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NON-NUTRITIVE ANTICARCINOGENS IN FOODS
State of the art and future developments.
A report of the international workshop
held on March 26-27 1990 in
Wageningen, The Netherlands.

M.G.L. Hertog and P.C.H. Hollman

State Institute for Quality Control of Agricultural Products
Bornsesteeg 45, 6708 PD Wageningen
P.O. Box 230, 6700 AE Wageningen
Tel. +31 8370-75400
Telex +31 75180 RIKIL
Telefax +31 8370-17717

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Participants workshop (see Annex III)
Agralin

ABSTRACT

NON-NUTRITIVE ANTICARCINOGENS IN FOODS. State of the art and future developments.
A report of the international workshop held on March 26-27 1990 in the International Agricultural Centre, Wageningen, the Netherlands

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M.G.L. Hertog and P.C.H. Hollman

State Institute for Quality Control of Agricultural Products (RIKILT)
PO Box 230, 6700 AE Wageningen, the Netherlands

3 annexes

On March 26-27 1990 an international workshop on non-nutritive anticarcinogens in foods was organised in Wageningen, The Netherlands. Aim of the workshop was to review progress in the research on natural occurring non-nutritive anticarcinogens and to set priorities for future analytical and epidemiological research. About fifteen experts from different countries were invited to hold lectures on experimental, analytical and epidemiological research on non-nutritive anticarcinogens. The total range of natural ocurring non-nutritive anticarcinogens was thus covered. In the final discussion it was concluded that polyphenols were the most promising group of anticarcinogens for future analytical and epidemiological research.

Keywords: anticarcinogens, review, polyphenols, indoles, glucosinolates, terpenes, cancer etiology, anticarcinogenesis

NON-NUTRITIVE ANTICARCINOGENS IN FOODS
State of the art and future developments

Report of the international workshop held on March 26-27 1990 in the International Agricultural Centre, Wageningen, The Netherlands.

Sponsor:
Dutch Commodity Board of Vegetables and Fruits, The Hague, The Netherlands

Organising committee:

Professor D. Kromhout,	National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
Professor J.G.A.J. Hautvast and professor M.B. Katan,	Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands.
Ir P.C.H. Hollman, drs M.G.L. Hertog and dr H. Herstel,	State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands

In collaboration with:

Professor L.W. Wattenberg,	University of Minnesota, Laboratory of Medicine and Pathology, Minneapolis, U.S.A.

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PREFACE

On March 26-27, 1990 an international workshop entitled " Non-nutritive anticarcinogens in foods. State of the art and future developments " took place in the International Agricultural Center in Wageningen, the Netherlands. The idea for such a workshop was born during discussion over the last several years between Dr. Kromhout and Dr. Wattenberg (Minneapolis, USA) about the role of different nutritive and non-nutritive substances in the etiology of cancer. Based on these discussions a pilot study was carried out in the Netherlands on the relation between flavonoids and cancer mortality using data collected in the Seven Countries Study. This pilot study was a collaborative effort of the State Institute for Quality Control of Agricultural Products, the Agricultural University (Wageningen) and the National Institute of Public Health and Environmental Protection (RIVM, Bilthoven). Due to the promising results of the pilot study the institutions decided to organise an international workshop. In close cooperation with Dr. Wattenberg 12 experts from different countries were invited.

After general introductions by Dr. Kromhout and Dr. Wattenberg the evidence of different non-nutritive anticarcinogens in experimental studies was reviewed. Thereafter the evidence of the protective effects of fruits and vegetables in relation to the occurence of cancer in epidemiologic studies was summarised. Also attention was paid to the analytic aspects of isolating these substances in foods. Finally research priorities were discussed.

This workshop was very useful because of the interaction between experimentalists, food chemists and epidemiologists. The suggestion was therefore done to have another workshop about three years from now.

The workshop could not have been realised without the financial support of the Dutch Commodity Board of Vegetables and Fruits. We are very grateful to this organisation, because through its support an unique interdisciplinary workshop could be held.

D. Kromhout

1 WORDS OF WELCOME

M. Heuver, General-Director, Agricultural Research Department; Ministry of Agriculture, Nature Management and Fisheries, The Netherlands

With great pleasure I use the opportunity to speak a few words to you at the opening session of this important workshop.
This workshop takes place within the framework of cooperation in the network of Human Nutrition Biology in which various university departments in the Netherlands work together with institutes for agricultural research. More specifically in which the Department of Human Nutrition of the Agricultural University cooperates with the State Institute for Quality Control of Agricultural Products, RIKILT. This cooperation, which formally started somewhat more than a year ago, concerns mutual analytical chemical support of the food and nutrition research and studies on the reliability of food analyses. Besides, and in future this type of cooperation will be strengthened, the fulfilment of terms of probation at the RIKILT institute, and the presentation of guest lectures by RIKILT coworkers, should be mentioned.
An important step forward in the cooperation has been made by the start of a study for a doctor's degree about anticarcinogens in foods, in which the Agricultural University, the National Institute of Public Health and Environmental Protection, and the State Institute for Quality Control of Agricultural Products cooperate. To perform this study optimally international contacts and collaboration with experts in this field of research are of utmost importance. Therefore I am very glad that Wageningen to day hosts this meeting. People hear more and more about harmful aspects of foods which may cause health problems like cardiovascular diseases, cancer, diabetics, allergy, food infections etc... Gradually the understanding is growing that bad food habits like eating too much, insufficient variation, too much fat, -salt, -alcohol, insufficient dietary fiber as well as carcinogens are jointly responsible for the development of cancer.

Far lesser known to the public, and scientifically not so strongly supported as yet, are data about naturally occurring compounds that may prohibit the development of cancer.
The workshop will deal with this subject. Thanks should be expressed to the Commodity Board for Vegetables and Fruit (of which a representative is present here), which financially made this meeting possible. Vegetables and fruit appear to be important food components that contain these anticarcinogenic compounds. This well explains the interest of the Commodity Board for Vegetables and Fruits in this subject. For the agricultural research it is not only important to get a better knowledge on the occurrence of these compounds in vegetables and fruits and in the role they play as anticarcinogens. No, the interest goes beyond that. The question that arises namely is, how through optimal composition of the daily menu, through selection of the most promising vegetable and fruit varieties and through breeding in the end the much feared illness, cancer, can de repelled.
Therefore I hope that this workshop will not only lead to good ideas, good tuning and new collaboration in the field of research on the occurrence and working-mechanisms of anticarcinogens in vegetable products, but will directly or indirectly contribute to an optimal attunement of the research of plant production to the needs for good and healthy food for the population, also seen from an international point of view. Being conscious that this is not a modest wish, I like to express confidence that this workshop will be fruitful and will contribute not only to good research, but in the end to still better food and thus health for human beings

B.H. Bijsterbosch. Deputy Rector Magnificus of the Wageningen Agricultural University. The Netherlands

It is a great pleasure to welcome you on behalf of the Board of Wageningen Agricultural University. Our University is in the Netherlands a unique University as we have only one Agricultural University. This University counts about 6000 students and offers more than 20 MSc- and PhD-programmes in disciplines covering a broad range of topics dealing with agriculture. Disciplines are of course topics like plantbreeding, soil sciences and forestry, to mention a few.

In addition we have developed in the past 20-30 years MSc- and PhD-programmes e.g. in Food Technology, Molecular-Biological Sciences, Environmental Sciences and also in Human Nutrition. For the MSc- and PhD-programmes in Human Nutrition, the Department of Human Nutrition under directorship of Professor Hautvast, has the main responsibility. This department is one of the organisers of this workshop which is done in collaboration with RIKILT, i.e. the State Institute for Quality Control of Agricultural Products and the RIVM, i.e. the National Institute of Public Health and Environmental Protection. The Department of Human Nutrition and the Department of Food Sciences and Food Technology have a linkage in organisation and policy issues. Both departments recently established the Wageningen Postgraduate School in Food Sciences, Food Technology and Human Nutrition. In this school more than 200 people are employed and 80 people are in training for a 4-years PhD-degree. The research concentrates around the issues:

- The role of nutrition in health; with special emphasis in lipoprotein metabolism, energy metabolism and vitamin A metabolism.
- The safety and quality of foods (food processing, food microbiology).
- The bio-process technology.

This University has a large knowledge of food and of food consumption and this knowledge is very carefully applied in several kinds of nutritional-health studies. This knowlegde about food gives our University and especially the Department of Human Nutrition, a relative advantage when carrying out nutrition studies.
This workshop concerns the topic of non-nutritive substances in foods, which might play a significant role in cancer prevention. We consider this topic as very relevant for study in the years ahead especially as cancer still causes high death-rates in Western populations. We are pleased that such a large group of very distinguished scientists in this field have travelled to Wageningen to discuss today and tomorrow this topic. I wish you all a very successful discussion. I am sure that after these two days you will have more clarity with regard to priorities for futher research in the rather broad area of non-nutritive substances in foods.

Finally I like to tell you that our University is very pleased with the existing cooperation with RIKILT and RIVM. This cooperation has not only a clear complementary character but it will lead to synergy. This means that the value of the three cooperating institutions will not be three, but may lead to a value four or five. I call on a further strenghtening of this cooperation in the future.

2 GENERAL INTRODUCTION

Kromhout (Utrecht, the Netherlands) presented a general introduction to the subject of the workshop, focussing on needs of epidemiologists. In epidemiological studies a relation is found between the consumption of fruits and vegetables and the occurrence of lung, stomach and colon cancer and recently also of pancreatic cancer. Until now, epidemiologists have explaned the protective effects of fruits and vegetables mainly by their β-carotene and vitamin C content. However, to take a only a few components of fruits and vegetables is much too simple because there are many components present in fruits and vegetables that deserve attention. These components and the evidence available on their cancer preventive potency are the subject of this workshop.

The well-known Western Electric Study of Shekelle and coworkers established the relationship between the "carotene index" and the occurrence of lung cancer after 19 years of follow-up. Persons with a high β-carotene index and who did not smoke, had an extremely low incidence of lung cancer. Persons with a low β-carotene index and a very long duration of cigarette smoking ($>$ 30 years) had a very high incidence of lung cancer. Shekelle and coworkers calculated a risk ratio of 7 for persons with a low carotene index compared to the ones with a high carotene index. Peto summarised results of about 20 epidemiological studies in this field in 1983. In general risk ratios were higher than 1 for those with a low β-carotene intake compared to those with a high β-carotene intake. But only one third had risk ratios higher than 2, making the Shekelle study the highest risk ratio ever reported.

The Zutphen Study in the Netherlands started in 1960 and followed 878, at that time middle-aged men during 25 years. Dietary data were collected at home using the cross-check dietary history method. During 25 years about 50% of the men died, 30% from coronary heart disease and 15% from lung cancer. The relation between fruit consumption and 25-year mortality from lung cancer was studied. The population was divided in four quartiles based on the fruit consumption, and risk ratios were calculated. A risk ratio of 1.0 was used for the lowest quartile. Risk ratios of the two middle quartiles were 0.4, and of the highest quartile 0.3. Thus a protective effect of fruits was evident. After adjusting for age, smoking, socio-economic status and vegetables, the protective effect remained. Furthermore, only persons with a very low fruit consumption level were at a higher risk, once they used a reasonable quantity of fruits no further protection was evident. The analysis was also done for vegetables, but no relationship between vegetables and the occurrence of lung cancer during this 25-year period was found.

Looking at the nutrient intake, vitamin C followed the same pattern as fruits consumption, and showed an inverse relationship with 25-years mortality from lung cancer. Only persons who had a vitamin C intake below 63 mg/day were at a higher risk. This relationship was still present after adjustment for confounding effects of smoking, socio-economic status and β-carotene. Comparing the Chi-square for trend, fruit consumption showed a stronger trend than vitamin C intake. This could mean that the relationship between fruit consumption and lung cancer mortality is of more importance than the relationship between vitamin C intake and lung cancer. So, the question remains to be resolved what component or components present in fruits are responsible for this relationship. Components like for instance flavonoids and terpenes could play a role in this relationship.

The aim of this workshop is to discuss the different anticarcinogens that are present in fruits and vegetables, and to review the evidence that is available from an experimental point of view. Also the analytical problems in isolating these components in foods will be discussed. At the end of the workshop, hopefully there is an idea about the importance of the different components and how to continue research in different areas. Epidemiologists could profit from

quantifying the different components in fruits and vegetables by comparing the intake of anticarcinogens by different groups in relation to the occurrence of cancer.

3 EXPERIMENTAL STUDIES

3.1 Anticarcinogens in the Diet: An Overview

Wattenberg (Minneapolis, USA) gave an overview of anticarcinogens in the diet. There is a large number of non-nutrients, members of at least 12 major chemical classes, that occur in foods and have profound inhibitory effects on carcinogenesis in experimental studies. Anticarcinogens can be classified according to the time in the carcinogenic process at which they are effective. First of all there is a group of inhibitors that prevent the formation of carcinogens. Once carcinogens are present, inhibitors called blocking agents can prevent the carcinogens from reaching or reacting with the target. Blocking agents act as a barrier, they prevent the target from ever being attacked. However, if carcinogenic agents already have reached the targets in sufficient concentration to produce a carcinogenic reaction, inhibitors called suppressing agents may prevent the evolution of the carcinogenic process.

In their protection against already formed carcinogens, blocking agents show three different mechanisms: preventing activation of the carcinogen; enhancing the detoxification; intercepting (trapping) the carcinogen.

Blocking of the activation of nitrosamines (f.i. diethylnitrosamine) was shown in mice by orange oil, lemon oil and d-limonene (major component of the oils), if these blocking agents were administered prior (1 h) to the carcinogen. Benzylisothiocyanate present in cruciferous vegetables, administered prior to the exposure of a carcinogen causing mammal tumours, showed also a protective effect. However, there was only a profound protective effect if the benzylisothiocyanate was given 4 or 2 hours before exposure to the carcinogen. Administration of benzylisothiocyanate 24 h prior or 4 h after exposure to the carcinogen had very little or no inhibitory effect.

All carcinogens undergo metabolic changes. Phase I and Phase II enzymes play an important role in this metabolism. Phase I enzymes can either activate or detoxify the carcinogens, while Phase II enzymes in general only detoxify. These enzymes are inducable, the level of some of these is totally dependent on the compounds present. Glutathione transferase is one of the Phase II enzymes. In a series of studies increases in the activity of glutathione transferase in the forestomach of mice were induced by addition to the diet of naturally occurring compounds like α-angelicolactone, coumarin and benzylisothiocyanate. These compounds were able to induce increased glutathione transferase activity (up to 250%) resulting in a marked decrease in the number of tumours in the forestomach, and thus are examples of blocking agents by enhancing the detoxification of carcinogens. In foods there are a large number of constituents that have both these blocking actions: prevent enzyme activation of carcinogens and induce increase in activity of phase II enzymes.
The third way in which blocking effects can occur is by trapping reactions. Thiosulphate, not a normal food constituent, is a very effective trapping agent. It forms an adduct with the direct acting carcinogen β-propiollactone, thus preventing tumour formation.
The following is a summary (not exhaustive) of chemical classes of non-nutritive constituents naturally occurring in foods that can act as blocking agents: nucleophiles (thiosulphate), organosulfides, aromatic isothiocyanates, indoles, flavones, ellagic acid, phenols, coumarins, terpenes and dithiothiones.
Suppressing agents act subsequent to the time that the carcinogen has hit the target. Elucidation of the mechanism by which the suppressing agents work is not yet possible, because the basic nature of cancer is still unknown. The following gives a summary of the groups of non-nutrititive compounds that can act as suppressing agents: retinoids, protease inhibitors, inhibitors of the arachidonic acid metabolism, selenium salts, and isothiocyanates. Studies on suppressing agents have been carried out with rats given one dose of 7,12-dimethylbenz-(a)anthracene (DMBA) and after waiting for 1 week (the carcinogen has disappeared from the animal) the suppressing agent is administered.

Studies with cabbage and broccoli give an inhibitory effect at 1 g cabbage or broccoli/day/rat. This dose level is somewhat high compared to human consumption, but is within a twofold range. However, results of broccoli studies are very difficult to reproduce. Attention is concentrated on the cabbage and efforts are made to identify the compound(s) that exert this suppressing effect. Studies with orange oil added to the diet also show a respectable inhibitory effect. Terpenes and benzylisothiocyanate also act as suppressing agents. These compounds are multifunctional. Maybe their action as blocking and suppressing agents is a coincidence to the fact that these are reactive compounds, or there is a single mechanism with multiple protective effects. This is still unknown.
An important aspect in the study of inhibitors is the fact that probably only a very small fraction of the agents has been identified. Until there is more known about the complete range of inhibitors in foods, epidemiological interpretation will be difficult. So, agent identification is really bedrock.
One of the biggest traps in the study of inhibitors is the fact that a compound that prevents cancer may have toxic effects or even promoting effects depending on the circumstances. So, a protective agent has to be tested under a number of different circumstances to asses the risk/ benefit ratio.

3.2 Modulators of the Arachidonic Acid Cascade

Fuerstenberger (Heidelberg, FRG) reported about the relationships between modulators of the arachidonic acid cascade and inhibition of carcinogenesis. The multistage mouse skin model has been used for the study of chemical carcinogenesis. It appeared that tumours develop through three stages, initiation, promotion and progression.
Initiation is normally performed by a single application of an initiating carcinogen. The carcinogen leads to genetic alterations in the target cells. This is an irreversible process. The cells remain for the rest of their life initiated. In 90% of the papillomas a point mutation of the ras-gene was observed. An initiated cell doesn't lead to a tumour unless it is stimulated by a long-term contact with promoting agents, such as forbolesters. With this contact a high

number of benign tumours are generated, some of them being malign or pre-malign. This spontaneous progression to malign tumours can be accelerated by some carcinogens yielding squamous carcinomas.
The mouse skin tissue is a very active proliferating tissue with a high turn-over rate of cells and an active arachidonic acid metabolism. The arachidonic acid metabolism could therefore play an important role in carcinogenesis.
Arachidonic acid is metabolized through three pathways into its oxygenated metabolites.

1. cyclo-oxygenase pathway generates prostaglandines and prostacyclines.
2. lipoxygenase pathway generates hydroperoxides which in turn are converted to leucotrienes.
3. P-450-mono-oxygenases generate thiols, epoxy-prostaglandines and hydroxy-eicosatetraeonic acid.

All pathways are active in the mouse epidermis. The main pathways are the cyclo-oxygenase and the lipoxygenase pathways. The arachidonic acid metabolism yields, besides the mentioned metabolites, byproducts such as reactive oxygen caused by peroxidase reactions. The reactive oxygen accepts hydrogen from donor components and can thus be potentially harmful to man.
In the above mentioned three stage model of carcinogenesis initiation is usually done with DMBA. DMBA is metabolically activated by oxygenation through the P450 enzymes. In turn the oxidation products are epoxygenated at the 3,4 position and finally oxidized to 1,2 epoxy-3,4 dihydrodiol. This is the ultimate carcinogen which binds to the NH2 group of adenine in the DNA which leads, at least in the ras-gene, to a A-T transversion mutation. There are some indications that the last step is catalyzed by peroxyradicals which could stem from the cyclo-oxygenase reactions. Furthermore it has been shown that arachidonic acid increased the initiating activity of DMBA.
Inhibition can occur at different stages. The release of arachidonic acid from phospholipids can be inhibited by agents that inhibit phospholipase a2 activity. This is the main enzyme responsible for this release. The cyclo-oxygenase pathway can be inhibited by antioxidants, some arachidonic acid analogues which are used as drugs (indomethacin) and phenols. Inhibition of the lipoxygenase reaction

can occur through inhibition of peroxidases or trapping of the reactive oxygens found in the peroxidase reactions.
Forbolesters are the common promotors applied in the two stage mouse skin model. They induce two major effects in mouseskin. First inflammatory processes like edemas, erythrea, activation and infiltration of leucocytes are induced. Secondly an increase in the epidermal thickness due to epidermal hyperplasia is induced. This is a common response of the epidermis to damaging mechanical or chemical influences. TPA (12-O-tetradecanoylphorbol-13-acetate) probably works through the stimulation of protein-kinase C receptors which in turn stimulates the release of arachidonic acid from membrane phospholipids. This, in turn, stimulates the synthesis of prostaglandins through the cyclo-oxygenase pathway and the synthesis of leucotrienes and others through the lipoxygenase pathway. Prostaglandins are involved in the induction of epidermal hyperplasia. The metabolites of the lipoxygenase pathway are involved in chromosomal alterations which play a crucial role in tumour promotion. Many studies have shown that promotion can be inhibited by inhibitors of phospholipase a2, like dexamethacin, dibromo-acetophenone and by inhibitors of the cyclo-oxygenase reactions, like indomethacin. This appears to be true for CD1-mice and NMRI mice, but not for SENCAR mice. In SENCAR mice tumour promotion was enhanced by indomethacin. However, inhibitors of the lipoxygenase and cyclo-oxygenase pathway like 5,8,11,14-eicosatetraynoic acid, phenidone and quercetin have shown to be potent inhibitors of forbolesters induced promotion in SENCAR mice.

3.3 Tannic Acid, Ellagic Acid and Catechin Derivatives

Mukhtar (Cleveland, USA) discussed the prevention of carcinogenesis by green tea polyphenols, tannic acid and ellagic acid. Ellagic acid, a degradation product of certain tannins, is an important polyphenolic acid present in the plant kingdom and more specific also in a variety of fruits and vegetables. Ellagic acid (EA) was found to have antimutagenic and anticarcinogenic capacities in different test systems. EA has been shown to inhibit the mutagenicity of the bay-region diol-epoxides of several polycyclic aromatic hydrocarbons

(PAHs) in Salmonella thyphimurium and in Chinese hamster V79 tests. Topically applied EA, before inititation, was also shown to inhibit PAHs and DMBA-induced skin carcinogenesis in mice. It has also been shown that intra peritonal or parenteral administration of EA in the diet protected against benzo(a)pyrene (BP)-induced lung tumour formation. Feeding of an ellagic acid supplemented diet for 3 weeks mounted in protection against nitroso-methyl-benzylamine (NMBA) induced methylation of O^6-Guanine in rat oesophageal DNA. EA in the diet of rats showed significant inhibition of NMBA induced oesophageal tumours. The effect of a chronic feeding of EA was studied using trace amounts of EA in drinking water of mice. This resulted in a significant increase of the latency period of skin tumours induced by 3-methylcholanthrene (MCA).

A study was carried out showing that dietary plant phenols, such as tannic acid, quercetin, myricetin and anthraflavic acid are capable of inhibiting PAH metabolism and PAH-DNA adduct formation in the epidermis of SENCAR mice. The same plant phenols protected against DMBA-, BP-, MCA and N-methyl-N-nitrosourea (MNU)-induced skin tumourigenesis in mice. In general the order of the anticarcinogenic potency of these plant phenols was found to be tannic acid>myricetin>-quercetin>anthraflavic acid. The possible mechanisms of protective effect of the plant phenols may be due to an inhibitory effect on the binding of the ultimate carcinogen to target tissue DNA, thus acting as trapping agents.

Green tea polyphenols, such as catechin, epicatechin, epicatechin-3-gallate and epigallocatechin and epigallocatechin gallate (EGCG) were found to be strong antimutagens. Green tea polyphenols were also able to give a substantial inhibition in both the initiation and promotion phase in the initiation-promotion skinmodel with DMBA-TPA in SENCAR mice. In other models with BP, BP-diolepoxides and photocarcinogenesis green tea polyphenols, especially EGCG showed potent inhibition against tumour development.

The water-soluble compounds of liqourice, present in candy, beer, chocolate and chewing gum showed a protective effect in the two stage mouse skin model with DMBA-TPA treatment. A strong antipromotor effect was observed and some anti-initiation effects could be seen.

Some phenolics discussed here have some very promising effects with regard to anticarcinogenesis and thus could be potentially beneficial to men. However, a word of caution should be mentioned. Besides their anticarcinogenic properties some substances may have other fysiological effects in man, some of them being harmful. Attention should therefore be paid to toxicological properties of the compounds studied.

3.4 Curcumin, Chlorogenic Acid, Caffeic Acid and Ferulic Acid

Huang (New Jersey, USA) reported about the inhibitory effects of curcumin and its structurally related compounds on tumour promotion. Curcumin was a well-known medicine that was used against inflammatory diseases. It is also used as a coloring agent and spice in many foods. Turmeric contains 1-5% curcumin. Recent studies have indicated that substances with anti-inflammatory and antioxidant activity were potent inhibitors of TPA induced tumour promotion on the mouse skin.
The effect of curcumin and the related compounds, chlorogenic acid, caffeic acid and ferulic acid were tested in several short-term bioassays. Curcumin and the related compounds inhibited in a dose-dependent way the induction of ornithine decarboxylase activity by TPA on the mouse skin. Curcumin and the phenolic acids also inhibited TPA-induced DNA synthesis and [3H] thymidine incorporation into epidermal DNA in a dose-dependent way. In both types of bioassays curcumin showed a stronger inhibitory effect.
Since arachidonic acid metabolism is believed to give rise to reactive oxygen species and other free radicals who may play an important role in tumour promotion, the effects of curcumin and phenolic acids were tested on TPA- and arachidonic acid induced edema of mouse ears. Only curcumin inhibited, again in a dose-dependent manner, the incidence of mouse ear edema when administered 30 mins before application of the inflammatory agent. The above mentioned inhibitory effect of curcumin on the ear edema may suggest that curcumin blocks arachidonic acid metabolism by inhibiting epidermal cyclo-oxygenase and/or lipoxygenase activity or that curcumin may act as a scavenger of reactive free radicals. In vitro studies showed that curcumin and the phenolic acids

inhibited the lipoxygenase activity, curcumin having the strongest effects. Curcumin also inhibited cyclo-oxygenases.
A long-term in vivo study with the initiation-promotion model on the mouse skin was carried out. The initiator used was DMBA with a topical application of TPA as a promotor. Curcumin was first tested for promoting activities but all the results were negative. Topical application of 10 umol of curcumin, chlorogenic acid, caffeic acid or ferulic acid in the mouse skin model resulted in a tumour inhibition of 100, 31, 28 or 7% respectively.
In summary curcumin seems to be a strong inhibitor of TPA promotion on the mouse skin. In this regard the structuraly related compounds such as chlorogenic acid, caffeic acid and ferulic acid are less potent inhibitors. The inhibitory effect of curcumin is possibly, at least partly due to an inhibition of the arachidonic acid metabolism.
Further studies in other tumour models have to be carried out in order to elucidate possible mechanisms. Also due to the fact that curcumin is present in food and therefore is ingested, inhibitory effects of curcumin on tumour formation in the digestive tract need more attention.

3.5 Indoles

Jongen (Wageningen, the Netherlands) showed some results of his studies on the effects of indoles on carcinogenesis. Glucobrassicin occurs in many plants where it functions as a plant hormone. Some breakdown products of this glucosinolate, the indole compounds, have shown to have anticarcinogenic potencies. Important indoles in this regard are indole-3-carbinol (I3C) and indole-3-acetonitril (I3A). Wattenberg et al. showed that gavaging indoles to animals, who were subsequently exposed to indirect acting carcinogens, resulted in a significant reduced tumour incidence. The carcinogens used were BP, DMBA and PAH. However, the use of DMH resulted in an increase of the tumour incidence. In a initiation-promotion model I3C acted as a promotor. The inhibitory capacities of indoles are thought to stem from their strong inducing capacity of the biotransformation enzymes.

Recently it has been shown that I3C was an active scavenger of electrophilic compounds. In summary, the anticarcinogenic effect of indoles seems to be rather a modulating effect and depends largely on the type of carcinogen applied.

The in-vitro effects of indoles were studied in a co-cultivation system. This co-cultivation system consisted of cultured chick hepatocytes co-cultivated with V79 chinese hamster cells. Chick hepatocytes showed to have a fairly stable biotransformation activity for at least 72 hrs. The genetic endpoints of this study are the Sister Chromatid Exchanges (SCE) in the V79 chinese hamster cells. The indole compounds were added to the cultured hepatocytes until the maximum activity was reached. I3C increased up to three-fold the P-450 activity. I3A induced a 60% increase of P-450 activity. The indoles were then removed, the V79 hamster cells added and co-cultured with the chick-hepatocytes. The co-cultivation system was exposed to BP for a certain amount of time. The V79 hamster cells were then screened for SCE's. It appeared that pretreatment with I3C and I3A resulted in a significant decrease of SCE's. Additional studies looked at the metabolite formation. It appeared that there was an increase in the formation of the mutagenic 7,8 -diol metabolite of BP due to the induction of P-450 enzymes by indoles. However, this increase was compensated by an increase of the detoxifying activity of glucuronyl-UDP transferase. The net result is a protective effect of I3C and I3A from BP and dimethylnitrosamine (DMNA). It was concluded that the balance between the activity of both the microsomal (P-450) and cytosolic enzym (G-UDP) systems are crucial for the observed effects. The type of modulation depends, as was already said, largely on the kind of chemical that is used.

Indoles, especially I3C, I3A and 4-chloro compounds in vegetables like Brassica's can be nitrosated with nitrite to form mutagenic N-nitroso compounds. Studies on nitrosation rate of indoles showed specific differences in chemical stability at different pH's. A strong correlation between the indolyl-glucosinolate content and the formation of N-nitroso compounds in cruciferous vegetables was observed. The nitrosated Brassica extracts were mutagenic in the Ames test, however, calculations showed that indolyl-glucosinolates contributed only approximately 2% to the mutagenicity of Brassica's after nitrosation.

3.6 Aromatic Isothiocyanates

Chung (New York, USA) commented the effects of aromatic isothiocyanates on carcinogenesis. In the late seventies and the beginning of the eighties Wattenberg tested some breakdown products of glucosinolates, the isothiocyanates, for their anticarcinogenic effects. Three isothiocyanates, phenylisothiocyanate (PITC), benzylisothiocyanate (BITC) and phenethylisothiocyanate (PEITC) were tested. PITC is a synthetic isothiocyanate, whereas BITC and PEITC are naturally occurring. It appeared that isothiocyanates could inhibit DMBA-induced mammary tumours in rats and DMBA- and BP-induced forestomach cancer and lung cancer in mice.

In this presentation the focus is on compounds that can inhibit nitrosamine induced tumours, especially NNK induced lung tumours. NNK is thought to be the most important lung carcinogen present in tobacco. NNK is derived from nicotine by nitrosation through tobacco smoking and chewing. In animal studies NNK was able to induce lung cancers independently from the administration route. NNK is an indirect acting carcinogen which requires metabolic activation. Two pathways are known. The first one is α-hydroxylation on the methylene carbon which generates DNA methylating agents forming 7-methylguanine, O^6-methylguanine or O^4-methylthymine. The second one is α-hydroxylation of the methylgroup generating formaldehyde. NNK can also be deactivated by N-oxidation to form an N-oxide.

In the early eighties a screening method was developed for potential inhibitors of nitrosamines. This screening methods consists of 3 stages:

1. In vitro metabolism
2. In vivo DNA-adductformation
3. Carcinogenicity bioassays

In the first stage the test compound is given to the diet of the animals, microsomes are then isolated, incubated with the carcinogen NNK and the metabolic activation is studied. It appeared that indoles were extremely potent inducers and aromatic isothiocyanates strong inhibitors. The second stage, with a comparable protocol showed that PEITC was the most potent inhibitor of NNK-DNA adduct formation among all of the isothiocyanates tested. In the third stage PEITC inhibited

50% of lung tumours in NNK induced rats, but it didn't inhibit the incidence of liver tumours and nasalcavity tumours. The inhibition was probably caused by the inhibition of 7-methylguanine formation. 7-methylguanine level was unchanged in the liver and nasalcavity but PEITC caused a 40% reduction in the lung. In a mouse bioassay system PEITC showed to inhibit in a dose dependent way lung adenomas induced by NNK. Looking at DNA-adduct formation it turned out that O^6-methylguanine was in a dose-dependent manner inhibited by PEITC. Comparable isothiocyanates, such as PITC and BITC failed to inhibit tumour formation and O^6-methylguanine formation. Studies with aromatic isothiocyanates, which had different CH-chain length, indicated that with increasing chain length the inhibitory capacities were enhanced. This is probably due to an increasing lipophilicity and stability of the isothiocyanates in mamalians.
C14 labelled PEITC was gavaged to A/J mouse. PEITC was rapidly distributed in all tissues. There was a major concentration in liver and kidneys. After 72 hrs all the radioactivity was gone. A peak of radioactivity in the lung was reached after 48 hrs. After 72 hrs 55% of the total dose was found in the urine and 23% in the feces. In the urine 3 major peaks of the metabolites of PEITC were found. Two of them were identified being the cyclic conjugate of mercaptol paravit (50% of radioactivity) and the N-acitiocysteine conjugate (25%) respectively. Metabolite 2 being approximately 25% of the total PEITC ingested could be a possible biochemical marker in human studies. In feeding tests with the glucosinolate gluconasturtiin it was found that 30% was hydrolyzed to PEITC in presence of the enzym myrosinase. Without the enzyme less than 5% was hydrolysed to PEITC.

3.7 Organosulfur Compounds

Wargowich (Houston, USA) summarised the effects of organosulfur compounds from allium species on carcinogenesis. The volatile sulfur containing compounds in allium species like onions and garlic are responsible for their typical odours. In general more unsaturated compounds are found in the garlic, whereas the straight chain compounds are more characteristic for onions. Besides the anticarcinogenic effects discussed here, the sulfur compounds of

allium species were reported to inhibit platelet aggregation, to reduce blood pressure and to have some antiviral and antifungal activities. The main substance that is discussed here is diallylsulfide (DAS), which is found in garlic.
Very little is known about the metabolism and distribution of DAS in mammals. DAS seems to be taken up quikly, rapidly distibuted but it doesn't last very long. Probably, rapid oxidation of DAS takes place. However, no tracer studies have been done.
DAS was tested in acute and sub-acute toxicity tests and was found to be virtually non-toxic up to grams/kg. Colon tumours induced by dimethylhydrazine in the rat are histologically comparable to colon tumours found in humans. So emphasis was placed on the anticarcinogenic effects of DAS on gastro-intestinal cancer. In the nuclear abberation assay in colon cells DAS inhibited in a dose-dependent way nuclear damage when given 3 hrs prior to induction by dimethylhydrazine (DMH). In a mouse study after 40 weeks a 75% inhibition by DAS, at a dose of 200 mg/kg, 3 hours before the weekly injection with DMH for 20 weeks, of all tumours in the gastro-intestinal tract was found. Interestingly, an almost complete inhibition of adenocarcinoma occured. So DAS seemed to inhibit the progression of tumours. Emphasis was also placed on the protective effect of DAS against NMBA-induced oesophageal cancer in the rat. NMBA is an indirect-acting carcinogen which needs metabolic activation by the liver. A complete inhibition of the 80% incidence of oesophageal tumours was found by administration of DAS 3 hrs prior to the carcinogen during 15 weeks. DAS was also tested with the carcinogen N-methyl-N'-nitro-N-nitrosoguadinine (MNNG) and it was found to inhibitit stomach cancer in the rat.
Different sulfur compounds of allium, for instance s-allylcysteine were studied and it appeared that the presence of an allyl-group was needed in the above mentioned test-systems in order to find inhibition. The straight-chain compounds had virtually no effect. Two allyl-groups, like DAS were more effective than compounds with one allyl-group.

In an epidemiological study in Japan, with a high stomach cancer incidence rate it was found that a chronic high consumption of garlic protected against stomach cancer. In a recently published epidemiological study in Italy a high consumption of citrus fruits and garlic in southern Italy compared to northern Italy was related to a lower colon cancer incidence.

3.8 Monoterpenoids

Gould (Madison, USA) reported about the effects of terpenes on carcinogenesis. Terpenoids are specially found in citrusoils and mints, but also in spices and other plant products. The most important one and most extensively studied is the monocyclic monoterpenoid d-limonene. Orange oil consists of up to 95% of this terpenoid. D-limonene was found to be an anticarcinogenic agent against chemically-induced mammary tumours in the rat. It inhibited mammary tumours induced by the indirectly acting DMBA (which requires metabolic activation) and the directly acting MNU in both, the initiation and promotion phase. It inhibited spontaneous mammary cancer and caused regression of frank mammary tumours. D-limonene was found non-toxic up to gram levels in animals and humans. It is at the present unclear whether d-limonene itself or a metabolite of d-limonene is responsible for its anticarcinogenic activities.
The metabolism of d-limonene was studied with C14-labelled d-limonene fed in the diet to rats. D-limonene was readily absorbed and reached its peak in the bloodserum after 12 hrs. After 48 hrs most of the d-limonene was excreted. D-limonene is well distributed throughout the various tissues; a peak is reached after 12-14 hrs and it decreases rapidly. In a GC-analysis of the serum two unknown peaks were found representing the metabolites of d-limonene. One of them was thought to be the methylester or the aldehydemethylester of d-limonene whereas the other remains unidentifiable up to now.
In order to establish a structure-relationship different terpenoids were tested on anti-initiation activity versus DMBA. Comparing the mono-, bi- and acyclic terpenoids it appeared that only the monocyclic terpenoid had anticarcinogenic activities. Within the group of the monocyclic terpenoids menthol had higher activity than d-limonene.

The activities of the urinary metabolites of d-limonene, uriterpenol and carveol, and an oxidation product of α-pinene, sobrero were compared to d-limonene and it appeared that the anti-initiation activities of these compounds were as follows: d-limonene<carveol<uriterpenol<sobrero. Sobrero was approximately 5 times more potent than d-limonene.
D-limonene reduced the DMBA-DNA adductformation up to 50%. Although d-limonene activated phase 1 enzymes e.g. P-450 and epoxide hydratase, the major inhibitory mechanism is an activation of glutathion transferase (GTA) activities (phase 2 enzymes). Looking at the direct acting NMU it appeared that d-limonene was also able to inhibit carcinogenesis at the promotion stage. D-limonene was given pair-fed to rats with frank mammary tumours induced by DMBA and a regression of approximately 90% occured in the experimental group compared to 30% spontaneous regression in the control group. The mechanism by which d-limonene inhibits the promotion/progression stage of carcinogenesis and causes the regression of carcinomas is not known. However, it is clear that this effect is not endocrine-modulated. Currently, the hypothesis is explored that the mono-terpenes may interfere in post-translational isoprenylation of key cellular membrane bound proteins such as ras p21 proteins.

3.9 Carcinogenicity of Human Diets in Rats

Kuiper (Wageningen, the Netherlands) showed some results of a study on the carcinogenicity of human diets in rats, the influence of heating and the addition of vegetables and fruit. Heat treatment of foods, especially meat, may cause formation of compounds with mutagenic and carcinogenic effects like quinolines, quinoxalines and imidazoquinolines. The influence of dietary factors such as total composition, thermal processing, and the addition of vegetables and fruits on the tumour rate in rats was studied in a long-term experiment. Groups of 50 male and 50 female Wistar rats were fed one of the following diets: a semi-synthetic animal diet (A, control); diet A to wich vegetables and fruits were added (B); an uncooked human diet (meat, bread and eggs) supplemented with semi-synthetic compounds

(C); diet C with fried or baked products (D); a complete human diet consisting of heated products, vegetables and fruits prepared according to mean consumption figures in the Netherlands (E). The animal diets (A and B) contained 26.0 energy (E)% protein, 21.6 E% fat, 52.4 E% carbohydrates and 10.7 % (w/w) fiber. The human diets contained 13.2 E% protein, 40.6 E% fat, 46.2 E% carbohydrate and 5 % (w/w) fiber. Care was taken that in all diets contents of vitamins and minerals were kept equal. The rats were fed ad lib. for 142 weeks. In male and female fed human diets (C, D or E) hepatocellular vacuolization was observed. Male rats (but not female) fed the human diet had a significantly ($p < 0.02$) higher incidence of epithelial tumours than those fed the animal diet. This increase was mainly due to tumours of the pituitary and thyroid. Frying and baking of food products (diet D) and the addition of vegetables and fruits (diet E) induced minor differences in tumour rate, but these were not statistically significant.

4 EPIDEMIOLOGY AND ANALYSIS

4.1 Vegetables and Fruits in Cancer Etiology

Riboli (Lyon, France) summarised the results of epidemiological studies on the role of vegetables and fruits in cancer etiology. There have been tremendous changes in cancer mortality rates in the world. A well-known example is the 3-fold decrease of stomach cancer throughout the world which is probably caused for a great deal by improvements in the diet and conservation practices. At the same time there was a modest increase in colorectal cancer incidence and a strong increase in lung cancer and breast cancer in women. The well-known epidemiologists Doll and Peto have made an estimation of the role of life-style factors on different cancers in man. The estimation for the impact of diet on cancer was 35% with a logical confidence interval of 10-70%. This means that there still is a lot of uncertainty about it. Current opinions among scientists on important etiological factors may also change in the course of time. A well-known example are fibers. Fibers were thought to play a major role in the etiology of colo-rectal cancer. Today the current belief is that there is rather a

protective effect of vegetables and fruits than from fibers alone. A problem in this context is that in epidemiological studies the effects of fibers cannot be adequately separated from the effects of fruits and vegetables, because the two are highly inter-correlated.

Gastric cancer

Ten (mainly small) case-control studies found a reduced risk for vegetables intake, comparing the highest vegetable intake with the lowest vegetables intake. Two early studies in the 1960's didn't find a reduced risk. Five out of seven studies who looked at fruits intake found a reduced risk for high fruits intake vs. low fruits intake. Five of these studies were able to look at micronutrient intake and again they all found a reduced risk for vitamin C and β-carotene but not for retinol.

Colorectal cancer

From the ten conducted case-control studies eight did evaluate vegetables intake. All but one found a reduced risk for vegetable intake comparing the highest quartile vs. de lowest quartile. One study found an increased risk, which was probably mainly due to the consumption of a rare vegetable in the Japanese diet. This result was never confirmed in other studies. No special reference was made to vitamin intake.

Breast cancer

There are only a few studies that investigated fruit and vegetable consumption in relation to breast cancer. In four case-control studies a reduced risk for a high fruits and vegetables intake was found. These results were only found in some specific subgroups and on the average the results are not very convincing.

In 1990 a pooled study was carried out by G. Howe (Toronto) to look at diet (especially fat) and breast cancer relations. In total there were some 10000 women enrolled in this meta-analysis, with approximately 4400 cases. A relative risk for fat intake (highest vs. lowest quartile) of 1.48 ($p<0.05$) was found. In addition a reduced risk for vitamin C, fiber an β-carotene intake for highest intake vs. lowest intake was found. No dose-response relationship was observed.

An international prospective study, coordinated at IARC (France), on diet and cancer relations has been started. This is a collaborative study involving 9 countries and a total of 450000 people. The countries are UK, France, Spain, Italy, the Netherlands, Germany (FRG), Greece, Sweden and Danmark. This study will start in early 1991 with data collection and will have a follow-up with cancer registries of at least 10 years. In this study biological samples, 24-hrs recall and food-frequency records will be used to estimate food and nutrient intake.

4.2 Pilot Study on Flavonoids

Hertog (Wageningen, the Netherlands) reported some preliminary results of an epidemiological evaluation of flavonols. Flavonoids are benzo-pyrone derivatives which share the common C6-C3-C6 skeleton. They are ubiquitous in plants, including foodplants in which they occur mainly as glycosides. Flavonoids contribute to colour and taste of plants and are often used in plant taxonomy. The average intake of flavonoids is estimated to be 1 gram/day. The flavonol quercetin (3,5,7,3',4'-pentahydroxyflavone) is the most frequently occurring flavonol and the most extensively studied. Quercetin is found in all common fruits and vegetables mainly in the skin and peel at concentrations varying from some milligrams up to a gram per kg fresh weight. An exception is the skin of coloured onions where concentrations up to 2% were found. The biological effects of quercetin have been subject of various studies. Quercetin showed anticarcinogenic effects in chemically induced tumours in rats and mice. It also reduced the mutagenicity of bay-region diol epoxides of BP. Quercetin was able to inhibit carcinogen-activating enzymes such as aryl-hydroxylases. More recently it has been shown that quercetin was a potent inhibitor of TPA and teleodicin induced tumour promotion in mice, probably through inhibition of the arachidonic acid cascade. However, no human data on the anticarcinogenic effects of flavonoids are available.

It was decided to look in the setting of the Seven Countries Study at the relation between quercetin intake and cancer mortality. The Seven Coutries Study is an epidemiological study which aim is to investigate the relation between diet, cardiovascular diseases and cancer.
Briefly, 13 000 men from 16 cohorts in seven countries aged between 40 and 59 at the baseline were selected around 1960. The countries involved were US, Japan, Finland, Yugoslavia, Italy, Greece and the Netherlands. Food composites representing the average intake of foods per day were sampled locally and sent to the Netherlands. A method of analysis based on HPLC was developed to determine the average daily quercetin intake per cohort. Quercetin intake was compared to the total cancer mortality data after 15 years of follow-up in each cohort. An inverse relation was found between quercetin intake and cancer mortality rates ($r = -0.58$, $p<0.05$). This relation could not be explained by the inverse relation between fruits and vegetables intake and cancer mortality rates.
However, the results presented here are not corrected for confounding by smoking behaviour and vitamin intake. Final analysis will be carried out using the 25 years mortality data and results will be presented subsequently.

4.3 Analysis of Potential Anticarcinogens in Cruciferous Vegetables and Allium Species

Fenwick (Norwich, UK) reported about the possibilities of analytical studies of potential anticarcinogens in cruciferous vegetables and allium species. Glucosinolates are found naturally in cruciferous plants such as Brassica vegetables, for instance cabbage, which are important in the human diet. Glucosinolates are thioglucosides. Important breakdown products of these thioglucosides and at the same time the active constituents in anticarcinogenesis are the isothiocyanates and the indoles. They are formed upon the hydrolytic activity of the enzyme myrosinase which is activated when the plant is macerated. Thus domestic/industrial processing which may totally or partially inactivate this enzym will have a great effect on the nature and amounts of the biologically active substances present.

The analytical possibilities to determine glucosinolate contents are vast but there is a need for more information about the biological effects of these compounds in animals, and specifically, in man. With more specific biological and toxicological evidence analytics could focus on individual glucosinolates rather than on total glucosinolate content.

Factors affecting glucosinolates content of vegetables are: species, variety (2-3 fold differences) and storage. Food preparation is also important, for instance fermenting decomposes glucosinolates and it is totally unknown which compounds are formed. Cooking and processing can give losses of 25-45% and isothiocyanates found at this stage will be volatilized or will undergo secondary reactions, whereas the indoles will probably be stable.

The average intake of glucosinolates in the UK is about 29 mg/day taking into account a loss of 25-45% due to cooking. Compared to Canada, the USA and the Netherlands, UK intakes are the highest. Within populations there are large differences in brassica consumption. Recent studies indicate that about 2 million people in the UK have an intake of 200-300 mg of glucosinolates per day. Glucosinolate intake is mainly due to the consumption of Brussels sprouts and cabbage. Approximately 1/3 of the total glucosinolate are indol-glucosinolates yielding the indole compounds. Indoles seem to be more stable than the isothiocyanates which underlines the importance they could play in cancer prevention. Over the year the intake of Brassicas and vegetables varies a lot; the highest intakes are recorded in the months October, January and February.

Most of the analytical studies on allium species have been conducted on the volatile compounds, which are the flavouring and biologically active compounds in the oils of Allium species. Quite recently, interest in these compounds has risen because some of them may play an important role in anticarcinogenesis (Wargowich). Bound γ-glutamylpeptide is the major component of the Allium species (100-200 mg/100g) and is enzymaticly degraded to, among others, cystein sulfoxide and the compound of interest in this context, diallylsulfide. A major problem for the determination of the flavouring compounds of garlic and onions is the fact that they are very unstable, variable from one allium sample to another, and

constantly changing. Practically no information is available on the occurrence of these sulfur substances in alliums. Diallylsulfide content of onions decreased with increasing cooking temperature and increasing cooking time length.
Clearly, there is information available, sometimes limited, on the occurrence of anticarcinogens in different products. The time has come to gather the available information from different laboratories. It should be possible to create a database on the occurrence of non-nutritive anticarcinogenic agents in foods. This database should take into account the following facts:

- Information on various components in foods that could cause synergistic interactions or have other effects. (e.g. some components increase permeability of the gut)
- By breeding of new varieties with a higher resistance to pests, plants are created with unknown components. Breeding could also affect the content and composition of known components.

5 RESEARCH PRIORITIES

General Discussion and Conclusions

The aim of this workshop was to establish priorities for epidemiologic and analytical research on anticarcinogens. In the following discussion and conclusions emphasis was placed on these types of research. It must be said that for other types of research, for instance elucidating mechanisms or application of anticarcinogens as therapeutic agents, other priorities may be relevant.

Polyphenols

Polyphenols were identified as a most promising group of anticarcinogens. Polyphenols are a vast class of different compounds present in a large variety of vegetables and fruits. Polyphenols prove to be effective in a number of experimental systems, and at least some of them affect the arachidonic acid cascade, which is clearly important in the promotional phase of carcinogenesis. A great deal of animal data show that these effects on the arachidonic acid cascade are associated with inhibition of carcinogenesis. There is sufficient

analytical support for small molecular polyphenols, however, only limited chemical information is available on so called "tannins". The group of polyphenols is very large, so guidance in the choice of the most promising polyphenolic anticarcinogens would be very helpful. Only structure-activity relations of inhibitory effects on ultimate carcinogens and the arachidonic acid cascade have been reported. However, extrapolation from these model systems to the human situation is very difficult.
The following promising compounds for epidemiological studies were suggested: flavonols, phenolic acids, catechin derivatives (green tea polyphenols).

Indoles

In experimental studies, indoles can either have an inhibitory effect or a stimulating effect on carcinogenesis depending on the type of carcinogen applied. Indoles originate from indolyl glucosinolates in cruciferous vegetables and, depending on the pretreatment of the vegetable, there is a direct exposure or an indirect exposure after metabolism in the digestive tract. Epidemiological studies on indoles are therefore difficult.

Aromatic isothiocyanates

Aromatic isothiocyanates could be interesting compounds in epidemiological studies on smokers, as in experimental systems these compounds were able to inhibit lung cancer induced by a tobacco specific nitrosamine. Only a minor fraction of the glucosinolates of vegetables, the parent compounds of isothiocyanates, release aromatic isothiocyanates after hydrolysis. So chemical analysis of total diets in order to determine the intake of aromatic isothiocyanates is not justified.
The intake could be determined by analysing specific vegetables relatively rich in the parent glucosinolate (gluconasturtiin). It was suggested to use a biological marker to be able to measure the human exposure. However, the thiocyanate ion as a metabolite of the isothiocyanate is not a specific marker for the exposure to aromatic isothiocyanates.

Terpenes

The major source for terpenes in the diet e.g. d-limonene are soft drinks. Part of the consumption of terpenes is due to flavouring agents that will be of increasing importance in the future. Terpenes are readily absorbed and their biological half life is rather long. Sensitive GC methods are available and determination of terpenes in serum is feasible. No data are available about amounts of terpenes ingested and their biological activity.
In conclusion, no top priority for epidemiological studies should be given to terpenes.

Sulfur compounds of allium species

Sulfur compounds of onions and garlic might be very potent, but they appear analytically to be a very difficult class of substances. As these compounds are restricted to onions and garlic, the need for chemical analyses is less urgent. For epidemiological studies it will be easy to determine the users and non-users of for instance garlic. However, this approach will not allow to quantify the intake of these compounds, so dose-response relations are not possible. A more promising approach compared to analyses of foods, would be to determine these sulfur compounds in the breath or sweat of the subjects, and use these data as indicators for the exposure.

Epidemiology

Between populations studies on the effects of anticarcinogens only can give indications of possible importance of certain anticarcinogens or types of foods. Compared to within population studies, between populations studies can be very valuable as they show more variation in dietary patterns and cancer rates. However, these studies never can prove causality. Individual based studies will always be necessary. If large cohorts are involved in individual based studies, individual food analyses are prohibited because hundreds of components have to be analysed. One solution would be to analyse only the average diet of individuals that developed cancer and the average diet of the controls.

It was recognised that the development of biological markers for the intake and or the effect of anticarcinogens in the human body should deserve a high priority. Especially markers for effects of anticarcinogens (polyphenols) on the arachidonic acid cascade in the human body would be very valuable. Possibilities of measuring these effects in for instance blood cells were discussed.

Concluding remarks

At the end of the discussion Wattenberg made the following concluding remarks. Each of the groups of compounds presented in this workshop have great potential. The importance of these compounds to the inhibition of human cancer remains to be determined. Epidemiology could be very important, but it is only one part of the whole picture. Nevertheless, positive epidemiological data would be very encouraging. The group of researchers present in this workshop represents almost the total range of the known natural occurring non-nutritive inhibitors. This shows how small the effort has been until now in identifying compounds that may have inhibitory effects. There may be many anticarcinogens that are more important than the ones that were discussed during this workshop.

LIST OF ABBREVIATIONS

BP	: benzo(a)pyrene
DMBA	: 7,12-dimethylbenz(a)anthracene
DMH	: dimethylhydrazine
DMNA	: dimethylnitrosamine
MCA	: 3-methylcholanthrene
MNNG	: N-methyl-N'-nitro-N-nitrosoguadinine
MNU	: N-methyl-N-nitrosourea
NMBA	: nitroso-methyl-benzylamine
NNK	: 4-(methylnitrosamino)-1-(3-pyridil)-1-butanone
PAH	: polycyclic aromatic hydrocarbon
TPA	: 12-O-tetradecanoylphorbol-13-acetate

ANNEX II

PROGRAMME WORKSHOP * 26 -27 MARCH 1990 WAGENINGEN, THE NETHERLANDS

NON-NUTRITIVE ANTICARCINOGENS IN FOODS
State of the art and future developments

First day

9.00- 9.30 Opening
M. Heuver, General-Director Agricultural Research Department, Ministry of Agriculture, Nature Management and Fisheries, The Netherlands.
B.H. Bijsterbosch, Deputy Rector Magnificus Wageningen Agricultural University, The Netherlands.

9.30- 9.45 General introduction
D. Kromhout, National Institute of Public Health and Environmental Protection, The Netherlands.

EXPERIMENTAL STUDIES

Chairman: D. Kromhout

9.45-10.05 Anticarcinogens in the diet: an overview
L. Wattenberg, University of Minnesota, USA.

10.05-10.20 Discussion

10.20-10.50 Coffee/tea break

10.50-11.10 Relationships between modulators of the arachidonic acid cascade and inhibition of carcinogenesis.
G. Fürstenberger, German Cancer Institute, Federal Republic of Germany.

11.10-11.25 Discussion

11.25-11.45 Prevention of carcinogenesis by green tea polyphenols, tannic acid and ellagic acid.
H. Mukhtar, Case Western Reserve University, USA.

11.45-12.00 Discussion

12.00-12.20 The inhibititory effects of curcumin and its structurally related compounds on tumor promotion.
M-T. Huang, The State University of New Jersey, USA.

12.20-12.35 Discussion

12.35-14.00 Lunch

Chairman: M.B.Katan

14.00-14.20 Effects of indoles on carcinogenesis.
W. Jongen, ATO Agrotechnology, The Netherlands.

14.20-14.35 Discussion

14.35-14.55 Effects of aromatic isothiocyanates on carcinogenesis.
F-L. Chung, American Health Foundation, USA.

14.55-15.10 Discussion

15.10-15.40 Coffee/tea break

15.40-16.00 Effects of organosulfur compounds from Allium species on carcinogenesis.
M. Wargovich, University of Texas, USA.

16.00-16.15 Discussion

16.15-16.35 Effects of terpenes on carcinogenesis.
M. Gould, University of Wisconsin, USA.

16.35-16.50 Discussion

16.50-17.05 Short Communication - A study on the carcinogenicity of human diets in rats: The influence of heating and the addition of vegetables and fruit
H. Kuiper/G. Alink, State Institute for Quality Control of Agricultural Products/ Wageningen Agricultural University, The Netherlands

17.05-17.15 Discussion

19.00 Dinner

Second day

EPIDEMIOLOGY AND ANALYSIS

Chairman: D. Kromhout

9.00- 9.20 Vegetables and fruits in cancer etiology
E. Riboli, International Agency for Research on Cancer, France.

9.20- 9.35 Discussion

9.35- 9.55 Preliminary results of an epidemiological evaluation of flavonols.
M. Hertog, State Institute for Quality Control of Agricultural Products/Wageningen Agricultural University, the Netherlands.

9.55-10.10 Discussion

10.10-10.30 Analytical studies of potential anticarcinogens in cruciferous vegetables and Allium species.
R. Fenwick, AFRC Institute of Food Research, United Kingdom.

10.30-10.45 Discussion

10.45-11.15 Coffee/tea break.

RESEARCH PRIORITIES

Chairman: M.B. Katan

11.15-11.30 Introduction
L. Wattenberg, University of Minnesota, USA.

11.30-13.00 General Discussion / Conclusions.

13.00-14.00 Lunch

ANNEX III

LIST OF PARTICIPANTS

Dr G.M. Alink
Department of Toxicology
Wageningen Agricultural University
P.O. Box 8129
6700 EV WAGENINGEN
The Netherlands

Ir. A. Bal
Ministry of Agriculture, Nature Management and Fisheries
P.O. Box 20401
2501 EK DEN HAAG
The Netherlands

Dr P.J. van Bladeren
CIVO Institutes
Utrechtseweg 48
3704 HE ZEIST
The Netherlands

Ir B. Bloemberg
National Institute of Public Health van Environmental Protection
P.O. Box 1
3720 BA BILTHOVEN
The Netherlands

Prof. B.H. Bijsterbosch
Department of Physical and Colloid Chemistry
Wageningen Agricultural University
P.O. Box 8129
6700 EV WAGENINGEN
The Netherlands

Dr F.-L. Chung
American Health Foundation
Section Nucleic Acid Chemistry, Division of Chemical Carcinogenesis
Institute Disease Prevention
VALHALLA, New York 10595
U.S.A.

Dr R. Fenwick
AFRC Institute of Food Research, Norwich Laboratory
Colney Lane
NORWICH, NR4 7UA
U.K.

Dr G. Fürstenberger
German Cancer Research Institute
Im Neuenheimer Feld 280
6900 HEIDELBERG
F.R.G.

Prof. M.N. Gould
University of Wisconsin-Madison Medical School
Department of Human Oncology
K4/310 Clinical Science Center
600 Highland Avenue
MADISON, Wisconsin 53792
U.S.A.

Prof. J.G.A.J. Hautvast
Department of Human Nutrition
Wageningen Agricultural University
Bomenweg 2
6703 HD WAGENINGEN
The Netherlands

Dr H. Herstel
State Institute for Quality Control of Agricultural Products
Bornsesteeg 45
6708 PD WAGENINGEN
The Netherlands

Drs M. Hertog
State Institute for Quality Control of Agricultural Products
Bornsesteeg 45
6708 PD WAGENINGEN
The Netherlands

Ir M. Heuver
Agricultural Research Department Central Management Authority
P.O. Box 59
6700 AB WAGENINGEN
The Netherlands

Ir P.C.H. Hollman
State Institute for Quality Control of Agricultural Products
Bornsesteeg 45
6708 PD WAGENINGEN
The Netherlands

Prof. M.-T. Huang
State Institute of New Yersey RUTGERS
Department of Chemical Biology and Pharmacology
College of Pharmacology
P.O. Box 789
PISCATAWAY, New Yersey 08855-0789
U.S.A.

Drs J.M.P. den Hartog
State Institute for Quality Control of Agricultural Products
Present address:
Randdijk 2
3435 PC NIEUWEGEIN
The Netherlands

Dr W. Jongen
Agrotechnological Research Institute (ATO)
P.O. Box 17
6700 AA WAGENINGEN
The Netherlands

Prof. M.B. Katan
Department of Human Nutrition
Wageningen Agricultural University
Bomenweg 2
6703 HD WAGENINGEN
The Netherlands

Dr J. Kleinjans
Department of Human Biology
Biomedical Centre State University of Limburg
P.O. Box 616
6200 MD MAASTRICHT
The Netherlands

Prof. D. Kromhout
National Institute of Public Health and Environmental Protection
P.O. Box 1
3720 BA BILTHOVEN
The Netherlands

Dr H. Kuiper
State Institute for Quality Control of Agricultural Products
Bornsesteeg 45
6708 PD WAGENINGEN
The Netherlands

Ing. C.G.M. van Leeuwen
Commodity Board of Vegetables and Fruits
Bezuidenhoutseweg 153
2509 LK DEN HAAG
The Netherlands

Prof. G.R. Mohn
National Institute of Public Health and Environmental Protection
P.O. Box 1
3720 BA BILTHOVEN
The Netherlands

Prof. H. Mukhtar
Case Western Reverse University
Department of Dermatology
CLEVELAND, Ohio 44106
U.S.A.

Dr W.H.M. Peters
St. Radboud Hospital
Division of Gastroenterology
Catholic University Nijmegen
P.O. Box 9101
6500 HB NIJMEGEN
The Netherlands

Dr E. Riboli
International Agency for Research on Cancer (IARC)
150, Cours Albert-Thomas
69372 LYON cedex 08
France

Ir G.S. Roosje
State Institute for Quality Control of Agricultural Products
P.O. Box 230
6700 AE WAGENINGEN
The Netherlands

Dr G.J.A. Speyers
National Institute for Public Health and Environmental Protection
P.O. Box 1
3720 BA BILTHOVEN
The Netherlands

Mrs. ir H.G.M. Tiedink
Department of Toxicology
Wageningen Agricultural University
P.O. Box 8129
6700 EV WAGENINGEN
The Netherlands

Prof. M.J. Wargovich
The University of Texas
MD Anderson Cancer Center
Section Gastrointestinal Oncology and Digestive Diseases, Box 78
1515 Holcombe Boulevard
HOUSTON, Texas 77030
U.S.A.

Prof. L.W. Wattenberg
University of Minnesota
Department of Laboratory Medicine and Pathology
6-133 Jackson Hall
321 Church Street S.E.
MINNEAPOLIS, Minnesota 55455-0315
U.S.A.

Dr M.I. Willems
CIVO-Institutes TNO
P.O. Box 360
3700 AJ ZEIST
The Netherlands

Workshop secretariate:
Mrs R. Hoogkamer
Department of Human Nutrition
Wageningen Agricultural University
Bomenweg 2
6703 HD WAGENINGEN
The Netherlands