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APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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Applicant:	Novo Nordisk
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1 Executive Summary

1.1 Introduction

Rybelsus® (semaglutide tablet) for oral administration is a glucagon-like peptide (GLP-1) analog co-formulated with salcaprozate sodium (SNAC), a novel absorption enhancer that transiently increases the permeability of the gastric epithelium to promote absorption of semaglutide in a size-selective manner. It is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes.

1.2 Brief Discussion of Nonclinical Findings

Semaglutide for subcutaneous injection was approved in 2017; therefore, the nonclinical program for oral semaglutide focused mainly on SNAC. As a novel excipient, SNAC was qualified in a full toxicology program.

Pharmacology
Semaglutide lowers fasting and postprandial blood glucose and reduces body weight by stimulating insulin secretion and lowering glucagon secretion in a glucose-dependent manner. In Rybelsus® (semaglutide tablet), semaglutide is co-formulated with the novel absorption enhancer SNAC. SNAC is a small fatty acid derivative that interacts with plasma membranes to promote transcellular absorption of semaglutide and facilitates localized decreases in pH to protect semaglutide from degradation by gastric enzymes.

Safety pharmacology studies were conducted with SNAC in vitro, in rats and in monkeys. Acute SNAC exposure in rats resulted in decreased touch response (≥16-fold higher than clinical exposure based on BSA), piloerection, decreased mean respiration rates and mortality (≥32-fold higher than clinical exposure based on BSA) which were due to inhibition of cellular respiration through inhibition of complex I in the electron transport chain.

Absorption, Distribution, Metabolism and Excretion
After oral administration, SNAC is rapidly absorbed and eliminated, with a C_{max} typically reached within 2 hours and a $T_{1/2}$ ranging between 1-3 hours. When SNAC is co-formulated with semaglutide, both products are absorbed due to a transient increase in permeability of the gastrointestinal epithelium in a highly localized area around the immediate vicinity of the tablet. SNAC-facilitated absorption of semaglutide showed very high inter-animal variability in rats, dogs, and monkeys and was influenced by the fasting state of the animals.

SNAC is highly bound to plasma proteins, predominantly albumin. The free fraction in animals is slightly higher than in humans. Radiolabeled SNAC rapidly distributes to all tissues at levels below plasma levels, except in the excretory system (kidney and urinary bladder wall and contents) and gastrointestinal system (cecum mucosa, small intestine mucosa, and stomach mucosa). Very low levels were observed in the CNS (~2%). Drug-related radioactivity was rapidly eliminated from most tissues within 24 hours, with quantifiable radioactivity limited to adipose tissue and skin at 168 hours

post-dose. SNAC crosses the placenta, distributes to all fetal tissues reaching peak levels at 4 hours and persists in the gastrointestinal tract up to 24 hours. SNAC is secreted into breast milk with a mean milk/plasma ratio that ranges between 7 and 12 in the 4 to 24 hour time frame following a single administration.

SNAC is rapidly metabolized via β-oxidation and glucuronidation mainly by UGT2B7 with additional contributions by UGT1A8 and UGT1A7. No unique metabolites were detected in humans. The primary route of excretion is the kidney, with negligible amounts recovered as unchanged SNAC. Metabolism is responsible for the majority of SNAC clearance in animals and humans.

General Toxicology
Pivotal repeat dose studies were conducted in mice up to 3 months, rats up to 12 months, and monkeys up to 9 months. Adverse clinical signs and mortality occurred in all species tested starting at low multiples to the clinical exposure. Generally, mortalities occurred within 3 hours after dosing and were not associated with histopathological or standard clinical chemistry findings. Some common clinical signs observed before death in animals included sedation/decreased activity, ruffled fur, abnormal respiration and salivation. Considerable inter-individual variability was observed for SNAC plasma concentrations with overlap of exposure between different dosing groups, making meaningful correlations between mean exposure levels and clinical signs challenging. Therefore, a series of mechanistic studies were performed where the onset of clinical signs was evaluated concurrently with SNAC plasma concentrations. The in vivo studies were conducted under fasting conditions to minimize variability in SNAC exposure, and mostly in female rats, as they appear to be more sensitive than males to SNAC effects.

Mechanistic Studies
SNAC caused a concentration-dependent inhibition of ATP biosynthesis in isolated mitochondria and submitochondrial particles and inhibited cellular respiration in several cell types with IC_{50} values that ranged between 175 and 1214 mcM. SNAC metabolites were at least 10-times less potent inhibitors of ATP biosynthesis in mitochondria indicating that metabolism could be important for detoxification of the parent compound. When human serum albumin was added in in vitro studies, the concentration of SNAC needed to inhibit cellular respiration increased indicating that albumin can sequester SNAC and prevent it from entering the inner mitochondrial membrane where it interacts with complex I and inhibits cellular respiration.

Following single high doses of SNAC (≥900 mg/kg), a dose-dependent increase in adverse clinical signs (e.g. apathy, abnormal respiration, reduced alertness and startle response, abnormal body carriage, abnormal gait, passivity, reduced body tone, salivation and convulsions) and mortality were observed at approximately >100-fold the clinical C_{max}. SNAC exposure also resulted in changes in clinical chemistry parameters including decreased blood and CSF glucose, and increases in plasma and cerebrospinal fluid (CSF) lactate levels. Decreases in blood pH, pO_2, sO_2, and HCO_3^- were also seen consistent with an effect of SNAC on cellular respiration. These findings generally occurred above 45-fold the clinical C_{max}, with only few animals showing changes in lactate levels without obvious clinical signs at lower exposures (between 3 to

30-fold the clinical C_{max}). In an investigative 13-week study, small but significant increases in CSF lactate levels (~ 25%) were observed at the clinical exposure in few animals.

Genetic Toxicology and Carcinogenicity
SNAC was not mutagenic or clastogenic in a standard battery of GLP-compliant in vitro and in vivo genetic toxicology studies and was not carcinogenic in a 2-year Sprague Dawley rat or 6-month rasH2 transgenic mouse study.

Reproductive and Developmental Toxicology
Fertility, early embryonic development and pre- and post-natal development was assessed in rats and rabbits at once daily dosing of 1,000 mg/kg. SNAC had no effect on mating, or male and female fertility indices in rats, and was not teratogenic in rats or rabbits at doses 32- or 65-fold clinical exposure, respectively (based on BSA). In a pre- and post-natal development study in rats, prolonged gestation, and an increased incidence of stillbirths and early pup mortality was observed at 32-fold clinical exposure (based on BSA). A NOAEL for maternal toxicity and postnatal mortality was not determined in this study. No remarkable effects on neurobehavioral development or on fertility and reproductive performance of the F1 generation were observed.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of Rybelsus® (semaglutide tablet).

1.3.2 Additional Nonclinical Recommendations

SNAC has been shown to inhibit cellular respiration in animals at high concentrations. Though SNAC exposure associated with toxicity in animals was not achieved in Phase 3 studies with semaglutide/SNAC, a risk for higher exposure to SNAC and/or its metabolites is plausible for individuals with weak UGT2B7activity (an enzyme involved in SNAC metabolism) or with compromised hepatic function. Similarly, pediatric patients and lactating infants may be at greater risk given the immaturity of UGT2B7 in this population and because it is unknown if SNAC and or its metabolites accumulate in milk.

1.3.3 Labeling

Pharm/Tox labeling recommendations for SNAC are shown below in red.

8 USE IN SPECIFIC POPULATIONS
8.1
Animal data
Salcaprozate sodium (SNAC), an absorption enhancer in TRADENAME, crosses the placenta and reaches fetal tissues in rats. In a pre- and postnatal development study in pregnant Sprague Dawley rats, SNAC was administered orally at 1,000 mg/kg/day

(exposure levels were not measured) on gestation day 7 through lactation day 20. An increase in gestation length, an increase in the number of stillbirths and a decrease in pup viability were observed.

8.2 Lactation

Risk Summary

There are no data on the presence of semaglutide in human milk, the effects on the breastfed infant, or the effects on milk production. Semaglutide was present in the milk of lactating rats. SNAC and/or its metabolites concentrated in the milk of lactating rats. When a substance is present in animal milk, it is likely that the drug will be present in human milk. *(see Data)*. Higher SNAC plasma levels may occur in neonates and infants, (b) (4) ecause of the potential for serious adverse reactions in the breastfed infant due to the possible concentration of SNAC (b) (4) advise patients that breastfeeding is not recommended during treatment with TRADENAME.

Data

In lactating rats, semaglutide was detected in milk at levels 3-12 fold lower than in maternal plasma. SNAC and/or its metabolites were detected in milk of lactating rats following a single maternal administration on lactation day 10. Levels of SNAC and/or its metabolites in milk were approximately 12-fold higher than those found in maternal blood based on C_{max}.

13.2 Animal Toxicology and/or Pharmacology

Increases in lactate levels and decrease in glucose levels in the plasma and cerebrospinal fluid (CSF) were observed in mechanistic studies with SNAC in rats. Small but statistically significant increases in lactate levels (up to 2-fold) were observed in a few animals at approximately the clinical exposure. At higher exposures (≥45-times clinical C_{max}) these findings were associated with moderate to marked adverse clinical signs (lethargy, abnormal respiration, ataxia, and reduced activity, body tone and reflexes) and marked decreases in plasma and CSF glucose levels. These findings are consistent with inhibition of cellular respiration and lead to mortality at SNAC concentrations ≥100-times the clinical C_{max}.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): RN910463-68-2

Generic Name: Semaglutide

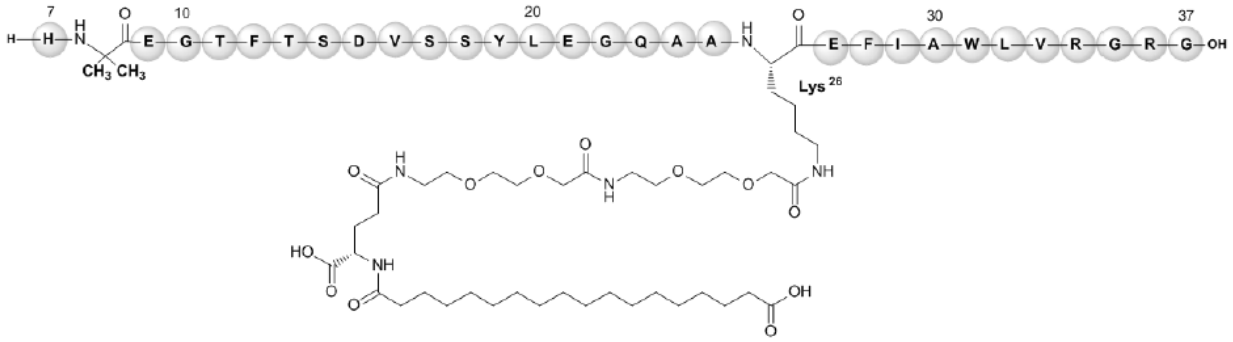
Code Name: NNC 0113-0217; NNC 0113-0000-0217; 0217; NN9535

Chemical Name: $N^{\varepsilon 26}$ [(S)-(22,40-dicarboxy-10,19,24-trioxo-3,6,12,15-tetraoxa-9,18,23-triazatetracontan-1-oyl)] [Aib^{8}, Arg^{34}] GLP-1-(7-37) peptide

Molecular Formula/Molecular Weight: $C_{187}H_{291}N_{45}O_{59}$ / 4,113.6 g/mole

Structure or Biochemical Description

Figure 1: Semaglutide chemical structure



Pharmacologic Class: Long acting GLP-1 receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

Semaglutide

NDA #	Drug Name	Sponsor	Indication	Status
209637	Ozempic (Semagllutide)	Novo Nordisk Inc	Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus	Approved

SNAC

IND #	Drug Name	Sponsor	Indication	Status
(b) (4)				

(b) (4)

2.3 Drug Formulation

Table 1: Composition of oral semaglutide used in the phase 3 clinical trial expressed as "per tablet"

Component	Composition (mg/tablet)			Function	Reference to standard
	3 mg tablet	7 mg tablet	14 mg tablet		
Drug substance					
Semaglutide[1]	3	7	14	Active ingredient	Novo Nordisk
Excipients					
SNAC	300	300	300	Absorption enhancer	Novo Nordisk
Cellulose, microcrystalline	(b) (4)				Ph Eur, USP
Povidone					Ph Eur, USP
Magnesium stearate[2]					Ph Eur, USP
Gross weight	400.7	404.7	411.7	N/A	N/A

[1] The amounts of semaglutide are shown (b) (4)

(b) (4)

[2] (b) (4)

2.4 Comments on Novel Excipients

Salcaprozate sodium (SNAC) is a novel excipient and will be the focus of this NDA review. It is a fatty acid derivative that is used as an absorption enhancer.

CAS Registry Number (Optional): 203787-91-1

Generic Name: Salcaprozate sodium (SNAC)

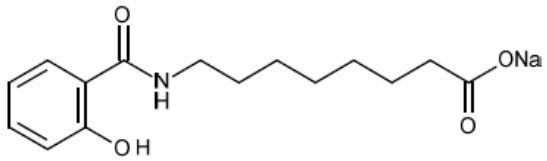
Code Name: SNAC, *N*-(salicyloyl)-8-aminooctanoic acid monosodium salt, monosodium *N*-{8-(2-phenoxybenzoyl)amino} octanoate, sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate E414 monosodium salt, EMIS000414 monosodium salt

Chemical Name: Sodium 8-[(2-hydroxybenzoyl)amino]octanoate

Molecular Formula/Molecular Weight: $C_{15}H_{20}NNaO_4$/301.32 g/mol

Structure or Biochemical Description

Figure 2: Chemical structure of SNAC



2.5 Comments on Impurities/Degradants of Concern

While semaglutide for oral administration is structurally identical to injectable semaglutide (Ozempic®), a different (b) (4) and optimized production process was introduced between Phase 2 and 3 (b) (4) The major semaglutide-related impurities in the degraded semaglutide drug product have been identified and found to be (b) (4) . All of the mentioned semaglutide-related impurities have been observed in nonclinical batches during development.
All impurities/degradants present in SNAC batches were qualified in toxicology studies or were present at levels below the reporting threshold described in ICH Q3A.

2.6 Proposed Clinical Population and Dosing Regimen

Rybelsus® is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. Rybelsus® has not been studied in patients with a history of pancreatitis and is not indicated for use in patients with type 1 diabetes mellitus or for the treatment of diabetic ketoacidosis.

Three, 7 or 14 mg of Rybelsus® is administered daily (dose escalation occurs at one-month intervals if additional benefits are needed).

2.7 Regulatory Background

Semaglutide is an FDA approved long acting GLP-1 analog with a high degree of homology to human GLP-1. Semaglutide injection was approved under NDA 209637 Ozempic® (semaglutide) on December 5, 2017. To facilitate long-term glycemic control using a convenient oral formulation, semaglutide was co-formulated with a novel absorption enhancer, salcaprozate sodium or SNAC. A full nonclinical program was completed to qualify this novel excipient under IND 114464 (submitted on September 26, 2013). On March 20, 2019, Novo Nordisk Inc. filed NDA 213051 to seek approval for Rybelsus® as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes. On the same day, Novo Nordisk also filed NDA 213182 to seek approval for Rybelsus® to reduce the risk of major adverse cardiovascular events (cardiovascular death, nonfatal myocardial infarction or non-fatal stroke) in adults with type 2 diabetes mellitus and established cardiovascular disease (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

Pivotal nonclinical studies addressing pharmacology, general toxicology, genotoxicity and carcinogenicity, and reproductive and developmental toxicology of SNAC were reviewed under IND 114464 and are summarized in this NDA review.

4 Pharmacology

Semaglutide binds the human GLP-1 receptor and is thought to lower blood glucose by stimulating glucose-dependent insulin secretion and insulin biosynthesis, inhibiting glucagon secretion and decreasing gastric emptying. Salcaprozate sodium (SNAC) is a small fatty acid derivative and absorption enhancer that facilitates the absorption of semaglutide across the gastrointestinal epithelium.

SNAC is currently available in a marketed 'medical food' in the US where 1,000 mcg vitamin B12 is formulated with 100 mg SNAC[1]. The amount of SNAC present at the maximum recommended dose for this product is 200 mg, which is less than the 300 mg/day dose proposed in Rybelsus®; therefore, SNAC has not been used in a marketed product at the proposed level.

4.1 Primary Pharmacology

In vitro and in vivo studies show that when SNAC is co-formulated with peptides it transiently increases the fluidity and permeability of cellular membranes to facilitate intracellular transport of peptides in a concentration-dependent and size-selective manner. In vitro studies with cell membranes and monolayers show that SNAC's ability to facilitate transport of compounds increased with increasing concentrations, decreased as the molecular weight of the compound increased over 4 kDa (absorption of compounds greater than 150 kDa is minimal) and occurred over a period of 20-90 minutes after SNAC was removed. Nuclear magnetic resonance studies show that SNAC does not associate with semaglutide in solution; therefore, increased absorption does not occur through an interaction between these two compounds. To demonstrate that semaglutide is pharmacologically active when administered orally and co-formulated with SNAC, male db/db rats were given a single oral dose of vehicle, semaglutide, SNAC, or semaglutide co-formulated with SNAC. When administered orally in an intraperitoneal glucose tolerance test, reduced glucose levels were observed when SNAC was co-formulated with semaglutide but not with semaglutide alone. These findings show that orally administered semaglutide co-formulated with SNAC can enter the blood stream and produce a pharmacological effect.

[1] https://eligenb12.com/wp-content/themes/eligen/pdfs/cccPC4745C_3c2.pdf

4.2 Secondary Pharmacology

Using an in vitro radioligand binding assay, SNAC was shown to inhibit human prostanoid DP and prostanoid EP_4 (49 and 34% at 30 mcM SNAC, respectively) and serotonin 5-HT_{2B} (>30% at 100 mcM SNAC) receptors. Additionally, SNAC caused reduction of spontaneous tone in a tracheal relaxation assay (21% at 10 mcM and 70% at 30 mcM) and inhibition of arachidonate-induced platelet aggregation (100% at 30 mcM), which may account for the moderate relaxation of spontaneous tone in guinea pig trachea (48% at 30 mcM). Since the highest recorded C_{max} value for SNAC after an oral dose of 300 mg in humans was 9,300 ng/mL or 31 mcM and albumin-mediated SNAC-binding will reduce the amount of free SNAC available to interact with these receptors in vivo, no significant inhibitory effects would be expected in humans.

4.3 Safety Pharmacology

Safety pharmacology studies were conducted in vitro, in rats and in monkeys to investigate the effect of SNAC on central nervous, cardiovascular and respiratory systems.

CNS
After a single oral dose of SNAC, male Sprague Dawley rats experienced generalized signs of CNS depression including a slight decrease in touch response (≥500 mg/kg), and decreased respiration and piloerection (≥1,000 mg/kg) that were transient in surviving rats. Transient decreases in mean body temperature from baseline were seen in female rats given a single 900 or 1,500 mg/kg dose of SNAC. Mortalities also occurred at doses ≤1,000 mg/kg.

Cardiovascular system
SNAC had no clear effects on the hERG tail current at doses up to 1 mM and other cardiac ion channels at concentrations up to 200 mcM. In female Sprague Dawley rats implanted with telemetry radiotransmitters that were administered 900 or 1,500 mg/kg SNAC orally on Day 1 and Day 8, transient increases in mean heart rate were observed. Consistent with increased heart rates, RR interval time decreased and QT and corrected QT interval times increased. Additionally, increases in atrial (pause and premature beat) and junctional (salvo) arrhythmias were observed at 900 mg/kg and increases in atrial (premature beat), ventricular (beat), junctional (beat), and other arrhythmias were observed at 1,500 mg/kg/day. Ultimately, 2/8 rats in the 900 mg/kg/day group (C_{max} values ranged between 240,593 - 445,346 ng/mL or 26- to 48-fold higher than clinical C_{max}) and 3/8 rats in the 1500 mg/kg/day group (C_{max} values ranged between 211,430 – 539,136 ng/mL or 23- to 58-fold higher than clinical C_{max}) died after experiencing marked decreases in diastolic arterial pressure. High levels of exposure found in this study would indicate that mortalities occurred due to SNAC inhibition of complex I in the electron transport chain and subsequent inhibition of cellular respiration; therefore, cardiovascular findings observed in this study may be a secondary response. Conscious monkeys instrumented with a telemetry transmitter did

not experience any treatment-related effects on cardiovascular parameters at doses up to 600 mg/kg for 6 months at 6 to 7-fold the clinical exposure based on C_{max}.

Respiratory system
A mechanistic study where female Sprague Dawley rats were given 900 or 1,500 mg/kg SNAC orally on Day 1 and Day 8 had increased mean respiration rates. Mortality also occurred at doses ≥1,000 mg/kg, with the lungs of decedent rats showing reddening with small hemorrhagic areas. A safety pharmacology study in male rats showed no statistically significant effects on mean respiration rates or tidal volumes at doses up to 1,000 mg/kg (32-fold clinical exposure based on BSA).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

SNAC Absorption
SNAC absorption was evaluated after oral administration in mice, rats, and monkeys. In all test species examined, SNAC was rapidly absorbed, typically reached C_{max} in under 2 hours and had a half-life that ranged between 1-3 hours. Although systemic exposure was highly variable, AUC and C_{max} values generally increased with increasing dose and female rodents tended to have a higher systemic exposure when compared to male rodents. In dogs and monkeys, the relative oral bioavailability of semaglutide in the presence of SNAC was estimated to range from 0.04 – 4.04%. SNAC absorption was influenced by the fasting state of the animal. Fasted Sprague Dawley rats given a single oral dose of ^{14}C-SNAC had $AUC_{(0\text{-}6h)}$ values 1.4 to 3-fold greater than unfasted rats.

Semaglutide Absorption After Co-formulation with SNAC
Absorption of semaglutide after co-formulation with SNAC has been investigated both in vitro and in vivo (rats, dogs and monkeys). In vitro, SNAC promoted trans-epithelial permeation of semaglutide across Caco-2 (human epithelial colorectal adenocarcinoma cell line) monolayers where increasing concentrations of SNAC (up to 80 mM) resulted in increased transport of semaglutide. Consistent with in vitro studies, a single oral administration of semaglutide co-formulated with SNAC to fasting beagle dogs resulted in absorption of semaglutide through the stomach. Systemic exposure to semaglutide over a 1-hour period was comparable between anesthetized dogs treated intragastrically after pyloric ligation and conscious dogs treated orally without pyloric ligation. Plasma levels of semaglutide were higher in the splenic vein 30 minutes after dosing when compared to the portal vein in anesthetized dogs dosed intragastrically with oral semaglutide. Optimal absorption of semaglutide occurred in the presence of 300 mg SNAC (higher levels of SNAC resulted in decreased bioavailability of semaglutide). Additionally, semaglutide exposure increased in a greater than dose proportional manner with increasing dose when administered with 300 mg SNAC.

Similar to SNAC absorption, SNAC-facilitated absorption of semaglutide showed very high inter-animal variability in rats, dogs, and monkeys and was influenced by the fasting state of the animal. When the time frame between dosing and feeding was shortened in beagle dogs, decreased T_{max} and C_{max} values for semaglutide were observed. Fasted beagles fed 240 minutes after dosing had a 3 to 5-fold higher systemic exposure to semaglutide when compared to fasted dogs fed 30 and 15 minutes after dosing (based on AUC values).

In rats and dogs, semaglutide and SNAC absorption was highly localized to the area in the immediate vicinity of the tablet. Immunohistochemical staining for semaglutide and SNAC was evident in the surface and cytoplasm of mucous epithelial cells of the stomach at the site of the tablet or blood vessels of the lamina propria mucosa in the area surrounding the tablet.

SNAC Distribution

Two different radiolabeled tracers (^{3}H and ^{14}C labeling of the alkyl chain) were used to examine the distribution of SNAC in mice and rats. After a single dose of ^{3}H-SNAC was administered orally to CD-1 mice, whole body autoradiography showed that SNAC was rapidly absorbed from the gastrointestinal tract and distributed to the liver and kidney. SNAC-related radioactivity was also detected in the gallbladder which is indicative of biliary secretion and enterohepatic recirculation.

When a single oral dose of ^{14}C-SNAC was given to male, non-pregnant female or pregnant female rats, quantifiable SNAC-related radioactivity was detected in the plasma 1 minute after dosing. Generally, SNAC plasma concentrations exceeded those in the tissue and were slightly higher in the portal vein when compared to the jugular vein. SNAC and its five major metabolites distributed to highly perfused tissues within 1.5 hours in rats. SNAC-related radioactivity present in tissues was considerably higher in females when compared to males up to 12 hours after dosing. Tissues containing the highest concentration of SNAC-related radioactivity included members of the excretory system (urinary bladder wall and contents) and gastrointestinal system (cecum mucosa, small intestine mucosa, and stomach mucosa), consistent with a product that passes through the digestive tract and is eliminated through biliary secretion. Negligible amounts of SNAC and SNAC metabolites (~2%) passed through the blood-brain barrier and were detected in the brain, CSF, choroid plexus, meninges and spinal cord by whole body autoradiography. No binding to melanin was observed. SNAC-related radioactivity was rapidly eliminated from most tissues by 1.5 or 24h post-dose. SNAC and up to 23 metabolites were detected in hepatic portal vein plasma, jugular vein plasma, and liver with the most abundant metabolite being a β-oxidation metabolite (E506). Metabolite concentrations generally peaked between 7-30 minutes after dosing and gradually declined thereafter, with the exception of E506 that persisted until the end of the 60 minute observation period. SNAC and/or its metabolites appear to be lipophilic because they persisted in white and brown fat and the skin at low levels for at least 168 hours after administration.

Placental Transfer and Fetal Distribution

Whole body autoradiography of pregnant rats showed that SNAC had a similar distribution profile to non-pregnant rats. In pregnant rats treated orally with ^{14}C-SNAC on gestation day 18, SNAC-related radioactivity crossed the placenta and was readily detected in all fetal organs examined. The levels of SNAC in fetal tissues peaked 4 hours after maternal administration and the highest levels were observed in the gastrointestinal tract, mammary tissue, and placenta. While most SNAC-related radioactivity had been eliminated from fetal tissue, SNAC-related radioactivity persisted in the fetal gastrointestinal tract 24 hours after maternal dosing.

When pregnant rats were allowed to litter and a single oral ^{14}C-SNAC dose was administered 10 day post-partum, SNAC-related radioactivity was detected in the milk of lactating females for up to 24 hours. Radiolabeled SNAC (500 mg/kg) was present at a milk/plasma ratio of 12 indicating that SNAC and/or its metabolites accumulate in the lipophilic milk of lactating rats.

Table 2: Placental transfer and lacteal secretion of ^{14}C-SNAC in the rat after an oral dose

	Dam - µg SNAC equivalents/gram of tissue				
Time	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**
Plasma	148	244	273	30.2	0.741
Aortic wall	58.6	137	122	38.3	4.74
Blood	93.5	156	201	22.3	BLQ
Bone marrow	18.7	28.9	53.9	4.64	1.83
Bone surface	24.0	24.6	48.4	10.4	14.5
Mandibular lymph nodes	25.9	59.6	96.3	9.09	BLQ
Spleen	26.5	54.1	63.7	4.85	BLQ
Bile ducts and contents	173	154	563	63.7	8.15
Kidney cortex	206	243	314	141	11.0
Kidney medulla	107	286	366	83.6	2.00
Liver	61.9	120	151	22.1	1.54
Urinary bladder wall	153[a]	1750[a]	1010[a]	225[a]	8.73
Urine	5190	5180	9240	4180	33.6
Lung	79.5	133	141	19.2	BLQ
Nasal mucosa	14.8	23.5	53.2	2.26	2.29
Trachea	33.6	38.3	148	7.43	20.6
Clitoris	49.9	50.2	70.2	4.78	2.27
Ovary	31.7	46.3	81.0	11.2	2.77
Uterus	24.6	56.8	72.8	56.5	8.28
Adrenal cortex	41.0	53.1	68.5	8.94	3.36
Adrenal medulla	58.4	91.8	98.6	15.2	BLQ
Pancreas	32.1	52.7	62.5	5.90	BLQ
Pineal body	28.3	64.9	87.2	9.48	BLQ
Pituitary gland	25.3	50.3	61.2	11.6	BLQ
Thymus	20.3	44.8	45.5	3.52	BLQ
Thyroid gland	29.4	83.2	65.2	18.5	1.37
Exorbital lachymal gland	15.6	37.6	31.1	4.02	BLQ
Harderian gland	18.4	64.6	135	7.52	BLQ
Intra-orbital lachymal gland	20.9	43.1	44.7	4.51	BLQ
Lens	BLQ	BLQ	BLQ	BLQ	BLQ
Uveal tract/retina	35.3	62.3	72.3	8.81	5.26
Brain	1.79	2.42	3.67	BLQ	BLQ
Choroid plexus	9.48	15.4	15.3	1.84	BLQ
Meninges	18.1	31.5	32.4	3.08	BLQ
Spinal cord	1.63	2.68	3.46	BLQ	BLQ
Caecum mucosa	49.7	79.3	380	403[a]	17.0
Large intestine mucosa	47.6	66.6	157	286	8.41
Oesophageal wall	80.6	74.7	104	17.1	2.14
Rectum mucosa	31.8	73.9	132	8.51	3.51
Small intestine mucosa	45.0	323[a]	258	119	2.01
Stomach mucosa (fundic)	345[a]	275[a]	92.9	7.97	BLQ
Stomach mucosa (non-fundic)	1200[a]	1440[a]	1150[a]	51.5	1.78
Brown fat	27.5	103	84.6	24.9	42.5
White fat	13.3	43.8	41.9	12.6	9.67
Periodontal membrane	31.1	45.6	71.6	5.80	BLQ
Salivary glands	20.9	54.6	54.3	6.64	2.96
Tongue	30.6	70.1	74.5	7.97	1.38
Tooth pulp	61.3	122	101	14.1	BLQ
Muscle	8.28	18.0	23.5	1.95	BLQ
Myocardium	35.4	80.2	89.2	9.09	BLQ
Skin	42.8	85.3	102	8.05	13.2

	Foetal Tissues- µg SNAC equivalents/gram of tissue				
Time	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**
Amniotic fluid	BLQ	24.3	2.49	4.19	1.67
Foetal adrenal	27.6	47.9	79.3	19.1	1.37
Foetal blood	23.4	61.0	80.3	16.4	BLQ
Foetal brain	2.39	10.3	26.0	6.42	BLQ
Foetal eye	11.7	50.4	69.0	14.3	2.39
Foetal gastrointestinal tract	9.90	59.0	79.3	19.7	84.6
Foetal kidney	35.1	53.9	78.6	25.9	2.63
Foetal liver	17.0	48.9	70.6	13.8	BLQ
Foetal lung	11.7	41.0	61.6	10.9	2.28
Foetal myocardium	6.43	45.6	66.6	12.6	BLQ
Foetal skin	14.3	87.3	83.6	21.2	3.22
Foetal uveal tract	10.1	49.2	68.8	15.7	2.76
Mammary tissue	55.6	130	52.9	34.7	25.4
Placenta	47.9	90.3	103	15.2	BLQ

Upper limit of quantification = 8376 µg equivalents/g

Lower limit of quantification = 1.34 µg equivalents/g

BLQ – Radioactivity concentration below lower limit of quantification

[a] Measurement affected by high level of radioactivity in adjacent tissue

Excretion into milk	**µg SNAC equivalents/g**					**h×µg SNAC equiv/g**
Time	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**	**AUC_{all}**
Milk:	413 ± 232	557 ± 277	702 ± 253	407 ± 69.7	8.36 ± 2.70	5760
Maternal Plasma:	263 ± 54.4	217 ± 53.7	100 ± 47.2	37.6 ± 12.5	0.831 ± 0.060	1080
Milk / plasma Ratio:	1.60 ± 0.946	2.69 ± 1.35	7.22 ± 0.836	12.1 ± 5.80	10.0 ± 3.15	5.3

Table excerpted from the Sponsor's Pharmacokinetic Tabulated Summary.

SNAC is thought to bind exclusively to albumin in human plasma based on in vitro ultrafiltration studies conducted with ^{14}C-SNAC and human plasma or purified human serum albumin. Although the majority of SNAC was bound by protein in all species examined, the free fraction of SNAC and its metabolites varied depending upon the species examined. Ultrafiltration studies conducted with 2-200 mcg/mL ^{14}C-SNAC and plasma from various species also show that human plasma most efficiently bound SNAC and had the lowest percentage of free SNAC (2-5%) when compared to monkey (2-6%), rabbit (8-11%), rat (11-17%), and mouse (16-30%) plasma. Pooled plasma from female mice, rats, rabbits, monkeys and human volunteers were used in dialysis studies in vitro to evaluate the ability of SNAC and each of its 5 major metabolites to bind plasma proteins. Consistent with ultrafiltration studies, dialysis studies show that human plasma contained the smallest free fraction of SNAC when compared to protein binding in plasma from mice, rats, rabbits, monkeys and humans.
In vitro studies performed with mouse, rat, and monkey plasma proteins showed that SNAC plasma concentrations above 30,000 ng/mL or 100 mcM resulted in an increase in the free fraction of SNAC. SNAC's 5 major metabolites had higher fractions of unbound material that approached 100% in some laboratory animal plasma; but, human plasma typically had the lowest percentage of unbound metabolites. In vivo, when plasma was examined from mice with free access to food, the majority of SNAC detected in whole blood was observed in the plasma.

SNAC Metabolism

SNAC had a similar in vitro metabolite profile in humans, monkeys and rats. SNAC quickly undergoes rounds of conjugation into glucuronide metabolites and β-oxidation

by phase II enzymes. In CD-1 mice with free access to food, β-oxidation and glucuronide metabolites were detected in mouse plasma between 5-60 minutes after dosing. Glucuronidation reactions were facilitated most efficiently by UGT2B7 with additional contributions by UGT1A8 and UGT1A7 using uridine diphosphate glucuronic acid as a substrate. The formation of all metabolites had a T_{max} of 0.33 hours. SNAC (250 mM) is also capable of neutralizing the pH of simulated gastric fluid and may have a stabilizing effect on semaglutide.

β-oxidized metabolites (E494 and E506) were at least 10-times less potent inhibitors of ATP biosynthesis in mitochondria and glucuronidated metabolites had minimal effect on cellular respiration indicating that metabolism could be important for detoxification of the parent compound. In rats, the major SNAC metabolite present is E506 and represents 54-70% of the total dose administered. In contrast, the E1247 metabolite is the most common metabolite observed in humans.

Figure 3: Proposed metabolic pathways in mouse, rat, monkey and human

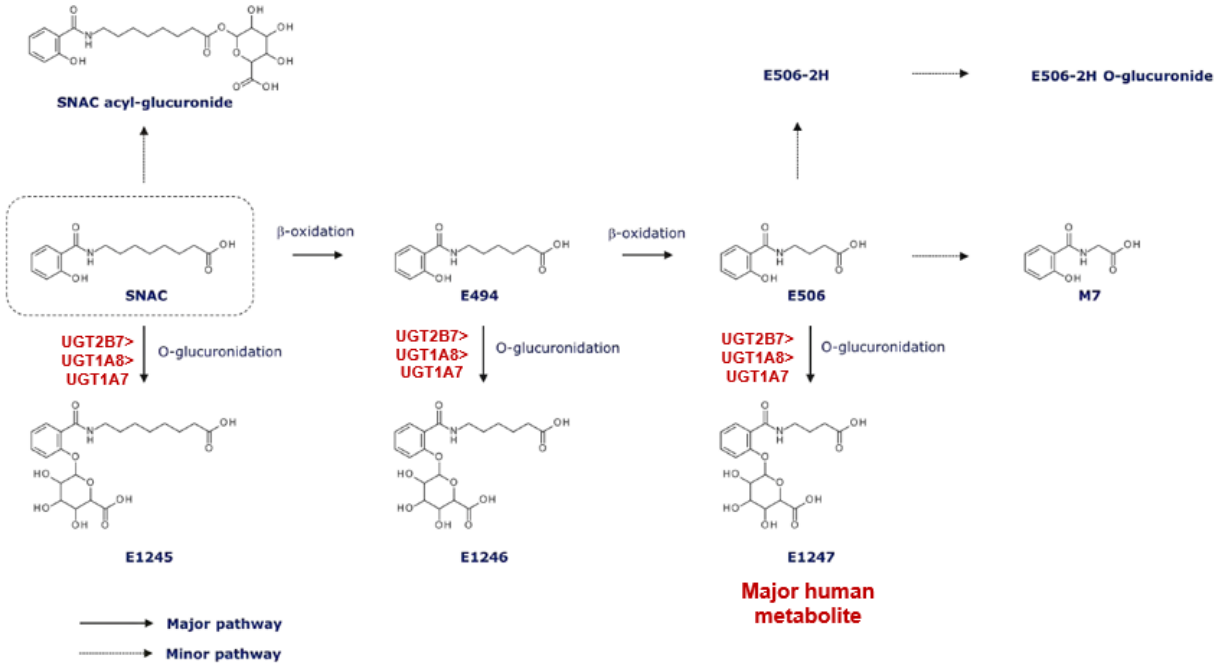


Figure modified by Elena Braithwaite from the Sponsor's submission (Figure 5, Nonclinical Overview).

Table 3: Percentage of Plasma Metabolites in Human and Rat

	% of Total Radioactivity					
	SNAC	**E494**	**E506**	**E1245**	**E1246**	**E1247**
Human[1]	5.1%	1.4%	7.9%	14%	13%	55%
Rat[2]	♂: 2.2% ♀: 0.9%	♂: 5.5% ♀: 5.0%	♂: 53.8% ♀: 70.2%	♂: 6.5% ♀: 1.6%	♂: 2.6% ♀: 1.4%	♂: 6.8% ♀: 3.5%

[1]Data from Study #: ERP23, Human AME trial characterizing the metabolic profile of SNAC following administration of 2.25 g ^{14}C-SNAC (3.7 MBQ) co-formulated with (b) (4) as a single

dose in nine healthy volunteers over 216 hours. 96% of total radioactivity was recovered. [2]Data from Study #: 8265975, Percent total of each peak area for Radio-HPLC profiles in male and female rat plasma. 96.1% of total radioactivity was recovered in males and 97.9% of total radioactivity was recovered in females.

SNAC Elimination

In mice, high levels of ^{3}H-SNAC or ^{14}C-SNAC-related radioactivity were seen in the kidney, urinary bladder and urine indicating that the major route of elimination for SNAC and/or its metabolites is through the kidney. Small amounts of ^{3}H-SNAC-related radioactivity were also detected in feces supporting the idea that SNAC and/or its metabolites undergo enterohepatic recirculation. Typically, 72 hours after dosing, SNAC-related radioactive material present in tissues was eliminated, as determined by whole body autoradiography studies in rodents.

In fasted female rats, clearance occurred more rapidly when SNAC was administered as a continuous intravenous infusion when compared to a single intravenous bolus dose.

Drug-drug interaction considerations

In vitro, SNAC inhibited human transporters OATP1B1, OAT1, and OAT3 by more than 50% resulting in IC_{50} values of 68, 28, and 5 mcM, respectively. SNAC's β-oxidized and glucuronidated metabolites also inhibited OAT1 and OAT3 at concentrations similar to plasma concentrations observed during clinical trials. However, a clinical drug-drug interaction trial (NN9924-4394) showed that oral co-administration of SNAC with inhibitors of OAT1 or OAT3 transporters did not impact systemic exposures to SNAC or the E494 and E506 β-oxidized metabolites and SNAC did not affect exposure/clearance of drugs that are substrates for OAT1 and OAT3. These findings indicate that the risk of drug-drug interactions would be predicted to be low. Additionally, clinical pharmacology studies showed that co-formulation of SNAC and semaglutide are required to increase gastric absorption, as co-administration of SNAC as a separate tablet does not increase systemic exposure to co-administered drugs.

Table 4: In vitro assessment of SNAC and metabolites as transporter substrates

Compound	P-gp	BCRP	OATP1B1	OATP1B3	OAT1	OAT3	OCT2	MATE1	MATE2-K	MRP2
SNAC	*No*	**Yes**	*No*	*No*	*No*	*No*	*No*	*No*	*No*	-
E494	*No*	**Yes**	*No*	*No*	*No*	**Yes**	*No*	*No*	*No*	-
E506	*No*	**Yes**	*No*	*No*	**Yes**	**Yes**	*No*	*No*	*No*	-
E1245	*No*	*No*	-	-	*No*	**Yes**	*No*	*No*	*No*	**Yes**
E1246	*No*	*No*	-	-	*No*	**Yes**	*No*	*No*	*No*	**Yes**
E1247	*No*	*No*	-	-	*No*	**Yes**	*No*	*No*	*No*	**Yes**

Compounds evaluated at 1, 3, 10 and 100 µM (MRP2 studies utilized a single concentration; 10 µM)
"-" denotes not evaluated

Table copied from the Sponsor's Nonclinical Overview (Table 10).

5.2 Toxicokinetics

Table 5: Pharmacokinetics for SNAC in Sprague Dawley rats after oral gavage

Week	Dose (mg/kg)	Sex	C_{max} (ng/mL)	$AUC_{0\text{-}4hr}$ (hr*ng/mL)
1	75	Male	404	904
		Female	670	919
	200	Male	1780	3420
		Female	27400	9070
	500	Male	94200	27600
		Female	110000	39800
50	75	Male	4880	3100
		Female	9520	3520
	200	Male	37800	17400
		Female	120000	26600
	500	Male	156000	55900
		Female	258000	82600
99	75	Male	29100	6800
		Female	1930	1520
	200	Male	82000	22100
		Female	9170	3970
	500	Male	197000	64600
		Female	160000	43700

2 year repeat dose carcinogenicity study of SNAC administered once daily by oral gavage to Sprague Dawley rats (Study #: 211519). Table copied from the Sponsor's submission (Table 6).

Table 6: Toxicokinetic parameters after oral administration of SNAC in Sprague Dawley rats

Week	Group	Dose (mg/kg/day)	Gender	t_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}24hr}$ (hr*ng/mL)	Rac_{Obs}
1	3	300	Male	2	1230	5120	NC
			Female	2	1800	6810	NC
1	4	900	Male	2	6210	23900	NC
			Female	2	2420	20200	NC
26	3	300	Male	2	1500	8970	1.75
			Female	6	1670	14300	2.10
26	4	900	Male	2	3250	18800	0.789
			Female	6	7420	42000	2.08

NC Not calculated.

26 week repeat dose toxicity study of SNAC administered once daily by oral gavage to Sprague Dawley rats (Study #: JLY0278/210196). Table copied from the Sponsor's submission (Table 5). Rac_{Obs}: apparent accumulation indexes.

Table 7: Pharmacokinetics for SNAC in Rhesus monkeys after oral gavage

Dose (mg/kg/d)	Day	C_{max} (ng/mL)		AUC_{0-8h} (ng•h/mL)		Half-Life (h)	
		M	F	M	F	M	F
200	1	50,500	17,400	29,300	23,900	0.99	5.56
	90	16,300	7,260	24,000	21,700	0.83	2.16
	180	12,500	7,810	20,100	18,700	1.35	4.08
300	1	39,400	37,500	46,500	50,500	1.58	1.96
	90	16,400	16,100	35,800	41,500	1.57	2.03
	180	13,400	21,500	30,200	38,100	2.20	1.95
600	1	142,000	114,000	130,000	188,000	2.25	1.34
	90	116,000	62,600	212,000	114,000	3.73	2.14
	180	55,200	62,500	114,000	86,100	2.17	1.85

9 month repeat dose toxicity study of SNAC administered once daily by oral gavage to Rhesus monkeys (Study #: 525-T-019 (209258 and 211503). Table copied from B. Timothy Hummer review (Brian T. Hummer, 04/02/2014, REV-NONCLINICAL-03 (General Review)).

6 General Toxicology

General toxicity studies were performed in mice, rats, and monkeys and were previously reviewed by Timothy Hummer under IND 114464.

6.1 Single-Dose Toxicity

When 2,000 mg/kg SNAC was administered via oral gavage, 3/5 female and 1/5 male ICR mice died within 4 hours after dosing. In Sprague Dawley rats, mortalities were observed at doses ≥900 mg/kg within 4 hours after dosing. Soon after exposure rats experienced clinical signs such as lethargy/apathy, ataxia/abnormal gait, and hunched posture that was transient in surviving rats. Gastrointestinal irritation including necrosis in the glandular stomach, duodenum, and cecum/colon in some cases was also observed.

6.2 Repeat-Dose Toxicity

SNAC

Repeat dose toxicology studies were performed in mice, rats, and monkeys. Mortalities were observed at clinical exposure in CD-1 mice and Sprague Dawley rats, and in monkeys at 117-fold the clinical exposure based on BSA. In general, considerable inter-individual variability was observed for SNAC plasma concentrations making meaningful correlations between mean systemic exposures and clinical signs difficult. Mortalities resulting from SNAC exposure typically occurred within 3 hours after dosing and were not associated with histopathological findings. But some common clinical signs observed before death in animals included sedation/decreased activity, ruffled fur, abnormal respiration and salivation.

Fasted rats tended to be more sensitive to SNAC when compared to rats with access to food ad libitum. A 13-week study in Wistar rats with free access to food identified a NOAEL that was 17-fold higher than clinical exposure based on $AUC_{(0-24h)}$ (no

treatment-related clinical signs were observed in this study). In contrast, a 13-week repeat dose study where rats were denied access to food 3 hours before dosing and 1 hour after dosing, one female exhibited adverse clinical signs including piloerection, reduced muscle tone, lethargic behavior and labored respiration and a statistically significant increase in mean cerebrospinal fluid lactate levels (a biomarker for decreased cellular respiration) in the 500 mg/kg dosing group (exposure multiple was 10-fold higher than the clinical exposure based on AUC). In a longer duration (2-year) carcinogenicity study where similar fasting conditions were used, a statistically significant increase in mortality occurred in females at doses below clinical exposure based on AUC.

In fasted monkeys, no mortalities occurred at doses 6-fold higher than clinical C_{max} values in a 9-month repeat dose study. However, transient lethargy was observed at these doses and resolved without intervention or following treatment for hypoglycemia.

SNAC and Semaglutide

A 26-week repeat dose toxicology study where fasted rats were orally administered SNAC alone or in combination with semaglutide resulted in clinical signs typically associated with exposure to each test article individually. When rats were orally administered 900 mg SNAC alone (below clinical exposure based on C_{max}), 7/20 female and 2/20 male rats died prematurely within 3 hours of dosing. When 900 mg SNAC was administered with 60 mg semaglutide, the incidence of unscheduled mortality and humane euthanasia decreased indicating that semaglutide administered in combination with SNAC does not exacerbate mortality in the rat at doses up to 5-fold clinical exposure based on AUC. Rats that received $\geq$20 mg/kg/day semaglutide in combination with SNAC experienced weight loss and decreased food consumption similar to that observed with semaglutide alone and consistent with the pharmacological activity of GLP-1 receptor agonists.

Decrease in body weight gain was also observed in monkeys administered up to 156.7 mg/kg/day SNAC (up to 18-fold human AUC) co-formulated in a tablet with 10 mg/kg/day semaglutide (6-fold human AUC) for 6 weeks. No clinical signs typically associated with SNAC were noted at these exposures.

Table 8: SNAC exposure multiples at the NOAEL in pivotal toxicology studies

	Study #	Sex	Dose (mg/kg/day)	C_{max} (ng/mL)	AUC (h*ng/mL)	Animal-to-human ratio	
						C_{max}	AUC
Human[2]	NN9924-4082		300 mg	9,300	6,405		
Mouse[3] (13 weeks)	209247	Male	500	2,720	7,240	0.3	1
		Female	500	1,720	8,980	0.2	0.7

[2] PK endpoints after 10 weeks of once-daily treatment, AUC_{0-24h}
[3] PK endpoints on Day 90, $AUC_{(0-24h)}$

Rat[4] (104 weeks)	JLY0366/ 211519	Male	500	197,000	64,600	21	10
		Female	75	1,930	1,520	0.2	0.2
Monkey[5] (39 weeks)	BNA00003/ 211503	Male	300	13,400	30,200	1	5
		Female	300	21,500	38,100	2	6

Mechanistic studies with SNAC

To further investigate the mechanism by which SNAC causes mortality in animals, a series of in vitro and in vivo studies were performed in rats. Based on the clinical signs observed in the general toxicity studies, the sponsor hypothesized that SNAC could affect cellular respiration, mitochondrial function, and ATP production.

To investigate SNAC's effect on cellular respiration in whole cells, oxygen consumption rates were measured in various cell lines and freshly isolated hepatocytes. Consistent with the proposed hypothesis, SNAC inhibited cellular respiration in all the cell lines examined (mouse myoblast C2Cl2 cell line IC_{50} = 175 mcM, mouse pre-adipocyte cell line IC_{50} = 666 mcM, human endometrial cell line HEC-1B IC_{50} = 199 mcM, human astrocyte cell line LN319 IC_{50} = 315 mcM, and rat peripheral blood mononuclear cells IC_{50} = 199 mcM). Inhibition was also seen in cryopreserved hepatocytes from different species (mouse IC_{50} =226 mcM, rat IC_{50} = 558 mcM, humans IC_{50} = 752 mcM, and monkey IC_{50} = 1215 mcM). SNAC's β-oxidized metabolites were 10 times less potent at inhibiting cellular respiration and SNAC's glucuronidated metabolites had a minimal effect on respiration. Additionally, when cellular respiration was evaluated in the presence of human serum albumin, SNAC's ability to inhibit cellular respiration was diminished.

[4] PK endpoints at Week 99, $AUC_{(0\text{-}4\ hr)}$
[5] PK endpoints on Day 180, $AUC_{(0\text{-}8h)}$

Additional studies were performed to identify the protein complex within the electron transport chain inhibited by SNAC. Permeabilized cells where SNAC had access to the mitochondria, were exposed to various complex inhibitors within the electron transport chain in the presence of SNAC. SNAC inhibited cellular respiration to the same level as rotenone (a known inhibitor of complex I), indicating that SNAC inhibits cellular respiration through a mechanism similar to rotenone. Concentration-dependent inhibition of ATP biosynthesis was also seen in isolated rat mitochondria.

Figure 4: Effect of SNAC on cellular respiration and the electron transport chain

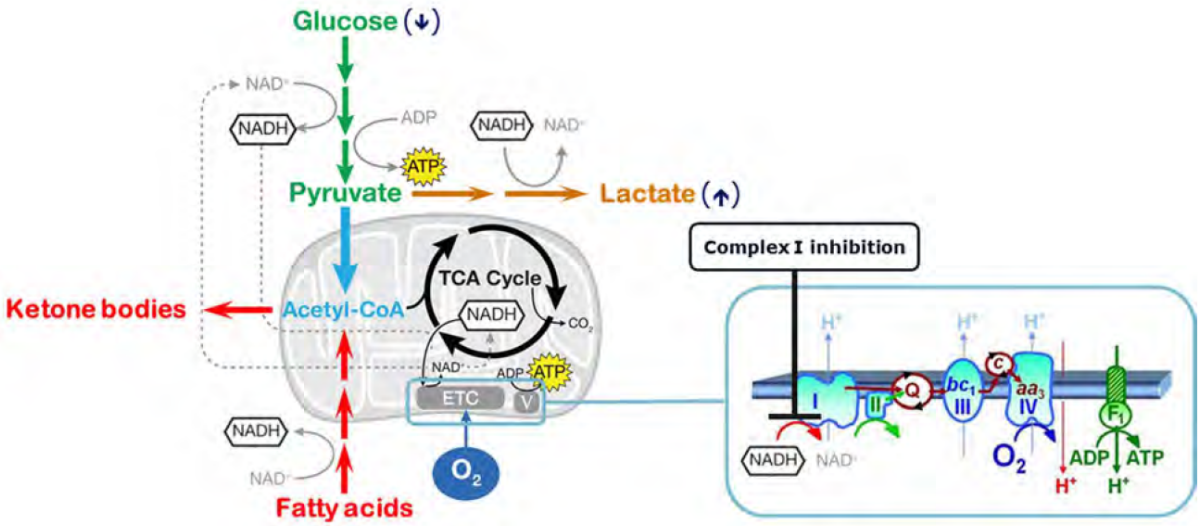


Figure copied from the Sponsor's Nonclinical Overview (Figure 4).

Several in vivo studies were performed to correlate exposure levels with changes in clinical chemistry parameters and the appearance of clinical signs. In vitro studies with permeabilized cells demonstrated that SNAC can inhibit cellular respiration at concentrations greater than 1,000 mcM or ~300,000 ng/mL. But, this inhibitory concentration is hard to correlate with oral doses and systemic plasma SNAC concentrations due to highly variable absorption of SNAC through the gastric epithelium. To obtain a better understanding of the correlation between systemic exposure and the onset of clinical signs, the Sponsor measured SNAC levels in individual animals while monitoring for clinical signs.

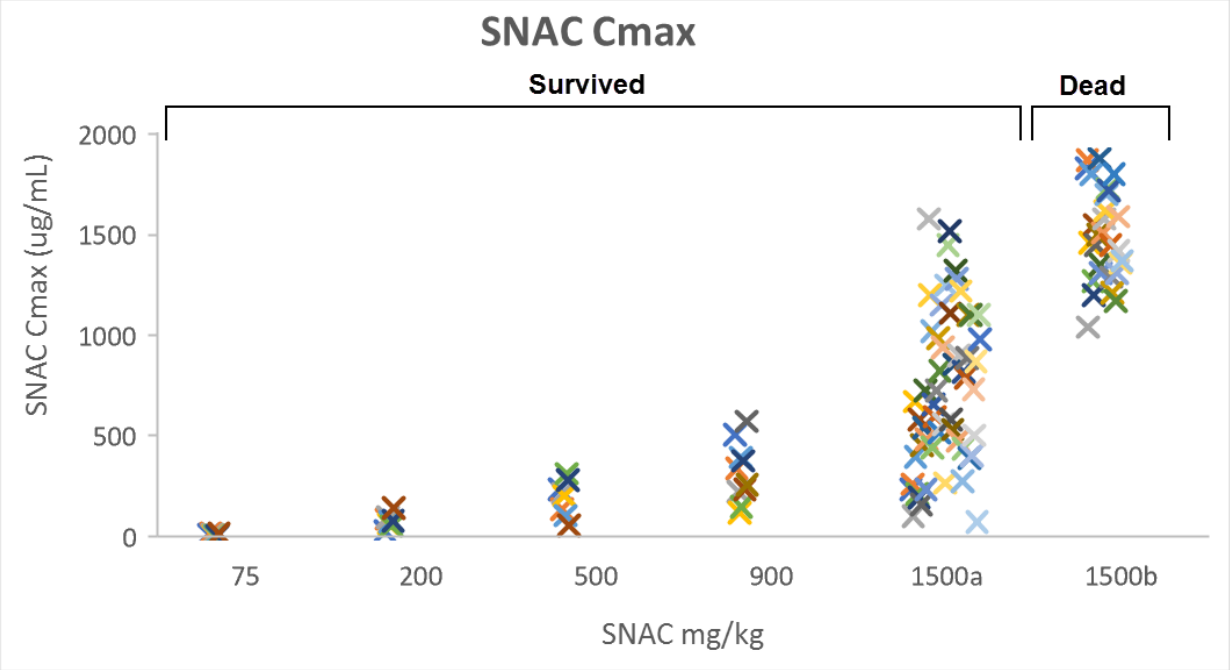
In single dose studies, fasted female rats (the most sensitive species examined in toxicology studies) were orally administered SNAC at doses of 75, 200, 500, 900, and 1500 mg/kg. Adverse clinical signs (apathy, abnormal respiration, reduced alertness and startle response, abnormal gait, reduced body tone, twitches, salivation, convulsions) and mortality were typically observed soon after dosing and roughly correlated with C_{max}. There was a large inter-individual variation where some animals showed marked clinical signs leading to death and other animals were completely unaffected at the same drug exposure range. The lowest identified C_{max} where mild

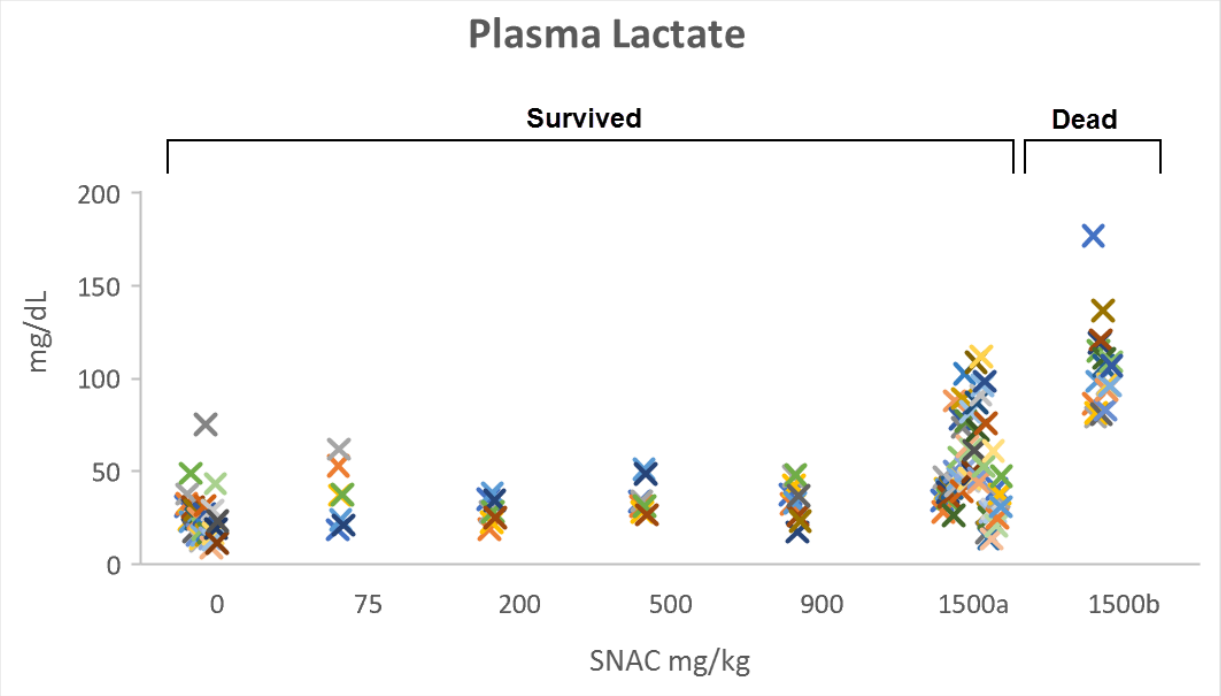
clinical signs were observed was 100,000 ng/mL (11-fold higher than clinical C_{max} levels). Moderate to marked clinical signs were observed starting at 415,000 ng/mL (45-fold higher than the clinical C_{max}) and mortality at $\geq$ 1,040,000 ng/mL (112-fold higher than the clinical C_{max}), with increased incidence in overnight fasted rats as compared to 3h fasted rats. Animals which exhibited clinical signs and survived had recovered within one hour after dosing. These noted C_{max} value for mortality greatly exceeds the 300,000 ng/mL concentration where SNAC caused inhibition of cellular respiration in vitro. The large margin between inhibition of cellular respiration in vitro and in vivo may exist because the in vitro study did not consider several variables (e.g. the large percentage of SNAC that is unable to enter the inner mitochondrial membrane where the electron transport chain (and more specifically complex I) is located due to metabolism, efficient excretion of SNAC and its metabolites or binding to albumin). Therefore, the amount of free, unbound SNAC is much lower than the total SNAC concentrations calculated in the in vivo studies.

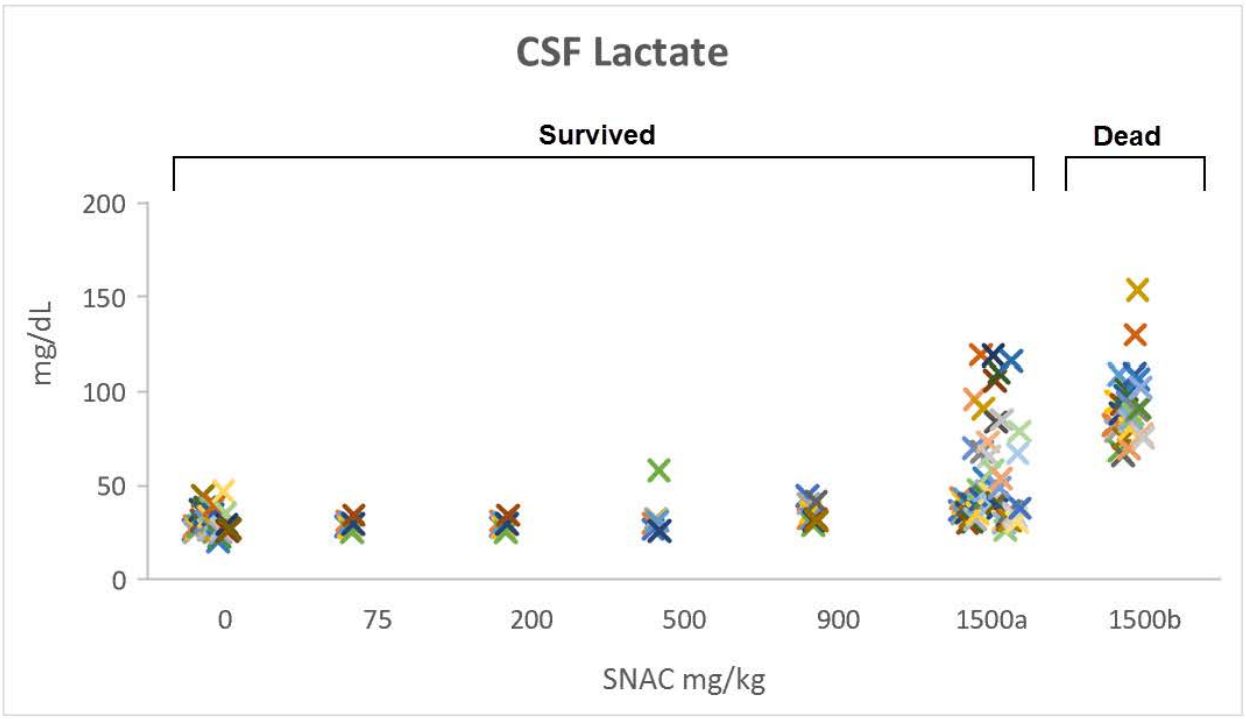
Statistically significant increases in plasma and CSF lactate and decreases in plasma glucose and ATP levels in heart and liver tissue were generally seen at $\geq$42X clinical C_{max}, with only few animals showing increases in plasma or CSF lactate at lower exposures (24.9 to 281 mcg/mL, 3-30-fold clinical C_{max}). Changes in lactate levels correlated with the presence of clinical signs and mortality. These findings were seen along with changes in acid-base status (↑pH), blood gas parameters (↑plasma O_2) and blood electrolytes (↑Na^+, ↑Cl^-).

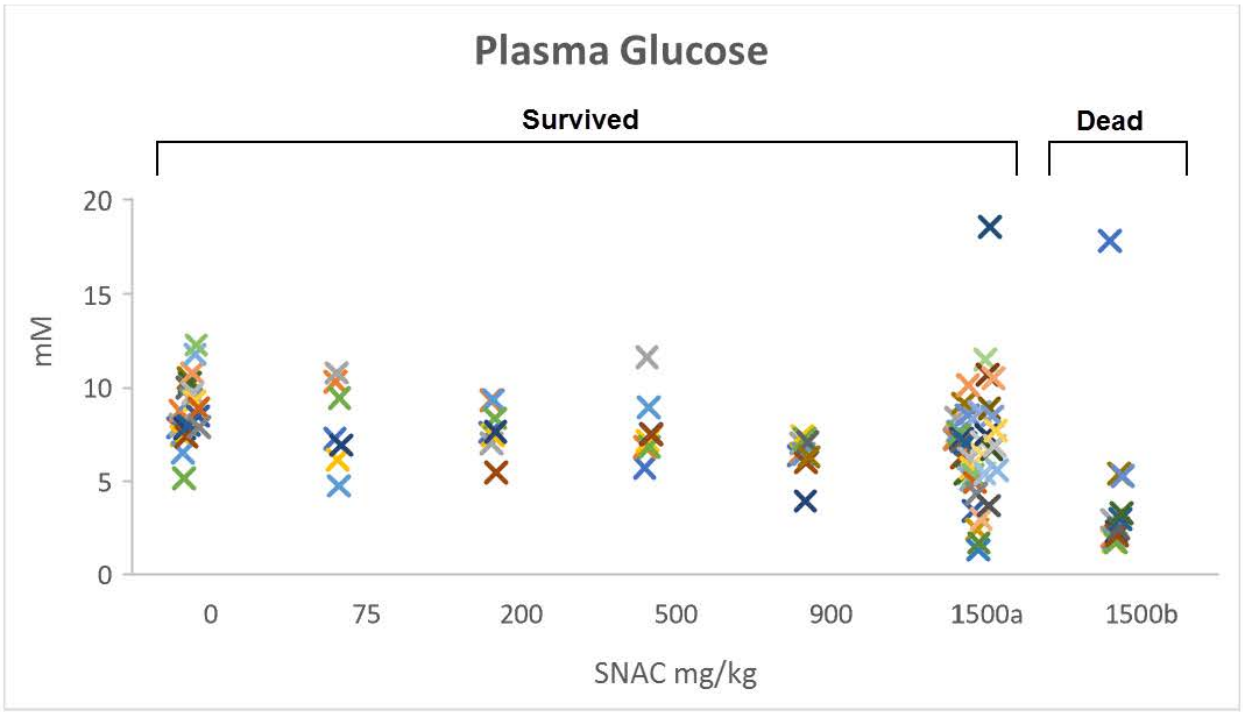
In a 13-week repeat dose investigative study in rats, small but significant increases in CSF lactate levels (up to 50%) in male (500 mg/kg) and female rats ($\geq$200 mg/kg) were generally observed at $\geq$10-fold the clinical C_{max}. However, an increase in CSF lactate of ~25% was noted in few animals at the clinical C_{max}. Although not statistically significant, increases in plasma lactate levels were also observed in few animals. These findings were considered treatment-related, even though they did not correlate with the appearance of clinical signs or decreases in liver glycogen levels.

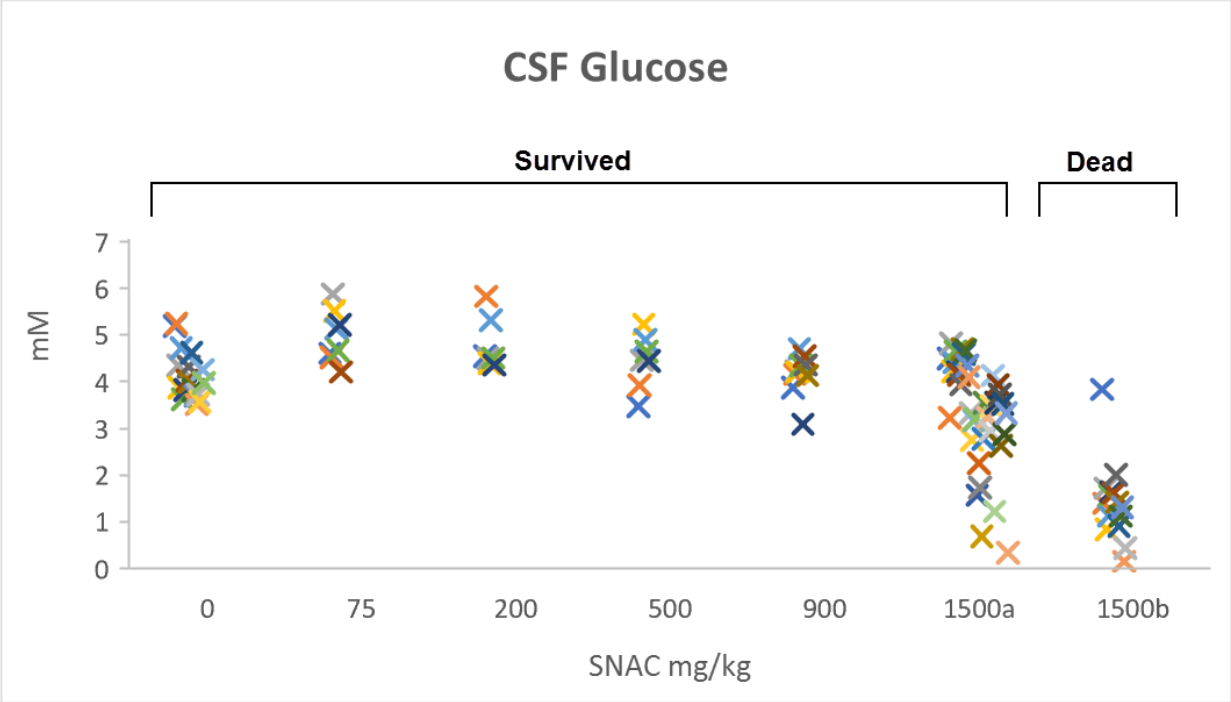
Figure 5: SNAC exposure (C_{max}) and clinical chemistry changes following administration of 75 to 1500 mg/kg SNAC in rats











Figures prepared by the reviewers and contains data from Studies JLY0468 and JLY0517. 1500a denotes rats that survived until the end of the study. 1500b indicates rats that died prematurely or were euthanized during the study.

Table 9: SNAC exposure multiples at the LOAEL in mechanistic studies

Findings	Severity	LOAEL (C_{max}, ng/mL)	Exposure Multiple
Mortality		1,040,000 ng/mL	112X
Clinical signs	Mild	100,000	11X
	Moderate-Marked	415,000	45X
Plasma Lactate	↑ 2x	24,900	3X
	↑ ≥3x	595,000	64X
CSF Lactate	↑ 25%	7,000	1X*
	↑ 2x	281,000	30X
	↑ ≥3x	780,000	84X
Plasma Glucose	↓ >2x	1,020,000	110X
CSF Glucose	↓ >3x	415,000	45X

Table prepared by the reviewers. LOAEL values were obtained from single dose mechanistic studies JLY0517 and JLY0468. One exception is noted with an * and includes data from a 13-week repeat dose study (JLY0517).

The ability of SNAC to cause toxicity in humans is predicted to be greatly diminished given that SNAC is highly bound to plasma albumin, which prevents it from entering the inner mitochondrial membrane where complex I is located, and because SNAC is rapidly oxidized and glucuronidated to metabolites that are less effective at inhibiting cellular respiration. In in vitro studies with permeabilized cells, concentrations of SNAC above 30,000 ng/mL can result in an increase in the free fraction of SNAC indicating that the number of albumin binding sites can be saturated. While plasma concentrations of SNAC should theoretically not reach the 30,000 ng/mL threshold in humans, situations where albumin has a reduced capacity to bind SNAC, when absorption exceeds the capacity of metabolizing enzymes to detoxify SNAC or when excretion has been compromised, may cause an increase in systemic exposure to free SNAC and ultimately affect cellular respiration. This hypothesis is supported by a study where rats were administered 200 mg/kg SNAC intravenously either by a single bolus dose or continuous infusion over 3.5 hours. In this study, rats that received the bolus dose experienced clinical signs (passivity, reduced body tone, apathy, abnormal gait) and mortality while no clinical observations were noted in rats receiving the continuous infusion. Additionally, the rats receiving a continuous infusion cleared SNAC at a 4-fold greater rate when compared to rats given the bolus dose. In the clinic, hepatic impairment can also affect systemic exposure to SNAC. Indeed, in the clinical program with semaglutide/SNAC, higher SNAC exposures were achieved in presence of severe hepatic impairment, as compared to normal hepatic function (clinical study NN9924-4082).

Patients are instructed to take Rybelsus® on an empty stomach at least 30 minutes before the first food, beverage or other medication. Presumably this dosing strategy will optimize semaglutide absorption and decrease inter-individual variability; but, will also provide conditions where SNAC will be most efficiently absorbed. UGT2B7, the main enzyme that glucuronidates SNAC in the gastrointestinal tract, liver and kidney, plays an important role in detoxifying SNAC before it enters the systemic circulation (this detoxification mechanism has been previously reported [1]). UGT2B7 exhibits substantial inter-subject variability in expression and glucuronidation activity in humans [2]. Variations in expression and activity can be influenced by sex [1], polymorphisms in the promoter region and within the allele [3-5], posttranslational modifications of the protein [6] and expression of the estrogen receptor alpha 1 [7]. These observations suggest that SNAC's ability to bind albumin, its metabolism and its excretion can become saturated at high exposures resulting in higher systemic exposure. To date, no increases in plasma lactate levels have been observed in the Phase 3 studies with semaglutide/SNAC 300 mg or after a single supratherapeutic SNAC dose (3600 mg) in a clinical pharmacology QTc trial, suggesting that SNAC exposures causing toxicity in animals are likely not achievable in the adult population at the recommended SNAC dose (300 mg).

Pediatric patients, however, may be at greater risk of higher SNAC exposures due to decreased detoxification of SNAC. At birth, it is estimated that UGT2B7 operates at approximately 3-10% of its maximal activity in adults and its maturation profile can vary significantly [2]. Therefore, pediatric patients might be at a greater risk of adverse

events due to immature detoxifying enzymes. However, UGT1A8 and UGT1A7 have also been shown to metabolize SNAC and may play a role in detoxifying SNAC when UGT2B7 is not present or highly active.

Another at risk population could be lactating infants. SNAC-related radioactivity following a single oral dose was detected in the milk of lactating female rats at levels up to12-fold higher than those found in maternal blood. Increased concentrations of SNAC in milk persisted for up to 24 hours post-dose. It is not known whether SNAC accumulates further in milk after repeated dosing.

7 Genetic Toxicology

SNAC was negative for genotoxicity in standard in vitro and in vivo genotoxicity tests (in vitro reverse mutation assay or Ames test, in vitro chromosome aberration test in cultured human peripheral lymphocytes, and in vivo micronuclei test in CD-1 mouse bone marrow erythrocytes).

8 Carcinogenicity

In a 2-year carcinogenicity study in Sprague Dawley rats, oral doses of 75, 200 or 500 mg/kg/day (males: 1-, 3-, or 10-fold the clinical $AUC_{0\text{-}24h}$, females: 0.2-, 0.6-, or 7-fold the clinical AUC_{max}) did not result in neoplastic findings that were related to oral SNAC exposure.

Increased mortality was observed in female rats receiving 200 or 500 mg/kg/day starting at ~ Week 80 and SNAC dosing was stopped for the remainder of the study at Week 103 for the 200 mg/kg/day group and at Week 99 for the 500 mg/kg/day group. No consistent cause of death was identified at the histopathological examination. Although the typical clinical signs associated with SNAC toxicity were not noted, relationship of mortality to SNAC exposure cannot be excluded.

In a 26-week carcinogenicity study in rasH2 mice, oral doses of 0, 30, 100, or 300 (males: 0.01-, 0.06-, or 0.3-fold the clinical $AUC_{0\text{-}24h}$, females: 0.04-, 0.1-, or 0.7-fold the clinical $AUC_{0\text{-}24h}$) did not result in neoplastic findings that were related to oral SNAC exposure.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Sprague Dawley rats were administered 1,000 mg/kg SNAC by oral gavage daily, 28 days before cohabitation continuing through a 21-day cohabitation period (males) or 15 days before cohabitation until gestation day (GD) 7 (females). Necropsies were performed on GD20. Excessive salivation and a slight decrease in body weight gain was observed in males. Due to a lack of adverse findings on fertility; the NOAEL for mating, fertility and early embryonic development was 1,000 mg/kg/day (32-fold the maximum recommended clinical dose based on body surface area or BSA).

9.2 Embryonic Fetal Development

Sprague Dawley rats were administered 1,000 mg/kg SNAC by oral gavage daily from GD 7 through GD 17. A C-section was performed on GD20. A decrease in maternal body weight gain (32% to 37%) was observed between GD7 and GD10; but there were no adverse effects on embryo-fetal viability, growth or development at doses 32-fold higher than clinical exposure based on BSA. New Zealand White rabbits were administered 1,000 mg SNAC by oral gavage once daily from GD 6 through GD 18. A C-section was performed on GD29. Maternal mean food consumption in rabbits receiving 1,000 mg/kg/day, was slightly reduced during the treatment period, especially from GD6 to GD9. The NOAELs for embryo-fetal survival, growth and development in rabbits were 1,000 mg/kg/day (65-fold the MRHD based on BSA).

9.3 Prenatal and Postnatal Development

In a pre- and post-natal study, Sprague Dawley rats were orally administered 1,000 mg/kg SNAC daily from GD 7 through GD24 or postnatal day 20. Maternal clinical signs included excess salivation and decreased food consumption. A statistically significant increase in the duration of gestation was also seen along with an increased number of stillbirths and an increased pup mortality rate between postnatal day 1 and 4. SNAC plasma levels were not measured in the dams or the pups; however, pup mortality does not appear to result from SNAC exposure through milk, as 10/13 pups found dead did not have milk in their stomach. In utero exposure to SNAC also did not result in adverse gross pathology findings (all pups appeared normal). No adverse developmental effects were observed in the surviving pups and no effects on fertility, mating, pregnancy performance or litter parameters were noted in the F1 generation.

Table 10: Summary of natural delivery observations - F_0 generation female rats

Nominal Dose Levels (mg/kg every day)	0 (Control)	1000
F0 FEMALES		
Initial Number of Animals	**25 F**	**25 F**
Noteworthy Findings		
Number Pregnant	24	24
Clinical signs[a]		
Gestation		
Excess salivation	0/0	67/22**
Lactation		
Excess salivation	0/0	24/13**
Red perivaginal substance	1/1	9/9**
Body weight change(g)		
GD7-10	12.1	9.3
GD18-20	27.2	20.9**
GD7-20	105.2	93.5**
Food consumption (g/kg/day)		
GD7-10	87.4	78.8**
GD18-20	67.8	62.8*
GD7-20	81.6	78.2*
PPD1-14	167.8	160
Pregnancy Performance		
Number of dams that delivered litters	24	24
Duration of gestation in days[b]	22.7	23.4**
Number of dams delivering		
Days 22.1-23	17	2**
Days 23.1-24	4	19**
Dams with stillborn pups	5	11**
Total pups delivered per litter	14.1	13.4
Liveborn per litter (%)	13.7 (97.3)	12.4(92.2)**
Stillborn per litter (%)	0.4 (2.7)	1.0(7.4)**

Nominal Dose Levels (mg/kg every day)	0 (Control)	1000
F1 LITTER DATA (preweaning)		
Noteworthy Findings		
Number of litters evaluated	24	24
Pups found dead/presumed cannibalised		
PPD2-4 (%)	9 (2.8)	18 (6.2)**
Live litter size		
PPD 1	13.3	12.2
PPD 4	13.5[c,d]	11.3**
PPD 7	13.5[c]	11.4**
PPD 14	13.6[c,e]	11.4**
PPD 21	13.6[c,e]	11.4**
Pup weight per litter (grams)		
PPD 21	33[c,e]	36.9**

* p<0.05, ** p<0.01 vs. control (pairwise test)
GD-Gestation Day
PPD *Post Partum* Day
a: Total occurrence/No. of animals
b: Calculated as the time (to the nearest tenth of a day) elapsed between confirmed mating (defined as 0 hour) and the time the first pup was delivered. Excludes litters in which delivery of first pup was not observed.
c: Excludes values for litters that had no surviving pups
d: Excludes values that were not recorded
e: Excludes value for litter 12620 since the dam was misdosed on PPD 7

Table copied from the Sponsor's Tabulated Toxicology Summary.

Table 11: SNAC exposure multiples at NOAEL in fertility and development studies

	Study Number	Sex	Dose	HED mg/m^2	Animal-to-human ratio based on BSA
Rat (Fertility)	EMISTOX98004/ 209251	Male	1,000 mg/kg/day	6,000	32
		Female	1,000 mg/kg/day	6,000	32
Rat EFD	EMISTOX98001/ 209253		1,000 mg/kg/day	6,000	32
Rabbit EFD	EMISTOX97008/ 209256		1,000	12,000	65
Rat PPND	EMISTOX98005/ 209254		1,000[1] mg/kg/day	6,000	32

Table prepared by Elena Braithwaite. [1] LOAEL (no NOAEL was identified in this study).

10 Special Toxicology Studies

Local Tolerance in the Gastrointestinal Tract

Local tolerance in the gastrointestinal tract of dogs was investigated by administering a single oral dose of 300 mg SNAC/dog (24-35 mg/kg) by oral gavage. An immediate necropsy was done to examine the stomach and intestinal tract for histopathological findings. In the areas where the SNAC tablet was located or where areas where SNAC liquid interacted with the gastrointestinal tract, some blood was observed but no correlating microscopic tissue damage was observed one hour after administration.

Immunotoxicology

To assess any potential effect SNAC had on the immune system, immune responses to keyhole limpet hemocyanin antigen was studied after exposure to up to 500 mg/kg SNAC on Days 8 and 22. SNAC had no remarkable impact on the total number of T and B lymphocyte cell numbers or T helper and cytotoxic T cell numbers or the ratio of lymphocyte populations. SNAC exposure did not influence the ability of rats to raise IgM or IgG responses after exposure to KLH. No SNAC-related changes in the M:E ratios, maturation sequence and morphology of the myeloid, erythroid and megakaryocytic cell lines were observed at doses 16-fold higher than clinical exposure based on BSA.

12 Appendix/Attachments

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

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Review number:	2
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Sponsor's submit date:	26 September 2013
CDER receipt date:	26 September 2013
Product:	Semaglutide, oral
Indication:	Type 2 diabetes
Sponsor:	Novo Nordisk
Review Division:	Metabolism and Endocrinology Products
Reviewer:	B. Timothy Hummer, PhD, DABT
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Table of Contents

1 Executive Summary

1.1 Introduction

Semaglutide (NNC0113-0217) is a long-acting glucagon-like peptide-1 (GLP-1) analog that is currently being developed by Novo Nordisk under IND 79,754 as a subcutaneously administered therapeutic for the treatment of type-2 diabetes. Under this IND, the sponsor is developing semaglutide reformulated with a novel excipient, N-(8-(2-hydroxybenzoyl) Amino) Caprylate (SNAC), to allow for oral administration.

1.2 Brief Discussion of Nonclinical Findings

Semaglutide (oral) plus SNAC

Semaglutide was orally administered to rats in a formulation containing SNAC for 6 months. Most findings were attributed to SNAC as the same findings were observed in the SNAC control groups. Effects that were attributed to semaglutide included decreased body weight and food consumption at ≥20 mg/kg/d for males and at 60 mg/kg/d for females; slightly increased adrenal weights in males receiving ≥20 mg/kg/d; distention of the duodenum, reduced adipose tissue, and thin uterus in females receiving 60 mg/kg/d; and hypertrophy of Brunner's gland in males (minimal at ≥20 mg/kg/d) and females (minimal to slight at ≥6 mg/kg/d). Effects on food consumption and body weight are expected for GLP-1 receptor agonists. Although hypertrophy of Brunner's gland is not always observed for this class, the same finding was observed in chronic rat and mouse studies in which semaglutide was administered subcutaneously. Therefore, this appears to be a pharmacological effect occurring after systemic exposure rather than specifically a local effect as a result of oral dosing.

Semaglutide (subcutaneous)

The toxicity profile of semaglutide has been evaluated in mice, rats, and monkeys and the potential effects of semaglutide on embryo-fetal development have been studied in rats, rabbits, and monkeys. The most noteworthy drug-related effect observed in toxicology studies is body weight loss / decreased body weight gain generally associated with decreased food and water consumption. When high dose levels are administered acutely, CNS-related effects such as decreased locomotor activity, abnormal gait (walking on tip toes), decreased touch response, passivity, lethargy, and/or piloerection have been observed in rats and mice. However, when dose levels are escalated slowly over a few weeks, CNS-related signs and effects on body weight and food consumption are diminished, indicating that tolerance occurs after repeated dosing. A dose-escalation approach was utilized in all of the pivotal toxicology studies to minimize the initial treatment-related effects on body weight.

Repeated dosing in mice for up to 3 months caused mild focal C-cell hyperplasia, C-cell nests, and dilated ultimobranchial ducts in association with detectable calcitonin levels at ≥1 mg/kg/d (≥32X clinical exposure). Female rats receiving daily subcutaneous injections of 0.6 mg/kg/d (51X clinical exposure) for up to 6 months showed possible effects on the estrus cycle, characterized by increased mean absolute uterine weights in conjunction with increased incidence of macroscopically-observed fluid distension and

microscopically-observed increased incidence of minimal to slight uterine luminal dilatation. Minimal Brunner's gland hypertrophy was observed in nearly all treated rats (≥0.03 mg/kg/d; ≥2.5X clinical exposure) but not in control animals. Treatment-related effects on the uterus and Brunner's glands were not observed after a 4-week recovery period. No treatment-related adverse microscopic lesions were observed in the pancreas or thyroid and no significant lesions were observed at the injection sites.

Monkeys receiving twice weekly semaglutide by subcutaneous injection for up to 12 months showed no definitive signs of toxicity other than the expected effects on body weight and food consumption. In a 3-month study, diffuse goblet cell hyperplasia was noted in the ileum and jejunum of monkeys receiving ≥0.086 mg/kg (≥12X clinical exposure) and pancreatic islet cell atrophy was noted in 1/4 HD females (0.467 mg/kg; 64X clinical exposure). In a 12-month study, ECG monitoring revealed that one female receiving 0.36 mg/kg semaglutide (44X clinical exposure) had a bigeminal rhythm, with two episodes of sinus tachycardia in Week 13 and a continuous left bundle branch block-like recording that persisted from Week 26 to Week 52. No cardiac lesions were identified in this animal. Because this is a rare background finding in Cynomolgus monkeys, a relationship to treatment could not be excluded. Microscopic evaluation of the heart showed one male receiving 0.36 mg/kg had slight multifocal myocardial vacuolation and degeneration, with karyomegaly, in the left ventricle. There was no significant ECG abnormality in this animal. Although this was likely an incidental finding, a possible relationship to treatment could not be excluded. Some differences in mean organ weights were observed in treated groups that were likely related to the treatment-related effects on body weights. No treatment-related microscopic findings were observed for the thyroid, pancreas, or Brunner's glands and treatment did not affect serum calcitonin levels. No significant histopathology lesions were observed at the injection sites.

The standard battery of in vitro and in vivo genetic toxicology studies indicated that semaglutide is devoid of mutagenic or clastogenic activity.

A complete development and reproductive toxicology (DART) program was conducted in rats, rabbits, and monkeys. In a combined fertility and embryonic development study, rats administered semaglutide at doses up to 0.09 mg/kg (2X clinical exposure) once daily by subcutaneous injection showed no effect on pre-coital intervals or mating performance in males or females, and no effect on fertility in males. Females receiving ≥0.03 mg/kg/d (≥0.6X clinical exposure) showed a slightly higher incidence of having an estrous cycle of 5 days or other irregular duration. A statistically significant decrease in the mean number of corpora lutea was also observed at ≥0.03 mg/kg/d, resulting in a decreased number of implantation sites and slightly fewer live young on GD 20. Mean weights for placentas, litters, and individual fetuses were statistically significantly decreased in the 0.09 mg/kg/d group compared with control. There was not a definitive treatment-related effect on pre- or post-implantation loss in the pivotal study, although increases in post-implantation loss (primarily due to early resorptions) were observed in a preliminary study that tested higher dose levels (≥4X clinical exposure).

In the 0.09 mg/kg/d group, a small number of major malformations were observed, including retroesophageal aortic arch, double aortic arch, membranous ventricular septal defect, and short tibia (malrotated hindlimb); absent cervical vertebral arch and

retroesophageal aortic arch were seen in the 0.03 mg/kg/d group. A slight increase in the incidence of some minor skeletal and visceral abnormalities/variants was observed at ≥0.03 mg/kg/d, and a slight increase in observations of incomplete ossification/ unossified cranial centers and sternebrae was noted at 0.09 mg/kg/d. Potential minor visceral abnormalities were observed at ≥0.03 mg/kg/d at low incidence, including subclavian artery (arising from aortic arch) and brain (dilated ventricles - 0.09 mg/kg/d only). These effects on embryo-fetal development occurred in the presence of maternal body weight loss/decreased body weight gain.

Pregnant rabbits were administered semaglutide once daily by subcutaneous injection at doses up to 0.0075 mg/kg (4X clinical exposure) from GD 6 through GD 19. Treatment resulted in profound effects on maternal body weight at all dose levels (≥0.1X clinical exposure), with the animals treated at ≥0.0025 mg/kg/d (≥0.6X clinical exposure) having weights that were below their GD 6 starting weights throughout nearly the entire dosing period. There was a trend for a slight increase in post-implantation loss due to early resorptions at ≥0.0025 mg/kg/d, which resulted in slightly lower mean litter sizes and weights; however these differences did not reach statistical significance. There was not a definitive treatment-related effect on the incidence of major abnormalities. There were marginally higher than expected incidences of fetuses/litters with minor skeletal abnormalities/variants (≥0.0025 mg/kg/d) and visceral abnormalities/variants (0.0075 mg/kg/d).

Segment 2 and 3 DART studies were conducted in Cynomolgus monkeys because of potential effects of semaglutide on nutrient uptake across the inverted yolk sac that may make rodents overly sensitive to nutrition-related in utero effects. In the embryo-fetal development study, pregnant Cynomolgus monkeys received up to 0.15 mg/kg semaglutide (24X clinical exposure at GD 50) every third day by subcutaneous injection from GD16 to GD50. Maternal effects at ≥0.075 mg/kg (8X clinical exposure) included dehydration, hypoactivity, and vomiting. Mean body weights for all treatment groups decreased by 12% to 18% between GD 15 to GD 50 compared with a 10% mean body weight increase for the control group during the same period. There was no apparent effect on fetal mortality, placental weights, or fetal weights. Mean absolute testis weights of males from the 0.015 mg/kg group were slightly increased (↑22%) compared with the control group. A single fetus from the 0.15 mg/kg group was noted as having a large thyroid, although the relationship to treatment is uncertain. Definitive treatment-related effects on fetal development were not observed; however single instances of a misshapen right brain hemisphere and fused kidneys and two instances of additional vertebrae were noted at ≥0.075, which exceeded the concurrent control values and the historical control range. Skin reddening, associated with blood vessel congestion and/or hemorrhages in the dermis and subcutis, was also observed in several fetuses including controls. Although this is not a common finding, it was observed in all study groups indicating that a relationship to the treatment of the mother animals is unlikely.

In the peri- and post-natal development study, pregnant Cynomolgus monkeys received up to 0.15 mg/kg semaglutide (12X clinical exposure at GD 140) every third day by subcutaneous injection from GD16 to GD140. Mean maternal body weights decreased between 6% and 16% from GD 16 to GD 50 for the treated groups, compared with a 3% weight increase for the control group. After GD 50 body weight gain was similar

between treated and control groups. The incidence of early pregnancy loss (GD 16 to GD 50) was higher for females receiving ≥0.075 mg/kg (5X clinical exposure) compared with control. Mean infant body weights were lower at birth for the 0.075 and 0.15 mg/kg dose groups, with the effect being statistically significant at 0.15 mg/kg (↓14%). In utero exposure to semaglutide had no apparent effect on fetal or infant development; skin reddening was not observed in this study.

Safety Margins for Subcutaneous Semaglutide (based on weekly AUC values at steady state)

General Toxicology Studies					
Species	**Duration**	**Exposure at NOAEL (nM•h)***	**Treatment Regimen (days/week)**	**Weekly Exposure (nM•h)**	**Safety Margin Based on Weekly Exposure‡**
Mouse	3 Months	11,400	7	79,800	32X†
Rat	3 Months	9,400	7	65,800	26X
	6 Months	18,100	7	126,700	51X
Monkey	3 Months	12,650	2	25,300††	10X
	12 Months	9,235	2	18,470††	7X
Developmental and Reproductive Toxicology Studies					
Species	**Endpoint**	**Exposure at NOAEL (nM•h)***	**Treatment Regimen (days/week)**	**Weekly Exposure (nM•h)**	**Safety Margin Based on Weekly Exposure‡**
Rat (Seg 1/2)	Male Fertility	≥590**	7	4,130	≥2X
	Female Fertility	72	7	504	0.2X
	Embryo-Fetal Loss	590	7	4,130	2X
	Embryo-Fetal Development	72	7	504	0.2X
Rabbit (Seg 2)	Embryo-Fetal Development /Loss	≥1,530**	7	10,710	≥4X
Monkey (Seg 2)	Embryo-Fetal Loss	≥30,000**	Every 3rd Day	60,000††	≥24X
	Embryo-Fetal Development	<2,000	Every 3rd Day	4,000††	<2X
Monkey (Seg 3)	Embryo-Fetal Loss	1,320	Every 3rd Day	2,640††	1X
	Fetal/Infant Development	≥14,400**	Every 3rd Day	28,800††	≥12X

*$AUC_{0\text{-}24h}$ or $AUC_{0\text{-}72h}$.

**Highest dose tested.

†C-cell hyperplasia was observed at this dose level. A NOEL for this finding was not established.

††$AUC_{0\text{-}72h}$ values were doubled to estimate weekly exposure.

‡Weekly human exposure = 2500 nM•h, based on an estimated $AUC_{0\text{-}24h}$ value of 475 nM•h for a 1 mg dose attained after repeated once weekly administrations (Clinical Study NN9535-1821). Weekly clinical exposure was further estimated based on a half-life of approximately 7 days.

SNAC

SNAC was shown to enhance the absorption of dextran in in vitro and ex vivo studies and enhance the absorption of semaglutide across Caco-2 monolayers. When administered orally with SNAC, semaglutide was shown to have glucose lowering activity in db/db mice. Bioavailability of semaglutide formulated as oral tablets in dogs ranged from 0.2% to 1.4% depending on the semaglutide to SNAC ratio. In monkeys, oral bioavailability ranged from 0.07% to 0.3%. In rats it was shown that oral bioavailability was greater in a fasted state.

SNAC is readily absorbed from the gastrointestinal tract, with a T_{max} of approximately 10 to 30 minutes. Major organs of distribution included kidney, bile ducts/gall bladder, blood, and liver. Data from bile-duct cannulated rats demonstrated evidence for enterohepatic recirculation. SNAC is highly bound to plasma proteins, ranging from approximately 84% to 98%, depending on the species. In Caco-2 and MDCKII-BCRP cell assays, SNAC inhibited human transporters P-gp, BCRP, OATP1B1, OAT1, and OAT3 by more than 50% resulting in IC_{50} values of 2620, 145, 68, 28, and 5 µM, respectively. SNAC is a weak inhibitor of CYP2C19, CYP3A4/5, and UGT1A9. One SNAC metabolite (E1245) was shown to be a weak inhibitor of CYP1A2, CYP2B6, CYP2C8, and CYP2C19. At the anticipated clinical exposure, significant drug-drug interactions due to metabolic interactions are not expected. SNAC is primarily metabolized by two levels of β-oxidation followed by glucuronidation. SNAC is not metabolized by Phase 1 enzymes. The major metabolites formed in humans were also identified in rats and/or monkeys. The elimination half-life of SNAC after oral administration was approximately 1.4, 2.7, 1.0, and 2.5 hours in CD1 mice, Wistar rats, beagle dogs, and Cynomolgus monkeys, respectively. SNAC is primarily excreted in urine, with a minor fraction in feces.

The primary toxicity observed with SNAC appears to be C_{max} driven, with high single oral doses causing significant depressant effects on respiration, piloerection, decreased touch response, weak reflexes, salivation, lethargy, twitching, ataxia, sedation, hunched posture, and death at ≥900 mg/kg. Decreases in diastolic and mean arterial pressures, increased heart rate, hypothermia, and increased respiratory rates were also observed for approximately 2 to 3 hours after dosing. ECG measurements showed an increase in QT and QTc values by up to 18 ms (Bazett) or 28 ms (Fridericia) for up to 4 hours after dosing. Increases in atrial (pause and premature beat) and junctional (salvo) arrhythmias were observed at 900 mg/kg and increases in atrial (premature beat), ventricular arrhythmias (beat), junctional (beat), and other arrhythmias were observed at 1500 mg/kg/d. Mean C_{max} values on Day 1 were 373,274 and 358,703 ng/mL at 900 and 1500 mg/kg/d, respectively, which are approximately 300- to 400-fold higher than the anticipated clinical C_{max} value. At necropsy, enlarged atria/hearts were noted at ≥1000 mg/kg and stomach irritation was also observed. In Cynomolgus monkeys, a single oral dose of up to 600 mg/kg SNAC caused no effects on clinical signs, body weight, food consumption, body temperature, heart rate, mean arterial blood pressure, or ECG intervals (including QTc) for up to 36 hours after dosing. The mean plasma concentration of SNAC at 600 mg/kg 2 hours after dosing was 18,123 ng/mL.

In repeat-dose toxicology studies, clinical signs and some deaths were also observed at high doses, similar to the single-dose studies. In a 6-month study in rats, necropsy findings included a dose-related increase in mean kidney weight at ≥300 mg/kg/d and a slight increase in the incidence and severity of microscopic lung findings (prominent number of alveolar macrophages, alveolitis, perivascular lymphoid aggregates, and increased cellularity of BALT) at ≥90 mg/kg/d. However, these findings were not noted in a 12-month rat study. An increase in epithelial hyperplasia of the limiting ridge of the stomach was noted for males at ≥90 mg/kg/d in the 6-month study. In the 12-month study, there were macroscopic (foci of glandular stomach) and microscopic findings noted in the stomach at the end of the treatment period for males treated with 900 mg/kg/d and females treated at ≥500 mg/kg/d. Microscopic findings noted in the stomach were generally minimal to mild and included hyperplasia of the nonglandular stomach; eosinophilic blebs in the keratin layer; erosive inflammation; and hemorrhage. Hyperplasia of the nonglandular stomach was also noted at the 6-month interim time point. The NOAEL for the 6-month study was considered to be 90 mg/kg/d SNAC because of a single male death at 300 mg/kg/d that was possibly related to SNAC treatment. In the 12-month study, mortalities were only observed at 900 mg/kg/d; the NOAEL for this study was 500 mg/kg/d, resulting in mean C_{max} and $AUC_{0\text{-}4h}$ values of 154,000 ng/mL and 41,050 ng•h/mL at Week 52, respectively.

Monkeys treated with ≥1000 mg/kg/d SNAC for 2 or 4 weeks showed signs of hypoglycemia (reduced motor activity, transitory recumbency, ptosis), progressing to loss of consciousness, no response to pain, severe hypothermia, and death/moribund sacrifice for some animals. Mean C_{max} on Day 14 was approximately 110,000 ng/mL. No treatment-related microscopic lesions were identified. In 13-week and 9-month studies in monkeys, signs of hypoglycemia (somnolence, hypoactivity, incoordination, and prostration) were noted at ≥800 or ≥500 mg/kg/d, respectively, requiring supplemental food (fruit) and/or an infusion of 50% dextrose. No definitive treatment-related adverse findings were noted upon microscopic examination. A slight imbalance in minimal, multifocal mineralization of brain parenchyma and minimal interstitial hemorrhage of the thymus was observed for the groups receiving 500 and 600 mg/kg/d SNAC. A relationship to treatment is uncertain. The NOAEL for the 9-month study was determined to be 300 mg/kg/d, resulting in mean C_{max} and $AUC_{0\text{-}8h}$ values of 17,450 ng/mL and 34,150 ng•h/mL on Day 180, respectively (~18-fold higher than clinical C_{max}).

SNAC was not mutagenic or clastogenic in standard in vitro and in vivo genetic toxicology assays. Mouse and rat 2-year carcinogenicity studies are currently ongoing.

Male and female rats treated with SNAC showed no adverse effects on mating or fertility indices. No effects on sperm motility, morphology, or number were noted for males. No effects on implantations, resorptions, fetal weight, fetal sex ratios, or fetal development were observed at doses up to 1000 mg/kg/d. In embryo-fetal development studies in rats and rabbits, no effects on embryo-fetal viability, growth, or development was observed at doses up to 1000 mg/kg/d. In a peri- and post-natal study in rats, slight decreases in maternal body weight gain (~11% less than control) were observed from GD7 to GD20 at 1000 mg/kg/d. A statistically significant, dose-related increase in

the duration of gestation was observed at ≥500 mg/kg/d. Increased stillbirths and decreased live litter sizes occurred at ≥750 mg/kg/d. An increased pup mortality rate with decreased viability and lactation indexes was also observed, primarily at 1000 mg/kg/d SNAC. No teratogenic effects were observed for the F1 generation. There were no apparent effects of treatment on survival, clinical signs, or development of the F1 generation after birth. There were no treatment-related effects for the F2 generation. The maternal NOAEL was considered to be <500 mg/kg/d SNAC due to an increased duration of gestation. The NOAEL for viability and growth for the F_1 offspring was 500 mg/kg/d SNAC due to increased incidence of stillbirths at ≥750 mg/kg SNAC. The NOAEL for developmental effects was ≥1000 mg/kg/d.

1.3 Recommendations

1.3.1 Clinical Study Safe to Proceed: Yes

1.3.2 Comments to sponsor: None

2 Drug Information

2.1 Drug

CAS Registry Number: NA

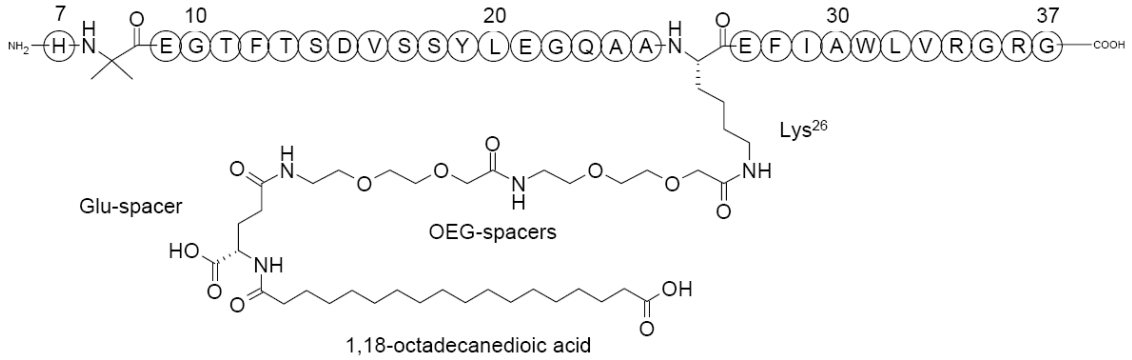
Generic Name: Semaglutide

Code Name: NNC 0113-0217; NNC 0113-0000-0217; 0217; NN9535

Chemical Name: *N*-Σ_{26}-[(21S)-9,18,23-trioxo-2,5,11,14-tetraoxa-8,17,22-triazanonatriacontane-21,39-dicarboxy-1-carbonyl] [8-(2-amino-2-methyl-propionic acid), 34-arginine]GLP-1-(7-37)-peptide

Molecular Formula/Molecular Weight: C_{187} H_{291} N_{45} O_{59} / 4,113.6 Daltons

Structure or Biochemical Description (semaglutide):



Pharmacologic Class: GLP-1 mimetic, GLP-1 receptor agonist

2.2 Relevant INDs and NDAs

GLP-1 Receptor Agonists
IND 79,754 - Semaglutide (SC), GLP-1 analog (Novo Nordisk)
IND 61,040 / NDA 22-341 - Liraglutide/Victoza, (Novo Nordisk)
IND 70,930 - Dulaglutide (Eli Lilly)
IND 57,725 / NDA 21-773 - Exenatide/Byetta (Amylin)
IND 67,092 / NDA 22-200 - Exenatide once weekly/Bydureon (Amylin/BMS)

(b) (4)

2.3 Drug Formulation

Table 1 Compositions of drug products expressed as "per tablet"

Component	Composition (mg/tablet) 2.5 to 60 mg semaglutide tablets	Function	Reference to standard
Drug Substance			
Semaglutide[1]	2.5 to 60	Active ingredient	Novo Nordisk A/S
Excipients			
SNAC	300	Absorption enhancer	Novo Nordisk A/S
Cellulose, microcrystalline	(b) (4)		Ph Eur
Povidone (b) (4)			Ph Eur
Magnesium stearate[2]			Ph Eur
Gross weight	400.2 to 457.7	N/A	N/A

[1] The amounts of semaglutide are shown (b) (4)

(b) (4)

[2] (b) (4)

2.4 Comments on Novel Excipients

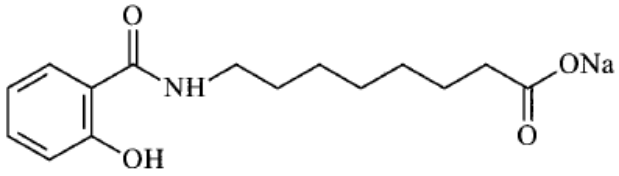
CAS Registry Number: 203787-91-1

Code Name: SNAC; NNC 0113-3363

Chemical Name: Sodium-N-[8-(2-hydroxybenzoyl)amino]caprylate, monosodium

Molecular Formula/Molecular Weight: 301.32 g/mol

Structure or Biochemical Description (SNAC):



Native GLP-1 and GLP-1 analogs generally have very low oral bioavailability (<0.01%) when administered alone. To enhance oral bioavailability, semaglutide will be formulated with SNAC, an absorption enhancing excipient based on the Eligen® Carrier concept developed by Emisphere Technologies Inc., to protect the peptide against acid and enzymatic degradation and facilitate increased transepithelial penetration. Because SNAC is a novel excipient, a complete toxicology program is being conducted for the excipient alone.

2.5 Comments on Impurities/Degradants of Concern

There are currently no impurities or degradants of concern that have been identified.

2.6 Proposed Clinical Protocol

Multiple dose trial examining dose range, escalation and efficacy of oral semaglutide in subjects with type 2 diabetes (Trial NN9924-3790)

As shown in the sponsor-generated figure below, the study will consist of 9 treatment arms; seven oral semaglutide treatment arms, an oral placebo arm, and a SC semaglutide arm. All treatment arms include subjects with type 2 diabetes who have failed on diet and exercise and/or metformin. Oral semaglutide doses will range from 2.5 mg to 40 mg, with varying dose escalation schemes and will be formulated as tablets containing 300 mg SNAC (see sponsor-generated Table 1 below for tablet composition). The placebo tablets do not contain SNAC.

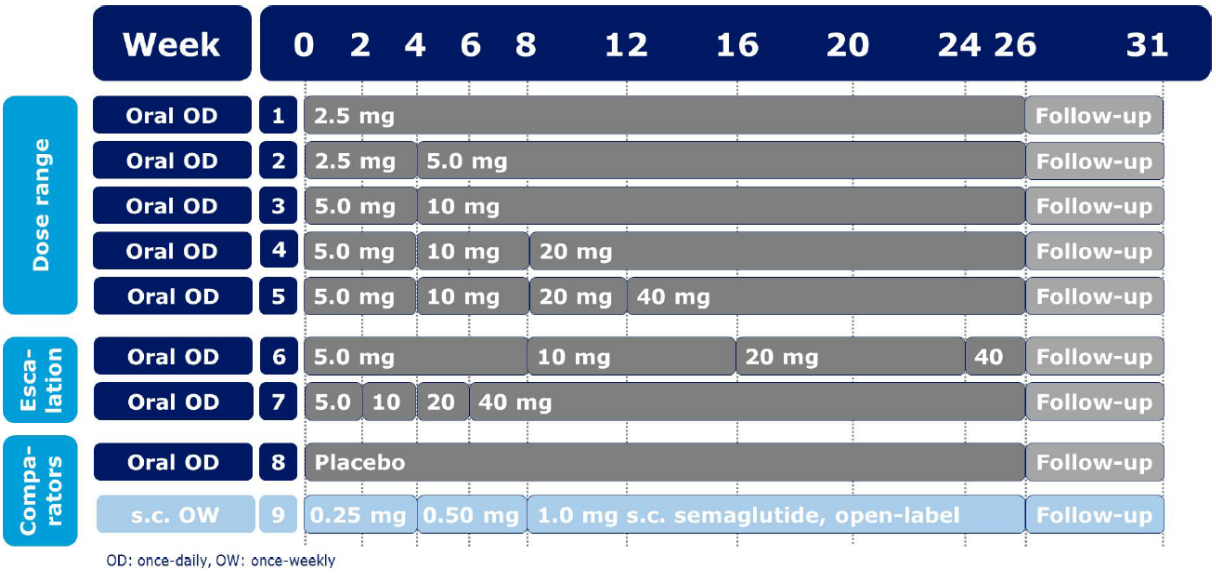


Figure 5–1 Trial design of NN9924-3790

2.7 Previous Clinical Experience

Five clinical studies have been conducted outside of the United States to characterize the pharmacokinetics, safety, and tolerability of oral semaglutide (summarized in the sponsor-generated table below).

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed/completed	Healthy or type 2 diabetes	Duration of treatment
NN9924-3691 United Kingdom	Safety, tolerability, PK including absolute bioavailability and PD	First human dose trial: a two-part, randomised, double-blind, single-centre and placebo-controlled trial with single escalating doses	Single escalating oral doses from 5 mg semaglutide w/ SNAC up to 20 mg semaglutide w/ SNAC 0.1 mg i.v. and 0.4 mg s.c. semaglutide Oral placebo including SNAC	155/154	Healthy male subject	1 day
NN9924-3692 Germany	Safety, tolerability, PK and PD	Multiple-dose, single-centre, placebo-controlled, randomised, semi-sequential trial	Multiple dosing for 10 weeks with end-doses of: - 5 mg oral semaglutide w/ 300 mg SNAC - 10 mg oral semaglutide w/ 300 mg SNAC - 2x(10 mg oral semaglutide w/ 300 mg SNAC) - 0.5 mg s.c. semaglutide - oral placebo w/ 300 mg SNAC	96/90	Healthy male subject	10 weeks

NN9924-3794 Germany	PK, safety and tolerability	Multiple-dose, single-centre, open-label, randomised, parallel group, eight armed, 2 x 4 factorial design, PK trial. Oral semaglutide w/ SNAC treatment at 8 different dosing conditions (post-dosing fasting period [15,30,60 or 120 min] and water taken with dosing [50 or 120 mL])	Multiple dosing for 10 days with 10 mg semaglutide w/ 300 mg SNAC.	158/151	Healthy male subject	10 days
NN9924-3957 United Kingdom	Whether the volume of water affects the anatomic location of tablet erosion and whether the PK of semaglutide are correlated to anatomic location and rate of tablet erosion.	Single-centre, randomised, open-label, single-dose, cross-over trial	Oral single dosing of 10 mg semaglutide w/ 300 mg SNAC	26/24	Healthy male subject	2 days
NN9924-3991 Germany	Safety, tolerability, PK and PD	Multiple-dose, single-centre, randomised, placebo-controlled, double-blind trial including four cohorts in a semi-parallel design	Multiple dosing for 10 weeks with end-doses of: - 20 mg oral semaglutide + 300 mg SNAC - 40 mg oral semaglutide + 300 mg SNAC - oral placebo with 300 mg SNAC - oral placebo	Healthy: 84/73 Subjects with type 2 diabetes: 23/19	Healthy male subjects and male subjects with type 2 diabetes	10 weeks

Human Pharmacokinetics for SNAC and Oral Semaglutide

SNAC is quickly absorbed, with T_{max} for a tablet containing 300 mg SNAC being between 0.4 and 1.5 hours. Mean exposure values (AUC_{0-24} and C_{max}) for subject arms receiving 300 mg SNAC ranged from 760 to 1900 ng•h/mL and 726 to 1400 ng/mL, depending on the dosing conditions, subject population, and amount of semaglutide. SNAC exposures tended to be slightly higher when food was eaten 15 minutes after dosing compared with longer post-dose fasting times. SNAC exposures slightly increased when administered with a large volume of water (240 mL) compared with 50 mL. Exposure differences were not noticeable between 50 mL and 120 mL of water. SNAC is quickly eliminated, with a mean half-life less than 1 hour.

Elimination half-life for semaglutide after a single oral dose ranged between approximately 58 and 95 hours, increasing with increasing dose level. The half-life of semaglutide after a single subcutaneous administration of 0.4 mg was approximately 158 hours. Mean half-life values ranged between 147 and 182 hours after 10 weeks of once daily oral dosing; mean half-life was 174 hours after 10 weeks of once weekly subcutaneous dosing of 0.5 mg semaglutide.

Because of the long half-life, semaglutide accumulated after repeated dosing. After a 6-week dose escalation scheme, steady state had not quite been reached after an additional 4 weeks at the target dose. Mean AUC_{0-24} values after 10 weeks were 61, 256, 448, 891, 1798, and 1473 nmol•h/L and mean C_{max} values were 3.85, 13.46, 23.03, 43.75, 89.23, and 72.74 nmol/L for healthy men receiving 5, 10, 20 (two 10 mg tablets), 20, and 40 mg doses and T2D men receiving 40 mg, respectively. Daily C_{ave} values were calculated to be 2.54, 10.69, and 18.66 hnmol/L for 5, 10, and 20 mg dose groups. In comparison, mean AUC_{tau}, C_{max}, and C_{ave} values at Week 10 for subjects receiving once weekly 0.5 mg semaglutide by subcutaneous injection were 2941 nmol•h/L, 21.46 nmol/L, and 17.51 hnmol/L, respectively.

T_{max} values for semaglutide were approximately 12 hours after a single dose of 5 or 10 mg semaglutide. After repeated dosing and steady state was nearly reached, T_{max} occurred between 2 and 3 hours. Across the different clinical PK studies, oral bioavailability for semaglutide relative to subcutaneous exposure was quite low, estimated to be between 0.02% and 0.90%, depending on the dose level and dosing conditions.

A Two-Part, Randomised, Double-blind and Placebo-controlled Trial of Oral Semaglutide, Formulated with the Absorption Enhancing Excipient SNAC, in Single Escalating Doses Exploring the Safety, Tolerability, and Bioavailability in Healthy Male Subjects (Trial NN9924-3691)

This was a two-part, randomized, double-blind, single-center and placebo-controlled trial with single escalating doses. Part 1 evaluated the safety, tolerability, and semaglutide plasma exposure of seven oral semaglutide formulations (5 mg semaglutide with 150 mg SNAC; 2, 5 and 10 mg semaglutide with 300 mg SNAC; 15 mg semaglutide with 450 mg SNAC; 10 and 20 mg semaglutide with 600 mg SNAC). Following each of the ascending dose escalation steps the Novo Nordisk A/S trial Safety Group decided on the dose for continuation based on an evaluation of safety and tolerability as well as of semaglutide exposure. Part 2 explored, in addition to safety, tolerability and semaglutide plasma exposure, the bioavailability of three oral semaglutide dose groups (2, 5 and 10 mg semaglutide with 300 mg SNAC) selected among those doses previously tested in Part 1, in a parallel single-dose manner. There were in total 13 different treatment groups in the trial: seven treated with oral semaglutide tablets, one with s.c. administered semaglutide (0.4 mg), one with i.v. administered semaglutide (0.1 mg) and four with placebo tablets (150, 300, 450 and 600 mg SNAC). Subjects were fasted for 10 hours before test article administration. Tablets were taken with 50 mL water and subjects were not allowed to eat for 5 hours.

The PK data for oral semaglutide were quite variable between subjects. For many subjects receiving oral semaglutide there was no measurable plasma concentration of semaglutide. The proportion of subjects with at least one quantifiable semaglutide plasma concentration was: 31 out of 70 subjects in Part 1 and 33 out of 72 subjects in Part 2 (pooled). Because of this, oral bioavailability of semaglutide could not be precisely calculated. Oral bioavailability relative to the subcutaneous route for the full analysis set (Part 2 pooled) was 0.00%, 0.02%, and 0.08% for the 2mg/300mg, 5mg/300mg, and 10mg/300mg dose groups, respectively. When calculating relative oral bioavailability using only subjects with at least one quantifiable semaglutide plasma concentration, the bioavailability was 0.49%, 0.62%, and 0.90% for the same 3 dose groups. SNAC exposure was slightly higher when formulated with lower doses of semaglutide. The majority of SNAC exposure occurs within the first 2 hours after dosing. The PK data for SNAC and semaglutide are presented in the sponsor-generated tables and figures below.

The most common AEs were headache and effects within the system organ class of gastrointestinal disorders (e.g. nausea and diarrhea). Approximately 90% of both the oral semaglutide and placebo treated subjects experienced one or more hypoglycemic episode (plasma glucose ≤ 3.9 mmol/L), while 100% of all subjects treated with i.v. and s.c. administered semaglutide experienced one or more hypoglycemic episode. Hypoglycemic episodes were primarily asymptomatic and no severe episodes were reported.

SNAC

Table 11–8 SNAC AUC_{0-5h}, t_{max} and C_{max} in Full Analysis Set – Part 1

	Sema 5.0 + SNAC 150	Sema 15.0 + SNAC 450	Sema 10.0 + SNAC 600	Sema 20.0 + SNAC 600	Sema 2.0 + SNAC 300	Sema 5.0 + SNAC 300	Sema 10.0 + SNAC 300
Number of subjects	10	10	10	10	10	10	10
AUC 0-5h							
AUC_0_5h (h*ng/mL)							
N	10	10	10	10	10	10	10
Mean	403.98	1405.67	1695.33	1910.29	1095.30	768.04	760.02
SD	124.45	353.13	867.63	541.75	252.45	254.63	293.07
CV	30.81	25.12	51.18	28.36	23.05	33.15	38.56
Geometric mean	383.49	1360.63	1520.00	1821.01	1071.13	723.79	703.91
Median	394.14	1358.62	1219.59	1904.21	1052.63	752.04	715.69
Min	162.22	700.15	761.65	756.50	722.19	295.97	300.75
Max	604.76	2029.82	3440.37	2567.59	1660.64	1222.46	1235.23
Time for maximal concentration							
t_max (hour)							
N	10	10	10	10	10	10	10
Mean	1.18	0.88	0.48	0.55	0.43	0.78	0.38
SD	1.28	0.71	0.22	0.35	0.24	0.98	0.24
CV	108.98	81.09	46.08	63.56	55.80	126.73	64.79
Geometric mean	0.65	0.66	0.44	0.49	0.38	0.53	0.33
Median	0.38	0.75	0.50	0.50	0.38	0.50	0.25
Min	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Max	3.00	2.50	1.00	1.50	1.00	3.50	1.00
Maximal concentration							
c_max (ng/mL)							
N	10	10	10	10	10	10	10
Mean	532.70	1056.00	1785.00	1936.00	1030.40	738.10	726.10
SD	327.53	547.62	1570.89	961.30	479.23	394.30	467.40
CV	61.49	51.86	88.01	49.65	46.51	53.42	64.37
Geometric mean	432.19	938.72	1323.35	1673.24	913.59	641.80	584.76
Median	510.00	842.50	1092.50	2036.50	1012.00	647.50	569.00
Min	105.00	505.00	441.00	469.00	276.00	246.00	211.00
Max	1190.00	1947.00	5367.00	3488.00	1988.00	1461.00	1514.00

Table 11–9 SNAC $AUC_{0\text{-}5h}$, t_{max} and C_{max} in Full Analysis Set – Part 2 (pooled)

	Sema 2.0 + SNAC 300	Sema 5.0 + SNAC 300	Sema 10.0 + SNAC 300
Number of subjects	24	24	24
AUC 0-5h			
AUC_0_5h (h*ng/mL)			
N	24	24	24
Mean	998.95	843.25	782.15
SD	261.62	290.52	239.75
CV	26.19	34.45	30.65
Geometric Mean	968.26	794.53	742.98
Median	992.77	816.62	752.93
Min	599.17	295.97	300.75
Max	1660.64	1537.05	1235.23
Time for maximal concentration			
t_max (hour)			
N	24	24	24
Mean	1.07	0.70	0.58
SD	1.35	0.87	0.96
CV	126.23	124.97	164.13
Geometric Mean	0.62	0.46	0.40
Median	0.50	0.50	0.38
Min	0.25	0.25	0.25
Max	5.00	3.50	5.00
Maximal concentration			
c_max (ng/mL)			
N	24	24	24
Mean	980.88	928.13	836.58
SD	460.39	621.69	416.74
CV	46.94	66.98	49.81
Geometric Mean	870.90	760.88	724.08
Median	920.00	793.50	807.00
Min	276.00	197.00	211.00
Max	1988.00	3012.00	1729.00

Sema: semaglutide, AUC0-5h: area under the curve from dosing up to 5 hours after dosing,
SD: standard deviation, CV: coefficient of variation

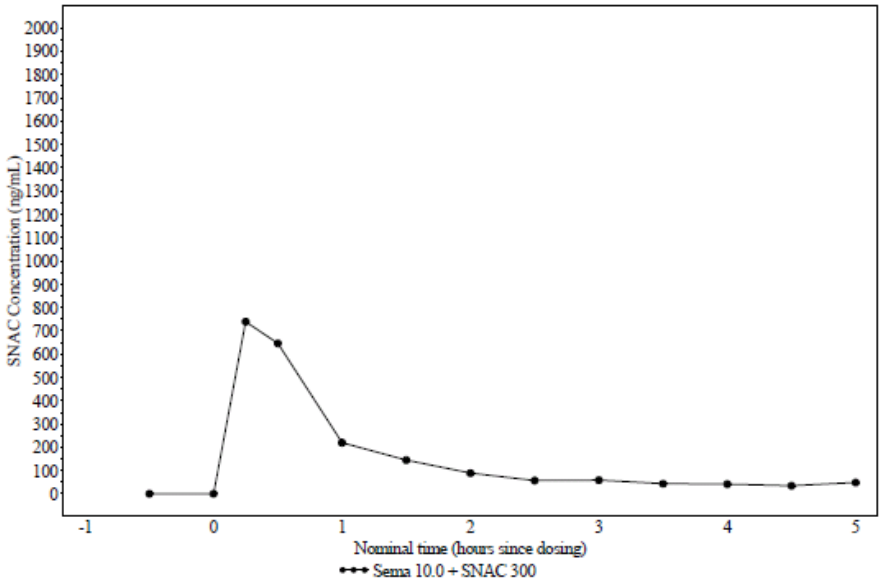
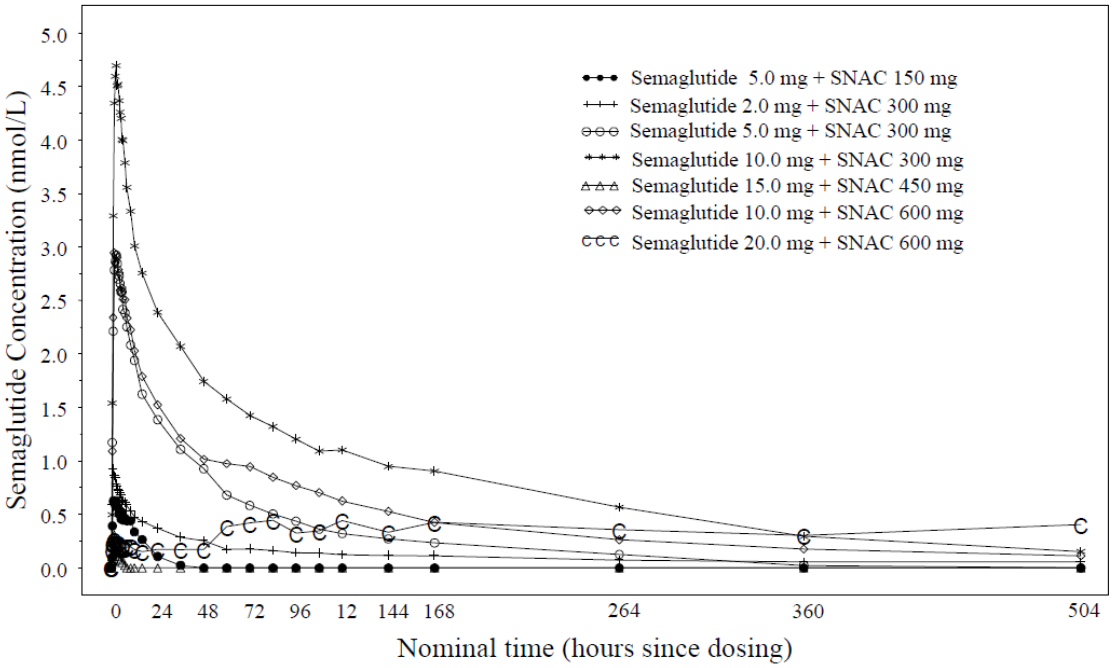


Figure 1. Mean plasma concentration – time profile in human (n=24) for SNAC (300 mg + semaglutide 10 mg)

Semaglutide



Cross-reference: EOT Figure 14.2.8

Figure 11–1 Mean Semaglutide Concentration over Time in Full Analysis Set – Part 1

Table 11–3 Semaglutide $AUC_{0\text{-}504h}$ and C_{max} in Full Analysis Set – Part 1

	Sema 5.0 + SNAC 150	Sema 15.0 + SNAC 450	Sema 10.0 + SNAC 600	Sema 20.0 + SNAC 600	Sema 2.0 + SNAC 300	Sema 5.0 + SNAC 300	Sema 10.0 + SNAC 300
Number of subjects	10	10	10	10	10	10	10
Responders	4	3	6	2	2	6	8
AUC 0-504h post dosing AUC_0_504h (h*nmol/L)							
N	10	10	10	10	10	10	10
Mean	9.33	0.98	241.09	174.86	61.25	151.05	414.47
SD	14.14	1.76	398.22	428.98	193.31	225.33	499.17
CV	151.51	178.72	165.17	245.33	315.59	149.17	120.44
Geometric mean	18.95	3.00	165.66	740.13	26.18	121.66	232.26
Median	0.00	0.00	65.14	0.00	0.00	59.45	329.51
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	35.46	4.98	1246.79	1339.69	611.41	699.62	1595.65
Maximal concentration c_max (nmol/L)							
N	10	10	10	10	10	10	10
Mean	0.73	0.32	3.03	0.49	0.93	3.03	4.80
SD	0.99	0.60	4.64	1.11	2.65	3.55	5.30
CV	135.32	189.48	152.88	226.12	284.63	117.30	110.56
Geometric mean	1.75	0.93	3.36	2.31	2.72	4.06	4.13
Median	0.00	0.00	1.34	0.00	0.00	2.32	3.51
Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Max	2.19	1.83	14.90	3.32	8.42	10.70	17.10

AUC0-504h: area under the curve from dosing up to 504 hours after dosing, SD: standard deviation, CV: coefficient of variation
The calculations of geometric mean only include subjects with at least one quantifiable semaglutide plasma concentration (i.e. responders).
Responders: Subjects with at least one quantifiable semaglutide plasma concentration

Table 11–4 Semaglutide $AUC_{0\text{-}504h}$ and C_{max} in Full Analysis Set – Part 2 (pooled)

	Sema 2.0 + SNAC 300	Sema 5.0 + SNAC 300	Sema 10.0 + SNAC 300	Sema 0.1 i.v.	Sema 0.4 s.c.
Number of subjects	24	24	24	10	10
Responders	6	11	16	10	10
AUC 0-504h post dosing AUC_0_504h (h*nmol/L)					
N	24	24	24	10	10
Mean	29.99	77.12	259.83	138.39	1178.24
SD	125.08	162.06	364.64	42.13	501.88
CV	417.02	210.14	140.34	30.45	42.60
Geometric mean	15.21	54.19	167.52	132.72	1109.19
Median	0.00	0.00	151.50	127.04	1069.38
Min	0.00	0.00	0.00	81.57	702.76
Max	611.41	699.62	1595.65	213.86	2506.62
Maximal concentration c_max (nmol/L)					
N	24	24	24	10	10
Mean	0.64	1.54	3.73	*	4.69
SD	1.79	2.65	4.21	*	0.87
CV	280.62	171.74	113.04	*	18.61
Geometric mean	1.67	2.21	4.18	*	4.62
Median	0.00	0.00	3.17	*	4.66
Min	0.00	0.00	0.00	*	3.80
Max	8.42	10.70	17.10	*	6.59

AUC0-504h: area under the curve from dosing up to 504 hours after dosing, SD: standard deviation, CV: coefficient of variation
The calculations of geometric mean only include subjects with at least one quantifiable semaglutide plasma concentration (i.e. responders)
Responders: Subjects with at least one quantifiable semaglutide plasma concentration
*Cmax of i.v. administered semaglutide was not an endpoint of the trial.

Table 11–5 Semaglutide $AUC_{0\text{-}inf}$, t_{max} and $t_{½}$ in Subjects with at Least one Quantifiable Semaglutide Plasma Concentrati 2 (pooled)

	Sema 2.0 + SNAC 300	Sema 5.0 + SNAC 300	Sema 10.0 + SNAC 300	Sema 0.1 iv.	Sema 0.4 sc.
Number of subjects	24	24	24	10	10
Responders	6	11	16	10	10
Total AUC AUC_0_inf (h*nmol/L)					
N	5	9	15	10	10
Mean	178.65	220.93	503.59	153.74	1564.69
SD	334.08	228.58	530.33	43.16	1514.72
CV	187.01	103.46	105.31	28.08	96.81
Geometric mean	31.30	98.76	283.56	148.31	1266.18
Median	13.72	125.73	373.92	144.68	1116.11
Min	3.73	4.93	4.69	96.69	718.11
Max	772.43	728.22	1816.25	223.42	5832.12
Time for maximal concentration t_max (hour)					
N	6	11	16	10	10
Mean	1.50	12.55	12.06	NA	54.60
SD	0.63	35.65	41.59	NA	10.75
CV	42.16	284.14	344.80	NA	19.69
Geometric mean	1.40	2.38	1.95	NA	53.71
Median	1.25	1.50	1.50	NA	48.00
Min	1.00	0.50	0.50	NA	42.00
Max	2.50	120.00	168.00	NA	72.00
Half-life t_half (hour)					
N	5	9	15	10	10
Mean	57.69	66.02	94.53	44.18	157.57
SD	109.24	74.55	133.89	16.88	162.69
CV	189.35	112.92	141.64	38.20	103.25
Harmonic mean	6.25	7.94	24.69	38.25	112.21
Median	5.87	53.30	73.52	43.01	103.70
Min	2.54	1.48	2.78	23.37	70.71
Max	252.63	242.70	567.52	67.09	617.33

Sema: semaglutide, AUC0-inf: area under the curve from dosing up to infinity, SD: standard deviation, CV: coefficient of variation
Responders: Subjects with at least one quantifiable semaglutide plasma concentration.
*Half-life of i.v. administered semaglutide was not an endpoint of the trial.

Investigation on Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Multiple Doses of a Long-acting GLP-1 Analogue in Healthy Male Subjects (Trial NN9924-3692)

This was a multiple dose, single-center, randomized trial including 3 oral and 1 subcutaneous semaglutide treatment arm in a semi-sequential design. The oral treatment arms (Oral 1, 2 and 3) included both active treatment and placebo allocated in a double-blinded manner. The subcutaneous treatment arm included only open-label active treatment. Oral treatment arms were initiated with a titration period to mitigate discomfort due to gastrointestinal AEs, which could potentially lead to withdrawal. Oral dose escalation started at 2 mg and was carried out in 2-week intervals until reaching the end-dose to be investigated. The final dose of the 3 oral treatment arms was 5 mg, 10 mg, and 20 mg (two 10 mg tablets) semaglutide, respectively. Oral dosing was once daily for 10 weeks and subcutaneous dosing was once weekly for 10 weeks. Each semaglutide tablet was formulated with 300 mg SNAC, meaning the 20 mg group received 600 mg SNAC per day for 4 weeks, once escalation reached the end-dose level. Subjects were fasted overnight prior to dose administration. Tablets were swallowed with 50 mL water and patients remained fasted for 2 hours after dosing.

Exposures increased in a greater than dose-proportional manner between 5 and 10 mg and in a slightly less than dose-proportional manner between 10 and 20 mg. Based on the calculated half-life, steady state should have been achieved by Day 68; however, both C_{max} and AUC appeared to continue to slightly increase on Day 69 and 70. The estimated oral bioavailability for the 2 x 10 mg dose group relative to the subcutaneous exposure was approximately 0.05% (reviewer calculation). The PK data for semaglutide are summarized in the sponsor-generated tables and figures below. PK measurements for SNAC were not conducted.

Treatment was generally well tolerated, with the percentage of AEs for the oral treatment arms (76% to 95%) only slightly higher than the pooled oral placebo arms (71%). The AEs reported during oral semaglutide treatment were primarily gastrointestinal-related (e.g., diarrhea, dyspepsia, abdominal distension, nausea, vomiting), with the frequency increasing with increasing oral semaglutide dose. Between 10 and 33% of subjects in the oral semaglutide treatment arms and 12% in the placebo arm experienced an asymptomatic hypoglycemic episode, defined as glucose ≤3.9 mmol/L by the ADA definition. No severe hypoglycemic events were reported.

Exposure after Administration of 0.5 mg Semaglutide by Subcutaneous Injection on Day 64

	AUC_{tau} (h•nmol/L)	C_{max} (nmol/L)	T_{max} (h)	Half-life (h)	C_{ave} (hnmol/L)
N	11	11	11	11	11
Mean	2941.3	21.46	34.46	173.71	17.51
Geometric Mean	2915.0	21.16	31.55	172.13	17.35
Median	3038.1	21.80	23.85	179.92	18.08
Min; Max	2090.6; 3551.2	13.90; 27.30	22.68; 71.73	139.04; 202.42	12.44; 21.14

(table reproduced by reviewer)

Table 11–1 Summary of semaglutide pharmacokinetic endpoints by oral treatment during last 3 days – full analysis set

	Oral 1 Sema 5 mg	Oral 2 Sema 10 mg	Oral 3 Sema 2x10 mg
Visit 12 Day 68			
AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	54.99 (77.07)	202.15 (145.96)	362.67 (227.93)
Geometric mean (CV)	15.55 (140.15)	151.94 (72.20)	320.60 (62.85)
Median	4.02	177.70	291.65
Min ; Max	2.86 ; 270.10	18.48 ; 553.44	132.18 ; 1156.8
Normalised AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	11.00 (15.41)	20.22 (14.60)	18.13 (11.40)
Geometric mean (CV)	3.11 (140.15)	15.19 (72.20)	16.03 (62.85)
Median	0.80	17.77	14.58
Min ; Max	0.57 ; 54.02	1.85 ; 55.34	6.61 ; 57.84
Cmax (nmol/L)			
N	17	21	18
Mean (SD)	3.53 (3.43)	10.72 (7.48)	18.74 (10.77)
Geometric mean (CV)	2.31 (97.11)	8.55 (69.80)	16.74 (57.48)
Median	2.14	8.67	16.10
Min ; Max	0.97 ; 12.60	1.98 ; 31.00	6.99 ; 55.20
tmax (h)			
N	9	21	18
Mean (SD)	2.56 (3.63)	2.32 (2.48)	2.51 (3.74)
Geometric mean (CV)	1.50 (141.68)	1.76 (107.07)	1.51 (148.67)
Median	1.02	2.00	1.00
Min ; Max	0.52 ; 11.98	0.00 ; 12.05	0.00 ; 12.10

Sema: Semaglutide, SD: standard deviation, CV: coefficient of variation
The calculations of geometric/harmonic mean only include subjects with at least one quantifiable semaglutide plasma concentration

Summary of Semaglutide PK Endpoints by Treatment - Oral Treatment - Full Analysis Set

(Continued)

	Oral 1 Sema 5 mg	Oral 2 Sema 10 mg	Oral 3 Sema 2x10 mg
Visit 12 Day 69			
AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	64.45 (81.84)	217.55 (122.93)	403.61 (261.65)
Geometric mean (CV)	23.41 (126.99)	186.92 (56.51)	348.51 (64.83)
Median	24.74	192.58	317.59
Min ; Max	4.02 ; 310.66	62.91 ; 483.95	96.67 ; 1304.5
Normalised AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	12.89 (16.37)	21.76 (12.29)	20.18 (13.08)
Geometric mean (CV)	4.68 (126.99)	18.69 (56.51)	17.43 (64.83)
Median	4.95	19.26	15.88
Min ; Max	0.80 ; 62.13	6.29 ; 48.40	4.83 ; 65.22
Cmax (nmol/L)			
N	17	21	18
Mean (SD)	4.02 (4.09)	11.22 (6.25)	20.81 (13.16)
Geometric mean (CV)	2.63 (101.77)	9.71 (55.70)	18.03 (63.22)
Median	3.23	10.10	16.25
Min ; Max	0.97 ; 17.00	3.57 ; 24.90	5.29 ; 65.80
tmax (h)			
N	10	21	18
Mean (SD)	2.26 (1.69)	2.00 (1.48)	1.65 (0.87)
Geometric mean (CV)	2.05 (74.97)	1.63 (73.71)	1.57 (52.70)
Median	2.01	2.00	1.98
Min ; Max	0.00 ; 6.00	0.50 ; 6.02	0.00 ; 3.03

Sema: Semaglutide, SD: standard deviation, CV: coefficient of variation
The calculations of geometric/harmonic mean only include subjects with at least one quantifiable semaglutide plasma concentration

Summary of Semaglutide PK Endpoints by Treatment - Oral Treatment
- Full Analysis Set

(Continued)

	Oral 1 Sema 5 mg	Oral 2 Sema 10 mg	Oral 3 Sema 2x10 mg
Visit 12 Day 70			
AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	61.01 (69.17)	256.48 (188.61)	447.78 (268.44)
Geometric mean (CV)	24.08 (113.37)	204.93 (73.54)	391.74 (59.95)
Median	54.62	189.61	359.63
Min ; Max	4.02 ; 252.93	70.30 ; 694.65	194.66 ; 1171.3
Normalised AUC tau (h*nmol/L)			
N	17	21	18
Mean (SD)	12.20 (13.83)	25.65 (18.86)	22.39 (13.42)
Geometric mean (CV)	4.82 (113.37)	20.49 (73.54)	19.59 (59.95)
Median	10.92	18.96	17.98
Min ; Max	0.80 ; 50.59	7.03 ; 69.47	9.73 ; 58.56
Cmax (nmol/L)			
N	17	21	18
Mean (SD)	3.85 (3.18)	13.46 (10.10)	23.03 (14.04)
Geometric mean (CV)	2.81 (82.66)	10.73 (74.99)	20.09 (60.95)
Median	3.23	9.39	17.65
Min ; Max	0.97 ; 13.10	3.48 ; 36.10	10.10 ; 61.30
tmax (h)			
N	12	21	18
Mean (SD)	1.91 (1.55)	2.87 (5.02)	1.81 (1.70)
Geometric mean (CV)	1.47 (80.84)	1.66 (174.78)	1.44 (93.75)
Median	1.50	1.97	1.04
Min ; Max	0.48 ; 5.98	0.00 ; 23.90	0.00 ; 6.05

Sema: Semaglutide, SD: standard deviation, CV: coefficient of variation
The calculations of geometric/harmonic mean only include subjects with at least one quantifiable semaglutide plasma concentration

Summary of Semaglutide PK Endpoints by Treatment - Oral Treatment
- Full Analysis Set

(Continued)

	Oral 1 Sema 5 mg	Oral 2 Sema 10 mg	Oral 3 Sema 2x10 mg
half life (h)			
N	6	20	18
Mean (SD)	146.76 (50.42)	181.56 (69.96)	153.29 (16.53)
Geometric mean (CV)	138.90 (34.35)	170.14 (38.53)	152.45 (10.79)
Harmonic mean	130.76	159.43	151.62
Median	147.44	171.81	150.14
Min ; Max	78.24 ; 205.72	80.81 ; 379.39	126.36 ; 185.75
Cave (hnmol/L)			
N	17	21	18
Mean (SD)	2.54 (2.88)	10.69 (7.86)	18.66 (11.18)
Geometric mean (CV)	1.00 (113.37)	8.54 (73.54)	16.32 (59.95)
Median	2.28	7.90	14.98
Min ; Max	0.17 ; 10.54	2.93 ; 28.94	8.11 ; 48.80

Sema: Semaglutide, SD: standard deviation, CV: coefficient of variation
The calculations of geometric/harmonic mean only include subjects with at least one quantifiable semaglutide plasma concentration

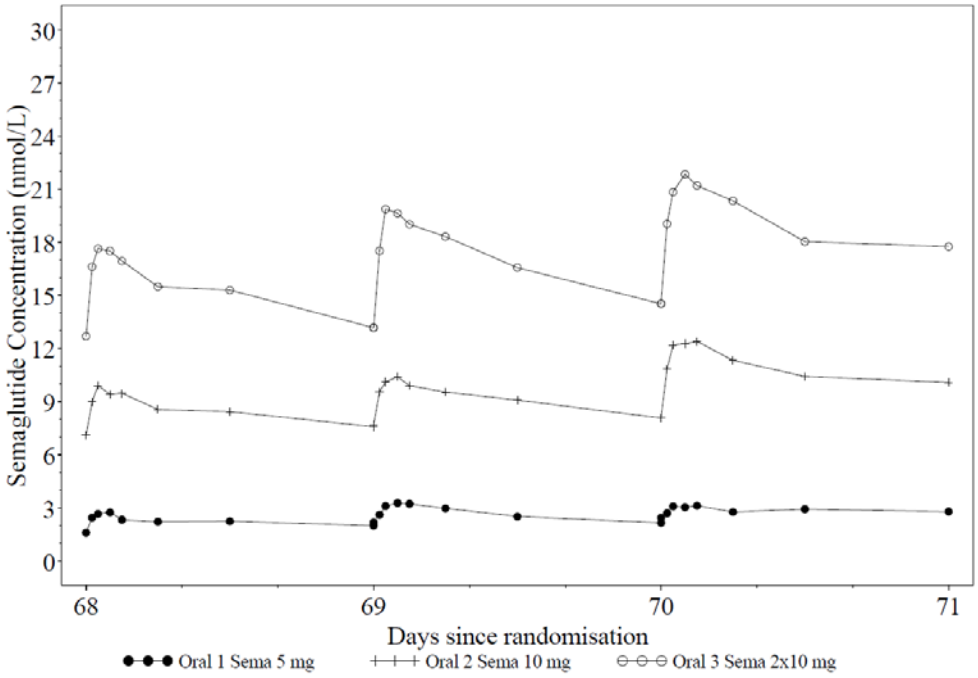


Figure 11-2. Mean oral semaglutide concentration over time - during last 3 days of treatment - full analysis set

A single-center, multiple-dose, open-label randomized trial to evaluate the effect of post-dose meal timings and the effect of volume of water with dosing on the pharmacokinetic properties of oral semaglutide in healthy male subjects (Trial NN9924-3794)

Subjects received 10 mg semaglutide formulated with 300 mg SNAC once daily for 10 days by oral administration. Subjects were divided between 8 arms, with each arm undergoing a different oral dosing procedure. Four of the groups swallowed the tablet with 50 mL water and the other four groups used 120 mL water. The four groups within each water volume condition were further subdivided into four different post-dose fast periods (15, 30, 45, and 60 minutes), after which time a standard breakfast was provided. All subjects were fasted over-night prior to dose administration each day.

The PK data demonstrated that the two dosing water volumes did not have a statistically significant effect on the exposure of semaglutide or SNAC. However, statistically significant increases in semaglutide exposures were observed with longer post-dose fasting periods. T_{max} values for semaglutide also increased with longer post-dose fasting periods. Semaglutide accumulation occurred over the dosing period.

In contrast, exposures for SNAC were highest for the shortest fasting time; T_{max} appeared to be independent of fasting time. There was no indication for SNAC accumulation, as there were no SNAC values above the LLOQ 24 hours after dosing. The PK data for SNAC and semaglutide are summarized in the sponsor-generated tables and figures below.

SNAC

Table 11-7. Summary of Mean SNAC Exposure ($AUC_{0\text{-}6h,\ Day\ 10}$ [h•ng/mL])

Estimated Least Square Means	N	Estimate
A: Sema/50mL/120min	19	754.18
B: Sema/50mL/60min	20	977.94
C: Sema/50mL/30min	20	1069.13
D: Sema/50mL/15min	20	919.23
E: Sema/120mL/120min	20	717.38
F: Sema/120mL/60min	20	828.74
G: Sema/120mL/30min	20	919.87
H: Sema/120mL/15min	19	1014.18

Table 11-9. Summary of Mean SNAC Exposure ($C_{max,\ Day\ 10}$ [ng/mL])

Estimated Least Square Means	N	Estimate
A: Sema/50mL/120min	19	504.98
B: Sema/50mL/60min	20	830.16
C: Sema/50mL/30min	20	1087.74
D: Sema/50mL/15min	20	1392.07
E: Sema/120mL/120min	20	599.88
F: Sema/120mL/60min	20	505.63
G: Sema/120mL/30min	20	819.88
H: Sema/120mL/15min	19	1279.08

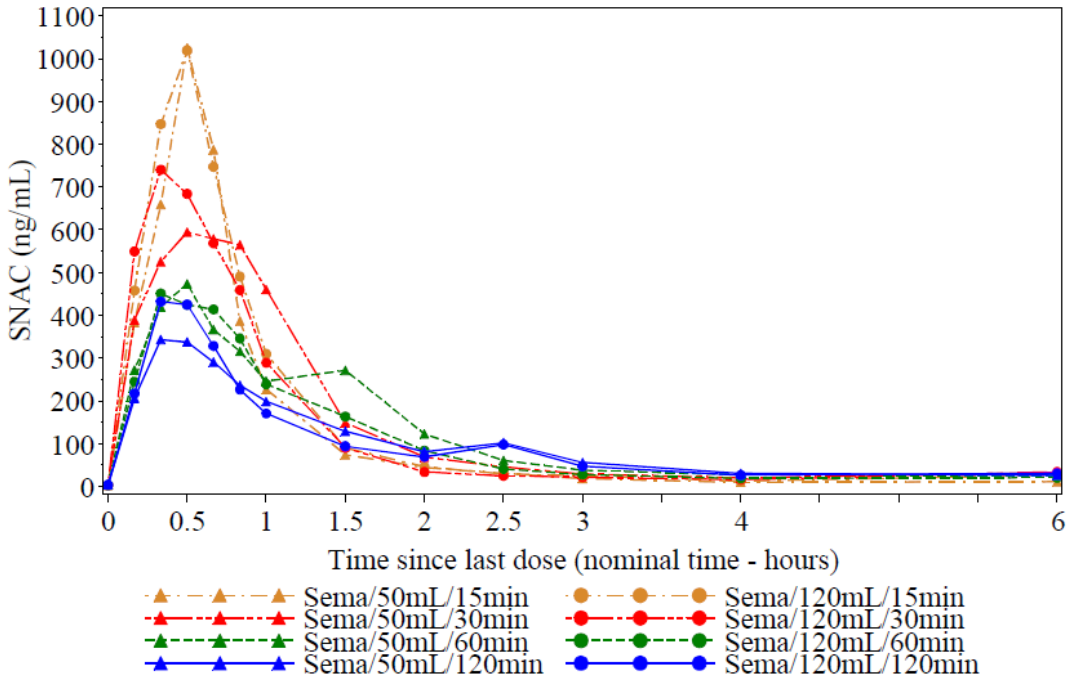


Figure 11–3 SNAC 6-hour concentration profile (ng/mL) - day 10 - geometric mean plot - Full Analysis Set

Semaglutide

Table 11-1. Summary of Mean Semaglutide Exposure ($AUC_{0\text{-}24h,\ Day\ 10}$ [h•nmol/mL])

Estimated Least Square Means	N	Estimate
A: Sema/50mL/120min	19	578.53
B: Sema/50mL/60min	20	376.04
C: Sema/50mL/30min	20	382.41
D: Sema/50mL/15min	20	160.51
E: Sema/120mL/120min	20	592.54
F: Sema/120mL/60min	20	499.12
G: Sema/120mL/30min	20	318.72
H: Sema/120mL/15min	19	192.94

Table 11-1. Summary of Mean Semaglutide Exposure ($C_{max,\ Day\ 10}$ [nmol/mL])

Estimated Least Square Means	N	Estimate
A: Sema/50mL/120min	19	27.81
B: Sema/50mL/60min	20	18.62
C: Sema/50mL/30min	20	19.48
D: Sema/50mL/15min	20	9.09
E: Sema/120mL/120min	20	29.44
F: Sema/120mL/60min	20	24.80
G: Sema/120mL/30min	20	15.78
H: Sema/120mL/15min	19	9.66

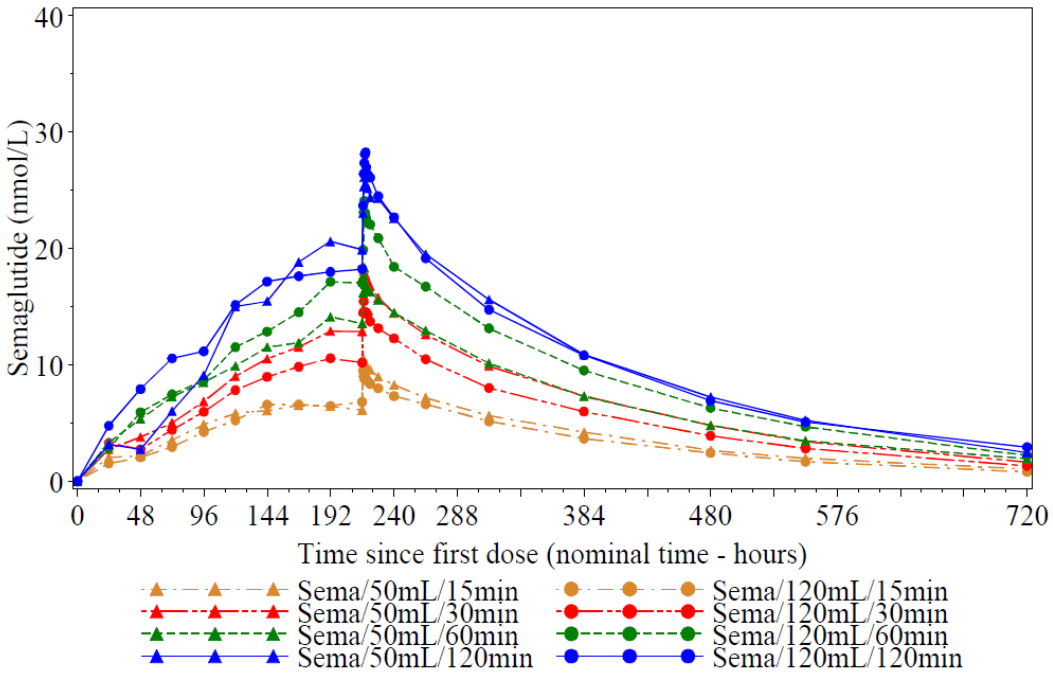


Figure 11-2. Semaglutide concentration profile (nmol/L) - geometric mean plot

Pharmacoscintigraphic investigation of a long-acting oral GLP-1 analogue in healthy male subjects (Trial NN9924-3957)

This was a single-center, randomized, open-label, single-dose, cross-over, PK trial to investigate whether the volume of water administered with oral semaglutide (formulated with SNAC) affects the anatomic location of tablet erosion and whether the pharmacokinetics of semaglutide are correlated to anatomic location and rate of tablet erosion.

Subjects were randomized 1:1 to one of two dosing sequences:

- Sequence A: 10 mg semaglutide formulated with 300 mg SNAC dosed with 50 mL water during dosing Period 1 and with 240 mL water during dosing Period 2.
- Sequence B: 10 mg semaglutide formulated with 300 mg SNAC dosed with 240 mL water during dosing Period 1 and with 50 mL water during dosing Period 2.

Subjects remained fasted until 5 hours after dosing.

The data showed that tablet erosion occurred completely in the stomach for all subjects, independent of dosing water volume. Time to complete tablet erosion appeared slightly longer when administered with 50 mL ((b) (4) minutes) compared with 240 mL (b) (4) minutes). SNAC exposure (AUC and C_{max}) was lower when administered with 50 mL of water compared with 240 mL. Semaglutide exposure (AUC and C_{max}) appeared higher when administered with 50 mL of water compared with 240 mL, which may correlate with the amount of time it takes for the tablet to completely erode. The PK data for SNAC and semaglutide are summarized in the sponsor-generated tables below.

The most frequent reported AEs were nervous system disorders (46%) and gastrointestinal disorders (42%). Most AEs were mild in severity (47/54 events), whereas few events (7/54) were moderate. No SAEs or severe AEs were reported. No severe of confirmed (severe or minor) hypoglycemic episodes were reported.

SNAC

Table 11-10. Statistical analysis of effect of volume of water on SNAC pharmacokinetic endpoints

	FAS	N	Estimate	95% CI	P-value
AUC SNAC, 0-6h, SD (ng*h/mL)					
LSMeans					
Sema 10 mg (50 mL)	26	24	610.69	[544.7; 684.71]	
Sema 10 mg (240 mL)	26	26	752.78	[674.5; 840.14]	
Treatment ratios					
Sema 10 mg: 50 mL / 240 mL			0.81	[0.70; 0.94]	0.0061
Cmax SNAC, SD (ng/mL)					
LSMeans					
Sema 10 mg (50 mL)	26	24	504.05	[407.9; 622.79]	
Sema 10 mg (240 mL)	26	26	811.20	[662.3; 993.51]	
Treatment ratios					
Sema 10 mg: 50 mL / 240 mL			0.62	[0.47; 0.82]	0.0018

Semaglutide

Table 11-3. Statistical analyses of effect of volume of water on semaglutide pharmacokinetic endpoints

	FAS	N	Estimate	95% CI	P-value
AUC semaglutide, 0-24h, SD (nmol*h/L)					
LSMeans					
Sema 10 mg (50 mL)	26	24	119.27	[58.65; 242.52]	
Sema 10 mg (240 mL)	26	26	46.57	[23.59; 91.94]	
Treatment ratios					
Sema 10 mg: 50 mL / 240 mL			2.56	[1.01; 6.47]	0.0471
Cmax semaglutide, SD (nmol/L)					
LSMeans					
Sema 10 mg (50 mL)	26	24	7.83	[4.92; 12.46]	
Sema 10 mg (240 mL)	26	26	4.42	[2.83; 6.90]	
Treatment ratios					
Sema 10 mg: 50 mL / 240 mL			1.77	[0.99; 3.17]	0.0531

Investigation on safety, tolerability, pharmacokinetics and pharmacodynamics of multiple doses of a long-acting GLP-1 analogue in healthy male subjects and male subjects with type 2 diabetes (Trial NN9924-3991)

Healthy or type 2 diabetic males received either placebo, SNAC placebo, or semaglutide once daily for 10 weeks. Subjects receiving semaglutide were dose escalated to avoid severe GI adverse events. The dose escalation scheme for 3 cohorts was 5 mg for 1 week, 10 mg for one week, 20 mg for 2 weeks, and then 40 mg for 6 weeks. The forth cohort received 5 mg and 10 mg each for one week followed by 20 mg for 8 weeks.

The PK data for both semaglutide and SNAC are shown below in the sponsor-generated figures and tables. Semaglutide and SNAC exposures were similar between healthy volunteers and subjects with T2D. Steady state for semaglutide was reached after approximately 22 days for the 20 mg cohort and after approximately 42 days for the 40 mg cohorts. SNAC has a short half-life, and therefore no accumulation was observed.

The number of subjects reporting TEAEs was similar between healthy subjects in the 20 mg and 40 mg (88% and 94%) semaglutide treatment groups and subjects in the placebo and SNAC-placebo groups (89% and 94%). The TEAEs, the majority of which were mild, reported during oral semaglutide treatment were primarily GI disorders with nausea, vomiting, eructation, abdominal pain/distension/discomfort, diarrhea, dyspepsia, and flatulence being the most common.

SNAC

Table 11-5. Summary of SNAC pharmacokinetic endpoints by treatment

	20 mg sema healthy	40 mg sema healthy	SNAC-placebo healthy	40 mg sema T2D	SNAC-placebo T2D
Number of subjects	16	32	18	11	6
Day 69					
AUC,tau (0-24h) (ng*h/L)					
N	15	25	17	8	6
Mean (SD)	1454.68 (517.78)	1892.55 (1255.54)	903.27 (222.78)	1375.80 (908.43)	977.57 (199.21)
Geometric Mean (CV%)	1382.66 (32.84)	1636.32 (54.12)	870.79 (30.83)	1206.22 (53.75)	960.21 (21.19)
Median	1252.78	1432.27	924.15	1176.14	965.88
Min; Max	827.5 ; 2861.5	938.9 ; 5554.4	340.9 ; 1250.6	722.0 ; 3528.5	691.0 ; 1265.0
Cmax (ng/L)					
N	15	25	17	8	6
Mean (SD)	820.87 (501.41)	753.12 (492.21)	981.35 (445.30)	1061.83 (1323.14)	1382.00 (565.15)
Geometric Mean (CV%)	657.91 (86.12)	606.56 (78.98)	876.25 (55.27)	578.82 (191.30)	1290.88 (42.09)
Median	899.00	605.00	973.00	711.00	1334.00
Min; Max	161.0 ; 1915.0	200.0 ; 2236.0	295.0 ; 1752.0	75.6 ; 4159.0	822.0 ; 2318.0
Tmax (h)					
N	15	25	17	8	6
Mean (SD)	0.99 (0.95)	1.34 (1.51)	0.61 (0.58)	1.51 (1.34)	0.54 (0.20)
Median	0.50	0.50	0.35	1.43	0.52
Min; Max	0.2 ; 2.5	0.2 ; 6.0	0.2 ; 2.5	0.2 ; 3.0	0.3 ; 0.9

N: Number of subjects, SD: Standard deviation, CV: Geometric coefficient of variation
sema: semaglutide, T2D: subjects with type 2 diabetes
40 mg semaglutide treated subjects are pooled cohorts 'Oral 40' and 'Oral 60'.
The SNAC content in all tablets except placebo was 300 mg.

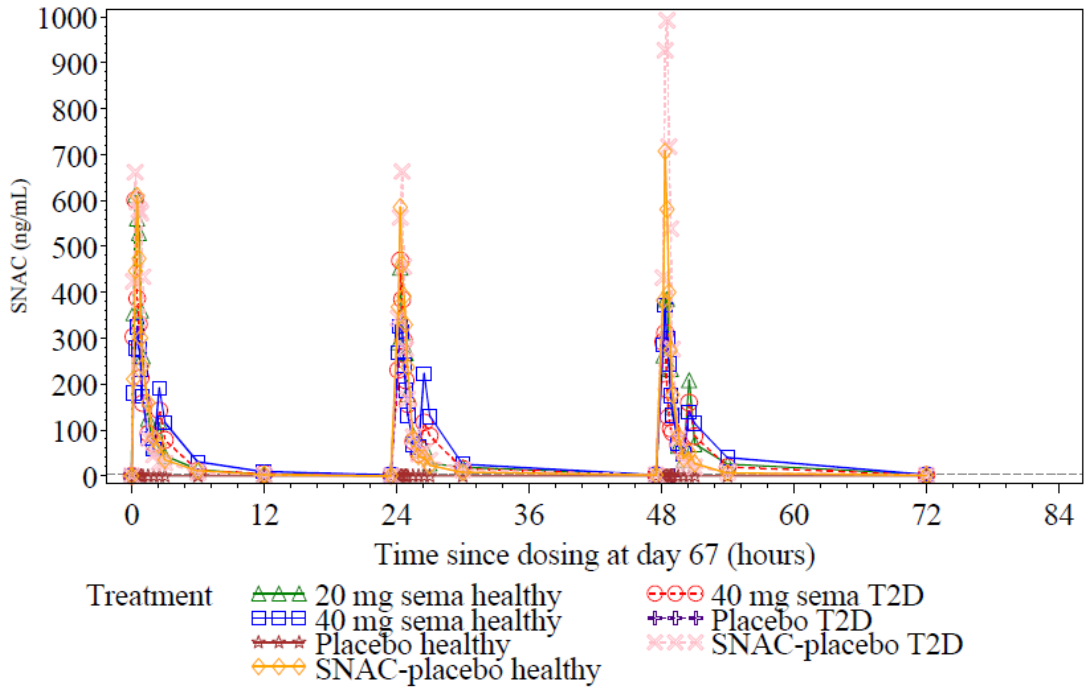


Figure 11-4. Mean plots of SNAC concentration time profiles - during last 3 days of treatment by treatment

Semaglutide

Table 11-1. Summary of semaglutide pharmacokinetic endpoints by treatment on last day of treatment (Day 69)

Day 69	20 mg sema healthy	40 mg sema healthy	40 mg sema T2D
Number of subjects	16	32	11
AUC, tau (0-24h) (nmol*h/L)			
N	15	25	8
Mean (SD)	890.55 (621.72)	1797.70 (1045.27)	1472.63 (849.62)
Geometric Mean (CV%)	741.66 (66.58)	1480.35 (77.99)	1300.82 (54.69)
Median	700.36	1547.47	1008.99
Min; Max	321.6 ; 2666.7	233.4 ; 4207.2	758.4 ; 3190.3
Cmax (nmol/L)			
N	15	25	8
Mean (SD)	43.75 (29.69)	89.23 (52.04)	72.74 (41.50)
Geometric Mean (CV%)	37.05 (62.21)	73.10 (79.68)	64.52 (53.66)
Median	34.70	84.90	51.50
Min; Max	17.2 ; 131.0	11.1 ; 205.0	35.8 ; 159.0
Tmax (h)			
N	15	25	8
Mean (SD)	1.91 (0.76)	3.09 (4.59)	2.63 (2.37)
Median	2.00	2.00	2.51
Min; Max	0.5 ; 3.1	0.0 ; 24.0	0.0 ; 6.0
Half-life (h)			
N	15	25	8
Mean (SD)	153.88 (13.55)	161.70 (14.06)	161.01 (35.36)
Geometric Mean (CV%)	153.34 (8.66)	161.08 (9.06)	158.18 (19.48)
Median	151.30	163.10	150.23
Min; Max	134.6 ; 182.6	129.0 ; 183.4	129.5 ; 242.5

N: Number of subjects, SD: Standard deviation, CV: Geometric coefficient of variation
sema: semaglutide, T2D: subjects with type 2 diabetes
40 mg semaglutide treated subjects are pooled cohorts 'Oral 40' and 'Oral 60'.
The SNAC content in all tablets except placebo was 300 mg.

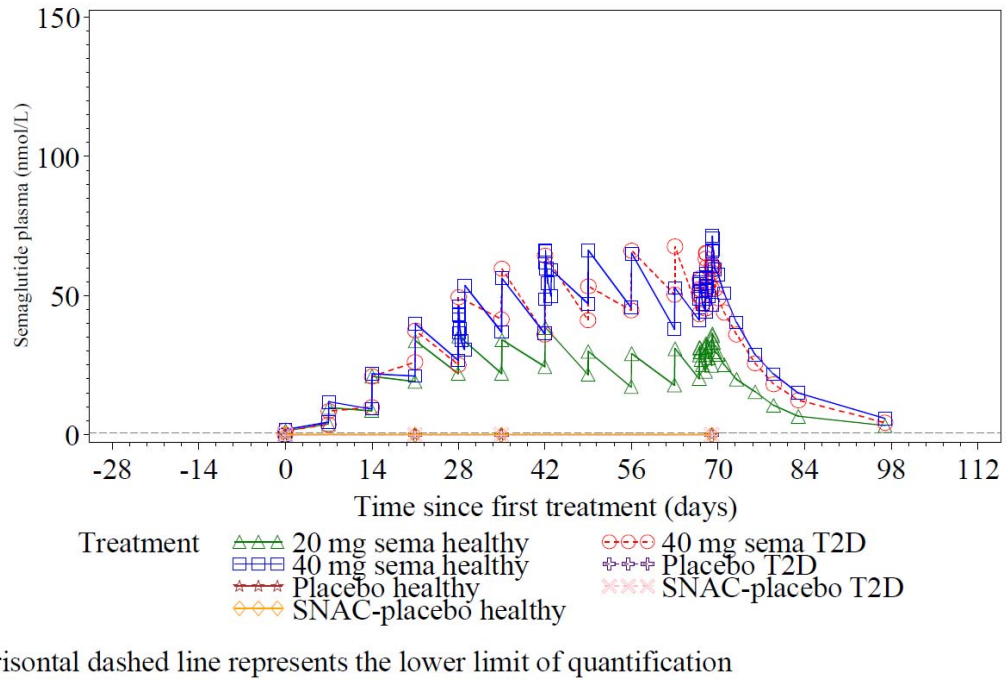


Figure 11-1. Mean plots of semaglutide concentration time profiles by treatment

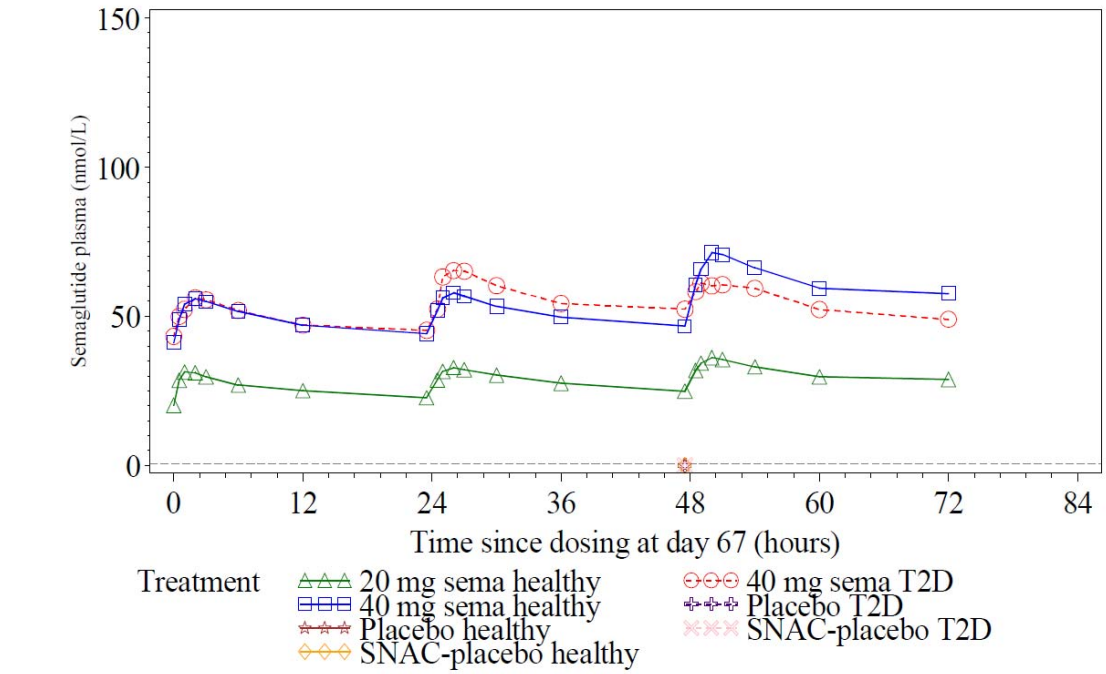


Figure 11-2. Mean plots of semaglutide concentration time profiles - during last 3 days of treatment by treatment

2.8 Regulatory Background

Semaglutide is currently being investigated by Novo Nordisk under IND 79,754 as a subcutaneously administered product for treatment of type 2 diabetes.

3 Studies Submitted

3.1 Studies Reviewed

Table 1. SNAC Nonclinical Program

Study Type and Duration	Route of Administration	Species
Mechanism of absorption		
Effect on lipid membranes	*In vitro* assay	Not applicable
ADME		
Single-dose Absorption	Oral	Mouse, Rat
Repeat-dose Absorption/TK		
13 weeks	Oral	Mouse, Rat
9 months	Oral	Rhesus monkey
12 months	Oral	Rat

Study Type and Duration	Route of Administration	Species
Distribution		
Tissue distribution (single dose)	*In vitro* assay	Not applicable
Plasma protein binding	*In vitro* assay	Not applicable
Metabolism		
In vivo plasma metabolite profile (single dose)	Oral	Mouse, Rat (×2), Rhesus monkey
In vitro metabolite profiles (3 studies)	*In vitro* assay	Mouse, Rat, Rabbit, Rhesus monkey and Human plasma
Cytochrome P450 inhibition (2 studies)	*In vitro* assay	Human
Excretion		
Excretion – Urine and feces	Oral	Mouse
Nonclinical Safety		
Pharmacology screening	*In vitro* assay	Not applicable
Safety Pharmacology		
Effect on CNS	Oral	Rat
Effect on cardiovascular system	*In vitro* assay	Rabbit
Effect on cardiovascular system	Oral	Rhesus monkey
Effect on respiratory system	Oral	Rat
Toxicology		
Single-dose toxicity	Oral	Mouse, Rat
Repeat-dose toxicity		
2 weeks	Oral	Rat, Rhesus monkey
4 weeks	Oral	Cynomolgus monkey
13 weeks	Oral	Mouse, Rat, Rhesus monkey
26 weeks*	Oral	Rat
9 months	Oral	Rhesus monkey
12 months	Oral	Rat
Reproductive and Developmental Toxicity		
Fertility	Oral	Rat
Embryo-fetal development	Oral	Rat, Rabbit
Pre- and post-natal development	Oral	Rat
Genotoxicity		
In vitro genotoxicity	*In vitro* assay	Ames test, cultured human peripheral lymphocytes
In vivo genotoxicity	Oral	Mouse

*Study sponsored by Novo Nordisk (Study no. JLY0278); all other studies sponsored by Emisphere Technologies or a partner to Emisphere.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Disclaimer: Some tables, figures, and/or text were taken from the Sponsor's submission, where indicated.

4 Pharmacology

NNC 0113-0217 (semaglutide) is a potent, long-acting GLP-1 analog discovered and developed by Novo Nordisk for the treatment of type 2 diabetes. The extended half-life occurs through the addition of 1, 18-octadecanedioic acid along with OEG- and Glu-spacers onto Lys^{26}. The mechanism of protraction is based on albumin binding, and the acylation technology is similar to that used for another Novo Nordisk GLP-1 analog product, liraglutide. Primary pharmacology studies, conducted in vitro and in vivo in diabetic mice, normal rats, and pigs, have shown NNC 0113-0217 to be a potent glucose and body weight lowering drug candidate.

4.1 Primary Pharmacology

Mechanism of action: Semaglutide (NNC 0113-0217) binds the human GLP-1 receptor. Therefore, the mechanism of action is believed to be the same as endogenous GLP-1. Endogenous GLP-1 lowers blood glucose by stimulating glucose-dependent insulin secretion and insulin biosynthesis, inhibiting glucagon secretion and gastric emptying. Studies in animals have also shown that GLP-1 stimulates beta cell proliferation and neogenesis while inhibiting apoptosis, resulting in an increased or maintained beta cell mass. Decreased gastric emptying is believed to result in enhanced fullness/satiety and decreased food intake, which in turn, is thought to contribute to decreased body weight.

Drug activity related to proposed indication:
In vitro assays have demonstrated that semaglutide is a GLP-1 receptor agonist with a potency comparable to liraglutide and approximately 8-fold less than natural GLP-1. In an isolated rat pancreas perfusion model, semaglutide-induced insulin secretion occurred at an EC_{50} of 13 to 14.5 nM semaglutide in the presence of 10 µM glucose. A single subcutaneous administration of semaglutide (30 nmol/kg) significantly stimulated plasma insulin and decreased blood glucose in male Wistar rats following a glucose challenge. In db/db diabetic mice, a single administration of semaglutide decreased blood glucose for 48 hours (49.1 nmol/kg for actual blood glucose AUC and 31.6 nmol/kg for delta blood glucose), and the ED_{50} for blood glucose lowering 6 hours post dosing was estimated to be 0.30 nmol/kg. Statistically significant decreases in body weights were noted in male db/db mice 48 hours after treatment with 30 and 100 nmol/kg semaglutide and 100 nmol/kg liraglutide. The potency to reduce food intake in normal mice for 45 hours was 3.3 nmol/kg for semaglutide and 45 nmol/kg for liraglutide. Food intake was reduced in female LYD pigs after single and repeated

administrations of semaglutide and food consumption returned to control values as drug plasma concentrations decreased. In beta-cell reduced Göttingen minipigs, pretreatment with semaglutide for 1 week resulted in increased glucose infusion rates on Days 1 and 3 and increased plasma insulin levels on Days 1, 3, and 7. The results of these studies showed that the effects on body weight, food consumption, plasma glucose reduction, and insulin secretion were similar to or more potent than that of liraglutide, and the effects tended to be prolonged compared with liraglutide due to the extended half-life of semaglutide.

Study title: Semaglutide dosed orally in combination with SNAC: Effects on blood glucose and body weight in db/db mice (mmla090602)

Male db/db mice received a single oral dose of vehicle, semaglutide, SNAC, or semaglutide formulated with SNAC. Glucose measurements were conducted 0.5 hours before dosing and 1, 2, 4, 6, 12, 24, 48, and 72 hours after dosing. Body weights were measured before dosing and at 24, 48, and 72 hours after dosing. For Groups 6 and 7, dosing occurred at time 0, an intraperitoneal glucose load was given 30 minutes after dosing, and blood glucose was measured 30 minutes before dosing and 30, 45, 60, 75, 90, 120, 150, 210, 240, and 360 minutes after dosing. The study design is shown in the table below.

Group*	Semaglutide (nmol/kg)	SNAC (mg/kg)	Number of Males/Group	Endpoints
1	0	0	6	Serial blood glucose measurements
2	1000	0	4	Serial blood glucose measurements Plasma semaglutide measurement at 72 hours postdose
3	0	200	6	Serial blood glucose measurements
4	1000	200	5	Serial blood glucose measurements Plasma semaglutide measurement at 72 hours postdose
5			6	Serial blood glucose measurements Plasma semaglutide measurement at 6 hours postdose
6			6	IPGTT at 30 minutes postdose Plasma semaglutide measurement at 6 hours postdose
7	0	0	6	IPGTT at 30 minutes postdose

*Dosing of Groups 1-5 was preceded by 6 hours of fasting; Dosing of Groups 6 and 7 was preceded by 18 hours of fasting.
IPGTT = intraperitoneal glucose tolerance test

Results

A single dose of oral semaglutide reduced glucose levels over the first 24 hours compared with vehicle, SNAC alone, or semaglutide alone (sponsor-generated Figure 2). The change in glucose levels during the IPGTT remained lower in animals that received oral semaglutide (sponsor-generated Figure 3). Treatment-related decreased body weight was also noted at the 24 hour time point, which is consistent with the pharmacology of semaglutide (sponsor-generated Figure 4). Plasma levels of

semaglutide were measurable 6 hours after dosing, but not 72 hours after dosing (sponsor-generated Figure 5). The results indicate that the bioavailability of orally administered semaglutide is sufficient to lower blood glucose levels.

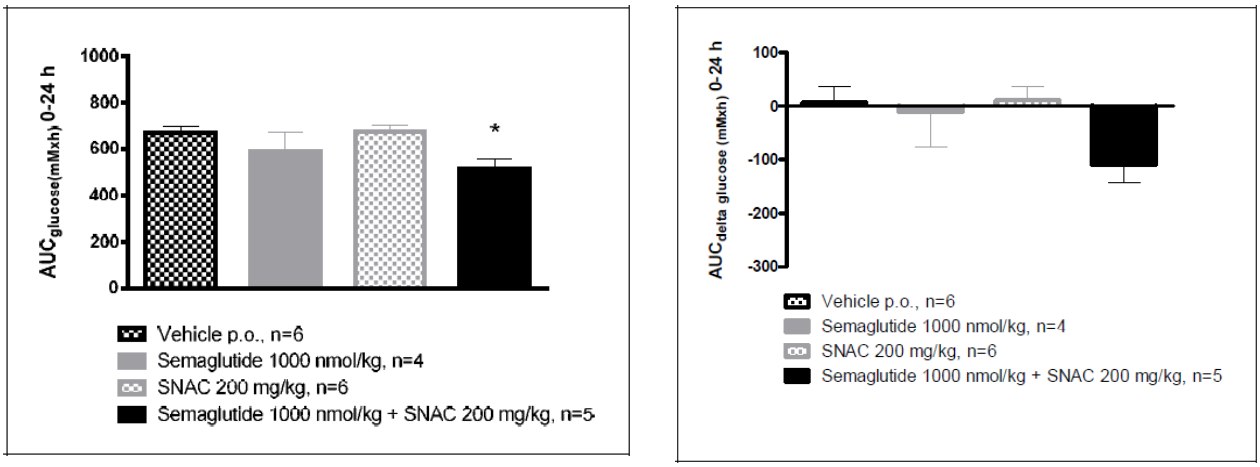


Figure 2 Effect of semaglutide and/or SNAC on $AUC_{glucose}$ (left panel) and $AUC_{deltaglucose}$ (right panel) during the first 24 hours of the study in db/db mice after oral dosing. n=4-6. *:p<0.05 vs. vehicle by post-test

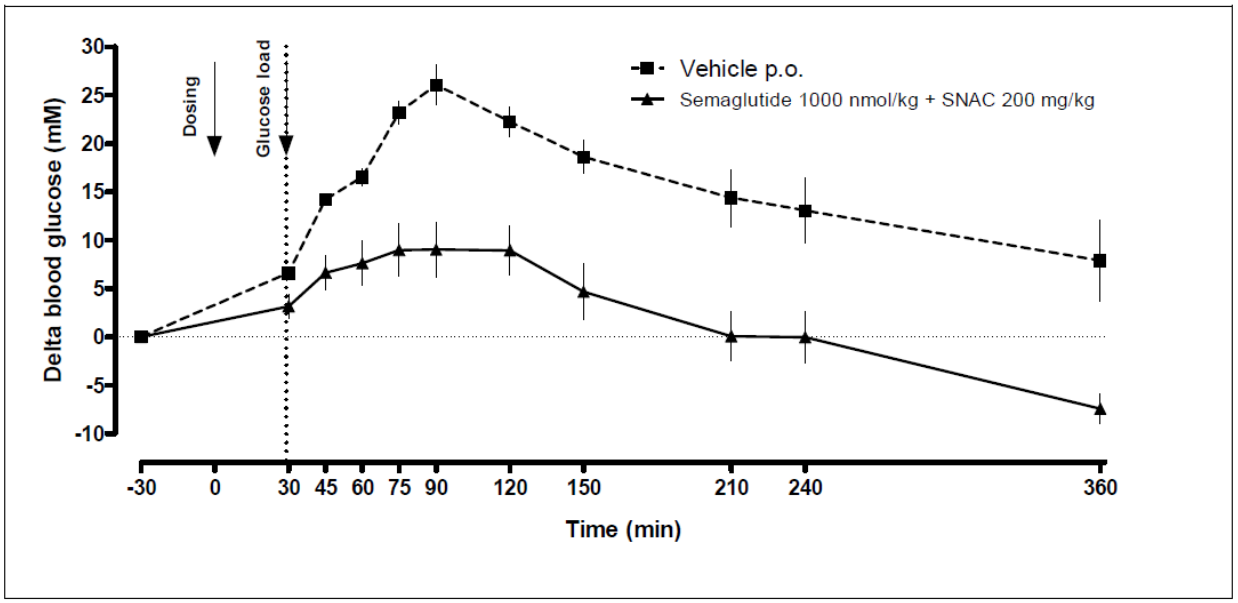


Figure 3 Effect of semaglutide and SNAC on blood glucose in db/db mice after oral dosing at time=0 and IPGTT at t=30 min. Dosing was preceded with an 18 hour fast. n=6.

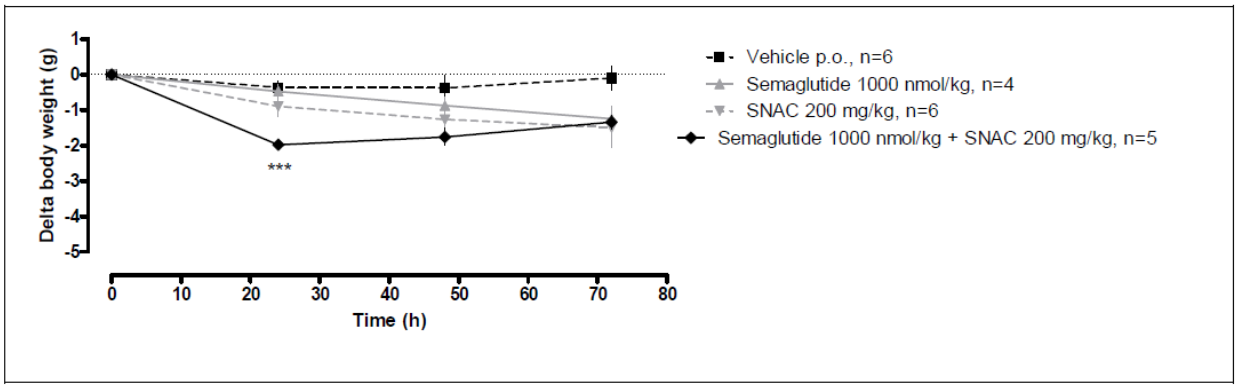


Figure 4 Delta body weight in db/db mice after oral dosing at time=0. Dosing was preceded with a 6 hour fast. n=4-6. ***:p<0.001 vs. vehicle by post-test

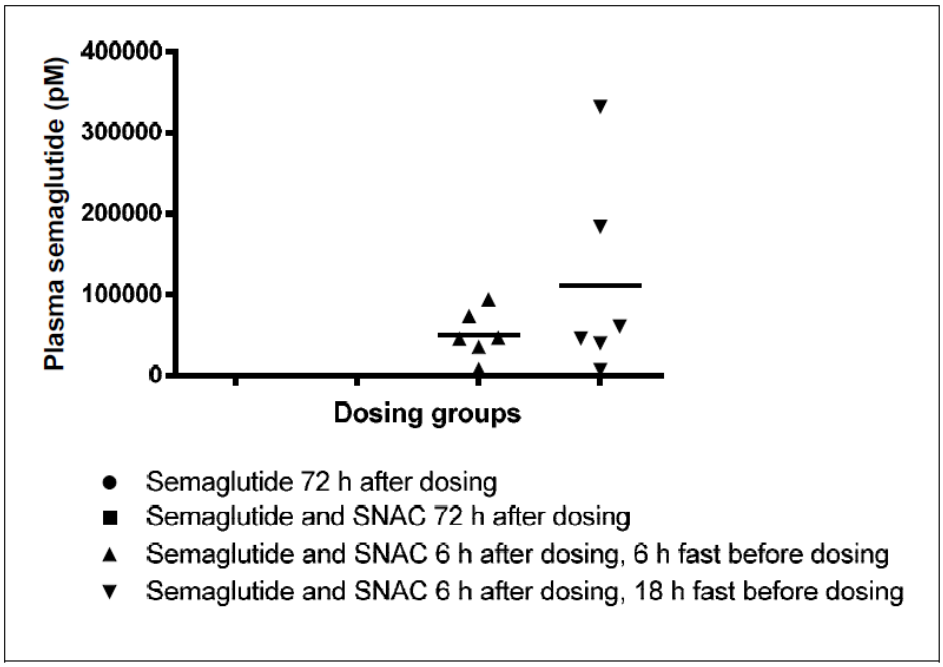


Figure 5 Plasma semaglutide concentrations in db/db mice after oral dosing at time=0.

Study title: Semaglutide and SNAC in tablets: Pharmacokinetic effects on food intake and body weight in rats (tbck081102)

Carotid artery cathetered male Sprague-Dawley rats (6/group) received a single oral dose of semaglutide formulated with SNAC in tablet form or SNAC alone. Pharmacodynamic effects were compared to a sham control group and a group receiving semaglutide by intravenous injection. Rats were fasted for 16 hours before dosing and food was returned 30 minutes after dosing. The dose groups are summarized in the sponsor-generated table below.

Table 1 Doses and route of administration in the groups

Group number	Group name	Route of administration	Total dose of semaglutide	Total dose of SNAC
1	Sham	-	0	0
2	1 Tablet	p.o.	41 nmol	35 mg
3	2 Tablet	p.o.	82 nmol	70 mg
4	SNAC only	p.o.	0	70 mg
5	i.v. dosing	i.v. infusion	4 nmol/kg	0

Plasma exposure of semaglutide was measured at 1, 2, 3, 4, 6, 24, and 30 hours after dosing. Body weights were recorded immediately before dosing and at 24 hours after dosing. Food consumption was measured by weighing food containers at 8, 24, and 30 hours after dosing.

Results

The PK profiles of 1 or 2 tablets of oral semaglutide and intravenous semaglutide are shown in sponsor-generated Figure 1. AUC values and oral bioavailability were not calculated. There was great variability in semaglutide exposure among the six rats receiving 2 tablets, with C_{max} values being 645, 4977, 9261, 30000, 445000, and 887000. The T_{max} was between 1 and 3 hours for four animals with the lowest C_{max} values and was 5 hours for the two animals with the highest C_{max} values. C_{max} values within groups were more similar for animals receiving one tablet and intravenous semaglutide.

Treatment-related effects on body weight gain or body weight loss was greatest in the two tablet group, followed by the intravenous semaglutide group, and then the single tablet group. The effects on body weight generally correlated with decreased food intake.

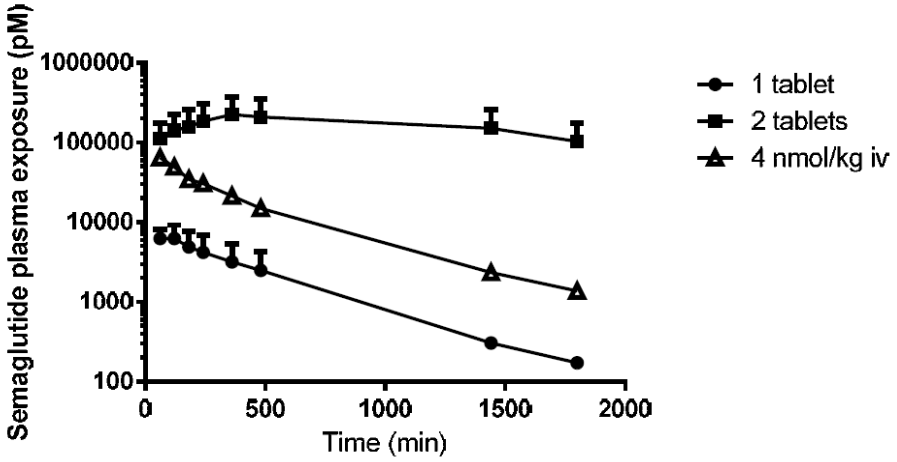


Figure 1. Plasma exposure of semaglutide in rats dosed by oral or intravenous routes

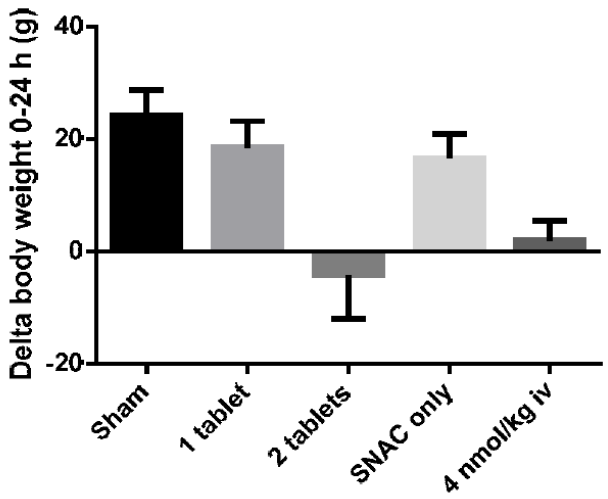


Figure 2. Change in body weight in rats dosed by oral or intravenous routes

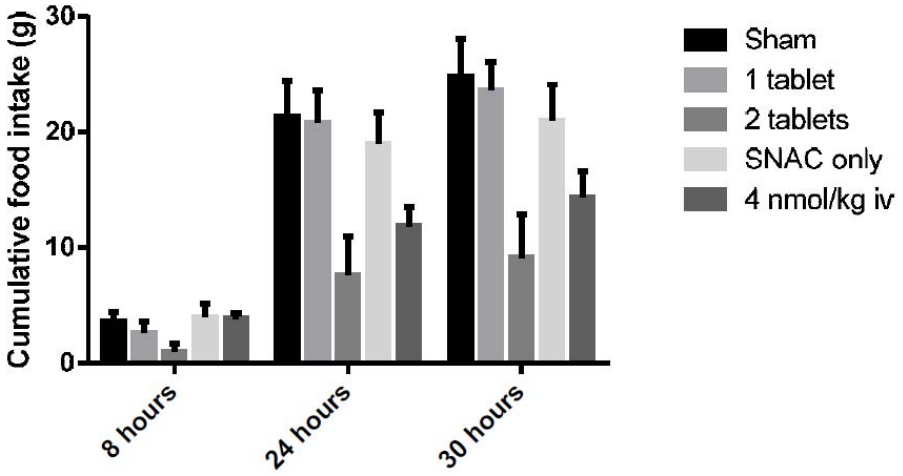


Figure 3. Cummulative food intake in rats dosed by oral or intravenous routes

4.2 Secondary Pharmacology

Study title: Pharmacology report: Radioligand binding assay with SNAC (211415)
The activity of SNAC was assessed in vitro for a panel of approximately 160 receptors and channels using a radioligand binding assay (SpectrumScreen). The assay was conducted at (b) (4) A response was considered to be significant when ≥50% inhibition or stimulation was observed for biochemical assays.

There were no results that met the criteria for a significant interaction. Results for the human prostanoid DP and prostanoid EP_4 receptors showed 49% and 34% inhibition, respectively, at 30 µM SNAC. All other interactions were modified by ±30% or less.

Study title: Pharmacology report: Radioligand binding assay with SNAC (211532)
Based on the results from the in vitro receptor binding assay, a subset of receptors was evaluated for SNAC activity in a tissue assay. The following receptors were evaluated: prostanoid EP_4, purinergic P_{2x}, serotonin (5-hydroxytryptamine) 5-HT_{2B}, tachykinin NK_3, and thyrotropin releasing hormone. The effect of SNAC on the activity of each of the receptors was assessed in tissues from rat trachea, rat vas deferens, rat stomach fundus, rat portal vein, and Taenia coli, respectively. A response was considered to be significant when ≥50% inhibition or stimulation was observed for biochemical assays.

No significant effects were noted in any of the assays. The greatest effect was noted for thyrotropin releasing hormone, which showed only 15% inhibition.

Study title: Pharmacology report: Radioligand binding assay with SNAC (213095)
The activity of SNAC was assessed in vitro for the cyclooxygenase-1 and cyclooxygenase-2 receptors using a radioligand binding assay (SpectrumScreen). The assay was conducted at (b) (4) A response was considered to be significant when ≥50% inhibition or stimulation was observed for biochemical assays.

SNAC showed 14% and 6% stimulation at the COX-1 and COX-2 receptors, respectively. Therefore, SNAC showed no significant activity at either of these receptors.

Study title: Pharmacology report: Radioligand binding assay with SNAC (212207)
The activity of SNAC was assessed in vitro for a panel of approximately 160 receptors and channels using a radioligand binding assay (SpectrumScreen). The assay was conducted at (b) (4) A response was considered to be significant when ≥50% inhibition or stimulation was observed for biochemical assays.

As shown in the sponsor-generated table below, 100 µM SNAC showed ≥50% inhibition for two receptors, human prostanoid DP and prostanoid EP_4. SNAC showed >30% inhibition for two other receptors, prostanoid EP_2 and serotonin 5-HT_{2B}. All other targets showed less than 30% inhibition or stimulation.

Cat #	Assay Name	Batch*	Spec.	Rep.	Conc.	% Inh.
(b) (4)	Prostanoid DP	316389	hum	2	100 µM	77
	Prostanoid EP_2	316390	hum	2	100 µM	34
	Prostanoid EP_4	316245	hum	2	100 µM	58
	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	316246	hum	2	100 µM	42

Study title: Pharmacology report: Radioligand binding assay with NNC 0113-3705, NNC 0113-3706, NNC 0113-3707, NNC 0113-3708, and NNC 0113-3709 (213078)

The activity of five SNAC metabolites was assessed in vitro for a panel of approximately 160 receptors and channels using a radioligand binding assay (SpectrumScreen). The assay was conducted at (b) (4) A response was considered to be significant when ≥50% inhibition or stimulation was observed for biochemical assays.

The following activity was noted for NNC 0113-3707:

Cat #	Assay Name	Species	Conc.	% Inh.
(b) (4)	Endothelin ET_A	hum	100 µM	86
	Melatonin MT_1	hum	100 µM	73
	Somatostatin sst2	hum	100 µM	66
	Somatostatin sst3	hum	100 µM	57
	Somatostatin sst4	hum	100 µM	61

No significant results were observed for compounds 3705, 3706, 3708, or 3709.

Study title: PharmaScreen® of E414 (211391)

SNAC (E414) was assessed in an array of 75 functional and receptor mediated in vitro and in vivo assays (PharmaScreen) to determine potentially important primary and/or secondary biological activities. Significant activity was considered to have occurred when >50% inhibition was observed.

Results:

Of the various assays conducted, there was a significant response reported for the tracheal relaxation assay (21% at 10 µM and 70% at 30 µM) and arachidonate-induced platelet aggregation assay (0% at 3 µM and 100% at 30 µM). The remaining 73 assays were negative.

The reduction of spontaneous tone in the tracheal relaxation assay and the inhibition of platelet aggregation upon induction with sodium archachidonate did not correlate with other similar assays. The sponsor concluded that there may be a possible relationship between these findings and cyclooxygenase inhibition; however, the large SNAC concentrations relative to the positive control suggest that these findings are not significant.

Study title: Pharmacology screen of EMS-17 (SNAC) (211392)

The inhibitory activity of SNAC on arachidonic acid-induced platelet aggregation and tracheal relaxation was evaluated using rabbit platelet rich plasma and guinea pig trachea.

Results:

Incubation with SNAC resulted in significant inhibitory activity (100% at 30 μM), which may account for the moderate relaxation of spontaneous tone in guinea pig trachea (48% at 30 μM). At 10 μM, SNAC did not show inhibitory activity on arachidonic acid-induced platelet aggregation.

Study title: Evaluation of the transport of fluorescein isothiocyanate-dextran (FD) 4, 10, 20, and 70 kDa across Caco-2 monolayers in the presence of SNAC (gihe130305)

The transport of varying molecular weights of fluorescein isothiocyanate-dextran (FD) in the presence of SNAC was evaluated in vivo using the Caco-2 cell culture model of intestinal epithelium. Caco-2 monolayers were incubated with FD plus 80 mM SNAC in the donor compartment. The amount of FD transported from the donor chamber (apical side) to the receiver chamber (basolateral side) was measured over 60 minutes.

The inclusion of SNAC in the donor chamber resulted in a clear size-dependent enhancement in absorption of FD, with a diminishing effect on the transport of molecules as they exceed 4 kDa. The results indicated that the capacity of SNAC to enhance the absorption of compounds with a molecular weight of 70 kDa or greater is minimal.

Study title: Evaluation of the duration of action of the permeation enhancing effect of SNAC in Caco-2 monolayers (gihe130401)

The transport of 4 kDa fluorescein isothiocyanate-dextran (FD4) in the presence of SNAC was evaluated in vivo using the Caco-2 cell culture model of intestinal epithelium. Caco-2 monolayers were incubated with FD4 with or without 80 mM SNAC, both concomitantly and at intervals of 20 and 40 minutes following removal of SNAC. The amount of FD4 transported from the donor chamber (apical side) to the receiver chamber (basolateral side) was measured over 120 minutes.

While concomitant incubation of FD4 with SNAC (for 20 minutes) resulted in marked enhancement in its cumulative transport across Caco-2 monolayers, a significant diminution of FD4 transport was observed following intervals of 20 and 40 minutes between SNAC treatment and FD4 addition (Sponsor-generated Figure 1). Moreover, a swift recovery in monolayer integrity was observed upon removal of SNAC, as illustrated by a near complete restitution in trans-epithelial electrical resistance (sponsor-generated Figure 2). The results of this study demonstrate that the window of action of SNAC is relatively short and supports the hypothesis that the effects of SNAC on intestinal permeability is temporary, with increases in permeability substantially reversed after as little as 20 minutes, under the conditions of this model.

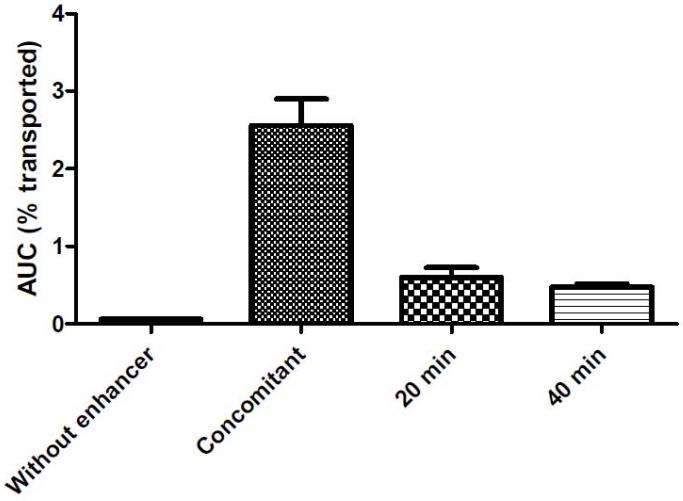


Figure 1 **Cumulative transport of FD4 across Caco-2 monolayers for 120 min upon concomitant incubation with SNAC (100 mM) for 20 min, and following intervals of 20 and 40 min between SNAC treatment and addition of FD4.Data are represented as means ±SEM, *n*=4-8.**

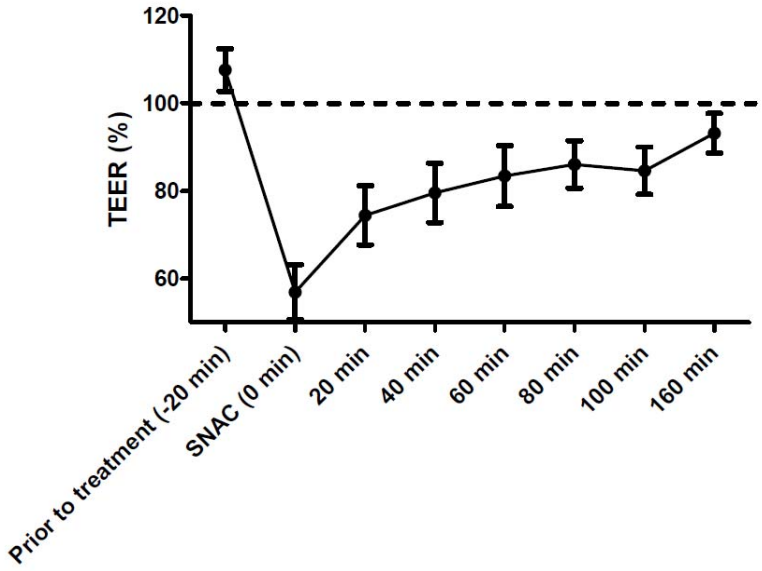


Figure 2 **Effect of SNAC removal on TEER depicted as % TEER relative to TEER in the absence of SNAC at each time point. Data are represented as means ±SEM, *n*=8.**

Study title: Evaluation of the transport of fluorescein isothiocyanate-dextran (FD) 4, 10, 20, and 70 kDa across the rat small intestine in the presence of SNAC (gbon130407)

The transport of varying molecular weights of fluorescein isothiocyanate-dextran (FD) in the presence of SNAC was evaluated ex vivo distal segments of rat small intestine mounted in Ussing chambers. The intestinal tissue (stripped of the muscle layer) was incubated with FD with or without 100 mM SNAC in the donor compartment. The amount of FD transported from the donor chamber (apical side) to the receiver chamber (basolateral side) was measured over 60 minutes.

The inclusion of SNAC in the donor chamber resulted in a clear size-dependent enhancement in absorption of FD, with a diminishing effect on the transport of molecules as they exceed 4 kDa. The results indicated that the capacity of SNAC to enhance the absorption of compounds with a molecular weight of 70 kDa or greater is minimal.

4.3 Safety Pharmacology

Neurological effects:

Study title: The effects of SNAC in the Irwin test in Sprague-Dawley rats (209222)

A GLP study was conducted to assess the potential neurological effects of SNAC in male rats. Rats (6/group) were administered a single oral dose of vehicle (deionized water), SNAC (500, 1000, or 1500 mg/kg), or 20 mg/kg chlorpromazine (positive control). A battery of Irwin test parameters were assessed prior to dosing and at 0.5, 4, and 24 hours postdose.

Results

At 500 mg/kg SNAC, the only abnormality noted was a slight decrease in touch response at the 0.5 hour time point. This finding was not observed at the later time points.

At 1000 mg/kg SNAC, significant depressant effects on respiration were observed following treatment together with piloerection and generalized signs of CNS depression (i.e., decreased touch response). One animal died at approximately 35 minutes after treatment. All surviving animals recovered by 24 hours postdose. The lungs of the decedent animal showed slight reddening with small hemorrhagic areas.

At 1500 mg/kg SNAC, significant depressant effects on respiration were observed following treatment together with piloerection and generalized signs of CNS depression (i.e., slightly decreased touch response, slightly decreased grooming). Two animals died within 30 minutes of treatment and a third animal died at approximately 50 minutes postdose. All surviving animals recovered by 24 hours postdose. The lungs of the decedent animals showed slight reddening with small hemorrhagic areas.

Chlorpromazine produced effects consistent with its known pharmacological activity.

The SNAC used in this study (batch #P144-121-1) was 97.6% pure and the dosing formulations were found to be within ±5% of nominal.

Study title: The effects of SNAC in the Irwin test in Sprague-Dawley rats (209223)

As a follow-up to a previously conducted Irwin test using higher dose levels, a second GLP study was conducted to assess the potential neurological effects of SNAC in male rats using lower doses to establish a NOEL. Rats (6/group) were administered a single oral dose of vehicle (deionized water), SNAC (250, 500, and 750 mg/kg), or 20 mg/kg chlorpromazine (positive control). A battery of Irwin test parameters were assessed prior to dosing and at 0.5, 4, and 24 hours postdose.

Results

Under the conditions of this study, oral treatment with SNAC at doses of 250, 500, and 750 mg/kg did not result in significant behavioral or physiological changes. Chlorpromazine produced effects consistent with its known pharmacological activity.

The SNAC used in this study (batch #P144-119-1) was 97.3% pure and the dosing formulations were found to be within ±4% of nominal. The test article purity and measured concentrations of dosing formulations were similar between this study and the previously conducted study (209222). The NOEL in this study was ≥750 mg/kg whereas the NOEL in the previous study was ≤500 mg/kg. Additionally, treatment-related death and adverse effects on respiration were noted at 1000 mg/kg in the previous study. It is not clear why treatment-related effects were not observed at 750 mg/kg in this study, as would be expected based on the results from the previous study. The results of these two studies suggest that SNAC has a steep toxicity curve.

Cardiovascular effects:

Study title: Effects of test articles on ion channels expressed in mammalian cells (213072)

The in vitro effects of SNAC and the main metabolite of SNAC (E506) on 12 cardiac ion channels (hCav1.2, hCAV3.2, hHCN2, hHCN4, hERG, hKir2.1, hKir3.1/hKir3.4, Kir6.2/SUR2A, hKvLQT1/mink, hKv1.5, hKv4.3, and hNav1.5) were evaluated in an automated parallel patch clamp system (QPatch) using CHO or HEK293 cell lines expressing the receptors via transfection (non-GLP study). A positive control was used for each of the channels.

Results:

After addition of 2, 20, or 200 µM SNAC, mean inhibition of the 12 selected cardiac ion channels did not exceed 16% and a clear dose-response relationship was not observed (Figure 1). After addition of 2, 20, or 200 µM E506, mean inhibition of 11/12 selected cardiac ion channels did not exceed 9.2% and did not display a clear dose-response relationship (Figure 2). Mean inhibition of hCAV1.2 did not exceed 21.3% at any of the E506 concentrations tested (2, 20, 70, 200, and 500 µM) and also did not exhibit a clear dose-response relationship, although the greatest inhibitory effects occurred at the three highest concentrations tested.

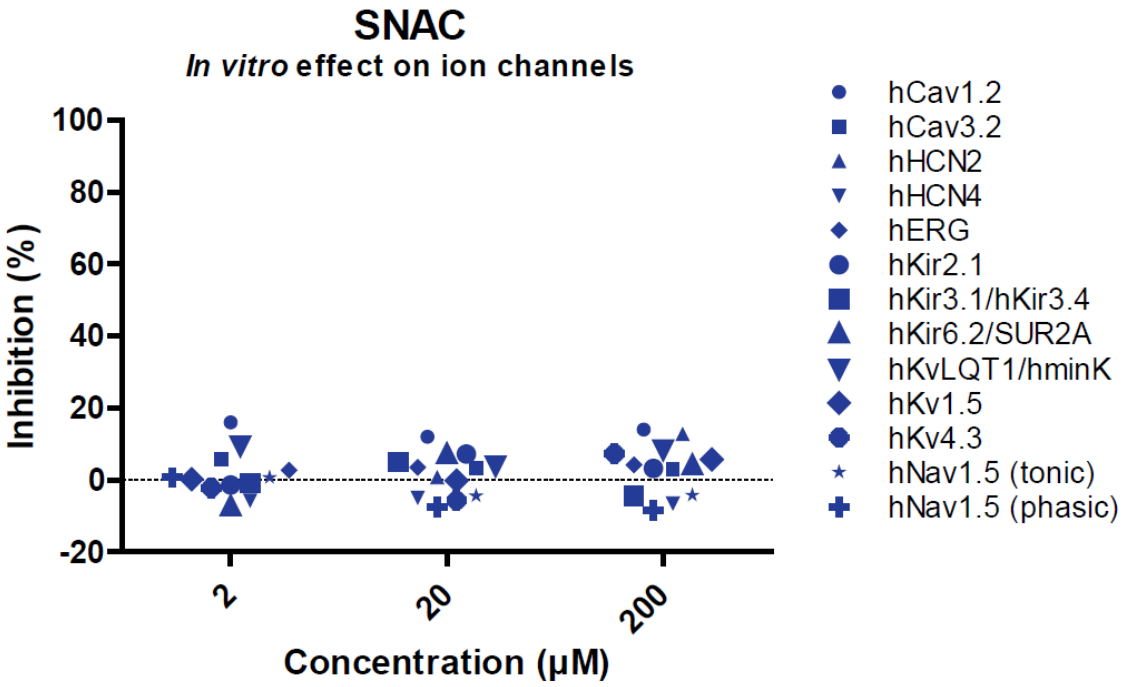


Figure 1. Overview of ion channel effects after SNAC application (sponsor-generated table)

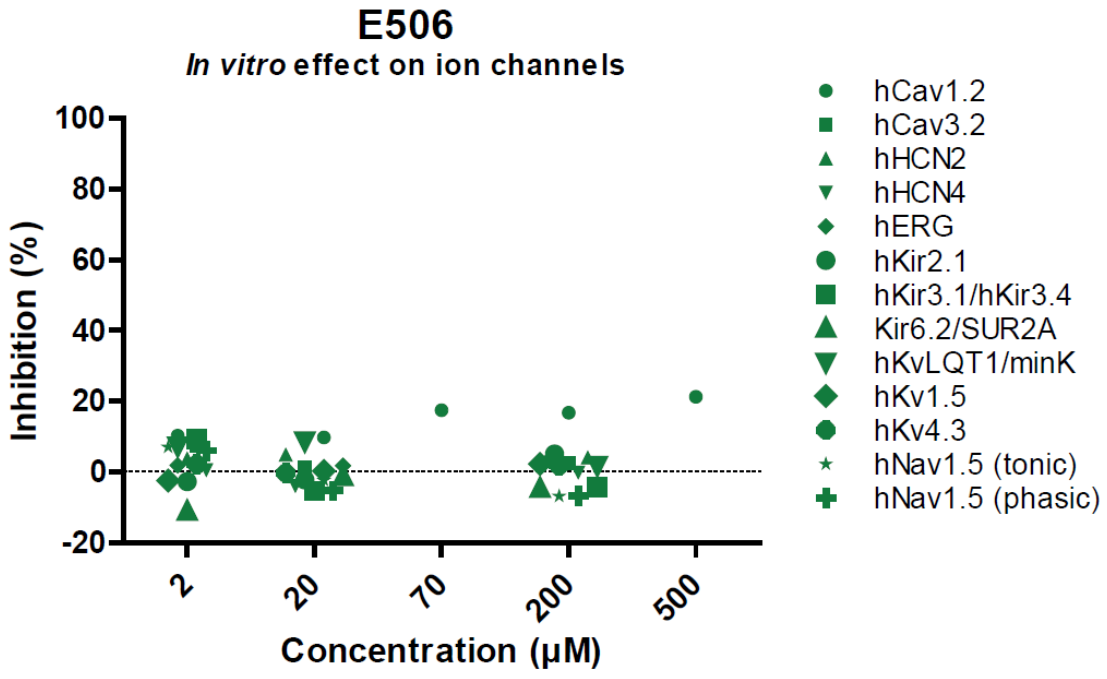


Figure 2. Overview of ion channel effects after E506 application (sponsor-generated table)

Study title: Effects of SNAC on hERG tail current recorded from stably transfected HEK293 cells (325-T-005, 209224)

This study was previously reviewed under IND (b) (4) *by Ke Zhang. Dr. Zhang's review is reproduced below.*

Methods: To investigate the effects of SNAC on hERG currents, human embryonic kidney 293 (HEK 293) cells transfected with hERG cDNA were perfused with bath solution at room temperature in a recording chamber. The currents evoked by stepping the membrane potential to +20 mV for outward current and then to –50 mV for a tail current were recorded in the presence and absence of SNAC at 1 mM using whole cell patch clamp technique. The magnitude of the hERG tail current was compared to the steady state current obtained during the control conditions and to the values from the vehicle treated cells. E-4031 (100 nM), a selective hERG current inhibitor, was used as a positive control.

Results: The results indicated that there were slight reductions of hERG tail currents (19.8%) after 15 minutes of exposure to SNAC. The slight reduction of the hERG tail current was also observed after 15 minutes of exposure to control bath solution (14.7%). Therefore, reductions of the hERG tail were not significantly different between the vehicle control and SNAC groups. The positive control significantly decreased the current by ~93%. The results suggest that SNAC had no clear effects on the hERG tail current in this model. A summary of the results is presented in the sponsor-generated table below.

Table 1. Effects of SNAC and E-4031 on HERG Tail Current

Treatment Group	Tail Current (% of Control)	Tail Current Corrected with Vehicle Effect (% of Control)
1 mM SNAC	80.2 ± 2.8 (n = 4)	94.1 ± 3.3
100 nM E-4031	6.7 ± 2.5 (n = 4)	—
Bath solution (vehicle)	85.3 ± 1.7 (n = 4)	—

Data are presented as mean ± standard error of the mean (SEM). Numbers of observations (n) in each group are provided in parentheses next to the data.

— indicates not applicable

Study title: Effects of single and repeat oral administration on cardiovascular and respiratory functions in the conscious rat using combined telemetry and whole body plethysmography (212471)

A non-GLP study was conducted with female Sprague-Dawley rats instrumented with implanted telemetry radiotransmitters. In the main experiment, animals (8/group) were administered vehicle or SNAC (900 or 1500 mg/kg/d) by oral gavage for 8 consecutive days. On Day 1 and Day 8, arterial blood pressure and heart rate were measured before administration and at 0.5, 1, 2, 3, 5, 12, and 20 hours postdose and respiratory

parameters were measured before administration and at 0.5, 1, 2, 3, and 5 hours postdose. As part of an amended experiment, additional groups of rats (6/group) received a single oral dose of vehicle or SNAC (900 or 1500 mg/kg/d). Arterial blood pressure, heart rate, temperature, and ECG parameters were measured before administration and at 0.5, 1, 2, 3, 4, 12, and 20 hours postdose and respiratory parameters were measured before administration and at 0.5, 1, 2, 3, and 4 hours postdose. Two satellite groups of 3 animals each were used for TK measurements.

Results - Main Experiment:

Mortality and Clinical Signs

At 900 mg/kg/d, 2/8 died during the first 2 hours of treatment on Day 1. At 1500 mg/kg/d, 3/8 rats died during the first hour of treatment on Day 1. Marked decreases in diastolic arterial pressure were noted in the decedent animals (up to -33% compared to baseline). Piloerection and tachypnea occurred on several occasions in surviving animals at both dose levels during the treatment period.

Hemodynamic Parameters

On Day 1 a dose-related decrease in diastolic and mean arterial blood pressure was observed for approximately the first hour after treatment compared with baseline and vehicle control (Figures 1 and 2). On Day 8, changes in diastolic and mean arterial pressure were not as noteworthy or dose related.

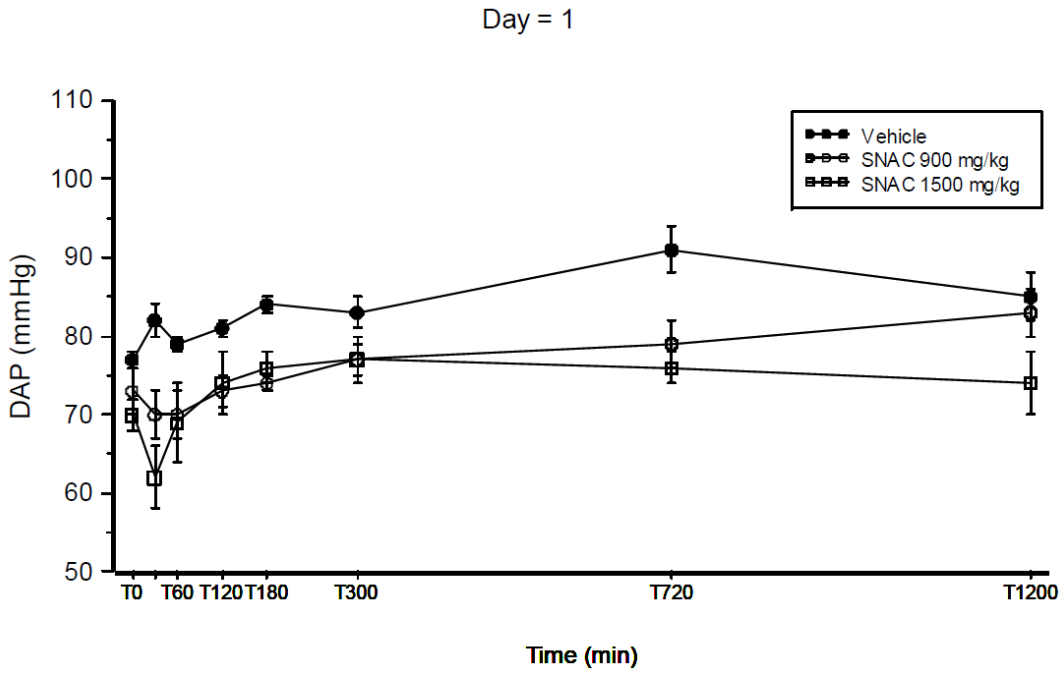


Figure 1. Diastolic arterial pressure after treatment with SNAC on Day 1 (sponsor-generated figure)

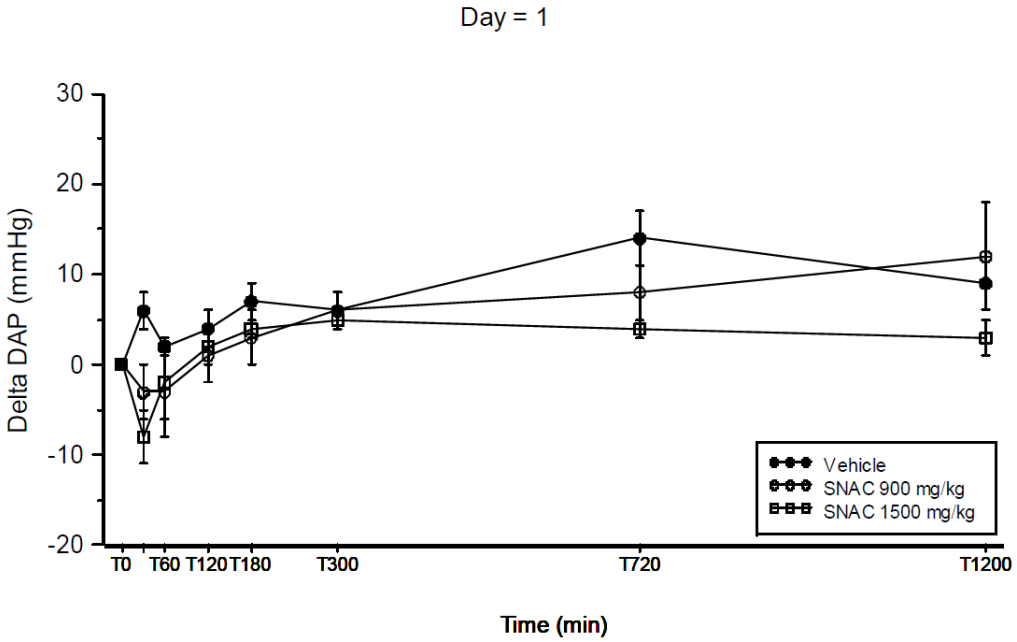


Figure 2. Change in diastolic arterial pressure from baseline after treatment with SNAC on Day 1 (sponsor-generated figure)

Heart Rate

A dose-related increase in mean heart rate was noted after dosing on Day 1 and Day 8 (Figures 3 and 4). On Day 1, the effect was noted for 2 to 3 hours at 900 mg/kg/d and over 6 hours at 1500 mg/kg/d. On Day 8, the effect was observed for 2 to 3 hours after dosing for both dose levels.

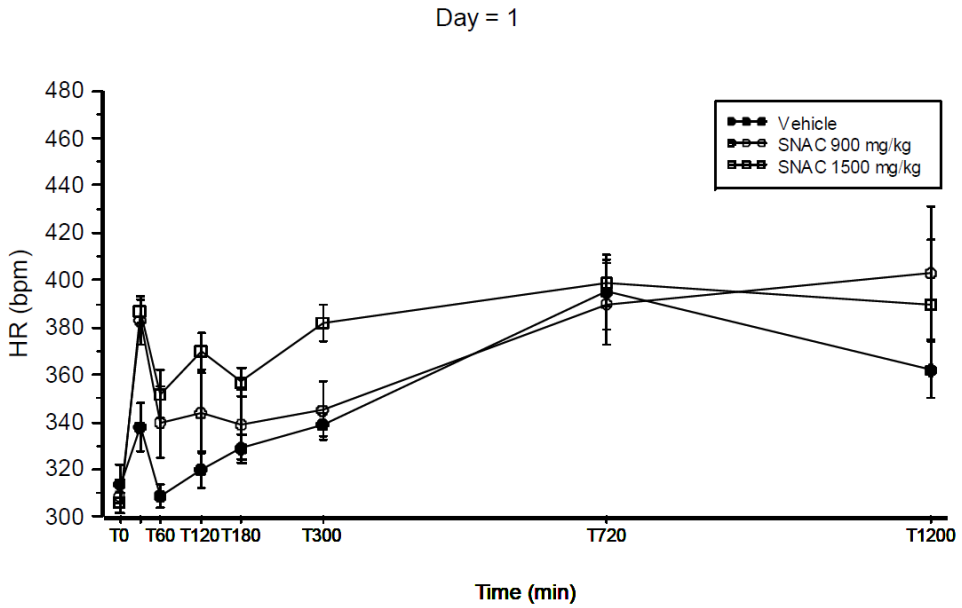


Figure 3. Heart rate after treatment with SNAC on Day 1 (sponsor-generated figure)

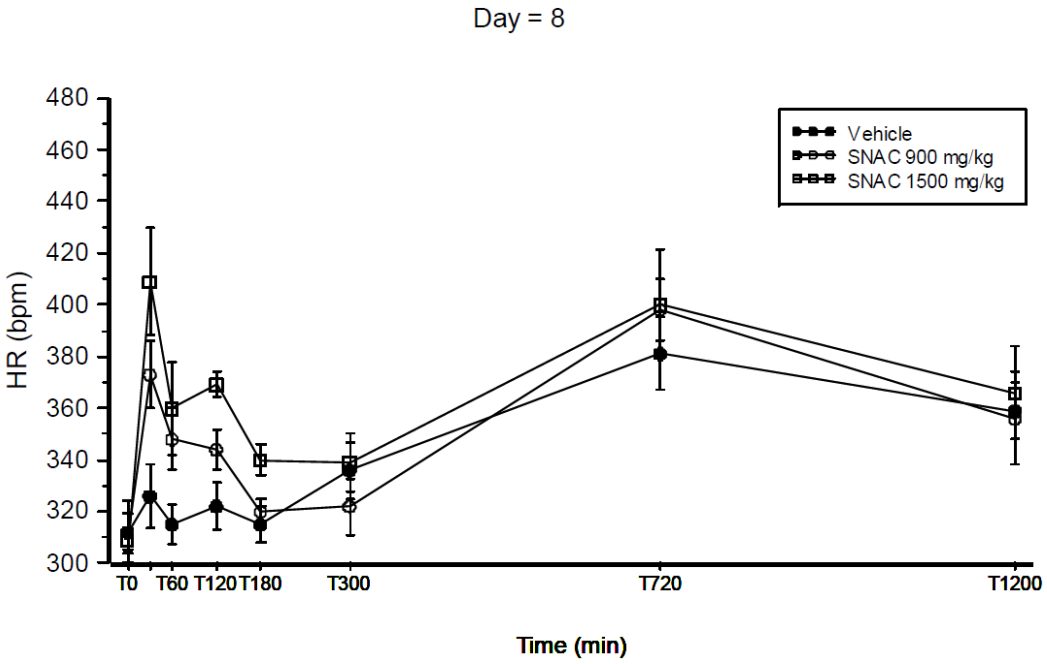


Figure 4. Heart rate after treatment with SNAC on Day 8 (sponsor-generated figure)

Respiratory Endpoints

Mean respiration rate was increased for 2 to 3 hours after treatment at both dose levels compared to baseline and vehicle control values (Figure 5). On Day 8, respiratory rate was increased at 30 minutes compared to baseline values but not significantly different from control values. Mean tidal volume and minute volume values were increased compared with baseline values for 0.5 to 1 hour at 1500 mg/kg/d and for 1 to 2 hours at 900 mg/kg/d (Figures 6 and 7). The magnitude of change from baseline was similar for both dose levels, although the duration of effect was slightly greater for the lower dose level. A similar trend for increased tidal and minute volume was also seen after dosing on Day 8. On Day 1, decreases in both mean inspiratory and expiratory times compared with baseline values were observed at both dose levels for approximately 2 to 3 hours after dosing; a similar effect was seen on Day 8 at the LD but not at the HD (data not shown). Increases in peak inspiratory and expiratory flow were also noted on Day 1 and Day 8 (LD only) during the first 3 hours after dosing.

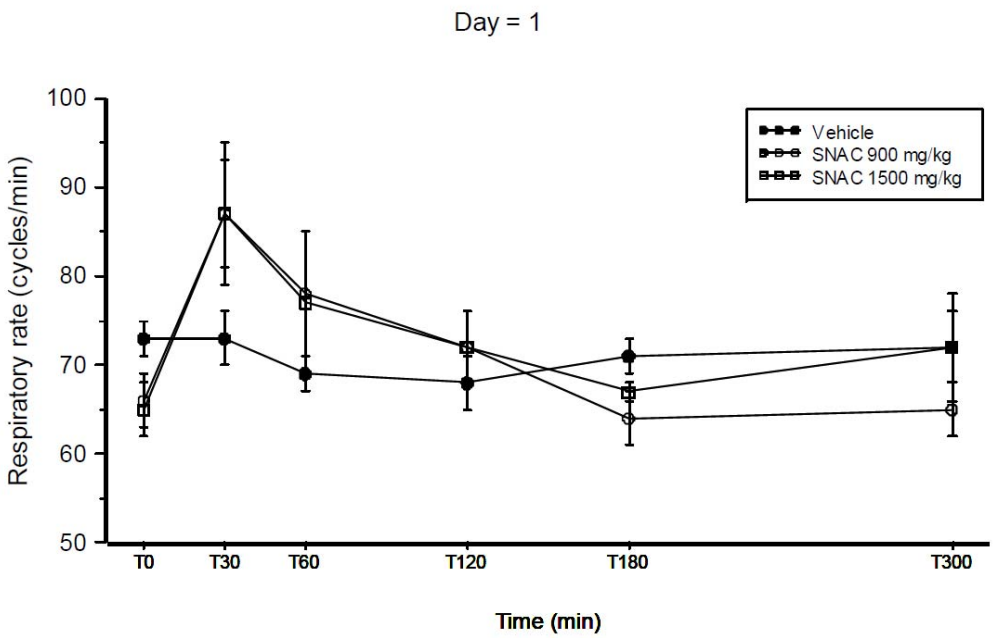


Figure 5. Effects on respiratory rate after treatment with SNAC on Day 1 (sponsor-generated figure)

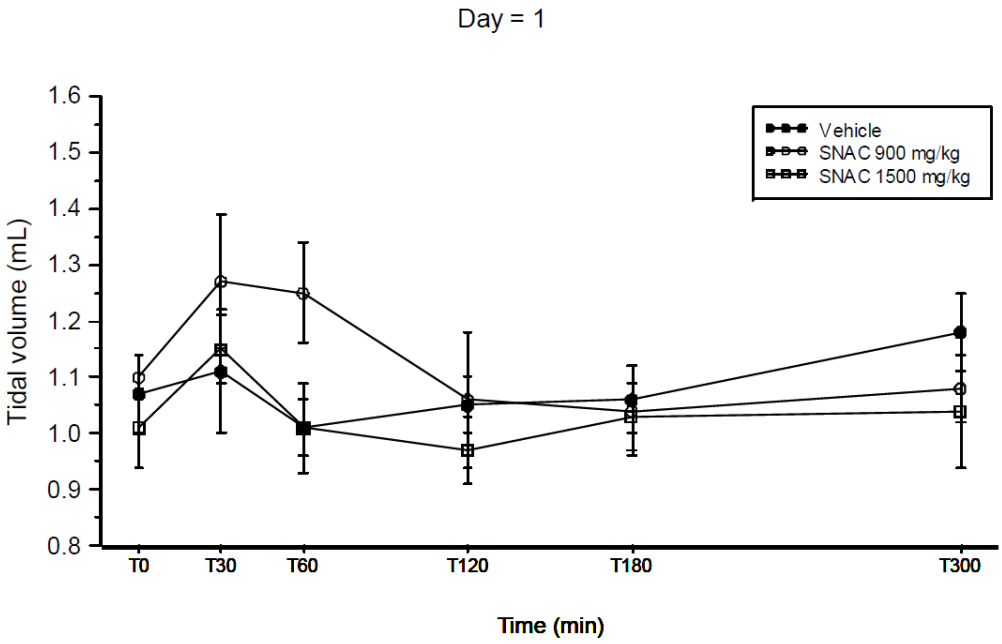


Figure 6. Effects on respiratory tidal volume after treatment with SNAC on Day 1 (sponsor-generated figure)

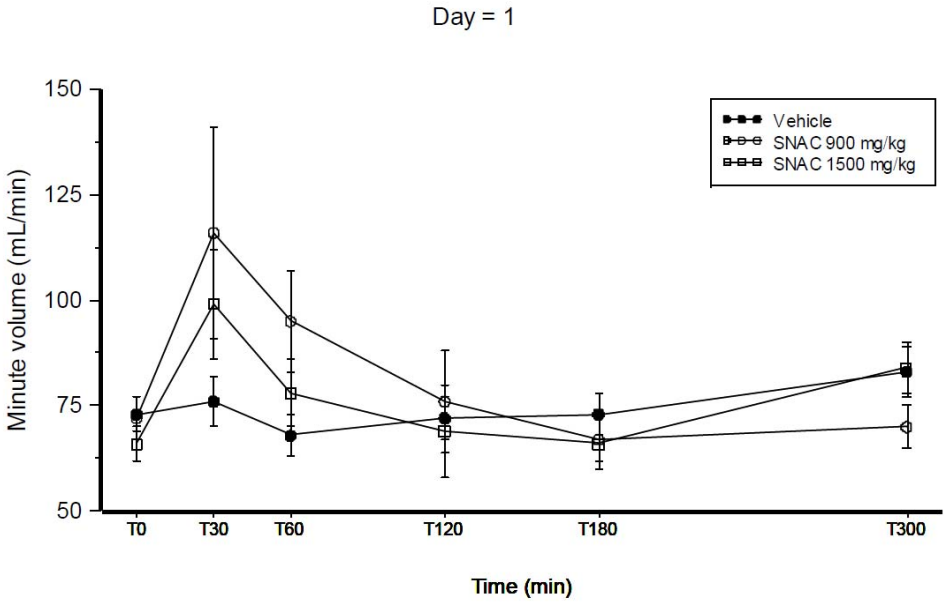


Figure 7. Effects on respiratory minute volume after treatment with SNAC on Day 1 (sponsor-generated figure)

Results - Amendment 1 Experiment:

Mortality and Clinical Signs

At 900 mg/kg/d, 2/6 died during the first 3 hours of treatment on Day 1. Prior to death, animals presented with excessive salivation, breathing difficulties, and abnormal posture. One surviving animal also showed excessive salivation immediately after dosing. No deaths or adverse clinical signs occurred at 1500 mg/kg/d

Hemodynamic Parameters

As with the animals in the main experiment, decreases in diastolic and mean arterial pressures compared to baseline and control values were observed at both dose levels for approximately 2 to 3 hours after dosing.

Heart Rate

Heart rates were increased at both dose levels compared with baseline and control values for up to 4 hours after dosing.

ECG Measurements

Consistent with increased heart rate, the RR interval times decreased from baseline for up to 4 hours after dosing in a dose-related manner (Figure 8). Treatment increased QT and corrected QT (QTc_{Bazett} and $QTc_{Fridericia}$) interval times for up to 4 hours after dosing. The maximum change from baseline for corrected QT values were approximately 18 ms (QTc_B) and 28 ms (QTc_F) at 0.5 hours after dosing. The degree of change was similar for both dose levels but the duration of effect was generally longer for the HD group. Increases in atrial (pause and premature beat) and junctional (salvo) arrhythmias were observed at 900 mg/kg and increases in atrial (premature beat),

ventricular arrhythmias (beat), junctional (beat), and other arrhythmias were observed at 1500 mg/kg/d. There were no meaningful changes in the PR or QRS interval times.

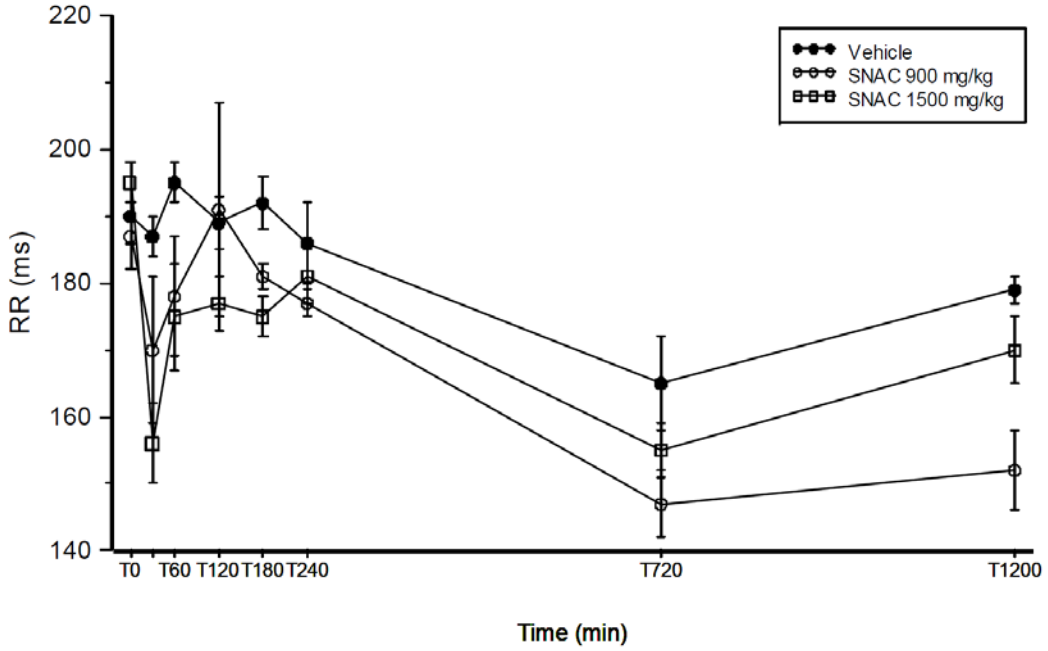


Figure 8. Effects on the RR interval after treatment with SNAC on Day 1 (sponsor-generated figure)

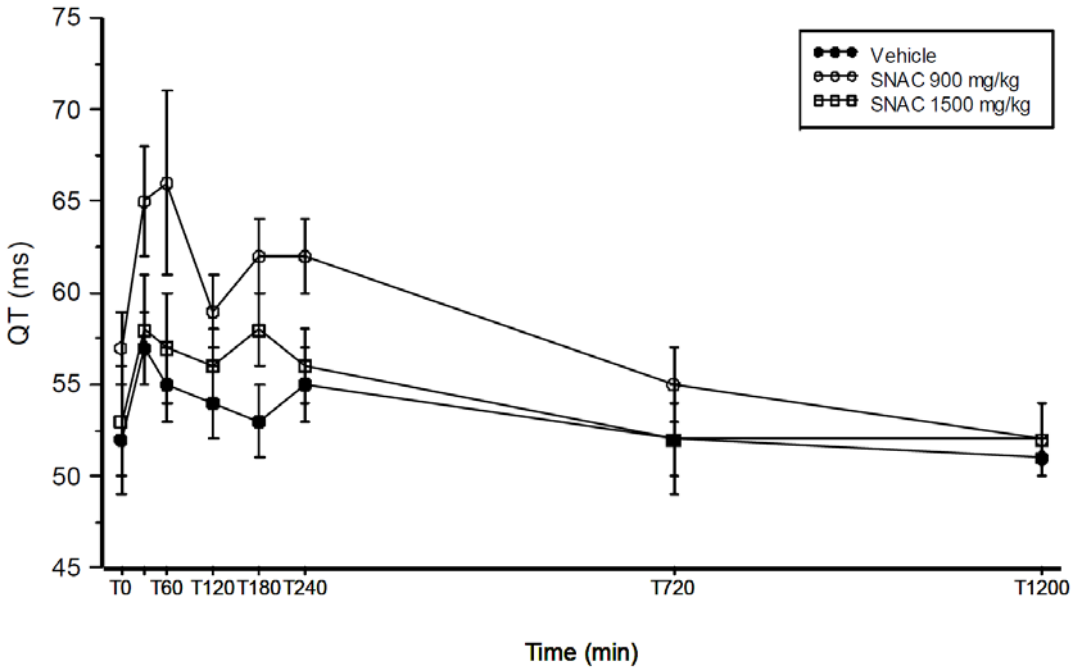


Figure 9. Effects on the QT interval after treatment with SNAC on Day 1 (sponsor-generated figure)

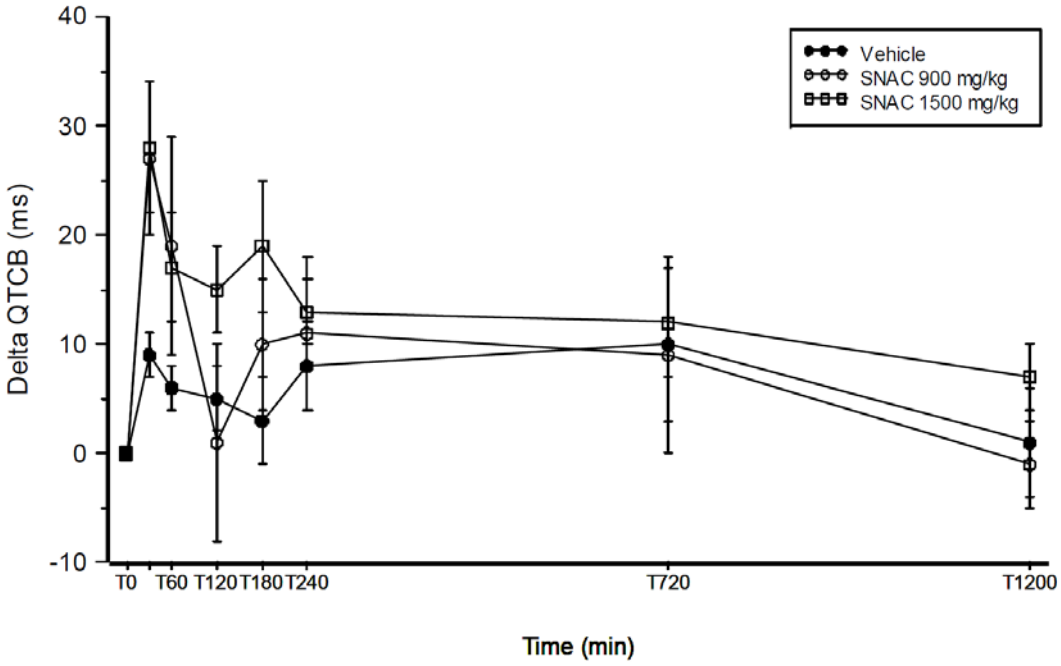


Figure 10. Change in QTc_B interval time relative to baseline after treatment with SNAC on Day 1 (sponsor-generated figure)

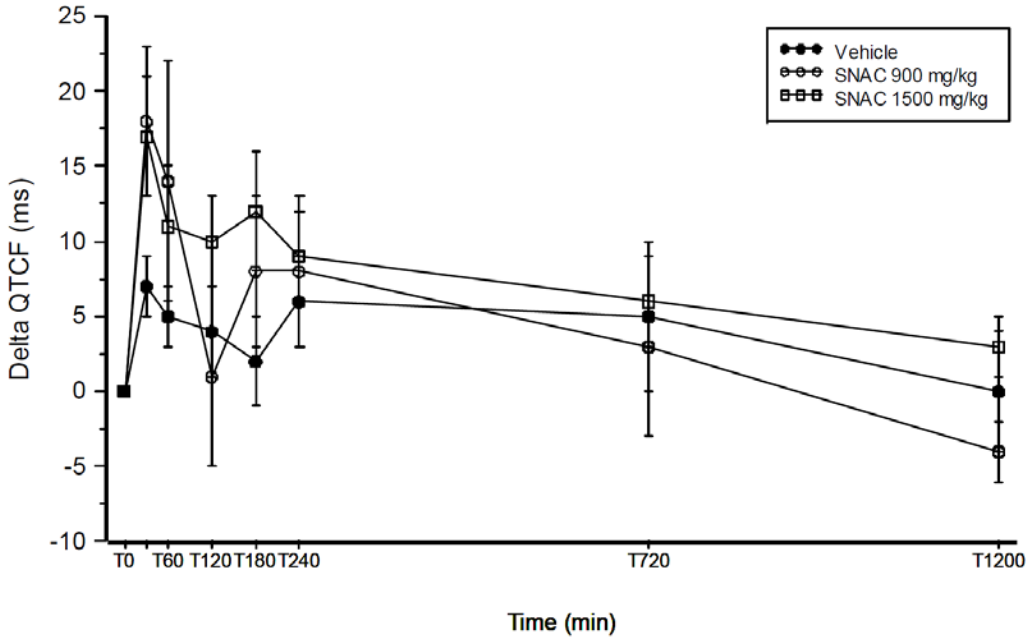


Figure 11. Change in QTc_F interval time relative to baseline after treatment with SNAC on Day 1 (sponsor-generated figure)

Number of Arrythmias Observed Before and After Treatment with SNAC

Dose (mg/kg)	Time of observation in relation to treatment	Atrial Arrythmias			Ventricular Arrhythmias		Junctional Arrythmia		Others
		2nd Degree AV Block	Pause	Premature Beat	Beat	Salvo	Beat	Salvo	
0	1h before dosing	0	0	0	4	0	0	0	1
	2h after dosing	2	6	0	0	0	9	0	0
900	1h before dosing	0	0	1	1	0	2	0	3
	2h after dosing	2	55	22	4	0	8	145	4
1500	1h before dosing	5	0	3	0	0	79	0	1
	2h after dosing	5	4	24	7	1	248	1	10

Respiratory Endpoints

Treatment resulted in increased respiratory rate for approximately 1 to 2 hours at both dose levels. An increase in mean tidal and minute volume was only observed at 900 mg/kg. Decreases in inspiratory and expiratory flow were observed at both dose levels.

Body Temperature

Mean body temperature were decreased from baseline for 2 to 3 hours after dosing at both dose levels, with a greater effect observed for the LD group (Figure 12). Body temperatures for the vehicle group were also slightly lower than baseline at 2, 3, and 4 hours postdose.

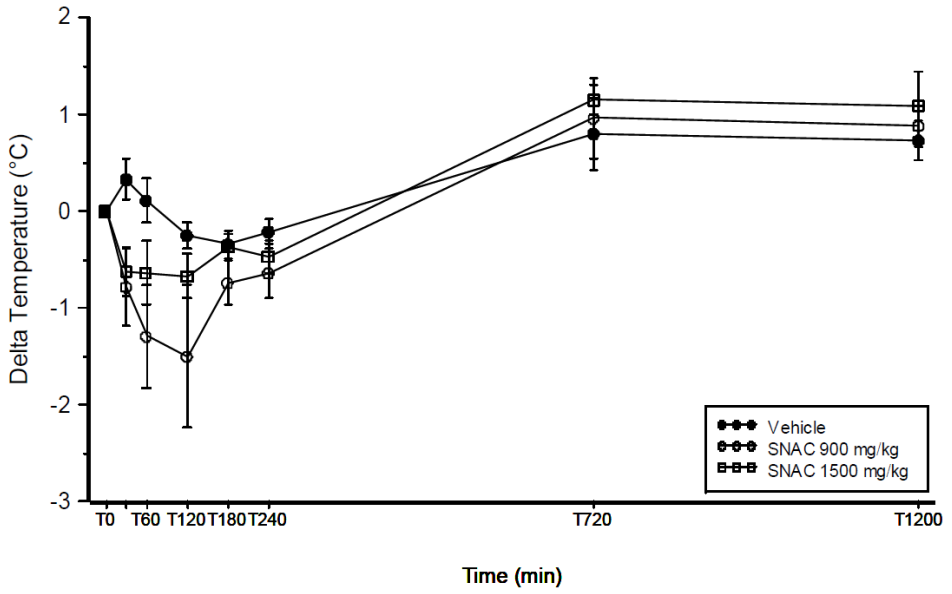


Figure 12. Change in body temperature relative to baseline after treatment with SNAC on Day 1 (sponsor-generated figure)

Toxicokinetics

The maximum concentration was variable on Day 1 among the animals in each group and the range of C_{max} values was similar between the 900 and 1500 mg/kg/d groups. On Day 8, C_{max} values for the 900 mg/kg/d animals were slightly lower and the values for the 1500 mg/kg/d group were slightly higher. The maximum concentration was generally observed at the first sampling time point (10 minutes), although T_{max} was 30 to 60 minutes for the 1500 mg/kg/d group on Day 8. The TK data are summarized in the table below.

Dose (mg/kg/d)	Day	Animal	C_{max} (ng/mL)	T_{max} (Minutes)
900	1	25	(b) (4)	
		26		
		27		
	8	25		
		26		
		27		
1500	1	28		
		29		
		30		
	8	28		
		29		
		30		

NS = no sample

Study title: A safety pharmacology study to assess potential cardiovascular effects of SNAC administered orally to rhesus monkeys (325-T-008, 209257)

Methods: To assess the potential effects of SNAC on the cardiovascular system, fasted Rhesus monkeys (3/sex) received increasing doses of SNAC sodium or placebo via naso-gastric tube on Days 1 (water), 3 (100 mg/kg), 5 (300 mg/kg), and 8 (600 mg/kg) at a volume of 10 mL/kg. A wash out period of 2 days was allowed between each dose level in the same group of animals. Each of the monkeys was instrumented with a telemetry transmitter approximately 4 weeks prior to the administration of the compound/placebo. In the present study, cage side observation, mortality, and clinical signs of toxicity were observed daily. The ECG tracings (for 30 seconds every 30 minutes), and cardiovascular data of heart rate and blood pressure (for 30 seconds every 5 minutes) and body temperature were obtained 24 hours prior to the administration of the compound, every 10 minutes for 30 seconds. Endpoints were collected for approximately 36 hours after dosing. Blood samples were collected at 2 and 18 hours post dose for toxicokinetics.

Results:
There were no treatment-related effects on clinical sign, body weight, food consumption, or body temperature. There were no treatment-related effects on heart rate, mean arterial blood pressure, or ECG intervals, including QTc. Descriptive TK parameters were not determined because of the minimal number of sampling points. Mean plasma concentrations at 2 hours after dosing are shown below.

Dose Level (mg/kg)	Plasma Concentration at 2 hours (ng/mL)	
	Males	Females
100	922	797
300	7,093	7,290
600	24,773	18,123

Pulmonary effects:

Study title: The effects of SNAC on respiration rate and tidal volume in Sprague Dawley rats (209225)

Groups of male Sprague-Dawley rats (minimum of 8/group) were administered a single oral dose of vehicle (water), SNAC (500, 750, or 1000 mg/kg), or 20 mg/kg baclofen (positive control) in Phase 1 of the study and 250 mg/kg SNAC or vehicle in Phase 2. Respiration rate and tidal volume were evaluated by plethysmography prior to dosing and at 0.5 and 4 hours postdose.

Results:
No statistically significant effects on mean respiration rate or tidal volume were noted at any SNAC dose level. Treatment with the positive control resulted in decreased respiration rate and increased tidal volume.

Between approximately 10 and 44 minutes postdose, six of nine animals treated with 1000 mg/kg SNAC and two of eight animals treated with 750 mg/kg SNAC showed signs of decreased activity. This observation was more severe in one animal from the HD group. This animal died approximately 44 minutes after dosing. The study director stated that there was no clear indication of a respiratory component, but the individual data for this animal was not presented in the report; it is not clear if respiration and tidal volume were measured for this animal at the 30 minute time point. At necropsy, this animal showed slight reddening of the lungs.

Study title: Effects of single and repeat oral administration on cardiovascular and respiratory functions in the conscious rat using combined telemetry and whole body plethysmography (212471)
See the cardiovascular safety pharmacology section for review of this study.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption
Semaglutide

The transport and uptake of semaglutide and [^{3}H]-mannitol in the presence and absence of 80 mM SNAC was evaluated in Caco-2 cells. The amount of compound transported from the donor chamber (apical side) to the receiver chamber (basolateral side) was measured over 60 minutes. Following the transport of the transport portion of the experiment, the amount of semaglutide that was inside the cells was measured.

SNAC was shown to elicit a significant increase in the trans-epithelial permeation of semaglutide across Caco-2 monolayers. Analysis of the non-membrane associated subcellular fraction revealed that the presence of SNAC significantly augmented the accumulation of semaglutide within the cellular monolayer, which is consistent with a transcellular transport mechanism. The permeation of mannitol was not markedly increased in the presence of SNAC, suggesting that SNAC has a limited effect on the paracellular route of absorption.

Study title: Evaluation of various ratios of SNAC and Semaglutide in tablets following oral administration to Beagle dogs (AnP090703)

Beagle dogs were administered a single oral tablet of varying ratios of SNAC plus semaglutide as shown in the table below to determine the ratio that provided the best oral bioavailability of semaglutide (compared with intravenous administration). Blood samples were collected for up to 240 hours after dosing. Semaglutide was measured by using a luminescent oxygen channeling immunoassay (LOCI) with an LLOQ of 100 pM.

Group/Round	Batch	Treatment	Dose (mg) per animal *	Animal Numbers
1/1	209G	SNAC/Semaglutide	150/10	1025-1032
2/1	210G	SNAC/Semaglutide	300/10	1033-1040
3/1	211G	SNAC/Semaglutide	600/10	1041-1048
4/1	208G	SNAC/Semaglutide	300/5	1049-1056
5/1	212G	SNAC/Semaglutide	300/20	1057-1064
6/1	218G	Semaglutide	2 nmol/kg BW	1065-1072
7/2	260G	SNAC/Semaglutide	300/15	1067-1072

*) The mean BW of the dogs was 9.3 and 8.8 kilograms for the males and females respectively
Groups 6: intravenous administration; 2 nmol/kg ~ 8.8 ng/kg

Results:

PK results for semaglutide are shown in the sponsor-generated tables below. PK results for SNAC are presented in the Absorption - SNAC section below. Increasing the amount of SNAC from 150 to 300 mg resulted in a 3.5-fold increase in semaglutide exposure, whereas increasing SNAC to 600 mg resulted in less exposure to semaglutide than 300 mg SNAC (Table 1). Based on these results, 300 mg SNAC was selected for the second part of the PK study in which 300 mg SNAC was administered with increasing amounts of semaglutide (Table 2). Semaglutide exposure increased in a greater than dose proportional manner when administered with 300 mg SNAC. When formulated with 300 mg SNAC, oral bioavailability ranged from 0.33% for 5 mg semaglutide to 1.6% for 20 mg semaglutide.

Table 1 (sponsor-generated)

Summary of Pharmacokinetic parameters for Semaglutide from simultaneously dosing of increasing SNAC amount while keeping Semaglutide amount constant at 10 mg.

Group/Round	SNAC/Semaglutide (mg/mg)	T_{max} (h)	C_{max} (pM)	AUC/D (h*kg/l)	T½ (h)	F(%)
1/1	150/10	0.6(0.5)	6222 (13130)	0.62(1.41)	29.3(2.3)	0.17(0.38)
2/1	300/10	0.8(0.5)	21871(31061)	2.35(3.49)	29.6(3.7)	0.63(0.94)
3/1	600/10	1.1(0.5)	9972(17298)	1.09(1.87)	42.7(115.6)	0.29(0.50)

mean (SD)

Table 2 (sponsor-generated)

Summary of pharmacokinetic parameters for Semaglutide from simultaneously increasing amount of Semaglutide while keeping SNAC amount constant at 300 mg.

Group/Round	SNAC/Semaglutide (mg/mg)	T_{max} (h)	C_{max} (pM)	AUC/D (h*kg/l)	T½ (h)	F (%)
4/1	300/5	0.5(0.5)	4446(5335)	1.22(1.42)	41.5(124.7)	0.33(0.38)
2/1	300/10	0.8(0.5)	21871(31061)	2.335(3.49)	29.6(3.7)	0.63(0.94)
7/2	300/15	1.0(0.8)	42612(43118)	4.61(6.00)	35.7(2.9)	1.2(1.6)
5/1	300/20	1.3(0.5)	93603(68667)	5.09(3.52)	34.8(1.9)	1.4(0.95)

Table 3. Summary of AUC values

SNAC/Semaglutide (mg/mg)	AUC_{last} (pmol•h/L)	$AUC_{inf\ obs}$ (pmol•h/L)
300/5	111,053	142,471
300/10	612,305	621,100
300/15	1,411,300	1,432,283
300/20	2,782,433	2,806,338
2 nmol/kg*	NC	737,900

*Semaglutide only by intravenous injection; NC = not calculated.

Study title: Oral bioavailability from SNAC semaglutide tablets in Beagle dogs (RkeA100803)

A head-to-head comparison of oral bioavailability in a multiple dose study comprising three dosing rounds in which 8, 16, and 24 Beagle dogs, respectively, were dosed with each formulation once daily for 5 consecutive days. The two oral formulations consisted of tablets manufactured using the current SNAC (b) (4) process and the new SNAC (b) (4) process. Each tablet contained 300 mg SNAC and 10 mg semaglutide (mean dose of 4.15 mg/kg semaglutide). The third formulation was an intravenous formulation containing 26 nmol/mL semaglutide (administered dose of 2 nmol/kg [~8.8 ng/kg]) to calculate absolute bioavailability. Blood samples were collected for up to 240 hours after dosing. Semaglutide was measured by using a luminescent oxygen channeling immunoassay (LOCI) with an LLOQ of ~500 pM.

Results:

The results indicated that the bioavailability of semaglutide is similar between the (b) (4) process tablet and the (b) (4) process tablet. However, there was high variability in exposure across individual animals for each dosing round, as demonstrated by the fact that the standard deviation was generally similar to or greater than the mean values for AUC. This variability led to a wide range in absolute bioavailability for the tablets. For example, bioavailability of semaglutide for the third dosing cycle ranged from 0.00% to 2.79% for the (b) (4) tablet and 0.00% to 1.71% for the (b) (4) tablet.

Summary of PK Data for Semaglutide after Oral and Intravenous Administration

Formulation/ Route	Dosing Cycle	Actual Mean Dose (mg/kg)	Number of Animals	AUC (pmol•h/L)	AUC/D (pM•h/(mg/kg)	$T_{1/2}$ (h)	F (%)
(b) (4)	1	4.15	8	4,540,639 (4,430,836)	1,062,008 (981,806)	50.0	0.56 (0.52)
	2	4.06	16	6,879,704 (8,933,330)	1,648,325 (1,978,974)	59.5	0.87 (1.05)
	3	3.95	24	2,918,880 (3,487,056)	749,271 (899,824)	50.9	0.40 (0.48)
Oral (b) (4)	1	4.32	8	8,589,177 (5,371,984)	2,046,374 (1,266,479)	55.8	1.08 (0.67)
	2	4.16	16	6,919,720 (6,402,089)	1,720,467 (1,662,732)	54.7	0.91 (0.88)
	3	3.76	24	4,536,554 (5,538,548)	1,234,783 (1,472,595)	55.9	0.65 (0.78)
Intravenous	1	0.0088	4	1,686,906 (207,845)	192,270,565 (23,938,389)	42.0	NA
	2	0.0083	4	1,544,128 (322,461)	186,002,585 (35,440,660)	56.5	NA

(Standard Deviation)

Study title: [^{3}H]-Oct-NNC 0113-000-0217 with carrier compound NNC 0113-3363: A study of disposition following intravenous and oral administration to the male Cynomolgus monkey (209115)

Male Cynomolgus monkeys received a single dose of [^{3}H]-Oct-NNC 0113-000-0217 by intravenous injection (0.01 mg/kg) or by oral gavage (15 mg/animal; tablet formulated with 450 mg SNAC). Plasma levels of parent semaglutide (0113-0217) were measured for up to 192 hours. Results are summarized on the sponsor-generated tables below. Radioactivity was measured in urine and feces; these data are presented in the Excretion section below.

Estimated individual pharmacokinetic parameters after intravenous and oral administration of NNC 0113-0217 to monkeys.

Treatment	Dose	Sex	Subject	C_{5min} (nM)	C_{max} (nM)	t_{max} (h)	AUC_{last} (h*nM)	AUC (h*nM)	$AUC_{\%extra}$ (%)	$t_{½}$ (h)	CL (L/h/kg)	Vz (L/kg)
IV Bolus	0.01 mg/kg	Male	101M	(b) (4)								
			102M									
			103M									
			Mean	37.7	NC	NC	997	1070	7.1	54	0.00227	0.177
			SD	4.23	NC	NC	68.1	73.8	1.9	3.7	0.000155	0.0192
Oral	15 mg	Male	201M	(b) (4)								
			202M									
			203M									
			Mean	NC	20.2	3.3	726	1040	16	51	NC	NC
			SD	NC	13.1	0.58	644	773	7.7	3.7	NC	NC

NR: Not Reported; NC: Not Calculated, *Not Reported as $AUC_{\%extra}$ was >20%.

Table 4 Estimated absolute oral bioavailability for NNC 0113-0217 after oral administration of 15 mg NNC 0113-0217.

Animal number[a]	Weight (kg)	AUC oral	Dose oral (mg/kg)	AUC iv	Dose iv (mg/kg)	F (%)
101/201	3.3	(b) (4)	4.55	(b) (4)	0.01	(b) (4)
102/202	3.1		4.84		0.01	
103/203	3.65		4.11		0.01	
Mean						0.16
SD						0.13

[a]The animal numbers correspond to Group A and Group B, respectively; animal no. 101 is the same as animal no. 201.
[b]AUClast was used in the calculation

SNAC

Study title: RO5045192 (SNAC, excipient for oral administration (b) (4)): Exploratory investigations on the transport of RO5045192 in wild type MDCKII cells and in MDCKII cells expressing the MDR1 (human) P-glycoprotein (212211)

The permeability of SNAC as well as its properties as a P-gp substrate and inhibitor were evaluated in vitro using MDCKII cells, with and without transfected human MDR1 P-gp.

Results:

The data indicated that SNAC has intermediate to high cell permeability. The permeability of SNAC was not affected by the P-gp inhibitor verapamil nor did SNAC affect the P-gp-mediated transport of digoxin. Therefore, under the conditions of this study, SNAC was not a substrate or inhibitor of human P-gp.

Study title: Salcaprozate sodium (SNAC), NNC 0113-3705, NNC 0113-3706, NNC 0113-3707, NNC 0113-3708, NNC 0113-3709: In vitro evaluation of SNAC and pool of five metabolites as inhibitors of human P-gp, BCRP, BSEP, MRP2, OATP1B1, OAT1, OAT3, OCT1, and OCT2 transporters (212524)

SNAC (30, 100, 300, 1000 and 4300 µM) and a pool of its metabolites (NNC0113-3705 (E1245), NNC0113-3706 (E494), NNC0113-3707 (E1246), NNC0113-3708 (E506) and NNC0113-3709 (E1247); all at 1, 10, and 100 µM) were evaluated for their ability to inhibit human efflux transporters (P-gp and BCRP) by measuring the bidirectional permeability of a probe substrate (digoxin or prazosin) across a monolayer of Caco-2 or MDCKII-BCRP cells, respectively. SNAC (0.1, 0.3, 1, 3, 10, 30 and 100 µM) and the pool of metabolites (1, 10 and 100 µM) were evaluated for their ability to inhibit human BSEP and MRP2 by measuring the accumulation of a probe substrate (taurocholate and estradiol glucuronide, respectively) in vesicles. The ability of SNAC (0.1, 0.3, 1, 3, 10, 30 and 100 µM) and the pooled metabolites (1, 10 and 100 µM) to inhibit human uptake transporters (OATP1B1, OATP1B3, OCT1, OCT2, OAT1 and OAT3) was evaluated by measuring the accumulation of probe substrates in transporter-expressing and control HEK293 cells in the presence of SNAC and its pooled metabolites. Additionally, the ability of each metabolite (0.1, 0.3, 1, 3, 10, 30 and 100 µM) to inhibit human uptake transporters OAT1 and OAT3 was evaluated by measuring the

accumulation of probe substrates in transporter-expressing and control HEK293 cells in the presence of each metabolite. Known inhibitors were included as positive controls.

Results:
Overall, SNAC inhibited P-gp, BCRP, OATP1B1, OAT1, and OAT3 by more than 50% resulting in IC_{50} values of 2620, 145, 68, 28, and 5 µM, respectively. The pooled metabolites at 100 µM inhibited OAT1 and OAT3 by 91% and 83%, respectively. Further investigation showed that the individual metabolites NNC0113-3705 (E1245), NNC0113-3706 (E494), NNC0113-3707 (E1246), and NNC0113-3708 (E506) inhibited OAT1 by more than 50% resulting in IC_{50} values of 32, 33, 77, and 7 µM, respectively. NNC0113-3705 (E1245), NNC0113-3706 (E494), and NNC0113-3708 (E506) also inhibited OAT3 by more than 50% resulting in IC_{50} values of 93, 21, and 10 µM, respectively.

Study title: RO5045192 (SNAC, excipient): Pharmacokinetics following oral (gavage) administration of RO5045192-001 in mice (05-6063, 209282)

Male CD-1 mice (3/time point) received a single oral dose of 30 mg/kg SNAC. Plasma samples were collected at 0.33, 0.66, 1, 3, 6, and 24 hours after dosing to determine the PK characteristics for SNAC and its metabolites by LC-MS/MS.

Results:
SNAC was quickly conjugated into the respective glucuronide (RO5081852) and was also rapidly transformed by β-oxidation into RO4729197. This intermediate was further oxidized by a subsequent β-oxidation into RO5086252. Both metabolites were further transformed into their respective glucuronides RO5090295 and RO5091748. The formation of all metabolites was fast with a T_{max} of 0.33 hours. TK data are summarized in the sponsor-generated table below.

gender: males

Parameter	Unit	RO5045192	RO4729197	RO5086252	RO5081852	RO5090295	RO5091748
Cmax	[ng/mL]	73.0	27.7	261	803	2710	6620
tmax	[h]	0.660	0.330	0.330	0.330	0.330	0.330
t1/2	[h]	1.40	0.234	4.05	1.46	3.18	3.03
AUC(0-inf)	[(ng·h)/mL]	92.5	16.2	630	1830	5150	15000
AUC(0-tlast)	[(ng·h)/mL]	79.0	12.4	364	1520	5130	15000
(0-h)		0-3	0-1	0-6	0-6	0-24	0-24

Study title: Pharmacokinetic profiling of the radiolabel following ^{14}C-E414 [SNAC] in fasted and unfasted Sprague-Dawley rats (209226)

Groups of fasted or unfasted Sprague-Dawley rats (3/sex/group) were administered a single oral dose of ^{14}C-SNAC (300 or 1000 mg/kg). Blood samples were taken for PK assessment of radioactivity levels at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, and 6 hours after dosing. Results are summarized in the sponsor-generated tables below, which show that absorption of SNAC was greater in the fasted state.

Exposure (AUC_{0-6h}) of ^{14}C-SNAC after oral administration to fasted and unfasted rats

Group 1 Fasted Animals (300 mg/kg E414)				Group 3 Unfasted Animals (300 mg/kg E414)			
Males	AUC(0-6h) (µg·min/mL)	Females	AUC(0-6h) (µg·min/mL)	Males	AUC(0-6h) (µg·min/mL)	Females	AUC(0-6h) (µg·min/mL)
1101A	(b) (4)						
1002A							
1003A							
Mean	38376	Mean	54112	Mean	12403	Mean	36133

Group 2 Fasted Animals (1000 mg/kg E414)				Group 4 Unfasted Animals (1000 mg/kg E414)			
Males	AUC(0-6h) (µg·min/mL)	Females	AUC(0-6h) (µg·min/mL)	Males	AUC(0-6h) (µg·min/mL)	Females	AUC(0-6h) (µg·min/mL)
2001A	(b) (4)						
2002A							
2003A							
Mean	134154	Mean	182622	Mean	94509	Mean	79812

PK profile of ^{14}C-SNAC after oral administration to fasted and unfasted rats

(b) (4)

Study title: Salcoprozate sodium (SNAC): A comparative study of pharmacokinetics in the rat following a single oral administration, single intravenous administration or daily repeated oral administration for 2 weeks (212150)

The PK characteristics of SNAC were evaluated in Sprague-Dawley rats after oral (single dose or once daily for 14 days) and intravenous (single dose) administration. The study design is shown in the table below. Plasma samples were assessed for SNAC concentration by using a validated LC-MS/MS assay with a LLOQ of 18.5 ng/mL.

Study Design

Dose Group	Dose Level (mg/kg)	Dose Route	Number of Animals	Number of Doses
A	200	Oral	3/sex	1
B	500	Oral	3/sex	1
C*	500	Oral	3/sex	1
D	200	Oral	6/sex	14
E	500	Oral	6/sex	14
F	100	Intravenous	6/sex	1

*Sampled in 0.45 M citrate buffer, pH 4.3. All other samples were placed in tubes containing K_2-EDTA.

Results:

Table 2 Estimated toxicokinetic parameters after oral administration of SNAC to rats.

Day	Group	Dose (mg/kg)	Gender	t_{max} (hr)	C_{max} (ng/ml)	AUC_{0-4hr} (hr*ng/ml)	AUC_{0-24hr} (hr*ng/ml)	Rac_{Obs}
1	A	200	Male	0.170	3330	3020	4930	NC
			Female	0.170	26700	8770	10900	NC
1	B	500	Male	0.170	44000	12800	18800	NC
			Female	0.170	257000	60900	65700	NC
1	C*	500	Male	0.170	13000	6040	11900	NC
			Female	0.170	50000	11700	17200	NC
14	D	200	Male	0.170	9460	4630	4630	0.939
			Female	0.170	22200	10400	10400	0.950
14	E	500	Male	0.170	19000	13100	13100	0.700
			Female	0.170	271000	64400	64400	0.980

*Group C blood tubes were pre filled with 10% 0.45M citrate buffer, pH 4.3. The plasma concentrations were not corrected for this dilution.

Table 3 Estimated toxicokinetic parameters after intravenous administration of SNAC to rats.

Day	Group	Dose (mg/kg)	Gender	C_0 (ng/ml)	C_{5min} (ng/ml)	AUC_{last} (hr*ng/ml)	AUC (hr*ng/ml)	AUC_{extrap} (%)	V_z (ml/kg)	CL (ml/hr/kg)
1	F	100	Male	924000	301000	69500	69600	0.137	4710	1440
1	F	100	Female	758000	349000	80300	80400	0.206	4420	1240

Study title: RO5045192 (SNAC, excipient): Pharmacokinetics following oral (gavage) administration of RO5045192-001 in Wistar rats (05-6064, 209283)

Methods: Male Wistar rats (2-6/time point) received a single oral dose of 30 mg/kg SNAC. Plasma samples were collected at 0.33, 0.66, 1, 3, 6, and 24 hours after dosing to determine the PK characteristics for SNAC and its metabolites by LC-MS/MS.

Results:

SNAC was conjugated into the respective glucuronide (RO5081852) and was also rapidly transformed by β-oxidation into RO4729197. This intermediate was further oxidized by a subsequent β-oxidation into RO5086252. Both metabolites were further transformed into their respective glucuronides RO5090295 and RO5091748. Among all metabolites, the highest systemic exposure was observed for the double oxidation product (RO5086252), its glucuronide, and the glucuronide formed from the parent compound (RO5081852). TK data are summarized in the sponsor-generated table below.

gender: males

Parameter	Unit	RO5045192	RO4729197	RO5086252	RO5081852	RO5090295	RO5091748
Cmax	[ng/mL]	273	62.1	2270	2130	328	725
tmax	[h]	0.660	0.660	0.660	0.660	0.660	0.660
t1/2	[h]	2.69	NC	NC	1.72	NC	NC
AUC(0-inf)	[(ng·h)/mL]	512	NC	NC	6580	NC	NC
AUC(0-6h)	[(ng·h)/mL]	383	101	4380	4660	735	1930

raw data ID: (b) (4)
NC not calculated

Study title: Evaluation of various ratios of SNAC and Semaglutide in tablets following oral administration to Beagle dogs (090703)

Beagle dogs were administered a single oral tablet of varying ratios of SNAC plus semaglutide as shown in the table below to determine the ratio that provided the best oral bioavailability of semaglutide. Blood samples were collected for up to 240 hours after dosing. SNAC was measured by LC-MS/MS with an LLOQ of 10 ng/mL or 25 ng/mL. The PK results for SNAC are shown in the sponsor-generated table below. PK results for semaglutide are shown in the Absorption - Semaglutide section above.

Group/Round	Batch	Treatment	Dose (mg) per animal *	Animal Numbers
1/1	209G	SNAC/Semaglutide	150/10	1025-1032
2/1	210G	SNAC/Semaglutide	300/10	1033-1040
3/1	211G	SNAC/Semaglutide	600/10	1041-1048
4/1	208G	SNAC/Semaglutide	300/5	1049-1056
5/1	212G	SNAC/Semaglutide	300/20	1057-1064
6/1	218G	Semaglutide	2 nmol/kg BW	1065-1072
7/2	260G	SNAC/Semaglutide	300/15	1067-1072

*) The mean BW of the dogs was 9.3 and 8.8 kilograms for the males and females respectively

Results:

Exposure to SNAC increased in a greater than dose proportional manner from 150 mg to 300 mg and in a nearly dose proportional manner from 300 mg to 600 mg when formulated with 10 mg semaglutide.

Summary of Pharmacokinetic parameters for SNAC from simultaneously dosing of increasing SNAC amount while keeping Semaglutide amount constant at 10 mg.

Group/Round	SNAC/Semaglutide (mg/mg)	T_{max} (h)	C_{max} (ng/mL)	AUClast (h*ng/ml)	T½ (h)
1/1	150/10	0.3(0.3)	393(273)	301(140)	0.8(0.4)
2/1	300/10	0.5(0.9)	1390(2290)	1030(510)	1.2(0.7)
3/1	600/10	0.4(0.8)	2540(2790)	2250(1000)	1.2(1.2)

mean (SD)

Study title: The disposition of E414 in cynomolgus macaques following intravenous and oral dosing (209235)

Three male cynomolgus monkeys received a single dose of SNAC by intravenous injection (15 mg/kg) or oral administration (300 mg/kg). Monkeys were provided food for 4 hours before dose administration. Blood samples were collected at 0.08, 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, and 23.5 hours after dosing. Plasma concentrations were determined by an LC-MS/MS assay.

Pharmacokinetics (noncompartment analysis) of SNAC after a single oral dose of 300 mg/kg

Estimated Parameter	UAN 21M	UAN 31M	UAN 12M	MEAN	±SD
Weight (kg)	(b) (4)			3.4	0.6
Tmax (hr)				-	-
Cmax (µg/mL)				19.7	6.1
β (1/hr)				0.279	0.042
t½β (hrs)				2.52	0.39
AUC (µg/mL/hr)				45.395	3.196
F (%)				14.9	1.8

Estimated pharmacokinetic parameters from three compartment nonlinear regression analysis of SNAC plasma concentrations following a single intravenous dose

Secondary Parameters	UAN 21M	UAN 31M	UAN 12M	MEAN	±SD
Cmax (µg/mL)	(b) (4)			143.8	45.9
V (L)				0.39	0.17
V_{ss} (L)				1.20	0.50
k_{21} (1/hr)				6.78	2.77
k_{31} (1/hr)				0.79	0.16
k_{10} (1/hr)				9.17	1.76
k_{12} (1/hr)				2.54	1.15
k_{13} (1/hr)				1.43	0.45
$t^1/_{2\alpha}$ (hr)				0.046	0.011
$t^1/_{2\beta}$ (hr)				0.161	0.054
$t^1/_{2\gamma}$ (hr)				1.055	0.178
CL (L/hr)				3.35	0.90
AUC (µg/mL/hr)				15.41	1.99

Distribution

Semaglutide:

Two whole body autoradiography studies were conducted in Wistar rats. These studies were previously reviewed under the IND for subcutaneous semaglutide (IND 79754). Tissue distribution studies were not conducted with oral semaglutide.

In Study NN206132, Wistar rats received a single dose of 0.1 mg/kg ^{3}H-semaglutide (322 µCi/mL) by intravenous (IV) or subcutaneous (SC) injection. One rat each was sacrificed for whole body radiography at 1, 8, and 24 hours post-dose (IV) and at 6, 24, and 30 hours post-dose (SC).

The radioactivity of ^{3}H-semaglutide showed a similar tissue distribution after administration by the IV or SC routes. Following IV administration, the blood concentration decreased to 10% after 24 hours post dosing, whereas the concentration only decreased to approximately 72% after SC administration. The concentration in the lung was similar to the concentration in blood after the first two time points and approximately twice the blood concentration after 24 and 30 hours. High levels of radioactivity were observed in kidney and urinary bladder, which is consistent with renal excretion of semaglutide. A high tissue:blood ratio was seen in the bone marrow, lung, pancreas, and mucosa of the gastrointestinal tract, which correlates with the presence of the GLP-1 receptor in these tissues. Radioactivity was poorly distributed to the brain and spinal cord at all time points.

In Study NN207267, 12 male and 3 pregnant female (~GD 18) Wistar rats were administered a single SC dose of 0.30 mg/kg ^{3}H-Oct-semaglutide (43 MBq/kg) and whole body radiography was conducted at 3, 6, 24, and 72 hours after treatment for males (3/time point) and at 3, 6, and 24 hours after treatment for females (1/time point).

In males, radioactivity was slowly absorbed from the injection site with about 30% of tissues attaining maximal levels at 6 hours after dosing and approximately 60% at 24 hours. Distribution of drug-related material became widespread and radioactivity was identified and measured in over 40 tissues at all time points, including the 72 hour post-dose time point. Overall, the highest levels of radioactivity were measured in the plasma, blood, lung, tooth pulp, kidney (cortex and medulla), and the adrenal medulla. Radioactivity was slowly eliminated by fecal and renal routes, suggesting that these are both key routes of elimination for semaglutide.

The distribution of radioactivity was similar in pregnant animals to that of male rats at comparable sampling times (3, 6, and 24 hours). In addition to those tissues specifically mentioned for the male animals above, the uterus also contained notable concentrations of radioactivity. However, although the placentas contained relatively high levels of radioactivity, concentrations in the fetal tissues were generally very low. No fetal tissues contained measurable levels of radioactivity at 3 hours and only the fetal liver contained radioactivity at 6 hours. Elevated levels were noted at 24 hours, with the brain, heart, liver, lung, and skin being identified at levels ranging between 15.3 and 36.1 ng equiv/g. Overall, a detectable, but very limited, amount of drug-related

radioactivity was distributed to the fetuses, (<4% of the radioactivity in the plasma of the dam).

SNAC:

Study title: In vitro evaluation of binding of ^{14}C-SNAC to mouse, rat, rabbit, monkey and human plasma proteins and to protein solutions (HAS and AAG) by ultrafiltration (209233; 993132)

The percent of specific binding of ^{14}C-SNAC to proteins in mouse, rat, rabbit, monkey, and human plasma and to human serum albumin (HAS) and α_1-acid glycoprotein (AAG) was determined by ultrafiltration. Plasma protein concentrations ranged from 2 to 200 µg/mL.

Results:

Binding of ^{14}C-SNAC in human plasma appeared to be exclusively the result of binding to human serum albumin. The SNAC concentrations tested in this assay are above those observed in rats at the MTD of 500 mg/kg/d. Additionally, SNAC plasma concentrations expected in humans were in the lower range of concentrations tested here. No data on plasma protein binding of the major SNAC metabolites are available. A summary of the binding results are shown in sponsor-generated Table 15.

TABLE 15. Summary of Percent Free and Bound in Mouse, Rat, Rabbit, Monkey and Human Plasma and Human Serum Albumin and α_1-Acid Glycoprotein Solutions.

	2 µg/mL		10 µg/mL		20 µg/mL	
Species	% Free	% Bound	% Free	% Bound	% Free	% Bound
Mouse	16.33	83.67	16.38	83.62	19.78	80.22
Rat	10.74	89.26	10.60	89.40	10.73	89.27
Rabbit	7.90	92.10	7.98	92.02	8.19	91.81
Monkey	2.54	97.46	2.63	97.37	2.92	97.08
Human	2.10	97.90	2.16	97.84	2.24	97.76
HSA	2.25	97.75	2.28	97.72	2.32	97.68
AAG	101.66	-1.66	102.54	-2.54	103.19	-3.19

	100 µg/mL		200 µg/mL	
Species	% Free	% Bound	% Free	% Bound
Mouse	24.31	75.69	30.35	69.65
Rat	13.46	86.54	17.16	82.84
Rabbit	9.24	90.76	10.53	89.47
Monkey	4.03	95.97	6.22	93.78
Human	3.23	96.77	4.72	95.28
HSA	2.96	97.04	3.80	96.20
AAG	103.88	-3.88	104.85	-4.85

Study title: A whole body autoradiography study following a single oral administration of ^{3}H-labeled E414 to mice (806-96, 209228)

CD-1 mice (5/sex) were administered a single oral dose of 300 mg ^{3}H-SNAC (E414). One animal/sex was euthanized at 0.25, 2, 4, 12, and 24 hours after dosing for whole body radiography.

Results:

15 minutes after administration: High levels of radioactivity were found in the stomach and small intestinal contents, liver, and kidney in both sexes and the urinary bladder in the male. In the kidney, radioactivity was more concentrated in the medulla than in the cortex. Small amounts of radioactivity were also observed in the lung for both sexes and gallbladder in the female.

2 hours after administration: High levels of radioactivity remained in the stomach and small intestine contents but at lower levels than at the 15-minute time point. Radioactivity in the liver was markedly decreased in both sexes and a slight decrease was noted for kidneys. In the male, an increased amount of radioactivity was observed in the gallbladder, large intestinal content and urine.

4 hours after administration: High radioactivity levels were detected in the small and large intestine, while little radioactivity was found in the rectum of both sexes. High levels of radioactivity were seen in the gallbladder of the female. Low levels of radioactivity remained in the liver and kidney in both sexes.

12 and 24 hours after administration: The amount of radioactivity in all tissues was reduced markedly compared to the previous time point. Low levels were still present in the small and large intestinal content of both sexes. Levels in other tissues decreased to a non-detectable level.

Conclusions:

The data indicate that SNAC is readily absorbed from the gastrointestinal tract and distributed to the liver. High levels of radioactivity in the gallbladder are indicative of biliary secretion. This observation in association with a small amount of radioactivity present in the feces in the rectum suggests enterohepatic recirculation of SNAC and/or its metabolites. High levels of radioactivity in the kidney in both sexes and the urinary bladder and urine in males indicate that the major route of elimination of radioactivity is through the kidney.

Study title: Salcaprozate sodium (SNAC): Tissue distribution of radioactivity in the rat by quantitative whole-body autoradiography (212181)

Nine Sprague-Dawley rats per sex were administered a single oral dose of 500 mg ^{14}C-SNAC to evaluate tissue distribution as well as the absorption and PK in plasma and whole blood. Animals were processed for whole body autoradiography at 0.5, 1, 1.5, 2, 4, 8, 12, 24, and 36 hours after dosing. Immediately before sacrifice, blood samples were taken.

Results:
Radioactivity was rapidly absorbed and widely distributed following oral administration. Tissues contained maximal levels of radioactivity at 1.5 hours after dosing. Concentrations decreased somewhat at 2 hours with a second, smaller peak at 4 hours. Radioactivity was present in the majority of tissues up to 12 hours post-dose, with levels declining thereafter. Concentrations of radioactivity in nearly all tissues were less than those in plasma. High levels were present in the kidney (cortex and medulla), bile ducts, blood, and liver. The difference in plasma concentrations between males and females was also observed in the tissues, with levels in females being considerably higher than in males up to and including the 12-hour time point. Tissue levels were generally higher in males than in females at the 24 and 36 hour time points. These results indicate that there are some gender-related differences in absorption, distribution, and/or elimination of SNAC-related material. Radioactivity in the bile ducts and urinary tract suggested that absorbed drug-related material was excreted by both the biliary and renal systems. The results are summarized in the sponsor-generated tables.

Table 3 Concentrations of radioactivity in the tissues of male albino rats after a single oral administration of [^{14}C]-SNAC at a nominal dose level of 500 mg/kg body weight (nmol SNAC/g of tissue)

		nmol/gram of tissue								
	Animal number and sex	101M	102M	103M	104M	105M	106M	107M	108M	109M
Tissue group	Sample Sampling time	0.5 hour	1 hour	1.5 hours	2 hours	4 hours	8 hours	12 hours	24 hours	36 hours
Blood-vascular	Plasma EDTA[1]	(b) (4)								
	Aortic wall									
	Blood									
	Blood EDTA									
	Bone marrow									
Lymphatic	Mandibular lymph nodes									
	Spleen									
Excretory	Bile ducts									
	Kidney cortex (whole)									
	Kidney medulla									
	Liver									
Respiratory	Lung									
	Nasal mucosa									
Reproductive	Bulbo-urethral gland									
	Epididymis									
	Preputial gland									
	Prostate									
	Seminal vesicles									
	Testis									
Endocrinal	Adrenal cortex									
	Adrenal medulla									
	Pancreas									
	Pineal body									
	Pituitary									
	Thymus									
	Thyroid									
Ocular	Exorbital lachrymal gland									
	Harderian gland									
	Intra-orbital lachrymal gland									
	Lens of the eye									
	Uveal tract/retina									
Central nervous system	Brain									
	Choroid plexus									
	Meninges									
	Spinal cord									
Gastrointestinal	Caecum mucosa									
	Large intestine mucosa									
	Oesophageal wall									
	Rectum mucosa									
	Small intestine mucosa									
	Stomach mucosa (fundus)									
	Stomach mucosa (non-fundic)									
Adipose	Brown fat									
	White fat									
Oral	Periodontal membrane									
	Salivary glands									
	Tongue									
	Tooth pulp									
Unclassified	Muscle									
	Myocardium									
	Skin									
	Upper limit of quantification =	17554.4	nmol/g for all measurements							
	Lower limit of quantification =	4.162	nmol/g for all measurements							

BLQ – Radioactivity concentration below lower limit of quantification

Table 6 Concentrations of radioactivity in the tissues of female albino rats after a single oral administration of [^{14}C]-SNAC at nominal dose level of 500 mg/kg body weight (nmol SNAC/g of tissue)

		nmol/gram of tissue								
	Animal number and sex	110F	111F	112F	113F	114F	115F	116F	117F	118F
Tissue group	Sample Sampling time	0.5 hour	1 hour	1.5 hours	2 hours	4 hours	8 hours	12 hours	24 hours	36 hours
Blood-vascular	Plasma EDTA [1]									(b) (4)
	Aortic wall									
	Blood									
	Blood EDTA									
	Bone marrow									
Lymphatic	Mandibular lymph nodes									
	Spleen									
Excretory	Bile ducts									
	Kidney cortex (whole)									
	Kidney medulla									
	Liver									
Respiratory	Lung									
	Nasal mucosa									
Reproductive	Clitoris									
	Ovary									
	Uterus									
Endocrinal	Adrenal cortex									
	Adrenal medulla									
	Pancreas									
	Pineal body									
	Pituitary									
	Thymus									
	Thyroid									
Blood-vascular	Plasma EDTA [1]									
	Aortic wall									
	Blood									
	Blood EDTA									
	Bone marrow									
Lymphatic	Mandibular lymph nodes									
	Spleen									
Excretory	Bile ducts									
	Kidney cortex (whole)									
	Kidney medulla									
	Liver									
Respiratory	Lung									
	Nasal mucosa									
Reproductive	Clitoris									
	Ovary									
	Uterus									
Endocrinal	Adrenal cortex									
	Adrenal medulla									
	Pancreas									
	Pineal body									
	Pituitary									
	Thymus									
	Thyroid									
	Upper limit of quantification =	17554.4	nmol/g for all measurements							
	Lower limit of quantification =	4.162	nmol/g for all measurements							

[1] – Radioactivity determined by liquid scintillation counting (LOD Plasma EDTA = 0.6970 nmol/g; plasma Sodium citrate = 0.6960 nmol/g)
BLQ – Radioactivity concentration below lower limit of quantification

Table 11 Pharmacokinetic parameters for the tissues of male albino rats after a single oral administration of [^{14}C]-SNAC at a nominal dose level of 500 mg/kg body weight

Tissue group	Sample Parameter	r^2	No points Lambda z	Lambda z lower (hr)	Lambda z upper (hr)	T_{half} (hr)	T_{max} (hr)	C_{max} (µg/g)	T_{last} (hr)	AUC_{last} (hr*µg/g)	AUC_{all} (hr*µg/g)	AUCall Tissue:blood ratio
Blood-vascular	Plasma LSC (EDTA)	0.976	3	12	36	7.53	1.0	177	36	866	866	1.70
	Plasma LSC (SC)	0.944	3	12	36	8.00	1.0	103	36	593	593	1.16
	Aortic wall	<0.850	4	1.5	8	NC	1.5	68.6	8	265	265	0.521
	Blood LSC	0.981	3	12	36	7.33	1.0	105	36	509	509	1.00
	Blood LSC (SC)	0.963	3	12	36	7.76	1.0	97.2	36	474	474	0.931
	Blood WBA	0.919	3	8	24	5.48	1.5	102	24	513	513	1.01
	Bone marrow	0.952	3	4	12	2.70	1.0	15.6	12	77.7	77.7	0.153
Lymphatic	Mandibular lymph nodes	<0.850	3	2	8	NC	1.5	33.1	8	98.9	98.9	0.194
	Spleen	0.852	3	2	8	2.20	1.5	42.6	8	109	109	0.214
Excretory	Bile ducts	0.944	4	2	12	1.86	1.0	1430	12	3913	3913	7.69
	Kidney cortex	0.950	3	12	36	6.19	1.5	228	36	1450	1450	2.85
	Kidney medulla	<0.850	3	12	36	NC	4.0	306	36	1898	1898	3.73
	Liver	0.908	3	8	24	4.59	1.5	140	24	745	745	1.46
Respiratory	Lung	<0.850	3	8	24	NC	1.5	85.5	24	388	388	0.763
	Nasal mucosa	<0.850	5	1.5	24	NC	1.5	24.1	24	125	125	0.245
Reproductive	Bulbo-urethral gland	0.951	3	4	12	2.52	1.0	37.0	12	143	143	0.282
	Epididymis	0.886	3	8	24	8.72	1.5	32.8	24	165	165	0.324
	Preputial gland	0.972	3	8	24	10.5	4.0	47.7	24	194	194	0.382
	Prostate	<0.850	4	1.5	8	NC	1.5	52.1	8	188	188	0.370
	Seminal vesicles	0.980	3	2	8	1.71	2.0	19.6	8	55.4	55.4	0.109
	Testis	<0.850	4	1.5	8	NC	1.5	19.1	8	64.9	64.9	0.128
Endocrinal	Adrenal cortex	<0.850	4	1.5	8	NC	1.5	29.3	8	150	150	0.294
	Adrenal medulla	0.863	4	1.5	8	2.54	1.5	39.9	8	158	158	0.311
	Pancreas	0.963	3	4	12	2.11	1.5	34.0	12	131	131	0.258
	Pineal body	0.961	3	2	8	2.89	1.5	37.4	8	124.	124	0.244
	Pituitary	0.970	3	4	12	2.67	1.0	29.4	12	148	148	0.291
	Thymus	0.765	4	1.5	8	2.35	1.5	17.7	8	57.3	57.3	0.113
	Thyroid	0.697	6	1.5	24	5.71	1.5	27.5	24	149	149	0.292
Ocular	Exorbital lachrymal gland	0.938	3	4	12	2.18	1.5	19.1	12	91.4	91.4	0.180
	Harderian gland	<0.850	3	2	8	NC	1.5	19.8	8	75.5	75.5	0.148
	Intra-orbital lachrymal gland	<0.850	3	8	24	NC	1.5	27.2	24	121	121	0.239
	Lens of the eye	NC	0	NC	NC	NC	2.0	1.59	2	NC	1.59	0.003
	Uveal tract/retina	1.00	3	4	12	2.24	1.0	36.3	12	181	181	0.356
Central nervous system	Brain	NC	0	NC	NC	NC	1.5	2.66	4	7.18	7.18	0.014
	Choroid plexus	NC	0	NC	NC	NC	4.0	25.1	8	104	104	0.204
	Meninges	0.998	3	4	12	4.00	1.5	50.9	12	104	104	0.205
	Spinal cord	NC	0	NC	NC	NC	1.5	4.23	4	10.1	10.1	0.020
Gastrointestinal	Caecum mucosa	0.998	3	8	24	5.19	4.0	337	24	2778	2778	5.46
	Large intestine mucosa	0.964	3	8	24	4.50	8.0	403	24	4519	4519	8.88
	Oesophageal wall	<0.850	6	1	24	NC	0.5	65.7	24	253	253	0.497
	Rectum mucosa	NC	0	NC	NC	NC	12.0	186	12	715	715	1.41
	Small intestine mucosa	0.887	3	4	12	2.33	2.0	1060	12	7710	7710	15.2
	Stomach mucosa (fundus)	0.916	3	4	12	1.01	1.0	591	12	2172	2172	4.27
	Stomach mucosa (non-fundic)	<0.850	7	1	24	NC	1.0	4670	24	12518	12518	24.6
Adipose	Brown fat	0.958	3	8	24	16.5	1.5	54.5	24	386	386	0.758
	White fat	0.738	4	4	36	13.8	1.5	13.7	36	215	215	0.422
Oral	Periodontal membrane	0.983	3	4	12	2.55	4.0	36.2	12	204	204	0.401
	Salivary glands	1.00	3	4	12	2.13	1.5	31.0	12	124	124	0.244
	Tongue	1.00	3	4	12	2.27	1.0	25.5	12	112	112	0.221
	Tooth pulp	0.983	3	4	12	2.05	1.5	66.5	12	268	268	0.527
Unclassified	Muscle	<0.850	3	2	8	NC	1.5	13.4	8	45.1	45.1	0.089
	Myocardium	0.997	3	4	12	1.92	1.5	46.7	12	192	192	0.378
	Skin	0.919	3	8	24	9.27	1.5	41.3	24	196	196	0.384

NC – Not calculated insufficient data available

Table 12 Pharmacokinetic parameters for the tissues of female albino rats after a single oral administration of [^{14}C]-SNAC at a nominal dose level of 500 mg/kg body weight

Tissue group	Sample Parameter	r^2	No points Lambda z	Lambda z lower (hr)	Lambda z upper (hr)	T_{Half} (hr)	T_{max} (hr)	C_{max} (µg/g)	T_{last} (hr)	AUC_{last} (hr*µg/g)	AUC_{all} (hr*µg/g)	AUCall Tissue:blood ratio
Blood-vascular	Plasma LSC (EDTA)	0.951	3	12	36	4.31	1	321	36	1819	1819	1.61
	Plasma LSC (SC)	0.937	3	12	36	4.72	1	180	36	980	980	0.867
	Aortic wall	0.896	3	4	12	2.22	1	197	12	640	640	0.566
	Blood LSC	0.951	3	8	24	2.50	1	213	24	1131	1131	1.00
	Blood LSC (SC)	0.952	3	8	24	2.47	1	196	24	1036	1036	0.916
	Blood WBA	0.961	3	8	24	2.84	1	222	24	1037	1037	0.917
	Bone marrow	<0.850	3	4	12	NC	1	90.8	12	363	363	0.321
Lymphatic	Mandibular lymph nodes	<0.850	3	8	24	NC	1	82.5	24	382	382	0.338
	Spleen	0.858	3	4	12	1.83	1	66.8	12	279	279	0.247
Excretory	Bile ducts	0.860	3	4	12	2.07	4	406	12	2836	2836	2.51
	Kidney cortex	0.989	4	4	24	4.60	1	261	24	2208	2208	1.95
	Kidney medulla	0.916	4	4	24	4.31	2	330	24	1695	1695	1.50
	Liver	0.947	4	4	24	3.39	0.5	132	24	865	865	0.765
Respiratory	Lung	0.915	3	8	24	3.12	1	121	24	733	733	0.648
	Nasal mucosa	<0.850	3	4	12	NC	1	34.1	12	192	192	0.170
Reproductive	Clitoris	0.996	4	4	24	4.49	1	74.2	24	452	452	0.399
	Ovary	0.906	3	12	36	13.7	1	70.5	36	596	596	0.527
	Uterus	<0.850	7	1	24	NC	1	126	24	556	556	0.491
Endocrinal	Adrenal cortex	<0.850	8	0.5	24	NC	0.5	103	24	541	541	0.479
	Adrenal medulla	<0.850	7	1	24	NC	1	77.2	24	541	541	0.478
	Pancreas	<0.850	3	4	12	NC	1	94.4	12	358	358	0.316
	Pineal body	<0.850	3	4	12	NC	1.5	76.7	12	396	396	0.350
	Pituitary	0.874	3	4	12	1.97	1	69.7	12	273	273	0.241
	Thymus	0.865	3	4	12	1.79	1	53.4	12	211	211	0.187
	Thyroid	<0.850	3	4	12	NC	1	73.2	12	240	240	0.212
Ocular	Exorbital lachrymal gland	0.864	3	4	12	2.24	1	61.5	12	213	213	0.189
	Harderian gland	<0.850	3	4	12	NC	1	82.4	12	316	316	0.279
	Intra-orbital lachrymal gland	<0.850	3	4	12	NC	1	99.8	12	360	360	0.318
	Lens of the eye	NC	0	NC	NC	NC	1	3.92	1.5	3.15	3.15	0.003
	Uveal tract/retina	<0.850	3	4	12	NC	1	83.3	12	381	381	0.337
Central nervous system	Brain	<0.850	5	1	8	NC	1	7.08	8	22.0	22.0	0.019
	Choroid plexus	<0.850	6	1	12	NC	1	29.8	12	220	220	0.194
	Meninges	0.926	3	4	12	3.93	1	32.8	12	153	153	0.135
	Spinal cord	<0.850	5	1	8	NC	1	5.45	8	20.1	20.1	0.018
Gastrointestinal	Caecum mucosa	NC	0	NC	NC	NC	8	359	12	2477	2477	2.19
	Large intestine mucosa	NC	0	NC	NC	NC	12	276	12	1479	1479	1.31
	Oesophageal wall	<0.850	3	4	12	NC	1	87.9	12	375	375	0.331
	Rectum mucosa	NC	0	NC	NC	NC	12	133	12	642	642	0.568
	Small intestine mucosa	0.999	3	4	12	3.30	1.5	1040	12	5514	5514	4.88
	Stomach mucosa (fundus)	0.950	3	4	12	1.15	0.5	1490	12	4048	4048	3.58
	Stomach mucosa (non-fundic)	0.852	3	4	12	3.20	0.5	8740	12	16791	16791	14.8
Adipose	Brown fat	0.970	3	8	24	3.36	1	229	24	719	719	0.636
	White fat	<0.850	7	1.5	36	NC	1	32.6	36	283	283	0.250
Oral	Periodontal membrane	0.927	3	4	12	2.68	1	72.1	12	333	333	0.295
	Salivary glands	0.886	3	4	12	2.13	1	79.5	12	280	280	0.247
	Tongue	0.854	3	4	12	2.13	1	89.5	12	309	309	0.273
	Tooth pulp	<0.850	3	4	12	NC	1.5	102	12	591	591	0.522
Unclassified	Muscle	<0.850	5	1	8	NC	1	36.2	8	116	116	0.103
	Myocardium	<0.850	3	4	12	NC	1	126	12	500	500	0.442
	Skin	<0.850	8	1	36	NC	1	96.2	36	444	444	0.392

NC – Not calculated insufficient data available

Table 9 Relative concentrations of radioactivity in the tissues of male and female albino rats after a single oral administration of [^{14}C]-SNAC at a nominal dose level of 500 mg/kg body weight

Animal number and sex	101M	102M	103M	104M	105M	106M	107M	108M	109M
Sample / Sampling time	0.5 hour	1 hour	1.5 hours	2 hours	4 hours	8 hours	12 hours	24 hours	36 hours
Kidney pyramid									(b) (4)
Oesophagus contents									
Stomach contents									
Small intestine contents									
Caecum contents									
Large intestine contents									
Rectum contents									

Animal number and sex	110F	111F	112F	113F	114F	115F	116F	117F	118F
Sample / Sampling time	0.5 hour	1 hour	1.5 hours	2 hours	4 hours	8 hours	12 hours	24 hours	36 hours
Kidney pyramid									(b) (4)
Oesophagus contents									
Stomach contents									
Small intestine contents									
Caecum contents									
Large intestine contents									
Rectum contents									

The assessment of the relative levels of radioactivity in the autoradiograms was made by visual inspection of the printed electronic autoradiograms and was performed for tissues for which a full quantification was not possible.
The relative levels of radioactivity in the tissues were then recorded in a simple scoring code thus:

3	Concentration described as "high"
2	Concentration described as "moderate"
1	Concentration described as "low"
RND	Radioactivity not detected on the autoradiogram

Metabolism

Semaglutide:

Study title: [^{3}H]-Oct-NNC 0113-0000-0217 with carrier compound NNC 0113-3363: Profiling of circulating metabolites in plasma following oral single dose administration of tablets to Sprague-Dawley rats (209112)

combination with 30 mg SNAC. Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 hours (3 animals/time point). Parent and metabolite profiles were determined by HPLC and radiochromatography.

Results:

Five components were detected in plasma (P1, P2, P3, P4, and parent). P2, P3, and parent were detected at 0.5, 2, and 8 hours. P2 and parent were detected at 1 and 4 hours. P1 and P4 were detected at 24 hours. The P2 and P3 metabolites were considered to be closely related to the parent compound. P4 eluted later than parent and is therefore much more hydrophilic than semaglutide. P1 eluted in front of the chromatogram and is expected to be tritiated water, as it disappeared after freeze drying. Tritiated water is formed as a consequence of total degradation of semaglutide into primary components, such as amino acids, water, and carbon dioxide, and therefore is not considered a metabolite of unlabeled semaglutide. A summary of the metabolite profile is shown in the sponsor-generated tables below.

Table 7 Relative peak areas and Rt_R of peaks detected in male rat plasma following single oral administration of a tablet containing 1 mg NNC 0113-0217 and 30 mg NNC 0113-3363

		Relative peak areas (%)					
Peak	**Mean Rt_R**	**0.5 h**	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**
P1	0.08	NA	NA	NA	NA	NA	90.96
P2	0.97	38.31	63.43	4.67	63.83	10.05	NA
P3	0.99	17.53	NA	51.95	NA	46.27	NA
NNC 0113-0217	1.00	44.16	36.56	43.38	36.17	43.27	4.02
P4	1.48	NA	NA	NA	NA	NA	5,01
Total	NA	100.00	99.99	100.00	100.00	99.59	99.99

NA = not applicable

Table 10 Pharmacokinetic parameters, AUC_{last} and fractions of total AUC obtained from male rats following single oral administration of a tablet containing 1 mg NNC 0113-0217 and 30 mg NNC 0113-3363

	AUC_{last} (h*µg equiv/L)	Fraction of total AUC	Fraction of total AUC (minus P1)
P1	1247	55	NA
P2	123	6	12
P3	401	18	40
NNC 0113-0217	410	18	41
P4	68	3	7
Total	2249	100	100
Total minus P1	1003	NA	100

NA = not applicable

Study title: [^{3}H]-Oct-NNC 0113-0000-0217 with carrier compound NNC 0113-3363: Profiling of circulating metabolites in plasma following oral single dose administration of liquid formulation to Sprague-Dawley rats (209157)

Eighteen rats were administered a single oral, liquid formulation containing 1.65 mg [^{3}H]-semaglutide in combination with 50 mg SNAC. Blood samples were collected at 0.08, 0.33, 1, 2, 6, and 24 hours (3 animals/time point). Parent and metabolite profiles were determined by HPLC and radiochromatography.

Five components were detected in plasma (P1, P2, P3, P4, and parent). The various peaks were detected at the various sampling time points. P2, P3, P4, and semaglutide were minor peaks when detected. All five peaks were detected at the 6-hour time point. P1 was the only peak detected at 24 hours. The P2 and P3 metabolites were considered to be closely related to the parent compound. P4 eluted later than parent and is therefore much more hydrophilic than semaglutide. P1 eluted in front of the chromatogram and is expected to be tritiated water, as it disappeared after freeze drying. Tritiated water is formed as a consequence of total degradation of semaglutide into primary components, such as amino acids, water, and carbon dioxide, and

therefore is not considered a metabolite of unlabeled semaglutide. A summary of the metabolite profile is shown in the sponsor-generated tables below.

Table 7 Relative peak areas and Rt_R of peaks detected in male rat plasma following single oral administration of the liquid formulation containing 1.1 mg/mL [^{3}H]-Oct-NNC 0113-0217 in combination with 33.3 mg/mL NNC 0113-3363

		Relative peak areas (%)					
Peak	**Mean Rt_R**	**5 min**	**20 min**	**1 h**	**2 h**	**6 h**	**24 h**
P1	0.08	NA	NA	85.11	NA	21.32	100.00
P2	0.97	23.34	63.68	NA	58.57	30.69	NA
P3	0.98	28.94	2.73	6.66	16.5	6.7	NA
NNC 0113-0217	1.00	47.72	33.59	8.24	24.93	15.77	NA
P4	1.48	NA	NA	NA	NA	25.52	NA
Total	NA	100.00	100.00	100.00	100.00	100.00	100.00

NA=not applicable

Table 10 Pharmacokinetic parameters, AUC_{last} and relative exposure obtained from male rats following single oral administration of liquid formulation containing nominally 1.1 mg/mL [^{3}H]-Oct-NNC 0113-0217 in combination with 33.3 mg/mL NNC 0113-3363

	AUC_{last} (h*µg/L)	**Relative peak exposure (Relative to total AUC)**	**Relative peak exposure (Relative to total AUC minus P1)**
P1	1684.8	80.6	NA
P2	213.6	10.2	52.8
P3	50.0	2.4	12.4
NNC 0113-0217	95.5	4.6	23.6
P4	45.6	2.2	11.3
Total	2089.5	100.0	100.0
Total minus P1	404.7	NA	NA

NA = not applicable

Study title: [^{3}H]-Oct-NNC 0113-000-0217 with carrier compound NNC 0113-3363: Metabolite profiling of plasma from male Cynomolgus monkey following single oral administration (209323)

Three male Cynomolgus monkeys received a single oral tablet containing 15 mg ^{3}H-semaglutide (405 µCi) and 450 mg SNAC. Metabolites were measured in plasma samples taken before dosing and at 2, 12, 24, 48, 72, 96, and 168 hours post-dose. Urine and feces were also collected but were not analyzed. Plasma metabolite profiling was conducted by HPLC and radiochromatography.

Results:

Three peaks were detected: P1, P2, and parent. P2 eluted approximately 20 minutes after semaglutide and is therefore much more hydrophobic than the parent molecule. P1 is expected to be tritiated water as it disappeared after freeze drying. Tritiated water

is formed as a consequence of total degradation of semaglutide into primary components such as amino acids, water, and carbon dioxide. P1 is therefore not considered a metabolite of unlabeled semaglutide. Results are summarized in sponsor-generated Tables 5 and 7 below.

Table 5 Peak concentration equivalents (ng equiv/mL) from peaks detected in male cynomolgus monkey plasma following single oral administration of a tablet containing 15 mg [^{3}H]-Oct-NNC 0113-0217 in combination with 450 mg NNC 0113-3363

	Concentration equivalents (ng equiv/mL)							
Peak	**predose**	**2 h**	**12 h**	**24 h**	**48 h**	**72 h**	**96 h**	**168 h**
P1	NA	NA	962.1	2009.8	2490.2	2473.5	2060	1425.2
NNC 0113-0217	NA	97.2	48.5	37.7	NA	NA	NA	NA
P2	NA	NA	81.9	56.2	NA	NA	NA	NA

NA= not applicable

Table 7 Pharmacokinetic parameters, AUC_{last} and fractions of total AUC obtained from male rats following single oral administration of a tablet containing 15 mg [^{3}H]-Oct-NNC 0113-0217 in combination with 450 mg NNC 0113-3363

	AUC_{last} (h*µg equiv /L)	Fraction of total AUC	Fraction of total AUC (minus P1)
P1	315486	99	50
NNC 0113-0217	1312	0.4	NA
P2	1310	0.4	50
Total	318109	100	100
Total minus P1	2623	NA	100

NA= not applicable

SNAC:

Study title: Determination of the potential for a new chemical entity to inhibit cytochrome P450 enzyme activity (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5) in vitro (983110, 209230)

Pooled human liver microsomal samples were incubated with SNAC (E414-sodium; 1 to 100 µM) with or without (b) (4) to evaluate the ability of SNAC to inhibit the cytochrome P450-mediated metabolism of ethoxyresorufin (CYP1A2), tolbutamide (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), chlorzoxazone (CYP2E1), and testosterone (CYP3A4/5).

Results:

In the absence of (b) (4), SNAC concentrations up to 100 µM did not inhibit the activity of the P450 isozymes tested in this study. In the presence o (b) (4), a slight inhibition of CYP2C19 activity by ~16% to 27% was observed at concentrations from 5 to 100 µM SNAC and a slight inhibition of CYP3A4 activity by ~25% was seen at 100 µM SNAC. The activities of the other P450 isozymes that were tested were not affected by SNAC in the presence of (b) (4).

Study title: Determination of the inhibition kinetics associated with the potential inhibition of human microsomal CYP2C19 and CYP3A4/5 activities by SNAC (with or without (b) (4)) (992933, 209232)

This study was previously reviewed by Ke Zhang under IND (b) (4) Dr. Zhang's review is reproduced below.

Methods: To determine the inhibition kinetics of SNAC in human microsomes, SNAC was incubated with human liver microsomes at six concentrations (25, 50, 100, 250, 500, and 1000 µM) in the presence and absence of (b) (4).

Results: The results indicated that SNAC with and without (b) (4) was a weak competitive inhibitor for CYP2C19 with apparent K_i of 200-450 µM and for CYP3A4/5 with apparent K_i of 500-1000 µM.

Study title: In vitro evaluation of SNAC as an inhibitor of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes in human liver microsomes (212422)

The ability of SNAC to inhibit the major CYP and UGT enzymes in human liver microsomes was evaluated in vitro. Human liver microsomes from a pool of 16 individuals were incubated with marker substrates in the absence or presence of SNAC (0.1 to 100 µM).

Results:

Under the conditions of this study, SNAC was found to directly inhibit CYP2C19 and UGT1A9 with approximately 25% and 33% inhibition, respectively, at the highest concentration tested (100 µM). The IC_{50} values for CYP2C19 and CYP1A2 were greater than 100 µM. There was little or no evidence that SNAC directly inhibited the other CYP and UGT enzymes tested. There was also little or no evidence of time- or metabolism-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 by SNAC. A summary of the results is presented in sponsor-generated Table 11 below.

Table 11: Summary of results: *In vitro* evaluation of SNAC as an inhibitor of human CYP and UGT enzymes

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (µM) [a]	Inhibition observed at 100 µM (%) [b]	IC_{50} (µM) [a]	Inhibition observed at 100 µM (%) [b]	IC_{50} (µM) [a]	Inhibition observed at 100 µM (%) [b]	
CYP1A2	Phenacetin *O*-dealkylation	> 100	13	> 100	15	> 100	14	Little or no
CYP2B6	Efavirenz 8-hydroxylation	> 100	NA	> 100	4.5	> 100	14	Little or no
CYP2C8	Amodiaquine *N*-dealkylation	> 100	15	> 100	17	> 100	13	Little or no
CYP2C9	Diclofenac 4′-hydroxylation	> 100	1.4	> 100	4.3	> 100	6.2	Little or no
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	> 100	25	> 100	18	> 100	26	Little or no
CYP2D6	Dextromethorphan *O*-demethylation	> 100	7.6	> 100	7.8	> 100	1.2	Little or no
CYP3A4/5	Testosterone 6β-hydroxylation	> 100	3.2	> 100	7.5	> 100	0.3	Little or no
CYP3A4/5	Midazolam 1′-hydroxylation	> 100	NA	> 100	NA	> 100	NA	Little or no
UGT1A1	17β-Estradiol 3-glucuronidation	> 100	NA	ND	ND	ND	ND	ND
UGT1A4	Trifluoperazine glucuronidation	> 100	0.3	ND	ND	ND	ND	ND
UGT1A6	1-Naphthol glucuronidation	> 100	0.4	ND	ND	ND	ND	ND
UGT1A9	Propofol glucuronidation	> 100	33	ND	ND	ND	ND	ND
UGT2B7	Morphine 3-glucuronidation	> 100	15	ND	ND	ND	ND	ND

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. IC_{50} values were calculated with XLFit.

b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures): Inhibition observed (%) = 100% − Percent solvent control.

c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

NA Not applicable. No value was obtained as the rates at the highest concentration of SNAC evaluated (100 µM) were higher than the control rates.

ND Not determined. UGT enzymes were not evaluated for time- or metabolism-dependent inhibition.

Study title: In vitro evaluation of NNC 0113-3705, NNC 0113-3706, NNC 0113-3707, NNC 0113-3708, NNC 0113-3709, as inhibitors of cytochrome P450 (CYP) and UDP-glucuronosyltransferase enzymes (UGT) in human liver microsomes (213026)

Five primary metabolites of SNAC, NNC0113-3705 (E1245), NNC0113-3706 (E494), NNC0113-3707 (E1246), NNC0113-3708 (E506), and NNC0113-3709 (E1247), were assessed as direct and time-dependent inhibitors of CYP activity and as direct inhibitors of UGT activity. Human liver microsomes from a pool of sixteen individuals were incubated with marker substrates, at concentrations approximately equal to their apparent K_m, in the presence or absence of each test article. To evaluate time-dependent inhibition, the test articles were preincubated with human liver microsomes and an NADPH-generating system for 30 minutes prior to the incubation with the marker substrate. The target concentrations of E1245, E494, E1246, E506, and E1247 were 0.1, 0.3, 1, 3, 10, 30 and 100 µM. Known direct inhibitors of CYP and UGT enzymes and metabolism-dependent inhibitors of CYP enzymes were included as positive controls.

Results:

E1245 was found to directly inhibit CYP1A2, CYP2B6, CYP2C8, and CYP2C19 by ~38%, 37%, 48%, and 32% at 100 µM, the highest concentration tested. The respective IC_{50} values were greater than 100 µM. Upon a 30 minute preincubation with NADPH, E1245 caused a time-dependent inhibition of CYP2B6 by at least a 2-fold shift

in IC_{50} values from >100 µM to 49 µM, when compared to IC_{50} values without preincubation. There was little or no evidence of direct inhibition by the other metabolites on any of the UGT enzymes or direct or time-dependent inhibition on any of the CYP enzymes evaluated.

Study title: RO5045192 (SNAC): Evaluation of human cytochrome P450 induction properties of RO5045192-001: In vitro experiments (212212)

Cytochrome P450 induction properties of RO5045192 (SNAC) were evaluated in vitro using monolayers of freshly isolated human hepatocytes. The primary CYPs involved in drug metabolism were studied: CYP1A2, CYP2C9, and CYP3A4/5. SNAC concentrations of 10, 30, and 100 µM were incubated with human hepatocytes for 72 to 96 hours to evaluate the potential effect on the metabolism of test compounds.

Results:

The activity of CYP1A2 on the O-deethylase of phenacetin was increased by 1.2- to 1.4-fold at 100 µM SNAC in two hepatocyte batches. However, a 20% decrease in CYP1A2 activity was observed at 10 and 30 µM for the third hepatocyte batch. The induction of activity was small compared to the positive control and therefore, the overall effect of SNAC on CYP1A2 activity is of questionable biological significance. Methylhydroxylase activity on tolbutamide (CYP2C9) was significantly and dose-dependently increased (1.2- to 1.6-fold) by SNAC at all tested concentrations. The induction was low compared to the positive control and therefore, SNAC is considered to be a weak inducer of CYP2C9. SNAC significantly reduced (70% to 80%) the oxidase activity on nifedipine (CYP3A4/5) at 100 µM.

Under the conditions of this study, SNAC was considered to be a weak inducer of CYP2C9 and CYP1A2, whereas SNAC showed an ability to decrease CYP3A4/5 activity.

Study title: In vitro evaluation of SNAC as an inducer of cytochrome P450 expression in cultured human hepatocytes (212430)

Three preparations of cultured human hepatocytes from three separate livers were treated once daily for 3 consecutive days with DMSO, SNAC (0.4, 4, 20, 40, 100, or 200 µM), or one of three known human CYP inducers (omeprazole, phenobarbital, and rifampin). After completion of treatment, cells were harvested for the isolation of microsomes and measurement of CYP activity using CYP-specific test compounds. mRNA was isolated from additional hepatocytes from the same treatment groups for measurement of gene expression by qRT-PCR. Spent media was assessed for the SNAC metabolic profile by LC-MS/MS.

Results:

Assessment of the metabolite profile in the tissue culture media showed that all five SNAC metabolites of interest were present (E1245, E1246, E1247, E506, and E494). The results indicated that there was no induction potential by SNAC (or its metabolites)

at test concentrations up to 200 μM (<2-fold or <20% of the prototypical inducer). Concentration-dependent increases (>2-fold) in CYP1A2 mRNA levels was observed at concentrations up to 40 μM with a decline at the higher concentrations. Hepatocytes from a single donor showed an increase of CYP2B6 and 2C8 mRNA levels by at least 2-fold.

Study title: Comparison of phase I and phase II metabolic profile of a new chemical entity in human, Rhesus monkey, Cynomolgus monkey, rabbit, rat, and mouse liver S9 incubations (209229; 983109)

Pooled liver S9 fractions isolated from mice, Sprague Dawley rats, Cynomolgus monkeys, Rhesus monkeys, New Zealand white rabbits, and human donors were used to determine if species differences exist in the metabolism, Phase I and Phase II, of SNAC when tested in vitro. Measurement of the parent and metabolite species was conducted by LC/MS.

Results:

No disappearance of parent compound and no metabolite formation occurred when SNAC was incubated with Phase I reaction mixtures. Biotransformation of SNAC occurred in Phase II reaction mixtures, for which species-related differences were observed. An unknown glucuronide metabolite was observed in both monkey species and mouse. The rate of parent compound disappearance by Phase II metabolism among the 6 species could be ranked from highest to lowest as: Rhesus monkey ~ Cynomolgus monkey > mouse > human > rat > rabbit. A summary of the Phase II reaction data is shown in the sponsor-generated table below.

PHASE II METABOLISM OF SNAC (E414-SODIUM) [10 µM] BY HUMAN, CYNOMOLGUS MONKEY, RHESUS MONKEY, RABBIT, RAT AND MOUSE LIVER S9 INCUBATIONS

Species S9	Incubation Time	Parent Compound Remaining		Relative Amount of Glucuronide Metabolite Formed
	(min)	pmol	%	
Human	0	5000	100	ND
	10	4383	88	ND
	30	3282	66	ND
Cynomolgus monkey	0	5000	100	ND
	10	2042	41	ND
	30	1204	24	555*
Rhesus monkey	0	5000	100	ND
	10	2382	48	208
	30	561	11	2652
Rabbit	0	5000	100	ND
	10	5702	114	ND
	30	5575	111	ND
Rat	0	5000	100	ND
	10	4996	100	ND
	30	4366	87	ND
Mouse	0	5000	100	ND
	10	3170	63	ND
	30	2562	51	553

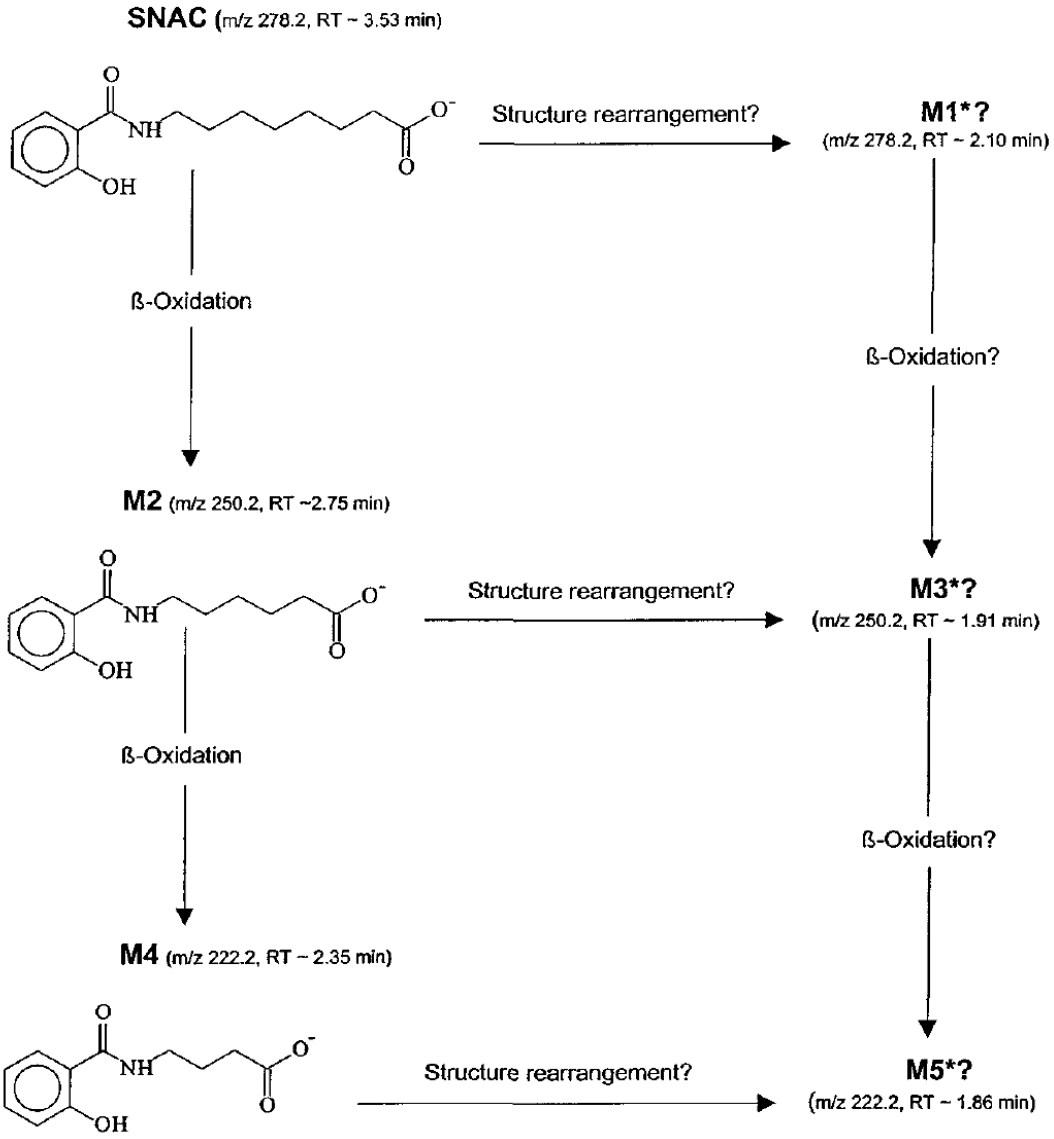
ND: not detected
P450 : 100 pmol
10µM of SNAC equal to 5000 pmol
* Pmol metabolite = mean area of metabolite x pmol / mean area of parent (0 min)

Study title: Species comparison of the metabolite profile of SNAC with and without (b) (4) using human, Rhesus monkey, and Sprague-Dawley rat primary hepatocytes (991596, 209234)

To compare the metabolic profile of SNAC in human, monkey, and rat primary hepatocytes, SNAC was incubated with human, monkey, and rat primary hepatocytes at 37° C for up to 6 hours. The samples were analyzed for SNAC and its metabolites using LC/MS/MS methods.

Results: The ranking order of SNAC substrate disappearance from the test medium from highest to lowest was: rat > monkey > human. For all incubations, SNAC was completely depleted by the end of the 6-hour incubation, indicating extensive hepatic metabolism. Metabolic profiles were similar in human and monkey hepatocytes, with a total of 5 metabolites identified (M1, M2, M3, M4, and M5). Only metabolites M2 and M4 were found in rats. M4 was the most abundant metabolite in rat and human hepatocytes. In monkey hepatocytes, a more polar from of SNAC (M1) was the most abundant metabolite. In all three species, SNAC went through two levels of β-oxidation. SNAC did not appear to undergo Phase I (mainly catalyzed by cytochrome P450 enzymes) or Phase II biotransformations in primary hepatocytes.

POSSIBLE METABOLIC PATHWAY OF SNAC IN *IN VITRO* INCUBATIONS WITH HUMAN, RAT AND MONKEY HEPATOCYTES



* Metabolites that were solely detected in human and monkey incubations

In Wistar rats, the essential metabolism pathway for SNAC is a stepwise degradation of the acid side chain, analogous to fatty acid degradation. This pathway involves β-oxidation where methylene moieties are removed from the SNAC 8-carbon chain to form the metabolites E494: N-[6-(2-hydroxybenzoyl)amino]caproate and E506: (N-[4-(2-hydroxy benzoyl)amino]butyrate). The proposed metabolic pathway for SNAC is shown in sponsor-generated Figure 2.

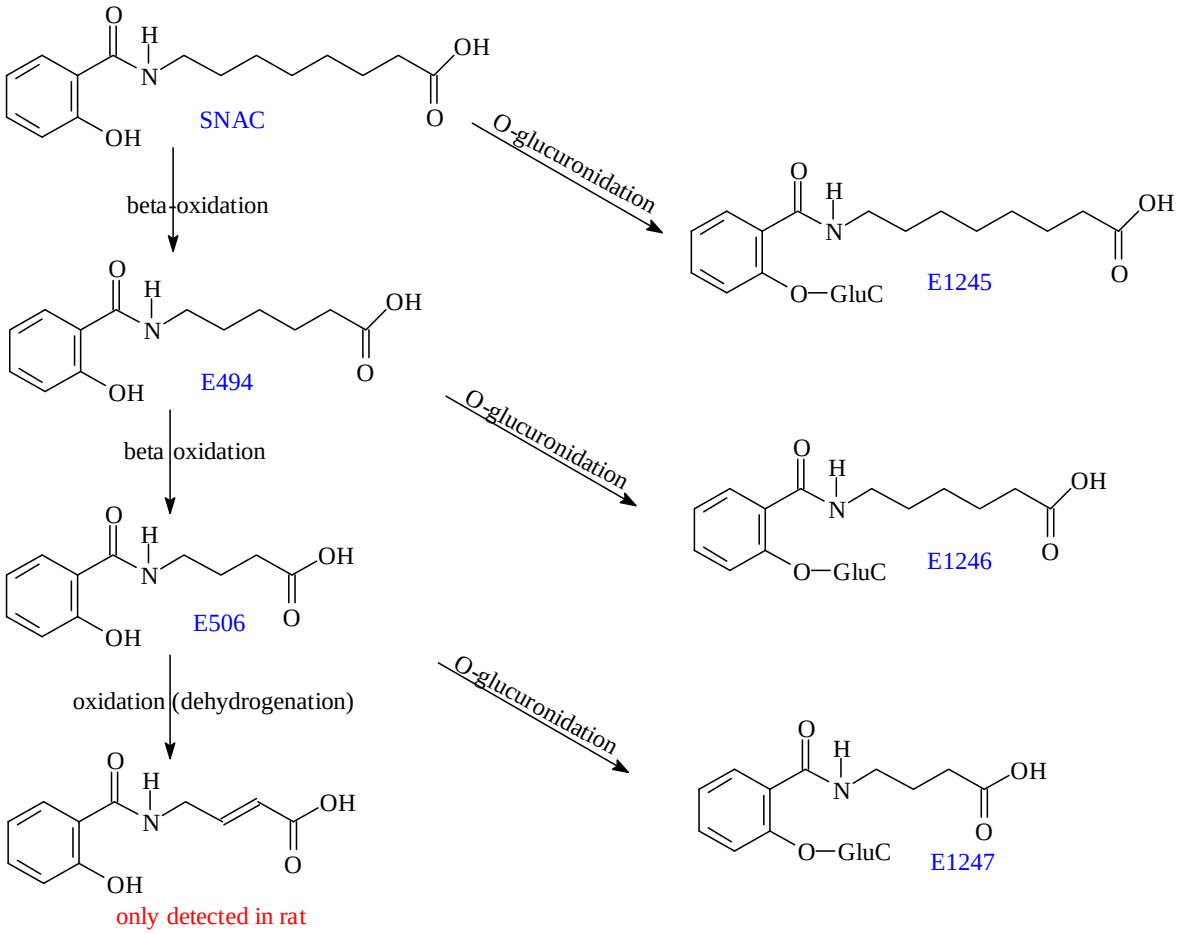


Figure 2. Proposed Metabolites of SNAC in Mice, Rats, and Monkeys

Study title: Pharmacokinetic profiling and metabolite identification of [^{14}C]-SNAC and [^{3}H]-SNAC derived radioactivity following oral administration in rats - feasibility study (M801, 209288)

Twenty-four rats received a single oral dose of 300 mg ^{14}C-SNAC (52 µCi/kg) or 300 mg ^{3}H-SNAC (63 µCi/kg). Plasma samples were collected at 0.167, 0.5, 0.75, 1, 2, 4, and 12 hours after dosing.

Results:

For the first 5 time points, there were approximately 10 detectible radiometabolites in the plasma of animals treated with ^{14}C-SNAC. The six most prevalent peaks were chosen for identification by LC-MS/MS. Metabolite 4 represented approximately 57% to 85% of the total radiometabolites found in the 0.167 to 2 hour samples. The concentration of radiometabolites decreased significantly from 2 to 4 hours post-dose. The radiometabolite profile and pattern were similar for animals treated with ^{3}H-SNAC, although Metabolites 3 and 5 were not detected. An additional metabolite, Metabolite 7,

was identified and eluted before Metabolite 4. LC-MS/MS data suggest that SNAC was metabolized by at least β-oxidation, cleavage at the amide bond, and sulfation. The PK results for each version of radiolabeled SNAC are summarized in sponsor-generated Table 5 below.

Table 5. Pharmacokinetic parameters of [^{14}C]SNAC or [^{3}H]SNAC in plasma of male rats after a single oral administration.

	[^{14}C]SNAC	[^{3}H]SNAC
Tmax (h)	0.75	0.50
Cmax (µg equiv/mL)	183.373	147.859
AUC $_{(0-last)}$ (µg equiv-h/mL)	514.733	1369.632

Study title: Salcaprozate sodium (SNAC): Investigation of metabolite profiles in plasma, urine, bile, and feces following oral administration to the rat (212270; 8265975)

Sprague-Dawley rats (9/sex) received a single oral administration of 500 mg SNAC (200 µCi/kg). Urine and feces were collected from intact animals and urine, feces, and bile were collected from bile-cannulated animals. Samples from 3 to 4 animals/sex were pooled (or 1 animal/sex/time point for plasma) and assessed for metabolites for the following post-dosing time periods:

Plasma:	0.5, 1, 1.5, 2, 4, 8, and 12 hours post-dose
Urine:	0-4, 4-8, 8-12, and 12-24 hours post-dose
Feces:	8-12 and 12-24 hours post-dose
Bile:	0-2, 2-4, 4-6, 6-12, and 12-24 hours post-dose
Cage wash:	0-4 hours post-dose

Metabolite profiles in urine, feces, and bile were assessed by using radio-HPLC followed by the characterization of notable metabolites by LC-MS/MS. Excreta and bile samples were also assessed for determination of excretion routes under (b) (4) Study Number 8264643 (212180; see Excretion section below).

Results:

^{14}C-SNAC was extensively metabolized, with the parent compound present as a minor component of the profile in plasma, urine, bile, and feces. In plasma the predominant metabolite was E506, which accounted for approximately 55% of the total drug related exposure (AUC) in male rats and 71% in female rats. All other detected metabolites each accounted for less than 10% of total drug related exposure.

The most abundant metabolites detected in urine were E506 and E1247 (glucuronidated E506). When the cage wash was taken into consideration, approximately 40% of the total administered radioactivity was excreted via the urine as E506, and 25% as E1247.

In bile, the glucuronide metabolites E1245, E1246, and E1247 were the most abundant metabolites, each accounting for approximately 4% to 14% of the total administered radioactive dose. Deconjugation experiments in bile indicated no acyl migration of the most abundant potential acyl glucuronide metabolite detected in bile.

In feces, the most abundant metabolite present in rat feces was E506, which typically accounted for 50% of the profile. In male and female rats, 7% and 2% of the total administered radioactivity was excreted via the feces as E506, respectively. The remaining detected metabolites each accounted for less than 1% of the administered dose.

There were no qualitative differences in metabolite profiles between male and female rats but there were some quantitative differences. In plasma, exposure to E506 was higher in females than in males. The proportions of the three identified glucuronide metabolites in bile showed some variation between sexes.

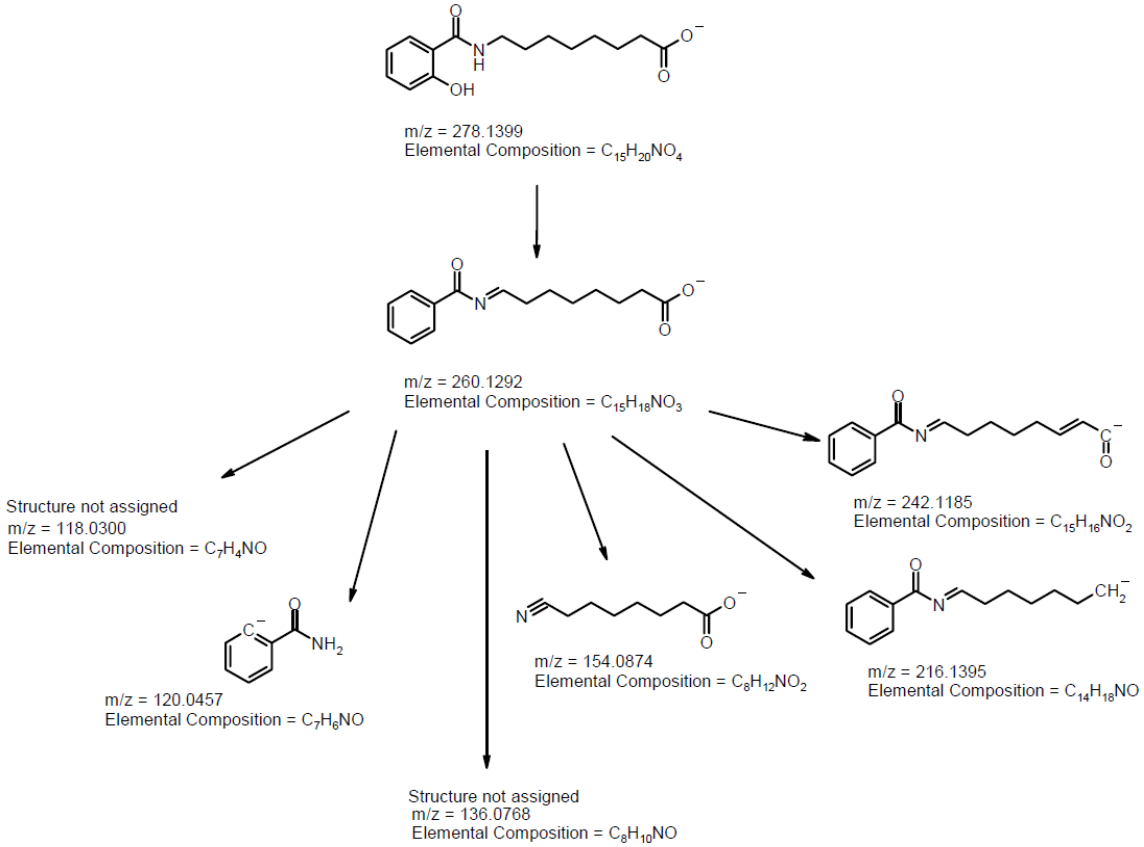
The metabolite summary and proposed fragmentation pattern for SNAC are presented in the sponsor-generated table and figure below.

Table 1 Metabolite Summary

Component	Matrix	Radio Peak	m/z	Proposed identity
R1	bile	RB1a	414.1042	E506 + O + glucuronide
R2	bile	RB1b	574.1415	E506 + 2 x glucuronide
E1247	plasma, urine and bile	E1247	398.1091	Consistent with E1247 reference standard
R3	plasma	RP1	302.0338	E506 sulphate
M4	urine and bile	RU1, RB2	396.0936	E506 -2H + glucuronide
E1246	plasma, urine and bile	E1246	426.1407	Consistent with E1246 reference standard
R5	plasma and urine	RP2, RU2a	238.0719	E506 + O
R6	plasma and urine	RP3a, RU2b	330.0649	E494 sulphate
R7	plasma* and urine	RP3b*, RU3	194.0457	SNAC – 6 (CH_2)
R8	urine and bile	RU4a, RB3	398.1093	E506 glucuronide (acyl)
R9	urine	RU4b	279.0985	E506 glycine conjugate
R10	bile	RB4	357.1123	E494 taurine conjugate
R11	faeces	RF4	266.1035	E494 + O
E1245	plasma, urine and bile	E1245	454.1717	Consistent with E1245 reference standard
E506	plasma, faeces, urine and bile	E506	222.0769	Consistent with E506 reference standard
R12	plasma and urine	RP4, RU5a	220.0611	E506 -2H
R13	plasma and urine	RP5, RU5b	426.1408	E494 glucuronide
R14	plasma, faeces and urine	RP6, RF5, RU6	220.0611	E506 -2H
R15	bile	RB5	385.1435	SNAC taurine conjugate
E494	plasma, faeces, urine* and bile*	E494	250.1084	Consistent with E494 reference standard
SNAC	plasma, faeces, urine* and bile*	SNAC	278.1399	Consistent with SNAC reference standard

* peak observed in LC-MS analysis but not present above limit of detection in the radio-HPLC chromatogram.

Proposed Fragmentation Pattern for SNAC



Study title: Pharmacokinetic profiling and metabolite identification of [^{14}C]-SNAC and [^{3}H]-SNAC derived radioactivity following oral administration in rhesus monkeys (M901, 209227)

This study was previously reviewed by Ke Zhang under IND (b) (4)*. Dr. Zhang's review is reproduced below.*

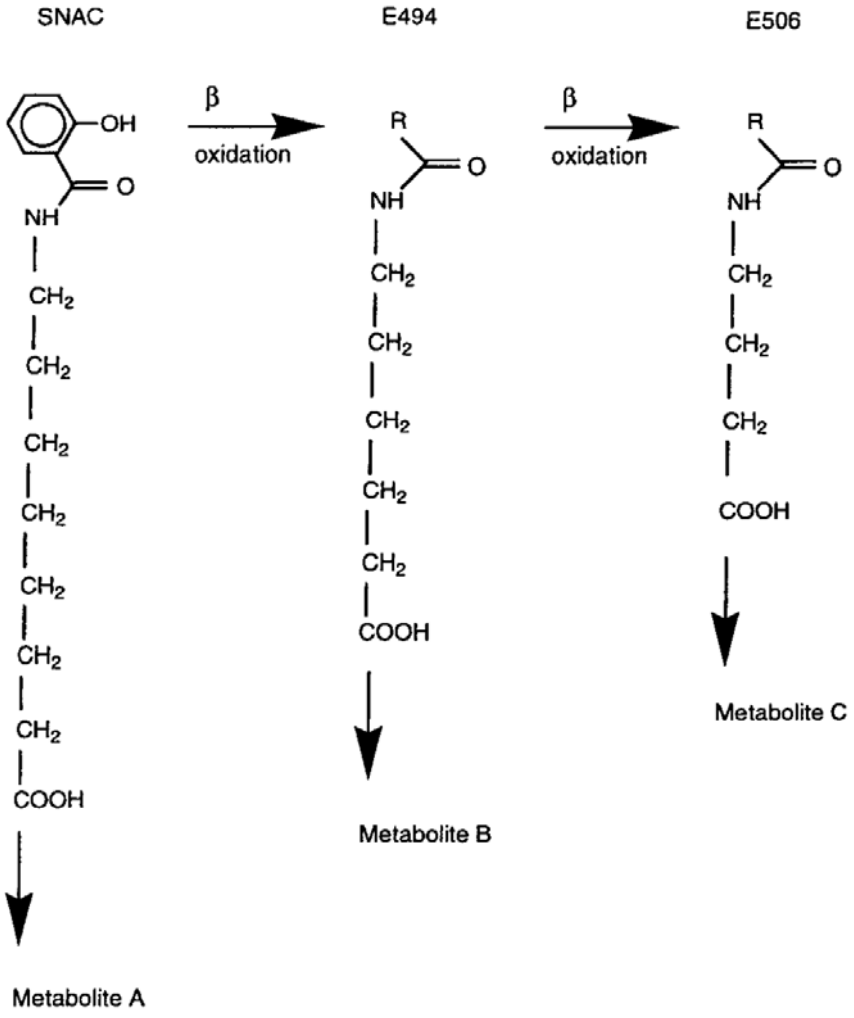
Methods: To study the pharmacokinetics and metabolism of SNAC in monkeys, SNAC was labeled with ^{14}C or ^{3}H. The ^{14}C-labeled SNAC was given to Rhesus monkeys (1/sex/group) by oral gavage at 300 mg/kg (~50 µCi/kg). After a washout period (~24 hours), the same monkeys were given ^{3}H-labeled SNAC at 300 mg/kg (~50 µCi/kg). Blood, urine, and feces samples were collected. The radioactivity was determined using liquid scintillation spectrometer. The metabolites were identified using HPLC with radiochemical detection and LC/MS/MS.

Results: The maximum plasma level of radioactivity (428 µg eqiv/mL in males or 286 µg eqiv/mL in females) was reached at 2 hours after oral dose of ^{14}C-SNAC. The radioactivity declined quickly with a terminal half-life of 0.9 to 1.7 hours. AUC values were 1377 µg equiv•h/mL in males and 1350 µg equiv•h/mL in females. Following an oral dose of ^{3}H-SNAC the maximum plasma level of radioactivity (389.7 µg equiv/mL for males and 282.1 µg equiv/mL for females) was reached at 2 hours after dosing. The plasma radioactivity declined with a terminal half-life of 8.3 to 11.3 hours. The sponsor

stated that the longer terminal half-life of 8.3 to 11.3 hours following the oral dose of ^{3}H-SNAC was unexpected. The sponsor did not provide a rationale for the use of two types of labeled drug (^{14}C and ^{3}H).

The metabolic profiles were similar in plasma and urine. Up to 5 metabolites were detected. Two of these had the same retention time as N-(hexanoic)-2-hydroxybenzamide (E506) and N-(butanoic)-2-hydroxybenzamide (E494). The results indicated that SNAC was metabolized by β-oxidation to metabolites E506 and E494. The parent compound (N-(octanoic)-2-hydroxybenzamide) and these two hydroxybenzamides were conjugated with glucuronic acid. This metabolic pathway is presented in sponsor-generated Figure 11 below.

Figure 11. Proposed Metabolism of SNAC in rhesus Monkeys



The human metabolic profile of SNAC was characterized in a clinical study in which 2.25 g ^{14}C-labeled SNAC was administered orally to 6 healthy volunteers (Study 259252). The primary metabolites are shown in Sponsor-generated Table 6. In

addition to the metabolites that were also identified in rats, one minor unique metabolite (a third beta-oxidation product obtained from three successive beta-oxidation steps) was found in plasma. This metabolite was only identified in 2 out of 6 subjects at a single time point (0.5 hours post dose).

Table 6. Plasma AUC ratio relative to SNAC for rat (Wistar) and human metabolites

	Metabolite					
	SNAC	E494	E506	E1245	E1246	E1247
Wistar rat (05-6064)	1	0.3	11	12	2	5
Human (b) (4) report no. 259252)	1	0.2	1	5	4	15

In human urine, 17 peaks were detected, 11 of which were new. A summary of metabolites detected in human urine are shown in the table below. Note that the dehydrogenated metabolite of E506 was detected in rat plasma suggesting that the same metabolite should be present in human plasma because the glucuronide of dehydrogenated E506 was observed in human urine.

Table 7. Human Urine Metabolites as % of Given Dose

SNAC	E1247	E1246	E1245	E506	E494	3rd beta oxidation product	Glucuronide of dehydrogenated E506	Other new metabolites
0.11%	42%	17%	9.5%	2.2%	0.31%	3.9%	3.1%	0.2% to 1.43%

In Vitro

Incubation of SNAC with liver S9 fractions showed no evidence of Phase I biotransformation, but Phase II glucuronidation reactions occurred. The rate of SNAC metabolism in liver fractions was Rhesus monkey ~Cynomolgus monkey > mouse > human > Sprague-Dawley rat > rabbit. The major human metabolite (E1247) was not detected in the rat S9 mix, but the second most abundant metabolite (E1245; only differing in the length of the acyl chain compared to E1247) was detected in mouse and monkey S9 liver preparations.

When incubated with primary hepatocytes, SNAC underwent beta-oxidation from Sprague-Dawley rat, monkey, and humans. Five metabolite peaks were observed in monkey and human hepatocyte incubations but only 2 peaks were found in rat hepatocytes; the extent of beta-oxidation differed by species. In human hepatocytes, E494 (single beta-oxidation) and E506 (double beta-oxidation) were highest in concentration following a 4-hour incubation, with E1245, E1246, and E1247 (the glucuronides of SNAC, E494, and E506, respectively) were found in lower amounts. The highest metabolic rate was observed in rat primary hepatocytes followed by the Rhesus monkey. SNAC metabolism was slowest in human primary hepatocytes.

Enzyme inhibition

The potential of SNAC to inhibit cytochrome P450 (CYP) enzymes was evaluated with the following CYP isozymes. CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5. SNAC was found to be a weak inhibitor of CYP2C19 and CYP3A4/5 with apparent K_i values of 200-450 µM and 1000 µM, respectively. Plasma concentrations of SNAC in humans are estimated to be approximately 3 µM based on a C_{max} of 980 ng/mL. Therefore, it is unlikely that the inhibition observed in this assay will translate into drug-drug interactions through hepatic CYP450 metabolism in vivo.

Excretion

Semaglutide:

Study title: [^{3}H]-Oct-NNC 0113-000-0217 with carrier compound NNC 0113-3363: A study of disposition following intravenous and oral administration to the male cynomolgus monkey (209115)

Male cynomolgus monkeys received a single dose of [3H]-Oct-NNC 0113-000-0217 by intravenous injection (0.01 mg/kg) or by oral gavage (15 mg/animal; tablet formulated with 450 mg SNAC). Semaglutide was radiolabeled in the octadecandioic acid moiety rather than on an amino acid. Radioactivity was measured in urine and feces for up to 192 hours. Plasma levels were also measured, the data for which are presented in the absorption section.

Cumulative recovery of radioactivity following a single intravenous administration of [^{3}H]-NNC 0113-0217 to the male cynomolgus monkey at a nominal dose level of 0.01 mg/kg (dose group A)

Sample	Collection interval (h)	Recovery (% administered dose)				
		101M	102M	103M	Mean	SD
Urine	0-24	(b) (4)			3.228	1.378
	24-48				5.585	1.188
	48-72				8.883	2.277
	72-96				11.96	3.186
	96-120				14.63	4.101
	120-144				16.76	4.298
	144-168				18.58	4.231
	168-192				20.10	4.155
Faeces	0-24				0.092	0.032
	24-48				1.125	0.294
	48-72				3.347	0.722
	72-96				5.543	0.337
	96-120				7.675	0.515
	120-144				9.523	0.990
	144-168				10.88	0.714
	168-192				12.46	0.973
Cage wash	0-24				0.290	0.166
	24-48				0.707	0.280
	48-72				0.974	0.397
	72-96				1.398	0.669
	96-120				1.852	1.046
	120-144				2.146	1.023
	144-168				2.540	1.269
	168-192 *				3.665	1.354
	Total	35.03	41.03	32.63	36.23	4.234

* includes final cage wash, cage debris and swabs

Cumulative recovery of radioactivity following a single oral administration of [^{3}H]-NNC 0113-0217 to the male cynomolgus monkey at a nominal dose level of 15 mg/animal (dose group B)

Sample	Collection interval (h)	Recovery (% administered dose)				
		201M	202M	203M	Mean	SD
Urine	0-24	[redacted]	[redacted]	[redacted] (b) (4)	1.926	0.971
	24-48				5.482	1.488
	48-72				7.308	1.847
	72-96				8.576	1.767
	96-120				10.54	2.714
	120-144				12.18	3.240
	144-168				13.84	3.763
	168-192				14.78	3.932
Faeces	0-24				6.906	11.92
	24-48				35.45	9.498
	48-72				46.03	8.649
	72-96				47.12	8.305
	96-120				47.37	8.257
	120-144				47.60	8.192
	144-168				47.84	8.124
	168-192				47.99	8.054
Cage wash	0-24				0.388	0.173
	24-48				0.652	0.183
	48-72				0.837	0.249
	72-96				0.978	0.322
	96-120				1.074	0.366
	120-144				1.244	0.477
	144-168				1.337	0.521
	168-192				1.793	0.603

* includes final cage wash, cage debris and swabs

SNAC

Study title: A study of the urinary and fecal excretion of radioactivity following a single oral administration of ^{14}C-labeled E414 to mice (768-95, 209237)

Methods: This study was conducted to determine the urinary and fecal excretion of radioactivity over a 5-day period after a single oral administration of 300 mg/kg [^{14}C]-E414 to male and female CD-1 mice. Urine and fecal samples were collected every 24 hours.

Results:

Results indicate that SNAC and its metabolites are primarily excreted in urine, with a minor fraction (~7%) in feces. The majority of radioactivity was excreted within the first 24 hours. No gender differences were observed. A summary of excretion data is shown in the sponsor-generated table below.

ORAL GAVAGE AT A DOSE OF 300 mg/kg OF E414 (including 8.9 mg of ^{14}C E414)

	ANIMAL NO.	HOURS 0-24	0-48	0-72	0-96	0-120	AT 120
MALES*							
	URINE	74.24	82.61	85.26	86.38	87.06	87.06
	FECES	6.41	6.85	7.08	7.20	7.26	7.26
	CARCASSES	-	-	-	-	-	0.06
	TOTAL	80.65	89.46	92.34	93.58	94.32	94.38
FEMALES**							
	URINE	79.61	83.77	85.22	86.14	86.74	86.74
	FECES	6.62	7.00	7.19	7.32	7.37	7.37
	CARCASSES	-	-	-	-	-	0.06
	TOTAL	86.23	90.77	92.41	93.46	94.11	94.17

* = The mean of 3 animals
** = The mean of 2 animals

Study title: Salcaprozate sodium (SNAC): A study in urine, feces, and bile following oral administration to the rat (212180; 8264643)

Sprague-Dawley rats (9/sex) received a single oral administration of 500 mg SNAC (200 µCi/kg). Urine and feces were collected from intact animals and urine, feces, and bile were collected from bile-cannulated animals during the following post-dosing time periods:

Bile: Pre-dose, 0-2, 2-4, 4-6, 6-12, 12-24, 24-48, 48-72 and 72-96 hours post-dose

Urine: Pre-dose, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72 and 72-96 hours post-dose

Faeces: Pre-dose, 0-4, 4-8, 8-12, 12-24, 24-48, 48-72 and 72-96 hours post-dose

Radioactivity in urine, feces, and bile was measured by using liquid scintillation counting. Excreta and bile samples were also transferred to (b) (4) Study Number 8265975 (212270) for metabolite profiling (see metabolism section above).

Results:

The data indicate that the principle route of elimination was via the renal system (> 85% in intact animals and >65% in bile duct cannulated animals) including significant levels in cage washing (assumed to be urinary in nature). In intact animals, approximately 12% of the administered dose was eliminated in the feces of the males and 5% from females. Following bile duct cannulation, 32% and 21% of the administered dose was recovered in bile from males and females, respectively, indicating that some degree of enterohepatic recirculation was occurring, with the material ultimately being eliminated via the renal system. Summaries of the excretion data are presented in sponsor-generated Tables 4, 5, 8, and 9 below.

Table 4 Cumulative recovery of radioactivity following a single oral administration of [^{14}C]-SNAC to intact male albino rats at a nominal dose level of 500 mg/kg Dose group A

Sample	Collection interval (hours)	Recovery (% administered radioactivity) 101M	102M	103M	Mean	SD
Urine	Pre-dose			(b) (4)	0.002	0.002
Urine	0-4				14.6	11.6
Urine	4-8				26.9	13.6
Urine	8-12				48.1	15.6
Urine	12-24				56.0	17.2
Urine	24-48				56.9	17.2
Urine	48-72				57.0	17.2
Urine	72-96				57.1	17.1
	Subtotal	45.9	48.6	76.8	57.1	17.1
Faeces	Pre-dose			(b) (4)	ND	NA
Faeces	0-4				NS	NA
Faeces	4-8				NS	NA
Faeces	8-12				5.57	5.02
Faeces	12-24				11.0	3.18
Faeces	24-48				12.0	3.15
Faeces	48-72				12.1	3.16
Faeces	72-96				12.1	3.16
	Subtotal	11.8	15.4	9.06	12.1	3.16
Cage Wash	Pre-dose			(b) (4)	ND	NA
Cage Wash	0-4				17.0	11.3
Cage Wash	4-8				21.5	12.0
Cage Wash	8-12				27.3	13.4
Cage Wash	12-24				28.9	13.9
Cage Wash	24-48				29.1	14.0
Cage Wash	48-72				29.2	14.0
Cage Wash*	72-96				29.5	14.2
	Subtotal	40.6	34.4	13.5	29.5	14.2
Cage Debris	0-96			(b) (4)	0.074	0.061
Carcass	96				0.349	0.088
	Total	98.9	98.7	99.7	99.2	0.717

ND: not detected
NA: not applicable, NS: no sample
*includes final cage wash

Table 5 Cumulative recovery of radioactivity following a single oral administration of [^{14}C]-SNAC to intact female albino rats at a nominal dose level of 500 mg/kg Dose group A

Sample	Collection interval (hours)	Recovery (% administered radioactivity) 104F	105F	106F	Mean	SD
Urine	Pre-dose			(b) (4)	ND	NA
Urine	0-4				34.8	10.5
Urine	4-8				55.5	9.08
Urine	8-12				63.1	9.63
Urine	12-24				66.9	8.31
Urine	24-48				68.2	7.73
Urine	48-72				68.7	7.57
Urine	72-96				68.9	7.46
	Subtotal	63.3	77.4	66.0	68.9	7.46
Faeces	Pre-dose			(b) (4)	ND	NA
Faeces	0-4				NS	NA
Faeces	4-8				NS	NA
Faeces	8-12				0.387	0.671
Faeces	12-24				3.94	0.300
Faeces	24-48				4.55	0.372
Faeces	48-72				4.67	0.325
Faeces	72-96				4.72	0.347
	Subtotal	5.02	4.34	4.80	4.72	0.347
Cage Wash	Pre-dose			(b) (4)	ND	NA
Cage Wash	0-4				12.2	5.21
Cage Wash	4-8				18.4	5.59
Cage Wash	8-12				21.4	6.08
Cage Wash	12-24				22.7	6.49
Cage Wash	24-48				23.2	6.45
Cage Wash	48-72				23.4	6.49
Cage Wash*	72-96				23.7	6.63
	Subtotal	28.3	16.1	26.7	23.7	6.63
Cage Debris	0-96			(b) (4)	0.089	0.070
Carcass	96				0.976	0.553
	Total	98.2	98.2	98.7	98.4	0.267

ND: not detected
NA: not applicable, NS: no sample
*includes final cage wash

Table 8 Cumulative recovery of radioactivity following a single oral administration of [^{14}C]-SNAC to bile duct cannulated male albino rats at a nominal dose level of 500 mg/kg-Dose group B

Sample	Collection interval (hours)	Recovery (% administered radioactivity) 201M	202M	204M	205M	Mean	SD
Urine	Pre-dose				(b) (4)	ND	NA
Urine	0-4					10.3	7.70
Urine	4-8					28.9	12.1
Urine	8-12					36.1	8.07
Urine	12-24					38.0	8.11
Urine	24-48					38.5	8.22
Urine	48-72					38.7	8.23
Urine	72-96					38.8	8.23
	Subtotal	28.9	36.5	41.5	48.4	38.8	8.23
Faeces	Pre-dose				(b) (4)	ND	NA
Faeces	0-4					0.004	0.008
Faeces	4-8					0.462	0.077
Faeces	8-12					1.43	0.415
Faeces	12-24					1.66	0.441
Faeces	24-48					1.83	0.634
Faeces	48-72					1.87	0.620
Faeces	72-96					1.92	0.591
	Subtotal	1.87	2.76	1.52	1.51	1.92	0.591
Cage Wash	Pre-dose				(b) (4)	0.002	NA
Cage Wash	0-4					15.7	5.59
Cage Wash	4-8					24.4	4.72
Cage Wash	8-12					26.4	5.48
Cage Wash	12-24					26.7	5.54
Cage Wash	24-48					26.8	5.53
Cage Wash	48-72					26.9	5.53
Cage Wash*	72-96					27.1	5.57
	Subtotal	32.6	30.2	25.7	19.9	27.1	5.57
Bile	Pre-dose				(b) (4)	NA	NA
Bile	0-2					8.80	1.82
Bile	2-4					18.5	5.06
Bile	4-6					26.1	4.67
Bile	6-12					31.3	5.69
Bile	12-24					32.0	5.24
Bile	24-48					32.0	5.23
Bile	48-72					32.1	5.23
Bile	72-96					32.1	5.22
	Subtotal	37.6	35.2	26.3	29.2	32.1	5.22
Cage Debris	96				(b) (4)	0.107	0.158
Carcass	96					0.502	0.159
	Total	101	106	95.9	99.4	101	4.02

ND: not detected, NA: not applicable, NS: no sample, *: includes final cage wash

Table 9 Cumulative recovery of radioactivity following a single oral administration of [^{14}C]-SNAC to bile duct cannulated female albino rats at a nominal dose level of 500 mg/kg-Dose group B

Sample	Collection interval (hours)	Recovery (% administered radioactivity) 207F	208F	210F	211F	Mean	SD
Urine	Pre-dose				(b) (4)	ND	NA
Urine	0-4					15.0	7.49
Urine	4-8					35.2	5.91
Urine	8-12					45.4	6.49
Urine	12-24					49.4	4.43
Urine	24-48					50.0	4.43
Urine	48-72					50.3	4.43
Urine	72-96					50.5	4.44
	Subtotal	50.8	54.0	53.0	44.1	50.5	4.44
Faeces	Pre-dose				(b) (4)	ND	NA
Faeces	0-4					0.003	0.006
Faeces	4-8					0.203	0.248
Faeces	8-12					0.499	0.379
Faeces	12-24					0.893	0.364
Faeces	24-48					1.15	0.240
Faeces	48-72					1.18	0.238
Faeces	72-96					1.22	0.249
	Subtotal	1.19	1.51	1.26	0.906	1.22	0.249
Cage Wash	Pre-dose				(b) (4)	ND	NA
Cage Wash	0-4					12.7	7.50
Cage Wash	4-8					20.3	9.50
Cage Wash	8-12					23.6	8.92
Cage Wash	12-24					24.4	8.99
Cage Wash	24-48					24.6	9.02
Cage Wash	48-72					24.7	9.04
Cage Wash*	72-96					24.9	9.01
	Subtotal	30.6	12.7	32.8	23.4	24.9	9.01
Bile	Pre-dose				(b) (4)	ND	NA
Bile	0-2					3.99	2.72
Bile	2-4					7.52	6.63
Bile	4-6					12.0	8.79
Bile	6-12					19.9	10.0
Bile	12-24					21.2	9.40
Bile	24-48					21.3	9.39
Bile	48-72					21.3	9.39
Bile	72-96					21.3	9.40
	Subtotal	15.4	29.6	11.2	29.0	21.3	9.40
Cage Debris	96				(b) (4)	0.098	0.077
Carcass	96					0.735	0.265
	Total	99.0	99.1	98.7	98.1	98.6	0.504

ND: not detected, NA: not applicable, NS: no sample, *: includes final cage wash

Human Excretion
Clinical trial data indicate that SNAC is also primarily eliminated by urinary excretion in humans.

Other Pharmacokinetic studies (drug-drug interactions)
Study title: Evaluation of absorption enhancer (SNAC) on absorption of metformin (flmn100105)
A study in male beagle dogs indicated that the absorption of 500 mg metformin (determined by AUC and C_{max} values) was not altered when co-formulated with 300 mg SNAC. It was not clear whether the co-formulation was administered as a solid tablet/ capsule or as a solution. The study results indicate that the co-administration of oral semaglutide with metformin should not affect the bioavailability of metformin.

5.2 Toxicokinetics

Mice
From 13-week toxicity study with SNAC, Study 209247 (sponsor-generated table)

Table 2 Toxicokinetic parameters of RO5045192 in plasma after oral (gavage) administration of RO5045192 to mice (from composite data)

group	dose [mg/kg]	day	gender	tmax [h]	Cmax [ng/mL]	AUC(0-24h) [h·ng/mL]	Cmax/dose [ng/mL]/[mg/kg]	AUC(0-24h)/dose [h·ng/mL]/[mg/kg]
G2	150	1	m	1	462	1630	3.08	10.9
G2	150	1	f	1	431	1320	2.87	8.78
G2	150	41	m	1	719	1820	4.80	12.1
G2	150	41	f	1	836	1710	5.57	11.4
G2	150	90	m	1	418	1810	2.79	12.1
G2	150	90	f	1	1040	2250	6.95	15.0
G3	500	1	m	1	2050	5110	4.11	10.2
G3	500	1	f	1	2310	11600	4.62	23.2
G3	500	41	m	1	3140	8450	6.28	16.9
G3	500	41	f	1	1710	4770	3.41	9.54
G3	500	90	m	1	2720	7240	5.43	14.5
G3	500	90	f	3	1720	8980	3.45	18.0
G4	1500	1	m	3	2770	14500	1.85	9.65
G4	1500	1	f	1	4340	20800	2.90	13.9

study no.: A16705
(b) (4)
raw data ID: (b) (4)
raw data ID:

Rats

TK results for Semaglutide (sponsor-generated table), Study 208301 (2-week study)

Day	Group	Dose (mg(kg)	Sex	C_{max} (nM)	t_{max} (h)	$AUC_{(0-6h)}$ (h*nM)	$AUC_{(0-24h)}$ (h*nM)	AUC (h*nM)
1	2	6.67	F	NR	NR	NR	NR	NR
			M	2.14	0.5	NC	NA	NR
	3	33.3	F	509	2	2090	4060	NR
			M	169	0.5	727	1470	1620
	4	66.7	F	1340	6	5940	14400	NR
			M	1640	2	6910	11900	12500
14	2	6.67	F	NR	NR	NR	NR	NR
			M	0.563	0.5	NR	NA	NR
	3	33.3	F	37.7	0.5	133	256	275
			M	7.68	0.5	17.7	NA	NR
	4	66.7	F	67.8	2	242	484	NR
			M	61.9	2	288	597	651

F – Female, M – Male, NC – not calculated, NA – not applicable, NR – not reported

TK results for Semaglutide (sponsor-generated table), Study 208301 (2-week study)

Day	Group	Dose (mg/kg)		Sex	C_{max} (ng/mL)	t_{max} (h)	$AUC_{(0-6h)}$ (h*ng/mL)	$AUC_{(0-24h)}$ (h*ng/mL)	AUC (h*ng/mL)
		NNC 0113-0217	NNC 0113-3363						
1	1	0	1000	F	44400	0.50	49200	NA	51200
				M	62500	0.50	66200	78100	78400
	2	6.67	100	F	819	0.50	1420	NA	NR
				M	707	0.50	1720	NA	NR
	3	33.3	500	F	1570	0.50	5650	NA	NC
				M	188[b]	0.50	231[b]	NA	NC
	4	66.7	1000	F	4720	2.0	12700	NA	NC
				M	3900	0.50	6110	NA	NR
14	1	0	1000	F	42900	0.50	55700	71200	72300
				M	14800	2.0	46800	NA	NC
	2	6.67	100	F	836	0.50	2820	NA	NR
				M	1210	0.50	3680	5060	NR
	3	33.3	500	F	3380	6.0	8170	26000	NC
				M	3110	2.0	14400	NA	NC
	4	66.7	1000	F	1090	2.0	5560	14700	17300
				M	3090	6.0	13000	30800	NC

TK results for Semaglutide (sponsor-generated table), Study 208300 (6-week study)

Day	Dose (mg/kg)	Sex	C_{max} (nM)	t_{max} (h)	AUC_{0-24h} (h*nM)	AUC_{last} (h*nM)	AUC (h*nM)	$AUC_{\%extra}$ (%)	Rac_{Obs}
1	0	Female	NC	NC	NC	NC	NC	NC	-
		Male	NC	NC	NC	NC	NC	NC	-
	6.67	Female	1.59	6.0	24.2	24.2	NR	NR	-
		Male	NC	NC	NC	NC	NC	NC	-
	33.36	Female	286	6.0	2200	2200	2280	3.3	-
		Male	141	6.0	1350	1350	1390	3.3	-
	66.67	Female	2130	2.0	20400	20400	NC	NC	-
		Male	705	2.0	6260	6260	6710	6.8	-
42	0	Female	NC	NC	NC	NC	NC	NC	NC
		Male	NC	NC	NC	NC	NC	NC	NC
	6.67	Female	10.9	6.0	41.6	41.6	NC	NC	1.72
		Male	NC	NC	NC	NC	NC	NC	NC
	33.36	Female	109	10	1050	1590	NR	NR	0.476
		Male	87.5	6.0	732	913	NR	NR	0.543
	66.67	Female	545	6.0	4720	5570	NR	NR	0.231
		Male	230	6.0	2930	4660	4670	0.20	0.468

TK results for SNAC (sponsor-generated table), Study 208300 (6-week study)

Day	Group	Sex	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (h*ng/mL)	AUC_{last} (h*ng/mL)	AUC (h*ng/mL)	$AUC_{\%extra}$ (%)	Rac_{Obs}
1	1 1000 mg/kg	Female	2450	6.0	22000	22000	22700	3.1	-
		Male	3690	6.0	32700	32700	33000	1.0	-
	2 100 mg/kg	Female	153	2.0	1820	1820	NC	NC	-
		Male	220	2.0	1120	1120	1330	16	-
	3 500 mg/kg	Female	818	24	6770	6770	NC	NC	-
		Male	1050	24	10400	10400	NC	NC	-
	4 1000 mg/kg	Female	1980	2.0	10400	10400	NR	NR	-
		Male	784	2.0	4350	4350	NR	NR	-
42	1 1000 mg/kg	Female	2520	2.0	23500	27000	NR	NR	1.07
		Male	2450	2.0	20100	25300	NR	NR	0.614
	2 100 mg/kg	Female	244	2.0	2430	2430	2580	6.0	1.33
		Male	569	6.0	3470	3470	NR	NR	3.09
	3 500 mg/kg	Female	2100	6.0	17600	18500	NR	NR	2.59
		Male	1600	10	16900	17700	NR	NR	1.63
	4 1000 mg/kg	Female	2850	10	39900	42100	NC	NC	3.82
		Male	4460	6.0	44600	46400	47200	1.6	10.3

Monkeys

Study 208302 (4-day dose range finding escalation, bioavailability, and 14-day range finder)

TK Data for Semaglutide - Phases 1 and 3

Dose (mg)	Day	Sex	C_{max} (nM)	AUC_{last} (nM•h)	T_{max} (h)
Phase 1					
10.69	1	Male[b]	NC	NC	NC
		Female	0.891	12.0	6.0
	4	Male	18.7	590	8.0
		Female	68.6	2090	6.0
15.63	8	Male	15.1	242	4.0
		Female	12.4	224	2.0
	11	Male	13.8	475	8.0
		Female	13.8	561	4.0
31.26	15	Male	10.9	192	4.0
		Female	139	2020	4.0
	18	Male	59.3	2550	14.0
		Female	204	9130	6.0
Phase 3					
31.26	1	Male	184	3040	4.0
		Female	104	1650	5.0
	14	Male	172	2960	4.0
		Female	229	4500	4.0

[a]Phase 1 - Day 1, 8, 15 AUC_{last}: 0-24 hours, Day 4, 11, 18 AUC_{last}: 0-72 hours. Phase 3 - Day 1 and 14: AUC_{tau}
[b]Animals did not receive their full dose due to dosing procedure problems.
NC = not calculated.

TK Data for Semaglutide (Phase 2)

Administration Route	Dose	Sex	C_{max} (nM)	AUC_{last} (nM•h)	t_{max} (h)	$t_{1/2}$ (h)	F_{rel} (%)
Subcutaneous	0.01 (mg/kg)	Male 1	(b) (4)				
		Male 2					
		Mean	10.6	1060	24	55	NA
		Female 1	(b) (4)				
		Female 2					
		Mean	13.9	1350	24	58	NA
Oral	31.26 (mg)	Male 1	(b) (4)				
		Male 2					
		Mean	320	16600	4.0	44	1.96*
		Female 1	(b) (4)				
		Female 2					
		Mean	397	21700	4.0	NC	1.67*

F_{rel} = relative oral bioavailability; NA = not applicable; NC = not calculated.
*Reviewer's calculation.

TK Data for SNAC - Phases 1 and 3

Dose (mg)	Day	Sex	C_{max} (nM)	AUC_{last} (nM•h)	T_{max} (h)
Phase 1					
160.31	1	Male[b]	216	433	4.0
		Female	603	2760	4.0
	4	Male	537	4110	4.0
		Female	1290	7870	4.0
234.37	8	Male	1050	7400	6.0
		Female	1730	8330	6.0
	11	Male	2560	11400	4.0
		Female	1620	9720	6.0
468.74	15	Male	1880	12500	6.0
		Female	3680	30100	4.0
	18	Male	7530	37100	4.0
		Female	5480	31900	4.0
Phase 3					
468.74	1	Male	1860	15000	5.0
		Female	2620	13500	4.0
	14	Male	6560	35000	6.0
		Female	3230	25800	5.0

[a]Phase 1 - Day 1, 8, 15 AUC_{last}: 0-24 hours, Day 4, 11, 18 AUC_{last}: 0-72 hours. Phase 3 - Day 1 and 14: AUC_{tau}
[b]Animals did not receive their full dose due to dosing procedure problems.
NC = not calculated.

TK Data for SNAC (Phase 2)
(sponsor-generated table; note that SNAC was not administered by SC injection, so oral bioavailability was not calculated)

Sex	Dose (mg)	Subject	C_{max} (ng/mL)	t_{max} (h)	AUC_{last} (h*ng/mL)	AUC (h*ng/mL)	$AUC_{\%extra}$ (%)	$t_{½}$ (h)
Female	468.74	706F	(b) (4)					
		708F						
		Mean	2870	4.0	17800	NC	NC	NC
		SD	1850	0.0	12200	9780	NC	NC
Male	468.74	705M	(b) (4)					
		707M						
		Mean	1840	6.0	19000	NC	NC	NC
		SD	161	2.8	900	NC	NC	NC

NC: Not Calculated

Study 209153 (6-week toxicity study)

TK Results for Semaglutide (sponsor-generated table)

Group	Dose (mg/kg)		Day	C_{max} (nM)			$AUC_{(0-24h)}$ (h*nM)		
	NNC 0113-0217	NNC 0113-3363		F	M	F/M	F	M	F/M
3	5	78.35	1	33.4	57.1	**0.58**	607	979	**0.62**
			42	25.4	64.5	**0.39**	445	1178	**0.38**
4	10	156.7	1	267	102	**2.62**	3580	1541	**2.32**
			42	281	160	**1.76**	5154	2873	**1.79**
					Mean	**1.34**		Mean	**1.28**
F- Females; M – Males.					SD	1.05		SD	0.93
					95% CI	**-0.33-3.01**		95% CI	**-0.2-2.76**

TK Results for SNAC (sponsor-generated table)

Group	Dose (mg/kg)		Day	C_{max} (ng/mL)			$AUC_{(0-24h)}$ (h*ng/mL)		
	NNC 0113-0217	NNC 0113-3363		F	M	F/M	F	M	F/M
3	5	78.35	1	2140.2	2681.8	**0.798**	8732	13296	**0.657**
			42	3419.3	1219.5	**2.80**	13286	10841	**1.23**
4	10	156.7	1	3654	3098	**1.18**	31148	23472	**1.33**
			42	3672	3144	**1.17**	36382	20267	**1.80**
					Mean	**1.49**		Mean	**1.25**
F- Females; M – Males.					SD	0.893		SD	0.469
					95% CI	**0.07-2.91**		95% CI	**0.50-2.00**

NNC0113-0217 = semaglutide; NNC0113-3363 = SNAC

6 General Toxicology

6.1 Single-Dose Toxicity

Single-Dose Toxicity (portions reproduced from sponsor summary)

Study Type Study No.	Species/strain Number/group Route	Dose Levels (mg/kg)	Maximum Non-Lethal Dose / Approximate Lethal Dose	Findings
Single dose with 14-day observation period EMIS/R96007 (209239)	Mouse/ICR 5/sex Oral gavage	0* 1000 1500 2000	1500 mg/kg 2000 mg/kg	• At 2000 mg/kg, 3 female mice died 1 hour post dosing and 1 male died within 4 hours post dosing. Three of 4 animals that died were noted as lethargic prior to death. • At 1500 mg/kg, 1 male appeared sedate at 1.5 hours post dosing but had recovered within the 4 hour observation period. • Significantly increased BW compared to control animals was observed for males receiving 1500 & 2000 mg/kg. • The NOAEL = 1500 mg/kg.
Single dose with 14-day observation period EMIS/R96009 (209238)	Rat/Sprague-Dawley 5/sex Oral gavage	0* 1000 1500 2000 3000	1000 mg/kg 1500 mg/kg	• At 3000 mg/kg, all the animals appeared lethargic immediately post dose and died within 30 minutes thereafter. • At 2000 mg/kg, 8/10 animals died 1 to 4 hours post-dose. • At 1500 mg/kg, 3/10 animals (1 M and 2 F) died 2 to 4 hours post dose. • At all dose levels except 1000 mg/kg treatment related clinical signs were weak reflexes, salivation, lethargy, twitching, ataxia, sedation, and hunched posture. • The BWs for animals administered 1000 & 1500 mg/kg were increased compared to the control group. • Necropsy findings at 1000 mg/kg were: enlarged atria (1M), enlarged mesenteric lymph nodes (2F), irritated small intestine (3F), rough texture on spleen (2F). At 1500 mg/kg: enlarged heart (1M), rough texture on spleen (1M, 1F), enlarged mesenteric lymph nodes (1M). At 2000 mg/kg: irritated stomachs (1M, 4F) and irritated intestines (3F), white patches on kidneys (1F), enlarged right atria (2M, 3F), and enlarged mesenteric lymph nodes (1F).

Study Type Study No.	Species/strain Number/group Route	Dose Levels (mg/kg)	Endpoints	Findings
Single dose with time points at 0.33, 1, and 24 h after dosing JLY0412 (212392)	Rat/Sprague-Dawley 33 F/group (11 F/time point) Oral gavage	0* 900 1500 Positive control: 800 sodium salicylate	-Neurobehavior (Irwin-like test) -Gastric pH and electrolytes -Plasma insulin, blood gases, glucose, and electrolytes -CSF glucose -Gross path. -Histopathology (limited tissues)	• 2 deaths occurred in the LD group and 8 deaths occurred in the HD group. All mortalities/moribund sacrifices occurred between 15 and 39 min postdose. • Tonic or aphyxial convulsions preceded most deaths. Other effects included abnormal gait, abnormal body carriage, hunched posture, shuffling, abnormal respiration, and increased pupil diameter. Additionally, at 1500 mg/kg, apathy, passivity, reduced alertness, startle response, or body tone were observed. • At 20 min. postdose, SNAC caused statistically significant decreases in both blood and CSF glucose. These effects were most pronounced in the decedent animals. • Statistically significant increases in blood levels of pCO_2, pO_2, oxygen saturation, blood pH, bicarbonate, and base excess between 20 and 60 min. Increased blood sodium and decreased blood potassium and calcium were also seen. • Treatment caused increased stomach content weight, pH, and volume. An increase in gastric damage scores at 1 hour postdose was also noted. • Red or dark areas were observed in the stomach corpus region that correlated with microscopic findings of peracute minimal to moderate mucosal necrosis in the glandular stomach and duodenum. 2 LD animals showed acute necrosis and inflammation of marked severity in the cecum/colon at 24 hours. • A NOAEL was not identified.

*Vehicle: 25% v/v aqueous propylene glycol; BW = body weight; CSF = cerebral spinal fluid; F = female; HD = high dose; LD = low dose; M = male; NOAEL = no observed adverse effect level.

6.2 Repeat-Dose Toxicity

Repeat-Dose Range-Finding Studies – SNAC alone or SNAC plus Semaglutide

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels		Endpoints	Findings
		SNAC (mg/kg/d)	Semaglutide (mg/kg/d)		
Oral 13 week A16705 (209247) GLP	Mouse/CD-1 Main: 10/sex TK: 12/sex	Group 1: 0 Group 2: 150 Group 3: 500 Group 4: 1500* *Dosing holiday on Days 3 and 4, and dose reduced to 1000 on Day 5. Group sacrificed on Day 9 due to excessive mortality.	Not applicable	Mortality Clinical signs Body weight Food cons. Ophthalmology Hematology Clinical chem. Urinalysis Toxicokinetics Organ weights Gross path. Histopathology	• 10/22 M and 13/22 F from the HD groups died during the study. • Group 4 M exhibited ruffled fur. • Group 4 F had lower mean BWs during Weeks 1 and 2. • Group 4 animals showed slightly to markedly reduced erythrocyte parameters with slightly increased relative reticulocyte counts. • Group 4 animals (at Week 2) showed minimally to slightly higher mean glucose and triglycerides and minimally lower total bilirubin, total protein, and globulins. • Group 4 M showed minimally decreased incidence of hepatic glycogen (centrilobular and diffuse). • See Section 5.2 for TK results • The NOAEL = 500 mg/kg/d.
Oral 2-week RF (208301) Non-GLP	Rat/Sprague-Dawley Main: 6/sex TK: samples taken from main study animals (2/sex/time point)	Group 1: 1000 Group 2: 100 Group 3: 500 Group 4: 1000	Group 1: 0 Group 2: 6.67 Group 3: 33.36 Group 4: 66.67	Mortality Clinical signs Body weight Food cons. Hematology Clinical chem. Organ weights (liver & kidney) Gross path. Histopathology (GI tract, liver, kidneys, testes) Toxicokinetics	• 0, 1, 1, and 2 unscheduled deaths occurred in Groups 1-4, respectively. No deaths were considered to be treatment related. • There were no treatment-related clinical signs. • Initial BW loss followed by decreased BW gain for Groups 3 & 4, which correlated with decreased FC. • A slight decrease in erythrocyte parameters was observed in female Groups 2-4 and male Groups 3-4. • Triglyceride levels were decreased in

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels Semaglutide (mg/kg/d)	Endpoints	Findings
					all semaglutide groups. • Mean relative liver weights for Group 4 males were 13% lower than Group 1 controls. • Minimal decreased hepatocellular rarefaction was seen for males and females from Groups 3&4. • See Section 5.2 for TK results
Oral 14-day study with SNAC from 2 different manufacture processes (B & C) (b) (4)-315004 (209241) GLP	Rat/Sprague-Dawley Main: 10/sex TK: None	Group 1: 0 Group 2: 500 (B) Group 3: 750 (B) Group 4: 1000 (B) Group 5: 500 (C) Group 6: 750 (C) Group 7: 1000 (C)	Not applicable	Mortality Clinical signs Body weight Food cons. Ophthalmology Hematology Clinical chem. Urinalysis Organ weights Gross path. Histopathology	• There were no unscheduled deaths, treatment-related clinical signs, or effects on BW or FC. • Minimal to mild infiltration of the submucosa of the glandular stomach was noted at low incidence for F in Groups 4, 6, & 7 and 1 M each from Groups 2 &4. • No toxicologically meaningful effects occurred at levels up to 1000 mg/kg SNAC regardless of the manufacturing process.
Oral 28-day immuno-toxicity 523561 (212329) GLP	Rat/Sprague-Dawley Main: 10/sex	Group 1: 0 Group 2: 75 Group 3: 200 Group 4: 500	Not applicable	Mortality Clinical signs Body weight Food cons. Water cons. Hematology Clinical chem. Immunopheno-typing KLH assay Organ weights Gross path. Histopathology (immune-related only)	• 1HD F showed hunched posture and piloerection immediately after dosing on day 2. Occasionally, excessive salivation and plowing through the cage shavings were observed at the HD. • No SNAC-related effects on total T and B lymphocyte cell numbers or T helper and cytotoxic T cell numbers. • SNAC had no effect on the animals' ability to raise IgM or IgG antibodies against KLH. • Bone marrow assessment showed no effects on M:E ratios, maturation sequence and morphology of the myeloid, erythroid, and

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels Semaglutide (mg/kg/d)	Endpoints	Findings
					megakaryocytic cell lines. • No adverse findings were observed in immune related tissues. • No signs of immunotoxicity were observed at doses up to 500 mg/kg/d.
Oral 6-week with 2-week recovery 208300 GLP	Rat/Sprague-Dawley Main: 10/sex Recovery: 5/sex TK: samples taken from main study animals (2/sex/time point)	Group 1: 1000 Group 2: 100 Group 3: 500 Group 4: 1000	Group 1: 0 Group 2: 6.67 Group 3: 33.36 Group 4: 66.67	Mortality Clinical signs Body weight Food cons. Ophthalmology Hematology Clinical chem. Organ weights Gross path. Histopathology Toxicokinetics	• There were no test article-related deaths. • Decreased BW gain (↓23%-54%) was seen for Groups 3&4, which correlated with decreased FC. A corrective increase in BW gain occurred during recovery for Group 3&4 M and Group 4 F. • Subdued behavior, piloerection, and hunched posture were seen in all HD animals on Days 1&2. • Decreased triglycerides were noted for Groups 2-4, especially for males. • Increased urine volume and pH was seen for Group 2-4 males. • An increased incidence in minimal Brunner's gland hypertrophy was observed for all semaglutide groups, with ≥78% of animals affected for Groups 3&4. Note that this finding is also observed when semaglutide is administered via SC injection. • No ADAs against semaglutide were detected after 6 weeks of treatment. • See Section 5.2 for TK results. • The NOAEL was considered to be the high dose level (Group 4).
Oral 14-day MTD JLY0186	Monkey/ Cynomolgus Phase 1: 2/sex	Phase 1: Days 1-4: 39.8 - 57.9 Days 8-11: 60.4 - 90.5 Days 15-18: 121.8 -	Phase 1: Days 1-4: 2.7 - 3.9 Days 8-11: 4 - 6 Days 15-18: 8.1 - 12.3	Phases 1, 2, 3 Mortality Clinical signs Body weight	Phase 1 • There were no unscheduled deaths or treatment-related clinical signs.

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels Semaglutide (mg/kg/d)	Endpoints	Findings
(208302) Non-GLP	Phase 2: 2/sex 4-week washout Phase 3: 4/sex	184.5 <u>Phase 2</u>: Day 1: NA Day 15: 99.9 - 178.2 (oral) <u>Phase 3 (14 days)</u>: 102 to 194	 <u>Phase 2</u>: Day 1: 0.01(SC) Day 15: 6.7 - 11.9 (oral) <u>Phase 3 (14 days)</u>: 6.8 to 13.0	Food cons. Toxicokinetics <u>Phase 3 only</u> Hematology Clinical chem. Organ weights Gross path.	• Small BW losses were observed during the dosing periods. • BW effects correlated with a slight decrease in FC. <u>Phase 2</u> • There were no unscheduled deaths or treatment-related clinical signs. • BW loss was observed for 3/4 animals up to Day 4 after SC administration and for 2/4 animals after oral administration. • BW effects correlated with a slight decrease in FC. <u>Phase 3</u> • There were no unscheduled deaths; clinical signs included hunched posture on Day 2 in two females, emesis, and salivation. • 7% to 13% BW loss occurred between Day 1 and 15, with the biggest effect occurring during the first 3 to 4 days. • BW effects correlated with a slight decrease in FC. • There was a decrease in erythrocyte parameter values compared with pre-treatment values. • There was a small reduction in plasma urea (M&F) and a slight increase in creatinine levels and reductions in phosphorous and triglyceride levels. • 2/4 males and 2/4 females had absolute thymus weights that were lower than the background range, which correlated with the observation

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels Semaglutide (mg/kg/d)	Endpoints	Findings
					of small thymus size at necropsy. • See Section 5.2 for TK results
Oral capsule for 6 weeks with 2-week recovery JLY0223 (209153) GLP	Monkey/ Cynomolgus Main: 4/sex Recovery: 2/sex (Groups 2-4)	Group 1: 0 Group 2: 156.7 Group 3: 78.35 Group 4: 156.7 (1 week lead in at 78.35 mg/kg/day for Groups 2, 3, &4)	Group 1: 0 Group 2: 0 Group 3: 5 Group 4: 10 (1 week lead in at 5 mg/kg/day for Groups 3 &4)	Mortality Clinical signs Neurobehavior Body weight Ophthalmology ECG/BP Hematology Clinical chem. Organ weights Gross path. Histopathology Toxicokinetics ADA	• There were no unscheduled deaths, adverse clinical signs, or effects on neurobehavior. • BW gain was decreased for groups receiving semaglutide; BW rebounded during recovery. • A slight increase in heart rate was noted for Group 4 males. • At Day 2, a decrease in reticulocytes was noted for Group 3 and 4, but was not noted at Week 6. • Increased plasma urea was observed for Group 4 females at Week 6. • A higher incidence of small thymus size was seen for Group 3 and 4. • A slight increase in the incidence of Brunner's gland dilatation/ eosinophilic cytoplasm was noted for Group 3 males and Group 4 animals. An increase in the incidence of thymic involution/atrophy was observed for Group 4 males and Group 2, 3, and 4 females; similar findings were observed after the 2-week recovery period (although there were no control recovery animals). • See ADME section for TK data. • No ADAs were detected. • The NOAEL was considered to be 10 mg/kg/d semaglutide with 156.7 mg/kg/d SNAC.

ADA = anti-drug antibody; BP = blood pressure; BW = body weight; ECG = electrocardiogram; F = female; FC = food consumption; GLP = Good Laboratory Practices; M = male; MTD = maximum tolerated dose; NOAEL = no observed adverse effect level; SC = subcutaneous; TK = toxicokinetics.

Repeat-Dose Range-Finding Studies – SNAC alone or SNAC plus [redacted]

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels: SNAC (mg/kg/d)	Dose Levels: [redacted]	Endpoints	Findings
Oral 14-day [redacted]315002 EMISTOX 97002 (211245)	Rat/Sprague-Dawley Main: 5/sex TK: None	Group 1: 0 Group 2: 1000 Group 3: 1667 Group 4: 2334 Group5: 3000 Group 6: 1000 Group 7: 1667 Group 8: 2334 Group 9: 3000	Group 1: 0 Group 2: 0 Group 3: 0 Group 4: 0 Group 5: 0 Group 6: [redacted] Group 7: Group 8: Group 9:	Mortality Clinical signs Body weight Food cons. Hematology Clinical chem. Organ weights (brain, kidney, liver) Gross pathology	• Treatment-related deaths were noted for two F each from Groups 4, 5, 8, & 9. Some of these animals exhibited impaired equilibrium and hypoactivity prior to death. • All treated groups showed red and yellow material on various body surfaces, most frequently observed at the 1-hour post-dose observation time point. Rales were occasionally observed at ≥1667 mg/kg/d SNAC with or without [redacted] • Mean BW gain was slightly lower (14% to 31%) for M receiving ≥1667 SNAC with or without [redacted]Decreased FC was seen for Group 5 & 9 M. • Decreased serum globulin was observed at ≥1667 mg/kg with or without [redacted] • Group 8 & 9 F that died had reddened mucosa in the glandular stomach. • Mean absolute (F only) and relative (M & F) liver weights were increased for Group 5, 8, & 9. Absolute and relative kidney weights were increased for Groups 4, 5, 8, & 9, with the effect generally being greater in F.
Oral 14-day EMISTOX 97004 (209244) GLP	Monkey/ Cynomolgus Main: 2/sex TK: 2/sex (Groups 5&7)	Group 1: 0 Group 2: 1000 Group 3: 1500 Group 4: 2500 Group 5: 1000 Group 6: 1500 Group 7: 2500	Group 1: 0 Group 2: 0 Group 3: 0 Group 4: 0 Group 5: [redacted] Group 6: Group 7:	Mortality Clinical signs Body weight Food cons. Ophthalmology Hematology Clinical chem. Urinalysis Toxicokinetics Organ weights Gross pathology Histopathology	• Treatment caused emesis, salivation, and signs of hypoglycemia, including reduced motor activity, transitory recumbency, and ptosis. Signs were seen in monkeys of both sexes in all 6 groups receiving SNAC or SNAC plus [redacted] generally in a SNAC dose-dependent manner. Upon the observation of severe hypoglycemia, individual animals were given fruit and/or dextrose (oral or IV). On Day 1, one TK female each from Groups 5 and 7 died from apparent severe hypoglycemia ~2 hours after dosing in spite of rescue with IV 50% dextrose. It is not

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels (b) (4)	Endpoints	Findings
					clear why the TK animals appeared to be affected the greatest. • Glucose values were within the pretreatment ranges (measurements were not taken during signs of apparent hypoglycemia). • There were no apparent treatment-related effects on any of the other parameters. • Mean C_{max} of SNAC on Day 1 was 208 µg/mL at 1 hour and 339 µg/mL at 45 minutes at 1000 and 2500 mg/kg/d SNAC (plus (b) (4) respectively. Mean C_{max} of SNAC on Day 14 was 110 µg/mL at 45 minutes and 76 µg/mL at 1 hour at 1000 and 2500 mg/kg/d SNAC (plus (b) (4) respectively. AUC was not calculated.
Oral 28-day EMIS/ R96014 (211248-1) GLP	Monkey/ Cynomolgus Main: 3/sex	Group 1: 0 Group 2: 800 Group 3: 1200 Group 4: 1800	Group 1: 0 Group 2: (b) (4) Group 3: Group 4: (b) (4)	Mortality Clinical signs Body weight Food cons. Hematology Clinical chem. Urinalysis Organ weights Gross path. Histopathology	• 1 HD M was sacrificed moribund 164 min after dosing on Day 3 and 1 MD M was sacrificed moribund 158 minutes after dosing on Day 27. The animals initially showed decreased activity that progressed to loss of consciousness, no response to pain, and severe hypothermia. The animals were suspected of having severe hypoglycemia and rescue with 50% dextrose IV was attempted prior to sacrifice. The MD animal also had a hypoglycemic episode on Day 26, which was corrected with dextrose. • Treated animals showed hypercellularity of the white pulp in spleen, in a non-dose-related manner. No other treatment-related toxicities were observed. • The NOAEL was 800 mg/kg/d SNAC based on severe hypoglycemia at the higher dose levels.
Oral 28-day EMIS/ R96014 (211248-2)	Monkey/ Cynomolgus Control: 2/sex SNAC: 3/sex	Group 1: 0 Group 2: 1800	Group 1: 0 Group 2: 0	Mortality Clinical signs Body weight Food cons. Hematology	• Clinical signs included emesis, decreased activity, recumbency, closed eyes, salivation, and one moribund sacrifice (Day 2). Moderate decreases in serum glucose were noted and animals exhibiting signs of hypoglycemia were

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels SNAC (mg/kg/d)	Dose Levels (b) (4)	Endpoints	Findings
GLP				Clinical chem. Urinalysis Organ weights Gross path. Histopathology	administered dextrose. • Urinalysis showed increased urinary sodium, protein, specific gravity, ketones, and acidity. • No treatment related macroscopic or microscopic findings were noted. • A NOAEL was not identified.

BW = body weight; F = female; FC = food consumption; GLP = Good Laboratory Practices; M = male; NOAEL = no observed adverse effect level; TK = toxicokinetics.

Oral Semaglutide with SNAC

Study title: Toxicity study by oral gavage administration to Sprague-Dawley rats for 26 weeks followed by a 4 week recovery period. Including bioanalysis of SNAC section report, toxicokinetic report (Draft) and pathology report (Draft)

Study no.:	JLY0278
Study report location:	Supporting Document #2, Volume 1 of 1, page 82
Conducting laboratory:	(b) (4)
Date of study initiation:	30 September 2010
GLP compliance:	Yes, but unsigned because of draft status
QA statement:	Yes, but unsigned because of draft status
Drug, lot #, and % purity:	NNC 0113-0217 (semaglutide) Batch #MP-0217-SD-Y002 (drug substance) Batch #s MP-9924-SD-Y007-A, -B, and -C (drug product) Purity: 91.0% to 91.2%
Novel Excipient, lot #, and % purity:	NNC 0113-3363 (sodium N-salicyloyl-8-aminocaprylate; SNAC) Batch #Y5U4425 Purity: 100.2% (HPLC), 98.4% (potency)

Key Study Findings

SNAC

- Possible treatment-related deaths occurred at 900 mg/kg/d (2 males and 8 females) and at 60/900 mg/kg/d (2 females). One male from the 300 mg/kg/d group died unexpectedly; the cause of death was not determined. Most animals died unexpectedly between 1 and 3 hours after dosing.
- There were no adverse clinical signs. Post-dose chin rubbing and salivation may suggest an issue with palatability of the dosing solution. The occurrence of deaths at the high dose shortly after dosing, in absence of anti-mortem signs, along with an increase in lung histopathology may suggest that the animals were struggling during dosing (because of unpalatability) thereby leading to small amounts of test article entering the lungs.
- There were no apparent effects on body weight for groups receiving SNAC alone.
- There were small but statistically significant increases in mean erythrocyte parameters and prothrombin time and decreases in reticulocytes at 0/900 mg/kg/d. Because of the small magnitude of changes, these effects were not considered adverse.
- At 900 mg/kg/d, statistically significant increases were noted for mean ALP (↑26%-31%) and phosphorus (↑14%; males only) and a statistically significant decrease was noted for total protein (↓7%-11%).
- Statistically significant increases in mean urine sodium were observed for all doses in males (↑54%-252%) and at 900 mg/kg/d in females (↑152%). Increased urine chloride was also noted at 900 mg/kg/d for males (↑70%) and females (↑127%). Urine volume was slightly increased for HD females (↑46%). Although mean kidney weights were increased at ≥20/300 mg/kg/d for males and females, there were no signs of overt kidney toxicity.

- In HD females, a slight increase in liver weights was also observed.
- A slight increase in incidence and severity of microscopic lung findings (prominent number of alveolar macrophages, alveolitis, perivascular lymphoid aggregates, and increased cellularity of BALT) was observed for all treatment groups, although not in a dose-dependent manner. An increase in epithelial hyperplasia of the limiting ridge of the stomach was noted at all male doses. An increase in some of the lung findings was still noted after recovery, but the findings did not occur in a dose-dependent manner.
- TK data were somewhat variable and values were lower than in the 12-month study, likely because the first sampling time was 2 hours post-dose rather than 0.17 hours post dose. Exposure generally increased with dose, but not always. SNAC concentration values at the 90 mg/kg/d could not be modeled because of insufficient data. SNAC exposure (C_{max} and AUC) tended to be higher when administered alone compared with when SNAC was formulated with semaglutide.
- The NOAEL for SNAC alone was considered to be 90 mg/kg/d SNAC because of a single male death at 300 mg/kg/d that was possibly related to SNAC treatment.

NNC 0113-0217 (semaglutide) with SNAC

- Possible treatment-related deaths occurred at 60/900 mg/kg/d (2 females). One of the females showed poor clinical condition prior to death; the cause of death for the other female was not determined. This was likely due to the SNAC component of the dosing solution based on the number of deaths occurring at 900 mg/kg/d SNAC.
- There were no adverse clinical signs. Post-dose chin rubbing and salivation may suggest an issue with palatability of the dosing solution.
- The mid-dose males and high-dose animals receiving SNAC and NNC 0113-0217 had lower body weights compared with the control group (up to 21% less for males and 16% for females), which is an expected effect for GLP-1 receptor agonists. Body weights rebounded during the recovery period. Lower body weights correlated with decreased food consumption.
- There were small but statistically significant increases in mean erythrocyte parameters and prothrombin time and decreases in reticulocytes at 20/300 and 60/900 mg/kg/d. Slight effects on prothrombin time were still noted after the recovery period. Because of the small magnitude of changes, these effects were not considered adverse.
- A statistically significant increase in mean serum ALP was noted at ≥20/300 mg/kg/d for males (↑23%-58%) and at ≥6/90 mg/kg/d for females (↑38% to 177%). A statistically significant increase in mean serum phosphorous was observed at ≥6/90 mg/kg/d for males (9%-16%) and 60/900 mg/kg/d for females (↑25%). A slight decrease in mean serum protein was observed for males at ≥20/300 mg/kg/d and females at 60/900 mg/kg/d. Correlative histopathology findings were not observed.
- Statistically significant increases in mean urine sodium were observed for all doses in males (↑48%-200%) and at 900 mg/kg/d in females (↑198%). Increased urine chloride was also noted at 900 mg/kg/d for males (↑40%) and females (↑153%). Mean urine volume was slightly increased for HD females (↑42%). Although mean kidney weights were increased at ≥20/300 mg/kg/d for males and at ≥6/90 mg/kg/d for females, there were no signs of overt kidney toxicity.

- An increase in absolute and relative adrenal gland weights was observed in males and females at ≥20/300 mg/kg/d. A slight increase in liver weights was observed in females at ≥20/300 mg/kg/d and decreases in mean salivary gland and ovary weights were seen in HD females. No correlative histopathology was observed.
- An increased incidence of distention of the duodenum, reduction in adipose tissue, and thin uterus were noted for HD females.
- A slight increase in incidence and severity of microscopic lung findings (prominent number of alveolar macrophages, alveolitis, perivascular lymphoid aggregates, and increased cellularity of BALT) was observed for all treatment groups, although not in a dose-dependent manner. Minimal hypertrophy of Brunner's gland in the duodenum was noted for males at ≥20/300 mg/kg/d and minimal to mild hypertrophy in females at 6/90 mg/kg/d. [note: hypertrophy of Brunner's gland was also noted after SC administration of semaglutide, so this does not appear to be a local effect due to oral dosing]. A slight increase in epithelial hyperplasia of the limiting ridge of the stomach was noted at all male doses. An increase in some of the lung findings was still noted after recovery, but the findings did not occur in a dose-dependent manner.
- Findings that were different from SNAC alone, and therefore are attributed to NNC 0113-0217 or the combination included: decreased body weight and food consumption, increased adrenal weights in MD and HD males, and macroscopic and microscopic findings in the duodenum. All other findings were attributed to SNAC.
- Exposure values for NNC 0113-0217 were variable, but generally increased with dose in a greater than dose-proportional manner between 20 and 60 mg/kg/d.
- The NOAEL for the combination of NNC 0113-0217 and SNAC was 20/300 mg/kg/d because of the deaths occurring at 60/900 mg/kg/d that were possibly related to SNAC treatment.

Methods

Species/Strain: Rat, Sprague-Dawley

Study design: (sponsor-generated Tables 1 and 2)

Table 2 Groups and dose levels

Group	Treatment	Dose NNC 0113-0217/SNAC (mg/kg/day)			Number of animals			
					Main study		Recovery phase	
		Week -2†	Week -1†	Week 1 - 26‡	Male	Female	Male	Female
1	Water Control	0/0	0/0	0/0	20	20	10	10
2	NNC 0113-0217/SNAC	0/90	0/90	0/90	20	20	10	10
3	NNC 0113-0217/SNAC	0/90	0/300	0/300	20	20	10	10
4	NNC 0113-0217/SNAC	0/90	0/300	0/900	20	20	10	10
5	NNC 0113-0217/SNAC	6/90	6/90	6/90	20	20	10	10
6	NNC 0113-0217/SNAC	6/90	20/300	20/300	20	20	10	10
7	NNC 0113-0217/SNAC	6/90	20/300	60/900	20	20	10	10

† Dose adaptation/escalation period

‡ Main study period

Table 1 Dosing regimen

Group	Dose NNC 0113-0217/SNAC (mg/kg/day)			Dose /SNAC (mg/kg/day)		
	Week -2†	Week -1†	Week 1 -26‡	Week -2†	Week -1†	Week 1 -26‡
1	0	0	0	0	0	0
2	0	0	0	90	90	90
3	0	0	0	90	300	300
4	0	0	0	90	300	900
5	6	6	6	90	90	90
6	6	20	20	90	300	300
7	6	20	60	90	300	900

† Dose adaptation/escalation period
‡ Main study period

Frequency of dosing: Once daily
Route of administration: Oral gavage
Dose volume: 5.4 mL/kg
Age: 40 to 46 days at start of the adaptation/escalation phase
Weight: 189 to 274 g (males) and 151 to 199 g (females)
Satellite groups: Recovery groups; TK samples were taken from main and recovery animals
Formulation/Vehicle: Water for injection, pH 8.5 ± 0.05, adjusted w/ HCl or NaOH
NNC 0113-0217 to SNAC concentrations were as follows:
Low dose: 1.11 mg/mL NNC 0113-0217, 16.67 mg/mL SNAC
Mid dose: 3.70 mg/mL NNC 0113-0217, 55.56 mg/mL SNAC
High dose: 11.11 mg/mL NNC 0113-0217, 166.67 mg/mL SNAC

Observations and Results:

Mortality:

There were 14 unscheduled deaths during the treatment period and none during the recovery period. Of those deaths, 11 were considered to be related to SNAC treatment; these included 1 male receiving 300 mg/kg/d SNAC, 2 males receiving 900 mg/kg/d SNAC, and 8 females receiving 900 mg/kg/d SNAC. Two females receiving 60/900 mg/kg/d NCC 0113-0217 / SNAC died prematurely, which may have been related to treatment. Most animals died unexpectedly between 1 and 3 hours after dosing. These animals did not exhibit adverse clinic signs before dosing, however macroscopic and microscopic exams could not identify the cause of death. Considering that these animals did not appear in poor health before dosing, died relatively soon after dosing, and showed signs of disliking the taste of the test article (chin rubbing and salivation), it is possible that the animals receiving the higher doses of SNAC struggled more during the gavage procedure leading to trauma or misdosing into the lungs, even though evidence for this was not observed during necropsy.

The female in the 90 mg/kg/d group died as a result of gavage error, which was confirmed by macroscopic and microscopic findings. A summary of premature deaths is shown in sponsor-generated Table 7 below.

Table 7 Group distribution of premature deaths

Group	1	2	3	4	5	6	7
Dose (mg/kg/day)	0	0/90	0/300	0/900	6/90	20/300	60/900
Males							
Number of deaths	0	0	1	2	0	0	0
Females							
Number of deaths	0	1	0	8	0	0	2

Clinical Signs:
An increase in chin rubbing was noted for all animals receiving SNAC, with the highest incidences at ≥300 mg/kg/d with or without NNC 0113-0217. Increased salivation was also noted for most dose groups, especially for those animals receiving 900 mg/kg/d SNAC with our without NNC 0113-0217. These observations were likely the result of a palatability issue with the dosing formulation, and therefore were not considered adverse.

One female in the 60/900 mg/kg/d dose group was noted as having a thin build, hunched posture, underactivity, piloerection, and pale skin color in the days before the moribund sacrifice. None of the other unscheduled decedents showed signs of poor health before dosing.

Body Weights:
SNAC

Dose (mg/kg/d)	Males				Females			
Sex	0	90	300	900	0	90	300	900
Final weight (g)	619	638	628	644	331	344	348	337
Diff from control (g)		19	9	25		13	17	6
% diff from control		↑3%	↑1%	↑4%		↑4%	↑5%	↑2%

NNC 0113-0217/SNAC

Dose (mg/kg/d)	Males				Females			
Sex	0/0	6/90	20/300	60/900	0/0	6/90	20/300	60/900
Final weight (g)	619	616	558	491	331	342	327	277
Diff from control (g)		-3	-61	-128		11	-4	-54
% diff from control		-	↓10%**	↓21%**		↑3%	↓1%	↓16%**

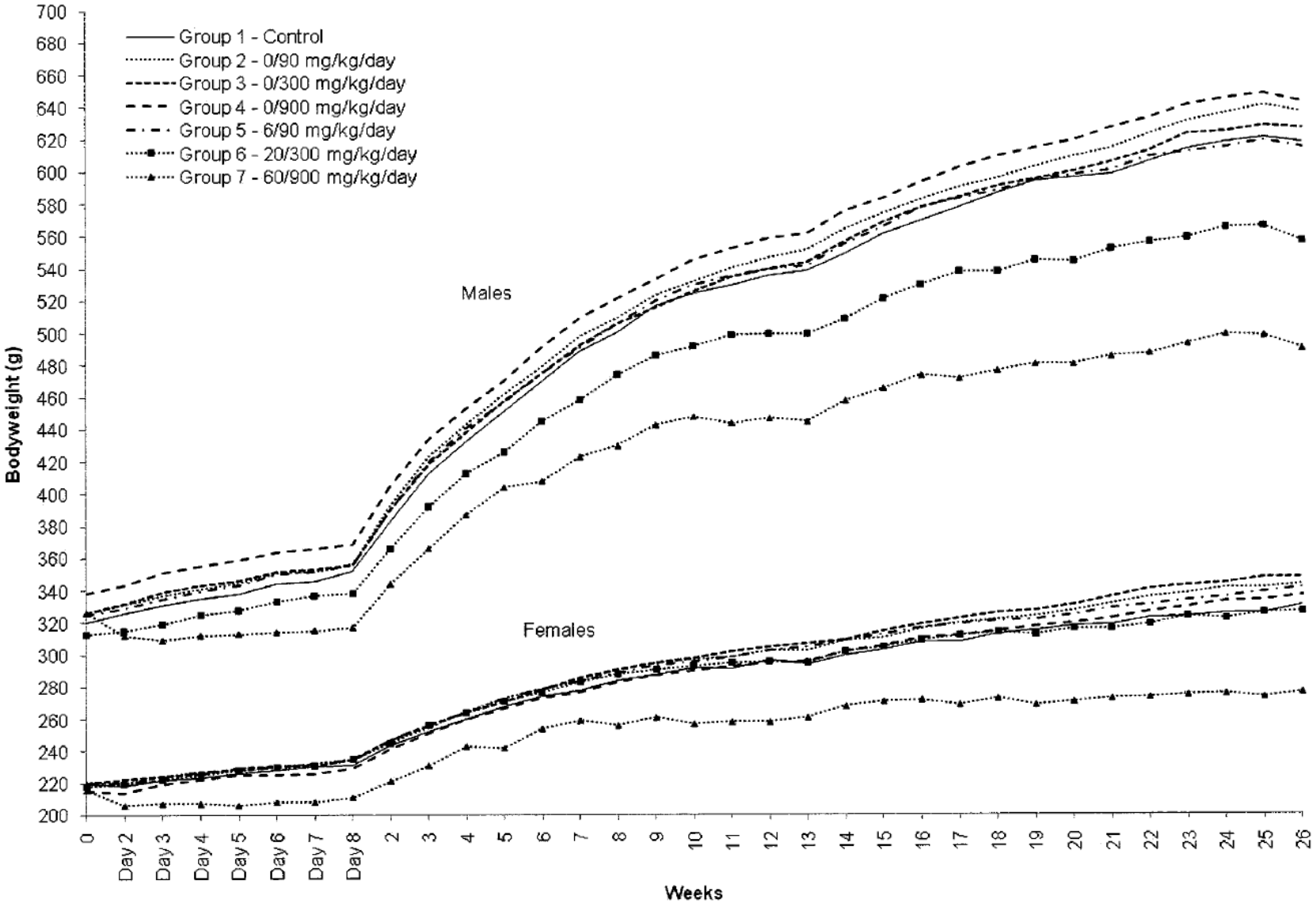
**p<0.01 (based on body weight change values calculated by sponsor)

Recovery: NNC 0113-0217/SNAC

Dose (mg/kg/d)	Males				Females			
Sex	0/0	6/90	20/300	60/900	0/0	6/90	20/300	60/900
Weight gain (g) - Week 0 to Week 4	27	15	39	81**	-6	2	9*	23**
Diff from control (g)		-12	12	54		8	15	29
% diff from control		↓44%	↑44%	↑200%		↑133%	↑250%	↑483%

**p<0.01 (based on body weight change values calculated by sponsor)

Bodyweight - group mean values during main study phase (g)



Food Consumption:
SNAC

Dose (mg/kg/d)	Males				Females			
Sex	0	90	300	900	0	90	300	900
Mean g/animal/week (Week 1 to 26)	199	202	202	212**	139	141	139	139
Diff from control (g)		3	3	13		2	0	0
% diff from control		↑2%	↑2%	↑7%		↑1%	-	-

**p<0.01

NNC 0113-0217/SNAC

Dose (mg/kg/d)	Males				Females			
Sex	0/0	6/90	20/300	60/900	0/0	6/90	20/300	60/900
Mean g/animal/week (Week 1 to 26)	199	199	190*	173**	139	142	139	112**
Diff from control (g)		0	-9	-26		3	0	-27
% diff from control		-	↓5%	↓13%		↑2%	-	↓19%

*p<0.05; **p<0.01

Ophthalmoscopy:
There were no ophthalmic findings attributed to NNC 0113-0217 or SNAC.

Hematology:
Small but statistically significant increases in erythrocyte parameters were observed for the 0/900, 20/300, and 60/900 mg/kg/d dose groups. These values were generally not statistically different from control after recovery. Statistically significant decreases in reticulocyte values were observed for males in the 20/300 and 60/900 mg/kg/d groups at 13 and 26 weeks, however these decreases did not correlate with a change in erythrocyte parameters. Overall, these findings were not considered to be toxicologically meaningful given the small magnitude of change. Small statistically significant increases in prothrombin time were observed for animals receiving 20/300 mg/kg/d and males receiving 60/900 mg/kg/d. Percent change for reticulocyte and prothrombin time values are summarized in the sponsor-generated tables below.

Group	1	2	3	4	5	6	7
Dose level (mg/kg/day)	0/0	0/90	0/300	0/900	6/90	20/300	60/900
Males							
Retic (%)Week 13	1.83	+6	+10	+5	+2	-22**##	-22**##
Retic (%)Week 26	1.94	0	-2	+9	+4	-15*#	-16**##
Retic (%)Week R4	1.87	+1	+11	+48	+15	-2	-1
PTP (sec) Week 13	19.1	-2	+2	+1	+2	+5*	+12**##
PTP (sec) Week 26	18.6	+1	+3	+5*	+3	+8**#	+19**#
PTP (sec) Week R4	18.8	+7*	+8*	+1	+5	+3	+5*

Group	1	2	3	4	5	6	7
Dose level (mg/kg/day)	0/0	0/90	0/300	0/900	6/90	20/300	60/900
Females							
Retic (%)Week 13	1.95	+4	-6	-3	+11	-5	-8*
Retic (%)Week 26	1.60	+14	+18	+9	+18	+4	-4
Retic (%)Week R4	1.84	+8	-13	-5	0	-11	-18
PTP (sec) Week 13	18.9	+4	+2	+6**	+6*	+7**#	+11**
PTP (sec) Week 26	18.9	+1	-4	+3	+2	+2##	+6**#
PTP (sec) Week R4	19.7	+5	+12*	+4*	+2	+3#	+8**

Clinical Chemistry:
Males at 26 weeks

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
ALP (U/L)	62	65	65	78* (↑26%)	62	76* (↑23%)	98**## (↑58%)
Cholesterol (mmol/L)	2.36	2.34	2.21	2.22	3.03	2.24	1.83*# (↓22%)
Triglycerides (mmol/L)	0.88	0.85	0.82	1.20	1.05	0.67	0.45**## (↓49%)
Phosphorus (mmol/L)	1.52	1.54	1.54	1.74** (↑14%)	1.65**# (↑9%)	1.75**## (↑15%)	1.76** (↑16%)
Total protein (g/L)	69	70	67	64** (↓7%)	70	66** (↓4%)	63** (↓9%)
Albumin/globulin	0.75	0.73	0.77	0.86** (↑15%)	0.72	0.79	0.91** (↑21%)

*p<0.05; **p<0.01 from vehicle control; # p<0.05; ##p<0.01 from matching SNAC control.
ALP = alkaline phosphatase.

Females at 26 weeks

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
ALP (U/L)	26	30	25	34** (↑31%)	36**# (↑38%)	42**## (↑62%)	72**## (↑177%)
Phosphorus (mmol/L)	1.32	1.37	1.38	1.39	1.45	1.46	1.65**## (↑25%)
Total protein (g/L)	73	73	76	68** (↓11%)	73	74	65** (↓11%)
Albumin/globulin	1.07	1.04	1.15* (↑7%)	1.22** (↑14%)	1.00	1.08	1.10

*p<0.05; **p<0.01 from vehicle control; # p<0.05; ##p<0.01 from matching SNAC control.
ALP = alkaline phosphatase.

Urinalysis: (26 Weeks)

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Males							
Chloride (mmol)	0.300	0.326	0.286	0.510** (↑70%)	0.316	0.235	0.421* (↑40%)
Sodium (mmol)	0.453	0.697* (↑54%)	0.785** (↑73%)	1.595** (↑252%)	0.672* (↑48%)	0.748** (↑65%)	1.358**# (↑200%)
Females							
Volume (mL)	3.8	3.9	4.1	5.6** (↑46%)	5.4*# (↑42%)	4.7* (↑24%)	5.4** (↑42%)
Chloride (mmol)	0.174	0.211	0.226	0.395** (↑127%)	0.267	0.255	0.440** (↑153%)
Sodium (mmol)	0.287	0.326	0.382	0.722** (↑152%)	0.354	0.325	0.856** (↑198%)

*p<0.05; **p<0.01 from vehicle control; # p<0.05; ##p<0.01 from matching SNAC control.

Organ Weights:
Males

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Adrenals (g)	0.052	0.054	0.053	0.056	0.055	0.061*# (↑17%)	0.058* (↑12%)
Relative to BW	0.0084	0.0085	0.0087	0.0088	0.0089	0.0109**## (↑30%)	0.0115**## (↑37%)
Kidneys (g)	3.81	3.91	4.08	4.62** (↑21%)	3.91	3.80	3.99## (↑5%)
Relative to BW	0.619	0.622	0.666* (↑8%)	0.723** (↑17%)	0.625	0.679** (↑10%)	0.797**## (↑29%)

*p<0.05; **p<0.01 from vehicle control; # p<0.05; ##p<0.01 from matching SNAC control. BW = body weight.

Females

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Kidneys (g)	2.02	2.18	2.27** (↑12%)	2.43** (↑20%)	2.29** (↑13%)	2.38** (↑18%)	2.33** (↑15%)
Relative to BW	0.626	0.642	0.650	0.743** (↑19%)	0.664	0.730**## (↑17%)	0.842**## (↑35%)
Liver (g)	11.83	12.54	12.75	13.35* (↑13%)	13.01	13.19* (↑11%)	11.59## (↓2%)
Relative to BW	3.66	3.70	3.64	4.08** (↑11%)	3.77	4.04**## (↑10%)	4.16** (↑14%)
Salivary glands (g)	0.445	0.452	0.429	0.418	0.475	0.429	0.333**## (↓25%)
Relative to BW	0.138	0.134	0.123* (↓11%)	0.128* (↓7%)	0.138	0.131	0.120** (↓13%)
Ovaries (g)	0.092	0.110	0.087	0.107	0.113	0.090	0.060**## (↓35%)
Relative to BW	0.0280	0.0335	0.0250	0.0334	0.0335	0.0275	0.0216 (↓23%)

*p<0.05; **p<0.01 from vehicle control; # p<0.05; ##p<0.01 from matching SNAC control.BW = body weight

Gross Pathology:
Duodenum

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Duodenum - Distension							
Males	-	-	-	-	-	-	-
Females	-	-	-	-	-	-	6
General appearance - thin/reduction in adipose tissue							
Males	-	-	-	-	-	-	-
Females	-	-	-	-	-	-	10
Uterus - thin							
Females	-	-	-	-	-	-	5

Histopathology:
Adequate Battery: Yes (see histopathology inventory table at end of section)
Peer Review: Yes, by a Novo Nordisk pathologist

Histological Findings:

Males

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Lung - prominent number of alveolar macrophages							
-minimal	2	11	9	7	10	6	11
-slight	0	0	1	1	1	0	2
Total	2	11	10	8	11	6	13
Lung - alveolitis							
-minimal	9	8	5	9	8	4	10
-slight	0	0	0	5	3	0	1
-moderate	0	0	0	1	0	0	0
Total	9	8	5	15	11	4	11
Lung - BALT, increased cellularity							
-minimal	6	6	6	10	6	6	7
-slight	0	3	2	3	2	0	0
-moderate	0	0	0	1	0	0	0
Total	6	9	8	14	8	6	7
Lung - perivascular lymphoid aggregates							
-minimal	12	5	9	3	4	6	12
-slight	5	12	9	8	13	13	7
-moderate	0	3	2	4	2	1	1
-marked	0	0	0	2	0	0	0
Total	17	20	20	17	19	20	20

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Lung - pleural fibrosis							
-minimal	0	0	0	0	0	0	2
-slight	0	0	0	0	0	1	0
Total	0	0	0	0	0	1	2
Duodenum - Brunner's Gland, hypertrophy							
-minimal	0	NE	NE	0	0	18	18
Lymph Node - mesenteric, ileum, mastocytosis							
-minimal	0			0			1
-slight	0			0			5
Total	0	NE	NE	0	NE	NE	6
Lymph Node - mesenteric, ileum, sinus histiocytosis							
-slight	0			1			3
Total	0	NE	NE	1	NE	NE	3
Stomach - epithelial hyperplasia, limiting ridge							
-minimal	0	2	3	3	0	1	2
-slight	0	0	4	0	1	0	0
Total	0	2	7	3	1	1	2
Stomach - submucosal inflammation, glandular							
-minimal	0	0	4	4	2	2	6
-slight	0	0	2	1	0	0	
Total	0	0	6	5	2	2	6
RECOVERY							
Lung - perivascular lymphoid aggregates							
-minimal	8	4	6	1	4	6	5
-slight	0	6	4	8	4	3	2
-moderate	0	0	0	0	1	0	0
Total	8	10	10	9	9	9	7
Lung - BALT, increased cellularity							
-minimal	3	5	4	5	4	2	4
-slight	0	0	0	2	0	0	1
Total	3	5	4	7	4	2	5

NE = not examined.

Females

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Lung - prominent number of alveolar macrophages							
-minimal	4	4	4	6	2	2	8
-slight	0	0	0	0	2	1	0
Total	4	4	4	6	4	3	8
Lung - alveolitis							
-minimal	4	12	5	7	5	6	5
-slight	0	1	0	1	1	0	0
Total	4	13	5	8	6	6	5
Lung - perivascular lymphoid aggregates							
-minimal	16	12	8	6	11	11	13
-slight	0	7	10	8	7	9	3
-moderate	0	0	1	0	0	0	0
Total	16	19	19	14	18	20	16
Lung - BALT, increased cellularity							
-minimal	2	6	5	3	7	4	5
-slight	0	0	1	1	0	1	0
Total	2	6	6	4	7	5	5
Lung - pleural fibrosis							
-minimal	0	1	0	0	2	1	1
-slight	0	0	0	0	0	0	0
-moderate	0	1	0	0	0	0	0
Total	0	2	0	0	2	1	1
Duodenum - Brunner's Gland, hypertrophy							
-minimal	0			0	1	8	17
-slight	0			0	0	0	3
Total	0	NE	NE	0	1	8	20
Lymph Node - mesenteric, ileum, mastocytosis							
-minimal	0			0			2
-slight	1			0			4
-moderate	0			0			1
Total	1	NE	NE	0	NE	NE	7
Lymph Node - mesenteric, ileum, sinus histiocytosis							
-slight	0	1		5			2
Total	0	1	NE	5	NE	NE	2
Stomach - epithelial hyperplasia, limiting ridge							
-minimal	1	1	0	2	2	2	1
-slight	0	0	0	0	0	0	0
Total	1	1	0	2	2	2	1

Dose (mg/kg/d)	0	0/90	0/300	0/900	6/90	20/300	60/900
Liver - hepatocyte hypertrophy, centrilobular							
-minimal	1	0	0	2	2	4	4
Uterus - general atrophy							
-slight	0			0			3
-moderate	0			0			1
Total	0	NE	NE	0	NE	NE	4
RECOVERY							
Lung - prominent number of alveolar macrophages							
-minimal	1	2	1	1	0	0	3
-slight	0	0	0	0	1	1	0
Total	1	2	1	1	1	1	3
Lung - perivascular lymphoid aggregates							
-minimal							
-slight	3	7	7	0	8	5	3
-moderate	1	1	2	0	0	3	1
Total	4	8	9	0	8	8	4

NE = not examined.

Toxicokinetics:
Blood samples were taken from main study group animals on Day 1 and Week 26 (following last dose) pre-dose and at 2, 6, 12, and 24 hours after dosing (4 animals per time point and 1 time point per animal). A single pre-dose sample was also taken from the first 4 animal numbers per treatment group on Day 91. Blood samples were also taken from all recovery animals at 336 and 672 hours after the final dose.

Note that T_{max} is around 10 minutes after dosing, so much of the exposure was not captured in this study, and therefore the TK data underestimate clinical safety margins. The TK data for semaglutide and SNAC are summarized in the sponsor-generated tables below.

Table 5 Estimated toxicokinetic parameters after oral administration of NNC 0113-0217

Week	Group	Dose Semaglutide (mg/kg/day)	Gender	t_{max} (hr)	C_{max} (nmol/l)	AUC_{0-24hr} (hr*nmol/l)	Rac_{Obs}
1	5	6	Male	NC	NC	NC	NC
			Female	6.0	6.01	53.7	NC
1	6	20	Male	2.0	28.8	316	NC
			Female	2.0	22.8	155	NC
1	7	60	Male	2.0	234	2650	NC
			Female	12.0	537	5540	NC
26	5	6	Male	NC	NC	NC	NC
			Female	6.0	9.37	86.0	1.60
26	6	20	Male	2.0	1500	3170	10.0
			Female	2.0	60.6	333	1.55
26	7	60	Male	2.0	383	3140	1.18
			Female	12.0	288	4250	0.767

NC Not calculated

AUC_{0-12hr} was used to calculate Rac_{Obs} for female Group 5 and 6

Table 6 Estimated toxicokinetic parameters after oral administration of SNAC

Week	Group	Dose SNAC (mg/kg/day)	Gender	t_{max} (hr)	C_{max} (ng/ml)	AUC_{0-24hr} (hr*ng/ml)	Rac_{Obs}
1	3	300	Male	2	1230	5120	NC
			Female	2	1800	6810	NC
1	4	900	Male	2	6210	23900	NC
			Female	2	2420	20200	NC
1	6	300	Male	2	693	7490	NC
			Female	6	754	5540	NC
1	7	900	Male	24	434	5010	NC
			Female	0	229	2890	NC
26	3	300	Male	2	1500	8970	1.75
			Female	6	1670	14300	2.10
26	4	900	Male	2	3250	18800	0.789
			Female	6	7420	42000	2.08
26	6	300	Male	24	495	9440	1.26
			Female	6	906	15300	2.76
26	7	900	Male	6	1390	11700	2.34
			Female	6	3740	33200	11.5

Antibody Analysis:
Blood samples were collected from recovery phase animals before treatment commencement and at the end of recovery to assess for antibody formation to NNC 0113-0217 (semaglutide).

There was no anti-NNC 0113-0217 antibody production detected in any animal treated with NNC 0113-0217.

Dosing Solution Analysis:
The concentration of NNC 0113-0217 and SNAC were within the ranges of 88% to 100% and 96% to 117%, respectively. Although some samples were outside of the applied limits of ±10% of nominal concentrations, the variations were considered to be negligible and did not impact the integrity of the study.

Study title: Toxicity study by oral capsule administration to Cynomolgus monkeys for 17 weeks followed by a 2 week recovery period

Study number:	JLY0239 (209428)
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4) (b) (4)
Date of study initiation:	16 December 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Semaglutide (NNC-0113-0217), Batch #LP0217K2X15 (97.46% pure) Batch #LP0217K2X07 (96.3% pure)

Key Study Findings

- **This study has characterized the toxicity profile of semaglutide when administered via the oral route; however, the formulation used for this study included (b) (4) rather than the novel excipient SNAC, so this study does not contribute to the toxicology qualification program for SNAC.**
- A reduction in BW gain or BW loss occurred throughout the treatment period for animals receiving semaglutide, but was greatest during the titration period at 10 mg/kg/day; no effect was observed at 5 mg/kg/day at the end of the study. BWs rebounded during the recovery period.
- There were no toxicologically meaningful changes in clinical chemistry, gross pathology, organ weights, or histopathology. No adverse effects within the gastrointestinal tract were observed.
- Exposures were approximately 2-fold lower on Day 119 than on Day 1; mean exposures for low-dose females were approximately 8-fold less on Day 119 than

on Day 1. Semaglutide exposure increased in a greater than dose-proportional manner, which was likely due to a higher amount of [redacted] at the high dose level allowing a greater degree of semaglutide absorption; although, the same ratio of semaglutide to [redacted] was used for both dose levels (1:15).

- The NOAEL for oral semaglutide was considered to be 20 mg/kg/day when administered with 300 mg/kg/day [redacted].

Methods

Species/Strain: Monkey/Cynomolgus

Study design: (sponsor-generated table)

Group/ Name	Treatment	Dose (mg/kg/day)			Number of animals			
		Dose Escalation		Main	Main study		Recovery phase	
		Days 1 to 4	Days 5 to 7	Phase Weeks 1 to17	Male	Female	Male	Female
1: Sham control	Empty capsules	0	0	0	4	4	0	0
2: [redacted] control	[redacted]	75	150	300	4	4	2	2
3: Low dose	NNC 0113-0217 [redacted]	5 75	5 75	5 75	4	4	2	2
4: High dose	NNC 0113-0217 [redacted]	5 75	10 150	20 300	4	4	2	2

Frequency of dosing: Once daily

Route of administration: Oral

Dose volume: Capsule was placed at the back of the throat so that the animal swallowed the capsule. Animals received up to 1 mL blackcurrent juice to aid swallowing

Formulation/Vehicle: Semaglutide powder was mixed at a 1:15 ratio with [redacted]

Age: 28 to 38 old

Weight: 1.94 to 2.51 kg (males), 1.93 to 2.61 kg (females)

Unique study design: As noted in the above table, HD group doses were escalated over the first week to reduce pharmacology-mediated clinical signs

Neurobehavioral assessments were integrated into the design of this study

Note [redacted]

Protocol deviations: No deviations were found that would impact the validity or interpretation of this study.

Observations and Results:

Mortality:
There were no treatment-related deaths. One control male was removed from the study during the escalation phase because of a fracture and dislocation to its right arm.

Clinical Signs: (at least twice daily)
There were no test article-related adverse clinical signs.

Neurobehavior: (detailed neurological exams were performed on all animals before treatment and 2 and 24 hours after dosing on Day 1 and on one day in Week 17)
There were no test article-related effects on neurobehavior.

Body Weights: (daily)

Group	Semaglutide (mg/kg/day)	(b) (4) (mg/kg/day)	BW Gain(kg)							
			Days 1 to 4		Days 5 to 7		Days 8 to 120		Recovery	
			M	F	M	F	M	F	M	F
1	0	0	0.030	0.023	0.063	0.088	0.28	0.32	NA	NA
2	0	75/150/300*	0.032	0.022	0.042	0.117	0.43	0.20	0.085	0.215
3	5	75	0.025 ↓17%	0.002 ↓91%	-0.047 ↓175%	0.027 ↓69%	0.33 ↑18%	0.42 ↑31%	0.175	0.100
4	5/10/20*	75/150/300*	0.017 ↓43%	-0.018 ↓178%	-0.073 ↓216%	-0.013 ↓115%	0.23 ↓18%	0.06 ↓81%	0.225	0.385

*Doses were escalated after Day 4 and Day 7. NA = not applicable (control group did not have recovery animals)

Feed Consumption:
Not reported

Ophthalmoscopy: (before treatment, at Week 17, and Week 2 of recovery)
There were no treatment-related changes.

Electrocardiography and Blood Pressure: (before treatment and 2 and 24 hours post-dose at Week 17 and Week 2 of recovery)
There were no treatment-related effects on ECG intervals or blood pressure.

Hematology: (before treatment, Day 2, Week 17, and Week 2 of recovery)
At Day 2, decreased reticulocytes were observed for semaglutide-treated animals. This effect was not noted at Week 17.

Clinical Chemistry: (before treatment, Day 2, Week 17, and Week 2 of recovery)
On Day 2, increases from control and pre-treatment values (23% to 48%) in mean urea and creatinine levels were observed in males and females at the high dose group. At Week 17, increases in mean urea and creatinine were still slightly elevated compared with control values, but the creatinine value was similar to the mean value at pre-

treatment. In the absence of increased severity in relation to increased dosing duration and a lack of correlative microscopic changes, these observations do not appear to be toxicologically meaningful.

Urinalysis: (before treatment, Week 17, and Week 2 of recovery)
There were no apparent treatment-related effects on urinalysis parameters.

Gross Pathology: (all animals)
An increased incidence of small thymus was noted for the HD groups. After a 2-week recovery period, one animal in each HD group was noted as having a small thymus.

Table 2 Summary of findings in the thymus for animals killed after 17 weeks of treatment

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
NNC 0113-0217 (b) (4)								
(mg/kg/day)	0	0/300	5/75	20/300	0	0/300	5/75	20/300
Small	0	0	0	2	2	1	0	4
Number of animals examined	3	4	4	4	4	4	4	4

(sponsor-generated table)

Organ Weights: (All animals)
There were no toxicologically meaningful effects on organ weights.

Histopathology: (All tissues from all animals)
Adequate Battery: Yes
Peer Review: Yes

Histological Findings
There were no definitive treatment-related microscopic findings. A slight increase in the incidence of thymic involution/atrophy was observed for the HD groups; however, this is a common background finding in stressed animals. The finding was considered to be unlikely related to treatment by the study pathologist.

Table 4 Summary of findings in the thymus for animals killed after 17 weeks of treatment

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
NNC 0113-0217 (b) (4)								
(mg/kg/day)	0	0/300	5/75	20/300	0	0/300	5/75	20/300
Involution/atrophy								
Minimal	0	1	0	1	1	1	1	2
Total	0	1	0	1	1	1	1	2
Number of tissues examined	3	4	4	4	4	4	4	4

Toxicokinetics: (collected from all animals before dosing and 2, 4, 8, 12, and 24 hours after dosing on Days 1 and 119)

Group	Dose (mg/kg/day)		Day	C_{max} (nM)			$AUC_{(0-24h)}$ (h*nM)		
	NNC 0113-0217	(b) (4)		F	M	F/M	F	M	F/M
3	5	75	1	38.3	13.2	**2.90**	627	256	**2.45**
			119	4.67	4.96	**0.942**	86.0	95.3	**0.902**
4	20	300	1	236	346	**0.682**	3370	5280	**0.638**
			119	101	165	**0.612**	1910	3250	**0.588**

Antibody Analysis: (collected from all animals before commencement of treatment, during Week 17, and all recovery phase animals during Week 2 of recovery)

Anti-semaglutide antibodies were not detected in any animal after 17 weeks of treatment or after the 2-week recovery period, a time point when semaglutide levels were very low and therefore would not interfere with the antibody assay.

Formulation Analysis:
The range of semaglutide in capsules prepared for Groups 3 and 4 was 79% to 102% of nominal and semaglutide was not detected in Group 2 formulations. The range of values for (b) (4) in capsules for Groups 2, 3, and 4 was 80% to 107% of nominal.

SNAC Alone (or SNAC with (b) (4))

Study title: 13-week oral toxicity study in rats

This study report was previously reviewed by Ke Zhang under IND (b) (4) The information from Dr. Zhang's review is reproduced below.

Study number: EMISTOX97007 (211246)
Study report location: Module 4.2.3.2
Conducting laboratory: (b) (4)
Date of study initiation: 26 November 1997
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SNAC (E414), #EM0066 and EM0067

Key Study Findings
E414 was tested orally in rats at 500, 1000, and 2000 mg/kg/day in combination with (b) (4) for 91 days. There is an additional high dose group (2000 mg/kg/day) without (b) (4) The dose of 1000 mg/kg/day or higher was lethal. Major treatment related clinical signs of toxicity were hypoactivity, prostration, unkempt appearance, pale extremities, and cool to touch observed prior to death. Other treatment related changes were decreased terminal body weight gain in mid and high

dose males (~9-12%), increased liver and kidney weights, and histopathological changes in stomach (neutrophil infiltration and hyperplasia in all treated females and mid and high dose males).

Methods

Species/Strain: Rat/Crl:CD(SD)BR

Study design: (sponsor-generated table)

Group Number	Treatment	Dosage Level (b) (4)	Dosage Volume (mL/kg)	Number of Animals Males	Females
Toxicology Groups ((b) (4)-315003)					
1	Vehicle	0/0	10	20	20
2	E414/(b) (4)	500/(b) (4)	10	20	20
3	E414/(b) (4)	1000/(b) (4)	10	20	20
4	E414/(b) (4)	2000/(b) (4)	10	20	20
5	E414	2000/0	10	20	20
Toxicokinetic Groups ((b) (4)-315003A)					
6/1[a]	E414/(b) (4)	500/(b) (4)	10	12	12
7/2[a]	E414/(b) (4)	1000/(b) (4)	10	12	12
8/3[a]	E414/(b) (4)	2000/(b) (4)	10	12	12

[a] = Computer protocol designation

Frequency of dosing: Once daily
Route of administration: Oral
Formulation/Vehicle: Water
Age: ~6 weeks old
Weight: 203-204 g (males) and 157-158 g (females)
Unique study design:
Protocol deviations:

Observations and Results:

Mortality: (daily)

Four females in the MD group, 2 males and 13 females in the HD group, and 4 males and 10 females in the SNAC alone group were found dead or euthanized in extremis. Clinical findings noted in these animals within 24 hours of death included hypoactivity, prostration, unkempt appearance, pale extremities, and body cool to touch. Due to the high mortality in the HD and SNAC alone groups, all remaining females were sacrificed during Week 10 of the study.

Clinical Signs: (daily)

Test article-related clinical signs were occasionally observed in the LD group but were predominantly in the MD, HD, and SNAC alone groups. Red and yellow material on various body surfaces (nose, mouth, forelimbs, and urogenital, anogenital areas) were noted in all treatment groups (more predominant in mid and high dose groups). Rales were occasionally noted in the mid and high dose groups. Following clinical signs of

toxicity were noted in the dead animals prior to death: hypoactivity, prostration, unkempt appearance, pale extremities, and cool to touch.

Body Weights: (weekly)
The initial and terminal body weights in the control group were 203 and 551 g (males) or 157 and 288 g (females). The terminal body weight gain in males was decreased by ~9% and 12% in the mid and high dose groups with (b) (4). The terminal body weight gain in males was decreased by ~10% in the high dose group without (b) (4). The terminal body weight gain was slightly increased in the female groups (~8-10%) as compared to the control.

Feed Consumption: (weekly)
The mean food consumption in the control group was 22-29 g/animal/day in males or 17-18 g/animal/day in females. The food consumption in the treatment groups (males = 22-27 g/animal/day and females = 17-20 g/animal/day) was comparable to the control.

Ophthalmoscopy: (before treatment and at Week 12)
There were no treatment-related changes.

Hematology: (Weeks 5, 10, and 13)
The percentage of neutrophil was increased in the high dose males group with (b) (4) (21%) as compared to the control (14%) during week 13. The percentage of lymphocyte was decreased in the high dose males group with (b) (4) (67%) as compared to the control (76%) during week 13. These changes were statistically significant ($P<0.01$). The increased neutrophil and decreased lymphocyte were also observed in other treatment groups but these changes were not statistically significant.

Clinical Chemistry: (Weeks 5, 10, and 13)
Decreases in total protein and globulin were noted mainly in the mid and high dose males. These were associated with increase in albumin to globulin (A/G) ratio. These changes were not very obvious in females. The absolute values with percentage changes from control (in parenthesis) of total protein, globulin, and A/G ratio during study week 5 were presented in the following table (similar changes were observed during study week 13).

The absolute values with percentage changes from control (in parenthesis) of total protein, globulin, and A/G ratio in males during study week 5

	Control	Low dose with (b) (4)	Mid dose with (b) (4)	High dose with (b) (4)	High dose w/out (b) (4)
Total protein (g/dl)	7.0	6.9	6.6 (5.7%)	6.3 (10%)	6.3 (10%)
Globulin (g/dl)	2.3	2.2	2.1 (8.7%)	1.7 (26%)	1.7 (26%)
A/G ratio	2	2.13	2.2 (10%)	2.66 (33%)	2.76 (28%)

Decrease in glucose (13%) was noted in the high dose males without (b) (4). Increases in alkaline phosphatase (21-25%) and cholesterol (30-31%) were noted in high dose females.

Urinalysis:
Not conducted

Gross Pathology: (all animals)
There were no treatment-related findings.

Organ Weights:
(All animals: adrenals, brain, kidneys, liver, spleen, ovaries, testes, thyroid, and thymus)

Increased liver and kidney weights were noted in the treatment groups. These changes were dose dependent. The percentage changes from control are presented in sponsor-generated Table 2 below.

TEXT TABLE 2: ORGAN WEIGHTS PERCENT CHANGE FROM CONTROL GROUP VALUES

GROUP:	2	3	4	5
E414 (mg/kg): [redacted] (b) (4)	500 5000	1000 5000	2000 5000	2000 0
Abs. Liver Weight				
Males	3	4	18**	22**
Females	11**	23**	29[a]	38[a]
Liver/Body Weight				
Males	4	10**	27**	28**
Females	5	15**	27[a]	37[a]
Liver/Brain Weight				
Males	4	6	19**	23**
Females	10**	22**	27[a]	35[a]
Abs. Kidney Weight				
Males	5	11*	18**	18**
Females	11**	18**	24[a]	21[a]
Kidney/Body Weight				
Males	7	18**	28**	25**
Females	5	11**	23[a]	22[a]
Kidney/Brain Weight				
Males	6	13**	19**	19**
Females	10**	18**	22[a]	19[a]
Abs. Thymus Gland Weight				
Males	-13	0	-1	-7
Females	2	-8	41[a]	26[a]
Thymus/Body Weight				
Males	-13	7	8	-3
Females	-3	-14	37[a]	25[a]
Thymus/Brain Weight				
Males	-13	2	1	-7
Females	2	-8	39[a]	23[a]

* = Significantly different from the control group at 0.05 using Dunnett's test.
** = Significantly different from the control group at 0.01 using Dunnett's test.
a = Organ weights obtained at study week 10 interim necropsy and compared with the study week 13 control group females. Statistical analysis not performed.

Histopathology: (All tissues from control, MD females, HD animals, and SNAC alone animals. Additionally, the lungs, liver, kidneys, and gross lesions (including stomach) were examined microscopically from all animals in the LD group and MD males. Pituitary was also examined from the LD males and MD animals and spleen from LD females)
Adequate Battery: Yes

Peer Review: No

Histological Findings
Histopathological examination revealed the increased severity of neutrophil infiltration in the mucosa and submucosa of the glandular stomach and a slightly increased incidence of hyperplasia of the squamous epithelial lining of the nonglandular stomach at the limiting ridge in all treated females and mid and high dose males. Vacuolation of cells in the pars distalis of the pituitary gland was also noted in the mid and high dose males. Necrosis of the red pulp of the spleen was found in two high dose females at study week 10.

Toxicokinetics: Data not included.

13-week oral toxicity (gavage) study in Wistar rats followed by a 4-week recovery period

Study no.:	A62807 (209242)
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	20 March 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO5045192 (E414 sodium salt; SNAC), Batch #SIS00516, 98.9% pure
Vehicle/Formulation:	Purified water

Key Study Findings
SNAC

- Note, in this study, Groups 2, 3, and 4 were administered an unrelated test article, and therefore, the results from those groups will not be presented in this review.
- Slight increases in mean absolute and relative liver and kidney weights were observed in MD males and HD males and females.
- An increase in minimal to slight inflammatory cell foci was observed for treated males, but not for females, as the control value for females was also high. Minimal renal mineralization of the outer medulla was noted for treated females. Stomach effects were also observed in longer duration studies conducted in Sprague Dawley rats, but treatment-related renal effects were not.
- The findings observed in this study were not considered to be toxicologically meaningful, and therefore, the NOAEL for SNAC was set at 1000 mg/kg/d.

Methods

Species/Strain:	Rat, Wistar
Dose levels:	0 (Group 1), 100, 500, 1000 mg/kg/day (Groups 5, 6, and 7)
Number/sex/group:	10
Number/sex/group (TK):	5
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5 mL/kg (Groups 1, 5, and 6), 10 mL/kg (Group 7)
Age:	7 weeks
Weight:	113.3 - 169.7 g (males) and 97.2 - 136.2 g (females)
Satellite groups:	No recovery groups included for SNAC

Observations and Results:

Mortality: (twice daily)

There were no deaths attributed to treatment with SNAC.

One HD male was euthanized on Day 71 due to a perforated esophagus caused by a gavage error. One HD female died spontaneously on Day 76; the cause of death was not determined.

Clinical Signs: (once daily and detailed exams once weekly)

There were no adverse clinical signs attributed to treatment with SNAC.

Body Weights: (twice weekly)

There were no apparent treatment-related effects on male body weight. LD and HD females had 15% and 9% lower body weight gains than control, respectively, which correlated with the differences observed with food consumption. Because this did not occur in a dose-related manner, the observed effect on body weight gain does not appear related to SNAC.

Food Consumption: (twice weekly)

There were no treatment-related effects on food consumption in males. A slight reduction in food consumption was noted for LD and HD females, with the greatest effect occurring for LD females.

Ophthalmoscopy: (pre-treatment and during Week 13)

There were no ophthalmic findings attributed to SNAC.

Hematology: (at Week 5 and 12; 18-hour fast)

At Week 12, an increase in hemoglobin concentration distribution width (↑7%) was observed in HD females; an increase in reticulocyte counts (↑18%) was observed in HD males; an increase in total lymphocyte count (↑29%) in HD males; and a prolongation of APTT in all treated male groups (↑12% to 22%). With the exception of prolonged APTT, similar trends were observed at Week 5. These changes are not considered to be toxicologically meaningful.

Clinical Chemistry: (at Week 5 and 12; 18-hour fast)
There were no changes in clinical chemistry parameters that were considered to be of toxicological concern.

Urinalysis: (at Week 5 and 12; 18-hour collection during fast)
There were no treatment-related effects on urinalysis parameters.

Organ Weights:
A slight increase in mean absolute and relative liver weights (↑13% to 22%) was observed for treated males and HD females. A slight increase in mean absolute and relative kidney weights (14% to 22%) was noted for MD and HD males and HD females.

Gross Pathology:
No treatment-related macroscopic findings were identified.

Histopathology:
Adequate Battery: Yes
Peer Review: Yes

Histological Findings:

	Male				Female			
Dose (mg/kg/d)	**0**	**100**	**500**	**1000**	**0**	**100**	**500**	**1000**
Stomach - inflammatory cell foci/mucosal -minimal	1	2	6	6	6	8	7	3
-slight	-	1	-	-	-	-	-	-
Total	1	3	6	6	6	8	7	3
Stomach - erosion/pyloric -minimal	-	-	1	-	-	-	-	1
-slight	-	-	-	1	-	-	-	1
Total	0	0	1	1	0	0	0	2
Kidney - mineralization/ outer medulla -minimal	-	-	-	-	-	4	9	8
-slight	-	-	-	-	-	1	-	-
Total	0	0	0	0	0	5	9	8

Toxicokinetics:

Table 2: SNAC exposure in Week 1 and Week 13 of treatment

	SNAC Dose [mg/kg/day]					
	100		500		1000	
Week 1	**males**	**females**	**males**	**females**	**males**	**females**
AUC [ng·h/ml]	5460	4990	25500	22500	65200	93100
C_{max} [ng/mL]	1530	1370	4830	7990	15000	41400
t_{max} [h]	1	0.5	1	0.5	0.5	0.5
Week 13						
AUC [ng·h/ml]	2560	8020	18500	38800	44400	108000
C_{max} [ng/mL]	1030	3530	3880	6770	5800	29100
t_{max} [h]	0.5	0.5	0.5	1	0.5	0.5

(sponsor-generated table)

Dosing Solution Analysis:
SNAC content was found to be within the accepted range of ±20% of the nominal content. Homogenous distribution of SNAC was demonstrated. Formulations were considered to be stable for at least 7 days under room temperature storage conditions.

Study title: A 12-Month Toxicity Study of SNAC and SNAC/ (b) (4) Administered by Oral Gavage to Rats with a 6-Month Interim Sacrifice

Study no.:	BNA00004 (sponsor #525-T-018 and 211504)
Study report location:	Module 4.2.3.2 and Module 4.2.2.2 (TK)
Conducting laboratory:	(b) (4)
Sponsor:	Emisphere Technologies, Inc.
Date of study initiation:	29 March 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Sodium N-salicyloyl-8-aminocaprylate; SNAC Lot # P146-72-1, 97.4% pure Lot # P189-191-1, 99.5% pure

Key Study Findings

- There were possible treatment-related deaths at 900 mg/kg/d SNAC.
- No adverse clinical signs were noted. Unlike the 6-month rat study, post-dose chin rubbing and salivation were not observed in this study.

- An increase in mean urine volume was observed for all male interim sacrifice SNAC treatment groups (up to 109% more than control) and mid- and high-dose females. Smaller increases were observed at that end of the scheduled dosing period.
- Kidney weights were increased at ≥500 mg/kg/d for both genders at Day 191 and Day 365. Small increases in liver weights were also observed at 900 mg/kg/d on Day 191 and at ≥500 mg/kg/d on Day 365.
- There were macroscopic (foci of glandular stomach) and microscopic findings noted in the stomach at the end of the treatment period for males treated with 900 mg/kg/d and females treated at ≥500 mg/kg/d; however, microscopic examinations were not conducted on the lower dose groups. Microscopic findings noted in the stomach included hyperplasia of the nonglandular stomach; eosinophilic blebs in the keratin layer; erosive inflammation; and hemorrhage. Hyperplasia of the nonglandular stomach was also noted at the 6-month time point.
- Exposures generally increased with dose in a slightly greater than dose-proportional manner. There was variability in values across the different sampling days with the same dose groups. There was no apparent gender difference.
- The NOAEL for this study was determined to be 500 mg/kg/d based on the mortalities observed at 900 mg/kg/d.

Methods

Species/Strain: Rat/Sprague Dawley

Study Design:

Group No.	No. of Animals						Test Material	Dose Level (mg/kg/d)	Dose Volume (mL/kg)	Dose Conc. (mg/mL)
	Interim		Toxicity		TK					
	M	F	M	F	M	F				
1	10	10	10	10	8	8	RODI	0	10	0
3	10	10	10	10	8	8	SNAC	250	10	25
4	10	10	10	10	8	8	SNAC	500	10	50
6	10	10	10	10	8	8	SNAC	900	10	90

Frequency of dosing: Once daily
Route of administration: Oral gavage
Formulation/Vehicle: Reverse osmosis deionized (RODI) water
Age: 8 weeks at randomization
Weight: 243 to 275 g (males) and 176 to 203 g (females)
Unique study design: This study also contained treatment Group number 2 and 5, which received SNAC plus (b) (4). These data are not presented here as they are not the focus of this review.

Observations and Results:

Mortality:

General health/mortality and moribundity checks were performed twice daily. A total of 4 males and 6 females were found dead or euthanized moribund prior to scheduled necropsy. The cause of death for two females in Group 6 was undetermined and may have been related to test article. A summary of unscheduled deaths is shown in the table below.

Gender	Day of Death/ Euthanasia	Probable Cause of Death
Group 1 (RODI Water)		
Female (TK)	Day 351 (e)	Pituitary mass
Group 3 (250 mg/kg SNAC)		
Male	Day 355 (e)	Marked cardiopathy, chronic progressive nephropathy, ulcerative inflam. of the stomach
Female	Day 321 (fd)	Histiocytic sarcoma
Group 4 (500 mg/kg SNAC)		
Female (TK)	Day 183 (e)	Fecal impaction
Group 6 (900 mg/kg SNAC)		
Male	Day 97 (fd)	Lymphoma
Male	Day 41 (fd)	Possible gavage error
Male	Day 212 (fd)	Possible gavage error
Female (TK)	Day 283 (e)	Subcutaneous mass
Female	Day 23 (fd)	Undetermined
Female	Day 141 (fd)	Undetermined

e = euthanized; fd = found dead; RODI = reverse osmosis deionized; TK = toxicokinetic satellite group.

Clinical Signs:
Cage-side observations were performed between 1 and 2 hours after dosing on Days 1 through 365. Detailed clinical observations were performed once prior to treatment and weekly during the interim and toxicity periods. There were no adverse clinical signs attributed to the test article.

Body Weights:
Body weights were measured weekly. There were no apparent treatment-related effects on body weight.

Feed Consumption:
Food consumption was measured weekly.

For males, the high-dose group consumed slightly less food than controls starting at around day 231. The mean difference from control from Day 231 to Day 364 was -6.5%, with weekly values ranging from -1.4% to -11.5%. These differences in food consumption did not correlate with effects on body weight.

Weekly food consumption data was more variable for females, however, on average, all SNAC-treated groups consumed less food than controls starting at Week 1 with some differences reaching statistical significance. Weekly differences were occasionally as great as -19.0%, -19.0%, and -20.4% for the 250, 500, and 900 mg/kg/d SNAC treatment groups, respectively. These differences in food consumption did not correlate with effects on body weight.

Ophthalmoscopy:
Ophthalmological examinations were performed by a board-certified veterinary ophthalmologist prior to in-life initiation, during the last week of the interim phase (Day 188), and during the last week of the main toxicity phase (Day 363). There were no ophthalmic findings considered to be treatment related.

Hematology:
Blood samples for hematology and coagulation were collected on Days 190 and 191 for the interim groups and on Days 365 and 366 for the main toxicity groups. Animals were fasted overnight. There were no treatment-related effects on hematology, coagulation, or red cell morphology parameters.

Clinical Chemistry:
Blood samples for clinical chemistry were collected on Days 190 and 191 for the interim groups and on Days 365 and 366 for the main toxicity groups. Animals were fasted overnight.

Mean serum phosphorous as statistically significantly increased for all groups receiving SNAC. Mean values for treated groups were up to 38% higher than control for males and up to 47% higher than controls for females. The degree of effect was similar between the three dose levels for each gender and did not generally occur in a dose-related manner.

Urinalysis:
Urine was collected overnight on Days 190 and 191 for the interim groups and on Days 365 and 366 for the main toxicity groups. Animals were fasted overnight.

Mean urine volume was increased for all male interim SNAC treatment groups, with the difference being statistically significant at the high dose (109% of control). Increases were also observed for the mid- and high-dose interim female groups, with the difference being statistically significant at the high dose (111%). Values were also increased at the end of the main treatment period for males and females, but the differences from control were not quite as large and did not occur in a completely dose-dependent manner. No changes in specific gravity, pH, or electrolytes were observed.

Organ Weights:

Dose (mg/kg/d)	0		250		500		900	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Day 191								
Kidneys (g)	3.45	2.12	3.68	2.26	3.98* (↑15%)	2.33 (↑10%)	4.22* (↑22%)	2.60* (↑23%)
Relative to BW	0.606	0.670	0.622	0.718	0.650	0.719	0.688* (↑13%)	0.798* (↑19%)
Liver (g)	14.65	8.91	15.52	8.97	16.18	9.12	16.41 (↑12%)	10.23 (↑15%)
Relative to BW	2.563	2.815	2.601	2.847	2.621	2.777	2.658 (↑4%)	3.135 (↑11%)
Day 365								
Kidneys (g)	3.87	2.54	4.05	2.81	4.72* (↑22%)	3.06* (↑21%)	4.74* (↑23%)	3.08* (↑21%)
Relative to BW	0.554	0.626	0.602	0.700	0.646* (↑17%)	0.724 (↑16%)	0.693* (↑25%)	0.753* (↑20%)
Liver (g)	16.79	10.45	16.96	10.97	19.90 (↑19%)	11.62 (↑11%)	18.42 (↑10%)	11.74 (↑12%)
Relative to BW	2.404	2.549	2.482	2.720	2.746 (↑14%)	2.745	2.663 (↑11%)	2.867 (↑13%)

*p<0.05; F = female; M = male.

Gross Pathology:

Dose (mg/kg/d)	0		250		500		900	
Finding **Sex**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Stomach, glandular - foci								
-Found dead/euthanized	-	-	1	1	-	-	1	-
-Day 190/191	-	-	-	-	-	-	1	-
-Day 365/366	-	-	-	-	-	4	1	4
-Total	**0**	**0**	**1**	**1**	**0**	**4**	**3**	**4**

Histopathology:
Adequate Battery: Yes (see tissue inventory table at end of section)
Peer Review: No

Histological Findings:

Stomach (sponsor-generated table)

	Males						Females					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
Dose Group in mg/kg/day	0	150/30	250	500	750/30	900	0	150/30	250	500	750/30	900
Stomachs examined	10	0	0	0	10	8	10	0	0	4	9	10
Finding												
Hyperplasia, non-glandular	**2**				**4**	**6**	**0**			**0**	**3**	**4**
minimal	2				2	6					3	3
mild					1							1
moderate					1							
Eosinophilic blebs in Keratin Layer	**0**				**1**	**3**	**0**			**3**	**2**	**2**
minimal						3				3	1	1
mild					1						1	1
Acute inflammation, glandular	**0**				**1**	**0**	**0**			**0**	**0**	**0**
minimal					1							
Erosive inflammation, glandular	**0**				**0**	**1**	**0**			**0**	**0**	**1**
mild						1						1
Hemorrhage	**0**				**0**	**1**	**0**			**1**	**0**	**3**
minimal						1				1		3
Acute inflammation, non glandular	**0**				**0**	**0**	**0**			**0**	**1**	**0**
minimal											1	
Fibrosis, glandular	**1**				**3**	**1**	**0**			**0**	**1**	**0**
minimal	1				1	1					1	
mild					2							
Atrophy, glandular	**0**				**1**	**0**	**0**			**0**	**0**	**0**
minimal					1							
Necrosis, glandular	**0**				**0**	**0**	**0**			**0**	**0**	**1**
minimal												1

Note that Groups 2 and 5 were treated with SNAC and (b) (4), respectively.
An increased incidence of hyperplasia in nonglandular stomach was also noted in the high-dose group at the 6-month interim sacrifice.

Neoplastic Observations in Terminal Euthanasia Animals

	Males						Females					
Dose Group	1	2	3	4	5	6	1	2	3	4	5	6
Dose Group in mg/kg/day	0	150/30	250	500	750/30	900	0	150/30	250	500	750/30	900
Cervix: Stromal cell sarcoma								1				
Colon: Adenocarcinoma						1						
Liver: Hepatocellular adenoma										1		
Liver: Hepatocellular carcinoma					1							
Mammary gland: fibroadenoma							1		1			
Pituitary gland: adenoma pars distalis	1				1		1	2	2		1	2
Lymphoma (spleen primary site)				1								
Skin, other: keratoacanthoma						1						

Note that Groups 2 and 5 were treated with SNAC and (b) (4) respectively.

Toxicokinetics:
Blood sampling for TK analysis was conducted prior to dosing and at 0.166, 0.5, 1, 2, and 4 hours after dosing on Days 1, 91, 273, and 364. Samples were collected from 4 animals per time point.

Estimated Toxicokinetic Parameters after Oral Administration of SNAC to Rats

Day	Dose (mg/kg/d)	Gender	t_{max} (hr)	C_{max} (ng/mL)	$AUC_{0\text{-}4hr}$ (ng•h/mL)	Rac_{Obs}
1	250	M	0.17	6,810	3,230	NC
		F	0.17	8,010	8,170	NC
	500	M	0.17	22,000	11,300	NC
		F	0.17	28,900	21,500	NC
	900	M	0.17	143,000	42,300	NC
		F	0.17	204,000	88,800	NC
91	250	M	0.17	13,600	7,150	2.21
		F	0.17	29,600	12,700	1.56
	500	M	0.50	66,700	31,900	2.82
		F	0.17	171,000	34,600	1.61
	900	M	0.17	90,000	27,500	0.65
		F	0.17	191,000	73,000	0.82
273	250	M	0.17	15,300	5,780	1.79
		F	0.17	19,600	6,110	0.75
	500	M	0.17	243,000	70,900	6.26
		F	0.17	144,000	39,200	1.82
	900	M	0.17	243,000	46,700	1.10
		F	0.17	288,000	88,200	0.99
364	250	M	0.17	28,900	12,100	3.75
		F	0.17	8,070	4,190	0.51
	500	M	0.17	177,000	51,600	4.55
		F	0.17	131,000	30,500	1.42
	900	M	0.17	229,000	47,400	1.12
		F	0.17	33,800	16,500	0.19

F = female; M = male.
Reproduced from sponsor Table 2; TK report (211504)

Dosing Solution Analysis:
Concentration verification analyses were performed on the dosing formulations for all dose groups at the following time points: Day 1, Day 7, Month 3, Month 6, Month 9, and Month 12.

No SNAC (or (b) (4) [from Groups 2 and 5 not discussed in this review]) was detected in the vehicle control group. All sample concentration results for SNAC were within ±10% of the theoretical concentration at each time point. Day 1 and 7 samples were also tested for stability, the results of which demonstrated that SNAC concentrations differed by no more than ±1.3%, demonstrating stability at room temperature.

Study title: 13-week oral toxicity study in rhesus monkeys

This study report was previously reviewed by Ke Zhang under IND (b) (4) *The information from Dr. Zhang's review is reproduced below.*

Study number: EMISTOX98006 (209245)
Study report location: Module 4.2.3.2
Conducting laboratory: (b) (4)
Date of study initiation: 23 December 1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SNAC (E414), #P121-157-2

Key Study Findings

The following treatment-related clinical signs of toxicity were observed: salvation, retching, vomiting, somnolence, hypoactivity, uncorordination, and prostration. Sponsor believed that these changes (somnolence, hypoactivity, uncorordination, and prostration) were due to "hypoglycemia" and intravenous administration of 50% dextrose was given to these monkeys. The liver weight was increased but there were no treatment-related histopathological changes in the liver.

Methods

Species/Strain: Monkey/rhesus
Study design: (sponsor-generated table)

Groups and Treatment	Dose Level		Dose Conc	No. of Animals	
	SNAC (mg/kg/day)	(b) (4)	SNAC/ (b) (4) (mg/U/mL)	Males	Females
1. Vehicle Control*	0		0/0	4	4
2. Carrier Control#	1800		180/0	4	4
3. Low Dose	800		80 (b) (4)	4	4
4. Mid Dose	1200		120	4	4
5. High Dose	1800		180	4	4

* The Vehicle Control animals received deionized water only.
\# The Carrier Control animals received SNAC in deionized water only.

Frequency of dosing: Once daily
Route of administration: Oral
Dose volume: 10 mL/kg
Formulation/Vehicle: Water
Age: ~2-3 years old
Weight: 3.0 to 4.1 kg (males), 2.4 to 3.9 kg (females)
Protocol deviations: There were no deviations that affected the integrity or interpretation of the study

Observations and Results:

Mortality: (daily)
There were no unscheduled deaths.

Clinical Signs: (daily)
The following treatment related clinical signs of toxicity were observed: salivation, retching, vomiting, somnolence, hypoactivity, incoordination, and prostration. Salivation, retching, and vomiting were observed in all groups including control but more frequent in the treatment groups. Somnolence, hypoactivity, incoordination, and prostration were observed in all groups receiving SNAC but not in control. Sponsor believed that these changes (somnolence, hypoactivity, incoordination, and prostration) were due to hypoglycemia and intravenous administration of 50% dextrose was given to these monkeys. Both the number of animals and number of days on which dextrose was administered occurred in a dose-dependent manner with relation to SNAC dose.

Body Weights: (weekly)
There were no clear treatment-related effects on body weight.

Feed Consumption: (weekly)
There were no clear treatment-related effects on food consumption.

Ophthalmoscopy: (before treatment and at Weeks 4, 8, and 13)
There were no treatment-related changes.

Electrocardiography: (before treatment and at Weeks 4, 8, and 13)
There were no treatment-related effects.

Hematology: (before treatment and at Weeks 4, 8, and 13)
There were no treatment-related effects.

Clinical Chemistry: (before treatment and at Weeks 4, 8, and 13)
There were no treatment-related effects. Serum glucose levels obtained during the treatment period (after an overnight fast) from animals treated with SNAC alone or a SNAC plus (b) (4) were comparable to those obtained from vehicle control animals and to values obtained during the pretreatment period. The study director stated that this suggests that the hypoglycemic effect, should it occur, is relatively short lasting and certainly not evident at approximately 24 hours after SNAC administration.

Urinalysis: (before treatment and at Weeks 4, 8, and 13)
When compared to values obtained during the pretreatment period or to values obtained from the vehicle control animals, marginal to slight increases in mean urine sodium levels were noted during Weeks 4, 8, and 13 in females receiving 1800 mg/kg/day SNAC alone or a combination of 1800 mg/kg/day SNAC and (b) (4) (b) (4).

Gross Pathology: (all animals)
There were no treatment-related macroscopic findings.

Organ Weights: (All animals: adrenals, brain, heart, kidneys, liver, lung, spleen, pituitary, ovaries, testes, prostate, salivary glands, thyroid, thymus, and uterus)

Increased liver weight was noted in the treatment groups. The percent change from control is presented in the sponsor-generated table below.

Table A: Increases in Mean Liver Weight

Dose Level		% Increase in Mean Liver Weight When Compared to Vehicle Control Animals			
SNAC (mg/kg/day)	(b) (4)	Absolute		Relative to Body Weight	
		Males	Females	Males	Females
1800	0	56	33	47	36
800	(b) (4)	37	15	24	14
1200		33	24	36	16
1800		45	35	43	33

Histopathology: (All tissues from all animals)
Adequate Battery: Yes

Peer Review: No

Histological Findings
There were no treatment-related microscopic findings.

Toxicokinetics:
Not conducted

Study title: A 9-month toxicity study of SNAC and SNAC/(b) (4) administered once daily by oral gavage to Rhesus monkeys

Study number:	525-T-019 (209258 and 211503 [TK report])
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	22 March 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SNAC (E414), Lot #P146-72-1 (97.4% pure) and P189-191-1 (97.0%)

Key Study Findings

- There were no unscheduled deaths or effects on body weight.
- Decreased activity and to a lesser extent, lethargy and recumbency, were observed in a few animals dosed with either 500/(b) (4) SNAC/(b) (4) or 600 mg/kg/day SNAC. On the four occasions when animals were observed to be recumbent, they either recovered spontaneously or following the feeding of fruit and/or IV administration of a 50% dextrose or lactate ringers solution. Similar observations have been made in other studies and have been attributed to hypoglycemia.
- There were no SNAC-related effects on ophthalmology, ECGs, clinical pathology, gross pathology, or organ weights.
- A slight imbalance in minimal, multifocal mineralization of brain parenchyma and minimal interstitial hemorrhage of the thymus observed for the groups receiving 500 and 600 mg/kg/d SNAC (sponsor-generated table below). The pathologist did not feel that the findings were related to treatment. In the absence of historical control data for these findings, a possible treatment-related effect cannot be ruled out. The majority of males from each group that were examined were immature based on the observation of no spermatogenesis in testes.
- The study director concluded that the clinical signs were sporadic and reversible and therefore not adverse and that the increase in brain parenchymal mineralization was not treatment related. Therefore the study director placed the NOAEL at 600 mg/kg/day SNAC. However, because on rare occasions the clinical signs resulted in intravenous intervention and a treatment-related effect on the brain parenchyma cannot be ruled out, a more conservative NOAEL would be 300 mg/kg/day SNAC.

Methods

Species/Strain: Monkey/rhesus

Study design: (sponsor-generated table)

		Dose Level		Dose Volume (mL/kg)	Dose Concentration		
Group No.	Number of M/F	SNAC (mg/kg)	(b) (4)		SNAC (mg/mL)	(b) (4)	Number Necropsied: Day 274
1	3/3	0 (control)	0	10	0	0	3/3
2	3/3	150	(b) (4)	10	15	(b) (4)	3/3
3	3/3	200	0	10	20	0	3/3
4	3/3	300	0	10	30	0	3/3
5	3/3	500	(b) (4)	10	50	(b) (4)	3/3
6	3/3	600	0	10	60	0	3/3

(b) (4)

Frequency of dosing: Once daily
Route of administration: Oral
Formulation/Vehicle: Water
Age: 2.6 to 3.8 years (males) and 3.1 to 4.8 years (females)
Weight: 2.2 to 3.4 kg (males), 2.4 to 3.8 kg (females)
Recovery group: None
Protocol deviations: There were no deviations that affected the integrity or interpretation of the study

Observations and Results:

Mortality: (once daily)
There were no unscheduled deaths.

Clinical Signs: (once daily plus post-dose observation)
Noteworthy clinical signs are shown below in the sponsor-generated table. Decreased activity and to a lesser extent, lethargy and recumbency, were observed in a few animals dosed with either 500/(b) (4) SNAC/(b) (4) or 600 mg/kg/day SNAC. On the four occasions where animals were observed to be recumbent, they either recovered spontaneously or following the feeding of fruit and/or IV administration of a 50% dextrose or lactate ringers solution.

Treatment-related Clinical Observations[a]

Dosage (SNAC or SNAC/ (b) (4)	Animal Number	Observation	Incidence	Study Day(s)
500 (b) (4) mg/kg/day	M 5001	Decreased activity	4	3-20
		Lethargy	0	-
		Recumbent	1[b]	3
	M 5002	Decreased activity	35	4-259
		Lethargy	4	188-191
		Recumbent	0	-
	M 5003	Decreased activity	2	186-245
		Lethargy	0	-
		Recumbent	0	-
600 mg/kg/day	M 6001	Decreased activity	10	139-221
		Lethargy	0	-
		Recumbent	1[c]	110
	M 6002	Decreased activity	0	
		Lethargy	0	-
		Recumbent	1[d]	-
	F 6501	Decreased activity	3	8-11
		Lethargy	0	-
		Recumbent	0	-
	F 6503	Decreased activity	4	2-6
		Lethargy	0	-
		Recumbent	1[e]	2

[a] Includes both post dose and cage-side observations; [b] Treated with dextrose; [c] Treated with lactate ringers; [d] No treatment; [e] given fruit

Body Weights: (weekly)
There were no treatment-related effects on body weight.

Feed Consumption: (daily qualitative assessments)
No effects on food consumption were noted.

Ophthalmoscopy: (prestudy and during Weeks 26 and 39)
There were no definitive SNAC or SNAC/ (b) (4) -related ophthalmic changes. The presence of conjunctival swelling in 1, 2, and 1 males from Groups 2, 3, and 4, respectively, during Weeks 26 and/or 39 was considered to have an uncertain relationship to SNAC or SNAC/ (b) (4) The uncertainty was predicated on the absence of the finding in females from any dose group or in males at the higher dose groups (Groups 5 or 6).

ECG: (prestudy and during Weeks 26 and 39, 1 to 2 hours postdose)
There were no test article-related qualitative or quantitative changes.

Hematology: (prestudy and during Weeks 27 and 38)
There were no apparent treatment-related effects on hematology or coagulation parameters.

Clinical Chemistry: (prestudy and during Weeks 27 and 38)
There were no definitive treatment-related effects. One animal each from Group 2, 5, and 6 had decreases in serum phosphorus (43% to 87%) on Day 185 or 262 compared with pretreatment values. A test article relationship is uncertain because of the sporadic nature of occurrence and absence of a dose response.

Urinalysis: (prestudy and during Weeks 27 and 38, bladder puncture during necropsy)
No treatment-related effects on urinalysis parameters were observed. Urine electrolytes were not measured (note that urine sodium was increased in rats).

Gross Pathology:
There were no macroscopic findings attributed to SNAC or SNAC/(b) (4).

Organ Weights:
There were no treatment-related effects in organ weights or organ weight ratios.

Histopathology: (Groups 1, 5, and 6 only)
Adequate Battery: Yes
Peer Review: No

Histological Findings
There were no definitive treatment-related microscopic findings. A slight imbalance in mineralization of brain parenchyma and interstitial hemorrhage of the thymus observed for the groups receiving 500 and 600 mg/kg/d SNAC (sponsor-generated table below). The majority of males from each group that were examined were immature based on the observation of no spermatogenesis in testes.

Observations: Neo-Plastic and Non Neo-Plastic	MALES						FEMALES					
Removal Reasons: All of those SELECTED	0	150	200	300	500	600	0	150	200	300	500	600 (b) (4)
Number of Animals on Study :	3	3	3	3	3	3	3	3	3	3	3	3
Number of Animals Completed:	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
BRAIN;												
Examined	(3)	(0)	(0)	(0)	(3)	(3)	(3)	(0)	(0)	(0)	(3)	(3)
Within Normal Limits	2	0	0	0	1	0	2	0	0	0	1	1
Mineralization; Parenchyma; multifocal	0	0	0	0	1	1	0	0	0	0	0	2
THYMUS;												
Examined	(3)	(0)	(0)	(0)	(3)	(3)	(3)	(0)	(0)	(0)	(3)	(3)
Within Normal Limits	3	0	0	0	0	0	2	0	0	0	1	0
Cyst; multifocal	0	0	0	0	0	0	0	0	0	0	1	2
Hemorrhage; Interstitium; multifocal	0	0	0	0	2	3	0	0	0	0	1	0

Toxicokinetics for SNAC:

Dose (mg/kg/d)	Day	C_{max} (ng/mL)		AUC_{0-8h} (ng•h/mL)		Half-Life (h)	
		M	F	M	F	M	F
150*	1	26,500	52,300	27,900	64,900	0.90	1.94
	90	9,000	13,100	15,000	16,800	1.19	1.71
	180	12,5000	11,700	15,500	16,400	0.95	1.29
200	1	50,500	17,400	29,300	23,900	0.99	5.56
	90	16,300	7,260	24,000	21,700	0.83	2.16
	180	12,500	7,810	20,100	18,700	1.35	4.08
300	1	39,400	37,500	46,500	50,500	1.58	1.96
	90	16,400	16,100	35,800	41,500	1.57	2.03
	180	13,400	21,500	30,200	38,100	2.20	1.95
500*	1	36,500	44,800	75,100	63,600	1.35	2.68
	90	29,400	43,400	58,900	83,100	2.42	4.21
	180	17,500	35,700	50,600	80,900	2.51	2.51
600	1	142,000	114,000	130,000	188,000	2.25	1.34
	90	116,000	62,600	212,000	114,000	3.73	2.14
	180	55,200	62,500	114,000	86,100	2.17	1.85

*Group also received (b) (4)

Dosing Solution Analysis:

All dosing solutions analyzed (Groups 2-6) were within the acceptance criteria of ±10% of the nominal concentration of SNAC from each time point evaluated (Days 1 and 9 and Months 3, 6, and 9). The results from Day 9 indicated that the dosing solutions were stable for at least 8 days under the conditions of use in this study. There was no SNAC or (b) (4) detected in the control group (Group 1).

Histopathology Inventory for IND #114,464

Study Number	210196	209243	209258
Duration/Species	**6 Month Rat**	**12 Month Rat**	**9 Month Monkey**
Adrenals	X*	X*	X*
Aorta	X	X	X
Bone Marrow - smear			
Bone (femur with joint)	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Colon	X	X	X
Duodenum	X	X	X
Epididymides	X*	X*	X*
Esophagus	X	X	X
Eyes	X	X	X
Gall bladder			X
Gross lesions	X	X	X
Harderian gland	X	X	
Heart	X*	X*	X*
Ileum	X	X	X
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland	X		
Larynx	X		
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, mandibular	X	X	X
Lymph nodes, axillary	X		
Lymph nodes, mesenteric	X	X	X
Lymph nodes, gastric	X		
Mammary Gland	X	X	
Nasal cavity	X		
Optic nerves	X	X	X
Ovaries	X*	X*	X*
Pancreas		X	X
Peyer's patches	X		X
Pharynx	X		
Pituitary	X*	X*	X*
Prostate	X*	X*	X
Rectum	X	X	X
Salivary gland (submandibular*, sublingual*, and parotid)	X*	X*	X (mandibular)
Sciatic nerve	X	X	
Seminal vesicles	X*	X*	X
Skeletal muscle	X	X	X
Skin with mammary glands	X	X	X
Spinal cord (cervical, lumbar, thoracic)	X	X	X
Spleen	X*	X*	X*
Sternum with bone marrow	X	X	X
Stomach	X	X	X
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid + parathyroid	X*	X*	X*
Tongue	X	X	X
Trachea	X	X	X
Ureters	X		
Urinary bladder	X	X	X
Uterus + cervix	X*	X*	X
Vagina	X	X	X
Zymbal gland			

X, histopathology performed; *, organ weight obtained;

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study reports for a reverse mutation assay and a chromosomal aberration assay were previously reviewed by Ke Zhang under IND (b) (4) *Dr. Zhang's reviews are reproduced below.*

Study title:	Ames test
Study report Number:	325-T-003 (209248)
Testing Laboratory:	(b) (4)
Date of study initiation:	January 31, 2002
Date of study report:	December 16, 2002
GLP Compliance:	Yes
QA statement:	Yes
Drug Batch Number:	P-145-116-1
Study Endpoint:	To determine the potential mutagenic effects of SNAC

Key Findings:
The results suggest that SNAC was not mutagenic in this test system.

Methods: To examine the potential mutagenic effects of SNAC, the reverse mutation assay (Ames test) was conducted using direct plate incorporation and preincubation methods in four strains Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and one strain of E. Coli (WP2uvrA) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations were tested: 313, 625, 1250, 2500, and 5000 µg/plate with and without S-9. Sponsor did not indicate whether the SNAC used was final clinical formulation (process C product). Positive controls (sodium azide, 9-aminoacridine, 2- nitrofluorene, methyl methanesulfonate, and 2-aminoanthracene) were tested. The results should be considered positive if the test substance induced a two fold increase in the mean revertant colonies as compared to the control and this increase should be a dose response to increasing concentrations of the test article.

Strain/species/cell line: Four strains of Salmonella typhimurium (TA98, TA100, TA1535, and TA1537) and one strain of E. Coli (WP2uvrA).

Dose selection criteria:

Basis of dose selection: Dose selection was based on the results of the dose ranging study.

Ranging finding studies: The toxicity range test was conducted in Strains TA100 and WP2uvrA at concentrations ranging from 6.67 to 5000 µg/plate. No cytotoxicity was observed in term of the bacterial background lawn and the number of revertants per plate.

Metabolic activation system:	Metabolic activation, S-9 mix, was from rat liver.
Controls:	
Negative control:	Distilled water.
Positive control:	Positive controls (sodium azide, 9-aminoacridine, 2-nitrofluorene, methyl methanesulfonate, and 2-aminoanthracene)) were tested.
Exposure conditions:	The reverse mutation assay (Ames test) was conducted using the direct plate incorporation and preincubation methods.
Dose used in defining study:	The following concentrations were tested: 313, 625, 1250, 2500, and 5000 µg/plate.
Counting method:	The condition of the bacterial background lawn was evaluated macroscopically and microscopically using a dissecting microscope.
Cytotoxic endpoints:	The condition of the bacterial background lawn was evaluated for evidence of cytotoxicity.
Genetic toxicity endpoints:	Number of revertant colonies.
Statistical methods:	Number of revertant colonies was averaged for each concentration.

Criteria for positive results: The results should be considered positive if the test substance induced a two fold increase in the mean revertant colonies as compared to the control and this increase should be a dose response to increasing concentrations of the test article.

Study Validity
The positive controls significantly increased the colonies compared to the solvent controls.

Results
SNAC did not significantly increase the colonies as compared to the solvent control in the presence and absence of S-9 mix. The results were reproducible.

7.2 *In Vitro* Assays in Mammalian Cells

Study title:	Test for Chemical Induction of Chromosome Aberrations in Cultured Human Peripheral Lymphocytes
Study number:	EMISTOX99001 (209249)
Laboratory:	(b) (4)
Study start date:	12 April 1999
Study end date:	21 July 1999
GLP:	Yes
QA statement:	Yes
Drug Batch Number:	P-121-136-2

Key Study Findings:
E414 was not inducer of chromosome aberration in this testing system

Methods:

Cells Employed:	Cultured human peripheral blood lymphocytes
Concentrations Employed:	400, 1000, 2000, 4000, and 5000 µg/ml
Solvent Control:	Water
Positive Controls:	Cyclophosphamide with S9 metabolic activation and mitomycin C without S9 metabolic activation

Criteria of Genotoxic Effect: The test article was considered to have a positive response if (1) the test article had a positive concentration-response trend and a statistically significant increase over that of the solvent controls in the proportion of cells with aberrations at one or more concentrations and (2) in the event of lack of positive concentration-response trend, at least two consecutive test concnentrations must have shown a statistically significant increase in the proportion of cells with aberrations.

In the first experiment, the cultures were treated with test drug for 3 hours with and without S9 followed by a 18 hour recovery period prior to harvest. Colcemid (0.1 µg/ml) was added to the culture 2 hours before the cells were harvested. At the end of the experiment, 100 metaphases were examined for chromosome aberration for each test concentration. Solvent and positive controls were also scored. The experiment was repeated only in the absence of metabolic activation.

Results:
In the first experiment with S9, positive control (mitomycin C 0.2 µg/ml) failed to increase percentage of cells with aberration. This experiment was then repeated with higher concentration of mitomycin C (0.4 µg/ml) and it significantly increased the percentage of cell with aberration. Treatment with E414 did not significantly increase the percentage of cells with aberration as compared to the control with and without metabolic activation in both experiments as well as the confirmatory experiment without S9.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells

Study number:	EMISTOX 95010 (209250)
Study report location:	Module 4.2.3.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	05 December 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SNAC (E414 sodium salt), E414-19S, >85% pure

Key Study Findings

- Under the conditions of this study, SNAC (E414 sodium salt) did not induce an increase in micronucleated polychromatic erythrocytes.

Methods

Species/Strain:	Mouse/CD-1
Doses in definitive study:	0, 200, 500, and 1000 mg/kg
Frequency of dosing:	Single dose on Day 1, with necropsy at 24, 48, and 72 hours after dosing
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	25% v/v propylene glycol
Number/Sex/Group:	5/sex/group for each time point
Satellite groups:	None
Basis of dose selection:	Range-finding study; mortality at 2000 mg/kg
Negative control:	25% v/v propylene glycol (vehicle)
Positive control:	1.0 mg/kg Triethylenemelamine by intraperitoneal injection

Study Validity

Criteria for a valid assay:

1. The average number of micronucleated polychromatic erythrocytes (MPCE) for the vehicle control group should not exceed 10 per 1000 polychromatic erythrocytes (PCE) evaluated.

2. For the positive control, the increase in the average number of MPCEs per 2000 PCEs over the average number of MPCEs for the vehicle control should be statistically significant.

3. At least 3 animals from each sex must be alive at the time of sacrifice for each dose level.

Criteria for a positive response:

1. The test article showed a positive dose-response trend and a statistically significant increase in the number of MPCEs at one or more dose levels over that of the concurrent vehicle control.

2. In the event there was no positive dose-response trend, at least two consecutive test doses should have produced a statistically significant increase in the number of MPCEs.

3. The test article was considered to have caused a negative response if none of the test doses showed a statistically significant increase in the number of MPCEs when compared to the vehicle control.

4. The test article was considered to have caused an equivocal response if the test article induced a statistically significant increase in the number of MPCEs when compared to the vehicle control at one of the test doses without a positive dose-response trend.

Results

A single oral dose of SNAC did not result in unscheduled deaths, adverse clinical signs, or meaningful effects on body weight gain. There was no apparent treatment-related bone marrow toxicity based on the PCE/NCE ratios. The PCE/NCE ratio was reduced for the male and female positive control groups. A summary of the mean number of MPCEs per group for each time point is shown in the sponsor-generated tables below. SNAC-treated groups did not have a statistically significant increase in MPCEs at any dose or time point. A statistically significant increase in MPCEs was observed for the positive control groups.

Summary of Micronuclei Results for Males

Time (hours)	Dose (mg/kg)	Cell Counts		PCE/NCE Ratio	MPCE per 2,000 PCEs
		PCE	NCE		
24	Vehicle	741	259	2.98	1.0
24	200	750	250	3.36	1.2
24	500	622	378	1.96	1.4
24	1000	731	269	2.80	0.8
24	TEM*	284	716	0.40	84.0 **
48	Vehicle	747	253	3.22	1.2
48	200	690	310	2.35	0.6
48	500	747	253	3.24	1.0
48	1000	745	253	2.97	1.2
72	Vehicle	774	226	3.47	0.6
72	200	762	238	3.38	0.6
72	500	759	241	3.19	0.8
72	1000	747	253	3.05	0.6

NOTE: Five animals were used per group.
* TEM was dosed at 1.0 mg/kg.
** Statistically significant response.

Summary of Micronuclei Results for Females

Time (hours)	Dose (mg/kg)	Cell Counts		PCE/NCE Ratio	MPCE per 2,000 PCEs
		PCE	NCE		
24	Vehicle	708	292	2.77	1.6
24	200	730	290	2.66	0.8
24	500	570	430	1.40	1.8
24	1000	767	233	3.58	1.6
24	TEM*	377	623	0.69	105.2 **
48	Vehicle	609	391	1.83	1.0
48	200	724	276	3.39	1.4
48	500	752	248	3.20	1.0
48	1000	701	299	2.44	0.8
72	Vehicle	743	257	2.99	1.0
72	200	756	244	3.33	1.0
72	500	731	269	2.81	1.2
72	1000	758	242	3.17	0.8

NOTE: Five animals were used per group.
* TEM was dosed at 1.0 mg/kg.
** Statistically significant response.

8 Carcinogenicity

Carcinogenicity studies are ongoing.

9 Reproductive and Developmental Toxicology

Dose Range-Finding Studies

Study Type Study No. GLP Status	Species/strain Number/group Route/Regimen	Dose Levels (mg/kg)	Endpoints	Findings
Segment 2 EMISTOX 97009 (209252) GLP	Rat/Sprague-Dawley 8 pregnant rats Oral/once daily from GD7 - GD17; necropsy on GD20	0, 1000, and 2000*	Mortality Body weight Food cons. Embryonic loss Fetal weight Fetal gross pathology	• One rat in each of the SNAC treatment groups died on GD 9 and GD 7, respectively. No adverse clinical signs preceded death and each female had 16/18 normally developing embryos. • Clinical signs included red perioral substance, chromorhinorrhea, and excess salivation at both dose levels and decreased motor activity at 2000 mg/kg. • Decreased maternal BW gain was noted for both dose levels from GD7 to GD10. BW gain for the entire treatment period and the entire gestation period were reduced for the HD group. BW effects generally correlated with decreased FC. • Decreased fetal BW at the HD. • There were no effects on litter averages for corpora lutea, implantations, dams with any resorptions, or percent live male fetuses. • Based on the degree of maternal toxicity at the HD, 1000 mg/kg was proposed as the HD for the definitive study.
Segment 2 EMISTOX 97003 (209255) GLP	Rabbit/New Zealand white 6 pregnant rabbits Oral/once daily from GD6 - GD18; necropsy on GD29	0, 1000, 1500, 2000, 2500, and, 3000*	Mortality Body weight Food cons. Embryonic loss Fetal weight Fetal gross pathology	• 1, 4, 5, and 6 does from the 1500, 2000, 2500, and 3000 mg/kg groups died or were sacrificed moribund during the study, respectively. • 1 doe each in the 1500 and 2000 mg/kg groups had complete resorptions. • Clinical signs included decreased motor activity at ≥1000 mg/kg; impaired righting reflex, ataxia, and labored breathing at ≥1500 mg/kg; loss of righting reflex, prostration, and excess salivation at 2000 mg/kg; excess salivation and vocalization at 3000 mg/kg. • Initial maternal BW loss was observed at ≥1500 mg/kg. • An increase in early resorptions was seen at 1500 and 2500 mg/kg. The percent of dead or resorbed conceptuses was increased at ≥1500 mg/kg. • 1000 mg/kg was proposed as the highest dose in the definitive study.

*Dose levels that included co-administration of (b) (4) are not included in this review.
BW = body weight; GD = gestation day; HD = high dose.

9.1 Fertility and Early Embryonic Development

Study title: Oral (gavage) fertility and general reproduction toxicity study of SNAC (b) (4) and SNAC in rats

Study number: EMISTOX98004 (209251)
Study report location: Module 4.2.3.5.1
Conducting laboratory: (b) (4)
Date of study initiation: 30 June 1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: SNAC (E414), E414-49S (EM0076), 92.9% pure

Key Study Findings

- There were no treatment-related mortalities. Treatment-related clinical signs were limited to excessive salivation.
- A slight decrease in body weight gain was noted for males receiving 1000 mg/kg/d SNAC, with or without (b) (4) and for females receiving 750 mg/kg/d SNAC, with or without (b) (4)
- There were no effects on mating or fertility indices for males or females. No effects on sperm motility, morphology, or number were noted. No effects on implantations, resorptions, fetal weight, fetal sex ratios, or fetal development were observed at any dose level.
- The NOAEL for mating, fertility, and early embryonic development was 1000 mg/kg/d SNAC.

Methods

Species/Strain: Rat/Sprague-Dawley
Design:

Dosage Group	SNAC/ (b) (4) Dosage (b) (4)	SNAC (b) (4) Concentration (b) (4)	Dosage Volume (mL per kg)	Number of Rats/Sex	Assigned Numbers	
					Male Rats	Female Rats
I	0 (Vehicle)	0	10	25	11101 - 11125	11226 - 11250
II	500/ (b) (4)	50/ (b) (4)	10	25	11126 - 11150	11251 - 11275
III	750/ (b) (4)	75/ (b) (4)	10	25	11151 - 11175	11276 - 11300
IV	1000/ (b) (4)	100/ (b) (4)	10	25	11176 - 11200	11301 - 11322, 6960[a], 11324 - 11325
V	1000/0	100/0	10	25	11201 - 11225	11326 - 11334, 7190[b], 11336 - 11350

a. Female rat 11323 was found dead on day 1 of dosing. Necropsy revealed a perforated lung. Rat 11323 was replaced with rat 6960.
b. Female rat 11335 was excluded from study because of a broken palate and was replaced with rat 7190.

Frequency of dosing:	Once daily Males: 28 days before cohabitation, continuing through a 21-day cohabitation period. Females: 15 days before cohabitation, through the cohabitation period, and until GD7, with necropsy occurring on GD20.
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Reverse osmosis, deionized water
Satellite groups:	None, no TK groups
Dosing Solution Analysis:	All test article solutions were found to be within the acceptance criteria.
Protocol deviations:	No deviations occurred that affected the quality of integrity of the study.

Observations and Results

Males

Mortality

One male rat from Group 4 (1000 mg/kg/d SNAC/ (b) (4)) was found dead on Day 26. The only adverse clinical observation for this rat was excessive salivation on Day 25.

Clinical Signs

An increased incidence of excess salivation was noted for groups receiving SNAC, with or without (b) (4) . This observation was first noted between the 3rd and 4th week of treatment.

Body Weight

Weight gains were slightly reduced for the entire treatment period at 1000 mg/kg/d SNAC, with or without (b) (4) (↓~7% of control). This effect was statistically significant on Days 56 to 63.

Feed Consumption

Absolute and relative feed consumption values for the males were generally comparable in each group.

Organ Weight

There were no treatment-related effects on reproductive organ weights.

Necropsy

There were no macroscopic findings that were attributed to treatment. Cauda epididymal sperm motility, total percentage of motile sperm, total number of nonmotile sperm, sperm counts, and sperm concentrations (density) did not differ significantly among the five dose groups.

Mating and Fertility Parameters

There were no apparent effects on mating or fertility indices.

Females

Mortality

There were no treatment-related mortalities. One rat administered 1000 mg/kg/d SNAC without (b) (4) was sacrificed moribund on Day 17 as a result of an injury.

Clinical Signs

Rats receiving SNAC, with or without (b) (4), had an increased incidence of excessive salivation, which was first noted between the 2nd and 3rd week of treatment.

Body Weight

No treatment-related effects were noted on body weight gain during pre-cohabitation. Maternal body weight gains were reduced from GD0 to 8 for 750 and 1000 mg/kg/d SNAC, with or without (b) (4) These effects correlated with decreased food consumption.

Necropsy

There were no maternal macroscopic findings that were attributed to treatment.

The only gross external fetal alteration was a cleft palate in a fetus from a dam treated with 1000 mg/kg/d SNAC plus (b) (4). This finding was not considered treatment related because it was only observed in a single fetus and the incidence was within the historical control range for the testing facility.

Fertility Parameters

One rat receiving 1000 mg/kg/d SNAC plus (b) (4) delivered early.

There were no apparent treatment-related effects on the number of corpora lutea, implantations, litter size, resorptions, fetal sex ratios, or fetal body weights.

There were no treatment-related effects on mating and fertility indices.

9.2 Embryonic Fetal Development

Study title: Oral (gavage) developmental toxicity study of E414/(b) (4) and E414 in rats

Study number:	EMISTOX98001 (209253)
Study report location:	Module 4 2 3 5 1
Conducting laboratory:	(b) (4)
Date of study initiation:	12 February 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SNAC (E414 sodium salt), E414-39-S1, 91.8% pure

Key Study Findings

- There were no treatment-related effects on mortality, clinical signs, C-section parameters, or fetal development.
- A decrease in maternal body weight gain (32% to 37%) was observed between GD7 and GD10 for the two groups receiving 1000 mg/kg/d SNAC, with or without (b) (4).
- No treatment-related adverse effects on embryo-fetal viability, growth, or development were observed at SNAC doses up to 1000 mg/kg/d, with or without (b) (4).

Methods

Species/Strain: Rat/Sprague-Dawley

Design:

Group	E414/ (b) (4) Dosage (b) (4)	E414 (b) (4) Concentration (b) (4)	Doage Volume (mL per kg)	Number of Rats	Assigned Numbers
I	0 (Vehicle)	0/0	10	25	5101 - 5125
II	500/ (b) (4)	50/ (b) (4)	10	25	5126 - 5150
III	750/	75/	10	25	5151 - 5175
IV	1000/	100/	10	25	5176 - 5200
V	1000/0	100/0	10	25	5201 - 5225

a. E414 was considered 100% active for the purpose of dosage calculation, dosage calculations for (b) (4) were adjusted for a (b) (4) potency.

Frequency of dosing: Once daily on GD7 through GD17; C-section on GD20
Route of administration: Oral (gavage)
Formulation/Vehicle: Reverse osmosis, deionized water
Satellite groups: None, no TK groups
Dosing Solution Analysis: All test article solutions were found to be within the acceptance criteria (±10% of nominal).
Protocol deviations: No deviations occurred that affected the quality of integrity of the study.

Observations and Results

Mortality:

No treatment-related deaths occurred. One Group 5 dam was found dead on GD17 about 1 hour after dosing. Necropsy findings in the lungs indicated that the death was due to gavage error.

Clinical Signs:

There were no treatment-related clinical observations.

Body Weight and Feed Consumption:

Maternal body weight gains were decreased from GD7 to GD10 for Groups 4 and 5. This effect correlated with decreased food consumption for Group 5 dams.

Necropsy:
Evaluation of dams did not reveal treatment-related macroscopic findings.

Cesarean Section Data:
One Group 5 dam had a unilateral pregnancy consisting of only 3 live fetuses; the study director stated that because such events tend to skew the distribution of data, values for this dam and litter were excluded from data summarization and statistical analyses.

The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable (did not statistically differ) among the five dose groups and the values were within the historical control ranges for the testing facility. All placentae appeared normal. No dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses.

Offspring:
There were no developmental variations or malformations that were considered to be related to treatment. As shown in the sponsor-generated table below, there was a slight increase in incomplete ossifications of the sternal centra and/or pelvis for Groups 3, 4, and 5. However, the incidence of these findings was within the historical control data range, and therefore, the findings were not considered to be related to treatment.

DOSAGE GROUP E414 (b) (4) DOSAGE a,b		I 0 (VEHICLE)	II 500/ (b) (4)	III 750/ (b) (4)	IV 1000/ (b) (4)	V 1000/0
LITTERS EVALUATED	N	24	25	21	24	24
FETUSES EVALUATED	N	174	184	154	171	172
LIVE	N	174	184	154	171	172
STERNAL CENTRA SUMMARIZATION (Includes incompletely ossified and not ossified sternal centra):						
LITTER INCIDENCE	N(%)	1(4.2)	4(16.0)	2(9.5)	5(20.8)	2(8.3)
FETAL INCIDENCE	N(%)	1(0.6)	6(3.3)*	2(1.3)	10(5.8)**	2(1.2)
STERNAL CENTRA: 1ST, NOT OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	2(8.0)	0(0.0)	2(8.3)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	2(1.1)	0(0.0)	3(1.8)e	1(0.6)
STERNAL CENTRA: 1ST, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	1(4.2)	3(12.0)	2(9.5)	4(16.7)	0(0.0)
FETAL INCIDENCE	N(%)	1(0.6)	4(2.2)c	2(1.3)d	6(3.5)	0(0.0)
STERNAL CENTRA: 2ND, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	1(4.2)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)g	1(0.6)h

DOSAGE GROUP		I	II	III	IV	V
E414 (b) (4) DOSAGE a,b		0 (VEHICLE)	500/ (b) (4)	750/ (b) (4)	1000/ (b) (4)	1000/0
LITTERS EVALUATED	N	24	25	21	24	24
FETUSES EVALUATED	N	174	184	154	171	172
LIVE	N	174	184	154	171	172
PELVIS: SUMMARIZATION (Includes incompletely ossified and not ossified pubes and ischia):						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	2(9.5)	4(16.7)	2(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	3(1.9)	4(2.3)	2(1.2)
PELVIS: PUBIS, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	2(9.5)	4(16.7)	2(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	3(1.9)d	4(2.3)e-g	2(1.2)h,i
PELVIS: ISCHIUM, INCOMPLETELY OSSIFIED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	2(8.3)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)f	2(1.2)h,i

PERCENTAGES ARE BASED ON THE NUMBER OF LIVE FETUSES EVALUATED

a. E414 dosage was mg/kg/day and (b) (4) dosage was (b) (4)
b. Test and control articles were administered on days 7 through 17 of gestation.
c. Fetus 5126-1 had other skeletal alterations.
d. Fetus 5172-8 had other skeletal alterations.
e. Fetus 5176-15 had other skeletal alterations.
f. Fetus 5181-9 had other skeletal alterations.
g. Fetus 5187-1 had other skeletal alterations.
h. Fetus 5214-14 had other skeletal alterations.
i. Fetus 5223-7 had other skeletal alterations.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Study title: Oral development toxicity study of E414 (b) (4) and E414 in rabbits

Study number:	EMISTOX97008 (209256)
Study report location:	Module 4.2.3.5.1
Conducting laboratory:	(b) (4)
Date of study initiation:	10 October 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SNAC (E414 sodium salt), E414-38S1, 91.2% pure

Key Study Findings

- There were no treatment-related effects on maternal mortality or clinical signs.
- Maternal body weight gains for does receiving 1000 mg/kg/d SNAC (Group 5) were slightly less than controls from GD12 to GD15 and GD19 to GD24 (post-treatment period), but slightly higher between GD15 and GD19. Mean food consumption for does receiving 1000 mg/kg/d, with or without (b) (4), was slightly reduced during the treatment period, especially from GD6 to GD9.
- Increases in early and late resorptions as well as a slight increase in the incidence of verterbrae/rib malformations were observed for litters derived from does treated with 1000 mg/kg/d SNAC with (b) (4). Similar effects were not observed for the group treated with SNAC alone.
- The NOAELs for embryo-fetal survival, growth, and development were 1000 mg/kg/d SNAC and 500 mg/kg/d SNAC plus (b) (4).

Methods

Species/Strain: Rabbit/New Zealand White

Design:

Group	E414 Dosage (b) (4)	E414 Concentration (b) (4)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Numbers
I	0 (Vehicle Control)	0/0	10	20	6401 - 6420
II	250 (b) (4)	25 (b) (4)	10	20 + 3b	6421 - 6440, 6391 - 6393b
III	500	50	10	20 + 3b	6441 - 6460, 6394 - 6396b
IV	1000	100	10	20	6461 - 6480
V	1000/0	100/0	10	20	6481 - 6500

a. E414 was considered 100% active for the purpose of dosage calculations; (b) (4) dosage calculations were adjusted for the (b) (4) potency.
b. Female rabbits assigned to a satellite group.

Frequency of dosing:	Once daily from GD6 through GD18; C-section on GD29
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Reverse osmosis, deionized water
Satellite groups:	3 TK animals for Groups 2 and 3 only for assessment of SNAC concentration.
Dosing Solution Analysis:	All test article solutions were found to be within the acceptance criteria.
Protocol deviations:	No deviations occurred that affected the quality of integrity of the study.

Observations and Results

Mortality:

There were no treatment-related mortalities. One Group 4 doe died shortly after dosing on GD17 due to a gavage error. Other than findings in lungs consistent with a gavage error, no other macroscopic findings were noted for this doe. Body weight gain and food consumption were within normal ranges. The litter consisted of 10 apparently normal embryos.

Clinical Signs:

There were no adverse clinical observations that were attributed to the test article.

Body Weight:

Maternal body weight gains for the Group 5 does were statistically significantly lower than control between GD12 and GD15, higher between GD15 and GD19, and lower for the first interval of the post-dosing period (GD19 to GD24).

Feed Consumption:
Mean feed consumption for Group 4 and 5 does was slightly reduced during the treatment period, being most noteworthy on GD6 to GD9.

Necropsy:
Macroscopic evaluation of the does did not reveal any test article-related findings.

Cesarean Section Data:
One control group dam litter consisted of only 2 live fetuses; the study director stated that because such unusually small litters tend to skew the distribution of data, values for this dam and litter were excluded from data summarization and statistical analyses.

The mean litter size was slightly smaller and the incidence of early and late resorptions and percent resorbed conceptuses were higher for Group 4 does compared with control values (see sponsor-generated table below).

The litter averages for corpora lutea, implantations, fetal body weights, and percent live male fetuses were comparable among the five dose groups and were within the historical control ranges for the testing facility.

DOSAGE GROUP E414/ (b) (4) DOSAGE a,b		I 0 (VEHICLE CONTROL)	II 250/ (b) (4)	III 500/ (b) (4)	IV 1000/ (b) (4)	V 1000/0
RABBITS TESTED	N	20	20	20	20	20
INCLUDED IN ANALYSES	N	19c	18	20	19	20
CORPORA LUTEA	MEAN±S.D.	8.8 ± 1.2	9.7 ± 1.8	9.2 ± 1.6	9.6 ± 2.2	9.6 ± 1.8
IMPLANTATIONS	MEAN±S.D.	8.3 ± 1.2	9.1 ± 1.6	8.5 ± 1.6	9.0 ± 2.1	8.8 ± 1.9
LITTER SIZES	MEAN±S.D.	8.2 ± 1.1	8.7 ± 1.7	8.4 ± 1.5	7.8 ± 2.3	8.7 ± 2.0
LIVE FETUSES	N	155	156	167	148	173
	MEAN±S.D.	8.2 ± 1.1	8.7 ± 1.7	8.4 ± 1.5	7.8 ± 2.3	8.6 ± 1.9
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.4 ± 0.8	0.2 ± 0.4	1.2 ± 1.9	0.2 ± 0.4
EARLY RESORPTIONS	N	1	5	2	13	2
	MEAN±S.D.	0.0 ± 0.2	0.3 ± 0.6	0.1 ± 0.3	0.7 ± 1.4	0.1 ± 0.3
LATE RESORPTIONS	N	2	3	1	10	1
	MEAN±S.D.	0.1 ± 0.3	0.2 ± 0.4	0.0 ± 0.2	0.5 ± 1.2	0.0 ± 0.2
DOES WITH ANY RESORPTIONS	N(%)	3(15.8)	6(33.3)	3(15.0)	8(42.1)	3(15.0)
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.8 ± 4.2	4.9 ± 8.7	1.6 ± 3.8	12.4 ± 19.4	1.7 ± 4.2

Offspring:
No definitive treatment-related effects on embryonic development were identified. A slight increase in the incidence of vertebrae/rib malformations (hemivertebrae and split, fused, 11 present, absent, proximate, or thickened ribs) was observed for Group 4 fetuses compared with the incidence for the control group; however, the incidence of each individual finding was within the historical control range for the testing facility. The litter and fetal incidences exceeded the historical ranges of the testing facility for the composite fetal malformation (see sponsor-generated table below). Because an

increase in the incidence of these findings did not occur in Group 5, the effects are attributed to the combination of 1000 mg/kg/d SNAC with (b) (4) but not SNAC alone.

DOSAGE GROUP E414/ (b) (4) DOSAGE a,b		I 0 (VEHICLE CONTROL)	II 250/ (b) (4)	III 500/ (b) (4)	IV 1000/ (b) (4)	V 1000/0
LITTERS EVALUATED	N	20	18	20	19	20
FETUSES EVALUATED	N	157	156	167	148	173
LIVE	N	157	156	167	148	173
VERTEBRAL/RIB: MALFORMATIONS (SUMMARIZATION OF THORACIC VERTEBRAE: HEMIVERTEBRAE, SMALL ARCH, FUSED ARCHES, AND RIBS: SPLIT, FUSED, 11 PRESENT, ABSENT, BASE ABSENT AND PROXIMATE)						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	3(15.8)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	5(3.4)**	1(0.6)
THORACIC VERTEBRAE: HEMIVERTEBRAE						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(5.0)	3(15.8)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(0.6)	3(2.0)j,k	1(0.6)l
THORACIC VERTEBRAE: ARCH, SMALL						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m	0(0.0)
THORACIC VERTEBRAE: ARCHES, FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m	0(0.0)
RIBS: SPLIT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	1(5.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m	1(0.6)l
RIBS: FUSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	3(15.8)**	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	3(2.0)**j,k	0(0.0)
RIBS: 11 PRESENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(10.5)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	2(1.4)k,m	0(0.0)
RIBS: ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m	0(0.0)
RIBS: BASE, ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)m	0(0.0)
RIBS: PROXIMATE						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)	0(0.0)
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.7)j	0(0.0)
RIBS: THICKENED						
LITTER INCIDENCE	N(%)	2(10.0)	0(0.0)	1(5.0)	3(15.8)	0(0.0)
FETAL INCIDENCE	N(%)	2(1.3)n	0(0.0)	1(0.6)i	4(2.7)	0(0.0)

Toxicokinetics:

Plasma concentrations of SNAC at the designated time points after dosing are shown in the sponsor-generated tables below. AUC values were not calculated.

Gestation Day 6, 250 mg/kg/d SNAC plus [redacted] (b) (4) (Group 2)

Sample Time Hr : Min	Rabbit Number		
	6391	6392	6393
00:00	(b) (4)		
00:10			
00:30			
01:00			
02:00			
04:00			
08:00			

Gestation Day 6, 500 mg/kg/d SNAC plus [redacted] (b) (4) (Group 3)

Sample Time Hr : Min	Rabbit Number		
	6394	6395	6396
00:00	(b) (4)		
00:10			
00:30			
01:00			
02:00			
04:00			
08:00			

Gestation Day 18, 250 mg/kg/d SNAC plus [redacted] (b) (4) (Group 2)

Sample Time Hr : Min	Rabbit Number		
	6391	6392	6393
00:00	(b) (4)		
00:10			
00:30			
01:00			
02:00			
04:00			
08:00			

Gestation Day 18, 500 mg/kg/d SNAC plus [redacted] (b) (4) (Group 3)

Sample Time Hr : Min	Rabbit Number		
	6394	6395	6396
00:00	(b) (4)		
00:10			
00:30			
01:00			
02:00			
04:00			
08:00			

NQ = non-quatifiable; NR = non-reportable.

9.3 Prenatal and Postnatal Development

Study title: Segment III oral (gavage) prenatal and postnatal reproductive toxicity study with SNAC in rats

Study number:	EMISTOX980005 (209254)
Study report location:	Module 4.2.3.5.3
Conducting laboratory:	(b) (4)
Date of study initiation:	28 July 1998
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SNAC, E414-47S (CCI-468/97), 93.2% free acid

Key Study Findings

- One high dose dam (with (b) (4)) was found dead on gestation day 21. Other signs of maternal toxicity included excess salivation observed for all groups treated with SNAC, with or without (b) (4) slight decrease in maternal body weight gain (~11%) during gestation days 7-20 in the high dose group without (b) (4), and decrease in food consumption (6-16%) in the high dose group with and without (b) (4).
- A statistically significant, dose-related increase in the duration of gestation was observed in all drug-treated groups.
- Treatment with SNAC increased stillbirths with decreased live litter sizes at 750 mg/kg/d SNAC with (b) (4) and 1000 mg/kg/d SNAC with or without (b) (4). Increased pup mortality rate with decreased viability and lactation indexes was also observed, primarily at 1000 mg/kg/d SNAC with and without (b) (4)
- No necropsy observations for the F1 generation pups were attributable to the test article. There were no apparent effects of treatment on survival, clinical signs, or development of the F1 generation after birth. There were no treatment related changes for the F2 generation.
- The maternal NOEL was considered to be less than 500 mg/kg/d SNAC combined with (b) (4) due to an increased duration of gestation. The NOEL for viability and growth for the F_1 offspring was 500 mg/kg/d SNAC combined with (b) (4) due to increased incidence of stillbirths at ≥750 mg/kg SNAC. No teratogenic effects were observed.

Methods

Species/Strain:	Rat/Crl:CDBR VAF/Plus (Sprague-Dawley)
Study design (F0 generation):	(sponsor-generated table)

Dose Group	SNAC/ [redacted] Dosage	SNAC/ [redacted] Concentration	Dosage Volume (mL per kg)	Number of Female Rats	Assigned Fo Generation Rat Numbers
I	0 (Vehicle)	0	10	25	12601 - 12625
II	500 [redacted]	50 [redacted]	10	25	12626 - 12650
III	750 [redacted]	75 [redacted]	10	25	12651 - 12675
IV	1000 [redacted]	100 [redacted]	10	25	12676 - 12700
V	1000/0	100/0	10	25	12701 - 12725

Frequency of dosing: Once daily from GD7 through GD24 or LD20
Route of administration: Oral
Formulation/Vehicle: Water
Weight at study assignment: 211 to 233 g
Study design (F1 generation): (sponsor-generated table)

Maternal Dose Group	SNAC/ [redacted] Dosage	Assigned F1 Generation Rat Numbers	
		Male Rats	Female Rats
I	0 (Vehicle)	15401 - 15418; 4360[a]; 4367[a]; 15421 - 15425	15526 - 15544; 4369[a]; 15546 - 15550
II	500/ [redacted]	15426 - 15450	15551 - 15562; 4359[b]; 15564 - 15575
III	750/ [redacted]	15451 - 15475	15576 - 15600
IV	1000 [redacted]	15476 - 15500	15601 - 15625
V	1000/0	15501 - 15525	15626 - 15650

a. Rats 15419, 15420 and 15545 (all from Fo generation rat 12620) were replaced by rats 4360, 4367 and 4369, respectively, on postweaning day 1 because the dam (12620) was administered 500/ [redacted] dosage of the test articles on DL 7.
b. Rat 15563 was found dead on postweaning day 1 and was replaced by rat 4359 .

Deviation from study protocol: No deviations were identified that would affect the quality or integrity of the study.

Observations and Results

F_0 Dams

Survival:
One dam treated with 1000 mg/kg/d with [redacted] was found dead on GD21.

Clinical signs:
Excess salivation occurred at statistically significant incidences for all groups administered SNAC either with or without [redacted]. Excess salivation tended to occur

late in the gestation period and was generally first observed on GDs 16, 17, or 18. Red perivaginal substance was found on the first 2 days of the lactation period in dams treated with 1000 mg/kg/d SNAC with or without (b) (4).

Body weight:
There was a slight decrease in maternal body weight gain (~11%) during GDs 7-20 in the HD group without (b) (4) as compared to control.

Feed consumption:
A slight decrease in feed consumption was observed for the HD group with or without (b) (4) from GDs 7-20.

Delivery and pup viability:
The mean duration of gestation was slightly longer for groups that received SNAC. Treatment also increased stillbirths with decreased live litter sizes and increased pup mortality rate with decreased viability and lactation indexes (summarized in the sponsor-generated tables below).

TABLE B10 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP SNAC/(b) (4) DOSAGE a,b		I 0 (VEHICLE)	II 500 (b) (4)	III 750 (b) (4)	IV 1000 (b) (4)	V 1000/0
RATS ASSIGNED TO NATURAL DELIVERY	N	25	25	25	25	25
PREGNANT	N	24	25	24	24	24
DELIVERED LITTERS	N(%)	24(100.0)	25(100.0)	24(100.0)	23(95.8)c	24(100.0)
DAMS WITH STILLBORN PUPS	N(%)	5(20.8)	5(20.0)	6(25.0)	14(60.9)**	11(45.8)**
DURATION OF GESTATION IN DAYS d	MEAN±S.D.	22.7 ± 0.3 [21]	23.2 ± 0.4** [18]	23.4 ± 0.3** [17]	23.4 ± 0.3** [20]	23.4 ± 0.3** [21]
NUMBER OF DAMS DELIVERING						
DAYS 22.1 - 23.0	N(%)	17(81.0)	6(33.3)**	3(17.6)**	3(15.0)**	2(9.5)**
DAYS 23.1 - 24.0	N(%)	4(19.0)	12(66.7)**	14(82.4)**	17(85.0)**	19(90.5)**
DURATION OF GESTATION IN WHOLE DAYS e	MEAN±S.D.	22.2 ± 0.4	22.8 ± 0.4**	22.8 ± 0.4**	22.9 ± 0.3**	22.9 ± 0.3**
NUMBER OF DAMS DELIVERING						
DAY 22	N(%)	20(83.3)	6(24.0)**	4(16.7)**	3(13.0)**	3(12.5)**
DAY 23	N(%)	4(16.7)	19(76.0)**	20(83.3)**	20(87.0)**	21(87.5)**
GESTATION INDEX f	%	100.0	100.0	100.0	95.8	100.0
	N/N	24/ 24	25/ 25	24/ 24	23/ 24	24/ 24

DAY(S) = DAY(S) OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. SNAC dosage was mg/kg/day and (b) (4) dosage was (b) (4)
b. Dosage occurred on day 7 of gestation through day 20 of lactation.
c. Rat 12697 was found dead on day 21 of gestation.
d. Calculated as the time (to the nearest tenth of a day) elapsed between confirmed mating (arbitrarily defined as 0 hour) and the time the first pup was delivered. Excludes litters in which the delivery of the first pup was not observed.
e. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
f. Number of rats with live offspring/number of pregnant rats.
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE B11 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
SNAC (b) (4) DOSAGE a,b		0 (VEHICLE)	500 (b) (4)	750 (b) (4)	1000 (b) (4)	1000/0
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	25	24	23	24
PUPS DELIVERED (TOTAL)	N	338	337	338	302	322
	MEAN±S.D.	14.1 ± 1.1	13.5 ± 2.2	14.1 ± 2.0	13.1 ± 2.2	13.4 ± 1.7
LIVEBORN	MEAN±S.D.	13.7 ± 1.5	13.2 ± 2.2	13.2 ± 2.8	12.1 ± 2.7	12.4 ± 2.1
	N(%)	329(97.3)	331(98.2)	318(94.1)*	278(92.0)**	297(92.2)**
STILLBORN	MEAN±S.D.	0.4 ± 0.9	0.2 ± 0.5	0.8 ± 2.5	1.0 ± 1.4*	1.0 ± 1.5
	N(%)	9(2.7)	6(1.8)	19(5.6)*	24(7.9)**	24(7.4)**
UNKNOWN VITAL STATUS	N	0	0	1	0	1
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED						
DAY 1	N/N(%)	9/329(2.7)	1/331(0.3)*	1/318(0.3)*	16/278(5.8)**	6/297(2.0)
DAYS 2- 4	N/N(%)	9/320(2.8)	12/330(3.6)	6/317(1.9)	14/262(5.3)**	18/291(6.2)**
DAYS 5- 7	N/N(%)	1/311(0.3)	6/318(1.9)**	1/311(0.3)	3/248(1.2)*	0/273(0.0)
DAYS 8-14	N/N(%)	0/300(0.0)e	4/312(1.3)	1/310(0.3)	8/245(3.3)**	0/273(0.0)
DAYS 15-21	N/N(%)	0/300(0.0)e	0/308(0.0)	0/309(0.0)	0/237(0.0)	0/273(0.0)
VIABILITY INDEX c	%	94.5	96.1	97.8	89.2	91.9
	N/N	311/329	318/331	311/318	248/278**	273/297
LACTATION INDEX d	%	99.7	96.8	99.4	95.6	100.0
	N/N	300/301e	308/318**	309/311	237/248**	273/273

DAY(S) = DAY(S) POSTPARTUM
a. SNAC dosage was mg/kg/day and (b) (4) dosage was (b) (4)
b. Dosage occurred on day 7 of gestation through day 20 of lactation.
c. Number of live pups on day 4 postpartum/Number of liveborn pups on day 1 postpartum.
d. Number of live pups on day 21 postpartum/Number of live pups on day 4 postpartum.
e. Excludes values for litter 12620, the dam was misdosed on day 7 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

TABLE B11 (PAGE 3): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV	V
SNAC/ (b) (4) DOSAGE a,b		0 (VEHICLE)	500 (b) (4)	750 (b) (4)	1000 (b) (4)	1000/0
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	25	24	23	24
LIVE LITTER SIZE AT WEIGHING						
DAY 1	MEAN±S.D.	13.3 ± 2.5	13.2 ± 2.3	13.2 ± 2.8	11.5 ± 3.5	12.2 ± 2.3
DAY 4	MEAN±S.D.	13.5 ± 1.7 [21]c,d	12.6 ± 2.2 [23]d	13.0 ± 3.1	11.3 ± 3.5 [22]c	11.3 ± 2.4** [23]d
DAY 7	MEAN±S.D.	13.5 ± 1.6 [23]c	12.5 ± 2.3	12.9 ± 3.1	11.1 ± 3.4* [22]c	11.4 ± 2.4**
DAY 14	MEAN±S.D.	13.6 ± 1.4 [22]c,e	12.3 ± 2.2	12.9 ± 3.0	10.8 ± 3.6** [22]c	11.4 ± 2.4**
DAY 21	MEAN±S.D.	13.6 ± 1.4 [22]c,e	12.3 ± 2.2	12.9 ± 3.0	10.8 ± 3.6** [22]c	11.4 ± 2.4**
PUP WEIGHT/LITTER (GRAMS)						
DAY 1	MEAN±S.D.	6.1 ± 0.4	6.2 ± 0.5	6.3 ± 0.4	6.3 ± 0.4 [22]c	6.3 ± 0.4
DAY 4	MEAN±S.D.	7.6 ± 0.8 [21]c,d	8.1 ± 1.0 [23]d	8.2 ± 0.9	8.2 ± 0.8 [22]c	8.3 ± 0.7 [23]d
DAY 7	MEAN±S.D.	10.6 ± 1.3 [23]c	11.2 ± 1.9	11.4 ± 1.4	11.2 ± 1.6 [22]c	11.7 ± 1.7
DAY 14	MEAN±S.D.	21.5 ± 2.3 [22]c,e	23.4 ± 3.1	22.4 ± 2.8	22.8 ± 3.0 [22]c	23.5 ± 3.1
DAY 21	MEAN±S.D.	33.0 ± 3.4 [22]c,e	36.6 ± 5.1*	34.8 ± 4.4	36.4 ± 4.6* [22]c	36.9 ± 5.1**

DAY = DAY POSTPARTUM
[ ] = NUMBER OF VALUES AVERAGED
a. SNAC dosage was mg/kg/day and (b) (4) dosage was (b) (4)
b. Dosage occurred on day 7 of gestation through day 20 of lactation.
c. Excludes values for litters that had no surviving pups.
d. Excludes values that were not recorded.
e. Excludes values for litter 12620, the dam was misdosed on day 7 of lactation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

F1 Generation:
No clinical observations in the F1 generation pups were attributed to the test article. No necropsy observations for the F1 generation pups were attributable to the test article. There were no apparent effects of treatment on survival or clinical signs of the F1 generation after birth. There were no effects on the timing of vaginal patency or preputial separation in the F1 generation rats. There were no biologically important differences in the values for learning, short-term retention, long-term retention, response inhibition, or watermaze performance.

Caesarean-sectioning (C-sectioning) observations were based on 24, 21, 20, 20, and 24 pregnant rats in the five respective dose groups. No C-sectioning or litter parameters for pregnant F1 dams were affected by administration of SNAC to the F0 generation dams as high as 1000 mg/kg/day alone or in combination with (b) (4)

F2 Generation:
Fetal evaluations were based on 342, 283, 302, 259, and 306 live, C-section delivered fetuses (GD20) in the five respective dose groups. Each fetus was examined from gross external alterations. There were no fetal malformations or effects on survival attributed to treatment of the F0 generation.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Semaglutide, a GLP-1 receptor agonist, is being developed for the treatment of type 2 diabetes through both the subcutaneous and oral routes of administration. A complete nonclinical toxicology program is being conducted for the subcutaneous formulation under IND 79754 and the sponsor is relying on that data to support the safety assessment of orally administered semaglutide. To allow for the oral administration of a peptide, semaglutide is formulated with a novel excipient, SNAC. SNAC is an absorption enhancing carrier molecule used to protect peptides against enzymatic and gastric acid degradation and to facilitate increased transepithelial penetration. Because SNAC is a novel excipient, a complete toxicology program is being conducted for this excipient, tested either alone or with semaglutide. Several of the toxicology studies for SNAC were conducted while formulated with (b) (4) In each of the studies, at least one dose level of SNAC alone was included. For the purpose of this IND, findings that are thought to be attributed to (b) (4) are not discussed here.

The ability of SNAC to allow for the absorption of pharmacologically active semaglutide via the oral route was evaluated in primary pharmacology studies. When orally administered with SNAC, a single administration of semaglutide to male db/db mice was

sufficient to lower blood glucose levels from baseline and during an IP glucose tolerance test. A single dose of oral semaglutide formulated in tablets had a dose-related decrease in food intake and body weight gain. These results indicate that when orally administered with SNAC, a sufficient amount of biologically active semaglutide is absorbed from the gastrointestinal tract for pharmacodynamic activity. The results of in vitro receptor binding assays did not suggest that SNAC has biologically meaningful interactions with the receptors included in these assays. In vitro and ex vivo transport assays indicated that SNAC causes a clear size-dependent enhancement in the absorption of dextran, with a diminished effect on transport as the size of the molecules exceed 4 kD, the approximate size of semaglutide; the ability of SNAC to enhance the absorption of molecules of 70 kD or greater is minimal. SNAC was also shown to elicit a significant increase in the trans-epithelial permeation of semaglutide across Caco-2 monolayers. The data from this study indicated that semaglutide permeates the cellular layer via transcellular transport rather than via a paracellular mechanism.

Bioavailability of semaglutide formulated as oral tablets in dogs ranged from 0.2% to 0.6%, with the amount of SNAC being 150, 300, or 600 mg and semaglutide remaining constant at 10 mg. The ratio of 300 mg and 10mg semaglutide gave the greatest bioavailability, although great inter-animal variation was observed. When 300 mg SNAC was formulated with 20 mg semaglutide, bioavailability was increased to 1.4%. Three male Cynomolgus monkeys administered an oral tablet containing 450 mg SNAC and 15 mg semaglutide (mean dose of 4.5 mg/kg semaglutide) had an oral bioavailability ranging from 0.07% to 0.3%, compared with intravenous dosing. Systemic exposure to SNAC was generally higher in fasted rats compared with fed animals.

Using Caco-2 and MDCKII-BCRP cells, it was shown that SNAC inhibited human transporters P-gp, BCRP, OATP1B1, OAT1, and OAT3 by more than 50% resulting in IC_{50} values of 2620, 145, 68, 28, and 5 µM, respectively. The elimination half-life of SNAC after oral administration was approximately 1.4, 2.7, 1.0, and 2.5 hours in CD1 mice, Wistar rats, beagle dogs, and Cynomolgus monkeys, respectively. Plasma protein binding was approximately 84% in mice, 90% in rats and rabbits, and 98% in monkeys and humans; nearly all binding appeared to be with serum albumin.

In mice, it was demonstrated that SNAC is readily absorbed from the gastrointestinal tract and distributed to the liver. Major organs of distribution in rats included kidney, bile ducts, blood, and liver. High levels of radioactivity in the gallbladder are indicative of biliary secretion. This observation, in association with a small amount of radioactivity present in feces in the rectum, suggests enterohepatic recirculation of SNAC and/or its metabolites. Data from bile-duct cannulated rats also demonstrated evidence for enterohepatic recirculation. High levels of radioactivity were detected in the kidney in both sexes and the urinary bladder and urine in males, indicating that the major route of elimination of radioactivity is through the kidney. Excretion studies in mice and rats confirmed that SNAC and its metabolites are primarily excreted in urine, with a minor fraction in feces. Clinical data have shown SNAC is also primarily eliminated by urinary excretion in humans.

In an in vitro study, SNAC was found to directly inhibit CYP2C19 and UGT1A9 with approximately 25% and 33% inhibition, respectively, at the highest concentration tested (100 μM). SNAC was also shown to be a weak inhibitor of CYP3A4/5. Plasma concentrations of SNAC in humans are estimated to be approximately 3 μM based on a C_{max} of 980 ng/mL. Therefore, it is unlikely that the inhibition observed in these assays will translate into drug-drug interactions through hepatic CYP450 metabolism in vivo. One SNAC metabolite (NNC0113-3705, E1245) was found to directly inhibit CYP1A2, CYP2B6, CYP2C8, and CYP2C19 by ~38%, 37%, 48%, and 32% at 100 μM, yielding IC_{50} values of greater than 100 μM. Using freshly isolated hepatocytes, SNAC was considered to be a weak inducer of CYP2C9 and CYP1A2, whereas SNAC showed an ability to decrease CYP3A4/5 activity. No significant induction of P450 enzymes was observed in human hepatocytes after incubation with the five primary SNAC metabolites.

Using liver S9 fractions, it was shown that the rate of SNAC disappearance by Phase II metabolism among the 6 species tested could be ranked from highest to lowest as: Rhesus monkey ~ Cynomolgus monkey > mouse > human > rat > rabbit. No biotransformation of SNAC occurred when incubated with Phase 1 reaction mixtures. When incubated with primary hepatocytes, SNAC substrate disappearance was greatest for rat, followed by monkey and then human. The metabolite profile was similar between monkeys and humans, with 5 primary metabolites identified. Only two of the five metabolites were observed in rats. The M4 metabolite was most abundant for rat and human whereas the M1 metabolite was most abundant for monkey. In all three species, SNAC went through two levels of β-oxidation with no apparent metabolism by Phase I reactions. After oral administration to rats, ^{14}C-SNAC was extensively metabolized, with the parent compound present as a minor component of the profile in plasma, urine, bile, and feces. In plasma the predominant metabolite was E506, which accounted for approximately 55% of the total drug related exposure (AUC) in male rats and 71% in female rats. All other detected metabolites each accounted for less than 10% of total drug related exposure. In monkeys, the metabolic profiles were similar in plasma and urine. Up to 5 metabolites were detected. Two of these had the same retention time as E506 and E494. The results indicated that SNAC was metabolized by β-oxidation to metabolites E506 and E494. The parent compound and these two hydroxybenzamides were conjugated with glucuronic acid. The human metabolic profile of SNAC was characterized in clinical study 259252. In addition to the metabolites that were also identified in rats, one minor unique metabolite (a third beta-oxidation product obtained from three successive beta-oxidation steps) was found in plasma.

In CNS safety pharmacology studies in rats, a single oral dose of SNAC led to significant depressant effects on respiration, piloerection, decreased touch response, and death at ≥1000 mg/kg. Clinical signs for all surviving animals were normal within 24 hours of dosing. The lungs of decedent animals showed slight reddening with small hemorrhagic areas. No significant CNS effects were noted at ≤750 mg/kg. In vitro studies showed no indication of biologically meaningful inhibition on 12 different cardiac

ion channels, including hERG, at concentrations up to 500 μM or 1 mM (hERG tail current assay). In a cardiovascular and respiratory safety pharmacology study, Sprague-Dawley rats treated with 900 or 1500 mg/kg/d SNAC resulted in mortality for 5/16 animals that was associated with marked decreases in diastolic arterial pressure. Piloerection and tachypnea occurred on several occasions in surviving animals at both dose levels. Surviving animals were also noted with decreased diastolic and mean arterial pressures, increased heart rate, and increased respiratory rates for approximately 2 to 3 hours after dosing. Increased respiratory rates were associated with increased mean tidal and minute volume and decreased mean inspiratory and expiratory times. ECG measurements showed an increase in QT and QTc values by up to 18 ms (Bazett) or 28 ms (Fridericia) for up to 4 hours after dosing. Increases in atrial (pause and premature beat) and junctional (salvo) arrhythmias were observed at 900 mg/kg and increases in atrial (premature beat), ventricular arrhythmias (beat), junctional (beat), and other arrhythmias were observed at 1500 mg/kg/d. Decreased mean body temperature was also noted for 2 to 3 hours after dosing. Mean C_{max} values on Day 1 were 373,274 ng/mL at 900 mg/kg/d and 358,703 mg/kg/d at 1500 mg/kg/d, suggesting absorption saturation at ≥900 mg/kg/d. In a second study in Sprague-Dawley rats, no effects on respiration were noted at doses up to 1000 mg/kg SNAC; however, C_{max} values were not reported. Decreased activity was observed in animals receiving 750 mg/kg and one death occurred in the 1000 mg/kg group; noteworthy effects were not observed at 500 mg/kg. In Cynomolgus monkeys administered a single oral dose of up to 600 mg/kg SNAC, no effects on clinical signs, body weight, food consumption, body temperature, heart rate, mean arterial blood pressure, or ECG intervals (including QTc) were observed for up to 36 hours after dosing. The mean plasma concentration of SNAC at 600 mg/kg 2 hours after dosing was 18,123 ng/mL.

Single dose toxicology studies in CD-1 mice and Sprague-Dawley rats resulted in deaths at 2000 mg/kg and ≥1500 mg/kg, respectively. Adverse clinical signs in rats included weak reflexes, salivation, lethargy, twitching, ataxia, sedation, and hunched posture. Enlarged atria/hearts were noted at necropsy at ≥1000 mg/kg. In a second study in rats, mortality occurred at ≥900 mg/kg accompanied by tonic or aphyxial convulsions, abnormal gait, abnormal body carriage, hunched posture, shuffling, abnormal respiration, and increased pupil diameter. Statistically significant increases in blood levels of pCO2, pO2, oxygen saturation, blood pH, bicarbonate, and base excess occurred between 20 and 60 minutes after dosing. Stomach irritation was observed at necropsy. TK data were not collected in these studies.

Repeat-dose toxicology studies have been conducted with SNAC in mice, rats, and monkeys. The primary findings in a 13-week mouse study were mortality and decreased erythrocyte parameters with increased reticulocytes at 1500 mg/kg/d (mean C_{max} values on Day 1 were 2770 and 4340 ng/mL for males and females, respectively [first time point at 1 hour post dose]). In a 2-week Sprague-Dawley rat study, increased mortality was observed at ≥1000 mg/kg/d, which was associated with impaired equilibrium and hypoactivity prior to death. In a 3-month study in Wistar rats, no treatment-related deaths occurred at doses up to 1000 mg/kg/d, resulting in mean C_{max} values of 15,000 and 41,400 for males and females, respectively.

In 6- and 12-month Sprague-Dawley rat studies, the most noteworthy toxicity noted for SNAC was increased mortality at 900 mg/kg/d and one death at 300 mg/kg/d (mean C_{max} of 1550 ng/mL). Generally, the cause of death was not determined and there were no apparent adverse clinical signs prior to the time the animal was found dead. There were small but statistically significant changes in some clinical pathology parameters, primarily at ≥300 mg/kg/d in the 6-month study, which were not considered to be dose limiting effects. Necropsy findings included a dose-related increase in mean kidney weight at ≥300 mg/kg/d. A slight increase in incidence and severity of microscopic lung findings (prominent number of alveolar macrophages, alveolitis, perivascular lymphoid aggregates, and increased cellularity of BALT) was observed for all treatment groups in the 6-month study, although not in a dose-dependent manner. These findings were not noted in the 12-month study. An increase in epithelial hyperplasia of the limiting ridge of the stomach was noted at all male doses in the 6-month study. In the 12-month study, there were macroscopic (foci of glandular stomach) and microscopic findings noted in the stomach at the end of the treatment period for males treated with 900 mg/kg/d and females treated at ≥500 mg/kg/d. Microscopic findings noted in the stomach were generally minimal to mild and included hyperplasia of the nonglandular stomach; eosinophilic blebs in the keratin layer; erosive inflammation; and hemorrhage. Hyperplasia of the nonglandular stomach was also noted at the 6-month interim time point. The NOAEL for the 6-month study was considered to be 90 mg/kg/d SNAC because of a single male death at 300 mg/kg/d that was possibly related to SNAC treatment. TK data could not be modeled at this dose level due to incomplete data. At the LOAEL, the mean C_{max} and $AUC_{0\text{-}24h}$ values at Week 26 were 1585 ng/mL and 11,635 ng•h/mL, respectively. Note that the exposure values for this study are underestimated because the first TK time point was 2 hours after dosing and T_{max} is around 10 minutes. In the 12-month study, mortalities were only observed at 900 mg/kg/d; the NOAEL for this study was 500 mg/kg/d, resulting in mean C_{max} and $AUC_{0\text{-}4h}$ values of 154,000 ng/mL and 41,050 ng•h/mL at Week 52, respectively. The first TK time point for this study was 10 minutes after dosing, which is why the C_{max} values are much higher than for the 6-month study.

Monkeys treated with ≥1000 mg/kg/d SNAC for 2 or 4 weeks showed signs of hypoglycemia (reduced motor activity, transitory recumbency, ptosis), progressing to loss of consciousness, no response to pain, severe hypothermia, and death/moribund sacrifice for some animals. Mean C_{max} values on Day 14 were approximately 110,000 ng/mL. No treatment-related microscopic lesions were identified. The NOAEL was determined to be 800 mg/kg/d. In a 13-week study in monkeys, signs of hypoglycemia (somnolence, hypoactivity, incoordination, and prostration) were noted at ≥800 mg/kg/d requiring intravenous administration of 50% dextrose. In a 9-month monkey study, signs of hypoglycemia were occasionally observed at ≥500 mg/kg/d, sometimes requiring supplemental food (fruit) and/or an infusion of 50% dextrose. No definitive treatment-related adverse findings were noted upon microscopic examination. A slight imbalance in minimal, multifocal mineralization of brain parenchyma and minimal interstitial hemorrhage of the thymus observed for the groups receiving 500 and 600 mg/kg/d SNAC. The pathologist did not feel that the findings were related to treatment.

However, in the absence of historical control data for these findings, a possible treatment-related effect cannot be ruled out. The NOAEL for this study was determined to be 300 mg/kg/d, resulting in mean C_{max} and AUC_{0-8h} values of 38,450 ng/mL and 48,500 ng•h/mL on Day 1 and 17,450 ng/mL and 34,150 ng•h/mL on Day 180, respectively. A summary of NOAEL values and associated exposures for the general toxicology program is presented in sponsor-generated Table 2 below.

The toxicity profile of semaglutide after oral administration was evaluated in a 6 month study in Sprague-Dawley rats at doses up to 60/900 mg/kg/d semaglutide/SNAC. There were no mortalities or adverse clinical signs attributed to semaglutide. Mean final body weights were lower than controls for males at ≥20/300 mg/kg/d (↓10%-21%) and females at 60/900 mg/kg/d (↓16%). Effects on body weight correlated with decreased food consumption and body weights rebounded during the recovery period. Other treatment-related findings that were attributed to semaglutide included an increase in absolute and relative adrenal gland weights in males and females at ≥20/300 mg/kg/d and an increased incidence of distention of the duodenum, reduction in adipose tissue, and thin uterus for HD females. Minimal hypertrophy of Brunner's gland in the duodenum was noted for males at ≥20/300 mg/kg/d and minimal to mild hypertrophy in females at 6/90 mg/kg/d. [note: hypertrophy of Brunner's gland was also observed after SC administration of semaglutide, so this does not appear to be a local effect due to oral dosing]. The NOAEL for the combination of semaglutide and SNAC was 20/300 mg/kg/d because of the deaths occurring at 60/900 mg/kg/d that were likely related to SNAC treatment (several deaths occurred in the 900 mg/kg/d SNAC alone group). The mean AUC_{0-24h} for semaglutide at the 60/900 mg/kg/d dose level during Week 26 was 3,695 nM•h.

A 6-week oral toxicology study was conducted in Cynomolgus monkeys at doses up to 10/157 mg/kg/d semaglutide/SNAC. Noteworthy effects attributed to semaglutide included decreased body weight gain, a slight increase in heart rate, increased incidence of small thymus, and a slight increase in the incidence of Brunner's gland dilatation/eosinophilic cytoplasm. The mean AUC_{0-24h} value for the high dose level of semaglutide on Day 42 was 4,014 nM•h. A 17-week study was also conducted in Cynomolgus monkeys, although the formulation used in this study included the (b) (4) rather than SNAC. The high-dose level was 20/300 mg/kg/d semaglutide/(b) (4). Body weight loss or a reduction in body weight gain occurred throughout the treatment period for animals receiving semaglutide, but was greatest during the titration period at 10 mg/kg/day. Body weights rebounded during the recovery period. There were no toxicologically meaningful changes in clinical chemistry, gross pathology, organ weights, or histopathology. No adverse effects within the gastrointestinal tract were observed. The NOAEL for oral semaglutide was considered to be 20 mg/kg/day when administered with 300 mg/kg/day (b) (4) resulting in a mean semaglutide AUC_{0-24h} value of 4,325 nM•h on Day 1 and 2,580 nM•h on Day 119.

SNAC did not exhibit mutagenic or clastogenic activity in standard in vitro and in vivo genetic toxicology assays. Mouse and rat 2-year carcinogenicity studies are currently ongoing.

A complete developmental and reproductive toxicology program has been conducted for SNAC. In the Segment 1 fertility study in Sprague-Dawley rats, no effects on mating or fertility indices for males or females were observed. No effects on sperm motility, morphology, or number were noted. No effects on implantations, resorptions, fetal weight, fetal sex ratios, or fetal development were observed at any dose level. The NOAEL for mating, fertility, and early embryonic development was 1000 mg/kg/d SNAC.

In a Segment 2 embryonic development study in rats, no treatment-related effects on maternal mortality, clinical signs, or C-section parameters were observed. A decrease in maternal body weight gain was observed between GD7 and GD10 for dams receiving 1000 mg/kg/d SNAC. The NOAEL for embryo-fetal viability, growth, and development was 1000 mg/kg/d, the highest dose tested. In a Segment 2 embryonic development study in rabbits, there were no treatment-related maternal mortalities or adverse clinical signs. Maternal body weight gains for does receiving 1000 mg/kg/d SNAC were slightly less than controls from GD12 to GD15 and GD19 to GD24 (post-treatment period), which correlated with a slight reduction in mean food consumption during the treatment period, especially from GD6 to GD9. No SNAC-related fetal malformations were observed. The NOAEL for embryo-fetal survival, growth, and development was 1000 mg/kg/d SNAC.

In a Segment 3 peri- and post-natal study in rats, excess salivation was noted in dams at ≥500 mg/kg/d SNAC and slight decreases in maternal body weight gain (~11% less than control) from GD7 to GD20 at 1000 mg/kg/d, which correlated with a slight decrease in food consumption. A statistically significant, dose-related increase in the duration of gestation was observed in all drug-treated groups (≥500 mg/kg/d SNAC). Treatment with SNAC increased stillbirths with decreased live litter sizes at ≥750 mg/kg/d SNAC. Increased pup mortality rate with decreased viability and lactation indexes was also observed, primarily at 1000 mg/kg/d SNAC. No necropsy observations for the F1 generation pups were attributed to the test article. There were no apparent effects of treatment on survival, clinical signs, or development of the F1 generation after birth. There were no treatment-related changes for the F2 generation. The maternal NOAEL was considered to be less than 500 mg/kg/d SNAC due to an increased duration of gestation. The NOAEL for viability and growth for the F_1 offspring was 500 mg/kg/d SNAC due to increased incidence of stillbirths at ≥750 mg/kg SNAC. No teratogenic effects were observed.

Table 2 NOAEL and mortality in oral toxicity studies with SNAC

Study duration	Study no.	NOAEL (mg/kg/day)	Lowest Dose with Possibly Treatment Related Death (mg/kg/day)	Exposure AUC [a] (h×ng/ml)	Exposure C_{max} [b] (ng/ml)
Mouse					
Single Dose	EMIS/R96007	1500	2000	n.a.	n.a.
13 weeks	A16705	500	1000	M: 6933 F: 8450	M: 2636 F: 1913
Rat					
Single Dose	EMIS/R96009	1000	1500	n.a.	n.a.
14 days	(b) (4)315002	Not established	2334	n.a.	n.a.
14 days	315004	1000 [c]	None observed	n.a.	n.a.
13 weeks	A62807	1000 [c]	None observed	M: 54800 F: 100500	M: 10400 F: 35250
13 weeks	(b) (4)315003	Not established [d]	2000	n.a.	n.a.
26 weeks	JLY0278	90	300	n.a.	n.a.
52 weeks	BNA00004	500	750	M: 41425 F: 31450	M: 127175 F: 118725
2 years (Week 52 data)	JLY0366	N/A[g]	200	M: 3420 F: 9070	M:1780 F: 27400
Non-human primates					
14 days	3007-97	Not established	1000 [f]	316000	185000
28 days	694-95	Not established [d]	1200 [f]	n.a.	n.a.
13 weeks	3060-98	Not established	None observed	n.a.	n.a.
39 weeks	BNA00003	600 [c]	None observed	M: 136333 F: 129367	M: 100733 F: 79700

[a] Average $AUC_{0\text{-}x\ h}$ over all time points at which TK was assessed in each respective study, where x = 24 h for studies A16705 and A62807, x = 4 h for study no. 211504 and x = 8 h for study no 211503 (data from Table 7.5). Because $t_{1/2}$ is generally in the range from 1 to 3 h across species, the calculated AUC values represents most of the $AUC_{0\text{-}24h}$. [b] Average C_{max} over all available time points in respective study; [c] Highest dose tested; [d] Doses below lowest dose causing mortality not tested; [f] Dosed together with (b) (4); effect of (b) (4) cannot be excluded; [g] NOAEL not yet assessed as study in on-going, n.a.: not available. (sponsor-generated table)

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/s/

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04/02/2014

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