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90-Day oral (gavage) study in rats with galactooligosaccharides syrup

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Abstract

A 90-day oral (gavage) study was conducted in male and female Sprague Dawley rats to investigate the safety of Vivinal® galactooligosaccharides (GOS) syrup at 2500 or 5000 mg/kg bw/day. A reference control containing fructooligosaccharides (FOS) was used to match the oligosaccharide and digestible sugars in the test material (approximately 45% and 30%, respectively) and to assess if these had an impact on food consumption. Measurements included clinical observations, body weights, food consumption, hematology, clotting parameters, blood chemistries, urinalysis, ophthalmologic examinations, gross necropsies, organ weights, and histological examinations. There were no effects of feeding GOS syrup at either concentration on any parameter except food consumption. Statistically significant decreases (7–13%) in food consumption were seen in both sexes in the GOS syrup-treated animals at 5000 mg/kg bw/day and animals treated with the FOS control when compared to the reverse osmosis deionized (RODI) water controls. Based on the lack of toxicological effects in the study, the NOAEL for Vivinal® GOS syrup is 5000 mg/kg bw/day when administered by gavage for 90 consecutive days.

Keywords: Prebiotics; Oligosaccharides; Galactooligosaccharides; GOS; Toxicity; Subchronic

1. Introduction

Breastfeeding is regarded as the gold standard for infant feeding (Vandenplas, 2002). In recognition of this fact, infant formula manufacturers have made changes in formulas to more closely match either human milk composition or breastfeeding performance (IOM, 2003). Human milk contains a complex mixture of more than 130 different oligosaccharides comprising a total concentration of 15–23 g/l in colostrum and 8–12 g/l in transitional and mature milk (Kunz et al., 2000). These oligosaccharides are resistant to enzymatic digestion in the upper gastrointestinal tract (Engfer, 2000) and thus, reaching the colon intact, they may serve as substrates for colonic microflora fermentation. It has been shown that human milk oligosaccharides induce an increase in the number of bifidobacteria in the colonic flora, along with a reduction in the number of potentially pathogenic bacteria (Kunz et al., 2000; Vandenplas, 2002; Chierici et al., 2003; Newburg, 1997).

Because of the variety, variability in levels, and complexity of the structure of human milk oligosaccharides, it is not feasible to add a similar oligosaccharide composition to infant formula (SCF, 2003). An alternative is to add other oligosaccharides that, while not duplicating the structure of those found in human milk, may mimic at least some of their functions. One such oligosaccharide is galactooligosaccharide (GOS), a chain of 3–8 galactose units with a glucose end-cap produced from lactose by the action of β-galactosidase. One commercially produced GOS product is Vivinal® GOS syrup, prepared by Bolculo Domo using β-galactosidase derived from *Bacillus circulans* and partially dried by evaporation to form a syrup containing approximately 45% GOS and 30% digestible sugars—lactose (15%), glucose (14%), and galactose (1%). This product is a candidate for

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addition to infant formula in the United States to mimic the functionality of the oligosaccharides that occur in human milk; a blend of FOS and GOS has been added to infant formulas in Europe for a number of years (SCF, 2001a,b, 2003).

A 90-day gavage study in rats was conducted according to Good Laboratory Practices to investigate the safety of Vivinal® GOS syrup. The details of this study are summarized in this paper. Oral administration was selected as the route of administration as this is the potential route of exposure to humans; gavage was chosen due to potential palatability issues.

2. Materials and methods

2.1. Test substance

Vivinal® GOS syrup was provided to Springborn Laboratories (now the Ohio Division of Charles River Laboratories) as a pale yellow viscous liquid and stored refrigerated at 2–8 °C.[1] As noted, GOS syrup contains approximately 45% GOS, 15% lactose, and 14% glucose. Reference controls, including fructooligosaccharides (FOS), lactose, and glucose, were also provided as white powders. The FOS served as a control to match the oligosaccharide in the test material; the lactose and glucose were included to match the digestible sugars in the test material. They were stored at room temperature.[1] The vehicle reference control was prepared weekly as a mixture comprising 70% w/v[2] FOS and 30% w/v suspension of equal amounts of lactose and glucose mixed with reverse osmosis deionized (RODI) tap water. This mixture was stored refrigerated and a sufficient amount was removed each day to prepare the solutions for dosing the vehicle reference control animals. The homogeneity and stability (over 10 days) of the reference controls were confirmed analytically by KAR Laboratories (Kalamazoo, MI). The concentration of each reference and vehicle control was verified analytically after dose preparation at weeks 0, 7, and 11.

2.2. Test animals and treatment

Sprague Dawley Crl:CD®(SD)IGS BR rats, male and female, were housed (2–3/sex/cage) for 6 days after receipt. During the remainder of the acclimation period, and while on study, the rats were housed individually in suspended stainless steel cages under a 12-h cycle of light and darkness. Over the course of the study, the room temperature and relative humidity were 19–23 °C and 38–64%, respectively. After acclimation for 13 days, on day 0 animals were weighed, examined for clinical observations, and randomized to 1 of 4 groups as noted below:

Study design

Dose group	Males	Females	Dose material	Dose level[a] (mg/kg/day)	Dose volume[b] (ml/kg bw)
1	15	15	RODI Water	0	14.7[c]
2	15	15	FOS Reference control	0	7.8
3	15	15	Vivinal® GOS syrup	2500	3.9
4	15	15	Vivinal® GOS syrup	5000	7.8

[a] Dosage levels were based on a prior range-finding/tolerability study (data not shown). The high-dose level was selected as it is an acceptable upper limit dosage level for longer term oral studies by regulatory agencies.

[b] Dosage volumes were calculated based on a density determination performed at Springborn Laboratories: GOS = 1400 mg/ml.

[c] The dose volume of RODI water was chosen to match the volume of the high dose of another oligosaccharide that was also tested, but later determined not to be a candidate for addition to infant formula at this time; results of this portion of the study are not reported.

Animals were approximately 6 weeks of age with mean body weights of 229.3 g (sd = 11.15 g) for males and 160.7 g (sd = 10.44 g) for females. The test article, reference control, and vehicle were administered each morning by oral gavage on study days 0–89 (first 5 animals/sex/group), days 0–90 (second 5 animals/sex/group) or days 0–91 (last 5 animals/sex/group). Individual doses were based on the most recent body weights. Reference controls were utilized at the same dose volume as the designated test article high-dose level to mimic caloric intake and oligosaccharide content.

2.3. Parameters evaluated

2.3.1. Clinical observations

Animals were checked in the morning and afternoon for general health/mortality and moribundity. Detailed clinical observations were performed once weekly prior to dosing and prior to scheduled euthanasia. Signs for overt toxicity were monitored approximately 2 h following dosing for each group.

2.3.2. Body weights and food consumption

Individual body weights were recorded on day 1 and weekly thereafter. A final body weight was taken prior to sacrifice on day 90, 91 or 92. Food consumption also was determined weekly (as g/animal/day) on the same days as body weight. Feed efficiency values were calculated.

2.3.3. Clinical pathology

Blood was collected on study day 90 (first 5 rats/sex/group), day 91 (second 5 rats/sex/group) or day 92 (last 5 rats/sex/group) from the orbital plexus of slightly anesthetized rats that were fasted overnight. Evaluations were conducted of hematological parameters (erythrocyte count [RBC], hematocrit [Hct], hemoglobin concentration [Hgb], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], mean corpuscular volume [MCV], platelet count, reticulocyte count and total differential leukocyte counts [including RBC morphology]), coagulation (activated partial thromboplastin time [APTT], prothrombin time [PT], and fibrinogen), and clinical chemistry parameters (alanine aminotransferase [ALT], albumin, albumin/globulin [calculated], alkaline phosphatase, aspartate aminotransferase [AST], blood creatinine, blood urea nitrogen [BUN], calcium, cholesterol, electrolytes [sodium, potassium and chloride], gamma glutamyl transferase [GGT], globulin [calculated], glucose, phosphorus, total bilirubin and total serum protein).

2.3.4. Urinalysis

Urine samples were collected overnight using urine collection cages prior to initiation of blood collection and qualitative analyses were

[1] Documentation concerning chemical identification, purity, strength, stability and other required data are maintained by Mead Johnson Nutritionals.

[2] w/v = weight to volume.

conducted for bilirubin, blood, glucose, ketones, leucocytes, nitrites, protein, specific gravity, and urobilinogen. Gross appearance, pH, and total volume were also recorded. Microscopy was done on the spun deposit. Feed was withheld during urine collection but water was available.

2.3.5. Ophthalmologic examinations

Examinations were performed on all rats prior to in-life initiation (day 2) and prior to the end of the study (day 83) by a board-certified veterinary ophthalmologist using a handheld slit lamp and ophthalmoscope.

2.3.6. Gross necropsy

After an overnight fast, surviving rats were euthanized by CO_2 inhalation followed by exsanguination. All animals were subjected to a complete gross necropsy examination upon death or scheduled sacrifice on day 90 (first 5 rats/sex/group), 91 (second 5 rats/sex/group) or 92 (last 5 rats/sex/group) by a board-certified veterinary pathologist. Gross necropsy examinations included evaluation of external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents.

Fresh organ weights were obtained at scheduled euthanasia for the adrenals, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes, and uterus. Paired organs were weighed together. Organs and tissues obtained at scheduled sacrifice were preserved in 10% neutral buffer for possible histological evaluation; these included accessory genital organs, adrenals, gross lesions, aorta, bone marrow smear (femur), brain, cecum, colon, duodenum, esophagus, exorbital lachrymal glands, eyes, femur and bone marrow, ileum, jejunum, kidneys, liver, lungs with bronchi, mammary glands, lymph nodes (mediastinal, mesenteric and submandibular), nasal cavity, pancreas, peripheral sciatic nerve, pituitary, prostate, rectum, skeletal muscle (thigh), skin, spinal cord (cervical, mid-thoracic and lumbar), spleen, stomach, submaxillary salivary gland, testes/ovaries, tongue, trachea and bladder. All tissues, excluding the bone marrow smear, collected at necropsy from Groups 1 and 4 and the lungs, liver, kidneys and gross lesions from Groups 2 and 3 were processed for histological examination. The tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The tissues were examined microscopically by a board-certified veterinary pathologist.

2.4. Statistical analyses

Body weights, body weight gain, and food consumption were analyzed by one-way analysis of variance (ANOVA; Gad and Weil, 1994). If significance was detected with ANOVA ($p < 0.05$), pair-wise group comparisons were conducted using the Tukey–Kramer test (Zar, 1999). Clinical pathology data and absolute and relative organ weights were analyzed for homogeneity of variance using Levene's test (Levene, 1960). If $p < 0.01$, multiple group comparisons proceeded using the Kruskal–Wallis non-parametric ANOVA (Siegel, 1956), followed by Dunn's test (Glantz, 1997) when $p < 0.05$. The following comparisons were made between the groups:

Group 1 vs. Groups 2, 3, and 4
Group 2 vs. Groups 3 and 4

Data for males and females were analyzed and reported separately.

3. Results

3.1. Analytical chemistry

Homogeneity analyses demonstrated that the lactose, sucrose, and FOS concentrations in the reference control mixtures were within 4.5% of the nominal concentrations for each component. Stability analyses of the individual sugars in the GOS syrup ranged from 2 to 12% of the homogeneity results, indicating that the syrup was stable through 10 days of refrigeration at 2–8 °C.

3.2. Survival and clinical observations

All animals survived until scheduled euthanasia with the exception of 2 deaths as a result of gavage error. Both deaths were females in Group 4 (days 6 and 11). Gavage error was confirmed at necropsy by findings of perforation of the esophagus, fluid contents in the thoracic cavity, and dark red lobes of the lung. Microscopic examination of these animals also revealed minimal to moderate chronic active esophageal inflammation. A low incidence of clinical signs including hair loss, ocular discharge, dark material around the eyes, dark material around the nose, malaligmment, trimmed incisor(s) and broken incisor(s) was observed sporadically throughout the RODI water control group, the reference control group, and the test article groups. These findings were not considered toxicologically meaningful since they occurred sporadically and did not follow a dose-related pattern.

3.3. Body weights (Figs. 1 and 2)

No statistically significant or toxicologically relevant differences were noted in mean body weights for either sex in the 2500 or 5000 mg GOS/kg bw/day groups when compared to the RODI or FOS control groups.

3.4. Food consumption

3.4.1. Group 1 (RODI water control) vs. Groups 2, 3, and 4

Mean food consumption of the FOS control group was significantly ($p < 0.05$) lower by approximately 7–14% than the RODI water control group during days 0–7, 14–21, 42–70 and 77–89 in males and lower than the RODI water control group by approximately 10–19% during days 0–7 and 35–89 in females. When compared to the RODI water control group, mean food consumption of the 5000 mg

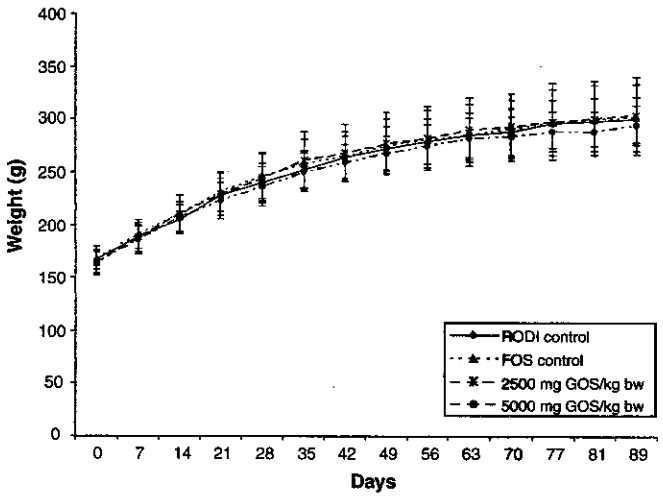

Fig. 1. Mean female body weight (±standard deviation).

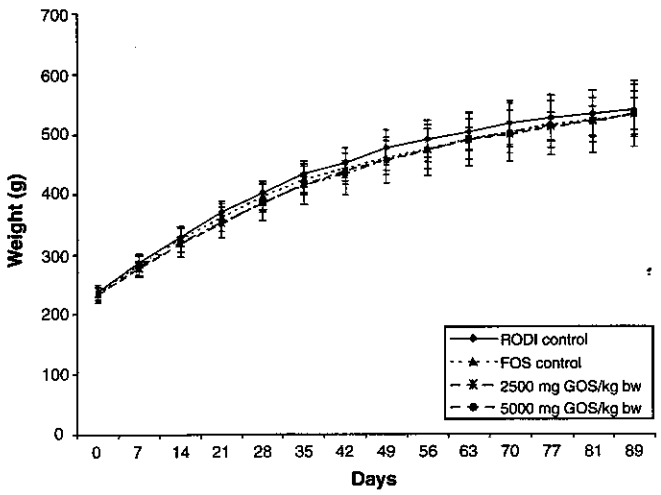

Fig. 2. Mean male body weight (±standard deviation).

GOS/kg bw/day males also was significantly lower by approximately 7–11% during days 14–21 and 28–89. No statistically significant or toxicologically meaningful differences in mean food consumption were observed for the 2500 mg GOS/kg bw/day males when compared to the RODI water control group. When compared to the RODI water control group, mean food consumption of the 2500 and 5000 mg GOS/kg bw/day females was significantly lower by approximately 10–14% during days 49–70 and 14–19% during days 0–7, 14–21 and 35–89, respectively.

3.4.2. Group 2 (FOS reference control) vs. Groups 3 and 4

No statistically significant or toxicologically meaningful differences in food consumption were observed in either sex in the 2500 and 5000 mg GOS/kg bw/day groups when compared to the FOS controls.

3.5. Feed efficiency

As shown in Table 1, feed efficiency was significantly increased for the 5000 mg GOS/kg bw/day males on days 56–63 ($p < 0.05$) and for both the 2500 ($p < 0.05$) and 5000 mg GOS/kg bw/day ($p < 0.01$) males on days 84–89 when compared to the RODI water control group. No other statistically significant changes were noted in the FOS control or test article groups when compared to the RODI water control group. No statistically significant or toxicologically meaningful differences were noted for males or females in the 2500 and 5000 mg GOS/kg bw/day groups when compared to the FOS controls.

3.6. Clinical pathology

3.6.1. Hematology and coagulation

There were occasional statistically significant observations (i.e., mean eosinophil value for the FOS control was higher than the RODI water control; mean hematocrit and hemoglobin values for 5000 mg GOS/kg bw/day females were higher that the FOS controls) as shown in Table 2, but these were minor, inconsistent, not related to dose, and within the intralaboratory historical control data. Therefore, none of these changes was judged to be of toxicological significance.

3.6.2. Clinical chemistry

There were occasional statistically significant observations noted (e.g., mean glucose value was statistically lower for the FOS control males compared to the RODI water control group; mean ALT value was statistically lower for the 5000 mg GOS/kg bw/day males compared to the FOS controls) as shown in Table 3, but these were minor, inconsistent, not related to dose, and within the intralabo-

Table 1
Mean feed efficiency (weight gain/food consumed)[a]

Days	RODI Control		FOS Control		2500 mg GOS/kg bw		5000 mg GOS/kg bw	
	Males $n = 15$	Females $n = 15$	Males $n = 15$	Females $n = 15$	Males $n = 15$	Females $n = 15$	Males $n = 15$	Females $n = 13$
0–7	0.3 ± 0.032	0.17 ± 0.038	0.31 ± 0.041	0.22 ± 0.03	0.28 ± 0.034	0.20 ± 0.045	0.31 ± 0.051	0.22 ± 0.050[b]
7–14	0.25 ± 0.022	0.14 ± 0.042	0.27 ± 0.031	0.17 ± 0.053	0.26 ± 0.037	0.19 ± 0.047	0.25 ± 0.034	0.18 ± 0.042
14–21	0.24 ± 0.024	0.18 ± 0.045	0.22 ± 0.030	0.17 ± 0.051	0.21 ± 0.030	0.15 ± 0.044	0.21 ± 0.026	0.14 ± 0.048
21–28	0.17 ± 0.031	0.09 ± 0.044	0.20 ± 0.024	0.12 ± 0.035	0.19 ± 0.031	0.12 ± 0.033	0.19 ± 0.039	0.11 ± 0.052
28–35	0.16 ± 0.030	0.09 ± 0.056	0.16 ± 0.028	0.09 ± 0.031	0.17 ± 0.042	0.12 ± 0.042	0.16 ± 0.043	0.11 ± 0.05
35–42	0.10 ± 0.029	0.08 ± 0.053	0.10 ± 0.023	0.06 ± 0.035	0.12 ± 0.033	0.05 ± 0.037	0.10 ± 0.066	0.07 ± 0.041
42–49	0.13 ± 0.040	0.05 ± 0.034	0.10 ± 0.031	0.08 ± 0.043	0.10 ± 0.048	0.07 ± 0.047	0.13 ± 0.039	0.07 ± 0.052
49–56	0.07 ± 0.030	0.05 ± 0.046	0.08 ± 0.063	0.05 ± 0.037	0.09 ± 0.021	0.04 ± 0.031	0.10 ± 0.040	0.05 ± 0.046
56–63	0.06 ± 0.033	0.04 ± 0.031	0.10 ± 0.045	0.03 ± 0.058	0.10 ± 0.021	0.07 ± 0.054	0.10 ± 0.032*	0.06 ± 0.043
63–70	0.07 ± 0.041	0.02 ± 0.056	0.07 ± 0.025	0.04 ± 0.063	0.05 ± 0.025	0.02 ± 0.055	0.07 ± 0.034	0.02 ± 0.055
70–77	0.05 ± 0.028	0.06 ± 0.048	0.08 ± 0.026	0.05 ± 0.053	0.07 ± 0.032	0.04 ± 0.044	0.07 ± 0.02	0.04 ± 0.041
77–84	0.04 ± 0.024	0.01 ± 0.035	0.04 ± 0.036	0.02 ± 0.047	0.05 ± 0.027	0.02 ± 0.025	0.03 ± 0.036	0.00 ± 0.044
84–89	0.04 ± 0.029	0.02 ± 0.061	0.07 ± 0.042	0.03 ± 0.051	0.09 ± 0.03*	0.03 ± 0.053	0.10 ± 0.029**	0.06 ± 0.037

* $p < 0.05$ when compared to RODI control.
** $p < 0.01$ when compared to RODI control.
[a] ±Standard deviation.
[b] $n = 13$ after day 7.

Table 2
Mean hematology and coagulation values (days 90–92)[a]

Parameter	RODI Control		FOS Control		2500 mg GOS/kg bw		5000 mg GOS/kg bw	
	Males $n = 15$	Females $n = 14$[b]	Males $n = 15$	Females $n = 15$	Males $n = 15$	Females $n = 15$	Males $n = 15$	Females $n = 13$
Erythrocytes (10^6/ml)	8.59 ± 0.373	7.92 ± 0.261	8.65 ± 0.390	7.96 ± 0.538	8.75 ± 0.489	7.83 ± 0.478	8.68 ± 0.748	8.21 ± 0.574
Hemoglobin (g/dl)	16.5 ± 0.78	16.2 ± 0.63	16.6 ± 0.71	15.9 ± 0.91	16.8 ± 0.71	15.8 ± 0.78	17.0 ± 1.78	16.8 ± 1.02*
Hematocrit (%)	39.8 ± 2.95	39.5 ± 3.05	39.7 ± 1.90	39.6 ± 1.77	40.4 ± 2.01	39.8 ± 2.49	40.5 ± 4.27	42.3 ± 3.17**
Mean corpuscular volume (fl)	46.4 ± 3.45	49.9 ± 3.33	45.8 ± 1.63	50.0 ± 1.53	46.3 ± 2.08	50.9 ± 2.50	46.7 ± 3.22	51.5 ± 1.10
MCH (pg)	19.3 ± 0.95	20.4 ± 0.71	19.2 ± 0.56	20.1 ± 0.65	19.2 ± 0.87	20.2 ± 0.90	19.6 ± 1.09	20.5 ± 0.74
MCHC (g/dl)	41.6 ± 2.08	41.0 ± 1.88	42.0 ± 1.41	40.1 ± 0.93	41.5 ± 1.36	39.7 ± 1.13	42.0 ± 1.87	39.8 ± 1.48
Platelets (10^3/cmm)	984 ± 204.0	1072 ± 135.3	1063 ± 230.9	1033 ± 172.7	1082 ± 126.3	998 ± 135.5	1044 ± 292.2	1095 ± 102.5
Nucleated RBCs (no./100 WBC)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
Reticulocytes (% RBC)	1.9 ± 0.38	1.9 ± 0.32	1.9 ± 0.50	2.1 ± 0.39	2.2 ± 0.32	2.0 ± 0.23	1.9 ± 0.47	1.9 ± 0.42
Prothrombin time (s)	12.1 ± 0.43	11.7 ± 0.43	12.0 ± 0.57	11.5 ± 0.33	12.1 ± 0.67	11.4 ± 0.38	11.9 ± 0.27	11.6 ± 0.41
Activated prothrombin time (s)	14.8 ± 1.03	15.7 ± 3.79	14.8 ± 1.2	15.2 ± 5.10	15.8 ± 1.63	15.2 ± 3.40	15.8 ± 3.37	14.1 ± 2.63
Fibrinogen (mg/dl)	266 ± 21.2	194 ± 30.4	263 ± 35.5	200 ± 31.2	274 ± 28.2	192 ± 29.9	277 ± 62.7	182 ± 27.8
Leukocytes (10^3/cmm)	12.57 ± 3.340	8.21 ± 2.355	12.71 ± 3.141	8.33 ± 2.985	10.75 ± 3.082	7.84 ± 1.643	13.54 ± 3.979	8.34 ± 1.853
Segmented neutrophils (10^3/ml)	2.66 ± 1.193	1.70 ± 0.667	3.70 ± 1.351	1.81 ± 0.461	3.13 ± 0.936	1.68 ± 0.352	4.03 ± 2.412	2.21 ± 0.621
Lymphocytes (10^3/ml)	9.30 ± 3.634	6.06 ± 2.065	8.19 ± 1.922	5.99 ± 2.806	6.95 ± 2.396	5.63 ± 1.365	8.66 ± 2.457	5.60 ± 1.543
Monocytes (10^3/ml)	0.59 ± 0.411	0.42 ± 0.231	0.73 ± 0.258	0.44 ± 0.221	0.61 ± 0.163	0.48 ± 0.116	0.78 ± 0.442	0.47 ± 0.116
Basophils (10^3/ml)	0.00 ± 0.005	0.00 ± 0.004	0.01 ± 0.015	0.00 ± 0.003	0.00 ± 0.004	0.00 ± 0.004	0.00 ± 0.006	0.00 ± 0.005
Eosinophils (10^3/ml)	0.02 ± 0.023	0.03 ± 0.054	0.08 ± 0.048***	0.09 ± 0.133	0.06 ± 0.034	0.05 ± 0.038	0.07 ± 0.051	0.05 ± 0.029

* $p < 0.05$ when compared to FOS control.
** $p < 0.01$ when compared to FOS control.
*** $p < 0.05$ compared to RODI control.
[a] ±Standard deviation.
[b] RODI female # 2622 had aberrant values on some hematology and clinical chemistry values on day 92; these values are not included in the means or standard deviations, but their exclusion did not affect the results of statistical comparisons among groups.

ratory historical control. Therefore, none of these changes was judged to be of toxicological significance.

3.6.3. Urinalysis

Mean specific gravity values were significantly lower by 1% for the 5000 mg GOS/kg bw/day males compared to the RODI water control group. Mean specific gravity for the 2500 mg GOS/kg bw/day females was significantly higher by 2% than the reference control. Since these findings were inconsistent and not related to dose, they were considered of no toxicological relevance.

3.6.4. Ophthalmology

No test article-related ocular abnormalities were noted.

3.6.5. Gross necropsy observation

No remarkable internal gross abnormalities were observed for any of the animals surviving to scheduled euthanasia at the end of the treatment period.

3.6.6. Organ weights

Mean spleen weight relative to final body weight in the FOS control and the 5000 mg GOS/kg bw/day males was significantly higher than the RODI water control group. Mean absolute and spleen weight relative to final body weight in the FOS control females was significantly higher compared to the RODI water control group. Mean absolute ovary weight in the 5000 mg GOS/kg bw/day females and mean liver weight relative to final body weight in the

Table 3
Mean clinical chemistry values (days 90–92)[a]

Parameter	RODI Control		FOS Control		2500 mg GOS/kg bw		5000 mg GOS/kg bw	
	Males $n=15$	Females $n=14$[b]	Males $n=15$	Females $n=15$	Males $n=15$	Females $n=15$	Males $n=15$	Females $n=13$
AST (IU/l)	97 ± 51.5	88 ± 32.9	93 ± 14.7	73 ± 15.6	84 ± 15.1	78 ± 15.6	85 ± 15.0	84 ± 24.8
ALT (IU/l)	37 ± 27.0	40 ± 21.7	30 ± 4.9	27 ± 8.1	27 ± 4.5	30 ± 8.3	25 ± 4.3*	37 ± 27.9
Alkaline phosphatase (IU/l)	84 ± 16.4	58 ± 14.4	81 ± 16.0	54 ± 13.9	86 ± 15.2	60 ± 16.9	78 ± 11.7	58 ± 22.5
Total bilirubin (mg/dl)	0.60 ± 0.112	0.57 ± 0.087	0.56 ± 0.103	0.58 ± 0.091	0.53 ± 0.070	0.54 ± 0.140	0.57 ± 0.108	0.59 ± 0.104
Total protein (g/dl)	6.46 ± 0.275	7.00 ± 0.514	6.37 ± 0.266	6.77 ± 0.245	6.45 ± 0.377	6.96 ± 0.333	6.53 ± 0.333	7.04 ± 0.544
Albumin (g/dl)	3.07 ± 0.136	3.52 ± 0.293	3.07 ± 0.122	3.36 ± 0.161	3.10 ± 0.160	3.45 ± 0.231	3.08 ± 0.169	3.49 ± 0.330
Globulin (g/dl)	3.40 ± 0.195	3.48 ± 0.247	3.29 ± 0.259	3.40 ± 0.170	3.36 ± 0.240	3.51 ± 0.180	3.45 ± 0.279	3.56 ± 0.258
A/G Ratio	0.91 ± 0.053	1.01 ± 0.048	0.94 ± 0.084	0.99 ± 0.066	0.92 ± 0.042	0.99 ± 0.071	0.90 ± 0.078	0.98 ± 0.064
Phosphorus (mg/dl)	6.3 ± 0.53	5.2 ± 0.93	6.8 ± 0.66	5.4 ± 0.87	6.5 ± 0.52	5.8 ± 0.56	6.6 ± 0.67	6.0 ± 0.61
Urea nitrogen (mg/dl)	15 ± 2.0	17 ± 2.6	16 ± 1.6	18 ± 3.5	15 ± 2.1	19 ± 2.3	16 ± 3.4	18 ± 3.3
Creatinine (mg/dl)	0.48 ± 0.058	0.58 ± 0.103	0.49 ± 0.069	0.58 ± 0.058	0.51 ± 0.055	0.54 ± 0.060	0.51 ± 0.057	0.56 ± 0.067
Glucose (mg/dl)	156 ± 25.0	138 ± 15.7	127 ± 19.0***	139 ± 18.4	141 ± 19.3	139 ± 12.3	139 ± 25.2	135 ± 12.9
Sodium (mmol/l)	141 ± 1.1	140 ± 1.4	140 ± 1.2	140 ± 1.3	141 ± 1.4	140 ± 1.0	141 ± 2.1	141 ± 1.3
Potassium (mmol/l)	4.76 ± 0.347	4.33 ± 0.372	4.91 ± 0.336	4.23 ± 0.477	4.98 ± 0.374	4.48 ± 0.325	4.81 ± 0.310	4.45 ± 0.347
Chloride (mmol/l)	104 ± 1.6	105 ± 1.7	103 ± 1.5	105 ± 1.7	104 ± 1.6	105 ± 1.8	103 ± 1.8	106 ± 1.5
Calcium (mg/dl)	9.98 ± 0.264	10.30 ± 0.324	10.23 ± 0.344	10.34 ± 0.349	10.18 ± 0.381	10.47 ± 0.314	10.33 ± 0.388	10.65 ± 0.438
Cholesterol (mg/dl)	32 ± 8.9	40 ± 9.3	28 ± 7.1	38 ± 7.5	32 ± 7.3	39 ± 6.1	28 ± 7.0	40 ± 14.3
Serum GGT (IU/l)	1.14 ± 0.634	2.10 ± 0.794	0.76 ± 0.516	1.62 ± 0.765	1.16 ± 0.659	2.14 ± 0.837	1.15 ± 0.497	1.85 ± 0.709

*$p < 0.05$ when compared to FOS control.
***$p < 0.01$ compared to RODI control.
[a] ±Standard deviation.
[b] RODI female # 2622 had aberrant values on some hematology and clinical chemistry values on day 92; these values are not included in the means or standard deviations, but their exclusion did not affect the results of statistical comparisons among groups.

2500 mg GOS/kg bw/day females were significantly lower than the FOS control group. Since these findings were inconsistent and not related to dose, they were considered to be of no toxicological relevance.

3.6.7. Histopathology

No test article-related changes were observed in this study. All changes were considered normal background lesions in this strain and age of rat. Histopathological findings of kidney, liver and lung from all the test and control groups are shown in Table 4.

4. Discussion

When GOS syrup was administered to rats by gavage at 2500 or 5000 mg/kg bw/day for 90 days, there were no significant adverse toxicological effects attributable to treatment. Clinical signs were unremarkable, and there were no ocular findings in any animal. Analysis of clinical pathologies, including blood biochemistries, hematology, urinalysis and coagulation revealed only random statistically significant effects. There were also occasional effects noted on absolute and relative organ weights. These differences were generally found only in the RODI water controls as opposed to the FOS reference controls, which were included in the study to account for the higher caloric and fiber intake in the treatment groups. There were no findings at study termination in either macroscopic or histopathologic examinations that indicated that any of these effects were related to treatment with the test material. In addition, the random occasional observations in hematology and blood chemistries were within the range of intralaboratory historical controls, were not consistent between sexes, and were not dose related. And, as noted above, none was corroborated by macroscopic or histological findings.

The only effects related to treatment were effects on food consumption. Decreased food consumption was noted in FOS control groups (both sexes) when compared to the RODI reference controls The only decreases in food consumption in the GOS syrup-treated animals were in both sexes at 5000 mg/kg bw/day when compared to the RODI water controls. Based on the lack of toxicologically relevant effects on other parameters in the study, the no-observable-adverse-effect level (NOAEL) for Vivinal® GOS syrup is 5000 mg/kg bw/day when administered by gavage for 90 consecutive days.

In September 2001, the EU's Scientific Committee on Food (SCF) reviewed the suitability and safety of a blend comprising 90% GOS (from Vivinal®) and 10% oligofructose (from chicory roots) for use at an addition level of 8 g/l in infant formula and follow-on formula (SCF, 2001a). The Committee noted the effect of the prebiotic blend on stool frequency and consistency, and expressed some concern regarding a potential for dehydration in young infants, although this was not a major concern for

Table 4
Incidence of selected histopathological findings[a]

Tissue	RODI Control		FOS Control		2500 mg GOS/kg bw		5000 mg GOS/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Kidney								
Cyst	–	–	–	–	–	1/15	–	0/13
Tubular degeneration	8/15	3/15	9/15	0/15	8/15	2/15	7/15	0/13
Pelvic dilatation	1/15	1/15	0/15	0/15	1/15	–	1/15	–
Hemorrhage	0/15	–	1/15	–	–	–	–	–
Tubular dilatation	–	1/15	–	1/15	1/15	0/15	1/15	1/13
Fibrosis	–	1/15	–	0/15	–	–	–	–
Epithelial hyperplasia	–	0/15	–	0/15	0/15	–	2/15	–
Infarct	1/15	2/15	2/15	2/15	2/15	1/15	2/15	0/13
Inflammation	6/15	4/15	7/15	5/15	4/15	4/15	8/15	3/15
Mineralization	0/15	0/15	0/15	1/15	–	–	–	–
Liver								
Extramedullary hematopoiesis	–	1/15	–	0/15	–	–	–	–
Hemorrhage	0/15	2/15	1/15	1/15	–	–	–	–
Epithelial hyperplasia	0/15	–	1/15	–	–	–	–	–
Bile duct hyperplasia	–	3/15	–	0/15	–	1/15	–	0/13
Chronic Inflammation	0/15	2/15	1/15	1/15	0/15	–	1/15	–
Granulomatous inflammation	–	–	–	–	–	0/15	–	1/13
Necrosis	0/15	2/15	1/15	1/15	0/15	–	1/15	–
Pigment	–	0/15	–	1/15		–	–	–
Vacuolar Change	1/15	0/15	0/15	2/15	–	1/15	–	0/13
Lung								
Hemorrhage	2/15	0/15	0/15	0/15	0/15	–	2/15	–
Histiocytosis	3/15	2/15	1/15	2/15	1/15	0/15	4/15	2/13
Acute inflammation	–	2/15	–	2/15	–	–	–	–
Chronic inflammation	1/15	1/15	0/15	2/15	1/15	0/15	2/15	1/13
Osseous metaplasia	1/15	–	1/15	–	–	–	–	–
Congestion	–	1/15	–	2/15	1/15	2/15	0/15	2/13

– = Not reported.
[a] No. of animals/no. of animals examined; based on tissues examined for all test and control groups.

older infants. Thus, the SCF found the potential for adverse effects of the addition of 8 g/l of the prebiotic blend to be low for older infants and approved its use in follow-on formula while recommending the submission of additional information regarding the safety of the blend for young infants.

Three months later, in December 2001, based on additional data from clinical studies that had not been previously available, the SCF determined that it no longer had concerns about the addition of 8 g/l of the prebiotic blend to infant formula intended for consumption by neonates (SCF, 2001b). It did, however, indicate a desire to continue to receive evidence regarding the suitability and safety of the addition of prebiotics to infant formula.

In its comprehensive review of infant formula composition in 2003 (SCF, 2003), the Committee reaffirmed its previous statement that it has no major concerns with the inclusion of up to 8 g/l of a combination of 90% GOS and 10% FOS in both infant formulas and follow-on formulas. The prebiotic addition approved by the Committee represents 7.2 g/l of GOS (and 0.8 g/l of FOS), or 16 g/l of Vivinal® syrup.

The current study, establishing a NOAEL of 5000 mg/kg bw/day for Vivinal® GOS syrup administered via gavage for 90 consecutive days, contributes to the evidence of the safety of current and proposed uses of GOS as a component of infant formulas. The totality of this evidence, which includes both animal and human studies, demonstrates that Vivinal® GOS syrup is Generally Recognized as Safe (GRAS) for its intended use as a prebiotic ingredient, alone or as one component of a blend, in infant formulas.

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