CENTER FOR DRUG EVALUATION AND RESEARCH

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Indication:	Induction and maintenance of procedural sedation in adults
Applicant:	Cosmo Technologies, Ltd (COSMO) Dublin, Ireland
Review Division:	Division of Pharmacology/Toxicology for Neuroscience supporting the Division of Anesthesiology, Addiction Medicine, and Pain Medicine
Primary Reviewer:	Katie Sokolowski, PhD, DABT
Supervisor/Team Leader:	R. Daniel Mellon, PhD (Tertiary Reviewer) / Newton Woo, PhD (Secondary Reviewer)
Division Director:	Rigoberto Roca, MD
Project Manager:	Taiye Adedeji

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1 Executive Summary

1.1 Introduction

COSMO is developing a new benzodiazepine, BYFAVO® (Remimazolam for Injection), for induction and maintenance of procedural sedation. Remimazolam is a short acting $GABA_A$ receptor agonist. The NDA was submitted as a 505(b)(1) application. However, because literature was necessary to support the safety of Dextran 40, a new excipient in the drug product formulation, and to support the adequacy of the male fertility assessment for this action, the application was converted to a 505(b)(2) application that relies in part on published literature.

1.2 Brief Discussion of Nonclinical Findings

The Applicant submitted genotoxicity studies, safety pharmacology studies, general toxicology studies in minipigs and monkeys, and developmental and reproductive toxicology studies in rat and rabbit to qualify the safety of remimazolam. Additional studies were submitted to qualify impurities and the novel excipient, Dextran 40. Extractable and leachables studies were submitted to justify the safety of the container closure system.

Genotoxicity
The potential for genetic toxicity has been adequately tested. Genetic toxicology battery for the API was negative in the Ames assay, mouse lymphoma assay, and in vivo rat micronucleus test.

Safety Pharmacology
Safety pharmacology studies were adequately conducted with the API in rats, rabbits, micropigs, and monkeys. Remimazolam produced typical benzodiazepine-related pharmacodynamic effects including sedation, respiratory depression, and cardiovascular depression.

General Toxicology
Adequate general toxicity studies were completed. In general, findings in the pivotal repeat-dose toxicity studies with the API in monkey and minipig were limited to expected pharmacology and local toxicity. There were no systemic toxicities identified in the repeat-dose toxicity studies that were not already identified in the safety pharmacology study up to 120 mg/kg/day in monkey and minipigs (safety margin >100x based on AUC at MRHD of 30 mg/kg/day). Local toxicities included thickened intima and partially recoverable endothelial fibrosis of blood vessels at 2.5 mg/mL in monkey. The clinical concentration after reconstitution is 2.5 mg/mL.

Developmental and Reproductive Toxicology
A full battery of developmental and reproductive toxicology (DART) studies for remimazolam in the rat and rabbit model were submitted in the NDA. The Applicant also conducted supplemental studies to characterize the effects of remimazolam on male reproductive tissues in minipigs. However, it should be noted that Agency

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expressed concerns with the adequacy of the planned studies during several meetings with the Applicant during development.

Female fertility was evaluated in both the rat and the rabbit. There were no adverse effects on fertility up to 20 mg/kg/day in rabbit (safety margin ~8x and ~13x MRHD[1] of 30 mg/day based on C_{max} and AUC, respectively) and rats (safety margin ~0.02x and ~0.01x MRHD of 30 mg/day based on C_{max} and AUC, respectively). Although the exposures in the rat study are well below the human exposures and therefore the study is considered inadequate, the rabbit female fertility study is considered adequate and no further studies are necessary.

Male fertility was evaluated in rats and minipigs. There were no adverse effects on male fertility in rats up to 30 mg/kg/day (safety margins were ~0.02x and ~0.01x MRHD of 30 mg/day based on C_{max} and AUC[2], respectively). When considering systemic exposures of the free compound exposure margins in the rat male fertility study are ~0.06x and ~0.03x MRHD of 30 mg/day based on C_{max} and AUC, respectively. Due to lack of exposure to the parent compound, the male fertility assessment in rats is not adequate. In sexually mature Gottingen minipigs, a comprehensive histopathological examination of the reproductive organs was conducted as part of a 28-day repeat-dose toxicity study. In this study, detailed histology of the testes and epididymis, sperm count, viability/motility, and morphology were analyzed. No adverse effects on sperm assessments and male reproductive tissue histopathology were observed in minipigs up to 120 mg/kg (safety margin >3000x and >400x MRHD of 30 mg/day based on C_{max} and AUC, respectively). These data support the conclusion that the potential for adverse effects of remimazolam on male fertility endpoints is low; however, reproductive performance has not been adequately assessed and a PMR for an adequate male fertility study is recommended. For an adequate study, the Applicant can either test a species other than rat or potentially modify the rat study design to ensure coverage for the proposed clinical exposures. The low exposure margins in rats are due to the rapid metabolism of remimazolam to its inactive metabolite by unidentified isoforms of carboxylesterases in the blood. The discrepancies in the safety margins reported in the 28-day rat study and the embryo-fetal toxicology rat study may have been due to a number of different reasons (e.g., an artifact of post-sampling metabolism or slight differences in study design, such as bolus duration, animal strain, etc). Further studies identifying the responsible enzyme in rat blood and PK studies utilizing an inhibitor post-blood sampling or using longer infusion times may result in more relevant exposure margins. Otherwise, mice, hamsters, or nonhuman primates should be further investigated as an alternate appropriate species to provide adequate exposure margins for reproductive and developmental toxicity testing with the clinical formulations.

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[1] As of this action, the MRHD will be 30 mg/day. However, as adequate human exposure data for 30 mg/day is not available, the Division is using the data from 33 mg/day for calculations of exposure margins in labeling. This is therefore an approximation (~) for the exposure data at 30 mg/day.
[2] Based on systemic exposure levels in male rats reported in the 28-day repeat-dose toxicity study. Systemic exposures were not evaluated in the male fertility study.

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Embryo-fetal development (EFD) was evaluated in an extended fertility study in rabbits and EFD studies in rats and rabbits. Rabbits appeared to have paradoxical pharmacological effects to remimazolam characterized by bucking and convulsions when touched leading to spinal damage and mortality. Regardless of these adverse effects to the pregnant female, in surviving animals, remimazolam had no adverse effects on embryo-fetal development up to 30 mg/kg/day in rat (exposure margin ~4.82x and ~0.32x MRHD of 30 mg/day based on C_{max} and AUC, respectively; and when considering systemic exposures of the free compound safety margins are ~17x and ~1.2x MRHD of 30 mg/day based on C_{max} and AUC, respectively at the beginning of the dosing interval) and 20 mg/kg/day in rabbits (safety margin 5x and 15x MRHD of 33 mg/day based on C_{max} and AUC, respectively) at the beginning of the dosing interval. By the end of dosing, exposure margins in the rat based on AUC were below 1. Therefore, the rat EFD study does not fully characterize the effects of remimazolam on EFD. The study should be repeated testing more prolonged infusions in the rat or in a different species. The rabbit EFD study is considered adequate.

Pre- and post-natal development (PPND) was evaluated in an extended fertility study in rabbits and a standard PPND study in rats. The rat PPND study included all recommended endpoints. However, the extended fertility study in rabbits did not evaluate learning and memory or reproductive function of the F1 kits and therefore, cannot be considered adequate to address all standard endpoints. Remimazolam had no adverse effects on pre- and post-natal development up to 30 mg/kg/day in rats (safety margin ~0.03-4.82x and ~0.11-0.32x MRHD of 30 mg/day based on C_{max} and AUC, respectively; and when considering systemic exposures of the free unbound parent safety margins are 0.09-17x and 0.3-1.2x MRHD of 30 mg/day based on C_{max} and AUC, respectively) and 20 mg/kg/day in rabbits (safety margin ~16x and ~19x MRHD of 30 mg/day based on C_{max} and AUC, respectively). The rat study did not test exposures comparable to the maximum recommended human dose (MRHD) of 30 mg/day over the full treatment period and the rabbit study did not evaluate all standard endpoints. Therefore, the rat PPND study does not fully characterize the effect of remimazolam on PPND and the study should be repeated testing more prolonged infusions in the rat or in a different species.

As noted above, the developmental and reproductive toxicology (DART) studies for remimazolam do not completely characterize the effects of the drug on these endpoints. Of specific concern, data from the rat studies did not provide acceptable safety margins as the DART studies were conducted with short boluses that resulted in systemic exposures below that in humans. However, the newly released ICH S5(R3) guidance states that the “intended patient population or therapeutic indication can influence the extent of the DART testing” and provides the example of patient populations in “hospitalized settings where pregnancy can be excluded.” As per the clinical team, women of childbearing potential undergoing planned procedural sedation are usually tested for pregnancy prior to administration of the sedative drug product thereby excluding pregnant women. As such the less than adequate battery of DART studies will not preclude approval of remimazolam. However, considering the clinical utility of

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this drug in pregnant females and given the possibility that a pregnant woman may be treated with the drug, PMRs are recommended to better characterize the effects of remimazolam on reproduction and development endpoints and to better inform labeling given the potential for inadvertent use in pregnant women, longer duration of clinical use than indicated in the labeling, and potential utility of this short acting drug in pregnant women. This will include the evaluation of remimazolam in a relevant species for 1) male fertility, 2) embryo-fetal development in a second relevant species (other than the rabbit), and 3) learning, memory, and reproductive function in the F1 offspring in a pre- and post-natal development study. The purpose of these PMRs is to support the safety of short procedural sedation indication in pregnant women and inform the label. In addition, a PMR for juvenile animal studies will be issued to further characterize the potential impact of the drug on the developing brain.

Impurities
Impurities that were above ICH qualification thresholds were evaluated in a genetic toxicology battery and a 4-week repeat-dose toxicology study in monkeys using a batch of remimazolam (YMK110831) spiked with 8 impurities at appropriate concentrations. Impurities were adequately evaluated in the submitted studies.

Excipients
Dextran 40 is listed in the Inactive Ingredient Database (IID) as being present in an FDA approved intravenous drug product (lyophilized powder for solution) with no maximum potency noted. Dextran of unknown MW is also listed in the IID as being present in an FDA-approved ophthalmic drop product. Because the dose of Dextran 40 in this drug product exceeds that of other FDA-approved IV drug products, Dextran 40 is considered a novel excipient that requires qualification. Per the excipient guidance, this generally includes repeat-dose general toxicology study in two species, standard genotoxicity battery, a fertility study, embryo-fetal development studies in two species, and a pre- and post-natal development study. The Applicant addressed the above requirements via conduct of nonclinical studies and submission of a literature based justification, which are discussed below.

Genetic toxicity of Dextran 40 was demonstrated to be negative in a published Ames assay, published mouse lymphoma TK assay, and in an in vivo mouse micronucleus assay that was conducted by the Applicant. Based on these data, Dextran 40 is considered adequately tested and nongenotoxic.

General toxicity of Dextran 40 was adequately evaluated after administration of remimazolam formulated with Dextran 40 in 1-month studies in rat and minipig. In the 1-month repeat-dose studies, rats had a NOAEL = 600 mg of Dextran 40/kg/day at 4 h daily infusions (43x safety margin to the MRHD of 33 mg API/day based on BSA) and minipig had a NOAEL = 360 mg Dextran 40/kg/day after 6 h daily infusions (>100x safety margin to the MRHD of 33 mg API/day based on BSA). **Note**: All approved products containing Dextran 40 have warnings for anaphylaxis in the package insert, one product includes a Boxed Warning for anaphylaxis.

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Female fertility was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits. In the female fertility study in rabbits, the NOAEL was 320 mg Dextran 40/kg/day (46x safety margin to the MRHD of 33 mg API/day based on BSA). The effects of Dextran 40 on female fertility has been adequately assessed.

Male fertility was assessed in a standard rat fertility study; however, exposures obtained were below that of the clinical exposures. In addition, male fertility endpoints were assessed in a 4-week minipig repeat-dose toxicity study evaluating remimazolam formulated with Dextran 40 that included sperm count, viability, and morphology in addition to histology of the testis and epididymis were evaluated after administration of remimazolam formulated with Dextran 40. The potential effects of Dextran 40 on male fertility and mating behaviors were not adequately evaluated in the provided studies. However, in addition to the submitted studies, the Applicant provided a literature-based safety justification emphasizing the bio-distributive properties of the glucose polysaccharide dextran, which is a hydrophilic polymer that will not traverse cell membranes. Therefore, minimal influence on any organ function is expected from exposure to macromolecular dextrans on male reproductive endpoints. Degradation products of dextrans are not expected to produce adverse effects on male reproductive tissues. These data support the conclusion that the risk to male fertility is low for this acute-use product and the limitations do not preclude approval.

Embryo-fetal development was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits. In this EFD study in rabbits, the fetal NOAEL was 320 mg/kg/day Dextran 40 (46x safety margin to the MRHD of 33 mg API/day based on BSA). The rabbit EFD assessment is considered adequate. In lieu of studies in a second species (rat), the Applicant provided a justification based on experimental evidence to demonstrate that Dextran 40 exposure to the embryo or fetus is negligible to support the safety of dextran. This justification included data suggesting that dextran does not cross the placental barrier, does not affect uterine blood flow in a manner that could negatively affect the embryo, is predominantly excreted unchanged, and any metabolites are well characterized low molecular weight carbohydrates. The combined animal data and literature-based safety justification suggest limited concern that Dextran 40 would negatively impact embryo-fetal development.

The effects of Dextran 40 on pre- and post-natal development was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits. The NOAEL in this study was 320 mg Dextran 40/kg/day (46x safety margin to the MRHD of 30 mg API/day based on BSA). However, this study did not include evaluation of the F1 kits for learning/memory, mating behavior, and reproductive indices, or any evaluation of F2 kits. To support the safety of Dextran 40 on pre-and post-natal development, the Applicant provided a literature-based safety justification that argues low exposure to the fetus and nursing infant. The combined animal data and literature-based safety justification suggest limited concern that Dextran 40 would negatively impact pre- and post-natal development.

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Although the existing reproductive and developmental toxicity studies with Dextran 40 are inadequate to fully qualify this new excipient, because the proposed indication is for procedural sedation and patients are tested for pregnancy prior to planned procedures, in accordance with ICH S5A(R3), the lack of adequate reproductive and developmental studies for Dextran 40 will not be considered an approval issue.

Although the existing data suggest limited concern, the lack of effects of Dextran 40 on reproduction and development will be confirmed by PMR studies testing the drug product formulation containing Dextran 40 that test exposures comparable to human exposures.

Container Closure System (CCS)
Remimazolam is formulated as a lyophilized powder in a glass vial and rubber stopper. Although lyophilized powders have been considered a relatively low risk for extracting compounds from container closure systems, new data from published extractable/leachable studies on CCS for lyophilized powders has demonstrated the potential for leachables, which may present a toxicological concern. In the Applicant's leachable studies, one batch was tested after cold, ambient, and accelerated storage for 6 months as a lyophilized powder. In a second leachable study, one batch stored ambient and accelerated conditions for 6 months was tested immediately after reconstitution. Under the conditions of the leachable studies, no compounds of concern were detected and no toxicological risk assessment were required, except for the one potential leachable that was not targeted: [redacted] (b) (4) mcg/day). In a risk assessment, the levels of [redacted] (b) (4) were determined to be acceptable. There is concern that the leachable studies did not fully characterize the leachable profile using three batches of drug product at multiple timepoints across the 36-month shelf-life; therefore, additional leachable studies may be required. The reader is referred to the CMC review for their assessment of the adequacy of the extractable leachable studies.

The package insert should include the 2016/2017 Anesthetic and Sedation Drugs Safety Labeling Change language for NMDA/GABAergic anesthetic agents and sedatives, comprehensive descriptions of the developmental and reproductive toxicity data with existing limitations, and warnings of anaphylaxis associated with Dextran 40.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical pharmacology/toxicology perspective, although there are technically inadequate reproductive and developmental toxicity studies for remimazolam and Dextran 40, because the indication is for planned procedural sedation where women of childbearing potential will be adequately screened for pregnancy prior to the procedure, the NDA may be approved at the discretion of the clinical team and with the recommended labeling. We also recommend the following postmarketing requirements (see below).

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1.3.2 Additional Nonclinical Recommendations

Postmarketing requirements (PMR)

1. Conduct a male fertility study testing the drug product formulation that evaluates reproductive behavior and fertility and obtains pharmacokinetic analysis in a species that provides adequate exposure to support the proposed clinical exposures for the proposed maximum duration of use based on the clinical indication.

2. Conduct an embryo-fetal development study testing the drug product formulation that results in adequate exposure to support the clinical indication in a species other than the rabbit that provides adequate exposures for the proposed clinical exposures for the proposed maximum duration of use based on the clinical indication.

3. Conduct a pre-and post-natal development study testing the drug product formulation that evaluates all standard endpoints including learning, memory, and reproductive function of the F1 offspring and obtains adequate toxicokinetic data in a species and provides adequate exposures to support the proposed clinical exposures for the proposed maximum duration of use based on the clinical indication.

4. Conduct a juvenile animal toxicology study in a rodent model to characterize the effects of remimazolam on the developing central nervous system to support clinical studies in pediatric patients under 3 years of age.

5. Conduct a juvenile animal toxicology study in a nonrodent model to characterize the effects of remimazolam on the developing central nervous system to support clinical studies in pediatric patients under 3 years of age.

6. Conduct a juvenile animal toxicology study in a rodent model to characterize the effects of remimazolam on the developing central nervous system to support a clinical indication for use in pediatric patients greater than or equal to three years of age and below 18 years age.

1.3.3 Labeling

Applicant's draft label section were taken from Remimazolam Prescribing Information Draft Label- Clean from Module 1.14. The following changes to the Applicant's draft labeling are recommended in the table below (deletions are shown in ~~strikethrough~~ and additions are shown in green). These labeling recommendations are preliminary and may be impacted by the required study outcomes. Refer to the action letter for final drug product labeling. The reader is advised that as of this action, the MRHD is 30 mg/day. However, because adequate human exposure data for 30 mg/day is not

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available, the Division is using the clinical data from 33 mg/day for calculations of exposure margins in labeling. This is therefore an approximation for the exposure data at 30 mg/day as noted in the labeling recommendations below.

Applicant's Draft Labeling	Reviewer's Recommended Edits	Reviewer's Comments
5 WARNINGS AND PRECAUTIONS	**5 WARNINGS AND PRECAUTIONS**	
	5.3 Pediatric Neurotoxicity	
	Published animal studies demonstrate that the administration of anesthetic and sedation drugs that block NMDA receptors and/or potentiate GABA activity increase neuronal apoptosis in the developing brain and result in long-term cognitive deficits when used for longer than 3 hours. The clinical significance of these findings is not clear. However, based on the available data, the window of vulnerability to these changes is believed to correlate with exposures in the third trimester of gestation through the first several months of life, but may extend out to approximately three years of age in humans. *[See Pregnancy]* Some published studies in children suggest that similar deficits may occur after repeated or prolonged exposures to anesthetic agents early in life and may result in adverse cognitive or behavioral effects. These studies have substantial limitations, and it is not clear if the observed effects are due to the anesthetic/sedation drug administration or other factors such as the surgery or underlying illness. Anesthetic and sedation drugs are a necessary part of the care of children needing surgery, other procedures, or tests that cannot be delayed, and no specific medications have been shown to be safer than any other. Decisions regarding the timing of any elective	A Safety Labeling Change issued on December 14, 2016 (and updated on April 27, 2017), requires general anesthetic and sedation drug products that block NMDA receptors and/or potentiate GABA activity to include standard language regarding the findings of developmental neurotoxicity associated with these drug classes. This language was added to the drug label because the drug is a benzodiazepine sedative that potentiates GABA receptors.

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	procedures requiring anesthesia should take into consideration the benefits of the procedure weighed against the potential risks.	
8 USE IN SPECIFIC POPULATIONS	**8 USE IN SPECIFIC POPULATIONS**	
8.1 Pregnancy	**8.1 Pregnancy**	
Risk Summary (b) (4)	Risk Summary	We defer to the clinical and maternal health teams regarding the adequacy of any "class labeling" for benzodiazepine use in pregnancy.
	In animal studies reduced fetal weights but no evidence of malformations or embryofetal lethality were noted in a study in which pregnant rabbits were treated intravenously with 4 times the MRHD of 30 mg during organogenesis. Adequate rodent reproductive and developmental toxicology studies have not been completed to fully evaluate the effects of remimazolam.	The risk summary statement proposed by the review team reflects the limitations of the existing nonclinical data to fully inform the risk to women of childbearing potential. Exposure margins are based on free plasma levels of remimazolam due to species differences in protein binding. Class labeling from the Anesthetic SLC should be included in the risk summary (see below); however, we note that the currently proposed duration of use will be lower than the warnings in the anesthetic SLC.
		Reviewer defers to DPMH and Clinical for appropriate language regarding Risk Summary of human data.

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(b) (4)		
	Published studies in pregnant primates demonstrate that the administration of anesthetic and sedation drugs that block NMDA receptors and/or potentiate GABA activity during the period of peak brain development increases neuronal apoptosis in the developing brain of the offspring when used for longer than 3 hours. There are no data on pregnancy exposures in primates corresponding to periods prior to the third trimester in humans.	SLC language for Anesthetic/Sedative drugs including GABAergic drugs
	The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.	Standard language for PLLR labeling should be included.
Data *Animal Data*	Data *Animal Data*	
(b) (4)		The language proposed by the Applicant in the initial submission is not in the typical format used by the Agency and lacks adequate detail to fulfill PLLR requirements. See revised text below.

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	Reduced fetal weights but no evidence of malformation or embryofetal lethality were noted in a study in which pregnant rabbits were treated intravenously with 5 mg/kg remimazolam (approximately 4 times the MRHD of 30 mg/day based on AUC) from Gestation Day 6 to 20 in the presence of maternal toxicity (reduced food intake and body weights).	Rabbits (EFD study (b) (4) 23507) were administered IV bolus doses of remimazolam (formulated with water and lactose monohydrate) at target doses of 0, 1.25, 2.5, and 5 mg/kg/day from GD6-20, laparotomies on GD29. Dams: pharmacological effect at all doses; stat. sig. decrease in bw gains and food consumption in all doses. Fetal NOAEL = 2.5 mg/kg/day, based on decreased fetal and placental weights at 5 mg/kg/day. *Rabbit conversion factor: 3.1* *SM_{free}: 4.0 based on GD6 AUC exposure levels* *SM_{free}: 5.2 based on GD20 AUC exposure levels*
	In a study that did not test exposures comparable to the maximum recommended human dose (MRHD) of 30 mg/day over the full period of organogenesis, there was an increase in early resorptions (embryolethality) but no evidence of malformations when female rats were treated from Gestation Day 6 through 17 with up to 30 mg/kg remimazolam via intravenous bolus (approximately 0.3 times the MRHD based on AUC by the end of the dosing interval) in the presence of maternal toxicity (convulsion in one mid dose and one high dose dam).	Rats (EFD study (b) (4) 23509) were administered IV bolus of remimazolam (formulated with water and lactose monohydrate) at target doses of 0, 3, 10, and 30 mg/kg/day from GD6-17, laparotomies on GD20. Dams: Pharmacological effect at all doses; convulsions occurred at MD and HD. Fetal NOAEL = 30 mg/kg/day based on slight but stat. sig. increased incidence of early and total resorptions observed at this dose level were within range of historical background. *Rat conversion factor: 6.2* *SM_{total}: 0.32 based on GD6 AUC exposure levels.* *$SM_{free\ fraction}$: 0.96 based on GD6 AUC exposure levels.* *SM_{total}: 0.073 based on GD17 AUC exposure levels.* *$SM_{free\ fraction}$: 0.27 based on GD17 AUC exposure levels.*
		Rabbits (extended fertility study 497736) were administered daily 1.2, 2.4, or 4h IV infusions of

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		remimazolam (CNS 7056B Lot [redacted]249, formulated with [redacted] (b) (4) Dextran 40 and [redacted] (b) (4) lactose monohydrate in saline) at target doses of 0, 12.5, 20, and 30 mg/kg/day from 14 days before mating until lactation day 30. The HD group was terminated due to high mortality rates. Does: pharmacological effect, convulsions and mortality at all doses. Fetal NOAEL = 20 mg/kg/day based on the lack of effect at this dose and termination of the next highest dose before does could litter. *Rabbit conversion factor: 3.1* *SM_{total}: 14.81 based on AUC during gestation*
[redacted] (b) (4)	In a pre- and post-natal development study that did not test exposures comparable to the maximum recommended human dose (MRHD) of 30 mg/day over the full treatment period, there were no adverse effects on survival or development of offspring when pregnant rats were treated with up to 30 mg/kg remimazolam (<0.3 times the MRHD by the end of the gestational period) by intravenous bolus injection from Gestation Day 6 through Lactation Day 20 with minimal evidence of maternal toxicity (sedation).	Rats (PPND study NO12230) were administered IV bolus doses of remimazolam (ONO-2745BS Lot SOL12621/6, formulated with [redacted] (b) (4) Dextran 40 and [redacted] (b) (4) lactose monohydrate in saline) at target doses of 0, 3, 10, 30 mg/kg/day from GD6-LD20. F0 Dams: Pharmacological effects at all doses. F1 offspring were weaned and mated, and C-sectioned on GD13. F2 offspring were not evaluated past GD13 for birth weights, sex ratio, or external malformations, but fertility of F1 offspring could be determined with evaluated parameters. F1 development/reproductivity NOAEL = 30 mg/kg/day based on no test article-related effect on growth, physical dev., sensory and reflex functions, FOB/open field, learning (Conditioned avoidance response/shuttle box), genital dev., mating ability, fertility, implantation or viability of F2 embryos. *Rat conversion factor: 6.2*

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		SM_{total}: 0.32 based on GD6 AUC exposure levels; 0.11 based on GD17 AUC exposure levels *$SM_{free\ fraction}$: 0.96 based on GD6 AUC exposure levels; 0.33 based on GD17 AUC exposure levels*
	No evidence of adverse effects on physical development, a functional observational battery of behavioral assessments, or fertility were noted in pups born to pregnant rabbits that were treated by intravenous infusion of up to 20 mg/kg/day remimazolam (approximately 19 times the MRHD based on AUC) from 14 days prior to mating until Lactation Day 30 despite the presence of maternal toxicity (sedation, convulsions, and mortality). Learning and memory of the first-generation offspring was not evaluated in this study.	Rabbits (extended fertility study 497736) were administered daily 1.2, 2.4, or 4h IV infusions of remimazolam (CNS 7056B Lot 10201249, formulated with (b) (4) Dextran 40 and (b) (4) lactose monohydrate in saline) at target doses of 0, 12.5, 20, and 30 mg/kg/day from 14 days before mating until lactation day 30. The HD group was terminated due to high mortality rates. Does: Convulsions and mortality at all doses. F1 development NOAEL = 20 mg/kg/day based on the lack of effect on kit body weight and open filed evaluations, reproductive indices at this dose and termination of the next highest dose before does could litter. *Rabbit conversion factor: 3.1* *SM_{total}: 14.8 based on AUC during gestation; 19.3 based on AUC during lactation* *SM_{free}: 18.8 based on AUC during gestation; 24.5 based on AUC during lactation*
	In a published study in primates, administration of an anesthetic dose of ketamine for 24 hours on Gestation Day 122 increased neuronal apoptosis in the developing brain of the fetus. In other published studies, administration of either isoflurane or propofol for 5 hours on Gestation Day 120 resulted in increased neuronal and oligodendrocyte apoptosis in the developing brain of the offspring. With respect to brain development, this time period corresponds to the third	SLC language for anesthetic/sedative drugs including GABAergic drugs

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	trimester of gestation in the human. The clinical significance of these findings is not clear; however, studies in juvenile animals suggest neuroapoptosis correlates with long-term cognitive deficits. *[See WARNINGS/Pediatric Neurotoxicity, Pediatric Use, and ANIMAL TOXICOLOGY AND PHARMACOLOGY].*	
8.2 Lactation	**8.2 Lactation**	
Risk Summary	Risk Summary (b) (4)	We defer to the clinical and maternal health teams regarding human lactation labeling.

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(b) (4)		
Data (b) (4) Remimazolam was not quantifiable in plasma samples obtained from rabbit kits taken in the morning on Day 10 or 11 of lactation. However, metabolite CNS7054 was present at low levels in 2 of the 5 kits sampled.	Data In rabbits administered daily intravenous infusions of remimazolam at 12.5, 20, and 30 mg/kg/day from 14 days before mating until Lactation Day 30, remimazolam and the metabolite CNS7054 were present in milk samples obtained after the end of an infusion on Day 10 or 11 of lactation. Remimazolam was not quantifiable in plasma samples obtained from rabbit kits taken in the morning on Day 10 or 11 of lactation. However, metabolite CNS7054 was present at low levels in 2 of the 5 kits sampled.	Rabbits (extended fertility study 497736) were administered daily 1.2, 2.4, or 4h IV infusions of remimazolam (CNS 7056B Lot 10201249, formulated with (b) (4) Dextran 40 and (b) (4) lactose monohydrate in saline) at target doses of 0, 12.5, 20, and 30 mg/kg/day from 14 days before mating until lactation day 30. Maternal milk Remimazolam: 475-3361 ng/mL CNS7054: 301-396 ng/mL Kit plasma Remimazolam: Not quantifiable CNS7054: 101-162 ng/mL
(b) (4)		

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[redacted] (b) (4)		
8.4 Pediatric Use	**8.4 Pediatric Use**	
Safety and effectiveness in pediatric patients have not been established. No studies are available in any pediatric population and extrapolation of adult effectiveness data to the pediatric population is not possible.	Safety and effectiveness in pediatric patients have not been established. No studies are available in any pediatric population and extrapolation of adult effectiveness data to the pediatric population is not possible.	
	Published juvenile animal studies demonstrate that the administration of anesthetic and sedation drugs, such as TRADENAME, that either block NMDA receptors or potentiate the activity of GABA during the period of rapid brain growth or synaptogenesis, results in widespread neuronal and oligodendrocyte cell loss in the developing brain and alterations in synaptic morphology and neurogenesis. Based on	SLC language for anesthetic/sedative drugs including GABAergic drugs

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	comparisons across species, the window of vulnerability to these changes is believed to correlate with exposures in the third trimester of gestation through the first several months of life, but may extend out to approximately 3 years of age in humans. In primates, exposure to 3 hours of ketamine that produced a light surgical plane of anesthesia did not increase neuronal cell loss, however, treatment regimens of 5 hours or longer of isoflurane increased neuronal cell loss. Data from isoflurane-treated rodents and ketamine-treated primates suggest that the neuronal and oligodendrocyte cell losses are associated with prolonged cognitive deficits in learning and memory. The clinical significance of these nonclinical findings is not known, and healthcare providers should balance the benefits of appropriate anesthesia in pregnant women, neonates, and young children who require procedures with the potential risks suggested by the nonclinical data. *[See WARNINGS/Pediatric Neurotoxicity, Pregnancy].*	
10 OVERDOSAGE	**10 OVERDOSAGE**	
Clinical Presentation Overdose may lead to CNS depression, associated with drowsiness, confusion and lethargy, possibly progressing to ataxia, respiratory depression and hypotension. Management of Overdosage Flumazenil, a specific benzodiazepine-receptor antagonist, is indicated for the (b) (4) reversal of the sedative effects of benzodiazepines and may be used in situations when an overdose with TRADENAME is	Clinical Presentation Overdose may lead to CNS depression, associated with drowsiness, confusion and lethargy, possibly progressing to ataxia, respiratory depression and hypotension. Management of Overdosage Flumazenil, a specific benzodiazepine-receptor antagonist, is indicated for the (b) (4) reversal of the sedative effects of benzodiazepines and may be used in situations when an overdose with TRADENAME is	

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known or suspected. Prior to the administration of flumazenil, necessary measures should be instituted to secure the airway, (b) (4) adequate ventilation, and (b) (4) intravenous access. Flumazenil is intended as an adjunct to, not as a substitute for, proper management of benzodiazepine overdose. Flumazenil will only reverse benzodiazepine-induced effects but will not reverse the effects of other (b) (4) medications. The mazenil package insert, including CONTRAINDICATIONS, WARNINGS, and PRECAUTIONS, should be consulted prior to use. Patients treated with flumazenil should be monitored for re-sedation, respiratory depression, and other residual benzodiazepine (b) (4) Re-sedation by TRADENAME has not been observed after administration of flumazenil in a clinical trial.	known or suspected. Prior to the administration of flumazenil, necessary measures should be instituted to secure the airway, (b) (4) adequate ventilation, and (b) (4) intravenous access. Flumazenil is intended as an adjunct to, not as a substitute for, proper management of benzodiazepine overdose. Flumazenil will only reverse benzodiazepine-induced effects but will not reverse the effects of other (b) (4) medications. The complete flumazenil package insert, including CONTRAINDICATIONS, WARNINGS, and PRECAUTIONS, should be consulted prior to use. Patients treated with flumazenil should be monitored for re-sedation, respiratory depression, and other residual benzodiazepine (b) (4) Re-sedation by TRADENAME has not been observed after administration of flumazenil in a clinical trial.	
12 CLINICAL PHARMACOLOGY	**12 CLINICAL PHARMACOLOGY**	
12.1 Mechanism of Action	**12.1 Mechanism of Action**	
TRADENAME is (b) (4) TRADENAME binds to brain benzodiazepine sites (gamma amino butyric acid type A [GABAA] receptors) (b) (4) , while its carboxylic acid metabolite (CNS 7054) has a 300 times lower affinity for the receptor. (b) (4)	TRADENAME is a benzodiazepine (b) (4) TRADENAME binds to brain benzodiazepine sites (gamma amino butyric acid type A [$GABA_A$] receptors) (b) (4) while its carboxylic acid metabolite (CNS 7054) has a 300 times lower affinity for the receptor. (b) (4)	Established Pharmacological Class (EPC), “benzodiazepine”, was used to replace the vague and promotional description of the drug. Reviewer deleted extraneous language.

(b) (4) [redacted] TRADENAME, like other benzodiazepines, did not show clear selectivity between subtypes of the GABAA receptor. (b) (4) [redacted]	(b) (4) [redacted] TRADENAME, like other benzodiazepines, did not show clear selectivity between subtypes of the GABAA receptor (b) (4) [redacted]	
13 NONCLINICAL TOXICOLOGY	**13 NONCLINICAL TOXICOLOGY**	
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**	
Carcinogenesis Long-term (b) (4) [redacted]	Carcinogenesis Long-term animal studies have not been performed to evaluate the carcinogenic potential of remimazolam (b) (4) (b) (4) [redacted]	Language was revised to be consistent with CFR recommendations
Mutagenesis TRADENAME was not (b) (4) [redacted]	Mutagenesis Remimazolam was not mutagenic or clastogenic when evaluated in an in vitro bacterial reverse mutation assay (Ames test), an in vivo rat micronucleus assay, mouse lymphoma cells, in vivo rat bone marrow micronucleus assay, or comet assay. (b) (4) [redacted]	Language was revised to be consistent with other labels from the Division
Impairment of Fertility (b) (4) [redacted]	Impairment of Fertility In a study that did not test exposures comparable to the maximum recommended human dose (MRHD) of 30 mg/day, there was no adverse effects on male or female fertility when male rats were treated for 28	Reviewer revised language to be consistent with other labels in the Division. Reviewer included human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels

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(b) (4)	days prior to mating and female rats were treated for 14 days prior to mating with up to 30 mg/kg remimazolam via intravenous bolus (approximately 0.03 times the MRHD based on AUC).	Male/Female Rats (Seg I study (b) (4) 24779) were administered IV bolus of remimazolam (formulated with lactose monohydrate in water) at target doses of 0, 3, 10, and 30 mg/kg/day. Males were treated daily for 4 wks prior to mating until 2 wks after the start of mating. Females were treated daily for 2 wks before mating until implantation (GD7). Laparotomies on GD13. Decreased motility of spermatozoa at HD (52.74%), compared to control (70.63%) but was transient and not considered adverse. NOAEL = 30 mg/kg/day *Rat conversion factor: 6.2* *SM_{total}: 0.01 based on AUC exposure levels reported in 28-day toxicity study.* *$SM_{free\ fraction}$: 0.03 based on AUC exposure levels reported in 28-day toxicity study.*
	There was no impact on female fertility when female rabbits were administered remimazolam by intravenous infusion (up to 4 hours/day) up to 20 mg/kg/day (approximately 17 times the MRHD of 30 mg/day based on AUC) from 14 days prior to mating.	Rabbits (extended fertility study 497736) were administered daily 1.2, 2.4, or 4h IV infusions of remimazolam (CNS 7056B Lot 10201249, formulated with 3.96% Dextran 40 and 2.64% lactose monohydrate in saline) at target doses of 0, 12.5, 20, and 30 mg/kg/day from 14 days before mating until lactation day 30. Does: pharmacological effect, convulsions and mortality at all doses. Fetal NOAEL = 20 mg/kg/day based on the lack of effect at this dose and termination of the next highest dose before does could litter. *Rabbit conversion factor: 3.1* *SM_{total}: 13 based on premating AUC exposures* *SM_{free}: 17 based on premating AUC exposures*

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	No adverse effect on histology of the testes and epididymides or evaluation of spermatid count, sperm motility, and sperm morphology were reported in a repeat-dose toxicity study in which male minipigs were administered remimazolam by intravenous infusion (6 hours) up to 120 mg/kg/day (approximately 400 times the MRHD based on AUC) for 28 days followed by a 14-day recovery period.	Male Minipigs (28-day repeat-dose toxicity study (b) (4) 32237) were administered 6h IV infusions of remimazolam (CNS 7056 Lot 10201238, formulated with 3.96% Dextran 40 and 2.64% lactose monohydrate in saline) at target doses of 0, 12, 40, 120 mg/kg/day. Spermatograms (spermatid count, sperm motility, and sperm morphology) and histology of the testes and epididymides was analyzed after 28-days and a 14-day recovery. NOAEL = 120 mg/kg based on lack of test article findings. *Minipig conversion factor: 1.1* *SM_{total}: 419.87 based on AUC exposures*
	13.2 Published studies in animals demonstrate that the use of anesthetic agents during the period of rapid brain growth or synaptogenesis results in widespread neuronal and oligodendrocyte cell loss in the developing brain and alterations in synaptic morphology and neurogenesis. Based on comparisons across species, the window of vulnerability to these changes is believed to correlate with exposures in the third trimester through the first several months of life, but may extend out to approximately 3 years of age in humans. In primates, exposure to 3 hours of an anesthetic regimen that produced a light surgical plane of anesthesia did not increase neuronal cell loss, however, treatment regimens of 5 hours or longer increased neuronal cell loss. Data in rodents and in primates suggest that the neuronal and oligodendrocyte cell losses are associated with subtle but prolonged cognitive deficits in learning and memory. The clinical significance of these nonclinical findings is not known,	SLC language for GABAergic drugs

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	and healthcare providers should balance the benefits of appropriate anesthesia in neonates and young children who require procedures against the potential risks suggested by the nonclinical data. *[See WARNINGS AND PRECAUTIONS, Pediatric Neurotoxicity; Use in Pregnancy, and Pediatric Use].*	

2 Drug Information

2.1 Drug

CAS Registry Number: 1001415-66-2

Generic Name: Remimazolam (Inn, Ph. Eur) or remimazolam besylate

Code Name:
Remimazolam; Remimazolam for injection; CNS7056; CNS7056X; ONO-2748; RMZ; CB-07-01; CNS 7056B; ONO-2745BS; ONO-IN-251; CNS 7056 besylate; RF10007; SP148.5; BIPRAZINE PF; GW502056X; BYFAVO®

Chemical Name:
Methyl 3-[(4S)-8-bromo-1-methyl-6-pyridin-2-yl-4H-imidazo[1,2-a][1,4]benzodiazepin-4yl] propanoate benzenesulfonic- acid; 4H Imidazo[1,2-a][1,4]benzodiazepine-4-propanoic acid, 8-bromo-1-methyl-6-(2-pyridinyl)-(4S)-methyl ester, benzenesulfonate (1:1)

Molecular Formula: $C_{27}H_{25}BrN_4O_5S$

Molecular Weight:
597.48 for remimazolam besylate
(439.31 for remimazolam free base, the active moiety)

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Structure or Biochemical Description:

Structure

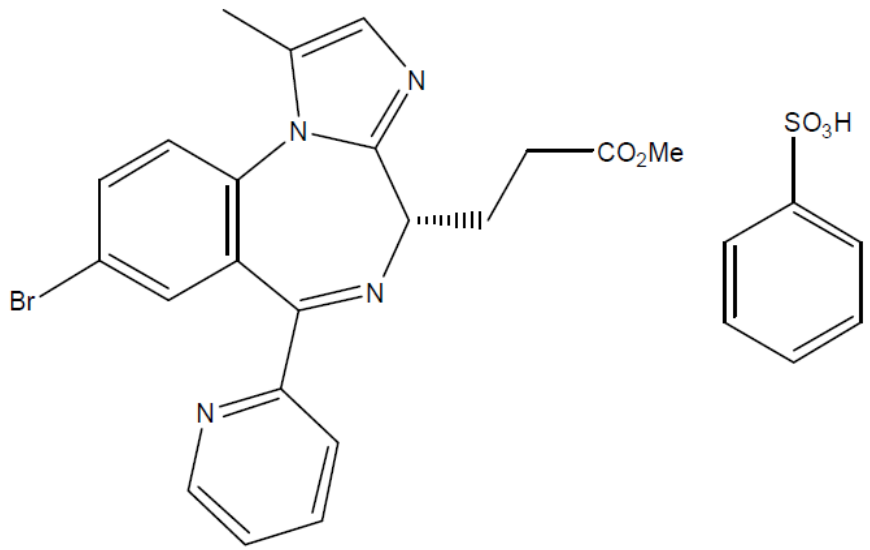

Figure 1 Remimazolam Besylate

Established Pharmacologic Class: Benzodiazepine (proposed)

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND/DMF No.	Holder	Subject	Comment
IND 102486	COSMO	Remimazolam	Nonclinical Review by Newton Woo, PhD 2/5/2013
DMF (b) (4)	(b) (4)		
DMF			
DMF Type III (b) (4)			
DMF (b) (4)			

2.3 Drug Formulation

Table 1 Drug Formulation of Remimazolam for Injection (20 mg)

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Component	Quantity per Vial	Percentage (W/W)	Function	Quality Standard
Remimazolam besylate (Remimazolam base)	(b) (4) mg[1] (20. (b) (4) mg)	(b) (4) %	Active ingredient	In-house
Lactose monohydrate	(b) (4) mg	%	Bulking agent / stabilizer	USP
Dextran 40	82. (b) (4) mg	%	(b) (4)	USP
(b) (4)	(b) (4)	-		USP
HCl/NaOH		-	pH adjustment	USP

1. Contains (b) (4) % overage

(b) (4)

Table 1 excerpted from M3.2.P.1 Description and Composition of the Drug Product

2.4 Comments on Novel Excipients

Dextran 40 is listed in the FDA inactive ingredient database (IID) but is not considered fully qualified by existing data because the approved drug product that contains this excipient is for a markedly different patient population, which is associated with a different benefit-risk consideration. No other novel excipients were identified in the proposed drug product as described in the table below.

Table 2 Excipient Assessment

Excipient	Conc.	MDE*	Comment
Lactose monohydrate	6.8 mg/mL	90 mg/day	Within limits of IID: Lactose monohydrate IV: Concentration 25 mg/mL, MDE = 6,750 mg/day
Dextran 40	10.3 mg/mL	136 mg/day	(b) (4)

			Inactive Ingredient Database (IID): There is one drug product listed in the IID that contains Dextran 40 as an excipient and is also intended for intravenous use. The maximum potency per unit dose is not listed in the IID. The proposed concentration in this drug product and MDE is within the limits of the IID. However, this product listed in the IID is an oncology product with positive genetic toxicology signals and therefore a different risk/benefit assessment compared to remimazolam. Therefore, although the levels are higher than remimazolam, the IID does not clearly support the proposed use in this NDA.
			Inactive Ingredient Database (IID): There is one product listed in the IID to contain dextran (MW not specified) as an excipient. The product is listed as an ophthalmic solution. The proposed concentration and MDE via remimazolam is above maximum potency for the ophthalmic product in the IID, therefore cannot be used to support safety of the excipient.

*MDE = maximum daily exposure assuming 33 mg/day; IV = intravenous. Lyophilized power is diluted to 2.5 mg/mL as per draft label DOSAGE AND ADMINISTRAITON.
AHFS Monograph for Dextran 40: https://www.drugs.com/monograph/dextran-40.html#ra

A safety justification for Dextran 40 was required as recommended in the *Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*. Specifically, the Division asked the Applicant to provide nonclinical data from 1-month repeat-dose general toxicology studies in two species, standard genotoxicity battery, a fertility study, embryo-fetal development studies in two species, and a pre-and post-natal development study.

In an Information Amendment submitted by the Applicant on 10/15/2019 SDN 0022 (23), the Applicant provided the following information and safety qualification for Dextran 40, which is described in the table below:

Required data for a new excipient as per FDA Guidance	**Applicant's proposal of safety**	**Reviewer comment**	**Adequacy as per SDN 22**
Safety Pharmacology	NA	Clinical data are available	Yes
Acute toxicology studies in two species (one nonrodent)	NA	May be omitted if repeat-dose studies are conducted	Yes
PK/ADME	Conducted in rats up to 2000 mg/kg/day in a 4-wk study (172.351.5266)	Clinical data are available	Yes
Genetic toxicology battery	Ames Assay (Seifried et al., 2006)[a] TA98, TA100, TA1535, TA1537, TA1538; with and without metabolic activation, 100-10,000 mcg/plate. Negative	Individual studies were not originally available for independent review. However, dextran (CAS 9004-54-0) was negative for mutagenicity and carcinogenicity as	No, require pdfs of cited study reports. Also require in vivo clastogenicity assay as per ICH S2B.
	Mouse Lymphoma TK assay (Seifried et al., 2006)[a], 1,000-5,000 mcg/plate with and without metabolic activation. Negative.		

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	Chinese hamster fibroblast (Ishidate et al., 1978)[a], Negative	identified in the CCRIS database consistent with the summaries provided by the Applicant.	IR was sent to resolve issues; see following table for an evaluation of the Applicant's response.
1-month repeat-dose toxicology study in two species (one nonrodent)	28-day rat, 4-h daily IV infusions (Study 172.351.5266) Dextran 40: 200, 600, 2000 mg/kg/day NOAEL = 600 mg/kg/day HED = 97 mg/kg/day = 5,806 mg/day for a 60 kg adult Safety Margin = 43x	Rat and minipig studies were reviewed during the NDA review period.	Yes
	4-week minipig, 6-h daily IV infusions Remimazolam with Dextran 40 (Study 32237) NOAEL = 360 mg/kg/day Dextran 40 (120 mg API/kg/day) HED = 327 mg/kg = 19,620 mg/day for a 60 kg adult Safety Margin >100x		
Fertility	Male Rabbit fertility study (Yakuri to Chiryo, 1975)[a] Dextran 70, IV, 91 days prior to mating, LOAEL = 3,640 g/kg/91-days (40 g/kg/day) • Effects on prostate, seminal vesicle, Cowper's gland, accessory gland HED = 13 g/kg/day = 774 g/day for a 60 kg adult Safety Margin > 5,000x	Male fertility study was not available for independent review. However, it is noted that there is a large safety margin in rabbit, and lack of histopathological effects on testes in repeat-dose minipig and rat studies.	No, require the submission of Yakuri article to satisfy the required fertility testing in males. Additional justification for the use of a surrogate compound is required. IR was sent to resolve issues; see following table for an evaluation of the Applicant's
	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg Dextran 40/kg/day (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day for a 60 kg adult Safety Margin = 46x		

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			response.
EFD (two species)	EFD study in Rabbit, (Oyo Yakuri 1972)[a] Dextran 70, IV, GD 8-16, LOAEL = 675 g/kg/9-days (75 g/kg/day) • Effects on extra-embryonic structures (e.g., placenta, umbilical cord) • Fetotoxicity (except death, e.g., stunted fetus) • Developmental abnormalities: musculoskeletal system HED = 24 g/kg/day = 1,451 g/day Safety Margin >10,000	Only one species was tested (rabbit). Oyo Yakuri study was not available for independent review. However, there is little concern based on the summary information provided, the large safety margins, and studies were conducted in a historically more sensitive nonrodent model (rabbit) compared to rat.	No, require EFD testing in second species or additional scientific justification of safety. Rodent species would suffice as a second species. IR was sent to resolve issues; see following table for an evaluation of the Applicant's response.
	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg/kg/day Dextran 40 (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day Safety Margin = 46x		
PPND	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg Dextran 40/kg/day (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day Safety Margin = 46x F0 females were treated from 2-wks prior to mating through lactation day 30. F1 pups were evaluated for growth and sensory/motor functional test (open field) up to PND35.	Evaluations of the F1 learning/memory, mating behavior, reproductive indices, and F2 pups was not conducted in this study.	No, require evaluations of reproductive parameters and learning behaviors are required for F1 pups required as per ICH S5(R3) or additional scientific justification of safety. IR was sent to resolve issues; see following table for an evaluation of the Applicant's response.
	No other preclinical data were found in literature on the effects of Dextran 40 in pregnancy. Applicant notes that high doses of low molecular dextran complex (i.e., iron dextran) were employed for decades and are currently used clinically	The Applicant is relying on clinical use data for the safety of this excipient. This will require the Applicant	No, these studies do not appear to evaluate endpoints similar to

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	in IV therapy of iron deficiency during pregnancy. As an example, a one-gram infusion of iron dextran has been proven to be safe and effective in pregnant women. Applicant referenced: 4. Henderson PA, Hillman RS. characteristics of iron dextran utilization in man. Blood, Vol 34, No.3 Sept 1969 5. Michael Auerbach, Jennifer Pappadakis, Huzefa Bahrain, Sandra Forrester, Wendy Capitano and Naomi V. Dahl. Rapid (Sixty Minutes) Infusion of One Gram of Low Molecular Iron Dextran: Safety and Efficacy Profile. Blood 2009 114:4054; 6. Michael Auerbach, Lilee Wong, Jessica McClintock, Steven Lenowitz, Nicola London, Sarah Auerbach and Samuel Smith Safety and Efficacy of Rapid (one hour) Single Intravenous Dose Low Molecular Weight Iron Dextran for Treatment of Oral Iron Intolerant Maternal Iron Deficient Anemia. Blood 2015 126:3356;	to submit the clinical data for review by the clinical team. PPND studies generally assess adverse effects on pregnant/lactating females and on development of the conceptus and the offspring following exposure of the female from implantation to weaning (ICH S5(R3)). Evaluations in a PPND study include: F0 females: clin obs/mortality, body wt, food consumption, parturition obs, necropsy. F1 pre-weaning: clin obs/mortality, litter size/live/dead, body wt, sex, physical development and reflex ontogeny. F1 post-weaning: clin obs/mortality, body wt, maturation (puberty), functional tests, reproductive performance, necropsy. Reviewer found: Auerbach et al., 2017 Wong et al., 2016	those required by ICH S5(R3), i.e., mating behavior, learning and memory, and reproductive indices of the children born to mothers treated with Iron Dextran. IR was sent to resolve issues; see following table for an evaluation of the Applicant's response.

[a]EMA/CHMP/187129/2016 Information for the package leaflet regarding dextrans used as excipients in medicinal products for human use. 19 November 2018. Committee for Medicinal Products for Human Use (CHMP).

The table above summarizes the deficiencies in the Information Amendment submitted 10/15/2019, which was also communicated to the Applicant in a Mid-Cycle teleconference and meeting minutes on 11/22/2019.

In a separate Information Amendment submitted by the Applicant on 12/06/2019, references and summaries to the existing genotoxicity assays in the literature, a statement of commitment to conduct an in vivo clastogenicity study with a projected

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submission date "before the Late Cycle Communication Meeting", and a safety justification for the use of surrogates were provided as described in the table below. In an additional Information Amendment submitted by the Applicant on 01/31/2020, further safety justification of male fertility and lactational exposures are provided and summarized in the table below.

Required data for a new excipient as per FDA Guidance	**Applicant's proposal of safety as per** Information Amendment 12/06/2019	**Reviewer comment**	**Adequacy following January 31 2020 submission**
Genetic toxicology battery	Ames Assay (Seifried et al., 2006 and Supporting Raw Data)[a] TA98, TA100, TA1535, TA1537, TA1538; with and without metabolic activation, 100-10,000 mcg/plate. Negative	Studies are reviewed in Appendix 12.5-12.7. As part of the NCI/NTP responsibilities to aid in the selection and prioritization of chemicals to be further evaluated in chronic 2-year rodent studies, Seifried et al. tested hundreds of compounds in the Ames and mouse lymphoma TK assay, including dextran (CAS 9004-54-0) and iron dextran (CAS 9004-66-4).	Yes, Ames, Mouse lymphoma TK, and rat micronucleus assay are adequate.
	Mouse Lymphoma TK assay (Seifried et al., 2006 and Supporting Raw Data)[a], 1,000-5,000 mcg/plate with and without metabolic activation. Negative.		
	Chinese hamster fibroblast (Ishidate et al., 1978)[a], Negative		
	Sponsor submitted the final report for a bone marrow micronucleus test (up to 2000 mg/kg) (b) (4) 38086 in SDN 0046 (47) 2/24/2020. Negative		
Justification for the use of surrogates	"Sponsor will not solely rely on the safety assessment of other compounds to establish safety of Dextran 40. As outlined below, we believe the genotoxicity and reproductive toxicity of Dextran 40 have been adequately assessed and results are favorable with considering the exposures estimated in the course of treatment with remimazolam drug product. Nevertheless we believe that data generated with higher or lower molecular mass dextrans, e.g. dextran 70 or dextran 10, respectively can further support the safety of Dextran 40. In fact all dextrans are a mixture of molecules with a wide range of molecular masses. The specification of Dextran 40 according to the Eur. Pharmacopoea indicates that the average molecular mass of the 10% higher fraction needs to be not more than 110,000 while the average	The Applicant's justification appears adequate from a pharmacology/toxicology perspective. A consultation for an evaluation of the surrogates was requested by the Division to Computational Toxicology Consultation Service on 1/15/2020. The evaluation concluded that Dextran 10, Dextran 70, and iron dextran are acceptable surrogates for Dextran 40.	Yes.

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	molecular mass of the 10% lower fraction of Dextran 70 needs to be not less than 15,000. This indicates are large overlap of dextran species within Dextran 40 and Dextran 70. The ADME of high molecular weight dextrans in general and Dextran 40 in particular is further discussed in response to Query #3."		
Fertility	Male rabbit fertility study (Kodera Yakuri to Chiryo, 1975) or (Maruoka Yakurito Chiryo, 1975) Dextran 70, IV, 91 days prior to mating, LOAEL = 3,640 g/kg/91-days (40 g/kg/day) • Effects on prostate, seminal vesicle, Cowper's gland, accessory gland HED = 13 g/kg/day = 774 g/day for a 60 kg adult Safety Margin > 5,000x	The Sponsor's justification is reviewed in Appendix 12.9. The English translation of Yakuri to Chiryo, 1975, does not adequately test dextran in a male fertility assay as these were general toxicity studies with no effect on male reproductive organ weight or histopathology. The extended fertility study (497736) adequately addresses female fertility, but male fertility is not addressed. The 4-week repeat-dose toxicity study in minipigs (32237) does not indicate adverse effects on male reproductive organ weight or histopathology or sperm count, motility, or morphology. Male fertility or reproductive behavior was not assessed in this study, however, the draft ICH S5(R3) indicates that if minipigs are used to assess male fertility it is adequate to assess histopathology as per note 1. There is little concern based on the summary information provided, the large safety margins in rabbit, and lack of	Yes.
	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg Dextran 40/kg/day (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day for a 60 kg adult Safety Margin = 46x		
	Minipig 4-week repeat-dose toxicity study (32237) Remimazolam with Dextran 40 IV 6h IV infusions for 4-weeks Evaluated sperm count, viability, and morphology from one testis and epididymis of all male animals in addition to histology of the testis and epididymis NOAEL = 360 mg Dextran 40/kg/day (120 mg API/kg/day) HED = 327 mg/kg = 19,620 mg/day for a 60 kg adult Safety Margin >100x		
	Literature-based safety justification as supplied in Information Amendment pages 9-19: "Given the bio-distributive properties of the glucose polysaccharide dextran, which as a hydrophilic polymer will not traverse cell membranes, minimal		

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	influence on any organ function is expected from exposure to macromolecular dextrans also on male reproductive endpoints. Likewise, the degradation products of dextrans bear no structural alert indicating adverse properties on organ functions including male reproductive tissues. Specifically, exposure to Dextran 40 as an excipient of remimazolam drug product will typically be single treatments and not extended over an appreciable portion of life time. Therefore, the long-term studies with excessively high exposures to dextrans and showing no effect on male reproductive endpoints provide a comfortable exposure margin supporting the use of Dextran 40 as an excipient in the remimazolam drug product."	histopathological effects on testis and epididymis in repeat-dose minipig and rat studies. Sperm count, viability, and morphology was analyzed in minipig repeat-dose toxicity assays without adverse effects.	
EFD (two species)	Sponsor provided a safety justification based on experimental evidence demonstrating that Dextran 40 exposure to the embryo or fetus is negligible or entirely absent (Information Amendment). Dextran 40: • Does not cross the placental barrier thus preventing prenatal exposures • Does not affect uterine blood flow in a manner that could negatively affect the embryo • Is excreted unchanged • Metabolites are well characterized low molecular weight carbohydrates such as glucose or nigerose. Larger oligosaccharide metabolites already exceed the limiting size (ME 500) for diffusional diaplacental transport	The Sponsor's justification is reviewed in Appendix 12.9. The submitted English translation of Oyo Yakuri 1972, does not adequately test Dextran in a rabbit EFD study. Only one species was tested (rabbit). However, there is little concern based on the summary information provided, the large safety margins, and studies were conducted in a historically more sensitive nonrodent model (rabbit) compared to rat.	Yes.
	EFD study in Rabbit, (Oyo Yakuri 1972)[a] Dextran 70, IV, GD 8-16, LOAEL = 675 g/kg/9-days (75 g/kg/day) • Effects on extra-embryonic structures (e.g., placenta, umbilical cord) • Fetotoxicity (except death, e.g. stunted fetus)		

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	• Developmental abnormalities: musculoskeletal system HED = 24 g/kg/day = 1,451 g/day Safety Margin >10,000		
	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg Dextran 40/kg/day (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day Safety Margin = 46x		
PPND	Extended Fertility/Mating study in Rabbits (Study 497736) Remimazolam with Dextran 40 IV NOAEL = 320 mg Dextran 40/kg/day (20 mg API /kg/day) HED = 103 mg/kg/day = 6,194 mg/day Safety Margin = 46x • F0 females were treated from 2-weeks prior to mating through lactation day 30. F1 pups were evaluated for growth and sensory/motor functional test (open field) up to PND35.	The Sponsor's justification is reviewed in Appendix 12.9. Evaluations of the F1 learning/memory, mating behavior, reproductive indices, and F2 kits was not conducted in this study. A similar product, Iron dextran, can pass into the breast milk and may harm a nursing baby	Yes, however, as noted in the comments column, this may impact the regulatory pathway for this application

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	Sponsor provided a safety justification based on experimental evidence demonstrating that Dextran 40 exposure to the embryo or fetus is negligible or entirely absent (Information Amendment). Sponsor provided additional safety justification of the safety of Dextran 40 exposures from maternal milk (Information Amendment page 19-21): • The probability of exposure of the infant to Dextran 40 or metabolites via the maternal milk is low since it is recommended to suspend breast feeding for (b) (4) hours after treatment with remimazolam" in the package insert. • If an infant is exposed to Dextran 40 via the maternal milk, the oral bioavailability is reported to be low. Koyoma and coworkers (Koyama et al, 1996) used an ex vivo system to evaluate the oral bioavailability of dextrans of different molecular sizes. Absorption was independent of the molecular size (potentially due to receptor-mediated uptake) and amounted to maximally 20% at low doses. At doses of above 1 mg this dropped to about 5%, indicating that the uptake mechanisms are saturable. • Dextran 40 has been classified by FDA as GRAS (CS/NF/DOS/7/ADD 3 FINAL) For Dextran 40, administered IV as a medicinal product for plasma volume expansion the recommended dose for pediatric patients is not to exceed 2 g/kg (total dose of >8 g), which provides a 50x safety margin.	(Drugs.com). Additionally, because it is not clear if it is the iron component or the dextran that is passed into breast milk to cause harm, the potential risk cannot be eliminated from consideration. This Reviewer agrees with the Sponsor's justification because of the lack of adverse findings in the extended fertility study in rabbits and the low risk of exposures to the fetus and infant. NOTE: (b) (4)	

In the table below are additional details on the studies conducted by the Sponsor to qualify the safety of Dextran 40.

Study	Key Findings
172.351.5266 GLP 4-week + 2 wk	28-Day Repeated-dose IV toxicity study of dextran 40 in rats, followed by a 14-day recovery period including TK

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Wistar Rat 4h IV infusion 0 (saline), 200, 600, 2000 mg/kg/day 10/sex/main group 5/sex/recovery group 9/sex/TK group	Evaluated clinical signs, detailed clinical examinations, body weight, food consumption, clinical pathology (hematology, clinical chemistry, and urinalysis), TK, ophthalmoscopy, gross pathology, organ weights, and histopathology. Recovery groups: control and HD only. • One non-test article-related death occurred in 1 HD male from procedure-related systemic bacterial infection and embolism • Dose-dependent increase in incidence and severity of swelling/edema in distal hind and fore limbs, snout, and scrotum occurring during infusion and subsided within hours of the end of infusion. Edema formation is a known anaphylactoid adverse reaction of rats to parenteral dextran administration. Study report suggests that this is a rat-specific reaction and is not relevant to humans; however, anaphylactoid reactions have been reported in humans after administration of drugs containing Dextran 40. • Body weight gains decreased 20% of the controls in the HD males (not statistically significant) • Statistically significant decreases in food consumption in HD males occurred during the first week of treatment • Increased liver weight (up to 18%) occurred in HD males and females on Day 29, in the recovery females there remained a mild increase in liver weights. This finding was correlated with fine microvesicular vacuolation in the cytoplasm of hepatocytes, statistically significant increase in AST (29%) • Increased liver weight (up to 8-12%) in MD males and females was associated with histopathological changes fine diffuse microvesicular vacuolation in hepatocyte cytoplasm in females and minimal increase in AST (31-72% not significant) and statistically significant increase in cholesterol in females (16%). Full recovery occurred on Day 43. • Increased spleen weight (up to 21%) occurred in HD males and females on Day 43. This finding was correlated with accumulation of foamy or vacuolated macrophages. • Increased adrenal weight (13%, not significant) in females on Day 29 was correlated with accumulation of foamy or vacuolated cells in the parenchyma. Recovery animals had slightly higher mean adrenal weights compared to controls. • Increased kidney weight (14%) in females on Day 43 without microscopic findings or clinical chemistry changes • Foamy and/or vacuolated cells occurred in adrenal inner cortex or medulla at HD in males and females and in MD females on Day 29. Full recovery occurred in both groups at Day 43 • Multifocal foamy alveolar macrophages in the lung occurred in the MD and HD groups. Partial recovery at Day 43 • Dose-related increase in incidence and severity of foamy and or vacuolated macrophages in the spleen and lymph nodes occurred in all dose groups. Partial recovery at Day 43. • The accumulation of foamy or vacuolated cells in parenchyma (liver and adrenals) or macrophages (lung, spleen, and lymph nodes) was considered test article storage and are not associated with sign of toxicity or inflammatory process. They are regarded as adaptive response of the host rather than an event of toxicological relevance. **NOAEL = 600 mg/kg** based on negative effects on body weight in males at the next highest dose of 2000 mg/kg and sparse reversible laboratory changes (increased cholesterol in MD females) *****all doses had anaphylactoid reactions**

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Study 32237 GLP 4-week Minipig 6h IV infusions [remimazolam with Dextran 40 0 (vehicle), 12, 40, 120 mg/kg] All groups received Dextran 40 at 360 mg/kg/day	28-Day Repeated-dose IV toxicity study of remimazolam with Dextran 40 in minipigs. Evaluated clinical signs, detailed clinical examinations, body weight, food and water consumption, scoring of sedative effects, hematology and coagulation, clinical chemistry, cytokine determination, urinalysis, ECG, blood pressure, body temperature, ophthalmological and auditory examinations, histopathology, sperm viability and morphology, and bone marrow. Key findings: • All groups received the same amount of Dextran 40 • No saline control groups were used • Dose-related increases in the mean sedative effect score occurred in both sexes. Sedative effects plateaued from 5 min after start of infusion until the end of the 6h infusion • No other test article-related changes were noted. NOAEL = 360 mg Dextran 40/kg/day (120 mg API/kg/day)
172.521.5589 Non-GLP 4-week Wistar Rat 4h IV infusion 0 (saline), 200, 600, 2000 mg/kg	Extended TK study of Dextran 40 administered IV for 28 days in Wistar rats This is a supportive study to 172.351.5266 (summarized above) because the previous study had unexplained variability of bioanalytical result which were difficult to interpret. Evaluated TK, clinical observations, body weight, gross pathology, organ weights, and histopathology (limited to kidney, liver, spleen, lung, cervical and mesenteric lymph nodes, site of administration, and any gross lesions in MD and HD; no controls were analyzed for histopathology) to bridge this study with the previous main toxicological study and to produce tissue samples to allow the determination of the tissue level of dextran in a separate supportive non-GLP investigation (reported separately in 172.103.5591) • 2 HD females died on Day 14 and 21, respectively. Test article-related death could not be ruled out; however, clinical signs and post mortem results suggested the deaths were secondary to the infusion. • Swelling of head/snout, fore and hind limbs, compulsive scratching, and ventral position in all treatment groups. These findings were considered anaphylactoid reactions of rats following parenteral dextran administration • Body weight gain were decreased (-35%) in HD males • Kidney weights were increased 18-20% in HD females compared to MD females, associated with fine microvesicular vacuolation in hepatocytes • Dose-related increase in incidence and/or severity of foamy or vacuolated cells were noted in the liver, lungs, spleen, cervical and mesenteric lymph nodes. These findings were considered adaptive and not adverse. • Procedure-related local tissue damage This study concluded that the findings were concordant with the previous study.
172.331.5600 GLP 1-wk + 1wk Renally impaired rats 4h IV infusion 200, 2000 mg/kg/day	7-Day Repeat-Dose toxicity study of Dextran 40 in renally impaired rats followed by a 7-day recovery period. Renal impairment was induced with cisplatin. Evaluated clinical observations, body weight, clinical pathology, urinalysis, gross pathology, kidney weights, and histopathology of the kidney Kidney injury achieved by 6.5 mg/kg cisplatin in male rats was adequate to investigate tolerability of Dextran 40 in males in a state of renal functional impairment. • Transient swelling of head/snout and extremities. Considered anaphylactoid reaction in rats administered parenteral dextran • Kidney weights were increased in HD groups on Day 12 and 19, associated with minimal to moderate vacuolated tubular epithelia with pyknotic nuclei (apoptotic cells) • Dextran 40 treatment did not exacerbate the degree of cisplatin-induced kidney injury nor did it influence the course of recovery from renal damage • Viscosity of urine was slightly increased in HD group on Day 11, but recovered by Day 19 to baseline

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During drug formulation optimization/development, a study was conducted to compare the sedative effects of the test article in different solvents (b) (4) and rat strains for optimization (E07CS022). This study was not informative for the safety of the proposed formulation, but is mentioned here for completeness.

Dextran 40 was adequately evaluated for genetic toxicity in a standard battery of tests. Dextran 40 was negative for genotoxicity in the Ames, mouse lymphoma TK assay, and in vivo rat bone marrow micronucleus test. Dextran 40 was adequately evaluated for general toxicity in 28-day repeat-dose toxicity studies in rat and minipig, female fertility in a standard Segment I study, and embryo-fetal development in an extended fertility study in one species (rabbits). Repeat-dose toxicity studies in minipig evaluated male reproductive organs with histology and sperm count, motility, and morphology with no adverse effects, suggesting that male fertility is not affected by Dextran 40. Further justification of the safety of Dextran 40 on male fertility was based on the distributive properties of hydrophilic macromolecules, such as dextrans, not to enter cell membranes thus not likely to influence organ functions. Degradation products of dextrans do not contain structural alerts for toxicity including male reproductive functions (however, QSAR evaluations for reproductive toxicity are not considered validated at this time). The Applicant concludes embryo-fetal as well as pre- and post-natal development is not likely to be adversely effected by Dextran 40 in the drug product because dextran does not cross the placental barrier, does not affect uterine blood flow in a manner that could negatively affect the embryo, is primarily excreted unchanged, and any metabolites are well-characterized low molecular weight carbohydrates such as glucose and nigerose, the probability of an infant exposure through maternal milk is low and the low exposures from maternal milk are not likely to cause harm because Dextran 40 has a low oral bioavailability and is GRAS.

In conclusion, based on the provided data and the additional safety justifications, Dextran 40 is not adequately evaluated for safety in the proposed drug product and indication. Definitive studies to characterize the safety of Dextran 40 on reproductive and developmental toxicity endpoints should provided to permit inclusion of Dextran 40 in the IID for a non-oncology indication; however, these data are not required to support approval of this application given the unique indication of procedural sedation where pregnancy testing can assure that pregnant women of child bearing potential will not be exposed to the drug product.

2.5 Comments on Impurities/Degradants of Concern

Drug Substance Impurities

Potential drug substance impurities are described in DMF (b) (4) M3.2.S.3.2 Impurities. The table below describes the specifications for the drug substance.

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Table 3 Drug Substance Impurity Specifications

Impurity	Structure	Structural alert? (Y/N)	Acceptance Criteria	TDI (mcg)	Qualified (Y/N)	Comment
(b) (4)						

CMC reviewer, Dr. Joseph Leginus, confirmed there were no structural alerts on the impurities in the table above, as communicated in an email on 2/20/2020.

As per ICH Q3A, the identification threshold is (b) (4)% and the qualification threshold for impurities is (b) (4)% for this drug product. A justification for the specification limits of (b) (4) is provided in DMF (b) (4) M 3.2.S.4.5. These impurities were tested in a repeat-dose toxicity rat study and in a battery of in vitro and in vivo genotoxicity studies, which are reviewed in the APPENDIX. All impurities with specifications at or above (b) (4)% were qualified in the repeat-dose

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toxicity rat study at higher concentrations as shown in the table below. It is noted that the genotoxicity studies were testing batches containing higher impurity levels than their proposed respective specifications and were not testing isolated impurities.

(b) (4)

Table 16: Information on Qualified Impurities in Drug Substance

Related Substances	Validation batch range	Proposed Specification	TDI based on 1 g MDD and specification	% in qualification batch	Confirmed safe level***	Confirmed safe level / 1g MDD based TDI	Qualified Yes/ No

(b) (4)

Source of Table 16 above: Nonclinical Overview. Report C11DA117 serves as the certificates of analysis of Lot YMK 110831 for the impurities as provided in a response to IR: SDN 0012 (13) 08/01/2019 Information Amendment/Response to IR #9, see page 14-16.

Drug Product Degradants

The table below describes the specifications for the drug product.

Impurity	Acceptance Criteria	TDI (mcg)	Qualified (Y/N)	Comment

(b) (4)

The two main degradation products are [redacted] (b) (4) and [redacted] (b) (4) The unknown degradation products were NMT [redacted] (b) (4)% and total degradation products were NMT [redacted] (b) (4)%, as described by the Applicant (M3.2.P.8.1 Stability Summary and Conclusion). The qualification threshold for degradants in this drug product is [redacted] (b) (4)

[redacted] (b) (4)

(b) (4)

[redacted]

Elemental Impurities

The Applicant conducted a Risk Assessment for the presence of elemental impurities in the drug product according to ICH Q3D, as stated in the Justification of Specifications. In this risk assessment, the Applicant considered 33 mg/day as the MRHD. Elemental impurity values for the drug product are described in the table below.

Table 5 Elemental Impurity Values for Remimazolam, 20 mg

(b) (4)

Elemental Impurities	[redacted]
Permitted Daily Exposure (µg/day)	
Control threshold – 30% of PDE (µg/day)	
Maximum Daily Intake (µg) for Remimazolam for injection 20 mg/vial	

Source of Table 5: M3.2.P.5.6 Justification of Specification

As shown in Table 5, the levels of elemental impurities are below the control threshold of 30% of the PDE as described in ICH Q3D.

2.6 Container Closure System

Lyophilized powder of remimazolam (20 mg) is packaged 12 mL (b) (4) glass vial fitted with (b) (4) rubber stoppers and capped with aluminum seals with (b) (4) flip-off caps, as detailed in the table below.

Table 6 Container Closure System

Component	Manufacturer	Product code	Description
Vial	(b) (4)		12 mL (b) (4) 22ø x 54 mm, clear glass vials
Stopper			(b) (4) rubber stopper with (b) (4)
Seal			Aluminum seal (20 mm) with (b) (4) (b) (4) flip-off cap

Source of Table 6 is M3.2.P.7 Container Closure System

Applicant summarized extractable and leachable studies in the Quality Overall Summary for Pharmaceutical Development. Submitted extraction and leachable studies are reviewed below.

(b) (4)

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2.7 Proposed Clinical Population and Dosing Regimen

Remimazolam is indicated for the induction and maintenance of procedural sedation in adults. Applicant proposes the following dosing regimen:

An initial dose of remimazolam administered IV as a 5 mg (2 mL) bolus injection over 1 minute followed by supplemental doses of 2.5 mg (1 mL) over 15 seconds as needed. At least 2 minutes must elapse from the initial dose prior to the administration of any supplemental doses. A limit of supplemental doses was not specified. In a response to IR, the Applicant stated that the maximum dose tested in Phase 3 clinical studies for procedural sedation was 33 mg/day (SDN 0012 (13) 08/01/2019 Information Amendment/Response to IR #9). For the purposes of this nonclinical review, this Reviewer is using 33 mg/day to represent the maximum daily dose (MDD) for this product indicated for sedation during procedures lasting approximately 24 minutes.

With this proposed maximum daily dose of 33 mg/day, elemental impurities, impurities, (b) (4) degradants, and extractables/leachables are qualified. Nonclinical repeat-dose toxicity studies provide adequate safety margins up to 1 g/day of the API. However, reproductive studies do not provide adequate coverage for the estimated MDD of 33 mg/day and is discussed further in the integrated summary.

2.8 Regulatory Background

A concise summary of the regulatory history of the product development, including major milestones/interactions, important nonclinical agreements, chronology of major meetings held with the applicant is presented in the table below.

Table 13 Regulatory Summary

Meeting	FDA Nonclinical Recommendations
Mid-Cycle Communication 12/12/2019	• **"Exposure Margins**: Comparison of human PK with exposure levels in the nonclinical studies to establish safety margins in the reproductive studies is ongoing. Conclusions regarding the maximum duration for the indicated procedures and human safety data will impact the final exposure margins and safety assessments." • **"Novel Excipient Qualification**: As communicated previously in a Type C written response (8/29/2014) and the EOP2 meeting (11/14/2013), Dextran 40 is considered a new excipient and has not been adequately qualified for safety given the proposed conditions of use with your product, remimazolam. As a new excipient, a safety justification is needed as recommended in the guidance for industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients. In response to our Information Request, you submitted an Information Amendment on 10/15/2019 SDN 0022 (23) to address the genotoxicity and reproductive/developmental concerns with Dextran 40. During our preliminary review of your Information Amendment, we have identified the following data gaps:" genetic toxicity and reproductive/developmental toxicity (more details are available in the communication document).
Cosmo's preNDA Type B Meeting Minutes 07/26/2018	• Provide justification for the species selected in the 28-day repeat-dose toxicity and the DART studies.

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	• Justify why rabbits are an appropriate species given their paradoxical response to remimazolam. Justify why their paradoxical response is not an issue when interpreting the results of the DART study. • Adequacy of the number of animals evaluated for fertility and assessment of postnatal development as per ICH S5A and S5B guidances will be determined during the review of the NDA. From the summaries in your pre-NDA package, it is unclear if male fertility was assessed in an appropriate species as per ICH S5B, including an evaluation of implantation sites and conceptuses. Additionally, it is unclear if your rabbit DART study adequately assessed postnatal developmental endpoints as per ICH S5A guidance, particularly learning/behavior/memory development and reproductive parameters (i.e., fertility) in the F1 kits. • Sponsor proposed submitting data in line with FDA's published expectations with respect to SEND data with NDAs • From a nonclinical perspective regarding PSP, studies and timelines proposed may require amendments as new data has emerged from published literature that raises concern for sedative-induced developmental neurotoxicity as indicated in FDA's Safety Labeling Changes issued December 14, 2016. Juvenile animal protocols should be discussed with the Division prior to initiating nonclinical juvenile animal studies. Additional Nonclinical Comments: • Submit all studies to NDA's EDR including paper studies submitted under relevant INDs • Justify a reasonable MDD • Include detailed discussion of nonclinical information in the published literature in Module 2; include copies of all referenced citations in Module 4; translate all journal articles to English • Label must be in PLLR; conduct a thorough review and integrated analysis of the existing clinical and nonclinical literature and propose a risk summary statement and test for Section 8 of the label • Impurities/degradants • Genotoxic impurities • Compare batches used in the clinical and nonclinical studies • Extractable/leachables

Paion's Type C Official Written Responses 11/21/2016	Abuse Potential Program:
	• The Sponsor's proposed rat study for physical dependence/tolerance were generally well-designed. FDA had the following comments:
	  o Typically, the route of administration of all drugs used in a physical dependence study is the same to achieve similar PK between drug groups. However, rats in this study will receive a single IV dose of remimazolam per day while the positive control drugs (morphine and diazepam) will be dosed orally twice daily. Differences in PK may produce differences in the ability of a drug to produce physical dependence.
	  o Drug discrimination period should last for at least two weeks. During this time, animals should be monitored every day for the first week and every other day for the second week. The behavioral observations should last for 10 minutes during each monitoring period.
	• As previously communicated by the Controlled Substance Staff (CCS) on January 26, 2015; a drug discrimination study should be conducted with dose that produce plasma levels of the drug that are similar to those produced by the therapeutic dose, as well as 2 to 3 times greater (if this can be done safely), in order for the study results to be meaningful. The timing

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	of the behavioral testing should occur at T_{max}. For the self-administration study, the dose should be high enough so that a drug with rewarding properties would require only a few bar presses to produce a cumulative dose that has a rewarding response. • A complete preclinical abuse-related assessment for an NDA submission includes chemistry, receptor binding for all major CNS-active sites, general behavioral studies conducted during toxicology testing, specific abuse-related behavioral studies (drug discrimination and self-administration) and a physical dependence evaluation. The clinical assessment of abuse includes an evaluation of abuse-related adverse events and a human abuse potential study. • Provide data for the solubility of remimazolam in 20% - 25% ethanol and with increased volume. • Rabbit behavioral study conducted with remimazolam, ethanol, and the combination showed that ethanol can potentiate the sedative effects of remimazolam, strongly suggesting that remimazolam has the potential to incapacitate a human for victimization purposes. This should be studied in humans and reviewed by CSS.
Paion's Type C Official Written Responses 08/29/2014	Advice on revised Phase3 development plan and nonclinical studies to support proposed indication: • Sponsor's planned 28-day repeat-dose toxicity study testing clinical formulations of remimazolam and literature-based justification to qualify the safety of Dextran 40 appears adequate but will be an NDA review issue. • We do not agree that a wide intraspecies margin between a dose that induces the desired **pharmacodynamic effect** compared to the dose causing toxicity is adequate justification for the rat as being a relevant nonclinical species. However, we concur that, if continuous IV infusion in rats produces systemic exposure levels equal to or greater than the maximum human exposure levels and can adequately define the toxicological potential of the drug, then the rat is a relevant nonclinical toxicology species. Note: If continuous infusions in rats cannot provide adequate coverage in terms of both AUC and C_{max}, it will not be deemed an adequate species for toxicological assessment. If that is the case, exploration of alternative rodent species will be required. • An adequate assessment of general toxicity in a rat repeat-dose toxicology study is not a substitute for reproductive and developmental toxicity studies. A full reproductive and developmental toxicity assessment is required for the NDA in an appropriate model.
Paion's EOP2 CMC Meeting Minutes 09/17/2014 and Amendments to Responses to Q 1, 3, 4, 9, and 12 12/09/2014	• Remimazolam **MDD = 60 mg** was used. No other nonclinical comments.
Paion's EOP2 Official Meeting Minutes 11/14/2013	Advice on Phase 3 development plan: • Provide adequate scientific justification or data to support the safety of Dextran 40 at a level associated with the maximal use of remimazolam. • Nonclinical studies in two species would be required to support the safety of an NME. Due to the lack of systemic exposure to the parent after bolus dosing, the rat does not appear to be a relevant species. Therefore, without scientific justification, the 4-week repeat-dose toxicology study as well as the reproductive and developmental studies conducted in rats must be repeated in an appropriate and relevant species prior to NDA submission. It was also discussed that the Sponsor will be required to

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	search and evaluate an appropriate species for the various reproductive toxicology studies. • Agreed that the nonclinical program appeared sufficient to initiate Phase 3, provided that women of child bearing potential are either excluded or are required to used two forms of contraception and that the informed consent stated that there was limited reproductive and developmental nonclinical data with remimazolam to date. • Provide data to adequately qualify any impurity or degradant that exceeds ICH Q3A and ICH Q3B thresholds. In Module 2 of the NDA, include a table listing drug substance and drug product impurity specifications, MDE to impurities, and how these levels compare to ICH Q3A/B qualification thresholds, and indicate if the impurity contains a structural alert for mutagenicity. NOTE: We may refuse to file your NDA if it does not contain adequate safety qualification data for any identified impurity or degradant that exceeds ICH qualification thresholds.

3 Studies Submitted

3.1 Studies Reviewed (in this NDA review)

Study Number	Title / Description
Repeat-dose tox	
RMN1047	"Intravenous Vehicle Tolerability and Dose Range Finding Study in the Rat with Toxicokinetic Evaluation" Rat, IV bolus, 1-wk, 15/30/50, GLP
RMN1017	"Intravenous Maximum Tolerated Dose (MTD) and 7 Day Repeat Dose Study in the Beagle Dog" Dog, IV bolus, 1-wk, 0.74/5 GLP
(b) (4) 015-149	"Single Intravenous Infusion Dose Toxicokinetics Study of ONO-2745BS in Cynomolgus Monkeys" Monkey, IV infusion 9h, Dosing once/wk total 4-wk, 6.75/9.0, 11.25/22.5, GLP
(b) (4) 015-100	"A 2-Week Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkeys Followed by A 2-Week Recovery Period" Monkey, IV infusion 9h, 2-wk, 0/6.75/9.0 11.25/22.5, GLP
(b) (4) 015-257	"A 4-Week Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkeys Followed by A 4-Week Recovery Period" Monkey, IV infusion 12h, 4-wk, 0/12/30/60, GLP, spiked with 8 impurities/degradants (Lot YMK110831)
13-0293RD	"Toxicity Experiment with 4 Weeks of Repeated Intravenous RF10007 Injections in Cynomolgus Monkeys and 2 Week Recovery Period" Monkey, IV infusion 2h, Dosing once/wk total 4-wk, 0/12/25/50, GLP
(b) (4) 015-133	"A 5-Day Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkeys"
RZA5000	"Intravenous (Bolus) Maximum Tolerated Dose (MTD) and 14 Day Repeat Dose Study in the Primate"
32237	"4-Week Subchronic Study of CNS7056 (Remimazolam) in Goettingen Minipigs Following Repeated Intravenous 6-Hour Infusion"
32236	"14-Day Dose Range Finding Study of CNS7056 (Remimazolam) in Goettingen Minipigs Following Repeated Intravenous Infusion up to 6 Hours"
Genotoxicity	
(b) (4) 015- 270	Ames, GLP, spiked with 8 impurities/degradants (lot YMK110831)
(b) (4) 015- 271	Mouse lymphoma assay, GLP, spiked with 8 impurities/degradants (lot YMK110831)

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E162 (083-235)	Rat micronucleus and Comet Assay, GLP, spiked with 8 impurities/degradants (lot YMK110831)
(b) (4) 38086	Rat micronucleus, GLP, Dextran 40
Reproductive and Developmental Toxicity	
(b) (4) 24779	Fertility and Early Embryonic Development/Segment I study, rat, 4-wks prior to mating until 2-wks after start of mating in males, 2-wks prior to mating through GD7 in females; GLP
497720	Fertility and Early Embryonic Development /Segment I study, rabbit, 4-wks in males, GD6-19 in females; non-GLP
497736	Extended fertility/Mating study, rabbit, 4-wk prior to mating until weaning, GLP
(b) (4) 23508	Extended fertility/Mating study, rat, 3/10/30 mg/kg, GD6-17, GLP
23509	EFD/Segment II study, rat, 3/10/30 mg/kg, GD6-17, GLP
23506	EFD/Segment II study, rabbit, 1.25/2.5/5/7.5 mg/kg, GD6-20, GLP
23507	EFD/Segment II study, rabbit, 1.25/2.5/5 mg/kg, GD6-20, GLP
NO12230	PPND/Segment II/III study, rat, 3/10/30 mg/kg, GD6-L20 (weaning), GLP
Local Tolerance	
514227	Rabbit irritation and local tolerance, IV and Perivenous
27962 (260880)	Blood compatibility, human, in vitro 1h, GLP
13-0296HE	Blood compatibility, rabbit in vitro, 3h, GLP
29972 (785899)	Blood compatibility, pig in vitro, non-GLP
Other Toxicity Studies	
F12XA058	Metabolites in rats, iv bolus, 2 days
F13XT009	Metabolites in rats, iv bolus/infusion, GD6-10/GD6
(b) (4) 015-309 (incl. PBC015-309-01)	Metabolites in cynomolgus, iv infusion, 2-4 days
(b) (4)	Impurities, Ames test, GLP
	Impurities, mouse lymphoma test, GLP
	Impurities, rat iv bolus/infusion, single dose/1h
	Impurities, rat iv bolus, single dose
	Impurities, rat iv bolus/infusion, single dose
	Impurities, rat, iv bolus/infusion, single dose
172.351.5266	Excipients, rat, iv infusion, 4-weeks, GLP
172.521.5589	Excipients, rat, iv infusion, 4-weeks, GLP
172.331.5600	Excipients, rat, iv infusion, 1-week, GLP
13-0295 AG	Active systemic anaphylaxis, guinea pig, ip sensitization and iv challenge, GLP
(b) (4) 015-142	Phototoxicity, Balb/c 3T3 cells, GLP

3.2 Studies Not Reviewed

All submitted studies were reviewed.

3.3 Previous Reviews Referenced

The following studies were reviewed previously under IND 102486 by Newton Woo, PhD.

Study Number	Title / Description	Reviewed
Repeat-dose tox		Nonclinical Review by Newton Woo, PhD 2/5/2013
RMN1018	"Four Week Intravenous (Bolus) Repeat Dose Toxicity Study in the Rat with a 2 Week Recovery Period" Rat, IV bolus, 4-wk, 0/0/10/20/30, GLP	
RXA5004	"Four Week Intravenous (Bolus) Repeat Dose Toxicity Study in the Cynomolgus Monkey with a 2 Week Recovery Period" Monkey, IV bolus, 4-wk, 0/5/10/20, GLP	

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Genotoxicity		
DPE0002	Ames, GLP	
DPE0003	Mouse lymphoma assay, GLP	
DPE0004	Rat micronucleus, GLP	
E161 (083-234)	Rat micronucleus and Comet Assay, non-GLP	

4 Pharmacology

4.1 Primary Pharmacology

Remimazolam, a benzodiazepine, is a chirally pure molecule (S-enantiomer), synthesized as a single enantiomer, as the R-enantiomer has 1/40 the potency of the S-enantiomer (E09QA002, E08CS009).

In Vitro Studies
High binding affinity of the parent for the benzodiazepine site of the $GABA_A$ receptor exceeded that of its metabolite, CNS 7054, as shown in competition experiments summarized in the table below. Sedative effects in rats of metabolite CNS 7054 were 1/200 that of the parent remimazolam (E09QA002, E08CS009).

Table 14 Competition for [^{3}H]flunitrazepam Binding

Species	Remimazolam pK_i	CNS 7054 pK_i	Separation
Human	7.53 ± 0.10	4.91 ± 0.22	410-fold
Rat	7.50 ± 0.09	4.99± 0.06	320-fold
Yucatan Micropig	7.56 ± 0.10	5.05 ± 0.04	326-fold

Source of table: Nonclinical Overview and SAIVS 04/001; remimazolam = parent; CNS 7054 = inactive metabolite

Remimazolam indiscriminately binds $GABA_A$ receptor subtypes similar to a benzodiazepine comparator, midazolam, in whole-cell patch-clamp assays (Study 99802) and electrophysiological recordings of neurons in anesthetized rats (SAIVS 03/004). As stated in the Nonclinical Overview, because the addictive properties, sedative, anterograde amnesia, anticonvulsant, myorelaxant effects, and tolerance of benzodiazepines are linked to individual alpha-subunits, these assay predicts that remimazolam will have similar pharmacological effects as compared to midazolam.

Multiple radioligand binding screens demonstrated that remimazolam binds to $GABA_A$ receptor isoforms with high selectivity and specificity (Study AL-5589G, 100043415, 146078, and 146079). These studies further demonstrated that the metabolite CNS 7054 is much less potent than the parent compound (Study AL-5589G), as summarized in the tables below.

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Table 15 IC_{50} Values for Benzodiazepine Binding Sites of $GABA_A$ Receptors

	ONO-2745BS	ONO-IN-252	Midazolam
IC_{50} Values (nmol/L)	30.5(28.3 - 33.0)	5130(4550 – 5790)	6.58(6.07 - 7.13)

Source of table: Study AL-5589G; ONO-2745BS = parent; ONO-IN-252 = metabolite

Table 16 Ki Values for Benzodiazepine Binding Sites of $GABA_A$ Receptors

	ONO-2745BS	ONO-IN-252	Midazolam
Ki Values (nmol/L)	26.3	4420	5.66

Source of table: Study AL-5589G; ONO-2745BS = parent; ONO-IN-252 = metabolite

In Vivo Studies

Potency of remimazolam to cause sedative effects was tested in mice, rats, and pigs using loss of righting reflex in rodents (E07CS024, E05EP006) and species-adapted sedation scores (081530, 081327, 080931, 080831). Sedative effects of remimazolam were similar when delivered to rats with different vehicles (E07CS022, 07.473/2). Amnesic effects of remimazolam were compared with that of propofol in rats by investigating light latency in the passive avoidance test. Amnesic effect of remimazolam occurred at lower doses (ED_{50} = 0.68 mg/kg) than propofol (ED_{50} = 2 mg/kg) (E13CS009). This finding suggests that remimazolam may have effects on short-term amnesia, similar to that of propofol.

In studies comparing the sedative effects of remimazolam to midazolam or propofol, the onset was more rapid with a shorter duration at the lowest dose resulting in sedation of all animals. A summary of these studies is presented in the tables below.

Table 17 Mean Duration of Sedation After Single IV Administration of Remimazolam, Midazolam, and Propofol In Different Species at the Lowest Dose Resulting in Sedation of All Animals Per Group or a Significant Increase of the Sedation Score

Species	Remimazolam		Midazolam		Propofol		Study No.
	Dose [mg/kg]	Duration [min]	Dose [mg/kg]	Duration [min]	Dose [mg/kg]	Duration [min]	
Mouse	30	6	50	51	20	10	05.709/2, 06.309/3
Rat	20 20 30 20 20	6.8 4.5 9.0 4.9 6.0	50 50 50 - / - /	49.2 37.4 28.5 - / - /	10 10 10 - / - /	10.7 7.9 10.5 - / - /	E08QA005, E07CS024, E07CS027, 07.473/2 (vehicle1), 07.473/2 (vehicle2)
Pig*	1 / 0.375	13 / 5	- / 0.375	- / 48	-	-	081327, [Mutter 2006]
Sheep	1.5	22	No sign. sedation up to 0.4 mg/kg		2	11	SAIVS 07-002

* - Data given for micropigs (NIBS) and Large White Pigs, respectively. Dose for Large White Pigs is the only one tested.

Source of table: Nonclinical Overview. Note: Mutter 2006 is a poster presentation.

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Table 18 Onset and Duration of Loss of Righting Reflex and Lethal Dose After Single IV Administration in Rats

Treatment group		Loss of righting reflex			Ataxia following LRR	Death
Substance	dose	No. with LRR/total animal	Latency [min]	Duration [min]	Duration [min]	No.deaths /total animal
Control$	-	0/10	-	-	-	0/10
ONO-2745BS [mg/kg]	1	1/10	0.8 (1)	2.1 (1)	3.1 (1)	0/10
	2	1/10	0.7 (1)	1.3 (1)	4.2 (1)	0/10
	5	6/10 **	0.9 ± 0.14 (6)	1.8 ± 0.33 (6)	5.1 ± 0.46 (6)	0/10
	10	9/10 ***	0.5 ± 0.06 (9)	4.6 ± 0.36 (9)	8.0 ± 0.70 (9)	0/10
	20	10/10 ***	0.8 ± 0.34 (10)	6.8 ± 0.46 (10)	9.6 ± 0.69 (10)	0/10
	50	10/10 ***	0.3 ± 0.02 (10)	10.8 ± 0.50 (10)	18.2 ± 2.11 (10)	0/10
	100	10/10 ***	0.3 ± 0.02 (10)	15.0 (1)	23.7 (1)	9/10
Midazolam [mg/kg]	5	1/10	3.3 (1)	0.8 (1)	62.9 (1)	0/10
	10	1/10	1.7 (1)	35.2 (1)	120.0# (1)	0/10
	20	8/10 ***	2.6 ± 0.87 (8)	22.1 ± 5.71 (8)	116.3 ± 3.75# (8)	0/10
	50	10/10 ***	0.6 ± 0.07 (10)	49.2 ± 9.69# (10)	120.0 ± 0.00# (10)	0/10
	100	10/10 ***	0.4 ± 0.06 (10)	87.5 ± 15.50# (6)	120.0 ± 0.00# (6)	4/10
Propofol [mg/kg]	1	0/10	-	-	-	0/10
	2	0/10	-	-	-	0/10
	5	9/10 ***	0.3 ± 0.03 (9)	1.2 ± 0.06 (9)	6.2 ± 0.75 (9)	0/10
	10	10/10 ***	0.3 ± 0.01 (10)	10.7 ± 0.72 (9)	14.0 ± 0.68 (9)	1/10
	20	10/10 ***	0.3 ± 0.04 (10)	16.6 ± 1.23 (7)	22.6 ± 0.91 (7)	3/10
	50	10/10 ***	0.3 ± 0.01 (10)	-	-	10/10
Dexmedetomidine hydrochloride [μg/kg]	2	0/10	-	-	-	0/10
	5	1/10	3.5 (1)	17.8 (1)	30.0 (1)	0/10
	10	6/10 **	5.8 ± 1.89 (6)	28.6 ± 5.66 (6)	40.6 ± 4.80 (6)	0/10
	20	10/10 ***	2.3 ± 0.52 (10)	61.9 ± 5.73 (10)	74.6 ± 8.91 (10)	0/10
	50	10/10 ***	1.1 ± 0.12 (10)	85.4 ± 3.41 (10)	112.0 ± 5.63# (10)	0/10
	100	10/10 ***	1.6 ± 0.45 (10)	115.0 ± 2.55# (10)	120.0 ± 0.00# (10)	0/10
	200	10/10 ***	0.9 ± 0.11 (10)	119.4 ± 0.56# (10)	120.0 ± 0.00# (10)	0/10
	500	10/10 ***	2.3 ± 0.53 (10)	120.0 ± 0.00# (8)	120.0 ± 0.00# (8)	2/10
	1000	10/10 ***	3.4 ± 0.70 (10)	95.6 ± 16.91# (7)	120.0 ± 0.00# (7)	3/10

$: Control animals received pH 3.3 saline solution.
The dose unit is presented below each substance.
#: Animals with no confirmed recovery from loss of righting reflex or ataxia within 120 minutes after onset of loss of righting reflex are included. Data from these animals were censored at 120 minutes.
Data on the number of animals with loss of righting reflex and dead animals are expressed as number of (dead) animals afflicted/total number of animals in each group (n=10). Data on the onset of loss of righting reflex, duration of loss of righting reflex, and duration of ataxia following loss of righting reflex are expressed as mean ± standard error in units of minutes. Values in parentheses are the number of animals included in summarization. Data on the duration of loss of righting reflex and ataxia following loss of righting reflex from animals that died after loss of righting reflex were excluded and data from the remaining animals were summarized.
Fisher's exact test was performed using a closed testing procedure to compare the number of animals with loss of righting reflex between the control and each substance groups. The test was one-sided, with a significance level of 5%. **: $p<0.01$, ***: $p<0.001$ vs. control group
A Cochran-Armitage trend test was performed to confirm the dose-response relationship with regard to the number of animals with loss of righting reflex in each substance group. The test was one-sided, with a significance level of 5%. In all substance groups, the response was dose-dependent with a P value of <0.001.

Source of table: E08QA005

The rat ED_{50} = 3.9 to 4.32 mg/kg (E09QA013, E08QA005). Flumazenil, a benzodiazepine antagonist, reversed the sedative effects in rats and mice (Study 05.709/2, E08QA010, 13-029IPD, E07CS027). Impurities spiked into lot no. YMK110831 exhibited the same sedative effects in rats as a comparison batch (E11CS033).

Other species including Yucatan micropig, sheep, and monkey also had a rapid-onset (immediately to within 5 min) and short duration (recovery within 30-60 min) of sedation after IV administration of remimazolam (SAIVS 04/003, 081327; SAIVS 07-002; (b) (4) 015-098); however, recovery was slower in these other species compared to the

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~10 minute recovery in rodents (SAIVS 04/002). As stated in the Nonclinical Overview, the slower recovery in these species was attributed to the fast metabolizing capacity in rodent plasma. Like non-rodents, humans lack the carboxylesterase type 1A in plasma, therefore, humans are likely to have longer sedation durations as compared to rodents.

In conclusion, primary pharmacology studies suggest that remimazolam produces sedative effects by acting at the benzodiazepine site on the $GABA_A$ receptor. Flumazenil prevents or inhibits the sedative effects of remimazolam. Onset of sedation and recovery from sedation was rapid in all species tested compared to midazolam. There is potential for abuse through the intranasal and oral route.

4.2 Secondary Pharmacology

Multiple radioligand binding assays demonstrated that remimazolam bound to the $GABA_A$ receptors with high selectivity and specificity (Study AL-5589G, 100043415, 146078, and 146079), with no affinity to other pharmacological targets.

4.3 Safety Pharmacology

Remimazolam has typical benzodiazepine-related pharmacodynamic effects including sedation, respiratory depression, and cardiovascular depression, as summarized in the sections below.

Central Nervous System

CNS effects (motor activity, behavioral changes, coordination, sensory/motor reflexes, and body temperature) of remimazolam were evaluated in an Irwin study and are summarized below.

Study	Results
Central Nervous System Safety Pharmacology	
DRMN1046 (GLP)* Effects of remimazolam on the Irwin Test in male rats 10, 20, 30 mg/kg IV	• Sedative effects 5 min post-dose at all doses; recovery by 45 min post-dose • Decrease body temperature at 45 min post-dose at 10 and 30 mg/kg

*Studies previously reviewed by Dr. Newton Woo for IND 102486

Sedation is the primary pharmacological effect of remimazolam and was reported in many of the primary pharmacology and toxicity studies. Decreased body temperature is likely a result from cardiovascular depression addressed in the sections below.

Cardiovascular

Three in vivo GLP studies in male rat, micropig, and monkey; two ex vivo GLP studies in guinea pig myocardial muscle, and an in vitro GLP hERG assay were conducted to investigate the effects of remimazolam on blood pressure, ECG, and repolarization and conductance abnormalities. The table below summarizes the results of these studies.

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Study	Results
Cardiovascular Safety Pharmacology	
1775-001 (GLP)* Cardiovascular safety pharmacology of remimazolam in male rats 0, 20, 30 mg/kg IV	• Clinical signs of salivation and stereotypic chewing movements as well as decreased activity observed at all doses with each animal remaining in a sedative state for approximately 5 to 10 min post-dose • Following 20 and 30 mg/kg, heart rates were elevated 18% and 34%, respectively and were not different from vehicle controls within 1.25 hours post-dose No other changes in parameters
080425 (non-GLP) Cardiovascular safety pharmacology of remimazolam in male micropigs (NIBS). Route: IV Dose: 12.5, 25, 50, 100 mcg/kg/min Dose volume: 1 mL/kg/15 min Dose duration: 15 min	Remimazolam had no effect on blood pressure, heart rate, ECG parameters (PR interval, QRS duration, RR interval, QT interval, or QTc), and arterial blood gas up to 50 mcg/kg/min. • 100 mcg/kg/min decreased blood pressure and heart rate • Propofol at doses that produced equivalent sedative effects as 50 mcg/kg/min of remimazolam, produced increased blood pressure and heart rate, prolonged QT interval and QTc interval
(b) (4) 015-105 (GLP) Cardiovascular safety pharmacology of remimazolam in telemeterized male Cynomolgus monkey after bolus and IV infusion IV Bolus Dose: 0.5, 1, 2, 5 mg/kg Dose volume: 2.5 mL/kg Rate: 3 mL/min Dose duration: 2 min bolus IV Infusion Dose: 6, 18, 30, 60 mg/kg Rate: 6 mL/kg/6 h Duration: 6 hours	**Bolus dosing**: Remimazolam had no clear effect on heart rate, ECG parameters (PR interval, QRS duration, RR interval, QT interval, or QTc), or respiratory rate up to 5 mg/kg, but transient and slight decreases in blood pressure occurred at 2 mg/kg and greater. • Statistically significant decreases in systolic, diastolic, and mean blood pressure immediately after dosing at 5 mg/kg compared to control but were within 10% of the pre-dose values. • Statistically significant decrease in systolic and mean blood pressure immediately after dosing at 2 mg/kg compared to control, but were within 10% the pre-dosing values • Statistically significant decrease in body temperature 20 minutes and 1 hour after dosing when compared to control at 2 mg/kg (-0.58°C) and 5 mg/kg (-1.28°C) • Effects of sedation (sitting position, difficulty standing, ataxic gait, decreased spontaneous activity) occurred at 0.5 mg/kg and greater; ananastasia, incomplete eyelid closing, or retching occurred at 1 mg/kg and greater; crouching lateral position, prone position, no touch response, eyelid closure occurred at 2 mg/kg and greater. **Infusion dosing**: Remimazolam had no clear effect on blood pressure, heart rate, PR interval, QRS duration, RR interval, QT interval, or respiratory rate up to 60 mg/kg, but decreased body temperature and prolongation of QTc occurred at 18 mg/kg and greater. • Statistically significant decreases in body temperature 1 h after start of the dosing to 0.5 h or 1 h after end of dosing at 18 mg/kg (-1.76°C), 30 mg/kg (-2.02°C), and 60 mg/kg (-2.50°C) • Statistically significant prolonged QTc during dosing at 18 mg/kg compared to control but were within 10% of the pre-dose values. Study argues that the change in QTc is

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	likely due to temperature changes. Effects of sedation (crouching, difficulty standing, ataxic gait, decrease in spontaneous activity, vomiting) occurred at 6 mg/kg and greater; siting position, lateral position, prone position, or suppressed response to stimulation occurred at 18 mg/kg and greater; supine position, no touch response, somnolence, retching occurred at 30 and 60 mg/kg.
Ex vivo Cardiovascular Safety Pharmacology	
F09PD004 (GLP) Effects of remimazolam on the myocardial action potential of isolated papillary muscle from male guinea pigs 10, 30, 100, 300 mcmol/L	Remimazolam had no effect on myocardial action potential at concentrations up to 30 mcmol/L. • The 30% and 50% action potential durations (APD_{30} and APD_{50}) were shortened at ≥ 100 mcmol/L. • Increased resting membrane potential (RMP) and prolongation of 90% action potential duration (APD_{90}) occurred at 300 mcmol/L.
F09PD006 (GLP) Effects of metabolite on the myocardial action potential of isolated papillary muscle from male guinea pigs 10, 30, 100, 300 mcmol/L	• A major metabolite of remimazolam is not considered to affect the myocardial action potential up to the concentration of 100 mcmol/L. • The 30% and 50% action potential durations (APD_{30} and APD_{50}) were shortened at ≥ 300 mcmol/L.
hERG Assay	
DRMN1024 (GLP)* hERG current recordings from stably transfected HEK293 cells Remimazolam: 10, 30, 100, 300 mcM CNS 7054: 10, 30, 100 mcM	• Remimazolam produced dose-dependent inhibition of hERG tail currents (IC_{50} > 207 mcM) • Metabolite (CNS 7054) had no effect on hERG tail current at concentrations up to 100 mcM • Positive control inhibited hERG tail currents
Preliminary hERG Assays	
SAIVS 05/001 (non-GLP) hERG current recordings from stably transfected HEK293 cells Remimazolam: 0.03, 0.3, 3 mcM CNS 7054x: 0.003, 0.03, 0.3 mcM E-4031: 0.003, 0.03, 0.3 mcM Midazolam: 0.1, 1, 10 mcM	Remimazolam had no effect on hERG tail currents at concentrations up to 3 mcM. Metabolite (CNS 7054x) produced insufficient inhibition of hERG to estimate IC_{50} E-4031 (positive control) IC_{50}= 16 nM Midazolam (comparator) IC_{50}= 7.6 mcM
SAIVS 05/007 (non-GLP) hERG current recordings from stably transfected HEK293 cells CNS 7054x: 0.03, 0.3, 3 mcM	Metabolite CNS 7054x had no effect on hERG tail current at concentrations up to 3 mcM.
SAIVS 05/010 (non-GLP) hERG current recordings from stably transfected HEK293 cells	E-4031 at 3 mcM: 99.7% inhibition Cisapride at 3 mcM: 98.8% inhibition CNS 7054X at 3 mcM: 18.8% inhibition Remimazolam at 3 mcM: 24.3% inhibition

*Studies previously reviewed by Dr. Newton Woo for IND 102486

QTc prolongation in conjunction with decreased body temperature was detected in monkeys at 18 mg/kg. As referenced in the Nonclinical Overview, prolongation of QT values in many species including humans has been associated with hypothermia. Furthermore, hERG potassium channel inhibition is the principle mechanism leading to QT prolongation. Concentrations of remimazolam inhibiting hERG channels are shown in the table below. Together, these data suggest a low risk for QT prolongation with

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remimazolam in the proposed clinical setting.

Table 19 Effects of Remimazolam on hERG Channel Currents and Papillary Muscle in vitro Compared with Clinical Free Plasma Concentrations

Parameter	Remimazolam	CNS 7054
Mean C_{max} (free), human reference study*	0.40 µg/mL (0.91 µM)	1.54 µg/mL (3.6 µM)
hERG IC_{25}	62 µM (68-fold)**	>100 µM (>28-fold) **
hERG IC_{50}	207 µM (227-fold)**	
Guinea-pig papillary muscle NOEL	30 µM (33-fold)**	100 µM (28-fold) **

*A Randomized, Single-blind Phase II Study Evaluating the Efficacy, Safety and Pharmacokinetics of Remimazolam in General Anesthesia in Adult Patients Undergoing Cardiac Surgery, Including Follow-up Sedation in the Post-anesthesia Care Unit/Intensive Care Unit (CNS7056-010). **compared to human reference study. Source of table: Nonclinical Overview

Transient slight decreases in blood pressure and heart rates occurred in micropigs (100 mcg/kg/min x 15 min) and monkeys (2 mg/kg bolus and greater), while increases in heart rate occurred in rats at 20 mg/kg and greater. Blood pressure and heart rates were unchanged in monkeys administered 60 mg/kg/6 h during IV infusion. Anesthetics are known to decrease heart rate and blood pressure, which should be monitored appropriately in the clinical setting.

Respiratory

Three in vivo studies in male rats, male monkeys, and female rabbits were conducted to investigate the effects of remimazolam on respiratory rate, tidal volume, arterial pH, oxygen partial pressure and hemoglobin oxygen saturation. The table below summarizes the results of these studies.

Study	Results
In vivo Respiratory Safety Pharmacology	
Pulmonary (rats)* 0.5, 1.5, 4.0 mg/kg IV	• LD and MD increased respiratory rate • HD decreased respiratory rate and tidal volume • Recovery by 75 min
(b) (4) 015-135 (GLP) Respiratory safety pharmacology of remimazolam in telemeterized male Cynomolgus monkey after bolus and IV infusion IV Bolus Dose: 0.5, 1, 2, 5 mg/kg Dose volume: 2.5 mL/kg Rate: 3 mL/min Dose duration: 2 min bolus IV Infusion Dose: 6, 18, 30, 60 mg/kg Rate: 6 mL/kg/6 h	**Bolus dosing:** • Arterial carbon dioxide tension increased slightly at 5 mg/kg compared to control; however, were within the range of physiological variation. • No test article-related changes in arterial blood pH, arterial oxygen tension, or hemoglobin oxygen saturation up to 5 mg/kg. • Sedative effects were noted in all dosing groups. **Infusion dosing:** • Arterial carbon dioxide tension increased slightly at 18 mg/kg and greater. • No other test article-related changes in arterial blood pH, arterial oxygen tension, or hemoglobin oxygen saturation up to 60 mg/kg.

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Duration: 6 hours	• Sedative effects were noted in all dosing groups
(b) (4) 24812 (non-GLP) Respiratory safety pharmacology in female rabbits with Remimazolam IV Bolus Dose: 0, 2.5, 5.0, 7.5 mg/kg Volume: 2 mL/kg Duration: 2 min IV Infusion Dose: 7.5 mg/kg Duration: 2 min	**Bolus dosing:** • Mean blood oxygen saturation decreased at 1 min post-dose (18%) and by 3 min post-dose (20%) after a single 2 min bolus of 7.5 mg/kg compared to pre-dose values. • No test article-related effect on blood oxygen saturation occurred up to 5.0 mg/kg. • No test article-related effect on heart rate occurred up to 7.5 mg/kg. • Respiratory rate decreased in 2/4 and increased in 1/4 animals given 2.5 mg/kg. Respiratory rate decreased in all animals dosed with 5.0 mg/kg or greater within 5 min post-dose with recovery by 4 hours. • Tremors occurred 30 min post-dose in 1/4 animals at 7.5 mg/kg. **Infusion dosing:** • Mean blood oxygen saturation decreased at 1 min post-dose (6%) and 3 min post-dose (4%) after a single 4 min infusion of 7.5 mg/kg compared to pre-dose values. • Heart rate was decreased by 16% at 1 min and 3 min post-dose and by 26% at 10 min post-dose compared to pre-dose values. • Respiratory rate decreased, and nystagmus and mydriasis occurred within 5 min post-dose with recovery by 4 hours. • Sedative effects were noted at 30 min post-dose.

*Studies previously reviewed by Dr. Newton Woo for IND 102486

Respiratory rate decreased in all species tested resulting in decreased blood oxygen saturation and increased arterial carbon dioxide in monkeys or rabbits. Respiratory depression is a class effect of benzodiazepines. As stated in the Nonclinical Overview, in comparative studies, remimazolam-induced respiratory depression at comparable degrees of sedation appeared less pronounced relative to that of propofol and midazolam.

Blood

Five in vitro studies in rat, dog, and human blood were conducted to investigate the effects of remimazolam or its major metabolite on hemolysis, platelet aggregation, coagulation, or fibrinolysis as this product is administered intravenously. The table below summarizes the results of these studies.

Study	Results
In vitro Hematological Assays	
Hemolysis (in vitro)* Rats, dogs, humans 1, 10, 30, 100 mcM	Hemolysis was not observed at all doses tested
F08XP008 (non-GLP) in vitro rats platelet aggregation / coagulation	Remimazolam had no effect on rat platelet aggregation at concentrations up to 167 mcg/mL and no effect on blood coagulation system at concentrations up to 1000 mcg/mL.

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Remimazolam: Platelet aggregation: 33-167 mcg/mL Blood coagulation: 100-1000 mcg/mL	
F09PD005 (GLP) in vitro human platelet aggregation/coagulation/fibrinolysis Remimazolam: 3, 10, 30, 100, 300 mcmol/L	Remimazolam had no effect on human platelet aggregation, coagulation, or fibrinolysis at concentrations up to 300 mcmol/L (equivalent to 132 mcg/mL).
F09PD007 (GLP) in vitro human plasma aggregation/coagulation/fibrinolysis (metabolite) Metabolite: 3, 10, 30, 100, 300 mcmol/L	Metabolite (ONO-IN-252) of remimazolam had no effect on human platelet aggregation, coagulation, or fibrinolysis at concentrations up to 300 mcmol/L (equivalent to 132 mcg/mL).
F09PD008 (GLP) in vitro hemolysis in human whole blood Remimazolam: 0.8, 1.2, 1.6, 2.4, 4.8 mg/L	Remimazolam had no hemolytic potential in human blood at concentrations up to 4.8 mg/mL.

*Studies previously reviewed by Dr. Newton Woo for IND 102486

Remimazolam or its major metabolite did not affect hemolysis, platelet aggregation, coagulation, or fibrinolysis under the conditions tested.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetics

Remimazolam and its major metabolite, CNS 7054, were evaluated in different species across different administration paradigms. Pharmacokinetic parameters were derived using non-compartmental and compartmental analysis, depending on the study, from plasma concentrations versus time profiles.

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Figure 1 Remimazolam PK curve in Cynomolgus during a 2-hour IV Infusion

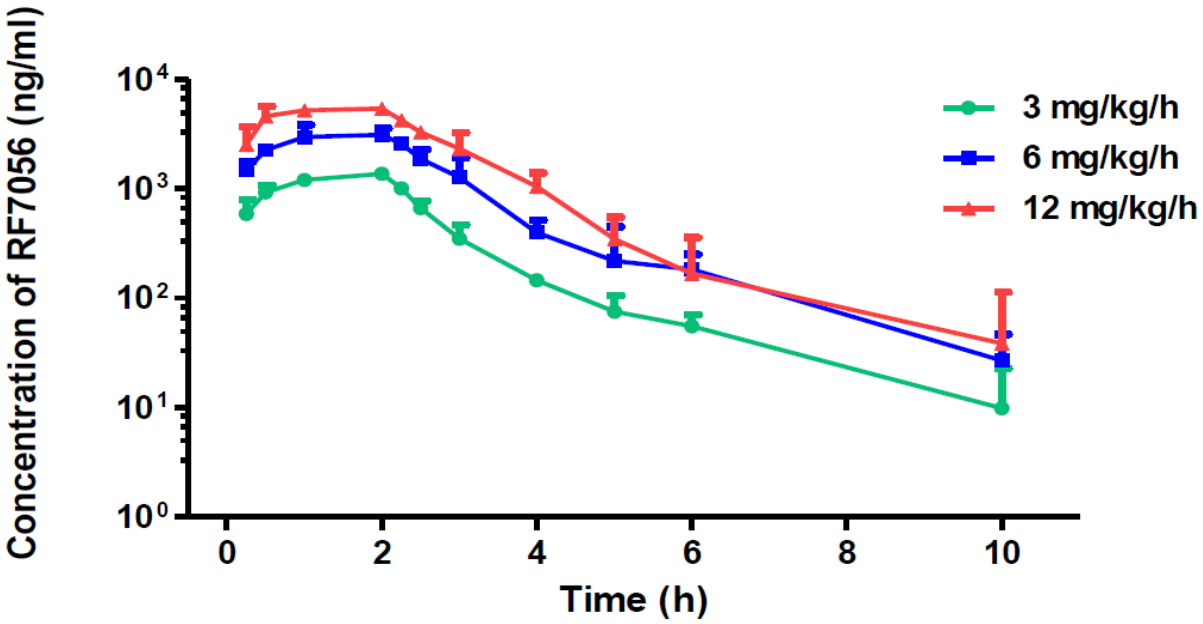

Fig.I-2.1 mean concentration-time curves of RF7056 in the second part, N=6

Source of figure: 13-0292PK

Figure 2 Major Metabolite (CNS 7054) PK curve in Cynomolgus during a 2-hour IV infusion with Remimazolam

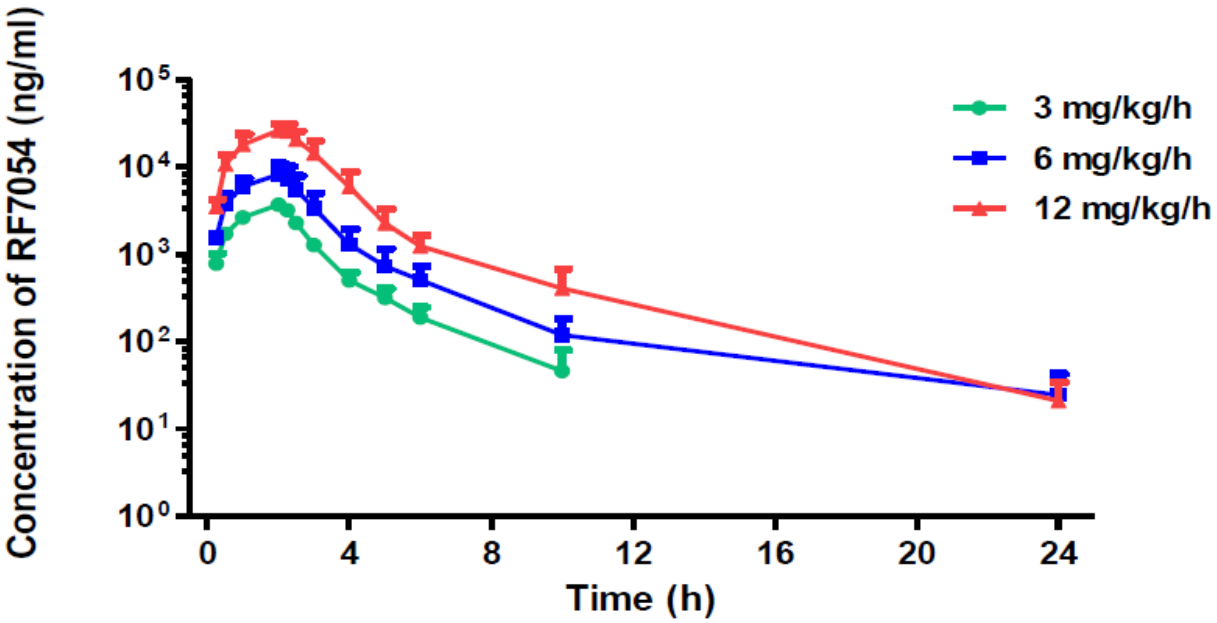

Fig.I-2.2 mean concentration-time curves of RF7054 in the second part, N=6

Source of figure: 13-0292PK

Reference ID: 4622909

Table 20 Summary of PK Parameters for the Parent (major metabolite CNS 7054 is presented in parentheses) Across Species

Single dose – IV bolus or short infusion						
Species	Rat	Rat	Micropig	Sheep	Monkey	Human
Study	DRMN1027 Table 3 and 4	AE-6094-G Table 1	Y08AG004 Tables 8 and 12	SAIVS 07-002 Table 18-20	AE-6095-G Table 1	Estimated PK*
Dosing	25 mg/kg	Radiolabeled 10 mg/kg bolus	0.1-1 mg/kg bolus	0.05-12 mg/kg bolus	Radiolabeled 10 mg/kg bolus	5mg + (9 x 3 mg) = 32 mg/day
C_{max} ng/mL	3975 (42535)	-	- (123-815)	1.20-5.48 (1.72-7.91)	-	945.85 (577.31)
T_{max} min	0 (4.00)	-	- (10-20)	1.84 (3.88)	-	
AUC mcg.min/mL	5.37 (1,547.905)	16.10	0.95-17.6 (4.16-80.6)	3.25-15.78 (37.62-161.84)	102.00	33.59 (720.16)
Half-life min	3.03 (27.2)	39	12-25 (78-150)	12.03 (24.43)	190	
CL mL/min/kg	4838 (-)	-	109-57.2 (56.7-60.2)	4768 (-)	-	
Vd L/kg	3.845 (-)	-	1.16-2.30 (1.37-2.40)	25.9 (-)	-	
Single dose – IV infusion						
Species	Micropig	Micropig	Minipig	Monkey	Monkey	
Study	Y10BG012 Effective concentrations	Y06AE004 Table 5	Y08AG004 Table 15 and 20	(b) (4) 015-149 Page 80	13-0292PK Page 8, second part	
Dosing		0.015 or 0.025 mg/kg for 15 or 25 min infusions	0.01-0.10 mg/kg 4 h infusion	6.75-22.5 mg/kg 9 h infusion	3-12 mg/kg/h 2 h infusion	
C_{max} ng/mL		-	189-1850 (730-4730)	450-2180 (954-4450)	1388-5605 (3730-26728)	
T_{max} min		-	4 (4)	540 (540)	108 (120)	
AUC mcg.min/mL		-	40.6-420 (211-1460)	238.8-1116 (587-2622)	208.8-970.5 (562.3-4633.8)	
Half-life min		-	20-47 (87-100)	-	78-80.4 (82.2-97.8)	
CL mL/min/kg		41.8	56.7-60.2 (-)	-	22.4-28.9 (-)	
Vd L/kg		0.631 and 1.01	1.37-2.41 (-)	-	2.82-3.36 (-)	
Multiple dose – IV bolus or short infusion						
Species	Rat	Rabbit			Monkey	
Study	RMN1018	(b) (4) 23507 Page 73-76			RXA5004	

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	Page 444-445 Table 13, 14				Page 274-275	
Dosing	10-30 mg/kg/day bolus, 4-wk	1.25-5.0 mg/kg IV bolus, GD6-20			5-20 mg/kg/day IV bolus, 4-wk	
C_{max} ng/mL	2.54-15.0 (22500-77700)	1977-5535 (1646-10792)			3810-11700 (7220-29700)	
T_{max} min	- (1.5-4.98)	0 (15-20)			- (5-20)	
AUC mcg.min/mL	0.01248-1.470 (1350-8640)	42.1-136.3 (90.7-561.8)			2750-12800 (930-4482)	
Half-life min	- (71.4-80.4)	16.8-19.0 (28-31)			41.88-58.80 (79.2-228)	
CL mL/min/kg	-	-			24.3-33.8 (-)	
Vd L/kg	-	-			0.995-1.470 (-)	
Multiple dose – IV infusion						
Species	Rat	Rabbit	Minipig	Monkey	Monkey	Monkey
Study	RAP17.0186 Page 14	497736 Page 14	(b) (4) 32237 Page 20-21	(b) (4) 015-100 Page 296-297	(b) (4) 015-257 Page 39	NO13199 Page 21-22
Dosing	30-60 mg/kg/day, IV bolus + 2h infusion, 28-day	12.5-30 mg/kg/day, IV bolus + infusion, 2-wk prior to mating until Day 30 of lactation	12-120 mg/kg/day, IV 6h infusion, 4-wk	6.75-22.5 mg/kg/day, IV 9h infusion, 2-wk	12-60 mg/kg/day, IV 12h infusion, 4-wk	24-120 mg/kg/day, IV 24-h infusion, 7-day
C_{max} ng/mL	39.9-223.7 (7008-17844)	4420-21300 (8910-14700)	496-9878 (-)	280-1270 (919-5420)	343-2220 (753-7670)	350-1480 (968-6900)
T_{max} min	-	2 (72-240)	100-355 (-)	-	-	-
AUC mcg.min/mL	-	319.2-696 (1194-3348)	155-2677 (-)	160-648 (521-3210)	235-1488 (592-4842)	-
Half-life min	-	-	18-47 (-)	-	-	-
CL mL/min/kg	-	18.5-44.8 (-)	-	-	-	-
Vd L/kg	-	-	-	-	-	-

Measurement of the parent is presented in a range for the study followed by the measurement of the metabolite in parentheses. * Estimated human PK parameters are based on Sponsor's provided exposures, submitted in the Nonclinical Information Amendment SDN 0038 (39) 01/31/2020.

Absorption

The clinical route for the proposed drug product is intravenous, which results in a 100% bioavailability. Bioavailability is significantly less when administered by other routes tested, as shown in the table below.

Route	Rat	Rabbit	Mini/Micropig
Oral	-	<10%	-
Intranasal	8-31%	-	5-10%
Intraperitoneal	5-9%	-	-
Studies referenced	33443, 673986	172-217-2582	(b) (4) 33659

Distribution

A number of distribution studies were conducted in adult rats (AE-6096-G), sheep (SAIVS 07-002), and monkey (AE-6095-G); pregnant rats (Y13AG004), and lactating rats (Y13AG005) and rabbits (497736). A summary of the data is below.

Radiolabeled distribution studies conducted in rat and monkey determined fast distribution to all tissues studied with peak radioactivity detected at the first timepoint sampled in monkey (5 min after dosing). Highest levels of radioactivity were detected in the organs of excretion, kidney and liver. Radioactivity was detected in the cerebrum and cerebellum at 5 min in both rat and monkey. At 72 h, cerebellum concentrations in the rat appeared to accumulate, while no radioactivity was detected in the monkey cerebellum at 168 h post dose. Radioactivity in skeletal muscle, skin, and fat was higher in the monkey compared to the rat.

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Table 21 Distribution in Rat: Tissue/plasma ratios of radioactivity concentrations after single IV administration of 10 mg/kg ^{14}C-Remimazolam to non-fasted male rats

	Tissue/plasma ratio of radioactivity concentration				
Tissue	5 min	1 h	8 h	24 h	72 h
Plasma	1.00	1.00	1.00	1.00	1.00
Blood	0.66	0.64	0.92	1.21	1.79
Cerebrum	0.04	0.02	0.11	NC	NC
Cerebellum	0.04	0.02	0.13	0.17	0.22
Pituitary gland	0.20	0.27	NC	NC	NC
Spinal cord	0.06	0.03	0.15	NC	NC
Eyeball	0.08	0.13	0.33	0.40	0.47
Harderian gland	0.24	0.13	0.97	1.62	1.74
Thyroid gland	0.29	0.34	0.58	NC	NC
Mandibular gland	0.25	0.21	0.86	1.24	1.38
Thymus	0.19	0.12	0.57	1.00	1.72
Heart	0.32	0.28	0.63	0.83	0.91
Lung	0.44	0.40	0.88	1.42	2.14
Liver	2.05	2.57	6.52	5.42	4.31
Kidney	3.35	3.87	4.83	3.25	3.25
Adrenal gland	0.39	0.24	0.90	1.33	NC
Spleen	0.21	0.18	0.78	1.18	1.52
Pancreas	0.21	0.27	0.94	0.82	0.94
Fat	0.08	0.07	0.15	NC	NC
Skeletal muscle	0.25	0.14	0.49	0.70	1.20
Skin	0.26	0.33	1.19	2.64	2.99
Thigh bone	0.08	0.08	0.58	1.18	2.06
Bone marrow	0.23	0.19	1.19	2.24	0.49
Mesenteric lymph node	0.28	0.33	1.02	0.85	0.66
Testis	0.13	0.21	0.34	0.52	0.24
Epididymis	0.16	0.19	0.65	0.89	0.80
Prostate gland	0.17	0.16	1.22	0.94	0.66
Seminal vesicle	0.09	0.38	0.87	1.02	2.19
Stomach	0.50	0.26	1.29	1.32	1.18
Duodenum	1.73	2.28	5.24	1.84	1.11
Small intestine	0.32	3.70	6.26	2.27	1.01
Cecum	0.19	0.40	128	23.2	1.86
Large intestine	0.20	0.32	115	24.0	1.64
Urinary bladder	0.60	1.28	7.39	1.71	1.29

NC: Not calculated

Source of table: AE-6096-G Page 38

Reference ID: 4622909

Table 22 Distribution in Monkey: Radioactivity concentrations after single IV administration of 10 mg/kg ^{14}C-Remimazolam to non-fasted male cynomolgus monkeys

	Radioactivity content (% of dose)	
Tissue	5 min	168 h
Blood	8.74	0.05
Cerebrum	3.36	0.00
Cerebellum	0.32	0.00
Pituitary gland	0.00	0.00
Eyeball	0.04	0.00
Thyroid gland	0.01	0.00
Mandibular gland	0.06	0.00
Thymus	0.04	0.00
Heart	0.78	0.00
Lung	0.79	0.00
Liver	10.34	0.08
Kidney	1.78	0.01
Adrenal gland	0.03	0.00
Spleen	0.24	0.00
Pancreas	0.22	0.00
Fat	2.49	0.12
Skeletal muscle	75.49	0.28
Skin	1.97	0.07
Testis	0.06	0.00
Epididymis	0.02	0.00
Seminal vesicle	0.01	0.00
Prostate gland	0.01	0.00
Stomach	1.84	0.00
Duodenum	0.52	0.00
Small intestine	1.73	0.01
Cecum	0.24	0.00
Large intestine	1.46	0.01
Urinary bladder	0.06	0.00
Gall bladder	0.02	0.00

n = 1

Blood, fat, skeletal muscle, and skin were assumed to be 6.0%, 7.8%, 50.0%, and 9.4% of body weight, respectively.

Source of table: AE-6095-G Page 46

In fetal blood and tissues and in female reproductive tissues, the radioactivity was detectable at all timepoints, as shown in the table below. The ovary, placenta, amnion, and fetal blood radioactivity was greater than maternal plasma at 48 h after a single IV bolus of 10 mg/kg.

Table 23 Distribution in Pregnant Rats: Tissue/plasma ratios of radioactivity concentrations after single IV administration of 10 mg/kg ^{14}C-Remimazolam to non-fasted pregnant rats.

Tissue	Tissue/plasma ratio of radioactivity concentration				
	5 min	1 h	8 h	24 h	48 h
Blood	0.71	0.68	0.95	1.11	1.42
Cerebrum	0.05	0.04	0.14	0.22	0.36
Heart	0.41	0.45	0.86	1.10	1.57
Lung	0.56	0.55	1.03	1.46	2.49
Liver	2.06	2.88	3.59	3.09	4.38
Kidney	4.13	6.25	2.98	2.78	4.37
Spleen	0.22	0.23	1.35	1.58	2.16
Pancreas	0.20	0.28	1.05	1.04	1.35
Thymus	0.26	0.22	0.87	1.58	2.87
Skeletal muscle	0.30	0.17	0.67	0.90	1.24
Thigh bone	0.09	0.06	0.29	0.40	0.76
Ovary	0.33	0.32	0.81	1.26	1.98
Uterus	0.30	0.74	0.98	1.50	2.12
Placenta	0.17	0.40	0.99	1.47	1.99
Amniotic fluid	0.01	0.05	0.83	0.96	0.48
Amnion	0.04	0.23	1.30	3.46	6.10
Fetus	0.05	0.17	0.43	0.67	0.88
Fetal blood	0.07	0.23	0.49	0.88	1.49

Tissue/plasma ratio of radioactivity concentration was calculated from the mean concentrations of three animals.

Source of table: Y13AG004 Page 27

Maternal milk in rats closely reflected the concentration of radioactivity in distribution studies as compared to plasma levels, as shown in the table below.

Table 24 Radioactivity concentrations in after single IV administration of 10 mg/kg ^{14}C-Remimazolam to non-fasted lactating rats

Time after administration (h)	Radioactivity concentration (ng eq./mL)		Milk/Plasma
	Plasma	Milk	
1	1090 ± 260	1040 ± 630	0.91 ± 0.33
8	51.7 ± 9.8	108 ± 19	2.10 ± 0.05
24	19.6 ± 3.9	45.5 ± 42.2	2.11 ± 1.63
48	9.53 ± 0.84	26.8 ± 10.4	2.89 ± 1.28

Data are expressed as the mean values ± SD of three animals.

Source of table: Y13AG005 Page 19

In rabbits, maternal milk was analyzed for remimazolam and the major metabolite, CNS7054, in a reproductive study dosing female rabbits from 2 weeks prior to mating to kit weaning. Milk bioanalysis was performed under non-GLP conditions. Results are summarized in the table below.

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Table 25 Concentration of Remimazolam and its major metabolite in the maternal milk of rabbits

Animal number	Day of sample collection (lactation)	Time of sample collection (minutes post end of infusion)	Remimazolam concentration (ng/mL)	CNS7054 concentration (ng/mL)
1509	11	37-44	NQ	NQ
1518	8	4-9	NQ	NQ
3512	11	36-42	474.88	396.2
3513	11	12-20	1198.02	323.1
3515	11	4-6	3324.22	256.9
3523	10	6-11	2005.11	301.1
3601	10	2-6	3360.78	374.3

NQ = not quantifiable

Source of table: 497736 page 43

Protein binding studies were conducted in multiple species. Results are shown in the table below.

Table 26 Summary of protein binding across species tested

Fraction Bound	Rat	Rabbit	Minipig	Monkey	Human
Parent (Remimazolam)	69-71%	86%-87%	76-79%	86-87%	89-92%
Metabolite (CNS7054)	75.4%	80-87%	49-50%	86.5%	79-92%
Studies referenced	(AE5641; AE-6097-G; AE-6505-G)	(ADM-18-2042)	(ADM-18-2042)	(AE5641; AE-6097-G; AE-6505-G)	(AE5641; ADM-18-2042; AE-6097-G; AE-6505-G)

The fraction of parent unbound in rat is about 30% and 10% in humans, by inference. Therefore, rats have three times the free and unbound parent of the humans, suggesting potentially higher availability to tissues in rats compared to humans.

Metabolism

The major metabolite of remimazolam is CNS7054, as identified in rat, monkey, and human metabolism studies (Y09AG009, Y09AE021, AE-6101-G, AE-6102-G, and Y10AE019).

Table 27 Metabolism of Remimazolam to CNS7054 when incubated with fresh frozen liver homogenates

Species	Mouse	Rat	Minipig	Dog	Monkey	Human
$t_{1/2}$ of parent (min)	<10	<10	<10	~40	~15	<10
Studies referenced	CNS-06	CNS-03, CNS-06	CNS-03,	CNS-03, CNS-06	CNS-07	CNS-03, CNS-06

Reference ID: 4622909

Table 28 Hydrolysis of Remimazolam in various tissues homogenates from species tested

Tissues	Mouse	Rat	Minipig	Dog	Monkey	Human
Liver		6	8	15	10	60
Lung	-	8	15	60	Stable	60
Kidney	-	12	2	60	8	Stable
Brain	-	15	Stable	Stable	Stable	Stable
Whole Blood	-	60	Stable	-	Stable	Stable
Plasma	-	15	Stable	-	Stable	Stable
Studies referenced	-	CNS-03; AE-5641	CNS-03; DRMN1042; DRMN1045	CNS-03	CNS-07; AE-5641	CNS-03; AE-5641

Table represents the time at which remimazolam was below the limits of quantification (approximately 0.8 mcM). Stable = indicates the levels of remimazolam were consistent across timepoints selected from 0-60 minutes incubation with tissue homogenates.

Data in the above tables suggest that, in humans, remimazolam is metabolized primarily in the liver. While in the rat, remimazolam is hydrolyzed in many other tissues including the plasma and whole blood. *Reviewer's Note: Repeat-dose toxicity studies were conducted in rat, minipig, and monkey; reproductive and developmental studies were conducted in rat and rabbit.* Initial studies identified carboxylesterases (CES) (AE-7176-G), and specifically CES1A1 and CES1A2 (Y10AG013), as the main enzyme hydrolyzing remimazolam in human plasma or lung, liver, and kidney homogenates.

As stated in the Nonclinical Overview, the main route of metabolism of remimazolam is via tissue carboxylesterases to CNS 7054, followed by hydroxylation and glucuronidation in all the species tested. Conversion to the major metabolite CNS7054 is mediated by carboxylesterases with no meaningful contribution of cytochrome P450 enzymes.

Excretion

Excretion studies were conducted in rat and monkey, results are presented in the table below.

Table 29 Summary excretion data from rat and monkey

Route of elimination	Rat	Monkey
Urine	44.8% by 24 h	65.6% by 168 h
Feces	48.3% by 24 h	17.5% by 168 h
(Bile)	(62.4% by 48 h)	-
Studies referenced	AE-6098-G, AE-6099-G	AE-6095-G

The primary route of elimination is renal and biliary excretion in rat and monkey, as shown in the table below. In rat, about 93.1% was eliminated by 24 hours. In monkey, 83.2% is eliminated by 168 hours. Distribution studies in lactating rats and rabbits also demonstrate that elimination occurs through the breast milk.

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6 General Toxicology

6.1 Single-Dose Toxicity

The intended marketing dosing regimen is for single dose, therefore, a single-dose monkey study (IV infusion 24 hours, (b) (4) 015-192) was reviewed in template format below. Other single-dose and acute studies were reviewed briefly and summarized in the table below.

Study	Key Findings
RMN1015 GLP, CD1 Mouse IV bolus Doses: 40, 50, 65, 100, 125 mg/kg	• Clinical signs (subdued behavior, prostration, piloerection, abnormal gait, lip licking, and abnormal breathing) occurred at 40-125 mg/kg were considered to be pharmacological effect of the test article. • Max. tolerated dose = 125 mg/kg
E08QA005 Non-GLP, SD Rat IV bolus Doses: 0, 1, 2, 5, 10, 50, 100 mg/kg	This is an efficacy and safety study evaluating clinical signs and mortality with comparison to midazolam, propofol, and dexmedetomidine. • ED_{50} = 5 mg/kg • LD = 100 mg/kg
E07CS024 Non-GLP, SD Rat IV bolus Doses: 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100 mg/kg	This is a preliminary study to determine efficacy endpoints (clinical signs and mortality) using midazolam, propofol, and dexmedetomidine. The following was determined for remimazolam: • Ataxia: ED >0.2 mg/kg, Latency = 0.3 min, Duration >2.8 min • LRR: ED >5 mg/kg, Latency <0.9 min, Duration >2 min • LD = 100 mg/kg
13-0291PD Non-GLP, SD Rat IV bolus Doses: 0, 0.5, 1, 2, 5, 10, 20, 40, 60, 80, 90, 100 mg/kg	This is an efficacy and safety study evaluating clinical signs and mortality comparison to midazolam. • ED_{50} = 4.32 mg/kg • LD = 90 mg/kg • LD_{50} = not calculated, gap between fatal dose and ED_{50} was ~20-fold • Sedation was reversed by Flumazenil

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32201 GLP, SD Rat Oral gavage Doses: 0, 3, 10, 30 mg/kg	Single-dose study with 2-week recovery. Evaluated mortality, clinical signs, body weight, food/water consumption, hematology, clinical chemistry, organ weights, macroscopic examination, histopathology. • NOAEL >30 mg/kg • No signs of sedation-like ataxia or reduced motility were noted. • No adverse test article-related effect were noted
32202 GLP. NZW Rabbit Oral gavage Doses: 0, 3, 10, 30 mg/kg	Single-dose study with 2-week recovery. Evaluated mortality, clinical signs, body weight, food/water consumption, hematology, clinical chemistry, organ weights, macroscopic examination, histopathology. • NOAEL>30 mg/kg • No signs of sedation-like ataxia or reduced motility were noted. • No adverse test article-related effects were noted
(b) (4) 015-098 GLP, Male Cyno. Monkey IV infusion (6h) Doses: 6, 18, 60, 150 mg/kg	Single-dose study with 2-week recovery. Evaluated mortality, clinical signs, body weight, food/water consumption, hematology, clinical chemistry, macroscopic examination, histopathology. **Males only (2/group)** • No mortalities occurred in this study • Clinical signs of sedation occurred at all dose levels • Decreased potassium in 2 animals at 150 mg/kg on Day 0 with recovery on Day 1 after dosing • Local histopathology: thrombus and inflammation of the vascular wall, thickening of the intima, fibrosis of the vascular wall, OR brown pigment in the vascular wall at the inj site occurred at all dose levels. • Study did not define NOAEL, but indicated that the LD >150 mg/kg
(b) (4) 015-138 GLP, Cyno. Monkey IV infusion (4h) Doses: 0, 3.2, 4.0, 4.8, 6.4 mg/kg	Single-dose study with 2-week recovery. Single-dose study with 2-week recovery. Evaluated mortality, clinical signs, body weight, food/water consumption, hematology, clinical chemistry, macroscopic examination, histopathology. **Males only (2/group)** • No mortalities occurred in this study • Clinical signs of sedation occurred at all dose levels • Local histopathology: desquamation of the endothelium, deposition of fibrinoid material on the intimal surface and necrosis of the vascular intima, very slight inflammation of the vascular wall and slight thrombus occurred at the injection site at 4.0 and 4.8 mg/kg. Desquamation of the endothelium at 4.0 mg/kg was very slight and considered toxicologically insignificant • NOAEL = 4.0 mg/kg
(b) (4) 015-192 GLP, Cyno Monkey IV infusion (24h) Doses: 0, 30, 60, 120 mg/kg	Single-dose study with 2-week recovery. Evaluated mortality, clinical signs, body weight, food/water consumption, hematology, clinical chemistry, organ weights, macroscopic examination, histopathology. Male and females (3/sex/group) • No mortalities occurred in this study • Clinical signs of sedation occurred at all dose levels • Local histopathology: slight thickening of the vascular intima at the inj site (vena cava) at 120 mg/kg with high grade and frequency compared to control.

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	• TK conducted Systemic NOAEL = 120 mg/kg Local NOAEL = 60 mg/kg [See below for detailed review of this study]

6.1.2 Monkey Single-Dose Toxicity

Study title: Single Intravenous Infusion Dose Toxicity Study of ONO-2745BS (Lot No. TFM110113-1) in Cynomolgus Monkeys

Study no.:	(b) (4) 015-192
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 21, 2011
GLP compliance:	Yes, this study was performed in compliance with GLP regulations for Nonclinical Safety Studies on Drugs (Ministry of Health and Welfare, 1997 and amendment 2008) and OECD Principles of GLP, FDA GLP (21 CFR 57)
QA statement:	Yes, QA signed August 9, 2011
Drug, lot #, and % purity:	Remimazolam (ONO-2745BS), Lot TFM110113-1, 98.4% pure

Key Study Findings

In this single-dose toxicity study with a 2-week observation period, monkeys (3/sex/group) were intravenously infused for 24 hours with remimazolam at target doses of 0 (saline), 30, 60, and 120 mg/kg/day. Evaluations included mortality, clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, gross lesions, organ weights, and histopathology. Ophthalmoscopic examinations and ECG recordings were not conducted in this study. Key study findings include the following:

- Pharmacodynamic effects were noted in all treatment groups
- Procedure-related findings caused by the indwelling catheter occurred as very slight to slight inflammatory response in the heart (mononuclear cell infiltration), caudal vena cava (perivascular inflammation with fibrosis, thrombus, and vasculitis), lung (thromboembolism, perivasculitis), and infusion site (thickened intima) of all test groups.
- Local tissue reactions characterized by slight thickened intima at the injection site were considered test-article related because there was an increase in severity and

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frequency at the HD (Control: 1/6 very slight; LD: 1/6 slight; MD: 1/6 very slight; HD: 2/6 slight).

- Target organ was the CNS based on the sedation occurring at all treatment doses
- **Local NOAEL = 1.25 mg/mL** (MD 60 mg/kg) based on the increased incidence and severity of thickened intima occurring at the next highest concentration of 2.5 mg/mL associated with the HD (120 mg/kg).
- **Systemic NOAEL = 120 mg/kg** based on the lack of test article-related toxicity findings in this study.
- Parent TK at the Systemic NOAEL:
    - Male AUC = 48,800 ng*h/mL
    - Female AUC = 55,300 ng*h/mL
    - Male C_{max} = 2,370 ng/mL
    - Female C_{max} = 2,700 ng/mL
- Metabolite TK at the Systemic NOAEL:
    - Male AUC = 312,000 ng*h/mL
    - Female AUC = 236,000 ng*h/mL
    - Male C_{max} = 18,300 ng/mL
    - Female C_{max} = 13,600 ng/mL

Group	Test and Control Articles	Dose Level (mg/kg)	Dose Volume		Concentration (mg/mL)	Number of Animals (Animal Number)	
			(mL/kg/h)	(mL/kg/24h)		Males	Females
1	Physiological saline	–	2	48	–	3 (1 – 3)	3 (4 – 6)
2	ONO-2745BS	30*	1	24	1.25*	3 (7 – 9)	3 (10 – 12)
3	ONO-2745BS	60*	2	48	1.25*	3 (13 – 15)	3 (16 – 18)
4	ONO-2745BS	120*	2	48	2.5*	3 (19 – 21)	3 (22 – 24)

*: as ONO-2745

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Methods

Doses:	0 (vehicle), 30, 60, 120 mg/kg Doses were selected based on available data obtained in a 7-day repeat-dose IV study in cynomolgus monkeys ([redacted] 015-121) where coma and other thoroughly sedative effects occurred at the first dosing at 5 mg/kg/h. Furthermore, a safety pharmacology study in cynomolgus monkeys ([redacted] 015-105) had 3/5 animals with body temperatures below 35ºC with 6h infusions of 10 mg/kg/h, therefore, it was considered that 24h infusions at 10 mg/kg/h would be difficult. Accordingly, the high dose was set at 120 mg/kg after a 24h infusion as the rate would be 5 mg/kg/h. Lower doses were selected at half increments.
Frequency of dosing:	Once, 24 hours a day (single dose) with 2-week observation period
Route of administration:	Intravenous 24-hour infusion into femoral vein and postcava via pre-implanted indwelling catheter
Dose volume:	24 or 48 mL/kg/day (1 or 2 mL/kg/h for 24 hours)
Formulation/Vehicle:	1.25 and 2.5 mg/mL / 0.9% saline
Species/Strain:	Cynomolgus monkey (purpose bred from [redacted]
Number/Sex/Group:	3/sex/group
Age:	3-6 years at dosing
Weight:	Males 3.52-4.48 kg at dosing Females 2.94-3.56 kg at dosing
Satellite groups:	Toxicokinetic analysis of the parent and major metabolite was performed on the main study animals.
Unique study design:	Ophthalmoscopy and ECG were not conducted for this study.
Deviation from study protocol:	Study report did not report any unexpected conditions that may affect the reliability of the study or any deviations from the protocol.

Mortality

No mortality occurred in this study.

Clinical Signs

Clinical signs for every animal were recorded at least 14 times on the day of dosing (before the start of dosing, 1, 4, 8, 12, 16, 20, and 24 hours after dosing, and 0.5, 1, 2, 4, 8, and 24 hours after the end of dosing) once daily thereafter. Cage-side observations were conducted daily.

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Pharmacodynamic effects of sedation occurred in a dose-related manner in both males and females. Sedation scores for males are presented in the tables below.

Table 1-2 Clinical signs in male cynomolgus monkeys (dosing day) Study No. (b) (4)015-192

Group	Animal No.	Clinical Signs	Pre	Time after initiation of dosing (h) 0*~1	1	4	4~8	8	12	16	20	24	Time after end of dosing (h) 0.5	1	2	4	8	24
ONO-2745BS 30 (mg/kg)$	7	Ataxic gait	-	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-
		Decrease in spontaneous activity	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
		Eyelid closure	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-
		Sedation	-	-	-	-	1	1	1	1	1	1	-	-	-	-	-	-
		Sitting position	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
	8	Ataxic gait	-	+	+	+	+	+	-	-	-	+	+	+	-	-	-	-
		Decrease in spontaneous activity	-	-	-	-	1	1	-	-	-	2	-	-	-	-	-	-
		Eyelid closure	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
		Sedation	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-
		Sitting position	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
	9	Ataxic gait	-	+	+	+	NE	+	-	-	-	+	+	-	-	-	-	-
		Decrease in spontaneous activity	-	-	-	-	NE	-	-	-	-	1	-	-	-	-	-	-
		Eyelid closure	-	-	-	-	NE	-	+	+	+	-	-	-	-	-	-	-
		Sedation	-	-	-	-	NE	-	1	1	1	-	-	-	-	-	-	-
		Sitting position	-	-	-	-	NE	-	+	+	+	-	-	-	-	-	-	-

Group	Animal No.	Clinical Signs	Pre	Time after initiation of dosing (h) 0*~1	1	1~4	4	4~8	8	8~12	12	16	20	24	Time after end of dosing (h) 0.5	1	2	4	8	24
ONO-2745BS 60 (mg/kg)$	13	Ataxic gait	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-
		Decrease in spontaneous activity	-	1	1	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-
		Sedation	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
		Somnolence	-	-	-	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-
		Sitting position	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-
		Lateral position	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-
		Supine position	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	14	Ataxic gait	-	+	+	NE	+	+	+	NE	+	-	+	+	+	-	-	-	-	-
		Decrease in spontaneous activity	-	-	-	NE	-	-	-	NE	2	-	1	1	-	-	-	-	-	-
		Eyelid closure	-	-	-	NE	-	-	-	NE	-	+	-	-	-	-	-	-	-	-
		Sedation	-	-	-	NE	-	1	-	NE	-	1	-	-	-	-	-	-	-	-
		Somnolence	-	-	-	NE	-	+	-	NE	-	-	-	-	-	-	-	-	-	-
		Sitting position	-	-	-	NE	-	+	-	NE	-	+	-	-	-	-	-	-	-	-
	15	Ataxic gait	-	+	+	+	+	NE	+	-	-	-	-	-	+	-	-	-	-	-
		Decrease in spontaneous activity	-	1	1	2	-	NE	-	-	-	-	-	-	-	-	-	-	-	-
		Eyelid closure	-	-	-	-	-	NE	-	-	-	+	-	-	-	-	-	-	-	-
		Sedation	-	-	-	-	-	NE	-	-	-	1	-	1	-	-	-	-	-	-
		Somnolence	-	-	-	+	-	NE	-	+	+	-	+	-	-	-	-	-	-	-
		Sitting position	-	-	-	+	-	NE	-	+	+	+	+	+	-	-	-	-	-	-

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Group	Animal No.	Clinical Signs	Pre	Time after initiation of dosing (h) 0*~1	1	1~4	4	4~8	8	12	16	20	24	Time after end of dosing (h) 0.5	1	2	4	8	24
ONO-2745BS 120 (mg/kg)$	19	Ataxic gait	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-
		Decrease in spontaneous activity	-	1	1	-	-	-	-	-	-	2	2	1	-	-	-	-	-
		Eyelid closure	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
		Sedation	-	-	-	1	-	1	-	-	1	-	-	-	-	-	-	-	-
		Somnolence	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-
		Sitting position	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-
		Lateral position	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
	20	Ataxic gait	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
		Decrease in spontaneous activity	-	1	-	-	-	-	1	-	-	-	-	1	-	-	-	-	-
		Eyelid closure	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
		Sedation	-	1	1	1	1	-	-	1	1	-	1	-	-	-	-	-	-
		Somnolence	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-
		Sitting position	-	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-
		Lateral position	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
	21	Ataxic gait	-	+	-	-	-	+	+	-	-	+	+	+	+	-	-	-	-
		Decrease in spontaneous activity	-	2	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-
		Eyelid closure	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
		Sedation	-	1	1	1	-	-	-	-	1	-	1	-	-	-	-	-	-
		Somnolence	-	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-
		Sitting position	-	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-
		Lateral position	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-

Notes) - : No abnormal signs + : Non-graded signs 1 : Slight 2 : Moderate 3 : Severe
NE: Not examined
* : Immediately after initiation of dosing
$: As ONO-2745

In both sexes, increasing levels of sedation occurred with increasing dose level. Sedation occurred within 4 hours of the start of infusion with recovery from sedation within 30 minutes and full recovery (no ataxia) within 2 hours after the end of infusion in the LD. Sedation occurred immediately after initiation of dosing with recovery from sedation within 30 minutes and full recovery (no ataxia) within 4 hours after the end of infusion in the HD.

No test article-related clinical sign of toxicity occurred in this study.

Body Weights

Body weights were recorded at least twice weekly. No test article-related findings occurred.

Food Consumption

Food consumption was calculated daily. Animals were fasted during the 24-hour infusion period. No test article-related findings occurred.

Ophthalmoscopy

Ophthalmological examinations were not conducted for this study.

ECG

Electrocardiography (ECG) recordings were not evaluated for this study.

Reference ID: 4622909

Hematology

For hematology, blood samples were collected from the femoral vein contralateral to the catheter once on Day 2 and Day 8 after the start of dosing. Parameters analyzed are shown in the table below.

Parameters	Unit	Method
Erythrocyte count	$10^6/mm^3$	Dual angle laser flow-cytometric measurement
Leukocyte count	$10^3/mm^3$	Dual angle laser flow-cytometric measurement
Hematocrit value	%	Calculation: (mean corpuscular volume × erythrocyte count) / 10
Hemoglobin concentration	g/dL	Modified cyanmethemoglobin method
Platelet count	$10^3/mm^3$	Dual angle laser flow-cytometric measurement
Mean corpuscular volume	fL	Dual angle laser flow-cytometric measurement
Mean corpuscular hemoglobin	pg	Calculation: (hemoglobin concentration/erythrocyte count) × 10
Mean corpuscular hemoglobin concentration	g/dL	Calculation: [hemoglobin concentration/(erythrocyte count × mean corpuscular volume)] × 1000
Reticulocyte ratio	%	Laser flow-cytometric measurement with RNA stain
Parameters	Unit	Method
Differential leukocytes[b)]	$10^3/mm^3$, %	Flow-cytometric measurement with peroxidase stain and dual angle laser flow-cytometric measurement
Prothrombin time	s	Coagulation method
Activated partial thromboplastin time	s	Coagulation method
Fibrinogen	mg/dL	Coagulation method

b) Parameters: eosinophils, basophils, neutrophils, monocytes, lymphocytes, and large unstained cells

No test article-related findings occurred.

Clinical Chemistry

For clinical chemistry, blood samples were collected from the femoral vein contralateral to the catheter once on Day 2 and Day 8 after the start of dosing. Parameters analyzed are shown in the table below.

Reference ID: 4622909

Parameters	Unit
Aspartate aminotransferase	IU/L
Alanine aminotransferase	IU/L
Alkaline phosphatase	IU/L
Parameters	Unit
Lactate dehydrogenase	IU/L
Creatine phosphokinase	IU/L
γ-glutamyltrans peptidase	IU/L
Total bilirubin	mg/dL
Total protein	g/dL
Albumin	g/dL
Total cholesterol	mg/dL
Triglycerides	mg/dL
Glucose	mg/dL
Blood urea nitrogen	mg/dL
Creatinine	mg/dL
Inorganic phosphorus	mg/dL
Calcium	mg/dL
Sodium	mEq/L
Potassium	mEq/L
Chloride	mEq/L
Protein fractions[b)]	%

b) Parameters: erythrocytes, leukocytes, epithelial cells, crystals, bacteria, sperm, casts, and others

No test article-related findings occurred.

Urinalysis

Urine for urinalysis was collected in stainless steel trays attached to the cages over 2 hours on Day 2 and Day 8 after the start of dosing. The urinalysis parameters are listed in the table below.

Reference ID: 4622909

Parameters	Unit	Method
Color	-	Visual
pH	-	Test paper
Ketone bodies	-	Test paper
Bilirubin	-	Test paper
Occult blood	-	Test paper
Urobilinogen	-	Test paper
Protein	mg/dL	Bromopyrogallol sulfonphthalein-In complex method

Parameters	Unit	Method
Glucose	mg/dL	GOD H_2O_2 electrode method
Urinary sediment[c)]	-	Microscopic examination of urine sediments stained with Sternheimer-Malbin stain

Parameters	Unit	Method
Urine volume	mL	Measuring cylinder
Specific gravity	-	Urinary refractometry
Sodium	mEq/L, mEq[e)]	Electrode method
Potassium	mEq/L, mEq[e)]	Electrode method
Chloride	mEq/L, mEq[e)]	Coulometric titration method

c) Parameters: erythrocytes, leukocytes, epithelial cells, crystals, bacteria, sperm, casts, and others
e) The total amount excreted was calculated from the concentration and urine volume. Only the total electrolyte excretion was evaluated.

No test article-related findings occurred.

Gross Pathology

No test article-related morphological changes occurred in any group.

Incidental findings included a white nodule in the liver in 1 LD male, and a white mass in the perivascular area of the left femoral vein to the caudal vena cava and in the intravascular area from the left femoral vein to caudal vena cava in 1 MD female. These findings were not dose-related.

Organ Weights

The following organs were weighed: lungs, submandibular glands, liver, heart, kidney, testes, epididymides, prostate, seminal vesicles, ovary, uterus, brain, spleen, thymus, pituitary, thyroids/parathyroids, and adrenals.

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No test article-related findings occurred.

Histopathology

Adequate Battery: Yes

Organs and tissues		Organ weight	Fixation	Histopathology (specimen preparation and examination)	Electron microscopy (specimen preparation)
Trachea		-	Y	Y	-
Lungs (including bronchi)	Left	Y	Y	Y	-
	Right			Y	-
Tongue		-	Y	Y	-
Submandibular glands	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Esophagus	Thoracic	-	Y	Y	-
Stomach	Body	-	Y	Y	-
	Pylorus	-		Y	-
Small intestine	Duodenum	-	Y	Y	-
	Jejunum	-		Y	-
	Ileum[a)]	-		Y	-
Large intestine	Cecum	-	Y	Y	-
	Colon	-		Y	-
	Rectum	-		Y	-
Pancreas		-	Y	Y	-
Liver		Y[b)]	Y	Y	Y[c)]
Gallbladder		-	Y	Y	-
Aorta	Thoracic	-	Y	Y	-
Heart		Y	Y	Y	-
Kidneys	Left	Y	Y	Y	Y[d)]
	Right	Y	Y	Y	-
Urinary bladder		-	Y	Y	-
Testes	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Epididymides	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Prostate		Y	Y	Y	-
Seminal vesicles	Left	Y	Y	Y	-
	Right			-	-
Ovaries	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Uterus		Y	Y	Y	-
Vagina		-	Y	Y	-

Reference ID: 4622909

Organs and tissues		Organ weight	Fixation	Histopathology (specimen preparation and examination)	Electron microscopy (specimen preparation)
Brain	Cerebrum[e)]	Y	Y	Y	-
	Cerebellum			Y	-
	Pons			Y	-
	Medulla oblongata			Y	-
Spinal cord	Thoracic	-	Y	Y	-
Sciatic nerves	Left	-	Y	Y	-
	Right	-	Y	-	-
Sternum/sternal bone marrow		-	Y	Y	-
Femurs/ femoral bone marrow	Left	-	Y	Y	-
	Right	-	Y	-	-
Submandibular lymph nodes	Left	-	Y	Y	-
	Right	-	Y	-	-
Mesenteric lymph nodes		-	Y	Y	-
Spleen		Y	Y	Y	-
Thymus		Y	Y	Y	-
Pituitary		Y	Y	Y	-
Thyroids/parathyroids*	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Adrenals	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Eyeballs/optic nerves	Left	-	Y	Y	-
	Right	-	Y	Y	-
Lacrimal glands	Left	-	Y	Y	-
	Right	-	Y	Y	-
Skeletal muscles (gastrocnemius)	Left	-	Y	Y	-
	Right	-	Y	-	-
Mammary glands/ skin (thoracic)	Left	-	Y	Y[f)]	-
	Right	-	Y	-	-
Skin (back)[g)]		-	Y	Y	-
Injection site[h)]		-	Y	Y	-
Gross lesions**		-	Y	Y	-

Y: Examined, - : Not examined
a) Including Peyer's patches
b) Including gallbladder
c) Left lobe
d) Cortex
e) Parietal lobe, temporal lobe, and diencephalons
f) Mammary glands: only females
g) Interscapular
h) Catheter site (postcava)
*: No specimen of the left parathyroid from No. 11 was prepared and examined.
**: Specimens of the left femoral vein and postcava from No. 18 and the liver from No. 9 were also prepared and examined.

Peer Review: No

Histopathological Findings

A dose-related increase in severity and frequency of thickened intima at the injection site could be related to the concentration of the test-article formulation at the HD (2.5 mg/mL) compared to the MD concentration (1.25 mg/mL) (Control: 1/6 very slight; LD:

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1/6 slight; MD: 1/6 very slight; HD: 2/6 slight). However, this change was not obviously distinct from the changes related to the physical stimulus of the catheter.

Procedure-related findings caused by the indwelling catheter occurred as inflammatory response in the heart, lung, and infusion site of all test groups. Slight to very slight changes occurring frequently across groups thromboembolism in the lung and mononuclear cell infiltration in the atrium of the heart.

Incidental lesion of the caudal vena cava and femoral vein identified in 1 MD female upon necropsy was characterized by marked perivascular inflammation with fibrosis, thrombus, and vasculitis.

A summary of histopathological findings of incidental and procedure-related findings are shown in the table below.

	Control		**LD (30 mg/kg)**		**MD (60 mg/kg)**		**HD (120 mg/kg)**	
	Male	Female	Male	Female	Male	Female	Male	Female
Heart								
Left atrium								
Mononuclear cell infiltration	1/3 very slight	1/3 very slight	1/3 very slight	1/3 very slight	2/3 very slight	1/3 very slight	-	-
Right atrium								
Mononuclear cell infiltration	-	-	1/3 very slight	-	-	-	-	1/3 slight
Intraventricular septum								
Mononuclear cell infiltration	-	-	-	-	-	1/3 very slight	1/3 very slight	-
Caudal vena cava (gross lesion)								
Perivascular inflammation with fibrosis	-	-	-	-	-	1/3 marked	-	-
Thrombus	-	-	-	-	-	1/3 marked	-	-
Vasculitis	-	-	-	-	-	1/3 marked	-	-
Injection site (vena cava)								
Thickening, intima	1/3 very slight	-	-	1/3 slight	-	1/3 very slight	-	2/3 slight
Femoral vein (left, gross lesion)								
Perivascular inflammation with fibrosis	-	-	-	-	-	1/3 marked	-	-
Thrombus	-	-	-	-	-	1/3 marked	-	-
Vasculitis	-	-	-	-	-	1/3 marked	-	-
Lung (left)								

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Thromboembolus	-	-	1/3 very slight	-	-	2/3 very slight	-	1/3 very slight
Lung (right)								
Thromboembolus	2/3 very slight	2/3 very slight	1/3 slight	1/3 very slight	1/3 very slight	2/3 very slight	1/3 very slight	-
Perivasculitis	-	-	1/3 slight	-	-	1/3 very slight	-	-
Kidney (right)								
Mononuclear cell infiltration	-	-	-	1/3 very slight	-	-	-	-

Toxicokinetics

Blood sampling for toxicokinetics occurred at 1, 3, 9, and 24 hours after the start of dosing and 0.5, 1, 6, and 24 hours after the end of dosing (8 timepoints) for analytes remimazolam (ONO-2745, parent) and CNS7054 (ONO-IN-252, metabolite).

Toxicokinetic analysis results are summarized in the tables below.

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Summary table 1 Mean TK parameters of ONO-2745 in cynomolgus monkey plasma

Group	Dosage (mg/kg)	Sex	C_{max} Mean ± SD (ng/mL)	AUC_{0-48h} Mean ± SD (ng·h/mL)
2	30	Male	669 ± 58	14500 ± 2300
		Female	749 ± 76	16300 ± 600
		Total	709 ± 75	15400 ± 1800
3	60	Male	1250 ± 270	29000 ± 5800
		Female	1540 ± 750	30600 ± 10200
		Total	1400 ± 530	29800 ± 7500
4	120	Male	2370 ± 100	48800 ± 3300
		Female	2700 ± 420	55300 ± 4900
		Total	2540 ± 330	52000 ± 5200

Summary table 2 Mean TK parameters of ONO-IN-252 in cynomolgus monkey plasma

Group	Dosage (mg/kg)	Sex	C_{max} Mean ± SD (ng/mL)	AUC_{0-48h} Mean ± SD (ng·h/mL)
2	30	Male	2490 ± 370	48100 ± 6300
		Female	2580 ± 1720	52100 ± 26400
		Total	2540 ± 1110	50100 ± 17300
3	60	Male	7010 ± 3210	128000 ± 43000
		Female	3790 ± 1480	88900 ± 37700
		Total	5400 ± 2850	108000 ± 42000
4	120	Male	18300 ± 7600	312000 ± 92000
		Female	13600 ± 3700	236000 ± 47000
		Total	16000 ± 5900	274000 ± 78000

Mean C_{max} and AUC of the parent (ONO-2745) increased approximately dose-proportionately in males and females.

Mean C_{max} and AUC of the major metabolite CNS7054 (ONO-IN-252) increased approximately dose-proportionally in females and in males up to 60 mg/kg. At 120 mg/kg, C_{max} and AUC in males increased more than dose-proportionately. No obvious sex differences occurred in mean C_{max} or AUC of the parent or metabolite.

Dosing Solution Analysis

Dosing solution analysis determined the concentrations were within 10% of the nominal values (values reported: 99.3-100.6%).

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6.2 Repeat-Dose Toxicity

The duration of repeat-dose toxicity studies to support marketing for an acute use product (up to 2 weeks duration of indicated treatment), is 4-week studies in two species (at least one non-rodent) as recommended per ICH M3(R2).

Two repeat-dose toxicology studies using bolus dosing in rat and monkey were reviewed previously by Dr. Newton Woo. Key study findings from these bolus-dosing studies and additional supplemental studies are summarized in the table below. Pivotal 4-week repeat-dose toxicity studies with 2-week recovery groups in minipig and monkey are reviewed in detail in sections 6.2.1 and 6.2.2. These two pivotal studies were selected for detailed review because the study investigated infusion dosing with the clinical formulation.

Study	Key Findings

RMN1047
GLP
1-wk Rat w/ TK
IV bolus
 	
0 (vehicle), 15, 30, 50 mg/kg
 	
Vehicle = 25 86%
(b) (4)
	"Intravenous Vehicle Tolerability and Dose Range Finding Study in the Rat with Toxicokinetic Evaluation"
	Evaluated clinical signs, body weights, food consumption, clinical pathology (hematology, blood chemistry, urinalysis), necropsy, organ weights, and histology
	
	• 1 HD male was found dead on Day 2, likely a result of respiratory depression.
	• Pharmacological effects of sedation occurred at all dose levels
	  o LD: subdued behavior, abnormal gait, lip licking, prostrate posture, irregular breathing, and pale pinnae; hyperactivity was limited to males; lasting 30 min after dosing.
	  o MD: prostrate posture, lip licking, abnormal gait, subdued behavior, irregular breathing, sporadic changes in respiration (slow or rapid breathing), pale pinnae/extremities, single incidence of reddened extremities and 1 incidence of pale whole body; hyperactivity occurred in 2 animals on separate occasions; protruding eyes; lasting 30 min after dosing and lasting over 1 hour on 2 occasions
	  o HD: prostrate posture, lip licking, abnormal gait, subdued behavior, irregular breathing, pale pinnae; change in breathing (labored, rapid, or slow breathing); hunched posture. Firm abdomen, pale extremities, purple extremities, partially closed left eye, dilated pupils, and black colored eyes observed in male only.
	• Hyperactivity, pale extremities, and incidence of red was observed in males on Day 3 and 7.
	• Liver weight (relative) was increased in HD females without correlation to clinical chemistry or histopathological changes
	• Kidney histology showed cortical tubular vacuolation in all animals, including vehicle controls with similar severity. This is likely a vehicle-related finding.
	• Lung histology showed a diffuse increase in alveolar macrophages in HD and vehicle controls, which was considered vehicle-related. Finding was present in LD and MD but at lower severity (these groups receive less vehicle).
	• Spleen histology showed a minor increase in hematopoiesis at the HD. LD was comparable to vehicle control, while MD was equivocal.

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	• Injection site histology showed minor increase in severity of inflammatory changes in MD females and HD males and females. Under the conditions of this study, 30 mg/kg/day was identified as a suitable high dose level for subsequent 4-wk studies in rats.
RXA5004 GLP 4-week Monkey 2-week recovery IV bolus 0 (vehicle), 5, 10, 20 mg/kg Vehicle = 25.86% (b) (4) pH 3.8	4-wk repeat-dose study with 2-wk recovery period. Evaluated mortality, clinical signs, body weight, ophthalmoscopy, ECG, TK, clinical chemistry/urinalysis/hematology, gross necropsy, organ weights, and histopathology. • Pharmacological effects of sedation occurred at all dose levels (recumbency, unresponsiveness, drowsiness, and unsteady gait). • ECG effect included prolongation of RR and PR interval at all doses associated with heart rate reduction related to pharmacology • Dose-dependent decrease in LDH in all groups • Histopathology o Kidney: focal or multifocal tubular vacuolation at all doses with a small increase in incidence in males of the MD and HD. Changes persisted in a few recovery animals. Potentially vehicle-related. o Liver: increased cytoplasmic rarefaction in MD and HD females, not present in recovery groups. Liver changes in females suggest a metabolic response in the liver related to glycogen storage and may be connected to the pharmacological effect of the test article. o Injection site: various inflammatory changes and hemorrhage, suggesting an irritant effect at all dose levels including vehicle controls. Changes were partially reversible during the recovery period. Potentially vehicle- or procedure-related. o Inguinal lymph nodes: pigmented macrophages considered related to the hemorrhage at the injection site in all groups including vehicle controls. NOEL = 5 mg/kg/day
RMN1018 GLP 4-week Rat 2-week recovery IV bolus 0 (vehicle), 1, 20, 30 mg/kg Vehicle = 25.86% (b) (4) pH 3.8	4-wk repeat-dose study with 2-wk recovery. Evaluated mortality, clinical signs, body weight, food consumption, ophthalmoscopy, TK, clinical chemistry/urinalysis/hematology, gross necropsy, organ weights, and histopathology. • One death from respiratory depression and smothered by sleeping cage mates • Blood in urine in all groups including vehicle controls considered procedural • Increased incidence of protein in urine in HD females • Yellow or dark yellow urine in HD females compared to straw color of controls • Histopathology o Local effects at the injection site (increased perivascular inflammation and increased proliferation of the intima was considered vehicle related) while other local effects were considered test article-related o Foamy macrophages in spleen was considered non-adverse because splenic function was not impaired o Other histopathological findings in kidney, lymph nodes, lungs, salivary glands, testes, and epididymides were considered vehicle-related Systemic NOEL = 10 mg/kg Systemic NOAEL = 20 mg/kg Local NOAEL = could not be determined based on the findings at the local tissues at all doses
RMN1017 GLP 1-wk	"Intravenous Maximum Tolerated Dose (MTD) and 7 Day Repeat Dose Study in the Beagle Dog"

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IV bolus Beagle dog 0, 0.75, 5 mg/kg Vehicle = 25.86% (b) (4)	Evaluated clinical signs, body weight, food consumption, TK, clinical pathology, terminal investigations. • **0.75 mg/kg:** Clinical signs of agitation, lip licking, vocalization, incoordination, impaired mobility, excessive resistance to dosing and salivation post-dosing occurred before the completion of dosing. Hyperactivity, lip licking, abnormal gait, salivation occurred 38 min after dosing with recovery by 2.5 hours after dosing. • **5 mg/kg:** Clinical signs after dosing included lateral recumbency, tremors, head shaking, noisy breathing, labored breathing, salivation, and muscular rigidity. Upon examination, no obvious signs of sedation were seen and the eye reflex was normal. The female appeared to be aware of its surroundings but was unable to physically respond and appeared in distress. Clinical signs were reversed with flumazenil. By 1 h 10 min after dosing, the animal was hyperactive. Approximately 2 h after dosing, female had watery feces. Full recovery was noted by 3.5 h after dosing. • No changes in body weight or food consumption were noted. • Because of the substantial clinical signs, TK, clinical pathology and terminal investigations were cancelled. Under the conditions of this study, it was determined that the beagle is not a good model for the toxicological assessment of remimazolam because of the substantial clinical signs preventing higher dosing.
(b) (4) 015-100 GLP 2-week Monkey 2-week recovery 9h IV infusion 0 (saline), 6.75, 9.0, 11.25, 22.5 mg/kg	"A 2-Week Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkeys Followed by A 2-Week Recovery Period" Evaluated clinical signs, body weight, food consumption, body temperature, blood pressure, ophthalmology, ECG, clinical pathology (hematology, blood chemistry, urinalysis), necropsy, organ weights, and histopathology. • Pharmacological effects of sedation occurred at all dose levels (incomplete eyelid opening, eyelid closure, decreased spontaneous activity, sedation, somnolence, ataxic gait, vomiting, sitting position, lateral position immediately after end of dosing) • No other test article-related adverse effects were noted NOAEL = 22.5 mg/kg/day
(b) (4) 015-133 Non-GLP Single and 4-day Repeat Monkey 4h IV infusion 0 (saline), 1.6, 3.2, 6.4 mg/kg/day Midazolam comparator (2.16 mg/kg left arm + 0.72 mg/kg right arm)	"A 5-Day Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkeys" Evaluated clinical signs, body weight, gross pathology, and limited histopathology (lungs, kidney, injection site, postcava) • Pharmacological effects of sedation occurred at all dose levels (incomplete eyelid opening, eyelid closure); ataxia, somnolence, and sedation occurred at the MD and HD. Similar effects were seen in midazolam comparator group • Erythema occurred at the injection site in 1 LD animal on Day 3, and swelling at the injection site in 1 HD animal on Day 3 and 4 • After single dosing, histopathology revealed desquamation of the endothelium, inflammation of the vascular wall, and thrombus in the HD Midazolam arm and HD test article group. Necrosis was also observed in the HD midazolam arm, which was absent in the HD test article group. • After 4-day repeated dosing, desquamation of the endothelium, hypertrophy/proliferation of the endothelium, inflammation of the vascular wall, necrosis of the vascular intima, and/or thrombus occurred at the LD and HD midazolam arm at the HD test article group.

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	• The changes in the HD test article group were more severe than those of the LD midazolam arm but similar in degree to that of the HD midazolam arm. • No effects of test article at the LD or MD were noted after single or 4-day repeated dosing
RZA5000 GLP 14-day Repeat Monkey IV bolus 1, 5, 7.5, 10, 12.5, 15, 17.5 mg/kg/day until the maximum tolerated dose was determined (20 mg/kg/day). Once 20 mg/kg/day was seen to be tolerated, the dosing period was extended out to 14 days. Vehicle = 25.86% (b) (4)	"Intravenous (Bolus) Maximum Tolerated Dose (MTD) and 14 Day Repeat Dose Study in the Primate" Evaluated clinical signs, body weight, and clinical pathology (hematology, blood chemistry, urinalysis) • Pharmacological effects of sedation occurred at 20 mg/kg/day (lateral recumbency, incoordination and unsteady gait) with recovery by 2 hours after dosing • Tremors, loose feces, hunched poster also occurred Under the conditions of this study, the maximum tolerated dose level for remimazolam when administered IV to monkey is 20 mg/kg/day. The Sponsor stated that for a subsequent study (e.g. 28-day repeat-dose), a dose escalation phase of approximately 2 weeks should be used before dosing at a HD of 20 mg/kg/day
RXA5004 GLP 4-week Monkey 2-week recovery IV bolus 0 (vehicle), 5, 10, 20 mg/kg/day (prior to start of treatment, animals were acclimated to the test article) Vehicle = 25.86% (b) (4) pH 3.8	"Four Week Intravenous (Bolus) Repeat Dose Toxicity Study in the Cynomolgus Monkey with a 2 Week Recovery Period" Evaluated clinical signs, body weight, ophthalmoscopy, ECG, TK, clinical chemistry, necropsy, limited histopathology (kidney, liver, injection site, inguinal lymph nodes) • Pharmacological effects of sedation occurred at all dose levels • Kidney histopathology revealed vehicle-related focal or multifocal tubular vacuolation in all groups, with partial recovery at the end of 2 weeks. • Liver histopathology revealed increased cytoplasmic rarefaction in MD and HD females, with full recovery after 2 weeks • Injection site histopathology revealed various inflammatory changes and hemorrhage, suggestive of an irritant effect in all groups with partial recovery after 2 weeks. This could be procedure- or vehicle-related effects. • Inguinal lymph node histopathology revealed pigmented macrophages in most animals across all groups and was considered related to the hemorrhage at the injection site. This could be procedure- or vehicle-related effects. • The MD was associated with slight increase in severity of the kidney changes with a minor change in the liver of females. NOEL = 5 mg/kg/day based on the slight increase in severity of the kidney findings and minor changes in the liver findings at the next higher dose of 10 mg/kg/day
32236 Non-GLP	"14-Day Dose Range Finding Study of CNS7056 (Remimazolam) in Goettingen Minipigs Following Repeated Intravenous Infusion up to 6 Hours"

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14-day Minipig 6h IV infusion 21, 24, 27, 30, 37.5, 45, 90 mg/kg/day until the maximum tolerated dose was determined (120 mg/kg/day). Once 120 mg/kg/day was seen to be tolerated, the dosing period was extended out to 14 days Midazolam comparator (15 mg)	Evaluated clinical signs, body weight, food consumption, TK, necropsy, and limited histopathology (infusion site only). • Pharmacological effects of sedation occurred during dose escalation (shivers, lateral position, slight to marked decreased motility, restlessness, chewing movements, apathetic behavior, salivation) • Pharmacological effects of sedation occurred at 120 mg/kg similar to midazolam, with no signs of systemic intolerance reactions. Full reversal of these effects occurred by 2 hours after dosing. • Midazolam showed signs of systemic intolerance reaction in 2 animals, both were found in lateral position on Day 1 and 2. One experienced emesis on Day 1. Based on the conditions of this study, 120 mg/kg/day remimazolam was considered the maximum feasible dose. The following dose levels were selected for the 4-week toxicity study (6h IV infusion of remimazolam) in minipigs: 0, 12, 40, 120 mg/kg/day

Two repeat-dose toxicology studies using infusion dosing of the clinical formulation (remimazolam with (b) (4) mg/mL lactose monohydrate and (b) (4) mg/mL Dextran 40 in saline) in monkey and minipig are reviewed below.

6.2.1 Minipig Repeat-Dose Toxicity

Study title: 4-Week Subchronic Study of CNS7056 (Remimazolam) in Goettingen Minipigs Following Repeated Intravenous 6-Hour Infusion

Study no.:	32237
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 9, 2017
GLP compliance:	Yes, this study was performed in compliance with GLP regulations of the EC enacted in (b) (4) and OECD Principles of GLP, which are compatible with FDA GLP
QA statement:	Yes, QA signed June 8, 2017
Drug, lot #, and % purity:	Remimazolam (CNS7056 besylate), Lot 10201238, 100.1% pure

Key Study Findings

In this repeat-dose toxicity study, minipigs (3-5/sex/group) were intravenously infused with remimazolam at target doses of 0 (vehicle), 12, 40, and 120 mg/kg/day for 6-hours/day for 4-weeks. Recovery animals (2/sex/group) in the 0 and 120 mg/kg/day dose groups were administered a treatment free period for 2 weeks after dosing for the recovery period. In addition to the expected battery of evaluations for a general

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toxicology study, this study also included auditory evaluations, body temperature, blood pressure, cytokine analysis, and spermiograms (spermatid count, sperm motility, and sperm morphology).

- Pharmacodynamic effects were noted in all treatment groups
- Procedure-related histopathological findings caused by the indwelling catheter were noted in all groups including controls: chronic vascular inflammation, neutrophilic perivascular infiltrations, perivascular hemorrhage, acute thrombosis, chronic and partially chronic-active thrombophlebitis and endothelial hyperplasia at the infusion sites (all groups); lymphohistocytic infiltrates in the epicardium (2/5 male and 1/5 female controls; 1/3 male and 1/3 female LD; 1/3 female MD; and 1/5 male and 1/5 female HD); neutrophilic infiltrations in the cardiac muscle (1/5 control males; 1/3 LD females; 1/3 MD males); edema (1/3 MD females; 1/5 HD males and 1/5 HD female) and hemorrhage (1/5 control males; 1/3 MD males; 1/5 HD females) in the heart.
- Histopathological findings secondary to the inflammation also occurred in all groups including controls: chronic-active glomerulonephritis in the kidney (1/5 HD males), chronic inflammation of the gall bladder (1/3 MD females), lymphohistiogranulocytic infiltrations in the liver (1/5 control males, 1/3 LD males, 1/5 HD females), and chronic and granulomatous inflammation of the lungs (all groups).
- TK was conducted for the parent CNS 7056, but not the metabolite CNS 7054
- Target organ was the CNS based on the sedation occurring at all treatment doses
- NOAEL = 120 mg/kg (Male AUC = 41,476 ng*h/mL, Female AUC = 27,229 ng*h/mL; Male C_{max} = 9879 ng/mL, Female C_{max} = 6385 ng/mL) based on the lack of test article-related toxicity findings in this study.

Remimazolam dose levels						
Group	Remimazolam dose# [mg/kg b.w.]	Administration volume [mL/kg b.w./h]	Remimazolam concentration [mg/mL]	Infusion duration [h]	Animal number MS	Animal number RP
1	0 (vehicle control)	4	0	6	1 m, 2 m, (3 m) 33 m*	4 m, 5 m
					6 f, 7 f, 8 f	9 f, 10 f
2	12 (low dose)	4	0.5	6	11 m, 12 m, 13 m	None
					14 f, 15 f, 16 f	
3	40 (intermediate dose)	4	1.67	6	(17 m), 18 m, (19 m) 34 m*, 35 m*	None
					20 f, 21 f, 22 f	
4	120 (high dose)	4	5	6	23 m, 24 m, 25 m	(26 m), 27 m 36 m*
					28 f, 29 f, 30 f	31 f, 32 f

m: male
f: female
MS: main study
RP: recovery period
#: all doses and concentrations indicated refer to the Remimazolam free drug base
*: male animals nos. 33 to 36 replaced animals nos. 3, 17, 19 and 26

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Methods

Doses:	0 (vehicle), 12, 40, 120 mg/kg Doses were selected based on available data obtained in a 14-day dose-range finding study in Gottingen minipigs following repeated intravenous 4-/6-hour infusions up to 120 mg/kg/day (32236). High dose for the current study was selected based on limitations set by the duration of sedation, the limit of local tolerability for concentration, and acceptable volume burden for this species (1 mL/kg/h within 24 h).
Frequency of dosing:	Once daily for 4 weeks
Route of administration:	Intravenous 6-hour infusion into vena jugularis via pre-implanted indwelling catheter
Dose volume:	24 mL/kg/day (4 mL/kg/h for 6 hours)
Formulation/Vehicle:	(b) (4) mg/mL (pH 3.0±0.1) / (b) (4) mg/mL lactose monohydrate and (b) (4) mg/mL Dextran 40 in 0.9% saline
Species/Strain:	Minipigs / Gottingen
Number/Sex/Group:	5/sex/main group – control and HD 3/sex/main group – LD and MD 2/sex/recovery group
Age:	6 to 8 months at dosing
Weight:	9.0 to 12.9 kg at dosing
Satellite groups:	Toxicokinetic analysis of the parent was performed on the main and recovery study animals. No metabolites were analyzed.
Unique study design:	Additional evaluations in this study were: • Auditory evaluation • Body temperature • Blood pressure • Cytokine (IL-6 and CRP) • Spermiogram (spermatid count, sperm motility, and sperm morphology)
Deviation from study protocol:	This study had minor deviations that were judged not to affect the validity of the results from this study, as described below: 1. The formulation samples were analyzed for the test article according to the validated analytical method (HPLC-UV). The method was validated in (b) (4) validation study no. 32238. As the HPLC equipment used in the (b) (4) study no. 32238 was replaced, a partial re-validation of the method was performed in

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order to verify the suitability of the new analytical equipment. Intra-day and inter-day precision were determined in the concentration ranges low (0.5 mg/mL), intermediate (1.67 mg/mL), and high (5 mg/mL). The acceptance criteria for accuracy and precision of (b) (4) study no 32238 were applied and confirmed. For the re-validation the following batch of remimazolam was used: batch no. 10201253.

2. On Test Day 17 (8/12/2016) animal no 11m was dosed 264 mL instead of 266.4 mL as a result of calculation error.
3. During ECG recording the processing speed of the ECG paper was 25 mm/sec for animal no. 1m during infusion and at the end of the infusion instead of 50 mm/sec.
4. Assessment of local skin reactions based on Draize were omitted as the test article was administered via an indwelling systemic central catheter. Thus, local tolerance was only assessed at necropsy at the tip of the catheter.
5. The body weight of two animals (animal no. 9 and 32) exceeded the predefined range of 10±2 kg b.w.

Mortality

No test article-related mortality occurred in this study.

Clinical Signs

Clinical signs for every animal were recorded before and after dosing. Cage-side observations were conducted daily.

Pharmacodynamic effects of sedation occurred in a dose-related manner in both males and females. A summary of the sedation scores for males are presented in the tables below.

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Sedative effect scores - Male animals Mean of scores noted across all measurement days, i.e. test days 1, 4, 7, 14, 21 and 28								
Time [min] after start of infusion	Group 1 vehicle control		Group 2 12 mg Remimazolam/ kg b.w.		Group 3 40 mg Remimazolam/ kg b.w.		Group 4 120 mg Remimazolam/ kg b.w.	
Symptom 1								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
predose	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00
5 min	0.00	±0.00	1.11	±0.58	1.56	±0.17	3.13	±0.24
180 min	0.00	±0.00	1.67	±0.42	2.00	±0.37	3.00	±0.22
350 min	0.00	±0.00	1.31	±0.63	1.86	±0.21	2.46	±0.27
Symptom 2								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
predose	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00
5 min	0.00	±0.00	0.22	±0.27	1.17	±0.28	1.93	±0.79
180 min	0.00	±0.00	0.28	±0.25	1.11	±0.17	2.00	±0.52
350 min	0.00	±0.00	0.22	±0.17	1.11	±0.40	1.87	±0.55

Symptom 1

Response when a sound was made by tapping on the side of the hammock frame caudal to the animal's head.

0 normal
1 slight latency in response to background stimulus
2 marked latency in response to background stimulus
3 no response to background stimulus
4 no response to direct stimulus (light)

Symptom 2

Response when the pad of the forelimb or hind limb was pinched.

0 normal
1 slight latency in response to stimulus
2 moderate latency in response to stimulus
3 marked latency in response to stimulus
4 no response to stimulus

Sedative effect scores - Male animals Mean of scores noted across all measurement days, i.e. test days 1, 4, 7, 14, 21 and 28								
Time [min] after end of infusion	Group 1 vehicle control		Group 2 12 mg Remimazolam/ kg b.w.		Group 3 40 mg Remimazolam/ kg b.w.		Group 4 120 mg Remimazolam/ kg b.w.	
Symptom 1								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
20 min	0.00	±0.00	0.22	±0.17	1.56	±0.17	2.83	±0.61
30 min	0.00	±0.00	0.00	±0.00	1.17	±0.18	2.43	±0.66
70 min	0.00	±0.00	0.00	±0.00	0.56	±0.46	1.30	±0.49
Symptom 2								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
20 min	0.00	±0.00	0.06	±0.14	1.67	±0.79	3.27	±0.56
30 min	0.00	±0.00	0.00	±0.00	1.17	±0.41	2.97	±0.53
70 min	0.00	±0.00	0.00	±0.00	0.89	±0.58	1.20	±0.54
Symptom 3								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
20 min	0.00	±0.00	0.06	±0.14	1.44	±0.34	2.60	±0.25
30 min	0.00	±0.00	0.00	±0.00	1.00	±0.21	2.10	±0.21
70 min	0.00	±0.00	0.00	±0.00	0.50	±0.28	0.77	±0.15
Symptom 4								
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
20 min	0.00	±0.00	0.44	±0.34	2.67	±0.52	3.43	±0.43
30 min	0.00	±0.00	0.00	±0.00	2.11	±0.17	3.10	±0.60
70 min	0.00	±0.00	0.00	±0.00	1.39	±0.39	1.50	±0.41

Symptom 1

Examined using the angle of gradient of the neck as index.

0 normal
1 slight decrease in muscle tone
2 moderate decrease in muscle tone
3 marked decrease in muscle tone
4 loss of muscle tone

Symptom 2

Response when an empty feeder was placed in the pen.

0 normal
1 decrease in tail-wagging or vocalization
2 decrease in exploratory behaviour
3 disappearance of exploratory behaviour
4 no response to feeders

Symptom 3

Response when a sound was made by tapping on the side of the pen caudal to the animal's head.

0 normal
1 slightly latency in response to background stimulus
2 marked latency in response to background stimulus
3 no response to background stimulus
4 no response to direct stimulus (pain)

Symptom 4

Response when the pig was disbalanced by slight pushing (gait analysis).

0 Normal
1 Slightly abnormal gait
2 Stumbling
3 Frequent falls
4 Supine or prone position

In both sexes, increasing levels of sedation occurred with increasing dose level. Sedation occurred within 5 minutes of the start of infusion and lasted for up to 20 minutes after the end of infusion in the LD and up to 70 minutes after the end of infusion in the HD.

No test article-related clinical sign of toxicity occurred in this study.

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Body Weights

Body weights were recorded weekly. No test article-related findings occurred.

Food Consumption

Food consumption was calculated daily. Animals were fed 1 hour after the end of infusion and any remaining food was collected the following morning. No test article-related findings occurred.

Ophthalmoscopy

Ophthalmological examinations for all animals occurred before first dosing, at the end of test week 4, and at the end of the recovery period. Eyes were examined with an Heine ophthalmoscope. After examining the pupillary reflex, Stulln® eye drops were used to produce mydriasis prior to examination of the following structures:

- Adnexa oculi (i.e. lids, lacrimal apparatus), conjunctiva
- Cornea, anterior chamber
- Lens, vitreous body, fundus (retina, optic disc)

No test article-related findings occurred.

Auditory Evaluations

Auditory evaluations for all animals occurred before first dosing, at the end of test week 4, and at the end of the recovery period. Auditory acuity was checked with a simple noise test. No test article-related findings occurred.

ECG

Electrocardiography (ECG) recordings were collected for all animals before first dosing, at the end of test week 4, and at the end of the recovery period. Recordings were made using the standard limb leads I, II, and III, augmented limb leads aVR, aVL, and aVF. A standardization of 10 mm = 1 mV was used and a paper speed of 50 mm/sec. The recordings were carried out for 1 minute. Recordings were examined visually for any abnormalities of the electrical complexes in addition to the following parameters:

Heart rate	(beats/min)
QRS interval	(ms)
QT interval	(ms)
PQ interval	(ms)
P segment	(ms)

In addition, the QTc values were calculated according to the following formula:

van de Water formula: **QTc = QT – 0.087 (R-R distance – 1000)**

No test article-related findings occurred.

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Blood pressure

Peripheral arterial systolic and diastolic blood pressure was evaluated in all animals before first dosing, at the end of Test Week 4, and at the end of the recovery period. Peripheral arterial and diastolic blood pressure was measured from the shaven tail of the conscious animal using a blood pressure monitor (PM-900 vet). No test article-related findings occurred.

Body Temperature

Body temperature was evaluated in all animals before first dosing, at the end of Test Week 4, and at the end of the recovery period. No test article-related findings occurred.

Hematology

For hematology, blood samples were collected from the vena jugularis from fasted animals in the main and recovery group during predose, on test day 28, and at the end of recovery period. Parameters analyzed are shown in the table below.

Haematological parameters		
Parameter	**Units**	**Instrument**
Differentia blood count[5] (relative)	%	ADVIA™120 Siemens Diagnostics GmbH 35463 Fernwald Germany
Differential blood count (absolute)	$10^3/\mu L$	
Erythrocytes (RBC)	$10^6/\mu L$	
Leucocytes (WBC)	$10^3/\mu L$	
Haematocrit value (HCT)	%	
Haemoglobin content	mmol/L	
Platelets (PLT)	$10^3/\mu L$	
Reticulocytes (RBC)	%	
Mean corpuscular volume (MCV)	fL	
Mean corpuscular haemoglobin (MCH)	fmol	
Mean corpuscular haemoglobin concentration (MCHC)	mmol/L	

Coagulation and ESR parameters		
Parameter	**Units**	**Instrument**
Thromboplastin time (TPT)	sec	Amax Destiny Plus Tcoag Deutschland GmbH 32657 Lemgo Germany
Activated partial thromboplastin time (aPTT)	sec	
Erythrocyte sedimentation rate (ESR)	mm/one hour	DISPETTE® (ICSH Reference Method) 6330 Cham (Zug) Switzerland

No test article-related findings occurred.

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Clinical Chemistry

For clinical chemistry, blood samples were collected from the vena jugularis from fasted animals in the main and recovery group during predose, on Test Day 28, and at the end of recovery period. Parameters analyzed are shown in the table below.

Biochemical parameters		
Parameter	**Units**	**Instrument**
Sodium	mmol/L	KONELAB 30i ThermoFisher Scientific 63303 Dreieich Germany
Potassium	mmol/L	
Calcium	mmol/L	
Chloride	mmol/L	
Triglycerides	mmol/L	
Total bilirubin	µmol/L	
Total cholesterol	mmol/L	
Glucose	mmol/L	
Total protein	g/L	
Blood urea (BUN)	mmol/L	
Creatinine	µmol/L	
Alanine amino-transferase (ALAT (GPT))	U/L	
Alkaline phosphatase (aP)	U/L	
Aspartate amino-transferase (ASAT (GOT))	U/L	
Gamma-glutamyl-transferase (γ-GT)	U/L	
Lactate dehydrogenase (LDH)	U/L	
Bile acids	µmol/L	
Albumin	g/L	Agarose gel electrophoresis with Hydasys LC Sebia GmbH 36043 Fulda Germany
Globulin	g/L	
Albumin/globulin ratio	non-dimensional	by calculation

Main study animals were tested for cytokines parameters listed in the table below.

Cytokine parameters			
Parameter	**Total amount of sample (serum)**	**Aliquots to be prepared**	**Analytical method**
Interleukin-6 (IL-6)	300 µL	2 × 150 µL	ELISA
C-reactive protein (CRP)	300 µL	2 × 150 µL	

No test article-related findings occurred.

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Urinalysis

Urine for urinalysis was collected in a metabolism cage for 3 hours following gavage of 50 mL/kg water. Urine was collected from main and recovery animals at predose, at the end of Test Week 4, and at the end of the recovery period. The urinalysis parameters are listed in the table below.

Urinary parameters		
Parameter	**Units**	**Instrument**
pH	non-dimensional	Digital pH-meter type WTW InoLab pH 720
Specific gravity	g/mL	Kern Refractometer type ORA 2PA Sample compared with water (nominal value of 1.000)

Urinary analytes						
Parameter	**Reporting convention**					
	Units	**Semi-quantitative determination level**				
Protein	g/L	neg	0.3	1	5	
Glucose	mmol/L	normal	2.8	5.5	17	55
Bilirubin	-	neg	+	+ +	+ + +	
Urobilirubin	µmol/L	normal	17	70	140	200
Ketones	-	neg	+	+ +	+ + +	
Haemoglobin (Hb) (approx. values)	Ery/µL	neg	10	25	50	250
Nitrite	-	neg	pos			

neg = negative
pos = positive
Ery = erythrocytes count
+ = 'small amount' of analyte
+ + = 'moderate amount' of analyte
+ + + = 'large amount' of analyte

Epithelial cells (E)
Leucocytes (L)
Erythrocytes (R)
Organisms (B)
Further constituents (i.e. sperm, casts) (C)
Crystalluria (A)

No test article-related findings occurred.

Gross Pathology

No test article-related macroscopic changes occurred in any group.

Procedure-related local findings cause by inflammatory reactions to the implanted indwelling catheter (i.e., thickened and induration around the catheter tip, including the traversed vein) occurred in individual animals of all groups, including controls. Swelling and thickening of the skin occurred around the infusion port of one control male and one LD male

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Procedure-related findings caused by the catheter implantation included increased fatty tissues and fibrin deposits in the heart, fibrin deposits, inflammatory reactions, and dark brown and partially clear fluid in the pericardium.

Reddened and indurated lobes of the lung and morphological changes in the gall bladder were considered incidental.

Organ Weights

Organ weights were determined prior to fixation. The following organs were weighed.

Adrenal gland (2)	Liver	Ovary (2)	Thymus
Brain	Lungs	Pituitary	Thyroid (1)
Epididymis (2)	Lymph nodes	Spleen	(incl. parathyroids)
Heart	(cervical (1), mesenteric (1))	Testicle (2)	
Kidney (2)			

No test article-related findings occurred.

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Histopathology

Adequate Battery: Yes

Tissues/organs collected at necropsy	
Adrenal gland (2)	Nerve (*sciatic*)
Aorta abdominalis	Oesophagus
Bone (*os femoris* with joint)	Ovary (2)
Bone marrow (*os femoris*)	Pancreas
Brain (*cerebrum*, *cerebellum*, brain stem, *hippocampus*, paraventricular parts	Pituitary
Caecum	Pulmonary thrombi (if present) with surrounding tissue
Epididymis (left)#	Prostate
Eye with optic nerve and lacrimal gland (2)	Salivary glands (*mandibular*, *sublingual* and *parotid gland*)
Gall bladder	Seminal vesicle (2)
Gross lesions observed	Skin (left flank)
Heart (left and right ventricle, septum)	Spinal cord (3 sections)
Infusion site (tip of the catheter in the vein)	Spleen
Intestine, large (colon, rectum)	Sternum
Intestine, small (duodenum, jejunum, ileum)	Stomach
Kidney (and ureter) (2)	Testicle (left)#
Liver (3 sections: left [medial], right [medial] and caudal lobe)	Thymus
Lungs (with mainstem bronchi and bronchioles; 3 sections, one through each of 3 different lobes)	Thyroid (1) (incl. parathyroids)
Lymph node (cervical (1))	Tissue masses or tumours (incl. regional lymph nodes)
Lymph node (mesenteric (1))	Tongue (incl. *larynx*)
Mammary gland	Urinary bladder
Muscle (skeletal, leg)	Uterus (incl. *cervix* and *oviducts*)
	Vagina

#: the second epididymis and testicle was not preserved but used for the sperm count

Bone marrow was obtained from the 7th rib (3 air-dried smears/animal) of all main and recovery animals and was stained according to Pappenheim. Cell differentiation (counting 200 nuclei-containing cells) was conducted in all animals for the determination of the myeloid:erythroid ratio.

Peer Review: No

Histopathological Findings

No test article-related histopathological changes occurred in the main or recovery group.

Procedure-related findings caused by the indwelling catheter occurred as inflammatory response in the heart and infusion sites of all test groups. Changes occurring more frequently included chronic vascular inflammation, neutrophilic perivascular infiltrations,

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perivascular hemorrhage, acute thrombosis, chronic and partially chronic-active thrombophlebitis and endothelial hyperplasia at the infusion sites (all groups); lymphohistocytic infiltrates in the epicardium (2/5 male and 1/5 female controls; 1/3 male and 1/3 female LD; 1/3 female MD; and 1/5 male and 1/5 female HD); neutrophilic infiltrations in the cardiac muscle (1/5 control males; 1/3 LD females; 1/3 MD males); edema (1/3 MD females; 1/5 HD males and 1/5 HD female) and hemorrhage (1/5 control males; 1/3 MD males; 1/5 HD females) in the heart.

Findings secondary to the spread of inflammation included chronic-active glomerulonephritis in the kidney (1/5 HD males), chronic inflammation of the gall bladder (1/3 MD females), lymphohistiogranulocytic infiltrations in the liver (1/5 control males, 1/3 LD males, 1/5 HD females), and chronic and granulomatous inflammation of the lungs (all groups).

Incidental pathological background findings included degeneration/atrophy of the seminiferous epithelium in the testis accompanied by decreased secretion in the seminal vesicles, which occurs spontaneously and frequently in Gottingen minipigs. Incidences of these findings are detailed in the table below.

Finding	Section	Group 1 Control	Group 2 12 mg/kg Remimazolam	Group 3 40 mg/kg Remimazolam	Group 4 120 mg/kg Remimazolam
Testis, left - Degeneration/atrophy					
Number of affected animals per section plane:	1	0/5	2/3	1/3	4/5
	2	1/5	2/3	1/3	3/5
	3	1/5	2/3	1/3	4/5
	4	1/5	2/3	2/3	4/5
	5	2/5	2/3	1/3	5/5
	6	3/5	1/3	2/3	3/5
Total number of affected animals per group:		5/5	2/3	2/3	5/5
Seminal vesicle, left - Decreased secretion					
Number of affected animals per section plane:	1	1/5	1/3	1/3	2/5
	2	1/5	1/3	1/3	2/5
	3	1/5	2/3	1/3	2/5
	4	1/5	1/3	1/3	2/5
	5	1/5	2/3	1/3	2/5
	6	1/5	2/3	1/3	2/5
Total number of affected animals per group:		1/5	2/3	2/3	2/5
Seminal vesicle, right - Decreased secretion					
Number of affected animals per section plane:	1	1/5	1/3	2/3	3/5
	2	1/5	2/3	1/3	3/5
	3	1/5	2/3	1/3	3/5
	4	1/5	2/3	1/3	3/5
	5	1/5	2/3	1/3	3/5
	6	1/5	2/3	1/3	3/5
Total number of affected animals per group:		1/5	2/3	2/3	3/5

(../..): number of incidences / number of animals affected

Histopathology report states the historical background rate of the testis findings from the test facility are between 25-83%. Therefore, differences in the incidences in the table above were considered incidental.

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Special Evaluation

Spermiogram
From one testis (right) and epididymis (right) of all male animals, sperm viability and sperm morphology were examined for the untreated and treated animals according to the method described by Chahoud et al.

No test article-related influences occurred on the number of spermatids, the sperm motility, or sperm morphology.

Toxicokinetics

Blood sampling for toxicokinetics occurred according to the schedule in the table below.

Blood sampling schedule for toxicokinetics				
Blood sampling on test day	Groups	Sampling times		Number of plasma samples
- Stagger 1 -				
1 3 14 28	1 to 4 (n = 4 main study animals per time-point)	10 min 60 min 360 min 15 min 60 min 120 min	after start of infusion after start of infusion after start of infusion (immediately before end of infusion) after end of infusion after end of infusion after end of infusion	4 × 24
- Stagger 3 to 7 -				
1 3 14 28	1 to 4 (n = 20 main study animals per time-point)	10 min 60 min 180 min 355 min 15 min 60 min 120 min	after start of infusion after start of infusion after start of infusion after start of infusion before end of infusion process, i.e. before the infusion pump stops after end of infusion after end of infusion after end of infusion	4 × 140
- Stagger 8 -				
1 3 14 28	33 – 35	10 min 60 min 180 min 355 min 15 min 60 min 120 min	after start of infusion after start of infusion after start of infusion after start of infusion before end of infusion process, i.e. before the infusion pump stops after end of infusion after end of infusion after end of infusion	4 × 21
			Total number of samples:	**740**

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Toxicokinetic analysis was performed by the analytical Test Site (b) (4)
Results of the toxicokinetic analysis are summarized in the tables below.

Toxicokinetic evaluation of CNS7056 (Remimazolam)
– Non compartmental analysis# –

Toxicokinetic parameter		Group 2 12 mg Remimazolam/ kg b.w.		Group 3 40 mg Remimazolam/ kg b.w.		Group 4 120 mg Remimazolam/ kg b.w.	
		males	females	males	females	males	females
Test day 1							
C_{max}	[ng/mL]	461.20 ±136.42	1375.90 ±397.64	3756.13 ±2298.47	1726.93 ±184.08	12756.13 ±8160.78	4618.27 ±493.61
C_{last}	[ng/mL]	85.87 ±79.07	35.33 ±19.21	141.80 ±63.13	57.67 ±13.05	489.80 ±532.57	245.53 ±97.53
t_{max}	[h]	4.97 ±1.71	2.06 ±1.63	2.64 ±2.84	4.28 ±2.84	4.28 ±2.84	3.31 ±2.47
t_{last}	[h]	7.00 ±0	7.33 ±0.58	7.57 ±0.58	8.00 ±0	8.00 ±0	8.00 ±0
$t_{½}$	[h]	0.65 ±0.58	0.38 ±0.17	0.50 ±0.24	0.50 ±0.06	0.65 ±0.33	0.65 ±0.16
K_{el}	[h^{-1}]	1.69 ±1.04	2.03 ±0.76	1.69 ±0.96	1.40 ±0.15	1.24 ±0.50	1.10 ±0.24
Test day 28							
C_{max}	[ng/mL]	496.97 ±89.12	661.77 ±82.63	2286.37 ±961.95	1398.40 ±117.90	9878.90 ±5743.13	6385.07 ±3142.31
C_{last}	[ng/mL]	30.43 ±6.38	48.10 ±28.63	115.10 ±42.33	84.47 ±35.57	353.73 ±43.07	301.73 ±207.11
t_{max}	[h]	1.67 ±1.16	5.92 ±0	3.31 ±2.47	1.67 ±1.16	3.31 ±2.47	4.95 ±1.69
t_{last}	[h]	7.00 ±0	7.67 ±0.58	8.00 ±0	8.00 ±0	8.00 ±0	8.00 ±0
$t_{½}$	[h]	0.30 ±0.04	0.74 ±0.53	0.75 ±0.35	0.69 ±0.24	0.78 ±0.32	0.59 ±0.06
K_{el}	[h^{-1}]	2.31 ±0.31	1.26 ±0.70	1.05 ±0.43	1.11 ±0.46	0.98 ±0.33	1.18 ±0.12

#: results partially rounded for reasons of clarity
C_{max}: maximum measured plasma concentration
C_{last}: last measurable plasma concentration >LLOQ
t_{max}: time of C_{max}
t_{last}: time of C_{last}
$t_{½}$: elimination half-life
K_{el}: elimination rate constant

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Toxicokinetic evaluation of CNS7056 (Remimazolam) – Non compartmental analysis# –						
Toxicokinetic parameter	Group 2 12 mg Remimazolam/ kg b.w.		Group 3 40 mg Remimazolam/ kg b.w.		Group 4 120 mg Remimazolam/ kg b.w.	
	males	females	males	females	males	females
Test day 1						
$AUC_{0-6\,h}$ [h*ng/mL]	2373.57 ±609.61	4116.61 ±820.95	15866.27 ±8448.05	8521.11 ±1735.86	50702.79 ±21104.12	21521.92 ±8905.68
$AUC_{6-8\,h}$ [h*ng/mL]	254.39 ±75.60	275.04 ±259.30	1177.34 ±511.94	722.32 ±228.60	4037.28 ±2583.37	1893.19 ±594.05
$AUC_{0-t\,last}$ [h*ng/mL]	2573.53 ±619.22	4389.87 ±906.79	17057.71 ±8871.10	9258.32 ±1953.35	54795.30 ±20210.16	23463.50 ±9260.89
$AUC_{0-\infty}$ [h*ng/mL]	2691.92 ±502.18	4413.30 ±920.64	17174.92 ±8966.52	9296.95 ±1959.95	55543.49 ±20232.55	23682.10 ±9183.43
$AUC_{0-\infty}$/dose [h*kg*ng/mL/mg]	224.33 ±41.85	367.78 ±76.72	429.37 ±224.16	232.42 ±49.00	462.86 ±168.61	197.351 ±76.53
Test day 28						
$AUC_{0-6\,h}$ [h*ng/mL]	2436.25 ±420.43	3076.20 ±589.85	11363.79 ±4146.69	7070.90 ±942.03	41475.71 ±14169.40	27229.23 ±8679.39
$AUC_{6-8\,h}$ [h*ng/mL]	147.48 ±41.40	305.64 ±187.16	1093.44 ±937.95	730.82 ±19.98	2731.72 ±1099.98	2473.94 ±1375.88
$AUC_{0-t\,last}$ [h*ng/mL]	2574.06 ±454.30	3379.31 ±743.25	12472.07 ±5077.00	7810.39 ±918.56	44237.32 ±13062.77	29759.55 ±10093.86
$AUC_{0-\infty}$ [h*ng/mL]	2587.13 ±455.77	3419.31 ±736.96	12582.67 ±5073.34	7915.26 ±858.10	44618.39 ±13167.48	30013.32 ±10297.30
$AUC_{0-\infty}$/dose [h*kg*ng/mL/mg]	215.59 ±37.98	284.94 ±61.41	314.57 ±126.83	197.88 ±21.45	371.82 ±109.73	250.11 ±85.81

#: results partially rounded for reasons of clarity
$AUC_{0-6\,h}$: area under curve from time zero (0) to six (6) hours after start of infusion (p.a.)
$AUC_{6-8\,h}$: area under curve from six (6) hours p.a. to eight (8) hours p.a.
$AUC_{0-t\,last}$: area under curve from time zero (0) to the last measurable concentration
$AUC_{0-\infty}$: area under the concentration-time curve from time zero (0) extrapolated to infinity

The results confirmed a dose-related exposure of all animals during the intravenous 6-hour infusion. Parent compound decreased rapidly after the end of infusion.

Dosing Solution Analysis

Dosing solution analysis determined the concentrations were within 10% of the nominal values.

6.2.2 Monkey Repeat-Dose Toxicity

Study title: Toxicity Experiment with 4 Weeks of Repeated Intravenous RF10007 Injections in Cynomolgus Monkeys and 2 Week Recovery Period

Study no.:	13-0293RD
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/6/2013
GLP compliance:	"This experiment was conducted based on a validated experiment plan, observing the standard operational procedures of (b) (4) under GLP conditions. The experiment complied with the requirements of the CFDA Criteria for the Quality Control of Non-clinical Study of Drugs (Julingdi no. 2, September 2003)" Statement not signed in this translated report
QA statement:	Yes, QA statement signed on 2/12/2014
Drug, lot #, and % purity:	Test sample: RF10007, lot S6130401, 106.2% pure Original reference product: RF10007P, lot CNS2501A, 96.0% pure

The objective of this study was to compare the original reference product to the clinical formulation in a 4-week repeat dose toxicity study. In this 4-week repeat-dose toxicity study, monkeys (2/sex/group) were intravenously administered the original reference product at a target dose of 25 mg/kg and remimazolam at target doses of 0 (vehicle), 12.5, 25, and 50 mg/kg/day using a 5 min bolus followed by a 2-hour infusion once weekly for 4-weeks (total of 5 doses). A recovery and group consisted of an additional 2/sex/group. Toxicokinetic analysis was performed on main and recovery animals (4/sex/group). Clinical observations, body weight and temperature, ECG, blood pressure, hematology, clinical chemistry, urinalysis, ophthalmology, and TK occurred in all animals during the dosing period. Necropsy and histopathological examination was performed on all animals. In general. The test article was well tolerated with a systemic LOAEL = 12.5 mg/kg/day and an acceptable local NOAEL = 1.25 mg/mL (MD).

Key Study Findings

- Pharmacodynamic effects of eyelid closing and muscle relaxation occurred 2-24 minutes after each administration in all animals administered the original reference product and all groups treated with the test article. These effect lasted 2 hours after the end of administration of the original reference product, and in the LD and MD groups. HD animals returned to normal 3 hours after the end of administration.

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- Heart rate decreased and QT and QTc intervals were prolonged in all treatment groups 1 hour after injection and generally returned to normal 2-4 hours after the end of administration.
    - Percent change compared to controls at 1 hour post dosing on Day 1:
        - Decreased HR: LD = -21%, MD = -24%, HD = -22%
        - Increased QT intervals: LD = +26%, MD = +26%, HD = +28%
        - Increased QTc intervals: LD = +15%, MD = +14%, HD = +17%
- Body temperature was decreased in all treatment groups below the range of normal (36.5^{o}C to 38.5^{o}C) 1 hour after the first administration with recovery by 2 hours after administration. Mean body temperature 1-hour post first dose: LD = 35.55 ^{o}C, MD = 34.66 ^{o}C, HD = 35.00 ^{o}C.
- Procedure-related histopathology included local findings characterized by slight to moderate mononuclear infiltration (Control: 2/4; Original Reference: 1/4; LD: 4/4; MD: 4/4; HD 4/4) and bleeding (Control: 3/4; Original Reference: 3/4; LD: 4/4; MD: 4/4; HD 3/4) at the injection sites and surrounding tissues. At the end of the 2-week recovery period, the local reactions were mostly recovered (Recovery Control: 4/4 normal; LD: 3/4 normal with 1/4 with slight bleeding; MD: 4/4 normal; HD: 3/4 normal with 1/4 with slight bleeding, mononuclear infiltration, and minor endothelial fibrosis).
- Treatment-related histopathology at the HD were local findings characterized by minor to moderate endothelial fibrosis (3/4) and minor thrombosis (1/4). Endothelia fibrosis was partially recovered (HD 1/4 with decreased severity) and thrombosis was not observed in the recovery animals.
- Target Organs: CNS (sedation was the pharmacodynamic effect at all doses), heart (cardiac rhythms were effected), and local tissue (various findings)
- Toxicokinetic results indicate a dose-proportional increase with no accumulation after repeated weekly administration, and no sex differences.

TK parameter	Parent	Metabolite (RF7054)
Half-life (h)	0.13 to 1.11	0.18 to 2.51
T-max (h)	0.00 to 1.04	0.00 to 2.33
C_{max} (ng/mL)		
LD (12.5 mg/kg/day)	614 to 4,263	5,050 to 13,465
MD (25 mg/kg/day)	2,029 to 7,453	4,897 to 36,662
HD (50 mg/kg/day)	2,427 to 17,616	15,031 to 90,692
AUC (ng*h/mL)		
LD (12.5 mg/kg/day)	1,422 to 12,059	7,616 to 45,199
MD (25 mg/kg/day)	4,124 to 21,560	21,150 to 121,931
HD (50 mg/kg/day)	4,449 to 46,534	72,017 to 400,775

- Systemic LOAEL = 12.5 mg/kg/day based on the prolonged QT and QTc intervals, decrease in heart rate, and decrease in body temperature 1 hour after the initial dose in this group and higher.
- Local NOAEL = 1.25 mg/mL (MD) based on the incomplete reversal of minor endothelial fibrosis of the blood vessel occurring at the next highest concentration 2.5 mg/mL (HD). Local reactions occurring at or below the NOAEL included slight bleeding and slight mononuclear infiltration at the blood vessel.

Reference ID: 4622909

Group		Drug administration dosage			Number of animals[a] Individuals/ gender	Animal no.	
		Induction mg/kg	Maintenance mg/kg	Total mg/kg/day		Male	Female
1	Negative control group	0	0	0	2+2	13-6401~13-6404	13-6405~13-6408
2	Original reference group	5	20	25	2+2	13-6409~13-6412	13-6413~13-6416
3	Low-dosage group	2.5	10	12.5	2+2	13-6417~13-6420	13-6421~13-6424
4	Medium-dosage group	5	20	25	2+2	13-6425~13-6428	13-6429~13-6432
5	High-dosage group	10	40	50	2+2	13-6433~13-6436	13-6437~13-6440

[a] The first 2 individuals/gender of Groups 1-5 were dissected on the day after the last drug administration, a further 2 individuals/gender were then dissected after a 2 week recovery period.

Group		Drug administration concentration mg/mL	Drug administration rate mL/kg/h		Drug administration time	
			Induction	Maintenance	Induction min	Maintenance h
1	Negative control group	0	48	8	5	2
2	Original reference group	1.25	48	8	5	2
3	Low-dosage group	0.625	48	8	5	2
4	Medium-dosage group	1.25	48	8	5	2
5	High-dosage group	2.5	48	8	5	2

Reference ID: 4622909

Methods

Doses:	0 (vehicle), 25 (original reference product), 12.5, 25, 50 mg/mL
Frequency of dosing:	Once a week for 4 weeks (5 doses)
Route of administration:	Intravenous bolus (48 mL/kg/h) over 5 minutes followed by 2-hour infusion (8 mL/kg/h)
Dose volume:	
Formulation/Vehicle:	0.625, 1.25, and 2.5 mg/mL / “a negative (auxiliary material) control product” in saline
Species/Strain:	Monkey / Cynomolgus
Number/Sex/Group:	2/sex/ main group 2/sex/ recovery group
Age:	3.5 to 5.5 years
Weight:	Male: 3.17 to 4.74 kg Female: 2.80 to 3.88 kg
Satellite groups:	TK was performed in all main and recovery animals (4/sex/group)
Unique study design:	Blood pressure and body temperature in addition to the typical battery of endpoints/parameters were analyzed in this study. Food consumption was not recorded in this study.
Deviation from study protocol:	Deviations were listed and were determined not to impact the reliability of the experimental results.

Mortality

No mortalities occurred in this study.

Clinical Signs

Clinical signs were recorded twice daily after cage-side observations. Detailed clinical observations occurred once weekly after the start of administration.

Pharmacodynamic effects of eyelid closing and muscle relaxation occurred 2-24 minutes after each administration in all animals administered the original reference product and all groups treated with the test article. These effect lasted 2 hours after the end of administration of the original reference product, and in the LD and MD groups. HD animals returned to normal 3 hours after the end of administration.

Local reaction characterized by wounds, swelling, and scabbing occurred in 1/4 LD and 1/4 MD in the left front limb where the catheter was implanted. The study report describes this as either as procedure-related or a result of fighting between animals. This Reviewer does not consider these adverse test article-related findings.

Reference ID: 4622909

Body Temperature

Body temperature was taken prior to 1st, 3rd, and 5th drug administration, 1 hour after drug administration, 2-4 hours after the end of drug administration, and prior to the end of the recovery period.

Results of body temperature measurements are summarized in the table below.

Table 30 Body Temperature Summary from a 4-Week Repeat-Dose (dosed once weekly) Toxicity Study in Monkeys

Group	Dosage (mg/kg/day)		Initial drug administration			3rd drug administration		
			pre-medication	1 hour after medication	2-4 hours after medication	pre-medication	1 hour after medication	2-4 hours after medication
Negative control group	0	Mean±SD	38.29±0.77	37.90±0.36	37.49±1.04	38.68±0.59	38.51±0.26	38.14±0.54
		n	8	8	8	8	8	8
Original reference group	25	Mean±SD	38.70±0.32	35.15±0.63*	35.49±1.31*	38.65±0.52	36.14±0.60*	37.43±0.94
		n	8	8	8	8	8	8
Low-dosage group	12.5	Mean±SD	39.06±0.50*	35.55±0.49*	37.48±0.70	39.19±0.29	36.55±0.38*	38.34±0.86
		n	8	8	8	8	8	8
Medium-dosage group	25	Mean±SD	38.96±0.46*	34.66±0.55*	37.64±0.48	39.16±0.29	36.08±0.37*	37.55±0.82
		n	8	8	8	8	8	8
High-dosage group	50	Mean±SD	39.00±0.36*	35.00±0.40*	36.39±1.33	39.74±0.35*	36.05±0.49*	35.03±1.18*
		n	8	8	7	8	8	8

Group	Dosage (mg/kg/day)		Last drug administration			D42
			pre-medication	1 hour after medication	2-4 hours after medication	
Negative control group	0	Mean±SD	39.38±0.40	38.10±0.45	38.53±0.50	38.80±0.63
		n	8	8	8	4
Original reference group	25	Mean±SD	39.34±0.52	36.06±0.32*	37.24±0.81*	38.85±0.42
		n	8	8	8	4
Low-dosage group	12.5	Mean±SD	39.21±0.32	36.65±0.38*	38.15±1.00	39.20±0.50
		n	8	8	8	4
Medium-dosage group	25	Mean±SD	39.23±0.40	36.29±0.34*	37.14±1.18*	39.28±0.34
		n	8	8	8	4
High-dosage group	50	Mean±SD	39.39±0.34	36.40±0.38*	35.51±1.07*	39.15±0.44
		n	8	8	8	4

Note: n indicates the number of animals in each group, * indicates a comparison with the negative control group over the same period, $P \leq 0.05$.

Decreased body temperature occurred 1 hour after injection with recovery after 2-4 hours. Core body temperature of free moving, radiotelemeterized, cynomolgus monkey ranged from 36.5°C to 38.5°C during a 22 hour period in a separate study (Gauvin et al., 2006). Therefore, body temperature decreases below 36.5°C are considered adverse by this Reviewer.

Reference ID: 4622909

Body Weights

Body weights were recorded weekly. No test article-related changes in body weight occurred.

Food Consumption

Animals were fed monkey chow twice daily (100 g/meal) and were provided vegetables and fruit once daily (50-100g/day). Study report does not indicate that food consumption was recorded, and no results of food consumption were located in the study report.

Ophthalmoscopy

Ophthalmoscopy was conducted at pre-dose period and after the dosing period at the time of sacrifice. Ophthalmologic examinations included animal eyelid, sclerae, cornea, conjunctiva, iris, pupil, lens, vitreous, and fundus. No test article-related changes were noted in the ophthalmology examinations.

ECG

ECG was recorded prior to 1st, 3rd, and 5th drug administration, 1 hour after drug administration, 2-4 hours after the end of drug administration, and prior to the end of the recovery period. ECG waveforms, heart rate, P-R interval, Q-T interval, and QRS duration was determined using limb II lead ECG indicators.

Results of the ECG are summarized in the table below.

Table 31 ECG Summary from a 4-Week Repeat-Dose (dosed once weekly) Toxicity Study in Monkeys

Test Time	Group	Dosage (mg/kg/day)		Heart rate (times/min)	P-R interval (s)	QRS duration (s)	Q-T interval (s)	QTc (s)
pre-D1 medication	Negative control group	0	Mean±SD	218.5±28.7	0.070±0.008	0.038±0.005	0.169±0.019	0.320±0.019
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	212.1±22.3	0.073±0.009	0.034±0.005	0.170±0.014	0.320±0.019
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	215.1±25.9	0.069±0.008	0.034±0.005	0.173±0.013	0.325±0.014
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	218.5±30.5	0.068±0.005	0.035±0.008	0.165±0.016	0.311±0.017
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	226.9±32.4	0.065±0.008	0.038±0.005	0.160±0.015	0.310±0.013
			n	8	8	8	8	8
1 hour after D1 medication	Negative control group	0	Mean±SD	231.9±39.1	0.066±0.007	0.036±0.005	0.169±0.022	0.326±0.019
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	195.6±20.0*	0.076±0.007	0.038±0.005	0.200±0.019*	0.360±0.020*
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	183.3±22.4*	0.075±0.005	0.039±0.004	0.213±0.018*	0.369±0.017*
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	175.4±19.0*	0.074±0.007	0.038±0.005	0.214±0.022*	0.365±0.020*
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	181.4±25.1*	0.076±0.011	0.039±0.004	0.216±0.021*	0.374±0.024*
			n	8	8	8	8	8

Reference ID: 4622909

Table 32 Percent change in HR, QT, and QTc from control at 1 hour post-dose on Day 1.

Group	Heart Rate	QT interval	QTc
Negative control	231.9 ± 39.1 bpm	0.169 ± 0.019 s	0.320 ± 0.019 s
Original reference	-15.6%	+18.3%	+12.5%
LD	-21.0%	+26.0%	+15.3%
MD	-24.4%	+26.0%	+14.1%
HD	-21.8%	+27.8%	+16.9%

Test Time	Group	Dosage (mg/kg/day)		Heart rate (times/min)	P-R interval (s)	QRS duration (s)	Q-T interval (s)	QTc (s)
2-4 hours after D1 medication	Negative control group	0	Mean±SD	216.3±25.8	0.071±0.008	0.038±0.007	0.178±0.013	0.336±0.009
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	196.5±17.5	0.075±0.005	0.038±0.005	0.200±0.014*	0.360±0.014*
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	219.9±25.9	0.068±0.007	0.038±0.005	0.176±0.013	0.336±0.017
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	204.8±14.5	0.071±0.006	0.035±0.008	0.180±0.009	0.334±0.015
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	193.9±31.4	0.071±0.008	0.039±0.004	0.198±0.031	0.350±0.027
			n	8	8	8	8	8
D14	Negative control group	0	Mean±SD	215.8±34.8	0.073±0.014	0.036±0.005	0.170±0.019	0.319±0.016
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	216.6±29.2	0.071±0.004	0.038±0.007	0.173±0.013	0.326±0.013
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	215.8±25.2	0.073±0.007	0.036±0.005	0.166±0.015	0.316±0.021
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	227.9±15.1	0.070±0.008	0.035±0.008	0.163±0.010	0.316±0.019
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	230.6±26.5	0.069±0.008	0.034±0.007	0.159±0.010	0.310±0.017
			n	8	8	8	8	8

Note: n indicates the number of animals in each group, * indicates a comparison with the negative control group over the same period, $P \leq 0.05$.

Test Time	Group	Dosage (mg/kg/day)		Heart rate (times/min)	P-R interval (s)	QRS duration (s)	Q-T interval (s)	QTc (s)
1 hour after D15 medication	Negative control group	0	Mean±SD	230.0±30.3	0.065±0.008	0.038±0.005	0.170±0.015	0.331±0.011
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	188.4±21.5*	0.073±0.009	0.038±0.005	0.201±0.022*	0.356±0.032
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	190.8±22.0*	0.071±0.006	0.035±0.005	0.200±0.013*	0.355±0.020
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	182.6±7.6*	0.071±0.006	0.036±0.005	0.209±0.010*	0.363±0.014
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	183.8±25.7*	0.074±0.005	0.034±0.005	0.205±0.024*	0.356±0.027
			n	8	8	8	8	8
2-4 hours after D15 medication	Negative control group	0	Mean±SD	190.0±23.0	0.079±0.008	0.031±0.008	0.185±0.014	0.329±0.014
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	216.5±28.8	0.070±0.009	0.038±0.009	0.180±0.015	0.340±0.016
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	228.9±26.8*	0.068±0.009	0.030±0.009	0.164±0.012*	0.320±0.030
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	222.8±20.8*	0.071±0.008	0.034±0.007	0.168±0.017	0.324±0.026
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	203.0±19.9	0.073±0.009	0.034±0.007	0.186±0.016	0.343±0.017
			n	8	8	8	8	8

Note: n indicates the number of animals in each group, * indicates a comparison with the negative control group over the same period, $P \leq 0.05$.

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Test Time	Group	Dosage (mg/kg/day)		Heart rate (times/min)	P-R interval (s)	QRS duration (s)	Q-T interval (s)	QTc (s)
D28	Negative control group	0	Mean±SD	221.1±36.9	0.073±0.007	0.034±0.007	0.164±0.013	0.311±0.011
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	222.0±32.6	0.073±0.005	0.034±0.007	0.161±0.011	0.309±0.017
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	230.4±23.6	0.066±0.009	0.035±0.005	0.156±0.009	0.304±0.019
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	222.8±30.3	0.066±0.007	0.036±0.009	0.164±0.015	0.311±0.010
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	227.3±36.0	0.069±0.010	0.036±0.005	0.163±0.015	0.315±0.009
			n	8	8	8	8	8
1 hour after D29 medication	Negative control group	0	Mean±SD	222.1±27.7	0.066±0.005	0.039±0.004	0.171±0.012	0.328±0.013
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	178.1±13.1*	0.076±0.007*	0.039±0.004	0.209±0.014*	0.360±0.017*
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	183.1±18.7*	0.071±0.008	0.034±0.005	0.203±0.010*	0.354±0.016*
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	185.0±22.6*	0.069±0.006	0.035±0.005	0.199±0.021*	0.346±0.024
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	183.3±27.6*	0.073±0.005	0.030±0.005*	0.195±0.025	0.338±0.025
			n	8	8	8	8	8

Test Time	Group	Dosage (mg/kg/day)		Heart rate (times/min)	P-R interval (s)	QRS duration (s)	Q-T interval (s)	QTc (s)
2-4 hours after D29 medication	Negative control group	0	Mean±SD	203.1±26.0	0.078±0.009	0.035±0.005	0.188±0.020	0.344±0.015
			n	8	8	8	8	8
	Original reference group	25	Mean±SD	203.6±21.0	0.076±0.005	0.034±0.005	0.183±0.015	0.335±0.018
			n	8	8	8	8	8
	Low-dosage group	12.5	Mean±SD	224.1±17.7	0.069±0.008	0.035±0.005	0.168±0.010*	0.323±0.013
			n	8	8	8	8	8
	Medium-dosage group	25	Mean±SD	240.8±11.1*	0.068±0.005*	0.038±0.005	0.161±0.012*	0.324±0.021
			n	8	8	8	8	8
	High-dosage group	50	Mean±SD	216.4±16.0	0.069±0.006	0.036±0.005	0.176±0.007	0.335±0.013
			n	8	8	8	8	8
D42	Negative control group	0	Mean±SD	204.5±46.6	0.075±0.006	0.033±0.005	0.170±0.027	0.310±0.012
			n	4	4	4	4	4
	Original reference group	25	Mean±SD	227.5±30.3	0.073±0.010	0.033±0.005	0.163±0.013	0.313±0.015
			n	4	4	4	4	4
	Low-dosage group	12.5	Mean±SD	217.3±34.7	0.068±0.005	0.035±0.006	0.168±0.025	0.313±0.022
			n	4	4	4	4	4
	Medium-dosage group	25	Mean±SD	205.8±17.2	0.073±0.010	0.040±0.008	0.170±0.008	0.315±0.013
			n	4	4	4	4	4
	High-dosage group	50	Mean±SD	217.0±31.8	0.070±0.008	0.033±0.005	0.163±0.010	0.308±0.010
			n	4	4	4	4	4

Note: n indicates the number of animals in each group, * indicates a comparison with the negative control group over the same period, $P \leq 0.05$.

Heart rate decreased and QT and QTc intervals were prolonged in all treatment groups 1 hour after injection and generally returned to normal 2-4 hours after the end of administration. Statistically significant decrease in PR interval occurring in the MD 2-4 hours after administration on Day 29 appears to be incidental.

Blood Pressure

Diastolic blood pressure, systolic blood pressure, and mean arterial pressure was recorded prior to 1st, 3rd, and 5th drug administration, 1 hour after drug administration, 2-4 hours after the end of drug administration, and prior to the end of the recovery period using a non-invasive blood pressure monitor (BP-2010E).

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No toxicologically significant test article-related effects on blood pressure occurred.

Hematology

Blood was collected for hematology at predose, the day after the third dose, the day after the 5th dose, and prior to the end of the recovery period. Adequate evaluation of hematology occurred as it included hematological parameters listed in the table below.

Test item	English acronym	Unit	Test method
White blood cell count	WBC	$\times 10^9$/L	Light scattering + sheath flow impedance method
Red blood cell count	RBC	$\times 10^{12}$/L	Light scattering + sheath flow impedance method
Hemoglobin	HGB	g/L	Cyanmethemoglobin colorimetry
Hematocrit	HCT	%	Calculated based on RBC and MCV
Mean red cell volume	MCV	fL	Calculated
average red cell hemoglobin	MCH	pg	Calculated
average red cell hemoglobin Concentration	MCHC	g/L	Calculated
blood platelet calculation	PLT	$\times 10^9$/L	Light scattering + sheath flow impedance method
Neutrophil	Neut	$\times 10^9$/L, %	Light scattering + RF method + sheath flow impedance method + light absorption method
lymphocyte	Lymph	$\times 10^9$/L, %	Light scattering + RF method + sheath flow impedance method + light absorption method
monocyte	Mono	$\times 10^9$/L, %	Light scattering + RF method + sheath flow impedance method + light absorption method
Eosinophilic granulocyte	Eos	$\times 10^9$/L, %	Light scattering + RF method + sheath flow impedance method + light absorption method
basophilic granulocyte	Baso	$\times 10^9$/L, %	Light scattering + sheath flow impedance method
reticulocyte count	Retic	%	Light scattering + DNA fluorescent staining method + sheath flow impedance method

Note: This experiment only analyzes the abovementioned indicators as detected by Advia2120 fully automatic blood cell analyzer, indicators other than other measured by this device are not analyzed.

Blood coagulation indicators

Item	English acronym	Unit	Test method
Prothrombin time	PT	s	Coagulation method
Activated partial thromboplastin time	APTT	s	Coagulation method

No toxicologically significant test article-related changes in hematology occurred.

Clinical Chemistry

Blood was collected for clinical chemistry at predose, the day after the third dose, the day after the 5th dose, and prior to the end of the recovery period. Adequate evaluation of clinical chemistry was conducted as it included parameters listed in the table below.

Reference ID: 4622909

Test item	English acronym	Unit	Test method
Alanine aminotransferase	ALT	U/L	Continuous monitoring method
Aspartate aminotransferase	AST	U/L	Continuous monitoring method
γ-glutamyl transferase	GGT	U/L	Continuous monitoring method
Total protein	TP	g/L	Biuret method
Albumin	Alb	g/L	Bromocresol green method
Albumin/globulin ratio	A/G	—	Calculated
Total bilirubin	TBil	μmol/L	Diazotization method
Lactate dehydrogenase	LDH	U/L	Continuous monitoring method
Alkaline phosphatase	ALP	U/L	Continuous monitoring method
Creatine phosphokinase	CK	U/L	Continuous monitoring method
Glucose	Glu	mmol/L	Hexokinase method
Blood urea nitrogen	BUN	mmol/L	Enzyme coupling rate method
Creatinine	Cre	μmol/L	Enzymatic method
Total cholesterol	TCH	mmol/L	COD-PAP method
Triglyceride	TG	mmol/L	GPO-PAP method
Total serum calcium	Ca	mmol/L	Arsenazo III method
Inorganic phosphate	P	mmol/L	UV method
Sodium ion concentration	Na^+	mmol/L	Electrode method
Potassium ion concentration	K^+	mmol/L	Electrode method
Chlorine ion concentration	Cl^-	mmol/L	Electrode method

No test article-related changes in clinical chemistry occurred.

Urinalysis

Samples for urinalysis were collected at predose and prior to sacrifice. Adequate evaluation of urine occurred as it included urinalysis parameters listed in the table below.

Test item	English acronym (unit)	Test method
Urine appearance check	-	Visual inspection
Weight ratio	SG	Urine dry chemistry analysis
Potential of hydrogen	pH	Urine dry chemistry analysis
Glucose in urine	GLU	Urine dry chemistry analysis
Urine protein	PRO	Urine dry chemistry analysis
Urine bilirubin	BIL	Urine dry chemistry analysis
Occult blood	BLD	Urine dry chemistry analysis
Leukocytes in urine	LEU	Urine dry chemistry analysis
Urobilinogen	UBG (mol/L)	Urine dry chemistry analysis
Ketone bodies	KET	Urine dry chemistry analysis
Nitrite	NIT	Urine dry chemistry analysis

No test article-related changes occurred in urinalysis.

Gross Pathology

As noted in the Clinical Signs, local reactions were characterized as wounds, swelling, and scabbing occurred in 1/4 LD and 1/4 MD in the left front limb where the catheter

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was implanted. The study report describes this as either procedure-related or a result of fighting between animals.

Organ Weights

Adequate assessment of organ weights was conducted. Organs considered are described in the table below.

Tissue/organ	Organ weighing	Collect and store	Microscope examination
Brain (cerebrum, cerebellum, brainstem)	X[1]	X	X
Spinal marrow (neck, chest, waist)		X	X
Pituitary		X	X
Thyroid (including parathyroid)	X	X	X
Salivary gland (parotid, submandibular, sublingual)		X	X
Thymus gland	X	X	X
Esophagus		X	X
Windpipe		X	X
Lung[2]	X	X	X
Heart	X	X	X
Aorta		X	X
Liver	X	X	X
Gallbladder		X	X
Spleen	X	X	X
Kidney	X	X	X
Adrenal gland	X	X	X
Stomach		X	X
Small intestine (duodenum, jejunum, ileum)		X	X
Large intestine (appendices, colon, rectum)		X	X
Intestinal lymph nodes and groin lymph nodes		X	X
Pancreas		X	X
Sciatic nerve		X	X
Eyeball[3]		X	X[4]
Optic nerve[3]		X	X[4]
Bladder		X	X
Testes[3]	X	X	X
Epididymidis	X	X	X

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Prostate	X	X	X
Ovary	X	X	X
Fallopian tube		X	X
Uterus and cervix	X	X	X
Female mammary gland		X	X
Bone and bone marrow, sternum and femur		X	X
Drug administration site and surrounding tissue		X	X
Abnormality discovered during gross observation		X	X

Note 1. "X" means an operation is required; 2. Infused with preservatives after weighing; 3. Davidson's preservatives; 4. Where an ophthalmologic examination discovered an abnormality potentially related to sample toxicity, a histopathology check was performed; otherwise no microscopy examination was required.

No test article-related changes on organ weight occurred.

Histopathology

Adequate Battery: Yes (See table under Organ Weights)

Peer Review: No

Histopathological Findings
Histopathological findings are summarized in the table below.

Dissection deadline		TN					RP				
Group		1	2	3	4	5	1	2	3	4	5
Dosage(mg/kg/day)		0	25	12.5	25	50	0	25	12.5	25	50
Number of animals		4	4	4	4	4	4	4	4	4	4
Drug administration site											
Observed cases		4	4	4	4	4	4	4	4	4	4
No abnormalities seen		(1)	(1)	-	-	-	(4)	(2)	(3)	(4)	(3)
Bleeding, blood vessel		(3)	(3)	(4)	(4)	(3)	-	(2)	(1)	-	(1)
	+	(2)	(2)	(1)	(1)	(3)	-	(2)	(1)	-	(1)
	++	(1)	(1)	(3)	(3)	-	-	-	-	-	-
Mononuclear infiltration, blood		(2)	(1)	(4)	(4)	(4)	-	(1)	-	-	(1)
	+	(1)	(1)	(4)	(3)	(3)	-	(1)	-	-	-
	++	(1)	-	-	(1)	(1)	-	-	-	-	(1)
Endothelial fibrosis, blood vessel		-	-	-	-	(3)	-	-	-	-	(1)
	++	-	-	-	-	(1)	-	-	-	-	(1)
	+++	-	-	-	-	(2)	-	-	-	-	-
Thrombosis	++	-	-	-	-	(1)	-	-	-	-	-

Note: TN = end of drug administration; RP = end of recovery period; - = 0; 4 degrees of classification are used: + = slight; ++ = minor; +++ = medium; ++++ = serious.

Pathology report describes slight to moderate degree of irritation reactions at the injection site characterized by mononuclear infiltration (Control: 2/4; Original Reference:

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1/4; LD: 4/4; MD: 4/4; HD 4/4) and bleeding (Control: 3/4; Original Reference: 3/4; LD: 4/4; MD: 4/4; HD 3/4) at the administration site. The HD had additional local findings characterized by minor to moderate endothelial fibrosis (3/4) and minor thrombosis (1/4). Injection sites in other groups, including controls, also exhibited visible endothelial mononuclear infiltration and bleeding at the injection sites and surrounding tissues, suggesting a procedure-related effect. At the end of the 2-week recovery period, the local reactions were partially recovered. Recoverable endothelial fibrosis (HD 1/4, decreased severity -– minor) and complete recovery thrombosis is considered related to the test-article in the HD.

All other findings were not considered test-article related.

Toxicokinetics

Blood sampling for toxicokinetic analysis were collected at prior to drug administration, 5 and 35 minutes after the start of administration, immediately after the end of administration, and 1, 2, 4, 6, 24 hours after the end of administration on the first dosing day (Day 1) and on the last dosing day (Day 29).

Toxicokinetic results indicate a dose-proportional increase with no accumulation after repeated weekly administration, and no sex differences. Below is a summary of mean TK parameters from both sexes.

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Table 33 Toxicokinetic Summary of the Parent (RF7056) in 4-Week Repeat-Dose (dosed once weekly) Toxicity Study in Monkeys on Day 1

Group	Drug administration day no.	Parameter	$t_{1/2}$ h	T_{max} h	C_{max} ng/mL	$AUC_{(0-26.083\ h)}$ h*ng/mL	AUC_{inf} h*ng/mL
Original reference group	D1	Mean	0.92	0.33	7762.66	21780.5	21924.9
		SD	0.25	0.71	1113.75	4342.8	4340.8
	D29	Mean	1.04	0.08	6237.94	15067.9	15176.8
		SD	0.15	0.00	1227.89	3130.4	3189.6
Low-dosage group	D1	Mean	0.91	0.83	4263.30	12059.5	12114.0
		SD	0.13	1.04	614.07	2084.2	2109.8
	D29	Mean	0.84	0.33	3542.88	9050.8	9080.0
		SD	0.14	0.71	699.03	1422.9	1417.5
Medium-dosage group	D1	Mean	1.11	0.58	7453.83	21560.3	21719.9
		SD	0.18	0.93	2184.02	5770.4	5812.1
	D29	Mean	1.00	0.33	6108.68	16094.6	16220.9
		SD	0.18	0.71	2029.22	4124.0	4159.7
High-dosage group	D1	Mean	1.24	0.58	17616.43	46534.1	47132.9
		SD	0.27	0.93	5810.61	5849.5	5986.5
	D29	Mean	1.07	0.89	11165.05	31263.9	31519.1
		SD	0.19	1.00	2427.59	4449.2	4439.9

Table 34 Toxicokinetic Summary of the Metabolite (RF7054) in 4-Week Repeat-Dose (dosed once weekly) Toxicity Study in Monkeys on Day 1

Group	Drug administration day no.	Parameter	$t_{1/2}$ h	T_{max} h	C_{max} ng/mL	$AUC_{(0-26.083\ h)}$ h*ng/mL	AUC_{inf} h*ng/mL
Original reference group	D1	Mean	1.18	2.08	31595.66	104966.3	105560.8
		SD	0.54	0.00	8400.44	30969.4	30988.1
	D29	Mean	1.98	2.08	24636.41	90013.0	90714.1
		SD	0.54	0.00	5447.66	31254.8	30679.8
Low-dosage group	D1	Mean	1.76	2.08	13465.91	45199.3	45629.5
		SD	0.69	0.00	5050.49	16580.9	16211.6
	D29	Mean	1.88	2.08	8458.86	25456.0	25937.0
		SD	1.01	0.00	2344.36	7616.3	7763.0
Medium-dosage group	D1	Mean	2.30	2.21	36662.17	121931.5	122021.4
		SD	0.69	0.35	6841.78	29603.5	29594.6
	D29	Mean	2.18	2.08	26398.27	87688.1	88094.4
		SD	0.68	0.00	4897.02	21150.4	21124.6
High-dosage group	D1	Mean	2.52	2.33	90692.65	400775.4	401119.3
		SD	0.20	0.46	26906.44	170571.9	170769.3
	D29	Mean	2.31	2.08	56677.93	213305.5	213406.5
		SD	0.18	0.00	15031.19	72017.1	72010.6

Dosing Solution Analysis

Dosing solution analysis of the original reference drug and test article demonstrated the concentrations were within 10% of the nominal concentrations.

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7 Genetic Toxicology

Genetic toxicology tests for remimazolam were previously reviewed by Newton Woo, PhD (DARRTS IND 102486 2/5/2013). Below is a summary of the findings previously reviewed.

Study	Study Conditions/Results	Conclusion
GLP Bacterial mutagenicity/Ames Assay (DPE0002)	CNS 7056 was tested up to 5000 mcg/plate in tester strains TA1535, TA1537, TA98, TA100 and WP2 *uvrA* with and without S9	Negative
GLP Mouse Lymphoma Assay (DPE0003)	Exp 1: 3 hours treatment with up to 2000 mcg/mL with S9 or up to 641 mcg/mL CNS 7056 without S9; statistically significant increase in small colony frequency at 600 mcg/mL and a positive linear trend without S9, but not with S9. Mutations were within historical controls. Exp 2: 3 hours treatment with up to 875 mcg/mL with S9 or up to 610 mcg/mL CNS 7056 without S9; statistically significant increase in mutation frequency and small colony frequency at 800 mcg/mL with S9, but no positive linear trends with S9. No significant changes without S9. Mutations were within historical controls. Exp 3: 24 hours treatment with up to 650 mcg/mL CNS 7056 without S9; statistically significant increase in mutation frequency and small colony frequency at 500 and 550 mcg/mL and positive linear trend at cytotoxic levels. Mutations were within historical controls.	Negative
GLP Rat Micronucleus Test (DPE0004)	Dose of 10, 20, 30 mg/kg/day were administered IV for 2 days and bone marrow was harvested 24 hours after second dose. No statistically significant reduction in the PCE/NCE ratio occurred. No evidence of clastogenicity or aneugenicity following CNS7056X.besylate up to the maximum tolerated dose of 30 mg/kg/day.	Negative

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

Study title: Bacterial Reverse Mutation Test of CNS 7056X

Study no.:	SAIVS-06/014
Study report location:	EDR
Conducting laboratory and location:	CENES Miyuki Saki, Yumi Yamamoto Fukui Safety Research Laboratories in Ono Pharmaceutical Co. Ltd
Date of study initiation:	December 8, 2005
GLP compliance:	Not stated[3]
QA statement:	No[3]
Drug, lot #, and % purity:	Not stated[3]

Key Findings: In the in vitro bacterial reverse mutation assay using the pre-incubation methods, remimazolam (CNS7056X), was tested in the absence and presence of metabolic activation in tester strains *S. typhimurium TA98, TA100, TA1535* and *TA1537*, and *E. coli WP2uvrA*. In the dose-finding test with or without metabolic activation, Remimazolam was tested at concentrations 39.1, 78.1, 156, 313, 625, 1250, 2500, and 5000 mcg/plate. In the main test with and without metabolic activation, Remimazolam was tested at concentrations 156, 313, 625, 1250, 2500, and 5000 mcg/plate. After a 48-hour incubation, the number of bacterial colonies were analyzed. This study generally followed the OECD test guidelines 471 for the bacterial reverse mutation test.

Results indicated that bacterial growth was absent in the sterility test, and number of colonies in vehicle control and appropriate positive control groups were within background values.

[3] Was not deemed a deficiency that would preclude approval because a GLP Ames assay with the parent compound was submitted and reviewed in the IND (see Newton Woo, PhD nonclinical review).

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Table 2-1.

Bacterial mutagenicity test of CNS-7056X (Mean number of revertant colonies in Main test)

S9Mix(-)

Concentration of the test article (μg/plate)		No. of plates	Revertant colonies / plate				
			Base substitution			Frameshift	
			TA100	TA1535	WP2uvrA	TA98	TA1537
Vehicle control (DMSO)		3	126	9	38	23	10
CNS-7056X	156	2	126	11	35	16	9
	313	2	133	13	34	18	10
	625	2	130	9	34	22	7
	1250	2	131	10	34	24	8
	2500	2	106 *	5 *	34	23 *	6 *
	5000	2	107 *#$	6 *#$	30 #$	17 *#	3 *#$
Positive control	Name	2	AF-2	SA	AF-2	4NQO	9AA
	Conc. (μg/plate)		0.01	0.5	0.01	0.2	80.0
	Revert./plate		697	428	246	139	557

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA: Sodium azide, 4NQO: 4-Nitroquinoline 1-oxide, 9AA: 9-Aminoacridine.

*: Growth inhibition of the bacteria was found. #: Precipitation of the test article was found. $: Revertant colonies were counted manually because of the severe precipitation of test article.

Table 2-2.

Bacterial mutagenicity test of CNS-7056X (Mean number of revertant colonies in Main test)

S9Mix(+)

Concentration of the test article (μg/plate)		No. of plates	Revertant colonies / plate				
			Base substitution			Frameshift	
			TA100	TA1535	WP2uvrA	TA98	TA1537
Vehicle control (DMSO)		3	130	9	34	33	15
CNS-7056X	156	2	136	11	33	18	11
	313	2	118	10	34	32	11
	625	2	149	12	37	29	15
	1250	2	136	15	41	25	9
	2500	2	108	7 *	43	21	16
	5000	2	133	8 *	36	22	11 *
Positive control	Name	2	2AA	2AA	2AA	2AA	2AA
	Conc. (μg/plate)		1.0	2.0	10.0	0.5	2.0
	Revert./plate		958	254	679	440	222

2AA: 2-Aminoanthracene.

*: Growth inhibition of the bacteria was found.

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In conclusion, remimazolam is not mutagenic under these test conditions.

7.4 Other Genetic Toxicity Studies

Genetic toxicology studies for 8 impurities/degradants are reviewed in the Appendix.

Genotoxicity	
(b) (4) 015- 270	Ames, GLP, spiked with 8 impurities/degradants (Lot YMK110831)
(b) (4) 015- 271	Mouse lymphoma assay, GLP, spiked with 8 impurities/degradants (Lot YMK110831)
E162 (083-235)	Rat micronucleus and comet assay, GLP, spiked with 8 impurities/degradants (Lot YMK110831)
E161 (083-234)	non-GLP, dose range finding test, rat micronucleus and comet assay

8 Carcinogenicity

Carcinogenicity studies were not conducted or required to support the safety of the proposed acute indication with remimazolam.

9 Reproductive and Developmental Toxicology

A justification for the use of rats was required as the parent compound is extensively metabolized in the rat and there was concern that adequate coverage would not be achieved using IV bolus dosing in rats. Rabbits appeared to have a paradoxical pharmacological effect and a justification for their use as a model was requested in the preNDA meeting. A justification for the use of rats and rabbits as models for reproductive and developmental toxicology was provided in the Nonclinical Overview in Module 2.4. Briefly the Applicants argues that the rat and rabbit are preferred species because both species are standardly used in reproduction studies, have extensive historical reference data, and that remimazolam produced pharmacological effects in both species.

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9.1.1 Fertility and Early Embryonic Development

Study title: Examination of the Influence of CNS 7056 on the Fertility and Early Embryonic Development to Implantation of Rats Following Intravenous Administration to the Animals of the F0 Generation

Study no.:	CRO Report No. 24779 (b) (4) 24779
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 9, 2011
GLP compliance:	Yes, this study was conducted in compliance with GLP regulations of the EC enacted in (b) (4) and OECD Principles of GLP, there were no deviations from the GLP regulations of US FDA 21 CFR 58 or Japanese Guidelines for Nonclinical Studies of Drugs Manual 1995.
QA statement:	Yes, QA statement was signed by (b) (4) on March 9, 2011
Drug, lot #, and % purity:	CNS 7056B, Lot 11084, and 99.0% pure

In this fertility and early embryonic development study, male and female Sprague Dawley rats (20/sex/group) were administered intravenous remimazolam at target dose of 0, 3, 10, and 30 mg/kg/day via bolus tail vein injections. Rats were treated once daily for 4 weeks before mating and continuing until 2 weeks after the start of mating in males and daily for 2 weeks before mating and continuing until implantation (GD7) in females. Laparotomies were performed on all treated females on GD13.

Key Study Findings

- The rat does not appear to represent an adequate nonclinical species for developmental and reproductive toxicology studies as systemic exposures were more than a 100-fold less than in humans.
- No mortality or change in body weight and food consumption occurred in this study
- Clinical signs of restlessness and agitation occurred immediately after injection, followed by reduced motility between occurring from 5 min at LD, MD, and HD. Reduced motility lasted for 5 min at the LD and 20 min at the MD and HD. Sedation was observed at the HD within 20 min after injection. This is considered the pharmacological action of the test article. Increased chewing and grooming occurred immediately after injection and lasted 5 min on the first five days of treatment at LD, MD, and HD.

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- Male fertility: no change in fertility index, sperm number and morphology or absolute testes or epididymides weight at any dose level. Decreased motility of spermatozoa occurred at the HD (52.74%), compared to control (70.63%) but was considered transient and not adverse.
- Female fertility: no change in fertility index, pre-coital time or estrous cycle, reproductive parameters, uterus weight
- Male and Female Fertility NOAEL = HD of 30 mg/kg. Note that the safety margin based on AUC is less than 0.01x when utilizing PK data from the 28-day repeat-dose toxicity study.

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Methods

Doses:	0, 3, 10, 30 mg/kg/day Doses were selected based on a dose range finding study (b) (4) 23508) in pregnant rats where the maternal NOEL was below 3 mg/kg/day. Pharmacodynamic effects (reduced motility) occurred in all animals from 3 mg/kg/day and above. Abdominal position occurred at 10 or 30 mg/kg, clonic convulsions occurred in one 10 mg/kg/day female on one test day, and tremor in one 30 mg/kg/day female on two test days. All symptoms started immediately after injection and lasted for up to 5 minutes. The NOEL for the fetus was above 30 mg/kg/day.
Frequency of dosing:	**Males**: once daily, beginning 4 weeks before mating and continuing until approximately 2 weeks after start of mating period. **Females**: once daily, beginning 2 weeks before mating and continuing until Gestation Day 7 (GD7)
Dose volume:	12 mL/kg
Route of administration:	Intravenous bolus injection into the tail vein
Formulation/Vehicle:	2.5, (b) (4) mg/mL / 0.9% saline solution
Species/Strain:	Rat / CD / Crl:CD(SD)
Number/Sex/Group:	20/sex/group
Satellite groups:	None. Toxicokinetic evaluations were not conducted for this study.
Study design:	This was a typical combined male/female fertility and early embryonic development study in rats with all recommended mating and fertility indices as described in ICH S5. **Male rats** were treated with the test article for 4 weeks prior to mating, during cohabitation with untreated females (1 male to 1 female), and until sacrifice (approximately 2 weeks after start of mating period). **Female rats** were treated with the test article for 2 weeks before mating, during cohabitation with untreated males (1 male to 1 female), and until GD7 (implantation). Laparotomies of all dams occurred on GD13. Mortality, clinical signs, body weight, food consumption, and gross examinations were performed on all control and test animals. Ovary, uterus, cervix, testis, epididymis, seminal vesicle/coagulation gland, and prostate gland

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	were preserved for possible microscopic examination. Mating and fertility indices included corpora lutea, implantation sites, placentae, resorptions, fetuses, resorption rate, fertility index, pre- and post- implantation loss. Control and treated males were evaluated for sperm count, sperm viability, and sperm morphology. This study used clinical route of administration, adequate frequency of dosing, number of animals, administration period, mating ratios and observations as recommended by ICH S5(R2). Dose selection is a concern because rats metabolizes the parent extensively. This is discussed in the Integrated Review.
Deviation from study protocol:	Minor deviations not likely to affect validity or integrity of the study results: - Person responsible for blood sampling as stated in the Study Plan was omitted because no blood sampling occurred in this study. - Humidity values were slightly below the 40% limit on several days. Acceptable range: 55%+15%.

Observations and Results

Mortality

No mortality occurred during this study.

Clinical Signs

Clinical observations occurred at least once daily during the study period until necropsy. Onset, intensity, and duration of clinical signs were recorded during the treatment period before and after dosing with attention in the first 1-2 hours after dosing. Local tolerance at injection sites was included in the clinical observations.

Expected pharmacological clinical signs of restlessness and agitation, reduced motility, increased chewing movements, increased grooming, and sedation occurred in treatment groups as summarized in the table below. Restlessness and agitation occurred immediately after injection; increased chewing movements and increased grooming started within 5 min after injection and lasted for up to 5 minutes; reduced motility started within 5 min after injection and lasted for up to 5 minutes in the LD or up to 20 min in the MD and HD; sedation started within 5 min after injection and lasted for up to 20 min.

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Table 35 Summary of Clinical Signs from Male/Female Rat Fertility Study

Clinical sign	Control	LD (3 mg/kg)	MD (10 mg/kg)	HD (30 mg/kg)
	n = 20/sex	n = 20/sex	n = 20/sex	n = 20/sex
Restlessness and agitation	None noted	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest
Reduced motility	None noted	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest
Increased chewing movements	None noted	20 M Pre 20 F Pre	20 F Pre	None noted
Increased grooming	None noted	20 M Pre 20 F Pre	None noted	None noted
Sedation	None noted	None noted	None noted	20 M Pre 20 M Post 20 F Pre 20 F Post 20 F Gest

Pre = during pre-mating period; Post = during post-mating period; Gest = during gestation; M = male; F = female.

Body Weight

Body weights were recorded twice weekly during the pre-mating and mating period. Beginning on GD0, females were weighed daily. No test article-related effects on body weight were noted in the study report.

Feed Consumption

Food consumption was recorded for both sexes once weekly until pairing for mating. Food consumption was recorded in mated females were recorded daily during gestation. No test article-related effects on food consumption were noted in the study report.

Estrous Cycle

Estrous cycle was monitored in treated females prior to and during the mating period by vaginal lavage. No test article-related effects on estrous cycle were noted in the study report.

Toxicokinetics

Toxicokinetic analysis was not conducted for this study.

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Dosing Solution Analysis

Dosing solution analysis revealed the test article concentrations were from 94.9% to 104.4% of the nominal value, which is within the admissible limits of 90% to 110%.

Necropsy

No test article-related effects on organ weight were noted in the study report. No macroscopic test article-related findings were noted in the study report.

Fertility Parameters

No aborted, dead, or malformed fetuses were noted in the study report. Female fertility parameters are described in the table below.

Table 36 Female Fertility Parameter Summary – Rat Fertility Study

	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
Female Fertility Index	100%	95%	95%	100%
Corpora lutea/litter	14.3	14.3	14.5	15.0
Implantation sites/litter	14.2	13.8	14.2	14.2
Preimplantation loss	0.6%	3.5%*	2.5%	6.1%*
Early resorptions	0 (0%)	3 (1.0%)	5 (1.7%*)	5 (1.5%)
Late resorptions	10 (3.6%)	15 (5.6%)	3 (1.2%)	15 (4.8%)
Total resorptions	10 (3.6%)	18 (6.6%)	8 (2.8%)	20 (6.3%)
Live fetuses/litter	13.7	12.9	13.7	13.2
Post-implantation loss	3.6%	6.6%	2.8%	6.3%
Dead fetuses/late resorptions	0	0	0	0

*P ≤ 0.05 by the Fisher test; (b) (4) Background Data for pre-implantation loss 4.2%±1.0-7.7% and early resorptions 3.6%±0.4-5.4%

All statistically significant changes in preimplantation loss and early resorptions were within background levels of this test facility. No test article-related effects on the number of corpora lutea, implantation sites, total, early, or late resorptions, placenta, or live fetuses occurred.

Male fertility parameters are described in the table below.

Table 37 Male Fertility Parameter Summary – Rat Fertility Study

	0 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg
Male Fertility Index	100%	100%	100%	100%
Sperm count (x 10^6)	80.01	89.01	84.88	84.97
Motile spermatozoa in epididymal cauda	70.63	61.13	68.54	52.74*
Morphologically normal spermatids in caudal epididymis	99.15	99.30	99.20	99.48

P ≤ 0.05 by Fisher test or Chi^2 test

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A statistically significant decrease in sperm motility occurred at the HD, but did not affect male fertility index. The study report suggests that the effect on sperm motility may be an exaggerated pharmacological effect. This Reviewer agrees and concludes that decreased sperm motility without effecting fertility is likely a non-adverse transient effect. No adverse test article-related effects on male or female fertility occurred in this study.

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9.1.2 Fertility and Early Embryonic Development

Study title: An Extended Fertility/Mating Study of Remimazolam Besylate by Intravenous Infusion and Intravenous (Bolus) Injection in Rabbits

Study no.:	CRO Study 497736
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	03/29/2018
GLP compliance:	Yes, with the exception of the bioanalyses, this study was performed in accordance with the OECD Principles of GLP as incorporated into the (b) (4) for GLP and as accepted by Regulatory Authorities throughout the European Union, US FDA and EPA, and Japan. Bioanalysis conducted in Italy was conducted in accordance with OECD Principles of GLP as accepted by the U.S. FDA and Japanese authorities. Lactation milk sample bioanalysis and method qualification were not GLP because they were performed under non-GLP conditions using qualified method.
QA statement:	Yes, QA statement was signed by (b) (4) on March 29, 2018
Drug, lot #, and % purity:	Remimazolam Besylate (CNS 7056B), Lot 10201249, 100% pure

In this extended fertility study, female rabbits (20/group) were intravenously administered remimazolam at target doses of 0, 12.5, 20, and 30 mg/kg/day. Rabbits were treated once daily for 2 weeks before mating and continuing until Lactation Day 30. Does were allowed to litter. Mortality, clinical signs, tactile stimulation, body weight, body weight gains, food consumption, mating performance, duration of gestation, maternal care, litter survival, kit body weights and open field evaluations, reproductive indices, plasma and milk bioanalysis, plasma TK of the does and kits, milk TK, gross necropsy findings and some histopathology examinations were recorded. Paradoxical effects in female rabbits at all doses in combination with the study procedures, resulted in a number of undesirable outcomes including death and euthanasia from treatment-related disinhibition injuries. The study report stated that rabbits after dosing were hypersensitive to stimulation, such as movement, and would buck/convulse in response to a slight movement. Because the rabbits were restrained, the bucking resulted in broken backs. The incidences of euthanasia noted in the report were the result of bucking of the restrained animals and are not believed to be a direct effect of the study

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drug. The HD group was terminated after mating performance and fertility because mortality occurred prior to reaching parturition.

Key Study Findings

- Mortalities occurred in all group (5/21 Control; 6/26 LD; 7/26 MD; and 5/5 HD). Mortalities described as test article-related mortalities (2/26 LD; 5/26 MD; and 4/5 HD) were attributed to the paradoxical pharmacodynamic effect of the test article on this species leading to convulsions and spinal injuries or procedure-related effects leading to thrombi. Mortalities occurred within the first four weeks of treatment.
- Convulsions occurred at all treatment groups (2/26 LD, 2/26 MD, 1/5 HD) and appeared to be a result of hypersensitivities to procedural manipulations in this species. All animals that convulsed died or were euthanized.
- Clinical signs included hypersensitivity immediately after dosing with the test article as well as sedative effects in most animals

NOAELs below were based on fertility and litter performance parameters and not on clinical signs observed in does as convulsions and mortality occurred at all dose levels tested.

- Female mating performance NOAEL = 30 mg/kg/day (AUC = 11600 ng•h/mL)
- Early embryonic development NOAEL = 20 mg/kg/day (AUC = 8290 ng•h/mL)
- Maternal care/kit survival NOAEL = 20 mg/kg/day (AUC = 10800 ng•h/mL)
- Kit behavior NOAEL = 20 mg/kg/day (AUC = 8290 ng•h/mL)

Group No.	Test Item	Dose Level Bolus + Infusion (mg/kg)	Dose Volume Bolus + Infusion (mL/kg)	Dose Concentration (mg/mL)	Duration of Infusion (h)	Dose rate for Infusion (mg/kg/h)	Volume rate for Infusion (mL/kg/h)	Number of Females
1	Control	0+0	2+10/6[#]	0	4/2.4[#]	0	2.5	Batch 1:5 Batch 2: 6 Batch 3: 11
2	Remimazolam Besylate	5+7.5	2+3	2.5	1.2	6.25	2.5	Batch 1: 10 Batch 2: 11 Batch 3: 5
3	Remimazolam Besylate	5+15	2+6	2.5	2.4	6.25	2.5	Batch 1: 10 Batch 2: 11 Batch 3: 5
4	Remimazolam Besylate	5+25	2+10	2.5	4	6.25	2.5	Batch 1: 5

Vehicle/Control = Lactose monohydrate, (b) (4)%/dextran 40, (b) (4)% in 0.9% sodium chloride
[#] Reduced for Batches 2 and 3 due to absence of Group 4 animals
All animals received a slow bolus injection: 0 (Group 1) or 5 mg/kg (Groups 2-4) using a 0 or 2.5 mg/mL concentration at 2 mL/kg body weight immediately prior to starting the infusion

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Methods

Doses:	0, 5+7.5, 5+15, and 5+25 mg/kg/day (bolus + infusion); total dose 0, 12.5, 20, and 30 mg/kg/day Treatment of 30 mg/kg was discontinued after deaths before or during gestation of 4/5 animals in Batch 1. As a result, investigations during gestation and lactation were restricted to the 12.5 and 20 mg/kg dose groups. However, fertility was evaluated in this group. Doses were selected based on a DRF study (497720) that administered a 4-hour infusion at levels of 15, 20, and 25 mg/kg/day and was generally well tolerated when dosed daily to females over GD 6-19. Reduced body weight gain associated with a reduction in food consumption in males during the first week of dosing and in females throughout the dosing period occurred at 25 mg/kg/day. Mated animals were initially administered doses of 30, 37.5, and 45 mg/kg/day over a 6-hour infusion period. The 6-hour infusion period was associated with premature deaths in two mated females (one at 30 mg/kg an another at 37.5 mg/kg).
Frequency of dosing:	Bolus + 1.2-, 2.4-, and 4-h infusion daily starting approximately 2 weeks prior to mating and continued until Day 30 of lactation.
Dose volume:	2 mL/kg bolus + 3, 6, and 10 mL/kg infusion
Route of administration:	Intravenous bolus + infusion via catheter in the right femoral vein. Catheter was protected by a customized jacket that carried the ambulatory pump system.
Formulation/Vehicle:	2.5 mg/mL / Dextran 40 (b) (4) and Lactose monohydrate (b) (4) in 0.9% saline
Species/Strain:	Rabbit/New Zealand White (HsdIf:NZW)
Number/Sex/Group:	21/sex/group
Satellite groups:	5/sex/group for Toxicokinetics
Study design:	This was an extended female fertility study in rabbits with all recommended mating and fertility indices as described in ICH S5. Female rabbits were treated with the test article for 2 weeks before mating, during cohabitation with untreated males (1 male to 1 female), throughout gestation, and until Lactation Day 30.

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	Females were allowed to litter normally. Mortality, clinical signs, tactile stimulation, body weight, body weight gains, food consumption, mating performance, duration of gestation, maternal care, litter survival, kit body weights and open field evaluations, reproductive indices, plasma and milk bioanalysis, plasma TK, gross necropsy findings and some histopathology examinations. This study used clinical route of administration, adequate frequency of dosing, number of animals, administration period, mating ratios and observations as recommended by ICH S5(R2). Species selection may be a concern because of the paradoxical pharmacodynamic effects occurring in this species that appeared to cause adverse effects and mortality.
Deviation from study protocol:	A number of protocol deviations occurred including concentration and homogeneity analysis, test system receipt, environmental acclimation, husbandry, environmental conditions, administration of test and control items, pre and post dose observations, body weights, food consumption, mating procedures, open field evaluations, bioanalytical sample collection and processing, terminal procedures, and unscheduled deaths. Body weights were not recorded at necropsy in the three unscheduled deaths, this was determined not to impact the study because organ weights on this study and these animals were weighted in-life on the day prior to necropsy. All protocol deviations were determined not to impact the study.

Observations and Results

Mortality

Mortality was monitored twice daily.

A total of 12 unscheduled mortalities were considered test-article related in this study as shown in the table below, excerpted from the study report.

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Dose level (mg/kg):	0 (Control)	12.5	20	30
Deaths/Total group size:	5/21	6/26	7/26	5/5
Percentage (%)	24	23	27	100
Test item-related	-	2	6	4
Not test item-related	5	4	1	1

Note: In addition, two unscheduled deaths occurred during surgical preparation for the study

The table below describes the test article-related mortalities, excerpted from the study report.

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Table 38 Summary of Mortalities for Rabbit Extended Fertility Study

Dose level (mg/kg/day) /Animal no.	Outcome	Principal observations
12.5/2504	FD D17	D16: appeared agitated and aggressive when paired for mating. D17: convulsed and died approximately 15 minutes after being checked for sexual receptivity (for pairing), approximately 22 hours post D16 dose. HP: femoral vein – small immature adherent thrombus; vena cava – small mature (well organised) nonadherent lumenal thrombus.
12.5/2520	FD GD10 (D26)	Convulsed, gasped and died when taken off infusion. Thymus – mottled discolouration. No HP.
20/3514	FD GD24 (D41)	Received 172% of intended infusion dose volume over 2.4 hours. Found dead at 1-hour post dose. Lungs: all lobes, discolouration, dark focus. No HP.
20/3516	UE D8	Approximately 2-3 hours after end of infusion (sedated): prostrate, shallow breathing, irregular respiration rate, pale skin and eyes. HP: femoral vein – canalised mural thrombus; vena cava – thrombus around catheter; heart - ventricular free walls and septum, mild interstitial fibrosis; focal minimal cardiomyocyte necrosis with mononuclear inflammatory cells.
20/3517	FD D5	Approximately 2 hours into infusion (sedated), during clinical observations check: sustained convulsion, animal died. Heart: left ventricle, abnormal consistency (firm); mottled discolouration (no HP correlation). HP: femoral vein and vena cava – thrombus around catheter.
20/3518	FD D12	Convulsed and died on removal from cage at end of infusion (sedated). Note: animal had fallen from cage 5 minutes into infusion – appeared unhurt, no evidence of damage to cranium, vertebrae or brain at necropsy. HP: femoral vein and vena cava – thrombus/debris around catheter.
20/3519	FD D11	D10: Lame on left forelimb 2 hours after end of infusion, given metacam. Found dead at morning check on D11. No findings at necropsy. No HP.
20/3520	UE GD15 (D31)	After bolus dose: extended forelimbs and neck. Immediately after end of infusion: decreased activity, head tilt, prostrate, lack of righting reflex, shallow breathing. No improvement over time. Abdomen: clear fluid accumulation; Kidneys: bilateral clear cysts; left, mottled discolouration. No HP.
30/4501	UE D4	Suspected damaged spine due to struggling during restraint for TK blood sampling procedure at 3 hours post dose (dragging hind limbs 30 minutes after last time point). Reduced muscle tone noted. Lungs: all lobes, mottled discolouration. No HP.
30/4503	UE D4	Spine fracture sustained whilst struggling during restraint for TK blood sampling procedure at 3 hours post dose (dragging hind limbs, no pain withdrawal reflex, no muscle tone 20 minutes after last time point). Spine: lumbar-thoracic region, fracture. No HP.
30/4504	UE D25	D23, 24: Abnormal gait after end of infusion and during pairing. D25: approximately 2 hours after end of infusion: abnormal gait, low carriage, hunched posture. Reduced muscle tone noted. Femoral vein: mild chronic inflammation (in surrounding tissue, associated with surgical procedure); vena cava: minimal intimal proliferation, thrombus; lungs: mild perivascular eosinophil infiltration.
30/4505	UE D27	Approximately 19 hours post D26 dose: lying on side, unwilling to move, decreased activity, shallow breathing, abnormal gait. Animal convulsed when returned to cage after weighing. HP: Femoral vein: minimal chronic inflammation (in surrounding tissue, associated with surgical procedure); lungs: mild perivascular eosinophil infiltration, moderate alveolar oedema.

FD = Found dead/died; UE = Unscheduled euthanasia
D = Day of study'; GD = Gestation Day; HP = Histopathology

Mortalities generally occurred within the first four weeks of treatment.

At the MD, one mortality (3514) described in the study report as test article-related was attributed to an excessive infusion dose rate error. While this may be considered a test article-related mortality, it should not be considered as a mortality at the nominal MD value of 20 mg/kg.

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Thrombi were noted in LD (2504) and MD (3516, 3517) mortalities. This Reviewer considers these findings procedure-related rather than test article-related causes of death.

Two mortalities at the HD (4503 and 4501) described in the study report as test article-related mortalities were euthanized with suspected spinal injuries sustained whilst struggling during restraint for TK blood sampling. Because spinal injuries were a result of struggling and not the test article, this Reviewer does not consider these two incidences to be test article-related mortalities.

MD animal 3519 presented with lameness in the left forelimb on day 10 two hours after end of infusion. Lameness can be indicative of a thrombus or spinal injury that, although was not noted in this animal upon necropsy, is a consistent finding with animals in this study.

Convulsions, where they occurred (LD 2/26; MD 2/26; HD 1/5), were associated with mortality at all dose levels, most directly associated with disturbing the animal to carry out study procedures during the post dose period or the next morning. One MD animal (3520) experienced convulsive-like episode (extended forelimbs and neck) after the bolus dose, from which it did not recover. Two animals at the MD and HD developed poor clinical condition in the post dose period that necessitated euthanasia. While the convulsions appear to be secondary to the paradoxical pharmacodynamic effect on this species, further investigations would be required to confirm this hypothesis.

Table 39 Summary of Mortalities and Clinical Signs Occurring in the Decedents

Clinical signs occurring in decedents	Control	LD	MD	HD
Test article-related mortalities/total group size	0/21	2/26	6/26	4/5
Percentage of animals	0	8%	23%	80%
Convulsions – Animal ID		2504 2520	3517 3518 (3520)*	4505
Thrombi – Animal ID		2504	3516 3517 3518	(4504) (4505)
Dosing Error – Animal ID			3514	
Spinal Injury – Animal ID				4501 4503
Undetermined – Animal ID			(3519)	
Additional mortalities not considered to be test item-related – Number of animals	5	4	1	1

*Animal 3520 had ‘convulsive-like’ clinical signs of extended forelimb and neck

None of the mortalities were considered to be directly related to the test article, meaning the paradoxical effects increased the susceptibility of rabbits to react to the study procedures and manipulations. Other findings, such as thrombi, appear to be procedure related.

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Clinical Signs

Pre and post dose clinical observations occurred for each animal throughout dosing day. Detailed clinical observations occurred once prior to the start of dosing and then weekly.

Sedative effects were similar at all dose levels, with unwillingness to move noted throughout the infusion and initially until 1 or 2 hours after the end of the infusion. This effect gradually decreased during the treatment period until the earliest recovery time point – 1 hour post dose was – achieve. Signs of sedation (e.g., abnormal gait) associated the pharmacodynamic effects of the test article included repetitive behavior defined as rapid eye movement and sneezing/nasal discharge occurred at the LD. The following clinical signs were only observed in the unscheduled deaths: ≥LD-shallow breathing, thin, pale eyes; ≥MD-irregular respiration rate, pale skin, convulsions, head tilt; HD- low carriage, hunched posture.

Convulsion-like episodes (abnormal head/neck movement, abnormal -stiff- limb movement) occurred in the LD and MD. Excessive licking at the LD and MD. Chewing action was noted at the HD only. Slightly aggressive/agitated behavior occurred on isolated occasions at LD and MD. Fur loss occurred from the start of mating/gestation onwards with 12/26 animals at the LD and MD and 4/21 in the control group.

Table 40 Summary of Clinical Signs for Rabbit Extended Fertility Study

	Control	LD	MD	HD
Convulsions non-sustained				1 (4505, Day 27)
Convulsions sustained			1 (3517, Day 5)	
Shallow breathing		1 (2521, Day 22)	3 (3516, Day 8; 3520; Day 1; 3602, Day 31)	1 (4505, Day 27)
Thin	1 (1521, Day 63 and 64)	1 (2521, Day 16, 19 to 22)		
Pale eyes		1 (2521, Day 20)		
Irregular respiration rate			1 (3516, Day 8)	
Pale skin			1 (3516, Day 8)	
Head tilt			1 (3520, Day 31)	
Low carriage				1 (4504, Day 25)
Hunched posture				1 (4504, Day 25)

Table represents number of animals affected (Animal ID, day of occurrence).

Tactile stimulus tests demonstrated reactions to a non-noxious stimulus. A test for a reaction to a blunt-ended device on the rump was performed on the first five available

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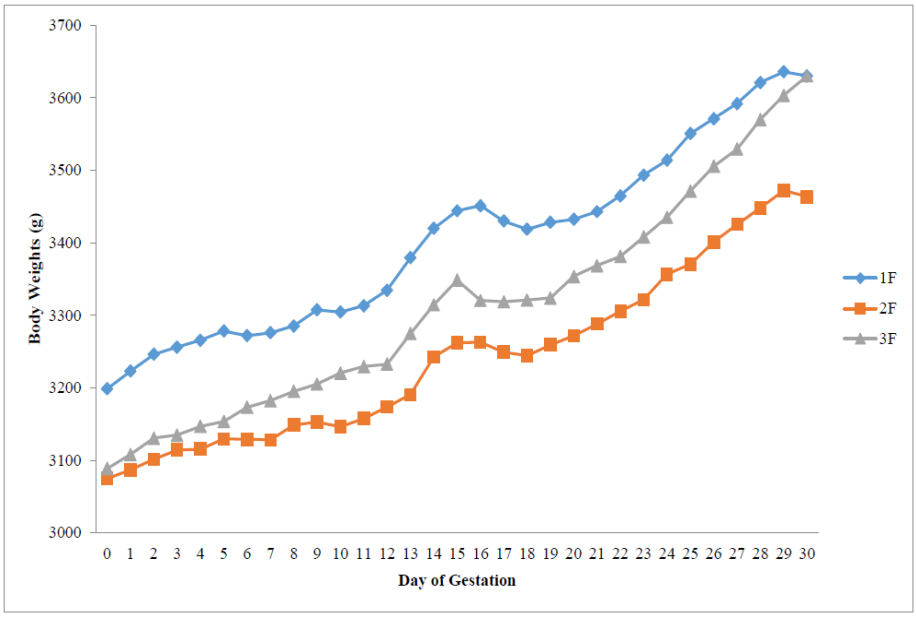
females in the control, LD, and MD groups. The test was performed once during the pre-mating period, once between Days 14 and 20 of gestation and once between Days 22 and 24 of lactation in the animal's cage at the following times: 1. Before bolus injection, 2. 5 and 45 minutes after the start of infusion, 3. Approximately 1.2 hours after the start of infusion, 4. Immediately before the end of infusion, and 5. 30, 60, and 180 minutes after the end of infusion. LD and MD animals were generally non-responsive at time points from 5 minutes after the start of infusion through to immediately before the end of infusion on the pre-mating occasion. When stimulus was applied during gestation, most animals were responsive before the end of infusion. During lactation, the treated animal was only non-responsive 5 minutes after the start of infusion. The period of non-responsiveness therefore decreased as the treatment period progressed.

Body Weight

Body weight was recorded weekly after surgical implantation of the catheter and then daily during the treatment period excluding PND1-3. Body weight results are summarized in the figures below excerpted from the study report.

Figure 3 Body Weight During Gestation for Rabbit Extended Fertility Study



1F = control; 2F = LD; 3F = MD

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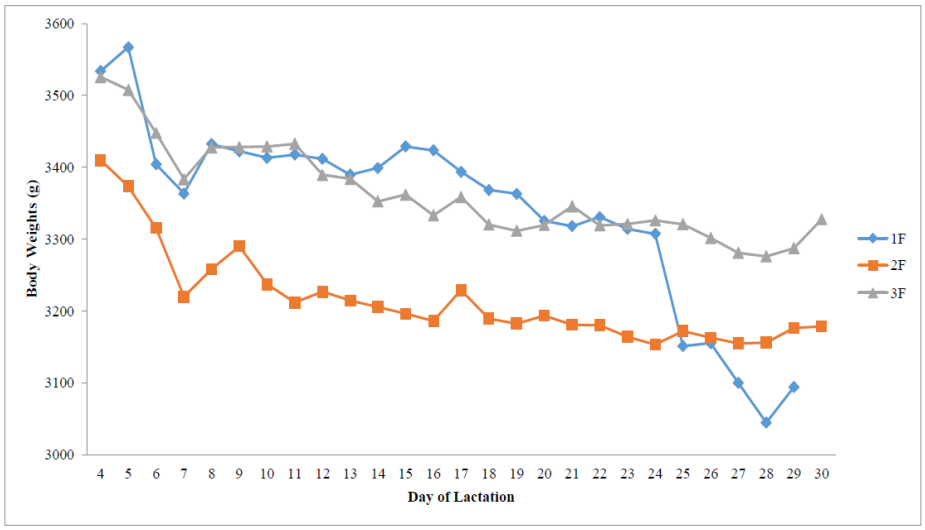
Figure 4 Body Weight During Lactation for Rabbit Extended Fertility Study



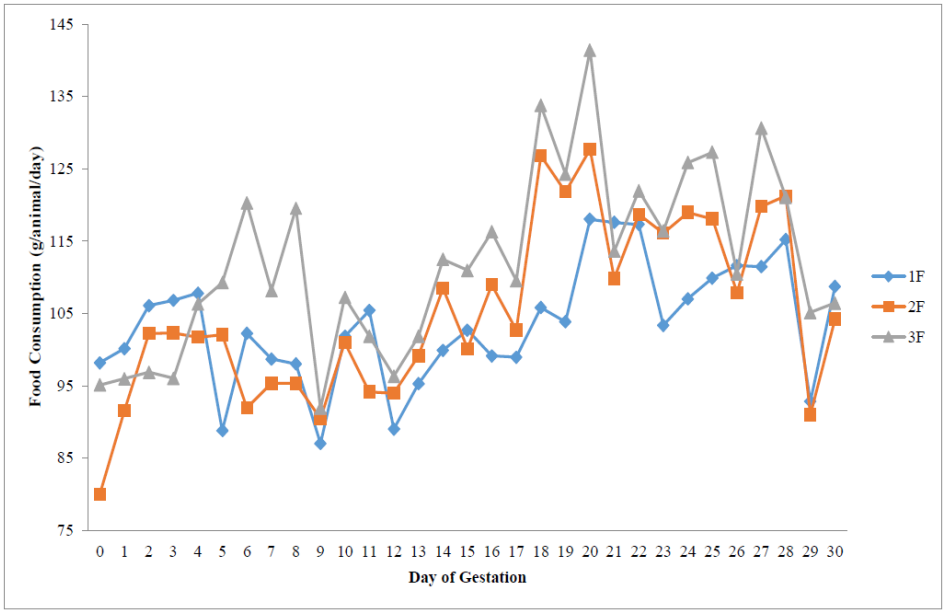
1F = control; 2F = LD; 3F = MD

Overall mean body weight gain for gestation was 23% higher at the MD compared to control. No adverse test article related effect on body weight occurred during gestation or lactation.

Food Consumption

Food consumption was recorded daily during treatment. Food consumption results are summarized in the figures below excerpted from the study report.

Figure 5 Food Consumption During Gestation for Rabbit Extended Fertility Study



1F = control; 2F = LD; 3F = MD

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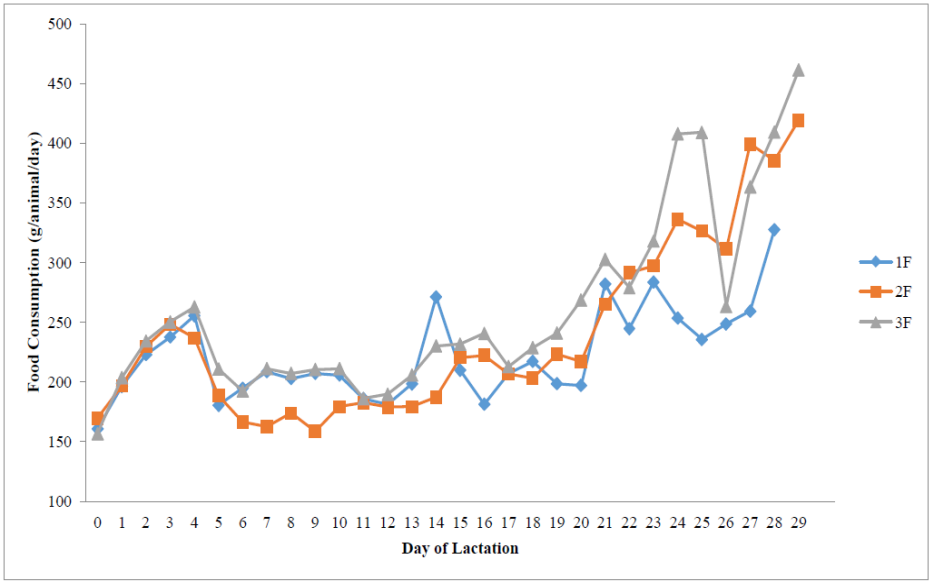
There was some small transient dose-related increase (17-26%) in mean food consumption during gestation at the LD and MD, the biggest effect occurring on GD 18, 19, and 20.

Figure 6 Food Consumption During Lactation for Rabbit Extended Fertility Study



1F = control; 2F = LD; 3F = MD

Dose-related increase (up to 38% at LD and 74% at MD) also occurred during lactation, the biggest effect occurring on Day 24 and 25 of lactation.

No adverse test article related effects on food consumption occurred during the gestation or lactation periods.

Toxicokinetics

Toxicokinetic analysis was conducted on plasma samples from F0 does during premating gestation, and lactation. Results are summarized in the table below.

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Table 41 Toxicokinetic Summary for Rabbit Extended Fertility Study

Analyte	Remimazolam			CNS7054		
Dose (mg/kg/day)	12.5	20	30	12.5	20	30
	Pre-mating					
Cmax (ng/mL)	5200	7330	4420	8910	10800	14000
Tmax (h)	0.033	0.033	0.033	1.2	2.4	4
AUC(0-t) (ng.h/mL)	6270	7430	11600	19900	21500	55800
CL (mL/h/kg)	1990	2690	2600	NA	NA	NA
	Gestation Period					
Cmax (ng/mL)	4740	4490	NR	12900	14700	NR
Tmax (h)	0.033	0.033		1.2	2.4	
AUC(0-t) (ng.h/mL)	5320	8290		25900	44300	
CL (mL/h/kg)	2360	2560		NA	NA	NA
	Lactation Period					
Cmax (ng/mL)	21300	15400	NR	9950	10200	NR
Tmax (h)	0.033	0.033		1.2	0.75	
AUC(0-t) (ng.h/mL)	11300	10800		16600	28400	
CL (mL/h/kg)	1110	2050		NA	NA	NA

NA = Parameter not applicable for analyte; NR = No result.

Table 42 TK Ratios for Exposure Comparisons from Rabbit Extended Fertility Study

Analyte	Remimazolam						CNS7054					
Comparison	Gestation/ Pre-mating		Lactation/ Pre-mating		Lactation/ Gestation		Gestation/ Pre-mating		Lactation/ Pre-mating		Lactation/ Gestation	
Dose (mg/kg/day)	12.5	20	12.5	20	12.5	20	12.5	20	12.5	20	12.5	20
C0 ratio	0.9	0.6	2.9	2.2	3.2	3.7	NA					
Cmax ratio	0.9	0.6	2.8	2.1	3.3	3.4	1.4	1.4	1.1	0.9	0.8	0.7
AUC(0-t) ratio	0.8	1.1	1.3	1.5	1.6	1.3	1.3	2.1	0.8	1.3	0.6	0.6

Comparison	CNS7054/Remimazolam						
	Pre-mating			Gestation Period		Lactation Period	
Dose (mg/kg/day)	12.5	20	30	12.5	20	12.5	20
Cmax ratio	1.7	1.5	3.2	2.7	3.3	0.7	0.7
AUC(0-t) ratio	3.2	2.9	4.8	4.9	5.3	2.0	2.6

NA = Parameter not applicable for analyte.

Plasma was collected from F1 kits on PND 9 and 11. Results are summarized in the table below.

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Table 43 Summary of Kit Plasma TK for Rabbit Extended Fertility Study

Group/Animal Number (mother of kit)	Day of Sample Collection (lactation)	Remimazolam concentration (ng/mL)	CNS7054 concentration (ng/mL)
3/3512	11	NQ	NQ
3/3513	11	NQ	100.9
3/3515	11	NQ	NQ
3/3523	10	NQ	162.0
3/3601	10	NQ	NQ

NQ = not quantifiable

Milk was collected on Day 8 and 11 of lactation using non-GLP conditions at the (b) (4) Results are summarized in the table below.

Table 44 Summary of Milk TK for Rabbit Extended Fertility Study

Animal number	Day of sample collection (lactation)	Time of sample collection (minutes post end of infusion)	Remimazolam concentration (ng/mL)	CNS7054 concentration (ng/mL)
1509	11	37-44	NQ	NQ
1518	8	4-9	NQ	NQ
3512	11	36-42	474.88	396.2
3513	11	12-20	1198.02	323.1
3515	11	4-6	3324.22	256.9
3523	10	6-11	2005.11	301.1
3601	10	2-6	3360.78	374.3

NQ = not quantifiable

Dosing Solution Analysis

All dose formulation analysis results were within acceptable criteria.

Necropsy

All females were subjected to a complete necropsy, which 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. The reproductive tract was examined for signs of implantation, the number of implantation sites was recorded where possible. Two male and two female kits from each litter were necropsied shortly after weaning and were subjected to external examination following macroscopic examination of the tissues and organs of the cranial, thoracic, and abdominal cavities in situ.

No test article related abnormalities occurred in surviving does or kits. Two non-pregnant animals in the LD group had reproductive tract findings (2509: clear cysts in one oviduct; 2514: dark red discoloration of both uterine horns), which may have

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negatively impacted the fertility index in this group but is not considered test article related.

Mortalities occurring in this study were necropsied. Necropsy and histopathology findings of thrombi in an around the vena cava and/or femoral vein were recorded in animals with observations consistent with this finding. Mastitis, characterized by large areas of thick mammary tissue, firm cream-colored and gelatinous red- or green-colored, sometimes exuding thick pale liquid and pale caseous material.

Fertility Parameters
Fertility and litter performance parameters are presented in the table below.

Table 45 Female Fertility and Litter Performance Parameter Summary – Rabbit Extended Fertility Study

Parameter	**Control** 0 mg/kg	**LD** 12.5 mg/kg	**MD** 20 mg/kg	**HD** 30 mg/kg
Group Fertility Index (number pregnant/number paired)	18/20 (90%)	21/26 (81%)	20/22 (91%)	1/3 (33%)
Group Gestation Index (number bearing live kits/number pregnant)	18/18 (100%)	18/21 (86%)	18/20 (90%)	NA
Mean Gestation Duration [a] (Days)	31.2	31.3	31.1	NA
Litter Birth Index (total number of kits born/number of implantation scars)	114%	152%	109%	NA
Litter Live Birth Index (number of live kits born/total number born)	89%	100%	94%	NA
Litter Viability Index (number of kits alive on PND 4/total number born alive)	41%	55%	53%	NA
Litter Lactation Index (number of kits alive on PND 29/number of kits alive on PND 4)	83%	94%	79%	NA
Litter Overall Survival Index (number of kits alive on PND 29/total number born)	27%	45%	38%	NA
Mean number of implantation scars [b] /birth	7.7±2.7	6.1±1.5	7.6±2.4	NA
Mean total number of kits born [b] /litter	7.4±1.5	5.7±1.2	7.6±2.1	NA
Mean number of live kits/litter on PND 0 [b]	6.9±1.9	5.7±1.2	6.8±2.4	NA
Mean number of live kits/litter on PND 4 [b]	6.9±1.9	5.4±1.0	6.8±2.4	NA
Mean number of live kits/litter on PND 29 [b]	4.7±2.9	4.3±1.7	4.6±2.1	NA

[a] Excludes animals 2511 and 2601; [b] Excludes litters where all kits died

Results at the HD should be viewed with caution because of the low number of animals. No test article related effects occurred up to 20 mg/kg/day.

Open Field/Functional Observational Battery Evaluations of F1 Kits

Full litters were maintained following scheduled euthanasia of the treated F0 does and selected kits were evaluated in the open field test once between PND 35-39. Two males and two females from each litter were evaluated.

There were no test-article related effects on most behavioral parameters tested (e.g., gait, handling reaction, urination, defecation, arousal, posture, lacrimation, salivation, piloerection, respiration, pupil response to light, toe-pinch reaction, auditory reaction) in

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kits after pregnant dams were administered remimazolam. However, visual and tactile tests were confounded as several animals were tested with a blunt-ended probe while other animals were tested with a finger, which elicited less reactions and therefore a NOAEL could not be identified for these specific parameters.

9.2.1 Embryonic Fetal Development

Study title: Study of Embryo-fetal Development in Rats with CNS 7056 by Intravenous Administration

Study no.:	(b) (4) 23509
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 12, 2011
GLP compliance:	Yes, this study was performed in compliance with GLP regulations of the EC enacted in (b) (4) and OECD Principles of GLP 2002. US FDA GLP 21CFR58 was considered
QA statement:	Yes, QA statement was signed by (b) (4) on July 12, 2011
Drug, lot #, and % purity:	CNS 7056B (CNS 7058, besylate salt), Lot 11084, 98.7%

In this embryo-fetal development study, female rats (25/group) were intravenously administered remimazolam at target doses of 0, 3, 10, and 30 mg/kg/day during organogenesis from implantation to hard palate closure (GD 6-17). Laparotomies occurred on GD 20.

Key Study Findings

- No mortalities or changes in body weight or food consumption occurred.
- Pharmacodynamic effects of reduced motility started 5 min after dosing and lasted for 5 min in the LD and 20 min at the MD. Reduced motility changed to sedation at the HD lasting up to 20 min after dosing.
- Convulsions occurred in one MD and one HD dam on GD 11.
- No effects on prenatal development (corpora lutea, implantation sites, resorptions, sex distribution, fetal and placental weights, number of live fetuses at birth and the values calculated for the pre- and post-implantation loss).
- No effects on malformations, variations, or retardations.
- Systemic exposures when taking into consideration protein binding was comparable to human levels at MRHD at the beginning of the study, but dropped below 1 by the end of the dosing period.
- Maternal NOAEL = 3 mg/kg/day based on the convulsions occurring at and above 10 mg/kg/day.

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- Fetal NOAEL = 30 mg/kg/day based on the slight but statistically significant increased incidence of early and total resorptions observed at this level was within range of historical background.

Group	CNS 7056 dose in mg/kg b.w./day, i.v.	Number of pregnant rats		Female rat no.[6] (treated animals)	
		Repro-duction[7]	Toxico-kinetics	Repro-duction	Toxico-kinetics
1	0 (control)	20	3	1 - 25	101 - 103
2	3 (low dose)	20	3	26 - 50	104 - 106
3	10 (intermediate dose)	20	3	51 - 75	107 - 109
4	30 (high dose)	20	3	76 - 100	110 - 112

Methods

Doses: 0 (vehicle), 3, 10, 30 mg/kg/day
Doses were selected based on a DRF study in rats ([redacted] (b) (4) 23508) where female rats were intravenously administered 3, 10, 30 mg/kg from GD 6-10. The NOEL for this study was below 3 mg/kg for dams and 30 mg/kg for the fetus. No malformations or variations were observed in this study.

Frequency of dosing: Once daily from GD 6-17

Dose volume: 12 mL/kg/day

Route of administration: Intravenous bolus injection over 3 minutes into tail vein

Formulation/Vehicle: [redacted] (b) (4) and 2.5 mg/mL / with lactose monohydrate in 0.9% saline

Species/Strain: Rat/CD®/Crl: CD (SD)

Number/Sex/Group: 20 females/group

Satellite groups: 3 females/group

Study design: This was a typical EFD study aligned with the OCED Test 414 and ICH S5(R2). In this study pregnant females were dosed once daily during organogenesis from implantation to hard palate closure (GD6-17). Laparotomies occurred on GD20. Mortality, body weight, and food consumption was monitored in dams during the treatment period. During laparotomy, dams' uterus and ovaries were weighted and macroscopic examination of the internal organs and placenta occurred. Corpora lutea,

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	implantation sites, and resorptions were evaluated in each dam. Fetuses were evaluated for viability, body weight, placental weight, macroscopic malformation, and skeletal and visceral retardations or variations. Toxicokinetic analysis occurred in maternal plasma for the parent and metabolite during treatment period.
Deviation from study protocol:	No major deviations from the study plan were noted. Minor deviation included the following, which were determined not to affect the validity or integrity of the results obtained in during this study: • Test item: the purity was 98.7% rather than 97.2% as stated in the study plan. • Housing and feeding: a malfunctioning air conditioning system resulted in relative humidity levels to exceed the maximum rang of 55%±15% on a few test days. The room temperature did not exceed the maximum range during this period. • TK evaluation: the AUc-value was calculated for the 10 mg/kg dose level on GD17 instead of an AUC-value as most of the plasma values were below LOQ.

Observations and Results

Mortality

No mortalities occurred in this study.

Clinical Signs

Clinical signs were monitored daily.

Restlessness and agitation occurred in all treatment groups and was attributed to an intense defense reaction against the administration procedure on all treatment days. This was not considered a pharmacodynamic effect but related to the low pH 3.1 of the test article.

Pharmacodynamic effects characterized by reduced motility occurred at all dose levels and on all treatment days, starting within 5 minutes after each injection and lasting for up to 5 minutes in the LD and up to 20 minutes in the MD. Reduced motility changed to sedation at the HD and lasted up to 20 minutes after injection.

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Increased chewing movements occurred on a few treatment days at the start of the treatment period at the LD, MD, and HD. Increased grooming occurred at the LD on one to two treatment days.

Clonic convulsions occurred in one MD dam (Animal 62) and tonic convulsions occurred and one HD dam (Animal 83) on GD 11. Convulsions started immediately after the injection and lasted for up to 5 minutes, suggesting a C_{max} effect of the parent or metabolite. The study report suggests the fast onset is indicative of a local reaction rather than an event mediated by the central nervous system. In agreement with published literature on infusion solutions, the findings may be related to the low pH of the administered solution, in conjunction with an extended administration time (3 min) and the required restraint.

Body Weight

Body weight was monitored daily starting on GD 0. No test article-related effect on body weights occurred in this study.

Food Consumption

Food consumption was monitored daily. No test article-related effect on food consumption occurred in this study.

Toxicokinetics

Toxicokinetic analysis of the parent (CNS 7056) and metabolite (CNS 7054X) were analyzed in plasma samples of dams at predose, 1, 2, and 8 minutes post dose on GD 6 and 17. Exposure levels were significantly lower on Day 17 compared to Day 6. It is also notable that exposure levels collected in this study were significantly higher than those reported in a 28-day IV bolus repeat-dose toxicity study conducted previously. Several factors that may account for these differences in exposure levels include animal status (pregnant versus non-pregnant), vehicle used (captisol vs saline), bolus infusion duration (3 min vs a shorter time), and volume administered (12 mL/kg vs 3 mL/kg).

A summary of TK results are presented in the two tables below.

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Table 46 Parent TK Summary for Rat EFD Study

Dosage [mg/kg b.w.], i.v.	CNS 7056: Non-compartmental analysis					
	C(0) [ng/mL]	$t_{1/2}$ [h]	$AUC_{0-0.18h}$ # [ng*h/mL]	$AUC_{0-\infty}$ # [ng*h/mL]	R	DPF
Gestation day 6						
3	-	-	-	-	n.a.	n.a.
10##	2.92	0.03	1.92	2.00	n.a.	-
30##	4489.78	0.03	178.83	179.71	n.a.	-
Gestation day 17						
3##	12.44	0.05	6.25	7.47	-	n.a.
10	69.40	0.07	3.31###	-	1.72	-
30##	279.28	0.10	41.00	64.35	0.23	0.66

-	Not computable or no reasonable interpretation from the available data as most of the plasma values were below LOQ
AUC	Area under the curve (0.18 h = 11 min)
C(0)	Calculated CNS 7056 plasma concentration at the end of the short-term infusion (3 min), corresponds to the C_{max} for CNS 7056
DPF	Dose proportion factor: [$AUC_{0-0.18h}$ (intermediate or high dose)/$AUC_{0-0.18h}$ (low dose)] / [(high or intermediate dose) / (low dose)] for the same day
n.a.	Not applicable
$t_{1/2}$	Elimination half-life after start of short infusion
R	Accumulation factor: ($AUC_{GD17\ 0-0.18h}$/$AUC_{GD6\ 0-0.18h}$)
#	Values obtained with an extrapolated value at the end of short-tem infusion of CNS 7056
##	Calculation of the TK-parameters by using the mean plasma levels and not the individual ones
###	$AUD_{0-0.083h}$ (Area under the data, 0.083 h = 5 min)

Table 47 Metabolite TK Summary for Rat EFD Study

Dosage [mg/kg b.w.], i.v.	Metabolite CNS 7054X: Non-compartmental analysis						
	C_{max} [ng/mL]	t_{max} [h]	$t_{1/2}$ [h]	$AUC_{0-0.18h}$ # [ng*h/mL]	$AUC_{0-\infty}$ # [ng*h/mL]	R	DPF
Gestation day 6							
3	7658.00	0.07	0.21	1032.63	2455.14	n.a.	n.a.
10	31020.33	0.07	0.20	4147.24	9539.65	n.a.	1.20
30	89879.00	0.08	1.72	13243.44	217275.82	n.a.	1.28
Gestation day 17							
3	7318.00	0.08	0.13	982.99	1761.38	0.95	n.a.
10	26489.00	0.07	0.27	3742.34	11018.54	0.90	1.14
30	76690.00	0.11	0.33	11566.51	40634.45	0.87	1.18

AUC	Area under the curve (0.18 h = 11 min)
C_{max}	Maximum metabolite plasma concentration
DPF	Dose proportion factor: [$AUC_{0-0.18h}$ (intermediate or high dose)/$AUC_{0-0.18h}$ (low dose)] / [(high or intermediate dose) / (low dose)] for the same day
n.a.	Not applicable
R	Accumulation factor: ($AUC_{GD17\ 0-0.18h}$/$AUC_{GD6\ 0-0.18h}$)
$t_{1/2}$	Elimination half-life after start of short infusion
t_{max}	Schedule sampling time to reach C_{max}
#	Values obtained with an extrapolated value at the end of short-tem infusion of CNS 7056

Dosing Solution Analysis

Dosing solution analysis revealed the formulations were within the expected range of theoretical concentrations. The measured actual concentrations ranged from 103.1% to 107.5%.

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Necropsy

Laparotomies and necropsy occurred on GD 20. Ovaries and uteri of dams were weighed and macroscopic examination of the internal organs and placenta of the dams occurred at necropsy.

A summary of the number of dams examined and the relevant reproductive parameters are presented in the next two tables below.

Table 48 Summary of Dams Examined in the Rat EFD Study

	Group 1 Control	Group 2 3 mg/kg	Group 3 10 mg/kg	Group 4 30 mg/kg
Treated dams	25	25	25	25
Not pregnant dams	0	2	0	0
Dams without viable fetuses	0	0	0	0
Not examined dams	5	3	5	5
Evaluated litters	20	20	20	20

No test article-related pathological findings occurred in this study. Gravid uterus weights and carcass weight were not affected by the test article.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Corpora lutea, implantations, and resorptions were counted per dam. Below is a summary of the findings from the study.

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Table 49 Summary of Reproduction Parameters in the Rat EFD Study

Parameter		Group 1 Control (n = 20)	Group 2 3 mg/kg b.w./day (n = 20)	Group 3 10 mg/kg b.w./day (n = 20)	Group 4 30 mg/kg b.w./day (n = 20)
Corpora lutea	total	284	262	298	292
per dam		14.2	13.1	14.9	14.6
Implantation sites#1	total	273	256	289	272
per dam		13.7	12.8	14.5	13.6
Resorptions#2	total	9	8	10	21*
per dam		0.5	0.4	0.5	1.1
Early resorptions#2	total	8	7	9	17*
per dam		0.4	0.4	0.5	0.9
Late resorptions#2	total	1	1	1	4
per dam		0.1	0.1	0.1	0.2
Live fetuses#3	total	264	248	279	251*
per dam		13.2	12.4	14.0	12.6
Dead fetuses at laparotomy	total	0	0	0	0
Pre-implantation loss	mean %	3.7	2.2	3.0	6.1
Post-implantation loss	mean %	3.4	2.9	3.6	7.4

* Significantly different from the controls at $p \leq 0.05$
** Significantly different from the controls at $p \leq 0.01$

The statistical comparison was conducted by

#1 comparing the ratio of implantation sites/corpora lutea of the test group with the ratio of implantation sites/corpora lutea of the control group

#2 comparing the ratio of resorptions/implantation sites of the test group with the ratio of resorptions/implantation sites of the control group.

#3 comparing the ratio of fetuses/implantation sites of the test group with the ratio of fetuses/implantation sites of the control group

Statistically significant increase in the early and total resorptions vs implantation sites at the HD were within the test facilitates historical background data (control range 0.0-1.8), therefore, the finding is not of toxicological significance.

No test article-related effects occurred on the prenatal fetal development with respect to the number of corpora lutea, implantation sites, resorptions, and the values calculated for the pre- and post-implantation loss.

Offspring (Malformations, Variations, etc.)

Placenta and fetuses were weighed. Fetuses were examined for viability, sex, distribution in the uterine horns, and runts. Fetuses were examined for the number and type of retardations, variations, and malformations. 50% of the fetuses were used for skeletal examination and the other 50% of the fetuses were used for soft tissue/visceral examinations.

No fetal mortality was noted at laparotomy. No test article-related effect on sex distribution, fetal weight, or placental weight occurred in this study. Runts identified in the control and MD groups were within test facility historical background data.

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Malformations of the fetus are summarized in the table below.

Table 50 Summary of Malformations in the Rat EFD Study

Group	Dam No.	Fetus No. and sex	Type of malformations
2: 3 mg CNS 7056/kg	35	1 m	Multiple malformations: Encephalocele (diameter approx. 2 mm), short snout, malrotated hind limbs

In the LD, multiple malformations such as encephalocele, short snout, and malrotated hind limbs occurred in a single fetus. These findings were considered incidental as these malformations are known to occur spontaneously in this rat strain and were within the test facilities historical background data.

An unclassified external variation characterized by hemorrhage between the cornea and the lens of the left eye in one HD fetus was considered spontaneous. No test article-related macroscopic variations in the fetuses occurred in this study.

Skeletal variations and retardations are summarized in the tables below.

Table 51 Summary of Skeletal Variations in the Rat EFD Study

Parameter	Mean value observed in this study [fetal incidence in%]##	LPT background data range of individual values [fetal incidence in mean %] (n = 48 control or n = 125 test item groups data taken from 2000 - 2010)#
Sternebra(e) misaligned (severity: slight)	Control: 0.0 Group 2: 4.8 ($p \leq 0.01$) Group 3: 2.1 Group 4: 3.2 ($p \leq 0.05$)	0.0 - 9.5 (control) 0.0 - 8.9 (test item groups)

data not audited by QAU

in each of the affected fetuses, absence of one ossification was observed in one (5th) of the metacarpal bones

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Table 52 Summary of Skeletal Retardations in the Rat EFD Study

Parameter	Mean value observed in this study [fetal incidence in%]##	LPT background data range of individual values [fetal incidence in mean %] (n = 48 control or n = 125 test item groups data taken from 2000 - 2010)#
Absence of ossification in metacarpalia 2 to 5	Control: 76.5 Group 2: 86.3 (p ≤ 0.05) Group 3: 85.7 (p ≤ 0.05) Group 4: 81.7	0.0 - 92.5 (control) 0.0 - 91.7 (test item groups)
Caudal vertebral bodies, no body ossified	Control: 7.6 Group 2: 5.6 Group 3: 2.9 Group 4: 1.6 (p ≤ 0.05)	0.0 - 27.6 (control) 0.0 - 23.3 (test item groups)
Skull incomplete ossification	Control: 14.4 Group 2: 14.5 Group 3: 6.4 (p ≤ 0.05) Group 4: 10.3	1.4 - 59.4 (control) 1.4 - 60.0 (test item groups)

data not audited by QAU

in each of the affected fetuses, absence of ossification was observed in one of the metacarpal bones

All skeletal variations and retardations were within test facility historical background data. No test article-related effect on skeletal development occurred in this study.

Soft tissue variations are summarized in the table below.

Table 53 Summary of Soft Tissue Variations in the Rat EFD Study

Parameter	Mean value observed in this study [fetal incidence in%] and proportion of fetuses affected (affected/examined)	LPT background data range of individual values [fetal incidence in mean %] (n = 48 control or n = 125 test item groups data taken from 2000 - 2010)#
Misplaced kidney	Control: 0.0 (0/132) Group 2: 1.6 (2/124) Group 3: 2.9 (4/140) (p ≤ 0.05) Group 4: 3.2 (4/126) (p ≤ 0.05)	0.0 - 3.0 (control) 0.0 - 3.6 (test item groups)

data not audited by QAU

All soft tissue variations were within test facility historical background data. No test article-related effect on soft tissue development occurred in this study.

No test article-related findings were noted after external/internal examinations, skeletal examinations, or soft tissue evaluations.

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9.2.2 Embryonic Fetal Development

Study title: Study of Embryo-Fetal Development in Rabbits with CNS 7056 by Intravenous Administration

Study no.:	(b) (4) 23507
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 9, 2011
GLP compliance:	Yes, this study was performed in compliance with GLP regulations of the EC enacted in (b) (4) and OECD Principles of GLP 2002. These principles are comparable with US FDA GLP 21CFR58.
QA statement:	Yes, QA statement was signed by (b) (4) on July 12, 2011
Drug, lot #, and % purity:	CNS 7056B (CNS 7058 besylate), Lot 11084, 98.7%

In this embryo-fetal development study, female rabbits (20/group) were intravenously administered remimazolam at target doses of 0, 1.25, 2.5, and 5.0 mg/kg/day during organogenesis from implantation to hard palate closure (GD 6-20). Laparotomies occurred on GD 29.

Key Study Findings

- No mortalities occurred.
- Pharmacological effects of sedation occurred in all groups. Sedation occurred within 5 minutes after dosing and lasted up to 1 hour in LD dams and up to 2 hours in MD and HD dams.
- Statistically significant decreases in body weights (up to 8.4% LD, 6.2% MD, and 11.1% HD) and body weight gains (C: +13.5%, LD: +10.6%, MD: +10.0%, HD: +10.5%) during the treatment period were correlated with a 55% decrease in food consumption (up to 44% LD, 49% MD, and 55% HD).
- No test article-related effect on corpora lutea, implantation sites, resorptions, number of live fetuses, and values calculated for the pre- and post-implantation loss and sex distribution of fetuses.
- Fetal and placental weights were decreased in the HD (-9% and -12%, respectively).
- No test article-related effect on malformations, variations, or retardations.
- Maternal NOAEL = 2.5 mg/kg/day, based on the >10% decrease in body weight and body weight gains at the next highest dose, 5 mg/kg/day
- Embryo-Fetal Development NOAEL = 2.5 mg/kg/day based on the decreased fetal and placental weights at the next highest dose, 5 mg/kg/day

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Group	CNS 7056 dose in mg/kg b.w./day, i.v.	Number[14] of evaluated litters ##	Rabbit no.[15] (treated animals)
1	0 (2 mL diluent/kg b.w./day)	20	1 f - 24 f
2	1.25 (low dose)	20	25 f - 48 f
3	2.5 (intermediate dose)	20	49 f - 72 f
4	5.0 (high dose)	20	73 f - 96 f

f = female
= Three (3) animals per group (groups 1 to 4) were used for blood sampling

Methods

Doses: 0, 1.25, 2.5, and 5.0 m/kg/day
Doses were selected based on a dose-range-finding EFD study (b) (4) 23506) where rabbits were intravenously administered 2.5, 5, or 7.5 mg/kg/day from GD 6-20. The NOEL was below 2.5 mg/kg/day based on pharmacodynamic effects of sedation in dams. The fetal NOEL was 5 mg/kg/day based on decreased female fetal and placental weights occurring at 7.5 mg/kg/day.

Frequency of dosing: Once daily from GD 6-20

Dose volume: 2 mL/kg/day

Route of administration: Intravenous administration (240 sec infusion or 4 min) into the middle ear vein

Formulation/Vehicle: (b) (4) 2.5 mg/mL / with lactose monohydrate in 0.9% saline

Species/Strain: Rabbit / Himalayan

Number/Sex/Group: 20/group

Satellite groups: None

Study design: This was a typical EFD study aligned with the OCED Test 414 and ICH S5(R2). In this study pregnant females were dosed once daily during organogenesis from implantation to hard palate closure (GD6-17). Laparotomies occurred on GD20. Mortality, body weight, and food consumption was monitored in dams during the treatment period. During laparotomy, does' uterus and ovaries were weighted and macroscopic examination of the internal organs and placenta occurred. Corpora lutea, implantation sites, and resorptions were evaluated in each doe. Fetuses were evaluated

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for viability, body weight, placental weight, macroscopic malformation, and skeletal and visceral retardations or variations. Toxicokinetic analysis occurred in maternal plasma for the parent and metabolite during treatment period.

Deviation from study protocol: No major deviations were noted. The following minor deviations were determined not to affect the validity and integrity of the results of this study:

- Dosing solutions: Two 5 mg/mL master stock solutions of 1500 mL or 500 mL were prepared. The three dosing solutions were divided into 80 portions or 27 portions of 20 mL instead of 45 portions (one for each dosing day) as stated in the study plan.
- Housing and feeding: A malfunctioning air conditioning system resulted in relative humidity levels to marginally exceed the maximum range of 55%±15% on a few test days. The humidity values were slightly below the 40% limit on one day. The room temperature did not exceed the maximum range during this period. The climate control plotting was interrupted on one day due to servicing.
- Evaluation/parameters: Carcass weight (terminal body weight minus Day 6 body weight) and net weight change from Day 6 (carcass weight minus Day 6 weight) were calculated although not stated in the study plan.
- Statistics: Statistical analysis (Student's t-test and Dunnett-test) were performed using an additional significance limit of $p \leq 0.05$. This was not fixed in the study plan or a study plan amendment.

Observations and Results

Mortality

No mortalities occurred in this study.

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Clinical Signs

Clinical signs were monitored once daily.

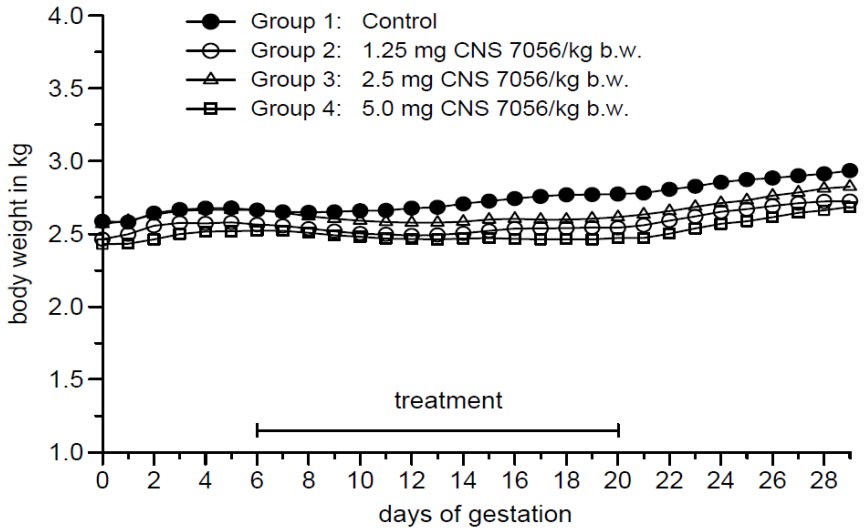
Pharmacodynamic effects of sedation occurred at all treatment doses on all treatment days, starting within 5 minutes after the start of each dose and lasting for 20-60 minutes in the LD and for up to 2 hours in the MD and HD.

Body Weight

Body weight was monitored daily during the treatment period.

A summary of body weights is described in the figure below.

Figure 1 Body weight of female animals
daily mean values per group



Body weight was slightly decreased (up to 8.4% LD, 6.2% MD, and 11.1% HD) from GD 11 or 12 onwards until laparotomy on GD 29. Body weight gain was slightly lower in the treatment groups (+10.6% LD, +10.0% MD, and +10.5% HD) compared to the control group (+13.5%). Body weight gains at the LD, MD, and HD were statistically significant on GD 12-15 and GD 15-18 in the HD compared to controls.

Food Consumption

Food consumption was monitored daily during the treatment period.

A summary of food consumption is described in the figure below.

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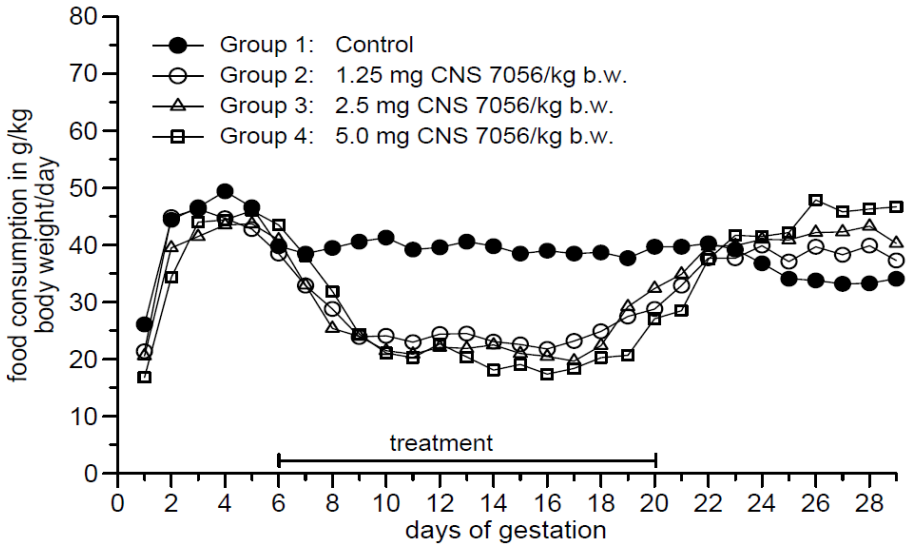
Figure 2 Food consumption of female animals
daily mean values per group



Absolute and relative food consumption was moderately to distinctly reduced during the treatment period at all dose levels. Statistically significant decreases in relative food consumption occurred in at the LD (44%) and MD (49%) on GD 8-18, and at the HD (55%) on GD 9-20. This is considered a test article-related decrease in food consumption, possibly secondary to the pharmacodynamic effects of sedation.

Toxicokinetics

Toxicokinetic analysis were conducted on plasma samples collected from does at 5, 15, 30, and 60 minutes after the start of dosing (i.e., 1, 11, 26, and 56 minutes after the end of dosing) on each treatment day (GD 6-20). Toxicokinetic analysis of the parent (CNS 7056) and its metabolite (CNS 7054X) in plasma was performed by the Test Site (b) (4)

Dosage [mg/kg b.w.] i.v.	CNS 7056: Non-compartmental analysis					
	C(0) [ng/mL]	$t_{1/2}$ [h]	AUC_{0-1h} # [ng*h/mL]	$AUC_{0-\infty}$ # [ng*h/mL]	R	DPF
Gestation day 6						
1.25	1496.155	0.337	507.081	576.494	-	-
2.5	2976.982	0.463	1069.306	1341.576	-	1.05
5.0	4319.183	0.287	1605.595	1767.320	-	0.79
Gestation day 20						
1.25	1977.795	0.280	645.333	701.317	1.27	-
2.5	3326.137	0.357	1315.800	1530.394	1.23	1.02
5.0	5535.251	0.317	2027.516	2272.032	1.26	0.79

C(0) Calculated CNS 7056 plasma concentration at the end of the short-term infusion (4 min), corresponds to the C_{max} for CNS 7056.
$t_{1/2}$ Elimination half-life
AUC Area under the curve
Values obtained with an extrapolated value at the end of the short-tem infusion
R accumulation factor: ($AUC_{GD20\ 0-1h}/AUC_{GD6\ 0-1h}$)
DPF dose proportion factor:
[AUC_{0-1h} (intermediate or high dose)/AUC_{0-1h} (low dose)] / [(high or intermediate dose)/(low dose)] for the same day

Dosage [mg/kg b.w.], i.v.	Metabolite CNS 7054X: Non-compartmental analysis							
	C(0) [ng/mL]	C_{max} [ng/mL]	t_{max} [h]	$t_{1/2}$ [h]	AUC_{0-1h} # [ng*h/mL]	$AUC_{0-\infty}$ # [ng*h/mL]	R	DPF
Gestation day 6								
1.25	737.431	1771.2	0.250	0.603	1161.965	1855.872	-	-
2.5	893.230	2533.4	0.250	0.940	1942.924	4161.857	-	0.84
5.0	6516.899	9436.5	0.193	0.600	6825.174	10578.685	-	1.47
Gestation day 20								
1.25	961.333	1646.4	0.333	0.513	1043.268	1511.467	0.90	-
2.5	1521.137	3331.3	0.333	0.560	2159.716	3399.596	1.11	1.04
5.0	5848.175	10792.1	0.250	0.480	6769.119	9364.372	0.99	1.62

C(0) Calculated CNS 7054X plasma concentration at the end of the short-term infusion (4 min) of CNS 7056
C_{max} Calculated maximum metabolite plasma concentration
Values obtained with an extrapolated value of the metabolite at the end of the short-tem infusion of CNS 7056
t_{max} Time-point of highest metabolite plasma concentrations
$t_{1/2}$ Elimination half-life
AUC Area under the curve
R accumulation factor: ($AUC_{GD20\ 0-1h}/AUC_{GD6\ 0-1h}$)
DPF dose proportion factor:
[AUC_{0-1h} (intermediate or high dose)/AUC_{0-1h} (low dose)] / [(high or intermediate dose)/(low dose)] for the same day

Dosing Solution Analysis

Dosing solution analysis revealed measured concentrations were from 95.3% to 102.4% of the theoretical value and were within acceptable limits.

Necropsy

Laparotomies occurred on GD 29. Ovaries and uteri were removed and weighed. Macroscopic examination of the internal organs of the does occurred on the same day as the laparotomy.

CNS 7056	Group 1 Control	Group 2 1.25 mg/kg	Group 3 2.5 mg/kg	Group 4 5.0 mg/kg
Treated dams	24	24	24	24
Not pregnant dams	3	2	2	3
Dams without viable fetuses	0	1	0	0
Dams with abortion	0	1 ##[1]	0	1
Not examined dams (spare animals)	1 ##[2]	0	1 ##[2] / 1 ##[3]	0
Evaluated litters	**20**	**20**	**20**	**20**

##[1] Incipient abortion was detected on gestation day 29.
##[2] One animal was excluded from evaluation as this rabbit was too restless for test item administration.
##[3] One animal injured its leg and was excluded from evaluation.

The abortions that occurred in one LD doe on GD29 and one HD doe on GD25 were considered spontaneous and not test article-related because abortions are known to occur spontaneously in rabbits of this strain and age.

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No test article-related pathological findings occurred in this study. Carcass weights of the dams were statistically decreased in the treatment groups (LD 7%, HD 10% below control values). No test article-related effect on the gravid uterus weight occurred.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Findings from macroscopic evaluation of the placenta for focal indurations; number of fetuses and placenta; sex and viability of the fetuses; viability of the fetuses after a 6- and 24-hour incubation at 34ºC; number and size of resorptions, corpora lutea, and implantations; and weight of the fetus and placenta were collected/recorded.

A summary of the relevant reproductive data is presented in the table below.

Parameter		Group 1 Control (n = 20)	Group 2 1.25 mg/kg b.w./day (n = 21/20)	Group 3 2.5 mg/kg b.w./day (n = 20)	Group 4 5.0 mg/kg b.w./day (n = 20)
Corpora lutea	total	151	163	147	151
	per dam	7.6	7.8	7.4	7.6
Implantation sites##[1]	total	132	146	126	142 *
	per dam	6.6	7.0	6.3	7.1
Resorptions##[2]	total	13	14	6	10
	per dam	0.7	0.7	0.3	0.5
Early resorptions##[2]	total	13	13	6	4 *
	per dam	0.7	0.6	0.3	0.2
Late resorptions##[2]	total	0	1	0	6 *
	per dam	0.0	0.0	0.0	0.3
Live fetuses##[3]	total	119	132 ##	120	132
	per dam	6.0	6.6	6.0	6.6
Dead fetuses at laparotomy	total	0	0	0	0
Pre-implantation loss	mean %	12.7	11.1	14.2	6.4
Post-implantation loss	mean %	9.7	7.6	4.8	7.1

* Significantly different from the controls at $p \leq 0.05$
** Significantly different from the controls at $p \leq 0.01$
No viable fetuses were noted in one dam in group 2: n = 21 females were examined for corpora lutea, implantation sites and resorptions, n = viable fetuses were found in 20 dams.
The statistical comparison was conducted by

##[1] comparing the ratio of implantation sites/corpora lutea of the test group with the ratio of implantation sites/corpora lutea of the control group

##[2] comparing the ratio of fetuses/implantation sites of the test group with the ratio of fetuses/implantation sites of the control group

##[3] comparing the ratio of resorptions/implantation sites of the test group with the ratio of resorptions/implantation sites of the control group.

Late and early resorptions were statistically significant in the HD, but were within the test facility historical background data. The statistically significant increase in ratio of implantation sites to corpora lutea was considered incidental as the pre-implantation loss occurred before the start of treatment with the test article.

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No test article-related effect on prenatal fetal development occurred with respect to corpora lutea, implantation sites, resorptions, number of live fetuses, and values calculated for the pre- and post-implantation loss and sex distribution of fetuses.

Fetal and placental weights are described in the table below.

Table 54 Summary of Fetal and Placental Weights in the Rabbit EFD Study

	Control	LD 1.25 mg/kg	MD 2.5 mg/kg	HD 5 mg/kg
PLACENTAL WEIGHTS				
of all viable fetuses	5.61 g	5.31 g	5.44 g	4.95 g* (-12%)
of male fetuses	5.56 g	5.29 g	5.49 g	4.84 g* (-13%)
of female fetuses	5.48 g	5.07 g	5.31 g	5.09 g
FETAL WEIGHTS				
of all viable fetuses	41.9 g	39.6 g* (-5%)	41.7 g	38.3 g** (-9%)
of male fetuses	42.1 g	39.4 g* (-6%)	41.6 g	38.2 g* (-9%)
of female fetuses	41.7 g	38.9 g	41.9 g	39.1 g

*p<0.05; **p<0.01 (Dunnett or Student)

Fetal and placental weight were decreased by 12% and 9% at the HD, with statistically significant decreases in the male and all viable fetuses.

Fetal mortality after laparotomy in an incubator is summarized in the table below.

Table 55 Summary of Fetal Viability After 6- and 24-Hours in an Incubator

Mortality	CNS 7056				(b) (4) background data range of individual total values (n = 57 control or n = 151 test item groups data taken from 2000 - 2011) ##[1], ##[2]	
	Group 1: Control	Group 2: 1.25 mg/kg b.w./day	Group 3: 2.5 mg/kg b.w./day	Group 4: 5.0 mg/kg b.w./day		
	Absolute number of fetuses that died ##[3] (Fetal incidence)				n	%
within 6 hours	5 of 119 (4.2%)	13 of 132 (9.8%)	9 of 120 (7.5%)	12 of 132 (9.1%)	0 - 18 (control) 0 - 22 (test groups)	0.0 – 15.0 (control) 0.0 – 19.5 (test groups)
within 6 to 24 hours	5 of 119 (4.2%)	2 of 132 (1.5%)	1 of 120 (0.8%)	3 of 132 (2.3%)	0 - 16 (control) 0 - 19 (test groups)	0.0 – 13.1 (control) 0.0 – 15.9 (test groups)

##[1] Treated groups not significantly influenced by any test compound
##[2] Data not audited by QAU
##[3] The statistical analysis was performed by comparing the number of dead fetuses per group and the total number of live fetuses at start of incubation in control and test item groups.

No dead fetuses were noted at laparotomy. Viability of all fetuses was within normal range during a 6- to 24-hour stay in an incubator.

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Offspring (Malformations, Variations, etc.)

Fetuses were inspected for malformations, variations and retardations of the skeletal and soft tissues.

Fetal malformations occurring in this study and historical controls are summarized in the tables below.

Table 56 Summary of Fetal Malformation in the Rabbit EFD Study

Malformed Fetuses - External Examination				
Group / Dose level	Dam No.	Fetus No.	Type of malformations	Fetal death after laparotomy
3 2.5 mg CNS 7056/kg	56	2 m	Malrotated fore paw (bilateral)	-
	61	1 f (runt)	Multiple malformations - Gastroschisis (approx. 22 x 15 mm) with prolapse of liver, stomach, intestines - Amelia of left hind limb - Malrotated right hind limb	30 min
4 5.0 mg CNS 7056/kg	73	3 f	Multiple malformations - Spina bifida - Gastroschisis with prolapse of liver, intestines - Malformed head	2 h
	79	4 f	Malrotated fore paw (bilateral)	-
	90	4 m	Malrotated fore paw (bilateral)	-

Table 57 Historical Control Data on Relevant Fetal Malformations

Indices	Data of control groups (obtained from mean values per group) fetal incidences per group		
	No.	Mean %	Range for control groups %
No. of groups examined	57	-	-
No. of fetuses examined	6886	-	-
No. of litters examined	1133	-	-
Extremities			
Malrotated limbs	15	0.23 ± 0.66	0.0 - 3.4
Micromelia	3	0.04 ± 0.19	0.0 - 0.9
Amelia (fore limb(s))	4	0.07 ± 0.24	0.0 - 0.9
Spina bifida	5	0.07 ± 0.30	0.0 - 1.8

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Multiple malformation including (thoraco)gastroschisis with prolapse of organs, amelia of fore- and/or hind limbs, and/or phocomelia of fore- and/or hind limbs	8	0.12 ± 0.40	0.0 - 2.4
Multiple malformation including (thoraco)gastroschisis with prolapse of organs, spina bifida, kyphosis, encephalo-cele, aplasia cutis, ablepharia, amelia of fore- and/or hind limbs, phocomelia	7	0.11 ± 0.38	0.0 - 2.1

Multiple malformations in the form of gastroschisis, spina bifida, amelia of the hind limb, malrotated hind limb or misshapen head were noted in one fetus each of the MD and HD. All findings were considered spontaneous because they were within range of the test facility background data.

A summary of the unclassified findings noted upon internal examination of the fetuses is described in the table below.

Table 58 Unclassified Fetal Observations in the Rabbit EFD Study

Fetuses with Unclassified Observations - Internal Examination				
Group / Dose level	**Dam No. and sex**	**Fetus No. and sex**	**Type of variations - classified or unclassified (U) -**	**Fetal death after laparotomy**
2 1.25 mg CNS 7056/kg	31	7 f	U: Urinary bladder: reddened	-
3 2.5 mg CNS 7056/kg	57	1 f	U: Urinary bladder: reddened	-
4 5.0 mg CNS 7056/kg	95	1 f	U: Region above left kidney: haematoma (diameter approx. 3 mm)	-

These reddened urinary bladder and the kidney hematoma were determined to be incidental because they were within the range of background incidences.

A summary of skeletal variations and retardations can be found in the tables below.

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Table 59 Summary of Skeletal Variations in the Rabbit EFD Study

Parameter	Mean value observed in this study [fetal incidence in%]	(b) (4) background data range of individual values [fetal incidence in mean %] (n = 57 control groups or n = 148 test item groups data taken from 2000 - 2011) ##
Sternebra(e) fused	Control: 2.5 Group 2: 0.0 (p ≤ 0.05) Group 3: 9.2 (p ≤ 0.05) Group 4: 4.5	0.0 - 13.0 (control groups) 0.0 - 19.5 (test item groups)
Total skeletal variations	Control: 6.7 Group 2: 0.8 (p ≤ 0.01) Group 3: 10.0 Group 4: 5.3	0.0 - 16.7 (control groups) 0.0 - 55.3 (test item groups)

Data not audited by QAU, test item groups data not significantly influenced by any test compound.

Table 60 Summary of Skeletal Retardations in the Rabbit EFD Study

Parameter	Mean value observed in this study [fetal incidence in%]	(b) (4) background data range of individual values [fetal incidence in mean %] (n = 57 control groups or n = 148 test item groups data taken from 2000 - 2011) ##
Less than 7 lumbar vertebral bodies ossified	Control: 2.5 Group 2: 0.0 (p ≤ 0.05) Group 3: 2.5 Group 4: 0.0 (p ≤ 0.05)	0.0 - 2.1 (control groups) 0.0 - 4.1 (test item groups)
Sternebra(e) not ossified	Control: 22.7 Group 2: 27.3 Group 3: 11.7 (p ≤ 0.05) Group 4: 34.1 (p ≤ 0.05)	6.4 - 41.2 (control groups) 5.7 - 43.6 (test item groups)
Total skeletal retardations	Control: 67.2 Group 2: 64.4 Group 3: 52.5 (p ≤ 0.05) Group 4: 72.0	36.7 - 81.3 (control groups) 27.9 - 89.7 (test item groups)

Data not audited by QAU, test item groups data not significantly influenced by any test compound.

All findings were within historical control ranges. No test article-related malformations, variations, or retardations occurred in this study.

9.3 Prenatal and Postnatal Development

Study title: Study of ONO-2745BS on Pre- and Postnatal Development, Including Maternal Function, by Intravenous Administration in Rats

Study no.:	NO12230
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 25, 2013
GLP compliance:	Yes, this study was conducted in accordance to GLP Standards for Non-Clinical Safety Studies on Drugs (Ministry of Health and Welfare Ordinance No. 21, 1997, (b) (4) partially revised in 2008 by the MHLWO Ordinance No. 114
QA statement:	Yes, a QA statement was signed by (b) (4) on November 25, 2013
Drug, lot #, and % purity:	ONO-2745BS, Lot SOL12621/6, 100.0% purity

In this pre- and post-natal development study, pregnant rats (20/group) were intravenously administered remimazolam at target doses of 0, 3, 10, and 30 mg/kg/day from implantation (GD 6) through lactation day 20 (weaning of F1 pups, PND 20). F1 offspring were weaned and mated. Male and female F1 offspring were necropsied after Caesarean section of the mated females on GD 13. F2 offspring were not evaluated past GD 13 for birth weights, sex ratio, or external malformations, but fertility of F1 generation can be determined with evaluated parameters.

Key Study Findings

- No test article-related effects on mortality occurred
- Pharmacodynamic effects of decreased movement, staggering gait, irregular respiration, prone position occurred in all F0 dosing groups and lateral position or sedation occurred in HD F0 females after dosing and lasting up to 1 hour.
- F0 general toxicity NOAEL = 30 mg/kg/day based on no test article-related effect on body weight, food consumption, or gross pathology
- F0 reproductivity NOAEL = 30 mg/kg/day based on no test article-related effect on duration of gestation, delivery, gestation index, birth index, and maternal behavior.
- F1 development/reproductivity NOAEL = 30 mg/kg/day based on no test article-related effect on growth, physical development, sensory and reflex functions, functional observational battery (open field test), learning (conditioned avoidance response/shuttle box), genital development, mating ability, fertility, implantation, or viability of F2 embryos.
- F2 embryos were assessed during Caesarean section at GD 13 without any test article-related findings.

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Group	Dosing article	Dose level* (mg/kg/day)	Concentration* (mg/mL)	Dose volume (mL/kg/day)
Control	Saline	0	0	12
Low	ONO-2745BS	3	0.25	12
Mid	ONO-2745BS	10	0.83	12
High	ONO-2745BS	30	2.5	12

* As ONO-2745

Methods

Doses: 0, 3, 10, and 30 mg/kg/day
Dose selection was based on an EFD study (b) (4) 23509) where pregnant rats were dosed at 0, 3, 10, and 30 mg/kg/day and decreased locomotor activity occurred at 3 mg/kg and above and sedation occurred at 30 mg/kg. Deaths were observed at 50 mg/kg in a 7-day IV study in rats (RMN1018). Based on these results, 30 mg/kg was selected as the HD for this study because no maternal deaths occurred, and toxicity evaluations would be appropriately feasible.

Frequency of dosing: Once daily from GD 6 to Lactation Day 20

Dose volume: 12 mL/kg

Route of administration: Intravenous (tail vein)

Formulation/Vehicle: (b) (4) 2.5 mg/mL pH 3.1/ 0.9% saline

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

Number/Sex/Group: 22/group

Satellite groups: No TK was performed as part of this study.

Study design: In this pre- and post-natal development study, pregnant rats (20/group) were intravenously administered remimazolam at target doses of 0, 3, 10, and 30 mg/kg/day from implantation (GD 6) through Lactation Day 20 (weaning of F1 pups, PND 20). F1 offspring were weaned and mated. Male and female F1 offspring were necropsied after Caesarean section of the mated females on GD 13. F2 offspring were not evaluated past GD 13.
F0 dams were evaluated for general toxicity parameters including mortality, clinical signs, body weights, food consumption, gross pathology as well as reproductive parameters including duration of gestation, delivery, gestation index, birth index, and maternal care behaviors.
F1 male and female offspring were evaluated for

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growth and development parameters including body weights, pinna unfolding, hair growth, incisor eruption, eye opening, righting reflex, negative geotaxis, visual/auditory/pain reflex, functional observational battery using the open field test, and learning/memory on the conditioned avoidance response test as well as reproductive parameters including genital development (preputial separation and vaginal opening), mating ability, fertility, implantation, and viability of F2 embryos.
F2 embryos were evaluated at GD 13. F2 offspring were not evaluated past GD 13 for birth weights, sex ratio, or external malformations.

Deviation from study protocol: An abnormal body weight (240 g) was noted in 1 F1 animal (1F21-1M) in the control group at 35 days of age as compared to the other 41 males (140-188 g) in the same group and an error in measurement was suspected. This instance was considered not to affect the evaluation of the study since data were collected from the other 41 animals in this group.
Mated females (F1) were prescribed to be weighed and undergo the observation at Cesarean section on GD 13. However, the measurement and observation of 1 control female (1F08-1F) were conducted on GD 14 instead. Therefore, mating data, the body weight on GD 13, and Caesarean section data on this animal were excluded from calculations. This instance was not considered to affect the evaluation of the study since data were collected from the other 19 animals in this group.

Observations and Results

F_0 Dams

Survival: No mortalities occurred in the F0 dams.

Clinical signs: Clinical signs from cage-side observations were recorded twice daily during the dosing period. Observations included nursing behavior (scattering of offspring in the cage, total litter loss, and cannibalization), delivery state (presence/absence of abnormal deliver and live newborns), and

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gestation duration.

Pharmacodynamic effects of decreased movement, staggering gait, irregular respiration, and prone position occurred in all treatment group after each dosing. Lateral position and sedation occurred in the MD and HD groups up to 1-hour post dosing.

No other test article-related effects on clinical signs, maternal behavior, gestation index, or duration of gestation occurred.

Body weight: Body weights were recorded at least twice weekly during the dosing period. No test article-related effects on body weight occurred.

Food consumption: Daily food consumption was calculated. No test article-related effects on food consumption occurred.

Uterine content: Implantation sites; total number of viable newborns; sex, body weight, and external morphology of newborns; dead newborns were preserved. No test article-related effects were noted.

Necropsy observation: Necropsy observations included gross pathology and number of implantation sites. Ovaries, uterus, mammary glands, and ear with ID number were preserved. No test article-related effects on implantation sites, total number of newborns, delivery index, number of live newborns, birth index, sex ratio of live newborns, or external malformations were noted.

Toxicokinetics: Not determined.

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Dosing Solution Analysis: Dosing solution analysis determined the measured concentration was 98.4-100.8% of the nominal concentrations, which is within acceptable limits.

F_1 Generation

Survival: Pup survival was monitored twice daily during the suckling period. No test article-related effects on viability index on PND 4 occurred.

Litters greater than 8 pups were culled to 8 (4 males and 4 females, where possible) at PND 4. One HD male died on PND 51. Pathologies in this animal included dilation of the right renal pelvis but a cause of death was not determined. This death was within range of historical background data and was determined not to be test article related. No test article-related effects on survival occurred.

Clinical signs: After weaning, F1 pups were assessed once daily for clinical signs.

Body weight: Body weights were recorded twice weekly from birth to weaning. After weaning, body weights of F1 pups were recorded weekly. No test article-related effects on F1 body weights occurred.

Food consumption: Milk in the stomachs of suckling F1 pups was noted. Adult F1 food consumption was not determined.

Physical development: Daily observations of physical development included pinna unfolding from PND 2-4, hair growth and eruption of incisors from PND 11-14, and eyelid opening from 14-18. No test article-related effects occurred.

Neurological assessment: Neurological assessment included observations of righting reflex and negative geotaxis on PND 11-14. No test article-related effects occurred.

After weaning, F1 pups were observed for sensory functions (visual, auditory, and pain) at PND 28 with the visual placing response and pupillary reflex, Preyer's reflex, and pain response. No test article-related effects occurred.

At PND 35, offspring were assessed in the open field test and conditioned avoidance response test. A

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statistically significant increase in frequency of defecation during the second trial of the open field in HD females was determined not to be of toxicological significance. No differences were noted in the conditioned avoidance in F1 males or females between the control and treatment groups.

Reproduction: Genital development was assessed in males (preputial separation at PND 42-49) and females (vaginal opening at PND 35-42). No test article-related effects on genital development occurred.

Reproduction was assessed in 2 males and 2 female pups from each litter. Mating ability was assessed at PND 70-84.
Females: Cesarean section occurred on GD 13 after observed mating. Implantations and number of corpora lutea, and number of live/dead embryos were recorded, ovaries, uterus, and ear with ID number were preserved.
Males: Necropsy occurred after Cesarean section of the mated females. Testes and epididymides from males that failed to mate or whose mating partners was not pregnant were preserved. Seminal vesicles including coagulating gland and prostate were examined.
No test article related effect on mating performance, fertility index, F1 male reproductive pathology, F1 female reproductive pathology (corpora lutea, implantation), or F2 embryos (live embryos or pre- or post-implantation loss) occurred.

Other: Gross pathology was assessed in offspring on PND 70 at necropsy. Kidneys, gross lesions, and ear with ID number was preserved. Unilateral dilation of the renal pelvis occurred in one LD and one HD male. The HD male with the dilated renal pelvis died. Incidence of dilated renal pelvis was within historical control ranges. No test article-related findings occurred.

F_2 Generation

Survival: F2 embryos were assessed for viability upon Cesarean section on GD 13. Number of implantations, live/dead F2 embryos, and classification of dead embryos as early, resorptions, or dead were the only parameters

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	collected for F2 embryos. No test article-related effects on F2 embryos occurred.
Body weight:	Not determined.
External evaluation:	Not determined.
Male/Female ratio:	Not determined.
Other:	None.

9.5 Juvenile Toxicology Studies

In the 2014 Agreed Initial Pediatric Study Plan (iPSP), the Applicant states the following:

> PAION plans to study the possible neurotoxicity of remimazolam and its possible sequelae when administered to juvenile animals in two species (rodent and non-rodent species). Despite an especially fast metabolism of remimazolam in adult rodents, juvenile rodents may be a suitable species due to their age-dependent lower expression of carboxylesterase. This will be evaluated in a preliminary study. It is planned to conduct these nonclinical studies before the enrollment of pediatric patients aged <3 years.

Because clinical studies in pediatric patients will be completed post-approval, the juvenile animal studies may also be permitted as post-marketing requirements under PREA.

In 2014, the Division agreed that studies should be completed in two species (rodent and nonrodent). Although unusual, this was based on the observations to date for general anesthetic and sedative agents and the resulting safety labeling changes in 2016 and 2017. Briefly, studies in rodents suggested that drugs that increase the activity of GABAergic neurotransmission or antagonize NMDA receptors were shown to result in widespread neuroapoptosis in the developing brain. To confirm these initial findings, studies in the nonhuman primate were completed by the FDA and subsequently by teams outside the FDA lead by Drs. (b) (4) Collectively, the results demonstrated that administration of these agents to pregnant animals or very young animals (PND 5) for approximately 3 hours or longer appeared to result in neuroapoptosis in brain structures which correlate with long-term cognitive deficits. The clinical significance of these findings is not clear; however, the risk assessment based on data in the NHP is more definitive and believed to be more relevant to human risk assessment for several reasons, including the facts that the NHP brain develops over a longer period of time and adequate control over physiological parameters is more easily accomplished in a larger animal model thereby avoiding potential confounding factors.

We recognize that the recently finalized ICH S11 suggests that the species selected should normally be the same species used in adult repeat-dose toxicity testing and when possible the rodent is the preferred model unless scientifically justified otherwise. In this case, the rodent does present some challenges. First, IV toxicity testing in a PND 7 rat is feasible though challenging; however, the study would have to be designed in a manner to assure adequate exposure given the rapid metabolism of the remimazolam in this species. Second, ideally the study would include adequate

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physiological support of the sedated animal, which usually would include supplemental oxygen and, if need be, nutritional support for prolonged sedation. This is more easily accomplished in a larger animal model. ICH S11 also states that studies in two species should be reserved for pediatric first indications or where multiple species concerns for postnatal development and one species alone is not able to address the concerns.

The proposed pediatric study plans were discussed again in the preNDA meeting in 2018. The minutes of that meeting note the following:

> From a nonclinical perspective, the studies and timeline proposed may require amendments as new data have emerged from published literature that raises our concern for sedative-induced developmental neurotoxicity as indicated by our Safety Labeling Change issued December 14, 2016. We strongly recommend that you discuss any juvenile animal protocol with the Division prior to initiating nonclinical juvenile animal studies.

(b) (4)

In the agreed 2014 PSP with FDA, the Division did not require nonclinical studies to support pediatric dosing in patients 3 years of age and older. Remimazolam is a centrally active drug product and the central nervous system is developing over the entire pediatric age range and into early adulthood. In 2014, the Division focused on the most vulnerable patient population (<3 years) given the class effect studies described above documenting concern for that age range. For the purposes of the current indication of procedural sedation of duration not more than 30 minutes, the existing data in very young animals suggests that procedures of less than 3 hours are not likely to result in neuroapoptosis. Although there is a second wave of synaptogenesis in adolescence, there are limited data to understand the potential vulnerability of neurons and impact of drugs on synaptic architecture during this developmental period. ICH S11 was recently finalized; although as of this review, the Federal Register notice has not yet been posted. This guidance clearly notes that the central nervous system is developing over the entire pediatric age range. Publications have identified continued development of brain structures through late childhood, adolescence, and young adulthood (Jernigan et al., 1991; Steen et al., 1997; Thompson et al., 2000). It is noted that the original iPSP did not address this period of development.

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Our preliminary review of the published literature identified several studies testing the potential neurotoxicity of benzodiazepines during early development. Although a thorough literature review has not been completed for benzodiazepine effects on brain development, the majority of studies suggest that administration of a single-dose (PND 14) or multiple doses (PND 7 to 11) of a benzodiazepine to juvenile animals during early brain development can result in long-term changes in the CNS that manifest in adult animals (Mikulecka et al., 2014a, Mikulecka et al., 2014b; Furukawa et al., 2016). These findings include changes in cognition, social behaviors, and drug taking behaviors. Although the window of vulnerability has been established from birth to early development for the effects of benzodiazepines there is much less known what happens when benzodiazepines are administered during the adolescence period even though it is well established that a second wave of synaptogenesis occurs in the adolescent brain (Arain et al., 2013). Many drugs that are substances of abuse (e.g., nicotine, alcohol, methamphetamine) when administered for short periods of time during the adolescent period can produce long-term changes in behaviors. The repeat-dose toxicology studies that were conducted by the Applicant during development evaluated fully mature animals that did not span the adolescent period.

Because remimazolam is a new molecular entity that is neuroactive, the entire period of brain development should ideally be studied in the juvenile animal studies. The age groups to be tested in the juvenile animal studies should include birth through adolescence exposures; however, the design of these studies is challenging for an acute use drug. Because there are other benzodiazepines approved for use in pediatric patients and the data to date suggesting adverse effects are either observed after treatments lasting 3 hours and longer or after repeated exposures, these studies can be done concurrent to the clinical studies. Negative results from a study in the rodent model testing the drug during peak synaptogenesis during adolescence would help to confirm the current clinical data that has not yet identified a risk for long-term changes in adolescent humans.

10 Special Toxicology Studies

Special toxicology studies and preliminary studies are summarized in the table below.

Study	Key Study Findings
13-0295 AG GLP Guinea pig sensitization test Sensitization/Challenge doses: Neg control: 0 IP / 0 IV (saline) Pos control: 20 mg IP / 40 mg IV human serum albumin LD: 0.2 mg/kg IP / 1.0 mg/kg IV	Title: **Active systemic anaphylaxis in guinea pig** Purpose: Evaluate anaphylaxis in guinea pig after repeated infusion of RF10007 (remimazolam). (3/sex/group) Experimental animals were sensitized by IP administration of the sensitization dosage of the control or test articles every other day for 3 times. Experimental animals were challenged by IV administration to the challenge dosage of the control or test articles via foot vein 14 days after the last sensitization.

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HD: 0.4 mg/kg IP / 2.0 mg/kg IV	Negative control (saline): no allergy symptoms Positive control (human serum albumin): 6/6 animals had a reaction to the allergen with symptoms of restlessness, scratching nose, piloerection, cough, shortness of breath, urination, defecation, wheezing, ataxia, spasm, et al. LD: 1/6 experienced trembling, sleep and ataxia HD: 6/6 experienced trembling, sleep and ataxia Trembling, sleep, and ataxia were observed in 2 unsensitized animals Conclusion: Remimazolam did not cause anaphylaxis in guinea pigs under the test conditions
(b) (4) 015-142 GLP Balb/c 3T3 Cells 0, 1.64, 4.10, 10.2, 25.6, 64, 160, 400, 1000 mcg/mL in the presence and absence of UVA irradiation	Title: **A phototoxicity study on ONO-2745BS (remimazolam) and ONO-IN-252 (metabolite CNS7054)** Study validation criteria: • Absorbance in the negative control = greater than 0.4 • Cell viability rate for the irradiated negative control group = 80% or greater when compared to the non-irradiated negative control group • Mean absorbance in both the negative and positive controls was within range of the background data of the test facility. The results were evaluated from the mean photo effect (MPE), because the IC_{50} could not be determined and because no cytotoxicity was observed with Remimazolam or CNS7054. • Remimazolam MPE = 0.013 • CNS7054 MPT = 0.009 • Criteria for phototoxicity is an MPE>0.1, therefore, the test article was judged to have no potential to induce phototoxicity Negative for phototoxicity
F12XA058 Non-GLP 2-day Metabolite Study IV bolus Rat 100, 200 mg/kg	Title: **Blood drug-exposure study by intravenous administration in ONO-IN-252 (metabolite CNS7054) rats** Purpose: this is a preliminary dose range-finding study for future rat micronucleus studies with the major metabolite of remimazolam, CNS7054. • After bolus administration of 100 or 200 mg/kg CNS7054 for 2 consecutive days, no changes in general condition, body weight, or necropsy findings. • 100 mg/kg: AUC24h = 287000 ng*h/mL • 200 mg/kg: AUC24h = 441000 ng*h/mL • The maximum tolerated dose or the maximum administrable dose is needed for the rat micronucleus assay
F13XT009 Non-GLP Metabolite DRF for an EFD (GD6-10) study IV bolus / IV infusion Crl:CD(SD) gravid rats BID dosing: 200 mg/kg	Title: **Dose Range-finding study by intravenous administration for the effects on embryo-fetal development in ONO-IN-252 (metabolite CNS7054) rats** • Ataxic gait, decreased motility, bradypnea, and sedation occurred on the first day of dosing with 200 mg/kg bolus. Excessive sedation at 200 mg/kg would prevent repeated dosing and this group was terminated.

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Vehicle pH 9	• Slow infusions of 200 mg/kg over 3 minutes (SID) resulted in an AUC_{24} = 684000 ng*h/mL • Established a dosage of 12 mL/kg at a dosing rate of total volume/3 min (1.2 mL/min) would be tolerated for repetitive administration of 200 mg/kg dose in gravid rats and exceed predicted clinical drug-exposure levels
(b) (4) 015-309 (incl. PBC015-309-01) Non-GLP 2- or 4-day Metabolite Study 24h IV infusion Male Monkey Two days: 120 mg/kg/day Four days: 240 mg/kg/day Vehicle = water for injection	Title: **Intravenous infusion dose finding study of ONO-IN-252 (metabolite CNS7054) in cynomolgus monkeys** Purpose: This is a dose range finding study for future 2-week repeat-dose toxicity studies in monkeys Evaluated clinical signs, body weight, food consumption, TK, clinical pathology (hematology, blood chemistry), and necropsy. • No mortalities • No changes in clinical signs, body weight, food consumption • No test article-related changes in hematology, blood chemistry, or necropsy • C_{max} and AUC increased dose-dependently No clear toxicity occurred at 120 or 240 mg/kg/day of CNS7054 and exposure was dose-dependent. Therefore, 240 mg/kg/day was considered appropriate to set as the highest dose for subsequent 2-week IV infusion dose studies.

11 Integrated Summary and Safety Evaluation

Cosmo Technologies, Ltd is developing BYFAVO® (Remimazolam for Injection), a benzodiazepine, for short procedural sedation.

Impurities

Impurities were evaluated in a genetic toxicology battery and a 4-week repeat-dose toxicology study in monkeys using a batch of remimazolam (YMK110831) spiked with 8 impurities at appropriate concentrations.

In this GLP repeat-dose toxicity study of impurities (b) (4) 015-257), monkeys (4/sex/group) were intravenously infused with ONO-2745BS spiked with 8 impurities/degradants at target doses of 0 (saline), 12, 30, 60 mg/kg/day once daily for 12 hours/day for 4 weeks. A separate recovery group (2/sex/group) was allowed to recover for 2-weeks after dosing. Toxicokinetics was conducted on main and recovery animals (6/sex/group). Key study findings support the conclusion that the NOAEL = 30 mg/kg/day in males and females. Transient increase in sodium (2x), potassium (3x), and chloride (2x) occurred in the HD females on Day 11. Transient increase in potassium (up to 3x) occurred in the MD and HD males on Day 11. These changes were transient and without a histopathological correlate. These findings were not considered adverse. Local findings included slight inflammation on the vascular wall with slight to moderate perivascular inflammatory granuloma, intimal thickening, and thrombus and necrotic tissue at the caudal vena cava (site of catheterization) in one HD

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male and one HD female. Local findings were reversible except for granuloma. Systemic NOAEL = 60 mg/kg/day and Local NOAEL = 1 mg/mL (LD and HD).

In an in vitro bacterial reverse mutation assay using the pre-incubation method ((b) (4)015- 270), remimazolam spiked with 8 impurities, in the presence or absence of metabolic activation tested negative for mutagenicity in *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 or *Escherichia coli* WP2*uvrA* at 156, 313, 625, 1250, 2500, 5000 mcg/plate. Key study findings support the conclusion that the test article is not mutagenic under these test conditions.

In a valid mouse lymphoma assay ((b) (4)015- 271), L5178Y ($Tk^{+/-}$-3.7.2c) mouse lymphoma cells were treated with 200-800 mcg/mL remimazolam in short-term (3 hour) treatments with and without metabolic activation and in 24-hour continuous treatment without metabolic activation. In the definitive experiment and confirmatory test, no statistically significant dose-dependent increase in total mutation frequency occurred with short-term treatment (3 hours) with metabolic activation or with 24-hour continuous treatment without metabolic activation. In the definitive experiment, a statistically significant dose-dependent increase in total mutation frequency occurred with short-term treatment (3 hours) without metabolic activation. This finding was not reproduced in the confirmatory experiment, which considered the increase in T-MF a nonspecific effect caused by cytotoxicity. Therefore, no statistically significant, dose-dependent, and reproducible increase in total mutation frequency occurred in this study. Study report concludes that the test article might have induced genotoxicity in the mouse lymphoma cells under these study conditions. However, because this finding was not reproducible in the confirmatory test, this Reviewer agrees with the Sponsor that the increase in T-MF was a nonspecific effect caused by cytotoxicity.

In this valid comet-micronucleus combination assay (E162 (083-235), male rats (5/group) were intravenously administered test article at target concentrations of 0, 18.8, 37.5, and 75.0 mg/kg once daily for 3 consecutive days. At 3 hours after the final administration, femur samples were collected for the micronucleus assay and liver samples were collected for the comet assay. The frequency of micronucleate immature erythrocytes and the ratio of immature erythrocytes to the total number of analyzed erythrocytes were calculated. In the comet assay, the % tail DNA, and indicator of DNA damage, was determined. Key study findings support the conclusion that the test article was negative for micronuclei induction (aneuploidy or clastogenicity) in rat bone marrow and DNA damage in the rat liver under the test conditions. In the micronucleus test, no statistically significant increases in micronucleated cells occurred. A statistically significant increase in the ratio of immature erythrocytes to total number of erythrocytes occurred at 75 mg/kg, indicating an influence of the test article on bone marrow cells; however, the value was within the standard ranges (mean ± 3SD) calculated from historical negative control data from the test facility. In the comet assay, the % tail DNA (%TD) in all test article groups were not significantly different than concurrent negative controls.

Impurities were adequately evaluated in the submitted studies.

Excipients

Dextran 40 is an excipient in the clinical formulation that has not been fully evaluated for safety in an approved product with a comparable patient population. As a novel

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excipient, a safety justification as recommended by the FDA guidance for industry*: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients* should include a 1-month repeat-dose general toxicology study in two species, standard genotoxicity battery, a fertility study, embryo-fetal development studies in two species, and a pre- and post-natal development study.

Genetic toxicity of dextran was negative in an Ames assay and in a mouse lymphoma TK assay (Seifried et al., 2006; Supporting Raw Data Ames; Supporting Raw Data MLTK). Dextran was negative in the Ames assay using tester strains TA98, TA100, TA1535, TA1537, TA1538 with and without metabolic activation up to 100-10,000 mcg/plate. Dextran was negative in the mouse lymphoma TK assay up to 1,000-5,000 mcg/plate with and without metabolic activation. A final study report for an in vivo rat micronucleus assay for Dextran 40 ((b) (4) 38086) was submitted by the Applicant late in the cycle, allowing for a clock extension. In the definitive study, male rats (5/group) were intravenously administered Dextran 40 EP at target doses of 0 (saline vehicle), 200, 600, and 2000 mg/kg/day for two days. Dextran 40 tested negative for clastogenicity in the rat bone marrow micronucleus assay under these test conditions. Therefore, Dextran 40 was adequately evaluated for mutagenicity and clastogenicity for the proposed drug product.

General toxicity was evaluated after administration of remimazolam formulated with Dextran 40 in 1-month studies in rat (Study 172.351.5266) and minipig (Study 32237). In the 1-month repeat-dose studies, rats had a NOAEL = 600 mg of Dextran 40/kg/day at 4 h daily infusions (43x safety margin to the MRHD of 33 mg/day) and minipig had a NOAEL = 360 mg Dextran 40/kg/day after 6 h daily infusions (>100x safety margin to the MRHD of 33 mg/day).

Female fertility was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits (Study 497736). In the female fertility study in rabbits, the NOAEL was 320 mg Dextran 40/kg/day (46x safety margin to the MRHD of 33 mg/day). In a 4-week minipig repeat-dose toxicity study (32237), sperm count, viability, and morphology in addition to histology of the testis and epididymis were evaluated after administration of remimazolam formulated with Dextran 40. The effect of Dextran 40 on male fertility and mating behavior was not adequately evaluated in the provided studies. The Applicant provided a literature-based justification of the safety of Dextran 40 on male fertility. This large hydrophilic polymer will not traverse cell membranes thus have minimal influence on any organ function including male reproductive endpoints. Furthermore, the degradation products of dextrans are metabolites to carbohydrates and therefore, are not expected to have adverse effects on fertility. Together, there appears to be low risk to male fertility with exposure to dextrans at the levels estimated with the proposed drug product and indication.

Embryo-fetal development was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits (Study 497736). In this EFD study in rabbits, the fetal NOAEL was 320 mg Dextran 40/kg/day (46x safety margin to the MRHD of 33 mg/day). To support the safety of Dextran 40 on embryo-fetal development, the Applicant provided a justification based on experimental evidence to demonstrate that Dextran 40 exposure to the embryo or fetus is negligible to support the safety of dextran. This justification included data suggesting that dextran does not cross the placental barrier, does not affect uterine blood flow in a manner that

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could negatively affect the embryo, is predominantly excreted unchanged, and metabolites are well characterized low molecular weight carbohydrates (glucose or nigerose). The combined animal data in one species (rabbit) and safety justification are adequate to qualify the safety of Dextran 40 on embryo-fetal development.

Pre- and post-natal development was evaluated after administration of remimazolam formulated with Dextran 40 in an extended fertility/mating study in rabbits (Study 497736). The NOAEL in this study was 320 mg Dextran 40/kg/day (46x safety margin to the MRHD of 33 mg/day). However, this study did not include evaluation of the F1 kits for learning/memory, mating behavior, and reproductive indices, or any evaluation of F2 kits. To support the safety of Dextran 40 on pre-and post-natal development, the Applicant provided a safety justification based on low exposure to the fetus and is the basis for the argument that there is a lack of concern for an adverse impact of dextran on pre-natal development. Furthermore, to justify the exposures to the infant that may occur from maternal milk, the Applicant provided further justification that there is a low risk of exposure based on low oral bioavailability, GRAS designation of dextrans from fermentation of sucrose by bacteria, and its use in an approved IV administration of Dextran 40 to pediatrics as a volume expander as well as to mitigate risk by labeling language that recommends interruption of breastfeeding (b) (4) hours after dosing. Together, there appears to be low risk to pre- and post-natal development with exposure to dextrans at the levels estimated with the proposed drug product and indication. However, reliance on literature makes this application a 505(b)(2).

Note: All approved products containing Dextran 40 have warnings for anaphylaxis in the package insert, some have Boxed Warnings, that should be reflected in the package insert of the proposed drug product in its current formulation.

Container Closure System (CCS)

Remimazolam is formulated as a lyophilized powder in a glass vial and rubber stopper. New data from published extractable/leachable studies on CCS for lyophilized powders has demonstrated that although previously these products were thought to have limited potential to extract compounds from the container closure, unique properties exist suggesting the potential for compounds to be extracted and therefore these should still be evaluated for potential toxicological concern. The Applicant submitted an extraction study and two leachable studies. The extraction study identified 6 potential leachables of which 5 were targeted in the leachable studies. The leachable studies analyzed two batches at a single timepoint and one batch across stability stored under different conditions. Under the conditions of the leachable studies, no compounds of concern were detected and no toxicological risk assessment were required, except for the one potential leachable that was not targeted: (b) (4) ((b) (4) mcg/day). In a risk assessment the levels of (b) (4) were determined to be acceptable. There is concern that the leachable studies did not fully characterize the leachable profile using three batches of drug product; therefore, additional leachable studies may be required. However, there was low variability between the two batches tested (20003 and TT280) and it is unlikely that a third batch would produce differing results. The reader is referred to the CMC review for adequacy of the methods used and timepoints tested in the extractable/leachable studies.

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Pharmacology
Primary pharmacology studies suggest that remimazolam produces sedative effects by acting at the benzodiazepine site on the $GABA_A$ receptor. Pharmacology studies suggest that remimazolam may produce short term amnesia similar to propofol. Flumazenil prevents or inhibits the sedative effects of remimazolam. Onset of sedation and recovery from sedation was rapid in all species tested compared to midazolam. There appears to be a potential for abuse through the intranasal and oral route (See CSS review for further details).

Multiple radioligand binding assays demonstrate the high electivity and specificity for remimazolam to bind $GABA_A$ receptors, suggesting a low risk of off-target pharmacological effects.

PK/ADME
PK/TK analysis was conducted in rat, rabbit, micropig, minipig, sheep, and monkey. All species had similar exposure levels with similar doses, with the exception of rat. Rat had significantly lower exposure levels of the parent after IV dosing attributed to the carboxylesterase 1A present in the plasma. In general, exposure was dose-proportional with no sex differences. T_{max} was 0-2 minutes after bolus dosing but increased after longer infusions suggesting accumulation during infusion. Half-life was shortest in rat (3 min), followed by micropig and sheep (12-25 min), and longest in the monkey (190 min) after bolus dosing.

Absorption is significantly lower by the oral (<10%), intranasal (8-31% in rat and 5-10% in minipig) or intraperitoneal (5-9%) routes compared to the clinical intravenous route. **Distribution** studies conducted in rat and monkey determined fast distribution to all tissues studied with peak radioactivity detected at the first timepoint sampled in monkey (5 min after dosing). Highest levels of radioactivity were detected in the organs of excretion, kidney and liver. Radioactivity was detected in the cerebrum and cerebellum at 5 min in both rat and monkey. At 72 h, cerebellum concentrations in the rat appeared to accumulate, while no radioactivity was detected in the monkey cerebellum at 168 h post dose. Radioactivity in skeletal muscle, skin, and fat was higher in the monkey compared to the rat. In fetal blood and tissues and in female reproductive tissues, the radioactivity was detectable up to the last sampling timepoint of 48 h. The ovary, placenta, amnion, and fetal blood radioactivity was greater than maternal plasma at 48 h after a single IV bolus of 10 mg/kg, suggesting higher exposures to the fetus. In rabbits, maternal milk was analyzed for remimazolam and the major metabolite, CNS7054, in a reproductive study dosing female rabbits from 2 weeks prior to mating to kit weaning. Parent and metabolite were detected in maternal milk from 2-42 minutes after the end of infusion. Protein binding was between 70-90% in rat, rabbit, minipig, monkey, and humans. **Metabolism** of the parent is primarily by tissue carboxylesterases to the major metabolite, CNS7054, followed by hydroxylation and glucuronidation in all species tested. In human plasma and whole blood, the parent is hydrolyzed primarily by carboxylesterases (CES), specifically CES1A1 and CES1A2. It is not clear which enzyme in the rat is responsible for the rapid metabolism of the parent

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compound. The primary route of **elimination** is renal and biliary excretion in rat and monkey. In rat, about 93.1% was eliminated by 24 hours. In monkey, 83.2% is eliminated by 168 hours. Distribution studies in lactating rats and rabbits also demonstrate that elimination occurs through the breast milk.

Genotoxicity

Genetic toxicology battery was negative in the Ames assay, mouse lymphoma assay, and in vivo rat micronucleus test. Remimazolam was tested in the Ames assay up to 5000 mcg/plate in tester strains TA1535, TA1537, TA98, TA100 and WP2 *uvrA* with and without S9. Remimazolam was tested in three separate mouse lymphoma assays. In Experiment 1, 3 hours treatment with remimazolam up to 2000 mcg/mL with S9 or up to 641 mcg/mL CNS 7056 without S9 resulted in a statistically significant increase in small colony frequency at 600 mcg/mL and a positive linear trend occurred without S9, but not with S9. Mutations were within historical controls. In Experiment 2, 3 hours treatment with remimazolam up to 875 mcg/mL with S9 or up to 610 mcg/mL CNS 7056 without S9 resulted in a statistically significant increase in mutation frequency and small colony frequency occurred at 800 mcg/mL with S9, but no positive linear trends with S9. No significant changes occurred without S9. Mutations were within historical controls. In Experiment 3, 24 hours treatment with remimazolam up to 650 mcg/mL CNS 7056 without S9 resulted in a statistically significant increase in mutation frequency and small colony frequency at 500 and 550 mcg/mL and positive linear trend at cytotoxic levels. Mutations were within historical controls. These three tests were considered negative. In an in vivo rat micronucleus test, remimazolam was administered intravenously for 2 days at target doses of 10, 20, and 30 mg/kg/day and bone marrow were harvested 24 hours after the second dose. No statistically significant reduction in the PCE/NCE ratio occurred. No evidence of clastogenicity or aneugenicity following remimazolam up to the maximum tolerated dose of 30 mg/kg/day.

Safety Pharmacology

Safety pharmacology studies in rats, rabbits, micropigs, and monkeys confirm that remimazolam has typical benzodiazepine-related pharmacodynamic effects including sedation, respiratory depression, and cardiovascular depression.

General Toxicology

In a single-dose toxicity study with a 2-week observation period ([redacted] (b) (4) 015-192), monkeys (3/sex/group) were intravenously infused with remimazolam at target doses of 0 (saline), 30, 60, and 120 mg/kg/day for 24-hours. Evaluations included mortality, clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, gross lesions, organ weights, and histopathology. Ophthalmoscopic examinations and ECG recordings were not conducted in this study. Key study findings were limited to expected pharmacological effects and local tissue reactions characterized by slight thickened intima at the injection site. Systemic NOAEL = 120 mg/kg based on the lack of test article-related toxicity findings in this study. Local NOAEL = 1.25 mg/mL (MD 60 mg/kg) based on the increased incidence and severity of thickened intima occurring at the next highest concentration of 2.5 mg/mL associated with the HD (120 mg/kg).

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In a repeat-dose toxicity study (32237), minipigs (3-5/sex/group) were intravenously infused with remimazolam at target doses of 0 (vehicle), 12, 40, and 120 mg/kg/day for 6-hours/day for 4 weeks. Recovery animals (2/sex/group) administered 0 and 120 mg/kg/day were allowed to recovery for 2 weeks after dosing. In addition to the expected battery of evaluations for a general toxicology study, this study also included auditory evaluations, body temperature, blood pressure, cytokine analysis, and spermiograms (spermatid count, sperm motility, and sperm morphology). Key study findings were limited to expected pharmacological effects and procedure related effects. NOAEL = 120 mg/kg based on the lack of test article-related toxicity findings in this study.

The objective of one study was to compare the original reference product to the clinical formulation in a 4-week repeat-dose toxicity study. In this 4-week repeat-dose toxicity study (13-0293RD), monkeys (2/sex/group) were intravenously administered the original reference product at a target dose of 25 mg/kg and remimazolam at target dose of 0 (vehicle), 12.5, 25, and 50 mg/kg/day by a 5 min bolus followed by a 2-hour infusion once weekly for 4 weeks (total of 5 doses). A recovery group consisted of an additional 2/sex/group. Toxicokinetic analysis was performed on main and recovery animals (4/sex/group). Clinical observations, body weight and temperature, ECG, blood pressure, hematology, clinical chemistry, urinalysis, ophthalmology, and TK occurred in all animals during the dosing period. Necropsy and histopathological examinations were performed in all animals. Key study findings were limited to expected pharmacological effects, class effects (i.e., QT and QTc prolongation, decreased HR, decreased body temperature), procedure-related findings and local findings. Test article related findings included local findings characterized by minor to moderate endothelial fibrosis and minor thrombosis, partially recoverable endothelial fibrosis, and recoverable thrombosis in the HD. Systemic LOAEL = 12.5 mg/kg/day based on the prolonged QT and QTc intervals, decreased heart rate, and decreased body temperature 1 hour after the initial dose in this group and higher. Considering the indication is for sedation under the supervision of a health professional, these adverse findings are likely clinically monitorable and manageable and likely the result of multiple injections. Local NOAEL = 1.25 mg/mL (MD) based on the incomplete reversal of minor endothelial fibrosis of the blood vessel occurring at the next highest concentration 2.5 mg/mL (HD). Local reactions occurring at or below the NOAEL included slight bleeding and slight mononuclear infiltration at the blood vessel, which was considered procedural-related.

In general, findings in the repeat-dose toxicity studies were limited to expected pharmacology and local toxicity. There were no systemic toxicities identified in the repeat-dose toxicity studies that were not already identified in the safety pharmacology study. Local toxicities included thickened intima and partially recoverable endothelial fibrosis of blood vessels at 2.5 mg/mL in monkey.

Developmental and Reproductive Toxicology

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Benzodiazepines as a class indicate concerns for developmental and reproductive toxicity. For example, some benzodiazepines have/were a Pregnancy Category C (NDA 202067, NDA 203993), others Category D (NDA 013263, NDA 018140, ANDA 074112, and ANDA 075494), others Category X (NDA 019080 and NDA 018163), while others are labeled with a description of the data rather than a pregnancy category (NDA 012750, NDA 017533, and NDA 017892) consistent with the new PLLR formats. As documented in a Safety Review by Gerard Boehm, Md, MPH in ODE1/DNP, neurodevelopmental studies demonstrated effects on learning, social behavior, and brain chemistry although these studies were inconsistent and in some cases were methodologically flawed. Despite the limitations, previous reviews concluded that some early studies raised the possibility of risk and there are positive animal findings, albeit in susceptible species with very high doses, which support current labeling from a conservative perspective. It should be noted that there is no standard class-labeling language that will be included in the proposed remimazolam package insert to warn of these class effects. Therefore, it is important to obtain all relevant developmental and reproductive data from remimazolam.

GABA agonists, such as benzodiazepines, along with NMDA receptor antagonists were identified as carrying potential risk to the developing brain in people less than 3 years of age when sedative exposures last longer than 3 hours (Drug Safety Communication 12/14/2016 and 04/27/2017). While the proposed drug product is not intended to be clinically used for procedural sedation lasting longer than 30 minutes, the package insert should include the Safety Labeling Change language as a warning for off-labeled use.

The existing data suggest some concern for the safety of reproduction and development with use of remimazolam, an NME in the benzodiazepine pharmacological class. Therefore, a full and complete battery of developmental and reproductive toxicity tests should be conducted in appropriate species with adequate exposure margins.

Fertility

In a fertility and early embryonic development study (24779 or (b) (4) 24779), male and female Sprague Dawley rats (20/sex/group) were administered intravenous remimazolam at target dose of 0, 3, 10, and 30 mg/kg/day via bolus tail vein injections. Rats were treated once daily for 4 weeks before mating and continuing until 2 weeks after the start of mating in males and daily for 2 weeks before mating and continuing until implantation (GD7) in females. Laparotomies were performed on all treated females on GD13. There were no changes in male fertility index, sperm number and morphology or absolute testes or epididymides weight at any dose level. Decreased motility of spermatozoa occurred at the HD (52.74%), compared to control (70.63%) but was considered transient and not adverse. There was no change in female fertility index, pre-coital time or estrous cycle, reproductive parameters, or uterus weight in this study. NOAEL female fertility = 30 mg/kg.

In a GLP 28-day repeat-dose toxicity study (32237), minipigs (3-5/sex/group) were intravenously infused with remimazolam at target doses of 0 (vehicle), 12, 40, and 120

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mg/kg/day for 6 hours/day for 4 weeks. Spermatograms (spermatid count, sperm motility, and sperm morphology) was not affected and detailed histopathological evaluation of seminiferous tubules of the testes and epididymis were within normal limits. A NOAEL = 120 mg/kg based on the lack of test article-related toxicity findings in this study. While, male fertility and mating behavior was not evaluated in this study, as per ICH S5(R3) pharmacologic effects of this general anesthetic are generally considered incompatible with measurement of mating behavior while the drug is having its pharmacological effects and mating evaluations are not generally feasible in non-rodents such as dogs, non-human primates, and minipigs.

In two GLP 28-day repeat-dose toxicity studies in monkeys testing IV remimazolam up to 60 mg/kg/day, testes and epididymides were weighed and evaluated for histopathology. In one GLP 28-day repeat-dose toxicity study ([redacted] 015-257), monkeys (4/sex/group) were intravenously infused with remimazolam without Dextran 40 and spiked with 8 impurities/degradants at target doses of 0 (saline), 12, 30, 60 mg/kg/day once daily for 12 hours/day for 4 weeks. Key study findings support the conclusion that the NOAEL = 60 mg/kg/day in males based on no adverse systemic findings. In the second GLP 28-day repeat-dose toxicity study (13-0293RD), monkeys (2/sex/group) were intravenously administered remimazolam at target dose of 0 (vehicle), 12.5, 25, and 50 mg/kg/day by a 5 min bolus followed by a 2-hour infusion once weekly for 4-weeks (total of 5 doses). A recovery group consisted of an additional 2/sex/group. Toxicokinetic analysis was performed on main and recovery animals (4/sex/group). Necropsy and histopathological examination were performed in all animals. Key study findings support the conclusion that the test article was well tolerated with a systemic LOAEL = 12.5 mg/kg/day based on prolonged QT and QTc intervals, decreased heart rate, and decreased body temperature 1 hour after initial dose in this group and higher. No adverse findings on male reproductive organs were noted in these studies up to 60 mg/kg/day.

In an extended fertility study (497736), female rabbits (20/group) were intravenously administered remimazolam at target doses of 0, 12.5, 20, and 30 mg/kg/day. Rabbits were treated once daily for 2 weeks before mating and continuing until Lactation Day 30. Does were allowed to litter. Mortality, clinical signs, tactile stimulation, body weight, body weight gains, food consumption, mating performance, duration of gestation, maternal care, litter survival, kit body weights and open field evaluations, reproductive indices, plasma and milk bioanalysis, plasma TK of the does and kits, milk TK, gross necropsy findings and some histopathology examinations were recorded. Paradoxical effects in female rabbits at all doses in combination with the study procedures, resulted in a number of undesirable outcomes including death and euthanasia from treatment-related disinhibition injuries. While these were not direct effects of the test article, they appear to be species-specific as they were not observed in humans. Note: paradoxical effects were also noted in dogs. The HD group was terminated after mating performance and fertility because mortality occurred prior to reaching parturition. Mortalities occurred in all group (5/21 Control; 6/26 LD; 7/26 MD; and 5/5 HD). **Mortalities** described as test article-related mortalities (2/26 LD; 5/26 MD; and 4/5 HD) were attributed to the paradoxical pharmacodynamic effect of the test article on this prey species leading to convulsions and spinal injuries or procedure-related effects

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leading to thrombi. Mortalities occurred within the first four weeks of treatment. **Convulsions** occurred at all treatment groups (2/26 LD, 2/26 MD, 1/5 HD) and appeared to be a result of hypersensitivities to procedural manipulations in this species. All animals that convulsed died or were euthanized. Clinical signs included hypersensitivity immediately after dosing with the test article as well as sedative effects in most animals. NOAELs below were based on fertility and litter performance parameters and not on clinical signs observed in does as convulsions and mortality occurred at all dose levels tested.

- Female mating performance NOAEL = 30 mg/kg/day (AUC = 11600 ng*h/mL)
- Early embryonic development NOAEL = 20 mg/kg/day (AUC = 8290 ng*h/mL)
- Maternal care/kit survival NOAEL = 20 mg/kg/day (AUC = 10800 ng*h/mL)
- Kit behavior NOAEL = 20 mg/kg/day (AUC = 8290 ng*h/mL)

Fertility was evaluated in male and female rats in a Segment I study, in female rabbits in an extended fertility study, and in sperm analysis in male minipigs and monkeys in a 28-day repeat-dose toxicity study. There are many dose selection endpoints that can be used for reproductive toxicity studies (ICH S5(R3)). Pharmacological response, including excessive sedation, is considered appropriate in terms of study design. Furthermore, the patient population and therapeutic indication, such as patient populations in hospitalized settings where pregnancy can be excluded, can influence the extent of the reproductive toxicity testing. Therefore, the female early embryonic development, EFD, and PPDN studies in rats, despite not providing adequate exposures to provide a safety margin to humans, are acceptable for approval of the NDA if the clinical team is convinced that the use of this drug will be limited to hospitalized settings where pregnancy can be excluded. PMRs to conduct the studies with PK analysis would be required to support the safety of the drug product in pregnant populations.

Female fertility was adequately evaluated in the submitted studies and determined that there were no adverse effects on fertility up to 30 mg/kg/day in rabbits and rats.

Male fertility was evaluated in a Segment I study in rats, which was supplemented with additional spermatograms in a 28-day repeat-dose toxicity study in minipigs, and histopathological evaluation of male reproductive organs in minipig and monkey in 28-day repeat-dose toxicity studies. Male fertility was not affected by remimazolam up to 30 mg/kg/day in the Segment I study in rats; however, rats did not provide adequate systemic exposures because of the rapid metabolism of the parent by carboxylesterases present in rat plasma. Toxicity in rats precludes attaining a systemic exposure relevant to the human exposure under conditions of use, which should be addressed as per ICH S5(R3). This was a concern communicated to the Applicant during development with the recommendation to find another rodent species or use infusion dosing to obtain multiples of human exposure levels. Histopathology of the reproductive organs from the repeat-dose toxicity studies is a sensitive method for detecting effects on male fertility, provided the animals are sexually mature. As per the ICH S5(R3), it is generally acceptable to assess the effects of a drug on fertility in dogs, minipigs, and monkeys using comprehensive histopathological examination of the reproductive organs in repeat-dose toxicity studies, if testes and epididymides are

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sampled and processed using methods that allow visualization of the spermatic cycles and a detailed qualitative microscopic evaluation with awareness of the spermatogenic cycles is conducted.

In the 28-day repeat-dose toxicity studies, Cynomolgus monkeys were 3-6 years of age and between 3.14-4.74 kg in body weight. The onset of sexual maturity in cynomolgus monkeys is difficult to predict and is subject to high interindividual variability. An age of 5 years and 5 months combined with a body weight of at least 5.3 kg is supposed to be associated with a 90% probability of testicular maturity. Furthermore, the duration of spermatogenesis cycle is about 1.2 months with full sperm counts only after 5-6 months after the first occurrence of spermatocytes (Mecklenburg et al., 2019). Therefore, the two 28-day repeat-dose toxicity studies in male monkeys are not sufficient to provide adequate safety data on the effects of remimazolam on male fertility.

In the 28-day repeat-dose toxicity study with a 14-day recovery group, Gottingen minipigs were 6 to 8 months of age at dosing, an age at which male Gottingen minipigs are considered mature with full spermatogenetic cycle in seminiferous tubules, including presence of spermatozoa in both the seminiferous tubules and epididymal lumens (Howroyd et al., 2016; Svendsen, 2006; Taberner et al., 2016). A NOAEL = 120 mg/kg based on the lack of test article-related toxicity findings in this study. While, male fertility and mating behavior was not evaluated in this study, pharmacologic effects of this general anesthetic are incompatible with measurement of mating behavior (ICH S5(R3)) and therefore assessment of mating behaviors must be timed to avoid confounding pharmacodynamic effects. The duration of spermiogenesis and spermatogenesis varies slightly depending on breed/species of pig (Almeida et al., 2006; Costa et al., 2010; Kangawa et al., 2016); however, in general spermiogenesis is about 9 days and spermatogenesis cycle is at least 40 days. While the 28-day toxicity study did not expose minipigs throughout the entire spermatogenesis cycle it exposed minipigs for two cycles of spermiogenesis, which is longer than the clinical context of single use. This suggests that the 28-day treatment would have exposed all stages of spermatogenesis and at the end of the recovery period (42 days after the first dose) evaluation of tissue and sperm should predict development all stages of spermatogenesis after a single exposure. It should be noted that adequate endpoints included detailed histopathological evaluation of the testes and epididymis to visualize spermatic cycles and a quantitative analysis of sperm number, viability/motility, and morphology.

In conclusion, while male fertility could have been tested in a Segment I study of male rabbits, the Segment I study in rats and supplemental histopathological evaluation of the minipig appears adequate for the short-term therapeutic indication in a highly controlled setting. There were no adverse effects on male fertility in rats up to 30 mg/kg/day or minipigs up to 120 mg/kg. Additional testing for the safety of male fertility will be required as a PMR.

Embryo-Fetal Development

In an embryo-fetal development study ((b) (4)23509), female rats (25/group) were intravenously administered remimazolam at target doses of 0, 3, 10, and 30 m/kg/day

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during organogenesis from implantation to hard palate closure (GD 6-17). Laparotomies occurred on GD 20. No mortalities or changes in body weight or food consumption occurred. Pharmacodynamic effects of reduced motility started 5 min after dosing and lasted for 5 min in the LD and 20 min at the MD. Reduced motility changed to sedation at the HD lasting up to 20 min after dosing. **Convulsions** occurred in one MD and one HD dam on GD 11. No effects on prenatal development (corpora lutea, implantation sites, resorptions, sex distribution, fetal and placental weights, number of live fetuses at birth and the values calculated for the pre- and post-implantation loss). No effects on malformations, variations, or retardations were reported.

- Maternal NOAEL = 3 mg/kg/day based on the convulsions occurring at and above 10 mg/kg/day.
- Fetal NOAEL = 30 mg/kg/day based on the slight but statistically significant increased incidence of early and total resorptions observed at this level was within range of historical background.

In an embryo-fetal development study ((b) (4) 23507), female rabbits (20/group) were intravenously administered remimazolam at target doses of 0, 1.25, 2.5, and 5.0 mg/kg/day during organogenesis from implantation to hard palate closure (GD 6-20). Laparotomies occurred on GD 29. No mortalities occurred. Pharmacological effects of sedation occurred in all groups. Sedation occurred within 5 minutes after dosing and lasted up to 1 hour in LD dams and up to 2 hours in MD and HD dams. Statistically significant decreases in body weights (up to 8.4% LD, 6.2% MD, and 11.1% HD) and body weight gains (C: +13.5%, LD: +10.6%, MD: +10.0%, HD: +10.5%) during the treatment period were correlated with a 55% decrease in food consumption (up to 44% LD, 49% MD, and 55% HD). No test article-related effect on corpora lutea, implantation sites, resorptions, number of live fetuses, and values calculated for the pre- and post-implantation loss and sex distribution of fetuses. Fetal and placental weights were decreased in the HD (-9% and -12%, respectively). No test article-related effect on malformations, variations, or retardations were reported.

- Maternal NOAEL = 2.5 mg/kg/day, based on the >10% decrease in body weight and body weight gains at the next highest dose, 5 mg/kg/day

- Embryo-Fetal Development NOAEL = 2.5 mg/kg/day based on the decreased fetal and placental weights at the next highest dose, 5 mg/kg/day

Embryo-fetal development was evaluated in an extended fertility study in rabbits and EFD studies in rats and rabbits. Remimazolam had no adverse effects on embryo-fetal development up to 30 mg/kg/day in rat and 20 mg/kg/day in rabbits. As stated in section 3.1.1 of the draft ICH S5 (R3), therapeutic indication, such as short-term therapies under highly controlled settings, can influence the extent of reproductive toxicity testing. Applicant states that rats and rabbits are typical species used in DART studies and dogs were not used because of the paradoxical pharmacological effects of remimazolam in dogs. There are many dose selection endpoints that can be used for reproductive toxicity studies (ICH S5 (R3), section 5.1). Pharmacological response, including excessive sedation (line 660), is considered appropriate in terms of study

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design. Rabbits appeared to have paradoxical pharmacological effects to remimazolam characterized bucking and convulsions when touched leading to spinal damage and death; therefore, rabbit studies used an adequate high dose. Despite having exaggerated pharmacological effects, rats did not provide exposure margins relevant to humans.

Pre- and Post-Natal Development
Applicant states that rats and rabbits are typical species used in DART studies and dogs were not used because of the paradoxical pharmacological effects of remimazolam in dogs. Remimazolam had no adverse effects on pre- and post-natal development up to 30 mg/kg/day in rats and 20 mg/kg/day in rabbits. It is noted that the extended fertility study in rabbits did no evaluate learning and memory or reproductive function of the F1 kits.

In the pre- and post-natal development study (NO12230), pregnant rats (20/group) were intravenously administered remimazolam at target doses of 0, 3, 10, and 30 mg/kg/day from implantation (GD 6) through Lactation Day 20 (weaning of F1 pups, PND 20). F1 offspring were weaned and mated. Male and female F1 offspring were necropsied after Caesarean section of the mated females on GD 13. F2 offspring were not evaluated past GD 13 for birth weights, sex ratio, or external malformations, but fertility of F1 generation can be determined with evaluated parameters. No test article-related effects on mortality occurred. Pharmacodynamic effects of decreased movement, staggering gait, irregular respiration, prone position occurred in all F0 dosing groups and lateral position or sedation occurred in HD F0 females after dosing and lasting up to 1 hour. F0 general toxicity NOAEL = 30 mg/kg/day based on no test article-related effect on body weight, food consumption, or gross pathology.

- F0 reproductivity NOAEL = 30 mg/kg/day based on no test article-related effect on duration of gestation, delivery, gestation index, birth index, and maternal behavior.

- F1 development/reproductivity NOAEL = 30 mg/kg/day based on no test article-related effect on growth, physical development, sensory and reflex functions, functional observational battery (open field test), learning (conditioned avoidance response/shuttle box), genital development, mating ability, fertility, implantation, or viability of F2 embryos.

Pre- and post-natal development was evaluated in an extended fertility study in rabbits and a standard designed PPND study in rats.

While benzodiazepines as a class have been known to have concerns for developmental and reproductive toxicity, ICH S5(R3) allows for flexibility in the extent of developmental and reproductive toxicity (DART) testing for products used in patient populations in hospitalized settings that can exclude pregnant women. Therefore, the DART studies in rats, despite the inadequate exposures to provide a safety margin to humans, does not preclude approval of this NDA. Given the short acting nature of

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remimazolam, clinicians may see a benefit use remimazolam during pregnancy and therefore it is recommended that additional DART evaluations along with TK evaluation are recommended as PMRs to support the safety of the drug product in pregnant populations and inform labeling.

It is important to note the PK in pregnant rats was not sampled during the full spectrum of vulnerable windows of development. Additionally, the rat exposures from GD 6 to GD 17 and in non-pregnant rats are extremely variable. The differences in PK across studies are inexplicably variable and do not appear to be a result of methods of sample handling. Together, the inconsistency in exposures across gestation along with the gaps in exposure analysis make it difficult to assess the safety margins in rats. The variability in PK within studies and across studies further supports the recommendation for TK evaluations in the DART studies as PMRs.

Safety Margins

The safety margin table below provided by the Applicant describes the safety margins for the EFD and PPND studies in rats and rabbits for total and free remimazolam exposures.

Table 4 Summary of TK-Data from Reproduction Toxicity Studies with Remimazolam

Species	Study	Dose Characterization	Treatment Period	Dose Level (mg/kg)	$C_{0/max}$ (ng/mL)	AUC_{0-t} (ng·h/mL)	Exposure Ratio[a]		Exposure Ratio Free Concentrations[b]	
							C_{max}	AUC	C_{max}	AUC
Rat	23509 Embryotoxicity Study	NOEL fetal	GD 6	10	2.9	1.9	0.003	0.003	0.01	0.01
			GD 17		69.4	3.3	0.073	0.006	0.27	0.02
		Highest dose studied	GD 6	30	4,490	179	4.7	0.320	17.29	1.16
			GD 17		280	41*	0.296	0.073	1.08	0.27
Rabbit	23507 Embryotoxicity Study	NOEL fetal	GD 6	2.5	2,977	1,342	3.1	2.4	4.0	3.0
			GD 20		3,326	1,530	3.5	2.7	4.5	3.5
		Highest dose studied	GD 6	5	4,319	1,767**	4.6	3.2	5.8	4.0
			GD 20		5,535	2,272**	5.9	4.1	7.4	5.2
	497736 Extended Fertility and Mating Study	Low - Highest dose studied (NOAEL)	Premating	12.5-30	5,200-4,420	6,270-11,600	5.5-4.7	11.2-20.7	7.0-5.9	14.2-26.6
			Gestation	12.5-20	4,740-4,490	5,320-8,290	5.0-4.7	9.5-14.8	6.4-6.0	12.1-18.8
			Lactation		21,300-15,400	11,300-10,800	22.5-16.3	20.2-19.3	28.6-20.7	25.7-24.5
Human	CNS7056-002	Highest dose administred	33.12		945.85	559.81**	n.a.			

C_0 Calculated CNS 7056 plasma concentration at the end of the short-term infusion
* $AUC_{(0-18h)}$
** $AUC_{(0-\infty)}$
*** $AUC_{(0-12)}$
GD gestation day
a reported animal values/observed human exposure
b corrected for differences in free plasma concentration (rat 29.5%; rabbit 14.5%; human 8.1% and 11.4%, respectively)

Source: IR SDN 0038 (39) 01/31/2020 in the Nonclinical Information Amendment, page 6.

The safety margin table below produced by this Reviewer (Table 61) is consistent with the Applicant's safety margins above.

Table 61 Safety Margins for Parent Compound

Safety Margins

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					Human dose of 5 mg + 9 top-up doses of 3 mg each = 33 mg/day	
		NOAEL			PARENT	
			PARENT		C_{max} (ng/mL)	AUC (ng*h/mL)
Study	Species	Dose	C_{max} (ng/mL)	AUC (ng*h/mL)	945.85	559.81
Single Dose Toxicity	Pivotal monkey	120 mg/kg	2700	55300	3x	99x
28-Day Repeat Dose Toxicity	Pivotal minipig with sperm analysis	120 mg/kg	9876	41476	3460x	420x
	Pivotal monkey	120 mg/kg (no dose-limiting toxicity)	17616	47133	19x	84x
Fertility						
Fertility and Early Embryonic Development	male and female rat	30 mg/kg/day	167	4.13	0.2x (0.6x*)	0.01x (0.03x*)
	female rabbits	20 mg/kg/day	7330	7430	8x (10x*)	13x (17x*)
Gestation						
Embryo-Fetal Development	rabbits	20 mg/kg/day	4490	8290	5x (6x*)	15x (19x*)
	rabbits	2.5 mg/kg/day	3326.1	1315.8	4x	2x
	rats	30 mg/kg/day	4490	179	4.8x (17x*) GD 6 (1x*) GD 17	0.32x (1.2x*) GD 6 (0.27x*) GD 17
Lactation						
Pre and Postnatal Development	rabbits	20 mg/kg/day	15400	10800	16x	19x
	rats	30 mg/kg/day	4490	179	0.03x to 4.8x (0.09x to 17x*) GD 6	0.11x to 0.32x (0.3x to 1.2x*) GD 6

*Safety margins (SM) corrected for differences in free plasma concentration (rat 29.5% and human 8.1%). Pink cells indicate estimated PK based on separate studies in the same species. Fertility SM in rats are based on estimated PK values from Study RMN1018 page 444-445; table4 13, 14; male C(0) and AUC after 4 weeks of 30 mg/kg IV bolus in rats. EFD SM in rats are based on estimated PK values from study (b) (4)23509 page 69; rat dam C(0) and AUC on gestation day 6 after 30 mg/kg IV bolus over 3 minutes. PPND SM in rats are based on estimated PK values from study (b) (4)23509 page 69; rat dam C(0) and AUC on gestation day 6 and 17 after 30 mg/kg IV bolus over 3 minutes.

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Human exposures (AUC and C_{max}) were provided in a response to IR SDN 0038 (39) 01/31/2020 in the Nonclinical Information Amendment.

As noted in the table above, taking into consideration free concentrations, although the exposure margin is equivalent to human exposures on GD6 in the rat EFD study, by GD 17 exposures were only one third of the human exposures. Because PK data were not obtained in the PPND study, data from the EFD study suggests a similar situation, that is, equivalence at GD 6 but even less than one third coverage by the end of the treatment period. As such, neither the rat EFD nor the PPND study adequately characterize the potential effects of the drug on these endpoints.

Table 62 Safety Margins for the Major Metabolite

					Safety Margins	
					Human dose of 5 mg + 9 top-up doses of 3 mg each = 33 mg/day	
		NOAEL			METABOLITE	
			METABOLITE		C_{max} (ng/mL)	AUC (ng*h/mL)
Study	Species	Dose	C_{max} (ng/mL)	AUC (ng*h/mL)	577.31	12002.72
Single Dose Toxicity	Pivotal monkey	120 mg/kg	18300	312000	32x	26x
28-Day Repeat Dose Toxicity	Pivotal minipig with sperm analysis	120 mg/kg	89879	217275.82	149x	936x
	Pivotal monkey	120 mg/kg (no dose-limiting toxicity)	90693	401119	157x	33x
Fertility						
Fertility and Early Embryonic Development	male and female rat	30 mg/kg/day	77700	144000	156	18
	female rabbits	20 mg/kg/day	10800	21500	19x	2x
Gestation						
Embryo-Fetal Development	rabbits	20 mg/kg/day	14700	44300	25x	4x
	rabbits	2.5 mg/kg/day	3331.3	2159.7	6x	0.18x
	rats	30 mg/kg/day	89879	217275.82	156x	18x
Lactation						
Pre and Postnatal Development	rabbits	20 mg/kg/day	10200	28400	18x	2x
	rats	30 mg/kg/day	89879	217275.82	156x	18x

*Pink cells indicate estimated PK based on separate studies in the same species.
Human exposures (AUC and C_{max}) were provided in a response to IR SDN 0038 (39) 01/31/2020 in the Nonclinical Information Amendment.

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Rabbits EFD and extended fertility and mating studies provide adequate safety margins for the estimated human exposures for a 30-minute procedure. In the rat EFD study, the NOAEL (highest dose tested) GD 6 exposures provided adequate safety margins for human C_{max} and AUC only when free remimazolam is considered. However, when considering the rat GD 17 exposures at the NOAEL, there is a 1.08x and 0.27x safety margin for C_{max} and AUC, respectively.

As noted in ICH S5(R3) step 4, page 9:

> "The intended patient population or therapeutic indication can influence the extent of DART testing. Studies evaluating all stages of reproduction and development are not warranted if the disease indicates that DART will have minimal impact on the risk of the pharmaceutical in the target population. For example, studies covering all stages are not necessarily appropriate for an exclusively post-menopausal female patient population, for use in the pediatric or juvenile pre-pubescent population, or for patient populations in hospitalized settings where pregnancy can be excluded." [emphasis added]

This Reviewer interprets the above excerpt from ICH S5(R3) as an allowance to accept DART studies that may not provide the safety coverage generally necessary to allow for approval. Therefore, the studies in rats, despite the inadequate exposures to provide a safety margin to humans, are acceptable for approval of the NDA because the clinical team has concluded that adequate pregnancy testing will occur prior to planned procedures consistent with the indication. PMRs to conduct the studies with PK analysis would be required to support the safety of the drug product in pregnant populations.

It is important to note the PK in pregnant rats was not sampled during the full spectrum of vulnerable windows of development. Additionally, the rat exposures from GD 6 to GD 17 and in non-pregnant rats are extremely variable. It is possible for the GD 17 exposures to be lower compared to GD 6 as a result of enzyme induction in animals treated daily on consecutive days. Likewise, exposures on Day 1 of the 28-day study were higher than Day 28 (Table 63). However, the differences in PK between these studies are inexplicably variable. The differences in PK between these study could be a result of differences in methodology in the 28-day study in rats (Study RMN1018) and the EFD study in rats (Study (b) (4) 23509). Both the 28-day study and EFD study collected samples in tubes containing EDTA/NaF to inhibit enzymes. The 28-day study samples were in tubes fortified with paraoxon and acetonitrile, while the EFD study then treated samples immediately with paraoxon and acetonitrile solution to further inhibit enzymes in the blood. It is unlikely that delaying the addition of the paraoxon and acetonitrile would contribute to the increased exposures in the EFD study. While the samples in the 28-day study were "placed immediately into ice before separation as soon as practical after collection", samples from the EFD study were cooled using an IsoTherm-Rack system until centrifugation, and then plasma was immediately frozen at -20°C or colder after centrifugation prior to analysis. Methodological differences in sample procurement do not elucidate the differences in PK across studies.

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Together, the inconsistency in exposures across gestation along with the gaps in exposure analysis make it difficult to assess the safety margins in rats. The variability in PK within studies and across studies further supports the recommendation for the PMR for the DART studies with PK analysis.

Table 63 Rat PK Comparison Across Studies

Study	C_0 (ng/mL)	C_{max} (ng/mL)	AUC_{0-12} (ng*h/mL)	AUC_{0-18} (ng*h/mL)	AUC_{0-inf} (ng*h/mL)
Study RMN1018; 30 mg/kg/day IV bolus					
Day 1 – female	46.1	19.4	1.67	-	-
Day 1 – male	131	27.6	3.57	-	-
Day 28 – female	119	4.19	3.65	-	-
Day 28 – male	167	15.0	4.13	-	-
Study (b) (4) 23509; 30 mg/kg IV bolus over 3 minutes					
GD 6	4489.78	-	-	178.83	179.71
GD 17	279.28	-	-	41.00	64.35

Source: Study RMN1018 page 444-445; table4 13, 14; male C(0) and AUC after 4 weeks of 30 mg/kg IV bolus in rats. EFD SM in rats are based on estimated PK values from study (b) (4) 23509 page 69.

Special Toxicology

Special toxicology studies included anaphylaxis in guinea pig, phototoxicity, and blood compatibility. Remimazolam did not affect hemolysis, platelet aggregation, coagulation, or fibrinolysis when tested in in vitro studies with rat, dog, and human blood. Remimazolam was negative for anaphylaxis in a guinea pig sensitization test. Remimazolam was negative for phototoxicity in Balb/c 3T3 cells up to 1000 mcg/mL in the presence of UVA irradiation.

12 Appendix/Attachments

12.1 Impurity Qualification Repeat-Dose Toxicity

To qualify impurities and degradation products the Sponsor conducted one 4-week repeat-dose toxicology study in monkeys using a batch of remimazolam spiked with 8 impurities (see table below) as required by ICH Q3B(R2) Attachment 3, page 17.

Impurity Name	Proposed Specification	Reported Content in Tested Sample	Repeat-dose toxicity study[1] (mg/kg)	High Dose Tested		
				Ames[2] (mcg)	Mouse Lymphoma Assay[3] (mcg/mL)	COMET/ Micronucleus Test[4] (mg/kg)
(b) (4)						

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(b) (4)

This study is reviewed in full below.

Study title: A 4-Week Repeated Intravenous Infusion Dose Toxicity Study of ONO-2745BS in Cynomolgus Monkey Followed by A 4-Week Recovery Period

Study no.:	(b) (4) 015-257
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 29, 2012
GLP compliance:	Yes, this study was conducted in compliance with the OECD Principles of GLP and GLP Practice Regulations of the Ministry of Health and Welfare of (b) (4)
QA statement:	Yes, signed June 25, 2012
Drug, lot #, and % purity:	ONO-2745BS spiked with 8 impurities/degradants, YMK110831, 94.6% pure

In this GLP repeat-dose toxicity study of impurities, monkeys (4/sex/group) were intravenously infused with ONO-2745BS spiked with 8 impurities/degradants (i.e. (b) (4)) at target doses of 0 (saline), 12, 30, 60 mg/kg/day once daily for 12 hours/day for 4 weeks. A separate recovery group (2/sex/group) was allowed to recover for 2 weeks after dosing. Toxicokinetics was conducted on main and recovery animals (6/sex/group). Key study findings support the designation of NOAEL = 60 mg/kg/day in males and females.

Key Study Findings

- Transient increase in sodium (2x), potassium (3x), and chloride (2x) occurred in the HD females on Day 11. Transient increase in potassium (up to 3x) occurred in the

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MD and HD males on Day 11. These changes were transient and without histopathological correlate. These findings were not considered adverse.

- Local findings included slight inflammation on the vascular wall with slight to moderate perivascular inflammatory granuloma, intimal thickening, and thrombus and necrotic tissue at the caudal vena cava (site of catheterization) in one HD male and one HD female. Local findings were recoverable except for granuloma.
- Systemic NOAEL = 60 mg/kg/day
- Local NOAEL = 1 mg/mL (LD and HD)

Group	Test and Control Articles	Dose Level (mg/kg/day)	Dose Volume		Concentration (mg/mL)	Necropsy	Number of Animals (Animal No.)	
			(mL/kg/h)	(mL/kg/12h)			Males	Females
1	Physiological saline	-	2.5	30	-	ERC EDA	2 (1, 2) 4 (3 – 6)	2 (7, 8) 4 (9 – 12)
2	ONO-2745BS	12*	1	12	1*	EDA	4 (13 – 16)	3 (17, 18, 20)[#]
3	ONO-2745BS	30*	2.5	30	1*	ERC EDA	2 (21, 22) 4 (23 – 26)	2 (27, 28) 4 (29 – 32)
4	ONO-2745BS	60*	2.5	30	2*	ERC EDA	2 (33, 34) 4 (35 – 38)	2 (39, 41)[#] 3 (42 – 44)

EDA: End of the dosing period

ERC: End of the recovery period

*: as ONO-2745

[#]: Animal Nos. 19 and 40 were excluded from the evaluation of the study results.

[#]HD female No. 40 was excluded from the study because the catheter was found to be detached from the blood vessel on Day 22 of dosing. LD female No. 19 was excluded from the study because the catheter was found to be detached from the blood vessel on Day 24 of dosing. It was unclear when the catheter became detached and the actual dose these animals last received. Reimplantation of the catheter was considered, but the results would be difficult considering the gap in dosing.

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Methods

Doses:	0 (saline), 12, 30, 60 mg/kg/day Doses were selected based on a previous 2-week repeat-dose IV infusion study in monkeys ([(b) (4)]015-100), where the systemic and local NOAEL was 22.5 mg/kg/day, based on slight irritation for large dose volumes MD and HD (12 h/day). In this DRF study, animals were administered: **0** (saline 2.5 mL/kg/h), **22.5** (2.5 mg/2.5 mL/kg/h x 9 h/day), **30** (2.5 mg/2.5 mL/kg/h x 12 h/day), **60 mg/kg/day** (5 mg/2.5 mL/kg/h x 12 h/day)
Frequency of dosing:	Once daily for 12 hours/day for 4 weeks
Route of administration:	Intravenous infusion into a catheter implanted into the femoral vein and postcava using a disposable syringe and infusion pump
Dose volume:	1 and 2.5 mL/kg/hour
Infusion Rate:	2 mL/hour for the first hour then rate was reset to 1 and 2.5 mL/kg/hour
Formulation/Vehicle:	0.02 0.2 10 mg/mL / saline
Species/Strain:	Monkey / Cynomolgus (purpose bred)
Number/Sex/Group:	Main 4/sex/group Recovery 2/sex/group
Age:	3-6 years
Weight:	Males 3.14 to 4.37 kg Females: 2.78 to 4.20 kg
Satellite groups:	Toxicokinetics was conducted for all animals (6/sex/group) Recovery animals (2/sex/group)
Unique study design:	This study was designed to test the toxicity of 8 impurities/degradants. The test article was spiked with the impurities prior to dosing animals.
Deviation from study protocol:	Two animals (1 LD and 1 HD female) were excluded from the study because their catheters were found to be detached late in the dosing period, but the exact time of detachment was unclear. This reduced the number of animals in the LD and HD groups, but there were no sex differences identified and with combined sexes sufficient sample sizes were achieve. This is not likely to influence the interpretation of the study results.

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Mortality

No mortality occurred in this study.

Clinical Signs

Clinical signs were recorded once daily after cage side observations.

Pharmacodynamic effects included clinical signs of sedation. Clinical signs of ataxic gait, decreased spontaneous activity, and incomplete eyelid opening occurred approximately 2 hours after the start of dosing and lasted for 1 hour after the end of dosing in all treatment groups. Sedation, sitting position, somnolence, and coma occurred approximately 2 hours after the start of dosing and lasted until immediately after dosing in the MD and HD.

Body Weights

Body weights were recorded once weekly during the dosing and recovery periods. Statistically significant increases in body weight gains were noted during the dosing period in HD females (+0.220) compared to controls (-0.67). The increased body weight appeared to be attributed to low body weight gains in the controls and is not considered an adverse test article-related finding. No adverse dose-dependent test article-related changes in body weight occurred in this study.

Food Consumption

Food consumption was calculated daily during the dosing and recovery periods. Statistically significant 12% increase in food consumption occurred in HD females but not males. The increased food consumption appears to be attributed to low food consumption in the control females and is not considered an adverse test article-related finding. No adverse test article-related changes in food consumption occurred in this study.

Body Temperature

Body temperature was recorded at predose, on Day 8 and 22 of dosing (before dosing), and on Day 22 of recovery. No test article-related changes in body temperature occurred in this study.

Blood Pressure

Diastolic and systolic blood pressure was recorded at predose, on Day 8 and 22 of dosing (before dosing), and on Day 22 of recovery using an automatic hemodynamometer at the brachium without anesthesia. No test article-related changes in blood pressure occurred in this study.

Ophthalmoscopy

Ophthalmoscopic examination occurred at predose, on Day 21 of dosing (before dosing), and once on Day 21 of recovery. Ophthalmoscopy consisted of evaluating pupillary light reflex with slit lamp, exanimating anterior ocular segment and optic media,

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and ocular fundus. No test article-related changes were detected with ophthalmoscopy in this study.

ECG

Electrocardiography (ECG) occurred at predose, on Day 8 and 22 of dosing (before dosing), and on day 22 of recovery. Heart rate, PR interval QRS duration, QT interval, and QTc, and waveforms in unanesthetized, caged, sitting animals were recorded using electrocardiograms (leads I, II, III, and a VR, aVL, and aVF) and an ECG processor using average continuous waveforms of lead II for seconds. No test article-related changes were detected with ECG in this study.

Hematology

Blood was collected for hematology at predose, Days 12 and 26 of dosing (before dosing), and Day 26 of recovery. The following parameters were included in hematological evaluations.

Parameter	Unit	Method
Erythrocyte count	$10^6/mm^3$	Dual angle laser flow-cytometric measurement
Leukocyte count	$10^3/mm^3$	Dual angle laser flow-cytometric measurement
Hematocrit value	%	Calculation: (mean corpuscular volume × erythrocyte count) / 10
Hemoglobin concentration	g/dL	Modified cyanmethemoglobin method
Platelet count	$10^3/mm^3$	Dual angle laser flow-cytometric measurement
Mean corpuscular volume	fL	Dual angle laser flow-cytometric measurement
Mean corpuscular hemoglobin	pg	Calculation: (hemoglobin concentration / erythrocyte count) × 10
Mean corpuscular hemoglobin concentration	g/dL	Calculation: [hemoglobin concentration / (erythrocyte count × mean corpuscular volume)] × 1000
Reticulocyte ratio	%	Laser flow-cytometric measurement with RNA stain
Differential leukocyte[b)]	$10^3/mm^3$, %	Flow-cytometric measurement with peroxidase stain and dual angle laser flow-cytometric measurement
Prothrombin time	s	Coagulation method
Activated partial thromboplastin time	s	Coagulation method
Fibrinogen	mg/dL	Coagulation method

b) Parameters: Eosinophils, basophils, neutrophils, monocytes, lymphocytes, and large unstained cells

Hematology results are summarized in the table below.

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Table 64 Hematology Summary for the 4-Week Monkey Study

Parameter (normal range*)	Con.	LD			MD			HD		
	Day 12	Pre	Day 12	Day 26	Pre	Day 12	Rec.	Pre	Day 12	Rec.
MALE										
RBC (4.5-7.2 x 10^6/mm^3)	5.48	5.54	5.46	5.37	5.63	5.40	5.37	5.56	5.03*	5.71
Ht (31-38%)	43.17	42.33	43.08	43.13	43.82	42.73	42.20	43.05	39.80*	44.15
Hb (10-12 g/dL)	12.75	13.43	12.78	12.85	13.50	12.47	12.75	13.52	11.62**	12.08
Ret (0-0.5 %)	2.02	1.58	1.85	1.75	1.45	2.10	1.10	1.53	2.77	0.90
FEMALE										
RBC (4.5-7.2 x 10^6/mm^3)	5.15	5.13	4.97	5.07	4.87	4.62 [Day 26 = 4.65*]	4.78	5.05	4.61 [Day 26 = 4.69*]	5.60
Ht (31-38%)	40.60	40.70	40.67	41.27	38.62	38.25	39.25	39.90	37.32	43.65
Hb (10-12 g/dL)	11.87	12.47	12.13	12.37	11.73	11.08	11.50	11.88	10.70	12.75
Ret (0-0.5 %)	1.77	1.47	2.27	1.87	2.22	2.73 [Day 26 = 2.53*]	1.90	1.94	3.42**	0.65

Day 12 = Day 12 of dosing; Day 26 is not included in this summary as the statistically significant findings were on Day 12, unless noted. There were no recovery data for the LD males
Ht = hematocrit; Hb = hemoglobin; Ret. = reticulocytes
*Normal range of cynomolgus monkeys aged 3-7 years, from The Toxicologist's Pocket Handbook, page 141 (Derelanko, 2008).

Decrease in erythrocyte count and hematocrit occurred on Day 12 of dosing and a decrease in hemoglobin concentration occurred on Day 12 and 26 of dosing in the HD males. Increase in reticulocyte ratio occurred on Day 12 of dosing and decreased erythrocyte count occurred on Day 26 of dosing in the HD females. These effects recovered after the 2-week recovery period. These findings suggest a recoverable drug-induced hemolysis. Because the erythrocyte count and hematocrit were within normal ranges this is not considered adverse.

Clinical Chemistry

Blood was collected for clinical chemistry at predose, Days 12 and 26 of dosing (before dosing), and Day 26 of recovery. The following parameters were included in clinical chemistry evaluations.

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Parameter	Unit	Method
Aspartate transaminase	IU/L	JSCC transferable method
Alanine transaminase	IU/L	JSCC transferable method
Alkaline phosphatase	IU/L	JSCC transferable method
Lactate dehydrogenase	IU/L	JSCC transferable method
Creatine kinase	IU/L	JSCC transferable method
γ-glutamyltrans peptidase	IU/L	JSCC transferable method
Total bilirubin	mg/dL	Vanadate oxidation method
Total protein	g/dL	Biuret method
Albumin	g/dL	BCG method
Total cholesterol	mg/dL	COD · HDAOS method
Triglycerides	mg/dL	GPO · HDAOS method, glycerol blanking method
Glucose	mg/dL	Hexokinase · G-6-PDH method
Urea nitrogen	mg/dL	Urease-GlDH method
Creatinine	mg/dL	Creatininase · F-DAOS method
Inorganic phosphorus	mg/dL	PNP · XDH method
Calcium	mg/dL	MXB method
Sodium	mEq/L	Electrode method
Potassium	mEq/L	Electrode method
Chloride	mEq/L	Electrode method
Protein fractions [b)]	%	Electrophoresis method

b) Parameters: Albumin, α_1-globulin, α_2-globulin, β-globulin, γ-globulin, and albumin/globulin ratios

No test article-related changes in clinical chemistry occurred in this study.

Urinalysis

Urinalysis was conducted at predose, on Days 11 and 25 of dosing, and on Day 25 of recovery. The following parameters were included in the urinalysis. No test article-related changes were detected with urinalysis in this study.

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Fresh urine

Parameter	Unit	Method	Apparatus
Color	-	Visual	-
pH	-	Test paper	Clinitek Atlas XL[a)]
Ketone bodies	-	Test paper	
Bilirubin	-	Test paper	
Occult blood	-	Test paper	
Urobilinogen	-	Test paper	
Protein	mg/dL	Pyrogallol red method	JCA-BM6070[b)]
Glucose	mg/dL	Hexokinase · G-6-PDH method	
Sediments[c)]	-	Microscopy after staining with Sternheimer-Malbin stain	-

a) Automated urine chemistry analyzer (Sparton Medical Systems)

b) Automatic analyzer (JEOL Ltd.)

c) Parameters: Erythrocytes, leukocytes, epithelial cells, crystals, bacteria, sperms (males only), casts, and others

Preserved urine

Parameter	Unit	Method	Apparatus
Urine volume	mL	Measuring cylinder	-
Specific gravity	-	Urinary refractometery	Uricon-JE[d)]
Sodium	mEq/L, mEq[e)]	Electrode method	JCA-BM6070[f)]
Potassium	mEq/L, mEq[e)]	Electrode method	
Chloride	mEq/L, mEq[e)]	Electrode method	

d) Urinary refractometer (Atago Co., Ltd.)

e) Total electrolyte excretion was calculated, from electrolyte concentration and urine volume. Only the total electrolyte excretion was evaluated.

f) Automatic analyzer (JEOL Ltd.)

Results of the urinalysis are summarized in the table below.

Table 65 Urinalysis Summary of 4-Week Monkey Study

Parameter	Con.	LD			MD			HD		
	Day 11	Pre	Day 11	Day 25	Pre	Day 11	Rec.	Pre	Day 11	Rec.
MALE										
Volume (mL)	88.5	76.3	262.5	195.8	69.5	199.8	49.0	75.3	182.2	40.0
Na (mEq)	13.5	8.9	10.9	11.3	7.9	16.7	4.8	7.5	16.9	5.3
K (mEq)	4.6	2.9	6.6	6.4	3.1	8.7**	2.3	3.1	8.9**	3.4
Cl (mEq)	11.4	6.2	9.7	10.2	5.5	14.4	2.0	4.4	15.8	2.5
FEMALE										
Volume (mL)	108.7	57.0	64.0	62.3	66.7	156.3	33.0	62.8	153.4	50.5
Na (mEq)	12.5	9.2	9.6	8.6	8.9	16.4	4.4	8.4	19.4*	7.5
K (mEq)	3.2	2.5	4.4	3.1	2.7	5.6	1.8	2.9	7.4**	2.7
Cl (mEq)	11.7	6.3	7.6	5.7*	7.1	15.5	2.6	7.8	18.9**	5.4

Day 11 = Day 11 of dosing; Day 25 of dosing is not included in this summary as the statistically significant findings were on Day 11 and Recovery, unless noted.

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Increased urine volume and increased potassium in the urine of treated males, without any histopathological correlate. This finding is not considered adverse because it is transient

Gross Pathology

All animals were evaluated at necropsy for gross pathology. No test article-related findings were detected at necropsy in this study.

Organ Weights

Organ weights were collected at necropsy as specified in the table below. No test article-related change in organ weights occurred in this study.

Organs and tissues		Organ weight	Fixation	Histopathology (specimen preparation and examination)	Electron microscopy (specimen preparation)
Trachea		-	Y	Y	-
Lungs (including bronchi)	Left	Y	Y	Y	-
	Right			Y	-
Tongue		-	Y	Y	-
Submandibular glands	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Esophagus	Thoracic	-	Y	Y	-
Stomach	Body	-	Y	Y	-
	Pylorus	-		Y	-
Small intestine	Duodenum	-	Y	Y	-
	Jejunum	-		Y	-
	Ileum[a)]	-		Y	-
Large intestine	Cecum	-	Y	Y	-
	Colon	-		Y	-
	Rectum	-		Y	-
Pancreas		-	Y	Y	-
Liver		Y[b)]	Y	Y	Y[c)]

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Gallbladder		-	Y	Y	-
Aorta	Thoracic	-	Y	Y	-
Heart		Y	Y	Y	-
Kidneys	Left	Y	Y	Y	Y[d)]
	Right	Y	Y	Y	-
Urinary bladder		-	Y	Y	-
Testes	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Epididymides	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Prostate		Y	Y	Y	-
Seminal vesicles	Left	Y	Y	Y	-
	Right			-	-
Ovaries	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Uterus		Y	Y	Y	-
Vagina		-	Y	Y	-
Brain	Cerebrum[e)]	Y	Y	Y	-
	Cerebellum			Y	-
	Pons			Y	-
	Medulla oblongata			Y	-
Spinal cord	Thoracic	-	Y	Y	-
Sciatic nerves	Left	-	Y	Y	-
	Right	-	Y	-	-
Sternum/Sternal bone marrow		-	Y	Y	-
Femurs/Femoral bone marrow	Left	-	Y	Y	-
	Right	-	Y	-	-
Submandibular lymph nodes	Left	-	Y	Y	-
	Right	-	Y	-	-
Mesenteric lymph nodes		-	Y	Y	-
Spleen		Y	Y	Y	-
Thymus		Y	Y	Y	-
Pituitary		Y	Y	Y	-
Thyroids/Parathyroids*	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Adrenals	Left	Y	Y	Y	-
	Right	Y	Y	Y	-
Eyeballs/Optic nerves	Left	-	Y	Y	-
	Right	-	Y	Y	-
Lacrimal glands	Left	-	Y	Y	-
	Right	-	Y	Y	-
Skeletal muscle (gastrocnemius)	Left	-	Y	Y	-
	Right	-	Y	-	-
Mammary glands/Skin (thoracic)	Left	-	Y	Y[f)]	-
	Right	-	Y	-	-
Skin (region of back)[g)]		-	Y	Y	-

Injection site h)**	-	Y	Y	-
Gross lesions***	-	Y	Y	-

Y: Examined

-: Not examined

a) Including Peyer's patches

b) Including gallbladder

c) Left lobe

d) Cortex

e) Parietal lobe, temporal lobe, and diencephalon

f) Mammary glands; only females

g) Interscapular

h) Catheter site (postcava)

*: Slide specimens of the left thyroid and left parathyroid from 1 female (No. 29) in the 30 mg/kg group could not be prepared or examined due to a congenital defect.

**: For No. 19, equivalent site was sampled, fixed, and prepared due to removed catheter.

***: Specimens of the postcava from 1 female (No. 11) in the control group, 1 female (No. 20) in the 12 mg/kg group, and 1 male (No. 38) in the 60 mg/kg group were also prepared and examined.
The traumatized section (the left femoral vein) from Animal No. 40 was also sampled, fixed, and prepared.

Histopathology

Adequate Battery: Yes, see list of tissues analyzed in the table in section titled Organ Weights

Peer Review: No

Histopathological Findings
All animals were evaluated for histopathological findings. Tissues evaluated are listed in the table under Organ Weights.

No test article-related changes in organ weights occurred in this study.

A summary table of the histopathological findings is presented below.

Table 13-1 Histopathological findings in male cynomolgus monkeys [H.E. staining] (End of the dosing per

Group	Physiological saline				ONO-2745BS				ONO-2745BS				ONO-2745BS			
Dose (mg/kg/day)					12$				30$				60$			
Findings / Animal No.	3	4	5	6	13	14	15	16	23	24	25	26	35	36	37	38
Caudal vena cava (site of catheterization)																
(gross lesion)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
Inflammation, vascular wall																+
Inflammatory granuloma, perivascular																2+
Thickening intima																2+
Thrombus/necrotic tissue																2+
Injection site (site of catheterization, caudal vena cava, transverse)																
Thickening, intima	+	-	-	+	+	-	-	-	+	-	-	2+	-	-	±	-

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Heart (atrium to ventricle, left)																	
Cellular infiltration, mononuclear cell	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-
Thickening, atrial endocardium	-	-	±	-	-	-	+	±	+	-	-	+	-	-	±	+
Heart (atrium to ventricle, right)																	
Cellular infiltration, mononuclear cell	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-
Heart (septum, interventricular)																	
Cellular infiltration, mononuclear cell	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-
Kidney (left)																	
Cellular infiltration, mononuclear cell	-	-	±	-	±	±	±	-	-	-	-	-	-	-	-	-
Kidney (right)																	
Cellular infiltration, mononuclear cell	-	-	-	-	-	-	±	-	-	-	-	-	-	-	±	-
Lung (including bronchus, right)																	
Accumulation, foam cell	-	-	-	-	-	-	-	±	±	-	-	-	-	-	-	-
Deposit, pigment, brown	-	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-
Granuloma, foreign body	-	-	-	-	±	-	-	-	±	-	-	-	-	-	-	-
Thrombus/organization, pulmonary artery	-	-	-	±	±	-	-	±	±	-	-	±	-	-	±	-

Notes) - : No abnormal changes ±: Very slight + : Slight 2+: Moderate 3+: Marked
P : Non-graded change NE: Not examined NA: Not applicable
$: As ONO-2745

Table 13-12 Histopathological findings in female cynomolgus monkeys [H.E. staining] (End of the d

Group	Physiological saline				ONO-2745BS			ONO-2745BS				ONO-2745BS		
Dose (mg/kg/day)					12$			30$				60$		
Findings / Animal No.	9	10	11	12	17	18	20	29	30	31	32	42	43	44
Caudal vena cava (site of catheterization)														
(gross lesion)	NA	NA		NA	NA	NA		NA	NA	NA	NA	NA	NA	NA
Inflammation, vascular wall			-				+							
Inflammatory granuloma, perivascular			-				2+							
Thickening intima			-				2+							
Thrombus/necrotic tissue			-				+							
Thrombus/organization			+				-							
Injection site (site of catheterization, caudal vena cava, transverse)														
Thickening, intima	-	-	-	-	-	-	-	-	-	-	-	-	+	-

Heart (atrium to ventricle, left)														
Cellular infiltration, mononuclear cell	-	-	-	-	-	-	±	-	-	-	-	-	-	-
Thickening, atrial endocardium	+	+	-	-	-	+	-	-	-	+	±	-	+	-
Heart (atrium to ventricle, right)														
Cellular infiltration, mononuclear cell	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Heart (septum, interventricular)														
Cellular infiltration, mononuclear cell	-	-	-	-	-	-	±	-	-	-	-	-	-	-
Kidney (left)														
Cellular infiltration, mononuclear cell	-	-	±	±	±	-	±	-	±	-	-	±	-	-
Regeneration, renal tubule	-	-	±	-	-	-	-	-	±	-	-	-	-	-
Thickening, intima, renal artery	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Kidney (right)														
Cellular infiltration, mononuclear cell	±	-	±	-	±	-	±	-	-	-	-	-	-	±
Regeneration, renal tubule	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Thickening, intima, renal artery	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Lung (including bronchus, left)														
Thrombus/organization, pulmonary artery	-	-	±	-	-	-	-	±	±	±	±	-	-	-
Lung (including bronchus, right)														
Thrombus/organization, pulmonary artery	±	-	±	-	±	-	-	-	-	±	±	±	-	-

Notes) - : No abnormal changes ±: Very slight + : Slight 2+: Moderate 3+: Marked
P : Non-graded change NE: Not examined NA: Not applicable
$: As ONO-2745

Table 13-25 Histopathological findings in male cynomolgus monkeys [H.E. staining] (End

Group	Physiological saline		ONO-2745BS		ONO-2745BS	
Dose (mg/kg/day)			30$		60$	
Findings / Animal No.	1	2	21	22	33	34
Heart (atrium to ventricle, left)						
Cellular infiltration, mononuclear cell	-	-	-	±	±	-
Heart (atrium to ventricle, right)						
Cellular infiltration, mononuclear cell	-	-	-	±	-	-
Heart (septum, interventricular)						
Cellular infiltration, mononuclear cell	-	±	-	-	-	-

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Injection site (site of catheterization, caudal vena cava, transverse)						
Inflammatory granuloma, perivascular	-	-	-	-	-	2+
Thrombus	-	P	-	-	P	-
Kidney (left)						
Cellular infiltration, mononuclear cell	-	-	-	±	±	-
Thickening, intima, renal artery	-	±	-	-	-	-
Kidney (right)						
Cellular infiltration, mononuclear cell	-	-	±	-	±	-
Thickening, intima, renal artery	-	-	-	±	-	-
Lung (including bronchus, left)						
Accumulation, pigmented macrophage	-	2+	-	-	-	-
Thrombus/organization, pulmonary artery	-	-	-	-	+	-
Lung (including bronchus, right)						
Accumulation, pigmented macrophage	-	2+	-	-	-	-
Thrombus/organization, pulmonary artery	-	-	+	-	±	-

Table 13-35 Histopathological findings in female cynomolgus monkeys [H.E. staining] (End

Group	Physiological saline		ONO-2745BS		ONO-2745BS	
Dose (mg/kg/day)			30$		60$	
Findings / Animal No.	7	8	27	28	39	41
Heart (atrium to ventricle, left)						
Cellular infiltration, mononuclear cell	-	-	-	±	-	-
Heart (atrium to ventricle, right)	-	-	-	-	-	-
Heart (septum, interventricular)						
Cellular infiltration, mononuclear cell	-	-	±	±	-	-
Ileum (including Peyer's patch)	-	-	-	-	-	-
Injection site (site of catheterization, caudal vena cava, transverse)						
Thickening, intima	±	+	-	-	-	+

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Kidney (left)						
Cellular infiltration, mononuclear cell	±	±	-	-	±	±
Kidney (right)						
Cellular infiltration, mononuclear cell	-	±	-	-	±	±
Inflammation, renal pelvis	±	-	-	-	-	-
Lung (including bronchus, left)						
Thrombus/organization, pulmonary artery	±	-	-	-	-	-
Lung (including bronchus, right)						
Thrombus/organization, pulmonary artery	-	-	-	-	-	±

No test article-related systemic histopathological findings occurred in this study.

Local findings included slight inflammation on the vascular wall with slight to moderate perivascular inflammatory granuloma, intima thickening, and thrombus and necrotic tissue at the caudal vena cava (site of catheterization) in one HD male one HD female. Local findings were recoverable except for the granuloma. All other local findings were not considered adverse because they were low severity (normal to slight) or appeared to be procedure related as they were similar to controls and were not concentration dependent.

Toxicokinetics

All animals, including controls, were evaluated in the toxicokinetic analysis. Blood for toxicokinetic analysis was collected according to the table below.

Sampling Days	Sampling Times	
	After the start of dosing	After the end of dosing
Day 0 of dosing	1, 3, 6, and 12 hours	1, 6, and d12 hours (13, 18, 24 hours after the start of dosing)
Day 27 of dosing	1, 3, 6, and 12 hours	1, 6, and 12 hours (13, 18, 24 hours after the start of dosing)

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Table 66 TK Summary of Parent (ONO-2745 spiked with metabolites) on Day 0 of a 4-Week Monkey Study

Group	Dosage (mg/kg/day)	Sex	Animal number	Concentrations of ONO-2745 (ng/mL) Time# (h) 1	3	6	12	13	18	24	Toxicokinetic parameters C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
2	12§	Male	13	257	327	327	437	103	10.9	6.53	437	4590
			14	258	292	265	284	84.5	12.0	6.68	292	3640
			15	182	310	315	392	93.7	10.1	6.21	392	4190
			16	262	263	274	333	70.3	10.2	8.60	333	3740
			Mean	240	298	295	362	87.9	10.8	7.01	364	4040
			SD	39	27	30	67	13.9	0.9	1.08	64	440
		Female	17	196	243	333	335	78.7	13.4	6.75	335	3900
			18	179	316	320	422	91.3	14.7	9.20	422	4360
			20	197	268	217	366	63.8	12.5	9.60	366	3510
			Mean	191	276	290	374	77.9	13.5	8.52	374	3920
			SD	10	37	64	44	13.8	1.1	1.54	44	430
		Total mean		219	288	293	367	83.6	12.0	7.65	368	3990
		SD		38	31	43	54	13.7	1.7	1.43	52	400
3	30§	Male	21	700	932	738	1030	268	16.4	9.95	1030	11200
			22	625	774	863	978	242	9.63	9.50	978	11000
			23	543	725	679	745	168	12.0	8.57	745	8890
			24	637	767	835	815	258	13.0	9.10	835	10400
			25	657	868	1020	1010	320	13.5	6.76	1020	12300
			26	808	1040	909	1290	302	39.5	12.8	1290	13600
			Mean	662	851	841	978	260	17.3	9.45	983	11200
			SD	88	120	122	191	53	11.1	1.98	188	1600
		Female	27	718	1020	994	996	173	16.7	10.6	1020	12200
			28	534	770	643	643	106	13.2	8.82	770	8290
			29	637	800	1060	1300	339	57.2	24.8	1300	13700
			30	818	960	1280	961	290	64.7	18.7	1280	14000
			31	558	712	811	882	182	13.7	10.1	882	10000
			32	625	691	813	984	262	17.4	9.14	984	10700
			Mean	648	826	934	961	225	30.5	13.7	1040	11500
			SD	105	135	225	212	86	23.8	6.6	210	2200
		Total mean		655	838	887	970	243	23.9	11.6	1010	11400
		SD		93	122	179	192	71	19.0	5.1	190	1900
4	60§	Male	33	1650	1600	1990	2040	343	25.5	16.3	2040	23800
			34	1650	1670	1640	2120	404	35.1	19.3	2120	22900
			35	2270	2090	2250	2230	901	52.6	20.4	2270	29600
			36	1600	1890	2030	1660	367	38.2	15.3	2030	23400
			37	1110	2660	1640	1620	291	19.7	15.2	2660	22400
			38	1380	1880	2170	1860	413	23.5	15.2	2170	24500
			Mean	1610	1970	1950	1920	453	32.4	17.0	2220	24400
			SD	390	380	260	250	224	12.1	2.3	240	2600
		Female	39	2230	1740	2320	1890	715	32.4	15.4	2320	27100
			41	2230	1630	1760	1980	476	46.6	19.9	2230	24000
			42	1520	1950	1710	1920	332	31.2	22.4	1950	22800
			43	2280	1860	1960	1620	790	46.6	15.3	2280	25200
			44	1440	1470	2030	1930	918	94.7	30.6	2030	25100
			Mean	1940	1730	1960	1870	646	50.3	20.7	2160	24800
			SD	420	190	240	140	238	25.9	6.3	160	1600
		Total mean		1760	1860	1950	1900	541	40.6	18.7	2190	24600
		SD		420	320	240	200	241	20.7	4.7	200	2100

#: Time after initiation of administration
§: As ONO-2745

Reference ID: 4622909

Table 67 TK Summary of Parent (ONO-2745 spiked with metabolites) on Day 27 of a 4-Week Monkey Study

Group	Dosage (mg/kg/day)	Sex	Animal number	Concentrations of ONO-2745 (ng/mL) Time# (h) 1	3	6	12	13	18	24	Toxicokinetic parameters C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
2	12§	Male	13	247	307	364	458	146	32.4	26.0	458	5090
			14	261	276	322	458	100	26.5	22.6	458	4660
			15	271	325	338	464	116	31.4	23.8	464	4970
			16	266	306	332	413	112	24.8	25.2	413	4660
			Mean	261	304	339	448	119	28.8	24.4	448	4850
			SD	10	20	18	24	20	3.7	1.5	24	220
		Female	17	288	292	234	325	115	36.2	32.8	325	4010
			18	249	312	264	365	133	38.9	29.4	365	4330
			20	153	239	243	339	154	34.7	24.8	339	3850
			Mean	230	281	247	343	134	36.6	29.0	343	4060
			SD	69	38	15	20	20	2.1	4.0	20	240
		Total mean		248	294	300	403	125	32.1	26.4	403	4510
		SD		44	29	52	60	20	5.1	3.5	60	470
3	30§	Male	21	817	832	962	1210	313	43.8	27.7	1210	13100
			22	671	764	794	922	248	45.1	34.1	922	10800
			23	579	679	709	1070	179	33.7	30.7	1070	10300
			24	575	870	936	1120	223	38.1	29.7	1120	12200
			25	626	747	763	862	248	34.6	23.1	862	10300
			26	537	652	794	828	274	50.1	29.3	828	10100
			Mean	634	757	826	1000	248	40.9	29.1	1000	11100
			SD	101	84	100	150	45	6.5	3.6	150	1200
		Female	27	803	992	887	718	193	41.1	33.2	992	11100
			28	418	566	615	792	269	89.8	25.2	792	8970
			29	663	728	988	871	343	84.8	43.7	988	12000
			30	623	723	791	833	312	67.4	36.1	833	10700
			31	401	648	550	664	170	31.4	27.8	664	7800
			32	523	785	687	803	244	45.4	27.1	803	9730
			Mean	572	740	753	780	255	60.0	32.2	845	10100
			SD	155	145	167	76	67	24.3	7.0	126	1500
		Total mean		603	749	790	891	251	50.4	30.6	924	10600
		SD		129	113	137	164	55	19.7	5.5	157	1400
4	60§	Male	33	1180	1420	2250	1650	446	67.6	43.2	2250	23100
			34	590	746	923	832	751	429 *	347 *	923	15600
			35	1050	1350	1680	2030	684	118 *	75.1	2030	22600
			36	974	1150	1480	1710	481	58.3	41.5	1710	18900
			37	1230	1300	1420	1700	472	60.4	36.8	1700	19300
			38	1250	1550	1430	1400	314	192 *	124 *	1550	19500
			Mean	1050	1250	1530	1550	525	154	111	1690	19800
			SD	250	280	430	410	162	144	120	460	2700
		Female	39	913	1110	1210	1640	710	273 *	196 *	1640	19600
			41	1070	1700	1350	1560	682	78.8	46.9	1700	20000
			42	1080	1270	1660	1540	410	49.8	41.2	1660	19300
			43	1290	1160	1200	1380	432	203 *	128 *	1380	17900
			44	1160	1280	1340	1630	485	68.8	50.7	1630	18700
			Mean	1100	1300	1350	1550	544	135	92.6	1600	19100
			SD	140	230	190	100	142	98	67.9	130	800
		Total mean		1070	1280	1450	1550	533	145	103	1650	19500
		SD		200	250	340	290	146	120	96	340	2000

#: Time after initiation of administration
*: Reanalysis data
§: As ONO-2745

Toxicokinetic data suggest that the parent C_{max} and AUC increase dose-proportionately in males and females. No sex differences or dose accumulation occurred. Parent was not detected in plasma from animals treated with saline control.

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Table 68 TK Summary of Metabolite (ONO-IN-252) on Day 0 of a 4-Week Monkey Study

Group	Dosage (mg/kg/day)	Sex	Animal number	Concentrations of ONO-IN-252 (ng/mL) Time# (h) 1	3	6	12	13	18	24	Toxicokinetic parameters C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
2	12§	Male	13	302 *	551	579	1310	406	73.2	33.1	1310	10700
			14	409 *	691	630	1170	584	124	38.1	1170	11800
			15	240 *	506 *	500	921	464	90.1	37.4	921	9100
			16	364 *	659	750	950	498	87.7	34.3	950	11000
			Mean	329	602	615	1090	488	93.8	35.7	1090	10700
			SD	74	88	105	190	74	21.5	2.4	190	1100
		Female	17	271	485	758	852	557	61.7	27.8	852	10100
			18	224	470	719	1090	518	85.8	33.2	1090	10700
			20	310	529	621	1550	866	63.8	25.9	1550	13000
			Mean	268	495	699	1160	647	70.4	29.0	1160	11300
			SD	43	31	71	350	191	13.3	3.8	350	1500
		Total mean		303	556	651	1120	556	83.8	32.8	1120	10900
		SD		66	86	96	250	149	21.1	4.6	250	1200
3	30§	Male	21	1320	2390	2710	4760	2690	412	111	4760	47500
			22	1150	1530	1760	2440	1380	217	59.4	2440	27500
			23	1430	2250	2710	3170	1040	192	59.6	3170	35400
			24	1450	1950	2400	2420	2330	313	114	2420	35400
			25	1370	3480	3780	4290	4300	264	88.3	4300	57400
			26	888	1800	2210	4180	3540	426	80.1	4180	43600
			Mean	1270	2230	2600	3540	2550	304	85.4	3550	41100
			SD	210	680	680	1010	1250	98	23.9	1010	10600
		Female	27	1000	1460	1950	2490	1020	133	65.9	2490	26600
			28	1090	1860	1690	2390	812	125	61.7	2390	25600
			29	1580	2770	3130	8840	6070	632	224	8840	76700
			30	1650	3120	3780	5090	2940	949	624	5090	61000
			31	1190	2100	2210	3730	1940	286	81.5	3730	37700
			32	789	1410	1580	2490	2030	214	45.4	2490	27900
			Mean	1220	2120	2390	4170	2470	390	184	4170	42600
			SD	340	700	880	2520	1920	331	225	2520	21400
		Total mean		1240	2180	2490	3860	2510	347	135	3860	41900
		SD		270	660	760	1860	1550	237	161	1860	16100
4	60§	Male	33	2690	6320	5830	8240	4750	450	200	8240	92200
			34	3090	5840	5620	4820	3260	362	140	5840	73600
			35	1940	4100	4720	7480	5030	377	133	7480	78100
			36	2470	4370	5350	6360	4720	394	88.2	6360	77600
			37	2760	5890	6580	6650	2550	470	141	6650	82400
			38	2840	4870	8890	6590	4010	401	117	8890	94100
			Mean	2630	5230	6170	6690	4050	409	137	7240	83000
			SD	390	910	1470	1150	980	42	37	1170	8400
		Female	39	2690	4640	6440	13000	9490	608	147	13000	122000
			41	3150	4740	6980	9720	7600	463	153	9720	108000
			42	2560	3290	3540	4630	2320	144	85.8	4630	52200
			43	1930	2900	5580	3560	1480	346	103	5580	54400
			44	2450	3900	4930	5420	2850	523	259	5420	66800
			Mean	2560	3890	5490	7270	4750	417	150	7670	80700
			SD	440	810	1350	3970	3560	180	68	3580	32200
		Total mean		2600	4620	5860	6950	4370	413	142	7440	81900
		SD		400	1080	1390	2660	2390	118	50	2420	21200

#: Time after initiation of administration

§: As ONO-2745

*: Reanalysis data

Reference ID: 4622909

Table 69 TK Summary of Metabolite (ONO-IN-252) on Day 0 of a 4-Week Monkey Study

Group	Dosage (mg/kg/day)	Sex	Animal number	Concentrations of ONO-IN-252 (ng/mL) Time# (h) 1	3	6	12	13	18	24	Toxicokinetic parameters C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)
2	12§	Male	13	451	860	753	1210	464	169	79.3	1210	13000
			14	740	738	808	1870	598	101	76.4	1870	15800
			15	469	763	763	1420	456	104	66.3	1420	13200
			16	836	1390	1020	1330	776	100	83.3	1390	17100
			Mean	624	938	836	1460	574	119	76.3	1470	14800
			SD	194	306	125	290	150	34	7.3	280	2000
		Female	17	543	733	583	722	433	86.7	111	733	9960
			18	571	883	772	677	521	100	67.0	883	11300
			20	347	608	499	642	393	74.9	53.8	642	8310
			Mean	487	741	618	680	449	87.2	77.3	753	9860
			SD	122	138	140	40	65	12.6	30.0	122	1500
		Total mean		565	854	743	1120	520	105	76.7	1160	12700
		SD		170	253	167	460	131	30	18.0	440	3100
3	30§	Male	21	2230	3040	3100	5800	1740	308	165	5800	52700
			22	1400	1830	2060	2410	1350	276	210	2410	30700
			23	1780	2280	2670	4200	1800	183	242	4200	42300
			24	2110	2620	2900	3990	2070	296	296	3990	45600
			25	1820	2740	2730	3040	1630	281	213	3040	39700
			26	1560	1780	2050	2400	1310	255	156	2400	30300
			Mean	1820	2380	2590	3640	1650	267	214	3640	40200
			SD	320	510	440	1300	290	45	52	1300	8700
		Female	27	1140	1780	1480	1730	920	129	87.9	1780	22700
			28	1310	1760	1720	2020	1080	400	107	2020	27000
			29	1620	2660	2320	3100	1610	559	380	3100	39600
			30	1380	2300	2420	3440	1970	380	131	3440	39200
			31	1580	2910	2430	3030	3090	178	137	3090	41900
			32	1140	2840	1530	1700	823	135	80.1	2840	25100
			Mean	1360	2380	1980	2500	1580	297	154	2710	32600
			SD	210	510	450	770	860	176	113	660	8500
		Total mean		1590	2380	2280	3070	1620	282	184	3180	36400
		SD		350	490	530	1180	610	123	89	1100	9100
4	60§	Male	33	3800	4700	5770	6580	3120	468	268	6580	79300
			34	1480	3420	2390	2540	2000	1120 *	889	3420	45700
			35	1580	3000	3850	8340	3570	671	380	8340	72100
			36	2510	3000	4040	5750	2010	519	281	5750	59400
			37	3630	5080	5500	6850	2460	365	427	6850	77800
			38	2570	5250	4180	4350	1620	577	299	5250	60100
			Mean	2600	4080	4290	5740	2460	620	424	6030	65700
			SD	980	1050	1230	2040	750	266	236	1660	13000
		Female	39	1870	3080	3440	4290	2620	1170 *	448	4290	56900
			41	2330	4770	4410	4250	2350	311	229	4770	59700
			42	2790	4570	3650	4390	2180	194	149	4570	55500
			43	1020	1470	1620	2640	1190	345	187	2640	27900
			44	1820	3260	3630	3910	1560	287	210	3910	47900
			Mean	1970	3430	3350	3900	1980	461	245	4040	49600
			SD	660	1330	1040	720	590	400	118	840	12900
		Total mean		2310	3780	3860	4900	2240	548	342	5120	58400
		SD		870	1170	1190	1790	690	326	205	1660	14900

#: Time after initiation of administration
*: Reanalysis data
§: As ONO-2745

Toxicokinetic data suggest that the metabolite C_{max} and AUC increase dose-proportionately in males and females. No sex differences or dose accumulation occurred.

Dosing Solution Analysis

Dosing solution analysis confirmed that the administered concentrations were within 5% of the nominal concentrations, which is acceptable. While the test article in this study was spiked with 8 impurities/degradants, the dosing solution analysis was not conducted to confirm the delivered amount of impurities.

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12.2 Impurity Qualification: *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

To qualify the identified impurities exceeding the qualification threshold, the Sponsor conducted an Ames assay and a mouse lymphoma assay with a batch of drug product spiked with the 8 impurities (i.e. [redacted] (b) (4)). This is in alignment with ICH Q3B(R2) Attachment 3, page 17. The Ames assay is reviewed below.

Study title: A Bacterial Reverse Mutation Test of ONO-2745BS (Lot number: YMK110831)

Study no.:	[redacted] (b) (4) 015- 270
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	June 25, 2012
GLP compliance:	Yes, this study was performed in compliance with GLP regulations and principles and in accordance with OECD Principles of GLP, OECD Test No. 471, among others.
QA statement:	Yes, a QA statement was signed by [redacted] (b) (4) on June 25, 2012
Drug, lot #, and % purity:	ONO-2745BS, Lot YMK110831 spiked with 8 impurities, 94.6% pure

In this in vitro bacterial reverse mutation assay using the pre-incubation method, remimazolam spiked with 8 impurities (i.e. [redacted] (b) (4)), in the presence or absence of metabolic activation tested negative for mutagenicity in *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 or *Escherichia coli* WP2*uvrA* at 156, 313, 625, 1250, 2500, 5000 mcg/plate. Key study findings support the conclusion that the test article is not mutagenic under these test conditions.

Key Study Findings

- This study was valid
- Test article precipitated at 2500 mcg/plate and greater upon addition of the test article formulation and on the plates after incubation for 48 hours with and without metabolic activation
- Growth was inhibited at 5000 mcg/plate in TA1537 without metabolic activation
- No statistically significant increase in mean revertant colonies per plate occurred in any test article condition.

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Methods

Strains:

Base Pair Substitution Mutations	Frameshift Mutation
S. typhimurium TA1537	*S. typhimurium TA100*
S. typhimurium TA98	*S. typhimurium TA1535*
E. coli WP2*uvrA*	

Concentrations in definitive study: 156, 313, 625, 1250, 2500, 5000 mcg/plate

Basis of concentration selection: In a preliminary dose range-finding assay using the pre-incubation method, test article was assessed for cytotoxicity and mutagenicity at doses of 5, 15, 50, 150, 500, 1500, 5000 mcg/plate in tester strains TA100, TA1535, TA98, TA1537, and WP2*uvrA* with or without metabolic activation. Cytotoxicity was assessed by examining bacterial lawn density and number of spontaneous revertants per plate. Test article at a dose level of 5000 mcg/plate was toxic to tester strain TA1537 without metabolic activation as evidenced by diminution of the bacterial lawn and decreased number of revertant colonies.

Negative control: Dimethyl sulfoxide (DMSO)

Positive control:

Test strain	Without metabolic activation		With metabolic activation	
	Positive control article	Dose (µg/plate)	Positive control article	Dose (µg/plate)
TA100	AF-2	0.01	2AA	1
TA1535	NaN_3	0.5	2AA	2
WP2*uvrA*	AF-2	0.02	2AA	10
TA98	AF-2	0.1	2AA	0.5
TA1537	9AA	80	2AA	2

AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
NaN3: Sodium azide
9AA: 9-Aminoacridine hydrochloride hydrate
2AA: 2-Aminoanthracene

Formulation/Vehicle: DRF: 0.05, 0.15, 0.5, 1.5, 5, 15, 50 mg/mL
Main: 1.56, 3.13, 6.25, 12.5, 25, 50 mg/mL
Vehicle: DMSO

Incubation & sampling time: For the pre-incubation method, tester strains were incubated with test article with and without metabolic activation for 20 minutes at 37°C before adding warm top agar and mixing. The mixture was poured onto a minimal glucose agar plate and incubated 48 hours at 37°C. Plates were scored following the 48-hour incubation. Cytotoxicity was assessed by examining bacterial lawn density and numbers of spontaneous revertants per plate.

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Metabolic activation consisted of an S9 fraction prepared from livers obtained from phenobarbital and 5,6-benzoflavone pretreated male Sprague-Dawley rats. Percentage of S9 was 25%, which is acceptable (Total pre-incubation mixture = 0.5 mL of S9 + 0.1 mL bacterial suspension + 0.1 mL test article + 2 mL of top agar).

Study Validity

This study is valid because the dose-finding test and the two main tests met the following conditions.

1. The number of revertant colonies in the negative and positive controls was within the range of the control background data of the test facility
2. No bacterial contamination in the sterility test was observed
3. Analysis (colony count) is possible for at least 5 dose levels or no inhibition is observed at least 4 dose levels in the two main tests
4. Selection of the bacterial tester strains was adequate
5. Dose selection was based on the limit dose (5000 mcg/plate) and precipitation occurred at 5000 mcg/plate in the DRF study.
6. The S9 concentration was acceptable

Results

In the dose-finding test and main tests, the test article precipitated at 2500 mcg/plate and greater upon addition of the test article formulation and the plates after incubation for 48 hours with and without metabolic activation. Growth inhibition occurred at 5000 mcg/plate in TA1537 without metabolic activation. Growth inhibition was not observed in other test strains up to 5000 mcg/plate. Below is a summary of the dose-finding test and two main test results.

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Table 1 Study No. (b) (4) 015-270

Results of dose-finding test

Test article: ONO-2745BS

Metabolic activation	Dose (µg/plate)	Number of revertant (colonies/plate)				
		Base pair substitution mutations			Frameshift mutations	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
Without metabolic activation	Negative control DMSO	102	8	20	23	6
	5	96	7	18	22	4
	15	107	8	22	26	3
	50	121	11	20	24	3
	150	95	8	21	14	3
	500	91	7	16	18	5
	1500	89	6	27	16	4
	5000 +	106	5	20	15	2 *
With metabolic activation	Negative control DMSO	111	10	19	31	8
	5	102	8	25	27	9
	15	113	10	24	23	9
	50	94	9	19	24	8
	150	93	5	29	29	9
	500	109	6	31	20	9
	1500	105	8	19	20	12
	5000 +	124	7	16	35	10
PC Without metabolic activation	Article	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (µg/plate)	0.01	0.5	0.02	0.1	80
	Colonies/plate	368	280	322	419	261
PC With metabolic activation	Article	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Colonies/plate	481	181	277	398	164

Remarks)

1. Dose : as ONO-2745
2. PC : Positive controls
   AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide NaN_3: Sodium azide
   9AA: 9-Aminoacridine hydrochloride hydrate 2AA: 2-Aminoanthracene
3. DMSO : Dimethyl sulfoxide
4. + : Test article precipitation (on the plates after incubation for 48 hours)
5. * : Density of the bacterial background lawn clearly lower than that of the negative control

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Table 2 Study No. (b) (4) 015-270

Results of first main test

Test article: ONO-2745BS

Metabolic activation	Dose (µg/plate)	Number of revertant (colonies/plate)				
		Base pair substitution mutations			Frameshift mutations	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
Without metabolic activation	Negative control	110 , 105	7 , 5	17 , 14	23 , 19	4 , 3
	DMSO	101 (105)	7 (6)	15 (15)	17 (20)	3 (3)
	156	99 , 105	7 , 5	18 , 16	21 , 25	5 , 4
		96 (100)	5 (6)	15 (16)	25 (24)	4 (4)
	313	103 , 96	8 , 5	17 , 20	14 , 16	5 , 4
		96 (98)	7 (7)	21 (19)	16 (15)	6 (5)
	625	96 , 108	8 , 7	21 , 19	18 , 24	5 , 4
		100 (101)	7 (7)	17 (19)	17 (20)	3 (4)
	1250	110 , 99	6 , 7	15 , 13	17 , 14	4 , 3
		98 (102)	6 (6)	19 (16)	16 (16)	3 (3)
	2500 +	105 , 103	4 , 5	18 , 15	17 , 15	4 , 3
		108 (105)	5 (5)	15 (16)	17 (16)	3 (3)
	5000 +	98 , 90	5 , 7	14 , 15	14 , 10	2* , 2*
		94 (94)	8 (7)	14 (14)	10 (11)	4* (3)
With metabolic activation	Negative control	100 , 116	6 , 7	18 , 19	27 , 21	11 , 6
	DMSO	112 (109)	8 (7)	23 (20)	20 (23)	7 (8)
	156	112 , 106	7 , 5	21 , 17	22 , 28	9 , 6
		104 (107)	6 (6)	18 (19)	24 (25)	9 (8)
	313	100 , 107	6 , 7	22 , 24	25 , 21	10 , 10
		96 (101)	6 (6)	26 (24)	22 (23)	7 (9)
	625	109 , 108	8 , 7	20 , 18	25 , 25	7 , 5
		118 (112)	6 (7)	21 (20)	21 (24)	5 (6)
	1250	98 , 95	9 , 6	15 , 17	21 , 18	6 , 8
		96 (96)	7 (7)	18 (17)	22 (20)	10 (8)
	2500 +	116 , 117	4 , 3	21 , 17	21 , 27	7 , 6
		112 (115)	5 (4)	20 (19)	25 (24)	8 (7)
	5000 +	99 , 105	8 , 7	20 , 18	22 , 21	6 , 5
		104 (103)	5 (7)	15 (18)	22 (22)	6 (6)
PC — Without metabolic activation	Article	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (µg/plate)	0.01	0.5	0.02	0.1	80
	Colonies/plate	493 , 503	393 , 365	517 , 496	523 , 557	368 , 560
		537 (511)	302 (353)	521 (511)	542 (541)	514 (481)
PC — With metabolic activation	Article	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Colonies/plate	560 , 539	209 , 199	336 , 281	404 , 442	149 , 159
		496 (532)	203 (204)	288 (302)	418 (421)	148 (152)

Remarks)

1. Dose : as ONO-2745
2. ( ) : Mean of three plates
3. PC : Positive controls
   AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide NaN_3: Sodium azide
   9AA: 9-Aminoacridine hydrochloride hydrate 2AA: 2-Aminoanthracene
4. DMSO : Dimethyl sulfoxide
5. + : Test article precipitation (on the plates after incubation for 48 hours)
6. * : Density of the bacterial background lawn clearly lower than that of the negative control

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Table 3 Study No. (b) (4)015-270

Results of second main test

Test article: ONO-2745BS

Metabolic activation	Dose (µg/plate)	Number of revertant (colonies/plate)				
		Base pair substitution mutations			Frameshift mutations	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
Without metabolic activation	Negative control DMSO	92 , 100 90 (94)	8 , 5 6 (6)	17 , 16 21 (18)	14 , 13 17 (15)	6 , 7 6 (6)
	156	98 , 99 91 (96)	6 , 6 5 (6)	17 , 17 23 (19)	14 , 11 15 (13)	6 , 5 7 (6)
	313	91 , 96 89 (92)	7 , 6 6 (6)	24 , 20 22 (22)	16 , 15 11 (14)	5 , 4 4 (4)
	625	97 , 90 98 (95)	5 , 6 3 (5)	24 , 26 20 (23)	20 , 19 21 (20)	5 , 4 3 (4)
	1250	96 , 93 102 (97)	4 , 3 4 (4)	22 , 21 25 (23)	16 , 17 13 (15)	5 , 3 4 (4)
	2500 +	91 , 86 89 (89)	8 , 5 6 (6)	20 , 16 18 (18)	16 , 17 11 (15)	4 , 3 5 (4)
	5000 +	86 , 82 87 (85)	7 , 6 7 (7)	20 , 18 18 (19)	14 , 17 13 (15)	3* , 2* 2* (2)
With metabolic activation	Negative control DMSO	104 , 95 90 (96)	10 , 7 6 (8)	20 , 24 24 (23)	20 , 24 27 (24)	7 , 6 6 (6)
	156	109 , 92 103 (101)	5 , 6 6 (6)	17 , 18 16 (17)	23 , 23 22 (23)	9 , 8 7 (8)
	313	100 , 102 110 (104)	10 , 11 9 (10)	23 , 22 21 (22)	21 , 25 20 (22)	5 , 9 7 (7)
	625	99 , 106 107 (104)	7 , 8 6 (7)	27 , 21 22 (23)	16 , 23 21 (20)	5 , 4 7 (5)
	1250	114 , 100 102 (105)	10 , 6 7 (8)	24 , 27 26 (26)	20 , 20 19 (20)	6 , 6 5 (6)
	2500 +	107 , 104 116 (109)	6 , 6 6 (6)	18 , 19 23 (20)	22 , 20 23 (22)	4 , 4 3 (4)
	5000 +	101 , 104 110 (105)	10 , 8 9 (9)	19 , 26 20 (22)	24 , 21 23 (23)	6 , 6 8 (7)
PC Without metabolic activation	Article	AF-2	NaN_3	AF-2	AF-2	9AA
	Dose (µg/plate)	0.01	0.5	0.02	0.1	80
	Colonies/plate	450 , 487 510 (482)	404 , 367 381 (384)	526 , 481 508 (505)	554 , 554 599 (569)	596 , 377 611 (528)
PC With metabolic activation	Article	2AA	2AA	2AA	2AA	2AA
	Dose (µg/plate)	1	2	10	0.5	2
	Colonies/plate	547 , 609 579 (578)	150 , 156 178 (161)	322 , 295 346 (321)	423 , 387 386 (399)	141 , 142 146 (143)

Remarks)

1. Dose : as ONO-2745
2. ( ) : Mean of three plates
3. PC : Positive controls
   AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide NaN_3: Sodium azide
   9AA: 9-Aminoacridine hydrochloride hydrate 2AA: 2-Aminoanthracene
4. DMSO : Dimethyl sulfoxide
5. + : Test article precipitation (on the plates after incubation for 48 hours)
6. * : Density of the bacterial background lawn clearly lower than that of the negative control

Positive test results are determined when the number of revertant colonies per plate (mean) in any test strain increases dose-dependently by 2 fold or greater, compared

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with that in the negative control, and the results are reproducible between the two main tests. The results are judged as negative when the number of revertant colonies per plate is less than 2 fold that of the negative control, and the results are reproducible between the two main tests.

As shown in the tables above, there was no statistically significant increase in mean revertant colonies per plate in any test article condition. Therefore, remimazolam spiked with 8 impurities (i.e. [redacted] (b) (4) tested negative and was not mutagenic under the conditions of this assay.

12.3 Impurity Qualification: *In Vitro* Assays in Mammalian Cells

To qualify the identified impurities exceeding the qualification threshold, the Sponsor conducted an Ames assay and a mouse lymphoma assay with a batch of drug product spiked with the 8 impurities (i.e. [redacted] (b) (4)). This is in alignment with ICH Q3B(R2) Attachment 3, page 17. The mouse lymphoma assay is reviewed below.

Study title: A Mouse Lymphoma TK Assay of ONO-2745BS (Lot number: YMK110831)

Study no.:	(b) (4) 015- 271
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	July 13, 2012
GLP compliance:	Yes, this study was performed in compliance with GLP regulations and principles and in accordance with OECD Principles of GLP, OECD Test No. 473, among others.
QA statement:	Yes, a QA statement was signed by [redacted] (b) (4) on July 13, 2012
Drug, lot #, and % purity:	ONO-2745BS, Lot YMK110831 spiked with 8 impurities, 94.6% pure

In a valid mouse lymphoma assay ([redacted] (b) (4) 015- 271), L5178Y ($Tk^{+/-}$-3.7.2c) mouse lymphoma cells were treated with 200-800 mcg/mL remimazolam in short-term (3 hour) treatments with and without metabolic activation and in 24-hour continuous treatment without metabolic activation.

Key Study Findings

- In the definitive experiment and confirmatory test, no statistically significant dose-dependent increase in total mutation frequency occurred with short-term

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treatment (3 hours) with metabolic activation or with 24-hour continuous treatment without metabolic activation.

- In the definitive experiment, a statistically significant dose-dependent increase in total mutation frequency occurred with short-term treatment (3 hours) without metabolic activation. This finding was not reproduced in the confirmatory experiment, which considered the increase in T-MF a nonspecific effect caused by cytotoxicity.
- Therefore, no statistically significant, dose-dependent, reproducible increase in total mutation frequency occurred in this study.
- Study report concludes that the test article might have induced genotoxicity in the mouse lymphoma cells under these study conditions. There were more instances of small colony mutations than in large colony mutations, indicative that the test article may have the potential for clastogenicity.

Methods

Assay Version:	Liquid media in 96-well plates
Cell line:	L5178Y (*Tk*$^{+/-}$-3.7.2c) mouse lymphoma cells This is an acceptable cell line because there is evidence that the assay detects gene mutations (point mutations) and chromosomal events (deletions, translocations, mitotic recombinations/gene conversion and aneuploidy).
Metabolic Activation:	S9 fraction from phenobarbital and 5,6-benzoflavone (also known as beta-naphthoflavone) induced male Sprague-Dawley rat livers was used at 10% v/v
Concentrations in definitive study:	Short-term treatment (3 hours): -S9: 200, 250, 300, 400, 500 mcg/mL +S9: 450, 500, 550, 600, 650, 700, 800 mcg/mL Continuous treatment (24 hours): -S9: 300, 350, 400, 450, 500, 550, 600 mcg/mL
Basis of concentration selection:	Dose-finding test used short-term (3-hour) treatments with and without S9 and 24-hour continuous treatment without S9. In a previous MLA (DPE0003) greater than 80% cytotoxicity occurred at 600 to 610 mcg/mL in short-term treatment without S9, 800 to 900 mcg/mL in short-term treatment with S9, and 550 mcg/mL in 24-hour treatment without S9. Based on this previous study, the dose-finding test used the following concentrations: 100, 200, 400, 600, 800, 1000, 1300 mcg/mL. Cytotoxicity results of the dose-finding test are shown in the table below.

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Treatment condition	Doses showing 80% cytotoxicity (RTG = 20%)*
Short-term treatment (−S9)	469 µg/mL
Short-term treatment (+S9)	729 µg/mL
24-hour continuous treatment (−S9)	558 µg/mL

*:as ONO-2745

Precipitation of the test article occurred at 1000 mcg/mL and greater at the start and end of treatment.
pH did not change when test article formulations were added.

Negative control: Dimethyl sulfoxide (DMSO)

Positive control:

Treatment condition	Positive control article	Prepared concentration (mg/mL)	Treated concentration (µg/mL)
Short-term treatment (−S9)	MMS	0.1	10
Short-term treatment (+S9)	CP	0.03	3
24-hour continuous treatment (−S9)	MMS	0.1	10

MMS = Methyl methanesulfonate
CP = cyclophosphamide

Formulation / Vehicle: Dose-finding test:10, 20, 40, 50, 60, 80, 100, 130 mg/mL in DMSO
Main and Confirmatory test: 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80 mg/mL in DMSO

Incubation & sampling time: Cultured L5178Y mouse lymphoma cells during the logarithmic growth phase are incubated with test article for 3- or 24-hour incubation with or without metabolic activation. After the 3- or 24-hour incubation, cells are washed of the test article and prepared for plating in a 96-well plate with trifluorothymidine (TFT) for the mutation expression period. Evaluation of colony growth occurs after a 2-day mutation expression period.

Study Validity

This study was valid because it conformed with the following acceptability criteria:

- The culture medium was monitored for changes in pH after test article formulation was added.
- All three experimental conditions were tested
- Adequate number of cells and concentrations were analyzable – at least three concentrations with appropriate cytotoxicity, number of cells (at least 300 well-spread metaphases)
- Selection of top concentrations were appropriate given the limitation of cytotoxicity
- Dosing solution analysis confirmed highest and lowest concentrations were within 10% of nominal using HPLC.

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- Concurrent negative and positive controls provided expected results:
  - PE in the negative control group was within range of 65-120%;
  - T-MF in the negative control group was within range of 50-170 x10^{-6};
  - T-MF in the positive control group was within range of mean±3SD of the control background data of test facility

Results

The test is considered positive if any of the three experimental conditions met all of the following criteria:

- At least one of the test concentrations exhibit a statistically significant increase compared with concurrent negative controls
- The increase is dose-dependent when evaluated with an appropriate trend test
- The response is required to exceed a defined value [the global evaluation factor (GEF) plus the control; 126 x 10^{-6}]

If results are neither clearly positive or clearly negative, the data should be evaluated by expert judgement and/or further investigations.

Definitive experiment

Three test conditions were tested in the definitive experiment. Two 96-well plates for each dose and 2000 cells/well (four plates for the negative control group because it was treated in duplicate). Results of the definitive experiment are summarized in the table below.

Compound	Conc.	PE (%)	RSG (%)	RTG (%)	Mutation Frequency (x10^{-6})		
					L-MF	S-MF	T-MF
-S9 (3 hours)							
Vehicle	0	94	100	100	49.6	49.6	102.5
Test article	200	95	106	107.6	48.8	64.1	116.1
	250	108	91	105.0	93.0	40.2	142.7
	300	95	101	102.6	73.4	76.6	158.8
	350	89	83	79.7	88.3	71.4	172.9
	400	95	66	66.8	76.6	76.6	166.3
	450	69	36	26.8	75.9	214.6	318.4*
	500	-	-	-	-	-	-
PC (MMS)	10	101	81	87.2	166.7	228.0	464.2
+S9 (3 hours)							
Vehicle	0	98	100	100	66.6	34.3	104.3
Test article	450	95	90	87.4	96.0	57.9	162.5
	500	105	87	93.4	81.2	36.2	124.4
	550	87	92	81.4	70.2	63.5	142.5
	600	**101**	**91**	**94.1**	**60.1**	**77.8**	**149.0**
	650	101	52	54.1	87.0	105.7	204.1*
	700	89	12	10.6	116.3	205.6	343.3*
	750	-	-	-	-	-	-
	800	-	-	-	-	-	-
PC (CP)	3	40	29	11.8	312.2	889.9	1471.1
-S9 (24 hours)							
Vehicle	0	96	100	100	67.7	45.1	119.3

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Test article	300	135	78	109.0	36.4	40.6	81.4
	350	125	72	92.7	83.1	60.6	149.9
	400	130	68	92.0	44.6	51.4	102.7
	450	98	77	78.4	93.0	56.1	161.1
	500	116	64	77.3	84.0	49.9	142.3
	550	82	31	26.3	167.7	171.9	375.3*
	600	-	-	-	-	-	-
PC (MMS)	10	89	85	78.5	201.4	359.7	680.0

RSG = relative suspension growth determined from the daily cell growth during a 2-day mutation expression period after test article treatment (RSG = [Daily cell growth (Day1) x Daily cell growth (Day 2) of test article group] / [Daily cell growth (Day1) x Daily cell growth (Day 2) of negative control group]).
PE = plating efficiency of cells at the completion of the mutation expression period (PE = -1n (the number of wells without colonies/total well number) / mean number of cells per well (1.6))
RTG = relative total growth (RTG = RSG x % relative survival at the completion of the mutation expression period)
MF = mutation frequency (-1n(the number of wells without colonies/total well number)/mean number of cells per well / PE2)
L-MF = large colony mutation frequency (wells containing only large colonies)
S-MF = small colony mutation frequency (wells containing only small colonies)
T-MF = total colony mutation frequency (wells containing both small and large colonies)
*Statistically significant increase from negative control ($p<0.05$, Dunnett's multiple comparison test).
Dose-dependency was noted in the short-term treatment without metabolic activation ($p<0.05$, Trend test with regression analysis).
Yellow highlight notes genotoxicity at a dose that did not induce 80% cytotoxicity (i.e., RTG<20%)

In the definitive experiment, a significant increase in the total mutation frequency (T-MF) occurred in the short-term treatment with metabolic activation and 24-hour treatment without metabolic activation; however, no statistically significant dose-dependency occurred.

A statistically significant dose-dependent increase in the T-MF occurred in the short-term treatment without metabolic activation. The highest dose (450 mcg/mL) had a statistically significant increase in T-MF (318.4×10^{-6}) compared to negative control (102.5×10^{-6}), which exceeded the criterion for a positive call after applying the global evaluation factor of 126. A criterion for a positive call states that at least one test concentration must exceed the control mutation frequency when added to the global evaluation factor (GEF) (Moore et al., 2006). In this experiment, at least one concentration must exceed 228.5×10^{-6} ($[102.5 + 126] \times 10^{-6}$), which occurred at 450 mcg/mL. Because the study report did not provide historical data to confirm this mutation frequency was within range of negative controls from this test facility, this test would be judged as positive.

Because of these findings, a confirmatory experiment was performed.

Confirmatory experiment
Three test conditions were tested in the definitive experiment. Two 96-well plates for each dose and 2000 cells/well (four plates for the negative control group because it was treated in duplicate). Results of the confirmatory experiment are summarized in the table below.

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Compound	Conc. (mcg/mL)	PE (%)	RSG (%)	RTG (%)	Mutation Frequency ($x10^{-6}$)		
					L-MF	S-MF	T-MF
-S9 (3 hours)							
Vehicle	0	95	100	100	51.8	56.4	112.7
Test article	200	98	89	92.1	59.1	71.1	139.7
	250	105	91	100.4	47.0	44.3	96.2
	300	98	98	101.6	38.6	74.2	119.1
	350	**105**	**87**	**96.2**	**58.2**	**81.2**	**150.9**
	400	**87**	**67**	**61.0**	**63.5**	**116.1**	**190.6**
	450	79	13	10.5	126.9	172.7	328.8*
	500	-	-	-	-	-	-
PC (MMS)	10	77	80	65.2	173.4	223.9	477.7
+S9 (3 hours)							
Vehicle	0	92	100	100	51.9	48.8	104.1
Test article	450	105	95	108.4	66.7	58.2	134.2
	500	92	94	93.6	72.5	105.8	195.3*
	550	92	81	81.0	72.5	69.3	148.7
	600	87	46	43.1	94.5	112.5	229.5*
	650	89	2	2.0	161.1	267.8	518.9*
	700	-	-	-	-	-	-
	750	-	-	-	-	-	-
	800	-	-	-	-	-	-
PC (CP)	3	30	31	10.2	427.7	1072.0	1805.4
-S9 (24 hours)							
Vehicle	0	94	100	100	54.2	49.6	109.3
Test article	300	92	88	86.7	62.9	38.1	105.8
	350	105	81	90.7	44.3	36.2	81.2
	400	98	82	85.6	56.1	41.5	102.6
	450	**98**	**82**	**85.6**	**53.1**	**74.2**	**136.2**
	500	89	51	49.1	71.4	112.7	197.2*
	550	-	-	-	-	-	-
	600	-	-	-	-	-	-
PC (MMS)	10	75	92	73.5	196.9	442.7	835.5

RSG = relative suspension growth determined from the daily cell growth during a 2-day mutation expression period after test article treatment (RSG = [Daily cell growth (Day1) x Daily cell growth (Day 2) of test article group] / [Daily cell growth (Day1) x Daily cell growth (Day 2) of negative control group]).
PE = plating efficiency of cells at the completion of the mutation expression period (PE = -1n (the number of wells without colonies/total well number) / mean number of cells per well (1.6))
RTG = relative total growth (RTG = RSG x % relative survival at the completion of the mutation expression period)
MF = mutation frequency (-1n(the number of wells without colonies/total well number)/mean number of cells per well / PE2)
L-MF = large colony mutation frequency (wells containing only large colonies)
S-MF = small colony mutation frequency (wells containing only small colonies)
T-MF = total colony mutation frequency (wells containing either small, large, or both colonies)
*Statistically significant increase from negative control ($p<0.05$, Dunnett's multiple comparison test).
No dose-dependency was noted in this confirmatory experiment
Yellow highlight notes genotoxicity at a dose that did not induce 80% cytotoxicity (i.e., RTG<20%)

In the confirmatory test, a significant increase in the T-MF occurred in the short-term treatment with metabolic activation and in 24-hour treatment without metabolic activation, however, no statistically significant dose-dependent increase was noted.

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In short-term treatment without metabolic activation, a significant increase in the T-MF occurred, however, the dose at which a significant increase was noted (450 mcg/mL) had an RTG below 20% and dose-dependency was not noted. Therefore, the statistically significant dose-dependent increase in the T-MF noted in the definitive experiment was not reproduced in the confirmatory experiment.

12.4 Impurity Qualification; *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Comet-Micronucleus Combination Assay with ONO-2745BS in Rats

Study no:	E162 (083-235)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 19, 2012
GLP compliance:	Yes, this study was conducted in compliance with FDA GLP under 21CFR58.
QA statement:	Yes, QA statement signed on September 19, 2012
Drug, lot #, and % purity:	ONO-2745BS, Lot YMK110831 spiked with 8 impurities, 94.6% pure

In this valid comet-micronucleus combination assay, male rats (5/group) were intravenously administered test article at target concentrations of 0, 18.8, 37.5, and 75.0 mg/kg once daily for 3 consecutive days. At 3 hours after the final administration, femur samples were collected for the micronucleus assay and liver samples were collected for the comet assay. The frequency of micronucleate immature erythrocytes and the ratio of immature erythrocytes to the total number of analyzed erythrocytes were calculated. In the comet assay, the % tail DNA, and indicator of DNA damage, was determined. Key study findings support the conclusion that the test article was negative for micronuclei induction (aneuploidy or clastogenicity) in rat bone marrow and DNA damage in the rat liver under the test conditions.

Key Study Findings

- In the micronucleus test, no statistically significant increases in micronucleated cells occurred.
- A statistically significant increase in the ratio of immature erythrocytes to total number of erythrocytes occurred at 75 mg/kg, indicating an influence of the test article on bone marrow cells; however, the value was within the standard ranges (mean ± 3SD) calculated from historical negative control data from the test facility.

Reference ID: 4622909

- In the comet assay, the % tail DNA (%TD) in all test article groups were not significantly different than concurrent negative controls.

Group	Dose (mg/kg)	Number of animals		Animal ID No.
		Animals treated	Animals examined	
Non-treatment	-	-	5	1001 to 1005
Negative control*	0	6	5	1101 to 1106
Test substance	18.8	6	5	1201 to 1206
	37.5	6	5	1301 to 1306
	75.0	6	5	1401 to 1406
Positive control**	200	6	5	1501 to 1506

*: physiological saline (pH 3) **: EMS

The administration volume was set at 20 mL/kg in the negative control and the test substance-treated groups, and at 10 mL/kg in the positive control group.

Methods

Doses in definitive study:	18.8, 37.5, and 75.0 mg/kg/day
Frequency of dosing:	Once daily for 3 consecutive days
Route of administration:	Intravenous, tail vein infusion (30 minutes)
Dose volume:	20 mL/kg
Formulation/Vehicle:	0.1 to 15 mg/mL/ 0.9% saline
Species/Strain:	Rat / Crl:CD (SD)
Number/Sex/Group:	5 males/group
Satellite groups:	None
Basis of dose selection:	Dose selection was based on a dose-finding study (E161 083-234) which dosed rats at 37.5, 75.0, 150, and 300 mg/kg. Two of three rats died at 300 mg/kg. Although no rats died at 150 mg/kg, one of three animals experienced coma at this does, which may lead to death. Therefore, 75.0 mg/kg was selected as the maximum tolerated dose.
Negative control:	0.9% saline (vehicle)
Positive control:	Ethyl methanesulfonate (EMS) 200 mg/kg, oral
Protocol:	In this comet-micronucleus combination assay, male rats (5/group) were intravenously administered test article at target concentrations of 0, 18.8, 37.5, and 75.0 mg/kg once daily for 3 consecutive days. At 3 hours after the final administration, femur samples were collected for the micronucleus assay and liver samples were collected for the comet assay.

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In the micronucleus test, bone marrow cells collected from femurs of treated animals were stained with acridine orange. The frequency of micronucleate immature erythrocytes and the ratio of immature erythrocytes to the total number of analyzed erythrocytes were calculated. The frequency of micronuclei was determined manually evaluating 2000 polychromatic erythrocytes (PCEs; i.e., immature erythrocytes) per animal. Further, 500 erythrocytes per animal were counted for determination of the ratio of PCE to all erythrocytes (normal chromatic erythrocytes, NCE). The PCE to NDE served as an index of bone marrow toxicity.

In the comet assay, DNA was extracted from homogenized liver cells. DNA was run in an electrophoresis chamber and visualized using staining solution (SYBR® Gold nucleic acid gel strain). 100 cells per organ (500 cells per group) were imaged and analyzed using the Comet assay analyzer (Comet Assay IV system, Perceptive Instruments Ltd.). The % tail DNA, and indicator of DNA damage, was determined.

Study Validity

Dosing solution analysis confirmed the highest and lowest concentrations were within 10% of the nominal concentration.

Micronucleus test was considered valid for the following reasons:

- Adequate numbers of immature erythrocytes (PCEs) per animal were scored for the presence of micronuclei.
- Acceptable positive control response: Positive controls had a statistically significant increase in frequency of micronucleated immature erythrocytes (MNPCEs).
- Acceptable high dose: The maximum tolerated dose was determined to be 75.0 mg/kg, based on a dose range finding study. In the dose-finding study, male rats were intravenously administered test article at target dose of 37.5, 75.0, 150, and 300 mg/kg/day for 3 consecutive days. Coma occurred in one of three animals immediately after administration 150 mg/kg. Mortality occurred in two of three animals administered 300 mg/kg during the second or third day. Staggering gait occurred in all groups just after administration and lasted for up to 30 minutes.

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Comet assay was considered valid for the following reasons:

- Acceptable negative control response: Negative control had an average DNA migration of 1-8% DNA in the tail.
- Acceptable positive control response: Positive controls had a statistically significant increase in % tail DNA compared to concurrent negative controls.
- Acceptable high dose: High dose was selected based on mortality and dose-limiting clinical signs of coma.

Results

Micronucleus

Micronucleus test is considered positive for clastogenicity if a statistically significant increase in the frequency of MNPCE occurred in the test article groups compared to the concurrent negative control group; however, final judgement was made considering the biological relevance (i.e., comparison with historical negative control data).

Results of the micronucleus test are summarized in the table below.

Summary of Micronucleus Test in Bone Marrow Cells from Rats following 3-day Treatment

Treatment	Sex (n=5)	Dose mg/kg	Harvest 3-hr after final treatment		
			Frequency of MNPCE (%) (mean ± SD)	Range of MNPCE per 2000 PCE (min – max)	Ratio of PCE (%) (mean ± SD)
Non-treatment	M	0	0.15 ± 0.10	1-6	49.2 ± 7.8
Negative Control	M	0	0.18 ± 0.11	1-7	46.7 ± 11.8
Test Article	M	18.8	0.19 ± 0.10	1-6	49.9 ± 9.8
	M	37.5	0.16 ±0.07	1-5	58.1 ± 5.5
	M	75.0	0.14 ± 0.07	2-5	61.1 ± 6.5**
Positive Control	M	200	2.59 ± 0.83*	34-75	17.3 ± 4.7***

PCE = polychromatic erythrocytes (immature erythrocytes); MNPCE = Micronucleated PCE
*Statistically significant compared to negative control using Kastenbaum and Bowman method (p<0.025)
**statistically significant compared to negative control using Dunnett test (p<0.05)
***statistically significant compared to negative control using Aspin-Welch t-test (p<0.05)

In the micronucleus test, as shown in the table above, no increases in micronucleated cells occurred. A statistically significant increase in the ratio of PCE (immature erythrocytes) to total number of erythrocytes at 75 mg/kg, indicating an influence of the test article on bone marrow cells; however, the value was within the standard ranges (mean ± 3SD) calculated from historical negative control data from the test facility.

Comet Assay

A comet assay is considered positive for DNA damage if there is a significant difference in the % tail DNA (%TD) between the negative control group and each test article group.

Reference ID: 4622909

Results of the micronucleus test are summarized in the table below.

Summary of Comet Assay in Hepatocytes from Rats following 3-day Treatment

Treatment	Sex (n=5)	Dose mg/kg	Total no. cells scored	% Tail DNA	
				Mean	SD
Non-treatment	M	0	500	2.39	0.66
Negative Control	M	0	500	1.86	0.25
Test Article	M	18.8	500	2.33	0.30
	M	37.5	500	1.97	0.30
	M	75.0	500	2.37	0.45
Positive Control	M	200	500	17.46	1.04*

*Statistically significant compared to negative control using Student's t-test (p<0.025)

In the comet assay, as shown in the table above, the % tail DNA (%TD) in all test article groups were not significantly different than concurrent negative controls.

Results from other nonclinical studies conducted for impurity qualification of one of the degradants, (b) (4) are summarized in a table below. In a study (F12FT002), it was demonstrated that IV bolus administration of (b) (4) at doses greater than or equal to 10 mg/kg caused deaths. Follow-up studies (F12XP004 and F12XT016) were conducted to further characterize the lethal and non-lethal doses. All studies identified a No Effect Level to death of 5 mg/kg for IV bolus administration, which yields a safety margin of 293x based on HED BSA and a MRHD of 33 mg.

Study	Key Study Findings
F12FT002 Non-GLP Single dose IV bolus/1h IV infusion Crl:CD (SD) rat 0 (saline), 0.2, 1, 5, 20 mg/kg IV bolus 0 (saline), 5, 20 mg/kg/h x 1h IV infusion 10 males/group	Title: **Single-dose intravenous toxicity study of a degradation product (b) (4) of remimazolam in rats** The purpose of this study was to determine the non-lethal dose, details of toxicities, and the non-toxic dose of the impurity (b) (4) • 1/10 animals died in the 20 mg/kg bolus group 7 min after dosing. Decedent showed loss of righting reflex, sedation, bradypnea, and discharge of red foamy fluid from the nasal cavity. Necropsy revealed dark red color and dilatation of the lungs, as well as foamy fluid in the trachea. Histopathology revealed alveolar and interstitial hemorrhage in the lungs with edema. The cause of death was determined to be pulmonary hemorrhage. • Surviving animals in the 20 mg/kg bolus group had irregular or deep respirations, bradypnea, loss of righting reflex, sedation, cyanosis, and decrease in spontaneous activity immediately after dosing. On the day after dosing, necropsy revealed dark red patches/spots, a dark red color, and dilatation of the lungs, and histopathological finings of alveolar and interstitial hemorrhage with edema. The weight of the lungs was increased, presumably from these changes. Hematology revealed a slight increase of the monocyte count, and blood chemistry revealed slight increase in total bilirubin.

Reference ID: 4622909

	• 5 mg/kg bolus group: Irregular or deep respirations immediately after dosing • 1h IV infusion groups: no signs of toxicity or sedation at any dose. Non-lethal dose: 5 mg/kg IV bolus NOAEL = 1 mg/kg IV bolus, based on irregular or deep respirations at the next highest dose of 5 mg/kg IV bolus Non-lethal dose: 20 mg/kg/h x 1h IV infusion • NOAEL = 20 mg/kg/h x 1h IV infusion
F12XP004 Non-GLP Single dose IV bolus/1h IV infusion Crl:CD (SD) rat IV bolus doses: 0 (V1), 0 (V2), 0 (V3), 20 (V1), 20 (V2), 20 (V3) mg/kg IV infusion doses: 0 (V2), 20 (V2) mg/kg V1 = saline V2 = saline pH3 V3 = Captisol, B-cyclodextran in saline pH3	Title: **Toxicity study in rats for impurities contained in ONO-2745BS (Remimazolam)** The purpose of this study was to determine the non-lethal dose for IV infusions of the impurity (b) (4). Evaluations during the 7-day observation period were general condition, body weight, food consumption, necropsy on Day 7. Limited histopathology (lung, trachea, and heart) was only conducted in groups with mortalities and their respective control groups. • **20 mg/kg bolus (V2 and V3):** mortality occurred immediately after dosing. In decedents, loss of righting reflex, convulsions, respiratory difficulties occurred prior to death. Histopathology revealed congestion of the lungs, deformation of the alveolar septum, pulmonary venal thrombosis, edema and hemorrhage of the pulmonary alveoli and the interstitium. In the surviving rats, irregular breathing occurred after dosing correlated with histopathological findings of brown pigment deposits in the lungs, pulmonary venal thrombosis, neutrophilic infiltration of the pulmonary stroma, aggregated foamy/pigmented macrophages or erythrophagia in the lungs. No abnormalities were found in the corresponding vehicle groups (V2 and V3). o The cause of death is thought to be respiratory failure from pulmonary edema and hemorrhage. • **20 mg/kg infusion (V2):** no mortalities or changes in general condition and necropsy occurred
F12XT016 Non-GLP	Title: **Study of the cause of death in rats from the formulation degradant of ONO-2745BS** Purpose: When studying the sedative action of the impurity (b) (4), rats died immediately after bolus dosing of 10 mg/kg or greater. The cause of death was thought to be respiratory failure from pulmonary hemorrhage with changes in the lung similar to anaphylactic shock. It is thought that there is a possibility of an immunoreaction related to pulmonary hemorrhage. The purpose of this study is to 1) characterized the systemic vascular permeability of (b) (4) using Evans blue and 2) characterized the protective effects of antihistamine or anti-PAF after (b) (4) administration. Experiment 1: Effects on the systemic vascular permeability Evans blue residue was found only in the lungs after dosing with 20 mg/kg IV bolus of the impurity. Experiment 2: Antihistamine and anti-PFA

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	Histamines or anti-PFA had no mitigating properties when administered prior to 20 mg/kg IV bolus of the impurity. <u>Experiment 3: systemic toxicity after bolus dosing</u> 0.5, 1, 5, 20 mg/kg IV bolus (3/group) • 5 mg/kg and greater: respiratory irregularities and reduced motility immediately after dosing. • 20 mg/kg: loss of righting reflex, dark reddening of the lungs, decreased red blood cell count, hemoglobin concentration, and hematocrit values, hemorrhage in the pulmonary alveoli and interstitium. <u>Experiment 4: infusion procedure</u> 10 males were infused for 1h IV with saline pH 3 to determine the effect of the infusion procedure on lungs. • No effects on the lungs occurred in any male Overall Conclusions The mortalities occurring at 10 mg/kg or greater after a single IV bolus of the impurity were not a result of blood coagulation or immunoreaction. After IV bolus dosing at 5 mg/kg or greater of the impurity, general conditions of the rats changed immediately and at 20 mg/kg pulmonary hemorrhage and edema occurred. After 1h IV infusion dosing at 20 mg/kg impurity, no changes occurred. The effects on the lungs found after IV bolus doses are due to high levels of blood exposure immediately after dosing.

Reference ID: 4622909

12.5 Excipient Qualification of Dextran 40: *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Publication title: A Compilation of Two Decades of Mutagenicity Test Results with the Ames Salmonella typhimurium and L5178Y Mouse Lymphoma Cell Mutation Assays

Study no.:	Seifried et al., 2006 Supporting Raw Data
Study report location:	EDR
Reference:	Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RH. Chem Res Toxicol. 2006 May;19(5):627-44. Review. Erratum in: Chem Res Toxicol. 2008 Feb;21(2):554-5. PMID: 16696565
Publication Date:	2006
GLP compliance:	No, however, this study was performed in accordance with accepted publications (Ames et al., 1975), which is consistent with OECD Test Guidance No. 471. This publication was associated with the following institutions. • Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20852 Dwight D. Eisenhower Army Medical Center, Fort Gordon, Georgia 30905, • BioReliance, Invitrogen Corporation, Rockville, Maryland 20850 • Technical Resources International Inc., Bethesda, Maryland 20817
QA statement:	No
Drug, lot #, and % purity:	Unknown. Tested agents were acquired by NCI contractors [Illinois Institute of Technology Research Institute, Chicago, IL and Midwest Research Institute (MRI), Kansas City, MO]

The National Cancer Institute (NCI) shares the responsibility of selecting the most significant chemicals for carcinogenicity testing by the National Toxicology Program (NTP) and has used data from Salmonella and mouse lymphoma mutagenicity assays to aid in the selection of prioritization of chemicals to be further evaluated in chronic 2-year rodent studies. In the NCI/NTP chemical selection process, it is no longer necessary to test chemicals prior to sending them to the NTP so the NCI program has ceased performing mutagenicity tests. Some of the testing data has been made available in summary form in the Chemical Carcinogenesis Research Information

Reference ID: 4622909

System (CCRIS), which is searchable on the NLM TOXNET system. The limitations in using this source are that only summary results are available and many negative test results are not included. This publication served to provide a summary table that presents the results for each compound and is provided in the Appendix with raw data in the Supporting Information. Two of the compounds tested were dextran and iron dextran. The results for dextran are summarized below.

In this *in vitro* bacterial reverse mutation assay using the plate incorporation method, Dextran (CAS 9004-54-0), in the presence or absence of metabolic activation tested negative for mutagenicity in *Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537 and TA1538 at 100, 333, 1000, 3333, and 10000 mcg/plate. Key study findings support the conclusion that the test article is not mutagenic under these test conditions.

Key Study Findings

- This study was valid
- No statistically significant increase in mean revertant colonies per plate occurred in any test article condition.

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Methods

Strains:

S. typhimurium TA98	*S. typhimurium TA100*
S. typhimurium TA102	*S. typhimurium TA1535*
S. typhimurium TA1537	*S. typhimurium TA1538*

Concentrations in definitive study: 100, 333, 1000, 3333, and 10000 mcg/plate

Basis of concentration selection: The doses tested in the mutagenicity assay were selected based on the levels of cytotoxicity observed in a preliminary dose range-findings study using strain TA100. Ten dose levels of the chemical, one per dose, were tested in both the presence and the absence of induced hamster S9. If no toxicity was observed, a total maximum dose of 10 mg of test chemical per plate was used.

Negative control: water

Positive control: unknown

Formulation/Vehicle: unknown

Incubation & sampling time: For the pre-incubation method, tester strains were incubated for an unknown amount of time with test article with and without metabolic activation at 45°C after adding warm top agar and mixing. The mixture was poured onto a minimal glucose agar plate and incubated 48 hours at 37°C. Plates were scored following the 48-hour incubation.

Metabolic activation consisted of an S9 fraction prepared from livers obtained from Aroclor 1254 (500 mg/kg) pretreated male Sprague-Dawley rats and Syrian golden hamsters. Percentage of S9 was 25%, which is acceptable (0.5 mL of S9 mix to 2.0 mL of molten top agar).

Study Validity

This study is valid because the dose-finding test and the two main tests met the following conditions.

1. The number of revertant colonies in the negative and positive controls was as expected
2. No bacterial contamination in the sterility test was reported

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3. Analysis (colony count) is possible for at least 5 dose levels or no inhibition is observed at least 4 dose levels in the two main tests
4. Each dosing group was tested in triplicate on each tester strain without metabolic activation and also with activation by induced rat and hamster liver S9 preparations
5. Selection of the bacterial tester strains was adequate. At least five strains of bacteria were used, including four strains of *S. typhimurium* that have been shown to be reliable and reproducibly responsive between laboratories for GC base pairs at the primary reversion site (TA1535, TA1537 or TA97a or TA97, TA98, and TA100). To detect certain oxidizing mutagens, cross-linking agents and hydrazines, either *E. coli* WP2 strains or *S. Typhimurium* TA102 with an AT base pair at the primary reversion site.
6. Dose selection was based on the limit dose (5000 mcg/plate) and precipitation occurred at 5000 mcg/plate in the DRF study.
7. The S9 concentration was acceptable

Results

The dose-finding test was not available for independent review. In the main tests, the test article was tested up to 10000 mcg/plate. Below is a summary of the dose-finding test and two main test results.

Tables

	TA98			**TA100**		
	no S9	rat S9	Ham'r S9	no S9	rat S9	Ham'r S9
Dose	Negative	Negative	Negative	Negative	Negative	Negative
H2O	18 ± 6	20 ± 3	16 ± 5	145 ± 17	197 ± 5	183 ± 4
100ug	14 ± 2	16 ± 4	21 ± 4	139 ± 6	203 ± 28	184 ± 22
333ug	16 ± 2	24 ± 11	20 ± 9	145 ± 19	208 ± 11	190 ± 23
1000ug	18 ± 5	29 ± 8	19 ± 3	130 ± 13	184 ± 6	182 ± 10
3333ug	17 ± 4	28 ± 1	19 ± 3	128 ± 5	183 ± 20	181 ± 10
10000ug	17 ± 1	25 ± 6	17 ± 3	129 ± 14	164 ± 17	184 ± 6
Positive	274 ± 39	611 ± 96	820 ± 81	586 ± 10	807 ± 80	1111 ± 115

	TA102			**TA1535**		
	no S9	rat S9	Ham'r S9	no S9	rat S9	Ham'r S9
Dose	Negative	Negative	Negative	Negative	Negative	Negative
H2O	286 ± 18	346 ± 32	308 ± 32	12 ± 5	11 ± 5	10 ± 1
100ug	301 ± 14	323 ± 15	405 ± 25	9 ± 3	15 ± 1	11 ± 3
333ug	299 ± 17	339 ± 7	353 ± 3	9 ± 3	9 ± 3	11 ± 4
1000ug	291 ± 11	327 ± 28	361 ± 29	10 ± 3	11 ± 6	12 ± 2
3333ug	284 ± 27	332 ± 10	325 ± 33	7 ± 1	11 ± 5	9 ± 3
10000ug	284 ± 17	322 ± 19	361 ± 29	8 ± 4	14 ± 2	7 ± 5
Positive	1122 ± 76	2447 ± 67	1400 ± 93	498 ± 20	47 ± 12	59 ± 6

	TA1537			**TA1538**		
Dose	no S9	rat S9	Ham'r S9	no S9	rat S9	Ham'r S9

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	Negative	Negative	Negative	Negative	Negative	Negative
H2O	5 ± 3	8 ± 2	6 ± 2	8 ± 1	12 ± 3	13 ± 4
100ug	4 ± 1	6 ± 1	7 ± 1	8 ± 1	13 ± 4	11 ± 2
333ug	3 ± 1	4 ± 2	5 ± 1	7 ± 2	15 ± 5	8 ± 3
1000ug	4 ± 1	4 ± 2	4 ± 3	8 ± 3	10 ± 2	6 ± 1
3333ug	4 ± 2	3 ± 2	4 ± 2	4 ± 2	8 ± 1	10 ± 4
10000ug	6 ± 4	5 ± 4	6 ± 2	2 ± 2	11 ± 2	4 ± 2
Positive	530 ± 99	193 ± 26	231 ± 18	291 ± 22	251 ± 27	681 ± 18

Criteria for a positive test results were determined when at least a doubling (TA98, TA100, and TA1535) in the mean number of revertants per plate occurred for at least one tester strain. This increase in the mean revertants per plate had to be accompanied by a dose response to increasing concentrations of the test chemical. If the study showed a dose response with a less than 3-fold increase on TA1537 or TA1538, the response had to be confirmed in a repeat experiment.

As shown in the tables above, there was no statistically significant increase in mean revertant colonies per plate in any test article condition.

Reference ID: 4622909

12.6 Excipient Qualification of Dextran 40: Mouse Lymphoma TK Assay

Publication title: A Compilation of Two Decades of Mutagenicity Test Results with the Ames Salmonella typhimurium and L5178Y Mouse Lymphoma Cell Mutation Assays

Study no.:	Seifried et al., 2006 Supporting Raw Data
Study report location:	EDR
Reference:	Seifried HE, Seifried RM, Clarke JJ, Junghans TB, San RH. Chem Res Toxicol. 2006 May;19(5):627-44. Review. Erratum in: Chem Res Toxicol. 2008 Feb;21(2):554-5. PMID: 16696565
Publication Date:	2006
GLP compliance:	No, however, this study was performed in accordance with accepted publications (Clive et al., 1979; Clive and Spector, 1975), which is consistent with OECD Test Guidance No. 490. This publication was associated with the following institutions. • Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20852 Dwight D. Eisenhower Army Medical Center, Fort Gordon, Georgia 30905, • BioReliance, Invitrogen Corporation, Rockville, Maryland 20850 • Technical Resources International Inc., Bethesda, Maryland 20817
QA statement:	No
Drug, lot #, and % purity:	Unknown. Tested agents were acquired by NCI contractors [Illinois Institute of Technology Research Institute, Chicago, IL and Midwest Research Institute (MRI), Kansas City, MO]

The National Cancer Institute (NCI) shares the responsibility of selecting the most significant chemicals for carcinogenicity testing by the National Toxicology Program (NTP) and has used data from Salmonella and mouse lymphoma mutagenicity assays to aid in the selection of prioritization of chemicals to be further evaluated in chronic 2-year rodent studies. In the NCI/NTP chemical selection process, it is no longer necessary to test chemicals prior to sending them to the NTP so the NCI program has ceased performing mutagenicity tests. Some of the testing data has been made available in summary form in the Chemical Carcinogenesis Research Information

Reference ID: 4622909

System (CCRIS), which is searchable on the NLM TOXNET system. The limitation s in using this source are that only summary results are available and many negative test results are not included. This publication served to provide a summary table that presents the results for each compound is provided in the Appendix with raw data provided in the Supporting Information. Two of the compounds tested were dextran and iron dextran. The results for dextran as summarized below.

In this *in vitro* mouse lymphoma TK assay using the soft agar method, Dextran (CAS 9004-54-0), in the presence or absence of metabolic activation tested negative for mutagenicity in *L5178Y TK*$^{+/-}$ cells after a four-hour incubation with at 1000, 2000, 3000, 4000, and 5000 mcg/mL. Key study findings support the conclusion that the test article is not mutagenic under these test conditions.

Key Study Findings

- This study was valid
- No biologically significant increase in mean revertant colonies occurred in any test article condition.

Methods:

Assay Version:	Soft Agar
Cell line:	L5178Y TK$^{+/-}$ -3.7.2C mouse lymphoma cells
Metabolic Activation:	S9 fraction from Aroclor-induced male Sprague-Dawley rats was used at 10% v/v.
Test agent solubility:	Soluble in water.
Concentrations definitive study:	1000 mcL/mL – 5000 mcL/mL
Basis of concentration selection:	A dose range experiment testing up to 10000 mcL/mL.
Negative control:	water
Positive control:	Without S9: Ethyl methylsulfonate or methyl methanesulfonate With S9: 3-Methylcholanthrene or dimethylbenz[α]-anthracene
Vehicle:	water
Historical controls:	Yes, provided for over 100 experiments with concurrent controls.
	Exposure conditions: Cytotoxicity of the test article was determined both with and without liver S9 prepared from Aroclor 1254-induced male Sprague-Dawley rats. While S9 typically is used at concentrations ranging from 1-2% and may be increased up to 10%, this study does not indicate the concentration of S9 used in the assay. Cells at a concentration of 6 x 10^5/mL (6 x 10^6 cells total) were exposed for 4 hours to a range of concentrations from

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0.0005 to 10000 mcg/mL. The cells were then washed and resuspended in growth medium and incubated at 37°C for 48 hours. The rate of cell growth was determined for each of the treated cultures and compared to the rate of growth of the solvent controls. The doses of chemical selected for testing were within the range yielding approximately 0-90% cytotoxicity. There were at least 2 solvent controls, a positive with and without S9.

In the mutagenicity assay, a total of 1.2 x 10^7 cells in duplicate cultures were exposed to the test chemical, positive control, and solvent control for 4 hour at 37°C, washed twice with growth medium, and maintained at 37°C for 48 hour in log-phase growth to allow recovery and mutant expression. Cells in the cultures were adjusted to 3 x 10^5/mL at 24-hour intervals. They were then cloned (1 x 10^6 cells/plate for mutant selection and 200 cells/plate for viable count determinations) in soft agar medium. Resistance to trifluorothymidine (TFT) was determined by adding TFT (final concentration, 3 mcg/mL) to the cloning medium for mutant selection. Plates were incubated at 37°C in 5% CO_2 air for 10-12 days and then counted with an Artek automated colony counter (Artek 982, DynaTech) or ProtoCol colony counter (Synbiosis, Frederick, MD).

Analyses:

Only colonies larger than ~0.2 mm in diameter were counted. Mutant frequencies were expressed as mutants per 10^6 surviving cells. Results were interpreted using a doubling of the mutant frequency over the concurrent solvent-treated control value as an indication of a positive effect, together with evidence of a dose-related increase. Cytotoxicity endpoints were Relative Suspension Growth for the dose range experiment and Relative Total Growth (RTG) for the definitive experiment.

The Mutant Frequency (Mut Freq) was determined by counting the number of TFT resistant colonies and correcting by the number of viable cells as determined by the plating efficiency (PE). That is, the Mut Freq = (number of mutants/number of cells plated) X PE.

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The size of the mutant colonies was determined using an Artek 982 colony counter/sizer or the ProtoCol colony counter. An internal discriminator was set to step sequentially to exclude increasingly larger colonies in approximate increments of 0.1 mm in colony diameter. The size range used was from ~0.2 to 1.1 mm.

Study Validity:
The study was deemed valid for the following reasons: 1) two experimental conditions for short-term treatments (4 hours) were tested (i.e., with and without metabolic); 2) adequate number of cells and concentrations were analyzed; 3) criteria for the selection of top concentrations utilize the highest test concentration above the recommended 2 mg/mL or 2 mcL/mL; 4) data included cytotoxicity and plating efficiency determination, colony counts and mutant frequencies for treated and control cultures and mutant colony sizing on appropriate cultures (although these may not have been reported); 5) mutant frequency (Mut Freq) in the untreated/vehicle controls fell between 30 – 140 X 10^{-6}, untreated/vehicle control plating efficiency (PE) at the time of mutant selection between 65 – 120% for the 4 hour treatment; 6) positive controls exhibited an increase above background of at least 300 X 10^{-6}.

However, the percentage of small colonies in the induced Mut Freq and an upper limit of cytotoxicity greater than a relative total growth (RTG) of 10% could not be determined with the provided data. Additionally, it is unclear if the maximum concentrations with relative total growth (RTG) values between 10 and 20% were achieved.

Results:
Data for the definitive experiments on Dextran are shown in the tables below.

Non-Activated Cultures (-S9)				
Dose (mcg/mL)	**Average TFT**	**Average VC (PE)**	**Mut Freq**	**RTG**
or mcL/mL				
1000 to 5000				
1000	15	148 (74%)	0.2	95 (48%)
	19	167 (84%)	0.23	106 (53%)
2000	23	151 (76%)	0.3	101 (51%)
	21	153 (77%)	0.27	101 (51%)
3000	23	164 (82%)	0.28	100 (50%)
	22	173 (87%)	0.25	113 (57%)
4000	18	158 (79%)	0.23	96 (48%)
	16	150 (75%)	0.21	95 (48%)
5000	20	151 (76%)	0.26	98 (49%)
	23	170 (85%)	0.27	110 (55%)
Solvent	19	167 (84%)	0.23	
Positive	350	93 (47%)	7.53	30 (15%)

Reference ID: 4622909

Average TFT = Number of mutant colonies; Average VC = Number of viable colonies; PE = plating efficiency; Mut Freq = mutant frequency; RTG = Relative Total Growth; Solvent = water (negative control); Positive = ethyl methylsulfonate or methyl methanesulfonate (positive control).

S9-Activated Cultures (+S9)				
Dose (mcg/mL)	**Average TFT**	**Average VC (PE)**	**Mut Freq**	**RTG**
or mcL/mL				
1000 to 5000				
1000	35	179 (90%)	0.39	112 (56%)
	36	177 (89%)	0.41	107 (54%)
2000	35	180 (90%)	0.39	105 (53%)
	24	157 (79%)	0.31	96 (48%)
3000	37	170 (85%)	0.44	99 (50%)
	28	179 (90%)	0.31	97 (49%)
4000	41	154 (77%)	0.53	94 (47%)
	36	149 (75%)	0.48	85 (43%)
5000	40	161 (81%)	0.5	93 (47%)
	37	173 (87%)	0.43	101 (51%)
Solvent	33	160 (80%)	0.4	
Positive	141	67 (34%)	4.21	14 (7%)

Average TFT = Number of mutant colonies; Average VC = Number of viable colonies; PE = plating efficiency; Mut Freq = mutant frequency; RTG = Relative Total Growth; Solvent = water (negative control); Positive = 3-methylcholanthrene or dimethylbenz[α]-anthracene (positive control).

Providing that all acceptability criteria are fulfilled, a test chemical is considered to be clearly positive if, in any of the experimental conditions examined, the increase in Mut Freq above the concurrent background exceeds a defined value [the global evaluation factor (GEF); 90 X 10^{-6} for the soft agar version of the assay] and the increase is concentration related using a trend test. The test chemical is then considered able to induce mutation in this test system.

For the non-metabolic activation arm (first table) of the experiment on Dextran, mutant frequency did not exceed the 257 X 10^{-6} (i.e., 167 X 10^{-6} + GEF); therefore, dextran is deemed non-mutagenic in the absence of metabolic activation.

For the metabolic activation arm (second table) of the experiment on dextran, mutant frequency did not exceed the 250 X 10^{-6} (i.e., 160 X 10^{-6} + GEF); therefore, dextran is deemed non-mutagenic in the absence of metabolic activation.

Reference ID: 4622909

12.7 Excipient Qualification of Dextran 40: In vitro Mammalian chromosome aberration test

Publication title: Cytotoxicity Test on Medical Drugs – Chromosome Aberration Test with Chinese Hamster Cells In Vitro

Study no.:	Ishidate et al., 1978
Study report location:	EDR
Reference:	Ishidate M Jr, Hayashi M, Sawada M, Matsuoka A, Yoshikawa K, Ono M, Nakadate M. [Cytotoxicity test on medical drugs--chromosome aberration tests with Chinese hamster cells in vitro (author's transl)]. Eisei Shikenjo Hokoku. 1978;(96):55-61. Japanese. PMID: 750044
Publication Date:	1978
GLP compliance:	No Note: this study appears to be inconsistent with the OECD Test Guidance No. 473 because exogenous source of metabolic activation was not used.
QA statement:	No
Drug, lot #, and % purity:	Unknown. The test substances in this study were supplied by the synthetic chemistry research department and the drug department of the institution conducting the study. In this connection, the test substances included additives such as pigments.

In this in vitro chromosome aberration assay in Chinese Hamster Lung Cells, dextran (CAS 9004-54-0), in the absence of metabolic activation tested negative for chromosome aberrations after a 24- or 48-hour incubation with three concentrations up to 15.3 mg/mL (further detail not provided in the translation). While the publication reports that dextran was negative for chromosome aberration under the test conditions, the provided English translation did not provide enough detail to determine the validity of the study. This study does not support the safety of Dextran 40 as recommended by this Reviewer.

Key Study Findings

- This study cannot be determined to be valid.
- No exogenous source of metabolic activation was used in this test.
- No positive controls were used.
- Three unknown concentrations of dextran were tested.
- Not enough detail to determine the test conditions were appropriate.

Reference ID: 4622909

Methods

Cell line:	Chinese hamster lung (CHL)
Concentrations in definitive study:	Without metabolic activation for 24 and 48 hours were three concentrations up to 15.3 mg/mL.
Basis of concentration selection:	Three concentrations were selected to reflect a concentration causing a 50% growth inhibition, one concentration 2-fold lower, and a concentration 2-fold higher than that causing 50% growth inhibition. Actual concentrations and data of growth inhibition were not provided.
Negative control:	Saline
Positive control:	Unknown
Formulation/Vehicle:	Formulated in saline, saline was used as control.
Incubation & sampling time:	The cells were seeded to 60-mm petri dishes at a rate of 2 x 10^4 cells per 5 mL of culture fluid. The cells were incubated without metabolic activation in a cell culture incubator at 37°C, 5% CO2. Test article was added 3 days after plating. At 24 and 48 hours after adding the test article, chromosome samples were prepared. On preparing the chromosome samples, each petri dish was first treated with a 0.2 mcg/mL colcemid solution for 2 hours to arrest cells in metaphase. Each sample was prepared by air drying and stained with the Giemsa staining fluid. At least 100 cells in metaphase mitoses were analyzed for polyploidy and the appearance of chromosome aberration. The morphological abnormality was classified into chromatid gap (g), breakage (b), transition (t), ring formation (r), and fragmentation (f), and the presence of chromosome aberration was judged as (-), (+/-), (+), (++), and (+++) when the percentage of cells with any abnormality was less than 5%, 5-10%, 10-20%, 20-50%, and more than 50%, respectively.

The table below is excerpted from the English translation of the publication. No further data was provided in the translation.

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Compound	Solvent*1	Dose*2 (mg/ml)	Polyploid % (48 h)
Dextran	S	15.30	2.0

Chromosome abberation %	(Time)	Type*3	Final judge	D_{20}*5	Note*6 C	Note*6 M
0.0	(24 h)		−			−

*1 Solvent : S : saline, E : ethanol, D : DMSO, C : CMC
*2 The maximum tolerated dose on cells.
*3 Aberration type : g : gap, b : break, t : exchange, r : ring, f : fragmentation
*4 Judgement : (−) less than 4%, (±) 5~9%, (+) 10~19%, (++) 20~49%, (+++) more than 50%

12.8 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title:

Study no: (b) (4) 38086
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: January 8, 2020
GLP compliance: Yes, there was a GLP certificate of the test facility, which certified that the study conformed with GLP standards according to (b) (4) and Directive 2004/9/EEC. These principles are compatible with GLP regulations specified by regulatory authorities throughout European Community, the U.S. EPA and FDA, and Japan (MHLW, MAFF, and METI).
QA statement: Yes, this study was conducted in accordance to the Council Regulation (EC) No. 2017/735 method B.12 and OECD guideline 474
Drug, lot #, and % purity: Dextran 40 EP, Lot DN324, purity information not available to the test facility. In a response to IR (SDN 0051 4/20/2020), the Applicant provided the following information regarding the purity of the Dextran 40: "The purity of Dextran 40 as defined in the USP is described by

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	the average weight and the size distribution of the molecules…" "In a Certificate of Analysis (CoA) that is amended to the study report (Appendix 1 of Report 38086) the relevant information for the Dextran 40 batch used in the study is provided." "The results of the batch analysis show that the Dextran 40 that was used in Study (b) (4) 38086 complies with the requirements of Dextran 40 as described in the EP and USP."

Key Study Findings

In the definitive study, male rats (5/group) were intravenously administered Dextran 40 EP at target doses of 0 (saline vehicle), 200, 600, and 2000 mg/kg/day for two days. Dextran 40 EP tested negative in the rat bone marrow micronucleus test under the test conditions.

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Methods

Doses in definitive study:	0 (saline vehicle), 200, 600, and 2000 mg/kg/day
Frequency of dosing:	Once daily for two consecutive days
Route of administration:	Intravenous infusion with injection cannula (WING-FLO Winged Infusion Set, Servoprax® GmbH, 46485 Wesel, Germany) into the tail vein. The cannula was connected to an infusion pump (Infusion pump 540220, UNO BV, The Neverlands) via a tube (Perfusor tube, B. BRAUN MELSUNGEN AG; e.g., 75 cm, article no. 872 2870)
Dose volume:	20 mL/kg
Duration of infusion:	10 minutes
Infusion Rate:	Approximately 35-43 mL/h
Formulation/Vehicle:	0.9% NaCl solution
Species/Strain:	Rat / CD / Crl: CD(SD)
Number/Sex/Group:	Definitive study: 5 males/group
Satellite groups:	Pretest: 3 males
Basis of dose selection:	Dose levels were selected following the limit dose concept for non-toxic materials as described in the OECD 474 guidance with a limit dose of 2000 mg/kg and intercepts of approximately 3-fold for the intermediate and the low dose groups. Prior to the main study, the tolerability of the high dose via 10-minute intravenous infusion was confirmed in three males with post-dose observations over 24 hours. Acute anaphylactoid reactions were noted, however, the reversibility of the symptoms suggested the tolerability and feasibility of the intended treatment conditions in the definitive study.
Negative control:	0.9% NaCl solution (vehicle)
Positive control:	Ethyl methane sulfonate (125 mg/kg oral)

Study Validity

This study is valid because the following acceptability criteria were achieved:

- The concurrent negative control data were within the historical controls from the laboratory
- The concurrent positive controls induced responses compatible with those generated in the historical positive control database and produced a statistically significant increase compared with the concurrent negative control
- The appropriate number of doses and cells have been analyzed

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- The criteria for the selection of the high dose is consistent with accepted guidelines (i.e., dosed up to 2000 mg/kg/day)

Results

All dose levels induced swelling of the extremities, nose, and application site at between 5 minutes to 6 hours post dosing on both days in all animals, suggestive of expected anaphylactoid reactions. No dose-dependency was noted in the incidence, duration, and severity of the findings.

Bone marrow was sampled on Test Day 3 (24 hours after the start of the second dose). Polychromatic erythrocytes (PCE; 4000 PCEs/animal) were scored for the incidence of micronuclei, and the ratio of PCE to NCE was determined for each animal. Summary group data are presented in the table below. Individual animal results were provided in the report but are not presented here.

Table 1 Summarised data of the bone marrow micronucleus test in rats

Dextran 40 EP [mg/kg b.w./day, i.v.]	Sampling time (h)[#1]	Number of polychromatic erythrocytes scored per group	Micronucleated polychromatic erythrocytes mean frequency per 4000 PCE	Micronucleated polychromatic erythrocytes mean frequency per 1000 PCE	Ratio PCE/NCE[#2]
			mean (n = 5)	mean (n = 5)	mean (n = 5)
0	24	20000	3.6	0.9	0.49
200	24	20000	2.6	0.7	0.35
600	24	20000	1.4	0.4*	0.48
2000	24	20000	2.0	0.5	0.57
Ethyl methane sulfonate					
125 mg/kg b.w., p.o.	24	20000	25.2	6.3*	0.3

* significant at $p \leq 0.05$ for de-/increases compared to the control
PCE polychromatic erythrocytes
NCE normochromatic erythrocytes
i.v. intravenous
p.o. per oral
[#1] after the last administration
[#2] per 500 counted cells

As summarized above, Dextran 40 EP did not increase the incidence of micronucleated PCE at any of the three tested dose levels. The incidence of micronucleated PCE was 0.5/1000 PCEs in the high-dose group. The corresponding micronucleus frequency of the vehicle control group was 0.9/1000 PCEs. Positive control (ethyl methane sulfonate) significantly increased the number of micronucleated PCE to 6.3/1000 PCEs.

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12.9 Excipient Qualification of Dextran 40: Male Fertility

Publication title: Toxicity studies of Dextran-40 formulations

Study no.:	(MaruokaH Yakuri to Chiryo, 1975)
Study report location:	EDR
Reference:	H. Maruoka, S. Koda, M. Mori, Y. Kawaguchi, Y. Takemoto, J. Nakagawa, M. Maruoka, H. Saito, Mioshi, et al. [Toxicity studies of dextran-40 formulations.] Japanese Pharmacology and Therapeutics. 1975, 3 (2): 25-78.
Publication Date:	1975
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Unknown

In this publication, the toxicity of long-term intravenous Dextran 40 administration up to 6 months was studied in Japanese white rabbits. Three different formulations of Dextran 40 were used as described in the table below. Histopathological analysis of the testes and epididymides of male rabbits indicated testicular atrophy after 1-, 3-, or 6-months of intravenous administration of dextran. This effect is considered secondary to hypervolemia rather than directly to dextran, as testicular atrophy also occurred in controls and without dose-relationship. However, this study did not evaluate the necessary endpoints of a male fertility test as recommended by ICH S5A Detection of Toxicity to Reproduction for Medicinal Products (i.e., assessment of maturation of male reproduction functions, development and maturation of gametes, mating behavior, and fertility) and ICH S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male fertility (i.e., case-by-case histopathological evaluation of testes and epididymides, and optional sperm analysis including sperm count, motility, and morphology). These guidances also point out that repeat-dose toxicity studies can provide important information regarding potential effects on male fertility.

Formulations of Dextran-40	Composition of formulation
Dextran and amino acids solution	10% dextran-40 (w/v) 0.27% L-arginine HCl 0.13% L-histidine HCl hydrate 0.18% L-isoleucine 0.41% L-leucine 0.62% L-lysine HCl 0.24% L-methionine 0.29% L-phenylalanine 0.18% L-threonine 0.06% L-tryptophan 0.20% L-valine 0.34% glycine
Dextran and glucose solution	10% dextran-40 (w/v) 5% glucose (w/v)

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Dextran and saline solution	10% dextran-40 (w/v) 0.9% sodium chloride (w/v)

In an acute toxicity test, male and female rabbits were administered dextran-40 intravenously at volumes of 150 (amino acid formulation), 180 (glucose formulation), and 240 mL/kg (saline formulation). Body weights reported as 2.1-2.4 kg. Toxicity symptoms were assessed in detail (clinical observations) during and immediately after dosing and continued for 7 days after dosing. Body weights and food intake were measured daily and necropsy occurred 7 days after dosing. LD_{50} was assessed using the Brownlee's up-and-down method. Results indicate that when large volumes of dextran formulations were administered to rabbits, some rabbits died after increased respiratory rate, exophthalmos, and cyanosis after dosing, marked weakening of the limbs, lateral recumbency, repeated opisthotonos and mouth opening, and ejection of blood-containing foamy liquid from the mouth and nostrils. Deaths occurred during or immediately to several hours after dosing. Necropsy result showed that there were large volumes of ascites and pleural effusion, marked pulmonary hyperemia and edema, and increased pericardial effusion indicative of complications with increased blood volume. The surviving animals increased body weights by 200 to 300 g after administration, which returned to baseline by 3 to 5 days. Food intake was decreased the day after dosing with recovery by 3 to 4 days after dosing. Necropsy determined the cause of death to be marked pulmonary hyperemia and edema with some animals showing hepatomegaly. Hydrothorax was found with 20 to 30 mL of pleural fluid and there was also 200 to 300 mL of bloody ascites. Many animals showed increased pericardial fluid. The surviving animals had pulmonary hyperemia, local hepatic changes with red coloration, and small volumes of ascites. All findings were similar across the three dextran formulations. LD_{50} = 178 (amino acid formulation), 212 (glucose formulation), and 208 in males or 229 mL/kg in females (saline formulation).

In a one-month toxicity test, male and female rabbits (6/sex/group) were administered one of three formulations of dextran-40 intravenously at target volumes of 20, 40, and 80 mL/kg. Body weights and food intake was measured daily. Cage side observations were assessed during the study. Hematology and urinalysis were assessed 2 days after the final dose. Organ weights (brain, pituitary gland, thymus, heart, lungs, liver, kidneys, adrenal gland, spleen, testes, epididymides, ovaries, fallopian tubes and uterus were collected at necropsy. Histopathology was performed on the tissues selected for organ weights as well as the pancreas, stomach, small intestine, large intestine, bone marrow, and mesenteric lymph nodes. Results included a decrease in body weights in males and females with all dextran formulations, but no dose-relationship. Mortality occurred in the saline control group (1 M, 1 F), dextran-amino acid groups (1 LD, 2 MD, 3 HD), dextran-glucose group (4 LD, 2 MD, 3 HD), and the dextran-saline group (0 LD, 2 MD, 5 HD). Food intake during the late phase of administration period had a tendency to decrease, which appeared to be dose-related. Diarrhea and deaths were noted without a dose-relationship (no further description of the deaths was provided). Hematology indicated a dose-related decrease in RBC, WBC, hemoglobin, and/or hematocrit, suggestive of anemia at 80 mL/kg dextran. Relative organ weights of liver and kidney were dose-dependently increased at the 80 mL/kg dextran groups compared

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to controls. Histopathology revealed panlobular pulmonary hyperemia and edema in both sexes with accumulation of ascites. Some animals showed hydrothorax, diffuse peritonitis, and/or emaciation thought to be due to diarrhea. Histopathology of surviving animals revealed marked pulmonary hyperemia and edema, scattered pulmonary abscesses, renal swelling and ischemia, splenar swelling, and hydrothorax. Females had uterine edema. Males had non-dose-related testicular atrophy.

Table 37 Macropathological findings of male rabbits administered intravenously dextran-40 for 1 month

Infusion		Control	Dextran-40 amino acids solution			Dextran-40 glucose solution			Dextran-40 saline solution		
Dose (ml/kg/day)			80	40	20	80	40	20	80	40	20
No. of animals		5	5	5	5	5	6	4	3	5	6
Lung	hyperemia		1		1						1
	abscess								1		
Hydrothorax			1								
Kidney	ischemia	1				1					
	atrophy										1
Adrenal	hypertrophy				1		1				
Spleen	swelling		1								
Testis	atrophy		1						1		1

In a three-month toxicity test, male rabbits (6 males/group) were administered one of three formulations of dextran-40 intravenously at target volumes of 20 and 40 mL/kg. Control animals received intravenous administration of saline at a target volume of 20 and 40 mL/kg. Mortality occurred in the dextran-amino acid group (2/6 LD, 3/6 HD), dextran-glucose group (2/6 LD, 3/6 HD), and dextran-saline group (2/6 LD, 1/6 HD). Body weights reported as 2.1-2.4 kg. Cage side observations were assessed during the study. Hematology and urinalysis were assessed 2 days after the final dose. Organ weights (brain, pituitary gland, thymus, heart, lungs, liver, kidneys, adrenal gland, spleen, testes, and epididymides) were collected at necropsy. Histopathology was performed on the tissues selected for organ weights as well as the pancreas, stomach, small intestine, large intestine, bone marrow, and mesenteric lymph nodes. Results indicate that body weights increased gradually with all dextran formulations with marked increases in the dextran saline formulation. Food intake was clearly decreased in all dextran groups except 20 mL/kg dextran-saline formulation in comparison with the control group in the late phase of administration. Hematology indicated a dose-related decrease in RBC, hemoglobin, and/or hematocrit indicative of anemia at 40 mL/kg dextran. Organ weights and relative organ weights showed a dose-related increase in liver and kidney weights at 20 and 40 mL/kg dextran compared to controls. Histopathology from animals that died on study revealed panlobular pulmonary hyperemia and edema associated with pericardial edema, pyothorax, and/or adhesive pleurisy. Histopathology of surviving animals revealed marked renal swelling, pulmonary hyperemia, pulmonary edema, scattered pulmonary abscesses, hydrothorax, renal cortical abscesses, adrenal swelling, splenar swelling, and testicular atrophy.

In a six-month toxicity test, male rabbits (6 males/group) were administered one of three formulations of dextran-40 intravenously at a target volume of 20 mL/kg. Control animals received intravenous administration of saline at a target volume of 20 mL/kg.

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Mortality occurred in 2/6 animals in the saline control group, 2/6 in the dextran-amino acid group, 2/6 in the dextran-glucose group, and 3/6 in the dextran-saline group. Body weights reported as 2.1-2.4 kg. Cage side observations were assessed during the study. Hematology and urinalysis were assessed 2 days after the final dose. Organ weights (brain, pituitary gland, thymus, heart, lungs, liver, kidneys, adrenal gland, spleen, testes, and epididymides) were collected at necropsy. Histopathology was performed on the tissues selected for organ weights as well as the pancreas, stomach, small intestine, large intestine, bone marrow, and mesenteric lymph nodes. Food intake was not significantly affected in any group. Hematology did not reveal any changes outside the range of normal. Organ weights showed an increase in liver weights in the dextran-glucose group compared to controls; however, relative organ weights were no different than controls. Histopathology revealed pulmonary hyperemia, pulmonary edema, pulmonary abscesses, and renal cortical abscesses. Histopathology of surviving animals revealed pulmonary hyperemia, pulmonary edema, scattered pulmonary abscesses, adrenal swelling, splenar swelling, and testicular atrophy.

Table 38 Macropathological findings of male rabbits administered intravenously dextran-40 for 3 and 6 months

Months	3 months							6 months			
Infusion	Control	Dextran-40 amino acids solution		Dextran-40 glucose solution		Dextran-40 saline solution		Control	Dextran-40 amino acids solution	Dextran-40 glucose solution	Dextran-40 saline solution
Dose (ml/kg/day)		40	20	40	20	40	20		20	20	20
No. of animals	6	3	4	3	4	5	4	4	4	4	4
Lung hyperemia		1						1	1	1	1
edema				1				1		1	
abscess			1		1	1				1	1
Hydrothorax				1							
Kidney hypertrophy		3		3		2	1				
cortical abscess		1	1								
Adrenal hypertrophy		1					1		1		
Spleen swelling					1					1	
Testis atrophy		1						1			

In the 1-, 3-, and 6-month studies, the adverse findings were primarily attributed to the large volumes of dextran solution administered. Anemia developed with administration of 80 mL/kg for 1 month and 40 mL/kg for 3 months, and the neutrophil nuclei showed a left shift. These changes are considered due to blood retention in the abdominal organ area. Decreases in fibrinogen, total protein, and total cholesterol levels were attributed to hypervolemia causing hepatic congestion, and thus decreased hepatic function. Necropsies suggested the cause of death to be pulmonary hyperemia and pulmonary edema as a result of hypervolemia. Diffuse vacuolar degeneration in the kidneys and renal tubules was marked at 80 mL/kg dextran for 1 month and 40 mL/kg for 3 months. However, no clear connection was found between vacuolar degeneration and renal dysfunction. Hyperplasia and vacuolation of cells in the reticuloendothelial system were marked with dextran administration at 80 mL/kg for 1 month and 40 mL/kg for 3 months.

Reviewer's Note: Because this study is potentially used as a male fertility assay, it is important to note the testicular atrophy that occurred in this study without dose-

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relationship. While this effect may be due to the complications from administering large volumes of intravenous fluids, this study is not designed in a way to mimic the clinical context of remimazolam and does not adequately assess male mating behavior, sperm count or motility.

Publication title: Toxicity studies of Dextran-70 formulations

Study no.:	(Koderak Yakuri to Chiryo, 1975)
Study report location:	EDR
Reference:	H. Hanai, A. Koide, and H. Nagae. Technology Department I, Meito Sangyo Co., Ltd. K. Kodera, H. Miyoshi, and T. Sugimoto Technology Department, Otsuka Pharmaceutical Factory, Inc.
Publication Date:	1975
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Unknown.

In this publication, the toxicity of long-term intravenous dextran-70 administration was studied in Japanese white rabbits up to 6 months. Three different formulations of dextran-70 were used as described in the previous study in Appendix 12.7 Toxicity Studies of Dextran-40 Formulations. Histopathological analysis of the testes and epididymides of male rabbits did not reveal any changes after 1-, 3-, or 6-months of large intravenous administration of dextran. However, this study did not evaluate the recommended male fertility endpoints per ICH S5A Detection of Toxicity to Reproduction for Medicinal Products (i.e., assessment of maturation of male reproduction functions, development and maturation of gametes, mating behavior, and fertility) and ICH S5B Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male fertility (i.e., case-by-case histopathological evaluation of testes and epididymides, and optional sperm analysis including sperm count, motility, and morphology). These guidances also point out that repeat-dose toxicity studies can provide important information regarding potential effects on male fertility.

12.10 Excipient Qualification of Dextran 40: Embryo-Fetal Development

The Applicant provided a safety justification based on reviews and experimental data demonstrating that Dextran 40 exposure to the embryo or fetus is negligible or entirely absent, to justify not conducting an embryo-fetal development study for Dextran 40 in a second species. Applicant's proposal of safety was provided in the Information Amendment 12/06/2019 and is summarized below.

Molecules of over a molecular weight of 500 Da are unable to traverse the placenta freely by passive diffusion but may pass via influx transporters or pinocytotic

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mechanisms (Griffiths and Campbell, 2014; Al-Enazy et al., 2017). Dextran 40 is a large molecule with a molecular weight of 40,000 Da and is not known as a substrate for transporters or interact with receptors on the surface of the placenta for pinocytosis (Griffiths and Campbell, 2014). While dextran is pinocytosed by cells (Roederer et al., 1989), it is expected to accumulate in the lysosomes (Nishimura et al., 2002; Maruoka et al., 1975) of the cell rather than being released. Dextran 40 was found not to cross the placental barrier in humans within 4 hours of intravenous administration of 400-500 mL of 10% Dextran 40. Dextran 40 concentrations in the maternal serum were detected at 0.76, 0.75, and 0.5 g/100 mL in three individual women, but Dextran 40 was not detected in cord blood taken at birth (Ricketts et al., 1966). These findings were replicated with more sensitive assays capable of detecting concentrations of 1.5 and 2 mg/100 mL (Falk et al., 1967). Furthermore, in vitro model of placenta perfusion using fluorescent Dextran 40 was established for a marker for viability and tightness (Mathiesen et al., 2010) and used by many investigators (Partanen 2012; Grafmuller et al., 2013; Karttunen, 2015; Aengenheister et al., 2018; and Freriksen et al., 2018).

While large dextran molecules are unable to pass the placenta, smaller fragments generated by cleavage of the 1,6-glycosidic bonds by dextranase may potentially pass through; therefore, the Applicant summarized the metabolism and elimination of dextran fragments. Elimination data for Dextran 40 came from published literature. The lowest molecular weight fraction of Dextran 40 is cleared by the kidneys within hours of administration, but the higher molecular weight fraction may take days to be excreted or metabolized (FDA, 2011). *Note: During a teleconference with the Applicant on 4/16/2020, the Agency communicated that the sources used to support the safety of Dextran 40 needed clarification. The Applicant was advised to provide the reference to "FDA, 2011" as a 505(b)(2) supportive reference, as documented by the Applicant in SDN 0053 (54) 4/29/2020. As of 6/10/2020, the Reviewer has not obtained the full reference to FDA, 2011. However, the Applicant indicated that they do not intend to rely upon any FDA-approved product; therefore, the above referenced FDA statements are not being used to support this action.* Dextran metabolism by various tissues, including the liver, is slow, suggesting that plasma concentrations of the evolving metabolites will remain low in the body. Oligosaccharides were detectable in human urine for up to 48 hours after intravenous administration of Dextran 40 (Esposito et al., 2014). Dextranase and isomaltase activity in the liver cleaves 1,6-bonds (Rosenfeld and Lukomskaya, 1957) to break di- and oligosaccharides to monosaccharides (Lee et al., 2016; Gericke et al., 2017). Glucose is the final cleavage product of dextranase activity (Gray, 1953; Rosenfeld and Saienko, 1964). Dextran metabolites are glucose, ready for entering the carbon pool, and shortened saccharides are more suitable for fast renal excretion. Finally, the 1,3-disaccharide, nigerose, is an ultimate product of dextran metabolism by dextranase, is found in human foods, probably absorbed by the intestine, and is metabolized by liver enzymes. The majority of Dextran 40 is eliminated unchanged (70%) in the urine. Possible metabolites other than glucose are nigerose and truncated dextran congeners, which can be further degraded or renally excreted. Oligosaccharides longer than two or three glucose units will still be excluded from diffusional placental transport. Therefore, it was concluded that exposure to low-

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molecular dextran degradation products will be low and short-lived and low risk to embryo-fetal development.

To assess the effects of Dextran 40 on placental blood flow, the Applicant summarized data from published literature. Uterine blood flow at term is approximately 600 mL/min, 80% of which passes to the placenta (Griffiths et al., 2014). There is no autoregulation in the uteroplacental circulation and therefore flow is directly related to the mean uterine perfusion pressure and inversely related to uterine vascular resistance. Blood flow in the uteroplacental circulation may consequently be reduced by maternal hypotension and increased uterine pressure during uterine contractions. As the uteroplacental arteries contain alpha-adrenergic receptors, sympathetic stimulation (e.g., by vasopressor drugs) may lead to uterine artery vasoconstriction. Dextran 40 is not known to cause hypotension (as a plasma expander, it will stabilize blood pressure), nor to possess vasoconstrictive properties. Dextran 40 has been used to enhance microcirculation in disorders of pregnancy, without apparent adverse effects (Wang and Young, 2006; Pawlak-Osinska et al., 2009; Xie and Wu, 2019). Finally, prompted by animal experiments showing beneficial effects of Dextran 40 on uterine blood flow and fetal oxygen supply, Dextran 40 has been studied in 11 pregnant women during parturition. After a short infusion of Dextran 40 (50 g over 10 minutes), the fetal blood PO_2 was normalized, that is, it was increased if it was low and decreased it if it was high before infusion (Kurz et al., 1985). While the volume and dose of dextran used in these studies are orders of magnitude higher than expected with the use of remimazolam, there is low risk for dextran in remimazolam to cause adverse effects on embryo-fetal development by effecting placental blood flow.

Reviewer's Note: this Reviewer agrees and accepts the scientific justification. The scientific justification is adequate to waive an embryo-fetal development study in a second species. None of the above-referenced literature mentions an FDA approved product by brand name. Reference to FDA

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Publication title: Studies on Hydroxyethyl Starch Solution (Hespander®) as a Plasma Substitute (VIII) Chronic Toxicity Tests by Three Months Administration in Rabbits

Study no.:	(IrikuraT Oyo Yakuri 1972)
Study report location:	EDR
Reference:	T. Irikura, K. Okada, R. Ishida, H., Arashima. [Studies on Hydroxyethyl Starch Solution (Hespander®) as a Plasma Substitute (VIII) Chronic Toxicity Tests by Three Months Administration in Rabbits]. Pharmacometrics. 1972, 6 (6) 1103-1117 (Special)
Publication Date:	1972
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Unknown.

Reviewer's Note: In this publication, Hespander® was the test article administered intravenously to male rabbits for 3 months at target doses of 15, 30, and 60 mL/kg at a rate of 100-150 mL/h. Dextran-70 and Ringer solution was administered intravenously at 60 mL/kg as the control articles. This study neither tests multiple doses of dextran-40 via the intravenous route or uses appropriate endpoints to assess embryo-fetal development. This reference cannot be used to supplement the safety of dextran-40 with regards to embryo-fetal development.

Publication title: Studies on Anti-atherosclerotic Agents (VIII) Toxicological Studies of Sodium Chondroitin Polysulfate (Part 1)

Study no.:	(TakeuchiM Oyo Yakuri 1972)
Study report location:	EDR
Reference:	Masaya Takeuchi and Masanobu Edanaga. [Studies on Anti-atherosclerotic Agents (VIII) Toxicological Studies of Sodium Chondroitin Polysulfate (Part 1)]. Unknown Journal and Issue.
Publication Date:	1972
GLP compliance:	No.
QA statement:	No.
Drug, lot #, and % purity:	Unknown.

Reviewer's Note: In this publication, sodium chondroitin polysulfate was the test article administered orally, subcutaneously, and intraperitoneally to mice and rats in acute (single), subacute (4 weeks), and chronic (24 weeks) toxicity studies. Sodium dextran sulfate was administered orally as the control article. This study neither tests multiple

Reference ID: 4622909

doses of dextran-40 via the intravenous route or uses appropriate endpoints to assess embryo-fetal development. This reference cannot be used to supplement the safety of dextran-40 with regards to embryo-fetal development.

12.11 Excipient Qualification of Dextran 40: Pre- and Post-Natal Development

The Applicant provided a safety justification based on experimental data demonstrating that Dextran 40 exposure to the embryo or fetus is negligible or entirely absent, to justify not conducting an embryo-fetal development study for Dextran 40. Applicant's proposal of safety was provided in the Information Amendment 12/06/2019 and is summarized in Appendix 12.8 and below.

The Applicant provided literature to suggest that 1) Dextran 40 does not cross the placental barrier and thus there is no prenatal exposure to Dextran 40, 2) uterine blood flow is not adversely affected by Dextran 40 in a manner that could negatively affect the embryo, and 3) that Dextran 40 is mainly excreted unchanged and metabolites are well-characterized low molecular weight carbohydrates such as glucose or nigerose larger oligosaccharide metabolites already exceed the limited size (MD 500 Da) for diffusional placental transport. Thus, there is minimal likelihood for the offspring to be exposed and consequently no detrimental effects of pre- or post-natal development are expected.

Reviewer's Note: I agree with the above justification; however, the PPND studies potentially expose pups to the drug through lactation. The Applicant's justification does not address if Dextran 40 is likely to be in maternal milk at a level that would cause risk to post-natal development. Drugs and Lactation Database (LactMed) states that limited data indicate trace amounts of iron dextran are excreted into breastmilk. No information is available on the clinical use of iron dextran during breastfeeding. However, intravenous iron dextran had been used in preterm infants for anemia of prematurity and would not be expected to cause any adverse effects in breastfed infants. An alternate intravenous drug with more published data available may be preferred. Pasteurization of milk by the Holder method reduces the concentration of iron in milk by about 6.5%. For this reason, the Applicant's justification for the safety of Dextran 40 with regards to post-natal development was not complete and required further justification.

Regarding exposure through maternal milk, the Applicant provided a safety justification based on experimental data and Agency's previous findings demonstrating that Dextran 40 has low oral bioavailability, is GRAS, will result in low exposures, and is not adverse in postnatal infants. The Reviewer agrees with the conclusion that there is low risk to postnatal development. The Applicant's proposal of safety was provided in the Information Amendment 01/31/2020 as shown below.

There is no data available on the potential transfer of Dextran 40 from blood to maternal milk. Nevertheless, we do not have safety concerns for Dextran 40 with respect to potential exposure of infants via maternal milk for the following reasons:

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- The probability of exposure of the infant to Dextran 40 or metabolites via the maternal milk is low since it is recommended to suspend breast feeding for (b) (4) hours after treatment with remimazolam. The following text is part of the proposed package insert: (b) (4)

- If an infant is exposed to Dextran 40 via the maternal milk, the oral bioavailability is reported to be low. Koyoma and coworkers (Koyama et al., 1996) used an ex vivo system to evaluate the oral bioavailability of dextrans of different molecular sizes. Absorption was independent of the molecular size (potentially due to receptor-mediated uptake) and amounted to maximally 20% at low doses. At doses of above 1 mg this dropped to about 5%, indicating that the uptake mechanisms are saturable.

  Reviewer's Note: This literature reference is necessary in understanding the oral bioavailability of dextrans, which can help in estimating the oral exposures in breast-feeding infants exposed through maternal milk from mothers administered remimazolam.

- Dextran 40 has been classified by FDA (Table 3. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe for Indirect Use in 21 CFR§186) as GRAS as a food additive which supports its safe use via oral route.

Table 3. Substances Derived from Microorganisms Affirmed by FDA as Generally Recognized as Safe for Indirect Uses in 21 CFR186

Section in 21 CFR	Substance
§186.1275	Dextrans, made by fermentation of sucrose by *Leuconostoc mesenteroides* strain NRRL B-512(F)
§186.1839	Sorbose, made by oxidation of sorbitol by *Acetobacter xylinum* or by *Acetobacter suboxydans*

The Scientific Commission on Food of the European Commission has also evaluated Dextran 40 as a food additive and concluded that it is safe up to levels of 5% (CS/NF/DOS/7/ADD 3 FINAL).
Reviewer's Note: Determination of GRAS adds to the support of safety of oral exposure to Dextran 40 from foods.

In summary, even if Dextran 40 passes to maternal milk and is transferred to an infant during breast feeding the uptake from the gastrointestinal tract can be considered low. Furthermore, oral Dextran 40 has been classified as GRAS if indirectly added to food and doses of at least 50-fold higher are safely administered to pediatric patients when Dextran 40 is used as a plasma expander. Consequently, the risk of harmful effects exerted by Dextran 40 to infants fed with maternal milk of remimazolam treated women can be regarded low.

Reference ID: 4622909

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/s/

KATIE B SOKOLOWSKI
06/10/2020 06:05:04 PM

NEWTON H WOO
06/10/2020 07:17:51 PM

RICHARD D MELLON
06/10/2020 07:29:05 PM
I concur with Drs. Sokolowski and Woo that NDA 212295 may be approved as noted. I also concur with the recommended post-marketing requirements and recommended labelling.