CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208032Orig1s000

PHARMACOLOGY 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

Application number:	208032
Supporting document/s:	1, 33, and 34
Applicant's letter date:	05/29/2015, 5/24/2016, and 6/13/2016
CDER stamp date:	05/29/2015, 5/24/2016, and 6/13/2016
Product:	Kovanaze® (Tetracaine HCl & Oxymetazoline HCl)
Indication:	Regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J
Applicant:	St. Renatus, LLC
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)
Reviewer:	Z. Alex Xu, PhD
Team Leader:	Jay H. Chang, PhD
Supervisor:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Mavis Darkwah, PharmD

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208032 are owned by St. Renatus, LLC or are data for which St. Renatus, LLC has obtained a written right of reference.
Any information or data necessary for approval of NDA 208032 that St. Renatus does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208032.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Kovanaze™ Nasal Spray is a fixed-combination drug formulation containing two active ingredients including 3% tetracaine hydrochloride (HCl) and 0.05% oxymetazoline HCl. Oxymetazoline is a vasoconstrictor (b) (4) The drug product is provided in a single-use, pre-filled nasal spray system that delivers 0.2 mL (containing 6 mg tetracaine HCl and 0.1 mg oxymetazoline HCl). The Applicant is seeking approval of Kovanaze for regional anesthesia when performing a dental restorative procedure on Teeth 4-13 and A-J. The recommended doses for adults and children are summarized in the Applicant's table below. The maximum daily dose for adults is 3 sprays (0.6 mL) containing 18 mg tetracaine and 0.3 mg oxymetazoline. (b) (4)

Age Group	Dose	Total Tetracaine HCl Content	Total Oxymetazoline HCl Content
Adults (≥ 18 years old)	2 sprays (0.2 mL per spray)	12 mg	0.2 mg
	1 additional spray (0.2 mL) if adequate anesthesia to initiate the dental procedure has not been achieved 10 minutes after the second spray	6 mg	0.1 mg
(b) (4)			
Children (b) (4) weighing ≥ 40 kg)	2 sprays (0.2 mL per spray)	12 mg	0.2 mg

Both tetracaine and oxymetazoline were approved by the Agency previously. Oxymetazoline was initially approved in 1964 (Afrin Nasal Spray; NDA 14717). An over-the-counter (OTC) monograph for oxymetazoline nasal solution was finalized in 1994. Tetracaine has been on the U.S. market since the 1930s. Tetracaine was approved by the Agency for local dermal anesthesia in combination with lidocaine in the Synera topical patch (70 mg lidocaine and 70 mg tetracaine; NDA 21623) and Pliaglis cream (7% lidocaine and 7% tetracaine; NDA 21717). However, tetracaine for intranasal administration has not been approved. During drug development, the Agency concluded that nonclinical studies were not needed prior to initiation of clinical studies due to the long history of use for both tetracaine and oxymetazoline; however, nonclinical studies were required for the NDA. The Applicant submitted the NDA as a 505(b)(2) referencing the final OTC monograph for oxymetazoline, and scientific literature providing relevant nonclinical pharmacological, pharmacokinetic, and toxicological data for oxymetazoline or tetracaine. In addition, the Applicant has a Letter of Authorization to cross-reference all relevant clinical or nonclinical information in NDA 21623 for Synera. The Applicant also conducted additional multiple nonclinical studies for the NDA.

1.2 Brief Discussion of Nonclinical Findings

General toxicity studies

The purpose of the 2-week study in dogs was to investigate the potential systemic and local toxic effects following co-administration of oxymetazoline and tetracaine via intranasal spray using a mucosal atomization device to mimic human dosing. The dosing levels were 0 (vehicle), 0.01/0, 0.01/0.6, 0.01/1.5 and 0.01/3.0 mg/kg/day of oxymetazoline/tetracaine (oxy/tetra). The vehicle control was similar to the clinical vehicle formulation as it contained the same concentration of benzyl alcohol and hydroxyethyl cellulose. The concentrations of oxymetazoline and tetracaine were 0.05% and 0.3%, respectively, the same as those in the clinical formulation. However, unlike the clinical formulation, the oxymetazoline solution and tetracaine solution were not mixed before administration. Oxymetazoline solution was sprayed first and immediately followed by different volumes of tetracaine solution (0.02, 0.05, 0.10 mL from low to high dose, respectively). The tetracaine doses provided 1.1, 2.8, and 5.6-fold margins for the maximal human dose, respectively, while the fixed oxymetazoline dose provided 1.1-fold margin based on body surface area (mg/m^2) comparison.

Oxymetazoline and tetracaine co-administration produced clinical signs including vocalization, salivation, nasal discharge, vomiting, soft and mucoid stools, red and swollen pinna shivering, shallow or labored breathing, impaired mobility, and wobbly gait primarily at the high-dose (0.01/3.0 mg/kg oxy/tetra) in a tetracaine dose-dependent manner. The clinical changes occurred immediately following dosing and recovered quickly on the same day, consistent with exposure profiles of tetracaine and oxymetazoline. These are common CNS changes associated with tetracaine or oxymetazoline as seen in publications of tetracaine studies in various animal models and an embryofetal dose range-finding study in nonpregnant female rats. ECG examination did not show significant adverse changes observed in any dosing group on Study Day (SD) 1, SD 14, and SD 28. There were no other treatment-related systemic toxic effects observed after 14 days of tetracaine and oxymetazoline administration. Tetracaine administration with the presence of 0.01 mg/kg oxymetazoline produced reversible cell infiltration and squamous metaplasia in the nasal cavity at ≥ 1.5 mg/kg in a dose-dependent manner after 14 days of consecutive daily dosing. These changes were likely to be due to repeated test article administration, and are not expected to occur following a single-use application for this indication.

The NOAEL was identified to be 0.01/0.6 oxy/tetra, the lowest combination dose tested. However, the high-dose may be considered an acceptable LOAEL (aLOAEL) since both the systemic and local findings were of minimal severity, reversible, and potentially monitorable clinically. Tetracaine exposure achieved in dogs at the NOAEL provided a sufficient safety margin to support the safety of the maximum recommended human dose (MRHD) (see table below). The average C_{max} and AUC in males and females on SD 14 was 570 ng/mL and 145 ng•h/mL, respectively, at the NOAEL. In comparison, the tetracaine plasma concentration was generally undetectable in the clinical studies after Kovanaze treatment at 18 mg or higher. At the NOAEL, the systemic exposure to p-butylamino benzoic acid (PBBA), a major human tetracaine metabolite, did not cover

the human exposure as shown below. In clinical studies, PBBA is used as a marker for tetracaine exposure when tetracaine plasma concentration is too low to be detected. In this dog study, the 0.01/3.0 mg/kg oxy/tetra dosing level provided a 1-fold exposure margin for PBBA compared to the MRHD by AUC comparison. Note that adequate safety margins for PBBA were established based on the aLOAEL of the 2-week dog study and the reproductive NOAELs from the reproductive and developmental toxicology studies, and the available genotoxicity data with tetracaine suggest that it is not mutagenic. Therefore, the metabolite is considered adequately qualified for safety from a systemic perspective. However, there are no nonclinical data to support the local tissue toxicity of PBBA in the nasal passages. In addition, there are no data available that compare the metabolic conversion rate of tetracaine to PBBA in the nasal mucosa between animal species and human. (b) (4) The lack of nonclinical support was discussed with the Applicant during the course of the review. Review of the clinical batches used in the clinical local tissue assessments that included evaluation of olfactory function indicate that the levels of up to 1.8% were tested clinically and there was no indication of differential adverse effects to the local tissue or olfactory function with these batches compared to batches with lower levels of PBBA (see the medical officer review). Taking into consideration the possibility for excursions in storage temperature, our chemistry, manufacturing, and controls (CMC) review team have concluded that, assuming the clinical safety assessment is adequate, a specification of NMT (b) (4) % with an (b) (4) month shelf-life could be supported (see the CMC review).

For oxymetazoline, the 2-week dog study did not establish a sufficient systemic safety margin for the MRHD by either C_{max} or AUC comparison as shown below. However, at the LOAEL (0.01/3.0 mg/kg oxy/tetra), the average C_{max} and AUC of oxymetazoline was 2.95-, and 0.7-fold greater than the human C_{max} and AUC at the MRHD, which is considered adequate to support the safety of the proposed clinical use given the previous human experience. The higher margin at this dose was because oxymetazoline exposure increased in a tetracaine dose-dependent manner when co-administered with tetracaine despite the fixed dose of oxymetazoline (0.01 mg/kg) (see **Table 8**). The reason for this observation is not known. The tetracaine solution spray volume may play a role, but cannot fully explain this phenomenon. There is no evidence that tetracaine may affect oxymetazoline absorption by drug-drug interaction pharmacokinetically or pharmacodynamically. Nevertheless, this result provided useful information to evaluate the C_{max} related effects of oxymetazoline and tetracaine co-administration. It should be noted that the oxymetazoline human dose level (0.3 mg) at the MRHD of Kovanaze is within the dose level considered to be safe for the nasal route per the referenced OTC monograph. According to the referenced OTC monograph, oxymetazoline is considered safe at up to two doses of 2-3 intranasal drops per nostril per day where a drop size is assumed to be 0.05 mL. This equates to a maximum daily dose of 0.3 mg (e.g., 0.050 mL/drop × 6 drops/dose × 2 doses × 0.5 mg/mL (0.05%)). Taken together, the proposed level of tetracaine and oxymetazoline at the MRHD of Kovanaze are considered safe from a general toxicity perspective and no additional studies are needed.

Safety margins for MRHD in the 2-week dog study			
Human exposure	**Tetracaine**	**PBBA**	**Oxymetazoline**
NOAEL = 0.01/0.6 mg/kg Oxy/Tetra			
C_{max}	570/-^	52.5/465 =0.11*	0.37/1.78 = 0.21
AUC $_{0\text{-}24h}$	145/-	156.9/960 = 0.16	0.75/3.67 = 0.20
LOAEL = 0.01/3.0 mg/kg Oxy/Tetra			
C_{max}	1508/-	242/465 =0.52	0.80/1.78 = 0.47[&]
AUC $_{0\text{-}24h}$	432/-	816/960 = 0.85[#]	0.80/3.67 = 0.22

^ -: undetectable
* Mean value in animals/mean value in human on SD 14 = exposure margin
[&] The mean C_{max} value at this dose was 3.42 ng/mL on SD 1, and exposure margin was 1.92
[#] The mean AUC value at this dose was 964.5 ng•h/mL on SD 1, and exposure margin was 1.0

For local toxic effects, the dog study provided useful information to predict potential changes in the local tissue in human if repeat-doses are needed. However, repeat-dose treatment is not likely to occur in the clinic very often for this indication. It appears, nevertheless, that the local changes observed in the dog study were volume related at the clinical concentration. The dose volume at the MRHD is 0.6 mL (3 sprays × 0.2 mL/spray) while the total volume administered to dogs at the high dose of this 2-week study was 0.12 mL (tetracaine solution + oxymetazoline solution). Note that dogs could not be administered the clinical volume of 0.6 mL because the maximum tolerated dose (MTD) volume was reached at the highest dose tested and largely attributed to the level of tetracaine. In a pilot dosing phase of this study, 4 mg/kg tetracaine with intranasal administration caused severe clinical signs including tremor, excessive salivation, sedation, and muscular rigidity in dogs, which were not considered tolerable. Using other animal models such as rats would be limited by difficulty of administration as well as the volumes that could be administered. Because the observed reversible local histopathology changes after 14-day treatment in dogs are not likely to be produced after a single dose and the MTD was achieved in this study, this study is considered to be appropriate.

Overall, this study in combination with the available information from the public domain supports the approval of Kovanaze with the proposed maximum recommended dose in adults.

There were no nonclinical juvenile studies conducted to support pediatric clinical studies or marketing for Kovanaze. During drug development, the Division did not require any animal juvenile studies to support the pediatric clinical trials. This was because both oxymetazoline and tetracaine have already been previously approved individually for pediatric use by the Agency. According to the oxymetazoline monograph, oxymetazoline nasal solution may be used for children ≥ 2 years old. For children 2 to under 6 years of age, a 0.025% aqueous solution in a container having either a

calibrated dropper or a metered-dose spray that delivers no more than 0.027 mg of oxymetazoline per three drops or three sprays. For both nostrils, it is 0.054 mg/dose, and 0.108 mg/day with a no more than 2 doses/day. The proposed dose for approval in pediatric patients of 3 to 5 years old is 0.1 mL which is 0.05 mg. For children > 6 years old, the oxymetazoline monograph recommends the same dose as for adults. In comparison, the proposed dose in pediatric patients of 6-17 is ≤ 2/3 of the proposed maximum recommended dose in adults. In addition, Synera, which contains 70 mg tetracaine for topical application as a dermal patch, was approved for children 3 years of age and older. The Applicant included the 3 completed pediatric clinical trials in children of ≥ 3 years old to support the approval of Kovanaze for pediatric use. The results of the pediatric clinical studies did not indicate significant safety issues that may need to be addressed by nonclinical investigation. In the pediatric study plan (PSP) included in this NDA, the Applicant requested a waiver of the pediatric 0 to 2 year old group because of no utility in this age group since these subjects do not normally receive dental care. This waiver request was granted by the Division with concurrence from the FDA Pediatric Review Committee (PeRC). Therefore, no juvenile animal studies are needed for the NDA or as post-marketing requirement (PMR).

Genotoxicity

No genotoxicity studies were conducted for oxymetazoline and tetracaine individually or in combination by the Applicant. At the pre-NDA meeting held on 8/21/2014 (meeting minutes dated 9/23/2015), the Division informed the Applicant that "Although we will not require these data for a 505(b)(2) NDA, in the interest of public health, we encourage you to obtain genotoxicity information for oxymetazoline to appropriately inform your drug product labeling". The Applicant indicated that "The genotoxic potential of oxymetazoline has not been evaluated". The Applicant referenced the genotoxicity studies for tetracaine in NDA 21623 for Synera. These studies were evaluated by the Division during the NDA review for Synera. The Division concluded that tetracaine tested negative in the in vitro bacterial reverse mutation assay and the in vivo mouse micronucleus assay. Although tetracaine tested negative in the absence of metabolic activation in the in vitro chromosome aberrations assay, in the presence of metabolic activation, tetracaine was equivocal. This information is included in the approved Synera label and will be incorporated in this drug product labeling via reference

Reproductive and development toxicity

Fertility and early embryofetal development study (Segment I), embryofetal development study (Segment II), and prenatal and postnatal development study (Segment III) were conducted in rats to investigate the reproductive and development toxicity of oxymetazoline and tetracaine combination by the Applicant. The embryo-fetal development toxicity study was required in accordance with the FDA guidance for industry titled *Nonclinical Safety Evaluation of Drug or Biologic Combinations*.

The doses tested were the same in all 3 of these studies with 3 different dose levels of oxymetazoline in combination with a fixed dose level of tetracaine. Animals (22 –

25/group) were administered drugs by SC injection at 0/0 [redacted] (b) (4) 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, and 0.1/7.5 mg/kg oxy/tetra. In the fertility and early embryofetal development study, both males and females were dosed from 28 days and 14 days before cohabitation, respectively. Males were dosed throughout mating period before euthanasia, and females were dosed through Gestational Day (GD) 7 and sacrificed on GD 13. In the embryofetal development study, pregnant females were dosed from GD 7 to GD 17, and C-section examination was conducted on GD 21 after sacrifice. In the prenatal and postnatal development study, pregnant females were dosed from GD 7 thought the gestational and lactation period until Lactation Day (LD) 20. The study designs appeared to be appropriate.

Across the 3 studies, incidences of observed tetracaine-related clinical observations included ataxia, impaired righting reflex, lost righting reflex, hunched posture, decreased motor activity and low carriage were observed at all doses except the oxymetazoline only group. These changes were significantly lower at the 0.01/7.5 and 0.03/7.5 mg/kg oxy/tetra as compared to the 7.5 mg/kg tetra only administration group. This was likely because oxymetazoline decreased or slowed tetracaine exposure to the systemic circulation by its vasoconstriction effect. Indeed, the systemic exposure to tetracaine was lower in the oxymetazoline co-administration groups as compared to the tetracaine only dosing group as indicated in the TK data.

Across all 3 studies, the slight decreases in body weight change were observed in female rats at all dosing groups as compared to the control (≤ 10%) with the highest incidences occurring at the 0.01 mg/kg oxymetazoline dosing level when administered alone or co-administered with 7.5 mg/kg tetracaine, suggesting an oxymetazoline effect. However, in males from the fertility study, the body weight change appeared to be a combined effect of both tetracaine and oxymetazoline, and the maximal change was 17% which occurred at 0.1/7.5 mg/kg oxy/tetra. Severe clinical signs such as hyperpnea, bradypnea, and clonic convulsion were only observed at 0.1/7.5 mg/kg/day oxy/tetra in males in the fertility study. Treatment-related mortality occurred in males from the 0.1 mg/kg oxy only and 0.1/7.5 mg/kg oxy/tetra groups in the fertility study. Body weight loss was observed without change in food consumption, and gross pathology examination found red or dark red lung lobes and mottled and dark red kidneys in some animals. A NOAEL for male general toxicity could not be identified due to clinical signs observed at all dose levels. However, the 0.03/7.5 mg/kg oxy/tetra dose level can be considered an acceptable LOAEL due to the low incidence and severity at this dose and below. Treatment-related mortality was also seen in females from the 0.1 mg/kg oxy only and 0.1/7.5 oxy/tetra groups in the pre- and post-natal development study. Gross necropsy examination revealed dark red fluid in thoracic cavity and mottled red and dark red regions in all lung lobes, and distended intestine with gas, which were also observed in female animals found dead at ≥ 0.6 mg/kg oxymetazoline in the embryofetal dose-range finding study. Treatment-related mortality in females was not seen in the fertility study and the embryofetal toxicity study. The NOAEL for maternal toxicity for all 3 studies is identified to be 0.03/7.5 mg/kg oxy/tetra.

In the fertility and early embryofetal development study, uterine content examination showed decreased numbers of corpora lutea, implantations, and viable embryos at 0.1 mg/kg oxymetazoline when administered alone (12, 14, and 16%, respectively) or in combination with 7.5 mg/kg tetracaine (16, 16, and 18%, respectively). In addition, decreased numbers of viable embryos were also seen at 0.01/7.5 (12%) and 0.03/7.5 (14%) oxy/tetra. The NOAEL for fertility and early embryofetal development in females could not be identified due to the impact on embryo viability observed at the lowest dose tested.

In the fertility study, sperm motility, total sperm count, and sperm concentration were decreased by 31%, 44%, and 25%, respectively, compared to control in males given 0.1 mg/kg oxymetazoline alone and by 18%, 33%, and 8.3%, respectively, compared to control in males given 0.1 mg/kg oxymetazoline in combination with tetracaine. At 0.03/7.5 mg/kg oxy/tetra, these fertility endpoints were decreased by 11%, 20%, and 7.7%, respectively, compared to control. The small decreases (<10%) in sperm concentration observed at the 0.03/7.5 and 0.1/7.5 mg/kg oxy/tetra dose levels were likely due to the decreased organ weight of cauda epididymis. Dose-dependent decrease was found in reproductive organ weights including cauda epididymis, seminal vesicles, and prostate. The NOAEL for male fertility was identified to be 0.01/7.5 mg/kg oxy/tetra.

In the embryofetal development study, Cesarean section examination did not show significant adverse changes in ovaries and uterus contents at any treatment level. Slight fetal body weight decrease (<10%) at 0.1 mg/kg oxy with or without tetracaine co-administration was likely due to maternal body weight change. External and skeletal alterations that were considered to be treatment-related were only seen in the 0.1 mg/kg oxymetazoline group but interestingly were not observed with the same oxymetazoline dose when tetracaine was co-administered. The observed malformations included short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs. The observed variations included irregularly shaped arches in thoracic vertebrae and increased bifid centra in thoracic vertebrae. In the embryofetal dose range-finding (DRF) study, a higher oxymetazoline dose was tested and higher incidences of external alterations were observed at the 0.3 mg/kg oxymetazoline dosing level compared to the 0.1 mg/kg level tested in the definitive study. In the DRF study, the findings were observed when oxymetazoline was given alone as well as in combination with 7.5 mg/kg tetracaine. Interestingly, the incidences were slightly lower when tetracaine was co-administered. This trend also applied to findings including jaw micrognathia, and forepaw digits short, absent or fused. Although it appears to be a possibility that the presence of tetracaine may ameliorate the teratogenic effect of oxymetazoline, the currently available data are not sufficient to make this conclusion. The NOAEL for embryofetal and development toxicity was identified to be at 0.03/7.5 mg/kg oxy/tetra.

In the prenatal and postnatal development study in rats, body weights were decreased compared to the control in the F1 fetuses on Lactation Day (LD) 20 (19% and 11% for the 0.1/0 and 0.1/7.5 mg/kg oxy/tetra maternal dosing groups, respectively). This change was not due to maternal body weight decrease. Oxymetazoline treatment

decreased the mean number of implant sites/litter at 0.1 mg/kg when administered with (approximately 9.2%) or without tetracaine administration (5.5%). At 0.03/7.5 mg/kg, this change was also 9.2%. Although these changes were not statistically significant, the values were outside the range of the historical control data, which suggests a treatment-related adverse change. This change resulted in reductions in the mean number of pups delivered, the mean number of liveborn pups, mean number of surviving pups per litter, and the mean live litter size at these doses compared to control. All of the values in each of these dose groups were below the historical range for the testing facility. The number of pups that died during the lactation period are 1, 4, 2, 3, 3, 8 at 0/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxy/tetra, respectively, which also suggested a treatment-related effect. However, these values were within the historical control range except the incidence at 0.1/7.5 mg/kg. The NOAEL for prenatal and postnatal toxicity is identified at 0.01/7.5 mg/kg oxy/tetra.

F1 male and female animals exhibited a >10% body weight decrease (statistically significant) compared to control 2 weeks after weaning (PND36). There were no other significant adverse changes in the F1 animals with respect to sexual maturation, reproduction, and nervous system development, suggesting that the development of the F1 generation was not significantly affected following maternal administration from the period of organogenesis through lactation.

In summary, reproductive studies in rats indicated toxic effects on fertility, embryofetal development, and postnatal development following administration of oxymetazoline and tetracaine in combination. The results of these studies support the conclusion that the toxic effects were caused by oxymetazoline, but not tetracaine at the doses tested.

The Applicant also referenced reproductive and development studies submitted in Synera NDA (NDA 21623) for tetracaine reproductive and development toxicity evaluation. These studies have been evaluated by the Agency, and the data indicated that tetracaine was not associated with reproductive toxicity, which are consistent with the observations in studies submitted in this NDA.

Overall, the Applicant conducted appropriate reproductive toxicity studies. These studies along with studies/information from Synera NDA and label provided sufficient information to enable appropriate labeling.

Other safety assessments

For excipients, the Applicant did not conduct any specific studies for excipient safety evaluation. All excipients that are used in Kovanaze have been approved for use in other products. Data in the Inactive Ingredient database (IID) for FDA-approved drugs covered the level, route, and treatment duration of all excipients in Kovanaze except that 1) the maximal potency for benzyl alcohol in previous approved nasal products (b) (4) %) is lower than the concentration in Kovanaze (0.9%), 2) hydroxyethyl cellulose has not been approved to be used in nasal drug products; though both are listed in approved products for other routes at levels that provide systemic safety coverage.

Notably, citric acid (b) (4)%), benzyl alcohol (b) (4)%), and hydroxyethyl cellulose (b) (4)%) were included in the vehicle formulation in the 2-week intranasal dog toxicity study. The high dose in the study provided (b) (4)-fold margin for the maximal human dose based on body surface area comparison. No significant adverse changes in the 2-week dog study were considered to be due to excipients. There is no safety concern for the excipients in Kovanaze.

The specification limit of impurities and degradants are appropriately within qualification thresholds in accordance with ICH Q3A(R2) and Q3B(R2), respectively. Bacterial reverse mutation assays were conducted with impurities (b) (4) and (b) (4) which contain genotoxicity structural alert groups, and the results indicated that these impurities are negative for mutagenicity. An oxymetazoline related impurity, (b) (4), also contains genotoxicity structural alert group. However, the Applicant controlled the daily exposure level of this impurity under (b) (4) mcg/day, which is acceptable due to the acute indication of Kovanaze according to ICH M7 guidance which allows a daily uptake level of 120 mcg/day for a mutagenic impurity in drug products with intended treatment of less than one month duration. The impurities and degradants in Kovanaze are considered to be adequately qualified, in part based on previous clinical experience (b) (4).

For leachables, 3 studies for extractables and leachables were included in this application. These included (1) a controlled extraction study, (2) a leachable-extractable correlation study in which older stability samples of drug product were examined for actual leachable compounds, and (3) a final study to quantitate leachable compounds in long-term storage samples from primary stability studies. The studies appeared to be acceptable from the CMC perspective per Dr. Xiaobin Shen, the CMC reviewer for this NDA. The analytical evaluation threshold (AET) of (b) (4) mcg/mL was calculated based on a Safety Concern Threshold (SCT) of (b) (4) mcg/day per the Agency's requirement. Four leachable compounds were identified in the leachable study of the aged drug product. Aged drug product from two lots were stored under refrigerated long-term storage conditions (2 - 8 °C) for nearly three years at the time of leachable testing, which is longer than the proposed (b) (4) month shelf-life of the drug product. The identified leachables were determined to be (b) (4) that are known to leach from similar packaging components (LDPE, plastic containers, coated glass, and stopper/plunger). Leachable study using 46 registration stability samples that appropriately represented different time intervals over the (b) (4) month shelf-life did not identify any of the leachables identified using the aged batches. Therefore, a toxicological risk assessment is not needed and the evaluations support the proposed expiry.

In summary, the nonclinical data provided by the Applicant along with the previous Agency findings support the safety of maximum recommended human dose in adults and pediatrics (b) (4). In addition, the data provided adequate nonclinical information for Kovanaze labeling. Furthermore, the safety of excipients, impurities/degradants, and leachables are considered to be adequately assessed from

nonclinical perspective. Therefore, this NDA may be approved from the nonclinical perspective.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, NDA 208032 may be approved.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The table below shows a comparison of the Applicant's proposed label language, this reviewer's recommended changes, and the rationale for the recommended changes. It should be noted that these recommended changes are not final. Subsequent revision is expected after discussion within the Division and negotiation with the Applicant.

Applicant's proposed label	Reviewer recommended changes	Rationale for changes
Highlights **Indications and Usage** Kovanaze is (b) (4) indicated for regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J.	**Highlights** **Indications and Usage** KOVANAZE contains tetracaine, an ester local anesthetic, and oxymetazoline, a vasoconstrictor. Kovanaze is indicated for regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J.	This section must include an appropriate established pharmacologic class (EPC) for the drug substance(s) if available per 21 CFR 201.57. Note, an EPC for oxymetazoline has not yet been established. According to the guidance for industry and review staff: *Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information*, the pharmacologic class of a drug can be defined on the basis of (1) the mechanism of action (MOA), (2) physiologic effect (PE), (3) chemical structure (CS). For oxymetazoline: • MOA = α1 and α2 adrenergic receptor agonist • PE = Vasoconstrictor • CS = Imidazoline The guidance notes that the EPC should be represented by a phrase that is scientifically and clinically meaningful. We agree with the Applicant's proposal to use vasoconstrictor as the EPC for oxymetazoline as this is the most clinically meaningful phrase in the context of its intended use in

		Kovanaze. Similarly, the EPC phrase vasoconstrictor is used for epinephrine in drug products when it is combined with local anesthetics with the intended effect of reducing local blood flow.
8.1 Pregnancy *Risk Summary* (b) (4) *Animal Data* (b) (4)	**8.1 Pregnancy** *Risk Summary* In animal reproduction and development studies, oxymetazoline given subcutaneously to rats during the period of organogenesis caused structural abnormalities at a dose approximately 7.6 times the level of oxymetazoline (0.3 mg) from the maximum recommended human dose (MRHD) of KOVANAZE. In a pre- and post-natal development study, oxymetazoline given subcutaneously to rats caused embryofetal toxicity manifested by reduced implantation sites and live litter sizes at doses approximately 1.5 times the MRHD level and greater. In addition, increased pup mortality was observed at oxymetazoline doses approximately 7.6 times the MRHD level. In animal reproduction studies, no adverse developmental effects were observed in pregnant rats and rabbits given subcutaneous doses of tetracaine equivalent to 5 times the MRHD of KOVANAZE during the period of organogenesis. [*see Data*] *Animal Data* In an embryofetal development study, pregnant rats were administered subcutaneous doses of oxymetazoline HCl at 0.1 mg/kg (7.6 times the amount of oxymetazoline from the maximum recommended human dose (MHRD) of KOVANAZE by AUC comparison), tetracaine HCl only at 7.5 mg/kg (32 times the amount of tetracaine from the MHRD as measured by PBBA	The draft label from the Applicant is already in PLLR format. This revision of the *Risk Summary* section should be completed by MHT and PT. (b) (4) (b) (4) (b) (4) Language regarding embryofetal development studies conducted with tetracaine alone is based on data from Synera NDA. Information of studies included in the Animal Data subsection per PLLR requirement • Types of studies • Animal species • doses or exposures in terms of human dose or exposure equivalents • Duration and timing Study findings • Maternal toxicity • Limitations of the data Proprietary name is used since the formulation of the drug product used in these studies was the same as the approved drug product.

(b) (4)	[major tetracaine metabolite] AUC comparison), and oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg/day (0.6, 1.5, and 7.6 times, respectively, the MHRD level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl during the period of organogenesis (Gestational Days [GD] 7-17). Oxymetazoline HCl treatment at 0.1 mg/kg (7.6 times the MHRD by AUC comparison) caused structural abnormalities including external and skeletal malformations (e.g., short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs), and variations (e.g., irregularly shaped arches and increased bifid centra in thoracic vertebrae, and un-ossified forelimb phalanx) in the absence of maternal toxicity. Similar fetal abnormalities were not observed when pregnant rats were co-administered the same dose of oxymetazoline HCl in combination with 7.5 mg/kg tetracaine HCl. The no-observed-adverse-effect-level (NOAEL) for fetal abnormalities is 0.03 mg/kg oxymetazoline HCl in combination with 7.5 mg/kg tetracaine HCl.	(b) (4)
(b) (4)	In other embryofetal development studies, tetracaine alone administered subcutaneously did not cause structural abnormalities in rats at doses up to 10 mg/kg/day (approximately 5.4 times the MRHD level by body surface area (BSA) comparison) or in rabbits at subcutaneous doses up to 5 mg/kg/day (approximately 5.4 times the MRHD level by BSA comparison).	This information is from the Synera label. Note the Applicant obtained a letter of authorization to cross-reference all nonclinical info, including the described embryofetal and development studies, contained in NDA 21623 for Synera. This language here is consistent with the Synera label. Since plasma exposure data for tetracaine were not available for these studies, the exposure margin calculations were based on body surface area comparison.
(b) (4)	In a prenatal and postnatal development study, pregnant rats were given subcutaneous	

(b) (4) There were no adverse effects on sexual maturation, neurobehavioral, or reproductive function in the offspring at any maternal dose.	doses of oxymetazoline HCl only at 0.1 mg/kg (7.6 times the MHRD level by AUC comparison), tetracaine HCl only at 7.5 mg/kg (12 times MHRD level by PBBA AUC comparison), and oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg (0.6, 1.5, and 7.6 times, respectively, the MHRD level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl from GD 7 to Lactation Day [LD] 20 (corresponding to the beginning of organogenesis through parturition and subsequent pup weaning). Oxymetazoline HCl treatment decreased the mean number of implant sites/litter at ≥ 0.03 mg/kg (≥ 1.5 times the MRHD by AUC comparison) when administered with 7.5 mg/kg tetracaine (approximately 9%) and without tetracaine administration (5.5%), which resulted in an overall reduction in live litter sizes in these groups. At the end of the lactation period, fetal body weights were significantly decreased at 0.1 mg/kg oxymetazoline dose (7.5 times the MRHD by AUC comparison) when administered alone (19%) and co-administered with 7.5 mg/kg/day tetracaine (11%). In addition, increased pup mortality was observed at the 0.1/7.5 mg/kg oxymetazoline/tetracaine dose (8 out of 22) compared to the control (1 out of 22). Maternal toxicity (decreased body weight and mortality) was observed in animals given oxymetazoline HCl alone at 0.1 mg/kg and when co-administered with 7.5 mg/kg tetracaine HCl. There were no adverse effects on sexual maturation, neurobehavioral, or reproductive function in the offspring at any maternal dose.	

8.2 Lactation ***Risk Summary*** (b) (4)	**8.2 Lactation** *Risk Summary* Detectable levels of oxymetazoline, tetracaine and its major metabolite, p-butylaminobenzoic acid (PBBA were found in the milk of lactating rats following subcutaneous administration of oxymetazoline HCl in combination with tetracaine HCl during the period of organogenesis through parturition and subsequent pup weaning [*see Data*].	The data from the pre- and post-natal studies will be included in 8.1 and the data from the rat milk excretion study will be described in more detail in this section.
	Data In a pre- and post-natal development study, rats were given subcutaneous doses of oxymetazoline HCl at doses of 0.01, 0.03, and 0.1 mg/kg/day (0.6, 1.5, and 7.6 times, respectively, the maximum recommended human dose (MRHD) level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl (12 times the MRHD level by tetracaine metabolite AUC comparison) from Gestational Day [GD] 7 to Lactation Day [LD] 20. Concentrations of oxymetazoline, tetracaine, and PBBA were measured in the milk of lactating rats at approximately 2 hours postdose on LD 15. The concentrations of oxymetazoline were generally dose dependent (2.5, 7.0, and 33.8 ng/mL at 0.01, 0.03, and 0.1 mg/kg, respectively). The concentrations of tetracaine and PBBA were generally similar across all 7.5 mg/kg tetracaine dosing groups regardless of the presence of oxymetazoline (54.2 – 72.9 ng/mL for tetracaine, and 100.5 – 131.2 ng/mL for PBBA).	A *Data* section must be included if milk secretion study was available per PLLR. For Kovanaze, milk secretion analysis was performed in the prenatal and postnatal development study

8.3 Females and Males of Reproductive Potential (b) (4)	**8.3 Females and Males of Reproductive Potential** Infertility *Females* Based on animal data, KOVANAZE may reduce fertility in females of reproductive potential. It is not known if the effects on fertility are reversible [*see Nonclinical Toxicology (13.1)*] *Males* Based on animal data, KOVANAZE may decrease sperm motility and sperm concentration [*see Nonclinical Toxicology (13.1)*].	(b) (4) Adverse findings in the fertility and early embryofetal study are included in this section, which is required per PLLR rule.

12 Clinical Pharmacology **12.1 Mechanism of Action** Tetracaine is a local anesthetic of the ester type and exerts its activity by blocking sodium ion channels required for the initiation and conduction of neuronal impulses. (b) (4)	**12 Clinical Pharmacology** **12.1 Mechanism of Action** Tetracaine is a local anesthetic of the ester type and exerts its activity by blocking sodium ion channels required for the initiation and conduction of neuronal impulses. Oxymetazoline is an imidazoline derivative with sympathomimetic activity. It is believed to be a mixed α1/α2-adrenoceptor agonist and by stimulating adrenergic receptors, it elicits vasoconstriction of dilated arterioles and reduces nasal blood flow.	(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility Long-term studies in animals have not been performed to evaluate the carcinogenic potential of tetracaine or oxymetazoline. Tetracaine base was negative in the in vitro Ames bacterial reverse mutation assay and the in vivo mouse micronucleus assay. In the in vitro chromosome aberration assay using Chinese hamster ovary cells, tetracaine base was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation. No studies have been conducted to evaluate the mutagenic potential of oxymetazoline.	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** *Carcinogenesis* Long-term studies in animals have not been performed to evaluate the carcinogenic potential of tetracaine or oxymetazoline. *Mutagenesis* Tetracaine base was negative in the in vitro Ames bacterial reverse mutation assay and the in vivo mouse micronucleus assay. In the in vitro chromosome aberration assay using Chinese hamster ovary cells, tetracaine base was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation. No studies have been conducted to evaluate the mutagenic potential of oxymetazoline. *Impairment of Fertility* (b) (4) Male and female rats were given subcutaneous doses of oxymetazoline HCl alone at 0.1 mg/kg/day (7.5 times the MRHD level by AUC comparison), tetracaine HCl alone at 7.5 mg/kg/day (33 times the MRHD level by tetracaine metabolite AUC comparison), or the combination of oxymetazoline HCl at 0.01, 0.03, or 0.1 mg/kg/day (0.7, 2.0, and 8.0 times, respectively, the MRHD level by AUC comparison) with 7.5 mg/kg/day tetracaine HCl prior to and during mating. (b) (4) Adverse effects related to oxymetazoline HCl were observed for male sperm parameters (reduced percentage of motile sperm, sperm counts, and sperm density). Sperm effects occurred at oxymetazoline doses equivalent to 2 times the	Subheadings were added to assist the reader. The proposed text was modified to reflect standard CFR language and to remove explanatory statements that could be deemed promotional rather than simply stating the findings. The Applicant provided a LOA to the Synera NDA, which contained tetracaine mutagenesis data. The proposed language by the Applicant is consistent with the language in the Synera label. It is acceptable that there is no oxymetazoline mutagenesis information in the label based on the Agency's previous findings of safety for oxymetazoline per the finalized OTC monograph. This section is revised to include the exposure margin based on plasma exposure comparison.

(b) (4) (b) (4) (b) (4). These effects were attributed to oxymetazoline HCl because similar effects were not observed in rats given tetracaine HCl alone. A no-effect level for fertility in female rats was not established in this study.	MRHD and higher (by AUC comparison), given alone or in combination with tetracaine HCl. In female rats, a reduction in the number of viable embryos was observed at oxymetazoline doses equivalent to 0.7 times the MRHD and higher (by AUC comparison), given alone or in combination with tetracaine HCl. Reduced numbers of corpora lutea and implantation sites occurred at an oxymetazoline dose equivalent to 7.5 times the MRHD (by AUC comparison) given alone or in combination with tetracaine HCl. These effects were attributed to oxymetazoline HCl because similar effects were not observed in rats given tetracaine HCl alone. A no-effect level for fertility in female rats was not established in this study.	

2 Drug Information

2.1 Drug

CAS Registry Number
Tetracaine: 136-47-0
Oxymetazoline: 2315-02-8

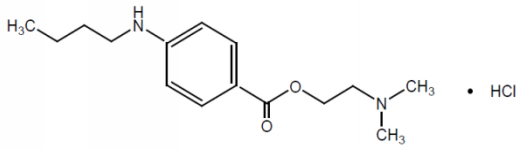
Generic Name
Tetracaine hydrochloride
Oxymetazoline hydrochloride

Code Name
Tetracaine hydrochloride
Oxymetazoline hydrochloride

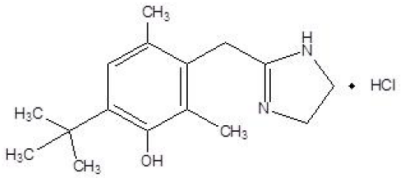
Chemical Name
Tetracaine Hydrochloride: Benzoic acid, 4-(butylamino)-, 2-(dimethylamino)ethyl ester, monohydrochloride
Oxymetazoline Hydrochloride: Phenol, 3-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimethylethyl)-2,4-dimethyl-,Hydrochloride

Molecular Formula/Molecular Weight
Tetracaine Hydrochloride: $C_{15}H_{24}N_2O_2 \cdot HCl/300.82$
Oxymetazoline Hydrochloride: $C_{16}H_{24}N_2O \cdot HCl/296.84$

Structure or Biochemical Description



Tetracaine Hydrochloride



Oxymetazoline Hydrochloride

Pharmacologic Class
Tetracaine: ester local anesthetic
Oxymetazoline: not established. Proposed EPC: vasoconstrictor

2.2 Relevant INDs, NDAs, BLAs and DMFs

This drug product was developed under IND 70868. The Applicant provided a right-of-reference to NDA 21623 (Synera®, lidocaine and tetracaine topical patch) and referenced the oxymetazoline relevant section of the final OTC monograph in Federal Register (Cold, Cough, Allergy, Bronchodilator, and Anti-asthmatic Drug Products for Over-the--Counter Human Use; Final Monograph for OTC Nasal Decongestant Drug Products, Volume 59, 1994) as well as published literature to support this NDA application. DMF (b) (4) and DMF (b) (4) were referenced for oxymetazoline hydrochloride and tetracaine hydrochloride drug substance, respectively.

2.3 Drug Formulation

Kovanaze Nasal Spray is a fixed-combination drug formulation with tetracaine and oxymetazoline as active ingredients, filled in unit-dose Accuspray® nasal spray systems. The composition of the drug product is shown below (**Table 1**). The maximum recommended human dose is 3 × 0.2 mL sprays/day. This results in a maximum daily dose of 18 mg of tetracaine hydrochloride and 0.3 mg of oxymetazoline hydrochloride per day.

Table 1: Composition of the drug product

Component	Function	Composition	Quantity per Spray	(b) (4)
		% w/v	0.2 mL spray	
Tetracaine hydrochloride, USP	Active	3.00	6.0 mg	
Oxymetazoline hydrochloride, USP	Active	0.05	0.10 mg	
Citric acid (b) (4) USP				
Sodium citrate (b) (4) USP				
(b) (4)				
Benzyl alcohol, NF				
Hydroxyethylcellulose, NF				
Sodium hydroxide, NF	pH adjustment	as needed	as needed	as needed
Hydrochloric acid, NF	pH adjustment	as needed	as needed	as needed
(b) (4) water, USP [1]				(b) (4)

2.4 Comments on Novel Excipients

All excipients in Kovanaze are listed in the Inactive Ingredient database (IID) for FDA approved drugs with comparable duration of treatment. As shown in the above table (**Table 1**), sodium hydroxide (NaOH) and hydrochloric acid (HCl) are listed as "as needed". Typically, there is no quantity provided for acid and base used for pH adjustment, according to Dr. Xiaobin Shen, the CMC reviewer for this product. NaOH

and HCl react and produce NaCl and H_2O in water solution. There is no safety concern for NaOH and HCl in Kovanaze.

Both citric acid [redacted] and sodium citrate [redacted]. The concentration of sodium citrate [redacted] proposed is technically novel for the intranasal route. The maximum potency of citric acid in FDA-approved nasal solutions is 0.28% (citric acid [redacted]; this covers the concentration of [redacted] citric acid concentration in Kovanaze [redacted]%). The maximum potency of sodium citrate is 0.44% (unspecified form) in nasal solutions and 1.83% for inhalation solutions [redacted]. The sodium citrate that is used in Kovanaze [redacted]. According to the IID, the maximum potency for [redacted] citric acid is 42.19% and 32 mg for IV injection solution. As a comparison, total amount of citric acid administered following the maximum dose of Kovanaze is [redacted] mg with 3 sprays/day, and the total citrate [redacted] daily amount is [redacted] in combination (citric acid and sodium citrate), which is covered by the previously approved drug products. Both sodium citrate and citric acid are used in drug products for various different routes of administration including buccal, intramuscular, oral rectal, inhalation, sublingual, topical, and transdermal, etc. This information from the IID indicates that [redacted] citrate [redacted] in Kovanaze [redacted]%) may not be associated with significant safety concern for local nasal tissue. Further, the concentration of these excipients was qualified via the intranasal toxicology studies conducted with the formulation.

The concentration of **benzyl alcohol** in Kovanaze is [redacted]%, and the total daily intake (TDI) is [redacted]mg based on the MRHD of Kovanaze. The systemic toxicity of benzyl alcohol may not be a significant safety concern unless it is administered to pre-term neonates. According to IID, the maximum potency of benzyl alcohol for a nasal sprayed drug product is 0.5%, therefore, the proposed use is technically novel. However, it has been used in FDA approved products at [redacted]% for auricular, epidural, intramuscular, intravenous, intra-articular, intrasynovial, intradermal, rectal, subcutaneous (SC), topical, and vaginal treatment. The safety concern for systemic effects of [redacted]% benzyl alcohol is minimal based on the IID experience. Although this is a novel concentration for the intranasal route, the concentration of the excipient was qualified via the intranasal toxicology studies conducted with the formulation. As noted in those studies, there was evidence of hyperplastic changes even after 14 days exposure, which may be due to the [redacted] benzyl alcohol. The proposed concentration; however, is acceptable for this acute use product.

The concentration of **hydroxyethyl cellulose** is [redacted]% in Kovanaze (0.3 mg TDI based on the MRHD of Kovanaze). This excipient has not been used in previously approved drug products for intranasal use according to the IID. It is included in approved drug products for buccal (up to 54 mg) and oral (up to 400 mg). Therefore, the systemic toxicity of hydroxyethyl cellulose appears to not be a safety concern with the maximum daily Kovanaze dose [redacted]mg hydroxyethyl cellulose). Hydroxyethyl cellulose is also included in drug products for auricular (0.25%), ophthalmic (1.6%), and topical (1.75%) administration. This information in the IID suggested that [redacted]% hydroxyethyl cellulose

may not impose significant safety in local tissue toxicity, although these tissues are different from nasal tissue. In addition, the concentration of the excipient was qualified via the intranasal toxicology studies conducted with the formulation

In summary, the novel excipients citric acid (b) (4) %), benzyl alcohol (b) (4) %), and hydroxyethyl cellulose (b) (4) %) were included in the vehicle formulation in the 2-week intranasal dog toxicity study conducted by the Applicant, and the results indicated that no significant adverse changes may be associated with the administration of these excipients for this acute indication.

Overall, the information from the public domain including levels in FDA-approved drugs and toxicity studies from the Applicant indicate that the excipients in Kovanaze are not likely to be associated with significant toxicity locally or systemically at the maximum daily dose of Kovanaze. The excipients are considered to be adequately qualified.

2.5 Comments on Impurities/Degradants of Concern

The Applicant referenced DMFs (b) (4) for tetracaine and (b) (4) for oxymetazoline. These DMFs have been deemed acceptable in the context of numerous FDA-approved drug product applications.

The proposed oxymetazoline drug substance specifications are listed in the table below:

Table 1: Oxymetazoline Drug Substance Specifications (MDD 0.3 mg/day)

Parameter	Proposed Specifications	Qualification Threshold	Comment on Adequacy
(b) (4)	NMT (b) (4) %	NMT (b) (4) mcg per day, (ICH M7)	(b) (4)
Each individual unspecified impurity	NMT (b) (4) %	(b) (4) % or (b) (4) mcg total daily intake, whichever is lower (ICH Q3A(R2))	**Adequate**, conforms with ICH Q3A(R2)
Heavy Metals	NMT (b) (4) ppm	N/A	**Adequate** at this time; however, the Applicant will be required to comply with ICH Q3D once the implementation period is completed.

Residual Solvents: [redacted]	NTM [redacted] ppm	NMT [redacted] ppm (ICH Q3C(R5))	**Adequate**. Complies with ICH Q3C(R5)

The oxymetazoline drug substance impurity, [redacted] also contains a structural alert. The Applicant is controlling this impurity with a specification of NMT [redacted]% which would result in exposure to NMT [redacted] mcg/day, which is acceptable due to the acute indication of Kovanaze according to ICH M7 which allows a daily uptake level of [redacted] mcg/day for a mutagenic impurity in drug products with intended treatment of less than one month duration. The proposed tetracaine drug substance specifications are listed in the table below:

Table 2: Tetracaine Drug Substance Specifications (MDD 18 mg/day)

Parameter	Proposed Specifications	Qualification Threshold	Comment on Adequacy
[redacted]	NMT [redacted]%	NMT [redacted]% or [redacted] mg per day, whichever is lower (Q3A(R2))	**Adequate**: Contains a structural alert for mutagenicity. [redacted] Negative in the Ames assay[1]. Complies with ICH Q3A(R2)
	NMT [redacted]%	NMT [redacted] mg per day, whichever is lower	Contains a structural alert for mutagenicity; however, [redacted] **Adequate**: Complies with ICH Q3A(R2)
	NMT [redacted]%	NMT [redacted] mg per day, whichever is lower for non-genotoxic impurity.	Contains a structural alert for mutagenicity. Negative in the Ames assay conducted by the Applicant. At this spec, would result in NMT [redacted] mcg/day. **Adequate**: Complies with ICH Q3A(R2) and M7
Each individual unspecified impurity	NMT [redacted]%	NMT [redacted] mg per day, whichever is lower	**Adequate**: Complies with ICH Q3A(R2)
Residual Solvents: [redacted]	NTM [redacted] ppm	NMT [redacted] ppm	**Adequate**: Complies with ICH Q3C(R5)

[1] Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, and Zeiger E. 1986. Salmonella Mutagenicity Tests: II. Results From The Testing Of 270 Chemicals. Environ. Mutagen. 8(Suppl. 7):1-119.

As noted in the tables above, the drug substance specifications either comply with ICH Q3A(R2) or M7 as appropriate.

Specifically, [redacted] contains a structural alert for mutagenicity. However, published studies have reported that the compound tests negative in the in vitro bacterial reverse mutation assay (Ames test). [redacted]. Therefore, this impurity may be regulated as a non-genotoxic impurity as per ICH Q3A(R2) and the proposed specification is acceptable.

The compound [redacted] and therefore is considered to be adequately qualified for safety from a systemic perspective. [redacted]

[redacted] also contains a structural alert for mutagenicity. The Applicant provided an adequate in vitro bacterial reverse mutation assay to demonstrate that the compound tested negative for potential mutagenicity. Therefore, the impurity can be regulated as non-genotoxic. The proposed specification is acceptable.

The drug product specifications are listed in the table below.

Table 3: Drug Product Specifications

Degradant	Proposed Specifications	ICH Q3B(R2) Qualification Threshold	Comment on Adequacy
[redacted]	NMT [redacted]% (originally proposed NMT [redacted]% but reduced following Agency discussion)	[redacted]% or [redacted] mcg total daily intake, whichever is lower.	• Exceeds ICH Qualification threshold [redacted] • **The proposed specification is not supported for local tissue toxicity by animal data. However, it is supported for safety** [redacted]

Other Single Unspecified	NMT [(b)(4)]% for tetracaine-related NMT [(b)(4)]% for oxymetazoline-related	[(b)(4)]% or [(b)(4)] mcg total daily intake, whichever is lower (tetracaine). [(b)(4)]% or [(b)(4)] mcg total daily intake, whichever is lower (oxymetazoline).	Adequate, conforms with ICH Q3B(R2)

The only compound that increases in the drug product to levels that exceed the qualification threshold is [redacted] of

The 14-day intranasal toxicology study in dogs tested only up to a 3% solution of tetracaine and the analytical data suggest that there was little to no [(b)(4)] in this drug product solution. Therefore, this study does not appear to provide local tissue data to qualify the originally proposed specification of NMT [(b)(4)]% [(b)(4)] The clinical batch analysis suggests that NMT [(b)(4)]% [(b)(4)] was present in a few batches of materials tested clinically, therefore, the originally proposed level of NMT [(b)(4)]% is also not adequately tested via the clinical studies. However, evaluation of the clinical batches used in the olfactory function studies that also included detailed evaluation of the local tissue effects suggest that there are clinical data to support a specification of NMT [(b)(4)]% (to account for potential excursions in storage temperatures).

Tetracaine is an ester linked local anesthetic, and the formation of [(b)(4)] is produced by hydrolysis of the ester linkage by plasma and tissue esterases. Therefore, it is likely that some degree of [(b)(4)] is formed in the nasal mucosa after administration of tetracaine. However, how the concentration formed compares to the levels in the drug product formulation (up to [(b)(4)]%) is not known. Taking into consideration the existing stability data and the levels present in clinical batches used for the human olfactory assessments, the existing human data support a specification of NMT [(b)(4)]% with a shelf life of 15 months (see the clinical and CMC reviews).

If the company wishes to increase the specification to more than [(b)(4)]% [(b)(4)] and extend their shelf-life, an intranasal toxicology study in a single species will be required.

2.6 Proposed Clinical Population and Dosing Regimen

Kovanaze is indicated to induce ipsilateral [(b)(4)] dental procedure on teeth 4 [redacted]-13 and teeth A [redacted]-J

when present. The recommended dose is as follows (**Table 5**). The drug product is supplied in a single-use BD Accuspray™ delivery system (sprayer). Each sprayer delivers a spray volume of 0.2 mL that contains 6 mg of tetracaine HCl (3% w/v concentration) and 0.1 mg of oxymetazoline HCl (0.05% w/v concentration). The dose may be delivered as a single spray of 0.2 mL (b) (4).

Table 5: Clinical dosage of Kovanaze

Age Group	Dose	Total Tetracaine HCl Content	Total Oxymetazoline HCl Content
Adults (≥ 18 years old)	2 sprays (0.2 mL per spray)	12 mg	0.2 mg
	1 additional spray (0.2 mL) if adequate anesthesia (b) (4) (b) (4) (b) (4) has not been achieved 10 minutes after the second spray	6 mg	0.1 mg
(b) (4)			
Children (b) (4) weighing ≥ 40 kg)	2 sprays (0.2 mL per spray)	12 mg	0.2 mg

The proposed maximum recommended human dose (MRHD) (18 mg or 0.3 mg/kg tetracaine and 0.3 mg or 0.005 mg/kg oxymetazoline) is for use in adults. At this dosing level, the C_{max} and $AUC_{0\text{-}inf}$ were 1.78 ± 0.59 ng/mL and 4.24 ± 2.09 ng·h/mL ($AUC_{0\text{-}t}$ was 3.67 ± 1.79 ng·h/mL), respectively, for oxymetazoline following 0.3 mg intranasal administration. Tetracaine exhibited quick metabolism as the plasma concentration was undetectable in human subjects. The concentration of the major metabolite of tetracaine, p-butylamino benzoic acid (PBBA), was measured as a marker for tetracaine exposure. The C_{max} and $AUC_{0\text{-}inf}$ were 465 ± 122 ng/mL and 973 ± 513 ng·h/mL ($AUC_{0\text{-}t}$ was 960 ± 509 ng·h/mL), respectively, after 18 mg tetracaine intranasal administration. These values were from the clinical PK study in 24 adults aged 18-48 (Study SR 2-06).

2.7 Regulatory Background

Both tetracaine and oxymetazoline are in drug products approved by the Agency previously. A 0.05% oxymetazoline solution (Afrin®) for nasal drop or spray was approved for relieving nasal congestion under NDA 14717 in 1964. The NDA was withdrawn in 1987 (withdrawn FR effective on 03/11/1987), but Afrin solution products are still available in the U.S. market as they are now covered by the final monograph. In addition, ophthalmic oxymetazoline solutions (0.025%) are available on the market for relieving eye redness (Ocuclear® under NDA 18471, Visine L.R. under NDA 19407). Oxymetazoline was included in the proposed rule for an over-the-counter (OTC) monograph for cold, cough, allergy, bronchodilator and antiasthmatic products, which was published in the Federal Register (FR) on September 9, 1976. The monograph was finalized and published in the FR (Volume 59, No 162) on August 23, 1994.

Tetracaine is an ester local anesthetic that has been on the market (unapproved) since the 1930s (Pontocaine®) (Altman *et al.* 1985). Tetracaine was first approved by the FDA for local dermal anesthesia in combination with lidocaine in a topical patch product (Synera®, 70 mg lidocaine and 70 mg tetracaine) and cream (Pliaglis®, 7% lidocaine and 7% tetracaine) under NDA 21623 and NDA 21717, respectively. Tetracaine has not been approved by the Agency for intranasal administration.

The Applicant submitted the NDA as a 505(b)(2) application referencing the Agency's previous finding of safety and efficacy of oxymetazoline (Federal Register (Volume 59, 1994, final monograph) and literature were referenced to support the NDA application. In addition, to support the safety of tetracaine, the Applicant submitted an authorization letter from Galen Specialty, the NDA holder for Synera NDA 21623, granting the Agency permission to cross-reference relevant nonclinical and clinical studies from the Synera NDA to support this application.

In communications between the Division and the Applicant during drug product development (IND 70968), the Division agreed no nonclinical studies were needed to support the clinical studies prior to NDA submission based on the available clinical experience with both oxymetazoline and tetracaine. A 2-week intranasal general toxicity dog study to investigate the combined effects of oxymetazoline and tetracaine was required by the Division for NDA submission. The Division informed the Applicant that studies to support the safety of oxymetazoline would not be required for the NDA because the proposed maximum oxymetazoline dose for Kovanaze administration is within the range that is recommended for oxymetazoline in the OTC monograph. For reproductive toxicity evaluation, the Division required that an embryofetal development toxicity study for oxymetazoline and tetracaine combination in one animal species must be conducted, consistent with the recommendations in the FDA guidance for industry: *Nonclinical Safety Evaluation of Drug or Biologic Combinations*.

3 Studies Submitted

3.1 Studies Reviewed

The following table extracted from the submission listed the nonclinical studied conducted for the NDA (**Table 6**).

Table 6: Toxicology studies conducted by St. Renatus with co-administration of oxymetazoline and tetracaine

Study Type and Duration	Route of Administration	Species	GLP
Repeat-dose toxicity			
14 days with 14 days recovery	Intranasal	Dog	Yes
Reproductive and developmental toxicity			
Fertility and early embryonic development	SC	Rat	Yes
Embryo-fetal development (pilot)	SC	Rat	No
Embryo-fetal development (pivotal)	SC	Rat	Yes
Prenatal and postnatal development, including maternal function	SC	Rat	Yes
Other studies			
In vitro reverse mutation assay of impurity: (b) (4)	*In vitro*	*S. typhimurium* & *E. coli*	Yes
In vitro reverse mutation assay of impurity: (b) (4)	*In vitro*	*S. typhimurium* & *E. coli*	Yes

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Because the Applicant obtained right-of-reference to the data in the Synera NDA, this review references the nonclinical review(s) for NDA 21623, the publically available versions of which are available on the Drugs@FDA website (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021623TOC.cfm).

4 Pharmacology

4.1 Primary Pharmacology

No nonclinical studies were available from the public domain or conducted by the Applicant to investigate the pharmacological effects of Kovanaze (oxymetazoline and tetracaine combination). The available literature describing the nonclinical pharmacology of oxymetazoline and tetracaine separately were summarized by the Applicant and submitted in this application.

Oxymetazoline is an imidazoline derivative. The pharmacologic effects have been well established in humans. It is an α-adrenoceptor agonist and causes vasoconstriction by activation of α-adrenergic receptors. Intranasal application of oxymetazoline results in constriction of dilated arterioles and reduction in nasal blood flow and congestion via the activation of both α_1 and α_2 adrenergic receptors (DeBernardis *et al.* 1987).

It is widely believed that oxymetazoline is a selective α_1 agonist and partial α_2-agonist. However, a recent in vitro study using a human embryonic kidney (HEK) 293 cell expression system suggested that oxymetazoline may be a partial α_1A but full α_2B agonist. In a study conducted in 2010, the affinity of oxymetazoline binding was evaluated to six cloned human α-adrenergic receptor subtypes transfected in HEK 293 cells (Haenisch *et al.* 2010). The affinities (K_i values) were determined from the IC_{50} values of oxymetazoline-induced inhibition of [^{3}H]prazosin binding to α1-adrenoceptors and [^{3}H]RX821002 binding to α_1 and α_2-adrenoceptors expressed in the plasma membrane of the transiently transfected cells. Receptor α_{1A} had the highest affinity to oxymetazoline followed by $\alpha_{2C} > \alpha_{1B} > \alpha_{2A} > \alpha_{1D} > \alpha_{2B}$, as shown in the table below (**Table 7**). To evaluate the potency of oxymetazoline on α-adrenergic receptors, HEK293 cells were transfected with the α-adrenoceptor subtype cDNA together with promiscuous G-protein (Gα16) cDNA. G-protein Gα16 was used to measure alpha-adrenoceptor agonist-mediated increase in cytosolic Ca^{2+} ions. Oxymetazoline acted as a full agonist at the α_{2B}-adrenoceptors. Oxymetazoline showed partial agonistic activity at the α_{1A} adrenoceptors. In the study, mRNA levels of alpha-receptors were also determined. Both α_{2B} and α_{1A} receptors were highly expressed in human nasal mucosa with the rank order of mRNA levels of a-adrenoceptor subtypes in human nasal mucosa was: $a_{2A} > a_{1A} \geq a_{2B} > a_{1D} \geq a_{2C} >> a_{1B}$.

Table 7: Affinity and potency of oxymetazoline to human α-adrenergic receptors (Haenisch *et al.* 2010)

COPYRIGHT MATERIAL WITHHELD

*E_{max} = mean% of the E_{max} of noradrenaline which was set as 100%
&nm = not measureable

The Applicant indicated that the results of these experiments may indicate that the calcium signaling in this heterologous expression system may differ from that observed in smooth muscle cells. However, the Applicant did not provide studies to demonstrate the mechanism of action of oxymetazoline in smooth muscle cells. It is possible that oxymetazoline produces the constrictive effect via other receptor subtypes which mediate different signaling pathways.

There are reports indicating that oxymetazoline activates other α_2 receptor subtypes. A study in mice revealed that oxymetazoline caused hyperglycemia (approximately 2-fold) and insulin release inhibition (approximately 67% decrease) at 0.3 mg/kg via intraperitoneal (IP) injection (Angel *et al.* 1990). These effects were antagonized by α_2

antagonist idazoxan but not α_{2B} preferential antagonists ARC-239, prazosin, or chlorpromazine.

The vasoconstrictive effect of oxymetazoline was indicated in multiple ex vivo studies. Oxymetazoline induced contractions in rat aorta (n=8) with an E_{max} of 0.41 and 0.93 of noradrenaline E_{max} (Ruffolo, Jr. and Waddell 1982). In another study in Beagle dogs, oxymetazoline induced contractions in the dog aorta with an ED_{50} of 0.28 $x10^7$ M and in Beagle dog saphenous veins with an ED_{50} of 9.3 x 10^7 M (DeBernardis *et al.* 1987).

The pharmacologic effects of tetracaine have been well established in human and animal models. Tetracaine is a potent local anesthetic which acts on the cell membrane by blocking sodium ion channels required for the initiation and conduction of nerve impulses. Blockade of neuronal conduction prevents the action potential of sensory neurons and therefore blocks the transmission of pain signals to the central nervous system (CNS). The Applicant summarized nonclinical pharmacology data from the public domain to demonstrate the analgesic efficacy of tetracaine in rats, rabbits, and dogs. Tetracaine was administered primarily via topical application, but also intranasal, intrathecal (IT) or intramuscular (IM) administration. There were no intranasal nonclinical pharmacology studies available the in public domain.

4.2 Secondary Pharmacology

Because oxymetazoline is an α-adrenergic agonist, the target organs for secondary pharmacology activities are related to the tissue localization of the α-adrenoceptors such as the CNS, gastrointestinal system, cardiovascular (CV) system, and skeletal muscle. Studies from published literature indicate that oxymetazoline is antinociceptive and hypothermic. Oxymetazoline also caused hyperglycemia, intraocular pressure decrease, skeletal twitch tension, as well as emesis.

In addition to blockade of sensory nerves, local anesthetics also interfere with the functioning of all organs which require the conduction of electrical impulses for their activity. These organs include the CNS, autonomic ganglia, neuromuscular junction and all forms of muscle, including cardiac. One publication, which was summarized by the Applicant, showed that tetracaine was found to inhibit the intracellular kinesin motor motility in human mammary epithelial cells, suggesting a mechanism for the observed action of tetracaine to reduce metastatic efficiency in circulating tumor cells (Yoon *et al.* 2011).

4.3 Safety Pharmacology

Published studies have shown that oxymetazoline caused cardiac effects in animal models. In rats, oxymetazoline intra-arterial infusion or IV injection (4 mcg/kg) produced bradycardia (up to 42% heart rate decrease) and pressor effect (up to 28% in mean arterial pressure) without subsequently evoking hypotension (Armstrong *et al.* 1982). In a dog study, oxymetazoline administered (0.000165 – 16.5 mg) using a humidifier containing the nebulized drug (1.65 mL over 5 minutes perfusion) increased blood pressure (maximum ~50-60%) and decreased heart rate (maximum ~30%) at 16.5 mg

oxymetazoline. Dogs treated with 1.65 mg showed approximately 15% blood pressure increase and 20% heart rate decrease in this study (DeBernardis *et al.*, 1987).

In the final monograph for OTC nasal decongestant drug products, the Agency noted cardiovascular effects such as bradycardia, tachycardia, hypertension, and hypotension have been reported for products containing topical nasal decongestants, particularly for oxymetazoline, in the adverse reaction reports. The Agency was concerned that certain individuals may be more vulnerable, and/or excessive use may occur. A warning for topical nasal decongestant products was included that stated "Do not use this product if you have heart disease, high blood pressure, thyroid disease, diabetes, or difficulty in urination due to enlargement of the prostate gland unless directed by a doctor."

Oxymetazoline may affect the CNS due to the expression of α-adrenoceptors in the CNS. Accidental ingestion of oxymetazoline in children has resulted in serious adverse events including coma, bradycardia, decreased respiration, sedation, and somnolence.

The primary target organs for tetracaine toxicity are the cardiovascular and CNS tissues. Tetracaine is also associated with respiratory depression according to studies in animal models. However, these effects are not expected to be evident unless the plasma levels exceed 5 mcg/mL, according to the nonclinical review for Synera NDA. Plasma levels of tetracaine following administration of Kovanaze were not detectable as indicated in the clinical studies.

The cardiovascular safety of intranasal oxymetazoline and tetracaine administered in combination was evaluated in dogs in a 14-day GLP toxicity study (Study No. 20010720). Male and female young adult Beagle dogs (3 or 5/sex/group) were administered oxy/tetra via intranasal administration for 14 consecutive days followed by a 14-day recovery period. The doses were 0/0 (vehicle), 0.01/0 (oxymetazoline only), 0.01/0.6, 0.01/1.5, and 0.01/3.0 mg/kg/day oxy/tetra. ECG measurements were recorded from conscious dogs prior to initiation of treatment and on Days 1, 14, and 28. On Days 1 and 14, the measurements were made at approximately 30 minutes postdose. No test article-related effects on ECG measurements or electrical rhythm were observed. All dogs maintained sinus rhythms throughout the study. No ventricular premature depolarizations were noted. There was no significant difference in heart rate between control and test-article treated groups except a significant increase at 0.01/1.5 mg/kg oxy/tetra as compared to the control on SD 1 (127 vs. 74 bmp). This is not considered to be treatment related due to the lack of dose-dependence. At 0.01/3.0 mg/kg on SD 1, the C_{max} of oxymetazoline in dogs was about 3-fold the C_{max} in human at the proposed maximal dose (3 sprays of 0.2 mL).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The information in this section includes summaries of nonclinical studies conducted by the Applicant and available literature from the public domain summarized by the Applicant.

Absorption

There were no literature references for pharmacokinetic (PK) data following the co-administration of oxymetazoline and tetracaine in animals by any route. Intranasal (IN) PK data for oxymetazoline alone was limited to a few studies in rats and rabbits. No intranasal studies of tetracaine were identified.

In 1995, Hayes and co-workers developed rapid HPLC-electrospray mass spectrometric (HPLC-ES-MS) assay for quantitation of oxymetazoline in whole rat blood (Hayes *et al.*, 1995). The assay permitted the analyses of nine samples per hour with the requisite sensitivity and selectivity and was used to determine the blood pharmacokinetics of oxymetazoline in rats dosed via IV and IN routes. The results showed that oxymetazoline was not extensively absorbed into the systemic circulation of rats from the intranasally dosed saline solution. The bioavailability of oxymetazoline delivered by the IN dosing (n = 4) was 6.8%. This was calculated using areas under the curves (0-90 min time points). This time range may be too short because oxymetazoline appeared to be detectable at later time-points (up to 6 hours) in other species such dogs and human. As the authors indicated, this publication was the first to report the use of non-radioisotopic method for the determination of oxymetazoline blood levels. The dose for both IV and IN administration was 40 mcg/animal without body weight adjustment. The body weight range was 200-300 g in animals.

In a study using a radiolabeled analytical method, the bioavailability of oxymetazoline administered via the IN route was estimated to be 48% in rabbit (Duzman *et al.* 1983). In this study, 20 mcL 0.25% [C^{14}]-oxymetazoline was administered by the nasal route, and radioactivity excreted in the urine was measured. During the first 48 hours, radioactivity excreted in the urine was approximately 48% for IV administration dose and approximately 23% for nasal administration, which suggested about 48% of oxymetazoline was absorbed by nasal administration (23/48 = 0.48). Although this is not the regular way to determine bioavailability, this study provided useful information to determine the rate of intranasal administration.

In the 2-week dog intranasal toxicity study conducted by the Applicant, the doses administered were 0.01 (oxy only), 0.01/0.6, 0.01/1.5 and 0.01/3.0 mg/kg of oxy/tetra in combination. The analytical method was validated under GLP conditions for the determination of oxymetazoline, tetracaine, and para-butylaminobenzoic acid (PBBA) concentration. The concentration range for oxymetazoline was 0.100 to 100 ng/mL, and the concentration range for tetracaine and PBBA was 1.00 to 1000 ng/mL. A summary of the systemic exposure of oxymetazoline in all treatment groups is shown in the table

below (**Table 8**). In general, the exposure of oxymetazoline decreased on SD 14 as compared to SD 1 in all treatment groups except the males of 0.01 mg/kg oxymetazoline alone group. Of note, peak plasma concentrations (C_{max}) of oxymetazoline increased with increasing doses of tetracaine when administered in combination despite the fixed dose of oxymetazoline. This trend was observed on SD 1 and SD 14 with greater magnitude observed on SD 1. This trend was also observed for oxymetazoline AUC levels on SD 1 albeit with a much lower magnitude of change (< 2 fold) with increasing doses of tetracaine. This trend was not seen in the AUC on SD 14. The reason for this phenomenon is not known. Individual variability could be part of the reason. The Applicant conducted a post-hoc analysis to determine if there was a statistically significant difference between the oxymetazoline treatment groups when administered with tetracaine or alone, and the results indicated that the oxymetazoline C_{max} was significantly higher in the 0.01/3.0 mg/kg oxy/tetra group compared to the 0.01 mg/kg oxymetazoline alone group on SD 1 ($p = 0.0014$) by the Dunnett's post-test. The remaining comparisons were not statistically significant. Therefore, the Applicant concluded that co-administration of tetracaine likely had no clinically meaningful impact on oxymetazoline disposition in dogs.

Table 8: Comparison of the exposure of oxymetazoline in the 2-week dog study

Doses Oxy/Tetra (mg/kg)	Males				Females			
	SD 1		SD 14		SD 1		SD 14	
	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
0.01/0 (n=3)	0.32	1.10	0.38	1.27	0.40	1.82	0.20	0.61
0.01/0.6 (n=3)	1.76	1.42	0.45	1.28	1.60	1.69	0.28	0.22
	5.5* ×	1.3 ×	1.2 ×	1.0 ×	4.0 ×	0.9	1.4 ×	0.4 ×
0.01/1.5 (n=3)	2.87	1.84	0.86	0.41	2.02	1.70	0.58	0.51
	9.0 ×	1.7 ×	2.3 ×	0.3 ×	5.1 ×	0.9 ×	2.9 ×	0.8 ×
0.01/3.0 (n =5)	3.94	2.23	1.10	1.37	6.57	2.89	0.49	0.22
	12.3 ×	2.0 ×	2.9 ×	1.1 ×	16.4 ×	1.6 ×	2.5 ×	0.36 ×

*value = C_{max} or AUC at oxy + tetra doses divided by C_{max} or AUC at oxy only

However, individual variability cannot fully explain this dose-dependent effect. Oxymetazoline plasma concentrations were generally quantifiable from 20 minutes until 6 hours postdose for both males and females in all oxymetazoline treatment doses. The mean T_{max} for oxymetazoline was 1.0 h (0.3 – 1.0 h) in males and 3.0 h (1.0 - 6.0 h) in females when oxymetazoline was given alone. In comparison, the mean T_{max} for oxymetazoline was 0.083 h (5 min) when oxymetazoline was given in combination with tetracaine, suggested oxymetazoline absorption may be enhanced by tetracaine. In this study, oxymetazoline solution and tetracaine solution did not mix before administration. Oxymetazoline solution was sprayed first (0.02 mL) and immediately followed by tetracaine solution with the volume of 0.02, 0.05, and 0.1 mL at 0.6, 1.5, and 3.0 mg/kg tetracaine dosing level. It is possible that a bigger volume further diluted oxymetazoline and therefore increased the oxymetazoline absorption. However, this cannot explain the 5.5-fold increase of C_{max} difference between the 0.01/0 and 0.01/0.6 mg/kg oxy/tetra

dosing levels because the volume sprayed were the same between these two groups, but the only difference was tetracaine. The $T_{1/2}$ of oxymetazoline was only estimable from a few animals and ranged from 1.03 hr to 2.96 hours in 0.01/3.0 mg/kg oxy/tetra group on SD 1.

The T_{max} was 0.083 hours (5 minutes) for tetracaine and 0.67 – 1.0 hour for the tetracaine major metabolite p-butylaminobenzoic acid (PBBA). Tetracaine plasma concentrations exhibited a biexponential decline with $T_{1/2}$ ranging from 0.19 to 2.2 hours. PBBA plasma concentrations displayed monoexponential decline with $T_{1/2}$ ranging from 1.17 to 5.45 hours. There was no significant difference in the C_{max} and AUC levels between SD 1 and SD 14. Both tetracaine and PBBA systemic exposure increased in a dose-proportional manner between 0.6 and 3.0 mg/kg dose level. It should be noted that tetracaine was not detectable in human subjects with the maximum recommended Kovanaze dose. A summary table of tetracaine and PBBA plasma exposure was available in the dog study review section (**Table 14**).

In the subcutaneous (SC) reproductive toxicity studies including the fertility and early embryofetal study (Study 20052281), embryofetal development study (Study 20013399), and the perinatal and postnatal development study (Study 20052282) (Segment I, II, and III studies, respectively) conducted by the Applicant, the doses were the same. Adult rats were injected via the SC route at 0 (b) (4) 0.1/0 (oxymetazoline only), 0/7.5 (tetracaine only), 0.01/7.5, 0.03/7.5, and 0.1/7.5 mg/kg of oxy/tetra. In the Segment I study, male and female rats were dosed for 28 days and 15 days, respectively, before cohabitation; blood samples were collected on SD 1 and 14 (females), or SD 1 and 28 (males). In the Segment II study, rats were dosed from Gestation Day 7 through Gestation Day (GD) 17; blood samples were collected on SD 1 and SD 11 (end of dosing). In the Segment III study, rats were dosed from GD 7 through Lactation Day (LD) 20; blood samples were collected from on LD 15 (SD 30). No samples were collected on SD 1 (GD 7). In all 3 studies, blood samples were collected from 3 rats/group/time point at 0 (predose), 5 and 20 minutes, and 1, 3, 6, and 24 hours postdose. The analytical methods were validated and the range for detection of oxymetazoline, tetracaine, and PBBA were the same as those in the 14-day dog study.

The exposure profiles were generally comparable for oxymetazoline, tetracaine, and PBBA, respectively, in females between the 3 studies. The T_{max} was 0.083 -0.33 h (5-20 min) for both oxymetazoline and tetracaine, and 0.33 – 3.0 h for PBBA. The $T_{1/2}$ was 1.8 - 2.48 h, 0.28 – 0.72 h, and 3.83 – 6.86 h for oxymetazoline, tetracaine, and PBBA, respectively. There was no significant difference in C_{max} and AUC after repeat-dose administration as compared to levels achieved on SD 1 (**Table 24 and Table 32**). The following table compares the C_{max} and AUC after repeat-dose administration in 3 studies (**Table 9**). The exposure of oxymetazoline (C_{max} and AUC) was increased in a dose-dependent manner. In contrast to what was observed in the 2-week dog study, co-administration of tetracaine did not appear to affect the exposure of oxymetazoline. However, co-administration of oxymetazoline appeared to decrease the systemic exposure of tetracaine generally, which is reasonable because oxymetazoline causes

vasoconstriction and slowness of blood flow. This effect was not oxymetazoline dose-dependent. This effect was most evident in the Segment III study with the values of tetracaine C_{max} and AUC at co-administration groups were approximately 24 – 39% and 54 – 88% of the tetracaine only administration, respectively. The exception at 0.03/7.5 mg/kg oxy/tetra in the Segment II study likely represented an outlier. At the same tetracaine dosing level, both the C_{max} and AUC were lower in the Segment III study as compared to the Segment I and Segment II study. This may reflect the difference in the metabolism level.

Table 9: Comparison of the systemic exposure in the reproductive studies in rats

Doses Oxy/Tetra (mg/kg)	**Segment I (SD 14)**		**Segment II (SD 11)**		**Segment III (SD 30)**	
	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
	Oxymetazoline (mean)					
0.1/0	21.0	27.5	11.8	28.0	16.1	22.2
0.01/7.5	1.37	2.68	1.21	2.26	0.84	1.85
0.03/7.5	4.43	7.37	3.67	5.61	3.11	5.50
0.1/7.5	18.8	29.4	11.5	19.4	13.4	22.8
	Tetracaine (mean)					
0/7.5	169	112	444	281	342	114
0.01/7.5	100	100	200	124	85.8	68.2
0.03/7.5	85.7	64.4	647	170	82.1	61.7
0.1/7.5	109	75.6	246	156	135	100
	PBBA (mean)					
0/7.5	7720	32000	6913	31085	3230	11900
0.01/7.5	4240	29800	4003	27324	2460	10100
0.03/7.5	3970	26300	3658	29042	2220	11500
0.1/7.5	5560	30000	4434	26179	2720	12900

Distribution

No nonclinical studies were conducted by the Applicant to evaluate the distribution of oxymetazoline or tetracaine following intranasal administration. This information is also not available from the public domain.

Metabolism

There is limited information available for oxymetazoline metabolism. In a published in vitro study, oxymetazoline metabolism was investigated in human, rat and rabbit homogenized liver tissue (S9) and their microsomes supplemented with nicotinamide adenine dinucleotide phosphate (NADPH) (Mahajan *et al.* 2011). The oxymetazoline

metabolites identified in S9 fractions are shown in the table below (**Table 10**). This in vitro assay indicated that the metabolism of oxymetazoline was generally similar between human, rat, and rabbit. However, it did not determine the specific enzymes that are responsible for oxymetazoline metabolism.

Table 10: Oxymetazoline metabolites identified in human, rat, and rabbit S9 fractions (Mahajan, 2011)

Metabolite	Retention Time (min)	Identification	Human Liver S9	Rat Liver S9	Rabbit Liver S9
COPYRIGHT MATERIAL WITHHELD					

An in vivo radiolabeled study in rabbits indicated that the excreted amounts of total radioactive oxymetazoline in urine following nasal instillation were about 23% (Duzman *et al.* 1983) . The proportion of oxymetazoline metabolites to unchanged oxymetazoline in urine was approximately 1.3 for intranasal administration.

The primary route of tetracaine metabolism is cleavage by pseudocholinesterase or nonspecific esterases to PBBA and dimethylaminoethanol. It is not known whether tetracaine may affect the metabolism of oxymetazoline or vice versa.

Excretion

There is limited information available about the excretion of oxymetazoline.
The urine excretion of [^{14}C]-oxymetazoline (6.3 mCi/mM) was investigated in female New Zealand white rabbits (3/group) (Duzman *et al.* 1983). Rabbits were given a dose of 0.25% oxymetazoline (20 mcL) by the intranasal (IN) route, 0.05% (50 mcL/eye) by topical ocular route, and 0.01% (1 mL) by IV administration. Urine was collected for 48 hours postdose, concentrated, and analyzed. The urinary excretion of radioactivity during the first 24-hour period following ^{14}C-oxymetazoline administration was twice as great when given by the IV route when compared to the topical ocular or intranasal routes as shown in the table below (**Table 11**). The excretion was very similar for the 24-48 hour time period for all three routes of administration.

Table 11: Urinary excretion of radioactivity after single-dose of ^{14}C-oxymetazoline in rabbits (Duzman, 1983)

Route of Administration[a]	Radioactivity Excreted in Urine, %	
	0-24 hour[b]	24-48 hour[b]
COPYRIGHT MATERIAL WITHHELD		

[a] Three rabbits were used for each route of administration

[b] Values are mean ± standard deviation

Information of tetracaine excretion was not available from the public domain.

The excretion of oxymetazoline, tetracaine, and PBBA in milk was examined in the perinatal and postnatal development study in rats (Study 20052282). Following SC injection of oxymetazoline and tetracaine to the pregnant rats from GD 7 through LD 15 at 0/0 (vehicle control), 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, and 0.1/7.5 mg/kg/day, milk samples (0.1 mL/dam) were collected from 5-6 rats/group at approximately 2 hours postdose. Each rat was administered 1 unit of oxytocin IV at least 5 minutes prior to collection. The milk samples were analyzed under non-GLP conditions using the modified rat plasma analysis method. Mean concentrations of oxymetazoline, tetracaine, and PBBA in maternal milk were generally dose dependent across the maternal dose groups (**Table 12**). In comparison to the plasma C_{max}, the milk concentration of oxymetazoline was higher at all treatment groups while both tetracaine and its major metabolites were significantly lower.

Table 12: Milk concentrations of oxymetazoline, tetracaine, and PBBA in rats

Dosing group (mg/kg oxy/tetra)	Oxymetazoline (ng/mL)	Tetracaine (ng/mL)	PBBA (ng/mL)
0/0	0	0	0
0.1/0	33.8	0	0
0/7.5	0	72.9	114.5
0.01/7.5	2.52	54.2	118.3
0.03/7.5	6.95	61.5	100.5
0.1/7.5	33.1	62.7	131.2

6 General Toxicology

6.1 Single-Dose Toxicity

The Applicant did not conduct any single-dose studies of oxymetazoline or tetracaine, alone or in combination. Acute toxicity studies for oxymetazoline in animals were not available from the public domain. Published studies have reported tetracaine acute toxicities examined in mice, rats, guinea pigs, and rabbits. Toxicities reported have included disturbance in coordination, salivation, loss of righting reflex, ataxia, spasm with convulsion, respiratory arrest, paralysis, and death. Pathological examinations revealed axonal degeneration in some studies. Histopathology examination of an adequate battery of tissues was not conducted in any of the studies. An appropriate NOAEL for tetracaine cannot be identified from these studies. In the 14-day repeat-dose intranasal toxicity study in dogs, a single 6 mg/kg tetracaine dose in combination with 0.01 mg/kg oxymetazoline caused animal death following moribund condition including tremors, excessive salivation, sedation, and/or muscular rigidity. Similar clinical signs were also observed following 4 mg/kg tetracaine in combination with 0.01 mg/kg oxymetazoline, which were considered to be intolerable by the Applicant. The high dose was eventually determined to be 3 mg/kg for the 14-day dog study.

6.2 Repeat-Dose Toxicity

Study title: A 14-day study of oxymetazoline and tetracaine by intranasal administration in dogs with a 14-day recovery period

Study no.:	20010720
Study report location:	eCTD submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	02/29/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Tetracaine: 10-002179, >99% Oxymetazoline: 10-002181, >99%

Key Study Findings

- Young male and female dogs were administered doses of 0 (vehicle), 0.01 (oxymetazoline only), 0.01/0.6, 0.01/1.5 and 0.01/3.0 mg/kg/day of oxymetazoline/tetracaine (oxy/tetra) in combination via intranasal spray using a mucosal atomization device (MAD). The vehicle was similar to the clinical formulation without the active ingredients.

- Dose-dependent reversible clinical observations included vomiting, reddened/swollen gums, vocalization, salivation, nasal discharge, soft and

mucoid stools, red and swollen pinna shivering, shallow or labored breathing, impaired mobility, and wobbly gait at 0.01/3.0 mg/kg oxy/tetra.

- Histopathological changes were primarily in the local tissue including cell infiltration and squamous metaplasia in nasal cavity at 1.5 mg and 3.0 mg tetracaine dosing group in a dose-dependent manner. These changes were reversible. No degenerate neuroepithelium was noted on Fluorojade B stain of the olfactory epithelium in the nasal cavity at all dosing levels of test articles.

- NOAEL = 0.01/0.6 mg/kg oxy/tetra. However, since all changes appear to be reversible, the exposure levels at the high-dose (0.01/3.0 mg/kg oxy/tetra) may be used to support safety of the drug product for human use.

- TK analysis revealed that the systemic exposure of oxymetazoline appeared to be increased when co-administered with tetracaine in a tetracaine dose-dependent manner.

Methods

Doses:	0/0, 0.01/0, 0.01/0.6, 0.01/1.5, 0.01/3.0 mg/kg oxy/tetra as shown in the table below.
Frequency of dosing:	daily
Route of administration:	Intranasal; Doses were administered using a standard plastic syringe with a mucosal atomization device (MAD) attached to the tip. This mimicked human administration.
Dose volume:	The total dose volume was various in different groups, as seen in the table below. These volumes were divided equally between the nares.
Formulation/Vehicle:	Intranasal solution similar to the clinical formulation

Component	Function	Amount
Citric acid (b) (4) USP		(b) (4)
Sodium hydroxide, NF	pH adjustment	As needed
Hydrochloric acid, NF	pH adjustment	As needed
Benzyl alcohol, NF		(b) (4)
Hydroxycellulose, NF (5000 CPS)		
(b) (4) water, USP		

Species/Strain:	Male and female Beagle dogs
Number/Sex/Group:	As shown in the table below (**Table 13**). The additional 2/sex/group in control and high-dose group were used for recovery observation.
Age:	7 months
Weight:	Male: 6.6 - 8.7 kg; Female: 5.7 - 7.5 kg
Satellite groups:	none
Unique study design:	The originally planned tetracaine HCl dose levels were 0.6, 3.0, and 6.0 mg/kg for Groups 3, 4, and 5, respectively. The high dose was reduced to 4.0 mg/kg after the 1st dosing due to adverse effects. The doses were ultimately changed to 0.6, 1.5, and 3.0 mg/kg due to adverse changes in the one dog after one-dose of 4 mg/kg.
Deviation from study protocol:	Deviations did not impact the quality of the study.

Table 13: Study design of the 2-week dog study

Group No.	No. of Main (Recovery) Animals		Dose Material(s)	Dose Level (mg/kg)		Total Dose Volume (mL/kg)[a]	Dose Concentration (mg/mL)	
	Males	Females		Oxy	Tetra		Oxy	Tetra
1	3 (2)	3 (2)	(b) (4)					(b) (4)
2	3	3						
3	3	3	Oxy + Tetra	0.01	0.6	0.02 Oxy + 0.02 Tetra	0.5	30
4[b,c]	3	3	Oxy + Tetra	0.01	1.5	0.02 Oxy + 0.05 Tetra	0.5	30
5[b,c]	3 (2)	3 (2)	Oxy + Tetra	0.01	3.0	0.02 Oxy + 0.10 Tetra	0.5	30

[a] Oxymetazoline HCl intranasal solution was administered first followed immediately by the formulation (b) (4) or tetracaine intranasal solution.

In-life Observations

Mortality

Two male animals from the high-dose group which were originally designated for 6.0 mg/kg tetracaine administration (with 0.1 mg/kg oxymetazoline) were euthanized right after the first dose due to moribund conditions including tremors, excessive salivation, sedation, and/or muscular rigidity salivation, according to the result summary. Other animals were not dosed in this group. The individual data of these 2 animals were not included in the study report. The dose at the high dose group was lowered to 4.0 mg/kg in the high-dose group, and two spare animals were added to this group.

Clinical Signs

After administration of 4 mg/kg to one male animal, clinical signs similar to those observed in the 2 euthanized animals were observed. The Applicant decided that the 4.0 mg/kg is not tolerable and the high dose was lowered to 3.0 mg/kg. At 3.0 mg/kg tetracaine, clinical observations included emesis, reddened/swollen gums, vocalization, salivation, nasal discharge, soft and mucoid stools, red and swollen pinna shivering, shallow or labored breathing, impaired mobility, and wobbly gait in both male and female animals. All these changes were reversible. Coughing and vomiting were also observed at 1.5 mg/kg tetracaine dosing group. At 0.6 mg/kg and 1.5 mg/kg tetracaine, the primary treatment related changes were soft stool and salivation. Soft stool was also observed in oxymetazoline only group, suggesting this effect was contributed to oxymetazoline treatment. No other treatment-related changes were observed in 0.01 mg/kg oxymetazoline group.

Body Weights and Feed Consumption

No test article-related changes were observed.

Ophthalmoscopy
Ophthalmic examination was conducted 8 days prior to dosing initiation and on SD 10 after dosing. No treatment-related ocular changes were reported.

ECG
ECG was recorded at predose, SD 1, SD 14, and SD 28. No test article-related effects on ECG measurements or electrical rhythm occurred during the study.

Clinical Pathology

Blood was collected by venipuncture of the jugular vein in all study groups at pre-dose and SD 15 (prior to necropsy) for hematology and clinical chemistry analysis. Urine was collected overnight on the same days. Clinical pathology analysis was also conducted at the end of recovery in control and high dose groups.

The values of hematology and clinical pathology parameters had large variability. There was a statistically significant increase in the number of leukocytes, lymphocytes, and monocytes in females at 3 mg/kg tetracaine dosing group (~50%) as compared to the control. However, all these values were within the range of historical control in the test facility, which may not be considered adverse. Other changes were not considered to be treatment related. In addition, no test article-related effects on parameters of urinalysis occurred.

Necropsy

Six animals (3/sex) from each study group were euthanized on SD 15 after 14-day administration. In addition, 2 animals/sex in control and high dose group were sacrificed on SD 29 following a 14-day recovery period.

Gross Pathology

Gross pathology examinations included evaluation of the carcass and musculoskeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Treatment-related changes were not observed.

Organ Weights

The following organs were collected and the organ weights were recorded. There was large variability in the individual values of organ weight. Greater than 10% changes were observed occasionally with statistically significant difference between control and treated groups. Dose-dependence was not observed. Overall, the results of the organ weights did not indicate adverse changes associated with oxymetazoline and tetracaine administration.

Brain	Liver
Epididymis[a]	Lung
Gland, adrenal[a]	Ovary[a]
Gland, pituitary	Spleen
Gland, prostate	Testis[a]
Gland, thyroid (including parathyroid)[a]	Thymus
Heart	Uterus
Kidney[a]	

[a] Paired organ weight.

Histopathology

Adequate Battery:
Yes

Peer Review:
No

Histological Findings
In addition to the tissues that are regularly collected for histopathology, nasal cavity and nasal turbinate tissues were also collected. All tissues were sectioned and stained with hematoxylin and eosin (H&E). The treatment-related changes are summarized in the table below (**Table 14**)

The primary changes that were considered to be tetracaine treatment-related were cell infiltration and squamous metaplasia in nasal cavity. Minimal to mild multifocal mixed-cell infiltration was seen in all 3 nasal cavity sections tested bilaterally at ≥ 0.01/1.5 mg/kg oxy/tetra in a dose-dependent manner. Squamous metaplasia was focal and multifocal in the 2 of the 3 nasal cavity sections at ≥ 0.01/1.5 mg/kg oxy/tetra with minimal and mild severity grade. These findings were seen in both males and females but the changes were more severe in males. These changes were not seen in the recovery animals at 0.01/3.0 mg/kg oxy/tetra, indicating reversibility of the findings.

The changes in the lung, trachea, and urinary bladder were only observed in animals from the 0.01 mg/kg oxymetazoline only treatment group. The treatment relationship was difficult to determine because similar changes were not seen in animals when the same dose oxymetazoline was treated in combination with tetracaine. There is no evidence to indicate that co-administration with tetracaine would alleviate the adverse effects of oxymetazoline. Therefore, these low-incidence changes may be considered incidental. The Applicant indicated that foreign body like plant material once aspirated into the lung induces inflammatory response and is occasionally encountered related to the experimental procedure. At the end of recovery, moderate subacute bronchoalveolar inflammation was noted in the lung of one dog at 0.01/3.0 mg/kg oxy/tetra. The Applicant indicated that even though aspirated material was not detected on microscopic examination the distribution of the lesion (multifocal, bronchoalveolar) was suggestive of aspiration and considered to be not likely directly related to tetracaine HCl. As a similar change was not seen at this dose level in animals from the main dosing phase and other animals from the recovery phase, I do not think this change

represents a delayed change in response to oxymetazoline and tetracaine administration.

Hypospermatogenesis and aspermatogenesis are not rare in normal dogs especially at a young age (7 month in this study). The change in this study was likely to be treatment related because the severity was dose-dependent. However, this change appeared to be reversible because similar change was not seen in recovery animals at the high dose.

Table 14: Summary of the histopathological changes in the 14-day dog study

Histological findings		Severity	Control		O/T 0.01/0		O/T 0.01/0.6		O/T 0.01/1.5		O/T 0.01/3.0	
			M	F	M	F	M	F	M	F	M	F
Nasal cavity (Section 1)	Infiltration, mixed-cell	Minimal (multifocal)									2	
Nasal cavity (Section 2)	Infiltration, mixed-cell	Minimal (focal)							1			
		Minimal (multifocal)							2	1	1	1
		Mild (multifocal)									2	2
	Metaplasia, squamous	Minimal (focal)							2		1	
		mild(focal)									1	
		Mild (multifocal)										1
Nasal cavity (Section 3)	Infiltration, mixed-cell	Minimal (multifocal)							1		1	3
		Mild (multifocal)									2	
	Metaplasia, squamous	Minimal (focal)							1		1	1
		mild(focal)									1	
Lung	Foreign body, plant material				1							
	Inflammation, bronchial	minimal			1				1			
	Inflammation, granulomatous	mild (multifocal)			1							
Trachea	Inflammation, mononuclear	minimal			1							
Urinary bladder	Degeneration/ regeneration, myofiber	mild			1							
Testis	Hypo/ aspermatogenesis	minimal					1					
		mild							1		1	

Special Evaluation

The nasal cavity was also stained with Fluorojade B and Bielschowsky's silver stain to assess for nerve terminal degeneration. The silver stain did not reveal details that were not identifiable by routine histology (hematoxylin & eosin staining). The laminaproprial nerve bundles associated with the olfactory epithelium were within normal limits. No degenerate neuroepithelium was noted on Fluorojade B stain of the olfactory epithelium in the nasal cavity at all test article dosing levels.

Toxicokinetics

Blood was collected by venipuncture of the jugular vein, blood samples were collected before dosing, 5 and 20 minutes, and 1, 3, 6, and 24 hours after dosing. Concentrations of oxymetazoline, tetracaine, and the tetracaine metabolite p-butylamino benzoic acid (PBBA) in plasma from SD 1 and SD 14 were measured, and TK parameters were calculated. The lower limit of quantitation (LLOQ) of the analytical method was 0.1 for oxymetazoline and 1.0 ng/mL for tetracaine and PBBA.

The animal that was dosed at 4.0 mg/kg tetracaine (Animal 5232) on SD 1 had tetracaine and PBBA plasma concentrations similar to those dosed at 3.0 mg/kg.

Oxymetazoline plasma concentrations were generally quantifiable from 20 minutes postdose until 6 hours postdose for 0.01/0 mg/kg oxy/tetra males and females. For Groups 3 and 4, and 5, oxymetazoline was generally quantifiable from 5 minutes postdose until 6 hours postdose. It appears the systemic exposure of oxymetazoline was higher in animals when co-administered with tetracaine. Individual variability in animals was large especially at 0.01/3.0 mg/kg oxy/tetra. For example, the range of C_{max} was 1.43 to 13.3 ng/mL in females from the 0.01/3.0 mg/kg oxy/tetra dose group on SD 1.

Table 15: Systemic exposure of oxymetazoline in the 2-week dog toxicity study

Doses Oxy/Tetra (mg/kg)	Males				Females			
	SD 1		SD 14		SD 1		SD 14	
	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h}* (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
0.01/0	0.31	0.68	0.55	1.50	0.46	1.65	0.23	0.61
	0.35	1.35	0.27	0.68	0.51	1.98	0.16	
	0.30	1.26	0.33	1.63	0.24		0.20	
Mean	0.32 ± 0.02	1.10 ± 0.36	0.38 ± 0.16	1.27 ± 0.52	0.40 ± 0.14	1.82	0.20 ± 0.4	0.61
0.01/0.6	1.45	1.63	0.69	2.46	0.61	1.55	0.42	0.26
	2.42	1.34	0.33	0.77	1.85	1.74	0.24	0.18
	1.42	1.29	0.34	0.60	2.34	1.79	0.18	
Mean	1.76 ± 0.57	1.42 ± 0.19	0.45 ± 0.21	1.28 ± 1.03	1.60 ± 0.90	1.69 ± 0.13	0.28 ± 0.13	0.22
0.01/1.5	1.00	0.60	0.52	0.27	2.37	2.28	0.88	0.82
	4.98	2.43	1.26	0.56	2.83	1.39	0.25	
	2.64	2.51	0.79		0.85	1.44	0.60	0.19
Mean	2.87 ± 2.00	1.84 ± 1.09	0.86 ± 0.38	0.41	2.02 ± 1.04	1.70 ± 0.50	0.58 ± 0.32	0.51
0.01/3.0	3.09	0.91	2.67	1.20	13.3	3.90	0.20	0.17
	4.87	4.29	0.92	0.73	1.43	1.19	0.38	
	2.92	2.31	0.67	1.89	4.82	2.93	0.29	0.19
	3.93	2.11	0.47	0.22	6.28	2.02	0.68	0.26
	2.84	1.43	0.78	2.80	7.01	4.41	0.88	0.26
Mean	3.94 ± 1.96	2.23 ± 1.49	1.10 ± 0.89	1.37 ± 1.01	6.57 ± 4.33	2.89 ± 1.32	0.49 ± 0.29	0.22 ± 0.05

* The AUC of some animal(s) cannot be calculated because the number of consecutive quantifiable plasma concentrations post dose was less than 3.

Both tetracaine and PBBA systemic exposure increased in a dose-proportional manner between 0.6 and 3.0 mg/kg dose level. The C_{max} levels of PBBA were significantly lower than that of tetracaine in all treatment groups while the AUC levels of PBBA were generally comparable at low- and mid-tetracaine dosing group but significantly higher in high-tetracaine dosing group, consistent with the longer half-life for PBBA.

Table 16: Systemic exposure of tetracaine/PBBA in the 2-week dog study

Tetra (mean)	**Males**				**females**			
	SD 1		SD 14		SD 1		SD 14	
	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)	C_{max} (ng/mL)	AUC_{0-24h} (ng•h/mL)
0.01/0.6	473	158	828	166	404	118	312	125
0.01/1.5	1431	335	1278	321	1060	278	627	197
0.01/3.0	3233	761	1506	445	3703	781	1509	419
PBBA (mean)								
0.01/0.6	75.2	198	40.2	87.8	61.6	197	64.6	226
0.01/1.5	112	301	123	454	128	424	149	486
0.01/3.0	321	1061	209	761	311	868	276	871

Dosing Solution Analysis

All values were within 10% of the theoretical concentration.

7 Genetic Toxicology

No genotoxic studies were conducted by the Applicant to evaluate the potential mutagenic and clastogenic effects of oxymetazoline and tetracaine from the public domain. The Applicant referenced the genotoxicity studies for tetracaine in NDA 21623 for Synera. These studies include an in vitro bacteria reverse mutation assay (Study 23841-0-409OECD), an in vitro chromosomal aberration in Chinese hamster ovary (CHO) cells (23841-0-437OECD), and an in vivo mouse micronucleus assay (Study 23841-0-455OECD). These studies were conducted under GLP regulation and determined to be valid studies by the Agency in the Synera NDA review. The Agency has concluded that tetracaine tested negative in the in vitro bacterial reverse mutation assay and the in vivo mouse micronucleus assay. Although tetracaine tested negative in the absence of metabolic activation in the in vitro chromosome aberrations assay, in the presence of metabolic activation, tetracaine was equivocal. This information is included in the current FDA-approved Synera label.

In addition, the Applicant referenced a standard plate incorporation in vitro bacterial reverse mutation assay for tetracaine that was published decades ago (Waskell 1978). This assay indicated that tetracaine up to 10 mg/plate did not induce increase in the number of revertant colonies compared to control values in the presence and absence of rat liver S9 metabolic activation. This study was conducted prior to the implementation of GLP regulation, and did not provide additional information regarding the genotoxicity of tetracaine. The results of this study are not recommended to be included in the label.

The Applicant indicated that the genotoxic potential of oxymetazoline has not been evaluated. This is consistent to the result of my literature search. The Division has informed the Applicant that this information is not required at the pre-NDA meeting.

The following studies were conducted to qualify tetracaine impurities, (b) (4) , which contain structure alert groups. (b) (4) was initially included in the drug product specification table as an (b) (4) drug substance and (b) (4) Revised drug product specifications submitted during the review cycle indicated that the drug product does not contain this impurity.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: (b) (4): Salmonella-E. Coli/mammalian microsome reverse mutation assay

Study no.:	(b) (4)
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	(b) (4)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), SYN-58283-32-21, 99.6%

Key Study Findings

In both the initial and confirmatory assays, the mean values of the revertant colonies per plate at all concentrations of the test article were not significantly higher than that of the DMSO vehicle control in any of the bacteria strain tested. These results indicated that (b) (4) is not mutagenic.

Methods

Strains:	Salmonella strains TA1537, TA98, TA100 and TA1535, *E. coli* strain WP2 uvrA
Concentrations in definitive study:	25, 50, 100, 250, 500, 1000, 2500 and 5000 mcg/plate
Basis of concentration selection:	Maximum concentration recommended by OECD protocol
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	For tests without metabolic activation, ICR-191 acridine was for TA1537; 2-nitrofluorene for TA98; sodium azide was for TA100 and TA1535; and 4-nitroquinoline-N-oxide was for WP2 uvrA. For testing with metabolic activation, 2-aminoanthracene was the positive control for all strains
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation method was used. Incubation time was 48 hours. The metabolic activation system was Aroclor™ 1254-induced rat liver S9 fraction.

Study Validity

Analytical data demonstrated that the concentrations of the test article formulations were within the ±10% of nominal, which is acceptable. Triplicate plates were tested at each concentration. The means of the vehicle control data were comparable to the historical data. The means of all positive control data were at least 3-fold greater than the means of the vehicle control data and comparable to the historical data. The study is therefore considered valid.

Results

Initial Assay

Cytotoxicity (i.e., reduction in the background lawn and/or mean number of revertant colonies) was not observed in any strain with or without metabolic activation. Precipitates were observed at ≥ 500 mcg/plate in all strains with and without metabolic activation. A summary of the results is shown in below (extracted from the study report). The values represented the mean revertant colonies per plate calculated from triplicate plates, and the values in the parenthesis represented the standard deviation. The mean value of the revertant colonies per plate at any concentration of the test article was not significantly higher than that of the DMSO vehicle control in any of the bacteria strain tested.

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
		WITHOUT ACTIVATION									
DMSO	50 µL	5	(3)	12	(2)	126	(21)	7	(1)	44	(6)
ICR	0.5	280	(29)								
2NF	2.5			530	(105)						
SA	1.0					469	(53)	279	(28)		
NQNO	2.0									798	(62)
[redacted]											(b) (4)
		WITH ACTIVATION									
DMSO	50 µL	6	(2)	17	(1)	131	(18)	7	(4)	53	(5)
2AA	2.5	115	(27)	1746	(191)	1868	(63)	172	(15)		
2AA	10.0									410	(51)
[redacted]											(b) (4)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
DMSO: Dimethylsulfoxide
[a] Calculated from triplicate plates
[b] Precipitates present

Confirmatory Assay

The results of the confirmatory plate incorporation assay are shown below (extracted from the study report). Based on precipitates observed in the initial assay, the concentrations tested in the confirmatory assay were 25, 50, 100, 250, 500, and 1000 mcg/plate using the plate incorporation method. The results in the confirmatory assay were comparable to those in the initial assay.

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	4	(1)	13	(4)	121	(10)	10	(4)	31	(10)
ICR	0.5	282	(10)								
2NF	2.5			613	(48)						
SA	1.0					447	(15)	361	(2)		
NQNO	2.0									983	(96)
[redacted]											(b) (4)
WITH ACTIVATION											
DMSO	50 µL	6	(3)	23	(3)	114	(5)	11	(5)	31	(6)
2AA	2.5	153	(29)	2267	(296)	1283	(187)	212	(10)		
2AA	10.0									430	(34)
[redacted]											(b) (4)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
DMSO: Dimethylsulfoxide
[a] Calculated from triplicate plates, unless otherwise noted
[b] Precipitates present
[c] Cytotoxicity: > 50% reduction in mean number of revertant colonies
[d] Plate discarded due to contamination, calculated from duplicate plates

Study title: [redacted] (b) (4): Salmonella-E. Coli/mammalian microsome reverse mutation assay

Study no.: [redacted] (b) (4)
Study report location: eCTD
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: [redacted] (b) (4)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: [redacted] (b) (4) SYN-63277-07-05, 99.0%

Key Study Findings

In both the initial and confirmatory assays, mean number of revertant colonies was comparable to the vehicle at all concentrations for all tester strains with and without metabolic activation. These results indicated that [redacted] (b) (4) is not mutagenic.

Methods

Strains:	Salmonella strains TA1537, TA98, TA100 and TA1535, *E. coli* strain WP2 uvrA
Concentrations in definitive study:	25, 50, 100, 250, 500, 1000, 2500 mcg/plate
Basis of concentration selection:	High concentration was limited by precipitation
Negative control:	Dimethylsulfoxide (DMSO)
Positive control:	For tests without metabolic activation, ICR-191 acridine was for TA1537; 2-nitrofluorene for TA98; sodium azide was for TA100 and TA1535; and 4-nitroquinoline-N-oxide was for WP2 uvrA. For testing with metabolic activation, 2-aminoanthracene was the positive control for all strains
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation method was used. Incubation time was 48 hours. The metallic activation system was Aroclor™ 1254-induced rat liver S9 fraction.

Study Validity

Analytical data demonstrated that the concentrations of the test article formulations were within the ±10% of nominal, which is acceptable. Triplicate plates were tested at each concentration. The means of the vehicle control data were comparable to the historical data. The means of all positive control data were at least 3-fold greater than the means of the vehicle control data and comparable to the historical data. The study is therefore considered valid.

Results

Initial Assay

Precipitates were observed at ≥ 250 mcg/plate in all strains with and without metabolic activation. Due to the density of the precipitates, plates at 5000 mcg/plate could not be evaluated. A summary of the results is shown below (extracted from the study report). Cytotoxicity was observed in the initial assay at ≥ 500 mcg/plate in TA1537, TA98, TA100, and TA1535 without metabolic activation. The values represented the mean revertant colonies per plate calculated from triplicate plates, and the values in the parenthesis represented the standard deviation. The mean value of the revertant colonies per plate at any concentration of the test article was not significantly higher than that of the DMSO vehicle control in any of the bacteria strain tested with or without the presence of the metabolic activation system.

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	2	(2)	17	(5)	94	(14)	6	(2)	33	(9)
ICR	0.5	225	(30)								
2NF	2.5			556	(38)						
SA	1.0					447	(32)	386	(21)		
NQNO	2.0									1014	(131)
[redacted] (b) (4)											
WITH ACTIVATION											
DMSO	50 µL	3	(2)	12	(2)	96	(8)	9	(4)	44	(4)
2AA	2.5	226	(14)	1514	(146)	1419	(93)	179	(31)		
2AA	10.0									419	(21)
[redacted] (b) (4)											

2AA – 2-Aminoanthracene
2NF – 2-Nitrofluorene
ICR – ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
DMSO: Dimethylsulfoxide
[a] Calculated from triplicate plates
[b] Precipitates present
[c] Due to density of precipitates, plates not scored and/or counted
[d] Slightly reduced background lawn
[e] Cytotoxicity: reduced background lawn, plates not counted

Confirmatory Assay

The results of the confirmatory plate incorporation assay are shown below (extracted from the study report). Based on precipitates observed in the initial assay, the concentrations tested in the confirmatory assay were 25, 50, 100, 250, and 500 mcg/plate using the plate incorporation method. The results in the confirmatory assay were comparable to those in the initial assay.

REVERTANT COLONIES PER PLATE—Mean (SD)[a]

Treatment Group	µg/plate	TA1537		TA98		TA100		TA1535		WP2*uvrA*	
WITHOUT ACTIVATION											
DMSO	50 µL	3	(1)	14	(3)	109	(12)	9	(3)	55	(5)
ICR	0.5	307	(13)								
2NF	2.5			613	(57)						
SA	1.0					560	(19)	318	(19)		
NQNO	2.0									1173	(103)
[redacted]											(b) (4)
WITH ACTIVATION											
DMSO	50 µL	6	(1)	17	(1)	108	(11)	12	(4)	58	(4)
2AA	2.5	206	(38)	2208	(104)	1858	(34)	209	(19)		
2AA	10.0									447	(3)
[redacted]											(b) (4)

2AA: 2-Aminoanthracene
2NF: 2-Nitrofluorene
ICR: ICR-191 Acridine
NQNO: 4-nitroquinoline-N-oxide
SA: Sodium azide
SD: standard deviation
DMSO: Dimethylsulfoxide
[a] Calculated from triplicate plates, unless otherwise noted
[b] Precipitates present
[c] Slightly reduced background lawn
[d] Plate discarded due to contamination, calculated from duplicate plates

8 Carcinogenicity

Chronic rodent carcinogenicity studies have not been conducted with oxymetazoline or tetracaine. Due to the proposed acute indication, carcinogenicity assessment for Kovanaze is not required according to the ICH S1A guidance.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A subcutaneous fertility and early embryo-fetal development study of oxymetazoline hydrochloride and tetracaine hydrochloride in rats

Study Number:	20052281
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/20/2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Oxymetazoline HCl, 018229AX22, 100% Tetracaine HCl, 0006093547, 100%

Key Study Findings

- Oxymetazoline and tetracaine alone or in combination were administered to male and female rats from 28 days and 14 days before cohabitation, respectively, at 0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, and 0.1/7.5 mg/kg oxymetazoline/tetracaine (oxy/tetra) by subcutaneous injection. Male rats were sacrificed after dosing for 64 to 67 days. Female rats were dosed through GD 7 and sacrificed on GD 13.

- Mortalities occurred in males during the dosing period when rats were treated with 0.1 mg/kg oxymetazoline alone or in combination with 7.5 mg/kg tetracaine. There was no treatment-related death or euthanasia in females.

- Clinical signs associated with tetracaine administration included ataxia, impaired righting reflex, lost righting reflex, hunched posture, decreased motor activity and low carriage in 7.5 mg/kg tetracaine treatment groups. However, the incidences of these changes were significantly lower when tetracaine was co-administered with 0.01 and 0.03 mg/kg oxymetazoline as compared to tetracaine only administration and co-administration with 0.1 mg/kg oxymetazoline. On the other hand, incidences of local changes in the injection sites were significantly higher at 0.01/7.5 mg/kg oxy/tetra groups.

- Incidences of clinical signs in males were higher than females. Hyperpnea, bradypnea, clonic convulsion were only observed at 0.1/7.5 mg/kg oxy/tetra in males. In addition, the body weight decrease at 0.1 mg/kg oxymetazoline only (10%) or in combination with 7.5 mg/kg tetracaine (17%) was more severe in males than females. In female, body weight decrease was less than 6.5% compared to control. These findings suggest there may be a sex difference in response to oxymetazoline and tetracaine.

- Necropsy examination detected treatment-related changes in both males (misshapen kidney with yellow area) and females (mottled red and dark kidney) at 0.1/7.5 mg/kg oxy/tetra.

- A NOAEL for male general toxicity could not be identified due to clinical signs observed at all dose levels. However, the 0.03/7.5 mg/kg oxy/tetra dose level can be considered an acceptable LOAEL due to the low incidence and severity at this dose and below. The NOAEL for maternal toxicity is identified to be 0.03/7.5 mg/kg oxy/tetra.

- In females, 0.1 mg/kg oxymetazoline administration significantly decreased number of corpora lutea (16%) and implantation (16%) when co-administered with 7.5 mg/kg tetracaine, and the values were outside of the range of historical control. In addition, a dose-dependent decrease in the number of viable embryos was observed at 0.01, 0.03, and 0.1 mg/kg oxymetazoline doses when co-administered with 7.5 mg/kg tetracaine (12%, 14%, and 18%), respectively. Although these values were within the range of historical control, the Applicant considered these changes as adverse effects related to treatment. I agree with this evaluation. Decrease in corpora lutea (12%), implantation (15%), and viable embryos (16%) were also observed in animals administered with 0.1 mg/kg oxymetazoline only without statistical significance.

- A NOAEL cannot be identified for female fertility and early embryofetal toxicity.

- In males, sperm motility was significantly decreased in the 0.1 mg/kg oxymetazoline treated groups with (18%) or without (31%) tetracaine co-administration. Decreases in total sperm count (44%) and sperm concentration (25%) in cauda epididymis were also observed in 0.1 mg/kg oxymetazoline only group. Total sperm count in the cauda epididymis was also significantly decreased at 0.03/7.5 mg/kg (20%) and 0.1/7.5 mg/kg oxy/tetra (33%). The lack of difference in sperm concentration observed in the 0.03 and 0.1/7.5 mg/kg oxy/tetra groups compared to control was likely due to the decreased cauda epididymis organ weight at these doses (11% and 26%, respectively). Reproductive organs including cauda epididymis, seminal vesicles, and prostate were found with lower organ weight after all doses of oxymetazoline administration alone or in combination with tetracaine in an oxymetazoline dose-dependent manner. This change was likely due to the direct effect of oxymetazoline and a secondary effect of the body weight change of animals. At low dose, the effect was not evident when the organ weight changes were adjusted with body weight.

- The NOAEL is identified as 0.01/7.5 mg/kg oxy/tetra for male fertility.

- TK analysis indicated that oxymetazoline systemic exposure was generally increased dose-dependently. The co-administration of tetracaine did not appear to affect the absorption of oxymetazoline and vice versa. There was no significant difference with respect to C_{max} and AUC between males and females.

Methods

Doses:	0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxy/tetra
Frequency of dosing:	Daily
Dose volume:	2 mL/kg
Route of administration:	SC. Injection sites were rotated in 6 approximately equal area on the dorsum to avoid injecting in the same site more than twice
Formulation/Vehicle:	Solution/ (b) (4) (not including clinical formulation excipient benzyl alcohol and HPC)
Species/Strain:	Crl:CD(SD) Sprague Dawley rats
Number/Sex/Group:	22/sex/group
Satellite groups:	TK groups were included with 6/sex/group in treatment groups and 3/sex/group in control
Study design:	Males were administered 28 days before cohabitation, through the day before euthanasia (SD 64 to 67). Females were administered beginning 15 days before cohabitation through GD 7. Males were sacrificed after dosing, and females were sacrificed during the mid-pregnancy (GD 13). The parameters and end points evaluated including viability, clinical signs, body weights, food consumption, estrous cycling, mating, ovarian and uterine examinations, sperm motility and concentration, gross necropsy findings, and organ weights.
Deviation from study protocol:	During the dosing period, some control animals were dosed with 0.03 mg/kg oxy/tetra formulation in one dosing day. The impact of this single occurrence of deviation to the study was minimal because 1) this represented a single occurrence; and 2) significance was not seen in these animals as compared to other animals in this group. This deviation does not appear to affect the quality and integrity of the study. Other deviations did not affect the quality and integrity of the study

Table 14: Study design for the Segment I study

Group No.	Dose Level (mg/kg)[a]		Concentration (mg/mL)		Dose Volume (mL/kg)
	Oxymetazoline HCl	Tetracaine HCl	Oxymetazoline HCl	Tetracaine HCl	
1	0 (Control)	0 (Control)	0	0	2
2	0.1	0	0.05	0	2
3	0	7.5	0	3.75	2
4	0.01	7.5	0.005	3.75	2
5	0.03	7.5	0.015	3.75	2
6	0.1	7.5	0.05	3.75	2

[a] The test articles were considered 100% pure for the purpose of dose calculations.

Observations and Results

Mortality

All unscheduled mortalities occurred at the oxymetazoline high dose groups administered alone or in combination with tetracaine.

In males, 2 rats (Animal 130 and Animal 143) at 0.1/0 mg/kg oxy/tetra were found dead on SD 15 and SD 18, respectively. In addition, one male animal at this dosing level in the TK satellite groups was also found dead on SD 26. Piloerection was observed in these animals, and red gelatinous material or red fluid was present in the thoracic cavity at necropsy examination. One animal had body weight loss (26 g from SD 3 to SD 17) prior to death, but food consumption change was not evident. Three males (Animal 219, 222, and 231) at 0.1/7.5 mg/kg oxy/tetra were found dead on SD 34, 55, and 64, respectively. Primary clinical observations included ataxic and exhibited clonic convulsions, decreased motor activity, impaired/lost righting reflex, low carriage, piloerection, and salivation. Body weight loss was seen in all 3 animals without change in food consumption. At necropsy, red or dark red lung lobes were observed in 2 animals, and mottled and dark red kidneys were found in one animal. Mortality in males was considered to be oxymetazoline treatment related.

Clinical Signs

Treatment-related clinical signs were observed in both males and females. Piloerection was observed in all oxymetazoline treated groups with comparable incidences. Ataxia, impaired righting reflex, lost righting reflex, hunched posture, decreased motor activity and low carriage in all tetracaine treatment groups. However, the incidence of these changes appeared to be significantly lower in 0.01/7.5 and 0.03/7.5 mg/kg groups as compared to 0/7.5 and 0.1/7.5 mg/kg groups in both males and females (see table below for males, female data were not sown). On the other hand, changes at the injection site including scabs and red/purple in color had much higher incidence in the 0.01/7.5 and 0.03/7.5 mg/kg groups as compared to 0 /7.5 and 0.1/7.5 mg/kg. Overall,

the incidences of these changes were higher in males than females. Hyperpnea, bradypnea, clonic convulsions, slight to extreme excess salivation, hyper-reactivity to touch, twitches, ptosis were only observed at 0.1/7.5 mg/kg/day oxy/tetra in males but not females. In addition, “tip/portion of tail black” was seen in males at 0.1/0 and 0.1/7.5 mg/kg/day oxy/tetra. There was no significant difference between males and females in tetracaine and oxymetazoline exposure. These more severe changes in males were likely due to the longer duration of treatment and/or a sex difference. The tables below are a summary of clinical signs in male and female rats (**Table 17a & 17b**).

Table 17a: Summary of the clinical signs in males in the Segment I study

GROUP	1	2	3	4	5	6
OXYMETAZOLINE HCl (MG/KG)a	0 (CONTROL)	0.1	0	0.01	0.03	0.1
TETRACAINE HCl (MG/KG)a	0 (CONTROL)	0	7.5	7.5	7.5	7.5
MAXIMUM POSSIBLE INCIDENCE	1440/ 22	1341/ 22	1441/ 22	1442/ 22	1442/ 22	1363/ 22
MORTALITY	0	2*	0	0	0	4**
FOUND DEAD	0	2b,c	0	0	0	3d,e,f
UNSCHEDULED EUTHANASIA	0	0	0	0	0	1g
PILOERECTION	0/ 0	1249/ 22**b,c	3/ 1	1234/ 22**	1345/ 22**	1281/ 22**d-g
ATAXIA	0/ 0	0/ 0	645/ 22**	99/ 16**	119/ 21**	384/ 22**d-g
IMPAIRED RIGHTING REFLEX	0/ 0	0/ 0	537/ 22**	53/ 10	116/ 21**	437/ 22**d-g
LOST RIGHTING REFLEX	0/ 0	0/ 0	365/ 22**	6/ 2	21/ 10	713/ 22**d-g
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	216/ 22**	8/ 3	3/ 2	195/ 22**d-g
LOW CARRIAGE	0/ 0	0/ 0	76/ 21**	1/ 1	1/ 1	28/ 14**d,e,g
HYPERPNEA	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0	35/ 14**d-g
BRADYPNEA	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	19/ 11**d,f,g
CONVULSION: CLONIC	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0	48/ 9**d-f
EXCESS SALIVATION: TOTAL	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	42/ 7**
SLIGHT	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	12/ 5**d,f
MODERATE	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	24/ 6**d,f
EXTREME	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	8/ 3**e
HYPERREACTIVITY TO TOUCH h	0/ 0	3/ 2	0/ 0	0/ 0	0/ 0	27/ 6**f
INJECTION SITE(S): TOTAL	2/ 2	0/ 0	39/ 4	494/ 20**	306/ 17**	25/ 4
SCAB(S)	0/ 0	0/ 0	39/ 4	490/ 20**	299/ 17**	24/ 3
RED OR PURPLE	0/ 0	0/ 0	0/ 0	5/ 4**	0/ 0	1/ 1
ABRASION(S)	0/ 0	0/ 0	0/ 0	2/ 1	3/ 3**	0/ 0
SWOLLEN	2/ 2	0/ 0	0/ 0	8/ 3	6/ 2	0/ 0
HUNCHED POSTURE	0/ 0	0/ 0	0/ 0	10/ 2*	0/ 0	7/ 4**d,g
TWITCHES	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	18/ 4**d,f
CHROMORHINORRHEA	0/ 0	1/ 1	1/ 1	4/ 2	2/ 2	7/ 3g
DEHYDRATION: TOTAL	0/ 0	0/ 0	1/ 1	15/ 2	0/ 0	11/ 3
MILD	0/ 0	0/ 0	1/ 1	12/ 2	0/ 0	9/ 3g
MODERATE	0/ 0	0/ 0	0/ 0	3/ 1	0/ 0	2/ 1
SEVERE	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
PTOSIS	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0	5/ 3**g
TIP/PORTION OF TAIL: BLACK	0/ 0	11/ 4**	0/ 0	0/ 0	0/ 0	26/ 3**e
VOCALIZATION TO TOUCH	51/ 2	92/ 4	0/ 0	69/ 3	69/ 4	36/ 2
SCANT FECES	0/ 0	0/ 0	0/ 0	2/ 2	0/ 0	3/ 2g

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION
* Significantly different from the control group value (p≤0.05).
** Significantly different from the control group value (p≤0.01).

Table 17b: Summary of the clinical signs in males in the Segment I study

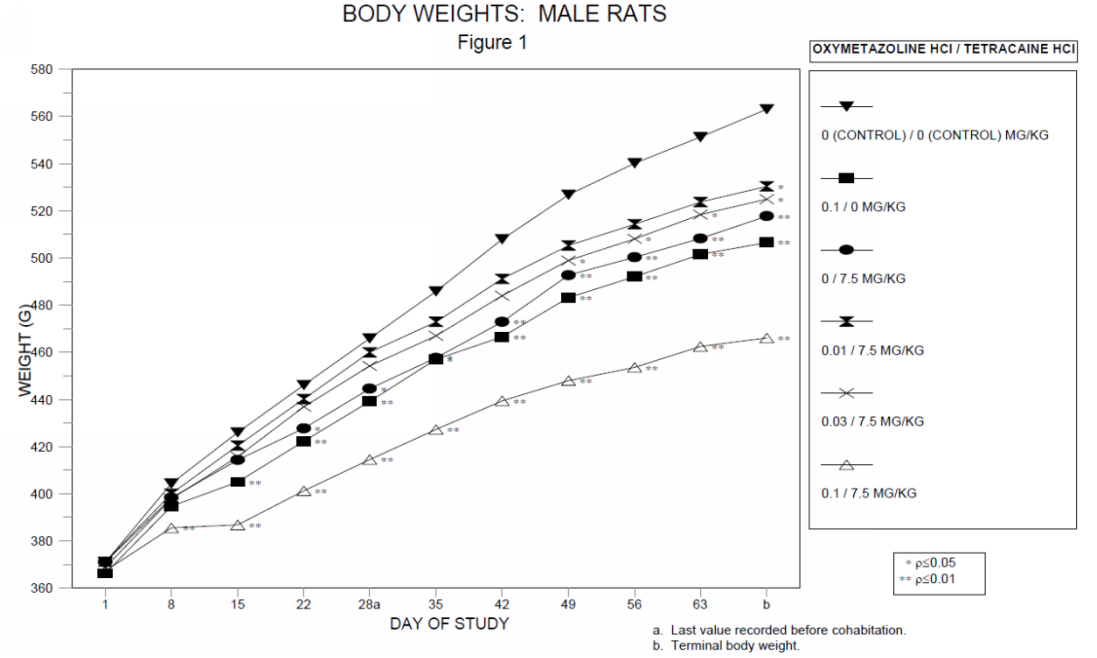
GROUP	1	2	3	4	5	6
OXYMETAZOLINE HCl (MG/KG)a	0 (CONTROL)	0.1	0	0.01	0.03	0.1
TETRACAINE HCl (MG/KG)a	0 (CONTROL)	0	7.5	7.5	7.5	7.5
UNSCHEDULED EUTHANASIA	0	1b	0	0	0	1c
DELIVERED AND EUTHANIZED	0	1d	0	0	0	0
PRECOHABITATION (DAY 1 OF STUDY TO THE DAY OF COHABITATION):						
MAXIMUM POSSIBLE INCIDENCE	330/ 22	330/ 22	330/ 22	330/ 22	330/ 22	330/ 22
PILOERECTION	8/ 8	277/ 22**b,d	0/ 0	282/ 22**	301/ 22**	256/ 22**c
ATAXIA	0/ 0	0/ 0	223/ 22**	5/ 4	22/ 9	231/ 22**c
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	112/ 21**	0/ 0	1/ 1	93/ 18**c
IMPAIRED RIGHTING REFLEX	0/ 0	0/ 0	70/ 18**	0/ 0	4/ 3	75/ 16**c
HUNCHED POSTURE	0/ 0	0/ 0	18/ 10**	0/ 0	1/ 1	34/ 15**c
LOST RIGHTING REFLEX	0/ 0	0/ 0	15/ 8**	0/ 0	0/ 0	21/ 12**c
INJECTION SITE(S): TOTAL	3/ 2	18/ 5	6/ 3	109/ 16**	133/ 17**	64/ 10*
DISCOLORATION e	3/ 2	18/ 5	6/ 3	22/ 6	23/ 5	33/ 8c
SCAB(S)	0/ 0	0/ 0	0/ 0	98/ 13**	124/ 15**	43/ 6
DEHYDRATION - MILD	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	2/ 2
SPARSE HAIR COAT: LIMB(S)	0/ 0	11/ 3	0/ 0	8/ 1	8/ 1	1/ 1
BOTH EYES: EXOPHTHALMOS	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
LOCALIZED ALOPECIA: LIMB(S)	0/ 0	0/ 0	0/ 0	8/ 1	1/ 1	0/ 0
BACK: SCAB	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
TACHYPNEA	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0	0/ 0

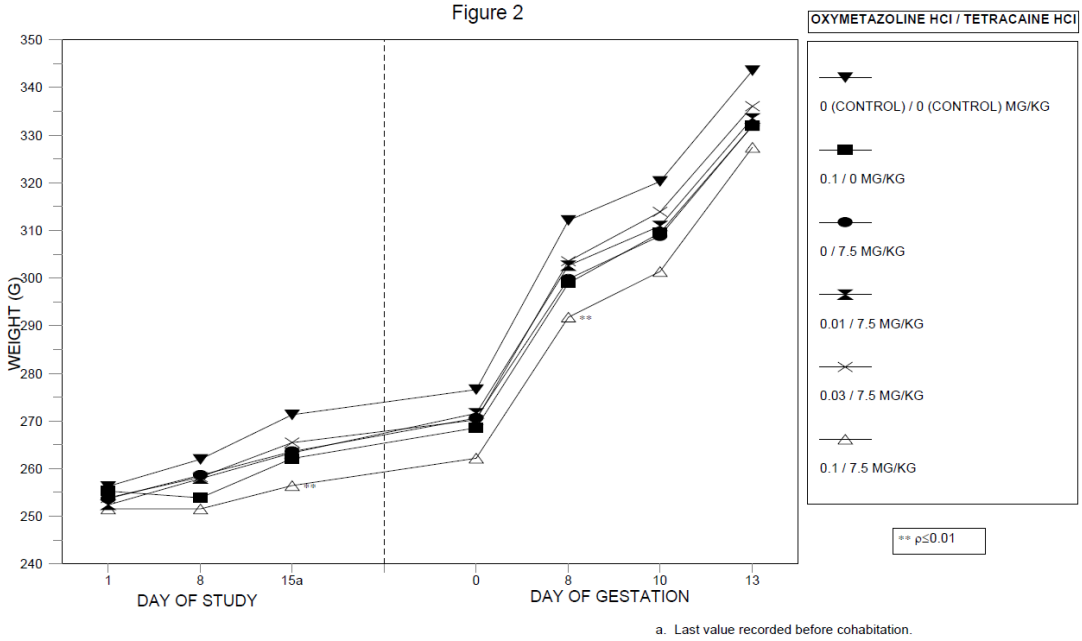
STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION

Body Weight

Significant body weight decrease was observed in both males and females in test-article treatment groups as compared to the control as shown in the figures below. In comparison, the body weight decrease in males was more severe than that in females. In males, statistically significant body weight decrease was seen in all treatment groups in most of the measurement time-points. In females, statistically significant body weight decreases were only observed at 0.1/7.5 mg/kg at two measurement time-points.

Figure 1: Body weight changes in Segment I study





At the end of treatment, statistically significant decrease was seen in all test-article treatment groups with the largest magnitude at 0.1/7.5 mg/kg oxy/tetra (17%). In females, statistical significant decrease was only observed at this dosing level.

Table 18: Body weight changes in the Segment I study

Dosing groups (O/T)	Males	females	
	End of treatment	Before cohabitation	End of treatment
0/0	563.1	271.3	312.1
0.1/0	506.6 (↓10%)*	262.1 (↓3.4%)	299.0 (↓4.2%)
0/7.5	517.8 (↓8%)*	263.6 (↓2.9%)	299.8 (↓4.0%)
0.01/7.5	530.5 (↓6%)*	263.3 (↓2.9%)	302.6 (↓3.0%)
0.03/7.5	525.0 (7%)*	265.5 (↓2.2%)	303.6 (↓2.7%)
0.1/7.5	466.1 (17%)*	256.4 (↓5.5%)*	291.8 (↓6.5%)

Food Consumption

Significant decrease of feed consumption was coincided with the body weight change.

Necropsy

A gross necropsy of the thoracic, abdominal, and pelvic viscera was performed at scheduled euthanasia for each rat assigned to the main study and for all rats that were found dead or euthanized prior to scheduled termination. Reproductive organs and tissues were collected for examination. Histopathology examination was not performed in this study.

No macroscopic observations were considered to be related to co-administration of the test articles except kidney lesions observed in 0.1 mg/kg oxy and 0.1/7.5 mg/kg oxy/tetra groups. In males, one animal that was on schedule euthanasia had a misshapen left kidney with yellow area present at 0.1/7.5 mg/kg oxy/tetra. Kidney lesions (both kidneys mottled red and dark red) were also observed in 1 of the 3 animals that were found dead. In females, kidney lesions (pitted areas and clear fluid-filled cyst) were observed in one animal at 0.1/7.5 mg/kg oxy/tetra group. These changes were considered to be treatment related.

Female mating, fertility, and early embryofetal development

There were no significant difference in the female mating and fertility parameters. Although some values such as the fertility index at 0.1 mg/kg oxymetazoline appeared to be lower than control (85.7 vs. 100%). All values were within the range of the historical control (e.g. the range for historical control is 78.3 – 100%). In addition, the estrous cycle of female rats was not affected by test article administration.

Table 19: Summary of female mating and fertility in Segment I study

GROUP		1	2	3	4	5	6
OXYMETAZOLINE HCl (MG/KG)a		0 (CONTROL)	0.1	0	0.01	0.03	0.1
TETRACAINE HCl (MG/KG)a		0 (CONTROL)	0	7.5	7.5	7.5	7.5
RATS IN COHABITATION	N	22	21b	22	22	22	21c
DAYS IN COHABITATION d	MEAN±S.D.	2.1 ± 1.2	3.4 ± 2.9 [20]	4.0 ± 3.5	2.8 ± 2.3	3.4 ± 2.4	3.0 ± 1.6
RATS THAT MATED	N(%)	22(100.0)	21(100.0)	22(100.0)	22(100.0)	22(100.0)	21(100.0)
FERTILITY INDEX e	N/N	22/ 22	18/ 21	21/ 22	20/ 22	22/ 22	19/ 21
	(%)	(100.0)	(85.7)	(95.4)	(90.9)	(100.0)	(90.5)
RATS WITH CONFIRMED MATING DATES	N	22	20	22	22	22	21
MATED BY FIRST MALE f							
DAYS 1-7	N(%)	22(100.0)	18(90.0)	20(90.9)	21(95.4)	21(95.4)	20(95.2)
DAYS 8-14	N(%)	0(0.0)	2(10.0)	0(0.0)	1(4.5)	1(4.5)	1(4.8)
MATED BY SECOND MALE f	N(%)	0(0.0)	0(0.0)	2(9.1)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT/RATS IN COHABITATION	N/N	22/ 22	18/ 21	21/ 22	20/ 22	22/ 22	19/ 21
	(%)	(100.0)	(85.7)	(95.4)	(90.9)	(100.0)	(90.5)

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 1 of study through Day 7 of presumed gestation.
b. Excludes rat 329, which was euthanized on Day 16 of study (first day of cohabitation) due to adverse clinical observations.
c. Excludes rat 419, which was removed from cohabitation with male 219 during the third day of cohabitation due to injuries and was placed with male 215 for approximately 44 minutes before being removed again and euthanized due to adverse clinical observations.
d. Restricted to rats with a confirmed mating date and rats that did not mate.
e. Number of pregnancies/number of rats that mated.
f. Restricted to rats with a confirmed mating date.

Due to a mis-timed pregnancy that resulted in delivery of a single live pup at 0.1/0 mg/kg/day oxy/tetra, ovarian/uterine observations on GD 13 were based on 22, 17, 21, 20, 22, and 19 pregnant dams, respectively. Statistically significant decrease in the mean number of corpora lutea, implantation, and viable embryos were observed in 0.1/7.5 mg/kg oxy/tetra group. In the 0.1 mg/kg oxy group, the values of these parameters were similarly decreased. Although there was no statistical significance, the treatment relationship cannot be excluded at this dose level. In addition, a statistically significant decrease in viable embryos was also observed in the 0.01 and 0.03 mg/kg oxymetazoline-treatment groups. These changes were considered to be treatment-related due to the statistical significance and dose-dependence. It was questionable whether these changes were adverse because the values of mean viable embryos at all doses were within the historical range.

Table 20: Summary of ovarian and uterine content evaluation in Segment I study

parameters/dam	Control	Oxy/Tetra 0.1/0 mg/kg	Oxy/Tetra 0/7.5 mg/kg	Oxy/Tetra 0.01/7.5 mg/kg	Oxy/Tetra 0.03/7.5 mg/kg	Oxy/Tetra 0.1/7.5 mg/kg
Rats examined	22	17	21	20	22	19
Corpora lutea (15.0 – 17.0)[&]	16.4±2.2	14.5 ± 3.3 (↓12%)	15.8 ± 1.9	15.1 ± 1.8	15.5 ± 2.8	13.8 ± 2.5* (↓16%)
Implantation (13.6 – 16.4)	15.9 ± 2.1	13.5 ± 3.9 (↓15%)	15.3 ± 1.8	14.4 ± 1.6	14.5 ± 2.6	13.4 ± 2.1* (↓16%)
Viable embryos (12.1 – 15.3)	15.2 ± 1.9	12.7 ± 3.6 (↓16%)	14.4 ± 2.4	13.4 ± 1.7* (↓12%)	13.1 ± 2.6* (↓14%)	12.5 ± 2.1* (↓ 18%)

[&]historical control range
*p < 0.05

Male mating and fertility

The results were summarized in the following table (**Table 21** extracted from the study report). There were no statistically significant difference between control and treated in all mating and fertility parameters examined. Although some values such as the fertility index at 0.1 mg/kg oxymetazoline appeared to be lower than control (84.2 vs. 100%). All values were within the range of the historical control (e.g. the range for historical control is 78.3 – 100%).

Table 21: Male mating and fertility parameters in Segment I study

TABLE 6 (PAGE 1): SUMMARY OF MATING AND FERTILITY: MALE RATS

GROUP		1	2	3	4	5	6
OXYMETAZOLINE HCl (MG/KG)a		0 (CONTROL)	0.1	0	0.01	0.03	0.1
TETRACAINE HCl (MG/KG)a		0 (CONTROL)	0	7.5	7.5	7.5	7.5
RATS IN COHABITATION	N	22	19b,c	22	22	22	21d
DAYS IN COHABITATION e,f	MEAN±S.D.	2.1 ± 1.2	3.3 ± 3.1 [18]	3.9 ± 3.3	2.8 ± 2.3	3.4 ± 2.4	3.0 ± 1.6
RATS THAT MATED f	N(%)	22(100.0)	19(100.0)	20(90.9)	22(100.0)	22(100.0)	21(100.0)
FERTILITY INDEX g,h	N/N	22/ 22	16/ 19	20/ 20	20/ 22	22/ 22	19/ 21
	(%)	(100.0)	(84.2)	(100.0)	(90.9)	(100.0)	(90.5)
RATS WITH CONFIRMED MATING DATES	N	22	18	20	22	22	21
MATED WITH FEMALE i							
DAYS 1-7	N(%)	22(100.0)	16(88.9)	20(100.0)	21(95.4)	21(95.4)	20(95.2)
DAYS 8-14	N(%)	0(0.0)	2(11.1)	0(0.0)	1(4.5)	1(4.5)	1(4.8)
RATS PREGNANT/RATS IN COHABITATION j	N/N	22/ 22	16/ 19	20/ 22	20/ 22	22/ 22	19/ 21
	(%)	(100.0)	(84.2)	(90.9)	(90.9)	(100.0)	(90.5)

[ ] = NUMBER OF VALUES AVERAGED
a. Dose administration occurred on Day 1 of study through the day before euthanasia.
b. Excludes rats that were found dead.
c. Excludes rat 129; cohort female rat 329 was euthanized on Day 16 of study (first day of cohabitation) due to adverse clinical observations.
d. Excludes rat 219, cohort female rat 419 was removed from cohabitation during the third day of cohabitation due to injuries and was placed with male 215 for approximately 44 minutes before being removed again and euthanized due to adverse clinical observations.
e. Restricted to rats with a confirmed mating date and rats that did not mate.
f. Includes only one mating for each male rat.
g. Number of pregnancies/number of rats that mated.
h. Includes only one pregnancy for each rat that impregnated more than one female rat.
i. Restricted to rats with a confirmed mating date.
j. Includes only one confirmed mating for each male rat.

The absolute weights of the reproductive organs include epididymis, seminal vesicles, and prostate were significantly decreased as compared to the control except the testis at all test-article treatment groups as shown in the table below. At low- and mid-dose oxymetazoline groups, this change was likely secondary to the body weight decrease. At 0.1 mg/kg oxymetazoline groups, the direct effect associated with test article treatment cannot be excluded because statistical significance was still observed at these groups when the organ weights were adjusted by the body weight. The absolute weight of testis was not changed as compared to the control, but the ratio of testis weight/body weight was increased statistically.

Table 22: Organ weights of male reproductive organs in Segment I study

Organ weight		Control	Oxy/Tetra 0.1/0 mg/kg	Oxy/Tetra 0/7.5 mg/kg	Oxy/Tetra 0.01/7.5 mg/kg	Oxy/Tetra 0.03/7.5 mg/kg	Oxy/Tetra 0.1/7.5 mg/kg
Epididymis (L)	Absolute	0.83	0.69* (↓17%)	0.79	0.72* (↓13%)	0.76* (↓8.4%)	0.66* (↓20%)
	Ratio to BW (%)	0.15	0.14	0.15	0.14	0.15	0.14
Cauda Epididymis (L)	Absolute	0.35	0.26* (↓26%)	0.36	0.31* (↓11%)	0.31* (↓11%)	0.26* (↓26%)
	Ratio to BW	0.063	0.051* (↓19%)	0.069	0.058	0.059	0.055* (↓17%)
Seminal vesicles with fluid	Absolute	2.18	1.75* (↓20%)	2.00	1.97* (↓11%)	1.95* (↓11%)	1.64* (↓25%)
	Ratio to BW (%)	0.39	0.34	0.39	0.37	0.37	0.36
Seminal vesicles	Absolute	1.12	0.92* (↓18%)	0.99* (↓12%)	1.03	1.05	0.86* (↓23%)
	Ratio to BW (%)	0.20	0.18	0.19	0.19	0.20	0.18
Prostate	Absolute	1.40	0.91* (↓35%)	1.40	1.20* (↓14%)	1.01* (↓18%)	0.88* (↓37%)
	Ratio to BW (%)	0.25	0.18* (↓28%)	0.27	0.23	0.21* (↓16%)	0.19 (↓24%)
Testis (L)	Absolute	1.87	1.85	1.85	1.84	1.89	1.85
	Ratio to BW (%)	0.33	0.37*	0.36	0.35	0.37	0.40*

In animals of scheduled euthanasia, sperm from each vas deferens were dispersed into appropriate medium for motility evaluation, and a homogenate was prepared from the left cauda epididymis to determine sperm concentration (sperm/g tissue). As shown in the table below, at 0.1 mg/kg alone or in combination with 7.5 mg/kg tetracaine, oxymetazoline significantly decreased the percentage of motile sperms in vas deferens, and the values at 0.1 mg/kg groups were outside of the range of historical control (77.1 – 96.4%). At 0.03 mg/kg oxymetazoline group, the percentage of motile sperms was decreased about 10% as compared to control, but this change was not statistically significant and the value was within the historical control range. There was no sperm count change in vas deferens. Total sperm count in cauda epididymis in 0.1 mg/kg oxymetazoline groups were statistically decreased as compared to control and the values were outside of the range of historical control (251.1-740.0). Statistically significant decrease in sperm concentration was only observed at 0.1 mg/kg oxymetazoline only treatment group. The lack of sperm density difference in 0.1/7.5 mg/kg oxy/tetra group was likely due to the deceased weight in cauda epididymis.

Table 23: Sperm motility and concentration in male rats in Segment I study

Doses Oxy/Tetra 9mg/kg)	Sperm motility (vas deferens)		Sperm concentration (cauda epididymis)	
	Total Count	Motile %	Total Count	concentration (/g)
0/0	537.7 ± 158.2	89.9 ± 5.0	279.8 ± 77.4	1031.57 ± 258.36
0.1/0	637.2 ± 210.3	62.8 ± 14.1* (↓31%)	155.5 ± 27.6* (↓44%)	777.37 ± 116.66* (↓25%)

0/7.5	550.3 ± 167.9	90.9 ± 3.3	335.4 ± 100.8	1202.15 ± 367.81
0.01/7.5	592.5 ± 124.6	90.8 ± 4.2	299.0 ± 115.6	1256.36 ± 488.51
0.03/7.5	497.5 ± 148.5	80.2 ± 19.8 (↓ 11%)	224.3 ± 59.7* (↓20%)	952.49 ± 188.15 (↓7.7%)
0.1/7.5	624.8 ± 194.9	73.6 ± 8.9* (↓18%)	186.1 ± 49.5* (↓33%)	945.33 ± 234.47 (↓8.3%)

* Statistically significant from the control, $p < 0.05$

Toxicokinetics

Blood samples (0.5 mL) were collected from the jugular vein on SD 1 and SD 28 (males) or SD 14 (females). The levels of oxymetazoline, tetracaine, and the tetracaine metabolite p-butylamino benzoic acid (PBBA) in plasma were analyzed. The lower limit of quantitation (LLOQ) was 0.1, 0.2, and 1.0 ng/mL for oxymetazoline, tetracaine, and PBBA. Oxymetazoline, tetracaine and PBBA plasma concentrations were below the LLOQ in all control samples as well as predose samples on Day 1. In test-article treatment groups, either alone or in combination with tetracaine, oxymetazoline plasma concentrations were generally quantifiable up to 6 hours postdose. Tetracaine plasma concentrations were generally quantifiable up to 3 hours postdose with marked inter-animal variability. PBBA plasma concentrations were quantifiable throughout the 24-hour sampling period. The following table summarizes the TK parameters in both males and females. In general, the systemic exposure of oxymetazoline increased in a dose-dependent manner when co-administered with tetracaine.

Table 24: C_{max} and AUC of oxymetazoline, tetracaine, and PBBA in the Segment I study

Doses Oxy/Tetra (mg/kg)	**Males**				**Females**			
	SD 1		**SD 28**		**SD 1**		**SD 14**	
	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)
	Oxymetazoline (mean)							
0.1/0	14.5	31.3	27.4	29.6	15.8	28.8	21.0	27.5
0.01/7.5	1.69	2.90	1.42	2.73	2.04	2.86	1.37	2.68
0.03/7.5	4.49	8.20	4.17	7.47	6.08	10.3	4.43	7.37
0.1/7.5	10.8	31.2	15.1	28.1	22.8	47.0	18.8	29.4
	Tetracaine (mean)							
0/7.5	224	151	94.2	76.3	215	124	169	112
0.01/7.5	148	126	79.5	77.7	240	106	100	100
0.03/7.5	128	114	79.8	65.1	120	150	85.7	64.4
0.1/7.5	148	258	284	168	335	285	109	75.6
	PBBA (mean)							
0/7.5	6280	24200	5440	26900	6660	31600	7720	32000

0.01/7.5	3720	23000	3560	25800	7310	36100	4240	29800
0.03/7.5	2640	18800	2750	21700	5730	33800	3970	26300
0.1/7.5	5500	26400	5130	25100	5460	41000	5560	30000

Dosing Solution Analysis:

The dosing solution was within acceptance criteria (± 10% of theoretical concentration).

9.2 Embryonic Fetal Development

Study Title: A subcutaneous dose range developmental toxicity study of oxymetazoline hydrochloride in rats, including a preliminary evaluation in non-pregnant rats

This study is a non-GLP study conducted by (b) (4). The purpose of this study was to provide information for selection of dose levels to be used in a subsequent embryo-fetal development study in rats and to provide a preliminary evaluation of the effects of oxymetazoline administered alone and in combination with tetracaine on pregnancy and embryo-fetal development.

Part A

In Part A of the study, oxymetazoline in (b) (4) was administered daily for 5 days by SC injection to nonpregnant female rats (3 animals/group) at 0.1, 0.6, 1.2, and 2.0 mg/kg. The dose selection was based on a published LD_{50} of 1.1-1.6 mg/kg in rats (ChemIDplus and MSDS). Parameters that were evaluated including viability, clinical observations, body weights and body weight changes, food consumption and necropsy observations. Dose levels of oxymetazoline at ≥ 0.6 mg/kg exceeded the maximum tolerated dose (MTD), as evidenced by mortality. In-life and postmortem observations of animals were summarized in the following table (**Table 25** extracted from the study report).

Table 25: Summary of in-life and postmortem observations in nonpregnant females in the Segment II dose-range finding study

Dose Level (mg/kg/day)	0.6	1.2			2.0		
Rat Number	6067	6070	6071	6072	6073	6074	6075
Doses Administered	1	1	3	3	1	2	1
Mode of Death	FD	UE	UE	UE	UE	UE	UE
Day of Death	DS 2	DS 1	DS 4	DS 4	DS 2	DS 2	DS 1
Clinical Observations – Found Dead or Euthanasia Due to Adverse Clinical Condition							
Piloerection	DS 1	DS 1	DS 1-3	DS 1-3	DS 1-2	DS 1-2	DS 1
Pale extremities, ears, fore/hind paws, and/or tail	DS 1	DS 1	DS 1-2	DS 1-2	DS 1-2	DS 1-2	DS 1
Cold to touch	DS 1	DS 1	-	-	DS 1-2	-	DS 1
Ataxia	DS 1	DS 1	DS 1	DS 1	DS 1	DS 2	DS 1
Decreased motor activity	DS 1	DS 1	DS 1	-	DS 1-2	DS 2	DS 1
Low carriage	DS 1	DS 1	-	-	DS 1	DS 1-2	DS 1
Hunched posture	-	-	DS 3	-	DS 1	-	DS 1
Impaired righting reflex	-	DS 1	-	-	-	-	DS 1
Prostrate	-	DS 1		-	-	-	DS 1
Hindlimbs, splayed	-	DS 1	-	-	DS 2	-	-
Increased motor activity	-	-	-	DS 1	-	-	-
Hyperactivity	-	-	-	DS 4	-	-	-
Vocalization to touch	-	-	DS 4	DS 3-4	-	-	-
Aggression	-	-	-	DS 4	-	-	-
Tachypnea	DS 1	DS 1	DS 1	DS 1-4	DS 1	DS 1-2	DS 1
Hyperpnea	-	-	-	DS 4	DS 2	DS 2	DS 1
Bradypnea	-	-	DS 2	-	DS 2	-	-
Rales	-	-	-	-	DS 2	-	-
Dyspnea	-	-	-	-	-	-	DS 1
Excess salivation, slight or moderate	DS 1	-	-	DS 2	DS 2	-	-
Dehydration, mild or moderate	-	DS 1	DS 2-4	DS 2,4	DS 2	DS 2	DS 1
Mucus membranes, discoloration, blue/gray	-	DS 1	-	-	-	-	DS 1
Lenticular opacity, right or both eyes	-	DS 1	-	-	-	-	DS 1
Eyes, discolored, dark red	-	DS 1	-	-	-	-	DS 1
Scabs on tail	-	-	-	DS 4	-	-	-
Portion of tail missing	-	-	DS 4	-	-	-	-
Red substance on tail and in cage	-	-	DS 4	-	-	-	-
Ungroomed coat	-	-	DS 2-4	DS 2	DS 2	-	-
Urine-stained abdominal fur	-	-	-	-	DS 2	-	-
Soft or liquid feces	-	-	-	DS 4	-	-	-

Body Weights – Found Dead or Euthanasia Due to Adverse Clinical Condition							
Body weight change (grams)	Not calculated	Not calculated	-32 (DS 1-4)	-18 (DS 1-4)	-12 (DS 1-2)	-13 (DS 1-2)	Not calculated
Food Consumption – Found Dead or Euthanasia Due to Adverse Clinical Condition							
Cumulative food consumption (grams)	Not calculated	Not calculated	8 (DS 1-4)	10 (DS 1-4)	1 (DS 1-2)	2 (DS 1-2)	Not calculated
Necropsy Observations – Found Dead or Euthanasia Due to Adverse Clinical Condition							
All tissues appeared normal	-	-	-	X	-	-	-
Thymus, red areas	X	-	-	-	-	-	-
Thoracic cavity, cloudy red or clear tan fluid	X	X	-	-	-	-	X
Kidneys, pelvic region red or dark red	-	X	-	-	X	-	X
Kidney, left or right, tan area present	-	-	X	-	-	X	-
Stomach distended with gas	-	-	-	-	X	-	-
Heart, dark red area	-	-	-	-	-	-	X

FD = Found dead UE = Euthanized due to adverse clinical condition DS = Day of study
X = Finding present - = Finding not present

Kidney lesions (mottled red and dark red) were also observed in one of the two animals that survived to the scheduled euthanasia at 0.6 mg/kg. Tachypnea and piloerection were the only adverse clinical signs observed in the 0.1 mg/kg dose group, and there was no decrease in body weight in this group. Necropsy examination revealed no gross lesions in the rats in the 0.1 mg/kg/day dose group. Therefore, 0.1 mg/kg is considered the NOAEL for Part A of the study.

Part B

In Part B of the study, 0.03, 0.1, 0.3 mg/kg oxymetazoline was administered to pregnant female rats (8 animals/group) from Gestational Day (GD) 7 to 17. An additional group of pregnant rats was dosed with 0.3 mg/kg oxymetazoline in combination with 7.5 mg/kg tetracaine with the same dosing regimen. Animals were euthanized on GD 21. The parameters evaluated included viability, clinical observations, body weights and body weight changes, food consumption, ovarian and uterine examinations, necropsy observations, fetal sex, fetal body weight and external abnormalities. For ovarian and uterine examinations, the reproductive tract was dissected from the abdominal cavity. The uterus was opened, and the contents were examined. The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape), live and dead fetuses, and early and late resorptions. An early resorption was defined as one in which organogenesis was not grossly evident. A late resorption was defined as one in which the occurrence of organogenesis was grossly evident. A live fetus was defined as a term fetus that responded to stimuli. Nonresponding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Fetuses were examined for sex and external abnormalities. Late resorptions and dead fetuses were also examined for external abnormalities and sex to the extent possible. The body weight of each fetus was recorded.

All rats treated with oxymetazoline presented with piloerection and tachypnea. Piloerection was observed after a single administration in each rat, and most rats presented with tachypnea following repeated administration. All rats at ≥ 0.1 mg/kg presented with pale extremities (ears, fore- and/or hindlimbs, and/or the tail), and decreased motor activity and slight excess salivation were also observed. Mild dehydration (based on skin turgor) and cold to touch occurred at 0.3 mg/kg/day alone or with tetracaine. Moderate excess salivation, impaired righting reflex; scabs on the

tail or tip of the tail; low carriage; red, purple, and/or black/purple discoloration of the tail; and lacrimation occurred in increased numbers of rats administered oxy/tetra. Single rats at 0.3/7.5 mg/kg had a portion of the tail missing, ptosis, and prostration. Increased motor activity was observed in 2 rats administered oxymetazoline at 0.1 mg/kg/day.

Mean body weight gains during the entire dose period (calculated as GDs 7 through 18) were 93%, 74%, 43%, and 36% of the vehicle control group value in Groups 2 through 5 (0.03, 0.1, and 0.3 mg/kg/day oxymetazoline and 0.3/7.5 mg/kg/day oxy/tetra, respectively), as shown below. After the cessation of treatment, the body weight gain was comparable between control and treatment-related groups (~60 g) from SD 18 - SD 21.

The body weight changes are shown in the following table. Over 10% body weight decrease was seen at 0.3 mg/kg groups after 11 days of administration. This change lasted until the end of gestation.

Table 26: Body weight changes in the pregnant rats in the Segment II dose-range finding study

Dosing level (mg/kg)	0 Oxy	0.03 Oxy	0.1 Oxy	0.3 Oxy	0.3/7.5 Oxy/Tetra
End of treatment (GD 17)	347.0	↓ 3.2%	↓4.4%	↓11.2%	↓11.6%
Day of sacrifice (GD 21)	425.4	↓2.8%	↓4.3%	↓11.0%	↓11.0%

Food consumption was reduced similarly to the body weight gain. As shown below, the mean absolute (g) and relative food consumption (g/kg body weight) values were both decreased, corresponding to the body weight gain change. Both absolute and relative food consumption values were generally comparable among the 5 dose groups during the postdose period (GDs 18 to 21).

Summary of Food Consumption During the Cumulative Dose and Gestation Periods[a,b]

Group Number	1		2		3		4		5	
Dose Level (mg/kg/day)	0 (Vehicle Control)		0.03 oxymetazoline		0.1 oxymetazoline		0.3 oxymetazoline		0.3/7.5 oxymetazoline /tetracaine	
Food Consumption Measure	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel	Abs	Rel
Dose Period (Days 7 through 18 of Gestation)	24.6	78.6	92%	94%	87%	89%	66%	72%	65%	71%
Gestation Period Following Dose Initiation (Days 7 through 21 of Gestation)	25.0	75.5	92%	95%	88%	91%	72%	79%	71%	78%
Entire Gestation Period (Days 0 through 21 of Gestation)	24.4	75.0	92%	95%	92%	95%	80%	87%	80%	87%

Abs = Absolute (g/day) food consumption; Rel = Relative (g/kg/day) food consumption

a. Percentage (%) of the Vehicle Control Group Value

b. For the vehicle control group, means are shown in grams. For groups treated with test article(s), percentages of the vehicle control group value are shown.

Gross pathology examination observed a tan area or multiple tan areas on one or both kidneys in 4 dams at 3 mg/kg Oxy and 1 dam at 0.3/7.5 mg/kg oxy/tetra. Ovarian and uterine examination on GD 21 was based on 8 pregnant dams from each group. Mean fetal body weight was decreased at > 10% at 0.3 and 0.3/7.5 mg/kg (17% and 20%, respectively), as compared to the control. There was a 5% fetal body weight decrease at 0.1 mg/kg Oxy. Other parameters for ovarian and uterine examination that were not affected included corpora lutea, implantation, litter size, resorption. Although the percent of post-implantation loss at >0.1 mg/kg Oxy groups was slightly higher than the control (~7.0% vs. 2.8%), these values were within the range of historical control.

Fetal gross external examination detected jaw micrognathia, forepaw digits absent, short, and/or fused, at 0.3 mg/kg oxymetazoline groups as shown in the following table (**Table 27**). At 0.1 mg/kg oxymetazoline, short pollex and digit 2-5 in the left forepaw were seen in one animal. Together, these data suggest that oxymetazoline is a teratogen.

Table 27: Summary of fetal gross external alterations in the Segment I dose-range finding study

Group Number		1	2	3	4	5
Dose Level (mg/kg/day)		0 (Vehicle Control)	0.03 oxymetazoline	0.1 oxymetazoline	0.3 oxymetazoline	0.3/7.5 oxymetazoline /tetracaine
Jaw: Micrognathia	Litter N(%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)	1 (12.5)
	Fetal N(%)	0 (0.0	0 (0.0	0 (0.0	3 (3.0)	2 (1.8)
Forepaws: Digit(s) absent	Litter N(%)	0 (0.0)	0 (0.0)	0 (0.0)	4 (50.0)	3 (37.5)
	Fetal N(%)	0 (0.0	0 (0.0	0 (0.0	8 (7.9)	5 (4.5)
Forepaws: Digit(s) short	Litter N(%)	0 (0.0)	0 (0.0)	1 (12.5)	7 (87.5)	5 (62.5)
	Fetal N(%)	0 (0.0	0 (0.0	1 (0.9)	26 (25.7)	23 (20.7)
Forepaws: Digit(s) fused	Litter N(%)	0 (0.0)	0 (0.0)	0 (0.0)	4 (50.0)	3 (37.5)
	Fetal N(%)	0 (0.0	0 (0.0	0 (0.0	5 (5.0)	3 (2.7)

Litter = Litter incidence N = Number % = Percentage Fetal = Fetal incidence

Study title: A subcutaneous developmental toxicity study of oxymetazoline hydrochloride and tetracaine hydrochloride in rats, including a satellite toxicokinetic evaluation

Study no.:	20013399
Study report location:	eCTD
Conducting laboratory and location:	(b) (4)
Date of study initiation:	05/07/2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Oxymetazoline: 008199AX11, >99% Tetracaine: 0003158361, >99%

Key Study Findings

- Pregnant rats were administered with 0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxymetazoline/tetracaine (oxy/tetra) from GD 7 to 17, and Cesarean sections were performed on GD 21.

- Clinical observations included piloerection, ataxia, decreased motor activity, limited use of limbs, impaired righting reflex, and sprayed limbs, which appeared attributable primarily to tetracaine treatment. These findings were consistent with those observed in the Segment I study.

- Slight body weight decrease (<10%) in maternal animals at 0.1 mg/kg when administered alone or in combination with 7.5 mg/kg tetracaine. Food consumption was also decreased in these animals.

- Necropsy examination found kidney changes (white area extending into the parenchyma) in one animal at 0.1/7.5 oxy/tetra.

- The LOAEL for maternal toxicity is identified as 0.03/7.5 mg/kg oxy/tetra.

- Cesarean section examination did not find significant adverse changes in ovaries and uterus contents.

- Slight fetal weight decrease (<10%) at 0.1 mg/kg when administered alone or in combination with 7.5 mg/kg tetracaine was likely associated with maternal body weight change.

- Malformations and variation were only observed at 0.1 mg/kg oxymetazoline when administered alone without tetracaine. Malformations included short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs. In addition, variations in skeletal development such as irregularly shaped arches in thoracic vertebrae and increased bifid centra in thoracic vertebrae also occurred at this group. Significant alterations were not observed in any treatment group of oxymetazoline in combination with tetracaine. Malformation was not observed in any dose of oxymetazoline in combination with 7.5 mg/kg tetracaine. The NOAEL for fetal malformations/variations is identified as 0.03/7.5 mg/kg oxy/tetra.

Methods

Doses:	0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxy/tetra
Frequency of dosing:	Daily from GD 7 to GD 17
Dose volume:	2 mL/kg
Route of administration:	SC with 6 injection sites rotated
Formulation/Vehicle:	Solution/ (b) (4) (not including clinical formulation excipient benzyl alcohol and HPC)
Species/Strain:	female Crl:CD(SD) Sprague Dawley rats
Number/Sex/Group:	25 female animals/group
Satellite groups:	TK groups (6/group except control with 3 animals) were sacrificed on GD 18 for TK analysis
Study design:	Pregnant female rats (spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be GD 0) were C-sectioned on GD 21 after 11-day administration during organogenesis period. Parameters that were evaluated included viabilities, clinical signs, body weights, body weight changes, food consumption, mating performance, bioanalytical

	chemistry and toxicokinetic parameters, gross pathology findings, ovarian and uterine examinations, and fetal sex, fetal body weights, and fetal gross external, visceral, and skeletal alterations, as well as ossification site averages
Deviation from study protocol:	The deviations did not appear to affect the quality of the study.

Table 28: Study design of the Segment II study

Group No.	Dose Level (mg/kg/day)[a]		Concentration (mg/mL)		Dose Volume (mL/kg)	No. of Animals
	Oxymetazoline HCl	Tetracaine HCl	Oxymetazoline HCl	Tetracaine HCl		
1	0 (Control)		0		2	25 + 3[b]
2	0.1	0	0.05	0	2	25 + 6[b]
3	0	7.5	0	3.75	2	25 + 6[b]
4	0.01	7.5	0.005	3.75	2	25 + 6[b]
5	0.03	7.5	0.015	3.75	2	25 + 6[b]
6	0.1	7.5	0.05	3.75	2	25 + 6[b]

[a] The test articles were considered 100% pure for the purpose of dose calculations.
[b] Animals assigned to toxicokinetic evaluation.

Observations and Results

Mortality

There was no test-article treatment related mortalities.

Clinical Signs

Piloerection was observed in in all treatment groups and the incidents appeared to increase with the elevation of oxymetazoline dosing level. Ataxia and motor activity decrease, limited use of limbs, impaired righting reflex, and splayed limbs were due to tetracaine treatment. These observations were less severe in tetracaine treatment groups when combined with 0.01 and 0.03 mg/kg oxymetazoline as compared to 7.5 mg/kg tetracaine only and in combination with 0.1 mg/kg oxymetazoline. In contrast, local changes such as scabs on the injection sites were observed at higher incidence at 0.01 and 0.03 mg/kg oxymetazoline groups as compared to 7.5 mg/kg tetracaine only and in combination with 0.1 mg/kg oxymetazoline. These findings were consistent with those observed in the Segment I and III studies.

Table 29: Summary of the clinical signs in the Segment II study

TABLE 1 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY

GROUP	1	2	3	4	5	6
TEST MATERIAL	CONTROL ARTICLE	OXY/TETRA	OXY/TETRA	OXY/TETRA	OXY/TETRA	OXY/TETRA
DOSE LEVEL (MG/KG/DAY)a	0	0.1/0	0/7.5	0.01/7.5	0.03/7.5	0.1/7.5
RATS TESTED	25	25	25	25	25	25
MAXIMUM POSSIBLE INCIDENCE	375/ 25	375/ 25	375/ 25	375/ 25	375/ 25	375/ 25
DELIVERED AND EUTHANIZED	0	0	1b	0	0	0
PILOERECTION	0/ 0	278/ 25**	14/ 14**	214/ 25**	235/ 25**	270/ 25**
ATAXIA	0/ 0	0/ 0	70/ 23**b	9/ 6	5/ 5	51/ 21**
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	35/ 15**b	3/ 3	0/ 0	21/ 12**
INJECTION SITE(S): PURPLE, GREEN, BLUE, BROWN AND/OR RED	62/ 10	154/ 22**	29/ 7b	81/ 14	40/ 10	81/ 16*
LIMITED USE OF BOTH FORELIMBS AND/OR HINDLIMBS	0/ 0	0/ 0	37/ 16**b	5/ 5	2/ 2	13/ 10**
LOW CARRIAGE	0/ 0	0/ 0	2/ 2	0/ 0	0/ 0	8/ 6**
MYDRIASIS	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0	4/ 4**
INJECTION SITE(S): SCAB(S)	15/ 3	6/ 1	14/ 2	157/ 19**	192/ 21**	16/ 3
BOTH FORELIMBS AND/OR HINDLIMBS: SPLAYED	0/ 0	0/ 0	5/ 5**	2/ 2	0/ 0	3/ 3**
IMPAIRED RIGHTING REFLEX	0/ 0	0/ 0	4/ 3b	1/ 1	0/ 0	3/ 3
BOTH HINDLIMBS: MUSCLE RIGIDITY	0/ 0	0/ 0	4/ 3**b	0/ 0	0/ 0	3/ 3**
NO USE OF BOTH FORELIMBS AND/OR HINDLIMBS	0/ 0	0/ 0	9/ 8**b	0/ 0	0/ 0	3/ 3

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP ON DAYS 7 THROUGH 21 OF PRESUMED GESTATION
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION
OXY/TETRA = OXYMETAZOLINE HCL/TETRACAINE HCL
a. Dose administration occurred on Days 7 through 17 of presumed gestation.
b. Dam 4167 was euthanized on Day 21 of gestation due to delivery.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

Body Weight

Body weight gain was significantly decreased in the 0.1 mg/kg oxymetazoline treatment groups as compared to the control during the treatment period (GD 7-18), which caused a slight but significant body weight decrease as compared to the control. After the cessation of treatment, body weight gain was comparable between control and test-article treatment groups, but the slight body weight decrease was still observed on the day of C-section. Although statistically significant, < 10% body weight decrease is generally not considered to be significant adverse change.

Table 30: Maternal body weight gains and body weight change in the Segment II study

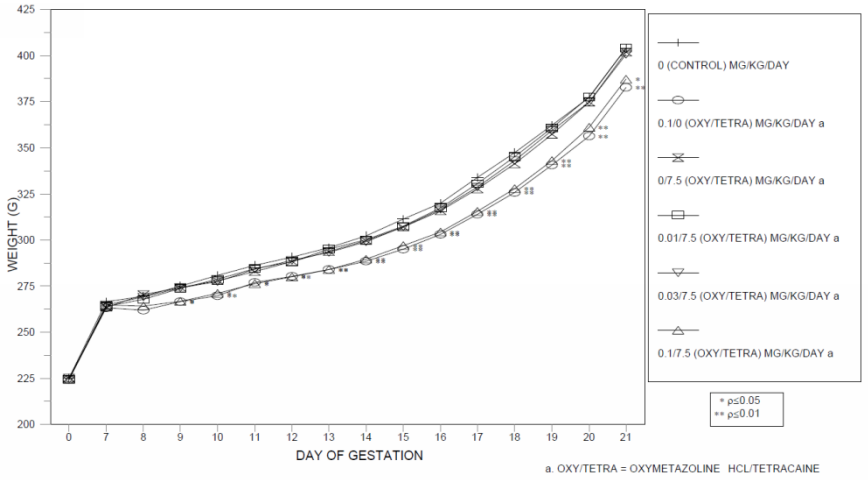
Dosing groups (Oxy/Tetra mg/kg)	Body weight gain		Body weight	
	GD 7-18	GD 18-21	End of treatment (GD 18)	Day of C-section (GD 21)
0/0	81.0	56.3	347.4	403.8
0.1/0	62.5 (↓23%)*	57.1	325.8 (↓6.2%)*	382.8 (↓5.2%)*
0/7.5	77.8	60.6	341.3	402.0
0.01/7.5	81.2	58.8	345.2	404.0

0.03/7.5	78.2	57.7	343.0	400.7
0.1/7.5	63.0 (↓23%)	59.5	327.8 (↓5.6%)*	387.2 (↓4.1%)*

*statistically significant p <0.05

The figure below shows the body weight change during the pregnancy period. The slight body weight decrease appeared after 2-day administration at 0.1 mg/kg oxymetazoline with or without tetracaine co-administration.

Figure 2: Maternal body weight change in the Segment II study



Maternal Food Consumption

Food consumption values were recorded on GD 0, 7, 10, 12, 15, 18, and 21. Significant decrease was observed during the treatment period. There was no difference between control and treatment groups in food consumption after the cessation of treatment.

Table 31: Maternal food consumption in Segment II study

Dosing groups (Oxy/Tetra mg/kg)	Mean Food Consumption (g)	
	GD 7-18	GD 18-21
0/0	23.7	27.2
0.1/0	20.6 (↓13%)*	26.8
0/7.5	22.1 (↓7%)*	26.3
0.01/7.5	22.8	26.8
0.03/7.5	22.8	27.3
0.1/7.5	21.1 (↓11%)*	27.6

Toxicokinetics

Blood samples (0.4-0.5 mL) were collected from the jugular vein on GD 7 and GD 17 at time points of pre-dose, 5 and 20 minutes, and 1, 3, 6, and 24 hours post-dose. Plasma samples were analyzed for concentrations of oxymetazoline, tetracaine, and PBBA

using a validated analytical procedure. Peak oxymetazoline and tetracaine concentrations generally occurred at 5 minutes post-dose, while peak PBBA concentrations occurred at 1 hour or 3 hours post-dose. Oxymetazoline was generally quantifiable up to 6 hours post-dose in the dosed rats. Tetracaine concentrations were generally quantifiable up to 3 hours post-dose. PBBA concentrations were quantifiable throughout the 24 hours sampling period in the tetracaine HCl administered groups. $T_{1/2}$ was estimated to range from 1.67 to 2.24 hours for oxymetazoline, 0.284 to 0.701 hours for tetracaine and from 4.78 to 6.86 hours for PBBA. Oxymetazoline and PBBA exposure remained generally unchanged whether the test articles were administered alone or in combination. The Applicant indicated that tetracaine exposure was approximately 1.8-fold higher when administered alone compared to its combination counterpart. There was no notable change in oxymetazoline, tetracaine, or PBBA exposure following daily dose administration from GD 7 to GD 17. Metabolite-to-parent-drug ratio showed markedly higher exposure of up to 24-fold for C_{max} and up to 218-fold for $AUC_{0\text{-}24h}$, for PBBA compared to tetracaine. The summary of the TK data for oxymetazoline, tetracaine, and PBBA is shown below.

Table 32: C_{max} and AUC of oxymetazoline, tetracaine, and PBBA in maternal rats in the Segment II study

Oxy (mean)	**GD 7**		**GD 17**	
	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)	C_{max} (ng/mL)	$AUC_{0\text{-}24h}$ (ng•h/mL)
0.1/0	13.7	17.1	11.8	28.0
0.01/7.5	1.31	2.04	1.21	2.26
0.03/7.5	4.57	4.30	3.67	5.61
0.1/7.5	14.0	15.4	11.5	19.4
Tetra (mean)				
0/7.5	472	190	444	281
0.01/7.5	178	141	200	124
0.03/7.5	350	111	647	170
0.1/7.5	245	119	246	156
PBBA (mean)				
0/7.5	5467	22797	6913	31085
0.01/7.5	4309	25168	4003	27324
0.03/7.5	1882	17261	3658	29042
0.1/7.5	3957	26056	4434	26179

Dosing Solution Analysis

Within acceptable criteria (± 10% of theoretical concentration)

Necropsy

Cervix, gross lesions/masses, heart, kidney, liver, lung, ovaries, spleen, stomach, and uterus were collected. In one animal at 0.1/7.5 mg/kg oxy/tetra, the left kidney contained a white area extending into the parenchyma. Because kidney lesions were observed in oxymetazoline treatment groups in other studies, this change may suggest an oxymetazoline associated effect. No other treatment-related changes were observed.

Cesarean Section Examination

The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, or shape), live and dead fetuses, and early and late resorptions.

There were no significant differences between vehicle control and test article groups in the litter averages for corpora lutea, implantations, percentage of preimplantation loss, litter sizes, live fetuses, early and late resorptions, percentage of resorbed conceptuses, and percent live male fetuses, which are consistent with the observations in the dose-range finding study.

Table 30: Summary of ovaries and uterus content evaluation in the Segment II study

GROUP		1	2	3	4	5	6
TEST MATERIAL		CONTROL ARTICLE	OXY/TETRA	OXY/TETRA	OXY/TETRA	OXY/TETRA	OXY/TETRA
DOSE LEVEL (MG/KG/DAY)a		0	0.1/0	0/7.5	0.01/7.5	0.03/7.5	0.1/7.5
RATS TESTED	N	25	25	25	25	25	25
PREGNANT	N(%)	24(96.0)	25(100.0)	25(100.0)	25(100.0)	25(100.0)	25(100.0)
DELIVERED AND EUTHANIZED	N(%)	0(0.0)	0(0.0)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 21 OF GESTATION	N	24	25	24	25	25	25
CORPORA LUTEA	MEAN±S.D.	14.4 ± 2.0	13.9 ± 1.9	14.3 ± 2.0	14.3 ± 1.7	14.1 ± 1.4	14.6 ± 2.1
IMPLANTATIONS	MEAN±S.D.	14.0 ± 1.9	13.6 ± 1.7	13.8 ± 2.7	14.1 ± 1.8	13.9 ± 1.5	14.2 ± 2.2
% PREIMPLANTATION LOSS	MEAN±S.D.	2.7 ± 4.2	2.1 ± 5.7	4.2 ± 13.1	1.8 ± 3.9	1.4 ± 3.9	3.0 ± 6.0
LITTER SIZES	MEAN±S.D.	13.4 ± 2.1	13.4 ± 1.8	13.3 ± 2.8	13.6 ± 1.9	13.5 ± 1.5	13.6 ± 2.2
LIVE FETUSES	N	321	336	319	339	337	341
	MEAN±S.D.	13.4 ± 2.1	13.4 ± 1.8	13.3 ± 2.8	13.6 ± 1.9	13.5 ± 1.5	13.6 ± 2.2
DEAD FETUSES	N	0	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.6 ± 0.8	0.2 ± 0.4	0.5 ± 0.7	0.5 ± 0.6	0.4 ± 0.7	0.5 ± 0.7
EARLY RESORPTIONS	N	15	4	13	13	10	13
	MEAN±S.D.	0.6 ± 0.8	0.2 ± 0.4	0.5 ± 0.7	0.5 ± 0.6	0.4 ± 0.7	0.5 ± 0.7
LATE RESORPTIONS	N	0	0	0	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
% POSTIMPLANTATION LOSS	MEAN±S.D.	4.5 ± 6.0	1.2 ± 2.7	4.8 ± 7.7	3.7 ± 4.7	2.8 ± 4.9	3.7 ± 5.2
DAMS WITH ANY RESORPTIONS	N(%)	11(45.8)	4(16.0)	10(41.7)	11(44.0)	7(28.0)	10(40.0)
DAMS WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE FETUSES	N(%)	24(100.0)	25(100.0)	24(100.0)	25(100.0)	25(100.0)	25(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	25(100.0)	25(100.0)	25(100.0)	25(100.0)	25(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS) / NUMBER OF CORPORA LUTEA] x 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES) / NUMBER OF IMPLANTATIONS] x 100
OXY/TETRA = OXYMETAZOLINE HCL/TETRACAINE HCL
a. Dose administration occurred on Days 7 through 17 of gestation.

The body weight of each fetus was recorded. Statistically significant decrease in fetal body weight was observed in Group 2 (0.1 mg/kg oxymetazoline) and Group 6 (0.1/7.5 mg/kg oxy/tetra) as compared to control (7.9% and 5.6%, respectively). This change was consistent with the maternal body weight change. In addition, there was no significant difference in body weight change between male and female.

Offspring Examinations

Fetuses were examined for sex and external abnormalities. Abnormalities were classified as malformations, variations, or skeletal ossification parameters. Approximately one-half of the fetuses in each litter were examined for visceral abnormalities by using a modification of the microdissection technique of Staples. The remaining fetuses (approximately one-half of the fetuses in each litter) were examined for skeletal abnormalities after staining with Alizarin Red S.

The following table summarizes changes that were considered to be treatment related and the values that were outside of the range of the historical control.

Fetal gross external malformations that were attributed to administration of the test article dose formulations were short digits. One or more digits on the left and/or right forepaw were short in 4 fetuses from 3 litters in the 0.1/0 mg/kg/day oxy/tetra dose group. Both the fetal and litter incidences for this malformation were significantly increased ($p \leq 0.01$) in this dose group, in comparison with the control article group values, and outside the range of historical control data for the testing facility. There were no other external malformations that could be considered treatment related

Skeletal malformations attributed to administration of 0.1/0 mg/kg/day oxy/tetra included fused arches in thoracic vertebrae (historical control 0-4.0%, 0-0.6%), fused ribs, and irregular number of ribs. Variations in skeletal development that were attributed to administration of 0.1/0 mg/kg/day oxy/tetra included bifid centra in thoracic vertebrae (historical control 0-25%, 0-3.3%), irregularly shaped arches in thoracic vertebrae, and not ossified phalanx in a forelimb. Lumbar vertebrae hemivertebrae was seen in one animal at 0.1/7.5 mg/kg oxy/tetra. This finding was not included in the historical data, but the range of thoracic vertebrae hemivertebrae incidences in normal animals was 0-4.3%/litter and 0-0.6%/fetus). Therefore, the incidence of lumbar vertebrae hemivertebrae may not represent a treatment-related adverse change.

Table 31 Fetal abnormalities observed in the Segment II study

Dosage (mg/kg)		Control	Oxy/Tetra 0.1/0	Oxy/Tetra 0/7.5	Oxy/Tetra 0.01/7.5	Oxy/Tetra 0.03/7.5	Oxy/Tetra 0.1/7.5
Examined Pups (letters)		321(24)	336(25)	319(24)	339(25)	337(25)	341(25)
Forelimb: Short Digits	L[&]		3 (12.0)*				
	F[&]		4 (1.2)*				
Thoracic vertebrae: Centrum, Bifid	L	4 (16.7)	7 (28.0)	1 (4.2)	2 (8.0)	2 (8.0)	4 (16.0)

	F	4 (2.4)	10 (5.7)	1 (0.6)	3 (1.7)	3 (1.7)	5 (2.8)
Thoracic vertebrae: Arches, fused	L		2 (8.0)				
	F		2 (1.1)				
Thoracic vertebrae: Arches, irregularly shaped	L		1 (4.0)				
	F		1 (0.6)				
Lumbar vertebrae: hemivertebrae	L						1 (4.0)
	F						1 (0.6)
Ribs: fused	L		3 (12.0)*				
	F		3 (1.7)*				
Ribs: 11 present	L		1 (4.0)				
	F		1 (0.6)				
Forelimb: phalanx, not ossified	L		3 (12.0)				
	F		4 (2.3)*				

[&] L = litter incidence; F = fetal incidence
* Statistically significant, $p < 0.05$

There was no difference in the ossification sites between control and test article-treated groups.

9.3 Prenatal and Postnatal Development

Study title: A subcutaneous developmental and perinatal/postnatal reproduction toxicity study of oxymetazoline hydrochloride and tetracaine hydrochloride in rats, Including a postnatal behavioral/functional evaluation

Study no.:	20052282
Study report location:	eCTD submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	12/16/2013
GLP compliance:	yes
QA statement:	yes
Drug, lot #, and % purity:	Oxymetazoline HCl, 018229AX22, 100.8%; Tetracaine HCl, 0006093547, 100/4%

Key Study Findings

- Pregnant female rats were dosed with oxymetazoline/tetracaine (oxy/tetra) at doses of 0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg from GD 7 to LD 20.

- Two F0 rats were found dead during lactation period. One at 0.1 mg/kg oxy only with dark red fluid in the thoracic cavity, mottled and dark red lung, and large spleen with black area. The other at 0.1/7.5 oxy/tetra with distended intestine with gas. Although the exact mechanism for the animal death was not clear, this finding is considered to be oxymetazoline treatment related.

- Clinical observations were similar to those observed in Segment I and Segment II studies.

- Slight body weight (approximately 10%) decreases in maternal dams (F0) were observed at 0.1 mg/kg oxy only and 0.1/7.5 mg oxy/tetra on the day of delivery correlating with decreased food consumption. At the end of lactation, mean body weight were 2.5% and 5.5% lower than control at 0.1 mg/kg oxy only and 0.1/7.5 mg oxy/tetra, respectively, suggesting a recovery.

- Reduction in the number of implantation sites was observed in the ≥ 0.03 mg/kg oxy groups without statistical significance but the values were outside of the range of historical control. The number of pups that died during the lactation period were 1, 4, 2, 3, 3, 8 at 0/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxy/tetra, respectively.

- The mean F1 fetal body weights were slightly decreased compared to control at 0.1 mg/kg oxy only (11%) and 0.1/7.5 mg oxy/tetra (4.5%) on LD 1. However, these fetal body weight changes were exacerbated during the lactation period as the body weight decreases for these treatment groups were 19% and 11%, respectively, on LD 21.

- TK analysis of F0 animals on LD 15 showed that the C_{max} and AUC of tetracaine were relatively lower in the tetracaine groups co-administered with oxymetazoline as compared to tetracaine only group.

- Significant adverse changes that were associated with maternal administration were not observed in the F1 generation animals including sex maturation, nervous system development, and reproduction.

Methods

Doses:	0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, 0.1/7.5 mg/kg oxy/tetra to F0 generation
Frequency of dosing:	Daily from GD 7 through LD 20
Dose volume:	2 mL/kg
Route of administration:	SC injection on dorsal skin. Six injection sites were rotated daily
Formulation/Vehicle:	Both oxymetazoline and tetracaine are powders dissolved in control vehicle (b) (4)
Species/Strain:	SD Rats
Number/Sex/Group:	22/group F0, 22/sex/group F1
Satellite groups:	TK animals (F0, 6/treatment group) dosed from GD 7 to LD 15, and TK evaluated was performed on LD 15
Study design:	Pregnant rats (F0) were allowed for natural delivery. Uterine contents were examined and delivered. Surviving pups were observed until the weaning period. Parameters that were examined in the F1 generation included viability, clinical signs, body weights, body weight changes, food consumption, sexual maturation, behavioral evaluations (passive avoidance and water maze), reproductive capacity, gross pathology findings, male reproductive organ weights, ovarian and uterine examinations and F2 generation fetal examinations (body weight, sex, and external abnormalities)
Deviation from study protocol:	Deviations did not affect the study quality

Table 35: Study design of the Segment III study

Group No.	Dose Level (mg/kg)[a]		Concentration (mg/mL)		Dose Volume (mL/kg)
	Oxymetazoline HCl	Tetracaine HCl	Oxymetazoline HCl	Tetracaine HCl	
1	0 (Control)	0 (Control)	0	0	2
2	0.1	0	0.05	0	2
3	0	7.5	0	3.75	2
4	0.01	7.5	0.005	3.75	2
5	0.03	7.5	0.015	3.75	2
6	0.1	7.5	0.05	3.75	2

[a] The test articles were considered 100% pure for the purpose of dose calculations.

Observations and Results

F0 Dams

Mortality

One rat (Dam 9751) in the 0.1/0 mg/kg/day oxy/tetra dose group was found dead on Day 8 of lactation (LD 8). Prior to death, this rat exhibited decreased motor activity, ataxia, mild to moderate dehydration, hunched posture, cold to touch, pale extremities, whole body pale, dyspnea, and bradypnea (LD 7). BW decrease (8%) was observed from LD 6 to 7 without food consumption decrease. Dark red fluid found in thoracic cavity, and all lung lobes were mottled red and dark red. The spleen was large and contained a black area. All 10 pups appeared normal.

One animal (Dam 9857) in the 0.1/7.5 mg/kg/day oxy/tetra dose group was found dead on LD 19. Clinical signs included ataxia, impaired/lost righting reflex, decreased motor activity. Intestines were distended with gas. The animal had BW loss from LD 16-17 (4.4%) without apparent food consumption decrease. Pups from this dam appeared normal. The mortality was likely due to oxymetazoline.

Clinical Signs

Oxymetazoline-related piloerection appeared to be dose-dependent. Ataxia, impaired/lost righting reflex, and decreased motor activity at all tetracaine treated groups with higher incidence at 0/7.5 and 0.1/7.5 mg/kg oxy/tetra as seen in the Segment I and Segment II studies. The table below is a summary of the clinical signs during gestation. The clinical signs during lactation were similar in general except that splayed hindlimbs and limited use of both hindlimbs were observed in 2 animals at 0/7.5 mg/kg during the lactation period (**Table 36**).

Table 36: Summary of clinical signs in F0 maternal rats in Segment III study

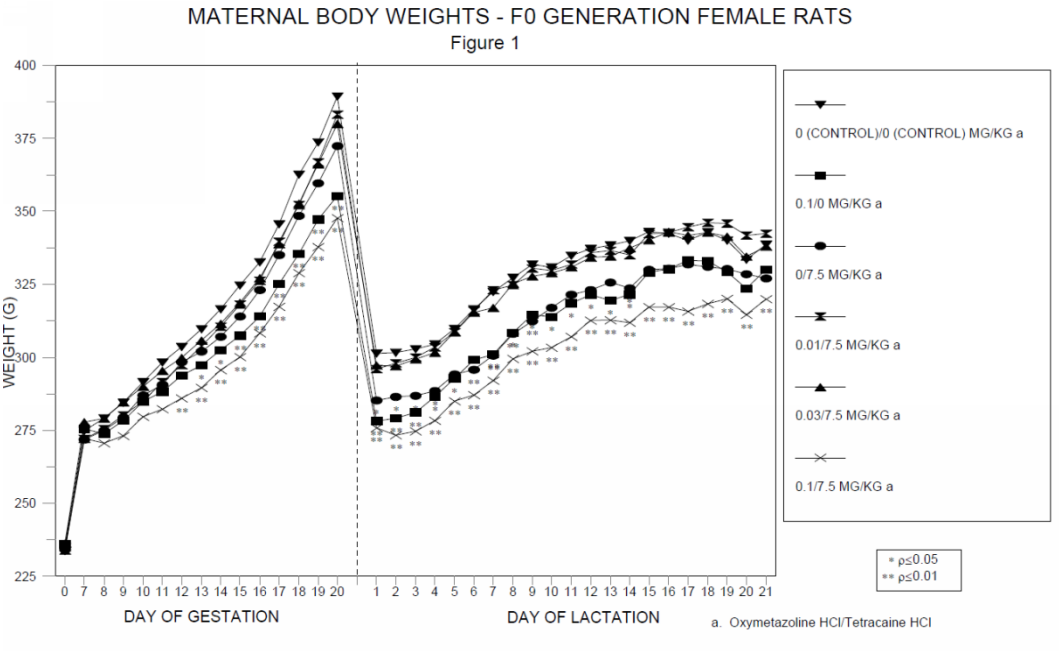
GROUP	1	2	3	4	5	6
OXYMETAZOLINE HCl (MG/KG)a	0 (CONTROL)	0.1	0	0.01	0.03	0.1
TETRACAINE HCl (MG/KG)a	0 (CONTROL)	0	7.5	7.5	7.5	7.5
FOUND DEAD	0	1b	0	0	0	1c
PRESUMED GESTATION:						
MAXIMUM POSSIBLE INCIDENCE	361/ 22	368/ 22	374/ 22	350/ 22	363/ 22	379/ 22
ATAXIA	0/ 0	0/ 0	206/ 22**	9/ 6	28/ 14**	190/ 22**c
IMPAIRED RIGHTING REFLEX	0/ 0	0/ 0	107/ 22**	5/ 4	11/ 9	103/ 22**c
LOST RIGHTING REFLEX	0/ 0	0/ 0	117/ 22**	1/ 1	5/ 5	113/ 22**c
PILOERECTION	0/ 0	315/ 22**b	0/ 0	55/ 20**	233/ 22**	277/ 22**c
DECREASED MOTOR ACTIVITY	0/ 0	0/ 0	38/ 20**	0/ 0	0/ 0	55/ 20**c
INJECTION SITE(S): PURPLE	4/ 2	145/ 19**b	10/ 2	44/ 8	35/ 6	68/ 11**
HYPERPNEA	0/ 0	5/ 2	0/ 0	0/ 0	1/ 1	2/ 2c
INJECTION SITE(S): SCAB(S)	0/ 0	0/ 0	0/ 0	60/ 8**	35/ 6**	12/ 1
SPARSE HAIR COAT: LIMB(S)	6/ 1	0/ 0	13/ 1	0/ 0	0/ 0	0/ 0
MOUTH: SCAB	4/ 1	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0
EXCESS SALIVATION - SLIGHT	1/ 1	0/ 0	0/ 0	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION
a. Dose administration occurred on Day 7 of presumed gestation through Day 20 of lactation or Day 24 of presumed gestation (rats that did not deliver a litter).
b. Dam 9751 was found dead on Day 8 of lactation.
c. Dam 9857 was found dead on Day 19 of lactation.
** Significantly different from the control group value (p≤0.01).

Body Weight

The body weight of F0 maternal animals were significantly lower at 0.1 mg/kg oxymetazoline with and without 7.5 mg/kg tetracaine combined during the administration period as shown in the figure below (**Figure 3**)

Figure 3 Maternal body weight change (F0) in the Segment III study



Statistically significant body weight gain decreases were observed in all treatment groups except the 0.01/7.5 mg/kg oxy/tetra group during the gestation dosing phase. However, the only significant decreases in mean body weight at the end of the gestation dosing phase were observed in the 0.1 mg/kg oxymetazoline treatment groups regardless of the presence of tetracaine (~10%). After delivery, body weight gain was slightly increased in all treatment groups as compared to control. At the end of dosing, the body weight decrease was in a trend of recovery.

Table 37 body weight change of F0 rats in the Segment III study

Dosing group (mg/kg oxy/tetra)	BW gain (GD 7-20)	BW (GD 20)	BW gain (LD 1-21)	BW (LD 21)
0/0	113.4	389.3	37.3	338.6
0.1/0	79.8* (↓30%)	355.3* (↓8.8%)	51.3	330.1 (↓2.5%)
0/7.5	100.5* (↓11%)	372.3	41.8	327.0
0.01/7.5	111.0	383.1	46.3	342.4
0.03/7.5	102.0* (↓10%)	379.9	40.7	337.9
0.1/7.5	75.5* (34%)	347.7* (↓10.7%)	44.2	319.9* (↓5.5%)

Food Consumption
Maternal food consumption was reduced approximately 10-15% within the dosing period in the 0.1/0 mg/kg/day oxy/tetra dose group and the 0.1/7.5 mg/kg/day oxy/tetra dose group, corresponding to the observed body weight changes.

Uterine Content
A reduction in the number of implantation sites was observed in the ≥ 0.03 mg/kg oxy groups. The values were below the historical range for the test facility. This change resulted in reductions in the mean number of pups delivered, the mean number of liveborn pups, mean number of surviving pups per litter, and the mean live litter size at these doses (only the pups delivered/litter was summarized in the following table). All of the values in each of these dose groups were below the historical range for the testing facility.

Litter observation
During LD 2-4, the incidences of pups found dead or presumed cannibalized were 0, 3 [1.2%], 1 [0.4%], 2 [0.7%] , 3 [1.2%], and 5 [2.1%], for the 0/0, 0.1/0, 0/7.5, 0.01/7.5, 0.03/7.5, and 0.1/7.5 oxy/tetra groups, respectively. The incidence appears to be related to the dose of oxymetazoline. However, these values were within the range of historical control (mean 0.9% [0-1.9%]), except the incidence at the high-dose group (0.1/7.5 oxy/tetra). Therefore, we cannot exclude the possibility that the high-dose group effect is treatment related. Although these findings may be treatment related, the clinical significance is not clear given the long clinical history of use of oxymetazoline..

The number of pups found dead or presumed cannibalized between LD 8 through 10 was statistically significantly increased in the 0.1/7.5 mg/kg/day oxy/tetra dose group (n=3 [1.3%]) in comparison to controls (n=0 [0.0%]), and exceeded the historical range of the Testing Facility. Lactation index was defined as the number of live pups on LD 21 divided by the number of live pups on LD 4. The lactation indices were significantly decreased in groups given 0.1 mg/kg oxymetazoline with and without tetracaine and the latter (oxy with tetra) being outside of the range of historical control data. Note, this change was primarily due to the loss of a whole litter in each group due to the death of the dams (one dam with 10 pups at 0.1 mg/kg oxymetazoline on LD 8, and one dam with 16 pups at 0.1/7.5 mg/kg oxy/tetra on LD 19). Aside from the apparently increased incidence of embryofetal lethality noted in the high dose group, no other abnormalities in the pups were observed on the day of dam euthanasia. From LD 4 to 21, no pups died in the 0.1 mg/kg oxymetazoline group. However, at 0.1/7.5 mg/kg oxy/tetra, additional 3 pups were died from LD 8-10, which was significantly different from control (no pup mortality) and the value was outside of the range of historical control (0-0.6% from LD 8-14 based on data from 12 studies). In the control group, one pup died between LD 5-7; there was no other pup death during the lactation period.

A significant decrease in fetal body weight was observed at 0.1 mg/kg oxy compared to control on LD 1 after delivery (11%), suggesting an effect of gestational administration. At the end of lactation (LD 21), there was a 19% body weight decrease at 0.1 mg/kg oxy

only compared to control. This suggested that fetal weight was continuously affected by oxymetazoline administration at 0.1 mg/kg in the absence of maternal body weight decrease. The change at 0.1/7.5 mg/kg oxy/tetra was similar but no statistically significant difference with a smaller magnitude.

Changes of the parameters of uterine content examination are summarized in the following table (**Table 38**).

Table 38: Summary of uterine content and litter examination of F0 rats in Segment III study

Dosing group (mg/kg oxy/tetra)	Implant sites/litter	pups delivered/litter	Viability index& % (n)	Lactation index# % (n)	Fetal weight (g) (LD 1)	Fetal weight (g) on (LD 21)
0/0	14.5	13.4	100	99.6	6.6	43.1
0.1/0	13.7 (↓5.5%)	12.7	98.4	96.0	5.9* (↓11%)	34.8* (↓19%)
0/7.5	14.2	13.5	99.6	99.6	6.5	40.7
0.01/7.5	14.2	13.1	98.9	100	6.6	42.8
0.03/7.5	13.3 (↓9.2%)	12.6	98.7	100	6.8	43.3
0.1/7.5	13.4 (↓9.2%)	12.6	97.9	91.9	6.3 (↓4.5%)	38.4 (↓11%)
Historical control range	14.1-16.2	13.0-15.7	97.7- 100	94.0 - 100		31.8 – 43.4

* Statistically significant; P < 0.05
& Number of live pups on PND4/PND1
# Number of liver pups on PND21/PND4

Necropsy
No test article-related maternal gross observations were reported at any dose level in animals that survived to scheduled euthanasia

Toxicokinetics:
For maternal plasma TK, 0.5 mL blood was collected from jugular vein at pre-dose, and post-dose at 5 and 20 minutes, 1, 3, 6, and 24 hours on LD 15. Tetracaine and oxymetazoline plasma concentrations were generally quantifiable up to 3 and 6 hours post-dose, respectively. PBBA plasma concentrations were quantifiable throughout the 24-hour sampling period. There was no significant difference in oxymetazoline exposure values when it was administered alone or in combination with tetracaine. However, the co-administration with oxymetazoline appeared to decrease tetracaine plasma exposure as shown in the table below. The level of PBBA did not appear to be affected by co-treatment of oxymetazoline.

Table 39: C_{max} and AUC of oxymetazoline, tetracaine, and PBBA in maternal rats (F0) in the Segment III study

Dosing group (mg/kg O/T)	Oxymetazoline		Tetracaine		PBBA	
	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)
0.1/0	16.1± 2.41	22.2 ± 1.64				
0/7.5			342 ± 203	114 ± 36.9	3230 ± 396	11900 ± 1800
0.01/7.5	0.84 ± 0.15	1.85 ± 0.20	85.8 ± 19.3	68.2 ± 9.17	2460 ± 658	10100 ± 667
0.03/7.5	3.11 ± 0.36	5.50 ± 0.16	82.1 ± 35.4	61.7 ± 7.79	2220 ± 397	11500 ± 437
0.1/7.5	13.4 ± 2.99	22.8 ± 2.26	135 ± 52.5	100 ± 25.0	2720 ± 358	12900 ± 1020

Milk samples were collected from each TK dam on LD 15 at 2 hours post-dose (5 minutes after oxytocin injection) and concentrations of oxymetazoline, tetracaine, and PBBA in maternal milk were determined. Results are shown below.

Dosing group (mg/kg oxy/tetra)	Oxymetazoline (ng/mL)	Tetracaine (ng/mL)	PBBA (ng/mL)
0/0	0	0	0
0.1/0	33.8	0	0
0/7.5	0	72.9	114.5
0.01/7.5	2.52	54.2	118.3
0.03/7.5	6.95	61.5	100.5
0.1/7.5	33.1	62.7	131.2

Dosing Solution Analysis
Sample concentrations were within ± 10% of theoretical concentration.

F1 Generation

Mortality and clinical signs:

During the pre-weaning period, pups in one litter (9751) were cold to touch in the 0.1 mg/kg oxymetazoline group, and the dam of this litter died the next day. Cold to touch was also occasionally seen in pups from the 0.1/7.5 mg/kg oxy/tetra group, which may also be due to maternal toxicity. There were no test article-related gross observations detected in the F1 generation pups that were found dead during the pre-weaning period.

The incidence of mild dehydration appeared to be higher at 0.1 mg/kg oxymetazoline groups when administered alone and co-administered as shown in the table below. Dehydration occurred transiently (less than 3 days) and primarily before Postnatal Day (PND) 36, which correlated with the body weight change as shown below. There were no treatment-related mortalities or other clinical signs during the post-weaning period.

Incidence of dehydration

Dosing group (mg/kg oxy/tetra)	Total incidence (litters)	
	Males	females
0/0	0	0
0.1/0	6 (2)	8 (4)*
0/7.5	2 (1)	0
0.01/7.5	0	3 (2)*
0.03/7.5	0	0
0.1/7.5	6 (3)	6 (3)*

*p < 0.05

Body weight and food consumption

After weaning from PND 22-29, body weight gain was significantly lower in both males (8.5%) and females (11%) in the 0.1 mg/kg oxymetazoline groups compared to control, which was likely responsible for the persistent body weight decrease in this dosing group after weaning. Statistically significant and > 10% body weight decrease was observed until PND 36 in both males and females (see table below). There were no significant changes in body weight changes in any other test article treatment groups as compared to control. Food consumption decrease was only seen in 0.1 mg/kg oxymetazoline group (15 – 7% decrease from PND 22 -43). Both the observed dehydration and body weight changes were related to maternal administration. However, these changes were reversed quickly and may not be considered to be adverse.

Body weight change

Dosing group (mg/kg O/T)	Body weight			
	Males		females	
	PND22	PND36	PND22	PND36
0/0	48.4	149.0	45.3	129.7
0.1/0	↓18%*	↓11%*	↓17%*	↓11%*
0/7.5	↓7.9%	↓2.4%	↓3.3%	↓1.0%
0.01/7.5	↓3.1%	↑1.1%	↓0.5%	↓1.0%
0.03/7.5	↓0.1%	↓2.7%	↑2.2%	↑0.6%
0.1/7.5	↓10%	↓5.4%	↓7.3%	↓4.8%

*p < 0.05

Sexual Maturation

The mean in days of male balano-preputial separation observation (43.6 to 45.3 days) were comparable for all maternal dose groups and were within the historical range of the testing facility.

The mean in days to female vaginal patency observation were statistically significant longer for the 0.1/0 (33.4 days) and 0/7.5 mg/kg/day (33.0 days) oxy/tetra maternal dose groups, in comparison to controls (31.4 days). However, this slight difference (<6.4%) was within the range of historical control (mean: 32.8 days; range: 30.1 – 35.3). Therefore, this change may not be considered adverse.

Neurological assessment

Passive avoidance: this test was conducted on PND 24, and included assessment of the following measures shown in the table below. There were no significant differences in the parameters assessed between the control and treated groups. Short-term and long-term retention did not appear to be affected in the F1 generation after maternal administration of oxymetazoline and tetracaine.

Parameter	Measurement
The number of trials to the criterion in the first session	compared groups for overall learning performance
The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the first test session	compared groups for activity levels and exploratory tendencies in a novel environment
The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session	compared groups for short-term retention
The number of trials to the criterion in the second test session	compared groups for long-term retention
The latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session	long-term retention

Water Maze: this test was conducted on PND 70 and included assessment of the following measures shown in the table below. There were no significant differences in the parameters assessed between the control and treated groups. Short-term and long-term retention did not appear to be affected in the F1 generation after maternal administration of oxymetazoline and tetracaine.

Parameter	Measurement
The number of trials to criterion on the first day of testing	compared groups for overall learning performance
The average number of errors (incorrect turns in the maze) for each trial on the first day of testing	compared groups for overall learning performance
The latency (in seconds) to reach the correct goal on trial 2 of the first day of testing	compared groups for short-term retention
The number of trials to criterion on the second day of testing	compared groups for long-term retention
The average number of errors for each trial on the second day of testing	compared groups for long-term retention
The latency (in seconds) to reach the correct goal on trial 1 of the second day of testing	long-term retention

Overall, these evaluations indicated that these neurologic functions in rats are not affected by maternal administration of oxymetazoline and tetracaine.

Reproduction

There were no significant changes in mating and fertility parameters and reproductive organ weights in both males and females between the test article-treated groups and the control. Gross examination of male and female reproductive organs did not reveal

significant treatment-related changes. Flaccid and small testes and epididymis were seen in the left side of one animal at 7.5 mg/kg tetracaine. This may not be considered treatment related because it occurred only in one side and the incidence was low. Ovarian and uterine content examination did not find treatment-related changes. There was no significant difference in sex ratios between control and treated groups. In addition, treatment-related abnormalities were not observed with fetal examination. Low incidences of changes are within the historical control ranges, and therefore not considered to be treatment related, as shown below.

Dosing group (mg/kg oxy/tetra)	External examination findings (n)
0/0	Body edema (1), thread-like tail (1)
0.1/0	Extra/long digits (1), unbilical hernia (1)
0/7.5	
0.01/7.5	Jaw agnathia and body edema (1), thread-like tail and short body trunk (1)
0.03/7.5	Exopthalmia, jaw agnathia, and mouth oral opening absent (1)
0.1/7.5	

Overall, the reproduction of rats was not affected by maternal oxymetazoline and/or tetracaine when administered alone or in combination.

Necropsy

Gross examination did not reveal treatment-related changes.

9.4 Other reproductive toxicity studies for oxymetazoline and tetracaine

The Applicant provided a summary of a published oxymetazoline fertility study in mice (Johns *et al.* 1975). In this study, 8-9 mice (20-30 mg body weight) were given oxymetazoline by IP injection for 5 days starting on the day of mating at 0, 1, 4, 50, and 200 mcg/day (0.03 - 0.05, 0.13 - 0.20, 1.67 - 2.50, 6.67 - 10 mg/kg/day, respectively) and the pregnancy rate was 85%, 78%, 63%, 22%, and 0%, respectively, as examined on Day 12. However, the average number of fetuses per pregnant dam was generally comparable between groups. This study was conducted prior to the implementation of GLP regulation. The paper did not provide much detail about the study procedures. The study was a simple examination that assessed the presence/absence of fetuses in the uterus to determine pregnancy rate. Since the reproductive toxicity studies submitted indicated oxymetazoline produced adverse effects on fertility and development, I do not recommend including the data of this study in the label as it may not provide additional information.

Another paper that was summarized in the submission was a published study that investigated the effect of tetracaine and other local anesthetics on the development of chick embryos (Lee and Nagele 1985). In this study, Stage 8 (four somite) chick embryos were removed from fertile White Leghorn eggs, and cultured on nutrient medium containing tetracaine HCl at concentrations of 100, 200, 300, and 400 mcg/mL

for up to 6 hours. Tetracaine treatment resulted in an increase in abnormal development. When incubated for 3 to 4 hours with 100 – 400 mcg/mL tetracaine HCl, reversible inhibition of the closure of the neural tube occurred in a dose-dependent manner. It was noted that after 3 to 4 hours of incubation, the neural folds of tetracaine-treated embryos appeared to be relaxed resulting in a neural tube that was open throughout its length. The results of the study are summarized below. The results showed potential adverse changes caused by tetracaine while all of the in vivo toxicity studies submitted to this NDA showed that tetracaine did not produce reproductive toxicity under conditions tested. However, It is not clear whether this reversible change represented potential adverse effects of tetracaine because the cultured tissue are very sensitive and subtle changes in the culture media such as pH or concentrations may cause significant adverse effects. Therefore, I do not recommend including this study in the label.

Effect of tetracaine on chick embryos explanted at Stage 8 and cultured for 6 hours			
Concentration (mcg/mL)	**No. of embryos examined**	**Little or no development (%)**	**Abnormal (%)**
0	36	0	3.0
100	18	0	77.8
200	12	0	88.3
300	14	7.1	85.7
400	6	66.7	33.3

The Applicant also referenced the embryo-fetal development studies in rabbits for tetracaine which were submitted for Synera under NDA 21623. These included the pilot non-GLP SC study for effects on embryofetal development in in rabbits (Study 925-013) and the pivotal GLP embryofetal study (Study 925-013). In the pilot rabbit study, pregnant New Zealand white rabbits (n=6/group) were administered tetracaine base via SC injection at doses of 0, 1, 2, 5, and 10 mg/kg/day on GD 7 through 20. Based on the Division's evaluation, tetracaine did not affect the pregnancy rate, delivery time, or maternal macroscopic pathology. In addition, there was no evidence of teratogenicity following tetracaine administration up to 10 mg/kg. In the pivotal SC rabbit embryofetal development study, tetracaine base was administered via SC injection to pregnant rabbits (n = 23/group) at doses of 0, 1, and 5 mg/kg/day on GD 7 through 20. The Division concluded that a dose of 5 mg/kg tetracaine caused body weight decrease in maternal animals, but did not produce embryofetal or teratogenic effects. Although the Applicant did not reference, SC embryofetal and development studies were also conducted in rats under NDA 21623. Similarly, tetracaine did not produce embryofetal and teratogenic effects in rats at doses up to 10 mg/kg. A fertility and early development toxicity study in rats indicated that tetracaine did not affect male or female fertility following SC injection at up to 7.5 mg/kg. In addition, a prenatal and postnatal development study in rats (Study 925-017) indicated that tetracaine did not produce

development effects on the offspring following maternal administration via SC injection at doses up to 7.5 mg/kg. This information has been included in the Synera label. I also recommend including this information in the Kovanaze label.

10 Special Toxicology Studies/Assessment

10.1 Support of the maximum recommended oxymetazoline dose in adults by the OTC monograph

Kovanaze contains 0.05% oxymetazoline HCl (w/v) and each spray delivers a volume of 0.2 mL that contains 0.1 mg oxymetazoline. The maximum recommended human dose (MRHD) is 3 sprays which delivers 0.6 mL Kovanaze and 0.3 mg in adults. During development of the drug product under IND 70868, the Division agreed with the Applicant that the proposed 0.3 mg oxymetazoline daily dose for marketing was within the dosing range that was determined to be safe per the final monograph on OTC Nasal Decongestant Drug Product in the FR published in 1994 (Volume 59, No. 162).

The recommended daily dose for oxymetazoline nasal decongestant published in the FR is that for adults and children 6 -12 years of age, 2 or 3 drops or sprays of 0.05% aqueous solution in each nostril with not more often than every 10 to 12 hours. This 0.05% oxymetazoline solution should not be used more than 2 doses in any 24-hour period, or consecutively for 3 days. This information is also available in the Code of Federal Regulations (CFR) Title 21 (Sec. 341.80 Labeling of nasal decongestant drug products).

The monograph did not indicate the exact daily milligram dose. In addition, it did not specify the drop (or spray) size (volume) of oxymetazoline in the monograph, which made it difficult to determine the exact dosing level. According to Dr. Xiaobin Shen in an email communication, a drop has been rounded to be 0.05 mL since metric measurement was used. Therefore, 6 drop/dose (2 or 3 drops in each nostril) are equal to 0.3 mL and 2 doses per day is 0.6 mL which is the same volume as the Kovanaze MRHD. Because the concentration of oxymetazoline in both Kovanaze and OTC nasal decongestant products are 0.05%, the recommended oxymetazoline daily dose for OTC use should be the same as that of Kovanaze in milligram. By this calculation, however, Kovanaze dosing regimen (2 × 0.2 mL/spray with 4 minutes apart +1 × 0.2 mL spray after 10 minute) may produce higher C_{max} than that the monograph recommended dosing regimen. Prior to the NDA submission, 13 clinical studies have been completed including 3 studies in pediatrics. The highest oxymetazoline dose tested was 0.6 mg in 12 subjects. In all these studies, 0.3 mg oxymetazoline in combination with tetracaine was tested in 198 subjects and the safety profile appeared acceptable. Refer to the medical officer's review for details.

In this NDA submission, the Applicant indicated that in the FDA's pharmacologist's summary of Afrin nasal spray 0.05% from NDA 14717 (approved in 1967), the human dose is described as follows:

"DOSAGE INFORMATION: Spray Solution: 2 or 3 sprays into each nostril. Repeat in 10 or 15 minutes (estimated maximum amount of drug is 0.53 mg/day or 0.01 mg/kg/day)."

This dosing level is significantly higher than the proposed (b) (4) kg for Kovanaze. (b) (4)

(b) (4)

Overall, I think the oxymetazoline monograph provides coverage for the oxymetazoline dosage in Kovanaze, and a plethora of clinical data are available to evaluate the safety of oxymetazoline in the proposed patient population.

10.2 Waiving of the nonclinical studies in juvenile animal models to support clinical trials in pediatrics

The proposed Kovanaze doses for pediatrics are as follows:

1) Children (b) (4), ≥ 40 kg): 2 × 0.2 mL/spray, (b) (4)

The Applicant completed multiple clinical studies in pediatrics to support the proposed pediatric dosage for the NDA. When the clinical protocols were submitted to the Division under IND 70868, an initial Pediatric Study Plan (iPSP) was not yet required. The Division decided that juvenile animal studies were not needed to support pediatric clinical studies at that time because of the following:

1) Synera, a topical patch containing 70 mg lidocaine and tetracaine was approved for children 3 years of age and older. (b) (4) In comparison, tetracaine concentration was not detectable in adults following 3 Kovanaze sprays.

2) Oxymetazoline is approved for children as an OTC nasal decongestant.

    a. According to the final monograph, a 0.025-percent aqueous solution in a container having either a calibrated dropper or a metered-dose spray that

delivers no more than 0.027 mg of oxymetazoline per three drops or three sprays should be used for children 2 to under 6 years of age. The recommended dose is 2 or 3 drops or sprays in each nostril not more often than every 10 to 12 hours within 24 hours. Therefore, it is calculated to be 0.054 mg/dose (0.027 mg/nostril ×2 nostrils), and 0.108 mg/day (0.054 mg/dose ×2).

b. This is higher than the proposed Kovanaze clinical dose at this age group (0.05 mg/day). For children at (b) (4), the OCT monograph recommended the same dose of oxymetazoline nasal administration as that for adults while the proposed Kovanaze clinical doses are lower than that for adults.

c. The Division recognized that the local exposure might be higher for Kovanaze than the monograph recommended dose, which could be a safety concern. However, Kovanaze has the same concentration of oxymetazoline as the approved via the monograph (0.05%). Therefore, the Division believed that the evaluation of the nasal mucosa in human adults would provide better prediction than the evaluation in animal models for the local toxicity in pediatrics.

In this NDA, the Applicant included the completed clinical studies in pediatrics to support the pediatric patient population. In the pediatric study plan (PSP) included in this NDA, the Applicant requested a waiver of the pediatric 0-2 year old group because of no utility in this age group since these subjects do not normally receive dental care. This waiver request has been granted by PeRC. Therefore, no juvenile animal studies were needed for the NDA or as post-marketing requirements (PMR).

10.3 Safety assessment of Leachables and extractables

Because Kovanaze is delivered by a single-use BD Accuspray™ sprayer, the Division required that the NDA submission must contain information on potential leachables and extractables from the drug container closure system and/or drug product formulation. At the pre-NDA meeting, the Division informed the Applicant that “From a genetic toxicology perspective, we will allow up to 120 mcg/day for an acute indication for most potentially genotoxic impurities. A toxicological risk assessment should be provided for any non-genotoxic leachable that exceeds 5 mcg/day.”

In the NDA submission, 3 studies for extractables and leachables were included. These included (1) a controlled extraction study, (2) a leachable-extractable correlation study in which older stability samples of drug product were examined for actual leachable compounds, and (3) a final study to quantitate leachable compounds in long-term storage samples from primary stability studies. These studies were considered to be acceptable during the filing review. However, the toxicological risk assessment was based on a Safety Concern Threshold (SCT) of 120 mcg per day because the Applicant

misinterpreted that the Agency's comments at the pre-NDA meeting regarding the acceptable daily intake for genotoxic compounds would apply to the concern for general toxicology of the compound due to the intended acute use of the product. This was identified as a filing review issue and a comment was sent to the Applicant in the 74-day letter to further clarify that a toxicological risk assessment is required for all leachable compounds identified from drug product batches on stability testing that exceed a total daily intake (TDI) of (b) (4) mcg per day, and any potentially genotoxic leachable must not exceed 120 mcg per day TDI for an acute indication or must be adequately qualified for safety from a genetic toxicology perspective.

The response to this issue from the Applicant was received on 10/19/2015 (SDN 13). The Applicant indicated that the initial evaluations in the leachable and extractable studies were originally performed using an SCT of (b) (4) mcg per day, and an updated assessment of all leachables above the (b) (4) mcg daily uptake level identified the leachable studies was included in the updated Toxicology Written Summary (Module 2.6.6).

The analytical evaluation threshold (AET) of (b) (4) mcg/mL was calculated using the (b) (4) mcg/mL SCT based on a delivered volume of 0.20 mL/sprayer, and an allowance of up to three sprayers per day. The AET used in the studies was then set to (b) (4) % of this value to obtain a final leachable AET of (b) (4) mcg/mL. Therefore, the AET employed actually represents an SCT of (b) (4) mcg/day.

In the extraction study, (b) (4) . Asymptotic extraction profiles were generated with the goal of estimating the largest amount or worst case extractable level from each component. Extraction samples were analyzed using different analytical screening techniques including HPLC-DAD-MS/ToF, GC-MS, polycyclic aromatic hydrocarbons (PAHs) by GC-MS-SIM, Anion and cation ion chromatography (IC), and atomic absorption (b) (4)

Table 40 Summary of controlled extraction study for Kovanaze container-closure components

Screening Type	Extraction Medium	Component	µg Extracted/ Component	Time (hr)	ID	Amount/ Component (µg)	Origin/ Use
General Screen (HPLC/ DAD)	(b) (4)						
Anions (IC)							
Cations (IC)							
Silicon (AA)							
GC/MS General Screen							
PAH by GC/MS							

(b) (4)

To determine if any of the extractable peaks identified in the initial study represent actual leachable peaks, the same generic analytical methods were applied to aged drug product samples. In addition, to distinguish actual leachable peaks from peaks related to excipients and active compounds, samples of each drug substance, newly manufactured bulk drug product, and solutions of the individual formulation components, and extracts of the container/closure components were also examined. The extractables/leachables correlation study analyzed aged drug product from two lots stored under refrigerated long-term storage conditions (2 – 8 °C) for nearly three years at the time of leachable testing, which is longer than the proposed (b) (4) month shelf-life of the drug product[2]. Using GC-MS method, of the seven potential leachables that were

[2] As per the product labeling the product should be stored between 2° and 8°C. Therefore, these

identified by GC-MS above the AET, three were determined to be related to the drug substance and the remaining four compounds were potential leachable compounds identified as common fatty acid-related compounds that are known to leach from similar packaging components (LDPE, plastic containers, coated glass, and stopper/plunger) as shown in the table below (**Table 38**, extracted from the submission).

Table 41: Potential leachables by GC-MS in the leachable study of the aged batches of Kovanaze

RT	RRT	Calc. Amount (µg/mL)	Tentative ID	Library Tentative ID	Probability[a]	Peak ID	Aged Drug Product	
							Lot 006773	Lot 006775
(b) (4)								

Note: X indicates the peak was present.

[a] A numerical probability value is designated by the GC Library match. The probability value is representative of the confidence in the identification based on ABC interpretation of the mass spectra.

All peaks identified using HPLC method and IC above (b) (4) mcg/mL were determined to be drug substance or drug product related but not leachables. There were no peaks above the (b) (4) mcg/mL AET identified by AA analysis.

A follow-up study was conducted to use 46 registration stability samples used GC-MS to quantify possible leachables. The samples tested included two product fill sizes (0.06 and 0.2 mL) from three lots stored at two temperatures (5°C and 25°C/60% RH)[3] and two orientations, all of which were collected at specified intervals over the 24-month study duration. The results showed that none of the 4 leachables observed in the expired samples were seen in the stability samples at levels above the AET in sprayers stored as long as 24 months at 5°C or 6 months at 25°C/60% RH.

---

conditions are relevant.

[3] Accelerated stability conditions

These extractable and leachable studies were determined to be sufficient to characterize potential leachables from CMC perspective by Dr. Xiaobin Shen, the CMC reviewer for this NDA. I agree with this evaluation. Detailed review of these studies can be found in Dr. Shen's review.

Because the leachable compounds above (b) (4) mcg/mL AET (b) (4) mcg SCT) observed in the aged Kovanaze batches were not identified in the stability samples up to 24 months at 5°C or 6 months at 25°C/60%RH, no toxicological risk assessment was needed. Overall, leachables were adequately qualified by the Applicant.

11 Integrated Summary and Safety Evaluation

Kovanaze drug product is an intranasal spray solution prefilled in a nasal spray system. It contains 3% tetracaine (6 mg/spray) and 0.05% oxymetazoline (0.1 mg/spray). The proposed maximum recommended human dose for adults is 3 sprays per day which delivers 18 mg tetracaine and 0.3 mg oxymetazoline.

Both oxymetazoline and tetracaine has been approved for marketing in the United States for decades. However, intranasal administration for tetracaine drug product has not been approved by the Agency.

This application was submitted as a 505(b)(2) referencing the final OTC monograph for nasal decongestant drug products published in the FR (Volume 59, 1994), which includes 0.05% oxymetazoline nasal solution. The NDA also included a letter of authorization to cross-reference all of the nonclinical information in the Synera NDA 21623 for tetracaine-related data. Synera is a dermal patch containing 70 mg lidocaine and 70 mg tetracaine. In addition, available literature for both oxymetazoline and tetracaine were also summarized.

Nonclinical studies were also conducted to support the NDA. These include a 2-week intranasal general toxicity study in dogs with 2-week recovery period to support the safety of the proposed clinical dose, and 3 reproductive and development toxicity studies for appropriate labeling.

General toxicity studies

In the previous communications during drug development, the Division agreed no nonclinical studies were needed to support the clinical studies during drug development, and a 2-week toxicity study in dogs using the clinical route of administration was considered to be appropriate for the NDA along with the available nonclinical information from the public domain.

The Applicant summarized the available published literature for acute toxicity studies with tetracaine in animal models. These studies indicated that a single-dose of tetracaine administration was associated with disturbances in coordination, salivation,

loss of righting reflex, ataxia, spasm with convulsion, respiratory arrest, paralysis, and death. An appropriate NOAEL for tetracaine could not be identified from these studies. There were no repeat-dose toxicity studies for tetracaine available in the public domain. In addition, acute or repeat-dose toxicity studies for oxymetazoline could not be identified from the public domain. In an embryofetal dose-range finding study conducted by the Applicant, nonpregnant female rats were injected with oxymetazoline SC for 5 days at 0.1, 0.6, 1.2, and 2.0 mg/kg. Animals did not tolerate doses ≥ 0.6 mg/kg, as evidenced by severe clinical signs and mortality. Clinical observations that were observed at ≥ 0.6 mg/kg included piloerection, could to touch, ataxia, motor activity decrease, low carriage, hunched posture, impaired righting reflex, vocalization, sprayed hindlimb, dehydration, tachypnea, hyperpnea, bradypnea, excess salivation, pale extremities, and portion of tail missing at ≥ 0.6 mg/kg. Gross pathology examination showed thymus with red area, thoracic cavity with cloudy red or clear tan fluid, kidney with dark red region, heart with dark area, and stomach distended with gas. Piloerection was the only clinical sign observed at 0.1 mg/kg in the reproductive toxicity studies the Applicant conducted.

In the 2-week dog study, young male and females were administered oxymetazoline and tetracaine via intranasal spray for 14 days using a mucosal atomization device at 0 (vehicle), 0.01/0, 0.01/0.6, 0.01/1.5 and 0.01/3.0 mg/kg/day of oxymetazoline/tetracaine (oxy/tetra). The vehicle buffer solution was similar to the clinical formulation which contained the same concentration of benzyl alcohol and hydroxyethyl cellulose. The concentrations of oxymetazoline and tetracaine were the same as those in the clinical formulation. However, the oxymetazoline solution and tetracaine solution were not premixed before administration. Oxymetazoline solution was sprayed first and immediately followed by a 0.020 mL buffer solution, 0.02, 0.05, or a 0.10 mL tetracaine solution. The tetracaine doses provided 1.1, 2.8, and 5.6-fold margins, respectively, for the level of tetracaine in the maximum recommended human dose of Kovanaze while the fixed oxymetazoline dose provided a 1.1-fold margin based on body surface area comparison.

The primary in-life observations were reversible clinical signs including, reddened/swollen gums (anesthetic effect), vocalization, salivation, nasal discharge, emesis, soft and mucoid stools, red and swollen pinna, shivering, shallow or labored breathing, impaired mobility, and wobbly gait, which were primarily observed at 0.01/3.0 mg/kg Oxy/Tetra. At a 1.5 mg tetracaine dose, coughing, vomiting, soft stool, and salivation were observed. Soft stool and salivation were also observed at 0.6 mg/kg tetracaine dose. Tetracaine administration was considered to be associated with these changes because of the observed tetracaine dose-dependency. In addition, similar changes were also seen in tetracaine toxicity studies available from the public domain as discussed above.

Similar changes were not observed in the oxymetazoline only dose group except soft stool. However, contribution from oxymetazoline to the apparent tetracaine-related changes cannot be excluded because the oxymetazoline exposure especially the C_{max} was increased when co-administered with tetracaine in a tetracaine-dependent manner

(**Table 8**). At 0.01/3.0 mg/kg oxy/tetra, the C_{max} was about 14-fold of the C_{max} at 0.01 oxymetazoline only on SD 1. However, I think the contribution of oxymetazoline may be minimal because at the end of 14-day dosing, oxymetazoline exposures were significantly decreased as compared to SD 1, but this did not appear to significantly affect the frequency of these clinical observations.

Furthermore, these clinical observations were evident immediately following dosing and the animals recovered quickly, suggesting that they are likely to be attributable to the parent compound tetracaine rather than the metabolites that were responsible for these changes because this coincided with tetracaine TK profile. As the TK data indicated, T_{max} for tetracaine was 5 minutes and the $T_{1/2}$ was about 0.19 to 2.2 hours. In comparison, the T_{max} for the tetracaine metabolite p-butylamino benzoic acid (PBBA) was primarily at 1 hour after dosing, although shorter T_{max} was also seen in some animals. The $T_{1/2}$ for PBBA was ranged from 1.17 to 5.45 hours.

ECG examination did not show significant adverse changes observed in any dosing group on SD 1, SD 14, and SD 28. Histopathological changes were primarily observed in the local tissue including cell infiltration and squamous metaplasia in the nasal cavity of animals from the 1.5 mg and 3.0 mg tetracaine dosing groups in a dose-dependent manner. These changes were reversible after the 14-day recovery. Because the concentration of tetracaine was the same cross all doses but the volumes of tetracaine solution at each dosing level was different, these histopathological changes may be tetracaine volume (amount)-dependent. These changes were not observed in the treatment groups of 0.01 mg/kg oxymetazoline alone. It is believed that changes in the local tissues were related to the local exposure of a test article but not the systemic exposure. Because similar histopathological changes were not seen at 0.01 oxy only, it is not likely that oxymetazoline plays an important role in the changes observed in the 0.01/3.0 mg/kg Oxy/Tetra group.

The NOAEL was identified to be 0.01/0.6 Oxy/Tetra. Tetracaine exposure provided sufficient safety margin to support the MRHD. The average C_{max} and AUC in males and females on SD 14 was 570 ng/mL and 145 ng•h/mL, respectively, at 0.6 mg/kg tetracaine as shown below. In comparison, the tetracaine plasma concentration was generally undetectable in the clinical studies after Kovanaze treatment at 18 mg or higher. At the NOAEL, the systemic exposure of PBBA did not cover the human exposure as seen below. In clinical studies, it is used as a marker for tetracaine exposure when tetracaine plasma concentration is usually not expected. In this dog study, 0.3 mg/kg tetracaine dose which is an acceptable NOAEL provided an approximately 1-fold exposure margin by AUC comparison for PBBA, and adverse findings observed in this study were not likely to be associated with PBBA. This is considered to be sufficient for PBBA safety evaluation in my opinion.

Table 42: Safety margin in the 2-week dog study for the maximal human dose

Safety margins for MRHD in the 2-week dog study

Human exposure	Tetracaine	PBBA	Oxymetazoline
NOAEL = 0.01/0.6 mg/kg Oxy/Tetra			
C_{max}	570/-^	52.5/465 =0.11*	0.37/1.78 = 0.21
AUC $_{0\text{-}24h}$	145/-	156.9/960 = 0.16	0.75/3.67 = 0.20
LOAEL = 0.01/3.0 mg/kg Oxy/Tetra			
C_{max}	1508/-	242/465 =0.52	0.80/1.78 = 0.47[&]
AUC $_{0\text{-}24h}$	432/-	816/960 = 0.85[#]	0.80/3.67 = 0.22

^ -: undetectable
* Mean value in animals/ mean value in human on SD14 = exposure margin
[&] The mean C_{max} value at this dose was 3.42 ng/mL on SD1, and exposure margin was 1.92
[#] The mean AUC value at this dose was 964.5 ng•h/mL on SD1, and exposure margin was 1.0

For oxymetazoline, this study did not provide sufficient safety margins based on either C_{max} or AUC comparison. Technically, there was no appropriate nonclinical data in this dog study to provide sufficient safety margin to support the safety of oxymetazoline at the maximal clinical dose. Useful information obtained in this study is that severe adverse changes were not seen at 0.01/3.0 mg/kg dose on SD 1 when the C_{max} of oxymetazoline was 2.95-fold human C_{max} at 0.03 mg. It should be noted that the maximal human oxymetazoline human dose (0.3 mg) for Kovanaze is the same as that was recommended by the OTC monograph. This calculation for the OTC monograph recommended oxymetazoline dose was based on the assumption that the drop size was 0.050 mL which is relatively conservative in my opinion. According to the oxymetazoline OTC monograph, two doses of 2-3 intranasal drops each nostril per day are considered to be safe. Then the maximal daily dose in milligram is 0.050 mL/drop × 6 drops/dose × 2 doses × 0.5 mg/mL (0.05%) = 0.3 mg. Taken all these together, I think no additional nonclinical are needed to further evaluate the safety of oxymetazoline.

For local toxic effects, it appeared that the local changes observed in this study were volume-related. The total daily volume was 0.6 mL (3 sprays × 0.2 mL/spray) in human at the MRHD while the maximal total volume administered to the dog was 0.12 mL (tetracaine solution + oxymetazoline solution). Therefore, this dog study is not appropriate to evaluate the potential local effects of Kovanaze at the maximal clinical dose (0.6 mL). However, a higher dose (larger volume attempting to reach 0.6 mL) is not possible in the dog because the maximum tolerable dose (MTD) for tetracaine was reached at 3 mg/kg. In a pilot dosing test at 4 mg/kg with intranasal administration, severe clinical signs including tremor, excessive salivation, sedation, and muscular rigidity were observed in dogs, which were not considered tolerable. Using other animal models such as rats will be limited by the difficulty of administration as well as the practicable volume. In addition, these observed histopathological changes were likely developed due to repeated dosing, and thus not likely to be associated with acute treatment as proposed for Kovanaze.

Overall, systemic adverse changes observed in the 2-week dog study with oxymetazoline and tetracaine co-administration were common findings associated with tetracaine. The local pathological changes were considered to be associated with repeated dosing but not acute treatment. The high tetracaine dosing level in this study (3.0 mg/kg) reached the MTD. At the NOAEL, there is a sufficient safety margin to support the safety of tetracaine in human at the maximal Kovanaze dose. Although this study did not provide sufficient safety margin for oxymetazoline exposure, results indicated that at an oxymetazoline plasma level of approximately 3-fold the human C_{max} at the MRHD, oxymetazoline in combination with tetracaine did not produce unexpected severe adverse changes. This study in combination with the available information from the public domain and the previous clinical experience (at the discretion of the clinical review team) supports the approval of Kovanaze.

Genotoxicity

No genotoxicity studies were conducted for oxymetazoline and tetracaine separately or in combination by the Applicant. At the pre-NDA meeting, the Division informed the Applicant that "Although we will not require these data for a 505(b)(2) NDA, in the interest of public health, we encourage you to obtain genotoxicity information for oxymetazoline to appropriately inform your drug product labeling" because oxymetazoline is generally recognized as safe according to the finalized OTC monograph. The Applicant indicated that "The genotoxic potential of oxymetazoline has not been evaluated". This is consistent with my search result. The Applicant referenced the genotoxicity studies for tetracaine in NDA 21623 for Synera. These studies were evaluated by the Agency during the NDA review for Synera. The Division concluded that tetracaine was tested negative in the in vitro bacterial reverse mutation assay and the in vivo mouse micronucleus assay. Although tetracaine tested negative in the absence of metabolic activation in the in vitro chromosome aberrations assay, in the presence of metabolic activation, tetracaine was considered "equivocal." This information is included in the approved Synera label. I do not think it is necessary to evaluate the genotoxicity potential of oxymetazoline and tetracaine combination. Since we have informed the Applicant that submission of genotoxicity information for oxymetazoline is not a requirement. The information included in this submission is considered to be sufficient.

Reproductive and development toxicity

The NDA included a fertility and early embryofetal development study, an embryofetal development study, and a prenatal and postnatal development study in rats to investigate the reproductive and development toxicity of oxymetazoline and tetracaine combination. The embryo-fetal development toxicity study was specifically required by the Division at the End-of-Phase-2 (EOP2) meeting according to the FDA guidance for industry: *Nonclinical Safety Evaluation of Drug or Biologic Combinations*.

The doses tested were the same for all 3 studies with 3 different dosing levels of oxymetazoline and a fixed dosing level of tetracaine. One oxymetazoline only dose group and one tetracaine only dose group were also included in these studies. Animals (22 - 25/group) were administered by SC injection at 0/0 (citric buffer vehicle), 0.1/0,

0/7.5, 0.01/7.5, 0.03/7.5, and 0.1/7.5 mg/kg oxy/tetra. To avoid local irritation, 6 injection sites on the dorsal area were rotated. In the fertility and early embryofetal development study, both males and females were dosed from 28 days and 14 days before cohabitation, respectively. Males were dosed throughout the mating period before euthanasia, and females were dosed through Gestational Day (GD) 7 and sacrificed on GD 13. In the embryofetal development study, pregnant females were dosed from GD 7 to GD 17, and C-section examination was conducted on GD 21 after sacrifice. In the prenatal and postnatal development study, pregnant females were dosed from GD 7 thought the gestational and lactation period until Lactation Day (LD) 20. The study designs appeared to be appropriate.

TK analysis showed that the exposures profiles of oxymetazoline and tetracaine were generally comparable across the 3 studies. The exposure of oxymetazoline was dose-dependent and not affected by the co-administration of tetracaine. However, the tetracaine administration appeared to be affected by co-administration of oxymetazoline. This effect was most evident in the prenatal and postnatal development study with the values of tetracaine C_{max} and AUC at co-administration groups were approximately 24 - 39% and 54 - 88% of those with tetracaine only administration, respectively. This is reasonable because oxymetazoline causes vasoconstriction and slowness of blood flow. These may, in part, explain why the incidences of observed tetracaine-related clinical observations including ataxia, impaired righting reflex, lost righting reflex, hunched posture, decreased motor activity and low carriage, were significantly lower at the 0.01/7.5 and 0.03/7.5 mg/kg oxy/tetra as compared to the 7.5 mg/kg tetra only administration group. Of note, at 0.1/7.5 mg/kg oxy/tetra, the incidences were not significantly different from those at 7.5 mg/kg tetra only. This suggested that oxymetazoline at 0.1 mg/kg also contributed to these changes because oxymetazoline alone were also associated with similar changes at higher doses (≥ 0.6 mg/kg) in an embryofetal development dose-range finding study.

Across all 3 studies, the body weight change in female rats at all dosing groups as compared to the control was slightly decreased (≤ 10%) and mainly occurred at the 0.01 mg/kg oxymetazoline dosing level when administered alone or co-administered with 7.5 mg/kg tetracaine. Tetracaine did not appear to affect female body weight. However, in males in the fertility study, body weight change appeared to be a combined effect of oxymetazoline and tetracaine with the magnitude of 10%, 7%, and 17% at 0.1/0, 0/7.5, and 0.1/7.5 mg/kg oxy/tetra. Severe clinical signs such as hyperpnea, bradypnea, and clonic convulsion were only observed at 0.1/7.5 mg/kg/day oxy/tetra in males in the fertility study, but not in females in any of the 3 studies. Treatment-related mortality occurred in males in 0.1 mg/kg oxy only treatment group (2 from SD 15-26) and 0.1/7.5 mg/kg group (3 from SD 34-64) in the fertility study. Body weight loss was observed without change in food consumption, and gross pathology examination revealed red or dark red lung lobes and mottled and dark red kidneys in some animals. Treatment-related mortalities were also seen in females from the 0.1 mg/kg oxy (one animal on SD 25) only group and 0.1/7.5 oxy/tetra group (on SD 34) in the prenatal and postnatal development study. Gross necropsy examination revealed dark red fluid in thoracic cavity and mottled red and dark red regions in all lung lobes, and distended intestine

with gas, which were also observed in female animals found dead at ≥ 0.6 mg/kg oxymetazoline in the embryofetal dose-range finding study. No treatment-related mortalities in females were seen in the fertility study and the embryofetal toxicity study.

In the fertility and early embryofetal development study, a NOAEL for male general toxicity could not be identified due to clinical signs observed at all dose levels. However, the 0.03/7.5 mg/kg oxy/tetra dose level can be considered an acceptable LOAEL due to the low incidence and severity of adverse effects at this dose and below. In females, the NOAEL for maternal toxicity is identified to be 0.03/7.5 mg/kg Oxy/Tetra. In females, the number of corpora lutea, implantation sites, and viable embryos were significantly decreased at 0.1 mg/kg oxymetazoline when administered alone (12, 14, and 16%, respectively) or in combination with 7.5 mg/kg tetracaine (16, 16, and 18%, respectively). In addition, a decreased number of viable embryos was also seen at 0.01/7.5 (12%) and 0.03/7.5 oxy/tetra. Therefore, a NOAEL for fertility and early embryofetal development cannot be identified.

In males from the fertility study, sperm motility, total sperm count, and sperm concentration were decreased at 0.1 mg/kg oxymetazoline when administered alone (31%, 44%, and 25%, respectively) or in combination with tetracaine (18%, 33%, and 8.3% respectively). At 0.03/7.5 mg/kg the values were 11%, 20%, and 7.7%. The less than 10% decrease in sperm concentration at 0.03/7.5, 0.1/7.5 oxy/tetra was likely due to the decreased organ weight of cauda epididymis at these dosing levels. Dose-dependent decrease was found in reproductive organ weights including cauda epididymis, seminal vesicles, and prostate. The NOAEL for male fertility was identified to be 0.01 mg/kg oxy/tetra.

In the embryofetal development study, the NOAEL for maternal toxicity was 0.03/7.5 mg/kg oxy/tetra. Caesarean section examination did not reveal significant adverse changes in ovaries and uterus contents for any treatment group. Slight fetal body weight decreases (<10%) were observed in the 0.1 mg/kg oxy with or without tetracaine co-administration groups and was likely due to maternal body weight changes. External and skeletal alterations that were considered to be treatment related were only seen in the 0.1 mg/kg oxymetazoline group when tetracaine was not co-administered. These include malformations such as short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs, and variations such as irregularly shaped arches in thoracic vertebrae and increased bifid centra in thoracic vertebrae. Similar changes were not seen in the same oxymetazoline dose when tetracaine was co-administered. In the embryofetal dose-range finding study, external alterations were observed at 0.3 mg/kg oxymetazoline dosing level when oxymetazoline was given alone or in combination with 7.5 mg/kg tetracaine. In comparison, the incidences were slightly lower when tetracaine was co-administered. These changes including jaw micrognathia, and forepaw digits short, absent, or fused. Although it appeared to be a possibility that the presence of tetracaine may ameliorate the teratogenic effect of oxymetazoline, the currently available data are not sufficient to make this conclusion. The NOAEL for embryofetal and development toxicity was identified to be at 0.03/7.5 mg/kg oxy/tetra.

In the prenatal and postnatal development toxicity, the NOAEL for maternal toxicity (F0 generation) was identified to be at 0.03 mg/kg oxy/tetra. During the lactation dosing phase, body weight gain was slightly higher at all dosing groups compared to control, which resulted in a recovery of the decreased body weight at 0.1/0 and 0.1/7.5 mg/kg oxy/tetra. The body weight decrease compared to control in F0 dams were 8.8% and 10.7% at 0.1/0 and 0.1/7.5 mg/kg oxy/tetra on GD 20, respectively, while the values at these doses were 2.5% and 5.5% on LD 20. As a comparison, the body weight decrease in the F0 fetuses was exacerbated to 19% and 11% on LD 20 from 11% and 4.5% on LD 1 at the 0.1/0 and 0.1/7.5 mg/kg oxy/tetra maternal dose levels, suggesting that the mean fetal body weight decrease was not associated with maternal toxicity. Oxymetazoline treatment decreased the mean number of implant sites/litter at ≥ 0.03 mg/kg when administered with (approximately 9%) or without tetracaine administration (5.5%). Although these changes were not statistically significant, the values were outside the range of historical control, which suggested a treatment-related adverse change. This change resulted in reductions in the mean number of pups delivered, the mean number of liveborn pups, mean number of surviving pups per litter, and the mean live litter size at these doses. All of the values in each of these dose groups were below the historical range for the testing facility.

In the F1 animals, after weaning, statistically significant and >10% body weight decrease was still observed in both males and females 2 weeks after weaning (PND 36). There were no other significant adverse changes in the F1 animals in sexual maturation, reproduction, and nervous system development via the endpoints examined, suggesting that the development of the F1 generation was not significantly affected following maternal administration from the organogenesis through lactation.

Overall, reproductive studies in rats indicated toxic effects in fertility, embryofetal development, and postnatal development following administration of oxymetazoline and tetracaine in combination. However, the results of these studies indicated that the toxic effects were caused by oxymetazoline, but not tetracaine. The following table shows the exposure margins of oxymetazoline and PBBA by AUC comparison in these studies. Exposure margin cannot be calculated because tetracaine was undetectable in the clinical studies.

Table 43: Exposure margin of oxymetazoline and PBBA for human dose in the reproductive studies

	Exposure margins by AUC comparison											
	Oxymetazoline (3.67 ng•h/mL in human)						**PBBA (0.97 mcg•h/mL in human)**					
O/T	**Seg I**		**Seg II**		**Seg III**		**Seg I**		**Seg II**		**Seg III**	
(mg/kg)	AUC*	EM	AUC	EM	AUC	EM	AUC	EM	AUC	EM	AUC	EM
0.1/0	27.5	7.49	28.0	7.63	22.2	6.05						
0/7.5							32.0	32.9	31.1	32.0	11.9	12.2
0.01/7.5	2.68	0.73	2.26	0.62	1.85	0.50	29.8	31.6	31.1	32.0	10.1	10.3
0.03/7.5	7.37	2.00	5.61	1.52	5.50	1.50	26.3	27.0	29.0	29.8	11.5	11.8
0.1/7.5	29.4	8.01	19.4	5.29	22.8	6.21	30.0	30.8	26.2	26.9	12.9	13.3

* AUC = $AUC_{0\text{-}24h}$, EM = exposure margin.

The Applicant also referenced reproductive and development studies submitted in Synera NDA (NDA 21623) for tetracaine reproductive and development toxicity evaluation. These studies included fertility and early development study in rats, embryofetal and development studies in rabbits, and prenatal and postnatal development study in rats. These studies have been evaluated by the Agency, and the data indicated that tetracaine when administered alone did not affect fertility and early embryofetal development at up to 10 mg/kg in rats, embryofetal development in both rats and rabbits at up to 10 mg/kg, and prenatal and postnatal development at 7.5 mg/kg, which are consistent with the observations in studies submitted in this NDA. The Applicant also identified 2 reproductive toxicity studies and included in this submission. One study was for investigation of the effect of oxymetazoline in fertility in female mice and the results showed that pregnancy rate was significantly decreased following IP injection of oxymetazoline at 50 and 200 ng/animal (1.7-2.5 and 6.7 -10 mg/kg). This study was conducted in 1975 and the results did not provide additional information for oxymetazoline fertility effect. I do not recommend including this information in the label. Another study was an ex vivo study in chick embryo by adding tetracaine in the culture media, and the results indicated that tetracaine caused reversible inhibition of the closure of the neural tube in a dose-dependent manner. Due to the uncertainly of the ex vivo assay to predict human findings, I also do not recommend including this study in the label.

Overall, the Applicant conducted appropriate reproductive toxicity studies. These studies along with studies/information from Synera NDA and label provided sufficient information to enable appropriate labeling.

Safety assessment of excipients, impurities/degradants, and leachables

For excipients, the Applicant did not conduct any specific studies for excipient safety evaluation. All excipients that are used in Kovanaze have been approved for use in other products for intranasal administration. Data in the Inactive Ingredient Database (IID) for FDA approved drugs covered the amount and treatment duration of all excipients in Kovanaze except that 1) which the maximal potency for benzyl alcohol in previous approved nasal products (0.05%) is lower than the concentration in Kovanaze (b) (4) %), 2) hydroxyethyl cellulose has not been used in previously approved drug products for intranasal use. However, citric acid (b) (4) %), benzyl alcohol ((b) (4) %), and hydroxyethyl cellulose (b) (4) %) were included in the vehicle formulation in the 2-week intranasal dog toxicity study. The high dose in the study provided 5.6-fold margin for the MRHD based on body surface area comparison. No significant adverse changes in the 2-week dog study were considered to be due to excipients. Overall, the safety of the excipients are considered to be appropriately assessed for this acute use indication.

The Applicant provided adequate safety justification for the proposed drug substance impurities and drug product degradation specifications. Bacterial reverse mutation assays were conducted for impurity (b) (4) which contain genotoxicity structural alert groups, and the results indicated that these impurities are negative for mutagenicity. An oxymetazoline-related

impurity, (b) (4) , also contains genotoxicity structure alert group. However, the Applicant controlled the daily exposure level of this impurity under (b) (4) mcg, which is acceptable due to the acute indication of Kovanaze according to ICH M7 guidance which allows a daily uptake level of 120 mcg/day for a mutagenic impurity in drug products with less than one month duration. The drug product specification for (b) (4) was qualified for local tissue safety via use of comparable concentrations in the clinical olfactory assessment studies. Should the Applicant wish to increase the specification to greater than (b) (4) %, an intranasal toxicology study will be required.

For leachables, 3 studies for extractables and leachables were included in this application. These included (1) a controlled extraction study, (2) a leachable-extractable correlation study in which older stability samples of drug product were examined for actual leachable compounds, and (3) a final study to quantitate leachable compounds in long-term storage samples from primary stability studies. The methodologies of these studies were reviewed Dr. Xiaobin Shen, the CMC reviewer for this NDA. The studies appeared to be acceptable from the CMC perspective. The analytical evaluation threshold (AET) of (b) (4) mcg/mL was calculated using the (b) (4) mcg Safety Concern Threshold (SCT) upon the Agency's requirement based on a delivered volume of 0.20 mL/spray, and an allowance of up to three sprays per day. The AET used in the studies was then set to 50% of this value as an added safety factor to obtain a final leachable AET of (b) (4) mcg/mL. Therefore, the AET actually represented a SCT of (b) (4) mcg. Four leachable compounds were identified in the leachable study using aged drug products. The aged drug product included two lots that were stored under refrigerated long-term storage conditions (2 - 8 °C) for nearly three years at the time of leachable testing, which is longer than the proposed (b) (4) -month shelf-life of the drug product. (b) (4) However, leachable studies using 46 registration stability samples that appropriately represented different time intervals over the (b) (4) month shelf-life did not identify any of the leachables identified using the aged batches. Therefore, a toxicological risk assessment is not needed.

Reference List

Altman R. S., Smith-Coggins R. and Ampel L. L. (1985) Local anesthetics. *Ann Emerg Med* **14,** 1209-1217.

Angel I., Niddam R. and Langer S. Z. (1990) Involvement of alpha-2 adrenergic receptor subtypes in hyperglycemia. *J Pharmacol Exp Ther* **254,** 877-882.

Armstrong J. M., Lefevre-Borg F., Scatton B. and Cavero I. (1982) Urethane inhibits cardiovascular responses mediated by the stimulation of alpha-2 adrenoceptors in the rat. *J Pharmacol Exp Ther* **223,** 524-535.

DeBernardis J. F., Winn M., Kerkman D. J., Kyncl J. J., Buckner S. and Horrom B. (1987) A new nasal decongestant, A-57219: a comparison with oxymetazoline. *J Pharm Pharmacol* **39,** 760-763.

Duzman E., Anderson J., Vita J. B., Lue J. C., Chen C. C. and Leopold I. H. (1983) Topically applied oxymetazoline. Ocular vasoconstrictive activity, pharmacokinetics, and metabolism. *Arch Ophthalmol.* **101,** 1122-1126.

Haenisch B., Walstab J., Herberhold S., Bootz F., Tschaikin M., Ramseger R. and Bonisch H. (2010) Alpha-adrenoceptor agonistic activity of oxymetazoline and xylometazoline. *Fundam Clin Pharmacol* **24,** 729-739.

Johns A., Chlumecky J., Cottle M. K. and Paton D. M. (1975) Effect of chemical sympathectomy and adrenergic agonists on the fertility of mice. *Contraception* **11,** 563-570.

Lee H. and Nagele R. G. (1985) Neural tube defects caused by local anesthetics in early chick embryos. *Teratology* **31,** 119-127.

Mahajan M. K., Uttamsingh V., Daniels J. S., Gan L. S., LeDuc B. W. and Williams D. A. (2011) In vitro metabolism of oxymetazoline: evidence for bioactivation to a reactive metabolite. *Drug Metab. Dispos.* **39,** 693-702.

Ruffolo R. R., Jr. and Waddell J. E. (1982) Receptor interactions of imidazolines: alpha-adrenoceptors of rat and rabbit aortae differentiated by relative potencies, affinities and efficacies of imidazoline agonists. *Br. J Pharmacol* **77,** 169-176.

Waskell L. (1978) A study of the mutagenicity of anesthetics and their metabolites. *Mutat. Res* **57,** 141-153.

Yoon J. R., Whipple R. A., Balzer E. M., Cho E. H., Matrone M. A., Peckham M. and Martin S. S. (2011) Local anesthetics inhibit kinesin motility and microtentacle protrusions in human epithelial and breast tumor cells. *Breast. Cancer Res Treat.* **129,** 691-701.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

ZENGJUN XU
06/16/2016

JAY H CHANG
06/16/2016

RICHARD D MELLON
06/17/2016
I concur.

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
Supervisory Secondary Review

Application number:	208032
Supporting document/s:	1, 33, and 34
Applicant's letter date:	05/29/2015, 5/24/2016, and 6/13/2016
CDER stamp date:	05/29/2015, 5/24/2016, and 6/13/2016
Product:	Kovanaze® (Tetracaine HCl & Oxymetazoline HCl)
Indication:	Regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J
Applicant:	St. Renatus, LLC
Review Division:	Division of Anesthesia, Analgesia, and Addiction Products (DAAAP)
Primary Reviewer:	Z. Alex Xu, PhD
Team Leader:	Jay H. Chang, PhD
Supervisor:	R. Daniel Mellon, PhD
Division Director:	Sharon Hertz, MD
Project Manager:	Mavis Darkwah, PharmD

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208032 are owned by St. Renatus, LLC or are data for which St. Renatus, LLC has obtained a written right of reference.
Any information or data necessary for approval of NDA 208032 that St. Renatus does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208032.

St. Renatus, LLC submitted NDA 208032 seeking approval of Kovanaze (tetracaine hydrochloride and lidocaine hydrochloride) nasal spray for induction of regional anesthesia for performing restorative procedures on Teeth 4-13 and A-J. Tetracaine is included in the drug product as the local anesthetic agent and oxymetazoline is included to serve as a vasoconstrictor [redacted] Dr. Alex Xu completed the primary pharmacology and toxicology review of NDA 208032. Dr. Jay Chang was the team leader for this application.

The NDA was submitted via the 505(b)(2) pathway with reliance upon an Agency previous finding of safety for intranasal oxymetazoline (21 CFR §341.20 Nasal decongestant active ingredients), published literature, a right-of-reference to the data in the Synera (lidocaine and tetracaine patch) NDA (21623), and by additional intranasal toxicity studies conducted in the dog model with the drug product formulation and reproductive and developmental toxicology studies for the combination of oxymetazoline and tetracaine.

As noted by Dr. Xu, the proposed drug substance and drug product specifications are acceptable from a nonclinical perspective. [redacted]. However, there are clinical data that includes assessment olfactory function and local tissue reaction for a drug product batch containing up to [redacted]% [redacted] These clinical data were reviewed by Dr. Amelia Luckett and found adequate to support a revised specification of NMT [redacted]%. The specification of NMT [redacted]% was deemed appropriate by our chemistry, manufacturing, and controls review team upon review of the stability data in order to take into consideration the distinct possibility of excursions in temperature on stability which could occur when the product is used based. As also noted by Dr. Xu, the container closure system has been adequately characterized for safety.

There are several excipients in this drug product formulation that have not been previously used in FDA-approved intranasal drug products, including citric acid [redacted]%), benzyl alcohol [redacted]%), and hydroxyethyl cellulose [redacted]%). However, the intranasal toxicology study in the dog was completed with a vehicle containing comparable or higher levels of these excipients; therefore, these studies and the clinical experience with the drug product to date, serve to characterize the local tissue effects of these excipients.

In terms of general toxicity, based on the previous clinical experience with the same dose and concentration (0.05%) of oxymetazoline as a nasal decongestant and tetracaine as a local anesthetic, the repeat-dose intranasal toxicology studies were not

required to support the proposed clinical studies for this acute-use indication. However, the Division required a repeat-dose intranasal toxicity study and an embryo-fetal development study with the combination to support the NDA submission. The Applicant also completed a fertility and early embryonic development, an embryo-fetal development, and a pre- and post-natal development studies in the rat model testing the combination. In addition, the Applicant has leveraged the data in the Synera NDA via right of reference to further support their application. Specifically, data from the Synera NDA included basic pharmacology; nonclinical absorption, distribution, metabolism, and elimination (ADME) data; genetic toxicity; and embryo-fetal development studies for tetracaine. These data further support the proposed drug product labeling, as appropriate.

The 14-day repeat-dose intranasal toxicology study in the dog model characterized the toxicological potential of oxymetazoline, oxymetazoline plus tetracaine in a vehicle containing citric acid (b) (4) %), benzyl alcohol (b) (4) %), and hydroxycellulose (b) (4) %) in water (concentrations similar to or higher than the final drug product formulation). Repeated-application of tetracaine and oxymetazoline resulted in clear treatment-related clinical signs (wobbly gait, salivation, shivering, vocalization, labored breathing, pupil dilation, impaired mobility, red swollen gums, nasal discharge, excessive barking, and excessive rolling/rubbing on the cage and face. These findings were observed immediately after dosing and resolved quickly. Microscopic changes were noted after 14-days of treatment, including reversible cell infiltration and squamous metaplasia in the nasal cavity. Although such findings would raise concerns for chronic administration, these changes are not expected to occur when used for the proposed acute indication.

The completed reproductive and developmental toxicology studies did suggest the potential for adverse effects via these drug substances. Specifically, in a fertility and early embryonic development study, oxymetazoline treatment of pregnant rats resulted in a decrease in the number of implantations (high dose), corpora lutea (high dose), and viable embryos (all doses). The effect appears to be largely driven by the oxymetazoline. A NOAEL was not identified in females. In males, oxymetazoline decreased sperm counts and concentration. A NOAEL of the low dose (0.01 mg/kg oxymetazoline and 7.5 mg/kg tetracaine) in males. The findings will be reflected in the drug product labeling.

An embryo-fetal development studies for the combination of oxymetazoline and tetracaine was completed in the rat model. Malformations were noted in the oxymetazoline alone control group (7.6 times the maximum recommended human dose based on AUC comparison), including short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs. Variations were also reported

following oxymetazoline treatment, including irregularly shaped arches, an increased incidence of bifid centra in thoracic vertebrae, and unossified forelimb phalanx. There were no clear malformations in the oxymetazoline plus tetracaine group. The effects were noted at doses that also demonstrated evidence of maternal toxicity, such as reduced maternal food consumption and maternal body weight. The maternal toxicity occurred with a similar magnitude in both the oxymetazoline alone and oxymetazoline plus tetracaine group. Therefore, it is not clear that the malformation can be attributed to maternal toxicity. These adverse effects will also be noted in the product labeling.

In a pre- and postnatal development study, there were reduced implantation sites and live litter sizes which were attributed to oxymetazoline (1.5 times the human dose). Fetal body weights were also reduced and there was an increase incidence of pup deaths during the lactation period which appear to be attributed primarily to oxymetazoline (6 times the maximum recommended human dose based on AUC comparison). These findings will also be noted in the product labeling.

The following table, prepared by Dr. Jay Chang, summarizes the exposure margins for oxymetazoline in the reproduction and development studies to appropriately inform the product labeling. These are not the same as reported in Dr. Xu's review, which this memo serves to correct.

Table 1: Exposure Margins for Oxymetazoline

Doses	**Segment I (SD 14)**		**Segment II (SD 11)**		**Segment III (SD 30)**	
Oxy/Tetra (mg/kg)	$AUC_{0\text{-}24h}$ (ng•h/mL)	**Exposure Margin***	$AUC_{0\text{-}24h}$ (ng•h/mL)	**Exposure Margin***	$AUC_{0\text{-}24h}$ (ng•h/mL)	**Exposure Margin***
Oxymetazoline						
0.1/0	27.5	**7.5x**	28.0	**7.6x**	22.2	**6.0x**
0.01/7.5	2.68	**0.7x**	2.26	**0.6x**	1.85	**0.5x**
0.03/7.5	7.37	**2.0x**	5.61	**1.5x**	5.50	**1.5x**
0.1/7.5	29.4	**8.0x**	19.4	**5.3x**	22.8	**6.2x**

*=Exposure margin based on oxymetazoline $AUC_{0\text{-}24h}$ levels from nonclinical reproductive and developmental toxicology studies relative to the mean oxymetazoline $AUC_{0\text{-}24h}$ (3.67 ng•h/mL) from patients given the maximum recommended human dose of Kovanaze (Clinical Study SR-2-06)

Recommendation: I agree with Drs. Xu and Chang that NDA 208032 may be approved from a nonclinical pharmacology and toxicology perspective. I also concur with the proposed revisions to the drug product labeling with a few corrections regarding the exposure margins and the **presence** of maternal toxicity (rather than the absence) in the embryo-fetal development study described in the labeling. Final labeling recommendations are listed below, reproduced, and edited from Dr. Xu's review:

Table 2: Revised Labeling Recommendations

Applicant's proposed label	Reviewer recommended changes	Rationale for changes
Highlights **Indications and Usage** Kovanaze is (b) (4) indicated for regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J.	**Highlights** **Indications and Usage** KOVANAZE contains tetracaine, an ester local anesthetic, and oxymetazoline, a vasoconstrictor. Kovanaze is indicated for regional anesthesia when performing a restorative procedure on teeth 4-13 and A-J.	This section must include an appropriate established pharmacologic class (EPC) for the drug substance(s) if available per 21 CFR 201.57. Note, an EPC for oxymetazoline has not yet been established. According to the guidance for industry and review staff: *Labeling for Human Prescription Drug and Biological Products — Determining Established Pharmacologic Class for Use in the Highlights of Prescribing Information*, the pharmacologic class of a drug can be defined on the basis of (1) the mechanism of action (MOA), (2) physiologic effect (PE), (3) chemical structure (CS). For oxymetazoline: • MOA = α1 and α2 adrenergic receptor agonist • PE = Vasoconstrictor • CS = Imidazoline The guidance notes that the EPC should be represented by a phrase that is scientifically and clinically meaningful. We agree with the Applicant's proposal to use vasoconstrictor as the EPC for oxymetazoline as this is the most clinically meaningful phrase in the context of its intended use in Kovanaze. Similarly, the EPC phrase vasoconstrictor is used for epinephrine in drug products when it is combined with local anesthetics with the intended effect of reducing local blood flow.

8.1 Pregnancy *Risk Summary* (b) (4) *Animal Data* (b) (4)	**8.1 Pregnancy** *Risk Summary* In animal reproduction and development studies, oxymetazoline given subcutaneously to rats during the period of organogenesis caused structural abnormalities at a dose approximately 7.6 times the level of oxymetazoline (0.3 mg) from the maximum recommended human dose (MRHD) of KOVANAZE. In a pre- and post-natal development study, oxymetazoline given subcutaneously to rats caused embryofetal toxicity manifested by reduced implantation sites and live litter sizes at doses approximately 1.5 times the MRHD level and greater. In addition, increased pup mortality was observed at oxymetazoline doses approximately (b) (4) 6 times the MRHD level. In animal reproduction studies, no adverse developmental effects were observed in pregnant rats and rabbits given subcutaneous doses of tetracaine equivalent to 5 times the MRHD of KOVANAZE during the period of organogenesis. [*see Data*] *Animal Data* In an embryofetal development study, pregnant rats were administered subcutaneous doses of oxymetazoline HCl at 0.1 mg/kg (7.6 times the amount of oxymetazoline from the maximum recommended human dose (MHRD) of KOVANAZE by AUC comparison), tetracaine HCl only at 7.5 mg/kg (32 times the amount of tetracaine from the MHRD as measured by PBBA [major tetracaine metabolite] AUC comparison), and oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg/day (0.6,	The draft label from the Applicant is already in PLLR format. This revision of the *Risk Summary* section should be completed by MHT and PT. (b) (4) (b) (4) Language regarding embryofetal development studies conducted with tetracaine alone is based on data from Synera NDA. Information of studies included in the Animal Data subsection per PLLR requirement • Types of studies • Animal species • doses or exposures in terms of human dose or exposure equivalents • Duration and timing Study findings • Maternal toxicity • Limitations of the data Proprietary name is used since the formulation of the drug product used in these studies was the same as the approved drug product. (b) (4)

(b) (4)	1.5, and (b) (4) 5.3 times, respectively, the MHRD level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl during the period of organogenesis (Gestational Days [GD] 7-17). Oxymetazoline HCl treatment at 0.1 mg/kg (7.6 times the MHRD by AUC comparison) caused structural abnormalities including external and skeletal malformations (e.g., short forelimb digits, fused arches in thoracic vertebrae, fused ribs, and irregular number of ribs), and variations (e.g., irregularly shaped arches and increased bifid centra in thoracic vertebrae, and un-ossified forelimb phalanx) in the (b) (4) presence of maternal toxicity, however, the findings cannot be clearly attributed to the maternal toxicity. Similar fetal abnormalities were not observed when pregnant rats were co-administered the same dose of oxymetazoline HCl in combination with 7.5 mg/kg tetracaine HCl. The no-observed-adverse-effect-level (NOAEL) for fetal abnormalities is 0.03 mg/kg oxymetazoline HCl in combination with 7.5 mg/kg tetracaine HCl. In other embryofetal development studies, tetracaine alone administered subcutaneously did not cause structural abnormalities in rats at doses up to 10 mg/kg/day (approximately 5.4 times the MRHD level by body surface area (BSA) comparison) or in rabbits at subcutaneous doses up to 5 mg/kg/day (approximately 5.4 times the MRHD level by BSA comparison). In a prenatal and postnatal development study, pregnant rats were given subcutaneous	(b) (4) This information is from the Synera label. Note the Applicant obtained a letter of authorization to cross-reference all nonclinical info, including the described embryofetal and development studies, contained in NDA 21623 for Synera. This language here is consistent with the Synera label. Since plasma exposure data for tetracaine were not available for these studies, the exposure margin calculations were based on body surface area comparison.
(b) (4)		
(b) (4)		

[redacted] (b) (4) There were no adverse effects on sexual maturation, neurobehavioral, or reproductive function in the offspring at any maternal dose.	doses of oxymetazoline HCl only at 0.1 mg/kg [redacted] (b) (4) 6 times the MHRD level by AUC comparison), tetracaine HCl only at 7.5 mg/kg (12 times MHRD level by PBBA AUC comparison), and oxymetazoline HCl at 0.01, 0.03, and 0.1 mg/kg [redacted] (b) (4) .5, 1.5, and 6 times, respectively, the MHRD level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl from GD 7 to Lactation Day [LD] 20 (corresponding to the beginning of organogenesis through parturition and subsequent pup weaning). Oxymetazoline HCl treatment decreased the mean number of implant sites/litter at ≥ 0.03 mg/kg (≥ 1.5 times the MRHD by AUC comparison) when administered with 7.5 mg/kg tetracaine (approximately 9%) and without tetracaine administration (5.5%), which resulted in an overall reduction in live litter sizes in these groups. At the end of the lactation period, fetal body weights were significantly decreased at 0.1 mg/kg oxymetazoline dose (6 times the MRHD by AUC comparison) when administered alone (19%) and co-administered with 7.5 mg/kg/day tetracaine (11%). In addition, increased pup mortality was observed at the 0.1/7.5 mg/kg oxymetazoline/tetracaine dose (8 out of 22) compared to the control (1 out of 22). Maternal toxicity (decreased body weight and mortality) was observed in animals given oxymetazoline HCl alone at 0.1 mg/kg and when co-administered with 7.5 mg/kg tetracaine HCl. There were no adverse effects on sexual maturation, neurobehavioral, or reproductive function in the	

	offspring at any maternal dose.	

8.2 Lactation ***Risk Summary*** (b) (4)	**8.2 Lactation** *Risk Summary* Detectable levels of oxymetazoline, tetracaine and its major metabolite, p-butylaminobenzoic acid (PBBA were found in the milk of lactating rats following subcutaneous administration of oxymetazoline HCl in combination with tetracaine HCl during the period of organogenesis through parturition and subsequent pup weaning [*see Data*]. *Data* In a pre- and post-natal development study, rats were given subcutaneous doses of oxymetazoline HCl at doses of 0.01, 0.03, and 0.1 mg/kg/day (b) (4) 0.5, 1.5, and (b) (4) 6 times, respectively, the maximum recommended human dose (MRHD) level by AUC comparison) in combination with 7.5 mg/kg tetracaine HCl (12 times the MRHD level by tetracaine metabolite AUC comparison) from Gestational Day [GD] 7 to Lactation Day [LD] 20. Concentrations of oxymetazoline, tetracaine, and PBBA were measured in the milk of lactating rats at approximately 2 hours postdose on LD 15. The concentrations of oxymetazoline were generally dose dependent (2.5, 7.0, and 33.8 ng/mL at 0.01, 0.03, and 0.1 mg/kg, respectively). The concentrations of tetracaine and PBBA were generally similar across all 7.5 mg/kg tetracaine dosing groups regardless of the presence of oxymetazoline (54.2 – 72.9 ng/mL for tetracaine, and 100.5 – 131.2 ng/mL for PBBA).	The data from the pre- and post-natal studies will be included in 8.1 and the data from the rat milk excretion study will be described in more detail in this section. A *Data* section must be included if milk secretion study was available per PLLR. For Kovanaze, milk secretion analysis was performed in the prenatal and postnatal development study

8.3 Females and Males of Reproductive Potential (b) (4)	**8.3 Females and Males of Reproductive Potential** Infertility *Females* Based on animal data, KOVANAZE may reduce fertility in females of reproductive potential. It is not known if the effects on fertility are reversible [*see Nonclinical Toxicology (13.1)*] *Males* Based on animal data, KOVANAZE may decrease sperm motility and sperm concentration [*see Nonclinical Toxicology (13.1)*].	(b) (4) Adverse findings in the fertility and early embryofetal study are included in this section, which is required per PLLR rule.

12 Clinical Pharmacology **12.1 Mechanism of Action** Tetracaine is a local anesthetic of the ester type and exerts its activity by blocking sodium ion channels required for the initiation and conduction of neuronal impulses. (b) (4)	**12 Clinical Pharmacology** **12.1 Mechanism of Action** Tetracaine is a local anesthetic of the ester type and exerts its activity by blocking sodium ion channels required for the initiation and conduction of neuronal impulses. Oxymetazoline is an imidazoline derivative with sympathomimetic activity. It is believed to be a mixed α1/α2-adrenoceptor agonist and by stimulating adrenergic receptors, it elicits vasoconstriction of dilated arterioles and reduces nasal blood flow.	(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility Long-term studies in animals have not been performed to evaluate the carcinogenic potential of tetracaine or oxymetazoline. Tetracaine base was negative in the in vitro Ames bacterial reverse mutation assay and the in vivo mouse micronucleus assay. In the in vitro chromosome aberration assay using Chinese hamster ovary cells, tetracaine base was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation. No studies have been conducted to evaluate the mutagenic potential of oxymetazoline.	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility** *Carcinogenesis* Long-term studies in animals have not been performed to evaluate the carcinogenic potential of tetracaine or oxymetazoline. *Mutagenesis* Tetracaine base was negative in the in vitro Ames bacterial reverse mutation assay and the in vivo mouse micronucleus assay. In the in vitro chromosome aberration assay using Chinese hamster ovary cells, tetracaine base was negative in the absence of metabolic activation, and equivocal in the presence of metabolic activation. No studies have been conducted to evaluate the mutagenic potential of oxymetazoline. *Impairment of Fertility* (b) (4) Male and female rats were given subcutaneous doses of oxymetazoline HCl alone at 0.1 mg/kg/day (7.5 times the MRHD level by AUC comparison), tetracaine HCl alone at 7.5 mg/kg/day (33 times the MRHD level by tetracaine metabolite AUC comparison), or the combination of oxymetazoline HCl at 0.01, 0.03, or 0.1 mg/kg/day (0.7, 2.0, and 8.0 times, respectively, the MRHD level by AUC comparison) with 7.5 mg/kg/day tetracaine HCl prior to and during mating. (b) (4) Adverse effects related to oxymetazoline HCl were observed for male sperm parameters (reduced percentage of motile sperm, sperm counts, and sperm density). Sperm effects occurred at oxymetazoline	Subheadings were added to assist the reader. The proposed text was modified to reflect standard CFR language and to remove explanatory statements that could be deemed promotional rather than simply stating the findings. The Applicant provided a LOA to the Synera NDA, which contained tetracaine mutagenesis data. The proposed language by the Applicant is consistent with the language in the Synera label. It is acceptable that there is no oxymetazoline mutagenesis information in the label based on the Agency's previous findings of safety for oxymetazoline per the finalized OTC monograph. This section is revised to include the exposure margin based on plasma exposure comparison.

(b) (4)	doses equivalent to 2 times the MRHD and higher (by AUC comparison), given alone or in combination with tetracaine HCl. In female rats, a reduction in the number of viable embryos was observed at oxymetazoline doses equivalent to 0.7 times the MRHD and higher (by AUC comparison), given alone or in combination with tetracaine HCl. Reduced numbers of corpora lutea and implantation sites occurred at an oxymetazoline dose equivalent to 7.5 times the MRHD (by AUC comparison) given alone or in combination with tetracaine HCl. These effects were attributed to oxymetazoline HCl because similar effects were not observed in rats given tetracaine HCl alone. A no-effect level for fertility in female rats was not established in this study.	
(b) (4)		
(b) (4) These effects were attributed to oxymetazoline HCl because similar effects were not observed in rats given tetracaine HCl alone. A no-effect level for fertility in female rats was not established in this study.		

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD D MELLON
06/17/2016