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FOOD ADDITIVES COMMITTEE
NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL

Application for Approval of
Calcium Sucrose Phosphates as
Food Additives

Applicant : Colonial Sugar Refining Company Ltd.,
1-3 O'Connell Street,
SYDNEY. AUSTRALIA.

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To : The Secretary,
National Health and Medical Research Council

For Attention :

The Chairman,
Food Additives Committee,
P.O. Box 93,
CANBERRA. A.C.T.

Dear Sir,

1 Name of Applicant

THE COLONIAL SUGAR REFINING COMPANY LIMITED of

2 Address

1-3 O'Connell Street, Sydney, N.S.W.,
AUSTRALIA, who is

3 Type of Business

both manufacturer of the proposed additive and processor of
some foods in which it is proposed to use

4 Chemical Name of Proposed Additives

calcium sucrose phosphates as food additives,
submits this application for approval by the Food Additives
Committee.

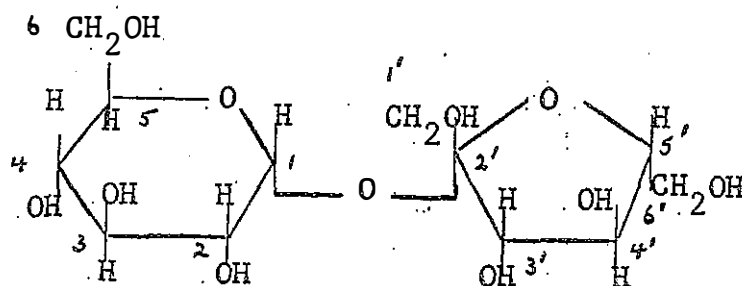
The detailed information supporting this application is
set out in items 1 to 4 above and 5 to 23 which follow, in
accordance with the requirements of Appendix 1 of the Food
Additives Committee.

It will be appreciated that this application differs from that normally received by the Food Additives Committee, in that the main purpose of the proposed additive is for the reduction of dental caries. For this reason evidence, establishing the need for such an additive (Item 10), that the additive will have its intended result (Item 20), that the same objectives cannot be obtained by existing approved additives (Item 21) and establishing the advantages which will accrue to the consumer (Item 22), will be presented in the form of reports of laboratory and clinical research, rather than in the form of requests and evidence from food manufacturers.

5 Chemical structure, and chemical and physical description of the additives

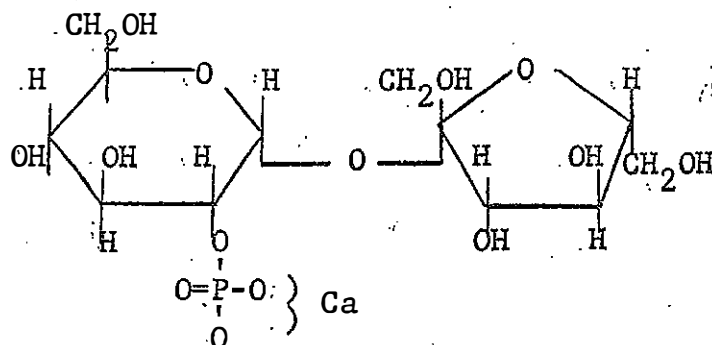
Calcium sucrose phosphates are the calcium salts of phosphate esters of sucrose. One or more of the eight hydroxyl groups of the sucrose molecule may be esterified by various means with orthophosphoric acid to give various orthophosphoric esters of sucrose. These esters are acidic and can, with suitable bases, form salts in the same way as orthophosphoric acid itself.

The structural formula of sucrose is shown below :



The six carbon atoms in the glucose moiety are numbered by convention 1 to 6, and those in the fructose ring 1' to 6'.

A monophosphoric acid ester of sucrose which has been positively characterised¹ is sucrose-2-phosphate. The structural formula of the calcium salt of this sucrose phosphate (which we have confirmed) is :



Sucrose monophosphates in which other hydroxyl groups of sucrose are esterified with orthophosphoric acid are also known. These include sucrose-6'-phosphate which has been identified in various plants^{2,3,4,5}. These sucrose phosphates belong to the general class of sugar phosphates which are known to occur widely in natural products, and during recent years many of them have been isolated and also prepared synthetically. The chemistry and properties of sugar phosphate esters have been reviewed by a number of authors, such as Leloir and Cardini (1963)⁶, Leloir (1951)⁷, Khorana (1961)⁸ and Van Wazer (1961)⁹.

Calcium sucrose phosphates can only be prepared economically as a mixture of calcium salts of different sucrose phosphate esters. The products which have been used in the work which is reported here are produced by the reaction of phosphorus oxychloride on an aqueous solution of sucrose in the presence of lime (See Item 8). This reaction produces the same calcium sucrose phosphates in spite of wide variations in reaction conditions. The consistent properties of these calcium sucrose

phosphates led previous workers to believe that the material was a definite homogeneous compound.

The following extract from one of Courtois' papers¹⁰ illustrates this :

"In 1909 Neuberg and Pollak described a mono-orthophosphoric ester of sucrose. This substance is obtained by phosphorylating sucrose by phosphorus oxychloride,

This ester, whose constitution still remains unknown, is always considered as a definite homogeneous product. We contribute results which support this homogeneity. The various fractions of calcium sucrose phosphate obtained in the course of various preparations have specific rotations very analogous to the commercial salt. (Hesperonal-calcium, Merck, has all the properties of the calcium sucrose phosphate of Neuberg and Pollak).

Attempts to fractionate these sucrose phosphates as their salts with calcium and strychnine have not enabled us to separate preparations of different optical rotations".

Courtois^{1,10,11} as well as Neuberg^{12,13,14}, Hatano¹⁵ and Sabetay and Rosenfeld¹⁶ have investigated the preparation and composition of sucrose phosphates made in this way.

The application of modern techniques such as chromatography and electrophoresis have enabled us to show that the product from the phosphorylation of sucrose by phosphorus oxychloride is not a single compound but consists of a mixture of various sucrose phosphates, the nature of which, however, is consistent.

Electrophoresis enables the product made by Neuberg's original method and our modified methods (described in Item 8) to be separated into four main sucrose phosphate zones, and if they are present, into zones due to inorganic ortho-phosphate and free sugars (See Item 9). In this figure zones (or bands) 1-4 represent the location of sucrose phosphates and band 5 the location of inorganic orthophosphate. If free sugars are present they occur below band 1.

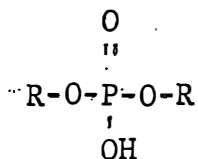
Quantitative electrophoretic analysis (See Item 9) of several different calcium sucrose phosphates indicates that the phosphorus is distributed among the various bands in the proportions shown in the following table :

Product	% total phosphorus in product				
	Band 1	Band 2	Band 3	Band 4	Band 5
CaSP* (C.S.R.C. product, Lot 6)	7.5	20.9	14.1	25.4	31.1
Calcium sucrose phosphate made by Neuberg's method	2.0	63.4	20.7	13.9	Nil
CaSP (Stauffer product)	3.8	13.8	14.9	30.7	36.8

* See definition later in this section.

Neuberg's method produces the same components but a considerable loss of calcium sucrose phosphates occurs in that method during the removal of the inorganic phosphate and excess lime. We have observed that proportionally more of the band 4 component is lost in that process. The overall yield of product in Neuberg's method is approximately one third that in our methods of manufacture.

We have found that the band 2 component is sucrose-2-phosphate. Of the other major components, band 4 has been shown to be a sucrose monophosphate in which one of the hydroxyls of the fructose moiety of sucrose is phosphorylated. The difficulty of preparing fructose esters of known configuration has prevented us to date from unequivocally defining the position of esterification. Present indications are that it is either hydroxyl 3' or 4'. The band 3 component appears to be a monophosphate of sucrose in which the hydroxyl on the glucose moiety is phosphorylated. The band 1 component from available evidence is a disucrose monophosphate probably of the following type of structure :



(where R = sucrose less one hydroxyl group)

Work is continuing in our Research Laboratories on the positive identification of these various sucrose phosphates which are produced by the phosphorylation of sucrose in aqueous solutions by phosphorus oxychloride.

The calcium sucrose phosphates which are the subject of this application should be defined as :

the calcium salts of phosphoric acid esters of sucrose produced by the phosphorylation of aqueous solutions of sucrose with phosphorus oxychloride.

These calcium sucrose phosphates may be associated with varying amounts of inorganic calcium phosphate which do not detract from the properties (in fact, the presence of inorganic calcium phosphates in some applications appears to be advantageous - see later Items). We will refer to these calcium sucrose phosphates containing various amounts of inorganic phosphates as CaSP in this application (Note : in some of the attachments reference may be made to "Anticay", which is the registered trade name of our product. This is equivalent to CaSP).

The inorganic calcium phosphate in the solid state in CaSP exists essentially as an amorphous tricalcium phosphate in association with a mixture of amorphous calcium salts of the sucrose phosphates. For example, C.S.R.C. CaSP, Lot 6, contains approximately 15% by dry weight of this calcium phosphate.

Inorganic calcium phosphates are either relatively insoluble in water or generally suffer incongruent dissolution, (that is, dissolution accompanied by reaction) as when monocalcium phosphate dissolves in water but then undergoes hydrolysis to form the less soluble dicalcium phosphate. In general it is well known that extended treatment of any calcium orthophosphate with excess water leads to the formation of an insoluble apatite.

CaSP, however, is very soluble in water in spite of the fact that it contains a proportion of inorganic calcium phosphate. These solutions are stable for long periods at concentrations exceeding about 5% by weight. Dilution of these solutions slowly precipitates an insoluble calcium phosphate associated with some sucrose phosphates. The precipitated material is highly dispersed and essentially amorphous; depending on concentration factors, it may form a gel or a viscous, hazy solution. Reconcentration of the solution redissolves the precipitate. We believe that these properties of CaSP solutions are due to complex association between inorganic calcium phosphate and calcium sucrose phosphates.

CaSP is a fine, white powder which is not hygroscopic but is readily soluble in water. The amount of CaSP which can be dissolved in water is apparently only limited by the extreme viscosity of solutions containing more than 80% solids. Unlike other phosphates of high solubility it has a bland taste, and can consequently be added to foods without any adverse affect on flavour.

Neutral aqueous solutions can be boiled without any more decomposition than corresponding sucrose solutions. However, hot acidic or alkaline conditions will cause hydrolysis. This behaviour is again analogous to the parent compound, sucrose; however in the case of CaSP, as well as hydrolysis of the glycosidic linkage occurring there is hydrolysis of the phosphate bond also.

The breakdown of CaSP by acids and alkalis as well as by various enzymes such as invertase, takadiastase and phosphatase has been studied by the previously mentioned authors¹⁰⁻¹⁶, as

well as by ourselves. These reactions can lead to a mixture of the free sugars, sucrose, glucose and fructose as well as their phosphate esters together with inorganic phosphate salts. Further mention of this is made in later Items of this application.

6 Nature and amounts of impurities present in the additives

The process of manufacture gives a reaction mixture which can contain various calcium sucrose phosphates, unreacted sucrose, unreacted lime, calcium chloride, inorganic calcium phosphate, trichlorethylene, ethanol, water and small quantities of glucose and fructose and their phosphates.

The calcium sucrose phosphates can be recovered from this reaction mixture in different ways as described in Item 8, and depending on these and the completeness of the reaction, the final product will contain varying amounts of these materials.

Considering these in turn :

Sucrose

Not all the sucrose is phosphorylated and there is always some free sucrose in the reaction mixture. In those manufacturing methods which employ alcohol precipitation or leaching to recover the product less sucrose remains in the final product than in the case where dialysis is used as a method of recovery. This sucrose can be regarded simply as a diluent to the active phosphorus and calcium containing compounds and has no deleterious properties in the use of the product.

The amount of sucrose in the product can vary from trace amounts up to about 20% by weight of the dry product.

Lime and calcium chloride

The reaction is controlled to give a pH which is less than 10.5. The bulk of the lime is converted to the calcium salts of the various phosphates and calcium chloride. Too great an excess of calcium chloride in the final product is undesirable, both because of adverse taste and the hygroscopicity which it produces. It is the main purpose of the recovery steps to reduce the calcium chloride to a level where these effects are eliminated.

The small amount of free lime remaining has little adverse effect on the final product apart from turbidity and pH of the solutions when the product is dissolved. The amounts of free lime and calcium chloride in the product are less than one percent of the dry product weight.

Inorganic calcium phosphate

Because the reaction is carried out in aqueous solution it is not possible to prevent the hydrolysis of some of the phosphorus oxychloride to phosphoric acid and its neutralization by lime to give inorganic calcium phosphates.

This calcium phosphate, provided it is not present in excessive amounts, is solubilized by association with the sucrose phosphates. This is a complex ionic association which is not well defined and which we are at present studying in our Research Laboratories. The presence of this solubilized inorganic calcium phosphate in the calcium sucrose phosphate product is not disadvantageous. The amount is, however, limited to about 20% by weight of the dry product in our specifications in order to ensure a product in which there is sufficient calcium sucrose

phosphate and in which the bulk of the inorganic calcium phosphate is soluble in aqueous solution.

Trichlorethylene, ethanol and water

We have found that the reaction can be carried out with or without trichlorethylene as a diluent for the phosphorus oxychloride. Where it is used the methods of recovery ensure that the concentration of this substance in the final product is well below (less than 10 p.p.m.) the level at which it could have toxic effects. In alcohol precipitation or leaching any trichlorethylene remaining after the separation of the bulk from the reaction mixture is removed in the large volumes of aqueous ethanol used, and in the final drying. In the dialysis purification any residual trichlorethylene is lost during the dialysis and spray drying steps.

The recovery of calcium sucrose phosphates may or may not involve the use of ethanol. Where ethanol is used any residual ethanol in the product is reduced by drying to an acceptable level. (less than 1% of the weight of the product) Water is present as moisture in the final product but is generally less than 15% of the weight of the product.

Glucose, fructose and their phosphates

Although we have not specifically detected all these substances in the products from the phosphorylation of sucrose it is possible that small quantities of these may occur due to the hydrolysis of sucrose during the reaction. They would be present in the product, if at all, in only trace amounts (less than 1% of the weight of the product).

Poisonous metals

The limits for these metals follow the general practice for food additives and food products. At the maximum level of use of calcium sucrose phosphates (say 2% by weight of the food) the level of poisonous metals introduced into the food is well below generally acceptable limits.

Lead in the calcium sucrose phosphate products is less than 10 p.p.m. Arsenic is less than 4 p.p.m. These levels would result in a maximum addition to foods of less than 0.2 p.p.m. lead and less than 0.08 p.p.m. arsenic on the weight of the food.

7 Standard of purity for the additives

From the information contained in Items 5 to 9 it can be seen that the product of various processes for the manufacture of calcium sucrose phosphates can range from a material which contains in excess of 95% of the dry weight as calcium sucrose phosphates to a product which contains about 50% of dry weight as calcium sucrose phosphates. This variation is due mainly to the presence of varying proportions of free sucrose and inorganic calcium phosphate. Sucrose is obviously a diluent to the cariostatic properties of the calcium sucrose phosphates, but the soluble inorganic calcium phosphate would be expected to make some contribution to the cariostatic properties (see Item 20 in this application).

Nevertheless for the purposes of the proposed regulations governing the use of calcium sucrose phosphates as cariostatic agents we assume that the cariostatic activity is due solely to the calcium sucrose phosphates.

The proposed standard of purity of calcium sucrose phosphates as food additives should be as follows :

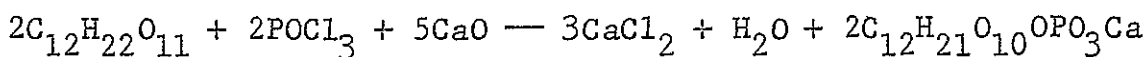
- (a) The cariostatic food additives designated as calcium sucrose phosphates shall contain at least 50% by weight of dry material as calcium sucrose phosphates.
- (b) Total free sugars (such as sucrose, glucose, fructose, etc) and inorganic calcium phosphates are permitted in the product provided that the concentration of each in the cariostatic food additive does not exceed 20% of the dry weight of the product.
- (c) The total calcium concentration in the additive shall be in the range 10 - 13% calculated on a dry and sugar free basis.
- (d) The total phosphorus concentration in the additive shall be in the range 8 - 11% calculated on a dry and sugar free basis.
- (e) The inorganic phosphorus content in the additive shall not exceed 3.0% calculated on a dry and sugar free basis.
- (f) The concentration of calcium chloride in the additive shall not exceed 0.5% determined as chloride on a dry and sugar free basis.
- (g) The concentration of trichlorethylene in the additive shall not exceed 10 p.p.m. determined on a dry and sugar free basis.

- (h) The content of lead in the additive shall not exceed 10 p.p.m. determined on a dry and sugar free basis.
- (i) The content of arsenic in the additive shall not exceed 4 p.p.m, determined on a dry and sugar free basis.
- (j) The content of ethanol in the additive shall not exceed 1% by weight on a dry and sugar free basis.
- (k) The pH of a 1% aqueous solution, one hour after making up, shall be between 7 and 10.5.
- (l) The colour and turbidity of a 25% queous solution shall be less than 420 E.C.U. (empirical colour units) and less than 260 E.T.U. (empirical turbidity units) respectively.

Methods for these determinations are given in Item 9.

8 Method of manufacture of the additives

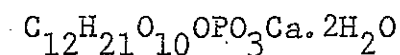
Calcium sucrose phosphates were first prepared in 1910 by Neuberg and Pollak^{12,13,14}, by the reaction of phosphorus oxychloride on an aqueous solution of sucrose in the presence of excess calcium oxide, by the following reaction :



After purification, the product had the following analyses :

Ca	8.02	Calculated values	8.07
P	6.31		6.25
C	28.85		29.03
H	5.29		5.04

and contained 2 moles of water of crystallization, and so approximated the following formula of the mono-orthophosphate ester of sucrose :



The above process for the manufacture of calcium sucrose phosphate was patented in 1910 by Chemische Werke vorm Dr. H. Byk¹⁷.

Sucrose phosphoric acid ester was manufactured in Germany and marketed under the name Hesperonal, and the salts as Calcium Hesperonal, etc., produced by E. Merck, Darmstadt, and used as a substitute for calcium glycerophosphate, which had become expensive under war conditions. A number of references concerned with the use of this material are given in Item 13(e) of this application.

Fischer (1914)¹⁸ also phosphorylated sucrose with phosphorus oxychloride, however in this case he used pyridine as

solvent at -20°C . The product obtained was different from that obtained by Neuberg, but is probably also a mixture containing isomers of mono-phosphorylated sucrose plus di- and tri-esters, with the possibility of cyclic phosphate esters. Very little work has been carried out on this product due to the difficulty in separating the different isomers.

Another method that has been used to prepare phosphate esters of sucrose was by the reaction of sucrose in anhydrous pyridine with phosphorus pentoxide at $90-100^{\circ}\text{C}$. Although this method was the subject of a patent¹⁹ no further work was carried out as the product was difficult to isolate and purify. The product would also be a mixture of phosphate esters of sucrose.

The calcium sucrose phosphates which are the subject of this application are produced by phosphorylation of sucrose with phosphorus oxychloride in aqueous solutions. We have introduced various improvements to the original method used by Neuberg. These modifications, which facilitate improved yields and recovery of the calcium sucrose phosphates, do not produce calcium sucrose phosphates different in kind from those produced by Neuberg's method. The differences between the products produced by the methods which we have developed and those of Neuberg lie in the relative proportions of the various calcium sucrose phosphates and inorganic calcium phosphate in the final product.

The main method which we have used for the production of calcium sucrose phosphates in Australia is outlined in reference²⁰. The phosphorylation of an aqueous solution of sucrose with phosphorus oxychloride in the presence of lime yields a reaction mixture which contains calcium sucrose phosphates, unreacted

sucrose, calcium chloride, lime, inorganic calcium phosphate, plus traces of glucose and fructose and hexose phosphates formed by the hydrolysis of sucrose during the reaction, together with the solvents used, trichlorethylene and water.

The product is isolated by first centrifuging off the small quantity of insoluble calcium phosphate formed during the reaction, then separating the insoluble trichlorethylene layer. The calcium sucrose phosphates are recovered from the reaction mixture by precipitation with ethanol. This precipitate contains co-precipitated calcium chloride and sucrose. It is further purified by repeated extraction with 80% ethanol solution. This removes calcium chloride, trichlorethylene and most of the sucrose from the product, which is then dried to remove ethanol. Thus the final product contains mainly calcium sucrose phosphates, inorganic calcium phosphate and water. Traces of sucrose, calcium chloride, lime, ethanol and some free sugars and hexose phosphates may remain in the product.

During the production of the three and one half tons of calcium sucrose phosphates which have been produced in Australia to date some further improvements have been made, which however, do not alter the essentials of the process. These improvements which are summarised in reference²¹ consist of slight modifications to the process flow sheet and the spray-drying of the alcohol precipitated product.

Further improvements to the basic process for the production of calcium sucrose phosphates by phosphorylating sucrose in aqueous solution with phosphorus oxychloride have been developed by our associates in the U.S.A. This method²² differs

from the previously described method in that the phosphorus oxychloride is not diluted with trichlorethylene. This facilitates the subsequent purification and recovery of the calcium sucrose phosphates without altering the nature and yield of the calcium sucrose phosphates in the reaction. A further difference is that the reaction mixture is purified by dialysis instead of by alcohol precipitation and leaching. The final product is recovered by spray drying.

The calcium sucrose phosphates in this product do not differ from those in the previously described methods. However the relative proportions of calcium sucrose phosphates, inorganic calcium phosphates and free sucrose are altered by the method of purification (see Table Item 5).

The original Neuberg method is not economical for the production of large quantities of calcium sucrose phosphates for food uses. The basic method outlined in reference²⁰ has been used to produce all material used in Australia. However for the clinical trials as a food additive the product was redissolved, centrifuged and spray-dried²¹ to give a more convenient product for food use. The method outlined in reference²², developed by the Stauffer Chemical Company U.S.A., is more economical than that described in references^{20,21} and will be used to produce the material commercially.

The calcium sucrose phosphates made by these related processes are equivalent in their properties and are covered by a common specification (see Item 7). They are also equivalent in their cariostatic properties based on their calcium sucrose phosphate content.

9 Analytical controls in manufacturing, processing and packing of the additives

The facilities for manufacturing and packaging calcium sucrose phosphates, (CaSP), both in Australia and the U.S.A. are of appropriate standards so that the products are of food grade and they are prepared and handled as food ingredients.

For research, animal tests, toxicity evaluations and clinical investigations in Australia over three and one half tons of CaSP have been manufactured by a subsidiary of the Colonial Sugar Refining Co. Ltd., by the method outlined in references^{20,21}. This company, C.S.R. Chemicals Pty. Ltd., produces a number of chemicals for food, including such materials as sorbitol and mannitol.

The raw materials which are used in the production of CaSP - sucrose, phosphorus oxychloride, lime and trichlorethylene - meet the specifications set out in reference²¹.

The CaSP product from each reaction batch, which is carried out on a scale of approximately one to two hundredweight, is analysed according to methods described later and falls within the prescribed specification (Item 7).

The CaSP product manufactured in the U.S.A. by the Stauffer Chemical Company by the process described in reference²², and which will be used in clinical trials to be carried out in the U.S.A., is subject to a similar quality control and falls within the same specifications as the CaSP product manufactured by C.S.R. Chemicals Pty. Ltd.

CaSP prepared by C.S.R. Chemicals Pty. Ltd. is packed in sealed polyethylene bags inside a fibre carton. For most purposes the material is packed in 56 pound lots which are forwarded to the manufacturers of the foods or toothpaste used in the clinical trials. For smaller consumers the material is repacked into polythene bags inside tins with press-fit lids. As CaSP is a free flowing, non-hygroscopic, white powder there are no difficulties in the handling and packaging of this material.

A complete schedule of all production by C.S.R. Chemicals Pty. Ltd., and use of calcium sucrose phosphates is maintained²³. Analyses are carried out on all production batches²⁴ to determine that the product falls within the required specification (see Item 7).

The determinations which are currently used for the control of manufacture of CaSP comprise the following :

- (1) Calcium
- (2) Total phosphorus
- (3) Inorganic phosphorus
- (4) Chloride
- (5) Free sugars
- (6) Water
- (7) Heavy metals as lead
- (8) Arsenic
- (9) Colour, turbidity and pH

The analytical methods which are used for these determinations are described in reference²⁵. Analyses (1) - (4) are normally carried out on each reaction batch before they are blended prior to packing. Reaction batches are normally blended

to comprise a larger batch before packing. In the case of the spray dried product several reaction batches are dissolved, centrifuged and spray dried in the one operation. This achieves a blending of these reaction batches. Complete analyses (1) to (9) are therefore carried out on the final uniform blended or spray dried product. These analyses are done on samples taken from the packed containers. These analyses are listed in reference²⁴.

Electrophoretic and anion exchange methods for the analysis of calcium sucrose phosphates are also described in reference²⁵. These are not used routinely for the quality control of CaSP but are used to compare the proportions of different calcium sucrose phosphates in the products prepared by different methods (see Table in Item 5). The variation in the proportions of these various components from batch to batch in a given method of production is not significant.

10 Evidence for the need for the additives

As pointed out at the beginning of this application the fact that this food additive is for use in the control of dental caries renders it different from those normally considered. For this reason evidence for the need for such an additive must come from the medical and dental facts concerning this public health problem²⁶. These matters will be more fully covered in Items 20, 21 and 22.

There are no differences of opinion on the fact that dental caries in modern, civilized communities is a major public health problem. No one preventive measure has been, or is likely to be, one hundred per cent effective. Such preventive measures

as regulation of diet, oral hygiene and fluoridation of water supplies have been shown to have some effect in reducing the incidence of dental caries. The present proposal for the use of a food additive which has also been shown to reduce the incidence of dental caries is consistent with the multi-pronged attack which must be made to alleviate this public health problem.

11 Stability and persistence of the additives in foods

As shown in Item 14 it is proposed to use calcium sucrose phosphates (CaSP), as additives in processed carbohydrate foods. Such foods include bread, flour, biscuits and cakes (or their pre-packaged mixes) breakfast cereals, sugar, sugar syrups, honey, jam, canned fruit, confectionery, soft drinks, fruit juices and cordials.

The stability of CaSP in these different foods varies. Like the sugars themselves, although stable to heat at the normal pH ranges of many foods, calcium sucrose phosphates can be broken down by acids and alkalies and various enzymes. (see Item 13(c)). Consequently in the processing of foods in which the acidity or alkalinity is high, or in which some enzymatic step is involved, there is some breakdown of CaSP.

For example, in sugars, sugar syrups, honey and flour CaSP is stable and there is no loss on processing. In bread there is some breakdown of CaSP in the yeast leavening step and also to a lesser extent in baking. In biscuits, cakes and other baked goods there is some breakdown of CaSP. In jams CaSP is added prior to packing in order to avoid prolonged boiling at low pH values, but there is nevertheless some breakdown at these low pH values. In acidic soft drinks and fruit juices there is also some hydrolysis of calcium sucrose phosphates.

The partial breakdown of calcium sucrose phosphates under these conditions involves both the hydrolysis of the glycoside linkage to form free sugars and hexose phosphates as well as hydrolysis of the phosphate ester linkage to form free sugars and inorganic phosphates. This same partial breakdown occurs with naturally occurring organic phosphates during the processing of foods. For example Pringle and Moran²⁷ estimate that flour loses of the order of 50% of its phytic acid during processing.

We have found that in some baked foods the breakdown of the sucrose phosphate can be of this same order. However the breakdown products, hexose phosphates and inorganic phosphates have also been shown⁵³ to be cariostatic in the diet of animals. We have shown (in animal experiments⁵³) that CaSP baked in bread and biscuits is equally, if not more effective, than CaSP incorporated in the diet after processing.

The accumulation of evidence suggests⁵³ that the intimacy of admixture of the phosphate and carbohydrate food is important in the cariostatic effect of phosphates in the diet of animals. With a readily soluble material such as CaSP a uniform incorporation in foods is facilitated. Moreover, any breakdown of CaSP to produce insoluble products would be expected to result in a high degree of dispersion of such products. We have also shown that inorganic calcium phosphates can be solubilised by the sucrose phosphates.

Analytical methods show that the major part of the added phosphorus and calcium remains in a water soluble form in the processed foods. Electrophoretic and chromatographic analysis²⁵ of the foods also shows the presence of all the sucrose phosphate components present in CaSP.

The clinical trial which we are carrying out (see Item 20) uses foods in which calcium sucrose phosphates have been added during processing. The consumption of these foods has been shown to reduce dental caries in consumers of such foods. This is conclusive evidence that the additive has its desired effect in spite of some breakdown which occurs during the processing of these foods.

12 Analytical methods to determine :

- (a) the amount of additives in foods
- (b) substances formed in such foods because of the use of the additives

(a) The analysis for calcium sucrose phosphates per se is not a simple procedure and electrophoretic or column chromatographic methods must be used for the separation of the sucrose phosphates from both other organic phosphates and inorganic phosphates which may be present in the sample. This can be carried out on a quantitative basis²⁵ but these techniques are not ideally suited to routine use on a large number of samples.

For this reason and the fact that the formulation and inspection of foods prepared for the trial can be adequately controlled by more simple analyses for total and soluble calcium and phosphorus, we have not used these techniques routinely. However they are quite capable of being used by properly equipped and trained laboratory personnel and we have used them as random checks on the other methods of analysis.

The amount of the additive added to the various foods used in the clinical trial was controlled by the analytical methods

described in reference²⁸. These methods involved the determinations in the food of :

Total phosphorus

Total calcium

Total soluble phosphorus

Total soluble inorganic phosphorus

Total soluble calcium

Moisture content

Knowing the levels of these in the untreated food this enables the content of added CaSP to be checked.

(b) Substances which may be primarily formed in foods because of the presence of the additive would be limited to the breakdown products of the calcium sucrose phosphates which may be formed during the processing or storage of such foods. As pointed out in Item 11 the three products of breakdown of the calcium sucrose phosphates are free sugars, inorganic phosphates and hexose phosphates.

The analysis for these products in foods can be carried out in various ways. For example free sugars can be estimated by the numerous methods listed in "Food Composition and Analysis"²⁹. However in carbohydrate foods obviously there could be interference from sugars naturally present. Inorganic phosphates can be determined by the method described in reference²⁸ or by other standard methods which are available for determining phosphates in foods. Hexose phosphates can be determined by the ion exchange chromatography method described in reference²⁸ or by other similar techniques which have been described³⁰. In all cases these breakdown products are similar to products which can occur

naturally in foods and these must be allowed for in any analysis.

The interaction of calcium sucrose phosphates and their breakdown products which have been described, with the foods themselves during cooking or processing would be similar and as complex as the interaction of the components of the foods themselves. Such reactions as caramelisation of sugars, formation of melanoidins by reaction of sugars and amino acids may also occur. It would not be possible to distinguish these from those products formed from the foods themselves during processing.

13 Pharmacological and toxicological investigations on the
the additives

(a) Acute, short-term and long-term (chronic) toxicity
studies

Acute and short term toxicity tests were carried out by Dr. B. Lilienthal, Melbourne University. The procedures were those outlined in the W.H.O. Report on safety of food additives³¹.

In the acute toxicity tests the animals employed were 20 male and 20 female rats, 20 male and 20 female guinea pigs, and 4 female dogs. CaSP was given to these animals in a single administration, by stomach tube in the case of rats and guinea pigs and mixed with minced meat in the case of the dogs. Controls were given an equivalent amount of water or untreated meat. There were no toxic signs nor mortalities at a dose of 5g/kg body weight. At this level there is no necessity for an accurate determination of the LD₅₀.

The short term toxicity tests used a single dosage level which was at the maximum level of 10% in the diet. The W.H.O. Report states that there is no useful purpose served by employing higher levels than 10% in the diet. At the time of this study, as we proposed to carry out long term (chronic) toxicity tests at several different levels, we were only interested in observing a maximum effect as an indication of what we could expect in the long term tests. The results of these tests showed no indication of any toxicity.

Again as a pointer to what might be expected in more extensive reproduction studies a short term breeding trial was carried out by Dr. Lilienthal. In this trial the fertility and gestation indices were 100% for the group of rats fed 2.0% CaSP in the diet, and no different from the control group.

A comprehensive study of the long-term (chronic) toxicity of calcium sucrose phosphates and their effects on reproduction over three generations was carried out by Hazleton Laboratories, Inc., Falls Church, Va., U.S.A., under the direction of Dr. O.E. Paynter. These studies were commenced in June 1963 and were completed in December 1965.

Dietary administration of calcium sucrose phosphates to albino rats was conducted over a 24 month period at levels of 2.0, 5.0 and 10.0%. The criteria evaluated were appearance, body weight, food consumption, food efficiency, clinical values, terminal body weights, organ weights, organ/body weight ratios, survival and gross and microscopic pathology. No toxic effects which could be related to the ingestion of the compound were observed³².

The dietary feeding of calcium sucrose phosphates to dogs at levels of 2.0, 5.0 and 10.0% for two years produced no observable effects with respect to behaviour, body weight, food consumption, haematology, clinical biochemical determinations, urine analyses, organ weights or gross and microscopic pathology³³.

The ingestion of calcium sucrose phosphates at a dietary level of 5.0% had no adverse effect on the reproductive performance of albino rats through three parental generations³⁴.

(b) Physiological effects and reactions in response to the additives

These studies comprise part of the work carried out by Hazleton Laboratories and reported in the preceding section (a). As reported in references ³²⁻³⁴ there were no physiological effects or abnormal reactions which could be ascribed to a response to calcium sucrose phosphates.

(c) Biochemical information and metabolic studies

The proposed use of calcium sucrose phosphates can be compared with the use of such food additives as calcium glycerophosphates and the sodium phosphate derivatives of mono- and di-glycerides from the glycerolysis of edible fats or oils, which are generally recognized as safe in foods (see (e)). As pointed out in the W.H.O. report³¹ on food additives, such types of food additives, and the examples which they give, glycerol monostearate and monosodium glutamate, have been accepted without much question because of the general knowledge in the relevant areas of carbohydrate, fat or protein absorption.

The role of sugar phosphates in intermediary metabolism is well established, as is the occurrence of these substances in foods. The biochemistry of sucrose phosphates is similar to that of the sugar phosphates generally^{6,7,8,9}. The phosphate and glycoside linkages in the sugar phosphates can be hydrolysed under suitable chemical conditions, or by enzymes, to produce free sugars, and inorganic phosphates. These products then behave according to the well known chemistry and biochemistry of sugars and inorganic orthophosphates.

The acid hydrolysis of sugar phosphates has been studied by a number of workers including Farrer³⁵. Factors which affect the rate of hydrolysis are temperature, pH, and structure. The vicinity of the phosphate group to the carbonyl group and whether the sugar exists in the furanose or pyranose form influence the rate of hydrolysis. For example glucose-2-phosphate hydrolyses 20 times faster than glucose-6-phosphate and fructose-6-phosphate hydrolyses 4 times as fast as glucose-6-phosphate.

The calcium sucrose phosphates behave similarly. Acids hydrolyse the -P-O-C- linkage to give free sucrose and inorganic phosphate. Hydrolysis of the glycoside linkage also occurs to produce hexose phosphates and free hexoses^{10,11,15,16}. We have shown³⁶ that the various components (bands 1-4) of the mixture of sucrose phosphates produced by Neuberg's methods and our modifications of it, hydrolyse at different rates to produce either free fructose or free glucose and a glucose phosphate or fructose phosphate depending on the position of phosphorylation of the original sucrose molecule. These glucose and fructose phosphates can in turn break down to the free sugars and inorganic phosphates.

The effect of enzymes on calcium sucrose phosphates leads to products similar to those obtained at the various stages of acid hydrolysis. We have shown³⁶ that invertase will hydrolyse the glycoside bond in calcium sucrose phosphates to produce the corresponding free hexose and hexose phosphate.

Tomita studied the action of phosphatases on hexose phosphates³⁷ and on sucrose phosphates³⁸ prepared by Neuberg's method. He showed that phosphatases from various organs break down sucrose phosphates in a manner similar to that in which they break down hexose phosphates. We have also confirmed that both acid and alkaline phosphatases are capable of hydrolysing calcium sucrose phosphates to sucrose and inorganic phosphate.

A study of the metabolism and excretion of calcium sucrose phosphates in animals has been carried out by Vickery and Wright³⁹. The work compares the excretion of CaSP with that of sucrose. The results show that at a level of about 70 mg/kg body weight CaSP is a readily metabolized nutrient whose behaviour does not differ significantly from that of sucrose. Moreover when CaSP was taken orally by humans at a dosage of as high as 300 mg CaSP/kg body weight, less than 1% of the dose could be detected in urine⁴⁰. (At 1% CaSP added to total carbohydrate intake the normal daily dose rate in the reference man would be 60 mg/kg body weight). These facts, together with the preceding biochemical information, suggest that at the levels proposed CaSP is a readily metabolized nutrient in man. The sucrose moiety is as capable of being utilized as sucrose is itself, leaving the calcium and phosphates available for body requirements in a manner similar to the calcium and phosphates present in naturally occurring carbohydrate foods.

It is relevant to compare the observations summarized above with the biological data on polyphosphates reported in W.H.O. Technical Report No. 281⁴¹. This report refers to studies which indicate that polyphosphates can be hydrolysed by enzymes with the formation of monophosphate and also to the occurrence of only trace amounts of labile phosphates in the urine after the oral administration of polyphosphates.

The conclusion of the report was as follows :

"The most important observation is that polyphosphates are not absorbed as such to any significant extent, but only in the form of monophosphates to which they are broken down in the intestine ...Thus for purposes of toxicological evaluation, polyphosphates may be considered equivalent to monophosphates".

The observations referred to previously, suggest that sucrose phosphates, likewise, can be considered to be equivalent to a mixture of sucrose and inorganic phosphate for toxicological evaluation. Results of the acute and chronic toxicity studies which have been carried out to date and reported in the previous sections do not contradict this (see also Item 18).

(d) Evidence of non interference of the additives with essential dietary constituents

Evidence that calcium sucrose phosphates do not interfere with essential dietary constituents is provided by the data contained in the preceding sections (a), (b) and (c), and also in the medical examinations of children in the food additive clinical trial described in Item 20 of this application.

The fact that rats and dogs fed on diets containing as high as 10% of calcium sucrose phosphates grow normally and have the same food efficiency as animals on a normal diet^{32,33}, shows clearly that calcium sucrose phosphates do not interfere with essential dietary constituents. Furthermore, the biochemical data and the comparison of metabolism and excretion of calcium sucrose phosphates with sucrose^{39,40} show that calcium sucrose phosphates in fact have food value in their own right.

Medical examinations of children receiving for one year an average of 4 gram calcium sucrose phosphates per day in the processed carbohydrate portion of their normal diet showed no difference in the normal criteria of nutritional status from children receiving a normal diet. (see Item 20).

(e) Summary of pertinent literature

Many phosphates are generally recognized as safe in foods. A list of these phosphates is shown in Table 1.

TABLE 1

Phosphates generally recognized as safe in foods
Data from Section 121.101 (d) of U.S. Code of Federal
Regulations Title 21 Revised as of January 1, 1963

<u>Phosphate</u>	<u>Use</u>
Ammonium phosphate, monobasic	M
Ammonium phosphate, dibasic	M
Calcium glycerophosphate	N
Calcium hexametaphosphate	S
Calcium phosphate monobasic	NSM
Calcium phosphate monobasic	NSM
Calcium phosphate tribasic	NSM
Calcium pyrophosphate	N
Calcium phytate	S
Ferric phosphate	N
Ferric pyrophosphate	N
Ferric sodium pyrophosphate	N
Magnesium phosphate, dibasic	N
Magnesium phosphate, tribasic	N
Manganese glycerophosphate	N
Monosodium phosphate derivatives of mono- and diglycerides from the glycerolysis of edible fats or oils	E
Phosphoric acid	M
Potassium glycerophosphate	N
Potassium phosphate, dibasic	S
Riboflavin-5-phosphate	N
Sodium acid pyrophosphate	M
Sodium aluminium phosphate	M
Sodium hexametaphosphate	S
Sodium phosphate, monobasic	NSM
Sodium phosphate, dibasic	NSM
Sodium phosphate, tribasic	NSM
Sodium tripolyphosphate	SM
Tetrasodium pyrophosphate	S

N - Nutrient and/or Dietary supplement

S - Sequestrant

M - Miscellaneous and/or general purpose food additives

E - Emulsifying agents

Studies which have been carried out on the toxicological properties of various inorganic phosphates include the recent work of Hodge (1956-1960)⁴² on the chronic toxicity of disodium phosphate, sodium tripolyphosphate, sodium hexametaphosphate, sodium trimetaphosphate and sodium tetrametaphosphate. Refer also to W.H.O. Technical Report No. 281⁴¹ which deals with the use of phosphates as food additives, and Item 18.

Less work has been done on the toxicity of sugar phosphates but their occurrence as essential metabolites of plants and animals suggests the unlikelihood of their having toxicities greater than those of inorganic phosphates. Work which has been done supports this, for example Abelles (1925)⁴³ has shown that sodium hexose diphosphate is less toxic than disodium orthophosphate when injected intraperitoneally into rats.

The evidence which is summarized in this application supports our contention that the sugar phosphates characterized by the methods of manufacture described in Items 4-9 are no less safe for use in foods than the phosphates which are included in the list as shown, and which are widely used in foods at the present time.

Calcium and sodium sucrose phosphates produced by the method of Neuberg^{12,13,14,17} were produced commercially in Germany by E. Merck, Darmstadt, initially during the 1914-18 War. These sucrose phosphate salts were used as a substitute for glycerophosphate salts^{44,45} and were used under the names Hesperonal-Calcium and Hesperonal-Sodium.

The following excerpt from reference⁴⁴ is quoted here as it adequately summarises their use :

"Application and Dosage

The physiological importance of carbohydrate phosphates points towards the application of sucrose phosphates as stimulants and strengthening agents.

Their application is greatly enhanced by the good solubility of Hesperonal-Calcium and Hesperonal-Sodium both preparations are given dissolved in water, milk or soups or mixed with paste-like foods. The dosage can be adjusted to that of the salts of glycerophosphoric acid: 0.2 - 0.5-1 g with or after meals. This dosage will correspond to the quantity which will fit on the tip of a table-knife 2 or 3 times daily for adults, or on the tip of a pocket-knife, twice a day for children.

The Hesperonal-preparations can be used instead of the presently hard to obtain glycerophosphates, unless they find an application on their own as nourishing or strengthening agents"

The calcium and sodium sucrose phosphates were for a time widely used in Europe but apparently the increasing availability of the glycerophosphates enabled the latter to resume their role as dietary supplements. To the best of our knowledge the calcium and sodium sucrose phosphates are not widely used in Europe at the present time. During the period of their use in Europe there is no record of any harmful effects produced by the regular ingestion of these substances.

Recent work by Leloir and Gardini² and Mendicino (1960)³ has shown the presence of enzymes in plant materials (wheat germ and spinach leaves) which produce sucrose-6'-phosphate from U.D.P. -glucose and fructose-6-phosphate. A sucrose phosphate ester was isolated from sugar beet leaves in 1953⁴ and more recently we have shown the presence of sucrose-6'-phosphate in sugar cane⁵. A phosphatase for sucrose-6'-phosphate has been shown to be present in a partially purified enzyme from wheat germ which breaks sucrose phosphate down to sucrose and inorganic phosphate. Intestinal phosphatase also has a similar action.

The adequacy of processed cereals and sugars in human nutrition has been questioned from many quarters in recent years⁴⁶. In this reference Feldburg gives some details on the calcium and phosphorus contents of typical cereal foods. In Table 2 the calcium and phosphorus contents of sugar cane, sugar beets and refined sugar are shown. In this table the proportions of inorganic phosphate and organic phosphate in the natural occurring substances are shown. The figures shown are average figures obtained, in the case of sugar cane, from work carried out in these laboratories as well as from the published work of Honig⁴⁷. In the case of sugar beet the data are those of Bougy⁴⁸ and Finkner⁴⁹. To facilitate comparisons the figures are all expressed as percent of the element based on sugar.

In cane juice the calcium content is generally in the range 2 - 6 mM, while the phosphate concentration is in the range 1 - 20 mM. As a percentage of the sugar contained in the juice this is in the range 0.05 - 0.2% Ca and 0.02 and 0.02 - 0.4% P. It will be seen from the tables that in the refining process

calcium and phosphorus have been reduced by over 90% to an insignificant level in refined sugar.

TABLE 2
Calcium and phosphate content of sugar materials
Concentrations expressed as % element on sugar

Substance	Total P	Inorganic P	Organic P	Ca
Immature Beet (roots)	0.4	0.25	0.15	-
Mature Beet (roots)	0.15	0.10	0.04	0.10
Sugar cane	0.10	0.08	0.02	0.15
Refined sugar	<0.005	<0.005	<0.005	<0.005
Refined sugar + 1% CaSP	0.10	0.03	0.07	0.13
Refined sugar + 2% CaSP	0.20	0.06	0.14	0.26

In Table 3 similar figures show the reduction in calcium and phosphate levels which occurs in the processing of wheat to white bread. In wheat the major organic phosphates are the salts of phytic acid, although other organic phosphates are present. In beet and cane the major organic phosphates appear to be the phosphate esters of sugars. The presence of sucrose phosphates in plants including wheat, sugar beet and sugar cane, has been shown in recent years. The calcium level in wheat is lower than in cane and beet but this is largely compensated for by an increased level of magnesium. The figures in Table 3 have been derived from those given by Pringle and Moran²⁷ and Feldburg⁴⁶. They are expressed as percent of element on dry weight to facilitate comparisons with levels in sugar sources.

TABLE 3

Calcium and phosphate content of wheat products
Concentrations expressed as % element dry weight

Substance	Total P	Inorganic P	Organic P	Ca
English Wheat	0.5	0.14	0.36	0.06
Manitoba Wheat	0.42	0.12	0.30	0.05
Purified Germ	1.55	0.80	0.75	0.10
White flour (85% extr.)	0.26	0.11	0.15	0.03
White flour (70% extr.)	0.13	0.09	0.04	0.015
White flour (70% extr.) + 1% CaSP	0.23	0.12	0.11	0.14
White flour (70% extr.) + 2% CaSP	0.33	0.15	0.18	0.27

It will be seen that in processing wheat to the form in which it is most generally consumed (low extraction white flour) the calcium and phosphate levels have been reduced by about 75%.

It has been recognized in recent years that the processing of many staple foods, particularly cereals, often decreases the nutritional value and the judicious supplementation of these foods by vitamins and other essential nutrients is now widely accepted. For example the National Research Council, U.S.A., in 1943⁵⁰ summed up the situation as follows :

"All the evidence from numerous surveys over the past ten years to the present among persons of all ages in many localities is without exception in complete agreement that inadequate diets are widespread in the nation Accordingly, there is widespread prevalence of moderately deficient diets ...

It would seem advisable to give further consideration to the program of judicious enrichment of appropriate foods since that would add much to the guarantee of "successful nutrition".

This has led in the United States to Federal Standards for cereal enrichment which approve the addition of between 300 and 1000 mg calcium/lb. of cereal product. This is equivalent to a range of about 0.05 - 0.25% on dry weight.

There does not appear to have been any major move to enrich these cereal products with phosphate which, as can be seen from the Tables 2 and 3, is reduced in processing in a manner similar to calcium.

As will be seen from the preceding tables, the supplementation of refined carbohydrate foods with calcium salts of sucrose phosphates at levels of 1% - 2%, will restore to the levels occurring in the natural raw material the calcium and phosphate concentrations in these processed foods.

The calcium sucrose phosphates (CaSP) which it is proposed to add to foodstuffs have the following approximate analysis :

Calcium	12% on dry weight
Total Phosphate (P)	9% on dry weight
Inorganic P	2% on dry weight

The effect on Ca and P levels of adding 1% and 2% of this material (CaSP) to the refined carbohydrates is shown in Tables 2 and 3.

14 Foods in which it is proposed to use the additives

As the purpose of the additives is to inhibit dental caries and the consensus of dental opinion points to carbohydrates

as the main cariogenic foods, it is proposed to use the additives in foods which contain significant proportions of carbohydrates. In particular it is proposed to use the additives in processed carbohydrates. A list of the main foods in which it is proposed to use calcium sucrose phosphates as additives is shown below :

List of foods in which it is proposed to use
calcium sucrose phosphates

Bread	Flour
Biscuits	Cake or biscuit mixes
Breakfast cereals	Sugar
Sugar syrups	Honey
Jam	Canned fruit
Confectionery	Soft drinks
Fruit juice (cordials concentrates or canned)	

Calcium sucrose phosphates can be readily added to these foods which are processed, predominantly carbohydrate foods. A considerable amount of work has been done both by ourselves and Stauffer Chemical Company, U.S.A., as well as by various food manufacturers in Australia, on the incorporation of CaSP into carbohydrate foods. There are few problems associated with the incorporation of CaSP into the majority of carbohydrate foods. The effect of approximately 1% of CaSP on the properties and acceptability of these foodstuffs is undetectable.

The methods of incorporating CaSP into these foods are summarized as follows :

Sugars

White crystal

An aqueous solution of CaSP is sprayed onto crystalline sucrose tumbling in a rotary drum mixer. The moist sugar is then dried in conventional drier - cooler units and packaged.

Soft brown sugar

Dry CaSP powder is mixed with soft brown sugar in a rotary drum mixer. This is then passed through a conventional brown sugar flaker and packaging unit.

Sugar syrups and honey

An aqueous solution of CaSP is run into the syrup in a stirred mixing vessel, before being conventionally packaged in tins or jars.

Flours

CaSP is blended with the flour using normal dry powder mixing equipment, used for the incorporation of leavening agents into the flour.

Bread and biscuits and cakes

CaSP is blended in with the other dry ingredients, using normal bakery equipment in the course of dough preparation.

Jams

CaSP is added as a twenty percent aqueous solution, at the final stages of jam cooking, just before packaging.

Canned fruits

CaSP is incorporated in the normal sugar syrups, and the fruits processed in the normal way.

Confectionery, soft drinks and fruit juices

CaSP can be added as a dry powder if solid sugar is used, or as an aqueous solution, or premixed in the sugar or sugar syrup.

15 Purpose of the additives in foods listed in Item 14

The main purpose of the additive in each of the foods listed in Item 14 is the same, namely to reduce the cariogenic properties of these foodstuffs. The evidence which shows that calcium sucrose phosphates will have this intended effect is presented in Items 20, 21 and 22 of this application.

An additional purpose achieved by the use of calcium sucrose phosphates is to increase the calcium and phosphorus levels in these foods. The addition of these minerals is permitted in some of these foods by various state Food and Drug Acts. For example, refer Regulation 3A, Pure Food Act, 1908, New South Wales⁵¹. Calcium sucrose phosphates being tasteless, readily-soluble and easily-assimilable sources of calcium and phosphorus offer obvious advantages over inorganic calcium and phosphate salts as sources of these elements. (refer other Items of this application).

Proposed regulations covering the use of calcium sucrose phosphates for these purposes in foods are put forward in reference⁵². These proposals are submitted as an indication of how we consider the regulation of use of these additives might be effected.

16 The minimum amounts of the additives necessary to achieve
the results required

The minimum amounts of calcium sucrose phosphates which are necessary to produce a significant reduction in dental caries when added to carbohydrate foods consumed by man are not easy to determine unequivocally. This can only be done by a number of extensive clinical trials. The cost and complexity of this would be prohibitive.

However, some indications of the expected minimum concentration in foods which should be effective can be obtained from the in vitro and animal tests outlined in Item 20. Animal tests⁵³ show that 0.5% calcium sucrose phosphates on total diet in rats produces a significant reduction in dental caries. This is a lower increase in the dietary calcium and phosphorus level (about 0.05%) than that which is needed to produce significant results with inorganic phosphates (0.3 - 0.5%).

The levels of calcium and phosphorus in natural carbohydrate foods, which are generally recognized^{54,55} as being less cariogenic than refined carbohydrate foods, are comparable to those which are obtained by the addition to the refined product of 1% CaSP in the case of sugar or 1 - 2% CaSP in the case of flour (see Item 13(e)).

In the clinical trial of calcium sucrose phosphates as cariostatic food additives we have used a concentration of approximately 1% of the carbohydrate content of the food (see Item 20). This level was arrived at after careful consideration of all the relevant data on levels likely to be needed to produce a

significant reduction in dental caries and increased levels of calcium and phosphorus which would be completely acceptable in the diet (see Item 18).

Our assessment of all available evidence suggests that an observable reduction in dental caries would result from the addition of about 0.5% of the carbohydrate content of the food to the bulk of the processed carbohydrate foods in the diet. We would not expect this level to be as effective as the 1% addition which we used in the trial. Furthermore we would expect additions of up to 2% of the carbohydrate content to be more effective than 1%. We do not believe that it would be advantageous or desirable to go beyond the level of 2% of the carbohydrate content of the food.

We therefore propose a minimum effective level of calcium sucrose phosphates as food additives of 0.5% of the carbohydrate content of the food, a proven effective level of 1.0%, and a maximum desirable level of 2.0%.

17 The amounts of additives expected to be used in each food listed in Item 14

For the reasons which have been outlined in Item 16, we used an amount of calcium sucrose phosphates in each of the foods used in the food additive clinical trial to give a level of about 1% calcium sucrose phosphates based on the carbohydrate content of the food. The consumption of these foods, which comprised about 70% of the carbohydrate intake of the children in the trial, has been proven to produce a reduction in dental caries in children.

For this reason we would expect the allowance of claims that processed carbohydrate foods containing approximately 1% of calcium sucrose phosphates based on the carbohydrate content of the foods will help to reduce dental caries in consumers of these foods.

Considering the foods listed in Item 14, their average carbohydrate content⁵⁶ and the addition of 1% of this carbohydrate content of calcium sucrose phosphates, we obtain the following table :

TABLE 4

Food (as purchased)	Per cent carbo- hydrate (average to nearest 5%)	Per cent calcium sucrose phosphates in food as purchased
Bread	50	0.5
Flour	75	0.75
Biscuits	70	0.7
Cake or biscuit mixes*	70	0.7
Breakfast cereals (precooked)	80	0.8
Oatmeal etc.	70	0.7
Sugar	100	1.0
Sugar syrups	80	0.8
Honey	75	0.75
Jam	70	0.7
Confectionery†	50 - 100	0.5 - 1.0
Canned fruit (sweetened)	20	0.2
Soft drinks	5	0.05
Fruit juice (cordials)		
full strength	40	0.4
diluted as consumed	10	0.1
Fruit juice (canned)	10	0.1
Fruit juice (concentrate)‡	40	0.4

* Not available in ref. ⁵⁶ (assume average same as biscuits)

† depends on very variable carbohydrate content

‡ assume average same as cordials

It was pointed out in Items 6 and 7 that commercial calcium sucrose phosphates can contain sucrose and inorganic calcium phosphates up to 20% of each on the weight of the dry product. The product which is being used in the food additive trial contains on the average about 15% of inorganic calcium phosphate but less than 1% of free sugars. In order to control the addition of various sucrose phosphate products in foods the amount of the additives shall be expressed in terms of the amount of calcium sucrose phosphates in the food. (excluding free sucrose and free calcium phosphate). This can be calculated from the analysis of the product for total phosphorus and inorganic phosphorus or from the electrophoretic or ion exchange chromatography analytical techniques outlined previously²⁵.

In the proposed regulation⁵² we suggest that a tolerance of $\pm 20\%$ should be allowed in the stipulated effective level of 1% calcium sucrose phosphates based on the carbohydrate content of the food. That is, in a food in which 1% calcium sucrose phosphate is required, 0.8 - 1.2% is an acceptable range.

In the regulation⁵² which we have proposed to cover the use of calcium sucrose phosphates as a cariostatic agent, we suggest that no claim as to the cariostatic properties of the food should be permitted unless the foodstuff contains at least this proven effective amount. For the addition of calcium and phosphorus to foods there is no similar necessity for a minimum effective amount and this can be covered by the existing regulations concerning the addition of these elements to foods.

18 Limits of the probable intake of the additives in the normal diet

As pointed out in previous Items the level of addition of calcium sucrose phosphates to foods is dictated by the amount required to achieve the intended cariostatic effect and the amount which would be completely acceptable in foods.

As we have shown from a consideration of the chemistry and biochemistry of calcium sucrose phosphates (see Item 13) these compounds can be broken down by normal metabolic processes to sugars and free calcium phosphates. The question of the safety of sugars does not arise; in order to consider reasonable limits of intake of calcium sucrose phosphates we can obtain guidance from the acceptable daily intakes of phosphates and calcium.

W.H.O. Technical Report No. 281 (1964)⁴¹ contains biological data on inorganic phosphates, diphosphates (pyrophosphates) and polyphosphates with respect to the use of these substances as food additives.

The findings of this report are summarised in the following main points :

- (1) The usual evaluation procedure of taking the highest dose level in animals that does not produce any toxicological effects and applying a safety factor to arrive at an estimate of acceptable daily intake for man is not appropriate in the case of phosphates.

- (2) Since nearly every food normally contains phosphates it is impossible to indicate acceptable intakes of these compounds as food additives without regard to the phosphate intake from the food itself.
- (3) For the purpose of estimating acceptable intake zones, diphosphates should be treated as if they were monophosphates on the basis of their phosphorus content.
- (4) For the purposes of toxicological evaluation, polyphosphates may be considered equivalent to monophosphates. The acceptable daily intake of polyphosphate is related to the total dietary phosphate.
- (5) Acceptable dose levels of phosphate intake depend on the amount of calcium in the diet.
- (6) The unconditional acceptable daily intake zone for man for the above classes of phosphates is -
30 mg/kg body weight as total dietary phosphorus intake from both food and food additives. This may be regarded as suitable for communities with a low calcium intake.
- (7) The conditional acceptable daily intake zone for man for the above classes of phosphates is -
30-70 mg/kg body weight.
This may be regarded as suitable for communities with a high calcium intake.

W.H.O. Technical Report No. 230 (1962)⁵⁷ discusses calcium requirements for man. Some findings of this report are :

- (1) Normal average daily intakes of calcium vary from as high as 1329 mg/day (Finland) to 347 mg/day (India).
- (2) In some limited groups intakes of over 2 to 3 g/day were without apparent detrimental effect on health.
- (3) There is no evidence that a daily intake as high as 1500 mg Ca/day is undesirable. However the raising of calcium intake beyond one gram/day is unlikely to serve any physiologically useful purpose.

Note: if reference man is 65 kg, a daily intake of 1500 mg Ca/day is equivalent to a daily intake of :

23 mg Ca/kg body weight.

The Commonwealth Bureau of Census and Statistics⁵⁸ gives the following figures for nutrients available for consumption by Australians in 1964-65.

Protein	91	gram	per	head/day
Fat	129	"	"	"
Carbohydrate	410	"	"	"
Calcium	910	mg	"	"

The average calcium content of CaSP used in the clinical trial is about 12%. The average phosphorus content of CaSP is about 9%. It is not possible to estimate accurately the actual intake of CaSP which would occur if it was widely used as a food additive. However if we assume for illustration that 1% CaSP is added to the total dry weight intake of carbohydrate, a daily intake of 4.1 gram CaSP/day would result. This for the reference

man of 65 kg is equivalent to a daily intake of 60 mg CaSP/kg body weight.

This in turn is equivalent to an additional daily intake of :

7.5 mg Ca/kg body weight

5.7 mg P/kg body weight.

A calcium intake of 827 mg per head/day for the average Australian is equivalent to :

14 mg Ca/kg body weight.

An increase of 7.5 mg Ca/kg body weight would bring the total intake of calcium to :

21.5 mg Ca/kg body weight

which is below the acceptable daily intake of 23 mg Ca/kg body weight reported in W.H.O. Tech. Report No. 230⁵⁷.

Similarly, although figures are not available for the total phosphorus intake, an increase of 5.7 mg P/kg body weight represents only 20% of the unconditional acceptable daily intake zone for man, 8% of the conditional acceptable intake zone for phosphates for man as reported in W.H.O. Tech. Report No. 281⁴¹. Moreover the Ca/P ration CaSP of 1/1 is more favourable in diet compared with the commonly used sodium phosphate food additives.

Data from the chronic toxicity studies^{32,33} which were carried out on rats and dogs cover observed daily intakes of CaSP ranging from 4,000 - 15,000 mg/kg body weight (10% of diet) to 700 - 3,000 mg/kg body weight (2% of diet). These ranges correspond to increases of approximately 70 - 400 mg/kg body weight for both calcium and phosphorus in the diet. No untoward

toxic effects have been observed even at the highest levels. These levels are from 10 to 250 times the maximum average level of CaSP intake in man, 60 mg/kg body weight, assuming 1% CaSP is added to the carbohydrate of all foods.

In actual practise however, only the processed carbohydrate foods will be treated. The proportion of the carbohydrate intake which represented by these foods can be estimated from the data in reference⁵⁸ to be about 80% of the total carbohydrate intake. At 1% calcium sucrose phosphate based on the carbohydrate content of these processed foods (Item 14) the average maximum level of intake of calcium sucrose phosphates would be 3.4 grams/day, (or 52 mg/kg body weight) at 2% the level would be 6.8 grams/day (or 104 mg/kg body weight).

19 Use of additives in packaging materials

There is no intention of using calcium sucrose phosphates as additives in packaging materials.

20 Evidence that the additives will have the intended effect in the foods listed in Item 14

Evidence establishing that calcium sucrose phosphates, when added to processed carbohydrate food listed in Item 14, will reduce dental caries in consumers of such foods is conclusively provided by the results of the clinical trial described later in this item.

However, it is pertinent to outline the evidence for the cariostatic effect of calcium sucrose phosphates which led to this trial.

The potential use of phosphates in the inhibition of dental caries has been extensively discussed in the "Conference on phosphates and dental caries" held at Cambridge, Mass., in October 1962⁵⁹.

Considerable attention has been given to the influence of refinement of carbohydrate foods on the incidence of dental caries. Osborn (1941)⁵⁴ postulated the existence of a "protective agent" in crude cereals and sugar cane juice to "account for the relative scarcity of carious teeth among primitive peoples and the prevalence of decay among the civilized". This was supported by the work of Jenkins et al. (1959)⁵⁵ who found that in treacle and cane juice and brown (wholemeal) flour there were substances which decreased the dissolution of calcium and phosphate from teeth and powdered calcium phosphate when incubated with saliva. Evidence was presented which suggested that these "protective agents" were organic phosphates which along with the inorganic phosphates present in natural occurring carbohydrate were largely removed during refining.

The aim of introducing phosphates into foodstuffs in a form similar to that in which they occur in nature led us in 1960 to undertake a systematic investigation of organic phosphates as cariostatic food additives in the diet. This work is summarized in a recent paper⁵³.

The investigation has employed three main methods of testing :

- (a) tests in vitro
- (b) tests in laboratory animals
- (c) tests in man.

(a) Tests in vitro

The initial work which we carried out in an attempt to assess the usefulness of various phosphates as cariostatic agents followed the approach⁶⁰ involving "white spot" formation in teeth in acidic buffer solutions containing small amounts of gelatin.

The procedure involved immersing areas of extracted cleaned human teeth in a buffered solution containing lactic acid and gelatine at a pH of 3.8, without agitation. After several hours immersion "white spots" which are similar to those preceding the formation of a carious lesion were observed in the tooth enamel.

The addition of amounts of CaSP as low as 0.5% inhibited the formation of these "white spots" under identical conditions (same pH, concentrations, temperature, time and agitation) to those in the control solutions in which "white spot" occurred.

This inhibiting effect has also been observed with other phosphates and it was realized that in the absence of a better understanding of the factors involved in "white spot" formation (and indeed in dental caries) the technique would not provide an effective method of screening a large number of different phosphates.

The carious process ultimately involves decalcification of the enamel, i.e. the dissolution of the hydroxyapatite constituting the enamel surface. So any compound which will prevent and/or retard this dissolution process could well possess prophylactic cariostatic activity. Equally a compound which deposited hydroxyapatite onto an eroded enamel surface would well possess curative cariostatic activity. CaSP has been shown both

to retard the acid dissolution of hydroxyapatite and to reharden softened human dental enamel.

Human dental enamel surfaces exhibit extremely variable behaviour. Because of this, the study of the dissolution kinetics of synthetic hydroxyapatite surfaces has been preferred. Exact definition of the hydrodynamic situation under which enamel dissolution occurs in vivo is difficult; indeed, it is probably variable. However the rate of enamel dissolution is undoubtedly slow due either to the slowness of mass transfer away from the dissolving surface and/or to low undersaturations. The effect of CaSP on the dissolution kinetics of hydroxyapatite under conditions likely to prevail in the mouth has thus been studied.

Synthetic hydroxyapatite was pressed into pellets which, even under vigorous stirring, did not undergo attrition⁶¹. The rate of dissolution of these pellets in an acetate buffer at pH = 4.0 was studied with and without CaSP as additive.

In unstirred systems a concentration of 0.5% CaSP was found to reduce the rate of dissolution of hydroxyapatite by upwards of a factor of 2. Most of this reduction was ascribed to the common ion effects of the calcium and inorganic phosphate ions from the added CaSP. The effect of the sucrose phosphate anion alone can be studied in these systems (containing 0.5M potassium chloride) by using the alkali metal salt of the organic phosphate. Dipotassium sucrose phosphate exerted no appreciable influence on the dissolution rate in unstirred systems.

Under stirred conditions⁶² even more dramatic reductions in rate were observed; for now the presence of even $5.0 \times 10^{-4} \text{ M}$

dipotassium sucrose phosphate (0.025%) reduced the rate of dissolution by a factor of 2. The maximum reduction in rate observed was by a factor of 2.5. The dependence of the reduction in dissolution rate on the concentration of sucrose phosphate anions suggest that adsorption of the sucrose phosphate anions on the hydroxyapatite surface leads to a reduction in the rate of detachment of constituent ions from the solid surface.

The onset of caries is signalled by sub-surface decalcification⁶⁰. This decalcification reduces the hardness of the enamel surface as measured by a surface penetrating device. We have carried out experiments⁶³ which have followed those described by Pigman⁶⁴ on the rehardening of softened tooth enamel. These experiments have shown that solutions of calcium sucrose phosphates reharden softened tooth enamel. Calcium sucrose phosphates containing some inorganic phosphate (CaSP) were more effective than the calcium sucrose phosphates alone. The rehardening process has the attributes expected of a calcium phosphate precipitation. In water calcium ions alone or phosphate ions alone did not reharden the enamel. Solutions of CaSP thus behave as stable supersaturated solutions of calcium phosphate which, in the presence of a suitable enamel surface, deposit calcium phosphate.

(b) Tests in laboratory animals

The effect of phosphates on experimental dental caries in hamsters and rats has been reviewed by Nizel and Harris⁵⁹. More than one hundred studies have shown that the addition of phosphates to the diet are effective anticaries agents in rodents.

The wide variations in the caries severity scores in both control and test groups of animals, which are inherent and difficult to avoid in these studies, have prevented the relative cariostatic efficacy of the various phosphate anions and their respective cations from being determined. There is a preponderance of evidence to suggest that the cariostatic effect of phosphates appears to be due largely to a local action on the tooth as these phosphates pass through the mouth. However, the purely systemic effect of phosphates on dental caries has not been investigated adequately.

We have carried out extensive tests⁵³ on a wide range of organic phosphates in the diet of laboratory animals. Osborne-Mendel strain, caries-active rats were fed a basic cariogenic diet from 21 days after birth. Three basic diets were used, a high sucrose diet, a white flour bread diet, and an enriched bread diet. Various inorganic and organic phosphates were added at levels ranging from 0.03% - 2% of the diet.

The results of these experiments showed that in all cases the addition of calcium sucrose phosphates to the cariogenic diets significantly reduced the caries severity score. Other phosphates, both organic and inorganic, also displayed cariostatic activity in these experiments, but, as in other animal experiments, it is not possible to determine unequivocally the relative cariostatic efficacy of the various phosphates.

Similar results on calcium sucrose phosphates have also been obtained by Dr. F.J. McClure of the National Institute of Dental Research, Bethesda, U.S.A.⁶⁵.

(c) Tests in man

In selecting a phosphate for testing in man one must assess the information which is available on

- (1) in-vitro experiments on the effect of phosphates on the dissolution and growth of hydroxyapatite in tooth enamel
- (2) the effect of phosphates in laboratory animals
- (3) the occurrence of phosphates in natural non-cariogenic carbohydrates
- (4) the chemical and physical properties of phosphates
- (5) the safety (non-toxicity) of phosphates, and
- (6) the incorporation, compatibility and acceptability of phosphates in foods.

The collective evidence on these points, which we have outlined in this application, clearly pointed to the desirability of carrying out clinical trials on the effect of calcium sucrose phosphates on dental caries in man.

Two trials have been undertaken in Australia. The first, to test calcium sucrose phosphates in toothpastes, gave us valuable experience in the more complex organization of the second trial to test calcium sucrose phosphates as cariostatic food additives.

The ability of solutions of CaSP to harden human teeth, together with the adsorption of sucrose phosphates on hydroxyapatite surfaces and the inhibition of dissolution of such surfaces, suggested that CaSP may be effective as an ingredient in toothpastes in the inhibition of dental caries in man.

The evidence which we have accumulated suggests that the most effective way of utilizing the cariostatic effects of calcium phosphates in dental caries is as additives to processed carbohydrate foods. Under these conditions the cariostatic agent is present at the same location and at the same time as the cariogenic agents which are produced from such foods. However a significant, but lesser effect may also be produced if the calcium sucrose phosphate is used in a toothpaste. A clinical trial of a dentifrice containing 5% CaSP was carried out by the University of Melbourne between May 1963 and May 1965 at Mount Scopus College, Melbourne.

The protocol⁶⁶ and the statistical analysis⁶⁷ of this trial show evidence that calcium sucrose phosphates as toothpaste ingredients produce a significant reduction in dental caries in certain groups of tooth surfaces in children using such toothpastes. These results, considering the lack of supervision to ensure that all the children actually used the dentifrice, support our laboratory evaluations of calcium sucrose phosphates as cariostatic agents.

The data which have been summarized clearly indicate the potential use of calcium sucrose phosphates as an inhibitor of dental caries. Moreover the data which have been accumulated on the chemistry and pharmacology of calcium sucrose phosphates do not in any way contra-indicate the carrying out of a clinical trial on the use of CaSP at a level of 1% based on the carbohydrate content of the diet in man.

A clinical trial to assess the safety and efficacy of a phosphate as a therapeutic food additive in the inhibition of dental caries should cover a wide range of such foods in which the additive is likely to be used. The foods which are generally regarded as being potentially cariogenic are those processed foods containing a large proportion of carbohydrates. Such foods are derived mainly from two types of natural sources, sugar cane and sugar beet on the one hand and cereal grains on the other. These naturally occurring carbohydrate foods are in the main processed and refined to produce a wide range of predominantly carbohydrate foods. Such foods which include table sugars and syrups, preserves, confectionery, bread, breakfast cereals, cakes, biscuits and the like have been listed in Item 14.

At our present state of knowledge there are no substantial grounds for incriminating any one of these foods as a more important cariogenic agent than another. Nor are there grounds for assuming that any one of these foods is a more satisfactory vehicle for a cariostatic agent than another. On the contrary, the evidence suggests that if processed carbohydrates generally contribute to the incidence of dental caries then the most effective vehicle for a cariostatic phosphate is carbohydrate foods generally.

Moreover from the viewpoint of establishing safety in the use of a cariostatic phosphate as a therapeutic food additive it is considered that this can only be adequately determined if the clinical trial covers the majority of carbohydrate foods in which the additive is likely, ultimately to be used.

We have therefore, in conjunction with the Dental Research Institute and the School of Paediatrics of the University of N.S.W., initiated a comprehensive trial in which calcium sucrose phosphates are added to carbohydrate foods in a concentration of 1% of the carbohydrate contents and consumed by a sufficient number of children for a sufficient time to determine whether the incidence of dental caries is reduced, and of equal importance, to determine that no harm is caused to any of the children by this substance administered in this form.

This trial, the protocol for which is given in reference⁶⁸ commenced in N.S.W. in March 1965. The trial will continue for three years, but the results of examinations carried out after one year⁶⁹ have already shown that calcium sucrose phosphates used as additives to processed carbohydrate foods significantly and markedly reduce dental caries in children consuming such foods without any adverse effects on the health of the children.

21 Evidence that the same objectives cannot be obtained by good manufacturing practices or existing approved additives

The aetiology of dental caries is imperfectly understood. Present knowledge suggests that it is a multi-causal disease depending on genetic factors, dietary factors and microbiological factors. As such the dental caries problem is capable of being attacked from many different angles. No one method of attack will necessarily exclude another.

In the present instance we are concerned with the control of dental caries through dietary factors. There is evidence that

such measures as the fluoridation of public water supplies and the consumption of only unprocessed natural foods provide ways of controlling (but not eliminating) dental caries. However these measures are not entirely acceptable to all members of the community. Moreover, the provision of large city populations with unprocessed natural foods would require an impracticable reorganization of the whole complex logistics of food supply.

The addition of an additive to processed carbohydrate foods which would minimise the generally accepted cariogenic properties of such foods is an objective which has been widely appreciated⁵⁹ but which to date has not been achieved. Such an attack on the public health problem of dental caries would provide an approach which would complement other measures such as the fluoridation of public water supplies. Moreover, the consumption of such foods would be a matter for individual choice, as it would be obvious to offer both treated and untreated foods to the public.

It is therefore clear that good manufacturing practices cannot achieve the same objective as the present proposal. In addition the use of existing approved additives has not been proven to achieve the same objective. The use of inorganic phosphates as potential cariostatic food additives has received much consideration⁵⁹. They have been shown generally to be effective in reducing dental caries in laboratory animals. However the evaluations⁷⁰ on the effect of inorganic phosphate supplementation of the human diet have produced equivocal results. We have outlined in Item 20 and its references, the many factors which must be considered in the choice of a phosphate as a cariostatic food additive in the human diet. It is apparent that

calcium sucrose phosphates meet these requirements more adequately than do the inorganic phosphates and in fact, to our knowledge, constitute the only proven safe and effective cariostatic food additive at the present time.

22 The advantages which will accrue to the consumer from the use of the additives

The principle advantage which will accrue to the consumer of foods containing calcium sucrose phosphates as cariostatic agents is of course dependent in the first place on whether the consumer has teeth.

The effect of the cariostatic agent would obviously be more important in children than in adults. During the development and eruption of the teeth and the period of maximum risk of dental caries⁷¹ which takes place at successive stages of childhood, the consumption of foods containing a caries inhibiting substance would be of greater benefit than in adulthood. Nevertheless there is no reason to believe that the cariostatic effect of calcium sucrose phosphates in foods will not continue into adulthood and, even in those adults and children who do not possess teeth, the supplementation of the diet with these small levels of calcium and phosphate would be expected to be generally beneficial.

In those diseases or conditions in which even this small increase in calcium or phosphorus may be contra-indicated the freedom of choice of consumption of the treated food can be exercised, as the fact that the additive is incorporated, and its nature, would be clearly indicated on the food according to the proposed regulations⁵².

The advantages of a decrease in the dental caries to the consumer and to general public health is far too obvious to enlarge on further.

23 Evidence of approval or rejection by any other statutory
body or authority

As the discovery of the cariostatic properties of calcium sucrose phosphates is an entirely Australian effort, and because the major part of the research and development on the use of these substances has been carried out in Australia, there has not been any application to any statutory body or authority for approval of calcium sucrose phosphates as cariostatic food additives.

An application to the Food Standards Committee of the Ministry of Agriculture, Fisheries and Food, U.K., for approval of calcium sucrose phosphates for general use in foods was made in January 1965 in order to meet a deadline for consideration of various types of food additives which are only reviewed periodically. There has been no action in this application to date.

It is also proposed to submit to the Food and Drug Administration of the U.S. Department of Health, Education and Welfare, a notice of claimed investigational exemption for a new drug. This is necessary in order to clear the way for clinical trials of calcium sucrose phosphates in the U.S.A. which we propose to commence in 1967 in conjunction with our associates in the U.S.A. in this venture, the Stauffer Chemical Company.

The Colonial Sugar Refining Company Limited

per

General Manager
December 1966.

24 References

Copies and translations of all references other than long reviews, text books, complete proceedings of conferences or theses are contained in separate folders which have been lodged with the fifteen copies of this application with the Food Additives Committee. Those references which are not included in these folders are underlined.

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