

Subchronic Oral Toxicity of Salcaprozate Sodium (SNAC) in Sprague-Dawley and Wistar Rats

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Salcaprozate sodium (SNAC) (sodium 8-((2-hydroxybenzoyl)amino) octanoate, CAS RN 203787-91-1) is classified as an oral absorption promoter, and its potential therapeutic applications as a delivery agent for oral forms of heparin and insulin have been explored in a number of clinical investigations. However, limited information about its nonclinical safety is available in the published scientific literature. As part of a larger study exploring the safety of SNAC in combination with heparin, Sprague-Dawley (SD) rats (20/sex/group) received SNAC alone at 2000 mg/kg/d orally (gavage) for 13 weeks (females were terminated after 10 weeks). In a separate study assessing the safety of SNAC in combination with ibandronate, Wistar rats (10/sex/group) received SNAC alone at levels of 100, 500, or 1000 mg/kg/d orally for 13 weeks. SNAC-related mortality was evident only at the 2000-mg/kg/d level, 20% among males and 50% among females; no clear cause of death was evident. No mortality was seen in

the Wistar rat study at doses up to 1000 mg/kg/d. Some differences in clinical pathology parameters, including slightly altered electrolyte levels and lower globulin levels, were seen in SD and Wistar rats. Although these differences reached statistical significance, parameters were within historical control ranges. Liver and kidney weights were slightly higher in SNAC-treated animals of both strains, with no corresponding histopathological changes. These changes may therefore constitute an adaptive response. Histopathological changes were seen in the stomach in both studies, probably secondary to irritation caused by the dosing method. Based on the results of these studies, a no-observed-adverse-effect level (NOAEL) cannot be given for SD rats. The NOAEL for SNAC in Wistar rats was considered to be 1000 mg/kg/d.

Keywords: CAS RN 203787-91-1; rat; salcaprozate sodium; SNAC; subchronic toxicity

Salcaprozate sodium (SNAC) (sodium 8-((2-hydroxybenzoyl)amino) octanoate, CAS RN 203787-91-1) is classified as an oral absorption promoter. The potential therapeutic applications of SNAC as a delivery agent for oral forms of heparin and insulin have been explored in a number of clinical investigations.¹⁻⁶ However, limited information about the nonclinical safety of SNAC itself

was found in the published scientific literature. A review article describing the development of an oral heparin formulation describes the results of SNAC toxicological studies in experimental animals but only in very general terms.⁷

The present report summarizes the findings of 2 separate studies that explored the subchronic oral toxicity of SNAC in Sprague-Dawley and Wistar rats. The first study, sponsored by Emisphere Technologies, Inc (Cedar Knolls, New Jersey), employed SNAC alone and in combination with heparin. A second study, exploring the toxicity of SNAC alone and in combination with ibandronate, was sponsored by F. Hoffmann-LaRoche Ltd under a development and license agreement with

From Emisphere Technologies, Cedar Knolls, New Jersey (MGIR, MCC); and Harlan Laboratories (formerly RCC Ltd), Itingen, Switzerland (EAP).

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Emisphere. For both studies, only the findings from groups treated with SNAC alone or the vehicle (deionized water) are included in this report because it would be difficult to distinguish SNAC-related effects from drug-related effects in SNAC-heparin and SNAC-ibandronate groups.

Materials and Methods

A study* conducted at WIL Research Laboratories (Ashland, Ohio) explored the safety of SNAC in Sprague-Dawley rats following oral (gavage) exposure for 13 weeks. The study was conducted from November 1997 to March 1998 in compliance with the US Food and Drug Administration (FDA) current good laboratory practice (GLP) standards (21 CFR Part 58). A second 13-week study** in Wistar rats was conducted at RCC Ltd Toxicology (Füllinsdorf, Switzerland), an American Association for Accreditation of Laboratory Animal Care (AAALAC)-approved laboratory, from March to July 2006 and in accordance with the Swiss Animal Protection Law; Organization for Economic Cooperation and Development (OECD) Guideline for the Testing of Chemicals, Number 408 (Repeated-Dose 90-Day Oral Toxicity Study in Rodents), adopted September 21, 1998; Environmental Protection Agency (EPA) Health Effects Test Guidelines, OPPTS 870.3100 (90-Day Oral Toxicity in Rodents); the Swiss Ordinance relating to good laboratory practice, adopted May 18, 2005 (RS 813.112.1), based on the OECD Principles of Good Laboratory Practice (C(97)186/Final); and other applicable European Community standards.

Animals

Male and female Crl:CD (SD) BR rats (Charles River Laboratories, Portage, Michigan) and Wistar HanRcc:WIST (SPF) rats (RCC Ltd, Füllinsdorf, Switzerland) were allowed to acclimate at least 7 days prior to treatment. Animals (6-7 weeks old) were then assigned to study groups: SD rats (20/sex/group) based on a body weight stratification block design and Wistar rats (10 sex/group) based

on a computer-generated random algorithm. An additional 5 animals/sex/group were included in the Wistar rat study for toxicokinetic analysis (satellite group). Some safety endpoints were measured in this satellite group, and the relevant data are included herein. SD body weights at the start of the study ranged from 154 to 244 g for males and from 127 to 195 g for females. Wistar body weights at the start of the study ranged from 113 to 170 g for males and from 97 to 136 g for females.

Test Article and Dosing

The purity of SNAC (E414, Sodium Salt, Lot No. EM0066 or EM0067) administered to SD rats ranged from 93% to 94%. The purity of SNAC (E414, Sodium Salt, Batch No. SIS00516) administered to Wistar rats was 98.9%. SNAC samples were stored at room temperature ($20 \pm 5^\circ\text{C}$) with desiccant.

For 13 weeks, SD and Wistar rats received a daily dose of the vehicle (deionized water) or SNAC by gastric intubation. SNAC was administered to SD rats at 2000 mg per kg body weight (2000 mg/kg/d) in a dose volume of 10 mL/kg of body weight. Wistar rats received SNAC at doses of 100 (5 mL/kg), 500 (5 mL/kg), or 1000 mg/kg/d (10 mL/kg) orally for 13 weeks.

Housing

SD rats were housed individually and Wistar rats were housed in groups of 5 in wire-mesh cages in an environmentally controlled room (22°C, 30%-70% relative humidity, 12-h light/dark cycle). Feed and drinking water were provided ad libitum, except during the period of fasting prior to blood collection, when feed was temporarily withheld. SD rats received Certified Rodent LabDiet 5002 from PMI Nutrition International, Inc and reverse osmosis-treated (on-site) water, delivered by an automatic watering system. Wistar rats received standard Provimi Klia 3433 rat maintenance diet from Provimi Klia AG and community tap water ad libitum.

Parameters Evaluated

Survival

Animals were observed twice daily for mortality and moribundity.

*OX97007: 13-week oral (gavage) toxicity study of E414/heparin and E414 in rats. WIL Study No. WIL-315003. July 23, 1999.

**1021150: 13-week oral (gavage) toxicity study in Wistar rats. Reference No. 8017P06, RCC Study No. A62807, RCC Study No. A62807. March 30, 2007.

Clinical Observations

Observations for clinical signs of toxicity were performed on SD rats at the time of dosing and approximately 1 to 2 hours postdosing. Detailed physical examinations were performed weekly, beginning 1 week prior to the start of dosing. Wistar rats were observed once daily. Detailed clinical observations were carried out once weekly during the treatment period.

Body Weights

The body weights of SD rats were measured weekly; Wistar rats were weighed twice weekly.

Mean body weights and mean body weight changes were calculated throughout the study, and final body weights were recorded on the day of scheduled necropsy.

Food Consumption

Food consumption was measured weekly in SD rats and twice weekly in Wistar rats.

Clinical Pathology

Clinical pathology parameters (hematology, serum chemistry) were measured at week 5 and at the end of the study. Due to high mortality (50%) among female SD rats receiving 2000 mg/kg/d of SNAC, all remaining females were euthanized, and evaluations were performed at week 10. Animals were fasted overnight prior to blood collection. In SD rats, blood was collected from a lateral tail vein at week 5 and from the vena cava at the time of necropsy. In Wistar rats, blood was drawn from the retroorbital plexus. Hematology parameters measured in both SD and Wistar rats included hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), platelets (PLT), red blood cell (RBC) count, white blood cell (WBC) count, and WBC differential count (neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells [LUC]). Red cell volume distribution width (RDW), hemoglobin concentration distribution width (HDW), and reticulocyte maturity index were measured in Wistar rats only. Activated partial thromboplastin time (APTT) and prothrombin time (PT) or thromboplastin time were measured in both strains. All animals were evaluated for albumin, globulin, albumin/globulin ratio (A/G ratio), alkaline

phosphatase (ALP), alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), total bilirubin, blood urea nitrogen (BUN), calcium (Ca), chloride (Cl), cholesterol, creatinine (Creat.), gamma-glutamyltransferase (GGT), glucose, potassium (K), phosphorus (P), total protein (Prot.), and sodium (Na). Triglycerides, phospholipids, glutamate dehydrogenase (GLDH), lactate dehydrogenase (LDH), and creatine kinase (CK) were measured in Wistar rats only.

Urinalysis

The urine of Wistar rats was collected and evaluated for volume (18-hour), specific gravity, color, appearance, pH, nitrite, protein, glucose, ketone, urobilinogen, bilirubin, erythrocytes, and leukocytes.

Organ Weights

Surviving SD rats were sacrificed by CO₂ asphyxiation, followed by exsanguination. Wistar rats were anesthetized by intraperitoneal pentobarbitone injection and sacrificed by exsanguination.

The weights of adrenal glands, brain, kidneys, liver, ovary (with oviduct in SD rats), spleen, testis, epididymis (with testis in SD rats), and thymus were recorded in all animals. The weights of thyroid with parathyroid glands were recorded for SD rats only. Paired organs were weighed together. Organ-to-final body weight ratios were calculated.

Macroscopic and Histopathological Examination

Complete necropsies were performed on all animals. Several major organs and tissues from control and high-dose animals were preserved in neutral buffered formalin or other suitable fixative (Bouin's or Davidson's solution) at the discretion of the study pathologist. Fixed tissue samples were processed and embedded. Sections were cut and placed on glass microscope slides, stained with hematoxylin and eosin (H&E), by a qualified pathologist.

Ophthalmological Examination

Ophthalmological examinations were conducted on all animals prior to treatment and toward the end of the study (weeks 12-13).

Table 1. Mortality Among Salcaprozate Sodium–Treated Sprague-Dawley Rats

Dosage, mg/kg/d	Number of Animals at Study Start		Days								
			0-4		20-40		50-80		88		
	M	F	M	F	M	F	M	F	M	F	
0	20	20	0	0	0	0	0	0	0	0	0
2000	20	20	1	1	0	5	2	4	1	0	

M, male; F, female.

Table 2. Mortality Among Salcaprozate Sodium–Treated Wistar Rats

Dosage, mg/kg/d	Number of Animals at Study Start ^a		Days								
			0-4		20-40		50-80		88		
	M	F	M	F	M	F	M	F	M	F	
0	20	20	0	0	0	0	0	0	0	0	0
100	15	15	0	0	0	0	0	0	0	0	0
500	15	15	0	0	0	0	0	0	0	0	0
1000	15	15	0	0	0	0	1	1 ^b	0	0	0

M, male; F, female.

^a Includes satellite group for toxicokinetic analysis.^b Death in satellite group; the only finding at necropsy was an enlarged eye.

Statistical Analyses

Statistical analyses of data from SD rats were performed by a Digital MicroVAX 3400 computer operating Wil Toxicology Data Management System software. Because the study involved multiple groups (vehicle, SNAC alone, and SNAC + heparin), body weight, food consumption, clinical pathology, and organ weight data were analyzed by 1-way analysis of variance (ANOVA), followed by Dunnett's test. Clinical laboratory values that occur at a low incidence (ie, monocytes, eosinophils, and basophils) were not subjected to statistical analysis.

A RCC-TOX LIMS computer was used to sort and process the Wistar rat study data. Dunnett's test (many-to-1 *t* test) based on a pooled variance estimate was used to compare the treated and control groups if the variables (body weight, food consumption, organ weights) could be assumed to follow a normal distribution. The Steel test (many-to-1 rank test) was used when the data could not be assumed to follow a normal distribution. Fisher's exact test was applied to the macroscopic and ophthalmoscopy findings. Clinical pathology data were analyzed by ANOVA when the variances were considered homogeneous according to Barlett. Alternatively, if the variances were heterogeneous ($P \leq .05$), a

nonparametric Kruskal-Wallis test was used. Treated groups were then compared to the control groups using Dunnett's test if the ANOVA was significant at the 5% level and by Dunn's test in the case of a significant Kruskal-Wallis test ($P \leq .05$). Ordinal data such as urine sediment were analyzed using the Kruskal-Wallis test, followed by Dunn's test, if significant ($P \leq .05$).

Results

Sprague-Dawley Rats

As Table 1 illustrates, 4 male and 10 female SD rats receiving 2000 mg/kg/d of SNAC were found dead or euthanized in extremis during the study (see also Table 2). Clinical signs noted in these animals within 24 hours prior to death included hypoactivity, prostration, unkempt appearance, pale extremities, and body cool to touch. Due to the high mortality rate (50%) among SNAC-treated females in this study, the remaining females in the SNAC group were euthanized during week 10 to obtain timely necropsy data and to harvest tissue for microscopic evaluation. All other animals survived to study end.

Clinical signs observed in SNAC-treated SD rats from day 1 and throughout the study included red

and yellow material on various body surfaces (most notably around the nose, mouth, forelimbs, and urogenital and anogenital areas), frequently at the 1-hour postdosing observation. Rales and the presence of wet, clear material around the mouth were also noted.

There were no significant differences between SD rats treated with 2000 mg/kg/d of SNAC and control animals in hematology values (Tables 3-6). Ophthalmological examination did not reveal any significant differences (data not shown). Similarly, there were no effects on food consumption (data not shown) or terminal body weights.

As shown in Table 7, male SD rats receiving 2000 mg/kg/d of SNAC had significantly lower ($P < .01$) circulating globulin levels at 5 and 13 weeks; chloride (Cl) was also lower in males, but these changes were minor and generally matched by changes in control animals (Tables 7 and 8), which suggested that technique-related stomach changes may have played a contributory role. Similar chloride trends were also noted in SNAC-treated female SD rats (Tables 9 and 10); however, in the absence of corresponding week 10 control animal data, statistical significance could not be established.

As Table 11 illustrates, SNAC-treated SD male rats had significantly higher relative liver and kidney weights than control animals. SNAC-treated SD female rats sacrificed at week 10 also had higher relative liver and kidney weights than control (week 13) females. However, in the absence of corresponding week 10 control animal data, the statistical significance of this difference could not be established. Other differences in organ weights were considered to be incidental.

Gross necropsy of SD rats that died or were euthanized in extremis, as well as those sacrificed at week 10 (SNAC females) or week 13, did not reveal any findings considered related to SNAC treatment; all findings were noted at a low incidence (typically in single animals) and/or were considered spontaneous or incidental, with no apparent relationship to treatment. Histopathological findings seen in all or nearly all animals, including the control group, included neutrophil infiltration in the glandular stomach and extramedullary hematopoiesis and brown pigment of the spleen (data not shown). In some instances, the severity of these effects was slightly greater (ie, mild vs minimal) in SNAC-treated males. Vacuolation of the cells in the pars distalis of the pituitary gland was seen in SNAC-treated

males only (Table 12). Affected cells were found singly or in small groups and contained large, clear, non-staining cytoplasmic vacuoles that frequently eccentrically displaced the nucleus. No such findings were present in females. All other microscopic findings were considered to be spontaneous, incidental, and unrelated to SNAC administration.

Wistar Rats

As Table 2 shows, SNAC had no effect on mortality or viability. One high-dose male Wistar rat was euthanized on day 71 following complications from an injury sustained during the gavage procedure. One high-dose Wistar female rat in the satellite toxicokinetics group died spontaneously on day 76; examination revealed an enlarged eye. Signs of stomach irritation (erosion, inflammatory cell foci) were noted in control and SNAC-treated animals. In some instances, the incidence or severity of these effects was slightly greater in SNAC-treated animals. Clinical signs noted throughout the study included skin and/or eye lesions commonly seen in experimental animals. All were noted in both the SNAC and control groups and therefore were not considered to be related to treatment with SNAC.

Treatment-related effects on body weights or on body weight gain were not observed in the study (data not shown). No statistically significant body weight effects were observed in any SNAC-treated male Wistar groups. In the females, no statistically significant effects on body weights were observed in the 500-mg/kg/d group or the 1000-mg/kg/d group. The exception was a sole statistically significant difference in the 1000-mg/kg/d group value for body weight during week 7 of the study. In the 100-mg/kg/d females, statistically significantly lower body weight was first recorded during the prestudy acclimatization period. Statistically significantly lower body weight gains were subsequently seen in this group during the study (27 of 27 body weight measurements). These differences in body weights of females, limited as they were to the low-dose group, were considered to be unrelated to SNAC treatment. Similarly, SNAC had no apparent effect on food consumption, urinalysis parameters, or ophthalmological findings (data not shown).

As Tables 13 through 18 show, some slight but statistically significant differences in hematology parameters were noted. For example, male Wistar rats receiving 1000 mg/kg/d of SNAC had slightly

Table 3. Hematology (RBC and Coagulation) Values in Salcaproate Sodium–Treated Male Sprague-Dawley Rats

Dosage, mg/kg/d	RBC, 10 ⁶ /μL	HB, g/dL	HCT, %	MCV, fl	MCH, pg	MCHC, g/dL	PLT, 10 ³ /μL	PT, s	APTT, s
0	Week 5 (n = 20)	7.96 ± 0.750	15.5 ± 1.15	44.7 ± 3.40	56.3 ± 2.13	19.5 ± 0.86	34.6 ± 0.50	1200 ± 133.5	NA
	Week 13 (n = 20)	9.05 ± 0.411	15.9 ± 0.38	47.3 ± 1.44	52.3 ± 1.68	17.6 ± 0.58	33.7 ± 0.42	1107 ± 168.7	16.7 ± 4.17
2000	Week 5 (n = 19)	8.13 ± 4.64	15.7 ± 0.71	45.3 ± 2.12	55.8 ± 1.49	19.4 ± 0.58	34.7 ± 0.39	1065 ± 144.0	NA
	Week 13 (n = 16)	8.67 ± 4.04	15.5 ± 0.42	45.9 ± 1.73	52.9 ± 1.05	17.8 ± 0.48	33.7 ± 0.47	1042 ± 121.8	16.8 ± 3.81

Values represent mean ± SD. NA, not available; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 4. Hematology (Total and Differential WBC Count) Values in Salcaproate Sodium–Treated Male Sprague-Dawley Rats

Dosage, mg/kg/d	WBC, 10 ³ /μL	Neutrophil		Lymphocyte		Monocyte		Eosinophil		Basophil	
		10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%
0	Week 5 (n = 20)	16.2 ± 3.22	2.4 ± 1.19	15 ± 5.4	12.3 ± 2.54	76 ± 6.4	1.2 ± 0.28	8 ± 1.7	0.2 ± 0.16	1 ± 1.0	0.0 ± 0.00
	Week 13 (n = 20)	13.7 ± 3.26	1.8 ± 0.55	14 ± 4.0	10.5 ± 3.01	76 ± 5.5	1.2 ± 0.45	9 ± 3.9	0.1 ± 0.07	1 ± 0.7	0.0 ± 0.00
2000	Week 5 (n = 19)	18.4 ± 3.17	2.8 ± 0.76	16 ± 4.8	14.0 ± 3.40	75 ± 7.8	1.4 ± 0.55	8 ± 3.1	0.2 ± 0.14	1 ± 0.8	0.0 ± 0.05
	Week 13 (n = 16)	14.9 ± 3.58	2.5 ± 0.68	17 ± 4.9	10.7 ± 3.36	71 ± 6.6	1.5 ± 0.43	11 ± 3.6	0.1 ± 0.07	1 ± 0.5	0.0 ± 0.00

Values represent mean ± SD. WBC, white blood cell.

Table 5. Hematology (RBC and Coagulation) Values in Salcaproate Sodium–Treated Female Sprague-Dawley Rats

Dosage, mg/kg/d	RBC, 10 ⁶ /μL	HB, g/dL	HCT, %	MCV, fl	MCH, pg	MCHC, g/dL	PLT, 10 ³ /μL	PT, s	APTT, s
0	Week 5 (n = 20)	7.82 ± 0.34	15.3 ± 0.59	44.8 ± 1.53	57.3 ± 1.07	19.6 ± 0.43	34.3 ± 0.57	1089 ± 154.3	NA
	Week 13 (n = 20)	8.18 ± 3.02	15.6 ± 0.58	45.7 ± 1.93	55.9 ± 1.69	19.0 ± 0.54	34.0 ± 0.42	1024 ± 105.5	16.0 ± 1.66
2000	Week 5 (n = 16)	8.11 ± 0.50	15.7 ± 0.92	45.5 ± 2.37	56.1 ± 1.36	19.4 ± 0.54	34.6 ± 0.40	1106 ± 140.5	NA
	Week 10 (n = 10)	8.17 ± 0.31	15.7 ± 0.51	46.2 ± 1.62	56.5 ± 1.44	19.3 ± 0.54	34.1 ± 0.68	1121 ± 141.2	14.5 ± 0.88
	Week 13	NA	NA	NA	NA	NA	NA	NA	NA

Values represent mean ± SD. NA, not available; RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 6. Hematology (Total and Differential WBC Count) in Salcaprozate Sodium-Treated Female Sprague-Dawley Rats

Dosage, mg/kg/d	Neutrophil		Lymphocyte		Monocyte		Eosinophil		Basophil			
	WBC, 10 ³ /μL	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	
0	Week 5 (n = 20)	12.9 ± 3.45	1.6 ± 0.61	12 ± 5.0	10.5 ± 3.29	81 ± 6.3	0.7 ± 0.33	6 ± 2.1	0.2 ± 0.09	1 ± 0.8	0.0 ± 0.00	0 ± 0.0
	Week 13 (n = 20)	11.2 ± 2.92	1.1 ± 0.38	11 ± 4.8	9.1 ± 3.04	79 ± 9.1	0.9 ± 0.39	9 ± 4.9	0.1 ± 0.07	1 ± 0.9	0.0 ± 0.00	0 ± 0.0
2000	Week 5 (n = 16)	13.6 ± 3.96	2.8 ± 0.76	13 ± 5.2	10.5 ± 3.23	77 ± 6.2	1.0 ± 0.53	8 ± 2.0	0.2 ± 0.14	2 ± 1.2	0.0 ± 0.00	0 ± 0.0
	Week 10 (n = 10)	10.0 ± 2.01	1.1 ± 0.42	11 ± 4.0	7.6 ± 1.58	76 ± 7.7	1.2 ± 0.58	12 ± 4.6	0.1 ± 0.09	1 ± 0.7	0.0 ± 0.00	0 ± 0.0
	Week 13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Values represent mean ± SD. NA, not available; WBC, white blood cell.

Table 7. Serum Chemistry Values in Salcaprozate Sodium-Treated Male Sprague-Dawley Rats

Dosage, mg/kg/d	Prot., g/dL	Albumin, g/dL	Globulin, g/dL	A/G, Ratio	Bilirubin, mg/dL	BUN, mg/dL	Creat., mg/dL	ALP, U/L	ALAT, U/L	ASAT, U/L	GGT, U/L	
												0
	Week 13 (n = 20)	6.8 ± 0.38	4.5 ± 0.28	2.3 ± 0.18	2.0 ± 0.17	0.1 ± 0.05	14.8 ± 2.69	0.3 ± 0.07	104 ± 24.4	42 ± 9.7	80 ± 14.1	0 ± 0.0
2000	Week 5 (n = 19)	6.3 ± 0.28**	4.6 ± 0.17	1.7 ± 0.19**	2.8 ± 0.32**	0.2 ± 0.08	12.9 ± 1.53	0.2 ± 0.07	181 ± 24.8	43 ± 6.7	110 ± 17.8	0 ± 0.2
	Week 13 (n = 16)	6.1 ± 0.46**	4.5 ± 0.31	1.6 ± 0.26**	2.8 ± 0.45**	0.1 ± 0.05	11.6 ± 1.71**	0.2 ± 0.05	110 ± 15.8	56 ± 28	99 ± 30.8	0 ± 0.3

Values represent mean ± SD. Prot., total protein; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Creat., creatinine; ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

**Significantly different from control, $P < .01$, Dunnett's test.

Table 8. Serum Chemistry Values in Salcaprozate Sodium-Treated Male Sprague-Dawley Rats

Dosage, mg/kg/d	Glucose, mg/dL	Cholesterol, mg/dL	Ca, mg/dL	Cl, mEq/L	P, mg/dL	K, mEq/L	Na, mEq/L	
								0
	Week 13 (n = 20)	240 ± 52.2	56 ± 13.2	11.3 ± 0.55	95 ± 1.7	9.3 ± 1.29	7.06 ± 1.12	144 ± 2.5
2000	Week 5 (n = 19)	104 ± 17.8**	45 ± 12.0	9.5 ± 0.28	98 ± 2.0**	8.8 ± 0.59	5.93 ± 0.48	140 ± 1.7
	Week 13 (n = 16)	230 ± 40.0	52 ± 12.8	11.1 ± 0.46	93 ± 2.1**	9.7 ± 0.87	6.72 ± 1.29	142 ± 1.7*

Values represent mean ± SD. Ca, calcium; Cl, chloride; P, phosphorus; K, potassium; Na, sodium.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

Table 9. Serum Chemistry Values in Salcaprozate Sodium-Treated Female Sprague-Dawley Rats

Dosage, mg/kg/d	Prot., g/dL	Albumin, g/dL	Globulin, g/dL	A/G, Ratio	Bilirubin, mg/dL	BUN, mg/dL	Creat., mg/dL	ALP, U/L	ALAT, U/L	ASAT, U/L	GGT, U/L
0	Week 5 (n = 20)	7.0 ± 0.38 ^a	4.9 ± 0.32 ^a	2.2 ± 0.25 ^b	2.2 ± 0.32 ^b	16.4 ± 2.01 ^a	0.3 ± 0.05 ^a	126 ± 26.4	38 ± 8.4 ^a	107 ± 27.4 ^a	0 ± 0.5 ^b
	Week 13 (n = 20)	7.0 ± 0.56	4.9 ± 0.51	2.3 ± 0.23	2.3 ± 0.36	15.9 ± 1.59	0.3 ± 0.05	60 ± 20.0	37 ± 18.6	77 ± 18.7	0 ± 0.5
2000	Week 5 (n = 16)	6.9 ± 0.28	5.0 ± 0.22	1.8 ± 0.22 ^{**}	2.8 ± 0.38	13.6 ± 2.90 [*]	0.3 ± 0.06	153 ± 19.1 [*]	38 ± 6.5	105 ± 23.0	1 ± 0.7
	Week 10 (n = 10)	6.7 ± 0.32	5.0 ± 0.23	1.6 ± 0.15	3.1 ± 0.30	12.5 ± 3.30	0.3 ± 0.07	97 ± 23.9	39 ± 10.3	72 ± 9.6	0 ± 0.5
	Week 13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Values represent mean ± SD. NA, not available; Prot., total protein; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Creat., creatinine; ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

^a n = 19.

^b n = 18.

*Significantly different from control, *P* < .05, Dunnett's test.

**Significantly different from control, *P* < .01, Dunnett's test.

Table 10. Serum Chemistry Values in Salcaprozate Sodium-Treated Female Sprague-Dawley Rats

Dosage, mg/kg/d	Glucose, mg/dL	Cholesterol, mg/dL	Ca, mg/dL	Cl, mEq/L	P, mg/dL	K, mEq/L	Na, mEq/L
0	Week 5 (n = 20)	118 ± 10.5 ^a	9.6 ± 0.40 ^a	103 ± 0.2	7.2 ± 1.03 ^b	5.75 ± 0.72	141 ± 2.2
	Week 13 (n = 20)	180 ± 41.7	11.2 ± 0.55	97 ± 2.3	8.7 ± 1.56	8.06 ± 1.28	142 ± 1.3
2000	Week 5 (n = 16)	108 ± 10.5	9.5 ± 0.44	99 ± 3.4 ^{**}	7.5 ± 1.18	5.77 ± 1.02	140 ± 4.4
	Week 10 (n = 10)	197 ± 25.8	11.4 ± 0.36	95 ± 1.8	7.3 ± 1.32	6.45 ± 0.70	141 ± 2.3
	Week 13	NA	NA	NA	NA	NA	NA

Values represent mean ± SD. NA, not available; Ca, calcium; Cl, chloride; P, phosphorus; K, potassium; Na, sodium.

^a n = 19.

^b n = 18.

*Significantly different from control, *P* < .05, Dunnett's test.

**Significantly different from control, *P* < .01, Dunnett's test.

Table 11. Final Body Weights and Relative Liver and Kidney Weights in Salcaprozate Sodium-Treated Sprague-Dawley Rats

Sex	Dosage, mg/kg/d	Number of Animals Examined	Final Body Weight, g	Liver, g/100 g Body Weight	Kidney, g/100 g Body Weight
Male	0	20	518 ± 59.1	2.98 ± 0.27	0.76 ± 0.05
	2000	16	496 ± 58.4	3.80 ± 0.27 ^{**}	0.95 ± 0.09 ^{**}
Female	0	20 ^a	269 ± 27.4	3.03 ± 0.31	0.80 ± 0.05
	2000	10 ^b	268 ± 24.6	4.15 ± 0.28	0.98 ± 0.06

Values represent mean ± SD.

^a Scheduled euthanasia (week 13).

^b Week 10 sacrifice (see Materials and Methods).

**Significantly different from control, *P* < .01, Dunnett's test.

Table 12. Histopathological Findings in Salcaprostate Sodium-Treated Sprague-Dawley Rats

Sex	Dosage, mg/kg/d	Number of Animals Examined	Pituitary ^a		Stomach ^b	
			(Vacuolation, Pars Distalis), n (%)	(Nonglandular Epithelial Hyperplasia), n (%)		
Male	0	20	0	3 (15)		
	2000	20 ^c	10 (50)	15 (75)		
Female	0	20	0	2 (20)		
	2000	20 ^d	0	5 (25)		

Only the findings for which the incidence and/or severity were of potential significance are listed.

^a Vacuolation was minimal in 7 animals and mild in 3 animals.

^b All hyperplasia was minimal.

^c Includes data from 4 animals that died prior to the end of the study.

^d Combined data from 10 animals that died prior to the end of the study and 10 animals sacrificed at week 10.

Table 13. Hematology (RBC and Coagulation) Values in Salcaprostate Sodium-Treated Male Wistar Rats

Dosage, mg/kg/d	RBC, 10 ⁶ /μL	HB, g/dL	HCT, %	MCV, fl	MCH, pg	MCHC, g/dL	PLT, 10 ³ /μL	PT, (rel.1)	APTT, s
0	Week 5 (n = 10)	8.51	46	54.3	18.84	21.55	1091	0.87	21.3
	Week 12 (n = 10)	9.26	47	51.0	17.72	21.54	950	0.95	20.7
	Week 5 (n = 10)	8.46	46	53.9	18.52	21.33	1033	0.88	21.2
100	Week 12 (n = 10)	9.36	47	50.3	17.40	21.51	960	1.02	23.1 [†]
	Week 5 (n = 10)	8.33	46	54.5	18.84	21.49	945 ^{**}	0.87	20.9
	Week 12 (n = 10)	9.10	46	51.0	17.88	21.80	926	0.97	25.1 [†]
1000	Week 5 (n = 10)	8.64	46	53.3	18.53	21.49	1013	0.81	23.0
	Week 12 (n = 9)	9.56	48	50.2	17.24	21.24	869	0.92	25.3 [†]

RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet (reported as a ratio of the normal activity); PT, prothrombin time; APTT, activated partial thromboplastin time.

^{**}Significantly different from control, $P < .01$, Dunnett's test.

[†] Significant, $P < .05$, Steel test.

Table 14. Hematology (Total and Differential WBC Count) Values in Salcaprozate Sodium-Treated Male Wistar Rats

Dosage, mg/kg/d	WBC, 10 ³ /μL		Neutrophil		Lymphocyte		Monocyte		Eosinophil		Basophil		LUC		
	(n)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)	(10 ³ /μL)	(%)
0	Week 5 (n = 10)	5.99	0.94	15.6	4.78	79.8	0.12	2.1	0.07	1.2	0.03	0.5	0.05	0.8	0.4
	Week 12 (n = 10)	7.03	1.31	18.6	5.36	76.2	0.14	2.0	0.12	1.6	0.08	1.1	0.03	0.4	0.7
100	Week 5 (n = 10)	6.82	1.03	15.1	5.49	80.4	0.14	2.0	0.09	1.4	0.03	0.5	0.05	0.7	0.4
	Week 12 (n = 10)	6.98	1.45	21.0	5.19	74.0	0.15	2.1	0.13	1.8	0.04**	0.6†	0.03	0.4	0.7
500	Week 5 (n = 10)	7.25	1.19	16.5	5.70	78.6	0.17	2.2	0.11*	1.5	0.04**	0.5	0.05	0.7	0.5
	Week 12 (n = 10)	7.66	1.46	19.3	5.82	75.8	0.14	1.9	0.16	2.0	0.04**	0.6†	0.04	0.5	0.8
1000	Week 5 (n = 10)	7.82**	1.26	16.1	6.20**	7.93	0.17	2.2	0.09	1.2	0.04	0.5	0.06	0.8	0.4
	Week 12 (n = 9)	9.06**	1.74	19.2	6.93**	76.6	0.15	1.7	0.14	1.5	0.05	0.6†	0.04	0.4	0.4

WBC, white blood cell; LUC, large unstained cells.

**Significantly different from control, $P < .01$, Dunnett's test.

† Significant, $P < .05$, Steel test.

Table 15. Hematology (Other) Values in Salcaprozate Sodium-Treated Male Wistar Rats

Dosage, mg/kg/d	RDW (rel.1)	HDW, mmol/L	Reticulocyte					
			RETI (rel.1)	RETI, G/L	L RETI (rel.1)	M RETI (rel.1)	H RETI (rel.1)	
0	Week 5 (n = 10)	0.122	1.58	0.023	199	0.604	0.342	0.054
	Week 12 (n = 10)	0.140	2.01	0.021	191	0.583	0.347	0.070
100	Week 5 (n = 10)	0.122	1.65	0.023	194	0.589	0.344	0.067
	Week 12 (n = 10)	0.143	2.14*	0.023	214	0.577	0.352	0.071
500	Week 5 (n = 10)	0.122	1.59	0.026	215	0.569	0.352	0.078
	Week 12 (n = 10)	0.145	2.12	0.021	193	0.570	0.351	0.079
1000	Week 5 (n = 10)	0.117	1.60	0.027†	230**	0.529†	0.381†	0.091†
	Week 12 (n = 9)	0.133	2.10	0.024	225*	0.512†	0.370	0.117†

RDW, red cell volume distribution width; HDW, hemoglobin concentration distribution width; RETI, reticulocyte count; L RETI, M RETI, H RETI, reticulocyte maturity index, low, medium, and high fluorescence, respectively.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

† Significant, $P < .05$, Steel test.

Table 16. Hematology Values (RBC and Coagulation) in Salcaprostate Sodium-Treated Female Wistar Rats

Dosage, mg/kg/d	RBC, 10 ⁶ /μL	HB, g/dL	HCT, %	MCV, fl	MCH, pg	MCHC, g/dL	PLT, 10 ³ /μL	PT (rel.1)	APTT, s
0	Week 5 (n = 10) Week 12 (n = 10)	9.6 9.4	44 40	53.5 49.0	18.85 18.53	21.86 23.43	1047 1045	0.84 0.86	23.9 30.7
100	Week 5 (n = 10) Week 12 (n = 10)	9.5 9.1	44 39	52.8 49.0	18.53 18.37	21.82 23.26	1093 1028	0.85 0.88	27.1 [†] 33.5
500	Week 5 (n = 10) Week 12 (n = 10)	9.6 9.3	43 40	53.8 49.7	19.17 18.69	22.15 23.23	1144 1084	0.81 0.87	32.3 30.4
1000	Week 5 (n = 10) Week 12 (n = 10)	9.7 9.4	44 40	51.7* 50.4	18.20 17.88	21.89 23.27	1193* 1054	0.86 0.85	27.1 [†] 32.8

RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet (reported as a ratio of the normal activity); PT, prothrombin time; APTT, activated partial thromboplastin time.

*Significantly different from control, $P < .05$, Dunnett's test.

[†] Significant, $P < .05$, Steel test.

Table 17. Hematology (Total and Differential WBC Count) Values in Salcaprostate Sodium-Treated Female Wistar Rats

Dosage, mg/kg/d	WBC, 10 ³ /μL	Neutrophil		Lymphocyte		Monocyte		Eosinophil		Basophil		LUC	
		10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%	10 ³ /μL	%
0	Week 5 (n = 10) Week 12 (n = 10)	4.80 4.81	0.81 0.78	16.8 16.7	3.78 3.84	78.4 79.2	0.10 0.08	2.1 1.6	0.06 0.07	1.3 1.6	0.03 0.02	0.6 0.5	0.04 0.02
100	Week 5 (n = 10) Week 12 (n = 10)	4.70 3.46**	0.70 0.70	15.2 20.0	3.80 2.58*	80.5 74.9	0.09 0.08	2.0 2.2	0.06 0.07	1.2 2.0	0.02 0.02	0.4 0.6	0.03 0.01**
500	Week 5 (n = 10) Week 12 (n = 10)	4.39 4.12	0.60 0.81	14.1 20.1	3.60 3.12	81.6 75.3	0.06** 0.07	1.4 [†] 1.7	0.07 0.09	1.6 2.1	0.03 0.02	0.5 0.5	0.04 0.01**
1000	Week 5 (n = 10) Week 12 (n = 10)	4.70 5.60	0.75 0.88	16.5 16.2	3.75 4.45	79.1 79.0	0.08 0.12	1.7 2.2	0.07 0.10	1.5 1.8	0.02 0.02	0.4 0.4	0.03 0.02

WBC, white blood cell; LUC, large unstained cells.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

[†] Significant, $P < .05$, Steel test.

Table 18. Hematology (Other) Values in Salcaprozate Sodium-Treated Female Wistar Rats

Dosage, mg/kg/d	Reticulocyte							
	RDW (rel.1)	HDW, mmol/L	RETI (rel.1)	RETI, G/L	L RETI (rel.1)	M RETI (rel.1)	H RETI (rel.1)	H RETI (rel.1)
0	Week 5 (n = 10)	0.115	1.47	0.028	231	0.589	0.354	0.057
	Week 12 (n = 10)	0.118	1.55	0.023	190	0.555	0.370	0.075
100	Week 5 (n = 10)	0.115	1.45	0.025	204	0.690 [†]	0.285 [†]	0.025 [†]
	Week 12 (n = 10)	0.121	1.63	0.025	203	0.564	0.373	0.063
500	Week 5 (n = 10)	0.121	1.49	0.025	201	0.668 [†]	0.301 [†]	0.031 [†]
	Week 12 (n = 10)	0.137	1.64	0.026	209	0.567	0.369	0.065
1000	Week 5 (n = 10)	0.118	1.57*	0.026	222	0.642	0.322	0.037
	Week 12 (n = 10)	0.124	1.66*	0.025	209	0.563	0.366	0.072

RDW, red cell volume distribution width; HDW, hemoglobin concentration distribution width; RETI, reticulocyte count; L RETI, M RETI, H RETI, reticulocyte maturity index, low, medium, and high fluorescence, respectively.

*Significantly different from control, $P < .05$, Dunnett's test.

[†] Significant, $P < .05$, Steel test.

Table 19. Serum Chemistry Values in Salcaprozate Sodium-Treated Male Wistar Rats

Dosage, mg/kg/d	Prot., g/dL	Albumin, g/dL	Globulin, g/dL	A/G, Ratio	Bilirubin, mg/dL	BUN, mg/dL	Creat., mg/dL	ALP, U/L	ALAT, U/L	ASAT, U/L	GLDH, U/L	CK, U/L	LDH, U/L
0	Week 5 (n = 10)	6.20	2.25	1.76	0.07	12.7	0.26	106	32.5	79.6	6.1	227	249
	Week 12 (n = 10)	6.86	2.54	1.72	0.10	13.4	0.31	60.7	37.2	82.7	10.6	183	300
100	Week 5 (n = 10)	6.27	4.07*	2.20	1.85	12.0	0.17**	87.7	35.5	83.8	4.8*	290	247
	Week 12 (n = 10)	6.85	4.32	2.53	1.71	12.9	0.28*	57.3	36.7	80.2	9.6	171	180
500	Week 5 (n = 10)	6.17	4.08*	2.09*	1.95 [†]	13.1	0.14**	115	34.3	86.8	6.2	226	274
	Week 12 (n = 10)	6.58*	4.25	2.34*	1.82	13.6	0.28	57.1	34.7	86.1	8.2	157	171
1000	Week 5 (n = 10)	6.07	4.11**	1.97**	2.09 [†]	13.0	0.13**	106	32.1	86.6	5.5	244	280
	Week 12 (n = 9)	6.50**	4.30	2.20**	1.97 [†]	13.7	0.30	64.3	32.1	87.0	5.7**	202	289

Prot., total protein; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Creat., creatinine; ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GLDH, glutamate dehydrogenase; CK, creatine kinase; LDH, lactate dehydrogenase. Gamma-glutamyltransferase (GGT) was 0.0 U/L in all animals.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

[†] Significant, $P < .05$, Steel test.

higher total leukocyte (WBC), lymphocyte, and reticulocyte counts, together with a shift toward immature reticulocytes; basophil counts were higher, and activated partial thromboplastin time was prolonged across all SNAC dosages. These differences were not evident in female Wistar rats. All the hematological effects described above were minor, within the range of historical controls for this strain at the testing facility, and were not considered to be toxicologically significant. There were no remarkable hematological effects in males at lower SNAC doses.

Slight differences in serum chemistry values were noted (Tables 19-22). Serum globulin values were reduced at the 5-week time point in males and females, but the change was transient, and there was a reversal of this trend by the end of the study in both SNAC and control animals of both sexes. All serum globulin values were also within historical control ranges. The relationship of this finding to SNAC treatment is not clear.

In SNAC-treated groups, there were in fluctuations in chloride and phosphorus levels, and sodium concentrations were higher across all SNAC dosages. These findings involving electrolytes showed no apparent relationship to dose or occurred only in one sex and were not considered related to SNAC treatment.

The relative liver weights of SNAC-treated animals were significantly higher at 500 (males only) or 1000 mg/kg/d of SNAC; relative kidney weights were significantly higher at 1000 mg/kg/d of SNAC. Kidney and liver weight changes were not associated with histopathological or clinical chemistry changes and were not considered toxicologically significant. Other differences in organ weights were considered to be incidental (see Table 23).

Discussion

In SD rats, oral administration of SNAC at 2000 mg/kg/d produced considerable mortality (50%) in females; mortality among males was much lower (20%). Examination of animals that died or were sacrificed in extremis revealed no clear cause of death. The clinical signs observed in SD rats (decreased activity, prostration, unkempt appearance, etc) in the present study are consistent with those seen in other SNAC experimental animal studies (data not shown). These effects are generally transient, occurring shortly after dosing and lasting a few hours. This is

consistent with the rapid absorption and elimination reported for SNAC.⁶ No hematological effects were reported in SD rats. Decreased globulin concentrations at 2000 mg/kg/d SNAC were observed at interim and terminal sampling points, and there were slight increases in liver and kidney weights.

No significant effects on food consumption, body weights, urinalysis parameters, or ophthalmological examinations were observed following administration of up to 1000 mg/kg/d SNAC to male and female Wistar rats for 13 weeks. Slight differences in some hematology parameters were observed with male Wistar rats at 1000 mg/kg/d SNAC, but these were within the historical control ranges for the testing facility and were not considered toxicologically significant.

In both subchronic toxicity studies described herein, high SNAC dosages were associated with slightly higher relative liver and kidney weights. This effect has also been seen in other experimental animal studies employing high doses of SNAC (≥ 500 mg/kg/d) and may constitute an adaptive response. Because this effect on relative liver and kidney weights was slight and was not associated with any significant corresponding clinical pathology or histopathological changes, it is not considered toxicologically significant.

The toxicological significance of the increased incidence of minimal to mild vacuolation of pituitary cells in SNAC-treated males at the 2000-mg/kg/d level is unclear because it was seen only in SD males and did not appear to have any significant effect on pituitary weight. Cells of the pars distalis produce various trophic hormones (growth hormone, prolactin, adrenocorticotrophic hormone [ACTH], follicle-stimulating hormone [FSH], luteinizing hormone [LH], etc) that influence the function of various organ systems. The production of these hormones by pituitary cells is subject to positive and negative feedback from target and nontarget tissues. In the present study, the affected cells were reported to lack staining, suggesting they may be chromophobes, which generally stain poorly with the standard H&E histological stains. Hyperstimulation of chromophobes might result in higher production (and cytoplasmic accumulation) of ACTH and/or prolactin. However, because there was no evidence of target organ effects (eg, gross or microscopic changes in adrenal glands, testis, prostate), it is possible that this effect was due to normal background variation. Pituitary cell vacuolation has not been observed in

Table 20. Serum Chemistry Values in Salcaprozate Sodium–Treated Male Wistar Rats

Dosage, mg/kg/d	Glucose, mg/dL	Cholesterol, mg/dL	Triglycerides, mg/dL	Phospholipids, mmol/L	Ca, mg/dL	Cl(mEq/l)	P, mg/dL	K, mEq/L	Na, mEq/L	
0	Week 5 (n = 10)	65.63	67.69	37.27	1.66	10.08	102.5	6.65	3.48	143.0
	Week 12 (n = 10)	85.09	70.38	42.72	1.60	11.28	105.8	5.88	3.89	146.2
100	Week 5 (n = 10)	81.27**	71.15	31.81	1.72	9.72	107.5**	5.51**	3.98**	147.7**
	Week 12 (n = 10)	106.00**	75.00	35.45	1.66	11.76**	108.5*	6.03	4.10	149.2*
500	Week 5 (n = 10)	69.45	80.00	49.09	1.90	10.28	106.2**	7.58**	4.09**	149.0**
	Week 12 (n = 10)	100.18	79.23	38.18	1.68	11.36	107.7	6.43*	3.98	149.7**
1000	Week 5 (n = 10)	69.45	63.84	52.72	1.59	10.12	103.8	7.52**	3.74	147.8**
	Week 12 (n = 9)	70.72	66.53	58.18	1.53	11.36	105.2	6.53*	3.79	149.0*

Ca, calcium; Cl, chloride; P, phosphorus; K, potassium; Na, sodium.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

Table 21. Serum Chemistry Values in Salcaprozate Sodium–Treated Female Wistar Rats

Dosage, mg/kg/d	Prot., g/dL	Albumin, g/dL	Globulin, g/dL	A/G, Ratio	Bilirubin, mg/dL	Creat., mg/dL	ALP, U/L		ASAT, U/L		GLDH, U/L		CK, U/L	LDH, U/L
							L	U/L	L	U/L	L	U/L		
0	Week 5 (n = 10)	6.42	4.35	2.07	2.11	0.12	15.7	49.5	26.5	74.2	5.6	218	265	
	Week 12 (n = 10)	7.40	5.08	2.32	2.20	0.14	16.6	24.2	31.8	77.1	10.9	152	201	
100	Week 5 (n = 10)	6.59	4.58	2.01	2.28	0.10	15.9	41.1	28.9	82.6	6.9	248	276	
	Week 12 (n = 10)	7.58	5.08	2.31	2.29	0.12	17.6	21.1	27.7	70.0	7.5	207	231	
500	Week 5 (n = 10)	6.47	4.44	2.02	2.20	0.09	15.4	54.7	26.0	81.4	5.0	242	278	
	Week 12 (n = 10)	7.57	5.22	2.35	2.23	0.09†	18.3	24.0	26.6	73.5	5.3	161	182	
1000	Week 5 (n = 10)	6.30	4.44	1.86*	2.39†	0.09	13.2	61.2	25.5	83.6	4.6	307	371	
	Week 12 (n = 10)	7.11	5.09	2.02**	2.53†	0.12	15.4	23.5	26.0	74.8	5.3	194	303**	

Prot., total protein; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Creat., creatinine; ALP, alkaline phosphatase; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GLDH, glutamate dehydrogenase; CK, creatine kinase; LDH, lactate dehydrogenase. Gamma-glutamyltransferase (GGT) was 0.0 U/L in all animals.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

† Significant, $P < .05$, Steel test.

Table 22. Serum Chemistry Values in Salcaproate Sodium-Treated Female Wistar Rats

Dosage, mg/kg/d	Glucose, mg/dL	Cholesterol, mg/dL	Triglycerides, mg/dL	Phospholipids, mmol/L	Ca, mg/dL	Cl, mEq/L	P, mg/dL	K, mEq/L	Na, mEq/L
0	Week 5 (n = 10) 79.81	50.76	27.27	1.48	9.92	102.8	5.26	3.03	141.3
	Week 12 (n = 10) 95.81	54.61	33.63	1.53	11.44	103.7	4.30	3.23	141.2
100	Week 5 (n = 10) 81.81	53.46	18.18**	1.45	9.92	108.3**	4.70	3.40**	146.9**
	Week 12 (n = 10) 101.81	58.07	28.18	1.67	11.76	107.5**	3.90	3.35	144.6**
500	Week 5 (n = 10) 78.54	54.23	26.36	1.50	10.28*	111.6**	5.88*	3.49**	147.3**
	Week 12 (n = 10) 95.63	56.53	34.54	1.72	11.72	108.1**	4.82	3.35	145.9**
1000	Week 5 (n = 10) 72.18	56.15	32.72	1.56	10.20	106.9	6.03**	3.47**	147.3**
	Week 12 (n = 10) 70.72**	59.61	47.27**	1.68	11.64	104.3	5.47**	3.29	145.4**

Ca, calcium; Cl, chloride; P, phosphorus; K, potassium; Na, sodium.

*Significantly different from control, $P < .05$, Dunnett's test.

**Significantly different from control, $P < .01$, Dunnett's test.

Table 23. Final Body Weights and Relative Liver and Kidney Weights in Salcaproate Sodium-Treated Wistar Rats

Sex	Dosage, mg/kg/d	Number of Animals Examined	Final Body Weight, g	Liver, g/100 g Body Weight	Kidney, g/100 g Body Weight
Male	0	10	432 ± 33.8	2.89 ± 0.20	0.51 ± 0.04
	100	10	447 ± 44.0	3.15 ± 0.33	0.52 ± 0.06
	500	10	449 ± 43.8	3.28 ± 0.34**	0.59 ± 0.04**
	1000	9	418 ± 10.0	3.45 ± 0.17**	0.62 ± 0.05
Female	0	10	250 ± 19.3	3.11 ± 0.26	0.59 ± 0.05
	100	10	236 ± 19.3	3.28 ± 0.19	0.58 ± 0.05
	500	10	248 ± 17.9	3.37 ± 0.29	0.58 ± 0.03
	1000	10	237 ± 13.4	3.78 ± 0.29**	0.71 ± 0.05**

Values represent mean ± SD.

**Significantly different from control, $P < .01$, Dunnett's test.

other toxicological studies employing SNAC and, as described herein, was not noted in Wistar rats.

SD and Wistar rats treated with SNAC exhibited signs of gastrointestinal tract irritation. These were noted in both control and SNAC-treated animals, occasionally with a slightly higher incidence or a slightly greater severity in animals receiving SNAC. Although other gastrointestinal effects (emesis and diarrhea) have been reported in other investigations with monkeys receiving high SNAC doses (≥ 1800 mg/kg/d), no irritation has been noted. Hyperplasia of the nonglandular stomach (forestomach) epithelium was noted in SD rats. This effect may constitute compensatory hyperplasia secondary to irritation caused by the repeated administration of high doses of the test compound via an oral gavage cannula.⁸ The occurrence of forestomach epithelial hyperplasia in vehicle control animals as well as in SNAC-treated animals would suggest that this is due in part to the gavage dosing method. In addition, it is important to consider that there is no functional analog of the rat forestomach in humans. The relevance of this finding to humans is therefore questionable.

A no-observed-adverse-effect level (NOAEL) for SNAC was not determined in SD rats. The single dose of 2000 mg/kg/d induced significant mortality and clinical signs in females and a lower mortality in males. Decreased globulin and increased liver and kidney weights appeared to be associated with SNAC dosing. These changes and others noted above in 2000-mg/kg/d SNAC animals all appeared to be of little or no toxicological significance or to be potentially related to local gastric effects probably attributable to the dosing technique.

The SNAC NOAEL, as identified by the Wistar study data, was considered to be the high dose of 1000 mg/kg/d. No mortality or clinical signs were observed. Changes in serum globulin were transient, and increases in liver and kidney weights were not toxicologically significant.

In summary, oral gavage administration of SNAC at 2000 mg/kg/d to rats for 13 weeks was associated with significant mortality. At 1000 mg/kg/d, SNAC was associated with several mild changes in clinical chemistry and organ weights. However, because

none of these changes was considered toxicologically significant, a NOAEL of 1000 mg/kg/d is proposed.

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