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## Sucralose: Assessment of Teratogenic Potential in the Rat and the Rabbit

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**Abstract**—The teratogenic potential of sucralose was examined following gavage administration to pregnant rats and rabbits during organogenesis. Groups of 20 mated rats were dosed on days 6–15 of gestation inclusive at 500, 1000 or 2000 mg/kg/day; groups of 16 to 18 inseminated rabbits were dosed on days 6 to 19 of gestation inclusive at 175, 350 or 700 mg/kg/day following preliminary studies at higher doses. Concurrent control groups received vehicle alone. Rats were killed on day 21 and rabbits on day 29 of gestation. Foetuses were evaluated at necropsy and after processing for possible soft tissue and skeletal alterations. There was no evidence of teratogenicity for either species. The only observed response to treatment in rats was a slight increase in water intake. Some adult rabbits receiving 700 mg/kg/day exhibited marked gastrointestinal disturbance, also seen at higher doses in preliminary studies. Gastrointestinal effects such as these occur non-specifically in response to high doses of poorly absorbed compounds, and in the present study were considered to be responsible for two maternal deaths and four abortions. Full evaluation of rabbit foetuses in the main study (up to 700 mg/kg/day) and necropsy of foetuses in a preliminary study with pregnant animals (up to 1000 mg/kg/day) showed no evidence of adverse foetal response to sucralose. These teratology studies in both pregnant rodent and non-rodent animal models demonstrate that maternal consumption of high levels of sucralose during the period of organogenesis has no effect on normal foetal development in the rat or rabbit. © 2000 Elsevier Science Ltd. All rights reserved

**Keywords:** sucralose; teratology; rats; rabbits; artificial sweeteners.

### INTRODUCTION

Previous evidence suggested that small amounts of sucralose may cross the placenta (McNeil, 1987). Because sucralose will be consumed by women of child-bearing potential, studies were conducted to evaluate the potential for this water-soluble molecule to adversely affect normal embryonic and foetal development.

The work reported here is an evaluation of the teratogenic potential of sucralose in pregnant CD rats and New Zealand White rabbits.

### MATERIALS AND METHODS

These studies were conducted in accordance with Good Laboratory Practice Regulations for non-clinical safety assessment studies (FDA, 1978).

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### Test material

Sucralose was supplied as a white powder (by Linson Ltd, Swords, Co. Dublin, Eire). Batch number 153002 MT 205 (purity >97.4%) was used for the rat teratology study; batch number 953011 (purity 99.3%) was used for the rabbit preliminary and main teratology studies. Uniformly radiolabelled <sup>14</sup>C- sucralose (batch number CFQ.3102) was supplied by Amersham International plc at a specific activity of 1.2835  $\mu$ Ci/mg.

### Animals and husbandry

**Rats.** Virgin female CD rats [CrI:CD<sup>R</sup>(SD) BR strain from Charles River (UK) Ltd, Margate, Kent, UK] at 8–9 wk of age were allowed 6 days' acclimatization and were then mated with proven stock males from the same strain and source.

Cages consisted of high-density polypropylene bodies with lids of stainless-steel grid. During acclimatization and pairing, the animals were in cages with stainless-steel grid floors but during gestation the females were housed individually in cages with solid polypropylene floors provided with autoclaved wood shavings as bedding. Cages were distributed

within batteries to minimize the effects of any spatially variable components of the environment. Animal rooms were maintained at positive pressure with filtered fresh air which was changed approximately 15 times per hr. The mean temperature and relative humidity ranges were 19–22°C and 36–60% relative humidity, respectively, and no excursions occurred that were considered to have adversely affected the study. The lighting cycle was 12 hr light/12 hr dark.

**Rabbits.** Virgin female New Zealand White rabbits (Ranch Rabbits, Crawley Down, Sussex, UK), approximately 16–24 wk old, were allowed a minimum of 3 wk acclimatization before insemination. Shortly after arrival, oestrus was synchronized by iv injection of 25 IU luteinizing hormone (Profasi, Serono).

The rabbits were housed singly in galvanized-steel cages with mesh floors and were randomly distributed within the caging array to minimize the effects of any environmental variations. The rabbitry had its own supply of filtered fresh air which was changed approximately 17–20 times per hr. The mean temperature and humidity ranges were 15–23°C and 40–70% relative humidity, respectively, and no excursions occurred that were considered to have adversely affected the study. A 14 hr light/10 hr dark cycle operated throughout.

**Food and water.** Tap water from the domestic supply and laboratory animal diet were freely available to the animals throughout. Rats received Spratt's Laboratory Diet 1, and rabbits received S.Q.C. Standard Rabbit Diet.

**Mating procedures.** Female rats were paired on a one-to-one basis with proven stock males from the same strain and source. The day on which evidence of mating was found was designated day 1 of gestation and, as far as possible, females mating on any one day were evenly distributed among the groups. Body weights on day 1 of gestation were 196 to 230 g.

Female rabbits were artificially inseminated with pooled semen from New Zealand White bucks of established fertility. Following insemination, each female was injected iv with 25 IU luteinizing hormone to ensure successful ovulation. The day of insemination was designated day 0 of gestation and body weights on that day were 3.52–4.83 kg.

**Treatment.** Sucralose was dispensed freshly each day as a solution in distilled water and was administered daily at a volume–dose of 10 ml/kg by the oral route (gavage) to rats from day 6 to day 15 of gestation inclusive and to rabbits from day 6 to day 19 of gestation inclusive. Control animals received distilled water at the same volume–dose. The volume administered daily to each animal was based on the animal's body weight on that day.

In the rat, doses administered were 500, 1000 and 2000 mg/kg/day. In the rabbit, however, a limited tolerance study indicated that 2000 mg/kg/day was

clearly too high, causing pronounced body weight loss and evidence of gastrointestinal tract disturbance, such as scouring and peri-anal faecal staining. In a subsequent preliminary teratology study at reduced doses of 500, 750 and 1000 mg/kg/day, rabbits receiving 1000 mg/kg/day showed marked scouring and one died. Females receiving 750 mg/kg/day were similarly, but less severely affected. Doses of 175, 350 and 700 mg/kg/day were therefore selected for the main rabbit teratology study.

**Blood plasma levels.** Blood plasma levels of <sup>14</sup>C-sucralose (prepared by Amersham International plc, sp. act. 0.541 mCi/mol) were determined by liquid scintillation spectrometry in the preliminary rabbit teratology study in order to demonstrate that sucralose was absorbed and blood levels increased with the dose.

Radiolabelled sucralose was administered by gavage to two pregnant rabbits per dose on day 19 of gestation at 100 or 750 mg/kg/day. The radiolabelled sucralose was administered following prior treatment daily for 18 days with non-labelled sucralose at the same doses. For dosing purposes, 20  $\mu$ Ci of <sup>14</sup>C-labelled sucralose were added to unlabelled sucralose. Radiolabelled sucralose was analysed for purity by thin layer chromatography (TLC) in three different solvent systems. The plates (Merck silica gel G60 F<sub>254</sub>, 0.25 mm on glass) were then dried and scanned with an Isomess IM-3000 Radio-TLC analyser. A specific activity of 1.2701  $\mu$ Ci/mg was obtained which was within 1% of the claimed figure. The radiolabelled material was not less than 98% pure.

Blood samples (2 ml) were taken immediately before dosing and at 1, 3, 6 and 24 hr after dosing. Blood was centrifuged to separate plasma, and accurately measured duplicate aliquots (0.24 or 0.25 ml) were mixed with Lumagel (10 ml) and analysed with a Packard 3300 liquid scintillation spectrometer using the channels-ratio method for quench correction.

**Observations.** Rats were examined once daily and rabbits twice daily throughout. Any visible signs of reaction to treatment or ill-health were recorded, with details of type, severity, time of onset and duration. Food and water intakes were recorded during gestation, at appropriate intervals before, during and after the treatment periods. Body weights were recorded for rats on days 1, 3, 6 to 16, 18 and 21 *post coitum* and rabbits were weighed daily after insemination.

Rats were killed by inhaled carbon dioxide on day 21 of gestation. Rabbits were killed by iv injection of pentobarbitone sodium on day 29 of gestation. Each animal was first examined macroscopically for evidence of disease or adverse reaction to treatment. The reproductive tract, complete with ovaries, was dissected and the following recorded: (a) number of corpora lutea in each ovary; (b) number of implantation sites—in appar-

Table 1. Rat body weights during gestation

Item	Dose (mg/kg/day)			
	Vehicle control	500	1000	2000
Number pregnant	20	19	20	20
Body weight (g) day 1	210 ± 9	210 ± 5	209 ± 5	209 ± 8
Body weight (g) day 6	237 ± 10	239 ± 7	239 ± 7	240 ± 12
Body weight (g) day 16	312 ± 14	310 ± 17	310 ± 16	312 ± 17
Body weight (g) day 21	374 ± 21	375 ± 21	376 ± 22	377 ± 23
Body weight change (g) day 6 to 16 <sup>a</sup>	75 ± 11	71 ± 13	72 ± 11	71 ± 8

<sup>a</sup>Period of dosing.

ently non-pregnant animals, a staining technique was used to check the uterus for implantation sites (Salewski, 1964); (c) number of resorption sites (classified as early or late); (d) number and distribution of live and dead fetuses in each uterine horn; (e) foetal examination—each foetus and placenta was weighed; external abnormalities were recorded, and sex was determined externally in rats and internally in rabbits. The neck and the thoracic and abdominal cavities were dissected and eviscerated for all rabbit fetuses and for two-thirds of each rat litter. The remaining one-third of rat fetuses and also the heads of one-third of each rabbit litter were fixed in Bouins' fixative for subsequent examination following free-hand serial sectioning (Wilson, 1965). Eviscerated carcasses were placed in industrial methylated spirit (74 o.p.) and were processed using a modification of Dawson's Alizarin staining technique for examination of the skeletons (Dawson, 1926; Tesh, 1968).

*Treatment of data.* Results have been presented as group values or as group mean values and standard deviations (SD) followed, where necessary, by sample size in brackets. Foetal and placental weight SD were calculated using combined inter- and intra-litter variance.

Foetal observations were assessed both in terms of the overall percentage incidence of fetuses presenting each finding and in terms of the numbers of litters with affected fetuses.

The significance of suggestive intergroup differences was tested using appropriate non-parametric or parametric statistical tests as indicated in the tables or text wherever significant differences were

found. Differences with an associated probability of  $P < 0.05$  were considered to be statistically significant.

**RESULTS**

*Rat—maternal condition*

Body weight gain and food consumption (Tables 1 and 2) were similar in all groups and no deaths occurred. Water intake was slightly increased during the treatment period at 2000 mg/kg/day, achieving statistical significance only during the first few days of treatment ( $P < 0.05$ ; Table 3). Water intake was unaffected at 500 and 1000 mg/kg/day. At necropsy on day 21 of gestation, no treatment-related macroscopic anomalies were observed in the dams.

*Rat—litters and foetal evaluation*

The numbers of implantation sites and live young, the extent of pre- and post-implantation losses, and foetal and placental weights were unaffected by treatment (Table 4). Examination of fetuses at necropsy by freehand serial sectioning and after skeletal processing revealed no indication of increasing incidence of observations with increasing dose (Table 5). The few sporadic occurrences of fetuses with combined anomalies were unrelated to treatment with sucralose.

After examination of fetuses at necropsy or at freehand serial sectioning or following skeletal processing, there was an apparent increase in the overall incidence of subcutaneous oedema. When this observation was broken out by severity, the inci-

Table 2. Rat food consumption during gestation

Item	Dose (mg/kg/day)			
	Vehicle control	500	1000	2000
	<b>Food consumption (g/rat/day)</b>			
Days 1–2	23 ± 2	23 ± 2	23 ± 2	23 ± 2
Days 3–5	23 ± 3	23 ± 3	23 ± 3	24 ± 4
Days 6–8	23 ± 2	22 ± 2	22 ± 2	22 ± 3
Days 9–11	25 ± 2	24 ± 2	26 ± 3	26 ± 4
Days 12–15	26 ± 3	26 ± 3	26 ± 2	26 ± 2
Days 16–17	28 ± 3	28 ± 3	28 ± 4	28 ± 3
Days 18–20	28 ± 3	27 ± 3	29 ± 3	28 ± 3

Table 3. Rat water consumption during gestation

Item	Dose (mg/kg/day)			
	Vehicle control	500	1000	2000
	<b>Water consumption (ml/rat/day)</b>			
Days 1–2	34 ± 5	34 ± 4	33 ± 4	34 ± 5
Days 3–5	40 ± 6	40 ± 4	39 ± 5	40 ± 4
Days 6–8	39 ± 7	39 ± 5	40 ± 5	42 ± 4*
Days 9–11	42 ± 8	42 ± 6	42 ± 5	46 ± 5
Days 12–15	45 ± 7	45 ± 5	46 ± 5	49 ± 4
Days 16–17	50 ± 10	51 ± 6	50 ± 6	52 ± 6
Days 18–20	54 ± 9	55 ± 10	52 ± 6	54 ± 6

\*Significantly different from vehicle control group,  $P < 0.05$  (Kruskal–Wallis followed by Dunn's test).

dence of slight subcutaneous oedema in treated groups was somewhat higher than in the concurrent control group, although all values were statistically insignificant ( $P > 0.05$  using chi-square with Yates' correction) and were within the laboratory background control range. Conversely, the incidence of foetuses with moderate subcutaneous oedema attained its highest value in the control group. No foetus was found to have severe subcutaneous oedema. There was clearly no dose relationship to the occurrence of subcutaneous oedema. The slight variations in the incidence of oedema were considered to reflect minimal differences in fixation of individual foetuses, and were therefore unrelated to treatment with sucralose.

One foetus in the highest dose group (2000 mg/kg/day) showed gross abnormalities including absence of intestines and anomalies of the vertebrae and ribs. As this was a single isolated occurrence out of 269 foetuses examined at this dose, and there were no other corroborating observations, no association with treatment was indicated.

#### Rabbit—maternal condition

The preliminary investigations demonstrated that radiolabelled sucralose was absorbed from the gut in a dose-related manner with maximum plasma

concentrations occurring approximately 1 hr after dosing. The ranges of concentrations (as radioactivity equivalent to sucralose) were 5.8–6.8  $\mu\text{g}$  equivalents/ml of plasma in animals dosed at 100 mg/kg and 28.6–31.6  $\mu\text{g}$  equivalents/ml in those dosed at 750 mg/kg, at 1 hr after dosing, declining to 1.0–1.3  $\mu\text{g}$  equivalents/ml and 8.0–9.4  $\mu\text{g}$  equivalents/ml, respectively, after 24 hr. Body weight records during the treatment phase did not show any marked differences between these animals and those dosed at up to 750 mg/kg used for detection of possible foetal abnormalities.

Also preliminary investigations indicated that pregnant females receiving 750 or 1000 mg/kg/day produced normal foetuses (external evaluation) but showed signs of gastrointestinal tract disturbance, such as peri-anal soiling, scouring and caecal enlargement. These effects were more severe at 1000 mg/kg/day than at 750 mg/kg/day, and one female died.

Based on this evidence, doses in the main study were reduced to 125, 350 and 700 mg/kg/day in order to avoid the GI tract distress. However, seven of 15 presumed pregnant females receiving the highest dose (700 mg/kg/day) exhibited gastrointestinal disturbance ranging from mild to severe. In the most extreme cases, this resulted in the deaths of two of the females. Three of the surviving females

Table 4. Rat study—necropsy and litter data

Item	Dose (mg/kg/day)			
	Vehicle control	500	1000	2000
Number examined at necropsy:	20	20	20	20
<b>Litter data:</b>				
Number of litters	20	19	20	20
Corpora lutea	14.9 ± 2.0	14.7 ± 2.0	14.9 ± 1.6	15.4 ± 1.9
Implantations	13.4 ± 3.0	14.2 ± 1.6	14.0 ± 1.6	14.3 ± 1.3
Live young: male	5.5 ± 1.7	6.9 ± 2.0	6.9 ± 1.5	6.3 ± 1.9
female	7.3 ± 2.1	6.4 ± 1.5	6.6 ± 1.8	7.2 ± 2.1
total	12.7 ± 2.6	13.3 ± 1.9	13.4 ± 1.5	13.5 ± 1.6
Resorptions: mean (number of litters affected)				
early: no foetal remnant	0.45 (8)	0.74 (10)	0.45 (8)	0.70 (11)
late: foetal and placental remnant	0.25 (3)	0.11 (2)	0.10 (2)	0.10 (2)
total	0.70 (11)	0.84 (11)	0.55 (9)	0.80 (12)
Pre-implantation loss (%) (calculated from implantations and corpora lutea)	9.8	3.6	6.4	7.5
Post-implantation loss (%) (calculated from live foetuses and implantations)	5.2	5.9	3.9	5.6
Foetal weight (g)	3.31 ± 0.08	3.28 ± 0.08	3.36 ± 0.07	3.36 ± 0.07
Placental weight (g)	0.50 ± 0.02	0.47 ± 0.02	0.48 ± 0.02	0.48 ± 0.02

Table 5. Rat study—incidence of specific foetal variations and anomalies at necropsy

	Dose of sucralose (mg/kg/day)			
	0	500	1000	2000
<b>External examination at necropsy</b>				
Number of foetuses (litters) examined:	254 (20)	253 (19)	268 (20)	269 (20)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
Small foetus (less than 2.70 g)	17 (6)	10 (9)	7 (7)	9 (6)
Large foetus (more than 4.00 g)	4 (2)	3 (1)	-	1 (1)
Foetus with shiny, immature skin	-	1 (1)	1 (1)	2 (2)
Gas-filled vesicles on head	-	1 (1)	-	-
Imperforate anus	2 (2)	-	-	-
Rudimentary tail—no skeletal elements	1 (1)	-	-	-
Agensis of tail	1 (1)	-	-	-
Abnormal foetus: oedematous: digits on fore-paws shortened, syndactyly on left fore-paw, hind-limbs malrotated, kink in tail	-	-	-	1 (1)
Small placenta (less than 0.30 g)	1 (1)	-	-	1 (1)
Large placenta (more than 0.70 g)	1 (1)	-	-	3 (3)
Abnormally thick placenta	-	-	-	1 (1)
<b>Internal examination at necropsy</b>				
Number of foetuses (litters) examined:	164 (20)	167 (19)	180 (20)	179 (20)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
Abnormal foetus: absence of small and large intestines and caecum; stomach joined directly to rectum	-	-	-	1 (1)
Unilateral hydronephrosis	1 (1)	5 (3)	2 (2)	1 (1)
Bilateral hydronephrosis	-	3 (2)	-	1 (1)
Unilateral hydroureter	16 (9)	14 (6)	19 (12)	7 (4)
Bilateral hydroureter	9 (4)	6 (4)	8 (5)	10 (5)
<b>Visceral examination following free-hand serial sectioning</b>				
Number of foetuses (litters) examined:	90 (20)	86 (19)	88 (20)	90 (20)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
<b>Head</b>				
Unilateral slightly folded retina	-	4 (3)	1 (1)	-
Bilateral slightly folded retina	1 (1)	-	2 (2)	-
Bilateral moderate microphthalmia	1 (1)	-	-	-
Perimeningeal cavitation	39 (15)	43 (18)	43 (18)	45 (19)
Subcutaneous cranial space	1 (1)	-	-	1 (1)
Slight dilatation of brain ventricles	2 (2)	3 (3)	4 (4)	1 (1)
Distended cerebral blood vessels	1 (1)	-	1 (1)	5 (4)
<b>Thorax and abdomen</b>				
Absence of convolutions from upper oesophagus	1 (1)	-	-	-
Lungs slightly reduced in size	1 (1)	-	-	-
Subcutaneous dorsal space	-	-	-	1 (1)
Space between body wall and organs	11 (7)	13 (11)	8 (6)	13 (5)
Small diaphragmatic hernia	-	-	-	1 (1)
Distended umbilical artery	3 (3)	2 (2)	3 (3)	2 (2)
Distended umbilical vein	1 (1)	-	1 (1)	-
Unilateral hydronephrosis	5 (5)	4 (3)	4 (3)	7 (5)
Bilateral hydronephrosis	5 (5)	7 (5)	-	2 (2)
Unilateral hydroureter	3 (3)	4 (4)	3 (3)	4 (4)
Bilateral hydroureter	12 (9)	5 (5)	4 (2)	8 (5)
Ectopic right testis	-	1 (1)†	-	-
Inguinal hernia	-	-	1 (1)	-
Slight subcutaneous oedema	2 (2)	10 (6)	11 (6)	15 (7)
Moderate subcutaneous oedema	12 (5)	8 (6)	7 (4)	8 (5)
Severe subcutaneous oedema	0	0	0	0
<b>Specific skeletal variations and anomalies</b>				
Number of foetuses (litters) examined:	164 (20)	167 (19)	180 (20)	179 (20)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
<b>Head</b>				
Supraoccipital bone (I0)	23 (10)	33 (13)	27 (13)	17 (11)
Interparietal bone (I0)	27 (12)	43 (15)	41 (15)	36 (16)
Parietal bones (I0)	2 (1)	3 (2)	5 (2)	3 (1)
Squamosal bones (I0)	-	-	-	2 (1)
Frontal suture enlarged	-	-	1 (1)	-
Frontal/nasal suture enlarged	2 (2)	-	3 (1)	3 (2)
Frontal bone (I0)	-	-	1 (1)	-
Hyoid bone (I0)	7 (6)	12 (8)	15 (7)	9 (7)
Hyoid bone absent	15 (9)	20 (8)	19 (9)	14 (8)

(continued)

[Table 5—*contd*]

Rib cage and vertebral column					
Additional (14th) rib		4 (3)	1 (1)	-	4(2)
Additional pair (14th) ribs		1 (1)	-	-	-
One or more ribs wavy		-	-	2 (2)	1 (1)
13th rib(s) reduced in length or floating		2 (2)	2 (1)	4 (2)	4 (4)
Incomplete ossification of sternbrae:					
	(1)	29 (13)	15 (10)	28 (11)	55 (19)
	(2)	104 (18)	107 (19)	121 (20)	105 (20)
Number of bones affected	(3)	20 (9)	40 (18)	21 (11)	15 (6)
	(4)	4 (4)	3 (3)	6 (4)	1 (1)
	(5)	1 (1)	-	1 (1)	-
	(6)	2 (2)	1 (1)	-	2 (2)
One or more sternbrae offset		4 (3)	2 (2)	2 (2)	3 (3)
Ossification of all cervical vertebral centra		-	3 (2)	-	1 (1)
Cervical vertebral arches (I0)		-	-	1 (1)	-
Thoracic vertebral centra (I0)		43 (16)	36 (16)	45 (16)	57 (18)
Lumbar vertebral centra (I0)		2 (2)	-	1 (1)	1 (1)
Lumbar vertebral arches (I0)		-	-	2 (1)	-
Sacral vertebral arches (I0)		3 (1)	3 (2)	5 (3)	2 (2)
Fewer than 5 caudal vertebrae, ossified		8 (3)	-	1 (1)	2 (2)
All vertebrae caudal to 6th lumbar absent, no tail		2 (2)	-	-	-
Anomalous vertebrae and ribs:		1 (1)	-	-	-
incomplete ossification of thoracic vertebral arches and thoracic and lumbar vertebral centra, slight lumbar scoliosis; head of 10th and 11th ribs closely apposed		-	-	-	1 (1)
Anomalous vertebrae and ribs:		-	-	-	-
incomplete ossification of thoracic vertebral arches and centra; left 1st and 4th rib absent, 2nd reduced		-	-	-	-
Limbs, pectoral and pelvic girdles					
Metacarpals/metatarsals (I0)		5 (4)	3 (3)	4 (1)	3 (2)
Ossification of one or more phalangeal bones		1 (1)	1 (1)	5 (3)	8 (4)
Scapulae (I0)		3 (3)	-	2 (2)	2 (2)
Pubic bones (I0)		12 (5)	14 (7)	7 (3)	7 (6)
Ischial bones (I0)		3 (1)	1 (1)	3 (1)	2 (2)

I0 = Incomplete ossification. <sup>a</sup>One foetus may have more than one observation. †38 male foetuses examined from 18 litters.

that showed evidence of gastrointestinal disturbance, and one other at this dose level which showed no obvious signs of gastrointestinal distress, aborted. Some degree of weight loss and reduced intake of food and water were recorded in all four of these females during the late treatment period. A few other deaths occurred across groups, but these either showed no association with treatment (one to respiratory infection, 700 mg/kg/day group) or were attributable to accidental tracheal intubation (Table 6).

In both the preliminary and main studies, rabbits which survived to term with live young showed

some inter-group variation in body weight (Table 6) and intake of food and water (Tables 7 and 8), but there was no association with treatment. Terminal necropsy of does on day 29 of gestation revealed no treatment-related macroscopic changes.

#### Rabbit—litters and foetal evaluation

The numbers of implantations and live foetuses, the extent of pre-implantation loss, and foetal and placental weights (Table 9) showed some inter- and intra-group variation in both the preliminary and main studies, but there was no indication of any adverse response to treatment. The mean number of

Table 6. Rabbit study—survival and body weights during gestation

Item	Dose (mg/kg/day)			
	Vehicle control	175	350	700
Number inseminated	16	18	16	18
Non-accidental deaths	1	2	1	3 <sup>b</sup>
Deaths from dosing intubation trauma <sup>a</sup>	0	2	1	3
Not pregnant	2	1	2	3
Abortion	0	0	0	4 <sup>c</sup>
Pregnant at term with live young	13	13	12	5
Body weight (kg) day 0	3.96 ± 0.29	4.12 ± 0.33	4.01 ± 0.29	3.97 ± 0.24
Body weight (kg) day 6	4.07 ± 0.37	4.24 ± 0.38	4.14 ± 0.35	4.07 ± 0.23
Body weight (kg) day 20	4.15 ± 0.38	4.39 ± 0.35	4.25 ± 0.31	4.17 ± 0.23
Body weight (kg) day 28	4.19 ± 0.36	4.42 ± 0.38	4.34 ± 0.36	4.21 ± 0.34
Body weight change (kg) day 6 to 20 <sup>d</sup>	0.08 ± 0.15	0.15 ± 0.14	0.12 ± 0.17	0.09 ± 0.15

<sup>a</sup>Deaths not attributed to treatment. <sup>b</sup>Two deaths following gastrointestinal distress, one due to respiratory infection. <sup>c</sup>Three with obvious signs of gastrointestinal distress. <sup>d</sup>Dosing period.

Table 7. Rabbit food consumption during gestation

	Dose (mg/kg/day)			
	Vehicle control	175	350	700
	<b>Food consumption (g/rabbit/day)</b>			
Days 1-5	167 ± 31	175 ± 23	188 ± 26	158 ± 63
Days 6-12	145 ± 37	141 ± 26	158 ± 22	145 ± 28
Days 13-19	151 ± 45	152 ± 27	143 ± 30	158 ± 22
Days 20-23	139 ± 40	155 ± 40	154 ± 34	129 ± 83
Days 24-28	109 ± 44	102 ± 47	138 ± 33	122 ± 79

resorptions and post-implantation loss were slightly higher than in the concurrent controls at 700 mg/kg/day in the main study, but the values fell within the laboratory historical control range and the differences were not statistically significant.

Necropsy of foetuses in the preliminary and main studies, examination of main study foetuses after skeletal processing, and evaluation of foetal heads after freehand serial sectioning revealed no remarkable findings (Table 10). Observed anomalies were of types, and occurred at incidences, seen historically in control rabbits of this strain in the laboratories of Pharmaco LSR. There were no terata, and no other evidence of adverse developmental response associated with exposure to sucralose.

**DISCUSSION**

The progress of pregnancy and foetal development in the rat were unaffected by sucralose. The only response to treatment was a marginal increase in water intake. Adult rats in this study showed no obvious untoward effects, raising the question of whether the potential for adverse developmental effects was tested at sufficiently high doses. In other sucralose toxicology studies, however, with more detailed examination of the adults, it has been shown that rats given sucralose at the levels in the present study exhibit the exaggerated physiologic responses that are typical in this species following exposure to poorly absorbed compounds (Lord and Newberne, 1990; Newberne *et al.*, 1988). In these separate studies, sucralose administered to rats by gavage at 2000 mg/kg/day (Goldsmith, 2000), or in the diet at a level of 3%, resulted in caecal enlargement and the concomitant renal sequelae relating to altered mineral absorption (Mann *et al.*, 2000).

Further elevations in dose, therefore, were not warranted as they would be expected to exacerbate these conditions and confound the interpretation of potential foetal effects.

In a preliminary dose-ranging study in pregnant rabbits none of the 51 foetuses (eight litters) receiving sucralose up to 1000 mg/kg/day showed any external evidence of teratogenic effect. However, scouring and other signs of maternal gastrointestinal distress, sometimes extensive, were observed at 750 and 1000 mg/kg/day. These extreme enteric responses to sucralose appear to be unique to the rabbit since they were not observed in mice, rats, dogs, marmosets or humans after consuming large doses of sucralose (McNeil, 1987). Such laxative effects are seen commonly in rabbits as a non-specific response following the ingestion of large amounts of osmotically active, poorly absorbed substances and are not to be confused with toxicity (WHO, 1987).

The radiolabel experiments in pregnant rabbits demonstrated that sucralose was absorbed into the systemic circulation following gavage administration, and that circulating levels increased with increasing dose. These results indicated that rabbit foetuses should be exposed to circulating levels of sucralose.

Although doses were lowered in the main rabbit teratology study in order to reduce confounding effects on foetal development due to maternal gastrointestinal distress, the maternal effects persisted at the 700 mg/kg/day level. These effects, and a number of gavage difficulties, reduced the total number of litters undergoing full developmental evaluation at this level to five. Following a thorough external, internal soft tissue, and skeletal evaluation, no indication of teratogenicity was detected in any of the foetuses in these five litters.

Table 8. Rabbit water consumption during gestation

	Dose (mg/kg/day)			
	Vehicle control	175	350	700
	<b>Water consumption (ml/rabbit/day)</b>			
Days 1-5	401 ± 212	423 ± 191	508 ± 219	394 ± 196
Days 6-12	341 ± 167	321 ± 159	334 ± 133	502 ± 313
Days 13-19	475 ± 261	378 ± 194	435 ± 227	352 ± 85
Days 20-23	581 ± 388	461 ± 262	494 ± 243	323 ± 194
Days 24-28	483 ± 284	373 ± 192	459 ± 218	350 ± 167

Table 9. Rabbit study—necropsy and litter data

Item	Dose (mg/kg/day)			
	Vehicle control	175	350	700
<b>Litter data:</b>				
Number of litters	13	13	12	5
Corpora lutea	10.5 ± 2.9	11.2 ± 2.0	10.3 ± 2.2	10.0 ± 2.4
Implantations	8.1 ± 4.0	10.2 ± 1.6	7.3 ± 2.1	7.4 ± 2.7
Live young: male	4.0 ± 2.2	4.9 ± 1.9	3.6 ± 1.6	2.8 ± 0.8
female	3.4 ± 2.5	4.5 ± 1.1	2.8 ± 1.7	3.2 ± 1.5
total	7.4 ± 3.6	9.5 ± 1.5	6.4 ± 2.0	6.0 ± 1.9
Resorptions: mean (number of litters affected)				
Early: no foetal remnant	0.2 (3)	0.3 (3)	0.7 (5)	0.8 (2)
Late: foetal and placental remnant	0.5 (5)	0.3 (3)	0.2 (1)	0.6 (2)
Total	0.7 (6)	0.6 (6)	0.8 (5)	1.4 (4)
Pre-implantation loss (%) (calculated from implantations and corpora lutea)	23.4	10.2	29.3	26.0
Post-implantation loss (%) <sup>a</sup> (calculated from live foetuses and implantations)	8.6	6.1	11.5	18.9
Foetal weight (g)	40.0 ± 1.5	39.6 ± 1.9	42.1 ± 1.5	39.9 ± 2.6
Placental weight (g)	5.7 ± 0.4	5.4 ± 0.2	5.6 ± 0.2	6.0 ± 0.4

<sup>a</sup>Background control study range, based on 108 studies/1380 litters: 1.0 to 20.5%; overall mean 10.3%.

Table 10. Rabbit study—incidence of specific foetal variations and anomalies

	Dose (mg/kg/day)			
	Vehicle control	175	350	700
<b>Examination at necropsy</b>				
Number of foetuses (litters) examined:	96 (13)	124 (13)	77 (12)	30 (5)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
Bilateral hind-limb flexure	-	-	-	1 (1)
Tail kinked/misshapen	1 (1)	-	1 (1)	-
Gall bladder variants	10 (9)	8 (6)	6 (5)	6 (3)
Agenesis of median lung lobe	-	-	-	1 (1)
Pale areas on liver	3 (3)	1 (1)	1 (1)	-
Small foetus (less than 32.0 g)	27 (4)	26 (10)	3 (3)	13 (3)
<b>Examination of heads following freehand serial sectioning</b>				
Number of foetuses (litters) examined:	29 (13)	37 (13)	23 (12)	10 (5)
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
Upper and lower incisors erupted	12 (9)	22 (10)	11 (7)	6 (4)
Lower incisors only erupted	8 (5)	7 (4)	6 (4)	-
Incisors not erupted	9 (6)	8 (5)	6 (5)	4 (2)
Unilateral slightly folded retina	2 (2)	3 (2)	2 (2)	-
Bilateral folded retina	-	-	1 (1)	-
Bilateral slightly dilated orbital sinus	-	1 (1)	-	-
<b>Specific skeletal variations and anomalies</b>				
Observations:	Foetal incidence <sup>a</sup> (number of litters)			
<b>Head</b>				
Number of foetuses (litters) examined:	67 (13)	87 (13)	54 (12)	20 (5)
Supra-occipital bone (I0)	-	2 (2)	1 (1)	3 (1)
Interparietal bone reduced, fissured or absent	3 (2)	-	-	1 (1)
Discrete unossified areas in parietal bones	-	-	-	1 (1)
Additional sutures in parietal bones	2 (2)	3 (2)	3 (3)	2 (2)
Additional sutures in nasal bones	-	-	1 (1)	-
Frontal suture enlarged at fronto-nasal junction	-	-	-	1 (1)
Lacrymal fossa enlarged	-	-	-	3 (1)
Minor cranial anomalies	-	1 (1)	-	-
Cervical vertebral centra (I0)	3 (3)	4 (3)	2 (2)	3 (1)
<b>Rib cage and vertebral column</b>				
Number of foetuses (litters) examined:	96(13)	124(13)	77(12)	30(5)
Incomplete ossification of (1) sternebra	20 (10)	57 (13)	22 (10)	12 (4)
Incomplete ossification of (2) sternebra	9 (7)	13 (9)	16 (6)	-
Incomplete ossification of (3) sternebra	-	1 (1)	2 (1)	1 (1)
Incomplete ossification of (4) sternebra	-	-	-	1 (1)
One or more sternebrae offset	2 (2)	2 (2)	-	-
Two or more sternebrae fused	-	-	2 (2)	-
Small additional 6th sternebra between 5th and xiphisternum	2 (1)	3 (3)	1 (1)	-
Ribs 12/12	21 (9)	47 (9)	25 (11)	16 (3)
Ribs 12/13	20 (8)	17 (10)	10 (6)	4 (3)

Ribs 13/13	55 (11)	60 (13)	42 (11)	10 (4)
Short 13th rib or ribs	26 (10)	27 (13)	23 (12)	8 (4)
Floating 13th rib or ribs	4 (3)	2 (2)	4 (4)	2 (2)
Rudimentary 13th rib or ribs	5 (3)	2 (2)	2 (2)	1 (1)
Rudimentary floating 13th rib or ribs	3 (3)	3 (3)	-	1 (1)
One or more ribs thickened at junction with costal cartilage	7 (5)	5 (2)	10 (6)	3 (3)
One or more ribs thickened medially	-	-	-	1 (1)
Anomalous rib cage and vertebral column	1 (1)	-	-	-
Thoracic vertebral centra (I0)	-	-	1 (1)	-
Incomplete or asymmetrical costal elements of sacral vertebrae	3 (3)	9 (7)	7 (6)	4 (3)
One or more caudal vertebrae offset, tail tip kinked	-	1 (1)	-	1 (1)
Caudal vertebrae, less than 16 ossified	1 (1)	-	-	5 (1)
Two or more caudal vertebrae fused, kinky tail	1 (1)	-	-	-
26 pre-sacral vertebrae	61 (12)	74 (13)	54 (12)	25 (5)
27 pre-sacral vertebrae	35 (11)	50 (12)	23 (9)	5 (3)
Limbs, pectoral and pelvic girdles				
Heads of limb long-bones (I0)	55 (11)	61 (13)	32 (9)	12 (3)
Centrales (I0)	7 (2)	-	-	5 (1)
Metacarpals and/or phalanges (I0)	30 (8)	13 (6)	15 (6)	11 (3)
Anomalous hind-limb flexure	-	-	-	1 (1)
Pubic bones (I0)	5 (2)	4 (2)	-	5 (1)
Asymmetric pelvis	2 (2)	5 (5)	6 (5)	2 (2)
Double association pelvis	7 (5)	5 (4)	1 (1)	2 (2)

In the low and mid-dose groups, however, there was little evidence of maternal gastrointestinal distress and a full complement of litters was produced by does receiving up to 350 mg sucralose/kg/day. These foetuses were also subjected to a thorough foetal evaluation which produced no evidence of teratogenicity or other indication of developmental toxicity. All results were comparable to control data.

As in other species, rabbits absorb sucralose poorly. Nevertheless, they absorb sucralose to a greater degree (30%) than either rats (10%) or humans (15%) (McNeil, 1987). Rabbits differ, however, in that their elimination of sucralose is prolonged relative to other species (John *et al.*, 2000). In the rabbit one would anticipate that, due to the greater absorption and protracted elimination, foetal exposure to systemic sucralose should be greater than in the rat at comparable doses. By the same token, moderate doses in the rabbit would be expected to produce circulating levels of sucralose comparable to higher administered doses in the pregnant rat. Therefore, although maximum tolerated administered doses of sucralose were quite different in the maternal rabbit (350 mg/kg/day) and rat (2000 mg/kg/day), foetal exposure at the same doses in these two species would be expected to be more comparable. Further studies undertaken to examine this specific hypothesis have shown it to be correct, that is, foetal exposure in the rabbit following administration of 350 mg/kg/day is comparable to foetal exposure in the rat following maternal administration of 2000 mg/kg/day. The results of these studies will be published separately.

In these and other studies on sucralose the responses of both the adult rat and rabbit have indicated that the limits of tolerability were achieved at the highest doses administered; the foetuses, however, were not affected. In the rat, no terata or other indications of foetal toxicity were seen at

doses producing distinct adult effects, (approx. 1250 times greater than the estimated daily human intake of 1.6 mg/kg/day; *Federal Register*, 1998). In the present rabbit study, full foetal evaluations likewise demonstrated no evidence of terata or developmental toxicity at dose levels up to 350 mg/kg/day or approximately 215 times estimated human exposure, and no indications of foetal effects at a 435-fold margin (based on a 700 mg/kg administered dose) where maternal sensitivity precluded a more quantitatively robust foetal accountability. Given the differences in absorption and elimination between rat and rabbit and the resultant comparability of foetal exposure when rats and rabbits are given 2000 or 350 mg/kg/day, respectively, foetal safety margins in the rabbit at 350 mg/kg/day for this study are considered likely to be as high as those in the rat at 2000 mg/kg/day.

These studies in both pregnant rodent (rat) and non-rodent (rabbit) animal models demonstrate that, at the high limit of maternally tolerated levels of sucralose (2000 mg/kg/day in the rat, in excess of 350 mg/kg/day in the rabbit), there are no adverse effects on foetal morphogenesis nor on any other parameters indicative of normal development.

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