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A Combined Chronic Toxicity/ Carcinogenicity Study of Sucralose in Sprague–Dawley Rats

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Abstract—The chronic toxicity and potential carcinogenicity of sucralose was evaluated by exposing Sprague–Dawley rats to dietary concentrations of this low-calorie sweetener both *in utero* and for up to 104 weeks following parturition. The rats assigned to the toxicity phase of this investigation were administered diets containing either 0% (control), 0.3% (3000 ppm), 1.0% (10,000 ppm) or 3.0% (30,000 ppm) sucralose. Each treatment group comprised 30 male and 30 female rats, of which 15 males and 15 females were sacrificed after 52 weeks of treatment. The surviving rats were killed following 78 weeks of sucralose administration. In the carcinogenicity phase of this investigation, groups of 50 male and 50 female rats were administered dietary sucralose at concentrations of 0% (control 1), 0% (control 2), 0.3%, 1.0% or 3.0% for 104 weeks. Evaluation of the data obtained from the two phases of this study showed that sucralose was not carcinogenic. Sucralose did not adversely affect the survival or clinical condition of the rats, and there were no toxicologically significant findings. Group mean body weight gain and food consumption were significantly decreased in a dose-dependent manner in sucralose-treated rats throughout the treatment period as compared to the controls. The primary effect of sucralose on food consumption, and secondarily on body weight gain, was established in later studies to be due to the fact that diets containing high concentrations of sucralose are unpalatable to rats. These subsequent studies established that the reduction of body weight gain seen in previous rat studies using sucralose in the diet at concentrations of 1% and below resulted from reduced food intake as a direct consequence of the unpalatable nature of sucralose. Similarly, at concentrations of 3% in the diet, it was shown that approximately 95% of the effect on body weight gain could be attributed to the reduction in food intake due to the reduced palatability of the diet, the remainder apparently due to a physiologic response to the high concentrations of non-digestible sucralose in the rats' diet. Complete toxicological evaluations of gavage studies with histopathological evaluations demonstrated that even at the 3% dietary level, toxicity was not responsible for the small body weight gain decrement. Gross and histopathologic examinations revealed that the administration of sucralose affected neither the types nor incidence of the tumours observed. The incidences of some non-neoplastic findings were statistically significantly increased in the sucralose treated groups relative to the controls. These included: renal pelvic epithelial hyperplasia in all female treatment groups, renal pelvic mineralization in females administered the intermediate or highest dietary concentrations of sucralose, adrenal cortical haemorrhagic degeneration in high-dose group female rats, and the histopathologic incidence of cataracts at necropsy in high-dose group male rats. The non-neoplastic findings that occurred were of no toxicological significance since they were either spontaneous findings commonly observed in aged rats of this strain or the physiological response to high dietary levels of a poorly absorbed compound. **© *2000 Published by Elsevier Science Ltd. All rights reserved***

Keywords: sucralose; Sprague–Dawley rats; carcinogenicity; toxicity; sweeteners.

INTRODUCTION

Sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside) is a unique, non-caloric sweetener that is made from, and tastes like, sucrose. Sucralose has a high quality of sweetness, approximately 600 times greater than that of sucrose, and has excellent stability. The functional and sensory properties of sucralose per-

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mit its inclusion in a broader range of food products than is possible for currently available high intensity sweeteners. A battery of short-term *in vitro* and *in vivo* tests for mutagenicity, chromosomal damage, and DNA damage found that sucralose is non-genotoxic (McNeil, 1987). Sucralose is minimally absorbed after oral administration with 5–10% of oral doses (10–1000 mg/kg) excreted in the urine and approximately 90% excreted in the faeces as unchanged parent compound (Sims *et al*., 2000). Biliary excretion was shown to be less than 10% of the orally administered dose (Sims *et al*., 2000). Two extremely minor radiolabelled urinary metabolites accounting for only 0.15%–0.25% of the oral dose are found in the rat (Sims *et al*., 2000). These two urinary components have similar chromatographic properties to the metabolites in dog urine and the two metabolites in human urine, which have been identified by mass spectrometry as glucuronide conjugates (Roberts *et al*., 2000, Wood *et al*., 2000). The absorption, distribution and metabolism pattern in the rat is similar to man (Roberts *et al*., 2000). Acute and subchronic toxicity studies demonstrated that sucralose is non-toxic (Goldsmith, 2000). Acute oral doses of 10 g/kg for rats and 16 g/kg for mice produced no adverse effects (Goldsmith, 2000). In a 4- or 8-wk dietary study, no sucralose-related untoward effects were observed in rats at dietary levels up to 2.5%, although several treatment-related effects occurred following administration of 5% sucralose (Goldsmith, 2000). High concentrations of sucralose in the diet result in decreased food consumption and body weight gain due to poor diet palatability (Goldsmith, 2000). Dietary concentrations of 3% or gavage doses of 3000 mg/kg/day for 6 months slightly affect nutrient utilization and growth. This small (4.9%) but statistically significant effect on nutrient utilization may affect growth in dietary studies where poor diet palatability inhibits correction for lower food efficiency by increasing food consumption, as noted in long-term gavage studies (Goldsmith, 2000). The data presented here are the results of an *in utero* combined chronic toxicity/carcinogenicity study in rats administered dietary sucralose at concentrations of 0.3%, 1.0% or 3.0%.

MATERIAL AND METHODS

Animals and diet husbandry

Healthy, 5–6-wk-old rats of Sprague–Dawley origin [CrL:CD®(SD) BR strain] were obtained from Charles River (UK) Ltd (Margate, Kent, UK), and assigned as the parental (F_0) generation. They were allocated randomly to cages and groups and allowed to acclimate for 13 days prior to pairing for mating. The study rooms were supplied with 15 changes of fresh filtered air per hour. Target values for temperature and relative humidity were 21°C and 55%, respectively. Deviation from these target values was small and did not affect the integrity of the study. During both the acclimatization period and early gestation (approx. 1–17 days after mating) rats of the F_0 generation were housed in groups of five per sex in polypropylene cages with stainless-steel lids and grid floors. Each female rat and her litter were then housed in a cage with wood shaving bedding. During late lactation (14–18 days *post partum*), each rat and litter were re-housed in a cage with a grid floor. F_1 generation rats were selected for the toxicity and carcinogenicity phases on day 23 *post partum*, and kept in the same environmental conditions as the parental generation. The cages were distributed within batteries to minimize the effects of any spatially variable components of the environment. The drinking water and each batch of diet were analysed by their respective suppliers for selected chemical and microbial contaminants. Wood shavings were analysed by Huntingdon Life Sciences for organochlorine pesticide and polychlorinated biphenyl residues. Results of these analyses did not reveal any contamination likely to affect the outcome of the study.

Test and control materials

A complete powdered rodent diet (Laboratory Animal Diet No. 2, Scientific Feeds, K and K Greef Chemicals Ltd, Croydon, UK), and potable water from the public supply were available for the rats to consume *ad lib*. The sucralose used was obtained in three batches from Linson Ltd (Swords, Co. Dublin, Eire). These batches of sucralose ranged in purity from 98–99%. Sucralose was incorporated into ground basal diet at concentrations of 0.3%, 1.0% or 3.0%. Control rats received the basal powdered diet only. The concentrations and stability of sucralose in the diet, as well as the homogeneity achieved by the mixing technique, were analysed and determined to be acceptable at 13-wk intervals.

Experimental design and conduct of the breeding phase

The breeding and all subsequent phases of this investigation were conducted in accordance with Good Laboratory Practice Regulations. Groups of 70 male and 70 female rats were administered sucralose in the diet at concentrations of 0% (control 1), 0% (control 2), 0.3%, 1.0% or 3.0% for 4 wk before pairing, and then throughout pairing, mating, gestation and lactation until termination of the F_0 generation, except as follows. The highest dose was determined on the basis of a previously conducted 4–8 wk dietary toxicity study which demonstrated that sucralose administered in the diet at concentrations of 5.0% produced a marked depression in body weight gain, while concentrations less than 3.0% produced minimal and inconsistent effects on this parameter (McNeil, 1987).

Sucralose at 3.0% in the diet produced moderate reductions in body weight gain that were associated with decreased food consumption. Further studies showed that decreased food consumption was causally related to reduced palatability of the diet (McNeil, 1987).

Preliminary work in lactating rats administered diets containing 1.0% sucralose showed that their food intake and body weight gain were comparable to those of control lactating rats. Food intake in both treated and control dams increased during lactation. The intake of sucralose increased correspondingly, while the body weight gain of the pups decreased in the treated group. Therefore, in order to minimize any potential severe adverse effects on the body weight gain of the offspring, and to maintain continuous exposure to the test material, the highest dietary concentration (3.0%) of sucralose was reduced to 1.0% during lactation only.

Data recorded during the breeding phase included clinical signs, mortality, food consumption and body weights. Rats were mated by housing a male and female of the same treatment group together for 7 days. The day on which a sperm-positive vaginal smear or vaginal copulation plug was found was designated day 1 of gestation. Maternal observations included the onset and duration of gestation and parturition. Observations on progeny (F_1) rats included litter size, clinical signs, mortality and body weight. For each group indices of mating performance, fertility and pup survival were calculated.

On day 23 *post partum*, offspring were randomly selected for the toxicity and carcinogenicity phases. For the carcinogenicity phase, one male and one female were selected (using random numbers) from 50 litters per group. One additional male and female were similarly selected from 30 of these litters per group, and assigned to the toxicity phase.

Experimental design and conduct of the toxicity phase

Groups of 30 male and 30 female rats were housed by sex, five per cage and administered sucralose in the diet at concentrations of 0% (control), 0.3%, 1.0% or 3.0%. 15 males and 15 females were sacrificed after 52 wk of treatment, while all surviving rats were killed after 78 wk. All rats were inspected twice daily for deaths, moribund condition and clinical signs. In addition, all rats were palpated and examined once weekly for the presence of superficial swellings. Rats were sacrificed if found moribund or for humane reasons. Each rat was weighed on the day that treatment commenced, at weekly intervals for the first 14 wk, and every 2 wk thereafter. Food consumption for the group-housed animals was measured weekly. Food conversion efficiency values were calculated after adjusting group mean food consumption for the concentration of sucralose in the diet.

Blood samples were withdrawn from the orbital sinus of 10 male and 10 female rats per group after 11, 25, 38, 51 and 77 wk of treatment. Rats were deprived of food and water for 24 hr prior to sampling, and were anaesthetized with ether immediately prior to the blood collection procedure. The samples were analysed for packed cell volume, haemoglobin concentration, prothrombin time and erythrocyte, leucocyte (total and differential), platelet and reticulocyte counts. Additional samples were collected and analysed for urea, glucose, alkaline phosphatase, alanine and aspartate aminotransferase, total protein, electrophoretic protein fraction, sodium, potassium, chloride, calcium, magnesium and inorganic phosphate levels. Triiodothyronine and thyroxine concentrations were measured in the wk 11 samples only. Urine samples were collected from these same rats for examination with respect to calcium, magnesium and inorganic phosphate concentrations.

Experimental design and conduct of the carcinogenicity phase

Groups of 50 male and 50 female rats were administered dietary sucralose at concentrations of 0% (control 1), 0%, (control 2), 0.3%, 1.0% or 3.0% for 104 wk. The parameters measured and the time intervals evaluated were generally the same as those of the toxicity phase. However, water consumption was recorded over a 24-hr period once each week for the first 13 wk of treatment. Prior to the commencement of treatment, both eyes of all rats were examined using a Fison's binocular indirect ophthalmoscope after the instillation of 0.5% tropicamide. Thereafter, the eyes of all surviving control and high-dose group rats were similarly re-examined approximately every 13 wk. After 103 wk of treatment, blood samples were withdrawn from 10 male and 10 female rats of the control 1 and each of the sucralose treatment groups, for the same determinations as performed on rats assigned to the toxicity phase.

Terminal observations in the toxicity and carcinogenicity phases

Gross examinations were performed on all rats, either killed or dying during the course of the study or sacrificed after 52, 78, or 104 wk of treatment. Adrenal glands, brain, caecum (full and empty), heart, kidneys, liver, gonads, spleen, thymus and uterus were weighed.

Specimens from the adrenal glands, aortic arch, bone (femur with marrow), brain (sectioned to include the cerebellum, cerebral cortex and medulla), caecum, colon, diaphragm, duodenum, fallopian tube, heart (auricular and ventricular levels), ileum, jejunum, kidneys, lachrymal gland, liver, lungs and mainstem bronchi, cervical and mesenteric lymph nodes, mammary tissue (anterior and posterior), oesophagus, ovaries, pancreas, pitu-

itary gland, prostate gland, rectum, salivary glands, sciatic nerves, seminal vesicles, skeletal and smooth muscle, skin, spleen, spinal cord and dorsal root ganglion from the cervical, thoracic and lumbar levels, stomach, testes, thymus, thyroid and parathyroid glands, trachea, lymph nodes including those draining the regions of tissue masses, turbinate bones, urinary bladder, uterus (corpus and cervix), vagina and Zymbal's gland were preserved in buffered 4% formaldehyde saline. Samples of tissue masses and other gross abnormalities were also retained. Eyes, optic nerves and Harderian glands were placed in Davidson's fixative, and bone marrow smears were air-dried and fixed in methanol before staining by a May–Grünwald–Giemsa procedure.

After embedding in paraffin wax, sections of the required tissues were stained with haematoxylin and eosin. Microscopic examination was performed on (i) the kidneys, liver, lungs and macroscopic abnormalities of all rats; (ii) the remaining tissues as specified above, from all control and high-dose group rats that survived until scheduled termination and (iii) the remaining tissues as specified above for all rats killed or dying during the treatment period.

Statistical analyses

Continuous variables were generally tested for statistical significance using the Student's *t*-test. Inter-group differences in mean absolute and relative organ weights were assessed using Dunnett's test (Dunnett, 1964). In view of inter-group differences in body weight gain, organ weights were also processed as a covariate with terminal body weight (Shirley, 1977) before analysis by Dunnett's test. Outliers were identified (Anscombe and Tukey, 1963) and excluded. Time-to-event analysis of mortality was by Cox's test (Cox, 1972), both as an overall test for homogeneity of survival curves, and for pairwise comparisons against the control group. The Cochran–Armitage test (Armitage, 1955) and Tarone's extension to Cox's test, (Tarone, 1975) were used to examine linear trend on dose, and to assess deviation from linearity. Macropathology and histopathology incidence data were analysed by the Fisher's exact test.

As treatment with sucralose had no adverse effect on survival, and there was no apparent increase in the incidence of any neoplasm, it was considered unnecessary to apply any time-to-event analysis of tumour data. For neoplastic findings, the probabilities presented are those related to higher incidences among treated rats as compared to the combined incidence of the two control groups. A one-tailed Fisher's exact test was employed for analyses of these data. In all other tests for significance, a two-tailed test was employed. The null hypotheses were rejected at probabilities of 5% or less.

Tests for the significance of differences were first conducted between the two control groups in the carcinogenicity phase of this investigation. In most cases, the two controls did not significantly differ ($P > 0.05$), and subsequent comparisons were made between each treatment group and the combined data for the two control groups. Wherever differences between the two control groups were established, the data from each treated group were compared to that of each control group.

RESULTS

During the week before pairing, mean calculated sucralose intake (Table 1) in the groups administered dietary concentrations of 0.3%, 1.0% or 3.0% were approximately 0.2, 0.7 or 2.2 g/kg/day in male rats, and 0.2, 0.9 or 2.6 g/kg/day in female rats, respectively. Male rats allocated to the carcinogenicity phase (F_1 generation) administered diets containing 0.3%, 1.0% or 3.0% of sucralose for 104 wk ingested average calculated doses of 0.1, 0.4 and 1.2 g/kg/day, respectively. The average calculated doses for female carcinogenicity phase rats (F_1 generation) administered these dietary concentrations during the 104-wk treatment period were 0.2, 0.5

Table 1. Achieved mean doses (g/kg/day)* for rats fed diets containing sucralose

Sucralose concn (%, w/w)	Week before pairing (F_0)	Carcinogenicity phase in F_1 rats (wk)**							
		1	2	3	4	1–4	1–14	15–104	1–104
Males									
0.3	0.2	0.6	0.5	0.4	0.3	0.4	0.3	0.1	0.1
1.0	0.7	1.9	1.6	1.2	0.9	1.4	0.8	0.3	0.4
3.0	2.2	5.7	4.7	3.7	2.9	4.2	2.6	1.0	1.2
Females									
0.3	0.2	0.6	0.4	0.4	0.3	0.4	0.3	0.1	0.2
1.0	0.9	1.9	1.5	1.2	1.1	1.4	0.9	0.4	0.5
3.0	2.6	5.5	4.6	3.5	3.1	4.2	2.8	1.4	1.6

*Derived from group mean body weight and food consumption values, and actual dietary concentrations of sucralose. **Each rat was weighed on the day that treatment commenced, at weekly intervals for the first 14 wk, and every 2 wk thereafter. Food consumption for the group-housed animals was measured weekly.

Table 2. Mean body weight change, food intake, and food conversion efficiency for F_0 rats fed diets containing sucralose

Sucralose concn (%, w/w)	Body weight change before pairing (g) wk 1–4		Food intake on wk 4 before pairing† g/rat/wk		Food conversion efficiency† (%)[a] wk 1–4
	Mean	(SD)	Mean	(SD)	
Males					
0	155	(20)	188	(6)	20.6
0	155	(24)	188	(7)	20.6
0.3	136***	(21)	179***	(8)	19.0
1.0	130***	(26)	178***	(7)	18.5
3.0	136***	(22)	182***	(6)	19.3
Females					
0	73	(13)	142	(6)	12.9
0	71	(15)	141	(7)	12.6
0.3	60***	(10)	135**	(5)	11.1
1.0	60***	(11)	135**	(6)	11.2
3.0	59***	(12)	134**	(7)	11.3

†Adjusted for concentration of sucralose. [a]Calculated from group mean body weight and food consumption values. Values marked with asterisks differ significantly (Student's *t*-test) from the corresponding combined control values: ** $P < 0.01$; *** $P < 0.001$.

and 1.6 g/kg/day. Overall, the estimated lifetime intakes were 150, 1000 and 1500 mg/kg body weight/day for the low, mid and high dose groups, respectively (Lehman, 1954).

Breeding phase, F_0 rats and offspring

Sucralose had no effect on the survival or clinical condition of F_0 generation rats. Group mean body weight gain of sucralose-treated F_0 rats, both before pairing (Table 2) and during gestation (Table 3), was statistically significantly decreased ($P < 0.05$, 0.01 or 0.001). However, overall group mean body weight gain of female rats was statistically significantly increased ($P < 0.05$ or 0.01) during lactation (Table 3). Food intake and food conversion efficiency before pairing (Table 2) were decreased in all sucralose treatment groups, although only food intake was decreased in a statistically significant ($P < 0.01$ or 0.001) fashion. These effects were most apparent in rats receiving the intermediate and highest dietary concentrations of sucralose during wk 1–3 since both food intake and food conversion efficiency were similar among treatment groups in wk 4. Sucralose did not effect any of the reproductive/developmental parameters evaluated (data not shown). The reproductive and developmental safety of sucralose is reported elsewhere (Kille *et al*., 2000). Offspring body weight gain from day 1 to day 21 *post partum* (Table 4) was statistically significantly ($P < 0.001$) lower in rats administered the intermediate or highest dietary concentrations of sucralose. This decrease in body weight gain occurred predominantly during the week immediately prior to weaning, when the pups began eating the dietary admixture.

Toxicity and carcinogenicity phases

Survival (Table 5) among male and female rats administered the intermediate or highest dietary concentrations of sucralose was higher than the controls. In addition, there were no sucralose-related clinical signs. Group mean body weight gain (Table 5) of rats administered sucralose in the diet was significantly lower ($P < 0.001$) than that of the respective combined control groups throughout the treatment period. Sucralose treated rats of either sex consumed significantly less food (Table 5), after adjustment for sucralose content, than their respective controls throughout the treatment period. However, no dose–response relationship was evident in food consumption. Water intake of the sucralose treatment groups (Table 5) was increased

Table 3. Body weight change of F_0 female rats fed diets containing sucralose

Sucralose concn (%, w/w)	Body weight change (g) days 1–21 of gestation		Body weight change (g) days 1–21 of lactation	
	Mean	(SD)	Mean	(SD)
0	142	(18)	33	(27)
0	142	(19)	32	(20)
0.3	141	(20)	46**	(20)
1.0	132**	(16)	42*	(28)
3.0	129***	(13)	45**	(23)

Values marked with asterisks differ significantly (Student's *t*-test) from the corresponding combined control values: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. Group mean body weight of offspring (F_1) and body weight gain *post partum* in rats fed diets containing sucralose

					Body weight gain (g)			
	Post partum day 1		*Post partum* day 21		*Post partum* days 14–21		*Post partum* days 1–21	
Sucralose concn (%, w/w)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
0	6.0	(0.7)	47.9	(5.5)	17.8	(3.6)	41.9	(5.2)
0	6.0	(0.6)	49.2	(4.5)	18.8	(2.2)	43.2	(4.1)
0.3	5.9	(0.7)	47.1*	(4.2)	16.9***	(2.1)	41.1*	(3.9)
1.0	6.0	(0.7)	44.5***	(4.5)	15.1***	(2.8)	38.5***	(4.3)
3.0	6.1	(0.7)	46.0***	(4.5)	16.2***	(2.5)	39.9***	(4.2)

Values marked with asterisks differ significantly (by Student's *t*-test) from the corresponding combined control values: $*P < 0.05$; $***P < 0.001$.

relative to controls (no statistical analysis performed), and the magnitude of the increases was generally related to the dietary concentration administered. No statistically significant changes in food conversion efficiency (Table 5) occurred in sucralose-treated rats during the rapid growth phase of wk 1–14, although the values were slightly lower than those of the controls.

There were no sucralose-related ophthalmologic findings. Haematological investigations after 11, 77 and 103 wk of treatment and just prior to termination revealed no inter-group differences related to sucralose. A significant decrease in packed cell volume was noted in male rats administered the 3% dietary concentration of sucralose for 25, 38 and 51 wk of treatment, and among female rats at this dietary level after 51 wk only. This finding was generally associated with a lowered haemoglobin concentration. The inter-group differences were small and the individual values were not abnormal. The variations were therefore not considered of toxicological significance. Alanine aminotransferase activity in high-dose group male rats was significantly decreased relative to controls after 11, 38, 51, 77 and 103 wk of treatment. This finding was sporadically observed in all other sucralose treatment groups, but did not appear to be a dose-dependent effect, particularly in females. These serum enzyme decreases unaccompanied by other hepatic findings are of no toxicological significance. A general trend towards a lower blood glucose level relative to the controls was noted in all treatment groups of both sexes. The changes were occasionally statistically significant (Table 6), but not dose dependent. After 103 wk of treatment, there were no statistically significant differences between the treated and control groups. These slight inter-group differences were not considered of toxicological significance. Thyroxine levels were significantly decreased in a dose-dependent manner (0.05 at 0.3% and 1.0%; $P < 0.01$ at 3.0%) in sucralose-treated male rats when compared to controls after 11 wk of treatment. However, no effect was observed in females and no histopathologically related changes were observed, therefore the change was not considered toxicologically significant. Urinalysis after 11, 25, 38 and 51 wk of treatment revealed that rats of either sex receiving the highest concentration excreted more magnesium than their respective controls. A higher magnesium excretion was also noted

Table 5. Survival, mean body weight gain, food consumption and food conversion efficiency for F_1 rats fed diets containing sucralose in the carcinogenicity study

Sucralose concn (%, w/w)	No. of rats at wk 0	Survival wk 104 (%)	Body weight gain wk 0–104 mean	(SD)	Food consumption† wk 0–104 (g/rat/wk)	(%)	Food conversion efficiency†[b] wk 0–14	Total water intake wk 1–13 ml/rat (%)	
Males									
0	50	40	902	(138)	178	-	18.9	390.7	-
0	50	48	908	(156)	177	-	18.9	386.7	-
0.3	50	68	788***	(147)	168**	95	18.6	406.0	104
1.0	50	62	731***	(121)	165***	93	18.4	408.8	105
3.0	50	70	721***	(116)	168**	95	18.4	449.2	116
Females									
0	50	58	533	(122)	133	-	12.3	362.0	-
0	50	56	581	(123)	134	-	12.5	361.2	-
0.3	50	50	448***	(110)	126***	94	11.9	368.2	102
1.0	50	80	426***	(119)	122***	91	11.9	414.2	115
3.0	50	80	410***	(105)	123***	92	12.1	417.4	115

†Adjusted for concentration of sucralose. % [a]As a % of control consumption. [b]Expressed as body weight gain per unit of food consumed ×100. $**P < 0.01$; $***P < 0.001$.

Table 6. Blood glucose changes relative to controls in rats administered sucralose for 103 wk

Dietary sucralose concn (%)	Wk 11 M	Wk 11 F	Wk 25 M	Wk 25 F	Wk 38 M	Wk 38 F	Wk 51 M	Wk 51 F	Wk 77 M	Wk 77 F	Wk 103 M	Wk 103 F
0.3	↓NS	↓NS	↓NS	↓b	↑a	↓c	↓NS	↓b	↓NS	↑NS	↓NS	↓NS
1.0	↓NS	↓NS	↓NS	↓a	↓NS	↓c	↓NS	↓a	↓NS	↓NS	↓NS	↓NS
3.0	↓NS	↓NS	↓NS	↓a	↑a	↓c	↓NS	↓b	↑NS	↓a	↑NS	↓NS

↓Mean value lower than control. ↑Mean value higher than control. NS = not statistically significant compared to the control group. a $P < 0.05$. b $P < 0.01$. c $P < 0.001$.

for female rats receiving the intermediate concentration after 25 and 51 wk of treatment; for male rats receiving the intermediate concentration after 51 wk of treatment, and for males receiving the low concentration after 25 and 51 wk of treatment. This trend was reversed after 77 wk of treatment, with rats receiving sucralose excreting less magnesium than their respective controls. The amount of phosphorus excreted by all treated rats was significantly higher than their respective controls after 77 wk of treatment. The volumes of urine excreted by female rats receiving sucralose at any concentration tended to be lower than those for controls. This difference achieved statistical significance after 39 and 77 wk of treatment. After 77 wk of treatment, urine volumes of treated males were lower than controls, the difference attaining statistical significance only for rats receiving the highest concentration. The urinary ion excretion patterns observed were variable and frequently non-dose related and were not considered of any toxicological significance.

Absolute caecal weights of the high-dose group rats both empty and with inclusion of all contents, were significantly increased in a dose-dependent manner over those of the controls after 52, 78 and 104 wk of treatment (Table 7). Because of the influence of lower body weight in the treated groups, covariate analysis, using the terminal body weight as the covariate, was undertaken as a means of adjusting organ weight for differences in body weight. Covariate analysis using the terminal body weight revealed a dose-dependent increase in kidney and brain weights of male rats with a significant difference at the high dose, and a dose-dependent increase in liver weights for sucralose-treated female rats during week 52 which was significantly greater than controls at all treatment levels (Table 8a). There were no sucralose-related differences in organ weights after 78 or 104 wk of treatment (Table 8b,c). The toxicological significance of the organ weight differences is discussed below in the Discussion.

Table 7. Absolute organ weight—group mean values (g) after 52, 78 or 104 wk of treatment

Group	1	2	3	4	5					
Compound	Control	Control	Sucralose							
Dietary level (%)	0	0	0.3%	1.0%	3.0%					
	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Wk 52										
Terminal body weight	811.7	-	733.0	704.9*	694.9*	469.3	-	363.8**	361.2**	351.4**
SD	±158.1	-	±92.7	±91.4	±97.9	±99.6	-	±74.6	±53.3	±43.4
Caecum (full)	4.8899	-	4.8206	5.7741**	9.1554**	3.2386	-	3.5201	3.8327*	6.6219**
SD	±0.7908	-	±1.0510	±.7240	±1.8951	±.6345	-	±.5455	±.7498	±1.6885
Caecum (empty)	1.2711	-	1.2187	1.1658	1.5365*	.8521	-	.7441	.8499	1.0619**
SD	±.3067	-	±.3472	±.2551	±.2586	±.1682	-	±.1273	±.1389	±.2272
Wk 78										
Terminal body weight	936.0	-	828.3	745.1**	749.4**	572.8	-	458.2*	437.8*	487.2
SD	±148.5	-	±142.6	±129.0	±117.1	±130.3	-	±84.0	±95.3	±134.1
Caecum (full)	5.9078	-	5.1985	5.9899	10.5565**	4.6606	-	4.0065	4.5734	6.6860**
SD	±.8446	-	±1.2585	±1.7922	±1.7115	±.9659	-	±.6410	±.9527	±2.1644
Caecum (empty)	1.4110	-	1.3641	1.2134	1.6099	1.0204	-	.8742	.9649	1.2229*
SD	±.2500	-	±.4112	±.1989	±.2398	±.1820	-	±.1203	±.2125	±.2339
Wk 104										
Terminal body weight	965.1	965.3	849.2**	790.0**	778.0**	594.5	640.1	515.1*	485.8**	471.9**
SD	±139.2	±154.3	±148.5	±126.2	±119.2	±127.7	±127.7	±111.8	±123.9	±105.6
Caecum (full)	5.7553	5.3613	5.9649	6.3902	9.9041**	4.6109	4.5432	4.6375	4.5453	7.5020**
SD	±1.1504	±1.5349	±1.6226	±1.4674	±1.6676	±1.2035	±1.0968	±.9489	±1.0064	±1.5429
Caecum (empty)	1.3247	1.2556	1.2216	1.3105	1.6917**	1.0311	1.0614	.9862	1.0096	1.2459*
SD	±.2063	±.2386	±.2381	±.2818	±.3063	±.3716	±.1865	±.1954	±.2636	±.2500

Values marked with asterisks differ significantly (by Dunnett's test) from the corresponding combined control values: $*P < 0.05$; $**P < 0.01$.

Table 8(a). Organ weight analysis of covariance-adjusted group mean values for animals killed after 52 wk of treatment (toxicity phase)

Group	1	3	4	5				
Compound	Control		Sucralose					
Dietary level (%)	0	0.3%	1.0%	3.0%				
	Group and sex							
	1 M	3 M	4 M	5 M	1F	3F	4F	5F
No. of rats examined	15	15	15	15	15	15	15	15
Organ								
Brain	2.17	2.18	2.21	2.28[a]	2.00	1.95	1.95	2.00
Heart	1.65	1.70	1.76	1.72	1.09	1.10	1.11	1.10
Thymus	0.59	0.53	0.52	0.48	0.39	0.41	0.36	0.34
Liver	22.99	22.94	24.56	25.37	11.07	12.21[a]	12.21[a]	13.00[b]
Spleen	0.87	0.92	0.91	0.94	0.52	0.54	0.54	0.52
Testes	3.43	3.62	3.62	3.39	-	-	-	-
Adrenal	0.046	0.046	0.052	0.048	0.052	0.063	0.063	0.062
Kidneys	4.03	4.34	4.45	4.64[b]	2.47	2.61	2.59	2.62
Ovaries	—	—	—	—	0.062	0.047	0.041[a]	0.046[a]
Uterus	—	—	—	—	0.704	0.739	0.735	0.745

Values without superscripts were not significantly different from controls, $P > 0.05$. Pairwise comparisons of the control group against the dosed groups were performed using Dunnett's test on the adjusted means; all P-values were two-tailed. [a]Significantly different from controls; $P < 0.05$. [b]Significantly different from controls; $P < 0.01$.

Table 8(b). Organ weight analysis of covariance-adjusted group mean values for animals killed after 72 wk of treatment (toxicity phase)

Group	1	3	4	5				
Compound	Control		Sucralose					
Dietary level (%)	0	0.3%	1.0%	3.0%				
	Group and sex							
	1 M	3 M	4 M	5 M	1F	3F	4F	5F
No. of rats examined	13	14	14	13	13	13	13	14
Organ								
Brain	2.314	2.319	2.223	2.276	2.045	2.064	2.005	2.072
Heart	1.856	1.870	1.778	1.893	1.318	1.251	1.224	1.225
Thymus	0.589	0.441[a]	0.530	0.561	0.461	0.427	0.314[a]	0.409
Liver	24.42	22.62	24.18	25.87	15.24	15.69	15.21	14.94
Spleen	1.129	1.102	0.962	1.006	0.642	0.624	0.591	0.632†
Testes	3.447	3.477	3.502	3.718	-	-	-	-
Adrenal	0.058	0.052	0.059†	0.051	0.076	0.076	0.065	0.072
Kidneys	4.811	4.526	4.473	4.836	3.092	2.887	2.761	3.102
Ovaries	—	—	—	—	0.055	0.061	0.053	0.055
Uterus	—	—	—	—	0.74	1.17	1.13	0.77

Values without superscripts were not significantly different from controls, $P > 0.05$. Pairwise comparisons of the control group against the dosed groups were performed using Dunnett's test on the adjusted means; all P-values were two tailed. †Outlier removed with no effect on original findings. [a]Significantly different from controls; $P < 0.05$.

Table 8(c). Organ weight analysis of covariance-adjusted group mean values for animals killed after 104 wk of treatment (carcinogenicity phase)

Group	1	2	3	4	5			
Compound	Control	Control		Sucralose				
Dietary level (%)	0	0	0.3%	1.0%	3.0%			
	Group and sex							
	1 + 2 M	3 M	4 M	5 M	1 + 2F	3F	4F	5F
No. of rats examined	44	24	34	31	56	24	38	40
Organ								
Brain	2.307	2.313	2.323	2.335	2.060	2.087	2.053	2.071
Heart	2.021†	2.023	1.981	2.050	1.390	1.435	1.384	1.414
Thymus	0.563†	0.511	0.564	0.519	0.488	0.492	0.485	0.427
Liver	25.00	25.98	24.67	27.03	17.33	19.07[a]	18.18	18.30
Spleen	1.177†	1.322	1.168	1.282	0.712	0.855	0.782	0.811
Testes	3.546	3.571	3.230	3.390	-	-	-	-
Adrenal	0.067	0.066	0.088	0.080	0.092	0.092	0.078	0.087
Kidneys	5.22†	5.70	5.40	5.80	3.41	5.04	3.19	3.62
Ovaries	—	—	—	—	0.079	0.087	0.199[a]	0.073
Uterus	—	—	—	—	0.763	0.825	0.793	0.735

Values without superscripts were not significantly different from controls, $P > 0.05$. Pairwise comparisons of the control group against the dosed groups were performed using Dunnett's test on the adjusted means; all P-values were two tailed. †Outlier removed with no effect on original findings. [a]Significantly different from combined controls; $P < 0.05$.

The administration of sucralose was not associated with any gross abnormalities. There were no statistically significant increases in the incidence of any neoplasm, and sucralose did not affect the types of tumours observed (Table 9a,b). In addition, there was no sucralose-related effect on the distribution or multiplicity of neoplasms (Table 10). The incidence of non-neoplastic findings among rats in the toxicity phase (treated for up to 78 wk) was also unaffected by treatment with sucralose. In the carcinogenicity phase, the incidences of several non-neoplastic findings (Tables 11a,b and 12) were statistically significantly increased in the sucralose-treated groups relative to the controls. These included: renal pelvic epithelial hyperplasia in all female treatment groups, renal pelvic mineralization in females administered the intermediate or highest dietary concentrations of sucralose, adrenal cortical haemorrhagic degeneration in high-dose group female rats, and cataracts in high-dose group male rats. The toxicological significance of the microscopic findings are discussed in the next section.

DISCUSSION

Sucralose did not adversely affect any of the reproductive/developmental parameters evaluated in the breeding phase of this investigation. Mating performance and fertility were similar in the sucralose-treated and control groups, and there was no effect on the survival of F_1 offspring on *post partum* days 1 and 4. Group mean body weight gain of pups administered the intermediate and highest dietary concentrations of sucralose was decreased during the week immediately prior to weaning as the pups began consuming diets containing sucralose. The survival and clinical condition of the F_O generation rats was unaffected by sucralose administration. However, group mean body weight gain of sucralose-treated F_0 rats, both before pairing and during gestation, was moderately decreased relative to controls. In addition, food consumption was initially decreased in the sucralose-treated rats when compared to controls, although not in a dose-related fashion.

Evaluation of the data obtained from both the toxicity and carcinogenicity phases of this investigation revealed that there were no sucralose-related untoward effects at the dietary concentrations administered. Survival was generally greater than or equal to that of the controls in the sucralose treatment groups, and there were no sucralose-related clinical signs. Sucralose administration did not affect the incidence of palpable swellings in rats assigned to the carcinogenicity phase of this investigation. The incidence and types of tumors observed were unaffected by sucralose administration, and all of the tumours which occurred in sucralose-treated rats were considered to be spontaneous in nature.

Group mean body weight gain was significantly decreased in sucralose-treated rats throughout the treatment interval. These decreases were associated with decreased food consumption in the sucralose-treated rats as compared to the controls, and are not judged to be an expression of sucralose-related toxicity. This effect of sucralose on food consumption, and secondarily body weight gain, is due primarily to the reduced palatability of diets containing high concentrations of sucralose, which is a poorly absorbed osmotically active inert material in the gut. This conclusion is supported by the fact that sucralose, administered to rats by gavage, did not adversely affect food consumption, food conversion efficiency or group mean body weight gain and had only a minor effect on adjusted body weight gain (linear covariance analysis) at a dose of 3000 mg/kg/day (McNeil, 1987). Special diet restriction studies examining the complex relationship between food consumption and body weight in groups of rats consuming diets containing high levels of sucralose have been performed (McNeil, 1987). These studies have demonstrated that at concentrations of 1% or less, all of the decrease in body weight gain observed can be attributed to the reduction in food intake, due to the poor palatability of the rats' diet containing sucralose. Approximately 95% of the decrease in body weight gain observed at the 3% dietary level was attributed to the reduction in food intake. In similar cases, the small remaining effect, not attributable to food intake, has been associated with the physiologic consequences of the high concentrations of poorly absorbed osmotically active non-digestible material in the diet (Lu, 1988; WHO, 1987). The increased water consumption, which occurred generally in a dose-related manner in the sucralose treatment groups, was not associated with increased urinary output. This additional water load was judged to be eliminated by faecal excretion, although no direct evidence of a significant increase in loose stool was observed in the study. However, an increased incidence of loose stool was observed in a separate study when a diet containing a higher concentration (5%) of sucralose was administered to rats (McNeil, 1987). This finding is of little toxicological significance since no adverse biological effects were associated with the increased fluid intake, and increased water consumption has been reported for rats administered other poorly absorbed carbohydrates such as lactose (Roe, 1984). However, the physiological adaptations in the gut to the presence of high levels of poorly absorbed materials is causally related to the caecal and renal changes observed in this study and discussed below.

There were several inter-group differences with respect to haematological and clinical chemistry parameters such as packed cell volume, alanine aminotransferase and glucose. These changes in haematology and clinical chemistry values were not

Table 9(a). Incidence of neoplastic findings in male rats of the carcinogenicity study

Group		1	2	3	4	5
Compound		Control	Control	Sucralose		
Dietary level (%)		0	0	0.3%	1.0%	3.0%
		No. and (%) of rats affected				
		Males				
Group		1	2	3	4	5
Sucralose (%, w/w)		0	0	0.3	1.0	3.0
Organ and finding						
Adrenals med. (L&R)	No. examined:	50	50	18	28	50
B–Medullary adenoma		4 (8)	3 (6)	1(6)	3(11)	4 (8)
M–Medullary carcinoma		0 (0)	0 (0)	0 (0)	1(4)	0 (0)
Duodenum	No. examined:	50	50	16	22	50
M–Intestinal carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Brain × 3	No. examined:	50	50	19	21	50
M–Ganglioneuroma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Eyes (L&R)	No. examined:	46	49	23	28	50
B–Intraocular fibroma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Femur and marrow	No. examined:	50	49	16	19	50
B–Osteogenic sarcoma		1 (2)	0 (0)	0 (0)	1(5)	0 (0)
Head - level 2	No. examined:	50	50	18	19	50
B–Papilloma of tooth alveolus		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M–Squamous-cell carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Kidneys (L&R)	No. examined:	50	50	50	50	50
B–Renal adenoma		0 (0)	0 (0)	1(2)	2(4)	0 (0)
M–Renal liposarcoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Squamous cell carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymph node mesenteric	No. examined:	50	50	18	21	50
B–Haemangioma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Liver	No. examined:	50	50	50	50	50
B–Hepatocytic adenoma		1 (2)	2 (4)	1(2)	1(2)	1 (2)
Lungs ×2	No. examined:	50	50	50	50	50
B–Pulmonary adenoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Histiocytic leukemia		0 (0)	0 (0)	0 (0)	1(2)	0 (0)
Mammary ×2	No. examined:	38	35	13	19	38
B–Mammary gland fibroadenoma		1 (3)	1 (3)	0 (0)	2(11)	1 (3)
M–Mammary gland carcinoma		0 (0)	0 (0)	1(8)	0 (0)	0 (0)
M–Sarcoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pancreas	No. examined:	49	50	24	23	50
B–Islet cell adenoma		4 (8)	7 (14)	3(13)	2(9)	3 (6)
M–Sarcoma		0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
Parathyroids (L&R)	No. examined:	49	48	17	18	47
B–Adenoma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Pituitary	No. examined:	50	50	23	33	49
B–Adenoma–pars distalis		18 (36)	13 (26)	11(48)	24(73)	19 (39)
B–Adenoma–pars intermedia		0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
M–Carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Prostate	No. examined:	50	50	16	20	50
M–Prostatic carcinoma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Skeletal muscle	No. examined:	50	50	17	20	50
B–Chondroma		0 (0)	0 (0)	0 (0)	1(5)	0 (0)
Skin	No. examined:	50	50	45	40	50
B–Basal cell adenoma		0 (0)	2 (4)	3(7)	2(5)	0 (0)
B–Fibroma		7 (14)	0 (0)	5(11)	2(5)	5 (10)
B–Keratoacanthoma		1 (2)	1 (2)	0 (0)	0 (0)	1 (2)
B–Lipoma		8 (16)	9 (18)	9(20)	9(23)	2 (4)
B–Papilloma		3 (6)	1 (2)	1(2)	1(3)	4 (8)
B–Sebaceous adenoma		1 (2)	0 (0)	0 (0)	0 (0)	2 (4)
M–Anaplastic carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M–Basal cell carcinoma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
M–Sarcoma		4 (8)	2 (4)	4(9)	2(5)	3 (6)
Spleen	No. examined:	50	50	19	19	50
M–Sarcoma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Testes (L&R)	No. examined:	50	50	27	33	50
B–Interstitial cell adenoma		2 (4)	2 (4)	6(22)	0 (0)	1 (2)
Thymus	No. examined:	42	47	16	19	47
B–Thymoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Thyroids (L&R)	No. examined:	49	50	20	20	49
B–Follicular cell adenoma		1 (2)	3 (6)	2(10)	0 (0)	0 (0)
B–Parafollicular cell adenoma		0 (0)	1 (2)	3(15)	0 (0)	3 (6)
M–Parafollicular cell carcinoma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
M–Follicular cell carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	1(2)
Urinary bladder	No. examined:	50	50	16	19	50
B–Transitional cell adenoma		0 (0)	0 (0)	0(0)	0 (0)	0 (0)
Zymbals gland	No. examined:	50	50	17	19	49

B–Adenoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Carcinoma		0 (0)	0 (0)	1(6)	0 (0)	0 (0)
Haematopoietic tissue	No. examined:	50	50	41	41	50
M–Granulocytic leukaemia		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
M–Histiocytic sarcoma		0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
M–Malignant lymphoma		0 (0)	1 (2)	1(2)	0 (0)	0 (0)

M = malignant; B = benign.

Table 9(b). Incidence of neoplastic findings in female rats of the carcinogenicity study

Group	1	2	3	4	5	
Compound	Control	Control	Sucralose			
Dietary level (%)	0	0	0.3%	1.0%	3.0%	

		No. and (%) of rats affected				
		Females				
Group		1	2	3	4	5
Sucralose (%, w/w)		0	0	0.3	1.0	3.0
Organ and Finding						
Adrenals med. L&R	No. examined:	50	50	50	50	50
M–Medullary adenoma		2 (4)	0 (0)	0 (0)	1(3)	0 (0)
M–Medullary carcinoma		0 (0)	0 (0)	0 (0)	0(0)	0 (0)
Duodenum	No. examined:	50	50	26	12	50
M–Intestinal carcinoma		0 (0)	0 (0)	1(4)	0 (0)	0 (0)
Brain ×3	No. examined:	50	50	31	21	50
M–Ganglioneuroma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Eyes (L&R)	No. examined:	50	50	29	16	50
B–Intraocular fibroma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Femur and marrow	No. examined:	49	50	26	12	50
B–Osteogenic sarcoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Head - level 2	No. examined:	50	50	26	12	48
B–Papilloma of tooth alveolus		1 (2)	0 (0)	1(4)	0 (0)	0 (0)
M–Squamous cell carcinoma		0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Kidneys (L&R)	No. examined:	50	50	50	50	50
B–Renal adenoma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
M–Renal liposarcoma		0 (0)	0 (0)	1(2)	0 (0)	0 (0)
M–Squamous cell carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Lymph node mesenteric	No. examined:	50	50	34	24	49
B–Haemangioma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Liver	No. examined:	50	49	50	50	50
B–Hepatocytic adenoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lungs ×2	No. examined:	50	50	50	50	50
B–Pulmonary adenoma		0 (0)	0 (0)	1(2)	0 (0)	0 (0)
M–Histiocytic leukemia		0 (0)	0 (0)	0 (0)	0(0)	0 (0)
Mammary ×2	No. examined:	50	50	44	38	50
B–Mammary gland fibroadenoma		27(54)	26 (52)	30(68)	22(58)	20 (40)
M–Mammary gland carcinoma		2 (4)	6 (12)	3(7)	3(8)	1 (2)
M–Sarcoma		0 (0)	0 (0)	1(2)	0 (0)	0 (0)
Ovaries (L&R)	No. examined:	50	50	31	22	50
M–Carcinosarcoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Pancreas	No. examined:	50	50	26	13	49
B–Islet cell adenoma		2 (4)	3 (6)	1(4)	1(8)	2 (4)
M–Sarcoma		0 (0)	0 (0)	0 (0)	0(0)	0 (0)
Parathyroids (L&R)	No. examined:	46	48	24	12	49
B–Adenoma		0 (0)	0 (0)	0 (0)	1(8)	0 (0)
Pituitary	No. examined:	50	50	41	34	50
B–Adenoma–pars distalis		32 (64)	30 (60)	33(80)	28(82)	26 (52)
B–Adenoma–pars intermedia		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Carcinoma		0 (0)	0 (0)	1(2)	0 (0)	0 (0)
Skeletal muscle	No. examined:	50	50	26	13	50
B–Chondroma		0 (0)	0 (0)	0 (0)	0(0)	0 (0)
Skin	No. examined:	50	50	43	26	50
B–Basal cell adenoma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
B–Fibroma		3 (6)	3 (6)	1(2)	3(12)	1 (2)
B–Keratoacanthoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
B–Lipoma		1 (2)	2 (4)	0 (0)	2(8)	1 (2)
B–Papilloma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
B–Sebaceous adenoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
M–Anaplastic carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Basal cell carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Sarcoma		1 (2)	1 (2)	2(5)	1(4)	0 (0)
Spleen	No. examined:	50	50	27	12	50
M–Sarcoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

(continued)

Thymus	No. examined:	44	45	27	14	39
B–Thymoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Thyroids (L&R)	No. examined:	49	50	27	13	49
B–Follicular cell adenoma		3 (6)	0 (0)	1(4)	0 (0)	0 (0)
B–Parafollicular cell adenoma		2 (4)	2 (4)	2(7)	0 (0)	0 (0)
M–Parafollicular cell carcinoma		0 (0)	1 (2)	0 (0)	1(8)	0 (0)
B–Follicular cell adenoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Urinary bladder	No. examined:	50	50	27	12	49
B–Transitional cell adenoma		0 (0)	0 (0)	1(4)	0 (0)	0 (0)
Uterus	No. examined:	50	50	31	19	50
M–Leiomyosarcoma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Vagina	No. examined:	49	50	27	12	50
M–Leiomyosarcoma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Zymbals gland	No. examined:	49	49	25	12	46
B–Adenoma		0 (0)	0 (0)	0 (0)	1(8)	0 (0)
M–Carcinoma		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Haematopoietic tissue	No. examined:	50	50	44	39	50
M–Granulocytic leukaemia		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
M–Histiocytic sarcoma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
M–Malignant lymphoma		0 (0)	0 (0)	0 (0)	2(5)	1 (2)

M = malignant; B = benign.

considered to be toxicologically significant due to the fact that they were generally not dose dependent, slight in degree, or in the case of serum alanine aminotransferase a decrease was observed, which has no toxicological importance alone. Furthermore, in some instances, for example packed cell volume, significant increases were also seen in some groups. In addition, the clinical appearance and general health of the rats administered dietary sucralose was unaffected by these differences, and they did not correlate with the gross and histopathologic findings observed.

Apparent increases in urinary magnesium levels noted during the first 52 wk of treatment were not considered to be toxicologically significant as the effect was completely reversed after 77 wk of treatment. Apparent decreases in phosphate excretion were only noted after 77 wk of treatment and likewise were considered not to be toxicologically significant.

Caecal weights of rats administered the highest dietary concentration of sucralose were generally heavier than the controls after 52, 78 and 104 wk of treatment. Both full and empty caecal weights were affected, and the increases noted were consistent with changes observed in rats fed high dietary levels of other poorly absorbed compounds. This finding is of no toxicological significance as there were no sucralose-related histopathological changes in this organ, and caecal enlargement in the rat is considered to be a physiological response to a diet containing high levels of poorly absorbed material (Leegwater *et al.*, 1974, Lord and Newberne, 1990).

Microscopic examination of tissues from rats assigned to the carcinogenicity phase of this investigation revealed a statistically significantly ($P < 0.05$) higher incidence of renal pelvic mineralization in female rats receiving the intermediate or highest dietary concentrations, and renal pelvic epithelial hyperplasia ($P < 0.001$) in all groups of sucralose-treated females. In most cases, these changes were graded as minimal or slight in severity. These two findings are related in that the epithelium of the renal pelvis frequently reacts to mineral crystals in the pelvis by physiological hyperplasia (Lord and Newberne, 1990). It is probable that mineralization is the single primary effect, visualized in histology sections only when crystals are

Table 10. Summary of neoplasms in all rats

	No. and (%) of rats affected									
	Males					Females				
Sucralose (%, w/w)	0	0	0.3	1.0	3.0	0	0	0.3	1.0	3.0
No. of rats	50	50	50	50	50	50	50	50	50	50
Finding										
No neoplasms	9 (18)	13 (26)	21 (42)	14 (28)	15 (30)	6 (12)	7 (14)	2 (4)	10 (20)	11 (22)
One or more benign neoplasms	37 (74)	35 (70)	28 (56)	34 (68)	30 (60)	43 (86)	41 (82)	46 (92)	38 (76)	34 (68)
One or more malignant neoplasms	7 (14)	8 (16)	7 (14)	7 (14)	7 (14)	5 (10)	10 (20)	8 (16)	5 (10)	7 (14)
One or more primary* neoplasms	41 (82)	37 (74)	29 (58)	36 (72)	35 (70)	44 (88)	43 (86)	48 (96)	40 (80)	39 (78)
No. of neoplasms										
Total no. of primary* neoplasms	64	67	57	60	60	99	109	95	88	70
Mean no. of primary* neoplasms	1.28	1.34	1.14	1.20	1.20	1.98	2.18	1.90	1.76	1.40

*Benign and/or malignant.

Table 11(a). Group distribution of non-neoplastic findings for all animals (oncogenicity phase)

Group	1	2	3	4	5
Compound	Control	Control		Sucralose	
Dietary level (%)	0	0	0.3%	1.0%	3.0%

		No. and (%) of rats affected					
		Males			Females		
Group		1	2	5	1	2	5
Sucralose (%, w/w)		0	0	3.0	0	0	3.0
Organ and finding							
Adrenals cortex (L&R)	No. examined:	50	50	50	50	50	50
Cortical fatty change		9 (18)	9 (18)	10 (20)	12 (24)	10 (20)	4 (8)a
Cortical haemorrhagic degeneration		1 (2)	3 (6)	0 (0)	29 (58)	27 (54)	37 (74)a
Cortical hyperplasia		1 (2)	0 (0)	1 (2)	0 (0)	2 (4)	0 (0)
Deep cortical pigmentation		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Extramedullary haemopoiesis		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Fibrosis		0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)
Vascular degeneration		0 (0)	0 (0)	0 (0)	1 (2)	2 (4)	2 (4)
Cortical necrosis		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Focal hyperplasia		0 (0)	1 (2)	3 (6)	1 (2)	0 (0)	1 (2)
Adrenals medullary (L&R)	No. examined:	50	50	50	50	50	50
Medullary hyperplasia		7 (14)	6 (12)	7 (14)	3 (6)	0 (0)	0 (0)
Aorta	No. examined:	50	50	50	50	50	50
Medial mineralization		4 (8)	2 (4)	3 (6)	0 (0)	3 (6)	0 (0)
Brain ×3	No. examined:	50	50	50	50	50	50
Basilar compression		8 (16)	5 (10)	7 (14)	25 (50)	20 (40)	12 (24)a
Cerebro-cortical focal spongiosus		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Chronic meningitis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cortex depression		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Embolic encephalitis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Encephalomalacia		0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)
Hemorrhage		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Internal hydrocephalus		3 (6)	2 (4)	2 (4)	5 (10)	3 (6)	2 (4)
Status spongiosus		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Thalamic mineralization		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Caecum	No. examined:	50	50	50	50	50	50
Arteritis		0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Submucosal granuloma		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Submucosal inflammation		0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)
Ulcer		0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)
Eyes (L&R)	No. examined:	46	49	50	50	50	50
Anterior synechia		0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Cataract		0 (0)	1 (2)	5 (10)a	3 (6)	1 (2)	1 (2)
Hypopion		0 (0)	1 (2)	2 (4)	0 (0)	1 (2)	0 (0)
Iris proliferation		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Keratitis		4 (9)	5 (10)	4 (8)	1 (2)	2 (4)	1 (2)
Phthisis bulbi		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Retinal degeneration		1 (2)	2 (4)	2 (4)	4 (8)	2 (4)	0 (0)
Retinal disorganization		1 (2)	1 (2)	0 (0)	1 (2)	1 (2)	0 (0)
Scleral focal proliferation		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Epithelial inclusion cyst in orbit		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Iritis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Fallopian tubes (L&R)	No. examined:	0	0	0	49	49	50
Fibrosis		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Femur and marrow	No. examined:	50	49	50	49	50	50
Fibrous osteodystrophy		4 (8)	2 (4)	3 (6)	0 (0)	3 (6)	0 (0)
Marrow hyperplasia		1 (2)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Stifle–synovial overgrowth		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Harderian gland (L&R)	No. examined:	48	49	50	50	50	50
Acinar atrophy		2 (4)	1 (2)	2 (4)	9 (18)	6 (12)	6 (12)
Inflammation		1 (2)	2 (4)	6 (12)	4 (8)	6 (12)	5 (10)
Head–level 1	No. examined:	49	50	50	50	50	49
Buccal cavity–focal inflammation		5 (10)	6 (12)	6 (12)	19 (38)	11 (22)	8 (16)
Facial oedema		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Folliculitis		3 (6)	3 (6)	2 (4)	4 (8)	2 (4)	0 (0)
Foreign body granuloma		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Myopathy		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Nerve fibre degeneration		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Oral cavity–foreign body granuloma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Head–level 2	No. examined:	50	50	50	50	50	48
Acantholytic focus in nasal epithelium		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Alveolitis		14 (28)	14 (28)	8 (16)	27 (54)	29 (58)	22 (46)
Aspergilloma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Buccal Cavity–focal inflammation		2 (4)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Cellulitis		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)

(continued)

Epithelial inclusion cyst(s)		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Folliculitis		2 (4)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Inhaled bone fragment embedded in mucosa		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pulpitis		1 (2)	2 (4)	1 (2)	8 (16)	13 (26)	5 (10)
Rhinitis		3 (6)	1 (2)	4 (8)	1 (2)	2 (4)	0 (0)
Sebaceous gland hyperplasia		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Subcutaneous granuloma		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Head–level 3	No. examined:	50	50	50	50	50	49
Arteritis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Middle ear disease		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Heart, auricle	No. examined:	50	50	49	50	50	49
Auricular thrombus		1 (2)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)
Myocarditis		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Heart, ventricle	No. examined:	50	50	50	50	50	49
Cartilaginous metaplasia		2 (4)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Endocardial proliferation		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Myocardial mineralization		2 (4)	2 (4)	0 (0)	0 (0)	1 (2)	0 (0)
Myocardiopathy		4 (8)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)
Myocarditis		27 (54)	25 (50)	27 (54)	12 (24)	12 (24)	12 (24)
Right ventribular myocarditis		2 (4)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Supperative thrombus		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ileum	No. examined:	48	50	50	50	50	49
Chronic active inflammation in peyers patch		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Jejunum	No. examined:	50	49	50	48	50	50
Peritonitis		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Submucosal chronic active inflammation		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)
Ulcer		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)
Lymph node, cervical	No. examined:	47	49	49	49	50	48
Congestion		1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Cyst(s)		1 (2)	1 (2)	3 (6)	0 (0)	1 (2)	1 (2)
Granuloma		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Lymphoid hyperplasia		1 (2)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Paracortical hyperplasia		14 (30)	8 (16)	16 (33)	17 (35)	19 (38)	21 (44)
Terminal haemorrhage		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Lymph node, mesenteric	No. examined:	50	50	50	50	50	49
Capsular focal inflammation		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Cysts(s)		0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	2 (4)
Increased pigmentation in syncytial macrophages		0 (0)	0 (0)	0 (0)	4 (8)	4 (8)	2 (4)
Lymphoid hyperplasia		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Paracortical hyperplasia		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Sinus erythrophagia		0 (0)	1 (2)	3 (6)	0 (0)	0 (0)	0 (0)
Sinus histiocytosis		0 (0)	1 (2)	1 (2)	0 (0)	0 (0)	1 (2)
Lachrymal gland	No. examined:	46	49	49	49	49	50
Acinar degeneration		10 (22)	9 (18)	5 (10)	0 (0)	0 (0)	1 (2)
Haemorrhage		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Harderian gland metaplasia		33 (72)	33 (67)	29 (59)	0 (0)	0 (0)	0 (0)
Inflammation		1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Periductular fibrosis		3 (7)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)
Mammary ×2	No. examined:	38	35	38	50	50	50
Acinar hyperplasia		0 (0)	0 (0)	1 (3)	25 (50)	23 (46)	22 (44)
Galactocoele(s)		0 (0)	0 (0)	1 (3)	18 (36)	19 (38)	19 (38)
Optic nerves (L&R)	No. examined:	37	45	47	48	43	45
Gliosis		0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	1 (2)
Polymorphonuclear leukocyte infiltrate		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Ovaries (L&R)	No. examined:	0	0	0	50	50	50
Bursal cyst		0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)
Cyst		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Luteal cyst		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Pancreas	No. examined:	49	50	50	50	50	49
Acinar atrophy		7 (14)	14 (28)	14 (28)	9 (18)	7 (14)	10 (20)
Acinar hyperplasia		0 (0)	2 (4)	0 (0)	0 (0)	1 (2)	0 (0)
Arteritis		2 (4)	0 (0)	2 (4)	0 (0)	0 (0)	2 (4)
Islet hyperplasia		2 (4)	2 (4)	1 (2)	1 (2)	1 (2)	3 (6)
Parathyroids (L&R)	No. examined:	49	48	47	46	48	49
Hyperplasia		5 (10)	2 (4)	1 (2)	0 (0)	4 (8)	0 (0)
Pituitary	No. examined:	50	50	49	50	50	50
Cholesterol clefts		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Craniopharyngeal cyst(s)		3 (6)	1 (2)	1 (2)	0 (0)	1 (2)	0 (0)
Pars Distalis–focal hyperplasia		7 (14)	12 (24)	9 (18)	6 (12)	7 (14)	2 (4)
Pars Distalis - haemorrhage		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Pars Nervosa–tubular forms		1 (2)	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)
Prostate	No. examined:	50	50	50	0	0	0
Chronic inflammation		0 (0)	4 (8)	1 (2)	0 (0)	0 (0)	0 (0)
Purulent prostatitis		2 (4)	5 (10)	3 (6)	0 (0)	0 (0)	0 (0)
Rectum	No. examined:	50	50	50	50	50	50
Arteritis		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Submucosal chronic active inflammation		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Submucosal fibrosis		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Sciatic nerve, LF	No. examined:	49	50	47	50	50	47
Axonal degeneration		5 (10)	6 (12)	3 (6)	3 (6)	2 (4)	3 (6)
Inter-fibre fibrosis		0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)
Seminal vesicles	No. examined:	50	50	50	0	0	0

Lack of secretion		0 (0)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)
Mural inflammation		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Skeletal muscle	No. examined:	50	50	50	50	50	50
Embolic myositis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Lymphocytic infiltration		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Myopathy		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Neurogenic atrophy		3 (4)	5 (10)	2 (4)	0 (0)	0 (0)	0 (0)
Skin	No. examined:	50	50	50	50	50	50
Abscess		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Acanthosis		0 (0)	1 (2)	2 (4)	0 (0)	1 (2)	0 (0)
Chronic inflammation		2 (4)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)
Dermal fibrosis		3 (6)	4 (8)	9 (18)	1 (2)	2 (4)	1 (2)
Epithelial adnexal atrophy		2 (4)	2 (4)	2 (4)	1 (2)	2 (4)	0 (0)
Epithelial inclusion cyst		11 (22)	7 (14)	10 (20)	2 (4)	1 (2)	1 (2)
Eschar		1 (2)	1 (2)	1 (2)	1 (2)	2 (4)	0 (0)
Focal epithelial hyperplasia		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)
Granulomatous dermatitis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Keloid		10 (20)	13 (26)	9 (18)	1 (2)	0 (0)	0 (0)
Mineralization of dermal collagen		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Perifolliculitis		0 (0)	2 (4)	0 (0)	1 (2)	0 (0)	0 (0)
Ulcer		2 (4)	2 (4)	0 (0)	1 (2)	1 (2)	1 (2)
Spinal cord ×3	No. examined:	50	50	50	50	50	50
Myelomalacia		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)
Vacuolation of sheathing nerves in cauda equina		4 (8)	7 (14)	6 (12)	1 (2)	1 (2)	0 (0)
Spleen	No. examined:	50	50	50	50	50	50
Extramedullary haemopoiesis		6 (12)	0 (0)	1 (2)	3 (6)	3 (6)	3 (6)
Focal lymphoid hypoplasia		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Haemosiderosis		2 (4)	4 (8)	3 (6)	17 (34)	15 (30)	8 (16)a
Lymphoid hyperplasia		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Stomach ×2	No. examined:	50	50	50	50	50	50
Arteritis		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Glandular dilatation		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Glandular zone–ulcer		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Keratin zone–acanthosis		5 (10)	2 (4)	0 (0)	3 (6)	5 (10)	2 (4)
Keratin zone–basal cell hyperplasia		0 (0)	0 (0)	1 (2)	1 (2)	1 (2)	0 (0)
Keratin zone–hyperkeratosis		1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Keratinized zone–submucosal oedema		2 (4)	3 (6)	2 (4)	2 (4)	2 (4)	0 (0)
Keratin zone–ulcer		2 (4)	1 (2)	1 (2)	1 (2)	4 (8)	2 (4)
Mineralization–mucosa		2 (4)	6 (12)	3 (6)	0 (0)	3 (6)	0 (0)
Mineralization–muscular wall		2 (4)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Mural purulent inflammation		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Submucosal fibrosis		4 (8)	1 (2)	1 (2)	0 (0)	4 (8)	1 (2)
Submucosal granuloma		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Testes (L&R)	No. examined:	50	50	50	0	0	0
Arteritis		9 (18)	8 (16)	9 (18)	0 (0)	0 (0)	0 (0)
Germinal epithelium degeneration		22 (44)	22 (44)	20 (40)	0 (0)	0 (0)	0 (0)
Interstitial cell hyperplasia		0 (0)	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)
Sperm granuloma		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tubular necrosis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tubule dilatation		0 (0)	2 (4)	2 (4)	0 (0)	0 (0)	0 (0)
Tubule mineralization		14 (28)	12 (24)	7 (14)	0 (0)	0 (0)	0 (0)
Thymus	No. examined:	42	47	47	44	45	39
Cyst(s)		0 (0)	0 (0)	0 (0)	4 (9)	4 (9)	2 (5)
Lymphoid hyperplasia		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Proliferation of the thyroglossal duct epithelium		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Thyroids (L&R)	No. examined:	49	50	49	49	50	49
Abnormality of follicular growth pattern		1 (2)	2 (4)	1 (2)	0 (0)	0 (0)	0 (0)
Follicular epithelial hypertrophy		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Large follicle with basophilic epithelium		3 (6)	1 (2)	3 (6)	0 (0)	2 (4)	2 (4)
Parafollicular cell hyperplasia		5 (10)	2 (4)	7 (14)	11 (22)	4 (8)	4 (8)
Hurthle cells		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Trachea	No. examined:	50	50	50	50	50	50
Tracheitis		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Urinary bladder	No. examined:	50	50	50	50	50	49
Cystitis		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Epithelial hyperplasia		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Focal submucosal fibrosis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Papillary hyperplasia		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Submucosal lymphocytic infiltration		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)
Uterus	No. examined:	0	0	0	50	50	50
Amyloid		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)
Arteritis		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)
Lumen dilatation		0 (0)	0 (0)	0 (0)	2 (4)	4 (8)	3 (6)
Stromal endometrial hyperplasia		0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	3 (6)
Telangiectasis		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Zymbals gland	No. examined:	50	50	49	49	49	46
Cystic ducts		1 (2)	0 (0)	2 (4)	1 (2)	0 (0)	0 (0)
Inflammation in diffuse gland		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)

[a]Significantly different from combined controls, $P < 0.05$.

Table 11(b). Group distribution of non-neoplastic findings for all animals (oncogenicity phase) liver, lungs and kidneys

Group	1	2	3	4	5
Compound	Control	Control		Sucralose	
Dietary level (%)	0	0	0.3%	1.0%	3.0%

		No. and (%) of rats affected									
		Males					Females				
Group		1	2	3	4	5	1	2	3	4	5
Sucralose (%, w/w)		0	0	0.3	1.0	3.0	0	0	0.3	1.0	3.0
Organ and finding											
Kidneys (L&R)	No. examined:	50	50	50	50	50	50	50	50	50	50
Arteritis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)
Cortical fibrosis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cyst(s)		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fatty tubular epithelium		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Hydronephrosis		2 (4)	2 (4)	5 (10)	6 (12)	3 (6)	5 (10)	6 (12)	13 (26)a	5 (10)	8 (16)
Mineralization of basement membranes		1 (2)	2 (4)	0 (0)	0 (0)	1 (2)	0 (0)	3 (6)	0 (0)	0 (0)	0 (0)
Nephrocalcinosis		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	3 (6)	5 (10)	5 (10)	2 (4)	2 (4)
Papillary necrosis		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)
Pelvic epithelial hyperplasia		2 (4)	2 (4)	2 (4)	4 (8)	1 (2)	2 (4)	0 (0)	6 (12)a	9 (18)c	10 (20)c
Pelvic mineralization		0 (0)	0 (0)	1 (2)	1 (2)	1 (2)	7 (14)	7 (14)	12 (24)	16 (32)a	15 (30)a
Progressive nephropathy		34 (68)	41 (82)	35 (70)	35 (70)	41 (82)	22 (44)	22 (44)	13 (26)a	17 (34)	16 (32)
Pyelitis		0 (0)	1 (2)	2 (4)	1 (2)	2 (4)	0 (0)	0 (0)	1 (2)	0 (0)	3 (6)
Pyelonephritis		1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)
Subcapsular haemorrhage		2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tubular epithelial vacuolation		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Interstitial fat infiltration		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Liver	No. examined.	50	50	50	50	50	50	49	50	50	50
Basophilic hepatocytic focus (I)		2 (4)	8 (16)	4 (8)	2 (4)	4 (8)	10 (20)	16 (33)	11 (22)	13 (26)	5 (10)a
Bile duct degeneration		0 (0)	1 (2)	3 (6)	2 (4)	1 (2)	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)
Bile duct hyperplasia		5 (10)	2 (4)	8 (16)	3 (6)	6 (12)	5 (10)	1 (2)	7 (14)	2 (4)	5 (10)
Bile duct lithiasis		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bile duct normal		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	0 (0)	1 (2)
Capsular fibrosis		1 (2)	1 (2)	1 (2)	0 (0)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Centriacinar glycogen vacuolation		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Centriacinar hepatocytic fatty vacuolation		9 (18)	9 (18)	7 (14)	8 (16)	11 (22)	19 (38)	23 (47)	10 (20)a	14 (28)	5 (10)c
Centriacinar pigmented macrophages		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clear-cell hepatocytic focus (I)		6 (12)	6 (12)	9 (18)	8 (16)	12 (24)	7 (14)	8 (16)	2 (4)	6 (12)	6 (12)
Coagulative necrosis		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Eosinophilic hepatocytic focus		3 (6)	1 (2)	2 (4)	0 (0)	5 (10)	6 (12)	0 (0)	0 (0)	1 (2)	0 (0)
Haemorrhagic necrosis		1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Hepatocytic hyperplasia		4 (8)	1 (2)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	3 (6)	2 (4)	0 (0)
Hepatocytic necrosis		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	1 (2)	1 (2)	0 (0)
Inter-biliary fibrosis		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)
Panacinar hepatocyte vacuolation		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Passive congestion		0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)

Peliosis hepatis		1 (2)	3 (6)	0 (0)	4 (8)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Periacinar agonal hepatocytic caseous necrosis		0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
Periacinar hepatocytic eosinophilia		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Periacinar hepatocytic fatty vacuolation		6 (12)	3 (6)	5 (10)	3 (6)	0 (0)	9 (18)	6 (12)	9 (18)	6 (12)	5 (10)
Periacinar hepatocytic necrosis		3 (6)	1 (2)	1 (2)	1 (2)	3 (6)	1 (2)	0 (0)	2 (4)	0 (0)	2 (4)
Subacute inflammation		1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	1 (2)	1 (2)
Telangiectasia		0 (0)	0 (0)	2 (4)	1 (2)	0 (0)	0 (0)	1 (2)	0 (0)	4 (8)	2 (4)
Agonal congestion haemorrhage		12 (24)	7 (14)	4 (8)	3 (6)a	6 (12)	1 (2)	0 (0)	2 (4)	1 (2)	1 (2)
Alveolar luminal bone		0 (0)	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alveolar macrophage proliferation		4 (8)	3 (6)	3 (6)	3 (6)	4 (8)	4 (8)	3 (6)	1 (2)	4 (8)	0 (0)
Alveolar type II cell proliferation		0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Alveolar wall mineralization		0 (0)	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Cholesterol granuloma		0 (0)	0 (0)	1 (2)	4 (8)	3 (6)	1 (2)	1 (2)	1 (2)	0 (0)	2 (4)
Diffuse pneumonitis		0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Focal pneumonitis		7 (14)	3 (6)	6 (12)	4 (8)	6 (12)	5 (10)	4 (8)	4 (8)	5 (10)	10 (20)
Focal vascular mineralization		2 (4)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)	0 (0)
Intra-alveolar granular pigmentation		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Lungs ×2	No. examined:	50	50	50	50	50	50	50	50	50	50
Mineralization alveolar wall		0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)
Pre-terminal haemorrhage		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)	0 (0)

[a]Significantly different from combined controls, $P < 0.05$. [c]Significantly different from combined controls, $P < 0.001$.

trapped in the pelvic epithelium. The presence of minerals in the renal pelvis (i.e. pelvic nephrocalcinosis), is related neither to the minerals deposited at the cortico-medullary junction deep in the renal parenchyma nor the mineralization of the renal basement membrane (which occurs secondary to marked progressive nephropathy). The incidence of renal disease, as exemplified by progressive nephropathy, was unaffected by sucralose administration. The incidence of some forms of renal mineralization is sex-related, and often occurs in control female rats (Cousins and Geary, 1966). No treatment-related effects were noted in male rats administered sucralose, and no significant inter-group differences were observed for either of these renal findings among rats assigned to the 78-wk toxicity phase of this investigation.

Pelvic epithelial hyperplasia in the rat is frequently associated with pelvic epithelial mineralization, and both of these events are often observed in rats with caecal hypertrophy (Lord and Newberne, 1990). The mechanism by which they occur is not clearly understood. However, caecal enlargement and pelvic mineralization have been observed as a consequence of feeding rats compounds that belong to a broad group of carbohydrates which include lactose, xylitol, sorbitol and chemically modified starches and bulking agents such as polydextrose (de Groot *et al.*, 1974; Hodgkinson *et al.*, 1982; Roe, 1984; Truhaut *et al.*, 1979). These findings are not considered to be toxicologically significant (Truhaut *et al.*, 1979). The increased incidence of renal changes and caecal enlargement described above are both believed to be a physiological response to high levels of poorly absorbed dietary substances leading to an increase in caecal intraluminal pressure and a compensatory distention of the organ. Thus, slight effects on water intake, lower urine output, increased soft stools at dietary levels of 5%, caecal and renal changes, and possibly other minor gut physiological effects are all judged related to consumption of high levels of a poorly absorbed material not unlike that observed with lactose, sorbitol or polydextrose. None of these effects are toxicologically relevant to man because humans will only consume minute amounts of sucralose as a highly intense sweetener.

Other non–treatment-related findings in which the incidence among high-dose group rats assigned to the carcinogenicity phase was higher than that of the controls were adrenal cortical haemorrhagic degeneration in females and cataracts in males. Adrenal cortical haemorrhagic degeneration occurs spontaneously at high incidence rates, is highly variable, and is commonplace in aged female rats. The incidence in a similar concurrently conducted 104 wk carcinogenicity study, on a related material, in this same strain of rat at the same testing facility found the female control incidence of cortical haemorrhagic degeneration to be 70% and ranged

Table 12. Incidence and severity of selected non-neoplastic findings of the kidney of rats of the carcinogenicity study fed diets containing sucralose

	No. and (%) of rats affected									
	Males					**Females**				
Sucralose (%, w/w)	0	0	0.3	1.0	3.0	0	0	0.3	1.0	3.0
No. of rats	50	50	50	50	50	50	50	50	50	50
Organ and finding										
Pelvic epithelial hyperplasia:										
Minimal >	2 (4)	-	-	4 (8)	-	1 (2)	-	5 (10)	7 (14)	8 (16)
Slight >	-	1 (2)	2 (4)	-	1 (2)	1 (2)	-	1 (2)	2 (4)	1 (2)
Moderate >	-	1 (2)	-	-	-	-	-	-	-	1 (2)
Total	2 (4)	2 (4)	2 (4)	4 (8)	1 (2)	2 (4)	-	6 (12)*	9 (18)***	10 (20)***
Pelvic mineralization:										
Minimal >	-	-	-	1 (2)	1 (2)	6 (12)	5 (10)	10 (20)	13 (26)	9 (18)
Slight >	-	-	1 (2)	-	-	1 (2)	2 (4)	2 (4)	3 (6)	6 (12)
Total	-	-	1 (2)	1 (2)	1 (2)	7 (14)	7 (14)	12 (24)	16 (32)*	15 (30)*

Incidences marked with asterisks differ significantly (Fisher's exact test) from the corresponding combined control incidence. $^{*}P < 0.05$; $^{***}P < 0.001$.

between 65–76% in the treated females. Therefore, it is unlikely to have been related to sucralose administration, and is not considered to be toxicologically significant. The apparently higher incidence of cataracts in microscopic sections of the eye taken from male rats administered the highest dietary concentration of sucralose as compared to the controls is also not considered to be a true reflection of treatment, because the incidence seen in histologic sections is not a true sampling of the actual incidence. Repeated in-life ophthalmological examinations are more exact and did not reveal any treatment-related ocular findings, and in several cases, the cataracts appeared to be secondarily related to keratitis or retinal disorganization with resultant nutritional damage to the lens. In addition, neither or these two findings were observed in rats assigned to the toxicity phase of this study.

In conclusion, sucralose is not carcinogenic when administered to both male and female rats prior to mating, female rats throughout gestation, and the F_1 offspring for 104 wk following parturition. The administration of sucralose affected neither the types nor incidence of tumours observed, and did not influence the multiplicity of tumours per rat. There were no treatment-related macroscopic abnormalities or neoplastic findings. The non-neoplastic findings that occurred were of no toxicological significance since they were either spontaneous findings commonly observed in aged rats of this strain or believed to be a physiological response to high dietary levels of a poorly absorbed compound. Finally, the in-life findings noted in this investigation were neither toxicologically significant nor manifestations of sucralose-induced toxicity.

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