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Scientific Committee on Food

Opinion
of the Scientific Committee on Food
on a request for the safety assessment of Salatrims
for use as reduced calorie fats alternative
as novel food ingredients

(expressed on 13 December 2001)

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1.   **TERMS OF REFERENCE**

With reference to the initial assessment carried out by the UK Competent Authority pursuant to Regulation (EC) 285/97 and in the light of the comments/objections presented by Member States the Scientific Committee on Food is asked to assess the safety from the point of view of consumer health of salatrims - a family of reduced calorie fat replacers as novel food.

2.   **BACKGROUND**

The Committee has received the initial evaluation of a petition for approval as novel food of salatrims, a family of reduced calorie triacylglycerides, for use as alternative fats. These materials are covered by article 1 of Regulation 258/97 and fall into category 2.1 - complex novel food from non-GM sources where the source materials have a history of use for food in the EU Community.

The Advisory Committee on Novel Food and Processes (ACNFP) of the UK authority was of the opinion that salatrims had no conventional counterparts and were therefore not substantially equivalent to other food fats and oils. Salatrims comprise a family of structured triacylglycerol derivatives constituted predominantly of mixtures of long-chain fatty acids (LCFA) and short-chain fatty acids (SCFA). The LCFAs are principally stearic acid, the SCFAs comprise acetic, propionic and butyric acid, which are all esterified with glycerol. They are prepared by interesterification of glycerol esters, a process routinely used in the edible oil industry[1].

The application is limited to use in bakery and confectionery food products such as chocolate, chocolate confectionery, other chocolate products, buns, pastries, cookies, and brownies. Salatrims are intended for sale to the food processing industry for use in manufactured products and not for direct sale to the consumer. Salatrims have been accorded GRAS status in 1994 by the US FDA[2] and products containing them are marketed in the US, Japan, Korea, Taiwan as foods, in Australia, New Zealand and Mexico as fats/oils. Salatrims have also been considered by the 49th FAO/WHO Joint Expert Committee on Food Additives in 1997[3] which at that time was of the opinion that adequate information was not available to evaluate their safety and nutritional effects.

On the basis of the totality of the data provided the ACNFP concluded that Salatrims are acceptable for use in baked goods and confectionery. It noted that any extension of use in other categories of food would require a further approval. As no specific safety data relating to possible effects of salatrims in children had been supplied the ACNFP remarked that on nutritional grounds foods containing salatrims should not be aimed at young children. As

salatrims provide fewer calories than conventional fats foods containing salatrims should be labelled with information on the true caloric value to supply consumers with appropriate information.

Following this positive result of the initial assessment by the UK Competent authority nine member states responded. Six member states agreed subject to some suggestions, three member states offered reasoned objections to the approval proposed by the UK competent authority.

The major concerns and suggestions raised by member states were as follows:

1.   Possible adverse effects in children.

2.   Appropriate labelling for consumers wishing to reduce their weight by restricting their calorie intake. The rise in saturated fat intake saves only 44 kcal out of the total energy intake hence it is doubtful whether this really contributes to weight loss when foods containing salatrims are consumed over a long time. Need for correct labelling with regard to the actual caloric value of foods containing salatrims.

3.   Tolerance of salatrims by obese adults with non-alcoholic liver disease.

4.   Not to be consumed by individuals with abnormal liver function in view of rise in serum transaminases.

5.   Determination of a NOAEL for gastrointestinal symptoms because of narrow margin between envisaged level of intake and level causing gastrointestinal symptoms.

6.   No data for specified material with 0.87 g stearic acid/g fat.

7.   One member state expressed general concern that nutritionally low grade salatrims fats are to be used to replace high grade fats and oils rich in essential fatty acids.

9.   Effect of postprandial lipaemia following salatrims ingestion on procoagulant and fibrinolytic activity requires careful assessment.

10. In the initial assessment concern was expressed over the absence of a NOAEL for the effects of salatrims on enzyme markers for liver dysfunction in humans.

3.   SPECIFICATION OF THE NOVEL FOOD

A generic specification has been provided. The fatty acid composition can be varied to confer the desired functional technological properties. The proportion of LCFAs varies from 33-70% and that of SCFAs from 30-67%. The triacylglycerides represent >87% of which a maximum of 70% is stearic acid. The long-chain fatty acids used in the manufacture of salatrims derive from hydrogenated edible oils such as canola, soya bean, cottonseed and sunflower seed oil. The short-chain fatty acids derive from triacetin, tripropionin and tributyrin[1].

Acetic, propionic and butyric acid are normal products of colonic bacterial fermentation. Phytosterols are carried over from the natural source materials used but their levels are reduced because of the dilution during manufacture. The tocopherols and unsaponifiable

matter also come from the natural source materials. Diacylglycerides and free fatty acids may be formed by hydrolysis during deodorisation but are physiologically formed also during fat digestion. Pesticide residues derive from the raw materials but do not concentrate during the manufacturing process and are present in amounts comparable to those found in commercial hydrogenated vegetable oils from which they are carried over.

Salatrims containing the possible maximum amount of stearic acid according to the specification would contain 10% tristearin and 90% monoacetyl-distearoylglycerol, equivalent to 0.1 x 0.881 g stearic acid + 0.9 x 0.784 g stearic acid = a total of 0.794 g stearic acid/g fat. However, because commercial stearin contains only 97% of its fatty acids as stearic acid, the contribution of commercial stearin reduces to 0.1 x 0.854 g stearic acid/g fat. This amount, when added to 0.9 x 0.784 g stearic acid now results in a possible total of 0.791 g stearic acid/g fat. In fact, the most recent specification of commercial salatrims limits the stearic acid content for technological reasons to <0.70 g stearic acid/g fat, which is the amount usually found in natural edible oils.

The specification also limits the amount of trans-fatty acids to <1%. The major source for trans-fatty acids would be the elaidic acid in partially hydrogenated soyabean oil. The latter is used in such low amounts as to keep the trans-fatty acid contribution to below 1%.

4.   PRODUCTION PROCESS APPLIED TO THE NOVEL FOOD

Salatrims are produced by interesterification, a process commonly used in edible oil production to modify the physical properties of the final product, the catalyst being sodium methoxide. The SCFAs are added in the desired molar excess ratios ranging from 1.5 - 12. The catalyst is deactivated by distilled water at the end of the reaction. The oil is then washed, bleached and filtered. Any residual mono- and diglycerides are converted to the corresponding triglycerides by acetic anhydride in the presence of anhydrous K_2CO_3, any residual short-chain triglycerides are reduced to <1% by vacuum and steam stripping followed by vacuum distillation. The final salatrims fat is then steam deodorised. All these processes are standard procedures in the production and refinement of edible oils and fats[1].

As the composition of the salatrims depends solely on the proportions of source materials used no unexpected reaction products or unique non-acylglycerol compounds are formed during manufacture.

5.   HISTORY OF THE SOURCE ORGANISMS

The source organisms have a history of safe use in the production of edible oils/fats in the Community and are all used as foods/food ingredients.

6.   ANTICIPATED INTAKE/EXTENT OF USE

Approval is requested for use in bakery and confectionery products only. Salatrims are not intended for use in infant formulas and they cannot be used as frying oils/fats because of their technological properties. They are also not available direct to the consumer but only for use by the food processing industry in products particularly for persons desiring a low calorie diet. Intakes may derive from chocolate, chocolate confectionery, brownies, buns, pastries and

cookies. Intake estimates are based on the UK National Dietary and Nutrition Survey of 1999 which used a 7-day record of all foods consumed by 2000 individuals aged 16-65 and in which the fat content of each food item was known[4]. These intake estimates are, however, comparable to similar estimates made in other EU member states because they are based on a review of the appropriate FAO Food Balance Sheets for the European countries for the years 1988 and 1997[25]. The application estimates the mean total fat intake for European countries to be 88 g/person/day (148 g/person/day calculated as the 97.5[th] percentile). The mean intake of salatrims would be 11 g/person/day (29 g/person/day calculated as the 97.5[th] percentile), if salatrims were to be used only as substitute for fats in bakery and confectionery products, the balance of 77 g/person/day (119 g/person/day calculated as 97.5[th] percentile) remaining as unreplaced food fats. If salatrims were to substitute all dietary fats the mean intake of salatrims would be 45 g/person/day (79 g/person/day calculated as the 97.5[th] percentile) with some 42 g/person/day (69 g/person/day calculated as 97.5[th] percentile) remaining as unreplaced food fat. However these data show considerable variability and uncertainties.

As salatrims-containing food products are aimed at persons intending to control their weight by choosing a restricted calorie diet and will also carry a price premium they are unlikely to be consumed by young children who generally do not need to restrict their energy intake. Hence no intake estimates for salatrims have been generated for young children but a recent JECFA estimate of salatrims intake by 3-5-year-olds at the 90th percentile level quotes 26 g/person/day[28]. A recent UK survey[5] has shown similarly, that children and adolescents (11-18 years) consume approximately the same amounts as adults of bakery products likely to contain salatrims, such as biscuits, cakes, pastries, and possibly somewhat more confectionery and chocolate so that the absolute intake estimates for adults would be also appropriate for older children and adolescents.

7. PREVIOUS HUMAN EXPOSURE TO THE NOVEL FOOD AND SOURCE MATERIALS

The short-chain fatty acids in salatrims are natural components of the fermentation products of the colonic bacteria. The fatty acids and glycerol liberated by the metabolism of salatrims are absorbed in a similar way to those formed during the normal metabolism of food fats. The phytosterols, tocopherols, unsaponifiable components and other organic constituents also present in salatrims occur at the same or lower levels as in the commercial hydrogenated edible fats and oils used as source materials. Salatrims have been on the US market since June 1995.

8. NUTRITIONAL INFORMATION

The replacement of conventional fats by salatrims will result in an increased dietary intake of saturated fats. Thus stearic acid intake will rise by 4.6 g/day for the mean consumer of fat. Only 3.1 g/day of these 4.6 g are absorbed with 1.5 g/day appearing in the faeces. These 4.6 g displace 3.3 g/day of the other saturated fatty acids (palmitic, myristic, lauric acid) from the diet. These latter fatty acids are known to be associated with an increase in serum cholesterol (each 1% increase in dietary intake of any of these acids is equivalent to a rise in serum cholesterol by 2 mg/dL) whilst stearic acid does not affect the serum cholesterol level, does not change the platelet phospholipids and reduces the activity of factor VII. Consequently there is no increase in thrombosis tendency if salatrims are ingested.

The original concern over the thrombogenic potential of salatrims was examined in 2 clinical trials. In one double blind crossover study 18 female and 17 male volunteers, all middle aged with moderately raised plasma cholesterol levels and moderately overweight were given a test meal with a single dose of 30 g salatrims or either 30 g oleate-rich sunflower oil or 30 g cocoa butter. The postprandial (after 3 hours) rise in serum triglycerides was smaller after salatrims compared to the control meals, the levels being 0.29 mmol/L for salatrims, 0.55 mmol/L for cocoa butter, 0.54 mmol/L for oleate. Markers of fibrinolytic activity, measured as tissue plasminogen activator activity and plasminogen activator type I activity, were not affected by the different fats. However plasma factor VII_c coagulant activity was raised after 6 hours with oleate by 11.3%, with cocoa butter by 9.9% and with salatrims by 2.1%. The plasma concentration of activated factor VII_a was increased by all 3 fats but less by salatrims[6]. As the half-life of factor VII is relatively short, chronic administration of salatrims would not represent a worse case than acute dosing[7]. In another 5-week randomised crossover study 9 males and 6 female hypercholesterolaemic subjects were pre-treated for 2 weeks with a low-fat diet (fat intake 20% of energy). They were then given for 3 weeks a high-fat diet (fat intake 40% of energy) either as 30 g stearic acid rich salatrims/day or as a 30 g palmitic acid rich diet/day (as palm oil in margarine). No increase in factor VII_c coagulant activity or fibrinogen was found after ingestion of salatrim[8]. There is thus no evidence that salatrims adversely affect the thrombogenic potential even in people at moderate risk from CHD.

The question of the possible impairment of the absorption of fat-soluble vitamins by humans ingesting salatrims was considered in the application. No impairment of the serum levels of vitamin A, E or D was considered to be likely for the following reasons[9]:

a)   The partitioning theory of fat-soluble vitamin absorption developed from the experimental data on olestra and the physicochemical properties of salatrims suggest that any unabsorbed salatrims fat component would sequester fat-soluble vitamins at a lower rate than normal undigested fat;

b)   The faecal fat originating from salatrims would represent no more than 3.6-4.8% of the total fat intake calculated from the proportion of unabsorbed stearic acid (1.5 g) formed from salatrims during digestion assuming the anticipated consumption of salatrims. This additional small amount would have a negligible sequestering effect on fat-soluble vitamin absorption and would be well within the normal variation in faecal fat content, observed in individuals with normal fat absorption;

c)   The 13-week studies in rats and the 28-day study in minipigs produced no evidence of adverse effects on vitamin A and E absorption thus supporting the likely absence of any effect on fat-soluble vitamin absorption.

Most triglycerides provide energy at 9 kcal/g. The salatrims are designed to provide 4-6 kcal/g due to the presence of the less energy dense SCFAs and the incomplete absorption of the stearic acid component. This was confirmed in a two-week study in groups of 10 Sprague-Dawley rats in which the growth and body weight gains in young growing rats fed 21% salatrims in their feed were compared with the effect of 5%, 10%, 15%, 21% corn oil in the feed. Assuming that the caloric availability of corn oil was 9 kcal/g over 2 weeks the average bodyweight increase of 80 g over 2 weeks, when 21% corn oil are present in the feed, yielded a calculated energy density of 5 kcal/g for salatrims[10, 11, 12]. Allowing for a mean daily intake of 11 g salatrims this would result in a daily energy saving of 44 kcal (99 minus 55 = 44 kcal).

In view of the variable composition of the salatrims it is not possible to determine a suitable absolute figure for their caloric value for the purpose of food labelling. The requirement to use the value of 9 kcal/g for fats, enshrined in the present labelling Directive EC 90/496, would seriously mislead the consumer with respect to the true caloric value of foods containing salatrims.

9.   MICROBIOLOGICAL INFORMATION

Salatrims are manufactured under GMP conditions and being an oil phase this precludes the growth of typical food-borne microorganisms.

When the caecal contents were checked in a 13-week study in groups of 20 male and 20 female Charles River rats, using 2 different salatrims at 10% in the diet, no changes in the bacterial activity due to the ingestion of salatrims and no alterations in the bacterial morphotypes were detected. There were also no changes in caecal pH and no changes in the extent of conversion of primary to secondary bile acids, in the conversion of primary to secondary phytosterols or the conversion of cholesterol to coprostanol suggestive of an effect on the gut microflora[13].

10.   TOXICOLOGICAL INFORMATION

10.1.   Studies in laboratory animals and in *in vitro* systems

The metabolism of salatrims has been studied using 4 different products under conditions of *in vitro* hydrolysis at pH 7.0 and 37°C in the presence of phosphatidyl choline, sodium taurocholate and sodium taurodesoxycholate during 2, 5, 10 and 30 minutes with porcine pancreatic lipase. The products were dissolved in chloroform and used at a concentration of 100 mg/mL. Salatrims containing 2 SCFAs were more rapidly hydrolysed than those with only 1 SCFA. Products containing butyrate were more rapidly hydrolysed than propionates and these more rapidly than acetates[14].

The absorption, distribution, metabolism and excretion (ADME) of salatrims was studied using salatrims labelled with ^{14}C in the acetate, stearate, propionate and 1 and 3 glycerol position. Groups of 5 male S-D rats were treated for 14 days with cold 10% salatrims in their feed and then given a gavage dose of 1.4 g/kg bw labelled material. Samples were collected for 72 hours. A large part of the SCFAs appeared as exhaled labelled CO_2. Between 1/3 to 1/2 of the ingested stearate appeared in the faeces. Of the absorbed stearate about 50% was converted to CO_2 the other 50% was converted to oleic acid[15].

Three 13-weeks feeding tests were carried out in groups of either 30 male and 30 female or 20 male and 20 female Charles River rats with 6 salatrims incorporated into the diet at levels of 0, 2, 5 and 10% with 10% corn oil as control. Males given 2% in the diet had an intake of 1.3 g/kg bw while females given 10% in the diet had an intake of 7.9 g/kg bw. Clinical chemistry and urinalysis showed no dose-related adverse effects but a trend to increased urinary phosphate clearance was noted. Growth and bodyweight gain were comparable to the corn oil controls but feed consumption was lower in males and females fed 10% salatrims. There was no effect on bone mineralisation while Zn and Sr deposition in bone was increased. Renal mineralisation was increased in females fed 5 and 10% salatrims without any accompanying functional, macroscopic or microscopic changes. Serum levels of AST, ALT and GGT

showed no changes. Organ weights of 5 major organs were determined and histopathology carried out on 42 tissues. The NOEL in these three studies was 2% in the diet equivalent to a dose of 1000 mg/kg bw[18, 19, 20].

Groups of 4 male and 4 female Hanford Charles River minipigs were fed 0, 3 or 10% salatrims for 4 weeks equivalent to 1.5 g/kg bw for males on 3% and 3.7 g/kg bw for females at 10%. The control group was fed 10% corn oil. No adverse effects due to treatment were noted, when a full range of serum chemistry parameters including vitamin A and E levels was examined. Bodyweight gain and feed consumption were comparable. Data on bone mineral content (Ca, P, Sr, Zn) and bone ash showed no significant differences between the controls and the groups fed either 10% corn oil or 10% salatrims in any of the measured mineral parameters. Nine organ weights and 27 tissues were examined. There was some degradation of the test material linearly with time at room temperature so that the test diet at the 3% and 10% incorporation level only contained about 92% of the intended dose. The loss in salatrims was probably due to hydrolysis by lipases present in the animal feed. The NOEL was 10% in the diet equivalent to about 3700 mg/kg bw[21].

The genotoxicity of 6 salatrims was examined in a microsomal reverse mutation test, using the 5 tester strains TA98, TA 100, TA 1535, TA 1537 and TA 1538 +/- S9. The test substances were dissolved in acetone and applied in concentrations ranging from 62.5 to 1000 µg/plate. Two independent experiments were carried out, the positive controls being dissolved in DSMO. No mutagenic activity of salatrims was observed in these tests[16]. Another salatrim, dissolved in acetone, was examined at concentrations of 250, 500 and 1000 µg/plate for induction of chromosomal aberrations in CHO cells +/- S9. 100 metaphases were examined. No effect on the mitotic index and no clastogenic potential of salatrims were noted[17]. The same salatrim was examined for the induction of gene mutations at the HPRT locus of CHO cells +/-S9. The concentrations used ranged from 31-1000 µg/mL. Two independent assays were performed. No gene mutations were induced by the salatrim used in this test[17]. The *in vitro* induction of UDS in F344 rat hepatocytes by salatrims was examined using concentrations ranging from 0.5-1000 µg/mL and sampling at 19 hours. No UDS was induced by salatrims[17]. Groups of 18 or 20 male and 18 or 20 female Charles River rats were fed 10% of two different salatrims in separate experiments for 13 weeks, with corn oil as control. No induction of micronuclei by salatrims in the bone marrow cells could be detected and there was no change in the MN/PCE ratio[17].

Carcinogenicity and developmental toxicity were not specifically tested for.

10.2. Human tolerance studies

In a clinical short-term study on 6 female and 12 male volunteers the 6 females were given as pre-treatment for 7 days 45 g/day hydrogenated coconut oil while the 12 males received as 7-day pre-treatment 60 g/day of hydrogenated coconut oil. This was followed by 7 days of treatment of the 6 females by 45 g/day salatrims or 60 g/day salatrims treatment for 7 days of the 12 males. For the 7 days post-treatment the 6 females received 45 g/day hydrogenated coconut oil and the 12 males 60 g/day hydrogenated coconut oil. As controls 8 females and 1 male were given 3 weeks of 45 g/day hydrogenated coconut oil and 1 female and 8 males for 3 weeks 60 g/day hydrogenated coconut oil. Complaints of lower abdominal cramps, diarrhoea, nausea and headache were recorded. There were a total of 72 complaints in the salatrims group against 7 complaints in the control group receiving hydrogenated coconut oil. In addition, their intake of Ca, Mg and Zn as well as their faecal excretion were measured. No significant increase (about 5%) in faecal mineral excretion by the salatrims-treated volunteers

compared to the controls on hydrogenated coconut oil was noted, although the dietary stearic acid intake in the salatrims-treated volunteers was four times as high as that of the control volunteers[22].

In another clinical study 12 males were kept on 2500 kcal/day diet and 12 females on 1800 kcal/day diet. During 4 days pre-treatment the 24 volunteers were given 60 g/day coconut oil followed by 4 days treatment with either 60 g/day salatrims, 30 g salatrims + 30 g hydrogenated soybean oil/day or 60 g/day hydrogenated soybean oil in a randomised triple crossover sequence, so that all subjects received all treatments. This was followed in all subjects by 4 days washout with 60 g/day coconut oil. With 60 g/day salatrims 58 complaints of lower abdominal pain, nausea, flatulence, and headache were recorded in 4 days. With 30 g/day salatrims + 30 g/day soybean oil 16 complaints were noted in 4 days. With 60 g/day soybean oil some 24 complaints were recorded in 4 days and with 60 g/day coconut oil 18 complaints were registered in 4 days. Women complained about 4 times as often as men. 60 g/day salatrims caused most complaints, while 30 g/day salatrims caused no more complaints than 60 g/ day soybean oil or 60 g/day coconut oil[22].

In another free living clinical study 2 control and 5 test groups of volunteers, each consisting of 12 males and 12 female subjects, were given as pre-treatment 1 week of 60 g/day partially hydrogenated soybean oil, followed by 4 weeks treatment with either 30 g/day, 45g/day or 60 g/day different salatrims, followed again by one week of 60 g/day partially hydrogenated soybean oil as wash out. The two control groups received partially hydrogenated soybean oil for 6 weeks. The test groups had their fat intake from salatrims made up to 60 g/day with partially hydrogenated soybean oil. Some 33 individuals dropped out of the test groups for various reasons including 20 complaining of abdominal colic and nausea. Also 4 individuals dropped out of the control groups including 2 with abdominal complaints. Most dropouts occurred in the groups receiving 60 g/day salatrims. The incidence of abdominal disturbances was as follows:

Treatment	No. of subjects entering	No. of subjects with abdominal complaints
Controls 1+2	48	2
30 g salatrims/day	24	1
45 g salatrims/day	24	3
60 g salatrims/day	72	16

There was no difference between controls and the 30 g/day group in the incidence of abdominal complaints. There was a marginal increase compared to controls in the 45 g/day group and a clear increase in the group receiving 60 g/day.

A major area of concern has been the finding of a rise in serum transaminase AST and ALT levels in the salatrims-treated groups after one week's administration in this clinical trial[23]. The rise was small but statistically significant for AST and ALT in all groups and persisted in 2 different groups even after the washout period. However the group means of none of the treatment groups fell outside the normal range for AST and ALT. Both AST and ALT rose in the same individuals and showed some temporal trends. There was no difference in the changes in enzyme activities between the subjects staying in the study and those dropping out of it because of gastrointestinal symptoms[23].

In the previously described 5-week randomised crossover study in 15 subjects, using either salatrims (high in stearic acid) or palm oil (high in palmitic acid) to investigate the thrombogenic potential of salatrims, additional data were recorded on the serum levels of AST, ALT, AP, LDH and GGT as markers of hepatic dysfunction. No changes in the activities of these enzymes were found between the values recorded at the start and at the end of the 3-week exposure to 30 g of either salatrims or palm oil. In fact the small increases in serum enzyme activities after palmitate were of a similar magnitude to those seen after salatrims in the free-living study[8, 23].

Butyrate and propionate have been shown to cause developmental abnormalities in *in vitro* tests. To determine whether salatrims can give rise to raised postprandial serum butyrate levels 5 male volunteers were given 30 g salatrims (containing 9 g butyric acid) in a milkshake and serum butyrate determined for the next 360 minutes. No butyrate could be detected in the serum using a method with a detection limit of 1 μmol/L. This was interpreted as rapid local use of butyrate by the gut mucosa preventing any spill-over into the general circulation[24].

11.   DISCUSSION

Increases in serum AST and ALT activities occurred in higher proportions of individuals consuming 30 g or 60 g salatrims per day for 28 days compared to controls. Small statistically significant increases were recorded in the mean activities of AST and ALT. However, only a few of these individual increases reached levels of clinical significance although the mean values for treatment groups were within the normal range of variation. Increases in hepatic enzyme activities of a similar magnitude were also seen in some individuals receiving the control fat. There was some concordance in increases in AST and ALT within individuals and some temporal trends. Whereas there were differences between individuals in respect of the magnitude of these increases, these observations might be interpreted as a weak treatment-related effect as the increases did not decline during the 28-day treatment. There were also concurrent increases in other serum enzymes related to liver dysfunction (AP, LDH, GGT) in a small number of individuals on salatrims. Individuals who withdrew from the study may have been atypical making the data rather variable but their enzyme activities were not significantly different from those remaining in the study. As there were some discrepancies in the recorded data their analysis could only be limited. A NOAEL with respect to clinical chemical markers of liver function could not be identified from the clinical studies nor was it possible to come to definite conclusions on the mechanism and the biological significance of these effects. However in the absence of any other indictors of liver damage and the absence of any enzyme changes or histopathological evidence of liver damage in the rat and minipig studies these changes in hepatic enzyme activities can be considered as not representing a clear toxic effect.

This view is supported by the results of the 5-week crossover study with palmitate in subjects with raised serum cholesterol[8] which showed no significant difference between palmitate and salatrims as regards levels of serum AST, ALT, GGT, AP and LDH. In only 2 out of the 15 subjects consuming 30 g of fat/day were the actual values of serum ALT and GGT above the reference levels, although the palmitate produced a slight rise in some values similar to those seen in the larger clinical study[23].

These results provide reassurance that this clinical chemistry finding is not a toxic effect although the studies only extended over short periods and involved comparatively few people.

Furthermore, the transaminase values outside the normal range were not systematically related with exposure to salatrims, occurred also in the control groups and were isolated findings devoid of clinical significance[26]. It has been suggested that the determination of a NOAEL for these markers of hepatic dysfunction might be desirable but this would require a large clinical study and reassurance that the methodological imprecisions in determining serum transaminase levels have been largely overcome.

The human clinical studies, however, provided no information on the potential effects in children or people with existing liver disease, because no special studies were carried out on obese people with impaired liver function. Such individuals would normally be excluded from clinical studies as not being representative of healthy consumers.

With regard to any possible atherogenic effects of salatrims a consideration of the effect of fat substitution by salatrims on total plasma cholesterol levels is useful. In the study on plasma lipids[8] 15 normolipaedemic subjects consumed 30 g salatrims for 5 weeks without showing any effect on plasma HDL levels but a concomitant slight lowering of plasma triglyceride, total cholesterol and plasma LDL levels. In the free living clinical study[23] groups of about 20 normolipaedemic subjects received for 1 week 60 g partially hydrogenated soyabean oil followed by 4 weeks either 30 g, 45 g or 60 g salatrims made up to a total fat intake of 60 g/day. In all cases plasma total cholesterol values were equal to or lower than those of the soya bean oil controls.

The Committee noted that intakes of 45 g or 60 g/day of salatrims had no deleterious effects in human volunteers on the mineral bioavailability, particularly of calcium and zinc, nor did the feeding of 10% salatrims for 4 weeks to minipigs lead to changes in their bone mineralisation.

The Committee also commented that for an accurate prospective estimate of the probable incidence of gastrointestinal complaints which have also occurred in healthy individuals the available human studies were too short, included too few individuals, and were not sufficiently representative of the total population likely to be exposed. Two of the available studies point to a dose without adverse effects of 30 g salatrims/day for these abdominal complaints. The Committee noted that this intake is close to the estimated exposure of 29 g/person/day for high consumers at the 97.5[th] percentile assuming that salatrims are used in all consumed bakery and confectionery products.

The occurrence of gastrointestinal symptoms after ingestion of large amounts of salatrims are probably caused by the shift from absorbable to partially unabsorbable intestinal content leading to a rapid release of cholecystokinin which is known to slow gastric emptying thereby producing nausea, bloating and abdominal cramps. These abdominal symptoms occur mostly with intakes of 60 g/day and are only little noticed with intakes of 30 or 45 g/day. Nothing is known about the incidence of these symptoms in children and people with compromised gastrointestinal function. An apparent NOEL of 30 g/day of salatrims for gastrointestinal effects was observable in the study on 168 free-living subjects[23]. The other study showing an absence of gastrointestinal effects after ingesting 30 g salatrims/day for 5 weeks was carried out in only 15 subjects[8].

The Committee has not been supplied with data on the effects of the consumption of salatrims-containing food products on children or people with defective gastrointestinal absorption and no suggestions have been offered on how to prevent consumption of these foods from occurring in view of the propensity of salatrims for causing gastrointestinal

complaints. At least in the UK, older children would consume amounts of food containing salatrims equivalent to those consumed by adults. Young children generally do not need to restrict their energy intake therefore energy-restricted foods containing salatrims should not be aimed at them but this consideration of applicability is a general one and not confined solely to foods containing salatrims.

Postmarketing surveillance was carried out in the USA over 14 months covering the introduction of fat-reduced products and over 20 months of marketing salatrims-containing sweet products. It showed a low incidence of adverse gastrointestinal effects as recorded from telephone, postal and Internet notifications. The incidence was approximately 266 notifications/million units fat-reduced items sold, of which 1% related to gastrointestinal adverse effects. For sweet products there were 122 notifications/million units sold, of which 0.5% related to gastrointestinal complaints. The survey included also possible consumption by children but no special issues had become apparent in this respect. A further postmarketing survey of enquiries/100 million units reduced-fat baked products sold showed that in 1998 for all such products about 2.2 enquiries were made of which 0.01 related to products containing salatrims. The corresponding figures for 1999 were 2.2 enquiries for all products and 0.4 related to salatrims-containing products. For the period January-October 2000 the number of enquiries were 2.0 for all products and 0 related to salatrims-containing products. None of the enquiries were concerned with abdominal symptoms[27]. These data were difficult to interpret because essential details of the intake including quantitative information had not been recorded. They therefore do not provide reassurance on the absence of adverse gastrointestinal effects after permitting broad use of these novel products.

Salatrims do not contain any structural alerts for mutagenicity or carcinogenicity and have been shown to be non-genotoxic in *in vitro* and *in vivo* studies for various genetic endpoints. There appears therefore no need for carrying out any assays for carcinogenicity.

There appears to be similarly no need for carrying out reproduction studies because:

1.   Triacylglycerides are not known to cause toxic effects on reproduction except if essential fatty acids are lacking;

2.   The ADME proceeds via the same steps known from other triglycerides;

3.   No lesions in the reproductive organs of rats and minipigs fed salatrims were noted;

4.   Salatrims ingestion does not cause any changes in serum levels of vitamin A and E.

Although *in vivo* developmental toxicity studies have not been carried out the concern over possible teratogenicity, because of the observations of developmental toxicity associated with high serum levels of butyrate in *in vitro* studies, is not considered relevant in the light of the study with ingested salatrims high in butyric acid, in which no butyrate was found in serum samples. Furthermore it is noted that butyrate is normally present in the body because it is a normal constituent of the diet and an intermediary of normal metabolism.

Because of their variable composition it is not possible to assign an appropriate figure for the caloric value of salatrims for labelling purposes. The actual caloric values are in conflict with the European Community rules on nutrition labelling which assign the value of 9 kcal/g to any fat while the value for salatrims, which are alternative fats, lies between 5-6 kcal/g. This problem requires solution to avoid misleading the weight-conscious consumer.

Direct data on the efficacy of foods containing salatrims in producing loss of weight in obese adults are not available.

Some concern has been expressed over the use of salatrims in food products which normally contain nutritionally valuable fats and oils. This is based on the following considerations. The chemical structure of the long chain fatty acids in natural fats correlates with various physiological functions. The saturated fatty acids (C 18:0, C 16:0) and monounsaturated fatty acids (C 18:1) furnish energy. The essential polyunsaturated fatty acids (C 18:2 linoleic, C 18:3 linolenic) are required for the synthesis of ω-6 and ω-3 acids from which tissue hormones are synthesised (prostaglandins , prostacyclins, thromboxane, leukotrienes). These essential fatty acids must be supplied in the diet. Hence partial substitution of unsaturated by saturated fatty acids (e.g. stearic acid) could potentially affect obesity, atherosclerosis, cardiovascular disease, cancer and certain autoimmune diseases and has to be considered when alternative fats are used. Additionally other nutritionally desirable objectives must be borne in mind, such as fat furnishing about 30% of the total energy intake, fatty acid composition to be about 1/3 saturated, 1/3 unsaturated and 1/3 polyunsaturated, and the P/S ratio to be >0.5. A desirable aim would therefore be the reduction of the intake of saturated fatty acids and this has to be taken into account in the evaluation of salatrims.

A further concern related to the possible formation of insoluble intestinal calcium salts as a consequence of the increased amounts of unabsorbed long-chain fatty acids, e.g. stearic acid, in the gastrointestinal tract. This could markedly reduce the bioavailability and cause increased faecal excretion of dietary calcium with possible untoward long-term effects on bone mineralisation. However, the mineral intake/excretion data in the study on 19 males and 17 females, dosed for 7 days with either 45 g or 60 g salatrims, showed no significant rise in faecal mineral excretion (about 5% for Ca). Thus untoward effects on bone mineralisation could be considered to be unlikely[22]. Additional support for this view was provided by the data on bone mineralisation in the minipig study[21], where no effect on bone mineralisation was noted, when 10% of salatrims was administered in their diet.

12.   CONCLUSION

The Committee noted that there were inadequacies in the database: an absence of chronic toxicity, reproduction and developmental toxicity studies, a paucity of information on the effect of the consumption of foods containing salatrims on children under the age of 16, and a comparatively low saving in daily energy intake, if salatrims were to be used as proposed.

The Committee noted, however, that salatrims contained no structural alerts for mutagenicity or carcinogenicity, were non-genotoxic, were easily hydrolysed in the gastrointestinal tract, and that the animal feeding studies showed no significant toxic effects.

The Committee had no concerns over reproductive or developmental toxicity because the structured triacylglycerols in salatrims were not known to cause such toxic effects and the feeding studies in rats and minipigs had not shown any toxic lesions in the organs of the reproductive systems in these species.

The only adverse effects observed in a number of human tolerance studies were gastrointestinal complaints when ingested doses exceeded 30 g/day/person.

The Committee was satisfied that the scattered rises in serum transaminase levels seen in human studies had no clinical significance and that any suspected thrombogenic potential or interference with mineral bioavailability, particularly of calcium and zinc, was absent.

The Committee noted that foods containing salatrims would provide fewer calories than foods containing conventional fats. It also drew attention to the fact that the specification supplied limits the presence of stearic acid to a maximum of 70% and of trans-fatty acids, originating mainly from elaidic acid in partially hydrogenated soyabean oil, to a maximum of 1%.

The Committee therefore considered the use of salatrims acceptable under the conditions proposed except in foods aimed at children, as adequate data had not been provided to evaluate the consequences in relation to gastrointestinal effects of the consumption by them of such salatrims-containing foods. Any extension of use would require a new assessment.

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