors for the disease.

As an initial step in setting up future studies to assess the effectiveness of dietary intervention to protect against colorectal cancer in particular, the development of output markers (i.e. markers associated with the disease process) is likely to be the most useful. Without these the impact of diet can still only be assessed either by waiting for tumours to appear, which takes too long to be of much practicable use, or by screening biological samples and seeking to match biomarkers with dietary constituents or types of diet or with eventual tumours in cases.

5.2 Future research

An important research need is to identify biomarkers for monitoring dietary intervention (either through animal studies or, if possible, through human population studies) and to develop biomarkers of susceptibility, (which will usually be genetic markers). However, development of such biomarkers requires a long-term research effort. Currently samples taken during the EPIC studies are being used for this purpose.

Future research should focus first on identifying and developing reliable output biomarkers for cancer, that is markers of the disease process that are closely associated with the disease endpoint, and preferably markers of early reversible stages of the disease process. For colorectal cancer, studies should focus on the identification of markers of premalignant lesions. The development of output biomarkers will depend, to some extent, on increased understanding of mechanisms. Input or exposure biomarkers, that is markers of for example dose or dietary components, should be developed to monitor how effectively intervention strategies are being adopted. It will be important to establish how input and output markers are linked mechanistically, to be sure that both are measuring different aspects of the same intervention strategy and disease process.

In the short term, cancer intervention studies should not be undertaken if only input (or exposure) biomarkers are available; suitable output biomarkers should always be used.

As is the case with cardiovascular disease, markers that could provide immediate objective feedback on the effectiveness of intervention, whether at the general population level or in high risk individuals, would be very useful, especially so for clinical applications.

It is probable that several biomarkers need to be developed and used rather than just one for each outcome; the development of profiles of markers can be envisaged, especially using new genomic techniques, that would be of benefit even at the individual level.

6 Food risk assessment in the future

This report on the fourth FORA workshop completes the first stage of the critical review and evaluation component of the FORA programme.

There is still much to be done on food risk assessment, particularly in the context of the whole diet. The EPIC study is now beginning to accumulate cancer cases and so will provide a vast bank of samples, from cases and controls, that can be used to develop biomarkers for several cancer endpoints and calibrate them to dietary factors. Particular goals should include the development of a clear understanding of what makes a good biomarker and the development of good reliable markers for colorectal cancer. The expectation should be that, from the EPIC study, biomarkers for dietary factors can be developed that could improve the power of epidemiological studies.

As the laboratory and epidemiological studies on food risk assessment continue over the next few years, it will be beneficial to continue a programme of expert workshops to provide the forum for interaction and collaboration between research groups, for maintaining awareness of developments in the field and associated disciplines, and for the development of new ideas and research directions that has been a prime objective of the FORA programme to date.

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IEH Publications

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Air Pollution and Respiratory Disease: UK Research Priorities	Report R2 (1994)
Natural and Man-made Mineral Fibres: UK Research Priorities	Report R3 (1995)
Perinatal Developmental Neurotoxicity	Report R4 (1996)
The Use of Biomarkers in Environmental Exposure Assessment	Report R5 (1996)
Health Effects of Waste Combustion Products	Report R7 (1997)
Factors Affecting the Absorption of Toxic Metals from the Diet	Report R8 (1998)
Recent UK Blood Lead Surveys	Report R9 (1998)
The Non-auditory Effects of Noise	Report R10 (1997)
Approaches to Predicting Toxicity from Occupational Exposure to Dusts	Report R11 (1999)
Benzene in the Environment: An Evaluation of Exposure of the UK General Population and Possible Adverse Health Effects	Report R12 (1999)

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Indoor Air Quality in the Home: Nitrogen Dioxide, Formaldehyde, Volatile Organic Compounds, House Dust Mites, Fungi and Bacteria	Assessment A2 (1996)
Oilseed Rape: Allergenicity and Irritancy	Assessment A3 (1997)
The Ecological Significance of Endocrine Disruption: Effects on Reproductive Function and Consequences for Natural Populations	Assessment A4 (1999)
Indoor Air Quality in the Home (2): Carbon Monoxide	Assessment A5 (1998)

Special Reports

Understanding Asthma (1995)

Health Effects of Ozone and Nitrogen Dioxide in an Integrated Assessment of Air Pollution (1997)

Fibrous Materials in the Environment (1997)

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Joint Research Programmes on Outdoor and Indoor Air Pollution (Review of Progress, 1999) (2000)

Airborne Particles: Exposure in the Home and Health Effects (2000)

Interdepartmental Group on Health Risks from Chemicals/ Risk Assessment and Toxicology Steering Committee reports

Developing New Approaches to Assessing Risk to Human Health from Chemicals	cr1 (1999)
Risk Assessment Approaches Used by UK Government for Evaluating Human Health Effects from Chemicals	cr2 (1999)
Risk Assessment Strategies in Relation to Population Subgroups	cr3 (1999)
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Exposure Assessment in the Evaluation of Risk to Human Health	cr5 (1999)
From Risk Assessment to Risk Management: Dealing with Uncertainty	cr6 (1999)
IGHRC First Report and Forward Plan to 2002	cr7 (2000)
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Food Risk Assessment (FORA)

Probabilistic Approaches to Food Risk Assessment	FORA 1 (2000)
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Diet–Gene Interactions: Characterisation of Risk	FORA 3 (2001)
Chemoprevention and Chemoprotection: The Role of Dietary Intervention and How to Measure its Effects	FORA 4 (2001)

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