REPORTS
OF THE SCIENTIFIC COMMITTEE
FOR FOOD

Ninth series

1979

Manuscript finished in October 1979
This publication is also available in

DE ISBN 92-825-1637-7
FR ISBN 92-825-1639-3
IT ISBN 92 825 1640-7
NL ISBN 92-825-1641-5

A bibliographical slip can be found at the end of this volume
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TERMS OF REFERENCE

To give an opinion on the appropriate approach for controlling the health hazards associated with the use of natural flavourings containing certain pharmacologically active principles.

BACKGROUND

The problem of the health control of flavourings has been under consideration for many years within the EEC, by the Council of Europe and by other international organisations. During this period the Council of Europe has established a list of natural flavouring materials, used either as flavouring substances per se or as source material from which flavourings are prepared. This list of acceptable source materials contains, additionally, directions regarding the relevant parts of the botanical species which may be used for flavouring purposes. The list also proposes limitations on the presence in the final food of certain active principles known to be present and known to possess pharmacological and toxic properties. These restrictions were based on a survey of existing information on the composition of natural flavouring materials.

The Commission has studied since 1967 the possibilities for harmonization of legislation on flavourings used in food in the Community. Eventually it is intended that a Directive, applicable in all Member States, will be prepared for the legislative control of these flavourings. In framing such a Directive due consideration is being given to the work already done by the Council of Europe in proposing positive lists of natural flavourings and of flavouring substances prepared by chemical synthesis acceptable for use in food.

At the present time the Committee has been asked to take particular note of the approach chosen by the Council of Europe for natural flavourings containing pharmacologically active principles. The Committee has not yet considered the specific question of the constituents of smoke flavourings, nor possible sources of contamination of natural flavourings.

The Committee understands that its advice will be sought on the approach to be adopted, and on the toxicological evaluation of substances and products to be included, in the future Directive. The Committee recognizes the complexities of classification, specification and toxicological evaluation of the large numbers of substances involved and the problems of enforcement entailed by such a list. Nevertheless the Committee sees no reason, on the information available to it, for departing from the traditional positive list approach to the control of food additives, and the Committee congratulates the Commission for its initiative in developing Community legislation in this manner. The Committee would wish to be consulted if an alternative though less encompassing approach were to be proposed so that it could assess the implications for human health.

CURRENT REVIEW

The Committee is aware that natural materials used as flavourings, or as source material for flavourings, have a complex composition. They contain pharmacologically active principles which have not necessarily intrinsic flavouring properties and which require limitation in the final food because of their biological properties. There are also many natural substances used as flavourings or as source materials for flavourings, the composition of which is not fully known. The Committee therefore agrees with the Council of Europe that a list of pharmacologically active principles, present in natural flavouring materials but requiring limitation, represents a practical approach to the problem and suggests that such a list be prepared. At present the Community has not developed a list of source materials. Should such a list be elaborated which differs from that of the Council of Europe it is possible that other pharmacologically active principles might have to be treated similarly. The compilation published by the Council of Europe is based on existing knowledge of the materials known to the Council of Europe as being in use commercially and is subject to amendment as circumstances demand (for instance if other materials were to be proposed).
The Committee examined the Council of Europe's list of active principles and the modifications proposed by the Commission on the basis of the Commission's own consultations. The Committee was unable to prepare a complete list of active principles requiring limitation in the absence of an appropriate compilation, agreed by the Community, of natural source materials or flavourings prepared from them. It understood, however, that the Commission had initiated the elaboration of such a Community list of natural source materials. As an interim measure the Committee therefore selected those active principles which in its opinion deserved particular attention as a risk to health e.g., if an active principle were likely to produce irreversible toxic effects. The Committee also agreed that these residues should be the smallest technologically achievable and their presence through transfer to food or beverages should arise entirely from the use of the natural source material as flavouring or of flavourings prepared from these natural source materials. These active principles are listed in Annex I to this report.

The Committee accepts the limits proposed by the Council of Europe for those active principles listed in Annex I except for some minor changes based on additional information which has become available to the Committee subsequent to the evaluation by experts of the Council of Europe. It stresses the desirability of lower limits on general health grounds but recognizes that the technologically achievable limitations generally dictated the limits proposed. The Committee notes that these limits must be determined by appropriate methods of analysis. The Committee considered it desirable to review the list of active principles at regular intervals, particularly once a list of natural flavouring materials has been adopted.

The Committee is aware that some pharmacologically active principles possessing flavouring properties may be synthesised or chemically isolated for direct addition to foodstuffs. The present evaluation does not cover this technological usage. Substances employed in this manner should be subjected to an appropriate toxicological evaluation based on the general principles applicable to the toxicological evaluation of any food additive.

Chemically synthesised or isolated analogues of their natural counterparts invariably contain isomers, homologues and impurities which differ significantly from those found in the natural material. The toxicological evaluation of one substance can be extrapolated to its analogue only if the concentrations of such isomers and contaminants are limited in the latter.

CONCLUSIONS

1. The Committee wishes to be consulted if an approach is proposed, which is less encompassing than a complete positive list of all flavourings acceptable for use in food.

2. The Committee accepts the suggestion of listing pharmacologically active principles as a means of controlling potential hazards to health. The present list is considered appropriate provided it is revised once a definitive positive list of natural flavouring materials has been established.

3. The Committee proposes a first list of limits for pharmacologically active principles in the final food (Annex I).
ANNEX I

Limits (in mg/kg) for certain pharmacologically active principles present in the final food arising entirely through transfer to food or beverages from the use of the natural source materials as flavouring or flavourings prepared from these natural source materials

<table>
<thead>
<tr>
<th>Substance</th>
<th>Food</th>
<th>Beverages</th>
<th>Exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta asarone</td>
<td>0.1</td>
<td>0.1</td>
<td>1 in alcoholic beverages</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 in seasonings used on snack food</td>
</tr>
<tr>
<td>Coumarin</td>
<td>2</td>
<td>2</td>
<td>10 in certain types of caramel confectionery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 in alcoholic beverages</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 in chewing gum</td>
</tr>
<tr>
<td>Total Hydrocyanic acid</td>
<td>1</td>
<td>1</td>
<td>1 for each alcoholic volume percent in alcoholic beverages</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 in stone fruit juices</td>
</tr>
<tr>
<td>Total Safrole and Iso-safrole</td>
<td>1</td>
<td>1</td>
<td>25 in confectionery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 in marzipan or its substitutes</td>
</tr>
<tr>
<td>Total Thujone (alpha and beta)</td>
<td>0.5</td>
<td>0.5</td>
<td>2 in alcoholic beverages with less than 2% alcohol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 in alcoholic beverages with less than 2% alcohol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 in alcoholic beverages with more than 2% alcohol</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35 in bitters</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50 in foods containing sage</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100 in sage stuffings</td>
</tr>
</tbody>
</table>
REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON ASBESTOS
(Opinion expressed 31 October 1979)

TERMS OF REFERENCE

To examine the potential hazards to human health from the presence of asbestos fibres in food, particularly liquids.

INTRODUCTION

The health hazard from occupational exposure to inhaled asbestos has been recognised for many years (1, 2). Awareness of the use of asbestos as a processing aid in food technology therefore raised concern over the possible presence of asbestos fibres in food, particularly liquids. In its appraisal of this complex subject the Scientific Committee for Food took cognisance of a number of major reviews on asbestos, particularly the comprehensive review of Zielhuis published by the Commission (3), the review prepared by the Bundesgesundheitsamt of Germany (4), the review paper by Vaille (5), the monograph on asbestos issued by IARC (6) and the review of the Water Research Centre, U.K. (7). The Committee was also informed about the point of view of the Food Additives and Contaminants Committee of the U.K. prepared at the request of the U.K. Ministry of Agriculture, Fisheries and Food.

PHYSICAL PROPERTIES OF ASBESTOS

Asbestos is the generic name for a group of naturally occurring mineral silicates. The material has been in use for more than 100 years, but only recently has industry fully exploited its technological properties. Chrysotile, one of the types of asbestos belonging to the serpentine group, - a silky and soft material - is by far the most abundant and commercially important type of asbestos, although anthophyllite, crocidolite and amosite have also some commercial use. The single fibre of chrysotile has a characteristic tubular appearance with a diameter of approximately 35 nm and a cavity in it of 5 nm. One gram of chrysotile separated into single fibres will account for approximately $10^{12}$-10$^{13}$ fibres.

Asbestos has a wide variety of uses from asbestos-cement pipes and sheets (including pasteboard and paper) to insulation, gaskets, seals, linings of ovens, and filtration materials. It is estimated that the main part of the mined asbestos (60%) is used to produce asbestos-cement pipes. Only 10% of the mined asbestos is used for paperboard, paper, seals and filters.

THE USE OF ASBESTOS IN THE FOOD INDUSTRY

Although filtration on kieselguhr and filtersheets is now performed without asbestos with satisfactory results, sterile filtration cannot be achieved at the moment without asbestos. The latter procedure is necessary for those products which cannot be heat-treated such as beer, wine, fruit juices, soft drinks and mineral water. The use of chrysotile is here unsurpassed. It has unique adsorptive properties caused by a high positive Zeta potential. This Zeta potential is responsible for the adsorption of colloids, micro-organisms, even viruses and perhaps pyrogens.

From the point of view of public health the removal of bacterial contaminants is clearly essential to keep food in standard hygienic condition and preserved. The asbestos filters used in food industry are made from cellulose pulp and chrysotile and may contain various filters like kieselguhr. The mass fraction of chrysotile of such filters varies between 3 and 50%. Before incorporation into filters chrysotile is treated to obtain elementary fibres of approximately 30 nanometers in diameter and a few millimeters in length. Mostly fibres are washed with a mineral acid (hydrochloric acid) in order to reduce the levels of calcium, magnesium, iron and tin. According to Bielig (8), there is no change in morphology of the fibres after acid treatment but the stiffness of the fibres is reduced up to 50%.
Although contradictory, the fact has several times been observed, that filtration through this type of cellulose-asbestos filters diminishes the number of asbestos fibres in the final product. Further decrease in the number of fibres can be achieved by using filters of which the down-stream surface bears a resin coated with a microporous film or by the installation of a cartridge filter with pore size 5 micrometers immediately prior to bottling. Nearly 100% of asbestos can be removed from liquids, but it is not possible to prevent asbestos entering the product during filling and canning (9). From the technological point of view it is not possible to use membrane filters alone.

Asbestos is also used in different types of processing equipment in the food industry because of its special properties. Examples are brake and clutch pads in evaporators and centrifuges, gland packings around rotating shafts in pumps, pipe-flange seals and in ovens where cans are lacquered and bottles sterilized. Contamination of the food occurs not only by direct contact but also, and especially, through transfer of abraded fibres. Even with beverages there is still no unequivocal evidence to identify asbestos-containing filters as a significant source of this contamination.

Because asbestos is also used as building material and in maintenance operations, this may be another source of contamination but only in cases where insufficient care is being exercised. The last, and sometimes most important, source of contamination is drinking water. Water, and food, may be contaminated with asbestos inevitably released into the environment from naturally occurring asbestos minerals or as a result of the long established use of asbestos-cement pipes for public water supplies, where the incorporation of 10-15% asbestos considerably strengthens the piping. No entirely suitable alternative material for use in the manufacture of mains pipes has so far been developed.

THE LEVELS OF ASBESTOS REPORTED IN BEVERAGES, WATER AND FOOD

It is well known and accepted that asbestos has adverse effects when inhaled. Nicholson and Pundack (10) refer to NAPCA measurements in 49 cities in the USA. These revealed that 87% of the results were between 3 600 - 18 000 fibres/m³. For New York a mean of 65 000 fibres/m³ was established. Rickards (11) found at different places in the UK 360 - 3 600 fibres/m³. In addition to inhalation of airborne asbestos, human exposure can occur through consumption of foods and beverages containing asbestos fibres and it is of interest to compare the figures by inhalation with those found in water and beverages. It should, however first be pointed out that analytical results obtained by different authors are different to compare not only because of differences in the methods of analysis but also in the conversion factor mass to number of fibres used. Cunningham and Pontefract (12) were the first who showed that drinking water was contaminated with asbestos fibres. A mean of 5 x 10⁶ fibres/l was found. Nicholson (13) found in Duluth, USA, 20 to 75 x 10⁶ amphibole fibres/l. These results were confirmed by Cook et al (14). Kay (15) established a mean of 1.1 x 10⁶ fibres/l in 22 cities. According to results published by the U.S. Environmental Protection Agency (16) nine out of 70 samples of drinking water accounted for more than 5 x 10⁶ fibres/l of which five had more than 10⁸ fibres/l. Badami and Rickards (17) found 72 000 fibres/l in the U.K. and Elzinga et al (18) in the Netherlands found 111 000 fibres/l.

Few data exist for beverages. According to information submitted to the Committee the number of fibres found in beverages can be of the same order as those found in water, varying from 0.6 to 24 x 10⁶ fibres/l. Maurer and Coors (9) doubted many of the results reported for beer. Levels for beer had been reported as varying from 1.1 - 6.6 x 10⁶ fibres/l, for gin 13 - 24 x 10⁶ fibres/l, port and sherry 2 - 4.1 x 10⁶ fibres/l and for vermouth 1.8 - 11.7 x 10⁶ fibres/l. Although Maurer and Coors' method was limited in its ability to assure absolute fibre-identification, they found levels of asbestos-like fibres of the order of 10⁴ less than previously reported. Most of the brands examined contained less than 1 000 asbestos-like fibres per litre. They conclude that containers apparently contribute many of the fibres. Asbestos-containing filters do not necessarily contribute large quantities of asbestos to beer. Maurer's conclusion agrees very well with that of Bielic (8) who came to the conclusion that products produced with asbestos-containing filters have concentrations of asbestos fibres similar or even lower than those products produced without asbestos filters.

At present no data are available for solid foods.

*Throughout this report it is assumed that 10⁶ fibres weigh 2.8 x 10⁷ which means that 10⁻⁶ (-1 µg) equals 36 x 10⁶ fibres. In the literature the weight of 10⁶ fibres varies between 1.3 x 10⁻⁷ to 1.7 x 10⁻⁷.
ASBESTOS AS AN ANALYTICAL PROBLEM

Much work has been done on the development of satisfactory analytical procedures for the identification and quantification of asbestos fibres in various media. The determination of the number, size and type of asbestos fibres requires advanced and expensive equipment, such as transmission electron microscopes, and highly experienced staff. For the effective detection and monitoring of asbestos in food and the environment, a screening method is required which can detect excessive quantities of asbestos with reasonable certainty, for example the direct examination of filtrates using scanning electron microscopy together with Energy Dispersive X-ray Analysis. A summary and description of the more important procedures is contained in the Annex and in the excellent review by Zielhuis (3).

DAILY EXPOSURE TO ASBESTOS FROM FOOD AND WATER

The findings of Cunningham (12) that drinking water and beverages also contain asbestos fibres drew much attention. From that study it became clear that airborne asbestos represents only a portion of the total environmental asbestos load. The calculated range of possible levels is very large and, consequently, conclusions as to its significance are difficult to draw. The very low levels generally found make analysis difficult, time consuming and expensive. The absence of reliable methods for analysis of solid food makes it impossible to estimate the contribution from this source. Assuming the average daily fluid consumption to be 2.5 l, the daily exposure may vary from $250 \times 10^3$ to $2.5 \times 10^4$ fibres ($3.6 \times 10^6$ to $3.6 \times 10^7$ fibres/kg body weight). For comparison, an industrially exposed person would ingest daily a number of fibres greater by several magnitudes. Furthermore, the now available data have indicated that, while many fibres are sometimes reported in water and beverages, these are mostly below 10 micrometers in length. The maximum level of ingestion from water and beverages is thus likely to be very much less than that of occupationally exposed persons. The Committee has been informed that the Commission Services have embarked on the preparation of a complete inventory of the uses of asbestos to assist in identifying the major sources of asbestos contamination of the environment. The Committee welcomes this attempt to clarify the situation.

ASBESTOS AS HEALTH HAZARD IN FOOD AND DRINK

It appears that many of the inhaled asbestos fibres are ultimately ingested after clearance from the lungs and it has been estimated that the amount could be about 50% of inhaled asbestos. In occupationally exposed individuals this is likely to be a major source of ingested asbestos. Several reports have shown an association between occupational exposure to asbestos mainly by inhalation, and a true increased incidence of cancer of the gastro-intestinal tract. It should be borne in mind, however, that swallowing the sputum is not the only transport system. Transport via blood or lymphstream from the lungs is a likely alternative. In a study on not-occupationally exposed people (19) Doniach found a high incidence of asbestos bodies in lung sections correlated with an apparent excess of stomach cancer in males and breast cancer in females but no excess bronchial carcinoma in either sex. These observations are difficult to interpret. Bignon and Bader have also reported on ingested asbestos and cancer (20).

From the public health point of view the publication of Pontefract (21) was important. It showed that asbestos fibres could migrate through the gastrointestinal wall into the blood stream and concentrate in various tissues. These results, however, have not been confirmed by others, although failure to find clear evidence of penetration may only reflect deficiencies in the detection methodology for asbestos fibres in tissue (22, 23). It is of interest that no penetration of fibres through the gut wall could be demonstrated by Gross et al in a 21 months long study. The paper presents the joint results of three laboratories and no evidence of tumour induction or of progression of any kind of lesion was found (22). Animal feeding studies to investigate the carcinogenicity of ingested asbestos have generally yielded negative results. Bolton and Davis (24) examined the effects of ingestion of asbestos in laboratory rats by feeding a diet supplemented with an asbestos/margarine mixture for up to 1 year. No evidence of asbestos retention was found within the gut or signs of cell penetration or damage to the intestinal mucosa in any of the animals tested. Direct injection of asbestos through the stomach wall of rats produced dissemination of fibres to other tissues but administration in the feed or by gavage did not. Intravenous injection of asbestos has been shown to lead to widespread dissemination (Pontefract and Cunningham, 25). Thus most experimental studies in rats investigating the
effect of asbestos administered orally in food or water have not produced statistically significant evidence of carcinogenicity for the gastrointestinal tract. However, several more animal ingestion studies are under way. The experiments of Usbel et al (26) in which pulverised asbestos filter material containing 53% chrysotile was fed to rats in their diet for 18 months, showed a significant increase in malignant tumours compared to controls. The significance of these findings is debatable since the composition of the filter materials was not specified and rats could have inhaled asbestos dust from their feed. A recent study by Sebastian et al (27), in which small numbers of rats were given either single doses of asbestos by gavage or were fed 1% asbestos in a synthetic diet for 3 hours to 12 days, claimed to have demonstrated the passage of chrysotile and crocidolite fibres across the gastrointestinal wall by the detection of fibres in the lymph fluid of the thoracic duct, using transmission electron microscopy. Although the results suffer from methodological uncertainties, other studies by Lefevre et al have apparently shown the passage of asbestos through Peyer's patches into the lymphatic system (28, 29).

In vitro studies on mammalian cell systems, including intestinal and respiratory mucosal cells showed crocidolite and chrysotile to have cytotoxic effects but gave no evidence for active cell penetration (30).

In 1973 the FDA concluded that "the evidence concerning the possible hazard from ingestion of asbestos particles is contradictory and inconclusive." This statement was confirmed by JECFA (22nd meeting) as follows: "Experimental studies in animals have reproduced the effects in the lung. These few studies involving ingestion of asbestos in chronic feeding studies are so far reported as negative". Reconsideration of the subject led JECFA (22nd meeting) to state "in the absence of unequivocal evidence relating to the ingestion of asbestos fibres to cancer, the conclusions and recommendations in the eighteenth report of the Committee, concerning possible hazards from oral ingestion of asbestos fibres, are still valid".

EPIDEMIOLOGICAL EVIDENCE ON ASBESTOS

Epidemiological studies in large populations exposed to inhaled asbestos from drinking water have shown no evidence of excess cancers in the gastrointestinal tract (31, 32, 33). The suggestion that the high rate of intestinal cancer incidence in Japan was caused by asbestos fibres originating from polished rice has not been proven (34, 35, 36).

CONCLUSIONS AND RECOMMENDATIONS

Although there is convincing evidence that occupationally exposed persons are at risk from inhaled asbestos fibres, there is at present no clear scientific evidence that persons not occupationally exposed to asbestos are at risk from asbestos fibres in air, water or food and other potential sources of contamination. However, a categorical answer to the question implicit in the terms of reference is not possible and therefore prudence leads the Committee to make a number of recommendations.

Animal feeding studies to investigate the carcinogenicity of ingested asbestos have generally yielded negative results. A number of other studies are, however, still in progress. There is no clear epidemiological evidence of increased incidence of cancer of the gastrointestinal tract attributable to asbestos in non-occupationally exposed populations. On the other hand, there is also no clear evidence that the exposure of the non-occupationally exposed population to asbestos in the environment is entirely free from hazard. Given the fact that asbestos is in the environment from natural and man-made sources, it would be unreasonable to require that no residues should be found in food and water. It also has to be borne in mind that fibres, which might be used as substitutes for asbestos, may have similar dimensions and stiffness and may give rise to similar adverse effects.

In view of all these considerations the Committee recommends:

1. That the preparation of the inventory of uses of asbestos should be given priority.

2. The development of improved and faster methods for the identification and determination of asbestos fibres in all types of matrices.
3. That the situation be kept under review so that account can be taken of the results of further studies.

4. That every effort be made to avoid using asbestos and related minerals except where their use is regarded as essential.

5. The development and evaluation, from the point of view of safety to health, of suitable alternatives to asbestos for use in the food industry.
REFERENCES

9. Maurer Ch.L., Coors J.H. (1975), Brewers Digest, November (54).
Asbestos is a generic term applied to a variety of commercially useful fibrous silicate minerals. These minerals include chrysotile in the serpentine group and crocidolite, amosite, anthophyllite and actinolite in the amphibole group. Although this is a widespread classification the inclusion of amosite as a mineral name highlights the chemistry problem. The Glossary of Geology refers to amosite as: "a commercial term .... may consist of anthophyllite or gedrite, cummingtonite or grunerite". Competent geologists have drawn attention to the fact that standard amphibole fibres have not been adequately characterized structurally. Four major polymeric configurations are found in nature: single and double chain sheet structure and 3-dimensional network. Of the 5 types of asbestos four have the double-chain structure (amosite, anthophyllite, crocidolite and tremolite). Chrysotile and talc are represented by a sheet like structure and see-sand has a 3-dimensional network.

A further problem in the absence of a universally accepted standard definition of an asbestos fibre. There is a general tendency to define - without regard to the fact that the amphibole group minerals can crystallize in either an asbestiform or a non-asbestiform - all particles of such minerals as 'asbestos' based solely on a 23:1 length to width ratio (A). The Commission Services are examining the suitability of the following definition "Asbestos fibres shall be those which are longer than 5 microns, with a diameter of less than 3 microns, and with a ratio of length/breadth greater than three". Other definitions used: a particle in an ashed residue with $A > 3 : 1$; a particle with $A > 3 : 1$ which has the correct image contrast and produces some diffraction spots; a particle of any aspect ratio which gives a fibrous crystalline diffraction pattern; a particle with $A > 3 : 1$ which gives a fibrous crystalline diffraction pattern and the correct elemental composition; a particle which has the correct electron contrast, gives a crystalline diffraction pattern and the correct elemental composition. It is clear that the application of each of these definitions to the same sample gives rise to a wide range of results.

Microfibres such as asbestos but also fibrous glass have become increasingly important because of their potential health hazard. Many of these fibres are smaller than 5 micrometers and advanced techniques are required to visualize and positively identify these fibres.

Microfibres may vary in size, shape, structure and elemental composition. The three identifying characteristics of a fibre are therefore: morphology, crystal structure and elemental composition. Microfibres are reported as the mass of fibres per unit of volume or the number of fibres per unit of volume. Since epidemiological studies of transport of fibres into the lungs and gastrointestinal tract show a significant relationship between penetration and the physical dimensions of the fibres, the fibre number concentration should be determined rather than the mass.

Many analytical techniques have been proposed for the identification and quantitation of fibrous minerals. Without being exhaustive these techniques include: optical and electron microscopy, microchemical analysis, differential thermal analysis and X-ray diffraction (both forms energy dispersive and wave length dispersive). All techniques have inherent instrumental limitations depending upon the morphology, the quantity and chemical composition of the sample. For a variety of reasons the identification and quantitation of fibres is difficult: asbestos and other fibrous minerals are generally present in low mass quantities (this does not exclude high quantities of fibre numbers); many instrumental analytical techniques cannot differentiate between fibrous and non-fibrous polymorphs; environmental modification of fibres may alter the elemental composition ratios; the different elemental composition of the same named mineral but from different geologic origin often do not compare with the standard fibre mineral; identification by morphology is extremely difficult.

The technique chosen depends on the definition of a fibre and other information wanted. In the USA the OSHA developed a standard analytical method based on light microscopy (LM). Since most of the fibres are transparent it is often difficult to "see" the fibres using conventional LM. Some analysts used polarizing LM because some types of asbestos fibres like chrysotile can then be detected, but other asbestos fibres and fibrous glass are invisible. Specialized LM techniques such as dispersion staining, which enables the
determination of the indices of refraction of the object in view, and phase-contrast can be used to visualize and to identify such microfibres. The possible carcinogenic effect of fibres smaller than the lower limit of resolution of the light microscope (0.7 micrometer) made urgent the need for more sensitive techniques.

However LM is often suited for a preliminary scan of materials when determining the overall degree of concentration, realizing that no reliable identification can be achieved and that only fibres with a diameter greater than approximately 1 micrometer can be detected. Electron microscopy techniques has a much better resolution than LM.

Both transmission (TEM) and scanning (SEM) electron microscopy are applied to the problem of counting and characterising fibres. SEM offers better observation of surface, while TEM offers superior image resolution. TEM has a resolution of 0.2 - 0.5 nm, The upper limit of fibre diameter viewable in TEM determined by the lowest magnification (1000 X) of the instrument is approximately 10 micrometer. The resolution of SEM is approximately 20 nm and has an upper limit of at least 1 000 micrometer. TEM has the advantage of showing the central canal in the chrysotile fibres and although this feature is not unique to the serpentine group asbestos type, it is an important identifying characteristic not evident in SEM. Disadvantages of TEM are the elaborate sample preparation required with the possibility of sample contamination as well as serious loss of fibres. Furthermore TEM examination requires a specimen supporting grid, the bars of which obscure fibres. Fibres lying under or over debris are not detected. These deficiencies result in an "accuracy" of the method which is at best qualitative.

As mentioned above another possibility to approach the estimation of asbestos is the use of Scanning Electron Microscopy (SEM) which became available in the late 1960's. SEM obtains an image by scanning the surface of a sample with a fine beam of electrons and simultaneously using the secondary electrons emitted at the point of incidence on the surface to modulate a scintillator. The image formed appears to have a three dimensional quality. The use of SEM alone is an unsatisfactory tool to be sure that the observed fibre is asbestos and only asbestos.

Both TEM and SEM give information only on the morphology of the fibre, which is not enough for positive identification of asbestos. The crystal structure of a fibre - the second identification characteristic of a fibre - can be determined with the help of Selected Area Electron Diffraction (SAED). A combination of TEM-SAED has been constructed and does give a combination of morphology and crystal structure information which seems to be sufficient, at least in principle, to identify chrysotile and to identify a fibre as an amphibole.

However it often happens that chrysotile and amphibole fibres do not give SAED patterns due to non-optimum orientation of the fibres. The SAED patterns for all amphiboles are similar and can only classify the fibre as a fibrous amphibole. In practice when SAED is obscured by debris or overlap 50-60% of the fibre can be identified.

The third characteristic of a fibre is its elemental composition. This can be measured with the help of Energy Dispersive X-ray Analysis (EDXA). SEM can be easily combined with EDXA. Analysis by SEM/EDXA is direct, it utilizes the filter originally used to collect the sample. The only sample preparation needed is vacuum deposition of a thin layer of carbon, making the method essentially non-modifying. The analysis can be undertaken within a few minutes after receiving the sample. Analysis time is rapid (0.5 - 1 hour). However SEM-EDXA technique is also not without problems. The EDXA spectrum of antigorite is indistinguishable from that of chrysotile as would be expected since they are polymorphs and therefore have the same chemical composition. Their SAED patterns are however completely different. The SEM-EDXA spectra of diopside and tremolite are also indistinguishable although the chemical composition of both minerals differ. Ratios of net-peak intensities of various elements are characteristic of a particular type of asbestos. Perhaps just as important is the elimination of non-asbestos fibres. For example, aluminium in hornblende distinguishes that amphibole from an actinolite fibre. SEM combined with EDXA is sufficient for fibre identification only when the mineralogy of the fibre is well-known. Further problems encountered with SEM-EDXA are: strong X-radiation from overlapping debris may produce unwanted EDXA results; analysis of unit fibres is difficult; the EDXA analysis is not specific due to lack of crystallographic data. This last point is grossly exaggerated as its main criticism. Although the method is non-specific, the method has only a small probability of mis-identifying asbestos fibres or, in a more positive way, the identification of asbestos occurs with reasonable certainty. Exceptions such as the nearly identical.
diffraction patterns of cleavage fragments of talc and anthophyllite and those mentioned above are over emphasized.

From the purely scientific point of view one might say that for an unambiguous identification of a fibre one can not rely on only one of these mentioned combinations (TEM-SAED and SEM-EDXA).

However adding EDXA to the TEM-SAED combination, which is technologically possible, provides a system in which all three identifying characteristics: morphology, crystal structure and elemental composition can be determined on the same fibre and many researchers regard this combination as the current "state of art" with regard to fibre studies although others believe that within three to four years Scanning Transmission Electron Microscopy (STEM) may be the cornerstone of research in this area. The combination TEM-SAED-EDXA is called analytical transmission electron microscopy and one could identify all fibrous particulates as follows:

1. Particulates must have a certain ratio length to width (commonly $\geq 3:1$).
2. SAED patterns have to be classified visually as
   (a) amphibole diffraction pattern
   (b) chrysotile diffraction pattern
   (c) non-asbestos diffraction pattern
   (d) ambiguous diffraction pattern
   (e) no SAED diffraction pattern (includes amorphous fibres)
3. EDXA micro chemical analysis is performed.

In the USA a computation of the cost of one analysis has been made. A complete identification of a sample will cost $1 200-1 500 and takes a week for the analysis.

From the foregoing it is clear that the identification of a fibre is a very difficult job, the quantitation being just as difficult. Sample collection is of equal importance to sample preparation and analytical analysis. Sample collection involves the separation of a small quantity of material from a parent population. Both the homogeneity of the parent population and the size of the sample collected greatly affect the final analytical result. The main point is that the sample must retain the contaminant without changing its physical and/or chemical characteristics.

Because most samples are initially concentrated by filtration the selection of the filter is important. Two types of filter are predominantly used Millipore (cellulose ester) and Nuclepore (poly carbonate) filters. The Nuclepore filter has a smooth collection surface which makes it advantageous for electron microscopy. A drawback of this filter is that redistribution and particulate loss from the surface often occurs during handling. The Nuclepore filter is used mostly in combination with TEM.

The physical behaviour of fibres with large length to diameter ratios during filtration makes some separation from non-fibres possible, but when large numbers of fibres begin to collect they become a filter in itself with retention capabilities in their own right. The effect can be controlled by proper selection of the sample volume to filtration area. Unfortunately the actual fibre and non-fibre solids in a sample are not known, so the selection of this ratio is a matter of chance. By processing a sample at three different dilutions this difficulty can be overcome. Small fibres and small particles are normally more common in a sample than large ones. A large sample volume to filter area provides a basis for assaying concentrations of large fibres but has too many small fibres and particles for accurate assay.

Subsequent manipulative steps often include the removal of a small area of the original filter for further processing. This sampling of the sample again raises the question of (in)homogeneity. The increase in measurement time by taking more than one such area is seldom rewarded, because the effect of final preparation for viewing cannot be ignored as a variable. In the preparation method for TEM the filter substrate must be removed and the particulate material deposited on a suitable TEM grid. This is a source for losses of particulates; the sample preparation step is a critical step in the whole procedure which is
very difficult to standardize. In general one might state that the main disadvantages of modifying steps during sample preparation may introduce foreign fibres or cause loss of fibres in a critical size range. TEM especially is time consuming (> 4 hours) in sample preparation. These facts are the reasons for the delay in implementing effective detection and monitoring for asbestos.

The method applied should be related to the problem. If one is interested in water samples coming from a contaminated river full of all sorts of minerals, or similarly, in air samples, this is a totally different problem from the question whether or not vinegar or beer or a parenteral drug has been filtered through an asbestos filter. In the USA chrysotile is used in more than 95% of the cases, this means that we need to know what interferences are common when analyzing chrysotile.

One has to realize also the fact that up to 5% of the earth's crust and upper mantle may be amphibole and for this reason amphibole fibres must be expected to occur in water and products like foods, made with water.
REPORT OF THE SCIENTIFIC COMMITTEE FOR FOOD ON NATAMYCIN

(Opinion expressed 31 October 1979)

TERMS OF REFERENCE

To give an opinion on the acceptability from the point of view of public health of the use of natamycin (pimaricin) in or on foodstuffs.

BACKGROUND

Natamycin is an antibiotic used in human and, to some extent, veterinary medicine. Its main uses in human medicine are in the treatment of fungal infection such as vaginitis due to Candida and certain ocular mycoses.

For a number of years natamycin has also been used as an antifungal agent on certain types of cheeses and sausages, which require a fairly long period of maturation and storage before marketing. Further uses on ham and in wine and other beverages have also been proposed. By inhibiting fungi it is also claimed that natamycin prevents the production of mycotoxins.

The use of other preservatives such as sorbic acid to achieve the same objectives have so far not proved entirely satisfactory with semi-hard cheeses and certain sausages.

CURRENT REVIEW

The Committee is strongly of the opinion that antibiotics used in human or veterinary medicine should not normally be used in food. Its reasons are similar to those given by the Joint FAO/WHO Expert Committee on Food Additives (Report of the 20th meeting). This principle has been borne in mind during the current review of the use of natamycin in foodstuffs. The Committee has taken note of the available toxicological data including a recent study on teratological effects in rabbits, a multigeneration reproduction study in rats as well as a long term study on natamycin and a 90 day study on certain degradation products. In relation to the present uses of natamycin on cheese and sausages these investigations are adequate and give rise to no cause for concern. Neither natamycin nor its principal degradation products are absorbed from the digestive tract and there is no evidence that natamycin has any significant effects on the bacterial flora of the human intestine. With regard to the microbiological properties of natamycin the Committee was advised by Prof. J.C. Gould and accepted the following conclusions:

(a) the range of sensitivities is relatively narrow;
(b) there are no naturally occurring highly resistant strains;
(c) no induced resistance has been demonstrated in patients treated with polyenes;
(d) the phenomena of cross resistance between polyenes are very limited;
(e) the dispersion of resistant strains in the population has not been demonstrated.

The Committee noted that experimental resistance to polyenes is not easily achieved in fungi, moreover that induced mutants grow only with difficulty in the normal environment.

The Committee has seen with interest recent publications concerning the production of mycotoxins by moulds liable to contaminate certain cheeses in the absence of preservatives and wishes to be provided with further information on this important aspect of the problem as and when it becomes available. The Committee accepts the case of need for natamycin for the surface treatment of certain semi-hard cheeses and certain sausages, applied to the finished product. The Committee understands that good manufacturing practice will ensure that at the time of sale the total residues, expressed in relation to the surface area of the casings or rind will not exceed 1 mg/dm² and that they will not be present at a depth greater than 5 mm. However, in view of the general principle with regard to the undesirability of using antibiotics in foodstuffs the Committee is strongly opposed to proposals for further food uses of Natamycin such as use on ham and in wine and other beverages.
CONCLUSIONS

1. Natamycin has a limited but important use in human medicine and is therefore not acceptable as a food additive for general use in and on foodstuffs.

2. Its use for the surface treatment of the rind of whole pressed cheese (semi-hard) ripened under aerobic conditions e.g. Gouda and Edam, and on the casings of certain sausages requiring maturation before marketing is acceptable, provided that:
   (i) the substance is applied only to the final product;
   (ii) the residues of natamycin in food at the time of sale, expressed in relation to the surface area of the casing or rind, do not exceed 1 mg/dm$^2$ and that they will not be present at a depth greater than 5 mm.

3. The use of natamycin on the casings of these foods should be clearly indicated by suitable labelling.

4. The position should be reviewed if there is any significant increase in the range of therapeutic uses.

Acknowledgement

The Committee is grateful for the assistance given by:

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The Scientific Committee for Food was established by Commission Decision 74/244/EEC of 16 April 1974 (OJ No. 31, 19 of 30.5.1974, page 1) to advise it on many problems relating to the protection of the health and safety of persons arising from the consumption of food, and in particular the composition of food, processes which are liable to modify food, the use of food additives and other processing aids as well as the presence of contaminants.

The members are independent persons, highly qualified in the fields associated with medicine, nutrition, toxicology, biology, chemistry, or other similar disciplines.

The present series relates to opinions on:

- the appropriate approach for controlling the health hazards associated with the use of natural flavourings containing certain pharmacologically active principles;
- the potential hazards to human health from the presence of asbestos fibres in food, particularly liquids;
- the acceptability from the point of view of public health of the use of Natamycin (Irganox) in or on foodstuffs.