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Estimation of daily intake of food preservatives

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The techniques that are available for estimating the intake of food preservatives are reviewed. These include *per-capita*, duplicate diet, diary records, food frequency, Total Diet and biomarker-based methods. Each approach has its merits and disadvantages and selection will depend upon a number of factors such as the reasons why the intake information is required, resource availability and the nature and extent of usage of the preservative. The application of these techniques is exemplified with reference to those that have been employed to estimate the dietary intake of nitrite and nitrate. It is concluded that such estimates are only as good as the analytical data upon which they are based and, in the case of nitrite, there is evidence to suggest that current data overestimate actual intake in the UK. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

As with other food additives there is a need to estimate the intake of food preservatives by consumers. Of primary importance is the requirement to ascertain that the consumption of preservatives poses a negligible threat to human health. In this context the intake by sub-sections of the population who may be at elevated relative risk through increased susceptibility, or greater than average consumption of foods containing the preservative, is a key issue. Additionally, exposure to food preservatives is unlikely to be constant and will change in the future as a consequence of technological developments by the food industry and alterations in dietary habits by the consumer. There will therefore be a continuing need to update exposure data to monitor the effects of these developments on preservative intake.

In order to assess the possible health risks, it is common practice in the UK, and elsewhere, to compare intakes with the Acceptable Daily Intake (ADI). The ADI is defined as 'an estimate by JECFA (the Joint Expert Committee on Food Additives) of the amount of a food additive, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk' (World Health Organisation, 1987). ADIs are based on the highest intake which does not give rise to observable adverse effects. The values are often derived from studies with laboratory animals and a safety factor is then applied to the highest level at which no adverse effect is observed. A factor of 100 is frequently chosen to allow for differences in susceptibility between humans and animals and also variations in susceptibility between different people. It is important to appreciate that ADIs refer to average daily intakes. Inevitably an individual's intake of a particular preservative will fluctuate from day to day. What is important, however, when making comparisons with the ADI is the average of these daily intakes.

In this paper, the various methods that may be employed to determine intake levels for food preservatives are discussed and their respective merits reviewed. The use of a number of these techniques are then exemplified with reference to their application in determining the intake of the preservatives nitrite and nitrate.

INTAKE MEASUREMENT

Methods available for estimating intake

A range of different procedures are available for estimating the dietary intake of food preservatives (for a review, see Lindsay, 1986; MAFF, 1993). The various methods are outlined in the following sections and summarised in Table 1.

Per-capita method

In this approach, information is obtained from the food preservative and food manufacturing industries on yearly production or usage of the preservative. This value is then divided by the number of people in the population. Thus in the case of benzoic acid (E210) and its salts (E211, E212 and E213), the total annual usage in the UK was estimated to be one million kg. For the total UK population of 56 million this corresponds to

an average intake of 48.9 mg/person/day (MAFF, 1993). The main advantage of the *per-capita* technique is that it is a very cost-effective means of obtaining estimates of average intake arising from preservative usage. There is no requirement for costly exercises such as the collection of foodstuffs and their subsequent analysis in the laboratory. On the other hand, however, no information is provided on actual exposure levels, including that of susceptible or high intake sub-sections of the population. In addition, the method does not take into account the effects of the reactivity of the preservative. If the compound reacts to a significant extent with the food matrix during manufacture and/or storage the consumer will, in practice, be exposed to a smaller quantity of the preservative than that predicted by the *per-capita* approach. In addition the approach does not account for exposure from sources other than the compound's use as a preservative.

Food diary records

In this technique, individuals keep a record of all the food items eaten during a study period, which is typically up to 10 days. This food consumption data is then combined with the expected concentrations of the preservative in each foodstuff and the resulting daily intake calculated. A number of variations on this theme are possible. The study participants may be asked to weigh each food item prior to consumption or, alternatively, food consumption may be estimated on the basis of standard portion sizes. The former approach will be more accurate but the overall costs will be higher. The concentration of the preservative in each food item may be estimated on the basis of information provided by the food manufacturer or, alternatively, from the results of published analytical surveys in the literature. As there is likely to be considerable day-to-day variation in an individual's diet, and consequent preservative intake, the greater the duration of the diary record study, the more accurate the estimate of average daily intake will be. Again, however, there is a trade off between accuracy and cost.

Dietary recall and food frequency

Dietary recall studies are retrospective and involve establishing preservative intakes on the basis of individuals' recollection of intake of specific food items. As with the food diary record approach, these data are then combined with expected concentrations of the preservative to estimate daily intake. In view of the difficulties associated with recalling details of food consumption, these studies are generally restricted to intake during the previous 24 h. In the food frequency technique, a checklist approach is adopted whereby participants are asked the daily, weekly or monthly frequency with which they consume particular foods. This again is combined with analytical data on the concentration of the preservative in the target foodstuffs and dietary intake calculated.

Total diet study

In this approach the types and quantities of food that make up the average British diet are calculated from the National Food Survey (Peattie *et al.*, 1983). The major items are prepared as if for consumption and then amalgamated into 20 separate food groups, e.g. bread and cereals, fish, milk, etc. Each group is chemically analysed and the average daily intake of the preservative calculated. The technique has the advantage that the information produced is derived from real foodstuffs prepared in a manner reflecting consumer practice. In addition, the relative contribution that each food group makes to the overall intake is readily quantified. The approach suffers, however, from a number of disadvantages. It is comparatively expensive to perform in relation to the techniques mentioned above. Furthermore, no information is provided on the intake of individual consumers. As practised in the UK, the Total Diet Study approach covers retail foods only and does not take into account food purchased in restaurants and take-away outlets, or indeed the contribution from alcoholic beverages, irrespective of whether they are consumed in the home or elsewhere. If the preservative is only present in a small number of products in a particular food group, its concentration in the composite sample will be correspondingly diluted. This will adversely influence the intake estimate if, as a consequence of such dilution, the preservative concentration in the food group drops below the detection limit of the analytical method employed. Finally, the estimation of preservative intake, arising from their specific use as preservatives, will be over-estimated if such compounds are also present in the diet arising from other sources, e.g. as environmental contaminants or natural constituents of some foods.

Table 1. Comparison of different methods for estimating intake

Method	Cost	All dietary sources of exposure covered	Reaction with food matrix allowed for	Analysis of samples required	Intake data on individuals provided
Per-capita	Low	No	No	No	No
Food diary	Medium	Possibly	Yes	No (literature data)	Yes
Food frequency	Medium	Possibly	Yes	No (literature data)	Yes
Total diet	High	No	Yes	Yes	No
Duplicate diet	High	Yes	Yes	Yes	Yes
Biomarker	High	Yes	Yes	Yes	Yes

Targeted surveys

In this method, analytical surveys are conducted on those food items which are known to contain the preservative of interest. This information may then be combined with data on national food consumption figures to provide an estimate of the average intake of the preservative. Alternatively, the analytical data may be utilised in combination with more detailed information, available from recent comprehensive studies on dietary habits of individual adults and children (MAFF, 1993), to provide an indication of actual intake.

Duplicate diet studies

In this approach duplicate portions of each component (or occasionally just particular items) of an individual's diet are purchased and prepared as for consumption. The duplicate samples are then analysed to provide an estimate of dietary intake of the preservative. The technique has the particular advantage of reflecting actual intake but is necessarily very expensive. Care needs to be taken to ensure that the act of taking part in such a study does not influence the normal eating and drinking behaviour of the participants. In addition, the accuracy of such studies will be adversely influenced if the size of the duplicate portion does not mirror that which is actually consumed (Pekkarinen, 1970).

Biomarker-based methods

The application of biomarkers to the intake estimation of preservatives, and other food chemicals, is an area of considerable current interest. The principle of the technique involves measuring the compound of interest, or one of its metabolites, in a body fluid such as blood or 24 h urine samples. Initially, developmental work is required to establish the quantitative relationship between the daily dietary intake of the compound and its biomarker, e.g. the amount of the biomarker excreted in urine in the following 24 h. Once this is known the concentration in a 24 h urine sample may be used to estimate the dietary intake of the compound in the preceding 24 h. This approach may then be employed in surveillance studies by collecting 24 h urine samples from the target population, measuring the biomarker concentration in each individual's sample and thence calculating dietary intake. Biomarker-based methods have been widely used to assess occupational exposure to potentially harmful chemicals in the work place (e.g. ECETOC, 1989; Lowry, 1995). Their application to food chemical intake is comparatively new and indeed was the subject of a recent symposium organised by the CSL Food Science Laboratory at Norwich (Crews & Hanley, 1995). Biomarker-based methods have to date been devised for a number of food chemicals including the contaminants aflatoxin B_1, di-(2-ethylhexyl) adipate and nitrate (see Massey, 1995 for a review). They are also currently under development for the artificial sweeteners saccharin and acesulfame K (Wilson & Crews, 1995). The major advantage of these biomarker-based approaches is that they enable the actual dietary intake of individual consumers to be quantified. The methodology is, however, expensive and analytically demanding. In addition it may not be applicable in all cases. For instance, if the extent to which the ingested food chemical is converted to the proposed biomarker varies significantly from person to person, as a consequence of cytochrome P450 polymorphism, for example, the accuracy of the estimated intake will be adversely affected. Similarly, the approach will be confounded if exposure to the biomarker also arises as a consequence of factors other than ingestion of the food chemical, e.g. via endogenous synthesis or occupational sources.

Method selection

The final selection of which method is employed to estimate preservative intake will depend upon a number of factors. These include: the purpose for which the information is required; the resources available; whether the preservative is used in a large number of food items or restricted to one or two foodstuffs; the concentration of the preservative in the food; the chemical characteristics and reactivity of the compound; the availability, or otherwise, of suitable analytical methods.

The strategy that has been employed in the UK (MAFF, 1993) is to undertake an initial screening using the *per-capita* approach. Comparison of the resulting estimated average intakes of preservatives with their corresponding ADIs provides a rational basis for deciding which compounds merit further investigation, e.g. quantification of actual intakes. Using this approach, it has been found that, of the 280 or so approved food additives in the UK, only five had intakes exceeding 10% of their respective ADIs, with the highest value being 44%. Of these compounds three were preservatives, namely sulphur dioxide and the sulphites (E220–E227), sodium nitrite (E250) and benzoic acid and the benzoates (E210–E212). The remaining two were the colours erythrosine (E217) and annatto, bixin and norbixin (E160b).

The *per-capita* strategy is not the only approach used in the UK for purposes of deciding whether more detailed intake information is needed. Where critical sub-sections of the population are concerned, such as young children or diabetics, for instance, additional measures are required. Such groups may be at increased risk relative to the overall population, either because they are inherently more vulnerable or as a consequence of higher than average intakes of particular foods. For instance, a special investigation has been undertaken on the intake of artificial sweeteners (Hinson & Nicol, 1992). This study utilised a food diary-based approach and revealed that, as a result of their increased consumption of soft drinks, intake of acesulfame-K was highest, on a mg/kg bodyweight basis, in children up to five years of age in comparison with other sections of

the population. It can be anticipated that a similar situation exists for sodium benzoate whose main use is as a preservative in carbonated soft drinks and carbonates.

To illustrate the application of these approaches, the following section reviews the current situation with regard to the estimation of the dietary intake of nitrite and nitrate.

ESTIMATION OF INTAKE OF NITRITE AND NITRATE

In comparison with some other preservatives, the use of nitrite and nitrate is restricted to a very limited number of foodstuffs. Both nitrite and nitrate are employed as preservatives in cured meats. In addition, nitrate is used in the manufacture of certain types of continental cheeses such as Edam and Gouda.

The concentration of nitrite (and nitrate) in food can be expressed in a number of different ways, e.g. mg (nitrite anion)/kg, mg (sodium nitrite)/kg or mg (N)/kg. To avoid confusion, nitrite is expressed in terms of mg (nitrite anion)/kg throughout the following sections.

Toxicological properties of nitrite and nitrate

The toxicological properties of nitrite and nitrate have been the subject of a number of investigations and these have been reviewed by Walker (1990). Nitrite has proved to be mutagenic in *in vitro* assays but when administered to laboratory animals has not demonstrated carcinogenic activity. Nitrite is a highly reactive species and forms carcinogenic N-nitrosamines on reaction with secondary amines. Whilst co-administration of nitrite and secondary amines results in carcinogenicity in animal studies, this only occurs at nitrite levels far in excess of the dietary intakes that humans are exposed to. The ADI set by the Scientific Committee for Food (SCF) for nitrite is 0 to 0.07 mg/kg bw/day (i.e. 0 to 0.1 mg/kg bw/day as sodium nitrite), which is equivalent to 0 to 4.2 mg/person/day for a 60 kg individual. Nitrate is considerably less toxic and this is reflected in the value of its ADI, 0 to 3.65 mg/kg bw/day (i.e. 0 to 5.0 mg/kg bw/day as sodium nitrate), equivalent to 0 to 219 mg/person/day for a 60 kg individual. The SCF have recommended that nitrate should not be used as an additive in infant foods in view of the acute effect of infantile methaemoglobinaemia, a condition which is extremely rare in the UK. Whilst the nitrate anion is chemically very stable, it is readily reduced microbially to nitrite and approximately 5% of ingested nitrate is so reduced to nitrite. This has led to concern that a small proportion of dietary nitrate may be converted to nitrite following consumption of nitrate-rich foods, with the subsequent endogenous formation of carcinogenic N-nitroso compounds. In practice, however, epidemiological investigations have failed to demonstrate an unequivocal link between nitrate exposure and cancer incidence. Long-term animal feeding studies with nitrate have similarly shown no evidence of carcinogenicity.

Dietary intake of nitrite

Per capita

The *per-capita* estimate for dietary intake of nitrite in the UK is 1.3 mg/person/day (MAFF, 1993). This, of course, assumes that all dietary nitrite derives from its use as a preservative in cured meats and that the tonnage production used by the food industry is consumed equally by the entire population of the UK.

Total diet study

The nitrite concentrations found in Total Diet samples obtained in 1985 (MAFF, 1992) are shown in Table 2. The total estimated intake was 4.2 mg/person/day. A number of samples were below the 1.0 mg/kg limit of detection of the analytical method employed. This is

Table 2. Total diet study on nitrite intake[a]

Food group	Average consumption (kg/person/day)	Nitrite concentration (mg/kg)	Nitrite intake[b] (mg/person/day)
Bread and cereals	0.24	1.4	0.34
Carcase meat, offal and poultry	0.059	1.0	0.059
Meat products	0.048	4.9	0.24
Fish	0.017	1.2	0.02
Oils, fats, eggs and dairy produce	0.12	1.0	0.12
Sugars and preserves	0.090	1.0	0.09
Green vegetables	0.050	3.4	0.17
Potatoes	0.16	11	1.8
Other vegetables	0.070	1.0	0.07
Canned vegetables	0.042	1.9	0.08
Fresh fruit and fruit products	0.091	1.0	0.091
Beverages (non alcoholic)	0.66	1.1	0.73
Milk	0.34	1.0	0.34
Total intake			**4.2**

[a]MAFF, 1992.
[b]Intakes calculated by assigning a value of 1.0 mg/kg to samples below the detection limit.

something of a problem when calculating the overall intake and the figure of 4.2 mg/person/day was estimated by assigning a value of 1.0 mg/kg to those samples below the limit of detection. If a figure of zero is assigned instead, the overall intake decreases to 2.4 mg/person/day. The true value is evidently in the range 2.4 to 4.2 mg/person/day. It should be noted that the Total Diet Study does not include the contribution from alcoholic beverages, drinking water or food purchased at restaurants and take-away outlets. The contribution from drinking water and alcoholic beverages is likely to be negligible. This is not necessarily the case for foods in restaurants and take-away outlets but at present no estimates are available on the nitrite intake arising from such sources.

Examination of the data in Table 2 reveals that preservative usage of nitrite makes a comparatively small contribution to overall intake from the diet. The Meat Products Group, which includes cured meats, only accounts for some 6% of the total. The largest contribution comes from potatoes, 43%. Whilst the reason for the presence of nitrite in potatoes is not certain, it may arise from microbial reduction of nitrate in the field or during storage prior to cooking. Other alternatives are also possible, as discussed below.

It is of interest to compare the nitrite intake figures attributable to cured meats with that derivable from the *per-capita* approach. The intake from the Meat Products Group of the Total Diet is 0.24 mg/person/day and this will be very largely due to the cured meat component of the group. The *per-capita* figure for intake from the whole diet is 1.3 mg/person/day (MAFF, 1993) and is, of course, calculated on the assumption that all of the dietary exposure to nitrite arises from its use as a preservative in cured meats. It is evident that the *per-capita* figure is some five times the higher of the two estimates. This is due to the reactivity of nitrite with other components of the cured meat matrix. It is well known that the concentration of nitrite in cured meats decreases rapidly during and after the manufacturing process as a consequence of interaction with other constituents of meat, particularly myoglobin, other proteins and thiol groups (Cassens *et al.*, 1977).

Duplicate diet study

Ellen and co-workers (1990) have conducted a series of 24 h duplicate diet studies in The Netherlands which are interesting from a number of perspectives. As shown in Table 3, investigations were conducted in summer 1976, winter 1978, autumn 1984 and spring 1985 and the mean intakes were found to be 4.2, 0.4, <0.1 and <0.1 mg/person/day, respectively. The authors comment that the summer 1976 results, in particular, may be erroneous. In the 1984 and 1985 studies, stringent measures were adopted, such that all samples were cooled down, frozen immediately and kept frozen prior to analysis. This was not the case for the 1976 and 1978 samples which were stored for a day in the participants' home *preferably in a cool place* (sic). As the 1976 sampling took place during a very warm period in the summer, the authors conclude that the high nitrite levels were probably an artefact, caused by microbial reduction of nitrate during storage of the duplicate samples prior to analysis.

Food frequency investigation

Knight *et al.* (1987) have conducted a food frequency study into the intake of nitrite in the UK. The average intake for some 747 people, resident in four geographically distinct regions, was found to be 1.4 mg/person/day.

Comparison of dietary intake of nitrite from different methods

Table 4 summarises the estimates of total dietary intake of nitrite as estimated by the *per-capita* (MAFF, 1993), Total Diet (MAFF, 1992), food frequency (Knight *et al.*, 1987) and duplicate diet (1984/1985 samples of Dutch study by Ellen *et al.*, 1990) approaches. The respective figures are: per-capita, 1.3 mg/person/day; Total Diet Study, 2.4 to 4.2 mg/person/day; food frequency, 1.4 mg/person/day; duplicate diet, <0.1 mg/person/day. These figures compare to an ADI of 0 to 4.2 mg/person/day for a 60 kg individual.

The range in these estimates is considerable, spanning between one and two orders of magnitude, at least. If the average intake as estimated by the Total Diet Study is correct, then a number of individuals must be exceeding the ADI. For instance the 97.5% percentile for potato consumption is 0.32 kg/person/day (Gregory *et al.*, 1990). This corresponds to an intake from potatoes alone of 3.5 mg/person/day, i.e. some 83% of the ADI. On the other hand, if the Dutch duplicate diet data more accurately reflect the true situation, then dietary intakes are likely to be substantially below the

Table 3. Duplicate diet estimates of nitrite intake in The Netherlands[a] (mg/person/day)

Sampling period	Nitrite intake
Summer 1976	4.2
Winter 1978	0.4
Autumn 1984	<0.1
Spring 1985	<0.1

[a]Ellen *et al.*, 1990.

Table 4. Estimates of average dietary intake of nitrite (mg/person/day)

Per-capita[a]	Total diet[b]	Food frequency	Duplicate diet
1.3	2.4 to 4.2	1.4	<0.1

[a]Derived from preservative usage of nitrite only.
[b]Excludes contributions from drinking water, alcoholic beverages and food consumed in restaurants and take-away outlets.

ADI. One of the problems with the Total Diet Study approach is that the samples are prepared in the same way irrespective of whether they are used to estimate the intake of nutrients, additives or contaminants. Bearing in mind the observations made by Ellen *et al.* (1990) concerning the risks of microbial reduction of nitrate to nitrite, it seems at least possible that the Total Diet Study estimate of nitrite intake is erroneously high. This, however, needs to be confirmed. On the basis of the currently available information, average nitrite intakes appear to lie somewhere in the range <0.1 to 4.2 mg/person/day.

Dietary intake of nitrate

Per-capita

The *per-capita* estimate for the intake of nitrate is 0.9 mg/person/day (MAFF, 1993).

Total diet studies

The estimated intake for nitrate derived from analysis of Total Diet samples collected in 1985 is 54 mg/person/day (MAFF, 1992). The single largest contribution comes from the Potatoes Group which accounts for 35% of the total followed by Green Vegetables (20%) and Other Vegetables (18%). The Meat Products Group, which contains cured meats, accounted for 6% whilst the Oils, Fats, Eggs and Dairy Produce Group, which includes cheeses, contributed less than 1% of the total intake. As discussed in the previous section on nitrite, the Total Diet Study does not include food consumed at restaurants and fast-food outlets, alcoholic beverages or drinking water. In a separate survey of some 172 beer samples, the mean nitrate concentration was determined to be 16 mg/kg (MAFF, 1992). Assuming an average consumption of 0.7 litres/day, this corresponds to a nitrate intake from beer of 11 mg/person/day (MAFF, 1993). The average intake from tap water is estimated to lie within the range 10 to 20 mg/person/day (MAFF, 1992). The intake from food purchased in restaurants and take-away outlets is unknown. Taking into account these expected contributions from beer and drinking water, the average intake of nitrate is likely to be in the range 75 to 85 mg/person/day.

Food frequency and food diary record studies

The same food frequency study (Knight *et al.*, 1987) referred to above was also used to calculate nitrate intake. The mean nitrate intake was estimated to be 108.5 mg/person/day and this included the mean contribution expected for drinking water, 13.5 mg/person/day. In view of the higher levels of nitrate found in vegetables, special studies have also been conducted on the dietary intake of vegetarians (MAFF, 1992). Using a three day weighed inventory diary record study, the dietary intake of nitrate, from vegetables alone, in lacto-ovo-vegetarians was found to be 194 mg/person/day. This, of course, excludes any contribution from alcoholic beverages or drinking water which may be expected to increase average intake for lacto-ovo-vegetarians to between 215 and 225 mg/person/day.

Duplicate diet studies

There do not appear to have been any duplicate diet studies on nitrate intake conducted in the UK. The 24 h duplicate studies performed in The Netherlands (Ellen *et al.*, 1990) indicate means ranging from 130 mg/person/day for the investigation conducted in summer 1976 to 50 mg/person/day for spring 1985. Individual intakes ranged from 2 to 750 mg/person/day. These figures are unlikely to reflect the lower and upper bounds of average intake as they only relate to a 24 h study period; the actual long-term average values will be higher than 2 mg/person/day and lower than 750 mg/person/day.

Biomarker-based investigations

Packer and co-workers (Packer & Leach, 1991; Packer *et al.*, 1995) have developed a biomarker-based approach involving measurement of nitrate excreted in the urine. Initial method development studies revealed that the amount, in mmol, of nitrate ingested in the preceding 24 h was described by the term $[(N_u-0.22)/0.55]$; where N_u is the amount of nitrate in the 24 h urine sample, the 0.22 figure corrects for urinary nitrate derived from endogenous synthesis of nitrate and slow clearance of body pools and the 0.55 factor corrects for the fact that, on average, 55% of dietary nitrate is excreted in urine within 24 h. Application of the methodology in a study involving over 300 subjects resident in seven geographically distinct regions of the UK revealed the average intake to be 157 mg/person/day. The mean intake in the seven different regions ranged from 117 to 190 mg/person/day. As expected there was a significant difference in the mean nitrate intake of vegetarians, 186 mg/person/day, and omnivores, 154 mg/person/day.

Comparison of UK dietary intake of nitrate from different methods

The estimates of UK nitrate intake derived from the different procedures are shown in Table 5. In summary, the average intakes are as follows: per-capita, 0.9 mg/person/day; Total Diet Study, 54 mg/person/day; food frequency, 108.5 mg/person/day; biomarker, 157 mg/

Table 5. Estimates of average dietary intake of nitrate in the UK (mg/person/day)

Per-capita[a]	Total diet[b]	Food frequency	Biomarker
0.9	54.0	108.5	157

[a]Derived from preservative usage of nitrate only.
[b]Excludes contributions from drinking water, alcoholic beverages and food consumed in restaurants and take-away outlets.

person/day. The *per-capita* figure clearly under-estimates the true intake as this only relates to exposure arising from usage of nitrate as a preservative. The Total Diet Study also under-estimates true intake as it does not take into account the contribution from food consumed at restaurants and other similar outlets, alcoholic beverages and drinking water. When the expected averages for these latter two sources are added to that of the Total Diet Study, the intake increases to between 75 and 85 mg/person/day. This is still some 25 to 35 mg/person/day lower than the food frequency-derived estimate. This difference is likely to be due in part to the nitrate exposure from foods consumed at restaurants, take-away outlets and the like. The food frequency estimate was derived from food consumption data combined with literature values for the nitrate content of foodstuffs. Almost all of the latter data were taken from the literature of the 1970s. These may not be wholly reliable as a consequence of improvements in analytical methodology and technological changes by the food industry. For instance, updating of legislation (The Preservatives in Food Regulations, 1979, 1989) has had the effect of reducing the maximum amounts of nitrate, and nitrite, that may be present in cured meats. Additionally, there has been a gradual move away from the more traditional hams and bacons, which need higher levels of nitrite and nitrate, to products manufactured by modern brine injection techniques. As a consequence of these changes, nitrate levels have decreased and the nitrate content of bacon used in the food frequency study, 69 mg/kg, is significantly higher than more recent estimates (43 mg/kg) (MAFF, 1992). The highest estimate of average nitrate exposure is obtained from the biomarker-based methodology, 157 mg/person/day. In principle, this may be expected to be the most accurate as it is based on actual exposure from all sources. However, data from studies with the rat (Mallett *et al.*, 1988; Ward *et al.*, 1989) suggest that a proportion of dietary protein may be converted *in vivo* to nitrate and excreted in the urine. If a similar phenomenon occurs to a significant extent in humans, it will confound the biomarker-based estimate of nitrate intake and result in an over-estimate. The contribution that dietary protein makes to urinary excretion of nitrate in humans has yet to be investigated.

In conclusion, the estimated average intake of nitrate in the UK varies, depending upon which method is employed. The true value is likely to lie in the range 75 to 157 mg/person/day. Such intakes are less than the upper limit of the ADI for nitrate, 219 mg/person/day.

Analytical methodology for nitrite and nitrate

The accuracy of intake data can only be as good as the analytical data upon which they are based and the methods that are available are reviewed in detail elsewhere (Massey, 1991).

Storage

When designing analytical protocols it is important to devise validated procedures for sample collection and storage as well as for the analytical measurement stage. The potential problems that may arise from microbial reduction of nitrate to nitrite, prior to laboratory analysis, have already been alluded to. Nitrite itself is a highly reactive species and care must be taken to ensure that the concentration does not decrease during storage. In comparison, nitrate is chemically stable and, provided microbial reduction does not occur, loss on storage is less of a problem. Room temperature storage of vegetables cannot, however, be recommended as, for instance, up to 50% of the nitrate in salad onions is lost after 4 days (MAFF, 1992).

Analytical measurement

The two major techniques employed for nitrate and nitrite analysis are colorimetry and HPLC. In the former method, nitrate, following extraction from the foodstuff, is reduced to nitrite using a cadmium column and then quantified colorimetrically after derivatisation with a diazotisation reagent. This gives a value for the combined concentration of the nitrate and the nitrite present in the food. In a separate analysis, nitrite is measured colorimetrically, i.e. without cadmium column reduction. The nitrate content of the food is then calculated from the difference in the two measurements. In the other commonly used method, nitrate and nitrite are extracted from the food matrix and, following clean up, measured simultaneously by HPLC with UV detection of the chromatographic peaks for the two anions.

In general, the agreement between the colorimetric and HPLC techniques is good for both nitrate and nitrite. This is exemplified by the results of a survey (Dennis *et al.*, 1990) of cured meats conducted in the UK as shown in Table 6. Where analyte concentrations are above 10 mg/kg, the agreement between the two methods is excellent for both nitrite and nitrate. At concentrations closer to the detection limit, 0.2 mg/kg nitrite and 1.0 to 2.0 mg/kg for nitrate, the results show less concordance. This is probably a consequence of two factors: the inherent poorer precision at levels close to

Table 6. Determination of nitrite and nitrate in cured meats by HPLC/UV and by colorimetry[a]

Sample	Nitrite (mg/kg)		Nitrate (mg/kg)	
	HPLC/UV	Colorimetry	HPLC/UV	Colorimetry
1	38.3	41.5	46.5	39.9
2	33.2	31.8	57.6	52.5
3	1.6	1.5	16.4	17.1
4	32.1	34.9	29.7	34.3
5	21.9	20.8	162	162
6	8.4	3.2	3.3	3.7
7	1.7	2.5	1.2	4.1
8	11.0	11.3	27.7	28.7
9	4.7	1.8	6.2	9.6

[a]From Dennis *et al.*, 1990.

the limit of detection and the proportionately greater influence of confounding factors associated with the analyses. This latter aspect is likely to have a more pronounced impact on the colorimetric assay. At low concentrations adequate correction for the contribution from the reagent/sample blank becomes crucial. This is analytically rather challenging as, in addition to the presence of traces of the analyte in the reagents, a problem common to both methods, one also needs to correct the results from the colorimetric analysis for (i) the background colour contribution from the diazotisation reagent itself and (ii) other UV absorbing/scattering species derived from the sample and/or reagents. For these reasons, the results of this procedure are likely to be prone to error at low concentrations for both nitrate and nitrite unless rigorous precautions are taken. This *caveat* applies not only to cured meats but other foodstuffs as well. In this context, it is of interest to note the reported presence of low levels of nitrite in vegetables used to calculate intake in the food frequency (Knight *et al.*, 1987) and Total Diet (MAFF, 1992) investigations. Whilst such data may be accurate, there is a possibility that they may, at least in part, be artefacts of the colorimetric method which was employed in both these studies. The presence, or otherwise, of low levels of nitrite in vegetables is of particular importance as they are a major component of the diet and have a predominant influence on overall dietary intake of this anion.

CONCLUSIONS

It is evident that there is a range of techniques that may be used to estimate the intake of food preservatives. Which approach is adopted will depend, amongst other things, upon what the information is required for, the resources available and the nature and usage of the preservative. In the UK, the strategy that has been employed involves using the *per-capita* methodology to provide an initial estimate of average intakes for the population as a whole. In addition, special investigations have focused on intakes of specific sub-sections of the population which may be at higher than average risk as a result of inherent vulnerability or extreme dietary consumption of certain foods. The results from the *per-capita* approach have been used to identify those preservatives whose average intakes appear to be in excess of 10% of their respective ADIs. Where this is the case, further studies are then undertaken using more accurate intake methodology such as Total Diet Studies, diary records, food frequency, duplicate diets and biomarker-based approaches. These procedures all have their merits but, on the other hand, none is without its disadvantages. With the exception of the *per-capita* approach, which can only be regarded as a preliminary screen, all of the methods rely on analytical data for estimating intake. In the case of nitrite, there are significant questions regarding the reliability of this data. The current estimates for nitrite exposure in the UK suggest that intakes are close to the ADI. Whilst this may be so, there are sound reasons for supposing that the actual intake may be a good deal lower. This, however, needs to be confirmed.

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