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**Opinion of the Scientific Committee on Food
on sucralose**

(Adopted by the SCF on 7 September 2000)

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Terms of reference

To update the Committee's opinion of 1989 on the safety evaluation of the artificial sweetener, sucralose.

Background

Sucralose is an artificial sweetener, 1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl-4-chloro-4-deoxy- β -D-galactopyranoside, known also as 4,1',6'-trichlorogalactosucrose (TGS). It is derived from sucrose by the selective replacement of three hydroxyl groups by chlorine atoms. It is a crystalline material readily soluble in water, lower alcohols and other polar solvents, giving solutions of neutral pH. Degradation occurs by simple hydrolysis. In acid solution, such as is found in some soft drinks, it hydrolyses slowly (e.g. 0.3% breakdown over 6 months at pH 3.0 and 20°C) to its constituent monosaccharides, 4-chloro-4-deoxygalactose (4-CG) and 1,6-dichloro-1,6-dideoxyfructose (1,6-DCF). It is therefore necessary to assess the safety of its hydrolysis products as well as the sweetener itself.

At room temperature in water at 4-5%, it has a sweetness potency around 600-650 times that of sucrose. Its proposed uses include soft drinks and other beverages, sugar substitute preparations, desserts, chewing gum, baking mixes, pre-sweetened breakfast cereals and salad dressings. If it were used as the sole sweetener, it has been estimated by the petitioner that intakes would be no more than 140 mg/day.¹

Following the allocation of an Acceptable Daily Intake (ADI) of 0-15mg/kg bw by the Joint FAO/WHO Expert Group on Food Additives (JECFA) in 1990,² sucralose has been approved as a sweetener in a large number of countries.

Previous SCF evaluations

The Scientific Committee for Food (SCF) first considered an extensive database on sucralose and its hydrolysis products in 1987 and further data submitted in 1988.^{1,3-7} The Committee's opinion was published in 1989.⁸ At that time the Committee considered sucralose to be toxicologically unacceptable due to unresolved questions concerning some of the observed treatment-related effects on body weight, organ weights and haematological parameters. It was unclear whether the effects observed in laboratory animals might be secondary to a cascade of events caused by impalatability of sucralose when given in the diet or might be due to a direct toxic action of sucralose itself. The Committee was particularly concerned about the relevance of potentially adverse findings relating to the immune system (thymus, spleen and white blood cell counts). There was also reference to the weak mutagenic activity of one of the hydrolysis products of sucralose, 1,6-DCF. The Committee was however satisfied that sucralose had not shown any serious target-directed organ toxicity and that the

sweetener as such possessed no carcinogenic or genotoxic potential. At its 70th meeting in December 1989, the Committee outlined the further work it considered would be necessary to resolve its outstanding questions.⁹

JECFA evaluations

Sucralose was first evaluated by JECFA at its 33rd meeting in 1989, when a temporary ADI of 0-3.5mg/kg bw was allocated, based on a no-observed-effect level of 750mg/kg bw/day in a 1-year dog study and a safety factor of 200. A toxicological monograph covering the studies available at that time was published.¹⁰

JECFA requested further data on absorption and metabolism in humans after prolonged oral dosing, studies to show sucralose produced no adverse effects on individuals with insulin-dependent and maturity-onset diabetes, studies on the elimination of sucralose from pregnant animals and the fetus to exclude bioaccumulation, and a short-term study on 6-chlorofructose (a potential intermediate in the metabolism of 1,6-DCF). Further studies on sucralose were subsequently submitted and although these were not the precise studies requested by JECFA in 1989, they were evaluated at its 37th meeting in 1991 and were considered sufficient to allocate a full ADI of 0-15mg/kg bw, based on the NOEL of 1500mg/kg bw/day from the long-term study in rats, which had included *in utero* exposure, and a safety factor of 100. JECFA noted that there were reductions in body weight gain in all sucralose-treated groups (150, 500 and 1500 mg/kg bw/day) compared with controls in this critical study. However, they also noted that food consumption was also reduced in all treated groups. They concluded that the reductions in weight gain were secondary to reduced food consumption and attributed this to impalatability of high concentrations of sucralose in the diet.

JECFA noted that studies on diabetics were underway. It further commented that additional immunotoxicity studies to assess the significance of observed weight changes in spleen and thymus and changes in lymphocyte counts were desirable and that, as yet, a causal relationship between these findings and high levels of exposure to sucralose could not be excluded. An addendum to the toxicological monograph was published.¹¹

Present evaluation

The present opinion considers further studies submitted to the SCF between 1994-2000 against the background of the studies seen earlier by the Committee. The data address the Committee's earlier unresolved questions concerning immune function and the theory that impalatability of sucralose given in the diet explains certain observations from earlier studies. The Committee has also considered other studies, on changes in food consumption and food conversion efficiency in relation to reductions in bodyweight gain in rats, on toxicokinetics of sucralose in man and laboratory animal species, on mutagenicity of 1,6-DCF, and on glucose homeostasis in healthy human volunteers and volunteers with diabetes. These studies were done at the request of the other regulatory and advisory bodies, but were also submitted to the SCF. Lastly, in response to reservations raised by some EU Member States, the Committee has reviewed the earlier teratology studies again and a new rabbit teratology study, and considered the stability of sucralose, its metabolism and whether repeated administration might alter the activity of the gut microflora. The Committee's conclusions on each of these aspects are set out below. A more detailed discussion of each topic can be found in Annex 1. Much of the data on which the evaluation of sucralose is based has recently been published.⁵⁹

Immunotoxicity aspects

The SCF expressed concern in 1989 about the possibility of effects on the immune system, based on observations in earlier studies of "effects on lymphoid organs, especially spleen and thymus weights, sporadic but not entirely random statistically significant reductions in peripheral white blood cell and lymphocyte counts".⁸ These observations related particularly to an earlier 4-8 week dietary study¹² in which sucralose had been given at up to 5% in the diet, and to other studies which showed slight effects^{13,14} (see Annex 1 for details).

In response to the question raised by the SCF of possible impairment of the function of the immune system, a 28-day, Tier I-type, study¹⁸ was conducted that specifically addressed immune aspects. No immunotoxicological effects were seen after either gavage or dietary administration of doses up to 3000mg/kg bw/day (see Annex 1 for details).

While this Tier I-type study was negative, it is not possible to entirely set aside the earlier findings. The data as a whole are compatible either with the view that there is no toxic effect on the thymus, the results of the earlier 4-8 week study being confounded by physiological thymic involution and reduced food intake/body weight, or with the view that there is a direct toxic effect of sucralose on the thymus, which is only apparent after several weeks of treatment at doses at or more likely above 3000mg/kg bw/day.

The question of whether some of the effects observed in earlier studies on thymus and spleen weight could be attributed to impalatability of sucralose at high levels in the diet has also been further addressed. In a gavage study,²¹ administration of sucralose covered the potentially sensitive period for induction of thymic changes around the time of normal physiological involution and provided sustained systemic exposure over 26 weeks. No significant effects were observed on lymphoid tissues. This study confirmed that the highest dose tested of 3000mg/kg bw/day is a no-effect level for lymphoid changes, which is consistent with the results of other dietary and gavage studies.

In the light of all the above considerations, the Committee has concluded that the data are now sufficient to establish a clear NOEL of 3000mg/kg bw/day for any effects on lymphoid organs and the immune system that might occur, whether caused directly by sucralose or indirectly.

Food consumption, food conversion efficiency and body weight

A study on food consumption and bodyweight gain was requested by the US Food and Drug Administration but was also submitted to the SCF²² (see Annex 1 for details). The results indicate that reductions in body weight at doses up to 1% in the diet are attributable solely to reductions in food intake, but at higher doses of 3% sucralose in the diet, while the reductions in body weight are mostly attributable to reduced food intake, a small part of the reduction (around 5%) is not. The Committee has concluded that the no-observed-effect level (NOEL) for this study was 1% in the diet at which intakes averaged 628-787mg/kg bw/day and that reductions in body weight not attributable to impalatability of the diet should be taken into account in determining the overall NOEL.

Comparative toxicokinetics

The Committee reviewed the available evidence on comparative toxicokinetics in order to ensure that any pivotal studies selected for determination of an overall NOEL, and hence an ADI, were conducted in a species relevant to man (see Annex 1 for details). Earlier studies had established that orally administered sucralose is poorly absorbed in mice, rats, rabbits, dogs and man.¹⁰ Amounts ranging from 8-22% are absorbed in man and that which is absorbed is excreted rapidly, essentially unchanged, in urine.¹⁰ The toxicokinetics for sucralose in blood was shown to be independent of the dose. Following administration of single oral doses, the terminal elimination half-life was around 5, 25, 39 and 79 hours for rat, man, rabbit and dog respectively.²³ The Committee concluded from the half-life data that accumulation in man is unlikely with repeat exposures. Since the half-life in man is intermediate to rat, dog and rabbit, any of these experimental species may provide relevant data for safety evaluation.

Teratology

Following reservations submitted to the SCF about the adequacy of the rabbit teratology study,²⁴ the Committee reviewed again the original reports on the rat and rabbit teratology studies conducted in the 1980s^{25,26} (see Annex 1 for details). Subsequently, in April 2000, the petitioner submitted a new range-finding study and a full teratology study in the rabbit.^{52,53}

The Committee concluded that the rat study²⁵ was well conducted and no effects were seen at any dose level, including the top dose of 2000mg/kg bw/day. While the earlier rabbit study,²⁶ which used 700mg/kg bw as a top dose, was of poorer quality, the Committee concluded that it raised no suspicion of developmental effects. In spite of the severe maternal gastrointestinal effects in this study, no developmental effects were seen in the surviving animals. The Committee also concluded there was no need for a further teratology study on the basis of exposure arguments. A comparative toxicokinetic study in pregnant rabbits and rats using oral gavage, showed that systemic exposure (AUC_{24h}) in the rabbit given 350mg/kg bw on days 6-19 of pregnancy was approximately double that of rats given 2000mg/kg bw/day on days 6-15.

The new rabbit teratology study⁵³ confirmed the absence of effects on foetal growth or development at doses up to 1000mg/kg bw/day, despite maternal gastrointestinal disturbance in a few animals at the top dose. Thus, in the rabbit teratology studies, the only effects of interest were maternal gastrointestinal reactions, seen at 700mg/kg bw/day in the earlier study and at 1000mg/kg bw/day in the recent study. Both studies showed that 350mg/kg bw/day was a NOEL for maternal gastrointestinal effects. These effects are discussed later in the section "Considerations of an ADI".

Stability, metabolism and effects on gut microflora

A review of biotransformation studies submitted to the SCF²⁸ raised the question of whether sucralose could be degraded, possibly to a toxic metabolite, by human gut microflora, particularly if there was prolonged exposure (see Annex 1 for details). In response, the petitioner submitted further information about the stability of sucralose²⁹ and a discussion of the metabolism and its potential for adaptation.³⁰ The Committee agreed with the evidence and arguments put forward that the structure of the molecule is such that it is extremely

resistant to hydrolysis, and its hydrolysis products, 4-CG and 1,6-DCF, are themselves also resistant to further degradation. The chlorination of sucrose to give sucralose also has the effect of changing the conformation of the molecule so that it is resistant to attack from glycosidic enzymes that normally degrade carbohydrates in the gut. The Committee therefore concluded that metabolic adaptation in humans was highly unlikely.

Mutagenicity of 1,6-DCF

In its opinion of 1989, the Committee did not express any reservations about the mutagenicity of sucralose but a question remained about one of its hydrolysis products, 1,6-DCF. At the request of another advisory body in an EU Member State, further *in vivo* and *in vitro* studies were carried out⁴⁰⁻⁴² (see Annex 1 for details). These additional studies have provided reassuring results and the SCF has no further concerns about 1,6-DCF.

Studies on glucose homeostasis in healthy humans and diabetic volunteers

None of the studies conducted in either animals or humans up to 1989 had indicated any effect on glucose homeostasis. However, no studies had been carried out in diabetic subjects. Such studies were initially requested by JECFA in 1989 and subsequently by the Canadian authorities, in view of the likelihood of chronic, above average intakes by this group of consumers. In 1997, five clinical studies were submitted by the petitioner to the SCF on glucose homeostasis in diabetic and non-diabetic human volunteers.⁴⁵⁻⁴⁹

In one of the studies, on Type II diabetic volunteers, a consistent increase above baseline was observed in blood levels of glycosylated haemoglobin, a measure of long-term glucose homeostasis, in subjects given 667mg sucralose daily for 6 months, compared to placebo treated subjects.⁴⁶ There were no changes in other indicators of glucose homeostasis. There was no effect of sucralose on the same indicators of glucose homeostasis in normal, non-diabetic subjects.⁴⁸ A subsequent study,⁴⁹ utilising the same dose of sucralose as in the previous study on diabetics, but given for a shorter period of 13 weeks and with a larger number of diabetic subjects, did not find any effect on glycosylated haemoglobin. However, the Committee considered that the two studies were not entirely comparable (see Annex 1 for details). Interpretation of the studies was further complicated by differing baseline values for glycosylated haemoglobin between sucralose and control groups in both studies. The Committee considered that regression to the mean from differing baseline values might account for the results, but noted that correction for regression to the mean still left a marginal significant difference between sucralose and control males on oral hypoglycaemic agents. Because of the differing baseline values, the possibility of a small effect cannot be definitively ruled out. However, bearing in mind that the amount of sucralose administered daily in these studies was greater than that likely to be consumed by high consumers, such as diabetics, the Committee concluded from consideration of all the evidence that any such effect would be so small as to be clinically insignificant.

Consideration of an ADI

After consideration of all the data on sucralose and its hydrolysis products, submitted both for the earlier evaluation⁸ and for this evaluation, the Committee has concluded that there are sufficient data with which to establish an ADI. The full range of the studies on sucralose and its hydrolysis products, which have now been submitted to the SCF, is listed in Annexes 2 and 3. Annex 2 also indicates, where appropriate and where established, the effects seen, the no-observed effect levels and lowest-observed effect levels for sucralose.

There is adequate evidence both for sucralose and its hydrolysis products, that there are no concerns about mutagenicity, carcinogenicity, developmental or reproductive toxicity. The critical studies which need to be considered in setting the ADI are those indicating effects on immune parameters in the rat, gastrointestinal effects in the rabbit and body weight effects in the rat. Each of these will be considered in turn.

Effects were observed at high doses, in some but not all studies, on one or several parameters of the immune system, notably thymus weight and histology, spleen weight, and reduced white blood cell and lymphocyte counts. However, the Committee has concluded from all the relevant studies, which included a specially enhanced study on cells, tissues and function of the immune system, that there is a clear NOEL of 3000mg/kg bw/day for any effects on lymphoid organs and the immune system that might occur, whether caused directly by sucralose, or indirectly via stress and/or dietary factors.

A lower NOEL of 350mg/kg bw/day for maternal gastrointestinal effects was observed in both rabbit teratology studies.^{26,53} In the earlier study, 700mg/kg bw/day caused markedly reduced food and water intake, gastrointestinal disturbance, including scouring, sufficient to cause weight loss and maternal death in two cases and abortion in four cases. In the more recent rabbit studies,^{52,53} no gastrointestinal disturbance was seen in the range-finding study up to 900 mg/kg bw/day, but effects were seen in a few animals in the full teratology study at 1000 mg/kg bw/day, albeit of less severity than in the earlier study (reduced food intakes, soft faeces, and abortion/total embryo-foetal loss in five cases, but no scouring or maternal deaths). The Committee therefore gave further consideration to whether the ADI should be set on the NOAEL for rabbit gastrointestinal effects.

Comparative oral toxicokinetic studies on pregnant rabbits and pregnant rats have confirmed that sucralose is absorbed in both species, but systemic exposure in the pregnant rabbit given 350mg/kg bw/day was about twice as high as that in the pregnant rat given a 6-fold higher dose of 2000mg/kg bw/day (AUC_{24h} 320 and 150 μ g.h/ml for rabbit and rat respectively). Even after 4000mg/kg bw/day, given orally by gavage to the non-pregnant rat, the AUC_{24} was only about 250 μ g.h/ml. These observations raised the question of whether the maternal gastrointestinal toxicity observed in the rabbit was due to systemic absorption of sucralose (the rabbit absorbs around 20% of an oral dose) or was a purely local effect on the gut.

The Committee noted that the rabbit is widely agreed to be particularly sensitive to disturbances of the gastrointestinal tract. In the rabbit teratology studies, sucralose was given in bolus doses by gavage, the mode of administration most likely to cause such problems with poorly absorbed substances that remain in the lower gut. The Committee considered comparative information about absorption of sucralose and gastrointestinal effects in other laboratory species. In rats, caecal enlargement reported in repeat-dose studies at 2000mg/kg

bw/day or more,^{14,21} is indicative of a local effect due to retention of poorly absorbed material in the gut. It was further noted that no gastrointestinal signs or histopathological lesions were reported in mice, which absorb 20-25% of an oral dose, when given up to 4500mg/kg bw/day for 2 years,¹⁶ nor in dogs, which absorb around 35%, given bolus doses up to 850 mg/kg bw/day via the diet for 1 year.¹⁷ No gastrointestinal symptoms were reported in human volunteer studies, in which doses up to 1000mg/person/day were given.⁴⁵⁻⁴⁹ The Committee also took into account further information submitted by the petitioner on toxicokinetics in the dog,⁵⁴ the species showing the highest absorption of sucralose. The petitioner had concluded that at high doses the dog would have had more systemic exposure to sucralose than the rabbit. It was noted that this was based on extrapolation from a single low-dose experiment. Nevertheless, given the passive absorption of sucralose from the gut, its lack of metabolism and its elimination by glomerular filtration, such extrapolation is acceptable. The Committee therefore considers that the totality of the data from the dog and other species are sufficient to answer the question the Committee raised earlier⁵⁵ and that it is reasonable to conclude that the effects observed on the gastrointestinal tract in the rabbit are most likely attributable to the sensitivity of that species to high doses of poorly digestible substances, exerting a local osmotic effect on the bowel. Such effects are not likely to occur in other species, including man, and so are not considered relevant to the setting of an ADI.

The pivotal effect on which to base an ADI is the consistent reduction in body weight gain observed in a number of studies in rats (see Annex 2). Effects on body weight gain were seen at particularly low doses (0.3% in the diet, equivalent to around 150mg/kg bw/day) in the 2-year chronic toxicity/carcinogenicity study¹⁵ and in the parental animals and offspring in the two-generation study.¹³ In each of these studies, in which doses of 0.3%, 1% and 3% were given in the diet, reductions in food consumption were around 10% or less compared with controls and the reductions were not dose-related, indicating that they were probably attributable to impalatability of sucralose-containing diets. Confirmation of this comes from a pair feeding/dietary restriction study,²² specifically designed to address the impalatability issue. This showed that, at doses up to 1% in the diet (628-787mg/kg bw/day), effects on food intake and body weight were caused by impalatability of the diet. Only at dietary doses of 3% (1973-2455mg/kg bw/day) and above, could a small proportion of the effects on body weight be attributed to direct toxicity of sucralose. These findings of direct toxicity at higher doses are further confirmed by the observation of reduced body weight in males at 3000mg/kg bw/day in the 26-week oral gavage study,²¹ in which impalatability cannot be a factor. Given that other dietary and gavage studies identify 1500mg/kg bw as a clear no-effect level for body weight changes, it is suggested that this is the critical NOEL for establishing an ADI. Application of a 100-fold safety factor to take account of possible inter-species and inter-human variability gives an ADI of 0-15mg/kg bw.

Conclusions

The Committee is satisfied that the range of studies now available is sufficient for a full safety evaluation of sucralose.

The studies on glucose homeostasis in both normal subjects and subjects with insulin-dependent and non-insulin-dependent diabetes have been considered in depth. The Committee considers that the possibility of a small effect on glucose homeostasis in diabetic persons cannot be definitively ruled out. However, there is no consistent evidence of any such

effect from the various studies. The Committee has concluded that if any such effect occurred, it would be so small as to be clinically insignificant.

There is adequate evidence, both for sucralose and its hydrolysis products, that there are no concerns about mutagenicity, carcinogenicity, developmental or reproductive toxicity. Effects have been observed in some experimental animal studies on immune parameters, the gastrointestinal tract and body weight gain. Consideration of the critical studies on these aspects have identified reduced body weight gain, where it is attributable to direct sucralose toxicity rather than secondary to reduced food intake because of impalatability of the diet, as the pivotal effect for establishing an ADI. The overall NOEL for such reductions in body weight gain was 1500mg/kg bw/day.

The Committee concludes that sucralose is acceptable as a sweetener for general food use and that a full ADI of 0-15mg/kg bw can be established, based on application of a 100-fold safety factor to the overall NOEL of 1500mg/kg bw/day.

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ANNEX 1

Immunotoxicity aspects

In earlier studies effects were reported on lymphoid organs, especially spleen and thymus weights, and sporadic but not entirely random statistically significant reductions in peripheral white blood cell and lymphocyte counts. Reductions in spleen and thymus weight had been seen in top dose males and females in a 4-8 week dietary study in which sucralose had been given at up to 5% in the diet.¹² Thymic weight reduction was accompanied by histopathological changes described as thymic lymphocytolysis and thymic cortical hypoplasia. A trend towards reduced thymus weights had also been observed in a multigeneration study at a dietary sucralose level of 3%¹³ and in a short-term mineral balance study in which doses of sucralose up to 8% in the diet had been given.¹⁴ No effects on the thymus or spleen had been observed in a 2-year rat study with *in utero* exposure at doses up to 3% in the diet,¹⁵ nor in a 2-year mouse study¹⁶ or 12-month dog study,¹⁷ in which the top doses were 3% in the diet. Reductions in total white blood cell and lymphocyte counts were also seen in the 4-8 week rat dietary study¹² and (questionably) in the 12-month dog study,¹⁷ but the Committee was satisfied in the case of the latter study that these were age-related changes characteristic of the beagle dog.

In response to the question raised by the SCF in 1989⁸ of possible impairment of the function of the immune system, a 28-day, Tier I-type, oral immunotoxicity study¹⁸ was conducted according to the US National Toxicology Program Guideline.¹⁹ The study used the same strain of rats as was used earlier in the 4-8 week dietary study that triggered the concerns and included both gavage and dietary administration. Separate toxicokinetic studies in the rat showed that overall systemic exposure to sucralose was similar following comparable doses via gavage or via diet, though peak concentrations were higher following bolus gavage doses.⁵¹ Thus, should any direct effects of sucralose of toxicological significance have occurred, it would be expected that they would be seen in both gavage and dietary groups in this study.

No immunotoxicological effects were seen after either gavage or dietary administration as judged by lack of changes in immunoglobulin concentrations in serum, lymphocyte subsets in the spleen, natural killer activity of spleen cells, bone marrow counts and pathology. No changes in spleen weight were observed. Changes in thymus weight were seen in the top dose gavage group receiving 3000mg sucralose/kg bw/day but they were of opposite direction in males and females and no changes were seen in the dietary groups receiving a similar dose of sucralose. No effects on thymic histopathology were observed. The normal process of thymic involution occurs at 6-11 weeks in the rat and so would have been occurring at the time these animals were being treated. Thus changes in thymus weight at the termination of this study at 8-9 weeks of age are difficult to interpret, but were unlikely to be due to sucralose. The Committee noted that in a previous rat gavage study, thymus weights were very variable.²⁰

It should be noted that daily intakes of sucralose in the dietary part of the oral immunotoxicity study, of 3150 and 3070mg/kg bw in males and females respectively, were lower than those achieved in the earlier 4-8 week dietary study,¹² which were around 6000mg/kg bw at the start falling to just under 3000mg/kg bw by week 8 in females. The

differing durations of the oral immunotoxicity study and the 4-8 week dietary study may also have been a factor contributing to the different results. In the earlier 4-8 week dietary study, reductions in spleen and thymus weights were seen, together with histopathological changes whose interpretation has varied among expert pathologists (e.g. direct effect of sucralose, undernutrition and/or stress). The reductions in thymus weight were seen in males after 4 weeks, but not in females until 8 weeks. For most immunotoxic agents an effect would be expected to show up at 28 days at high doses but there are some known immunomodulators which show a delay in onset of immunotoxicity.

Thus the results of the oral immunotoxicity study and the earlier 4-8 week dietary study are not necessarily in conflict but do raise the question of whether a longer periods of treatment at higher dose levels might produce thymic changes. The Committee therefore looked at the database as a whole with respect to thymic changes. There were only two studies, other than the 4-8 week dietary study, in which the thymus was examined after 8 weeks' exposure. In the mineral balance study,¹⁴ the top dose was 8% in the diet, compared to 5% in the 4-8 week dietary study, and an apparent trend to reduced thymus weight was reported in females but it did not achieve statistical significance. Histopathological examination of the thymus was not conducted. In the gavage study of unusual design,²⁰ one group received 3000mg/kg bw for 8 weeks and there was no effect on thymic weight or histology. The data as a whole are thus compatible either with the view that there is no toxic effect on the thymus, the results of the 4-8 week study being confounded by physiological thymic involution and reduced food intake/body weight, or with the view that there is a direct toxic effect of sucralose on the thymus which is only apparent after several weeks of treatment at doses at, or more likely above, 3000mg/kg bw/day.

In its earlier review⁸ the Committee had also been concerned about decreases in white blood cell and lymphocyte counts. The petitioner and others have argued that these were inconsistent and of no toxic significance or were secondary to reductions in food intake and bodyweight.⁶ In the oral immunotoxicity study, increases not decreases were observed in white blood cell count, lymphocytes and neutrophils in rats given sucralose in the diet and these changes were not seen in the gavage groups. The Committee has concluded that any changes in white blood cells are unlikely to be directly attributable to sucralose itself.

The question of whether some of the effects observed in earlier studies, such as those on thymus and spleen weight, could be attributed to impalatability of sucralose at high levels in the diet has been further addressed. The results of the oral immunotoxicity study,¹⁸ showed decreases in food intake and body weights in rats given around 3000mg/kg bw/day of sucralose in the diet, but no such effects in rats given comparable doses by gavage. This supports the suggestion made, following earlier studies, that sucralose is impalatable at high doses in the diet. However, the oral immunotoxicity study does not give any further insight into the possible causes of the effects observed in earlier studies on lymphoid organs and cells, because conflicting evidence on thymus weight was obtained.

A new 26-week gavage study²¹ has also been carried out to specifically address the point raised by the SCF in 1989⁸ that the earlier, high dose gavage study,²⁰ which was designed to exclude any confounding nutritional effects of impalatability of sucralose when given in the diet, gave uninterpretable results. This was due to its unusual design, its failure to achieve the same high doses over time as in the earlier 4-8 week dietary study, the occurrence of decreased spleen weights, variable thymus weights, increased kidney weights, and the limited

clinical chemistry which did not enable liver function to be properly assessed. The design of the new gavage study²¹ ensured administration of sucralose covered the potentially sensitive period for induction of thymic changes around the time of normal physiological involution and provided sustained systemic exposure over 26 weeks. No significant effects were observed on lymphoid tissues. This study confirmed that the highest dose tested of 3000mg/kg bw/day is a no-effect level for lymphoid changes, which is consistent with the results of other dietary and gavage studies.

In the light of all the above considerations on both immunotoxicity and impalatability aspects, the Committee has concluded that the data are now sufficient to establish a clear NOEL of 3000mg/kg bw for any effects on lymphoid organs and the immune system that might occur, whether caused directly or indirectly.

Food consumption, food conversion efficiency and body weight

In a study on food consumption and bodyweight gain,²² rats aged 27-29 days at the start of the study, were fed basal diet or diet containing sucralose for 26 weeks. Those given basal diet were fed *ad libitum* or restricted to 95%, 90% or 85% of the *ad libitum* intake. Others were fed with diet containing 1% or 3% sucralose *ad libitum* or were given 90% of the *ad libitum* intake containing the same concentrations of sucralose. A consistent decrease was seen in overall mean food conversion efficiency in all sucralose and restricted basal diet groups compared with *ad libitum* controls, which was generally related to the degree of food restriction. Statistical comparison of regression curves of observed and expected body weights showed no significant differences between 1% sucralose groups and controls in the relationship of initial body weight, average weekly food consumption, final bodyweight or expected bodyweights. The 3% sucralose groups however showed statistically significant differences, bodyweights being 4-6% lower than expected.

Overall the results suggest that reductions in body weight at doses up to 1% in the diet are attributable solely to reductions in food intake but at higher doses of 3% sucralose in the diet, while the reductions in body weight are mostly attributable to reduced food intake, a small part of the reduction (around 5%) is not. The study authors suggested this residual reduction in bodyweight was due to a physiological response to the high concentrations of non-digestible sucralose in the diet and considered 3% to be a no-observed-adverse-effect level. However, the Committee has concluded that the no-observed-effect level (NOEL) for this study was 1% in the diet at which intakes averaged 628-787mg/kg bw/day and that reductions in body weight not attributable to impalatability should be taken into account in determining the overall NOEL.

Comparative toxicokinetics

The Committee reviewed the available evidence on comparative toxicokinetics in order to ensure that any pivotal studies selected for determination of an overall NOEL, and hence an ADI, were conducted in a species with kinetic relevance for man. The data initially available to the Committee indicated that there could be substantial differences between species in the half-life of sucralose in the blood and this raised the question of whether accumulation might occur in man with repeat exposure. This could occur, for example, if there was enterohepatic

recirculation of sucralose or prolonged absorption over time from poorly absorbable sucralose in the gut.

In response to these questions, the petitioner submitted further analytical and modelling data, derived from the earlier studies and from new kinetic studies, on rat, dog, man and pregnant rats and rabbits.²³ The terminal elimination half-life for sucralose in blood, which is independent of the dose, following administration of a single oral dose, indicated approximate values of 5, 25, 39 and 79 hours for rat, man, rabbit and dog respectively. Steady-state body load will be 1.44 times the half-life divided by the dosing interval. Since the terminal half-life in man is approximately the same as one day, body load following single daily repeated exposures can be estimated to be around 1.44 times that found following a single dose. This will overestimate actual potential for accumulation since the terminal half-life is only a minor component of the overall area under the kinetic curve. The Committee has therefore concluded that accumulation in man is unlikely with repeat exposures. Since half-life in man is intermediate to rat, dog and rabbit, the Committee further concluded that any of these experimental species may provide relevant data for safety evaluation.

Teratology

Following reservations submitted to the SCF about the adequacy of the rabbit teratology study raised by the UK Committee on Toxicity (COT),²⁴ the Committee reviewed again the original reports on the rat and rabbit teratology studies.^{25,26} The comments below address the specific reservations raised by the COT.

The rat study was well conducted and no effects were seen at any dose level including the top dose of 2000mg/kg bw/day. In the rabbit study, the top dose level of 700mg/kg bw/day was toxic causing maternal deaths, reduced food intake, gastrointestinal disturbance and scouring, and the results from that dose level cannot readily be used for developmental toxicity risk assessment. However, the Committee noted that there was no indication of teratogenicity in the top-dose litters that survived. There were no signs of maternal toxicity in the mid-dose group (350mg/kg bw/day). Particular attention was paid to whether there was any effect on post-implantation losses at the mid-dose (controls 8.6%, low dose 6.1%, mid dose 11.5%, historical controls average 10.3%, range 1.0 - 20.5%). It was concluded there was no clear indication of an effect. The issue of gall bladder size and agenesis was also examined. It was noted that there were reductions in size in 2 fetuses in the controls and in 5 fetuses in the mid-dose group. In some cases, fetuses with reduced gall bladder size also had reduced body weight so a reduction was to be expected. The Committee noted that this parameter can be variable and is not really an indicator of teratogenicity. The Committee did agree with the COT that the rabbit study is of reduced quality, mainly because of the number of maternal deaths at the top dose, but considered it was possible to draw certain conclusions from this sub-optimal study. It provided useful information in a second species. The committee also noted that a subsequent kinetic study had shown that maternal plasma levels following 350mg/kg bw/day in the rabbit were twice as high as those in the rat after 2000mg/kg bw/day.²⁷ It was considered unlikely that any repeat study in the rabbit would be able to utilise a dose much higher than 350mg/kg bw/day.

The Committee concluded that there was no need to ask for a further teratology study on the basis of exposure arguments, nor because of any suspicion of effects from the existing rabbit

study. Since no treatment-related developmental effects were seen in the rabbit study, the only effect of interest was maternal gastrointestinal disturbance and the mid-dose of 350 mg/kg bw/day was a NOEL. It was noted that the COT had also commented that for a compound with the potential for widespread use, adequate teratology studies carried out in at least two species are required. The Committee agreed that if that view were taken, then it could not be said that two entirely satisfactory studies were available for sucralose. However, the Committee considered that enough information could be derived from the rat and rabbit teratology and kinetic studies to satisfy safety considerations with respect to developmental toxicity and it does not consider it necessary to recommend any further work in this area.

Nevertheless, subsequent to the above review, new range-finding and teratology studies in the rabbit were submitted by the petitioner.^{52, 53} The new teratology study showed absence of effects on foetal growth or development at doses up to 1000 mg/kg bw/day, despite maternal gastrointestinal disturbance in a few animals at the top dose, as evidenced by markedly reduced food intake (0-17g/day compared with more than 100g/day in controls) and body weight loss, followed by abortion or increased intrauterine deaths. No abortions were seen in unaffected animals in the top dose group. Very similar results have been found in other rabbit studies in which antibiotics that disturb the gut flora have been given, i.e. abortion or total resorption of foetuses, particularly in those animals with severe reductions in food consumption and consequent body weight loss, and also in studies in which food intake has been restricted to 15-60g/day during organogenesis.⁵⁶⁻⁵⁸

The absence of teratogenicity is in agreement with the earlier (1987) study in which similar, but more severe gastrointestinal disturbances, maternal death and abortion were seen at a lower top dose of 700mg/kg bw/day. Both this and the earlier teratology study show that 350mg/kg bw/day is a NOEL for maternal gastrointestinal effects.

Stability, metabolism and effects on gut microflora

A review of biotransformation studies submitted to the SCF by the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Germany, raised the question of whether sucralose could be degraded, possibly to a toxic metabolite, by the human gut microflora, particularly if there was prolonged exposure.²⁸ Sucrose is readily hydrolysed to its component monosaccharides in the gut, but studies in experimental animals show that sucralose is excreted mainly unchanged in faeces and urine following oral administration. However, glucuronide formation can occur, and in man up to 5% of an administered dose may be excreted in urine other than as parent compound. On the other hand, no changes in the metabolism of sucralose were seen during the first 18-months of the chronic rat study.¹⁵ In response, the petitioner submitted further information about the stability of sucralose²⁹ and a discussion of the metabolism and its potential for adaptation.³⁰ The Committee agreed with the evidence and arguments put forward that the structure of the molecule is such that it is extremely resistant to hydrolysis, even at high temperature and in acidic pH. Its known hydrolysis products, 4-CG and 1,6-DCF, are themselves also resistant to further degradation. The chlorination of sucrose to give sucralose also has the effect of changing the conformation of the molecule so that it is resistant to attack from glycosidic enzymes that normally degrade carbohydrates. The Committee therefore concluded that metabolic adaptation in humans was highly unlikely.

Mutagenicity of 1,6-DCF

In its opinion of 1989, the Committee did not express any reservations about the mutagenicity of sucralose but a question remained about one of its hydrolysis products, 1,6-DCF. A new study on 1,6-DCF was submitted to the SCF in 1994.³¹ In order to place it in context, the history of the testing of 1,6-DCF is reviewed below, along with the new study.

1,6-DCF appeared to be a weak alkylating agent *in vitro*. In two separate studies conducted in one laboratory it was positive in *Salmonella typhimurium* strain TA 1535 with dose-related increases in reversion up to the maximum level tested of 5000µg/plate, both in the presence and absence of metabolic activation.^{32,33} It was negative in strains TA 98, 1538, 1537 and 100. Similar results were obtained in another laboratory.³⁴ It was also positive in two separate studies conducted in one laboratory in the L5178Y TK +/- mouse lymphoma assay in the absence of metabolic activation; the presence of metabolic activation greatly reduced its mutagenic activity.^{35,36} 1,6-DCF did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.³⁷ 1,6-DCF was negative in an *in vitro* cytogenetics test for chromosome aberrations in cultured human lymphocytes.³⁸ It was also negative in an *in vivo* rat bone marrow assay for chromosome damage.³⁹

At the request of another advisory body, the UK Committee on Mutagenicity (COM), further *in vivo* studies were carried out. Studies on sister chromatid exchanges and micronuclei in bone marrow cells following oral administration of 1,6-DCF in the mouse were both negative.^{40,41} In a covalent binding study, tissue radioactivity levels and covalent binding were studied in liver, kidneys, stomach, small intestine, colon and bone marrow.⁴² In this study, an association between radiolabelled 1,6-DCF and DNA was found in all tissues examined, except the bone marrow in which no DNA was identified, but the nature of the association could not be established. The COM considered that although the protocol for the study had been adequate, the study itself had been poorly conducted and the results were uninterpretable. All the above mentioned *in vitro* and *in vivo* studies were submitted and reviewed by the SCF prior to issuing its opinion in 1989.

In the light of the low level of DNA binding, the COM decided it would be preferable to request an *in vitro* unscheduled DNA synthesis (UDS) assay in rat liver.⁴³ In this study, no reproducible induction of UDS was seen following 1,6-DCF treatment.³¹ The COM considered this study to be done to an acceptable protocol and that it was negative.⁴⁴ Neither that Committee nor the SCF have any further concerns about 1,6-DCF.

Studies on glucose homeostasis in healthy humans and diabetic volunteers

Five clinical studies were submitted to the SCF on glucose homeostasis in diabetic and non-diabetic human volunteers.⁴⁵⁻⁴⁹ Only one study included subjects with both insulin-dependent (Type I) and non-insulin dependent (Type II) diabetes.⁴⁵ They were given a single, high dose of 1000mg sucralose and no effects on short-term glucose homeostasis were seen. The remaining studies were on normal subjects and subjects with Type II, non-insulin dependent diabetes.^{46,47,49}

In a 6-month study on Type II diabetics, a consistent increase above baseline was observed in blood levels of glycosylated haemoglobin (HbA1C), a measure of long-term glucose

homeostasis, in subjects given 667mg sucralose daily for 6 months, compared to placebo treated subjects.⁴⁶ There were no changes in other indicators of glucose homeostasis, i.e. C-peptide, insulin or plasma/blood glucose. There was no effect of sucralose, given at a 1000mg daily for 12 weeks, on the same indicators of glucose homeostasis in normal, non-diabetic subjects.⁴⁸ The Committee considered whether these results in Type II diabetics might indicate a sucralose-induced shift in the distribution of glucose between red blood cells and plasma and consequently potential for altering glucose control. Equally, it was also possible that the diabetes of the patients in the study was not well controlled at the start of the study but became better controlled as the study progressed; a reduction was seen in HbA1c in the placebo group, which started off with a higher value than the sucralose group, with the sucralose group showing a statistically significant increase in HbA1c levels compared to placebo controls.

A subsequent study,⁴⁹ utilising the same dose of sucralose, 667mg, as in the earlier study on diabetics,⁴⁶ but given for a shorter period of 13 weeks and with a larger number of diabetic subjects, did not find any effect on glycosylated haemoglobin. However, the Committee considered that the two studies were not entirely comparable; there were potentially important differences between the two study populations in both sex and racial composition. There were too few subjects to analyse results by race, but in the second, larger study there were sufficient to analyse separately the outcome by gender and by type of diabetes treatment (insulin or oral hypoglycaemic agents). In this analysis, there were no effects in males or females taking insulin, but in males taking oral hypoglycaemic agents, levels of C-peptide, a biomarker for insulin, significantly increased after the start of sucralose treatment and HbA1c levels started to fall significantly after about 5 weeks on sucralose. This was compatible with several possible explanations, such as artefact or that the diabetes became better controlled because subjects were enrolled in a study, but it was also necessary to rule out the possibility that sucralose could interfere with the efficiency of oral hypoglycaemic treatments.

In response to these questions, the petitioner provided further expert commentary and re-analysis of the statistical aspects of the second larger study.⁵⁰ This argued that the changes observed were a reflection of the well-known phenomenon of regression to the mean from differing baseline values, which can occur when subjects are randomly assigned, double-blind to treated and control groups. The Committee considered this a reasonable suggestion, but noted that correction for regression to the mean still left a marginal significant difference between sucralose and control males on oral hypoglycaemic agents. Because of differing baseline values, seen in both the Type II diabetic studies,^{46,49} the possibility of a small effect could not be definitively ruled out. However, bearing in mind that the amount of sucralose administered daily in these studies was greater than that likely to be consumed by high consumers, such as diabetics, the Committee concluded from consideration of all the evidence that any such effect would be so small as to be clinically insignificant.

ANNEX 2

Studies submitted on sucralose and the no-effect and effect levels

STUDY	NOEL mg/kg	LOEL mg/kg	EFFECTS
Acute oral toxicity in mice	>16000		
Acute oral toxicity in rats	>10000		
4-8 week dietary study in rats	500	1250 2500	↓ ♂ bw gain, ↓ ♂ & ♀ bw gain, ↓ spleen wt, ↓ thymus wt and cellular changes
26-week dietary administration and dietary restriction in rats	628-787	1973-2455	↓ Bw gain, ↓ food intake
12-month dietary study in dogs	>874		Highest dose NOEL
Teratology oral gavage study in rats	>2000		Highest dose NOEL
Teratology oral gavage study in rabbits	350	700	Maternal gastro- intestinal effects
Teratology oral gavage range-finding study in rabbits	900		
Teratology oral gavage study in rabbits	350	1000	Maternal gastro- intestinal effects
Two-generation dietary reproduction study in rats	Not established	150	↓ Bw gain, ↓ food intake
2-year dietary chronic toxicity/carcinogenicity study in mice	1500	4500	↓ Bw, ↑ Liver wt, ↓ erythrocytes, nephropathy
2-year dietary chronic toxicity/carcinogenicity study in rats with <i>in utero</i> exposure	Not established	150	↓ Bw gain, ↓ food intake
Mineral bioavailability with dietary administration in rats	1000	2000	↓ Bw gain, ↓ food intake, ↑ caecal wt
21-day oral gavage neurotoxicity study in mice	>1000		Highest dose NOEL
4- to 13-week oral gavage study in rats	Not established	2000	↑ Bw gain, ↑ food intake, ↑ kidney wt, ↓ spleen wt, variable thymus wt

26-week oral gavage study in rats	1500	3000	↓ ♂ bw gain, ↑ caecal wt
28-day Tier I oral immunotoxicity in rats by oral gavage and diet	1500	3000	↓ Bw gain
Gene mutation in bacteria			Negative
Gene mutation in mammalian cells <i>in vitro</i> (mouse lymphoma assay)			Negative
Chromosome aberrations in human lymphocytes <i>in vitro</i>			Negative
<i>In vivo</i> rat bone marrow cytogenetics			Negative
Mouse micronucleus test			Negative
Absorption, distribution, metabolism and excretion in rat, dog and man, and metabolism in rabbit			
Pharmacokinetics after oral administration to pregnant rats and rabbits			
Comparative pharmacokinetics, dietary and oral gavage administration in rats			
8-week palatability study in rats			
Acceptability in diet in rats			
Acceptability in water in rats			
Glycolysis in various rat tissues <i>in vitro</i>			
Insulin secretion in rats <i>in vivo</i>			
Glycolytic activity of rat sperm			
Liver enzyme induction in rats			
Short-term clinical study in normal human volunteers			See Annex 1
13-week clinical study in normal human volunteers			See Annex 1
Insulin secretion and sucrose absorption in normal human volunteers			See Annex 1
Glycaemic effect of a single high oral dose in diabetic patients			See Annex 1
6-month study of glucose homeostasis in non-insulin-dependent diabetes			See Annex 1
Specific clinical chemistry parameters in 6-month study of glucose in non-insulin-dependent diabetes			See Annex 1
12-week study of glucose homeostasis in normal volunteers			See Annex 1
3-month study of glucose homeostasis in non-insulin-dependent diabetes			See Annex 1

ANNEX 3

Studies submitted on hydrolysis products of sucralose, 4-CG and 1,6-DCF

Absorption, distribution metabolism and excretion in rats
Acute toxicity in mice and rats
Gene mutation in bacteria (*S. typhimurium*)
Gene mutation in mammalian cells *in vitro* (mouse lymphoma assay)
Chromosome aberrations in mammalian cells *in vitro* (human lymphocytes)
In vivo rat bone marrow cytogenetics
Dominant lethal mutations in mice
Sex-linked recessive lethal mutations in *Drosophila melanogaster* (1,6-DCF only)
In vivo sister chromatid exchange in the mouse (1,6-DCF only)
In vivo mouse micronucleus test (1,6-DCF only)
Covalent binding to DNA *in vivo* in rats (1,6-DCF only)
In vitro unscheduled DNA synthesis in rat hepatocytes (1,6-DCF only)
Teratogenicity in rats
Two-generation reproduction study in rats
Rat 2-year chronic toxicity/carcinogenicity study
90-day dietary study in rats
6-month dietary study in the dog