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# **EVALUATION OF THE TOXICITY OF A NUMBER OF ANTIMICROBIALS AND ANTIOXIDANTS**

**Sixth Report  
of the Joint FAO/WHO Expert Committee  
on Food Additives**

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**WORLD HEALTH ORGANIZATION**

**GENEVA**

**1962**

## JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Geneva, 5-12 June 1961

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# EVALUATION OF THE TOXICITY OF A NUMBER OF ANTIMICROBIALS AND ANTIOXIDANTS

## Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives

### INTRODUCTION

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva from 5 to 12 June 1961. The meeting was opened by Dr L. Verhoestraete, Director of the Division of Health Protection and Promotion of WHO, on behalf of the Directors-General of the Food and Agriculture Organization of the United Nations and the World Health Organization. Dr H. C. Spencer and Professor R. Truhaut were unanimously elected Chairman and Vice-Chairman respectively. Professor A. C. Frazer agreed to act as Rapporteur.

As a result of the recommendations of the Joint FAO/WHO Conference on Food Additives held in September 1955 <sup>1</sup> five Joint FAO/WHO Expert Committees on Food Additives have met and issued the following reports: "General Principles Governing the Use of Food Additives: First Report", <sup>2</sup> "Procedures for the Testing of Intentional Food Additives to Establish their Safety for Use: Second Report", <sup>3</sup> "Specifications for Identity and Purity of Food Additives (Antimicrobial Preservatives and Antioxidants): Third Report", <sup>4</sup> "Specifications for Identity and Purity of Food Additives (Food Colours): Fourth Report", <sup>5</sup> and "Evaluation of the Carcinogenic Hazards of Food Additives: Fifth Report". <sup>6</sup>

<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1956, No. 11; *Wld Hlth Org. techn. Rep. Ser.*, 1956, 107.

<sup>2</sup> *FAO Nutrition Meetings Report Series*, 1957, No. 15; *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129.

<sup>3</sup> *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.

<sup>4</sup> Unpublished working document WHO/Food Add./15.

<sup>5</sup> Unpublished working document WHO/Food Add./17.

<sup>6</sup> *FAO Nutrition Meetings Report Series*, 1961, No. 29; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

This meeting was convened on recommendations made in the third, fourth, and fifth reports of the Joint FAO/WHO Expert Committee on Food Additives, its terms of reference being to consider the evaluation of the evidence on the toxicological aspects of a number of antimicrobials and antioxidants used as food additives.

## GENERAL CONSIDERATIONS

### Objectives

One of the major problems in connexion with food additives is the satisfactory control of their use. In many countries, special agencies or departments are responsible for such control and in some, supporting scientific facilities are available. In many others, however, there is no adequate machinery by which those responsible for public health can usefully handle these problems. Furthermore, the solutions that may be effective in one country are not necessarily applicable to another, since the nature of foods consumed, pattern of the diet, and environmental conditions of life vary greatly. It was therefore recommended in earlier reports of the Joint FAO/WHO Expert Committee on Food Additives<sup>1, 2, 3</sup> that FAO and WHO should carry out a survey of the literature dealing with the biological properties of the more important chemical substances that might be proposed for use as food additives and submit this information to an expert committee for evaluation. This has been done and on the basis of the available data the Committee has now been asked to evaluate the toxicities of a selected group of substances. Before considering the substances individually, however, there are a number of general considerations that require discussion.

### Substances to be considered

The substances evaluated by this Committee have been chosen from a number that might be used as antimicrobial preservatives or as antioxidants. These substances were chosen because they are widely used in many countries, because they can play an important part in reducing food wastage by improving storage efficiency and distribution, and because provisional specifications for most of them had already been prepared by previous Committees. The use of antimicrobial preservatives and anti-

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.

<sup>2</sup> Unpublished working document WHO/Food Add./15.

<sup>3</sup> Unpublished working document WHO/Food Add./17.

oxidants may have special importance in tropical countries where the climatic conditions particularly favour some of the deteriorative changes in food that can be controlled by these agents.<sup>1</sup>

There are many other important food additives that require toxicological evaluation, for example: emulsifying and stabilizing agents; important preservatives not included in the present report, such as carbon dioxide; antibiotic substances; and food colours.

Reference was also made to acetaldehyde, chloramine T, ethylene oxide, propylene oxide, and hydrogen peroxide. Since acetaldehyde is a flavouring agent, chloramine T is mainly used for the treatment of water, and ethylene oxide, propylene oxide and hydrogen peroxide are non-residual agents, it was felt that they did not fit appropriately into the groups dealt with in this report and might be more usefully considered on some future occasion.

### **Specifications for substances considered**

The importance of specifications for substances used as food additives was fully discussed in the third report of the Committee.<sup>2</sup> In each monograph of the present report, only such information is given as is necessary for identification of the substance under consideration, together with a few properties that might be of interest to the toxicologist. In special cases, a mixture currently used as a food additive has been made the subject of a monograph; this has been done only where investigations in animals or in man have been carried out on the same mixture. The principle that each substance used as a food additive should be considered separately has been followed as far as identification is concerned; in some cases, however, no differences in toxicology have been found between closely related substances—for example, acids and their esters or salts—and one experimental study may sometimes include work on several different compounds belonging to the same group. The Committee therefore decided that although a separate specification should be given for each substance, in appropriate cases related substances might be considered jointly in the toxicological evaluation.

### **Use in food**

It is the common practice in many countries nowadays to work on the basis of "permitted lists", that is to say, for each main category of food

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1957, No. 15; *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129.

<sup>2</sup> Unpublished working document WHO/Food Add./15.

additives a list of permitted substances is prepared. It is also a growing practice to relate the inclusion of a substance in a permitted list to some specified use or uses and, in certain cases, to specify a particular tolerance level or levels.<sup>1</sup> While this may have much to commend it from a national point of view, it is not possible to adopt such a practice internationally, since there is no international law on which permission could be based nor is there any international machinery for enforcement of such legislation. Furthermore, the needs for food additives and their possible uses and applications vary so widely from country to country that a single pattern of legislation is not feasible on a world-wide basis. It is, of course, clear that groups of countries may agree on joint permitted lists. Such developments are welcome and may represent a valuable contribution to the promotion of international trade.

The Committee therefore decided not to relate acceptable levels to any specific use or uses, except in those cases where particular hazards might be involved. Nevertheless, consideration has been given to likely levels of usage to ensure, if possible, that the acceptable intake zones proposed will allow effective technological use. While no attempt has been made to compile permitted lists or to propose tolerance levels, an acceptable intake zone has been defined in those cases in which this could be assessed on the basis of published scientific evidence. This information is intended to assist those concerned with food additive problems in taking decisions on the use of food additives, in making appropriate laws or regulations to control such use, in establishing appropriate tolerance levels, or in planning any further investigations that may seem desirable in relation to individual national needs and within the framework of national legislation.

### **Baby food**

While the use of food additives is not a point of primary consideration in this report, there is one class of foods to which special reference must be made. Foods that are specifically prepared for babies require separate consideration from all other foods as regards the use of food additives and toxicological risks. The reason for this is that the detoxicating mechanisms that are effective in the more mature individual may be ineffective in the baby. The Committee strongly urges that baby foods should be prepared without food additives, if possible. If the use of a food additive is necessary in a baby food, great caution should be exercised both in the choice of additive and in the level of use.

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<sup>1</sup> These tolerance levels are usually established on the basis of the estimated acceptable human intake and the average per capita consumption of the food items to which the chemical compound may be added.

## BIOLOGICAL DATA PRESENTED IN THE MONOGRAPHS

In order to give as much assistance as possible to national groups concerned with food additive problems, the main biological information made available to the Committee is summarized at the end of each monograph. This may help individual scientists to examine the original papers if they wish to do so. With the exceptions mentioned, only published work has been used in compiling this report. It is one of the great problems in this field that, for a variety of reasons, much of the experimental work remains unpublished. Nevertheless, the Committee wishes to re-emphasize that, as stated in earlier reports,<sup>1, 2, 3</sup> there is an urgent need for more complete publication of the experimental work carried out in this field and also for an improvement in the facilities necessary to achieve this.

The biological data are presented under four heads, as set out in the second report of the Committee.<sup>1</sup>

### Acute toxicity

Since this information is of limited use in the evaluation of the toxicological risk of a food additive, it is presented only in tabular form. The LD<sub>50</sub> gives some indication of the general toxicity class<sup>4</sup> and the difference between the oral and the parenteral LD<sub>50</sub> levels may sometimes be of interest. The studies are usually performed on several species, and this may give some indication of species differences.

### Short-term studies

These include all investigations other than those continued for most of the animal's life span; they are commonly carried out over 10% of the life span or less. As a rule, a number of different species are used. It is probable that the majority of toxic effects can be demonstrated in such studies. The dosage level at which deleterious effects occur, the time taken to cause them, and the nature of the effects produced are all of interest and potential importance. Relevant observations in man may be helpful in revealing gross species differences.

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.

<sup>2</sup> Unpublished working document WHO/Food Add./15.

<sup>3</sup> *FAO Nutrition Meetings Report Series*, 1961, No. 29; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

<sup>4</sup> Spector, W. S. (1956) *Handbook of toxicology*, Philadelphia & London, Saunders.

### Long-term studies

In this category the most important tests are those carried out over the greater part of the animal's life span. Such tests are essential for the assessment of the carcinogenic risk as discussed in detail in the Committee's fifth report.<sup>1</sup> They are also important for the evaluation of the acceptable level of a food additive, since it may be consumed daily for the whole life span. Long-term studies are almost always done in the rat, but with some food additives life-span studies have also been done in the mouse. Unfortunately, long-term studies in other animals are rare.

### Biochemical studies

It is important to know whether a substance is absorbed, what factors may affect its absorption, how the substance is distributed in the body, where and how it is metabolized, and the route by which it is eliminated. Such information is by no means always available.

In evaluating the toxicological status of a substance it may be helpful to know the metabolic pathways that it follows in the body, and whether the changes in structure that take place during metabolism result in any significant change in the biological effects of the substance. The information available on the metabolism of many groups of substances is rapidly increasing. One aspect of metabolic studies that has not received enough attention so far is the study of the metabolic pathways in man. Although this presents considerable difficulties, information on the main pathways of metabolism in man might be helpful in choosing the most appropriate animal for long-term studies. There are often quite marked differences in metabolism between animal species; if a choice of experimental animal for long-term studies of a substance is made without knowing in which species (if any) the metabolism of the substance is substantially similar to that in man, there is a danger that the choice may be an inappropriate one and the results of the studies consequently of little relevance to the problem of assessing the human hazard involved in the use of the substance as a food additive. It may be noted, however, that the Committee has taken into account the paucity of information on metabolism in man in assessing the acceptable intakes. The rates of metabolism and elimination are also important, since they give an indication of the likelihood of cumulation. In general, cumulative substances must be considered unsuitable for use as food additives.

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<sup>1</sup> *FAO Nutrition Meetings Report Series* 1961, No. 29; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

## ASSESSMENT OF THE SIGNIFICANCE OF THE BIOLOGICAL DATA

### Comments on the evidence presented

In each monograph, the comments of the Committee are recorded following the presentation of relevant biological data. This section is a brief appraisal of the main evidence. The Committee is fully aware that great difficulties face investigators in this field and that methodology has advanced considerably in recent years and is still developing. As already stated, other work on a substance may often have been done but not be generally available, and this may have been used by some national agencies in arriving at their assessment of toxicological risk. Any criticisms put forward in the report should not be regarded as being directed at any individual investigator or group but as a statement of the problems experienced by the Committee in their attempt to make an evaluation of the toxicological status of the substance. As might be expected, the Committee would have liked more evidence in most cases. It may be stated, however, that one thorough investigation planned and executed along the general lines indicated in the second and fifth reports of this Committee<sup>1, 2</sup> should provide a satisfactory basis for evaluation. There is no doubt that one of the main difficulties is the serious lack of personnel capable of carrying out the investigations needed in this field, particularly pharmacologists with training in toxicology, biochemists with special training in mechanisms of detoxication, and pathologists with training in animal pathology.

### Evaluation

#### *Estimation of the dosage level causing no significant toxicological effect*

The essential basis of evaluation in almost every case considered by the Committee was one or more long-term studies in rats. The most useful quantitative index was considered to be the highest dosage level that caused no significant toxicological effect, either in acute, short-term or long-term studies. In some cases a particular dosage level was found to have only one effect, the significance of which from the toxicological viewpoint might be doubtful. Some effects, such as osmotic action at high dosage levels, may be properly disregarded in an evaluation of the toxicity of a lower dosage range. The decision to disregard a particular

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1957, No. 15 ; *Wld Hlth Org. techn. Rep. Ser.*, 1957, 129.

<sup>2</sup> *FAO Nutrition Meetings Report Series*, 1961, No. 29 ; *Wld Hlth Org. techn. Rep. Ser.*, 1961, 220.

effect in a series of studies must be made by scientists experienced in this field. In general, it is advisable, when in doubt, to err on the side of regarding as significant an effect that may later be found to be unimportant, rather than to disregard an effect that may later be shown to be relevant. It has been a fairly common practice in the past to regard interference with weight gain as of no significance if a corresponding reduction in food intake was demonstrated, on the grounds that the effect was due to interference with palatability. The Committee considers that statistically significant interference with weight gain should not be lightly disregarded. The dosage level at which no significant toxicological effect was observed is expressed as mg per kg of body weight per day for a stated animal species.

*Estimate of acceptable daily intake zones for man*

Many factors are concerned in deriving from the dosage level that causes no significant toxicological effect in an experimental animal an estimate of the acceptable intake level in man. It is necessary to take into account species differences, individual variations, incompleteness of available data, and a number of other matters. It must be remembered that food additives may be consumed by people of all ages throughout the whole life span, that they are eaten by the sick as well as the healthy, and that there are wide variations in individual dietary patterns. Each case must be judged on its merits.

The Committee considered these various factors in relation to the biological data on each substance upon which a monograph is included in this report. As might be expected, it was not possible to deal with each of the substances considered in exactly the same way. The principle that guided the Committee throughout its work has been stated in the second report, in which the principles for interpreting biological data were outlined.<sup>1</sup> Although there must always be some element of doubt in extrapolation from animal studies to man, the Committee felt that it would be helpful to define an unconditional intake zone for as many substances as possible. The important questions were whether such an unconditional zone could be regarded as acceptable from the toxicological point of view and whether the levels allowed within the zone were likely to be technologically useful.

The method of converting actual levels of use to a figure that can be compared with those given in this report is discussed in detail later.

The highest level stated for this unconditional acceptable intake zone is not, however, the maximum amount of the food additive that might be

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<sup>1</sup> *FAO Nutrition Meetings Report Series*, 1958, No. 17; *Wld Hlth Org. techn. Rep. Ser.*, 1958, 144.

regarded as acceptable on toxicological grounds. As already stated, many countries have special problems, different patterns of dietary intake, and special groups of the population that may require consideration. The Committee therefore considered that it would be helpful to include a conditional acceptable intake zone in many instances. A final decision on whether intakes that fall within this range may be considered acceptable in particular circumstances should be taken by a group of scientists experienced in this field, including a toxicologist. It is expected that the scientific group consulted will examine the proposition in relation to the special needs of the country, the population groups concerned, the foods to be treated, the levels and patterns of consumption of these foods and other relevant considerations. The necessary control, such as limitation to specified use or uses, special labelling or other requirements, can then be exercised.

Wherever possible the Committee estimated for each compound both unconditional and conditional acceptable daily intake zones for man, as shown in the table on p. 12.

Several substances in the table occur naturally and in many instances the top level of acceptability shown in the table has not been determined by toxicological considerations. The Committee felt that it was desirable to propose finite acceptability zones, even for substances that occur abundantly in nature. The levels proposed in the table are in some cases arbitrary limits, but they should allow the substances to be used effectively as food additives.

In the case of butylated hydroxytoluene and of formic acid and formates the Committee was not prepared to propose an unconditional acceptable intake zone, as they did not feel that they had sufficient scientific evidence to enable them to make a toxicological evaluation. For these substances only a conditional acceptable intake is given, and it is proposed that they be used under scientific supervision. For nordihydroguaiaretic acid it was considered by the Committee that, on the data available, it was not possible to establish any estimate of an acceptable daily intake for man. The Committee wished to make it clear, however, that this does not necessarily imply that this substance is toxic or undesirable as a food additive. Indeed, it has been in use as a food additive for a considerable time. It may have been judged to be acceptable for use in some countries because of unpublished evidence not available to this Committee or because it meets some special need in the country concerned.

Finally, the Committee decided that the use of boric acid, borax, hexamethylenetetramine, and salicylic acid as food additives should be objected to on toxicological grounds. In this case, the words "not considered suitable for use as a food additive" have been inserted in the table as a footnote, the grounds for the decision being given in the monograph. The Committee wished to point out, however, that although the use of

ACCEPTABLE DAILY INTAKE ZONES FOR MAN  
IN MG/KG BODY WEIGHT

Compounds considered	Unconditional	Conditional
Ascorbic acid <sup>1</sup> . . . . .	0-2.5	2.5-7.5
Sodium ascorbate <sup>1</sup> . . . . .	0-2.5	2.5-7.5
Isoascorbic acid . . . . .	0-2.5	2.5-7.5
Sodium isoascorbate . . . . .	0-2.5	2.5-7.5
Ascorbyl palmitate . . . . .	0-0.25	0.25-0.5
Benzoic acid <sup>1</sup> . . . . .	0-5	5-10
Methyl <i>p</i> -hydroxybenzoate . . . . .	0-2	2-7
Ethyl <i>p</i> -hydroxybenzoate . . . . .	0-2	2-7
Propyl <i>p</i> -hydroxybenzoate . . . . .	0-2	2-7
Boric acid <sup>3</sup> . . . . .	—	—
Borax <sup>3</sup> . . . . .	—	—
Butylated hydroxyanisole . . . . .	0-0.5	0.5-2
Butylated hydroxytoluene . . . . .	—	0-0.5
Citric acid <sup>1</sup> . . . . .	0-60	60-120
Isopropyl citrate mixture . . . . .	0-7	7-20
Sodium diacetate . . . . .	0-15	15-30
Diphenyl . . . . .	0-0.05	0.05-0.25
Formic acid <sup>1</sup> . . . . .	—	0-5
Propyl gallate . . . . .	0-0.2	0.2-0.5
Octyl gallate . . . . .	0-0.2	0.2-0.5
Dodecyl gallate . . . . .	0-0.2	0.2-0.5
Gum guaiac . . . . .	0-2	2-4
Hexamethylenetetramine <sup>3</sup> . . . . .	—	—
Nitrates of sodium and potassium <sup>1</sup> . . . . .	0-5	5-10
Nitrites of sodium and potassium <sup>1</sup> . . . . .	0-0.4	0.4-0.8
Nordihydroguaiaretic acid <sup>2</sup> . . . . .	—	—
Ortho-phenylphenol . . . . .	0-0.2	0.2-1
Sodium ortho-phenylphenol . . . . .	0-0.2	0.2-1
Phosphoric acid <sup>1</sup> . . . . .	0-5	5-15
Propionates of sodium, potassium and calcium . . . . .	0-10	10-20
Salicylic acid <sup>3</sup> . . . . .	—	—
Sorbic acid <sup>1</sup> . . . . .	0-12.5	12.5-25
Sulfur dioxide . . . . .	0-0.35	0.35-1.5
Sodium sulfites (calculated as SO <sub>2</sub> ) . . . . .	0-0.35	0.35-1.5
Sodium metabisulfite (calculated as SO <sub>2</sub> ) . . . . .	0-0.35	0.35-1.5
Sodium hydrogen sulfite (calculated as SO <sub>2</sub> ) . . . . .	0-0.35	0.35-1.5
Tartaric acid <sup>1</sup> . . . . .	0-3	3-10
Thiodipropionic acid . . . . .	0-3	3-15
Dilauryl thiodipropionate . . . . .	0-3	3-15
Distearyl thiodipropionate . . . . .	0-3	3-15
Tocopherols <sup>1</sup> . . . . .	0-1	1-2

<sup>1</sup> Naturally occurring substances; the estimated acceptable intakes listed above do not include the amounts occurring naturally.

<sup>2</sup> Available scientific evidence inadequate for evaluation.

<sup>3</sup> Not considered suitable for use as a food additive.

these substances should be discontinued as soon as possible, danger to health might occur if this were done before suitable and economic alternatives can be made available.

In all cases where the available scientific information was inadequate the Committee has indicated some further studies that might be carried out.

### NOTE ON THE USE OF THE MONOGRAPHS

It cannot be too strongly emphasized that food additives should only be used when necessary and the level of use should not exceed the lowest levels that are certain to be effective in good technological practice. The considerations required to establish the acceptable human intakes set out in these monographs are different in certain ways from those needed by the food legislator or administrator at national level in deciding upon the tolerance level of a particular food additive with, perhaps, some particular use in mind.

The following procedure is one way in which the information given in this report may be effectively used ; it is set out in detail for the benefit of those who may not be particularly familiar with these problems.

1. Decide upon the effective level of the food additive under consideration that would be needed in good technological practice.
2. Examine the possible uses and list all the foods in which the food additive might be used.
3. Calculate the daily intake level that might occur if the food additive was used in all the foods for which it might be a useful additive, working on the basis of the average intake of the food materials containing the additive. This average intake for appropriate population groups<sup>1</sup> is obtained from national food consumption surveys.<sup>2</sup>
4. Obtain the necessary information from which to calculate the average body weight of the population group concerned (usually between 50 and 70 kg).
5. From this information calculate the intake of the additive in mg per kg of body weight per day.

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<sup>1</sup> For certain kinds of food, consideration should be given to relatively large variations in consumption between individuals or between special groups of the population. Such individuals or groups might be exposed to excessive amounts of the additive if the calculation is based on average levels derived from food consumption surveys. Examples of this are beverages and sweets, which may be consumed by children in much larger quantities than the average.

<sup>2</sup> In many countries, food intake information is inadequate at the present time.

6. Check this figure against the acceptable intakes given for the substance in the table. If it falls within the unconditional intake zone the situation is satisfactory and the level proposed may be accepted. If it falls within the conditional intake zone, further scientific advice is required before the level of use proposed is accepted.

7. The Committee does not advise the use of the food additives considered in amounts that exceed the acceptable intake zones put forward in this report.

#### *Example*

1. A substance X is proposed as a food additive in several foods at a level of treatment of 100 p.p.m. of the food as it is eaten.

2. The foods in which it might occur are listed and the amounts of these foods that would be eaten daily on the basis of national food consumption survey are calculated.

3. The total average intake of treated food of an average man is found to be 500 g a day. The daily intake of X is therefore estimated as 50 mg.

4. The body weight of an average man in the population under consideration is 70 kg.

5. Therefore the intake of X would be 0.7 mg/kg body weight per day.

6. From examination of the table on p. 12 the acceptable daily intakes for substance X are :

unconditional zone : 0.1 mg/kg body weight

conditional zone : 1-7.5 mg/kg body weight

Thus, the suggested use of substance X gives an intake in the unconditional zone. It is therefore acceptable without further advice. (Substance X is an imaginary substance and the figures given here are only illustrative.)

**The Committee wished to emphasize that opinions recorded in this report are based upon the scientific evidence collected by or made available to its members at the time of the meeting. Every effort was made to obtain all the information on each substance but it is almost inevitable that some published work has been missed. The Committee offered its apologies to any authors whose work may not have been taken into consideration ; information on such work should be forwarded either to the Nutrition Division of FAO or to Food Additives, Nutrition, WHO. In any case, it is certain that new work on many of the substances dealt with in the monographs will be published in the future and it may be necessary to modify the opinions in this report in the light of new knowledge.**

### GENERAL RECOMMENDATIONS TO FAO AND WHO

In conclusion, the Committee recommends the following further action by FAO and WHO :

1. FAO should extend the scope of the food balance sheets to give more details on food consumption in member countries, since this information is essential for the effective control of the use of food additives ; the governments of member countries should be strongly urged to give every assistance in this work.

2. FAO and WHO should use all means available to promote further research in the food additive field, more adequate training facilities for scientists engaged in assessing the toxicological status of food additives, and more complete publication of the results of investigations in this field ; they should also publish additional information as necessary to keep the existing reports of the joint FAO/WHO Expert Committee on Food Additives up to date.

3. A Joint FAO/WHO Expert Committee on Food Additives should be convened as soon as practicable to draw up specifications for emulsifiers, stabilizers and related substances used as food additives, and to evaluate the toxic hazards involved in their use.

4. FAO and WHO should give early consideration to the need for extending the work of the Joint FAO/WHO Expert Committee on Food Additives to other groups of food additives, such as antibiotics, other preservatives, and food colours.

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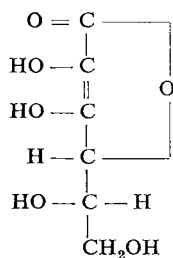
## MONOGRAPHS

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### ASCORBIC ACID \*

<b>Chemical names</b>	Ascorbic acid; L-ascorbic acid; 3-keto-L-gulofuranolactone
<b>Synonym</b>	Vitamin C
<b>Empirical formula</b>	$C_6H_8O_6$
<b>Structural formula</b>	

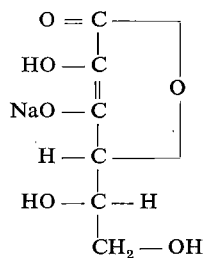


<b>Molecular weight</b>	176.13
<b>Definition</b>	Ascorbic acid, after drying over sulfuric acid for 24 hours, contains not less than 99% of $C_6H_8O_6$ .
<b>Description</b>	A white or almost white, odourless, crystalline solid. 1 g is soluble in 3.5 ml of water or 30 ml of ethanol. Insoluble in oils and fats.
<b>Natural occurrence</b>	Ascorbic acid is present in significant amounts in a number of fruits and vegetables. It also occurs in milk and in small quantities in certain other foods.
<b>Uses</b>	As an antioxidant in emulsions of fats and oils and as a browning inhibitor in unprocessed cut fruits, fruit pulps and juices.

### SODIUM ASCORBATE

<b>Chemical names</b>	Sodium ascorbate; sodium L-ascorbate; 3-keto-L-gulofuranolactone sodium enolate
<b>Empirical formula</b>	$C_6H_7O_6Na$

\* For biological data and toxicological evaluation see pp. 20-23.

**Structural formula****Molecular weight** 198.11**Definition** Sodium ascorbate, after drying over sulfuric acid for 24 hours, contains not less than 99% of  $\text{C}_6\text{H}_7\text{O}_6\text{Na}$ **Description** Sodium ascorbate is a white or almost white, odourless, crystalline solid. 1 g is soluble in 2 ml of water.**Uses** As an antioxidant in meat packing, the bottling industry, frozen fruit, and spice manufacturing.**Biological Data****Acute toxicity**

Animal	Route <sup>a</sup>	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	oral	> 5000	1
"	i.v.	> 1000	1
Rat	oral	> 5000	1
"	i.v.	> 1000	1
Guinea-pig	oral	> 5000	1
"	i.v.	> 500	1

**Short-term studies***Mouse*

Mice given ascorbic acid orally, subcutaneously and intravenously in daily doses of 500-1000 mg/kg body weight for 7 days, showed no difference in appetite, weight gain and general behaviour from controls receiving the same amount of the biologically inactive galacturonic acid. Histological examination of various organs showed no definite changes.<sup>1</sup>

*Guinea-pig*

Guinea-pigs given ascorbic acid orally, subcutaneously and intravenously in daily doses of 400-2500 mg/kg body weight for 6 days, showed

<sup>a</sup> The substance was administered using an aqueous solution which was neutralized with sodium hydroxide shortly before use.

no difference in appetite, weight gain and general behaviour from controls receiving the same amounts of the biologically inactive galacturonic acid. Histological examination of various organs showed no definite changes.<sup>1</sup>

#### *Man*

One woman and 3 men were each given 1000 mg of ascorbic acid a day for 3 months. The ascorbic acid levels in the serum and in the white blood cells and the urinary excretion of this acid did not show any progressive changes. Further, no harmful effects were observed in these four subjects during the three months.<sup>2</sup>

On the other hand, after a daily dose of 5 mg/kg for 3 days a significant increase in urinary volume was observed in 30 children (10 active rheumatic, 10 convalescent rheumatic patients and 10 controls).<sup>3</sup> This diuretic effect was confirmed in another study on 9 patients with heart failure, given 300 mg of ascorbic acid daily.<sup>4</sup>

Doses up to 6000 mg of ascorbic acid were given to 29 infants, 93 children of pre-school and school age, and 20 adults for more than 1400 days. With the higher doses, toxic manifestations were observed in 5 adults and 4 infants. The signs and symptoms in adults were nausea, vomiting, diarrhoea, flushing of the face, headache, fatigue and disturbed sleep. The main toxic reactions in the infants were skin rashes.<sup>5</sup>

#### **Long-term studies**

No information available.

#### **Biochemical aspects**

It is well established that ascorbic acid is readily absorbed and metabolized. However, after oral administration of large quantities only small amounts are excreted in the urine, while there is a steady rise in the level of ascorbic acid in the plasma. If the oral ingestion is continued for a sufficient period, the plasma concentration rises to a maximum, after which a rapid urinary excretion of a large part of the ingested ascorbic acid occurs.<sup>6</sup>

Using <sup>14</sup>C-labelled ascorbic acid, it was found that in the rat after intraperitoneal injection of 1.5-5.9 mg approximately 19-29% was converted to CO<sub>2</sub> and only 0.4% was excreted as oxalate within 24 hours.<sup>7</sup>

#### **Comments on experimental studies reported**

From the animal studies it is evident that ascorbic acid is not apparently toxic after a single or a few repeated administrations of relatively large doses. However, no long-term studies have been carried out in animals to substantiate its acceptability at high dose levels.

Studies in man indicate that ascorbic acid has a diuretic effect at 5 mg/kg body weight in children and adults.<sup>2</sup> Certain toxic manifestations were observed in infants and adults after doses of up to 6000 mg.<sup>4</sup> The significance of these findings is doubtful.

### Evaluation

#### Level causing no significant toxicological effect

A daily dose of 1000 mg taken by 4 human subjects over a period of 3 months was found to have no deleterious effect.<sup>5</sup> A dietary allowance of 30 mg of ascorbic acid was recommended by the League of Nations in 1938.<sup>8</sup> The same recommendation has been made by the Medical Research Council of Great Britain.<sup>9</sup>

The United States Food and Nutrition Board recommended 30 mg for infants, 75 mg for men, 70 mg for women and higher values during pregnancy and lactation. It is stated that these recommended allowances are so ample that they cover all needs of healthy individuals, even in cases where these are enhanced.<sup>10</sup> The minimum requirement of ascorbic acid in adults was estimated to be between 5 and 10 mg per day.<sup>11</sup> It is estimated that the daily intake of ascorbic acid is between 30-100 mg from natural sources. The usual therapeutic dose is 1000 mg. However, the safety of large doses of ascorbic acid over long periods of time has not been unequivocally demonstrated.

#### Estimate of acceptable daily intakes for man \*

	mg/kg body weight
Unconditional acceptance . . . . .	0-2.5
Conditional acceptance . . . . .	2.5-7.5

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\* The estimated daily intakes of ascorbic acid listed above do not include the ascorbic acid that is present naturally in foods.

**ISOASCORBIC ACID \***

<b>Chemical names</b>	Isoascorbic acid; D-isoascorbic acid; 3-keto-D-glucofuranolactone
<b>Synonym</b>	Erythorbic acid
<b>Empirical formula</b>	$C_6H_8O_6$
<b>Structural formula</b>	$  \begin{array}{c}  \text{O} = \text{C} \text{---} \text{O} \\    \qquad \qquad \qquad   \\  \text{HO} \text{---} \text{C} \qquad \qquad \text{C} \\     \qquad \qquad \qquad   \\  \text{HO} \text{---} \text{C} \qquad \qquad \text{C} \\    \qquad \qquad \qquad   \\  \text{H} \text{---} \text{C} \qquad \qquad \text{C} \\    \qquad \qquad \qquad   \\  \text{H} \text{---} \text{C} \text{---} \text{OH} \\    \\  \text{CH}_2 \text{---} \text{OH}  \end{array}  $
<b>Molecular weight</b>	176.13
<b>Definition</b>	Isoascorbic acid, after drying for 24 hours over sulfuric acid, should contain not less than 98% of $C_6H_8O_6$ .
<b>Description</b>	A white or almost white, crystalline solid. 1 g is soluble in 2.5 ml of water or 20 ml of ethanol.
<b>Uses</b>	As an antioxidant in emulsions of fats and oils and in meat and meat products, and as an inhibitor of enzymatic browning. It has no vitamin C potency.

**SODIUM ISOASCORBATE**

<b>Chemical names</b>	Sodium isoascorbate; sodium D-isoascorbate; 3-keto-D-glucofuranolactone sodium enolate monohydrate
<b>Synonym</b>	Sodium erythorbate
<b>Empirical formula</b>	$C_6H_7O_6Na \cdot H_2O$
<b>Structural formula</b>	$  \begin{array}{c}  \text{O} = \text{C} \text{---} \text{O} \cdot \text{H}_2\text{O} \\    \qquad \qquad \qquad   \\  \text{HO} \text{---} \text{C} \qquad \qquad \text{C} \\     \qquad \qquad \qquad   \\  \text{NaO} \text{---} \text{C} \qquad \qquad \text{C} \\    \qquad \qquad \qquad   \\  \text{H} \text{---} \text{C} \qquad \qquad \text{C} \\    \qquad \qquad \qquad   \\  \text{H} \text{---} \text{C} \text{---} \text{OH} \\    \\  \text{CH}_2 \text{---} \text{OH}  \end{array}  $

\* For biological data and toxicological evaluation see pp. 24-25.

<b>Molecular weight</b>	216.13
<b>Definition</b>	Sodium isoascorbate contains not less than 98% of $C_6H_7O_6Na \cdot H_2O$
<b>Description</b>	Sodium isoascorbate is a white, almost odourless crystalline solid. 1 g is soluble in 2 ml of water.

### Biological Data

#### Acute toxicity

No information available.

#### Short-term studies

##### *Rat*

Groups of 10 male rats were fed for 36 weeks on diets containing 1% of isoascorbic acid, and on diets without isoascorbic acid. There was no difference between the treated rats and the controls with respect to rate of growth and mortality. Gross post-mortem examination and microscopic studies of various organs revealed no lesion attributable to isoascorbic acid.<sup>1</sup>

#### Long-term studies

##### *Rat*

Groups of rats were fed on diets containing 1% of isoascorbic acid and diets without isoascorbic acid for 2 years. The growth rate, mortality, and histopathology were not affected by the treatment.<sup>2</sup>

#### Biochemical aspects

Isoascorbic acid is readily absorbed and metabolized. Following an oral dose of 500 mg of isoascorbic acid to human subjects the blood level curves for ascorbic acid and isoascorbic acid showed a similar rise. In 5 human subjects, an oral dose of 300 mg was shown to have no effect on the urinary excretion of ascorbic acid.<sup>3</sup> Isoascorbic acid was found to have no antagonistic effect on the action of ascorbic acid.<sup>4</sup>

#### Comments on experimental studies reported

The short-term and long-term studies in rats were thorough. However, no long-term studies have been carried out in other species. The biochemical studies in man indicate that isoascorbic acid is readily metabolized and does not affect the urinary excretion of ascorbic acid.

### Evaluation

#### Level causing no significant toxicological effect in the rat

1% (= 10 000 p.p.m.) in the diet, equivalent to 500 mg/kg body weight per day.

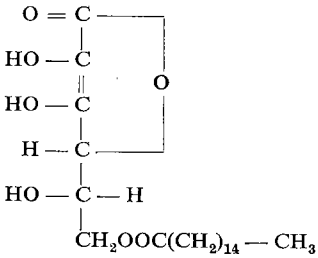
#### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-2.5
Conditional acceptance . . . . .	2.5-7.5

### References

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### ASCORBYL PALMITATE

<b>Chemical names</b>	Ascorbyl palmitate ; L-ascorbyl palmitate ; 6-palmitoyl-3-keto-L-gulofuranolactone
<b>Synonym</b>	Vitamin C palmitate
<b>Empirical formula</b>	$C_{22}H_{38}O_7$
<b>Structural formula</b>	 <p style="text-align: center;"> <math display="block">  \begin{array}{c}  \text{O} = \text{C} \text{---} \\    \\  \text{HO} \text{---} \text{C} \text{---} \\     \\  \text{HO} \text{---} \text{C} \text{---} \\    \\  \text{H} \text{---} \text{C} \text{---} \\    \\  \text{HO} \text{---} \text{C} \text{---} \text{H} \\    \\  \text{CH}_2\text{OOC}(\text{CH}_2)_{14} \text{---} \text{CH}_3  \end{array}  </math> </p>
<b>Molecular weight</b>	414.55
<b>Definition</b>	Ascorbyl palmitate contains not less than 98% of $C_{22}H_{38}O_7$
<b>Description</b>	A white or yellowish-white solid, with a citrus-like odour. 1 g is soluble in 4.5 ml of ethanol.
<b>Use</b>	As an antioxidant.

### Biological Data

#### Acute toxicity

No information available.

#### Short-term studies

##### *Rat*

Groups of 10 rats each were fed for 9 months on normal diets and diets containing 2% and 5% of ascorbyl palmitate. At the 5% level the growth rate was significantly retarded, and 2 of the 10 rats had numerous bladder stones and hyperplasia of the bladder epithelium. Another rat in this group showed an inflammatory condition in the kidney. There was a slight retardation of growth in the rats on the diet containing 2% of ascorbyl palmitate, but there were no significant differences between these rats and the controls in respect of mortality and histopathology.<sup>1</sup>

#### Long-term studies

##### *Rat*

Heat-treated lard containing 1% or 5% of L-ascorbyl palmitate (0.05% or 0.25% of the total diet) was fed for 2 years to groups of 10 rats each. No adverse effects were observed in any of the experimental animals as determined by growth rate, mortality and pathological examination.<sup>1</sup>

#### Biochemical aspects

No information available.

#### Comment on experimental studies reported

The significance of the damage to the urinary tract in rats on the diet containing 5% of ascorbyl palmitate is difficult to assess. However, at the 2% level, there was no sign of toxicity, except a slight retardation of growth, and there was no demonstrable deleterious effect at the 0.25% level.<sup>1</sup>

### Evaluation

#### Level causing no significant toxicological effect in the rat

0.25% (= 2500 p.p.m.) in diet, equivalent to 125 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.25
Conditional acceptance . . . . .	0.25-0.50

**Further work considered desirable**

1. Studies in other species of animals.
2. Biochemical studies in man and animals.

**Reference**


1. Fitzhugh, O. G. & Nelson, A. A. (1946) *Proc. Soc. exp. Biol.*, **61**, 195

**BENZOIC ACID \***

<b>Chemical names</b>	Benzoic acid ; benzenecarboxylic acid ; phenylformic acid
<b>Empirical formula</b>	$C_7H_6O_2$
<b>Structural formula</b>	$\begin{array}{c} \text{COOH} \\   \\ \text{C}_6\text{H}_5 \end{array}$
<b>Molecular weight</b>	122.12
<b>Definition</b>	Benzoic acid contains not less than 99.7% of $C_7H_6O_2$ .
<b>Description</b>	A white crystalline solid. It may have not more than a faint characteristic odour. 1 g is soluble in 350 ml of water or 3 ml of ethanol. Soluble in volatile and fixed oils.
<b>Natural occurrence</b>	Most berries contain appreciable amounts (approx. 0.05%).
<b>Use</b>	As a preservative. Its efficiency is increased in acid media and it is more effective against yeasts than moulds. It is usually combined with sodium benzoate.

\* For biological data and toxicological evaluation see pp. 28-30.

**SODIUM BENZOATE**

<b>Chemical names</b>	Sodium benzoate ; sodium salt of benzenecarboxylic acid ; sodium salt of phenylformic acid
<b>Empirical formula</b>	$C_7H_5O_2Na$
<b>Structural formula</b>	<div style="text-align: center;"> <math>COONa</math>   </div>
<b>Molecular weight</b>	144.11
<b>Definition</b>	Sodium benzoate, after drying for 4 hours at 105°C, contains not less than 99% of $C_7H_5O_2Na$ .
<b>Description</b>	Sodium benzoate is a white, almost colourless, crystalline solid. 1 g is soluble in 2 ml of water or 90 ml of ethanol.
<b>Use</b>	As an antimicrobial preservative.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	References
Rat	oral	2700	1
Rabbit	oral	2000	2
Dog	oral	2000	2

**Short-term studies****Rat**

Groups of 10 rats (5 male and 5 female) were fed sodium benzoate for 30 days at levels ranging from 16 to 1090 mg/kg body weight. There was no observable effect on body weight, appetite or mortality, nor any micro-pathological changes in the organs.<sup>3</sup>

Ninety-day feeding tests were carried out on groups of 8 or 10 rats on diets containing 1%, 2%, 4% and 8% of sodium benzoate. In the group on the 8% diet there were 4 deaths (average number of days to death, 13); the gain in weight of the 4 survivors was two-thirds of that of the control rats on an identical food intake. Kidney and liver weights were significantly higher than those of the control group. At the lower levels no demonstrable effect was observed.<sup>1</sup>

*Dog*

Feeding tests on 17 dogs over 250 days with sodium benzoate or benzoic acid at the rate of 1g/kg body weight had no effect on growth, appetite and well-being. Above this level ataxia, epileptic convulsions and death occurred.<sup>4</sup>

*Man*

Nine patients receiving penicillin treatment were given 1200 mg of benzoic acid divided into 8 doses over a period of 5 days in 8 of the subjects and 14 days in one case. No effect was observed; in particular, in no case did the endogenous creatinine clearance show significant changes, nor did routine urine analyses show any abnormality.<sup>5</sup>

**Long-term studies***Rat*

Experiments were carried out over 4 generations, two of which were fed benzoic acid for the whole of the life span. There were 40 animals in each group and the diets contained 0.5% and 1% benzoic acid. No harmful effects were observed on growth, fertility, life span, or mortality. No abnormalities were detectable post-mortem.<sup>6</sup>

**Biochemical aspects**

Benzoic acid is rapidly and completely excreted in the urine.<sup>7</sup> Cumulation does not occur, as shown by experiments on the distribution and elimination of <sup>14</sup>C-benzoic acid in the rat.<sup>8</sup>

Two urinary metabolites of benzoic acid are known, namely hippuric acid and benzoyl glucuronic acid. In man, rat and rabbit, benzoic acid is almost entirely excreted as hippuric acid, whereas dogs excrete more conjugated glucuronic acid than hippuric acid.<sup>9</sup>

Normal urinary excretion of hippuric acid in man may be 1-2.5 g/day, which is equivalent to 0.7-1.7 g of benzoic acid.<sup>10</sup> The maximum rate of the hippuric acid excretion after ingestion of benzoic acid was observed to be about 17 mg per min. (24 g per day) calculated as benzoic acid.<sup>7</sup>

**Comment on experimental studies reported**

The metabolic data on benzoic acid and sodium benzoate in experimental animals and man are very good. The rat seems to be close to man so far as metabolism of these compounds is concerned.

**Evaluation****Level causing no significant toxicological effect in the rat**

1% (= 10 000 p.p.m.) in the diet, equivalent to 500 mg/kg body weight per day.

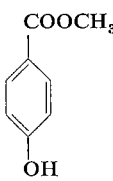
## Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-5
Conditional acceptance . . . . .	5-10

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METHYL *p*-HYDROXYBENZOATE

<b>Chemical names</b>	Methyl <i>p</i> -hydroxybenzoate ; methyl ester of <i>p</i> -hydroxybenzoic acid
<b>Synonyms</b>	Methyl <i>p</i> -oxybenzoate ; methylparaben
<b>Empirical formula</b>	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>
<b>Structural formula</b>	
<b>Molecular weight</b>	152.15
<b>Definition</b>	Methyl <i>p</i> -hydroxybenzoate, after drying for 2 hours at 80°C, contains not less than 99% of C <sub>8</sub> H <sub>8</sub> O <sub>3</sub> .
<b>Description</b>	Methyl <i>p</i> -hydroxybenzoate is a white, almost odourless, crystalline solid. 1 g is soluble in 400 ml of water or 3.5 ml of ethanol.
<b>Use</b>	As a preservative.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)		Reference
Mouse	oral	free acid :	> 8000	1
		Na salt :	2000	1
	i.p.	free acid :	960	1
		Na salt :	760	1
	i.v.	Na salt :	170	1

Oral doses of 3000 mg/kg body weight are reported to be lethal in the dog and rabbit, and doses of 2000 mg/kg body weight caused harmful effects.<sup>2</sup>

#### Short-term studies

##### *Guinea-pigs*

Daily doses of 11-100 mg for 120 days showed no effect.<sup>3</sup> Forty animals were given intradermal injections of a 0.1% solution in physiological saline, 3 times weekly up to 10 injections. There was no sensitivity reaction 2 weeks later.<sup>1</sup>

##### *Rat*

0.5-5.0 mg daily for 80 days had no effect. The blood picture was not influenced.<sup>3</sup>

##### *Dog*

Three mongrel puppies were fed 1000 mg/kg body weight daily and 2 were fed 500 mg/kg daily for 6 days a week. Those in the lower dose group were on test for 313 days, and those on higher dose levels for periods longer than a year. At the end of the experimental period all the animals appeared in excellent condition with reasonable gains in weight. One animal receiving 500 mg/kg produced a normal litter of puppies near the end of the experimental period. At a late stage of the experiment, blood samples were analysed for presence of the drug and for metabolic end-products, but there was no evidence of cumulation. Blood counts and urine examination were normal. All dogs were killed and autopsied; no abnormalities were found on microscopic or macroscopic examination of the organs.<sup>1</sup>

##### *Man*

Solutions in propylene glycol, in concentrations up to 5%, were applied to the skin of 50 human subjects for 4 to 8 hours every other day up to 10 applications without evidence of irritation. Higher concentrations than this produced some irritation. There was no evidence of development of

sensitization.<sup>1</sup> A local anaesthetic effect on the buccal mucosa has been reported after ingestion of a 0.1% aqueous solution.<sup>4</sup>

### Long-term studies

#### *Rats*

Groups of 12 male and 12 female rats received 2% and 8% methyl *p*-hydroxybenzoate in the diet respectively and were compared with equal numbers of control animals over a period of 96 weeks. At the 2% level, the animals did not show significant differences from the controls, but at the 8% level there was a reduction in growth rate during the earlier part of the experiment, with a tendency to return to normal later. Food intake remained fairly constant throughout the experiment. All animals dying during the course of the experiment or killed at the end were autopsied and examined macroscopically and microscopically; no significant changes were found in the organs.<sup>1</sup>

### Biochemical aspects

Chemical methods are available for the determination of *p*-hydroxybenzoic acid and its methyl ester in tissues and body fluids. Dogs were given either the free acid or the methyl ester in doses of 1000 mg/kg body weight orally or 50 mg/kg body weight intravenously and excretion in the urine was measured. The total material recovered (i.e., *p*-hydroxybenzoic acid, *p*-hydroxybenzoates and metabolic conjugates) represented from 60% to 95% of that ingested. Plasma ester concentrations rarely reached measurable levels, but high plasma levels and high urinary output of *p*-hydroxybenzoic acid and conjugated products indicated hydrolysis of the ester linkage. Enzymatic hydrolysis of the ester was demonstrated *in vitro* using preparations of liver and kidney. Studies on one man given 70 mg/kg orally suggested that metabolism in man is similar to that in the dog.<sup>5</sup>

### Comment on experimental studies reported

The long-term toxicity studies in rats<sup>1</sup> are adequate for an assessment when taken in conjunction with the evidence from the feeding experiments lasting for a year with dogs.<sup>5</sup> The metabolic studies are less extensive, particularly in man; extrapolation from rat to man must therefore be made with caution. The local anaesthetic action of *p*-hydroxybenzoic acid esters should be emphasized.

### Evaluation

#### Level causing no significant toxicological effect in the rat

2% (= 20 000 p.p.m.) in the diet, equivalent to 1000 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-2
Conditional acceptance . . . . .	2-7

**Comment**

Attention should be paid to a possible local anaesthetic effect in the buccal mucosa if this preservative is to be used.


**Further work considered desirable**

1. Biochemical studies in animals and man.
2. Studies of the local anaesthetic action using different vehicles.

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1. Matthew, C., Davidson, J., Bauer, E., Morrison, J. L. & Richardson, A. P. (1956) *J. Amer. pharm. Ass., Sci. Ed.*, **45**, 260
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**ETHYL *p*-HYDROXYBENZOATE**

<b>Chemical name</b>	Ethyl <i>p</i> -hydroxybenzoate ; ethyl ester of <i>p</i> -hydroxybenzoic acid
<b>Synonyms</b>	Ethyl <i>p</i> -oxybenzoate ; ethylparaben
<b>Empirical formula</b>	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>
<b>Structural formula</b>	$\text{COOCH}_2\text{CH}_3$ 
<b>Molecular weight</b>	166.18
<b>Definition</b>	Ethyl <i>p</i> -hydroxybenzoate, after drying for 2 hours at 80°C, contains not less than 99% of C <sub>9</sub> H <sub>10</sub> O <sub>3</sub> .
<b>Description</b>	A white, almost odourless, crystalline solid. 1 g is soluble in 2 ml of ethanol.
<b>Use</b>	As an antimicrobial preservative.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	oral	Na salt : approx. 2500	1
"	i.p.	Na salt : 520	1

Doses of 5 g/kg were lethal in dogs, and rabbits and 4 g/kg caused harmful effects.<sup>2</sup>

**Short-term studies***Rat*

Feeding experiments similar to those reported under "Long-term studies" for the methyl and propyl esters were done at the 2% and 8% levels, but the duration was only 12 weeks. Concentrations of 2% were without effect, but there was reduced growth rate and evidence of toxicity at 8%.<sup>1</sup>

*Guinea pigs*

Similar experiments on skin sensitivity to those done with the methyl ester revealed no skin sensitivity.<sup>1</sup>

*Man*

Tests for skin irritation and sensitivity were made similar to those done with the methyl ester. At a concentration of 7%, no evidence of irritation or sensitivity was observed, but irritation occurred at higher concentrations.<sup>1</sup> Ingestion of 0.05% aqueous solutions caused local anaesthesia of the buccal mucosa.<sup>3</sup>

**Long-term studies**

No information available.

**Biochemical aspects**

Similar metabolic experiments in the dog to those described with the methyl ester have been reported.<sup>4</sup> The results were essentially similar except that detectable but very low levels of the ethyl ester were found in the plasma 2 hours after oral administration, and at 5 and 15 minutes after intravenous administration. No human metabolic studies are available.

**Comments on experimental studies reported**

The feeding experiments with ethyl *p*-hydroxybenzoate reported above are less extensive than those with the methyl and propyl esters. Recent information, however, supports the conclusion that this ester is no more toxic than the others, and that the acceptable daily intakes may be similarly evaluated.<sup>5</sup>


**Evaluation**

As for methyl *p*-hydroxybenzoate.

**References**

1. Mathews, C., Davidson, J., Bauer, E., Morrison, J. L. & Richardson, A. P. (1956) *J. Amer. pharm. Ass., sci. Ed.*, **45**, 260
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**PROPYL *p*-HYDROXYBENZOATE**

<b>Chemical names</b>	Propyl <i>p</i> -hydroxybenzoate; <i>n</i> -propyl ester of <i>p</i> -hydroxybenzoic acid
<b>Synonym</b>	Propylparaben
<b>Empirical formula</b>	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>
<b>Structural formula</b>	$\text{COO} - \text{CH}_2 - \text{CH}_2 - \text{CH}_3$ 
<b>Molecular weight</b>	180.21
<b>Definition</b>	Propyl <i>p</i> -hydroxybenzoate, after drying for 2 hours at 80°C, contains not less than 99.5% of C <sub>10</sub> H <sub>12</sub> O <sub>3</sub> .
<b>Description</b>	Propyl <i>p</i> -hydroxybenzoate is a white, almost odourless, crystalline solid. 1 g is soluble in 2 ml of ethanol.
<b>Use</b>	As an antimicrobial preservative.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	oral	free acid : > 8000	1
"	"	Na salt : 3700	1
"	i.p.	free acid : 640	1
"	"	Na salt : 490	1
"	i.v.	Na salt : 180	1

Doses of 6 g/kg were lethal in dogs and rabbits, and 3-4 g/kg caused harmful effects.<sup>2</sup>

**Short-term studies***Guinea pig*

Similar experiments on skin sensitivity to those done with the methyl ester revealed no skin sensitivity.<sup>1</sup>

*Dogs*

Dogs were fed propyl *p*-hydroxybenzoate at the rate of 1000 mg/kg body weight daily for one year, as with the methyl ester, and were investigated in the same way. No harmful effects were found.<sup>1</sup>

*Man*

Tests for skin irritation and sensitivity were made similar to those done with the methyl ester. At a concentration of 12%, no evidence of irritation or sensitivity was observed, but irritation occurred at higher concentrations.<sup>1</sup> Ingestion of 0.03% aqueous solutions caused local anaesthesia of the buccal mucosa.<sup>3</sup>

**Long-term studies***Rat*

Experiments were done with groups of 12 animals, similar to those done with the methyl ester. No harmful effect was observed with a diet containing 2% of propyl *p*-hydroxybenzoate, but the growth rate was depressed at the 8% level. The feeding lasted for 96 weeks. There were no significant pathological findings.<sup>1</sup>

**Biochemical aspects**

Similar metabolic experiments in the dog to those described with the methyl ester have been reported, with essentially similar results.<sup>4</sup> No metabolic studies in man are available.

**Comment on experimental studies reported**

Similar comments to those made for the methyl ester also apply here. The rat and dog feeding data are essentially similar, but the metabolic data are less complete, since no studies have been done in man. It must be noted that the local anaesthetic action of the aqueous solution is present at a lower concentration (0.03%) than with the methyl ester.

**Evaluation**

As for methyl *p*-hydroxybenzoate.

**References**

1. Mathews, C., Davison, J., Bauer, E., Morrison, J. L. & Richardson, A. P. (1956) *J. Amer. pharm. Ass., sci. Ed.*, **45**, 260.
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**BORIC ACID \***

<b>Chemical names</b>	Boric acid ; boracic acid ; orthoboric acid
<b>Empirical formula</b>	$\text{H}_3\text{BO}_3$
<b>Molecular weight</b>	61.8
<b>Description</b>	Boric acid is a white or colourless, odourless solid with a slightly acid and bitter taste. It is soluble in water and ethanol.
<b>Use</b>	As a preservative, particularly for fish and crustaceans.

**BORAX**

<b>Chemical names</b>	Sodium baborate ; sodium tetraborate ; sodium pyroborate
<b>Empirical formula</b>	$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{H}_2\text{O}$
<b>Molecular weight</b>	381.4
<b>Description</b>	Hard, transparent, colourless, odourless crystals or white granules, efflorescent in dry air. Freely soluble in boiling water.
<b>Uses</b>	As an antimicrobial preservative, either alone or in combination with other substances ; antiseptic.

\* For biological data and toxicological evaluation see pp. 38-41.

**Biological Data****Acute toxicity (*boric acid*)**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	oral	3450	1
"	s.c.	2070	1
"	i.v.	1780	1
Rat	oral	2660	1
"	i.v.	1330	1
Guinea pig	s.c.	1220	1

**Short-term studies***Rat*

Groups of 20-24 immature rats received varying concentrations of boric acid in drinking water for 30 days. After 20-30 days a definite inhibition of growth occurred at a concentration of 0.25% (about 330 mg/kg per rat per day). There were no significant pathological changes in the blood and no characteristic lesions of the organs examined.<sup>1</sup>

Four groups of 12 weanling male rats were fed on a commercial diet containing 19 p.p.m. of boron. Boric acid was added to give 3 higher levels of 73, 104 and 198 p.p.m. of boron. Seven rats were killed at the start to give data on initial boron content. Six rats in each group were sacrificed after 4 weeks and 6 after 8 weeks. Boron added to the diet significantly decreased the growth of young rats.<sup>2</sup>

Twelve weanling rats, weighing 49 g each were injected subcutaneously each day for 30 days with 2 ml of a solution containing 1.5% of boric acid (equal to 30 mg) and 0.1% riboflavin. No significant differences in haemoglobin levels, erythrocyte or leucocyte counts were found. No gross pathological change or irritation at the injection site was observed. The heart, adrenals, kidney and testes were normal. The liver showed moderate cloudy swelling and some fatty infiltration but no necrosis.<sup>3</sup>

In a two-generation study, 14 rats were injected subcutaneously 3 times a week with a boric acid solution providing the equivalent of 0.15 g boric acid per day. Rats were mated at 75 days. Rate of growth, reproduction, average number of offspring in litters, and survival of young were about the same as in control rats.<sup>3</sup>

*Cat*

Six male cats were fed for 133 days on borated diets at six different levels, ranging from 0.54 to 0.86 g borax per day per animal. Autopsy showed kidney lesions of varying intensity in the 5 animals given more than 0.54 g of borax per day.<sup>4</sup>

*Dog*

Three adult dogs were given intravenous injections of 38-50 mg/kg body weight of boric acid each day for 30 days. There was no significant change in weight or blood picture during the experiment. At autopsy the livers showed a moderate cloudy swelling. The kidney of one dog showed some exudate in the glomerular spaces and the tubules, with some degenerative changes in a few glomeruli. The kidney of another dog showed mild hyperaemia.<sup>3</sup>

Four dogs given subcutaneous injections of 100 ml/kg body weight of boric acid in a 5% solution twice daily for 45 days showed no significant haematological changes.<sup>1</sup>

Five young dogs were given intramuscular injections of 1.5 mg/kg body weight of boric acid 3 times a week for 12-18 months. There was no irritation or itching, and no difference was observed in growth or in general behaviour between test dogs and control dogs.<sup>3</sup>

*Man*

The following instances of boric acid poisoning have been chosen to illustrate the main features.

*Infants and children.* Six infants were given by mistake 60-150 ml of a boric acid solution, the amounts being equivalent to 1-2 g/kg body weight. All cases proved fatal. The highest concentrations of boric acid were found in the brain and liver. The heart, lungs, diaphragm, stomach, kidney and bowel contained smaller amounts.<sup>5</sup>

Four infants died after receiving 10-13 g of boric acid in the form of a 5% solution over a period of 4 days. At autopsy, all infants showed a more or less extensive generalized exfoliative dermatitis. There was extensive haemorrhagic oesophagitis with necrosis and sloughing of large patches of epithelium.<sup>6</sup>

A baby 11 days old was given in error 85 ml of a 5% boric acid solution orally in 2 doses (total of 4.25 g boric acid). The baby died after 3 days.<sup>7</sup>

Six babies (6-11 days old) died after a single feed with milk containing 1.25% of boric acid. The amount of boric acid ingested must have been 1-2 g, assuming the quantity of milk per feed to have been 50-100 ml. Deaths occurred 19 hours to 5 days after ingestion of the milk.<sup>8</sup>

*Adults.* Twelve young males ate various amounts of boric acid and borax for periods of 30 to 70 days. After an intake of 4-5 g of boric acid per day, loss of appetite and general malaise were noticed. After an intake of 3 g per day, the same symptoms occurred but less quickly. An intake of 0.5 g per day for 50 days had a similar adverse effect.<sup>9</sup>

**Biochemical aspects**

Boric acid is absorbed from unbroken skin after fairly prolonged contact, and is readily taken up from skin affected by eczema, superficial ulcers or wounds. It is generally slowly excreted by the kidneys. Cumulation apparently occurs and there is some evidence from published records that cumulation in the central nervous system may be an important feature. In sufficiently high concentration, borate ions inhibit oxygen uptake,<sup>10</sup> ammonia formation and glutamine synthesis in brain tissue.<sup>11</sup> On the other hand, it is well known that boric acid forms complexes with polyhydroxy compounds and if present in sufficient concentration inhibits the oxidation of adrenaline by this mechanism; this action may be responsible, at least in part, for the toxic effects of boric acid.<sup>12</sup>

**Comment on experimental studies reported**

The main evidence on which to base an evaluation of toxicity is from studies in man, both infants and adults.

Acute and short-term studies in animals of several species have yielded a fair amount of toxicological data, but in most cases they have not been conducted in accordance with recently recommended procedures for toxicological studies. No long-term studies have been made.

The main pathological actions of boric acid appear to be irritation of the gut and skin, and kidney damage. Growth inhibition seems to be the earliest sign of intoxication in chronic tests.

**Evaluation**

No information is available on long-term studies either in animals or man. There are some indications that boric acid and related substances tend to cumulate; one of the sites of cumulation may be the central nervous system. It is apparent that large doses of boric acid are highly toxic and that infants are particularly vulnerable. It is recognised that boric acid and related substances have been used as food additives for a considerable period and that no-one has presented evidence of toxic effects resulting from their use for this purpose. In the absence of long-term studies and in view of the possibility of cumulation, it is not possible to establish the level that does not cause any significant toxicological effect in an experimental animal. It is equally impossible to make use of the human data since these are derived from acute poisoning. If cumulation does occur, boric acid must be considered unsuitable for use as a food additive. If it can be shown that no cumulation occurs, further evidence based on long-term studies will be required before it is possible for the non-toxic dose to be satisfactorily determined.

### Further work considered desirable

If the use of boric acid as a food additive is continued, long-term studies should be made, with particular attention to the possibility of cumulation.

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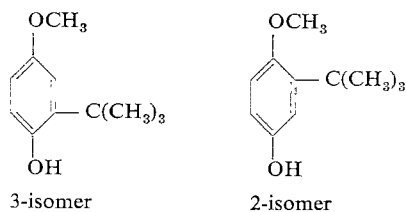
### BUTYLATED HYDROXYANISOLE

**Chemical name** A mixture of 3- and 2-*tert.*-butyl-4-hydroxyanisole

**Synonym** BHA

**Empirical formula**  $C_{11}H_{16}O_2$

**Structural formula**



**Molecular weight** 180.25

**Definition** Butylated hydroxyanisole is a mixture of 85% or more of 3-*tert.*-butyl-hydroxyanisole and 15% or less of 2-*tert.*-butyl-hydroxyanisole<sup>1</sup> and should contain not less than 98.5% of  $C_{11}H_{16}O_2$ .

<b>Description</b>	A white or slightly yellow, waxy crystalline solid with an aromatic odour. Insoluble in water but soluble in fats. 1 g is soluble in 4 ml of ethanol.
<b>Use</b>	As an antioxidant for fats and oils and in packaging material for fat-containing foods; its activity is enhanced in combination with other phenolic antioxidants and synergists.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	References
Mouse	oral	2000	2,3
Rat	oral	2200 to > 5000	2,3

#### Short-term studies

##### Rat

No effect on potassium excretion, as described below for the rabbit, was observed in the rat.<sup>4</sup>

Groups of 7 recently weaned rats were fed for six months on rations containing 0%, 0.5%, 1%, 2% and 3% of BHA. The rats at the 3% level did not eat enough to gain weight and were put on to the 2% diet for a time, then returned to 3%. Even at the 2% level, food consumption was not optimal. Histopathological examination revealed no pathological condition attributable to BHA.<sup>5</sup>

Combinations of BHA with other food additives, such as chlorine dioxide, sodium propionate, propyl gallate, or polyoxyethylene-8-stearate, at fifty times the normal levels of use in bread, had no deleterious effects when they were fed in bread to groups of 26 rats for a period of 32 weeks. The treated bread formed 75% of the animals' diet. The daily dosage levels of BHA were from 3.3-7.0 mg/kg body weight.<sup>6, 7</sup>

##### Rabbit

In rabbits, a dose of 1 g given daily for 5 to 6 days by stomach tube caused a tenfold increase in sodium excretion and a 20% increase in potassium excretion in the urine. Extracellular fluid volume fell, and this prevented any marked change in the plasma sodium level. The serum potassium fell after 5 days' treatment and potassium was being replaced by sodium in muscle cells. In heart muscle the changes occurred later than in skeletal muscle and were less marked. The antioxidant may have a direct effect on the kidney; the adrenal cortex showed changes in the *zona glomerulosa* and there was increased excretion of aldosterone in the urine, associated with the sodium and potassium loss.<sup>8</sup>

*Dog*

When BHA was fed to dogs at dose levels of 0, 0.3, 30 and 100 mg/kg body weight for one year, no ill effects were observed. Renal function, haematology and histopathology of the main tissues were normal. Organ weights were within normal limits and there was no demonstrable storage of BHA. The urine did not contain a demonstrable increase of reducing substances, even when 100 mg/kg body weight of BHA was fed. Groups of 3 dogs were used at each dose level for these experiments.<sup>9</sup>

**Long-term studies***Rat*

Groups of 15 or more newly weaned rats were placed on diets containing 0%, 0.05%, 0.5% and 1% BHA in lard (0%, 0.003%, 0.03% and 0.06% of the total diet) for 22 months. Weight gain was comparable in all groups. Reproduction was normal, and young rats kept on the same ration grew normally. Number, size, weight, weight gain and mortality of the litters were comparable for animals of all groups. After one year on test, the colony suffered from an infectious respiratory disease and many died. There was no significant difference in mortality among the groups. After 22 months, the remaining animals were killed; histopathological examination revealed no changes attributable to the antioxidant.<sup>5</sup>

A similar series of tests was undertaken, with an additional group on a diet of 2% BHA in lard (0.12% of the total diet). There were 17 rats in each group. After 21 months, the survivors were killed. Histopathological examination revealed no significant differences compared with the control animals. The rate of gain in weight during the growing period was unchanged, and all rats appeared normal in every respect.<sup>5</sup>

In another rat feeding test carried out over a period of 2 years on groups of 40 rats, there was a small reduction in the mature weight and an increase in relative liver weight in some cases with the highest level of BHA used (0.5% of the diet), but there were no effects on any of the following: the reproductive cycle; histology of the spleen, kidney, liver, or skin; ratio of weight of heart, spleen, or kidneys to total body weight; mortality. The toxicity of BHA was not affected by the dietary fat load.<sup>10</sup>

**Biochemical aspects**

BHA was absorbed from the gastrointestinal tract, and there was some evidence that the feeding of amounts 100-500 times the levels generally permitted in fats for human consumption (in the USA 200 mg/kg fat) caused deposition in depot fat, the stability of which was thereby increased.<sup>11</sup> However, there was no evidence of cumulation in other tissues.<sup>1, 2, 9</sup> In the rabbit, BHA was conjugated mainly with glucuronic acid or sulfuric

acid;<sup>12</sup> a small amount of unchanged BHA was excreted in the urine. In rats the 2-*tert.*-butyl isomer was chiefly excreted as glucuronide, while the 3-*tert.*-butyl isomer was excreted mainly as ethereal sulfate.<sup>1</sup> Thus, these animals effectively detoxicated BHA. The changes described occurred in the liver. No evidence has been found to suggest that BHA produces any adverse biochemical or metabolic effect in the animal body.<sup>4</sup>

#### Comment on experimental studies reported

Most of the studies reported have been carried out in rats and there is little work on other species. Negative results in groups of 3 dogs<sup>9</sup> fed for one year at each dose level are of limited use for evaluation. The metabolic studies have been done mainly in rabbits; differences between the rabbit and rat have been reported.

Long-term studies in rats have been carried out by several groups of workers. Some reservations can be made about each of these studies. However, taken together, the two long-term studies in rats<sup>5, 10</sup> provide a basis for evaluation. It is presumed that man metabolizes butylated hydroxyanisole similarly to the rat.

#### Evaluation

##### Level causing no significant toxicological effect in the rat

From consideration of the long-term studies in rats, the level causing no toxicological effect on feeding over a period approximating to the life span is 0.5% of the diet. An inconstant effect on relative liver weight and body weight gain in one series is disregarded.

0.5% (=5000 p.p.m.) in the diet is equivalent to 250 mg/kg body weight per day.

##### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.5
Conditional acceptance . . . . .	0.5-2.0

#### Comment

For levels of use in the conditional dosage zone attention should be paid to the presence of other phenolic antioxidants in the diet or environmental conditions involving significant exposure to phenolic substances.

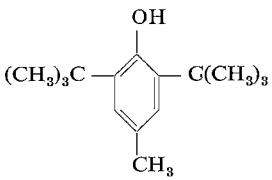
#### Further work considered desirable

1. Long-term studies in a species other than the rat, with careful evaluation of tumour incidence.
2. Biochemical studies in human subjects.

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## BUTYLATED HYDROXYTOLUENE

<b>Chemical names</b>	2 : 6-di- <i>tert.</i> -butyl- <i>p</i> -cresol ; 4-methyl-2 : 6-ditertiary-butylphenol
<b>Synonym</b>	BHT
<b>Empirical formula</b>	$C_{15}H_{24}O$
<b>Structural formula</b>	
<b>Molecular weight</b>	220.36
<b>Definition</b>	Butylated hydroxytoluene should contain not less than 99.0% of $C_{15}H_{24}O$ .
<b>Description</b>	A white, crystalline, odourless solid. It is insoluble in water but soluble in fats ; 1 g is soluble in 4 ml of ethanol.
<b>Use</b>	As an antioxidant for fats and oils or in packaging material for fat-containing foods. Its activity is enhanced in combination with other antioxidants and synergists.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Approx. lethal dose (mg/kg body weight)	Reference
Rat	oral	1700-1970	—	1
Cat	oral	—	940-2100	1
Rabbit	oral	—	2100-3200	1
Guinea-pig	oral	—	10 700	1

**Short-term studies***Rat*

Feeding experiments were carried out on 45 pairs of weanling male rats for 5-8 weeks with diets containing 0, 10 and 20% lard supplements to which 0.001%, 0.1% or 0.5% BHT had been added. 0.001% caused no changes in any of the serum constituents studied. 0.5% produced increase in the serum cholesterol level within 5 weeks. Female rats fed for 8 months on a diet containing a 10% lard supplement with 0.1% BHT showed increased serum cholesterol levels, but no other significant changes. 0.5% BHT in 10% and 20% lard supplements fed to female rats for the same period increased serum cholesterol, phospholipid, and mucoprotein levels.<sup>2</sup>

0.3% BHT in the diet of pregnant rats that had been kept for 5 weeks on a diet deficient in vitamin E produced no toxic symptoms. 1.55% caused drastic loss of weight and foetal death.<sup>3</sup>

BHT fed to rats in groups of 12 for a period of 7 weeks at a dietary level of 0.1% in conjunction with a 20% lard supplement significantly reduced the initial growth rate and mature weight of male rats. No effect was noted in female or male rats with a 10% lard supplement. A paired feeding experiment showed that this inhibition of growth was a direct toxic effect of BHT and could not be explained by a reduction in the palatability of the diet. At this level BHT produced a significant increase in the weight of the liver, both absolute and relative to body weight. Rats under increased stress showed significant loss of hair from the top of the head. The toxic effect of BHT was greater if the fat load in the diet was increased. Anophthalmia occurred in 10% of the litters.<sup>4</sup>

Feeding experiments conducted for 20 and 90 days respectively indicated that rats do not find food containing 0.5% or 1% BHT palatable. However, the animals ingest foods so treated more readily if these concentrations are attained gradually. Paired feeding experiments with groups of 5 or 10 rats for 25 days demonstrated that diets containing 0.8% and 1% BHT will reduce the daily intake of food below control values. A level of 1% in the diet retarded weight gain.<sup>1</sup>

*Rabbit*

Acute effects on electrolyte excretion similar to those described for large doses of BHA were also obtained following administration of doses of BHT of 500-700 mg/kg body weight (about 2% in the diet). No such effects were observed at lower dosage levels.<sup>5</sup>

*Dog*

A mild to moderately severe degree of diarrhoea was induced in a group of 4 dogs fed doses of 1.4-4.7 g/kg body weight every 2-4 days over a period of 4 weeks. Post-mortem examination revealed no significant gross pathological changes. No signs of intoxication and no gross or histopathological changes were observed in dogs fed doses of 0.17-0.94 g/kg body weight 5 days a week for a period of 12 months.<sup>1</sup>

*Fowl*

When BHT was fed at a level of 0.125% for 34 weeks to a group of 10 pullets, no differences in fertility, hatchability of eggs, or health of chicks in comparison with a similar control group were found. The eggs of the antioxidant-treated birds contained more carotenoids and vitamin A than those of the controls.<sup>6</sup>

**Long-term studies***Rat*

Groups of 15 male and 15 female rats given diets containing 1% lard and 0.2, 0.5 or 0.8% BHT for 24 months showed no specific signs of intoxication, and micropathological studies were negative. For one group given a diet containing 0.5% BHT, the BHT was dissolved in lard and then heated for 30 minutes at 150°C before incorporation in the diet. There were no effects on weight gain or blood constituents and micropathological studies of the main organs were negative. The feeding of 1% BHT was followed in both male and female rats by a subnormal weight gain and by an increase in the weight of the brain and liver and some other organs in relation to body weight. Micropathological studies were negative in this group also. BHT in these concentrations had no specific effect on the number of erythrocytes and leucocytes, or on the concentration of haemoglobin in the peripheral blood. A number of rats of both sexes died during this experiment, but as the fatalities were in no relation to the concentration of BHT fed, it was believed that the cause of death was unrelated to the feeding of this substance. Micropathological studies support this observation.<sup>1</sup>

When fed at the 0.5% level in the diet, BHT had no effect in rats on the reproductive cycle, the histology of the spleen, kidney, liver and skin,

or on the weight of the heart, spleen or kidney. There was no significant increase in mortality of rats fed a diet containing 0.1% BHT and 10% hydrogenated coconut oil for a period of 2 years. The effects on weight gain have already been described.<sup>4</sup>

#### **Biochemical aspects**

BHT is readily absorbed. Some deposition in adipose tissue has been described following high dosage in the rat, and this may cause increased stability of the extracted perineal fat.<sup>7</sup> The metabolism of BHT is complicated by the presence of the butyl groups on each side of the hydroxyl. Preliminary modification is necessary before conjugation can occur. This takes the form of either oxidation of one of the butyl groups to a 2-hydroxy-2 : 2-dimethyl-2-ethyl group, or oxidation of the methyl group to a carboxy acid. The former yields glucuronide conjugates, while the latter becomes conjugated with glucuronic acid or glycine. Some of the oxidized material is also excreted unconjugated.<sup>8, 9</sup>

#### **Comment on experimental studies reported**

Experimental studies have been carried out in several species. There are, however, some important discrepancies between the results obtained by earlier and by more recent observers. Short-term and long-term studies<sup>1, 2, 4</sup> in rats and metabolic studies will be discussed under evaluation.

#### **Evaluation**

##### **Level causing no significant toxicological effect in the rat**

One extensive series of studies<sup>1</sup> indicates that the level of BHT that would cause no significant deleterious effect in the rat would be 0.8% of the diet. However, even in short-term studies two other groups of investigators<sup>2, 4</sup> have shown more recently that as little as 0.1% resulted in an increase of various lipid components of the blood and significant reduction in weight gain. This latter effect was shown to be due to a toxic action of BHT. Long-term studies by these same investigators did not reveal significant differences between groups receiving 0.5% BHT and controls. Several investigators<sup>1, 2, 3, 4</sup> have shown that 1% and upwards of BHT will cause quite marked toxic effects and, in this respect, it appears to differ rather strikingly from BHA. A possible explanation of this discrepancy is that the toxicity of BHT is materially affected by the level of fat in the diet. In the experiments showing that 0.8% of BHT in the diet caused no significant deleterious effect in the rat, the fat content of the diet used was less than 5%. The addition of 10% or 20% of lard was found

to enhance the deleterious action of BHT but it did not modify the effect of BHA. With a 20% lard supplement, fat provides about 33% of the calorie intake. Human diets frequently contain this level of fat. The effect of such levels of dietary fat on BHT toxicity is therefore relevant to its use as a food additive.

For the 0.1% level to be accepted as the level causing no significant toxicological effect in the rat, it would be necessary to disregard both the effect on weight gain, which was carefully checked by paired feeding experiments, and also the effect on blood lipids. Notice must also be taken of the fact that the structure of BHT suggests the possibility of some delay in metabolism and the finding that the dietary fat load enhances BHT toxicity. It would be inadvisable to disregard these observations. The next lower dose studied was 0.01% (= 100 p.p.m.) in the diet in a group of 26 rats,<sup>4</sup> which is equivalent to 5 mg/kg body weight per day.

#### Estimate of acceptable daily intake for man

	mg/kg body weight
Conditional acceptance . . . . .	0-0.5

#### Further work considered desirable

1. Further long-term studies, with particular reference to the effect of BHT on lipid metabolism and the relationship between the dietary fat load and toxicity.
2. Metabolic studies in human subjects.

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## CITRIC ACID

<b>Chemical names</b>	Citric acid ; 2-hydroxy-1,2,3-propanetricarboxylic acid ; $\beta$ -hydroxytricarballic acid
<b>Empirical formula</b>	<i>Anhydrous</i> : $C_6H_8O_7$ <i>Monohydrate</i> : $C_6H_8O_7 \cdot H_2O$
<b>Structural formula</b>	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <i>Anhydrous</i>  <math display="block">\begin{array}{c} CH_2 - COOH \\   \\ OH - C - COOH \\   \\ CH_2 - COOH \end{array}</math> </div> <div style="text-align: center;"> <i>Monohydrate</i>  <math display="block">\begin{array}{c} CH_2 - COOH \\   \\ OH - C - COOH \cdot H_2O \\   \\ CH_2 - COOH \end{array}</math> </div> </div>
<b>Molecular weight</b>	<i>Anhydrous</i> : 192.13 <i>Monohydrate</i> : 210.15
<b>Definition</b>	Citric acid may be anhydrous or it may contain one molecule of water. Citric acid contains not less than 99.5% of $C_6H_8O_7$ , calculated in terms of the anhydrous compound.
<b>Description</b>	A white or colourless, odourless, crystalline solid. 1 g is soluble in 0.5 ml of water and in 2 ml of ethanol.
<b>Natural occurrence</b>	Widely distributed in plants and in animal tissues.
<b>Use</b>	Antioxidant, sequestrant, and acidulant.

## Biological Data

## Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	i.v. (rapid injection)	42	1
Mouse	i.p.	961	1
Rat	i.p.	884	1

## Short-term studies

*Dog*

A daily oral dose of 1380 mg/kg body weight given to 3 dogs for 112 to 120 days produced no symptoms or evidence of renal damage.<sup>2</sup>

## Long-term studies

*Rat*

Diets containing 1.2% of citric acid had no harmful effect on the growth of two successive generations of rats over a period of 90 weeks. No detrimental effect could be observed on reproduction. No significant change could be noted in the blood picture nor was there any other pathological

finding that could be attributed to the diet. Loss of calcium or other fixed bases was not observed. The dental attrition was found to be slightly more marked than in the control groups.<sup>3</sup>

### Biochemical aspects

In man, citric acid is an important intermediate in the *Krebs citric acid cycle*, which represents the pathway of aerobic oxidation of pyruvic acid in the body.

### Comment on experimental studies reported

In evaluating the acceptance of citric acid, emphasis is placed on its well-established metabolic pathways. Toxicological studies on animals supplement this information.

### Evaluation

#### Level causing no significant toxicological effect in the rat

1.2% (= 12 000 p.p.m.) in the diet, equivalent to 600 mg/kg body weight per day.

#### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-60
Conditional acceptance . . . . .	60-120

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## ISOPROPYL CITRATE MIXTURE

<b>Definition</b>	A mixture consisting of approximately 27% monoisopropyl citrate 9% diisopropyl citrate 2% triisopropyl citrate 62% mono and diglycerides
<b>Description</b>	More readily soluble in oils than citric acid.
<b>Use</b>	As a sequestering agent in antioxidant mixtures and in fatty foods.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Rat	oral	2800-3700	1
Dog	oral	2250	1

**Short-term studies***Rat*

A group of rats were fed isopropyl mixture in the diet at the rate of 1500-2000 mg/rat per day for 6 weeks. There was no demonstrable effect on growth or mortality, and there were no pathological findings.<sup>1</sup>

*Rabbit*

A group of rabbits were fed isopropyl mixture at an average level of about 3600 mg/rabbit per day for 6 weeks. There was no demonstrable effect on growth or mortality and there were no abnormal findings post mortem.<sup>1</sup>

*Dog*

A group of dogs were fed isopropyl mixture in the diet at the level of 0.06% for 6 weeks with no demonstrable effect on growth or mortality and no pathological findings.<sup>1</sup>

**Long-term studies***Rat*

Groups of rats were fed isopropyl mixture in the diet at the levels of 0%, 0.28%, 0.56% and 2.8% for a period of two years. No deleterious effects were noted in any of the treated groups with respect to growth rate, mortality or histopathology of the tissues.<sup>1</sup>

Multigeneration studies likewise indicated that isopropyl mixture at a level of 2.8% in the diet was innocuous.<sup>1</sup>

**Biochemical aspects**

Studies in the rat showed that isopropyl mixture was readily absorbed when it was incorporated in the diet up to the 10% level.<sup>2</sup>

**Comments on the experimental studies reported**

No deleterious effects were observed in the short-term studies in rats, rabbits and dogs, or in the long-term studies in rats.

### Evaluation

#### Level causing no significant toxicological effect in the rat

2.8% (= 28 000 p.p.m.) in the diet, equivalent to 1400 mg/kg body weight per day.

#### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-7
Conditional acceptance . . . . .	7-20

### References

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### SODIUM DIACETATE

<b>Chemical name</b>	Sodium hydrogen diacetate
<b>Empirical formula</b>	$C_4H_7O_4Na \cdot H_2O$
<b>Structural formula</b>	$CH_3COONa \cdot CH_3COOH \cdot H_2O$
<b>Definition</b>	Sodium diacetate is a molecular compound of sodium acetate and acetic acid, containing not less than 39% of free acetic acid.
<b>Description</b>	A white, hygroscopic crystalline solid with an acetic odour. 1 g is soluble in 1 ml of water.
<b>Use</b>	Mould and rope inhibitor in baked goods.

### Biological Data

#### Acute toxicity

There is no direct information on the  $LD_{50}$  of sodium diacetate in animals. It is probably similar to that of neutralized acetic acid.

Animal	Route	Neutralized acetic acid	
		$LD_{50}$ (mg/kg body weight)	Reference
Mouse	oral	3310	1
Rat	oral	4960	1
Rat	oral	3530	2

**Short-term studies****Rat**

Since there are no data on sodium diacetate as such, studies with acetic acid may be considered. Rats given drinking water containing 0.25% of acetic acid were not affected; at a concentration of 0.5% growth was inhibited.<sup>3</sup>

**Long-term studies**

No studies in animals are available. Acetic acid from vinegar and from other sources is normally consumed by man in amounts of about 1 g daily, apparently without adverse effects.

**Biochemical aspects**

There are no studies with sodium diacetate as such, but it may be expected to be metabolized in the same way as other salts of acetic acid after absorption.

**Comments on experimental studies reported**

In the absence of long-term studies, an acceptable level of human intake of sodium diacetate has been estimated from the observations of the effect of acetic acid in animals and in man.

**Evaluation****Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-15
Conditional acceptance . . . . .	15-30

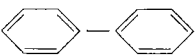
**Comment**

Since acetic acid is sufficiently acid to limit the amount used in foods, it is not necessary to indicate a maximum acceptable dose level or a tolerance. Consumption of sodium diacetate, however, is probably not as self-limiting, and it is therefore desirable to set limits for this compound, so that no more than about 1 g will be consumed per day.

**References**

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3. Sollmann, T. (1920-21) *J. Pharmacol. exp. Ther.*, **16**, 463

**DIPHENYL**

<b>Chemical names</b>	Diphenyl; biphenyl; phenylbenzene
<b>Empirical formula</b>	$C_{12}H_{10}$
<b>Structural formula</b>	
<b>Molecular weight</b>	154.2
<b>Description</b>	A white crystalline powder with an unpleasant smell; sublimes easily; insoluble in water but soluble in fats.
<b>Use</b>	As a fungistatic in wrapping material to inhibit growth of moulds causing decay of citrus fruits.

**Biological Data****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	References
Rat	oral	3500-5000	1,2
Rabbit	oral	2400	1

**Short-term studies***Rabbit*

Five rabbits were each given doses of 1g/kg body weight by stomach tube in a 25% dilution in olive oil 2 or 3 times a week until they died. Deaths occurred within 5 to 20 weeks. The blood picture showed no significant change, but the gain in body weight was less than in the controls, and retention of urea occurred near the terminal stage of intoxication.<sup>1</sup>

*Rat*

Eleven young female rats were fed for 32 days on a diet low in casein and containing 1% diphenyl. A marked growth retardation was observed. Histological examination of 2 animals showed the beginning of fatty changes in the liver and mild toxic changes in the kidney.<sup>3</sup>

In a small group of weanling rats fed with a diet containing 0.3% diphenyl, weight increase was less than in control animals.<sup>4</sup>

Administration of diphenyl to young rats for 4 weeks in doses of 2, 20 and 200 mg/kg body weight per day induced neither growth retardation nor significant change in the blood picture.<sup>5</sup>

Oral administration of 0.3 g/kg body weight daily to 10 rats for 12 days did not induce growth retardation.<sup>6</sup>

Groups of young rats were fed for 3 months on rations containing 0%, 0.01% and 0.1% of diphenyl. No significant differences were observed in the growth rates, food efficiency, organ weights, blood urea or histology of tissues between treated and control rats. At the level of 0.1% only a slight polyuria was noted.<sup>7</sup>

#### *Monkey*

Small groups of monkeys were fed for a year on rations containing 0.01%, 0.1% and 1% diphenyl. The only significant change was a slight increase in the weight of the liver at the intake level of 1%.<sup>7</sup>

#### *Dog*

Nine dogs divided into 3 groups received diphenyl in corn oil orally at the rate of 0, 2.5 and 25 mg/kg body weight 5 days a week for 52 weeks. One dog on 2.5 mg/kg and one on 25 mg/kg lost about 1 kg in weight each. The other animals gained in weight. Blood and urine were unchanged. No gross or microscopic pathological changes were found in any of the tissues examined.<sup>8</sup>

### **Long-term studies**

#### *Rat*

A long-term feeding experiment with rats made use of dietary levels of 0.01%, 0.1% and 1% diphenyl for a two-year period.<sup>7</sup> Unfortunately this study failed to supply the desired information for two reasons: (a) a severe respiratory infection caused a high mortality among the controls; (b) rats receiving 0.1% and 1% diphenyl exhibited tubular dilatation of the kidneys, as did two of the control rats.

Groups of 15 weanling rats of each sex were fed 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5% and 1% diphenyl in the diet. Growth was inhibited, particularly at the 0.5% and 1% levels, apparently because of decreased food consumption during the first 100 days of the test. During 750 days there was a decrease in longevity in rats on the 0.5% and 1% diets. At the other levels there was no significant evidence of toxicity. With the diet containing 1% diphenyl, haemoglobin values of male and female rats were lower in comparison with controls when measured at 300 and 400 days respectively. The histopathological changes observed in the kidneys of rats on the 0.5% and 1% diets were irregular scarring, lymphocytic infiltration, tubular atrophy and patchy tubular dilatation to the point of cyst formation, in association with hydronephrosis and sometimes albuminuria and haemoglobinuria. These phenomena did not occur at a concentration of 0.1% diphenyl or less.<sup>9</sup>

In another long-term study, the onset and reversibility of kidney damage in rats on diets containing 0.1%, 0.25% and 0.5% diphenyl were investigated, and the findings quoted under reference 9 were confirmed.<sup>10</sup>

In one experiment on 13 rats fed daily with a diet containing 0.025%-0.05% diphenyl, after two months one animal had squamous cell carcinoma and two had papillomas of the forestomach.<sup>2</sup>

### Biochemical aspects

Diphenyl is metabolized in rats<sup>3, 11</sup> and rabbits<sup>11</sup> to various phenolic compounds, mainly 4-hydroxydiphenyl which is excreted in the urine in both free and conjugated form (ethereal sulfate and glucuronate). This demonstration of a common mechanism in 2 species of animals suggests that human metabolism would follow similar paths, but no report of this has been found in the literature.

Diphenyl was found to be less toxic when given with a diet containing a supplement of l-cystine or dl-methionine, but diphenyl is not highly conjugated with cystine.<sup>12</sup>

### Comment on experimental studies reported

Most of the studies reported have been carried out in dogs and rats. The value of negative results of short-term studies in groups of 3 dogs at levels of 2.5 and 25 mg/kg body weight is limited.<sup>8</sup> Long-term studies in rats provide a basis for evaluation.<sup>9, 10</sup>

Metabolic studies on 2 species reveal a common mechanism of detoxication, but no data are available on metabolism in man.

## Evaluation

### Level causing no significant toxicological effect in the rat

From consideration of the long-term studies in rats, the level causing no significant toxicological effect when ingested over a period approximating to the life-span may be assessed at 0.1%.

0.05% (= 500 p.p.m.) in the diet is equivalent to 25 mg/kg body weight per day.

### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.05
Conditional acceptance . . . . .	0.05-0.25

### Comment

Diphenyl presents certain peculiar problems since it is primarily used as a treatment for wrappers for fruit. Nevertheless, diphenyl penetrates into the skin of the fruit and may consequently be included in food or

drink prepared from it. Fruit drinks are commonly consumed in considerable quantities by children and by sick people. For these reasons caution should be exercised and the pattern of use should be closely studied.

#### Further work considered desirable

1. Long-term studies in a species other than the rat, with careful evaluation of tumour incidence.
2. Biochemical studies in man.

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#### FORMIC ACID \*

Chemical name	Formic acid
Empirical formula	CH <sub>2</sub> O <sub>2</sub>
Structural formula	$\begin{array}{c} \text{O} \\ \parallel \\ \text{H} - \text{C} \\ \diagdown \\ \text{OH} \end{array}$
Molecular weight	46.0

\* From the toxicological point of view formic acid and formates may be considered together.

<b>Description</b>	A colourless, highly caustic liquid, having a very pungent odour. Soluble in water and ethanol.
<b>Use</b>	As a preservative.

### Biological Data

#### Acute toxicity

Exact LD<sub>50</sub> values are not available. In dogs, sodium formate in oral doses of 4000 mg/kg and intravenous doses of 3000 mg/kg body weight produced toxic effects such as methaemoglobinaemia and heart congestion.<sup>1</sup> About 50 mg/kg in 10% aqueous solution given orally to dogs or 6 mg/kg given subcutaneously to rabbits produced methaemoglobinaemia which lasted about 10 days.<sup>2</sup>

This slow disappearance may be due to the inhibition of catalase by formic acid.<sup>3</sup> 4.6 mg per kg intravenously given to 6 dogs produced no ill effect and 13.8 mg per kg only slight hypertension.<sup>4</sup>

#### Short-term studies

##### *Dog*

0.5 g of formic acid daily in the food has been tolerated by dogs without effect.<sup>10</sup>

##### *Man*

2-4 g of sodium formate daily did not produce toxic manifestations in human subjects, even if they were suffering from kidney disease. It has been stated that a daily intake of 2-4 g for therapeutic purposes could be tolerated for months without untoward effects.<sup>5</sup>

#### Biochemical aspects

Formate is an intermediate in normal metabolism. It takes part in the metabolism of one-carbon compounds and its carbon may appear in methyl groups undergoing transmethylation. It is eventually oxidized to carbon dioxide.<sup>6</sup> When formate is administered it could also be expected to enter one-carbon metabolism. However, there is a species difference in the extent of this metabolism, for in rabbits no administered formate is excreted, whereas in dogs about half the administered formate is excreted unchanged in the urine.<sup>2</sup> Its metabolism in human beings is probably somewhere between that in dogs and that in rabbits, judging from the relative amounts of formate excreted by man, dogs and rabbits receiving methanol.<sup>7, 8</sup> Formic acid (or formate) is apparently more toxic than other fatty acids, possibly owing to its enzyme-inhibiting activity.<sup>9</sup> However, no cumulative toxic effects are known.

**Comments on experimental studies reported**

Since long-term toxicity studies are lacking, it is not possible to give guidance on an unconditional acceptable daily intake level in man.

**Evaluation****Level causing no significant toxicological effect**

Short-term studies in dogs and man indicate that formic acid has no significant toxicological effect at a dosage of about 50 mg/kg body weight per day.

**Estimate of acceptable daily intake for man**

	mg/kg body weight
Conditional acceptance . . . . .	0-5

**Further work considered desirable**

Long-term toxicity studies in animals and metabolism studies in man.

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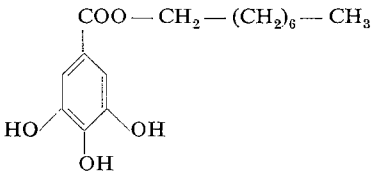
**PROPYL GALLATE \***

<b>Chemical names</b>	Propyl gallate ; <i>n</i> -propyl ester of 3,4,5-trihydroxybenzoic acid
<b>Empirical formula</b>	C <sub>10</sub> H <sub>12</sub> O <sub>5</sub>
<b>Structural formula</b>	$\begin{array}{c} \text{COO} - \text{CH}_2 - \text{CH}_2 - \text{CH}_3 \\   \\ \text{C}_6\text{H}_2\text{O}_3 \\   \quad   \quad   \\ \text{HO} \quad \text{OH} \quad \text{OH} \end{array}$
<b>Molecular weight</b>	212.21

\* For biological data and toxicological evaluation see pp. 62-65.

<b>Definition</b>	Propyl gallate contains not less than 99% of $C_{10}H_{12}O_5$ after drying at 110°C for 4 hours.
<b>Description</b>	A white to creamy-white, crystalline, odourless solid, with a slightly bitter taste. 1 g is soluble in 300 ml of water or in 3.5 ml of ethanol; freely soluble in fat.
<b>Use</b>	As an antioxidant; more effective combined with citric acid as synergist.

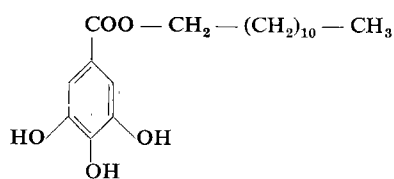
**OCTYL GALLATE \***

<b>Chemical name</b>	Octyl gallate; <i>n</i> -octyl ester of 3,4,5-trihydroxybenzoic acid
<b>Empirical formula</b>	$C_{15}H_{22}O_5$
<b>Structural formula</b>	$COO-CH_2-(CH_2)_6-CH_3$ 
<b>Molecular weight</b>	282.34
<b>Definition</b>	Octyl gallate contains not less than 98.5% of $C_{15}H_{22}O_5$ after drying at 60°C for 4 hours.
<b>Description</b>	A white to creamy-white, odourless solid, which may have a slightly bitter taste. 1 g is soluble in 2.5 ml of ethanol. It is insoluble in water but freely soluble in fats.
<b>Use</b>	As an antioxidant; more effective combined with citric acid as synergist.

**DODECYL GALLATE**

<b>Chemical names</b>	Dodecyl gallate; <i>n</i> -dodecyl (or lauryl) ester of 3,4,5-trihydroxybenzoic acid
<b>Synonym</b>	Lauryl gallate
<b>Empirical formula</b>	$C_{19}H_{30}O_5$

\* For biological data and toxicological evaluation see pp. 62-65.

**Structural formula****Molecular weight** 338.45**Definition** Dodecyl gallate contains not less than 98.5% of  $\text{C}_{19}\text{H}_{30}\text{O}_5$  after drying at  $60^\circ\text{C}$  for 4 hours.**Description** A white or creamy-white, odourless solid, which may have a slightly bitter taste. 1 g is soluble in 3.5 ml of ethanol. Insoluble in water but freely soluble in fats.**Use** As an antioxidant; more effective combined with citric acid as synergist.**Biological Data****Acute toxicity**

	Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Propyl gallate	Rat	oral	3800	1
	"	"	3600	2
	"	i.p.	380	1
	Mouse	oral	2000-3500	2
Octyl gallate	Rat	oral	4700	3
	"	i.p.	60-80	4
Dodecyl gallate	"	oral	6500	3
	"	i.p.	100-120	4

**Short-term studies****Rat**

Levels of propyl gallate of 1.2% and 2.3% in the diet caused interference with weight gain, the bitter taste of the gallate apparently making the diet unpalatable. The higher dose level caused some deaths (about 40%) during the first month; the survivors continued to eat the diet for 10-16 months and showed retarded growth, but no pathological lesions. The animals that died exhibited renal damage.<sup>1</sup>

Weanling rats were given diets containing 2.5% and 5% dodecyl gallate. All animals fed the smaller quantity were dead within 10 days, and all animals fed the larger quantity died within 7 days.<sup>5</sup>

Rats fed for 70 days on a diet containing 7% fat and 0.2% dodecyl gallate showed no effect on body weight.<sup>6</sup>

*Guinea-pig*

Propyl gallate fed to guinea-pigs in groups of 20 at a level of 0.0117% in the diet for 14 months caused no detectable ill effects.<sup>1</sup>

*Dog*

A level of 0.0117% of propyl gallate in the diet was well tolerated by a group of 7 dogs over a period of 14 months.<sup>1</sup>

*Pig*

Diets containing 0.2% of propyl, octyl or dodecyl gallate were fed to pigs without demonstrable ill effect; no anaemia was observed.<sup>7</sup>

**Long-term studies***Rat and mouse*

A level of 5% propyl gallate in the diet in a two-year chronic toxicity test on rats and mice gave rise to patchy hyperplasia in the proventriculus. At a level of 1% no difference was noted between test and control animals.<sup>2</sup>

Rats in groups of 10 males and 10 females were fed for 2 years on diets containing 0%, 0.00117%, 0.0117%, 0.117%, 1.17% and 2.34% of propyl gallate. The groups receiving 1.17% and 2.34% of propyl gallate showed stunted growth and evidence of renal damage. In the other groups, there was no detectable effect on haemoglobin, erythrocyte or leucocyte levels in the blood, nor on the histological appearance of the organs examined.<sup>1</sup>

Propyl, octyl and dodecyl gallates were fed to rats at concentrations of 0.035%, 0.2% and 0.5% in the diet. Growth was affected only at the 0.5% level of dodecyl gallate; there was significant retardation, particularly in the second generation. At this level of dodecyl gallate, some litters were lost in the second generation because they were not sufficiently fed by the mothers. A slight hypochromic anaemia was noticed in the groups on diets containing 0.2% octyl and dodecyl gallate. No abnormalities were observed in the organs or tissues of the rats at autopsy.<sup>7</sup>

Young rats in groups of 12 males and 12 females were fed diets containing 7% fat and 0.2% octyl or dodecyl gallate. There was no significant difference between test and control animals over 3 generations.<sup>3</sup>

**Biochemical aspects**

The available evidence indicates that the esters are hydrolysed in the body. Most of the gallic acid is converted into 4-O-methyl gallic acid. Free gallic acid or a conjugated derivative of 4-O-methyl gallic acid is

excreted in the urine. Conjugation of the 4-O-methyl gallic acid with glucuronic acid was demonstrated.<sup>8</sup> The detailed metabolic pathways for propyl gallate have been described.<sup>9</sup> There is no evidence to suggest that other esters of gallic acid differ greatly in their metabolism from the pattern described for propyl gallate.

#### **Comment on experimental studies reported**

The acute and short-term studies reported are fairly extensive and cover a wide range of animal species. Several long-term studies have been made and the levels of feeding varied widely. There are some discrepancies in the results reported. However, the long-term studies on rats<sup>1, 2, 3, 7</sup> provide a basis for evaluation.

#### **Evaluation**

##### **Level causing no significant toxicological effect in the rat**

With one exception, in long-term studies in rats gallates caused no demonstrable ill effects when fed at a level of 0.2%; in one investigation, however, this level resulted in hypochromic anaemia. It seems likely that this may have been due to interference with iron absorption, but the cause was not established. Haematological effects were carefully examined in a number of other investigations and no abnormalities were seen. It seems probable, therefore, that this effect was related to the particular circumstances of one study and can be properly disregarded in arriving at the level that causes no significant effects in the rat. The level that causes no significant toxicological effect in the rat is 100 mg/kg body weight per day.

##### **Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.2
Conditional acceptance . . . . .	0.2-0.5

#### **Comment**

Attention should be given to the amounts of other phenolic antioxidants in the diet.

#### **Further work considered desirable**

1. Long-term studies in other species than the rat, with special attention to possible interference with iron metabolism and haematopoiesis.
2. Metabolic studies in human subjects.

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### GUM GUAIAIC

<b>Synonyms</b>	Resin guaiac ; gum guaiacum ; guaiacum
<b>Description</b>	Brown or greenish brown, irregular lumps. Consists of about 70% $\alpha$ - and $\beta$ -guiaiconic acids, about 10% guaiaretic acid, and 15% guaiac yellow, vanillin, etc. Insoluble in water, freely soluble in alcohol, sparingly soluble in fat.
<b>Natural occurrence</b>	Resin from wood of <i>Guajacum officinale</i> L. or <i>G. sanctum</i> L., Zygophyllaceae.
<b>Use</b>	As an antioxidant for oils and fats, especially rendered animal fat or a combination of such fat and vegetable oils.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Rat	oral	> 5000	1
Mouse	oral	> 2000	1
"	i.p.	> 2000	1
Guinea pig	oral	1120	1

Six human subjects took a total of 10 doses of 2 or 3 g at a time. The only untoward sequel of ingesting these large quantities was the passage of one or two loose stools in some instances.<sup>2</sup>

**Short-term studies***Rat*

Four groups of 10 rats received diets containing 10% lard with 0%, 0.05%, 0.5% and 5% gum guaiac (0%, 0.005%, 0.05% and 0.5% of the total diet). None of them showed any effect on growth rate over a period of 41 weeks.<sup>2</sup>

*Dog*

Gum guaiac in doses of 500 or 1000 mg was administered to 11 fully grown dogs daily for 62 to 103 weeks. Body weight, general behaviour, appearance, red and white blood cell counts, and haemoglobin remained normal. Histological sections from three dogs (fed 1000 mg guaiacum daily for 75 weeks) showed a normal intestinal mucosa in each case, with no suggestion of irritation or injury. The lungs, kidney, liver and spleen from these dogs were normal upon microscopic examination.<sup>2</sup>

*Cat*

Eight full-grown cats received a daily dose of 500 or 1000 mg of gum guaiac for 34 to 117 weeks. Body weight, general behaviour, appearance, red and white blood cell counts, and haemoglobin remained normal. Two of the cats fed for 74 weeks showed no irritation of intestinal mucosa; upon histological examination the lungs, kidney, liver and spleen were found to be normal.<sup>2</sup>

*Man*

Eleven human subjects (4 female and 7 male) received 50 or 100 mg of gum guaiac daily for periods of 18 to 104 weeks. Red and white blood cell counts, haemoglobin, kidney function, body weight, number and consistency of stools, and general physical condition were unchanged throughout the experimental period.<sup>2</sup>

**Long-term studies***Rat*

Forty rats were divided into 4 groups and fed diets containing lard with 0%, 0.05%, 0.5% and 5% gum guaiac (0%, 0.005%, 0.05% and 0.5% of the total diet) for a lifetime study. The second and third generation descendants (80 in number) of the original rats were maintained throughout their lifetime on the same diet as their parents. No differences were observed between the experimental groups and the controls in regard to body weight, growth rate, life span, reproduction, or pathological examination.<sup>2</sup>

Groups of 10 rats each were maintained on diets containing 0% and 0.5% gum guaiac for a period of two years. No discernable difference

was observed between the two groups as determined by growth rate, mortality, and pathological examination.<sup>1</sup>

#### Biochemical aspects

Very little, if any, gum guaiac is absorbed, much is passed out in the faeces, and the remainder is destroyed in the colon.<sup>2</sup>

#### Comment on experimental studies reported

The studies reported in the literature <sup>1, 2</sup> are good and provide a basis for estimating the acceptable intake for man.

In addition, further assurance of safety is given by toxicological studies on dogs, cats and human subjects as well as biochemical studies on the action of guaiacum on the gut and its fate in the organism.

#### Evaluation

##### Level causing no significant toxicological effect in the rat

0.5% (= 5000 p.p.m.) in the diet, equivalent to 250 mg/kg body weight per day

##### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-2.0
Conditional acceptance . . . . .	2.0-4.0

#### Further work considered desirable

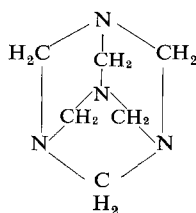
Consideration should be given to the establishment of a food-grade product.

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#### HEXAMETHYLENETETRAMINE

<b>Chemical names</b>	Hexamethylenetetramine ; methenamine ; methamin ; hexamethyleneamine
<b>Empirical formula</b>	C <sub>6</sub> H <sub>12</sub> N <sub>4</sub>

**Structural formula**

**Molecular weight** 140.2

**Description** White odourless crystals, which sublime at about 263°C. 1 g yields 1.2 g of formaldehyde on hydrolysis. 1 part is soluble in 1.5 ml of water or 12.5 ml of alcohol.

**Use** As a preservative for fish, meat and pickles.

**Biological Data****Acute toxicity**

Animal	Route	Lethal dose (mg/kg body weight)	Reference
Mouse	s.c.	450	1
Rat	s.c.	200	1
Guinea-pig	s.c.	300	1
Cat	s.c.	200	1

**Short-term studies**

No information available.

**Long-term studies****Rat**

Repeated subcutaneous injections of 35-40% solutions of hexamethylenetetramine produced local sarcomas in 8 out of 14 rats.<sup>3</sup>

**Biochemical aspects**

Under acid conditions or in the presence of proteins, hexamethylenetetramine decomposes gradually, yielding ammonia and formaldehyde.<sup>4</sup> Its toxicological properties must therefore be similar to those of formaldehyde.

**Special studies**

Both hexamethylenetetramine<sup>5</sup> and formaldehyde<sup>6</sup> have been shown to act as mutagens in *Drosophila*.

### Evaluation

The toxicological data are considered to be insufficient for evaluation in terms of a safe level for use in man. In addition, hexamethylenetetramine is suspect because it produces sarcomas in rats after subcutaneous injection and has a mutagenic action in insects. The deodorizing action of hexamethylenetetramine in some food products may mask a state of advanced decomposition.

It is concluded that hexamethylenetetramine should not be used as a food additive in human foodstuffs.

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### NITRATE OF SODIUM \*

<b>Chemical name</b>	Sodium nitrate
<b>Empirical formula</b>	$\text{NaNO}_3$
<b>Molecular weight</b>	85.01
<b>Definition</b>	Sodium nitrate contains at least 99.0% of $\text{NaNO}_3$ .
<b>Description</b>	Colourless transparent crystals, or white granules or powder. Deliquescent in moist air; freely soluble in water, very soluble in boiling water, slightly soluble in ethanol.
<b>Uses</b>	As an antimicrobial preservative and colour fixative in meat and meat and fish products and in some kinds of cheese; often used in combination with nitrites.
<b>Natural occurrence</b>	See Nitrate of Potassium.

### NITRATE OF POTASSIUM

<b>Chemical name</b>	Potassium nitrate
<b>Empirical formula</b>	$\text{KNO}_3$

\* For biological data and toxicological evaluation see pp. 70-72.

<b>Molecular weight</b>	101.1
<b>Definition</b>	Potassium nitrate contains about 99.5% of $\text{KNO}_3$ .
<b>Description</b>	Colourless transparent prisms, or white granular or crystalline powder, having a cooling, saline, pungent taste. Freely soluble in water, very soluble in boiling water, slightly soluble in ethanol.
<b>Uses</b>	As an antimicrobial preservative and colour fixative in meat and meat and fish products and in some kinds of cheese; often used in combination with nitrites.
<b>Natural occurrence</b>	Nitrates are normal constituents of many soils and are added to the soil in fertilizers. Nitrates are found in most growing plants: while values as high as 3600 p.p.m. have been reported for spinach and 4500 for rhubarb, tomatoes do not contain any nitrates. There are also wide variations in the figures obtained for particular vegetables, <sup>1</sup> e.g., from 50 to 4500 p.p.m. for asparagus. In strained baby foods, concentrations varying from 9 in peas, tomatoes and squash to 33 p.p.m. in spinach were found. It would be possible for a person to consume, from fresh vegetables, as much as 1-2 g of nitrate daily. <sup>2</sup>

#### Biological Data

##### Acute toxicity

Animal	Route	Min. lethal dose (mg/kg body weight)	LD <sub>50</sub> (mg/kg body weight)	Reference
Rat — male	oral	190-2000	—	3
Rat — female	oral	460-1200	—	3
Rat	oral	—	3236	4

Poisoning in man may result from a total oral daily dose in excess of 4 g or from a single dose of more than 1 g. 8 g may be fatal and 13-15 g are generally fatal.<sup>5</sup>

##### Short-term studies

###### Cattle

Most data have been obtained from livestock fed various forage crops with a high nitrate content. Poisoning depends upon the conversion of the nitrate to nitrite by the intestinal flora. The lowest level that may result in fatal poisoning in cattle has been reported to be 1.5% of potassium nitrate in the forage.<sup>6</sup>

*Dogs*

Two dogs were fed 2% of sodium nitrate in their diets for 105 days and for 125 days without any adverse effects.<sup>7</sup>

*Man*

Numerous cases have been reported of poisoning in small children and infants from the use of well water containing nitrates. Among these, there were 26 cases in which the nitrate nitrogen content of the well water was 21-50 p.p.m. (93-221 p.p.m. as  $\text{NO}_3$ ), 54 cases in which it was 51-100 p.p.m. (221-443 p.p.m. as  $\text{NO}_3$ ), and 52 cases in which it was over 100 p.p.m. (443 p.p.m. as  $\text{NO}_3$ ).<sup>8</sup>

In one instance, a level as low as 50 p.p.m. (as  $\text{NO}_3$ ) in tap water produced 72% methaemoglobinaemia in a dyspeptic child.<sup>9</sup> Healthy babies, however, have tolerated quantities up to 21 mg/kg body weight (as  $\text{NO}_3$ ) for one week without any disturbance.<sup>10</sup>

**Long-term studies***Rat*

Sodium nitrate was fed to 4 groups of 20 rats each at dosages of 0.1%, 1%, 5% and 10% of the diet for 2 years. Slight growth depression occurred at the 5% level, and additional morphological changes due to inanition occurred at the 10% level.<sup>7</sup>

**Biochemical aspects**

In certain circumstances reduction of nitrate to nitrite can take place in the digestive tract by the activity of the intestinal flora. If appreciable reduction occurs before the normal rapid elimination of the nitrate, poisoning can result. This appears to have occurred in cattle,<sup>6</sup> and in babies less than 6 months old, especially in dyspeptic infants.<sup>9</sup>

In experiments with rabbits about one half of the ingested amount of nitrate was excreted in the urine, and only 0.5% was recovered as nitrite in the urine.<sup>11</sup>

**Evaluation****Levels causing no significant toxicological effect in animals**

From consideration of the long-term study in rats, the level of sodium nitrate causing no demonstrable effect over a period approximating to the life span is assessed at 1% of the diet, or 500 mg/kg body weight daily. In the short-term study with dogs fed 2% sodium nitrate in the diet for 105 days, the level producing no demonstrable effect likewise corresponds to 500 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight (as sodium nitrate)
Unconditional acceptance . . . . .	0-5
Conditional acceptance . . . . .	5-10

**Comment**

In establishing tolerances for added nitrate it is important to take into account the amount of nitrate already present in other foods.

The sensitivity of normal babies and the apparent sensitivity of dyspeptic babies makes it impossible to make an estimate of an acceptable dose for babies of 6 months of age or less on the basis of animal experimentation or clinical experience. Nitrate should on no account be added to baby foods. Water with high nitrate content is unsuitable for the preparation of baby foods.

It is recommended that, if possible, sodium nitrate should be used as a 2.5% mixture with common salt.

**References**

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**NITRITE OF SODIUM \***

<b>Chemical name</b>	Sodium nitrite
<b>Empirical formula</b>	NaNO <sub>2</sub>
<b>Molecular weight</b>	69.0

\* For biological data and toxicological evaluation see pp. 73-75.

<b>Definition</b>	Sodium nitrite contains 96-98% of $\text{NaNO}_2$ .
<b>Description</b>	White or slightly yellow, hygroscopic granules or powder. Freely soluble in water, very soluble in boiling water, slightly soluble in ethanol.
<b>Uses</b>	As a colour fixative in pickling and curing meat, sometimes in combination with potassium nitrite; as a preservative for fish products, in fish brine and frozen fish, and for meat and meat products. Often used in combination with nitrates.

### NITRITE OF POTASSIUM

<b>Chemical name</b>	Potassium nitrite
<b>Empirical formula</b>	$\text{KNO}_2$
<b>Molecular weight</b>	85.1
<b>Definition</b>	Potassium nitrite contains 85% of $\text{KNO}_2$ , the rest being chiefly nitrate.
<b>Description</b>	White or slightly yellow, deliquescent granules or rods. Very soluble in water, slightly soluble in ethanol.
<b>Uses</b>	As a colour fixative in pickling and curing meat, sometimes in combination with sodium nitrite; as a preservative for fish products, in fish brine and frozen fish, and for meat and meat products. Often used in combination with nitrates.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Mouse	oral	220	1
Mouse — female	oral	175	2
Rat — female	oral	85	2

Many cases have been reported of accidental poisoning resulting from the presence of sodium nitrite in food products. From this information it is possible to deduce that the oral lethal dose in man varies from 0.18 to 2.5 g, the lower figures being those for children and old people.<sup>3, 4, 5, 6</sup> Sodium nitrite has been used for therapeutic purposes as a vasodilating agent in dosages of 30-120 mg.

**Short-term studies***Rat and cat*

Rats were fed a sodium nitrite supplement for a period up to 168 days. One rat received a total of 167 mg of sodium nitrite in 121 days. This represents 93 p.p.m. in the daily diet. No effects on growth or on the weights of important organs were noted. In a similar experiment with cats, one animal received a total of about 4100 mg of sodium nitrite during a period of 105 days. This represents approximately 390 p.p.m. in the daily diet. No effects on the growth rate or on the weight of important organs were noted. No histopathological examination has been reported on any animal fed with nitrite.<sup>7</sup>

**Long-term studies***Rat*

The continuous administration of sodium nitrite in the drinking water at the rate of 100 mg/kg body weight daily over the whole life span and in three successive generations (95 rats) resulted in spite of the high dosage (67% of the acute LD<sub>50</sub>) in only a slight inhibition of growth (10-20%) and in a shortening of the median life span from 740 to 640 days. Reproduction was normal. Neither the blood picture nor the organs showed any ill effects. The number of tumours observed in the test group (1 thymoma and 1 hepatoma) was not greater than in the control group. Cumulative toxic effects were not observed.<sup>8</sup>

Since nitrous acid reacts easily with secondary amines yielding dialkyl-nitrosamines which are highly toxic and produce cancer of the liver in rats, additional experiments have been made in which nitrite was administered together with diethylamine. Thirty hybrid rats were given sodium nitrite in the drinking water at a daily dosage of 100 mg/kg body weight and diethylamine in the food at the rate of 500 mg/kg body weight daily. The same slight shortening of the median life span (to 625 days) was observed as in the experiments with nitrite alone, but there were no tumours.<sup>8</sup>

**Biochemical aspects**

Following absorption of nitrites, the most important biochemical reaction that occurs is the conversion of haemoglobin to methaemoglobin. There is some controversy concerning the molar ratios involved in this reaction. Making an extreme assumption, it may be stated that 1 g of sodium nitrite could convert as much as 1855 g of haemoglobin to methaemoglobin.<sup>9</sup>

The subacute hazard of nitrites rests on the amount of methaemoglobin formed and on the ability of the body to reconvert this methaemoglobin back to haemoglobin.

### Comments on experimental studies reported

Though in the long-term studies cited only a slight inhibition of growth occurred, the dose causing this effect appears to give the best approximation to the threshold dose level.

### Evaluation

#### Level causing no significant toxicological effect in the rat

From consideration of the long-term studies it can be concluded that this level will be somewhat below 100 mg/kg body weight per day.

#### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.4
Conditional acceptance . . . . .	0.4-0.8

### Comment

It is recommended that if possible sodium nitrite should be used mixed with common salt, the amount of nitrite in the salt being not more than 0.6%. Food for babies should not contain added nitrite.

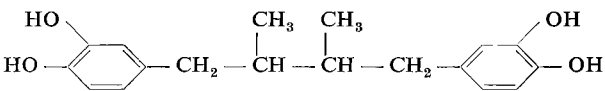
### Further work considered desirable

1. The estimation of nitrite in vegetables.
2. Chronic toxicity experiments in non-rodent species.

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### NORDIHYDROGUAIARETIC ACID

<b>Chemical names</b>	Nordihydroguaiaretic acid ; $\beta,\gamma$ -dimethyl- $\alpha,\delta$ -bis-(3,4-dihydroxyphenyl)butane ; 4,4'-(2,3-dimethyl-tetramethylene) dipyrocatechol
<b>Synonym</b>	NDGA
<b>Empirical formula</b>	$C_{18}H_{22}O_4$
<b>Structural formula</b>	
<b>Molecular weight</b>	302.37
<b>Description</b>	Nordihydroguaiaretic acid is a white to greyish-white crystalline solid, which may be prepared from an evergreen desert shrub, <i>Larrea divaricata</i> . 1 g is soluble in 4 ml of ethanol.
<b>Use</b>	As an antioxidant in oils and foods.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Rat	oral	2000-5500	1
Mouse	oral	2000-4000	1
Mouse	i.p.	550	1
Guinea pig	oral	830	1

#### Long-term studies

##### Rat

Chronic toxicity experiments were conducted over a period of 2 years, in which NDGA was compared with phenol, catechol and gum guaiac in concentrations of 0.5% in rats. NDGA had little or no effect on growth or food intake, except in the highest concentration, where there was a temporary decrease in growth associated with a decreased food intake. Histological study of the liver, spleen and kidneys showed no significant effect. Necrosis of the liver was noted occasionally in all groups, including controls.<sup>2</sup>

Concentrations of 0.1%, 0.5% and 1.0% of NDGA were used in another series of rats. Haemorrhage into the caecum was observed in 50% of the

animals. In a larger series of tests on rats at the same concentrations of NDGA, this haemorrhage did not occur. Cysts in the mesentery were found in several rats on the higher concentrations of NDGA.<sup>2</sup>

Two-year toxicity tests on groups of 10 male rats at 0%, 0.1%, 0.25%, 0.5% and 1% NDGA in the diet showed that 0.5% was the lowest level causing inflammatory caecal lesions and slight cystic enlargement of lymph nodes near the caecum. Growth inhibition occurred after the first 6 months at levels of 0.5% and 1%.<sup>1</sup>

In another experiment lasting 2 years, 0.5% of NDGA in the diet caused massive caecal haemorrhages, with single and multiple cysts in the mesentery in the angle of the junction between the small and large intestines. During the first 6 months, inhibition of the growth rate occurred only at the 1% level.<sup>1</sup>

#### *Mouse*

Long-term studies were carried out using 0.25% and 0.5% levels in mice. No deleterious effects on weight gain were observed, nor any important histological changes.<sup>2</sup>

#### **Biochemical aspects**

There does not appear to be any information on the metabolism of NDGA.<sup>3</sup> The effect of NDGA on enzyme systems has been studied. Specific inhibition of peroxidase, catalase and ethyl alcohol dehydrogenase occurs with a concentration of  $2 \times 10^{-4}$  M of the antioxidant. Non-specific inhibition of ascorbic acid oxidase, D-amino-acid oxidase, the cyclophorase system and urease at a concentration of  $2 \times 10^{-3}$  M has been described.<sup>4</sup>

#### **Comment on experimental studies reported**

The acute toxicity studies indicate that the guinea pig is more sensitive than the rat. All the long-term studies have, however, been carried out in the rat or mouse. This cannot be regarded as satisfactory. The long-term studies provide little detailed information and seem to have left some of the investigators in doubt about the acceptability of NDGA.

#### **Evaluation**

##### **Level causing no significant toxicological effect in the rat**

From the limited information available, it is not possible to evaluate the toxicological status of NDGA. No guidance can therefore be given at present on the use of NDGA as an antioxidant in food.

### Further work considered desirable

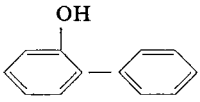
If the use of NDGA in food is to continue, the following work is considered desirable :

1. Further long-term studies in the rat and other species.
2. Metabolic studies in animals and man.

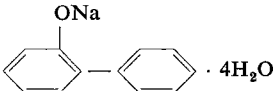
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### ***o*-PHENYLPHENOL \***

<b>Chemical names</b>	<i>o</i> -phenylphenol ; <i>o</i> -hydroxydiphenyl
<b>Empirical formula</b>	$C_{12}H_{10}O$
<b>Structural formula</b>	
<b>Molecular weight</b>	170.2
<b>Description</b>	White, crystalline, free-flowing powder with a mildly phenolic odour. Less than 0.1 g is soluble in 1 ml of water ; freely soluble in ethanol ; soluble in fats and oils.
<b>Use</b>	For the post-harvest treatment of fruits and vegetables to protect against microbial damage.

### **SODIUM *o*-PHENYLPHENOL**

<b>Chemical name</b>	Sodium <i>o</i> -phenylphenate
<b>Empirical formula</b>	$C_{12}H_9ONa \cdot 4H_2O$
<b>Structural formula</b>	

\* For biological data and toxicological evaluation see pp. 79-81.

<b>Molecular weight</b>	264.3
<b>Description</b>	A buff-coloured solid. Very soluble in water and ethanol ; practically insoluble in oils.
<b>Use</b>	As for <i>o</i> -phenylphenol.

### Biological Data

#### Acute toxicity

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	References
Rat	oral	2700-3000 (approx.)	1,2
Cat	oral	500 (approx.)	2

#### Short-term studies

##### *Rat*

Over a period of 32 days, groups of 15 male rats were fed *o*-phenylphenol in daily doses of 2, 20 and 200 mg/kg body weight. No harmful effect was demonstrable in any of the groups.<sup>2</sup>

5 male and 5 female rats in each group were given by stomach tube doses of 50, 100, 200 and 500 mg/kg body weight for 5 days a week over a period of 6 months. The only abnormality observed was a slight increase in average liver and kidney weights in the animals at the 500 mg/kg dosage.<sup>1</sup>

When diets containing 0.1%, 0.3%, 1.0% and 2% of *o*-phenylphenol were fed for 3 months to groups comprising 12 males and 12 females, slight retardation of growth was observed in the 2% group. There was no significant difference between the mortality of control and test animals. There were doubtful increases in weight of liver, kidney and spleen of certain rats of the 1% and 2% groups. No tissue changes were observed.<sup>1</sup>

##### *Dog*

Daily doses of 1000 mg/kg of *o*-phenylphenol killed 2 dogs within a month. Groups of 2 dogs each were fed *o*-phenylphenol for a period of 1 year in daily amounts of 20, 200 and 500 mg/kg body weight ; no effect related to the administration of *o*-phenylphenol was observed. Haematological values, urinary sugar and protein values, organ weights and histopathological examination of the various tissues did not differ from the normal range.<sup>1</sup>

##### *Man*

A 5.0% solution of *o*-phenylphenol in sesame oil and a 0.1% aqueous solution of the sodium salt tested on 200 subjects caused neither primary skin irritation nor skin sensitization.<sup>1</sup> The sodium salt is slightly irritating

in 0.5% aqueous solution and decidedly irritating in 1.0% and 5% solutions.

### Long-term studies

#### *Rat*

Male and female rats (25 of each sex per group) maintained for 2 years on diets containing 0.02% and 0.2% of *o*-phenylphenol showed no adverse effects when compared with a control group, as judged by growth, mortality, gross appearance, haematology, urinary sugar and protein values, organ weights, tissue content of *o*-phenylphenol, and histopathological examination of various tissues. A similar group of rats maintained for 2 years on a diet containing 2% of *o*-phenylphenol differed from the controls by exhibiting slight retardation of growth, histological kidney changes (marked tubular dilatation), and the presence of small amounts of *o*-phenylphenol in the kidney tissues.<sup>1</sup>

### Biochemical aspects

Storage of *o*-phenylphenol has not been observed in rats. In feeding experiments lasting 2 years, average values of 22 mg/100 g tissue were found in the kidneys of rats on a 2% diet, and approximately 1 mg/100 g tissue in the kidneys of rats on a 0.2% diet.<sup>1</sup>

It is known that the *o*- and *p*-hydroxydiphenyls are highly conjugated with glucuronic acids in the rabbit, but whether they form ethereal sulfates is not known.<sup>3</sup>

### Comment on experimental studies reported

From experiments on rats and dogs, *o*-phenylphenol does not seem to be a very toxic substance. The noted difference in acute toxicity between these animals and cats might be explained by the known high sensitivity of cats to phenolic compounds. The long-term study in rats is taken as a basis for the evaluation.<sup>1</sup>

### Evaluation

#### Level causing no significant toxicological effect in the rat

0.2% (= 2000 p.p.m.) in the diet, equivalent to 100 mg/kg body weight per day.

#### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.2
Conditional acceptance . . . . .	0.2-1.0

**Further work considered desirable**

Metabolic studies in experimental animals and man.

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**PHOSPHORIC ACID**

<b>Chemical names</b>	Phosphoric acid ; orthophosphoric acid
<b>Empirical formula</b>	$\text{H}_3\text{PO}_4$
<b>Molecular weight</b>	98.0
<b>Definition</b>	Phosphoric acid contains not less than 85% of $\text{H}_3\text{PO}_4$ .
<b>Description</b>	Clear, colourless, odourless liquid of syrupy consistency. Miscible with water and ethanol.
<b>Natural occurrence</b>	Phosphorus-containing substances occur very widely in natural foods, usually as free phosphoric acid or as the potassium, sodium or calcium salts. Phosphate is found in highest concentrations (0.1-0.5% or more, in terms of phosphorus) in such foods as milk, cheese, nuts, fish, meat, poultry, eggs (yolk), and certain cereals.
<b>Uses</b>	As a sequestrant, an antioxidant and a "synergist" for other antioxidants ; also as an acidulant and flavour in beverages and fruit products.

**Biological Data****Short-term studies***Rat*

Pathological effects in the parathyroids, kidneys and bones have been observed in mature male rats fed a diet containing an excessively high level (8%) of sodium orthophosphate for 7 months or until the animals succumbed.<sup>1</sup> Histological and histochemical changes in the kidneys have been found in rats fed for 24 to 72 hours on a diet containing an excess of inorganic phosphate (10% disodium acid phosphate).<sup>2</sup>

There are many other reports of adverse effects produced in rats and other laboratory animals by an excessive intake of inorganic phosphate.<sup>3, 4, 5, 6, 7</sup>

Three groups of 12 rats each were fed diets containing added dibasic potassium phosphate so that the calcium and phosphorus concentrations in the experimental diets were as follows :

Diet	Calcium %	Phosphorus %
Control	0.56	0.42
" Normal orthophosphate "	0.47	0.43
" High orthophosphate "	0.50	1.30

The experiment was conducted in three stages, with experimental observations made when animals had consumed the test diets for 50, 60 and 150 days. No adverse physiological effects were observed clinically, at autopsy or on histological examination. All the data obtained from this study indicated that there was probably adequate absorption and utilization of calcium, phosphorus and iron with both high and normal levels of orthophosphate.<sup>8</sup>

#### *Man*

Studies on 15 students, who drank 2000-4000 mg of phosphoric acid in fruit juices every day for 10 days, and on 2 males who received 3900 mg of phosphoric acid every day for 14 days revealed no observable change in urine composition indicative of a disturbed metabolism.<sup>9</sup>

#### **Long-term studies**

##### *Rat*

Three successive generations of rats were fed diets containing 0.4% and 0.75% of phosphoric acid for 90 weeks. No harmful effect on growth or reproduction could be observed. No significant differences were noted in the blood picture in comparison with control rats and there was no other pathological finding which was attributable to the diets. There was no acidosis nor any change in the calcium metabolism. The dental attrition was somewhat more marked than that in the control rats.<sup>10</sup>

#### **Biochemical aspects**

Phosphoric acid is an essential constituent of the human organism, not only in the bones and teeth, but also in many enzyme systems. Phosphorus plays an important role in carbohydrate, fat and protein metabolism.

The daily intake of phosphate necessary for man lies between 1 and 2 g. Insufficient supply of phosphate produces deficiency in the bones. Since the phosphate concentration of serum and tissues is maintained by physio-

logical regulations, the intestinal absorption depends on requirements and is therefore limited. Doses of 2 to 4 g act as weak saline cathartics. Excretion takes place mainly in the faeces as calcium phosphate, so that the continuous use of excessive amounts of sodium phosphate and phosphoric acid may cause a loss of calcium.

There have been a great many publications on phosphorus metabolism,<sup>11</sup> on the interrelationships of calcium and phosphorus in foods and nutrition,<sup>12</sup> and on the impact thereon of the use of phosphate as a food additive.<sup>13</sup>

#### Comments on experimental studies reported

Phosphoric acid is a material that should not be evaluated solely on the basis of toxicological studies in animals.

There are strong indications that phosphoric acid should not be used in such a manner as to result in excessively high phosphorus levels in the total diet, adverse alterations in the mineral balance of the diet (i.e., Ca/P ratio), or an appreciable increase in the total mineral content of the diet as a whole.

However, there is ample evidence to support the safety of the addition of small quantities of phosphoric acid to food. Thus, the use of 0.01%-0.02% as a sequestrant, an antioxidant or "synergist" in antioxidant mixtures should present no health hazards, whatsoever.

Moreover, the use of phosphoric acid to compensate for deficiency of fruit acidity, as a flavour component, and in other ways, essentially within the "normal" concentration of phosphates naturally occurring in foods, should present no problems.

#### Evaluation

##### Level causing no significant toxicological effect in the rat

0.75% (= 7500 p.p.m.) in the diet, equivalent to 375 mg/kg body weight per day.

##### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-5
Conditional acceptance . . . . .	5-15

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### PROPIONATES OF SODIUM, POTASSIUM AND CALCIUM

Chemical names	Sodium propionate	Potassium propionate	Calcium propionate
Empirical formula	$C_3H_5O_2Na$	$C_3H_5O_2K$	$C_6H_{10}O_4Ca$
Structural formula	$CH_3 \cdot CH_2 \cdot COONa$	$CH_3 \cdot CH_2 \cdot COOK$	$\begin{array}{l} CH_3 \cdot CH_2 \cdot COO \\ CH_3 \cdot CH_2 \cdot COO \end{array} \rangle Ca$
Molecular weight	96.06	112.17	186.23
Definition	Not less than 99% in the dry state.		
Description	White or colourless, crystalline solids; soluble in water to about 30%		
Natural occurrence	Propionates occur naturally in fermented foods, as well as in human perspiration and in the products of rumen digestion.		
Use	As mould inhibitors in foodstuffs.		

### Biological Data

#### Acute toxicity

The  $LD_{50}$  of propionic acid administered orally to the rat was found to be 2600 mg/kg body weight.<sup>1</sup> The sodium and potassium salts were well tolerated.<sup>2, 3</sup> Solutions of propionates applied to the eye in concentrations up to 15% in man and up to 20% in rabbits had no irritating effect.<sup>4</sup>

**Short-term studies***Rat*

4 groups of 8 rats were kept for 4 weeks on diets containing 1% and 3% of sodium or calcium propionate. No effect on growth was observed.<sup>5</sup>

*Man*

In an adult male, daily oral doses of 6000 mg of sodium propionate rendered the urine faintly alkaline but had no other effect.<sup>3</sup>

**Long-term studies***Rat*

Sodium propionate fed to large numbers of rats in the diet at a concentration of 3.75% for one year had no effect on growth or mortality rate, nor any other toxicological effect as judged by the organ weights and histological findings in the organs studied.<sup>6, 7</sup>

**Biochemical aspects**

Propionates are metabolized and utilized in the same way as a normal fatty acid.<sup>8</sup> Isotopic sodium propionate fed to fasted rats gave rise to glucose in the liver.<sup>9</sup> In the mouse, <sup>14</sup>C-tagged propionate has been shown to act as a precursor of body fat,<sup>10</sup> and to be incorporated into the odd-numbered fatty acids of milk fat.<sup>11</sup> Furthermore, the labelled carbon has been found in CO<sub>2</sub>, glucose, succinate, malate, fumarate and proteins.<sup>12</sup> Propionate combines with coenzyme A in the same way as acetic acid, becoming carboxylated to methyl-malonyl-coenzyme A and undergoing a quantitative conversion to succinate which then enters the Krebs cycle.<sup>13</sup> According to *in vitro* experiments, acetate metabolism may be inhibited by propionate. The production of <sup>14</sup>CO<sub>2</sub> from labelled acetate by rat liver homogenates was almost abolished by propionate in only one tenth the concentration of the acetate.<sup>14</sup> Inhibition of catalase activity has been reported.<sup>15</sup>

**Comments on experimental studies reported**

There are no toxicological studies of longer duration than one year. *In vivo* studies bearing on the possibility of interference with acetate metabolism are lacking, but there is sufficient evidence that propionate is metabolized as a normal fatty acid.

**Evaluation****Levels causing no significant toxicological effect in the rat**

3.75% (= 37 500 p.p.m.) in the diet, equivalent to 2000 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-10
Conditional acceptance . . . . .	10-20

**Further work considered desirable**

Biochemical studies on interference of propionate with acetate metabolism *in vivo*.

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**SALICYLIC ACID**

**Chemical names** . . . . . *o*-Hydroxybenzoic acid ; *o*-oxybenzoic acid

**Empirical formula** . . . . .  $C_7H_6O_3$

**Structural formula** . . . . .

**Molecular weight** . . . . . 138.1

**Definition** . . . . . Salicylic acid contains not less than 99.5% of  $C_7H_6O_3$  after drying over sulfuric acid for 3 hours.

<b>Description</b>	A white or colourless, odourless, crystalline solid with a sweetish, ultimately acrid taste. 1 g is soluble in 460 ml of water, 2.7 ml of ethanol or about 80 ml of fats or oils.
<b>Use</b>	As an antimicrobial preservative ; its efficiency is increased 100 times in strongly acid solutions.

### Biological Data \*

#### Acute toxicity

	Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Salicylic acid	rat	oral	1500-2000	1
	rabbit	i.v.	600	2
Sodium salicylate	rat	oral	1600	3
	"	s.c.	650	4
	mouse	oral	900	3
	rabbit	oral	1700	3

#### Short-term studies

##### Rat

Rats received 0.4, 0.5 and 0.6 g of salicylic acid per kg body weight each day for 4-21 days. 0.5 g/kg body weight proved fatal to 50% of the animals within 2 weeks. 0.6 g/kg body weight usually caused death (of non-hepatic origin) within 10 days. The lowest total dose causing moderate necrosis of the liver and kidney was 1.6 g/kg, and that causing severe necrosis 2.8 g/kg.<sup>5</sup>

Rats were fed 2, 10, and 100 mg of salicylic acid for 60 days. All animals survived. Mild hypoprothrombinaemia was observed at the 10-mg level, and severe hypoprothrombinaemia developed after 20 days on 100 mg. At 300 mg per day, rats developed severe hypoprothrombinaemia within 5 days, and the average survival time was 10 days. Haemorrhagic manifestations developed.<sup>6</sup>

Young male rats (weighing 20-35 g) were fed for 6 weeks on basal diets containing 0.1%, 0.25% and 0.5% of sodium salicylate. Growth was not affected by concentrations of 0.1% and 0.25%, but was definitely retarded by 0.5%.<sup>7</sup>

For 8 weeks, 20 young rats were fed on diets to which was added 1% or 5% of salicylic acid. The 5% level was definitely toxic. The 1% level appeared to be toxic only near the end of the experiment. During the first 3 weeks of feeding there was a stimulation of growth.<sup>8</sup>

\* From a toxicological point of view salicylic acid and salicylates may be taken together.

*Mouse*

Ten mice were given, by stomach tube, 0.1 g of salicylic acid per kg body weight daily for 57 days. There were no significant changes in weight gain or food intake compared with control animals. Histopathological examination showed no change in the liver and only an insignificant change in the kidney. Ten other mice received 0.3 g/kg body weight by stomach tube each day for 34 days. Six animals died during this period. Histopathological examination of all animals showed significant degenerative changes of the liver and kidney, necrosis of the liver cells, and fatty infiltration of the liver.<sup>9</sup>

*Rabbit*

When salicylic acid was given to rabbits by mouth, one animal died after ingesting 700 mg/kg, another survived a dose of 1100 mg/kg. Autopsy showed fatty degeneration of the heart muscle, liver and kidney, and haemorrhagic manifestations, especially in the lung.<sup>10</sup> Nineteen rabbits received sodium salicylate subcutaneously in amounts varying from 0.15 to 1 g/kg body weight per day. A correlation was found between the severity of hypoprothrombinaemia and the level of salicylate in the plasma.<sup>11</sup>

*Dog*

Necrosis of the liver and kidneys was found in dogs after administration of 0.3 g/kg body weight per day for 2 weeks.<sup>5</sup> The lethal dose in 10 dogs varied from 900 to 1540 mg/kg when sodium salicylate was administered by slow intravenous injection.<sup>12</sup>

*Man*

The therapeutic use of salicylic acid and the salicylates has yielded a great amount of information on its action. The ingestion of large amounts of the acid may irritate the gastric mucosa and produce headache, nausea, and vomiting.<sup>13, 14, 15</sup> Long-term intake of salicylic acid may result in kidney damage of a reversible nature. As a side effect of the therapeutic use, reversible auditory disturbances have been observed in many patients.<sup>16</sup> The lethal dose for man seems to lie between 20 and 30 g.

In many instances, patients who have been given large doses of sodium salicylate have shown increased prothrombin times. In a study on 113 men with rheumatic fever, 57 served as controls while the other 56 received orally 3.2 g of sodium salicylate for the first week, 6.4 g daily for the second week, 9.6 g daily for the third week, and 12 g daily for the fourth week. There was an increase in prothrombin time. No haemorrhagic manifestations were observed, nor was there any deleterious effect on the hepatic parenchyma, but slight reductions in the haemoglobin content and erythrocyte count were noted.<sup>17</sup>

In another experiment, no significant prothrombin change was observed in 15 non-rheumatic patients given 1, 2, 3, and 6 g of sodium salicylate daily. It was generally found that small doses of salicylate had no clinically significant thrombopenic effect.<sup>18</sup> Sodium salicylate was given orally to patients in amounts varying from 0.12 to 0.29 g/kg at 4-hourly intervals over periods of 4 to 16 weeks, so that the plasma levels remained in the range 300-400 µg/ml.

There was no evidence of marked liver damage. The prothrombin time was lengthened. Haemorrhagic manifestations occurred in only one patient. Above 500 µg/ml plasma salicylate level, 23 patients showed more or less severe disturbances of the central nervous system. Among 17 patients the carbon dioxide combining power of the plasma was reduced in 14 cases.<sup>19</sup> In another study where 10 g of sodium salicylate were given to 35 patients for 4-10 days, severe delirium occurred in 6 patients.<sup>20</sup>

#### Long-term studies

No information available

#### Biochemical aspects

Salicylic acid is rapidly absorbed and distributed throughout the whole body. It has been detected in brain tissues, eyes, milk, etc. It may take several days to eliminate the acid after a single dose. It appears in the urine both in free and in conjugated form.

#### Comment

On the basis of published evidence salicylic acid is considered unsuitable as a food additive.

#### Further work considered desirable

Long-term studies in animals.

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### SORBIC ACID

<b>Chemical names</b>	Sorbic acid ; <i>trans-trans</i> -2,4-hexadienoic acid
<b>Empirical formula</b>	$C_6H_8O_2$
<b>Structural formula</b>	$  \begin{array}{c}  \text{CH}_3 \quad \quad \text{H} \\  \diagdown \quad \diagup \\  \text{C} = \text{C} \\  \diagup \quad \diagdown \\  \text{H} \quad \quad \text{H} \\  \quad \quad \quad \diagdown \quad \diagup \\  \quad \quad \quad \text{C} = \text{C} \\  \quad \quad \quad \diagup \quad \diagdown \\  \quad \quad \quad \text{H} \quad \quad \text{COOH}  \end{array}  $
<b>Molecular weight</b>	112.13
<b>Definition</b>	Sorbic acid contains not less than 99% of $C_6H_8O_2$ after drying for 4 hours in a vacuum desiccator over sulfuric acid.
<b>Description</b>	White crystalline solid with a mildly acid odour. Soluble 0.25% in water at 30°C, 12.9% in absolute ethanol, and 0.6-0.8% in glycerides.
<b>Natural occurrence</b>	Sorbic acid is present, in association with parasorbic acid, in fruits of <i>Sorbus aucuparia</i> L.
<b>Use</b>	As an antimicrobial preservative ; it is an active agent against moulds and yeasts and to a lesser degree against bacteria. The optimum pH is 4.5. Fungistatic activity is increased by addition of acids and sodium chloride.

**Biological Data \*****Acute toxicity**

Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Rat	oral	10 500	1

**Short-term studies***Rat*

Tests with two different strains of rats in two separate laboratories showed that ingestion of diets containing 4% and 8% of sorbic acid for a period of 90 days did not affect the rate of weight gain. The animals receiving 4% of sorbic acid showed no abnormality of renal, hepatic or other tissues. Rats on the 8% diet showed a slight but statistically significant increase in relative liver weight. The histopathological appearance of the liver was, however, normal.<sup>1</sup>

*Dog*

In dogs fed for 3 months on a diet containing 50% of cheddar cheese to which 4% of sorbic acid or 4% of caproic acid had been added, the response was similar to that in dogs on the same cheese diet without such supplements. No histopathological differences were observed in tissues obtained from any of the 3 groups.<sup>1</sup>

**Long-term studies***Rat*

Feeding 5% of sorbic acid in the diet to groups of 100 rats for 1000 days in two generations had no effect on weight gain or reproduction. No ill effects were observed, and no sorbic acid could be found in the urine.<sup>2</sup>

**Biochemical aspects**

Sorbic acid did not act as an antimetabolite for essential fatty acids in the rat. The incorporation of sorbic acid into the diet of rats did not decrease the efficiency of utilization of calories. Sorbic acid is used by the animal organism as a source of calories.<sup>1</sup> Sorbic acid is metabolized similarly to caproic acid.<sup>3, 4, 5</sup>

Enzyme studies have shown that a concentration of 0.112% of sorbic acid inhibited catalase activity by 72-77%.<sup>6</sup> Sulfhydryl enzymes in ficin and alcohol dehydrogenase were inhibited at a concentration of  $10^{-4}$ M.

\* From a toxicological point of view, sorbic acid and sorbates may be taken together.

Aldolase and urease were not significantly inhibited by sorbic acid. Irradiated sorbic acid was a stronger inhibitor of ficin than sorbic acid.<sup>7</sup> This inhibition of dehydrogenase is the main basis for the fungistatic activity of sorbic acid. It appears to be impossible to give sufficient sorbic acid to inhibit the dehydrogenase enzyme systems in the animal body.<sup>8</sup>

When sorbic acid is incorporated into food it may undergo oxidation, with the formation of peroxides and secondary oxidation products. In the presence of sufficient metabolizable carbohydrates the end-products are carbon dioxide and water. If metabolizable carbohydrates are not present, acetoacetate and acetone are also produced.<sup>9</sup>

#### Comment on experimental studies reported

Sorbic acid is a relatively simple compound that appears to be readily metabolized in the animal body. This and the long-term studies reported<sup>2</sup> form the basis for evaluation.

#### Evaluation

##### Level causing no significant toxicological effect in the rat

5% (= 50 000 p.p.m.) in the diet, equivalent to 2500 mg/kg body weight per day.

##### Estimate of acceptable daily intakes for man

	mg/kg body weight
Unconditional acceptance . . . . .	0-12.5
Conditional acceptance . . . . .	12.5-25.0

#### Further work considered desirable

1. Further long-term studies, especially in some other species than the rat.
2. Metabolic studies in man.

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### SULFUR DIOXIDE \*

<b>Chemical names</b>	Sulfur dioxide ; sulfurous acid anhydride
<b>Empirical formula</b>	SO <sub>2</sub>
<b>Molecular weight</b>	64.1
<b>Definition</b>	Sulfur dioxide contains not less than 95% SO <sub>2</sub> .
<b>Description</b>	A colourless, non-inflammable gas with a strong, pungent, suffocating odour. Soluble in water and ethanol.
<b>Use</b>	As an antimicrobial preservative and as an anti-browning agent.

### SODIUM SULFITE \*

<b>Chemical name</b>	Sodium sulfite
<b>Empirical formula</b>	<i>Anhydrous</i> : Na <sub>2</sub> SO <sub>3</sub> <i>Heptahydrate</i> : Na <sub>2</sub> SO <sub>3</sub> .7H <sub>2</sub> O
<b>Molecular weight</b>	<i>Anhydrous</i> : 126.05 <i>Heptahydrate</i> : 252.16
<b>Definition</b>	Anhydrous sodium sulfite contains not less than 95.0% Na <sub>2</sub> SO <sub>3</sub> . Sodium sulfite heptahydrate contains not less than 48.0% Na <sub>2</sub> SO <sub>3</sub> .
<b>Description</b>	Anhydrous sodium sulfite is a white powder, with not more than a faint odour of sulfur dioxide ; 1 g is soluble in 4 ml of water. Sodium sulfite heptahydrate is a transparent or white crystalline solid, with not more than a faint odour of sulfur dioxide ; 1 g is soluble in 2 ml of water.
<b>Use</b>	As an antimicrobial preservative and as an anti-browning agent.

### SODIUM METABISULFITE \*

<b>Chemical name</b>	Sodium pyrosulfite
<b>Empirical formula</b>	Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub>
<b>Molecular weight</b>	190.1

\* For biological data and toxicological evaluation see pp. 94-97.

<b>Definition</b>	Sodium pyrosulfite contains not less than 95.0% of $\text{Na}_2\text{S}_2\text{O}_5$ .
<b>Description</b>	A white crystalline solid, with an odour of sulfur dioxide. 1 g is soluble in 2 ml of water.
<b>Use</b>	As an antimicrobial preservative and as an anti-browning agent.

### SODIUM HYDROGEN SULFITE

<b>Chemical names</b>	Sodium hydrogen sulfite ; sodium bisulfite ; sodium acid sulfite
<b>Empirical formula</b>	$\text{NaHSO}_3$
<b>Molecular weight</b>	104.06
<b>Definition</b>	Sodium hydrogen sulfite contains not less than 95% of $\text{NaHSO}_3$ .
<b>Description</b>	A white crystalline or granular solid, with an odour of sulfur dioxide. 1 g is soluble in 2.5 ml of water.

### Biological Data

#### Acute toxicity

In rabbits, the oral  $\text{LD}_{50}$  of sulfite, measured as  $\text{SO}_2$  was found to be between 600 and 700 mg/kg body weight.<sup>1</sup>

Animal	Route	$\text{LD}_{50}$ (mg/kg body weight)		Reference
		Sodium bisulfite	Sodium sulfite	
Mouse	i.v.	130	175	2
Rat	i.v.	115	—	2
Hamster	i.v.	95	—	2
Rabbit	i.v.	65	—	2

In man, a single oral dose of 4 g of sodium sulfite caused toxic symptoms in 6 of 7 persons. In another subject, 5.8 g caused severe irritation of the stomach and intestine.<sup>1</sup>

#### Short-term studies

##### Rabbit

One rabbit given 3 g of sodium sulfite by stomach tube each day for 185 days lost weight, but all organs were normal post-mortem. Two rabbits given 1.08 g daily for 127 days gained weight. Autopsy showed haemorrhages in the stomach. Three rabbits given 1.8 g daily for between 46 and 171 days lost weight and autopsy showed stomach haemorrhages.<sup>1</sup>

*Dog*

A dose of 3 g of sodium sulfite daily was given by stomach tube to a dog weighing 17 kg for 23 days. Another dog weighing 34 kg was given 6-16 g of sodium sulfite daily for 20 days (total dose 235 g). No abnormalities were observed on autopsy in the first dog, but the second dog had haemorrhages in several organs. Sodium sulfite was given by stomach tube to 16 growing dogs in daily doses of 0.2-4.8 g for 43-419 days; no damage was observed in any of the dogs. Sodium bisulfite was given to 2 dogs by the same method and for the same length of time as in the preceding experiment in daily doses of 1.08-2.51 g. Examination of heart, lungs, liver, kidney, and intestine showed no damage. A total of 91-265 g of sodium sulfite fed to 5 pregnant dogs over a period of 60 days had no effect on the weight of the mothers or on the weight gain of the litters.<sup>1</sup>

*Rat*

In thiamine-deficient rats, daily oral administration of fruit syrup containing 350 p.p.m. of sulfur dioxide in a dose of 0.5 ml/150 g rat for 8 weeks failed to influence growth.<sup>3</sup>

**Long-term studies***Rat*

Groups of rats numbering from 18 to 24 per group were fed sodium bisulfite in dosages of 0.0125%, 0.025%, 0.05%, 0.1%, 0.25%, 0.5%, 1% or 2% of the diet for periods ranging from 1 to 2 years. The rats fed 0.05% sodium bisulfite (307 p.p.m. as SO<sub>2</sub>) for 2 years showed no toxic symptoms. Sulfite in concentrations of 0.1% (615 p.p.m. as SO<sub>2</sub>), or more, in the diet inhibited the growth of the rats, probably through destruction of thiamine in the diet.<sup>4</sup>

**Biochemical aspects**

Sulfite is oxidized in the body to sulfate. Bisulfite reacts with aldehydes and ketones, including aldehydic sugars. This is a reversible reaction; the equilibrium concentrations depend on temperature. The acute effects of sulfite in foods are related to the amount and concentration of free sulfur dioxide and to the speed at which the additive compounds liberate the bound sulfur dioxide. Sulfite may also react reversibly with disulfide linkages in proteins. The disulfide is split into one part containing a thiol group and another part with an S-sulfonic acid group.<sup>5</sup>

**Comment on experimental studies reported**

Sufficient data are not available to indicate the lowest dosage causing acute effects in man, nor the highest dosage that will normally be tolerated

without producing harmful effects. The position of the lowest level at which sulfite produced a significant effect in the long-term experiments in rats<sup>4</sup> may have been determined by the destruction of thiamine in the diet, rather than by a direct action of sulfite on the animals.

### Evaluation

#### Level causing no significant toxicological effect in the rat

0.05% of sodium bisulfite (= 307 p.p.m. as  $\text{SO}_2$ ) in the diet, equivalent to 15 mg/kg body weight per day, calculated as  $\text{SO}_2$ .

#### Estimate of acceptable daily intakes for man (calculated as $\text{SO}_2$ )

	mg/kg body weight
Unconditional acceptance . . . . .	0-0.35
Conditional acceptance . . . . .	0.35-1.5

#### Comment

1. Acute effects, limited principally to irritation of the gastrointestinal tract, are serious in some instances, particularly in persons consuming treated beverages. In this connexion, off-flavour of the treated food has been considered as a deterrent to excessive  $\text{SO}_2$  treatment.<sup>6</sup> However, it has been pointed out<sup>7</sup> that stomach acidity plays a part in recognition of this off-flavour in wine, and since some persons may consume a toxic level before becoming aware of the off-flavour, tolerance levels need to be established.

2. Sulfite preserves colour and restores the redness of dull-coloured meat; it may thus serve to mask any putrefaction. Since it does not prevent putrefactive processes in meat, it may lead to deception regarding freshness and to possible injury from the consumption of tainted meat. For this reason, sulfite should not be used for meat.

3. Thiamine, which is an essential element of the human diet, is destroyed by treatment with sulfites. For this reason, foods that serve as a significant source of thiamine, such as meat, cereal grain, dairy products and nuts, should not be treated with sulfites.

#### Further work considered desirable

1. More data should be collected on the acute effects of sulfites in man.
2. Further studies in a second species are needed to confirm and/or to elucidate the connexion between the toxic action of sulfites and the destruction of thiamine.

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### TARTARIC ACID

<b>Chemical names</b>	Tartaric acid ; 2,3-dihydroxysuccinic acid
<b>Empirical formula</b>	$C_4H_6O_6$
<b>Structural formula</b>	$HOOC-CHOH-CHOH-COOH$
<b>Molecular weight</b>	150.09
<b>Definition</b>	Tartaric acid contains not less than 99.5% of $C_4H_6O_6$ after drying to constant weight at 105°C.
<b>Description</b>	A colourless or translucent, odourless, crystalline solid with an acid taste. 1 g is soluble in 0.8 ml of water or 3 ml of ethanol.
<b>Natural occurrence</b>	Tartaric acid occurs in many fruits, either free or combined with potassium, calcium or magnesium. It may constitute from 60-80% of the non-volatile acid in wine.
<b>Use</b>	As a synergist with antioxidants and as an acidulant. Acidic constituent in some baking powders.

### Biological Data

#### Acute toxicity

In the mouse, the  $LD_{50}$  of the sodium salt administered by mouth was found to be 4360 mg/kg body weight.<sup>1</sup>

Tartaric acid administered by stomach tube in a dose of 5000 mg/kg was fatal to a dog.<sup>2</sup>

Three out of 7 male rabbits died following oral administration of disodium tartrate in an average dose of 5290 mg/kg ; while 6 male rabbits survived an average oral dose of 3680 mg/kg.<sup>1</sup>

**Short-term studies***Rabbit*

Three rabbits survived 17 consecutive daily feedings of disodium tartrate in an average dosage of 1150 mg/kg; whereas average dosages of 3680 mg/kg killed 3 out of 6 rabbits in 6 to 19 consecutive daily feedings.<sup>1</sup>

*Dog*

Tartaric acid was administered in daily oral doses of 990 mg/kg to each of 4 dogs for 90-114 days. Casts appeared in the urine of 3 dogs; the blood chemistry remained normal except in one dog in which azotaemia developed with death in 90 days. Weight changes varied from a gain of 30% to a loss of 32%.<sup>3</sup>

**Long-term studies***Rat*

Groups of 24 rats (12 of each sex) were fed diets containing 0.1%, 0.5%, 0.8% and 1.2% of tartaric acid for a period of 2 years. A group of 48 rats served as controls. No significant toxic effects were observed in any of the groups as determined by growth rate (for the first year), mortality throughout the experiment, and gross and microscopic findings at the end of the 2-year period. An exceptionally thorough microscopic pathological examination was carried out.<sup>4</sup>

**Biochemical aspects**

Tartaric acid is metabolically inert in the human body.<sup>5, 6, 7, 8</sup> When taken by mouth, only about 20% of ingested tartrate is eliminated in the urine; the remainder is not absorbed but destroyed in the intestinal tract by bacterial action.

**Comments on experimental studies reported**

The 2-year feeding experiment on rats and the metabolic studies in man provide a good basis for estimating the safe intake in man.

**Evaluation****Level causing no significant toxicological effect in the rat**

1.2% (= 12 000 p.p.m.) in the diet, equivalent to 600 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-3
Conditional acceptance . . . . .	3-10

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## THIODIPROPIONIC ACID \*

<b>Chemical names</b>	$\beta,\beta'$ -thiodipropionic acid ; 3,3'-thiodipropionic acid
<b>Empirical formula</b>	$C_6H_{10}O_4S$
<b>Structural formula</b>	$\begin{array}{c} CH_2 - CH_2 - COOH \\   \\ S \\   \\ CH_2 - CH_2 - COOH \end{array}$
<b>Molecular weight</b>	178.21
<b>Description</b>	Leaflets from hot water. 1 g dissolves in 26.9 ml of water at 26°C. Freely soluble in hot water and ethanol.
<b>Use</b>	As an antioxidant for fats and other foodstuffs.

## DILAURYL THIODIPROPIONATE \*

<b>Chemical names</b>	Dilauryl ester of $\beta,\beta'$ -thiodipropionic acid ; dilauryl ester of 3,3'-thiodipropionic acid
<b>Empirical formula</b>	$C_{30}H_{58}O_4S$
<b>Structural formula</b>	$\begin{array}{c} CH_2 - CH_2 - COO - (CH_2)_{11} - CH_3 \\   \\ S \\   \\ CH_2 - CH_2 - COO - (CH_2)_{11} - CH_3 \end{array}$
<b>Molecular weight</b>	514.8
<b>Use</b>	As an antioxidant for fats and other foodstuffs.

\* For biological data and toxicological evaluation see pp. 100-101.

**DISTEARYL THIODIPROPIONATE**

<b>Chemical names</b>	Distearyl ester $\beta,\beta'$ -thiodipropionic acid ; distearyl ester of 3,3'-thiodipropionic acid
<b>Empirical formula</b>	$C_{42}H_{82}O_4S$
<b>Structural formula</b>	$\begin{array}{c} \text{CH}_2 - \text{CH}_2 - \text{COO} - (\text{CH}_2)_{17} - \text{CH}_3 \\   \\ \text{S} \\   \\ \text{CH}_2 - \text{CH}_2 - \text{COO} - (\text{CH}_2)_{17} - \text{CH}_3 \end{array}$
<b>Molecular weight</b>	683.18
<b>Use</b>	As an antioxidant for fats and other foodstuffs.

**Biological Data****Acute toxicity**

	Animal	Route	LD <sub>50</sub> (mg/kg body weight)	Reference
Thiodipropionic acid	mouse	oral	2000	1
	"	i.p.	250	1
	"	i.v.	175	1
	rat	oral	3000	1
	"	i.p.	500	1
	"	i.v.	> 300	1
Dilauryl thiodipropionate	mouse	oral	> 2000	1
	"	i.p.	> 2000	1
	rat	oral	> 2500	1
Distearyl thiodipropionate	mouse	oral	> 2000	1
	"	i.p.	> 2000	1
	rat	oral	> 2500	1

**Short-term studies***Rat*

Mixtures of thiodipropionic acid and dilauryl thiodipropionate were heated with lard in concentrations at least 10 times that recommended for use and fed to rats. There was no detectable toxic effect.<sup>1</sup>

*Guinea-pig*

Thiodipropionic acid was fed to guinea-pigs at a level of 0.5% in the drinking water for a period of 120 days. No significant effect was noted on weight or mortality.<sup>1</sup>

*Dog*

Dogs were fed a mixture of 10 parts by weight of dilauryl thiodipropionate and one part of thiodipropionic acid in the diet at concentrations of 0.1% and 3.0% of the acid-ester mixture. No untoward effects were noted over a period of 100 days.

**Long-term studies***Rat*

Thiodipropionic acid or its dilauryl or distearyl ester was fed to groups of 20 rats each at levels of 0%, 0.5%, 1.0%, and 3.0% in the diet for a period of 2 years. No discernable adverse effects were observed in any of the experimental animals, as determined by growth rate, mortality, and pathological examination.<sup>1</sup>

**Biochemical aspects**

No information available.

**Comment on experimental studies reported**

It would be desirable to have further details on the experimental work that has been carried out on thiodipropionic acid; the published report gives only a summary. Biochemical studies and observations on human subjects are not available.

**Evaluation****Level causing no significant toxicological effect in the rat**

3.0% (= 30 000 p.p.m.) in the diet, equivalent to 1500 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-3.0
Conditional acceptance . . . . .	3.0-15.0

**Further work considered desirable**

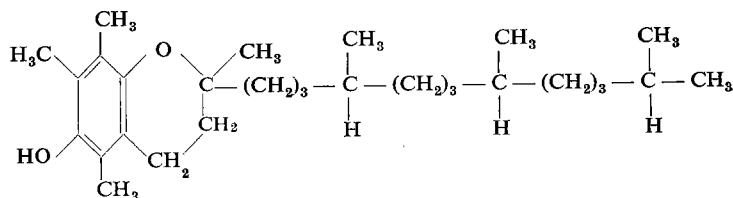
1. Long-term studies in a species other than the rat.
2. Biochemical studies in animals and in man.

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**$\alpha$ -TOCOPHEROL \***

<b>Chemical name</b>	DL- $\alpha$ -Tocopherol ; D- $\alpha$ -tocopherol ; 2,5,7,8-tetramethyl-2-(4',8',12'-trimethyldecyl)-6-chromanol
<b>Synonym</b>	Vitamin E
<b>Empirical formula</b>	$C_{29}H_{50}O_2$
<b>Structural formula</b>	



<b>Molecular weight</b>	430.72
<b>Definition</b>	$\alpha$ -Tocopherol may be obtained by vacuum distillation of edible vegetable oils, or it may be prepared synthetically. It contains not less than 96% of $C_{29}H_{50}O_2$ .
<b>Description</b>	A yellow, nearly odourless, clear, viscous oil. It oxidizes and darkens in air and on exposure to light.
<b>Natural occurrence</b>	Natural $\alpha$ -tocopherol is the D-form. About 90% of the tocopherol of animal tissues is D- $\alpha$ -tocopherol which has a higher vitamin E activity than its congeners. The synthetic form is DL.
<b>Use</b>	As an antioxidant in edible oils and fats.

**MIXED TOCOPHEROLS CONCENTRATE**

<b>Synonym</b>	Vitamin E concentrate
<b>Definition</b>	Mixed tocopherols concentrate contains not less than 34% total tocopherols ; of the total tocopherols present not less than 50% consists of DL- $\alpha$ -tocopherol, $C_{29}H_{50}O_2$ .
<b>Description</b>	A brownish-red to red, nearly odourless, clear viscous oil. It oxidizes and darkens slowly in air and on exposure to light. Insoluble in water and miscible in ethanol
<b>Use</b>	As an antioxidant.

\* For biological data and toxicological evaluation see pp. 103-104.

## Biological Data

### Acute toxicity

LD<sub>50</sub> values are not known.

### Short-term studies

#### *Rat and mouse*

Rats receiving  $\alpha$ -tocopherol in a dosage of 100 mg/rat/day for 19 weeks showed an increase in phosphorus metabolism, but no effect was found when the dose was 10 mg/rat/day.<sup>1</sup>

It has been found that mice will tolerate oral doses of 50 g/kg and rats 4 g/kg daily for 2 months.<sup>2</sup>

#### *Man*

Adult humans have tolerated 1 g/day for months or larger doses for shorter periods with no undesirable effects. Therapeutically, daily doses of 20-600 mg of  $\alpha$ -tocopherol or its acetate are often taken with no toxic effects.<sup>3, 4, 5</sup> The clinical literature contains references to complaints of gastric distress and other symptoms in patients receiving much smaller dosages; these symptoms are probably attributable to fatty substances present in tocopherol concentrates or, in some instances, to psychic factors.<sup>3</sup>

### Biochemical aspects

The metabolic fate of  $\alpha$ -tocopherol is not fully known. When rats are given 3.5 mg daily by mouth 3-15% appears in the faeces. With doses larger than this, up to 25% may appear in the faeces. There is practically no urinary excretion of tocopherols, but from studies with labelled material it appears that one or more metabolites of tocopherols are excreted in the urine.<sup>6</sup> When more than the daily requirement is administered, there is some storage of tocopherol in the liver.<sup>3</sup>

### Comment on experimental studies reported

Though the toxicological studies are less than would normally be required for foreign substances used as food additives, it is considered that the clinical experience with this vitamin gives some compensation for the incompleteness of the animal data. Only a provisional estimate of an acceptable daily intake level for man could be made.

## Evaluation

### Level causing no significant toxicological effect in the rat

10 mg/rat/day in the diet, equivalent to about 50 mg/kg body weight per day.

**Estimate of acceptable daily intakes for man**

	mg/kg body weight
Unconditional acceptance . . . . .	0-1
Conditional acceptance . . . . .	1-2

**Comment**

The average American diet provides a daily intake of about 20 mg of tocopherol.<sup>3</sup> The level of 70 mg/day is thus about 4 times this natural intake.

**References**

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