# COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

## STATEMENT FOR THE ADVISORY COMMITTEE ON NOVEL FOODS AND PROCESSES ON SHORT AND LONG CHAIN TRIACYL GLYCEROL MOLECULES (SALATRIMs) - A FAMILY OF LOW CALORIE FATS

### Introduction

1. We have been asked by the Advisory Committee on Novel Foods and Processes (ACNFP) to comment on specific aspects of a large submission of data received by the ACNFP in respect of Salatrims, a family of low calorie fat products. The ACNFP reviewed the available toxicological and clinical safety data on Salatrim at its 32nd meeting on 26 September 1996. The ACNFP requested advice from the Committee (COT) in respect of:

i) the adequacy of the animal toxicological database and, in particular, the arguments proposed by Cultor Food Science, who wish to market these products in the UK, regarding limited testing of Salatrims in animals.

ii) an evaluation of the increases in serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) documented in human clinical studies and, for comments regarding the lack of predictivity of the animal toxicology studies in respect of these effects on the liver.

2. Salatrims comprise a family of structured glycerides composed predominantly of mixtures of long chain fatty acids (LCFAs; principally stearic acid) and short chain fatty acids (SCFAs; acetic, propionic and/or butyric) which are intended as low caloried fats for use in soft sweets, coatings (eg wafers and confections), dairy products, shortening and potentially in table spreads. Predicted intakes will vary according to the uses of Salatrims, level of substitution for existing fats, and the extent to which these fatty acids are consumed. We have considered several reports provided by Cultor Food Science which present calculations regarding potential intakes. The estimated intake for the whole population assuming selected uses varied between 11-29 g/day (mean), and 18-65 g/day (97.5th percentile). Estimates of intake using all potential uses varied between 18-46 g/day (mean) and 30-88 g/day (97.5th percentile). We also *note* that the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives estimated intakes in children aged 3-5 years to be approximately 26 g/day (90th percentile). We

*note* that there are considerable uncertainties regarding the methods and precision of the calculated estimates of potential Salatrim intake and that the various figures provided by the company show a wide variation. We are also *aware* that children have a caloric intake which on a body weight basis is higher than that in adults, on which basis their exposure to Salatrim would also be higher. Thus it would have facilitated interpretation of the intake data if the latter had been calculated and expressed in terms of grams Salatrim/kg bw/day and grams Salatrim/energy intake/day. However, we *consider* that the available figures provided by the company can be used as a guide to the evaluation of the clinical and toxicological data provided in the submission.

3. An abbreviated nomenclature has been used by the manufacturers and also throughout this statement to describe the various Salatrims which have been evaluated in toxicological and clinical safety studies. An account of this nomenclature and details regarding all Salatrims tested are given in tables 1 and 2 of the Annex to this statement. (For example, in Salatrim 43SO tributyrin and tripropionin are the SCFAs and the LCFA source is hydrogenated Soybean Oil.)

4. The specific questions raised by the ACNFP with regard to Salatrims were considered by the COT during 1996 and at a joint ACNFP/COT Working Group in early 1997 where representations from the company were heard. Following this latter meeting, additional information regarding intakes and evaluation of the animal toxicity and human clinical studies was submitted to the COT. A short summary of the available animal and human toxicology data on Salatrims is given below for information so that our consideration of the questions raised by the ACNFP can be placed into context.

**Animal Metabolism/Toxicity Data**

5. Initial *in-vitro* studies of the hydrolysis of Salatrim products using porcine pancreatic lipase demonstrated that a wide range of Salatrim triacylglycerides underwent rapid hydrolysis. *In-vivo* metabolism experiments in rats were designed to compare the metabolism of a specified Salatrim (23CA) with triolein and the results showed that Salatrim 23CA was metabolised in an analogous way to triolein (a normal dietary fat).

6. Five 90-day feeding studies were undertaken in rats using a range of Salatrim products selected to include different combinations of short chain fatty acids (ie acetate, propionate, butyrate) and different sources of stearate (ie canola oil, cottonseed oil and soybean oil). Details regarding the Salatrim products tested are given in table 2 of Annex 1. The results of the toxicity studies were consistent, showing no toxicologically significant effects at up to 10% w/w in the diet (ie approximately 6-8 g fat/kg bw/day). The observed changes in clinical chemistry and histopathological findings noted in these studies, which occurred predominantly at 10% w/w in the diet and consisted of alterations in bone mineral levels (eg increased bone zinc concentrations), and of renal mineralisation in female rats, were considered by the authors to be consistent with alterations in mineral metabolism induced by a reduced proportion of polyunsaturated fats in the diet. The authors of the animal

toxicology studies noted that published evidence is available which reported diet-induced alterations in mineral metabolism, and in particular reduced bone zinc concentrations in rats following administration of diets containing high levels of polyunsaturated fatty acids. (Lusak & Johnson, 1992) Dietary administration of 10% w/w Salatrim 23SO to rats for up to 17 days had no effect on serum activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) or γ-glutamyltransferase (GGT) activities, although increases in the levels of serum enzyme have been documented in the human clinical studies. No toxicologically significant effects were documented in a 28-day study in minipigs fed a diet containing 10% w/w Salatrim 23SO. Samples of caecum were taken during routine necropsies of the rats fed Salatrim 23CA or 32CA for 13 weeks, frozen and then subsequently analysed for gut microflora and for evidence of any Salatrim induced changes in caecal pH, and primary/secondary metabolites of bile acids and phytosterols and for cholesterol and coprostanol levels. There was no evidence of any effects on gut microflora from these experiments. We *note* that the morphological methods used in these investigations to assess effects on gut microflora were insensitive. Salatrim products do not contain any structural alerts for potential mutagenicity. There was no evidence of genotoxicity in an adequate range of *in-vitro* or *in-vivo* studies. No studies to evaluate potential carcinogenicity or effects on reproduction are available.

**Human Toxicity Data**

7. Four clinic-based studies using adult volunteers have been undertaken which utilised double blind study designs. The experimental protocols used exposures up to 60 g Salatrim/day for 1, 4 or 7 days and study designs which included a 4 day triple cross-over experiment. This type of design allowed the effects of three different fat sources on clinical chemistry and recording of symptomatology to be compared in the same individuals, but we *note* that only a very short exposure period of 4 days was used. The results of the clinic based studies with tested Salatrim products showed similar effects on gastrointestinal function (ie nausea, stomach cramps, diarrhoea, flatulence were reported) at 60 g/day. These effects subsided when the Salatrim administration was stopped. Overall, the authors concluded that 30 g Salatrim/day in the clinic-based studies did not result in any gastrointestinal effects. We *note*, however, the limited duration of these studies and that clinical investigations were only performed on healthy adults and thus potential effects in children or individuals with compromised gastrointestinal function were not considered.

8. A 28-day free living study was undertaken using groups of at least 12 male and 12 female volunteers consuming a range of food products containing one of three Salatrim products (23SO, 43SO, 4SO) at levels of up to 60 g Salatrim/day. The purpose of this study was to allow an extended evaluation of the Salatrim products and to confirm the effects seen in the clinic based studies and also to determine if any of these effects were reversible. The adverse effects reported in this study, predominantly at 60 g Salatrim/day, were consistent with those documented during the clinic based studies (ie gastrointestinal disturbances and increases in serum transaminase levels). The

authors considered that the effects on serum transaminase levels were transient with noticeable decreases in serum activities of AST and ALT occurring towards the end of the study period. We *note*, however, that convincing evidence of a decline in the activities of serum AST and ALT to baseline levels was not provided. Although increases in serum AST and ALT activities rarely reached clinical significance in the 28 day free living study, we *note* that there were concomitant increases in the activities of some liver function enzymes (ie AST and ALT) and reduced serum cholesterol documented in the clinic-based studies at 60 g Salatrim/day. There is limited published evidence to support the view that an increased load of high caloric materials such as proteins or sucrose in the diet may induce alterations in serum transaminase enzyme levels (Schimke, 1962; Porikos & Van Itallie, 1983).

9. The authors noted that none of the subjects in the 28-day free living study reported severe gastrointestinal effects which might impair normal function. The effects were considered to be "mild" or "annoying". They considered that 30 g Salatrim/day would have little or no effect on the health of individuals. We *note* that complaints of adverse effects on gastrointestinal function persisted for at least 10 days in a small number of individuals at all dose levels, including two who consumed 30 g Salatrim/day.

10. There are a number of proposals concerning the mechanism(s) for the effects of Salatrim on gastrointestinal function. Increased stool weight and faecal water content as reported in the 4 day triple cross over study could have resulted in a bulking effect and hence may have contributed to the gastrointestinal symptoms. (Besselaar Clinical Research Unit, 1993). The authors have also proposed that the introduction of Salatrim, a poorly digested fat, into the diet may have caused transient gastrointestinal disturbance since similar effects have been documented following an abrupt increase in dietary fibre intake. (Finley *et al.*, 1994a; Pilch, 1987). The authors of the clinic-based studies have also speculated that the level of short chain fatty acids at high Salatrim doses (ie 60 g/day) might temporarily overwhelm the ability to utilise acetate which might be sufficient to induce some adverse gastrointestinal symptoms. (Finley *et al.*, 1994b). Overall, we *conclude* that there is no convincing explanation regarding the mechanism of the adverse gastrointestinal effects of Salatrims seen in the clinical studies. Additionally there is no information available regarding the likelihood of adverse gastrointestinal effects in children or individuals with compromised gastrointestinal tract function.

**Consideration of the adequacy of the animal toxicological database**

11. We *consider* that the animal toxicity studies were adequately conducted and can be used in the safety assessment of Salatrims. However, we *note*, that there are inadequate data available in respect of the potential effects of bolus doses of SCFAs on reproduction following their release from Salatrims during the metabolism of Salatrims in the gastrointestinal tract. We are *aware* that butyrate and propionate have been shown to have teratogenic potential *in-vitro* ( Coakley *et al.* 1986, Brown *et al.* 1987). We reviewed the additional data provided by Cultor Food Science including calculations regarding potential blood

levels of butyrate following consumption of a meal containing 30 g of Salatrim 4SO and *conclude* that additional pharmacokinetic studies to evaluate blood levels of SCFAs in volunteers following consumption of individual Salatrim products are required before any conclusions regarding the teratogenic risk of Salatrim(s) can be drawn. We *agree* that there is no requirement for additional mutagenicity studies or for the provision of carcinogenicity bioassays with Salatrim(s).

**Consideration of the increases in aspartate aminotransferase and alanine aminotransferase documented in human clinical studies.**

12. We have considered all of the data available from the clinical studies with regard to the potential effects of Salatrim(s) on serum enzymes which can be used to evaluate potential adverse effects on liver function and have also considered the further information supplied by Cultor Food Science regarding an evaluation of the individual clinical chemistry for all of the clinical studies. We *conclude* that increases in both AST and ALT occurred in a higher proportion of individuals consuming 30 g or 60 g Salatrim per day for 28 days compared to controls, although only a few of these reported increases can be regarded as reaching the level of clinical significance. Whereas there were differences between individuals in respect of the magnitude of the increase in AST and ALT in response to the ingested dose level of Salatrim and also between that induced by different Salatrim products, we *conclude* that the evidence is consistent with a weak treatment related effect which did not appear to decline during the 28 day treatment period. The analysis of individual clinical chemical data for all of the clinical studies is complicated by the absence of detailed background information on the individuals included in the clinical studies and also, in respect of the 28 day study, by the identification of inconsistencies and transcription errors both in the original and published reports of this study. There was also additional evidence of concurrent increases in other serum enzymes consistent with liver dysfunction, such as alkaline phosphatase, lactate dehydrogenase and GGT, in a small number of individuals who ingested Salatrims. Overall, we *consider* that a No Observable Adverse Effect Level (NOAEL) with respect to clinical chemical markers of liver function cannot be identified from the clinical safety studies undertaken with Salatrims and that no conclusions can be drawn with regard to the mechanism or biological significance of the Salatrim-induced effects on AST and ALT.

13. We *consider* that there are insufficient data available to derive any firm conclusions regarding the reasons for an absence of effects on serum liver enzymes in the animal studies. We *conclude* that it would be appropriate to proceed on the basis that the effects on serum liver enzymes documented in the clinical safety studies were treatment-related and thus require additional evaluation, particularly with respect to the identification of an appropriate NOAEL. In this regard the discrepancies in the reporting of the results from the 28 day free living study limit the value of this investigation. We *recommend* that a suitable long term clinical evaluation study of the individual Salatrims to be marketed should be undertaken with particular reference to the identification of a NOAEL.

Discussion and Recommendations

14. We have, during our consideration of the questions raised by the ACNFP, evaluated all of the available toxicological and clinical safety studies on Salatrim and have considered the representations made by Cultor Food Science at the joint ACNFP/COT Working Group. The following two conclusions respond to the specific requests made by the ACNFP. An additional conclusion based on a consideration of all the toxicological and clinical data submitted to the COT is given in paragraph 15.

i) Animal toxicological data alone on Salatrims are insufficient to evaluate the proposed use of these materials as fat replacers. We *recommend* that additional pharmacokinetic studies to evaluate blood levels of SCFAs in volunteers following consumption of individual Salatrim products are required before any conclusions regarding the teratogenic risk of Salatrim(s) can be drawn.

ii) The clinical safety studies demonstrate a weak treatment related effect of Salatrims on serum levels of marker enzymes for liver dysfunction. There are insufficient data available to derive any firm conclusions regarding the reasons for an absence of effects on serum liver enzymes in the animal studies. The documented discrepancies in the reporting of the results from the 28 day free living study limit the value of this investigation with regard to evaluating the potential effects of Salatrims on liver function. We *recommend* that a suitable long term clinical evaluation study of the individual Salatrims to be marketed should be undertaken with particular reference to the identification of a NOAEL for this effect.

15. Regarding overall conclusions, we *note* that evidence of adverse effects on gastrointestinal function and on marker enzymes for liver dysfunction in humans were reported following the consumption of 30 g Salatrim 23SO for 28 days and that there was clear evidence of adverse effects at 60 g/day regarding several Salatrim products. We are *concerned* that there would appear to be no margin of safety between these levels of consumption and the calculated potential intakes reported above in paragraph 2. Additionally children and individuals with compromised gastrointestinal function might be more susceptible to these particular effects associated with Salatrim consumption. We therefore *recommend* that the additional clinical studies requested in paragraph 14 (ii) should further investigate the potential effects of the Salatrim(s) to be marketed on gastrointestinal function with a view to also identifying a NOAEL for this effect.

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Table 1. Typical molar ratios of short- and long-chain acid sources used to prepare the SALATRIM family of edible oils*

| SALATRIM family | short-chain source | long-chain source | molar ratio |
|---|---|---|---|
| SALATRIM 4CA | tributyrin | hydrogenated canola oil | 2.5:1 |
| SALATRIM 4SO | tributyrin | hydrogenated soybean oil | 12:1 |
| SALATRIM 23CA | triacetin/tripropionin | hydrogenated canola oil | 11:1:1 |
| SALATRIM 23SO | triacetin/tripropionin | hydrogenated soybean oil | 11:1:1 |
| SALATRIM 32CA | tripropionin/triacetin | hydrogenated canola oil | 11:1:1 |
| SALATRIM 43SO | tributyrin/tripropionin | hydrogenated soybean oil | 11:1:1 |
| SALATRIM 234CS | triacetin/tripropionin/ tributyrin | hydrogenated cottonseed oil | 4:4:4:1 |
| SALATRIM 234CA | triacetin/tripropionin/ tributyrin | hydrogenated canola oil | 4:4:4:1 |
| SALATRIM 234SO | triacetin/tripropionin/ tributyrin | hydrogenated soybean oil | 4:4:4:1 |

* The SALATRIM family name defines the sources of the short-chain and long-chain fatty acids with the numerals representing the carbon chain lengths of the short chain acids in decreasing proportion in the mix; the letters define the oil which provides the source of the long-chain fatty acids. (E.g. in SALATRIM 43SO tributyrin and tripropionin are the SCFAs and the LCFA source is hydrogenated Soybean Oil. The molar ratio of the mix that is used to prepare the SALATRIM is 11 parts tributyrin : 1 part tripropionin : 1 part hydrogenated soybean oil).

A listing of those products which have been used in safety evaluation studies is also given below. There are only very minor differences in composition between Salatrim products prepared from different long chain fatty acid sources. However, different batches of a product may have used differing molar ratios of the starting products.

Table 2. Materials used in metabolism and toxicity studies

| | |
|---|---|
| Ames tests | 4CA, 23CA, 23SO, 32CA, 234CA, 234CS |
| In vitro mammalian tests | 23CA |
| In vivo bone marrow micronucleus assays | 234CA, 234SO |
| In vitro metabolism (Porcine pancreatic lipase) | 4CA, 23CA, 32CA, 234CA |
| Metabolism in rats | 23CA |
| 90 day feeding studies (rats) (*and supplementary 17 day test of effects on transaminases) | 4CA, 23CA, 32CA, 23SO, 234CA, 234CS* |
| 28 day mini-pigs | 23SO |
| Effects on gut microflora: rats | 23CA, 32CA |
| Studies I & II in volunteers | 23CA |
| Studies III & IV in volunteers | 23SO |
| Free living study in volunteers | 4SO, 23SO, 43SO |

References

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