NTP TECHNICAL REPORT

ON THE

PRENATAL DEVELOPMENTAL TOXICITY STUDIES

OF VINPOCETINE

(CAS No. 42971-09-5)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

AND NEW ZEALAND WHITE (Hra:NZW SPF) RABBITS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NTP DART-03

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

Although the NTP has conducted numerous Developmental and Reproductive Toxicology (DART) Studies since the inception of the Program, it was only in 2009 that the Program formulated levels of evidence criteria for drawing conclusions as to the developmental and/or reproductive toxicity of a compound based on the conditions employed in the study. The studies described in this DART Report series are designed and conducted to characterize and evaluate the developmental and/or reproductive toxicity of selected substances in laboratory animals. Substances selected for NTP DART studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP DART Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's developmental or reproductive toxicity potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*). Additional information regarding this study may be requested through Central Data Management (CDM) at cdm@niehs.nih.gov. Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://www.niehs.nih.gov/research/resources/databases/cebs/index.cfm

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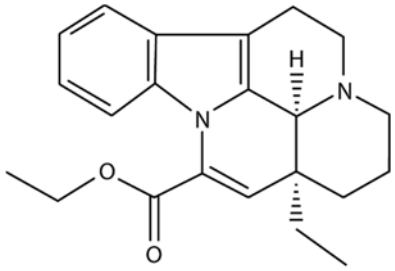
ABSTRACT



VINPOCETINE

CAS No. 42971-09-5

Chemical Formula: $C_{22}H_{26}N_2O_2$ Molecular Weight: 350.46

Synonyms: Apovincaminic acid ethyl ester; cis-apovincaminic acid ethyl ester; ethyl (+)-apovincaminate; ethyl apovincamin-22-oate; ethyl (+)-cis-apovincaminate; ethyl (3α,16α)-eburnamenine-14-carboxylate
Trade names: Bravinton, Cavinton, Ceractin, Intelectol, RGH-4405, TCV-3B, Vinporal

Vinpocetine is mainly marketed as a dietary supplement for cognitive enhancement, Alzheimer's, dementia, and ischemic stroke; however, several products are marketed towards students as brain supplements for increased cognitive performance. Additionally, vinpocetine is used by bodybuilders to enhance visual acuity, memory, and focus and to rapidly reduce body fat. Human exposure to vinpocetine typically occurs through oral consumption. Marketed as a dietary supplement in the United States, vinpocetine is regulated by the Food and Drug Administration (FDA) under the Dietary Supplement Health and Education Act of 1994. Analysis of vinpocetine supplements have shown that in a significant number of products, the actual vinpocetine content varied from what was stated on the label which could result in higher doses than what is recommended by the product labels. Due to limited literature indicating that vinpocetine may not be safe for use during pregnancy and the possibility for widespread exposure to women of childbearing age, the National Toxicology Program (NTP) conducted prenatal developmental toxicology studies. In these studies, time-mated Sprague Dawley (Hsd:Sprague Dawley SD) rats and New Zealand White (Hra:NZW SPF) rabbits received vinpocetine (99.3% pure) in 0.5% methylcellulose by gavage

from implantation on gestation day (GD) 6 (rats) or 7 (rabbits) to the day before expected parturition (GD 20 for rats; GD 28 for rabbits). Evidence of vinpocetine-related maternal and fetal toxicity was examined in the dose range-finding study in rats followed by the standard prenatal developmental toxicity study in rats. A dose range-finding study in rabbits was conducted to see if effects occurred in a second species, but a standard prenatal developmental toxicity study was not performed in rabbits.

DOSE RANGE-FINDING PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Groups of 10 time-mated female rats were administered 0, 20, 40, 80, 160, or 320 mg vinpocetine/kg body weight per day, based on the most recent body weight, in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control (0 mg/kg) animals received aqueous methylcellulose.

All animals survived to the end of the study. Clinical observations were limited to red and/or brown vaginal discharge, discoloration of the nares, and piloerection. There were dose-related decreases in mean maternal body weight and mean body weight gains from GD 6 to GD 21 in groups administered 40 mg/kg or greater. When adjusted for gravid uterine weight, maternal body weights of the 160 and 320 mg/kg groups were significantly lower than those of the vehicle controls. Concomitant treatment-related, dose-dependent decreases in maternal feed consumption in groups receiving 40 mg/kg or greater was also noted. There was a significant, treatment-related effect on percent post-implantation loss in all dose groups. At doses of 80 mg/kg or greater, dams exhibited total resorption of their litters with the exception of one dam in the 160 mg/kg group with live fetuses.

No external malformation or variations attributed to vinpocetine administration in fetuses were observed.

PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Due to the post-implantation loss observed at doses of 80 mg/kg and greater in the dose ranging-finding study, 60 mg/kg was chosen as the high dose for the prenatal developmental toxicity study. Groups of 25 time-mated female rats were administered 0, 5, 20, or 60 mg vinpocetine/kg body weight per day, based on the most recent body

weight, in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control (0 mg/kg) animals received aqueous methylcellulose.

All animals survived to the end of the study. Treatment-related clinical findings were red and/or brown vaginal discharge in the 20 and 60 mg/kg groups. There were significantly decreased mean maternal body weights and mean body weight gains in the 60 mg/kg group which were associated with a significant increase in post-implantation loss, including total litter resorption in 12 dams. There was also a treatment-related decrease in feed consumption in the 60 mg/kg group. Due to the increased post-implantation loss in the 60 mg/kg group, there was a significant decrease in the number of live fetuses per litter and in gravid uterine weight.

There were a small number of viable litters and fetuses for evaluation at 60 mg/kg. In the viscera, there were treatment-related increased incidences of ventricular septum defect in all exposed groups. In the skeleton, treatment-related findings included significantly increased incidences of incomplete ossification of the thoracic centrum in the 20 and 60 mg/kg groups and full supernumerary thoracolumbar ribs in the 60 mg/kg group.

DOSE RANGE-FINDING STUDY IN RABBITS

Groups of 8 time-mated female rabbits were administered 0, 25, 75, 150, or 300 mg vinpocetine/kg body weight per day, based on the most recent body weight, in 0.5% aqueous methylcellulose by gavage from GD 7 to GD 28. Vehicle control (0 mg/kg) animals received aqueous methylcellulose.

All rabbits survived until the end of the study except one 150 mg/kg female that was removed on GD 25 due to abortion. There were no clinical observations related to vinpocetine treatment. There were significant decreases in mean maternal body weights gains in the 150 and 300 mg/kg groups. The decreased maternal body weight gains were consistent with a treatment-related decrease in feed consumption in these groups.

There was an exposure-related effect on embryo-fetal survival in the 300 mg/kg group with a significant decrease in the number of live fetuses per litter and an increase in early resorptions per litter resulting in an increase in percent post-implantation loss. These findings in the 300 mg/kg group were also associated with a significant decrease in

mean gravid uterine weight. There were no exposure-related effects on embryo-fetal survival in any group administered 150 mg/kg or less. There were no external malformations or variations attributed to vinpocetine exposure.

Data from this rabbit dose range-finding study supported findings observed in the rat dose range-finding study and rat prenatal developmental toxicity studies (increase in post-implantation loss) with exposure to vinpocetine.

CONCLUSIONS

Under the conditions of the rat prenatal study, there was *clear evidence* of developmental toxicity of vinpocetine in Hsd:Sprague Dawley SD rats based on increased post-implantation loss and increased incidences of ventricular septum defects, thoracolumbar ribs (full), and incomplete ossification of the thoracic centrum in the absence of overt maternal toxicity.

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* Explanation of Levels of Evidence of Prenatal Developmental Toxicity is on page 11.

Summary of Exposure-Related Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Maternal Parameters				
Animals on study	25	25	25	25
Number pregnant	21	20	22	20
Number died or euthanized moribund	0	0	0	0
Clinical Observations	None	None	Red or brown vaginal discharge	Red or brown vaginal discharge
Body Weight and Feed Consumption[a]				
Terminal body weight	385.7 ± 34.2**	368.5 ± 8.2	370.0 ± 5.5	296.1 ± 8.2**
Body weight change GD 6 to 21	142.8 ± 3.4**	128.7 ± 7.4	130.0 ± 5.1	55.3 ± 7.9**
Feed consumption GD 6 to 21	22.0 ± 0.3**	21.6 ± 0.4	22.2 ± 0.3	19.9 ± 0.4**
Necropsy Observations	None	None	None	None
Developmental/Fetal Parameters				
Number of litters examined	21	19	21	8
Number of live fetuses evaluated	293	239	261	51
Number of live fetuses per litter[b]	13.95 ± 0.55**	11.95 ± 1.06	11.86 ± 0.88	2.5 ± 1.00**
Number of early resorptions	7	12	19	208
Number of late resorptions	1	0	2	0
Number of dead fetuses	1	0	0	0
Number of whole litter resorption	0**	1	1	12**
Percent post-implantation loss[b]	3.29 ± 1.33**	10.67 ± 5.29	11.13 ± 4.65	83.13 ± 6.47**
Fetal body weight per litter[a]	5.15 ± 0.07	5.29 ± 0.16	5.21 ± 0.12	5.11 ± 0.10
Male fetal weight per litter[a]	5.28 ± 0.06	5.49 ± 0.21	5.35 ± 0.12	5.18 ± 0.08
Female fetal weight per litter[a]	5.03 ± 0.07	5.10 ± 0.10	5.09 ± 0.12	4.63 ± 0.06
Gravid uterine weight[a]	97.79 ± 3.11**	83.89 ± 6.59	85.07 ± 5.28	19.52 ± 6.53**
External Findings	None	None	None	None
Visceral Findings[c]				
Heart				
Ventricle, ventricular septum defect — [M]				
Fetuses	0 (0.00)	3 (1.26)	8 (3.07)	2 (3.92)
Litters	0 (0.00)	3 (15.79)	7 (33.33)**	2 (25.00)
Skeletal Findings				
Thoracic centrum				
Incomplete ossification, total — [V]				
Fetuses	1 (0.34)##	1 (0.42)	6 (2.31)#	8 (17.02)##
Litters	1 (4.76)**	1 (5.26)	5 (23.81)	3 (42.86)*
Supernumerary rib				
Thoracolumbar, full, total — [M]				
Fetuses	1 (0.34)##	5 (2.09)	12 (4.62)	12 (25.53)##
Litters	1 (4.76)*	3 (15.79)	4 (19.05)	3 (42.86)*
Level of evidence of developmental toxicity: Clear evidence				

* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); ** P≤0.01.

\# Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) for fetuses; ##P≤0.01.

[a] Results given in grams. Data are displayed as mean ± standard error.

[b] Data are displayed as mean ± standard error.

[c] Upper row denotes the number of affected fetuses and (%) and lower row the number of affected litters and (%).

GD = Gestation Day; [M] = Malformation; [V] = Variation

Summary of Exposure-Related Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Maternal Parameters					
Animals on study	8	8	8	8	8
Number pregnant	8	7	8	8	8
Number died or euthanized moribund	0	0	0	0	0
Number euthanized –abortion	0	0	0	1	0
Clinical Observations	None	None	None	Red vaginal discharge	Red vaginal discharge
Body Weight and Feed Consumption[a]					
Necropsy body weight	3,499.4 ± 64.6*	3,406.5 ± 58.0	3,467.7 ± 95.0	3,358.4 ± 105.6	3,271.4 ± 34.2
Body weight change GD 7 to 29	460.2 ± 33.8**	458.2 ± 46.2	399.3 ± 58.4	256.7 ± 40.9**	304.1 ± 33.2**
Feed consumption GD 7 to 29	137.6 ± 4.3	131.8 ± 5.7	125.2 ± 4.0	101.3 ± 11.4**	113.8 ± 8.9*
Necropsy Observations	None	None	None	None	None
Developmental/Fetal Parameters					
Number of litters examined	8	7	8	7	8
Number of live fetuses evaluated	73	54	72	53	52
Number of live fetuses/litter[b]	9.13 ± 0.44*	7.71 ± 0.42	9.00 ± 0.53	7.57 ± 0.81	6.50 ± 0.73*
Number of early resorptions	1	0	0	1	13
Number of late resorptions	0	1	2	0	2
Dead fetuses	0	1	0	0	0
Number of whole litter resorption	0	0	0	0	0
Percent post-implantation loss[b]	1.39 ± 1.39	3.37 ± 2.18	2.53 ± 1.66	3.57 ± 3.57	20.42 ± 9.05
Fetal body weight per litter[a]	39.72 ± 1.33**	41.47 ± 0.95	37.53 ± 0.90	39.36 ± 1.74	35.78 ± 1.15
Male fetal weight per litter[a]	40.87 ± 1.59**	42.70 ± 0.97	38.50 ± 1.15	38.06 ± 1.62	36.49 ± 2.00
Female fetal weight per litter[a]	38.76 ± 1.57*	40.37 ± 1.12	36.35 ± 1.01	39.29 ± 1.75	34.65 ± 0.95
Gravid uterine weight[a]	515.25 ± 14.70**	470.05 ± 20.66	483.91 ± 32.24	421.86 ± 39.25*	340.94 ± 27.73**
External Findings	None	None	None	None	None

* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); ** P≤0.01.

[a] Results given in grams. Data are displayed as mean ± standard error.

[b] Data are displayed as mean ± standard error.

GD = Gestation Day

EXPLANATION OF LEVELS OF EVIDENCE FOR DEVELOPMENTAL TOXICITY

The NTP describes the results of individual studies of chemical agents and other test articles, and notes the strength of the evidence for conclusions regarding each study. Generally, each study is confined to a single laboratory animal species, although in some instances, multiple species may be investigated under the purview of a single study report. Negative results, in which the study animals do not exhibit evidence of developmental toxicity, do not necessarily imply that a test article is not a developmental toxicant, but only that the test article is not a developmental toxicant under the specific conditions of the study. Positive results demonstrating that a test article causes developmental toxicity in laboratory animals under the conditions of the study are assumed to be relevant to humans, unless data are available that demonstrate otherwise. In addition, such positive effects should be assumed to be primary effects, unless there is clear evidence that they are secondary consequences of excessive maternal toxicity. Given that developmental events are intertwined in the reproductive process, effects on developmental toxicity may be detected in reproductive studies. Evaluation of such developmental effects should be based on the NTP Criteria for Levels of Evidence for Developmental Toxicity.

It is critical to recognize that the "levels of evidence" statements described herein describe only developmental **hazard**. The actual determination of **risk** to humans requires exposure data that are not considered in these summary statements.

Five categories of evidence of developmental toxicity are used to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence** and **some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major design or performance flaws **(inadequate study)**. Application of these criteria requires professional judgment by individuals with ample experience and an understanding of the animal models and study designs employed. For each study, conclusion statements are made using one of the following five categories to describe the findings. These categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

Levels of Evidence for Evaluating Developmental System Toxicity

- **Clear evidence** of developmental toxicity is demonstrated by data that indicate a dose-related[a] effect on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation, or functional deficits) that is not secondary to overt maternal toxicity.
- **Some evidence** of developmental toxicity is demonstrated by dose-related effects on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation, or functional deficits), but where there are greater uncertainties or weaker relationships with regard to dose, severity, magnitude, incidence, persistence, and/or decreased concordance among affected endpoints.
- **Equivocal evidence** of developmental toxicity is demonstrated by marginal or discordant effects on developmental parameters that may or may not be related to the test article.
- **No evidence** of developmental toxicity is demonstrated by data from a study with appropriate experimental design and conduct that are interpreted as showing no biologically relevant effects on developmental parameters that are related to the test article.
- **Inadequate study** of developmental toxicity is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of developmental toxicity.

When a conclusion statement for a particular study is selected, consideration must be given to key factors that would support the selection of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of developmental toxicity studies in laboratory animals, particularly with respect to interrelationships between endpoints, impact of the change on development, relative sensitivity of endpoints, normal background incidence, and specificity of the effect. For those evaluations that may be on the borderline between two adjacent levels, some factors to consider in selecting the level of evidence of developmental toxicity are given below:

- Increases in severity and/or prevalence (more individuals and/or more affected litters) as a function of dose generally strengthen the level of evidence, keeping in mind that the specific manifestation may be different with increasing dose. For example, malformations may be observed at a lower dose level, but higher doses may produce embryo-fetal death.
- Effects seen in many litters may provide stronger evidence than effects confined to one or a few litters, even if the incidence within those litters is high.
- Because of the complex relationship between maternal physiology and development, evidence for developmental toxicity may be greater for a selective effect on the embryo-fetus or pup.
- Concordant effects (syndromic) may strengthen the evidence of developmental toxicity. Single endpoint changes by themselves may be weaker indicators of effect than concordant effects on multiple endpoints related by a common process or mechanism.
- In order to be assigned a level of "clear evidence" the endpoint(s) evaluated should normally show a statistical increase in the deficit, or syndrome, on a litter basis.
- In general, the more animals affected, the stronger the evidence; however, effects in a small number of animals across multiple, related endpoints should not be discounted, even in the absence of statistical significance for the individual endpoint(s). In addition, rare malformations with low incidence, when interpreted in the context of historical controls, may be biologically important.
- Consistency of effects across generations in a multigenerational study may strengthen the level of evidence. However, if effects are observed in the F_1 generation but not in the F_2 generation (or the effects occur at a lesser frequency in the F_2 generation), this may be due to survivor selection for resistance to the effect (i.e., if the effect is incompatible with successful reproduction, then the affected individuals will not produce offspring).
- Transient changes (e.g., pup weight decrements, reduced ossification in fetuses) by themselves may be weaker indicators of an effect than persistent changes.
- Uncertainty about the occurrence of developmental toxicity in one study may be lessened by effects (even if not identical) that are observed in a second species.

- Insights from supportive studies (e.g., toxicokinetics, ADME, computational models, structure-activity relationships) and developmental findings from other *in vivo* animal studies (NTP or otherwise) should be drawn upon when interpreting the biological plausibility of an effect.
- New assays and techniques need to be appropriately characterized to build confidence in their utility: their usefulness as indicators of effect is increased if they can be associated with changes in traditional endpoints.

http://ntp.niehs.nih.gov/go/10003

[a] The term “dose-related” describes any dose relationship, recognizing that the test article-related responses for some endpoints may be non-monotonic due to saturation of exposure or effect, overlapping dose-response behaviors, change in manifestation of the effect at different dose levels, or other phenomena.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

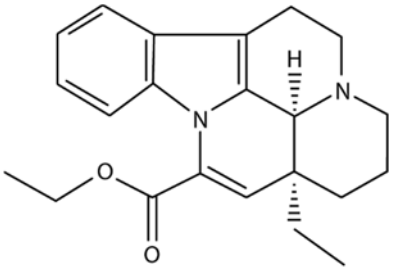
The members of the Peer Review Panel who evaluated the draft NTP Prenatal Developmental Toxicity Study Report on vinpocetine in 2019 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

SUMMARY OF PEER REVIEW PANEL COMMENTS

A summary of the Peer Review Panel's remarks will appear in a future draft of this report.

INTRODUCTION



VINPOCETINE

CAS No. 42971-09-5

Chemical Formula: $C_{22}H_{26}N_2O_2$ Molecular Weight: 350.46

Synonyms: Apovincaminic acid ethyl ester; cis-apovincaminic acid ethyl ester; ethyl (+)-apovincaminate; ethyl apovincamin-22-oate; ethyl (+)-cis-apovincaminate; ethyl (3α,16α)-eburnamenine-14-carboxylate
Trade names: Bravinton, Cavinton, Ceractin, Intelectol, RGH-4405, TCV-3B, Vinporal

CHEMICAL AND PHYSICAL PROPERTIES

Vinpocetine is a white crystalline solid with a molecular mass of 350.45 g/mol. It has an estimated boiling point of 420° C, melting point of 147° to 153° C, vapor pressure of 3.02×10^{-7} mm Hg at 25° C, and log K_{OW} of 4.3 (WOC, 2017).

PRODUCTION, USE, AND HUMAN EXPOSURE

Vinpocetine can be synthesized in several ways from vincamine, an alkaloid extract from the periwinkle plant. One method described by Szabó *et al.* (1983) involves heating (+)-14-oxo-15-hydroxyimino-*E*-homo-eburnane with ethanol and sulfuric acid. The resulting solution is cooled and brought to a pH of 9 with ammonium hydroxide. The organic phase is extracted with methylene chloride, then dried, filtered and evaporated. The residual oil is

recrystallized in ethanol, which yields 67.6% vinpocetine. A "one-pot" synthesis method describes two synthesis pathways for vinpocetine production from vincamine (Kuge *et al.*, 1994). With this method, vinpocetine is produced (80% product yield) through either transesterification or dehydration of vincamine in ethanol using Lewis acids; ferric chloride catalyzed both processes. Tabersonine, an alkaloid extract from voacanga seeds found mostly in West Africa, can also serve as a source from which vinpocetine can be derived (Linnea SA, 2017). Additionally, there are patents for synthetic methods of vinpocetine production that can result in higher yields (~90%) than the semi-synthetic methods described above. For example, one method described the reaction of apovincaminic acid with ethanol in the presence of 2-fluoro-1,3,5-trinitrobenzene and 4-dimethylaminopyridine (Mondelo, 1989).

Since the late 1970s, vinpocetine has been widely available as a pharmaceutical agent in Hungary, Germany, Poland, Russia, China, and Japan for use in cerebrovascular and cognitive disorders (Bereczki and Fekete, 2008). In the United States, vinpocetine is mainly marketed as a dietary supplement with the primary purported indication of cognitive enhancement, including use for Alzheimer's, dementia, and ischemic stroke (Manconi *et al.*, 1986; Peruzza and DeJacobis, 1986; Thal *et al.*, 1989; Feigin *et al.*, 2001; Bereczki and Fekete, 2008; Szatmári and Whitehouse, 2009). Though original indications for vinpocetine promoted its use in the elderly, several products are currently available that are specifically marketed towards students as brain supplements for increasing cognitive performance (Ley, 2000). Additionally, vinpocetine is used among healthy athletes within the bodybuilding community for reported enhancement of visual acuity, memory, and focus in addition to rapid reductions in body fat (South, 2007). Other reported uses are for vertigo, urinary incontinence, tinnitus, Meniere's disease, visual impairment, menopause symptoms, chronic fatigue syndrome, seizure disorders, and prevention of motion sickness (Gedeon Richter, 1984; Taiji and Kanzaki, 1986; Truss *et al.*, 2000; Thorne Research, 2002; Sitges *et al.*, 2016). There are several patents claiming additional applications for vinpocetine, including topical use for enhanced female sexual response (Crosby and Bennett, 2004, 2012), as a primary ingredient in a supplement for the improvement of sleep and lucid dreaming (Luciano, 2012), and as an ingredient (either alone or in combination with stimulants, anti-motion drugs, or nootropics) for intranasal administration to treat dyslexia in children (Misra *et al.*, 2011).

Human exposure to vinpocetine typically occurs through oral consumption. As reported by the Physicians' Desk Reference for Nutritional Supplements, vinpocetine doses may range from 5 to 20 mg per day (Hendler and Rorvik,

2001). In the United States, vinpocetine products are available in dosages ranging from 5 to 30 mg, with recommended uses of 1 to 3 times daily, equaling daily doses of 5 to 90 mg. However, a recent analysis of vinpocetine supplements demonstrated a common problem with botanical dietary supplements, where six out of the 23 (17%) sampled supplements contained no vinpocetine and, in those that did contain vinpocetine, the actual vinpocetine content varied from what was stated on the label (Avula *et al.*, 2015). This results in differences in total daily consumption rates, and could potentially result in higher doses than what is recommended by the product labels.

REGULATORY STATUS

Vinpocetine is often marketed as a dietary supplement in the United States and, therefore, regulated by the Food and Drug Administration (FDA) under the Dietary Supplement Health and Education Act of 1994. Vinpocetine was submitted in several notifications to the FDA as a new dietary ingredient by manufacturers beginning in 1997. However, the FDA has recently published a notice in the Federal Register requesting comment as to the regulatory status of vinpocetine as a dietary ingredient. Specifically, FDA tentatively concluded that vinpocetine does not meet the definition of a dietary ingredient and is excluded from the definition of a dietary supplement in the Food, Drug, and Cosmetic (FD&C) Act (Federal Register, 2016). This administrative proceeding has not been finalized.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

In rats, vinpocetine was rapidly absorbed following a single oral administration with peak plasma and tissue concentrations occurring within 2 hours (Vereczkey, 1985; Xia *et al.*, 2010; Sozański *et al.*, 2011). Following administration of [3H]vinpocetine, approximately 47% and 34% of the dose was recovered in the urine and feces, respectively, at 48 hours; less than 5% was recovered in bile within 9 hours of administration (Vereczkey and Szporny, 1976). The highest radioactivity was recovered in the liver and small intestine, followed by the lung, stomach, kidney, and adrenal glands. With the exception of the liver and kidneys, residual radioactivity in tissues returned to minimal levels within 48 hours of administration. Urinary and fecal excretion of vinpocetine was similar, following a 5-day repeated oral exposure. The plasma elimination half-life of vinpocetine following a

single gavage administration of 10 mg (Vereczkey *et al.*, 1979a) or 1 to 2 mg/kg (Xia *et al.*, 2010; Sozański *et al.*, 2011) was ≤ 3 hours. The C_{max} and AUC following a single gavage administration of 2 mg/kg were 135.33 ng/mL and 504.03 ng.h/mL (Sozański *et al.*, 2011) and 1 mg/kg were 23.8 ng/mL and 57.4 ng.h/mL (Xia *et al.*, 2010), respectively. Oral bioavailability of vinpocetine in rats was 52% suggesting extensive first pass metabolism (Vereczkey *et al*., 1979a). The main metabolite of vinpocetine identified in rat urine was apovincaminic acid (approximately 75% of urinary excretion), arising from deesterification of vinpocetine (Vereczkey and Szporny, 1976; Vereczkey *et al*., 1979a). Formation of apovincaminic acid following oral administration of vinpocetine in rats was rapid with the highest plasma concentration observed approximately 1 hour after administration with an elimination half-life of 3 to 10 hours (Vereczkey *et al*., 1979a; Xia *et al*., 2010).

The National Toxicology Program (NTP) conducted a study to investigate the toxicokinetics of vinpocetine and apovincaminic acid in pregnant rats and to estimate gestational transfer following gavage administration of 5 and 20 mg/kg vinpocetine to dams from gestational days 6 through 18 (Waidyanatha *et al*., 2018). Both vinpocetine and apovincaminic acid were detected in dam plasma. Vinpocetine was absorbed rapidly in dams with the maximum plasma concentration (C_{max}) occurring ≤ 1.4 hours after dosing. The predicted C_{max} and area under the concentration versus time curve (AUC) increased less than proportionally to the dose. Vinpocetine was rapidly distributed to the peripheral compartment. More importantly, a significant transfer of vinpocetine from dams to fetuses was observed with fetal C_{max} and AUC ≥ 55% of that of dams. Vinpocetine was rapidly cleared from dam plasma with a half-life of ≤ 4.02 hours with no apparent dose-related effect. Vinpocetine was rapidly and highly metabolized to apovincaminic acid with apovincaminic acid C_{max} reached ≤ 1.5 hours. Based on the C_{max} and AUC values, apovincaminic acid levels were ≥ 2.7-fold higher than vinpocetine levels in dams, although in the fetuses, apovincaminic acid levels were much lower than those of vinpocetine.

Absorption of vinpocetine was also rapid in New Zealand White rabbits following oral administration, with peak plasma concentration reached within 2 hours; maximum plasma concentrations varied with 209 ng/mL, 163 ng/mL and 61.5 ng/mL reported for 10 mg, 10 mg/kg, and 40 mg, respectively (Nie *et al*., 2006; Ribeiro *et al*., 2007; Xu *et al*., 2009). The plasma elimination half-life was 2 to 6.5 hours, depending on the study. In dogs, the elimination half-life was longer (approximately 9 hours) (Vereczkey, 1985).

The NTP investigated the systemic exposure from vinpocetine and apovincaminic acid in pregnant New Zealand White rabbits, using plasma samples collected in the current study. Doses of 0, 20, 40, 80, 160, or 320 mg/kg vinpocetine were administered by gavage from gestational days 7 through 19 and doe plasma was collected 1 and 2 hours following the last dose (Catlin *et al*., 2018). Vinpocetine and apovincaminic acid were detected at both time points and the concentrations of both increased less than proportionally to the dose. Unlike in rats, the plasma concentration of apovincaminic acid was much higher than that of vinpocetine, suggesting significant species difference in metabolism. These species differences in metabolism were previously reported *in vivo* (Vereczkey, 1985) and *in vitro* (Szakács *et al*., 2001). In addition to apovincaminic acid, hydroxyvinpocetine, hydroxyl-apovincaminic acid, and dihydroxy-vinpocetine-glycinate are other minor metabolites that have been identified in dogs and humans (Vereczkey, 1985).

Humans

In humans, similar to animals, absorption of vinpocetine following ingestion was fast with a maximum plasma concentration reached within 2 hours after ingestion and a plasma elimination half-life of ≤ 2 hours (Vereczkey *et al*., 1979b; Grandt *et al*., 1989; Miskolczi *et al*., 1990; Lohmann *et al*., 1992: Elbary *et al*., 2002). The reported oral bioavailability in humans varies from 6.7% to 57% (Vereczkey *et al*., 1979b; Grandt *et al*., 1989). Vinpocetine bioavailability has been shown to be largely influenced by its administration with or without food, which is a likely factor in the differences in reported values. The relative bioavailability was 60% to 100% higher in individuals who were administered vinpocetine under non-fasting conditions, in comparison to fasting conditions (Lohmann *et al*., 1992). In the same study, food intake did not impact the rate of absorption with maximum serum concentrations observed at 1 hour following vinpocetine administration, similar to other observed peak plasma levels. Gulyás *et al.* (2002a,b) examined the tissue distribution of orally administered ^{11}C-vinpocetine in humans through the use of positron emission tomography and found that vinpocetine rapidly enters the blood stream and liver through the stomach and gastrointestinal tract. These studies also demonstrated radioactivity uptake and distribution of vinpocetine in the brain, indicating that the compound is able to cross the blood brain barrier.

Metabolism of vinpocetine is extensive in humans similar to animals, with undetectable levels of unchanged vinpocetine in the urine 24 hours after administration (Vereczkey *et al.*, 1979b). *In vitro* studies with human

hepatocytes have demonstrated that human metabolism of vinpocetine is similar to that in dogs, in that metabolism occurs almost exclusively in the liver (Szakács *et al*., 2001). The main metabolite measured in humans is apovincaminic acid (Miskolczi *et al*., 1987, 1990; Vlase *et al*., 2005).

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Experimental Animals

Data in the publicly available literature regarding the developmental and reproductive toxicity of vinpocetine are limited to one publication that summarized 14 safety studies (Cholnoky and Dömök, 1976). Vinpocetine was tested in multiple animal species (rats, dogs, and rabbits) at doses ranging from 2 to 150 mg/kg, depending on the route of exposure (oral, intraperitoneal, intravenous, or intramuscular). Maternal findings observed in these studies were limited to decreased maternal body weight gain and uterine bleeding. Fetal findings ranged from no adverse fetal outcomes (noted in the litters that survived to term) to fetal growth retardation and malformations; however, little detail on the types of malformations is available. Fetal loss, including whole litter resorptions, was noted in all studies. Study findings and details were minimal in the publication; but based on the data presented, the authors concluded that vinpocetine was safe for use in adults but recommended avoidance for pregnant women.

Humans

There are no studies that examined vinpocetine exposure and adverse reproductive (or prenatal) outcomes in humans in the literature.

GENERAL TOXICITY

Experimental Animals

The oral LD_{50} value for vinpocetine is approximately 500 mg/kg in rats and mice, the intravenous LD_{50} is approximately 50 mg/kg in rats and mice, and the intraperitoneal LD_{50} ranges from 134 to 240 mg/kg in rats and mice (Cholnoky and Dömök, 1976; Pálosi and Szporny, 1976). Rodents that were administered lethal doses displayed the clinical observations of ataxia and clonic convulsions (Cholnoky and Dömök, 1976).

Summary data from subchronic toxicity tests of vinpocetine in animals were published with limited details by Cholnoky and Dömök (1976). Rats exposed orally to 100 mg/kg for 4 weeks displayed increased liver and thyroid gland weights and clinical observations of increased salivation. Contrary to these findings, no vinpocetine-related toxicity was noted in a separate rat study with doses up to 100 mg/kg by oral gavage. A 3-month intraperitoneal injection study in rats resulted in mortality (38% of the males, 25% of the females) due to severe confluent fibroblastic peritonitis and ascites with vinpocetine doses of 25 mg/kg. No general toxicities were noted in a study performed in dogs with vinpocetine doses up to 25 mg/kg administered orally through capsules.

Humans

Vinpocetine exposure in humans has been associated with nausea, dizziness, insomnia, drowsiness, dry mouth, transient hypotension and tachycardia, pressure-type headache, and facial flushing (Ebi, 1985; Hendler and Rorvik, 2001). Long-term use of vinpocetine has also been associated with slight reductions in systolic and diastolic blood pressure, as well as slight reductions in blood glucose (Hendler and Rorvik, 2001).

STUDY RATIONALE

The dietary supplement vincamine was nominated by the National Cancer Institute for genotoxicity, subchronic toxicity, and mechanistic studies due to a lack of information on potential toxicity. However, vincamine is no longer widely marketed as a dietary supplement in the United States and has been replaced by its semisynthetic derivative, vinpocetine. Due to limited literature indicating that vinpocetine may not be safe for use during pregnancy and the possibility for widespread exposure to women of childbearing age, the developmental toxicity of vinpocetine in rats was investigated. Given the adverse responses on prenatal development that were observed in the rat, a dose range-finding rabbit study was included to provide information on vinpocetine in a second species, to assess species-specific developmental effects.

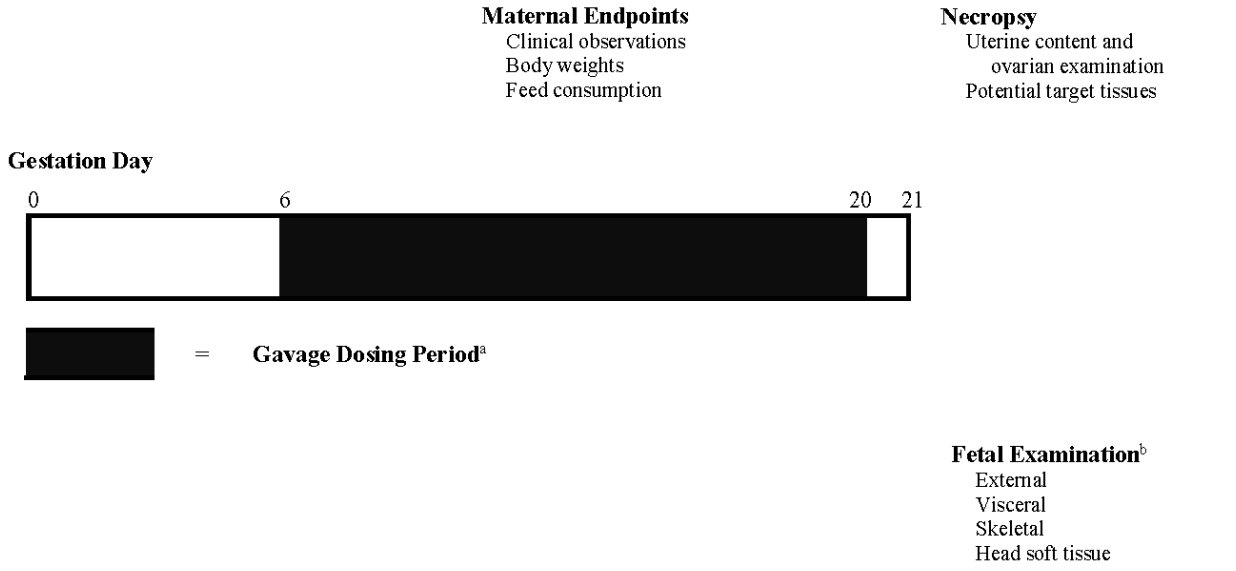
MATERIALS AND METHODS

OVERVIEW OF PRENATAL DEVELOPMENTAL TOXICITY STUDY DESIGNS

Prenatal developmental toxicity studies are conducted to ascertain if *in utero* exposure to a test agent results in embryo-fetal death, structural malformations/variations, growth retardation, or functional deficits that are not secondary to overt maternal toxicity. Overt maternal toxicity has been shown to impact normal embryo-fetal growth and development (e.g., excessively lower maternal body weight gains and lower fetal weights, increased maternal stress in mice, and cleft palate) (Chernoff *et al.*, 1990; USEPA, 1991; Tyl, 2012). However, the presence of maternal toxicity should not *a priori* negate an apparent fetal response. Rather, given the maternal/embryo-fetal interrelationship, fetal findings should be interpreted considering the maternal responses. Conversely, pregnant animals should be administered dose levels of test agent, to the extent feasible (or limit dose) to obtain maximal dam and fetal exposure thereby sufficiently challenging the test system to identify potential developmental hazards (OECD, 2001).

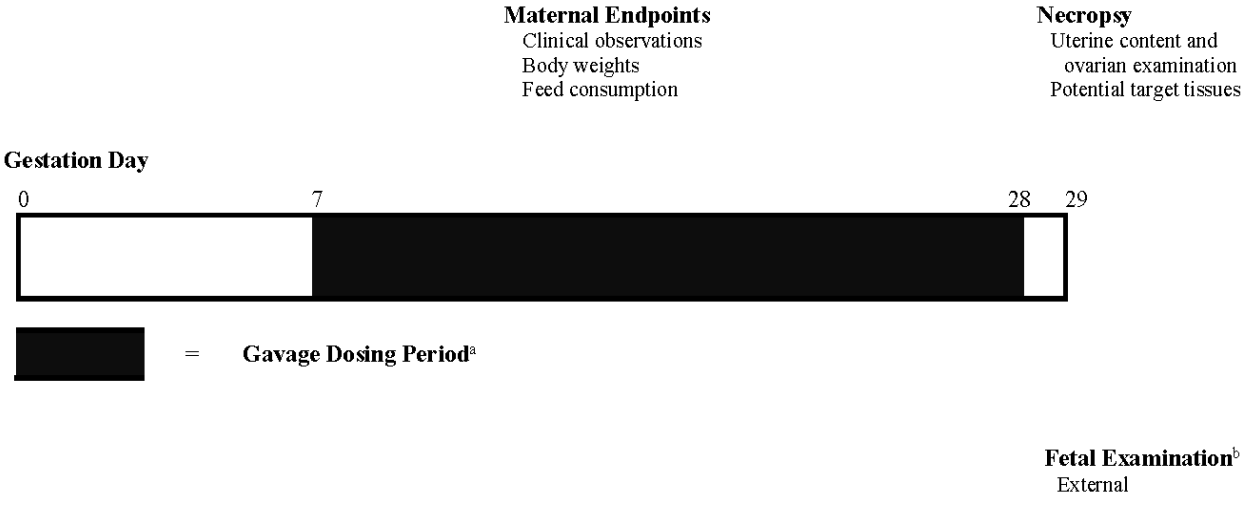
The conduct of a dose range-finding study aids in the determination of dose selection when the potential for test agent-induced maternal toxicity is unknown, and can provide preliminary information on embryo-fetal outcomes (e.g., post-implantation loss, changes in fetal weight, external defects) and inform the prenatal developmental toxicity study design. In the prenatal developmental toxicity study, fetal examination is expanded to include examination of the fetal viscera, head (soft tissue and skeletal components), and the skeleton for osseous and cartilaginous defects. Abnormalities are separated into malformations that are permanent structural changes that may adversely affect survival, development, or function or variations that are a divergence beyond the usual range of structural constitution that may not adversely affect survival or health (USEPA, 1991), consistent with that described by Makris *et al.* (2009). The general study design for the dose range-finding and prenatal developmental toxicity studies in the rat is presented in Figure 1, and the general study design for the dose range-finding rabbit study is presented in Figure 2.

FIGURE 1
Design of the Dose Range-Finding and Prenatal Developmental Toxicity Studies in the Rat



[a] Animals were gavaged once daily from gestation day (GD) 6 to 20 and necropsied on GD 21.
[b] All fetuses were given an external examination (including inspection of the oral cavity). Fetuses in the prenatal developmental toxicity study were also subjected to visceral and skeletal examinations with approximately 50% of the heads examined for soft tissue alterations.

FIGURE 2
Design of the Dose Range-Finding Prenatal Developmental Toxicity Study in the Rabbit



[a] Animals were gavaged once daily from gestation day (GD) 7 to 28 and necropsied on GD 29.
[b] All fetuses were given an external examination (including inspection of the oral cavity).

PROCUREMENT AND CHARACTERIZATION

Vinpocetine

Vinpocetine was obtained from Maypro Industries, LLC (Purchase, NY) in one lot (VA201211001). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH) (Appendix D). The chemical, a white crystalline powder, was identified as vinpocetine using Fourier transform infrared (FTIR) and proton and carbon-13 nuclear magnetic resonance spectroscopy and gas chromatography (GC) with mass spectrometry detection. The optical activity analysis indicated an average rotation of +131.6°, which is consistent with the optical rotation of vinpocetine. Purity of the test article was determined by elemental analyses, proton-induced X-ray emission (PIXE) spectroscopy, differential scanning calorimetry, melting point analysis, high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection, and GC with flame ionization detection (FID).

Karl Fischer titration indicated less than 0.07% water. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for vinpocetine; PIXE analyses indicated no inorganic impurities greater than 0.1%. Melting point analysis averaged 149.88° C and differential scanning calorimetry indicated a purity of 99.9%. HPLC/UV indicated one major peak (99.5% of the total peak area) and two impurities greater than 0.1% of the total peak area (0.17% and 0.28%); the larger impurity peak was tentatively identified as apovincamine. GC/FID indicated one major peak (99.3% of the total peak area) and one reportable impurity (0.67% of the total peak area); this impurity was tentatively identified as apovincamine. Screening for volatiles using a second GC/FID system indicated the presence of 0.018% methylene chloride. The overall purity of lot VA201211001 was determined to be greater than 99.3%.

Stability studies of the bulk chemical were performed using GC/FID. These studies indicated that vinpocetine was stable as a bulk chemical for at least 14 days when stored in sealed amber glass vials at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed double plastic bags in a plastic bucket. Reanalysis of the bulk chemical was performed twice during the studies with GC/FID, and no degradation of the bulk chemical was detected.

Methylcellulose

Methylcellulose was obtained from Spectrum Chemical Manufacturing Corporation (Gardena, CA) in two lots (2CB0045 and 2DH0326); lot 2CB0045 was used in the dose range-finding study in rats, and lot 2DH0326 was used in the prenatal developmental toxicity study in rats and the dose range-finding study in rabbits. Lot 2DH0326 was identified as methylcellulose using FTIR spectroscopy. Duplicate determinations of the methoxy content (30.9% and 31.1%) were within the acceptance limits of 26.0% to 33.0%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for each study by mixing vinpocetine with 0.5% aqueous methylcellulose solution. The analytical chemistry laboratory performed homogeneity studies of 0.1 and 200 mg/mL formulations, syringeability studies for 18- and 22-gauge gavage needles using the 200 mg/mL formulation, resuspendability studies of the 200 mg/mL formulation, and stability studies of the 0.1 mg/mL formulation; all of these analyses were conducted using GC/FID. Homogeneity, syringeability, and resuspendability were confirmed, and stability was confirmed for at least 42 days for dose formulations stored in clear glass bottles with Teflon®-lined lids packaged in sealed amber plastic bags at room temperature and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of vinpocetine were conducted by the analytical chemistry laboratory using GC/FID. During the dose range-finding study in rats, the dose formulations were analyzed once; all five dose formulations analyzed and used were within 10% of the target concentrations (Table D3). Animal room samples of these dose formulations were also analyzed; four of five were within 10% of the target concentrations. During the prenatal developmental toxicity study in rats, the dose formulations were analyzed once; animal room samples of these dose formulations were also analyzed (Table D4). All three dose formulations and all three animal room samples were within 10% of the target concentrations. During the dose range-finding study in rabbits, the dose formulations were analyzed once (Table D5). Of the dose formulations analyzed during the study, all eight were within 10% of the target concentrations; two of four animal room samples were within 10% of the target concentrations.

ANIMAL SOURCE

Female Sprague Dawley (Hsd:Sprague Dawley SD) rats for use in the dose range-finding and prenatal developmental toxicity studies were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN) (Table 1). This stock is routinely used in NTP studies for toxicity evaluation. Sexually mature (12 to 13 weeks old) females were time-mated overnight at the vendor and were received on gestation day (GD) 1 or 2 for both the dose range-finding and prenatal developmental toxicity studies. GD 0 was defined as the day that positive evidence of mating was observed.

Female New Zealand White (Hra:NZW SPF) rabbits for use in the dose range-finding study were obtained from Covance Research Products (Greenfield, IN) (Table 2). Sexually mature females (5 months old) were time mated at the vendor and were received on GD 1 or 2.

ANIMAL HEALTH SURVEILLANCE

In accordance with the NTP Sentinel Animal Program (Appendix G), 10 female rabbits randomly selected from among the study groups were evaluated at the end of the dose range-finding study. Antibodies to Rotavirus were detected in several samples. Rotavirus is a common virus in rabbits that was not considered to have impacted the current study (Sukow *et al*., 2012). All other test results were negative. Disease screening was not conducted in the rats; however, rats were obtained from a commercial colony free of the following rat pathogens: Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan's H1 virus, rat minute virus, reovirus, rat theilovirus, lymphocytic choriomeningitis virus, hantavirus, mouse adenovirus, rat parvovirus, *Mycoplasma pulmonis*, and *Pneumocystis carinii*.

ANIMAL WELFARE

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals and the U.S. Animal Welfare Act and Regulations. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the Southern Research Animal Care and Use

Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

EXPERIMENTAL DESIGN

In the dose range-finding and prenatal developmental toxicity studies, time-mated rats were housed individually, provided NIH-07 feed and water *ad libitum*, and observed at least twice daily for viability (morning and afternoon). Clinical observations were performed on GD 3 (prenatal developmental toxicity study only) and on GD 6 through 21 until removal, typically twice daily (at the time of dose administration and cageside post-dose). Females in the dose range-finding study were weighed daily from GD 3 through 21, and those in the prenatal developmental study were weighed on the day of arrival, on GD 3, and on GD 6 through 21. Feed consumption was recorded for GDs 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, and 18 to 21. Details of the study design including animal source and identification, diet, water, husbandry, environmental conditions, euthanasia, necropsy, and fetal evaluations are summarized in Table 1. Information on feed composition and contaminants is provided in Appendixes E and F.

In the vinpocetine rabbit dose range-finding study, time-mated animals were housed individually, provided Purina 5322 5LMO and/or Teklad 2031C feeds and water *ad libitum*, and observed at least twice daily for viability (morning and afternoon). Clinical observations were recorded on GD 3 and on GD 7 through 29 until removal, typically twice daily (at the time of dose administration and cageside post-dose). Females were weighed daily on GD 3 through 29. Feed consumption was recorded for GDs 3 to 7, 7 to 9, 9 to 12, 12 to 15, 15 to 18, 18 to 21, 21 to 24, 24 to 27, and 27 to 29. Details of the study design including animal source and identification, diet, water, husbandry, environmental conditions, euthanasia, necropsy, and fetal evaluations are summarized in Table 2.

On GD 21, rats were weighed, euthanized by CO_2 inhalation, and examined for gross lesions of the thoracic and abdominal cavities. On GD 29; rabbits were weighed, euthanized with intravenous injection of sodium pentobarbital-containing solution, and examined for gross lesions of the thoracic and abdominal cavities. The ovaries and gravid uterus were excised and weighed (ovaries for prenatal developmental toxicology study only) and placental findings were recorded. The numbers of implantation sites and corpora lutea visible on the surface of each ovary were recorded. Uterine contents were examined for pregnancy status and the number and location of all live

and dead fetuses (a live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and is not markedly autolyzed) and resorptions were recorded. Resorptions were classified as early or late. Early resorptions included a conceptus characterized by a grossly necrotic mass that had no recognizable fetal form or presence of nidation sites ("pregnant by stain"). Late resorptions were characterized by grossly necrotic but recognizable fetal form with placental remnants visible (Suckow *et al.*, 2006; Hayes and Kruger, 2014). Post-implantation loss was calculated as the number of dead plus resorbed conceptuses divided by the total number of implantations (multiplied by 100). For each uterus with no macroscopic evidence of implantation, the uterus was stained with 10% (v/v) ammonium sulfide to visualize any possible early implantation sites (Salewski, 1964).

Adult females that were euthanized moribund, delivered early, or found dead received a gross necropsy that included an examination of the thoracic and abdominal viscera for evidence of dosing trauma or toxicity. The uterus of each female was examined and stained, if necessary, to determine pregnancy status. Females were not retained for further examination.

All rabbits that aborted (defined as delivering before GD 29), were euthanized moribund, or found dead received a gross necropsy that included examination of the thoracic and abdominal viscera for evidence of dosing trauma, toxicity, and gross lesions. The uterus of each female was examined and stained, as necessary, to determine pregnancy status. Females were not retained for further examination.

Dose Range-Finding Study in Rats

Time-mated rats were individually identified by tail marking and randomized by GD 3 body weight stratification into six groups (vehicle control, low, low-mid, mid, mid-high, or high) using Southern Research's Instem™ Provantis® (version 8) electronic data collection system.

Groups of 10 time-mated female rats were administered 0 (vehicle control), 20, 40, 80, 160, or 320 mg vinpocetine/kg/day, based on the most recent body weight, in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control animals received aqueous methylcellulose alone; the dosing volume was 5 mL/kg/day.

Given the limited data in one publication and potential strain differences, the high dose of 320 mg/kg was chosen to ensure that the animals were sufficiently challenged. It was recognized that excessive toxicity may be observed at the higher dose levels. Data from this study were used to inform a prenatal developmental toxicity study.

On GD 21, fetuses were removed from the uterus, individually weighed (live fetuses only), and examined externally for alterations, including inspection of the oral cavity for cleft palate. Live fetuses were euthanized by decapitation or with intraperitoneal injection of a commercially available solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation. Fetuses were not retained following completion of the external examination.

Prenatal Developmental Toxicity Study in Rats

On receipt (GD 1 or 2), time-mated rats were individually identified by tail marking and randomized, based on GD 3 body weight stratification, into four groups (vehicle control, low, mid, or high) using Southern Research's Instem™ Provantis® (version 9) electronic data collection system. Dams were delivered over a 4-day period to allow for a staggered study start.

Groups of 25 time-mated female rats were administered 0 (vehicle control), 5, 20, or 60 mg vinpocetine/kg/day, based on the most recent body weight, in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20 (15 days). Vehicle control animals received the aqueous methylcellulose vehicle alone; the dosing volume was 5 mL/kg.

On GD 21, fetuses were removed from the uterus, and live fetuses individually weighed. The uteri of animals that did not appear pregnant were examined for nidations (implantation sites) by staining with 0.5% ammonium sulfide (Salewski, 1964; Tyl and Marr, 2006). All fetuses were examined externally for alterations, including inspection of the oral cavity for cleft palate. Live fetuses were subsequently euthanized by oral administration of sodium pentobarbital. Fetal sex was confirmed by inspection of gonads *in situ*. All fetuses were examined for soft tissue alterations under a stereomicroscope (Staples, 1974; Stuckhardt and Poppe, 1984). The heads were removed from approximately half of the fetuses in each litter and fixed in Bouin's solution and subsequently examined by free-hand sectioning (Thompson, 1967). Fetuses were eviscerated, fixed in ethanol, macerated in potassium hydroxide,

stained with alcian blue and alizarin red, and examined for subsequent cartilage and osseous alterations (Marr *et al.*, 1992; Tyl and Marr, 2006). External, visceral, and skeletal fetal alterations were recorded as developmental variations or malformations.

Dose Range-Finding Study in Rabbits

Groups of eight time-mated female rabbits were administered 0 (vehicle control), 25, 75, 150, or 300 mg vinpocetine/kg/day based on the most recent body weight in 0.5% aqueous methylcellulose by gavage from GD 7 to GD 28. Vehicle control animals received aqueous methylcellulose alone; the dosing volume was 5 mL/kg. The high dose of 300 mg/kg was chosen based on data from the rat range-finding study and the limited toxicokinetic data in the literature on rabbits, suggesting similar disposition of vinpocetine between rats and rabbits (Vereczkey *et al.*, 1979a; Nie *et al.*, 2006; Ribeiro *et al.*, 2007; Xu *et al.*, 2009; Xia *et al.*, 2010; Sozański *et al.*, 2011).

On GD 21, live fetuses were removed from the uterus, individually weighed (live fetuses only), and examined externally for alterations, including inspection of the oral cavity for cleft palate. Live fetuses were euthanized by intraperitoneal injection of a commercially available solution containing sodium pentobarbital. Fetuses were not retained following completion of the external examination.

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Vinpocetine in Rats

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Study Laboratory Southern Research (Birmingham, AL)	Southern Research (Birmingham, AL)
Strain and Species Sprague Dawley (Hsd:Sprague Dawley SD) rats	Sprague Dawley (Hsd:Sprague Dawley SD) rats
Animal Source Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)	Enviro (formerly Harlan Laboratories, Inc., Indianapolis, IN)
Day of Arrival February 19, 2014 [gestation day (GD) 1 or 2]	January 14 or 16, 2015 (GD 1 or 2)
Average Age on Arrival 12 weeks	12 to 13 weeks
Weight Range at Randomization 201.7 g to 256.4 g on GD 3	190.4 g to 260.2 g on GD 3
Calendar Day of First Dose (GD 6) and Last Dose (GD 20) GD 6 (February 23 or 24, 2014) and GD 20 (March 9 or 10, 2014); staggered start	GD 6 (January 18 to 21, 2015) and GD 20 (February 1 to 4, 2015); staggered start
Duration of Dosing GD 6 to 20, once daily	GD 6 to 20, once daily
Size of Study Groups 10 time-mated females	25 time-mated females
Method of Randomization and Identification Time-mated animals were uniquely identified on day of receipt by tail marking. Animals were assigned to exposure groups by GD 3 body weight stratified randomization using Instem Provantis® (version 8) electronic data collection system. Each animal was assigned a unique animal number in Provantis®. This number was linked to the respective tattoo and all data collected during the study was associated with the Provantis® animal number.	Same as dose range-finding study; Instem Provantis® (version 9) electronic data collection system.
Animals per Cage 1	1
Diet Irradiated NIH-07 Certified Rodent Diet wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as dose range-finding study
Water Tap water (Birmingham Water Works Co., Birmingham, AL, municipal supply) via automatic watering system, available *ad libitum*	Same as dose range-finding study
Cages Solid bottom polycarbonate cages (Lab Products, Seaford, DE), changed weekly	Same as dose range-finding study
Bedding Certified irradiated Sani-Chips® hardwood cage bedding (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Same as dose range-finding study

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Vinpocetine in Rats

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Cage Filters Spunbonded Remay (Andico, Birmingham, AL), changed every 2 weeks	Same as dose range-finding study
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as dose range-finding study
Animal Room Environment Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Same as dose range-finding study
Doses 0, 20, 40, 80, 160, or 320 mg/kg in 0.5% methylcellulose (dosing volume 5 mL/kg)	0, 5, 20, or 60 mg/kg in 0.5% aqueous methylcellulose (dosing volume 5 mL/kg)
Type and Frequency of Observation of Dams Observed for viability twice daily from GD 3 through GD 20. Clinical observations were recorded twice daily from GD 6 until necropsy [prior to dosing (out of cage) and at 1 to 3 hours post-dose (cageside)]. Animals were weighed daily beginning on GD 3. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.	Observed for viability twice daily from GD 3 through GD 20. Clinical observations were recorded on GD 3 (out of cage) and at 1 to 3 hours post-dose (cageside) from GD 6 through GD 20. Animals were weighed on the day of arrival, on GD 3, and on GD 6 through 21. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.
Primary Method of Euthanasia 100% C`O$_2$ (adults) or intraperitoneal injection of a solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation (fetuses)	Same as dose range-finding study
Necropsy and Postmortem Evaluation of Females On GD 21, terminal body and gravid uterine weights were recorded and the uterine contents examined. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses (live or dead) and resorptions (early or late) and the total number of implantation sites were recorded; if no macroscopic evidence of pregnancy, the uterus was stained to visualize potential evidence of implantation sites. For animals removed early, gross necropsy including an examination of the thoracic and abdominal viscera was performed. The uterus of each female was examined to determine pregnancy status or, if no evidence of pregnancy, stained to visualize possible early implantation sites.	On GD 21, terminal body, ovarian, and gravid uterine weights were recorded. Uterine contents were examined. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses (live or dead) and resorptions (early or late) and the total number of implantation sites were recorded; if no macroscopic evidence of pregnancy, the uterus was stained to visualize potential evidence of implantation sites. For animals removed early, gross necropsy including an examination of the thoracic and abdominal viscera was performed. The uterus of each female was examined to determine pregnancy status or, if no evidence of pregnancy, stained to visualize possible early implantation sites.
Fetal Evaluation Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate.	Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate. Placental morphology was also evaluated. Live fetuses were euthanized and then examined for visceral morphologic abnormalities by fresh dissection. The sex of each fetus was confirmed by internal examination. The heads from approximately one half of the fetuses in each litter were fixed, sectioned, and examined. All fetuses were eviscerated, fixed, stained, and examined for skeletal developmental variations, malformations, or other morphologic findings.

TABLE 2
Experimental Design and Materials and Methods in the Dose Range-Finding Gavage Study of Vinpocetine in Rabbits

Study Laboratory
Southern Research (Birmingham, AL)

Strain and Species
New Zealand White (Hra:NZW SPF) rabbits

Animal Source
Covance Research Products (Greenfield, IN)

Day of Arrival
April 24, 2015 (GD 1 or 2)

Average Age on Arrival
5 to 6 months

Weight Range at Randomization
2,676.0 g to 3,561.6 g on GD 3

Calendar Day of First Dose (GD 7) and Last Dose (GD 28)
GD 7 (April 29 or 30, 2015) and GD 28 (May 20 or 21, 2015); staggered start

Duration of Dosing
GD 7 to 28, once daily

Size of Study Groups
8 time-mated females

Method of Randomization and Identification
Time-mated animals were individually identified by ear marking and randomized by GD 3 body weight stratification into five groups using Instem Provantis® (version 9) electronic data collection system.

Animals per Cage
1

Diet
Irradiated Purina 5322 5LMO (Purina, Richmond, IN) and Teklad 2031C (Harlan, Madison, WI) Certified Rabbit Diets, available *ad libitum*; timothy hay (BioServe, Flemington, NJ) once daily as consumable enrichment

Water
Tap water (Birmingham Water Works Co., Birmingham, AL, municipal supply) via automatic watering system, available *ad libitum*

Cages
Perforated-bottom stainless steel cages (Allentown Caging Equipment Co, Allentown, PA), changed every 2 weeks

Bedding
Paper cage liners (Manufacturer, City, ST), changed 3 times per week

Racks
Stainless steel (Allentown Caging Equipment Co, Allentown, PA), changed every 2 weeks

Animal Room Environment
Temperature: 61° to 72°F
Relative humidity: 30% to 70%
Room fluorescent light: 12 hours/day
Room air changes: at least 14/hour

Doses
0, 25, 75, 150, or 300 mg/kg in 0.5% methylcellulose (dosing volume 5 mL/kg)

TABLE 2
Experimental Design and Materials and Methods in the Dose Range-Finding Gavage Study of Vinpocetine in Rabbits

Type and Frequency of Observation of Does Observed for viability twice daily from GD 3 through GD 29. Clinical observations were recorded twice daily on GD 3 (out of cage) and at 1 to 3 hours post-dose (cageside) beginning on GD 7. Animals were weighed daily beginning on GD 3. Feed consumption was recorded at 3-day intervals from GD 3 through GD 29.
Primary Method of Euthanasia Intravenous injection (adults) or intraperitoneal injection (fetuses) of a solution containing sodium pentobarbital
Necropsy and Postmortem Evaluation of Does On GD 29, terminal body and gravid uterine weights were recorded and the uterine contents examined. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses (live and dead) and resorptions (early or late) and the total number of implantation sites were recorded; if no macroscopic evidence of pregnancy, the uterus was stained to visualize potential evidence of implantation sites. For animals removed early, gross necropsy including an examination of the thoracic and abdominal viscera was performed. The uterus of each female was examined to determine pregnancy status or, if no evidence of pregnancy, stained to visualize possible early implantation sites.
Fetal Evaluation Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included examination of the oral cavity for cleft palate.

STATISTICAL METHODS

In the dose range-finding studies and the prenatal developmental toxicity study, statistical analyses were performed on data from pregnant females that survived until the end of the study and were examined on GD 21 (rats) or GD 29 (rabbits) and from live fetuses. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary NC).

Descriptive Statistics

Maternal Parameters: Disposition of pregnant females is presented as the number of animals that were moribund, found dead, or survived to the end of the study (Tables 3, 7, and 13). Summaries of maternal clinical observations are presented as the total number of animals with the observation and the first day of onset (Tables A1 and B1).

Maternal body weights were measured daily starting at GD 3. Mean body weights are shown in Figures 3 and 4 (Tables A2 and B2). Body weight gains were calculated over each three-day interval and from GD 6 to GD 21 (rats) or GD 29 (rabbits). Terminal maternal body weights were adjusted for gravid uterine weight by subtracting the gravid uterine weight from the dam's body weight. Daily feed consumption was averaged over each three-day

interval and from GD 6 to GD 21 (rats) or GD 29 (rabbits). These continuous variables, in addition to gravid uterine weights and other organ weights, were summarized with means and standard errors.

Placental and Fetal Parameters: Data on uterine contents are reported as means and standard errors of counts per dam/litter (corpora lutea, implants, resorptions, dead fetuses) and as total numbers of occurrences (resorptions, dead fetuses) and are presented in Tables 6 and 10. Data from females that were not pregnant or that did not survive to the end of the study were not included. Post-implantation loss is calculated as a percentage of the number of implants per dam. Fetal findings are reported as means and standard errors of counts per litter (numbers of live fetuses, male fetuses, female fetuses), means and standard errors of litter means (fetal weight, male fetal weight, female fetal weight) and total numbers of occurrences (total number of live fetuses). In addition, several calculated variables are reported, including the percentage of live male fetuses per litter.

Incidences of morphologic findings from the gross, external, visceral, skeletal and head examinations of pathology of placentae and fetuses are presented as number and percentage of affected fetuses and as number and percentage of affected litters. Fetal findings listing dam and fetus identification number are provided in Table B6.

Analysis of Maternal Parameters and Uterine Contents

Maternal organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Non-normally distributed variables, such as food consumption and uterine content endpoints, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). For normally distributed and non-normally distributed variables, Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-related trends at $P < 0.01$ to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis.

Fetal body weights were analyzed using mixed effects linear models, with litter as a random effect to account for potential within-litter correlations. To test for a linear trend, dose was entered into the model as its numeric value and its significance was evaluated. For pairwise comparisons with the control group, a second mixed effects model with dose entered into the model as a categorical variable was estimated, followed by the Dunnett (1955)-Hsu (1992) multiple comparisons test.

Analysis of Incidences of Gross Pathology and Morphology Findings

Incidences of gross findings, malformations, and variations in the fetuses were summarized and analyzed as number of litters affected and as number of fetuses affected. Incidences of gross findings, malformations and numbers of litters affected were analyzed using the Cochran-Armitage trend test (Armitage, 1955) and Fisher's exact test (Gart *et al.*, 1979). Incidences of numbers of fetuses affected were analyzed using mixed effects logistic regression in which the litter was a random effect in order to account for potential litter effects (Zorilla, 1997; Pendergast *et al.*, 2005; Li *et al.*, 2011). For each fetal finding, an initial mixed effects logistic regression model incorporated dose as its numeric value to assess the significance of a dose-related trend; a subsequent logistic regression model incorporated dose as a categorical variable to assess the significance of contrasts of each dose group with the control group. To conduct the mixed effects logistic regression analyses, at least one finding was required per dose group and the correlation matrix describing the relationship between litters was required to be "positive definite." If the mixed effects logistic regression failed to converge or did not meet the specified criteria, two separate analyses were used to bracket the true P value. The Cochran-Armitage trend test and Fisher's exact test were used with litter as the experimental unit to calculate the upper limit for the true P value and with fetus as the experimental unit to calculate the lower limit for the true P value.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP developmental and reproductive toxicity studies. However, historical control data are often helpful in interpreting potential exposure-related effects, particularly for uncommon fetal findings that occur at a very low incidence. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors that may affect the background incidences of fetal findings at

a variety of sites are diet, sex, strain/stock, route of exposure, study type, and/or laboratory that conducted the study. The NTP historical control database for teratology studies contains all fetal evaluations (e.g., teratology studies or modified one generation studies) for each laboratory. In general, the historical control database for a given study includes studies using the same route of administration and study design. Historical control data for rats in this Prenatal Developmental Toxicity Study Report represents data from gavage studies conducted at Southern Research Institute. The concurrent controls are included in the historical control data set. NTP historical control data are available online at https://ntp.niehs.nih.gov/go/historical_controls.

QUALITY ASSURANCE METHODS

The dose range-finding and prenatal developmental toxicity studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). Records from these studies were submitted to the NTP Archives. The prenatal developmental toxicity study was audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the final study data tables for the dose range-finding and prenatal developmental toxicity studies and a draft of this NTP Prenatal Developmental Toxicity Study Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Prenatal Developmental Toxicity Study Report.

RESULTS

DOSE RANGE-FINDING STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

All rats survived until the end of the study (Table 3). Clinical observations of red and/or brown vaginal discharge occurred in all groups (4, 5, 7, 10, 10, and 9 dams in the 0, 20, 40, 80, 160, and 320 mg/kg groups, respectively; Table A1). Other observations included brown discoloration of the nares (1, 2, 8, and 10 dams in the 40, 80, 160 and 320 mg/kg groups, respectively) and piloerection in all dams administered 160 or 320 mg/kg, which occurred beginning on gestation day (GD) 7 through the end of the dosing period.

TABLE 3
Maternal Disposition of Rats in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Time-mated females	10	10	10	10	10	10
Pregnant (on GD 21)	8	10	8	10	10	9
Non-pregnant (on GD 21)	2	0	2	0	0	1

Body Weights and Feed Consumption

Dose-related decreases in mean maternal body weights and mean body weight gains were observed in groups administered 40 mg/kg or greater, relative to those of the vehicle controls, from GD 6 to 21 (Figure 3, Tables 4 and 6). Maternal body weights were 28%, 31%, and 35% lower than those of vehicle controls in the 80, 160, and 320 mg/kg groups, respectively (Table 4). When adjusted for gravid uterine weight (at necropsy), maternal body weights were 4.5%, 11%, and 14% lower than those of vehicle controls in the 80, 160, and 320 mg/kg groups, respectively (Table 6) and were associated with the embryo fetal loss also observed in these groups. Daily mean body weights for dams in each dose group are available in Table A2.

Concomitant treatment-related, dose-dependent decreases in maternal feed consumption were observed with doses of 40 mg/kg or greater from GD 6 to GD 21 (Table 5) and were 8%, 19%, 28%, and 38% lower than that of the vehicle controls in the 40, 80, 160, and 320 mg/kg groups, respectively.

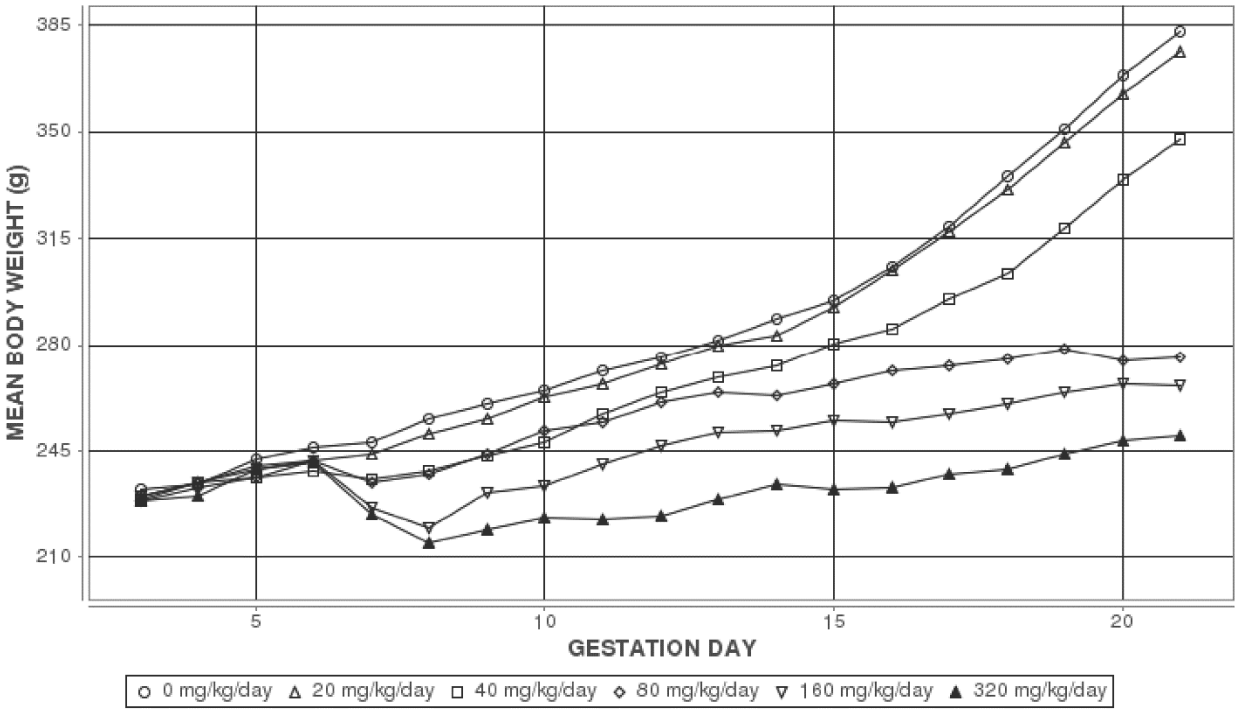


FIGURE 3
Maternal Growth Curves for Pregnant Rats Administered Vinpocetine by Gavage in the Dose Range-Finding Study
Information for statistical significance in maternal weights is provided in Tables 4 and A2.

TABLE 4
Summary of Maternal Body Weight Gains of Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Gestation Day Interval						
6 to 21	137.1 ± 5.3** (8)[b]	134.5 ± 8.5 (10)	109.1 ± 14.3* (8)	34.3 ± 3.1** (10)	24.8 ± 4.3** (10)	9.3 ± 2.4** (9)
3 to 6	13.9 ± 3.4 (8)	11.9 ± 0.8 (10)	9.3 ± 1.9 (8)	13.6 ± 2.3 (10)	11.6 ± 1.7 (10)	12.2 ± 1.7 (9)
6 to 9	14.6 ± 1.5** (8)	13.8 ± 1.6 (10)	5.2 ± 4.8* (8)	2.1 ± 2.2** (10)	–10.2 ± 2.2** (10)	–21.6 ± 3.0** (9)
9 to 12	15.0 ± 1.2* (8)	18.0 ± 1.0 (10)	20.6 ± 3.6 (8)	16.9 ± 2.3 (10)	15.3 ± 1.9 (10)	4.4 ± 2.6** (9)
12 to 15	19.0 ± 1.9** (8)	18.8 ± 1.8 (10)	16.1 ± 2.6 (8)	6.2 ± 1.5** (10)	8.0 ± 2.7** (10)	8.3 ± 2.0** (9)
15 to 18	41.1 ± 1.4** (8)	38.7 ± 2.8 (10)	23.4 ± 6.7** (8)	8.5 ± 1.3** (10)	5.6 ± 1.3** (10)	7.2 ± 2.2** (9)
18 to 21	47.4 ± 2.2** (8)	45.2 ± 3.4 (10)	43.8 ± 6.7 (8)	0.6 ± 2.3** (10)	6.0 ± 2.7** (10)	11.0 ± 2.8** (9)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Body weight gains for pregnant animals are given in grams. Data are displayed as mean ± standard error.
[b] Number of dams weighed is given in parentheses.

TABLE 5
Summary of Maternal Feed Consumption of Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Gestation Day Interval						
6 to 21	21.2 ± 0.4** (8)[b]	21.2 ± 0.6 (10)	19.5 ± 0.8 (8)	17.1 ± 0.4** (10)	15.2 ± 0.5** (10)	13.0 ± 0.4** (9)
3 to 6	18.9 ± 0.8 (8)	19.0 ± 0.5 (10)	18.5 ± 0.8 (8)	18.8 ± 0.8 (10)	18.7 ± 0.4 (10)	18.9 ± 0.7 (9)
6 to 9	18.4 ± 0.7** (8)	17.3 ± 0.6 (10)	14.0 ± 1.0** (8)	11.5 ± 0.3** (10)	6.8 ± 0.5** (10)	5.3 ± 0.7** (9)
9 to 12	20.8 ± 0.5** (8)	20.4 ± 0.5 (10)	18.8 ± 0.9 (8)	18.1 ± 0.5** (10)	15.7 ± 0.5** (10)	11.7 ± 0.9** (9)
12 to 15	21.2 ± 0.5** (8)	21.6 ± 0.6 (10)	21.5 ± 1.0 (8)	19.8 ± 0.4 (10)	18.7 ± 0.8* (10)	16.3 ± 0.6** (9)
15 to 18	22.5 ± 0.4** (8)	23.8 ± 0.9 (10)	21.0 ± 1.7 (8)	19.7 ± 0.7 (10)	18.4 ± 0.8** (10)	16.1 ± 0.7** (9)
18 to 21	22.8 ± 0.5** (8)	22.8 ± 0.8 (10)	22.4 ± 1.5 (8)	16.7 ± 0.7** (10)	16.3 ± 0.6** (10)	15.6 ± 0.4** (9)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Feed consumption for pregnant animals is given in grams/day. Data are displayed as mean ± standard error.
[b] Number of dams with feed consumption measured is given in parentheses.

Maternal and Litter Observations

At necropsy, no gross observations related to vinpocetine administration were observed.

There was an exposure-related effect on percent post-implantation loss as a result of increased early resorptions across all groups (Table 6). Although not statistically significant at 20 and 40 mg/kg, these values were greater than those of the vehicle controls. At doses of 80 mg/kg and above, dams exhibited total resorption of their litters, with the exception of one dam in the 160 mg/kg group. As a result of the increased post-implantation loss, there was a decrease in the number of live fetuses per litter in the 40 mg/kg group in comparison to the vehicle controls, and there were no live fetuses at doses of 80 mg/kg and above with the exception of the one litter in the 160 mg/kg group that contained 12 live fetuses. These findings were associated with reductions in mean gravid uterine weights (13.7%, 26.0%, 97.6%, 88.8%, and 96.8% less than vehicle controls at 20, 40, 80, 160, and 320 mg/kg, respectively).

There were no exposure-related effects on fetal weight or fetal sex ratio in the 20 or 40 mg/kg groups; fetal weight and sex ratio could not be evaluated at 80, 160, or 320 mg/kg due to the presence of only one litter among these groups (Table 6).

TABLE 6
Summary of Uterine Content Data for Rats in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Pregnancy Summary						
Mated females	10	10	10	10	10	10
Pregnant females	8	10	8	10	10	9
Pregnant females examined on GD 21[a]	8	10	8	10	10	9
Corpora lutea per female[b]	15.63 ± 0.50 (8)	16.20 ± 0.49 (10)	17.25 ± 1.08 (8)	16.50 ± 0.70 (10)	17.00 ± 0.54 (10)	15.44 ± 0.50 (9)
Implantations[b] per female	14.38 ± 0.42 (8)	14.10 ± 0.74 (10)	14.00 ± 0.65 (8)	13.40 ± 1.19 (10)	14.50 ± 0.76 (10)	14.56 ± 0.73 (9)
Percent post-implantation loss[b]	5.30 ± 1.78** (8)	18.41 ± 11.70 (10)	27.55 ± 12.35 (8)	100.00 ± 0.00** (10)	90.77 ± 9.23** (10)	100.00 ± 0.00** (9)
Total resorptions per litter[b]	0.75 ± 0.25** (8)	2.60 ± 1.65 (10)	3.88 ± 1.79 (8)	13.40 ± 1.19** (10)	13.30 ± 1.56** (10)	14.56 ± 0.73** (9)
Early resorptions per litter[b]	0.75 ± 0.25** (8)	2.60 ± 1.65 (10)	3.88 ± 1.79 (8)	13.40 ± 1.19** (10)	13.30 ± 1.56** (10)	14.56 ± 0.73** (9)
Late resorptions per litter[b]	0.00 ± 0.00 (8)	0.00 ± 0.00 (10)	0.00 ± 0.00 (8)	0.00 ± 0.00 (10)	0.00 ± 0.00 (10)	0.00 ± 0.00 (9)
Dead fetuses per litter[b]	0.00 ± 0.00 (8)	0.00 ± 0.00 (10)	0.00 ± 0.00 (8)	0.00 ± 0.00 (10)	0.00 ± 0.00 (10)	0.00 ± 0.00 (9)
Number of early resorptions[c]	6	26	31	134	133	131
Number of late resorptions[c]	0	0	0	0	0	0
Number of whole litter resorptions[a]	0**	1	1	10**	9**	9**
Number of dead fetuses[c]	0	0	0	0	0	0
Live Fetuses[b]						
Number of live fetuses	109	115	81	0	12	0
Live fetuses per litter	13.63 ± 0.53** (8)	11.50 ± 1.78 (10)	10.13 ± 1.85 (8)	0.00 ± 0.00** (10)	1.20 ± 1.20** (10)	0.00 ± 0.00** (9)
Live male fetuses per litter	5.88 ± 0.55** (8)	5.70 ± 0.96 (10)	6.13 ± 1.08 (8)	0.00 ± 0.00** (10)	0.40 ± 0.40** (10)	0.00 ± 0.00** (9)
Live female fetuses per litter	7.75 ± 0.73** (8)	5.80 ± 1.04 (10)	4.00 ± 1.16* (8)	0.00 ± 0.00** (10)	0.80 ± 0.80** (10)	0.00 ± 0.00** (9)
Percent live male fetuses per litter	43.35 ± 4.01 (8)	48.83 ± 4.82 (9)	64.60 ± 7.92 (7)		33.33 (1)	
Fetal Weight[c]						
Fetal body weight per litter (g)	5.18 ± 0.07 (8)	5.26 ± 0.16 (9)	5.06 ± 0.16 (7)	—[d]	4.98 (1)	—[d]
Male fetal weight per litter (g)	5.33 ± 0.07 (8)	5.41 ± 0.16 (9)	5.16 ± 0.16 (7)		5.06 (1)	
Female fetal weight per litter (g)	5.06 ± 0.07 (8)	5.11 ± 0.14 (9)	4.92 ± 0.19 (6)		4.95 (1)	

TABLE 6
Summary of Uterine Content Data for Rats in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Gravid Uterine Weight[e]						
Gravid uterine weight (g)	96.41 ± 3.22** (8)	83.23 ± 11.81 (10)	71.35 ± 12.39* (8)	2.35 ± 0.20** (10)	10.78 ± 8.21** (10)	3.09 ± 0.56** (9)
Terminal body weight (g)	383.2 ± 4.7** (8)	376.2 ± 10.7 (10)	347.5 ± 13.9** (8)	276.1 ± 3.4** (10)	266.3 ± 5.4** (10)	250.2 ± 5.3** (9)
Adjusted body weight (g)	286.83 ± 3.30** (8)	292.96 ± 8.77 (10)	276.18 ± 4.45 (8)	273.76 ± 3.31 (10)	255.53 ± 10.69** (10)	247.10 ± 5.39** (9)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to the end of the study.
(g) = grams
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
** P≤0.01
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] Statistical analysis performed using a mixed effect linear model with litter as a random effect
[d] No live fetuses in dose group
[e] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight

Fetal Findings

External

There were no external malformations or variations attributed to vinpocetine exposure at 20, 40, 80, 160, or 320 mg/kg per day (Tables A4 and A5). External findings in exposed fetuses were limited to a singular occurrence of subcutaneous hemorrhage in the 20 mg/kg group, which was considered to be unrelated to vinpocetine exposure.

Dose Selection Rationale for the Prenatal Development Toxicity Study in Rats

In the dose range-finding study, embryo-fetal loss occurred at all doses (20, 40, 80, 160, and 320 mg/kg per day), and an increase in the number of fetal resorptions was observed between 40 and 80 mg/kg per day (28% and 100% post-implantation loss, respectively). Based on these findings, 60 mg/kg per day was chosen as the top dose for the prenatal developmental toxicity study and nearly half-log dose spacing was used to provide adequate spacing for evaluation of potential dose-response relationships and to ideally capture the no-observed-effect level (NOEL). The doses selected for the prenatal developmental toxicity study were 0, 5, 20, and 60 mg/kg per day.

PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

No animals were removed from the study prior to scheduled necropsy (Table 7). There were test article-related clinical observations at ≥ 20 mg/kg, which were limited to a dose-related increase in the incidence of red and/or brown vaginal discharge (6, 4, 13, and 17 dams in the 0, 5, 20, and 60 mg/kg groups, respectively; Table B1). Observations of abnormal vaginal discharge generally began on GD 13 and continued until GD 19. There were no treatment-related clinical observations in the 5 mg/kg animals.

TABLE 7
Maternal Disposition of Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Time-mated females	25	25	25	25
Pregnant (on GD 21)	21	20	22	20
Non-pregnant (on GD 21)	4	5	3	5

Body Weights and Feed Consumption

Significant decreases in mean maternal body weights and mean body weight gains during gestation occurred in the 60 mg/kg group (Figure 4; Tables 8 and 10). Relative to the vehicle controls, administration of 60 mg/kg vinpocetine resulted in a 23% reduction in maternal body weight on GD 21 (Table 10) and a 61% reduction in maternal body weight gain from GD 6 to 21 (Table 8). These maternal body weight decreases in the 60 mg/kg group were associated with 83% post-implantation loss (compared to 3% in vehicle controls), which included total litter resorptions in 12 dams and resulted in fewer fetuses (Table 10). There were no significant body weight changes in the 5 or 20 mg/kg groups. Daily mean body weights for dams in each dose group are available in Table B2.

Treatment-related effects on feed consumption were limited to the 60 mg/kg group and consisted of a slight decrease in overall feed consumption from GD 6 to 21 in this dose group (9.5% lower than vehicle controls; Table 9). Feed consumption in the 5 and 20 mg/kg dose groups was similar to that of the vehicle controls.

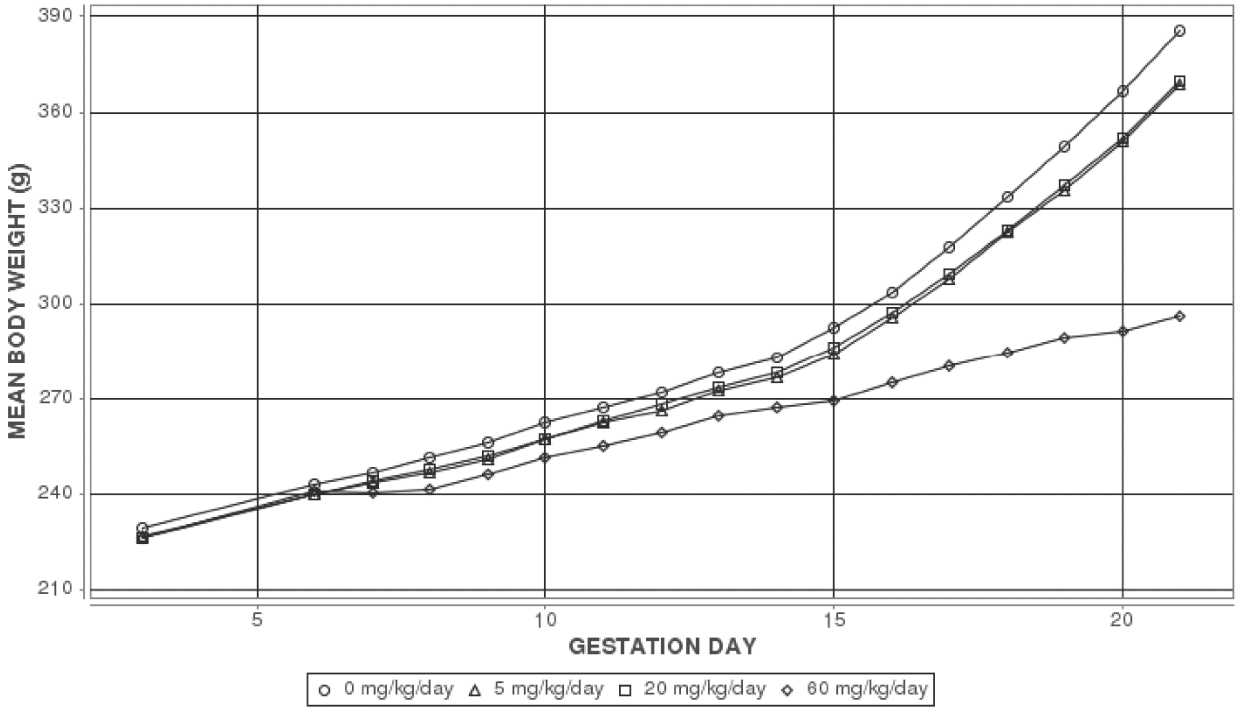


FIGURE 4
Maternal Growth Curves for Pregnant Rats Administered Vinpocetine by Gavage in the Prenatal Developmental Toxicity Study
Information for statistical significance in maternal weights is provided in Tables 8 and B2.

TABLE 8
Summary of Maternal Body Weight Gains of Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Gestation Day Interval				
6 to 21	142.8 ± 3.4** (21)[b]	128.7 ± 7.4 (20)	130.0 ± 5.1 (22)	55.3 ± 7.9** (20)
3 to 6	13.7 ± 1.0 (21)	13.1 ± 1.0 (20)	13.5 ± 1.2 (22)	14.5 ± 2.0 (20)
6 to 9	13.2 ± 0.7** (21)	11.3 ± 0.8 (20)	12.2 ± 0.9 (22)	5.2 ± 1.0** (20)
9 to 12	16.0 ± 0.6 (21)	15.4 ± 0.9 (20)	16.0 ± 0.8 (22)	13.4 ± 1.2 (20)
12 to 15	20.7 ± 0.8** (21)	17.7 ± 1.3 (20)	17.8 ± 0.8 (22)	9.9 ± 1.7** (20)
15 to 18	40.8 ± 1.4** (21)	38.1 ± 2.4 (20)	37.0 ± 2.0 (22)	15.4 ± 2.5** (20)
18 to 21	52.2 ± 1.7** (21)	46.2 ± 3.3 (20)	47.0 ± 2.4 (22)	11.5 ± 4.5** (20)

** Statistically significant (P≤0.01) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
[a] Body weight gains for pregnant animals are given in grams. Data are displayed as mean ± standard error.
[b] Number of dams weighed is given in parentheses.

TABLE 9
Summary of Maternal Feed Consumption of Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Gestation Day Interval				
6 to 21	22.0 ± 0.3** (21)[b]	21.6 ± 0.4 (20)	22.2 ± 0.3 (22)	19.9 ± 0.4** (20)
3 to 6	19.6 ± 0.6 (16)	19.5 ± 0.4 (17)	19.8 ± 0.4 (18)	20.4 ± 0.6 (16)
6 to 9	20.0 ± 0.4** (21)	19.6 ± 0.3 (20)	19.3 ± 0.3 (22)	17.2 ± 0.4** (20)
9 to 12	20.7 ± 0.3* (21)	20.1 ± 0.4 (20)	20.9 ± 0.3 (22)	18.8 ± 0.5** (20)
12 to 15	21.2 ± 0.3 (21)	21.0 ± 0.5 (20)	21.5 ± 0.4 (22)	21.5 ± 0.6 (20)
15 to 18	23.5 ± 0.3 (21)	23.3 ± 0.5 (20)	24.7 ± 0.4 (22)	21.7 ± 0.5* (20)
18 to 21	24.4 ± 0.4** (21)	24.1 ± 0.6 (20)	24.7 ± 0.4 (22)	20.2 ± 0.7** (20)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Feed consumption for pregnant animals is given in grams/day. Data are displayed as mean ± standard error.
[b] Number of dams with feed consumption measured is given in parentheses.

Maternal and Litter Observations

There were no notable maternal necropsy findings. The number of pregnant females and the mean numbers of corpora lutea and implantation sites were similar across groups.

There was a significant effect on percent post-implantation loss in the 60 mg/kg group (83.1% compared to 3.3% in vehicle controls) as a result of resorption of entire litters in 12 of the dams and increased incidences of resorptions in 7 of the dams (Table 10). As a result of the increased post-implantation loss, there was a decrease in the number of live fetuses per litter in the 60 mg/kg group (2.6 compared to 14.0 in vehicle controls), which was associated with an 80% decrease in gravid uterine weight in this group. Mean percent post-implantation loss was higher than concurrent vehicle controls and the NTP historical control values (2.9% to 8.0%), but not significant in the 5 and 20 mg/kg groups (3.3% versus 10.7% and 11.1%, respectively). This higher percent loss is due to one dam each in the 5 and 20 mg/kg groups with whole litter resorptions.

There were a smaller number of litters at 60 mg/kg. While there were no exposure-related effects on male fetal body weights noted; female body weights were 8% lower than vehicle controls (Table 10) and the fetal sex-ratio appeared to be higher in the 60 mg/kg group (82% males compared to 46% males in vehicle controls). These observations are likely confounded by the small number of viable litters and fetuses available for assessment and thus was considered a spurious finding.

TABLE 10
Summary of Uterine Content Data for Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Pregnancy Summary				
Mated females	25	25	25	25
Pregnant females	21	20	22	20
Pregnant females examined on GD 21[a]	21	20	22	20
Corpora lutea per female[b]	15.86 ± 0.58 (21)	16.00 ± 0.70 (20)	15.41 ± 0.42 (22)	16.70 ± 0.44 (20)
Implantations per female[b]	14.38 ± 0.49 (21)	12.55 ± 0.92 (20)	12.82 ± 0.85 (22)	12.95 ± 1.04 (20)
Percent post-implantation loss[b]	3.29 ± 1.33** (21)	10.67 ± 5.29 (20)	11.13 ± 4.65 (22)	83.13 ± 6.47** (20)
Total resorptions per litter[b]	0.38 ± 0.15** (21)	0.60 ± 0.21 (20)	0.95 ± 0.27 (22)	10.40 ± 1.21** (20)
Early resorptions per litter[b]	0.33 ± 0.14** (21)	0.60 ± 0.21 (20)	0.86 ± 0.27 (22)	10.40 ± 1.21** (20)
Late resorptions per litter[b]	0.05 ± 0.05 (21)	0.00 ± 0.00 (20)	0.09 ± 0.06 (22)	0.00 ± 0.00 (20)
Dead fetuses per litter[b]	0.05 ± 0.05 (21)	0.00 ± 0.00 (20)	0.00 ± 0.00 (22)	0.00 ± 0.00 (20)
Number of early resorptions[a]	7	12	19	208
Number of late resorptions[a]	1	0	2	0
Number of whole litter resorptions[a]	0**	1	1	12**
Number of dead fetuses[a]	1	0	0	0
Live Fetuses[b]				
Number of live fetuses	293	239	261	51
Live fetuses per litter[b]	13.95 ± 0.55** (21)	11.95 ± 1.06 (20)	11.86 ± 0.88 (22)	2.55 ± 1.00** (20)
Live male fetuses per litter[b]	6.38 ± 0.42** (21)	4.95 ± 0.62 (20)	5.50 ± 0.59 (22)	1.80 ± 0.69** (20)
Live female fetuses per litter[b]	7.57 ± 0.57** (21)	7.00 ± 0.74 (20)	6.36 ± 0.60 (22)	0.75 ± 0.42** (20)
Percent live male fetuses per litter[b]	46.47 ± 2.99 (21)	41.63 ± 3.55 (19)	45.74 ± 3.44 (21)	82.19 ± 8.29* (8)
Fetal Weight[c]				
Fetal weight per litter (g)	5.15 ± 0.07 (21)	5.29 ± 0.16 (19)	5.21 ± 0.12 (21)	5.11 ± 0.10 (8)
Male fetal weight per litter (g)	5.28 ± 0.06 (21)	5.49 ± 0.21 (19)	5.35 ± 0.12 (21)	5.18 ± 0.08 (8)
Female fetal weight per litter (g)	5.03 ± 0.07 (21)	5.10 ± 0.10 (19)	5.09 ± 0.12 (21)	4.63 ± 0.06 (4)
Gravid Uterine Weight[d]				
Gravid uterine weight (g)	97.79 ± 3.11** (21)	83.89 ± 6.59 (20)	85.07 ± 5.28 (22)	19.52 ± 6.53** (20)
Terminal body weight (g)	385.7 ± 4.2** (21)	368.5 ± 8.2 (20)	370.0 ± 5.5 (22)	296.1 ± 8.2** (20)
Adjusted body weight (g)	287.89 ± 2.33* (21)	284.58 ± 3.60 (20)	284.90 ± 2.20 (22)	276.60 ± 2.79* (20)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to the end of the study.
(g) = grams
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
** P≤0.01
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] Statistical analysis performed using a mixed effect linear model with litter as a random effect (trend and pairwise)
[d] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight.

Fetal Findings

External

Fetal external abnormalities were unrelated to vinpocetine exposure and limited to singular occurrences of generalized subcutaneous edema in the 5 and 20 mg/kg groups, a singular occurrence of omphalocele in the 20 mg/kg group, and singular occurrences of a bent or short tail in two separate fetuses in the 20 mg/kg group (Table B4).

Visceral

An exposure-related effect was observed in the heart with increased incidences of ventricular septum defects (VSDs), a malformation, which occurred in 0%, 1.3%, 3.1%, and 3.9% of the fetuses (and 0%, 15.8%, 33.3%, and 25.0% of litters) in the 0, 5, 20, and 60 mg/kg groups, respectively (Tables 11 and B4). The NTP historical control range for VSDs is 0% to 0.5% for affected fetuses and litters. Several other visceral and skeletal abnormalities were noted in two and four fetuses with VSDs each in the 5 and 20 mg/kg groups and in one fetus in the 60 mg/kg group; however, no other fetal malformations were observed in the remainder of the fetuses with VSDs.

Other malformations in the heart included misshapen aortic valves, a large right atrium, and thick left ventricle wall (Table 11), but these findings were not considered to be exposure-related due to the high background incidence (misshapen aortic valves) or occurrence in a single fetus (large right atrium and thick left ventricle wall).

In the major vessels and thoracic viscera, there were singular incidences of a supernumerary right carotid artery, patent ductus arteriosus, absent lung lobe accessory, fused right cranial lung lobe, thin diaphragm, and a diaphragm hernia and multiple incidences of absent innominate arteries and short innominate arteries (Table B4). These findings were incidental or are a common background finding (absent or short innominate arteries) (Scott *et al.*, 1997) with no significant dose-response and were not considered to be exposure related.

TABLE 11
Summary of Selected Visceral Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Visceral								
Number of fetuses examined	293		239		261		51	
Number of litters examined	21		19		21		8	
Heart								
Aortic valve, misshapen — [M]								
Fetuses	19	(6.48)	14	(5.86)	17	(6.51)	0	(0.0)*
Litters	12	(57.14)**	11	(57.89)	10	(47.62)	0	(0.00)**
Atrium, right, large — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Ventricle, ventricular septum defect — [M][a]								
Fetuses	0	(0.0)**	3	(1.26)	8	(3.07)**	2	(3.92)*
Litters	0	(0.00)	3	(15.79)	7	(33.33)**	2	(25.00)
Ventricle, left, thick wall — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Major Vessels								
Carotid artery, right, supernumerary — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.96)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(12.50)
Ductus arteriosus, patent — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Innominate artery, absent — [V]								
Fetuses	4	(1.37)	7	(2.93)	8	(3.07)	1	(1.96)
Litters	4	(19.05)	6	(31.58)	5	(23.81)	1	(12.50)
Innominate artery, short — [V]								
Fetuses	3	(1.02)	4	(1.67)	2	(0.77)	1	(1.96)
Litters	3	(14.29)	4	(21.05)	2	(9.52)	1	(12.50)

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis for litter data and for fetal data (without the litter effects) performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
** Statistically significant ($P \leq 0.01$) trend (denoted in the vehicle control column) or pairwise comparison (denoted in the dosed group column)
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models found no statistically significant trend or pairwise comparison.
[a] Historical incidence for all routes: fetuses- 2/1,326 (0.15%), range 0.00%-0.48%; litters – 2/104 (1.92%), range 0.00%-5.26%
[M] = Malformation
[V] = Variation

Head

Malformations observed in vinpocetine-treated groups included a single incidence of hydrocephaly in one fetus in the 5 mg/kg group (Table B4). Additionally, there was a single fetus in the 20 mg/kg group that had the variation of dilated ventricles. These findings were incidental and were not considered to be exposure related.

Skeletal

In the fetal vertebrae, there was a significant trend for increased incidences of incomplete ossification throughout the thoracic centra that was considered exposure related due to dose-dependent increases by pairwise comparison in the 20 and 60 mg/kg groups (Tables 12 and B4). In addition, the incidence of this variation at 60 mg/kg (17%) exceeded the historical control range (0% to 0.82%). Additional exposure-related findings included supernumerary ribs that occurred in a dose-dependent manner and were present in multiple litters per group. Significantly increased trends were noted for incidences of full (malformation) and short (less than 1/3 the length of the rib above it; variation) thoracolumbar ribs (Table 12). There were increased numbers of fetuses with full supernumerary thoracolumbar ribs on the left, right, and bilaterally, which culminated in total incidences of full thoracolumbar ribs in 4.6% and 25.5% of the fetuses in the 20 and 60 mg/kg groups, respectively. This increased incidence was statistically significant by pairwise comparison at 60 mg/kg ($P \leq 0.01$). Although increased incidences of short supernumerary thoracolumbar ribs are a common background lesion in this strain of rat, the findings were statistically significant for both the trend test and pairwise comparison at 20 and 60 mg/kg ($P \leq 0.05$) and provide supporting evidence that the dose-dependent increases of full supernumerary thoracolumbar ribs were exposure-related.

In the 60 mg/kg group, there was an increased incidence of greater than 26 presacral vertebrae (Table 12). It is unclear whether this increased incidence is related to exposure; however, it should be noted that the incidences were outside of the historical control range (0%), and all the fetuses with this variation also had either bilateral full supernumerary ribs or left full supernumerary ribs.

TABLE 12
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Skeletal: Body								
Number of fetuses examined	293		239		260		47	
Number of litters examined	21		19		21		7	
Vertebrae								
Cervical arch, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic arch, 6th right, misshapen — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic arch, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 1st, hemicentric — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 12th, hemicentric — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 1st, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Thoracic centrum, 5th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Thoracic centrum, 6th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, 9th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, 10th, incomplete ossification — [V]								
Fetuses	1	(0.34)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	1	(4.76)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, 11th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	3	(1.15)	2	(4.26)*
Litters	0	(0.00)**	0	(0.00)	3	(14.29)	2	(28.57)
Thoracic centrum, 12th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	1	(0.38)	3	(6.38)**
Litters	0	(0.00)**	0	(0.00)	1	(4.76)	2	(28.57)
Thoracic centrum, 13th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	0	(0.0)	2	(4.26)*
Litters	0	(0.00)**	0	(0.00)	0	(0.00)	2	(28.57)
Thoracic centrum, multiple sites, incomplete ossification — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, incomplete ossification, total — [V][a]								
Fetuses	1	(0.34)**##	1	(0.42)	6	(2.31)*#	8	(17.02)**##
Litters	1	(4.76)**	1	(5.26)	5	(23.81)	3	(42.86)*
Lumbar arch, 5th left, fused — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Lumbar centrum, 5th, fused — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)

TABLE 12
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Skeletal: Body (continued)								
Number of fetuses examined	293		239		260		47	
Number of litters examined	21		19		21		7	
Vertebrae (continued)								
Lumbar centrum, 1st, incomplete ossification — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Lumbar centrum, 3rd, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Presacral vertebrae, greater than 26 — [V]								
Fetuses	0	(0.0)**	0	(0.0)	0	(0.0)	4	(8.51)**
Litters	0	(0.00)**	0	(0.00)	0	(0.00)	2	(28.57)
Sacral centrum, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Supernumerary rib								
Thoracolumbar, left, full — [M]								
Fetuses	0	(0.0)**	1	(0.42)	2	(0.77)	3	(6.38)**
Litters	0	(0.00)	1	(5.26)	2	(9.52)	1	(14.29)
Thoracolumbar, right, full — [M]								
Fetuses	1	(0.34)	2	(0.84)	1	(0.38)	1	(2.13)
Litters	1	(4.76)	2	(10.53)	1	(4.76)	1	(14.29)
Thoracolumbar, bilateral, full — [M]								
Fetuses	0	(0.0)**	2	(0.84)	9	(3.46)**	8	(17.02)**
Litters	0	(0.00)	2	(10.53)	1	(4.76)	2	(28.57)
Thoracolumbar, full, total — [M][b]								
Fetuses	1	(0.34)**##	5	(2.09)	12	(4.62)**	12	(25.53)**##
Litters	1	(4.76)*	3	(15.79)	4	(19.05)	3	(42.86)*
Thoracolumbar, left, short — [V]								
Fetuses	21	(7.17)*#	10	(4.18)	22	(8.46)	7	(14.89)
Litters	13	(61.90)	6	(31.58)	13	(61.90)	5	(71.43)
Thoracolumbar, right, short — [V]								
Fetuses	2	(0.68)*	11	(4.6)**#	16	(6.15)**##	2	(4.26)#
Litters	2	(9.52)	6	(31.58)	12	(57.14)**	2	(28.57)
Thoracolumbar, bilateral, short — [V]								
Fetuses	6	(2.05)	9	(3.77)	17	(6.54)**#	1	(2.13)
Litters	4	(19.05)	5	(26.32)	11	(52.38)*	1	(14.29)
Thoracolumbar, short, total — [V]								
Fetuses	29	(9.9)**#	30	(12.55)	55	(21.15)**#	10	(21.28)*#
Litters	14	(66.67)	10	(52.63)	17	(80.95)	5	(71.43)

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis for litter data and for fetal data (without the litter effects) performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
* Statistically significant (P≤0.05) trend (denoted in the vehicle control column) or pairwise comparison (denoted in the dosed group column)
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression
\# Statistically significant (P≤0.05) trend (denoted in the vehicle control column) or pairwise comparison (denoted in the dosed group column)
P≤0.01
[a] Historical incidence for gavage studies: fetuses – 3/1,325 (0.23%), range 0.00%-0.82%; litters – 3/104 (2.88%), range 0.00%-11.11%
[b] Historical incidence for gavage studies: fetuses – 14/1,324 (1.06%), range 0.34%-3.35%; litters – 13/104 (12.50%), range 4.76%-31.58%
[M] = Malformation
[V] = Variation

Additionally, singular incidences of incomplete ossification were noted in the lumbar centrum in the 5 and 20 mg/kg groups. However, as these incidences in the lumbar centrum were incidental, they were not considered exposure-related. All of the malformations present in the vertebrae (misshapen cervical arch, misshapen sacral centrum, misshapen thoracic arch, fused lumbar arch, and fused lumbar centrum) were limited to three fetuses and as such were not considered related to vinpocetine exposure.

Dose Selection Rationale for the Dose Range-Finding Study in Rabbits

Dose selection for the range-finding study in rabbits was based on both the results from our dose range-finding study in the rat and on toxicokinetic data on vinpocetine in rabbits from the literature. Toxicokinetic data on vinpocetine in rats and rabbits demonstrate similar plasma AUC and C_{max} levels between the species (Vereczkey *et al.*, 1979a; Nie *et al.*, 2006; Ribeiro *et al.*, 2007; Xu *et al.*, 2009; Xia *et al.*, 2010; Sozański *et al.*, 2011). Therefore, the doses chosen for the rabbits were similar to those chosen for the dose range-finding study in rats (0, 25, 75, 150, and 300 mg/kg per day).

DOSE RANGE-FINDING STUDY IN RABBITS

Maternal Findings

Viability and Clinical Observations

All vehicle control and dosed rabbits survived until the end of the study (Table 13), with the exception of one female in the 150 mg/kg group that was removed on GD 25 due to abortion. This doe also had clinical observations beginning on GD 21 of red abnormal vaginal discharge and red substance present in the cage pan that were consistent with the abortion (Table C1). Clinical observations of red vaginal discharge and red discoloring of the vagina also occurred in one animal each from the vehicle control and 300 mg/kg groups beginning on GD 22 and 20, respectively. These clinical observations were not accompanied by abortions; however, post-implantation loss was noted in the doe from the 300 mg/kg group. There was an additional incidence of red substance in the cage pan observed on GD 20 in a doe from the 300 mg/kg group that also had 66.7% post-implantation loss.

TABLE 13
Maternal Disposition of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Time-mated females	8	8	8	8	8
Pregnant (on GD 29)	8	7	8	7	8
Euthanasia aborted-pregnant	0	0	0	1[a]	0
Non-pregnant (on GD 29)	0	1	0	0	0

[a] Doe removed on GD 25

Body Weights and Feed Consumption

Treatment-related decreases in maternal body weights were noted from GD 12 to 29 in the 300 mg/kg group, relative to the vehicle controls (Figure 5; Table 16). Decreases in mean maternal body weight gains, compared to vehicle controls, were 44% and 34% for the 150 and 300 mg/kg groups, respectively (Table 14). The decreased maternal body weight gains in the 150 and 300 mg/kg groups were consistent with decreased feed consumption in these groups (Table 15) and an increase in embryo-fetal loss (20.4% compared to 1.4% in vehicle controls) that occurred in the 300 mg/kg group (Table 16). Daily mean body weight changes for does in each dose group are available in Table C2.

There was a treatment-related decrease in maternal feed consumption in the 150 and 300 mg/kg groups during gestation (Table 15). Decreases in feed consumption across several dosing intervals (11% to 30% in both high dose groups compared to the vehicle controls) culminated in overall decreases of 26% and 17%, respectively, during the GD 7 to GD 29 interval.

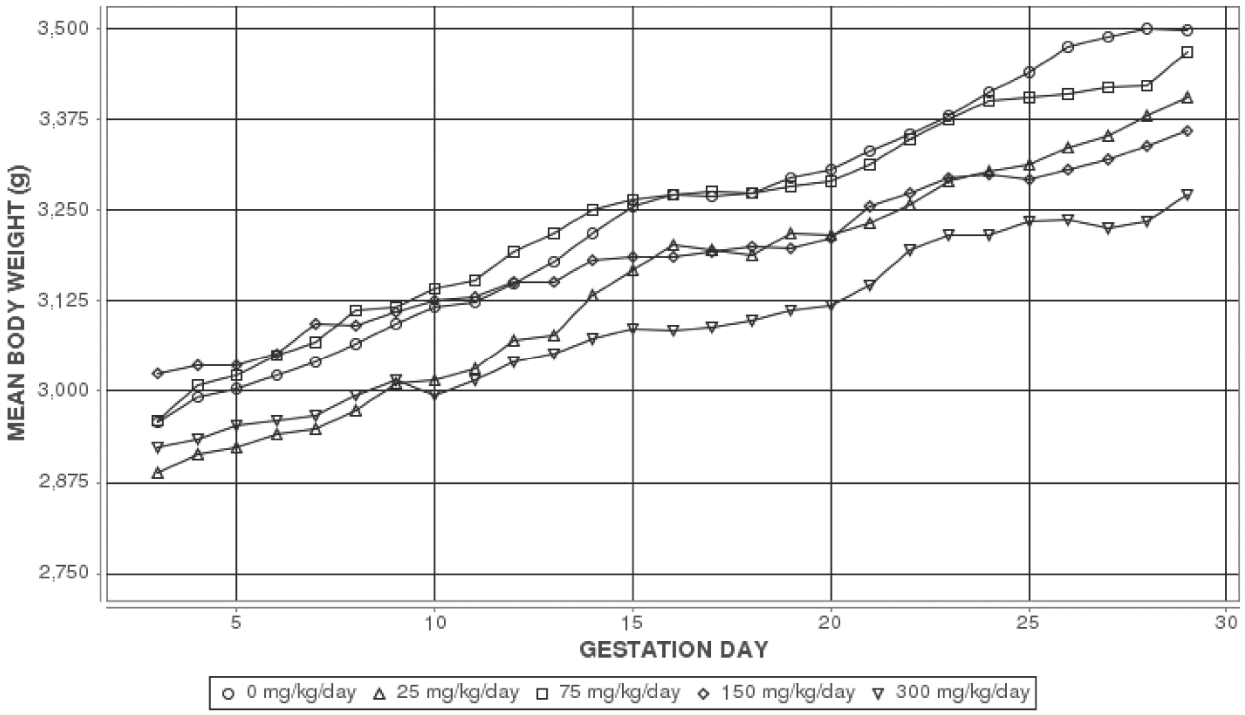


FIGURE 5
Maternal Growth Curves for Pregnant Rabbits Administered Vinpocetine by Gavage in the Dose Range-Finding Study
Information for statistical significance in maternal weights is provided in Tables 14 and C2.

TABLE 14
Summary of Maternal Body Weight Gains of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Gestation Day Interval					
7 to 29	460.2 ± 33.8** (8)[b]	458.2 ± 46.2 (7)	399.3 ± 58.4 (8)	256.7 ± 40.9** (7)	304.1 ± 33.2** (8)
3 to 7	81.5 ± 17.7 (8)	61.6 ± 20.1 (7)	108.0 ± 10.7 (8)	69.2 ± 19.3 (8)	45.3 ± 13.1 (8)
7 to 9	53.2 ± 8.2 (8)	61.6 ± 11.1 (7)	49.1 ± 10.8 (8)	14.9 ± 19.9 (8)	47.3 ± 29.3 (8)
9 to 12	56.8 ± 15.1 (8)	60.8 ± 13.4 (7)	74.9 ± 22.4 (8)	40.9 ± 16.0 (8)	26.8 ± 19.6 (8)
12 to 15	105.3 ± 11.1** (8)	97.7 ± 10.2 (7)	71.5 ± 17.8 (8)	36.1 ± 36.7 (8)	46.0 ± 20.7 (8)
15 to 18	20.0 ± 9.5 (8)	19.4 ± 9.3 (7)	11.0 ± 10.2 (8)	12.5 ± 9.9 (8)	11.0 ± 11.8 (8)
18 to 21	56.3 ± 12.6 (8)	43.8 ± 10.0 (7)	39.3 ± 11.3 (8)	55.5 ± 23.8 (8)	47.4 ± 23.6 (8)
21 to 24	82.2 ± 6.7 (8)	71.5 ± 18.1 (7)	88.0 ± 12.7 (8)	44.0 ± 10.8 (8)	71.4 ± 15.3 (8)
24 to 27	76.0 ± 11.5** (8)	48.4 ± 14.6 (7)	18.4 ± 18.6** (8)	24.9 ± 11.7* (7)	7.3 ± 12.4** (8)
27 to 29	10.3 ± 16.2 (8)	55.5 ± 8.0 (7)	47.1 ± 9.6 (8)	38.7 ± 15.4 (7)	46.9 ± 17.6 (8)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Body weight gains for pregnant animals are given in grams. Data are displayed as mean ± standard error.
[b] Number of does weighed is given in parentheses.

TABLE 15
Summary of Maternal Feed Consumption of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Gestation Day Interval					
7 to 29	137.6 ± 4.3** (8)[b]	131.8 ± 5.7 (7)	125.2 ± 4.0 (8)	101.3 ± 11.4** (7)	113.8 ± 8.9* (8)
3 to 7	149.1 ± 0.8 (8)	143.2 ± 7.1 (7)	149.8 ± 0.5 (8)	139.6 ± 7.6 (8)	147.4 ± 2.6 (8)
7 to 9	148.9 ± 2.0* (8)	143.0 ± 5.3 (7)	148.7 ± 2.1 (8)	131.1 ± 9.0 (8)	118.7 ± 15.4 (8)
9 to 12	145.1 ± 4.3* (8)	141.1 ± 5.5 (7)	137.4 ± 6.7 (8)	107.3 ± 13.4 (8)	106.1 ± 18.6 (8)
12 to 15	128.6 ± 9.4 (8)	131.8 ± 8.1 (7)	117.6 ± 9.9 (8)	92.5 ± 20.9 (8)	102.9 ± 18.3 (8)
15 to 18	142.9 ± 4.1* (8)	138.7 ± 6.7 (7)	118.5 ± 10.9 (8)	98.7 ± 20.0 (8)	109.6 ± 15.9 (8)
18 to 21	148.0 ± 2.4** (8)	137.9 ± 6.3 (7)	132.5 ± 6.7 (8)	109.3 ± 8.7** (8)	131.2 ± 6.0* (8)
21 to 24	142.6 ± 5.2 (8)	127.8 ± 10.8 (7)	137.1 ± 6.1 (8)	117.2 ± 8.6 (8)	127.3 ± 5.9 (8)
24 to 27	134.7 ± 5.6* (8)	126.9 ± 9.1 (7)	106.6 ± 8.8 (8)	89.8 ± 10.6** (7)	110.3 ± 3.2 (8)
27 to 29	96.1 ± 14.4 (8)	100.7 ± 18.4 (7)	95.0 ± 24.2 (8)	97.1 ± 14.3 (7)	102.0 ± 16.2 (8)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Feed consumption for pregnant animals is given in grams/day. Data are displayed as mean ± standard error.
[b] Number of does with feed consumption measured is given in parentheses.

Maternal and Litter Observations

At necropsy, there were no notable maternal gross pathology findings (Table C3). There was an exposure-related effect on embryo-fetal survival in the 300 mg/kg group (Table 16). Uterine examination revealed fewer live fetuses per litter in the 300 mg/kg group (6.5 compared to 9.1 in vehicle controls), which was associated with an increase in early resorptions per litter (1.6 compared to 0.1 in vehicle controls). Overall, these findings in the 300 mg/kg group led to an increase in percent post-implantation loss (20.4% compared to 1.4% in vehicle controls). These findings in the 300 mg/kg group were also associated with a 34% reduction in mean gravid uterine weight. There were no exposure-related effects on embryo-fetal survival in any group administered 150 mg/kg or less.

Mean fetal weights were reduced for both males and females in the 300 mg/kg group (10.7% and 10.6% less than vehicle controls, respectively). No effects were noted at the lower doses.

Fetal Findings

External

There were no external malformations or variations attributed to vinpocetine exposure at 25, 75, 150, or 300 mg/kg per day (Tables C4 and C5). External findings were limited to a singular occurrence of localized subcutaneous edema in the 75 mg/kg group and one incidence of subcutaneous hemorrhage in each of the vehicle control, 25, and 150 mg/kg groups and were considered to be incidental and unrelated to vinpocetine exposure.

TABLE 16
Summary of Uterine Content Data for Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Pregnancy Summary					
Mated females	8	8	8	8	8
Pregnant females	8	7	8	8	8
Pregnant females examined on GD 29[a]	8	7	8	7	8
Corpora lutea per female[b]	9.50 ± 0.38 (8)	8.71 ± 0.47 (7)	9.63 ± 0.53 (8)	8.86 ± 0.40 (7)	9.13 ± 0.61 (8)
Implantations per female[b]	9.25 ± 0.41 (8)	8.00 ± 0.44 (7)	9.25 ± 0.56 (8)	7.71 ± 0.68 (7)	8.38 ± 0.56 (8)
Percent post-implantation loss[b]	1.39 ± 1.39 (8)	3.37 ± 2.18 (7)	2.53 ± 1.66 (8)	3.57 ± 3.57 (7)	20.42 ± 9.05 (8)
Total resorptions per litter[b]	0.13 ± 0.13 (8)	0.14 ± 0.14 (7)	0.25 ± 0.16 (8)	0.14 ± 0.14 (7)	1.88 ± 0.85 (8)
Early resorptions per litter[b]	0.13 ± 0.13* (8)	0.00 ± 0.00 (7)	0.00 ± 0.00 (8)	0.14 ± 0.14 (7)	1.63 ± 0.71 (8)
Late resorptions per litter[b]	0.00 ± 0.00 (8)	0.14 ± 0.14 (7)	0.25 ± 0.16 (8)	0.00 ± 0.00 (7)	0.25 ± 0.25 (8)
Dead fetuses per litter[b]	0.00 ± 0.00 (8)	0.14 ± 0.14 (7)	0.00 ± 0.00 (8)	0.00 ± 0.00 (7)	0.00 ± 0.00 (8)
Number of early resorptions[a]	1	0	0	1	13
Number of late resorptions[a]	0	1	2	0	2
Number of whole litter resorptions[a]	0	0	0	0	0
Number of dead fetuses[a]	0	1	0	0	0
Live Fetuses[b]					
Number of live fetuses	73	54	72	53	52
Live fetuses per litter	9.13 ± 0.44* (8)	7.71 ± 0.42 (7)	9.00 ± 0.53 (8)	7.57 ± 0.81 (7)	6.50 ± 0.73* (8)
Live male fetuses per litter	3.50 ± 0.94 (8)	3.86 ± 0.63 (7)	4.63 ± 0.38 (8)	3.86 ± 0.91 (7)	3.38 ± 0.73* (8)
Live female fetuses per litter	5.63 ± 0.68* (8)	3.86 ± 0.70 (7)	4.38 ± 0.68 (8)	3.71 ± 0.42 (7)	3.13 ± 0.67 (8)
Percent live male fetuses per litter	36.30 ± 8.71 (8)	50.68 ± 8.87 (7)	52.67 ± 5.63 (8)	45.15 ± 9.80 (7)	50.80 ± 9.02 (8)
Fetal Weight[c]					
Fetal weight per litter (g)	39.72 ± 1.33** (8)	41.47 ± 0.95 (7)	37.53 ± 0.90 (8)	39.36 ± 1.74 (7)	35.78 ± 1.15 (8)
Male fetal weight per litter (g)	40.87 ± 1.59** (8)	42.70 ± 0.97 (7)	38.50 ± 1.15 (8)	38.06 ± 1.62 (6)	36.49 ± 2.00 (8)
Female fetal weight per litter (g)	38.76 ± 1.57* (8)	40.37 ± 1.12 (7)	36.35 ± 1.01 (8)	39.29 ± 1.75 (7)	34.65 ± 0.95 (8)

TABLE 16
Summary of Uterine Content Data for Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Gravid Uterine Weight[e]					
Gravid uterine weight	515.25 ± 14.70** (8)	470.05 ± 20.66 (7)	483.91 ± 32.24 (8)	421.86 ± 39.25* (7)	340.94 ± 27.73** (8)
Terminal body weight (g)	3,449.4 ± 64.6* (8)	3,406.5 ± 58.0 (7)	3,467.7 ± 95.0 (8)	3,358.4 ± 105.6 (7)	3,271.4 ± 34.2 (8)
Adjusted body weight (g)	2,984.19 ± 64.86 (8)	2,936.41 ± 42.60 (7)	2,983.75 ± 75.64 (8)	2,936.53 ± 110.63 (7)	2,930.44 ± 46.81 (8)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to the end of the study.
(g) = grams
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
** P≤0.01
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] Statistical analysis performed using a mixed effect linear model with litter as a random effect (trend and pairwise)
[d] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests. Adjusted body weight = terminal body weight minus gravid uterine weight

DISCUSSION AND CONCLUSIONS

Vinpocetine is a semi-synthetic derivative of vincamine, an alkaloid extract derived from the periwinkle plant *Vinca minor*. Vinpocetine has been widely available as a pharmaceutical in Europe, Russia, China, and Japan for treatment of cerebrovascular and cognitive disorders (Bereczki and Fekete, 2008). However, in the United States it is available as a dietary supplement with claims of cognitive enhancement (Manconi *et al.*, 1986; Peruzza and DeJacobis, 1986; Thal *et al.*, 1989; Feigin *et al.*, 2001; Szatmári and Whitehouse, 2009). Interest in memory enhancement has shifted its use from a primarily older population to use by all ages, including women of childbearing potential (WOCBP) and the one publication available for review (Cholnoky and Dömök, 1976) provides insufficient details to effectively evaluate the safety of vinpocetine in a younger population that includes WOCBP.

The National Toxicology Program (NTP) conducted developmental studies with vinpocetine in the rat based on the possibility of widespread exposure to pregnant women and WOCBP and limited literature indicating that vinpocetine may not be safe for use during pregnancy. Additionally, a dose range-finding study in rabbits was included to see if effects occurred in a second species. The current Prenatal Developmental Toxicity Report presents the findings of the dose-range finding and prenatal developmental toxicity studies of vinpocetine in HSD rats and the dose range-finding study of vinpocetine in NZW rabbits.

There was clear evidence of developmental toxicity in rats based on findings in the prenatal toxicity study, with supportive evidence from the dose range-findings studies in rats and rabbits. In the dose range-finding study in rats, daily oral gavage exposure of 0, 20, 40, 80, 160, or 320 mg/kg resulted in lowered maternal body weight and body weight gains, decreased maternal feed consumption, and clinical observations of abnormal vaginal discharge at ≥ 80 mg/kg and significant embryo-fetal loss in all exposed groups.

As a result of the increased incidences of fetal resorptions between the 40 and 80 mg/kg groups in the dose range-finding study, 60 mg/kg of vinpocetine was chosen as the high dose in the prenatal developmental toxicity study in rats. Maternal effects at 60 mg/kg were similar to the dose range-finding study and included lowered maternal body weights and body weight gains, decreased feed consumption, and an increase in red vaginal discharge. These findings are consistent with a review of the industry studies published by Cholnoky and Dömök (1976), where oral gavage administration of vinpocetine to rats over the major period of organogenesis resulted in lowered maternal body weight gain at 50 mg/kg and uterine bleeding at 50 and 150 mg/kg. Additionally, significant embryo-fetal loss was observed at 60 mg/kg.

In both the dose range-finding and prenatal developmental toxicity studies in rats, there was a dose-related increase in post-implantation loss that occurred at doses ≥ 40 mg/kg. In the prenatal developmental toxicity study in rats, post-implantation loss of 83% occurred in dams administered 60 mg/kg. This increased post-implantation loss was a result of 12 dams with whole litter resorptions. Of the limited data in the literature (one paper reviewing 14 studies), high fetal mortality was noted following administration of 150 mg/kg vinpocetine to the dams in one study and complete litter resorptions were observed in 55% of the dams administered 135 mg/kg vinpocetine in another study (Cholnoky and Dömök, 1976).

Additional evidence of teratogenicity in the rat prenatal developmental toxicity study consisted of exposure-related increased incidences of ventricular septum defects (VSDs). The incidences of VSDs were significantly increased between the 5 and 20 mg/kg dose groups, but not in the 60 mg/kg group. The lack of a dose-responsive increase in the number of VSDs at 60 mg/kg was likely a result of the significant post-implantation loss observed in this dose group (there were only 51 fetuses available for evaluation); however, the percent of affected fetuses was increased at both 20 and 60 mg/kg (3.1% and 3.9%) and was outside the NTP historical control range (0.0% to 0.5%) for SD rats. VSDs are a malformation that arise as a result of a disruption in the developmental processes that lead to partitioning of the ventricles and manifests as an opening in the interventricular septum (IVS). Development of the IVS is typically complete by GD 15 in rats, and consists of both muscular and membranous segments (DeSesso, 2006). VSDs can occur spontaneously, have been identified as the most common type of congenital heart disease in humans, and have been shown to close during postnatal development in both rats and humans (Roguin *et al.*, 1995;

Solomon *et al.*, 1997; Du *et al.*, 1998; Paladini *et al.*, 2000; Hoffman and Kaplan, 2002). Membranous VSDs can also be induced in rats as a result of toxicant exposure (Solomon *et al.*, 1997; Fleeman *et al.*, 2004). Administration of trimethadione on GD 9 and 10, resulted in a high incidence of membranous VSDs that were morphologically similar to spontaneously occurring VSDs, albeit larger in size (Solomon *et al.*, 1997, Fleeman *et al.*, 2004). These toxicant-induced small membranous VSDs in rats have also been shown to close postnatally, indicative of a potential delay in cardiac development (Solomon *et al.*, 1997; Fleeman *et al.*, 2004). The increased incidences of VSDs seen in the current studies with vinpocetine exposure could be indicative of a developmental delay; however, signs of delay (decreases in fetal weight and delays in ossification) were only observed in the 60 mg/kg group and the incidences of VSDs were noted in the 5 and 20 mg/kg dose groups. Additionally, Fleeman *et al.* (2004) found no association between the occurrence of VSDs and decreased fetal weight, suggesting that VSDs are independent of overall fetal growth as measured by fetal weight. Therefore, the presence of VSDs at all doses were likely related to the administration of vinpocetine and not a secondary effect of delayed development.

Additional evidence of teratogenicity associated with vinpocetine exposure in the rats included significantly increased incidences in the formation of full supernumerary thoracolumbar ribs (SNR). This malformation was present in 25.5% of the fetuses in the 60 mg/kg dose group where significant fetal mortality occurred (compared to 0.3% in the vehicle control group and 0.3% to 3.4% in the available historical control reports for fetuses from SD rats). The formation of supernumerary ribs in the thoracolumbar region are indicative of an alteration in early embryonic development of the axial skeleton (Branch *et al.*, 1996) and have been observed from exposure to a wide range of dissimilar chemicals in a dose-dependent manner, including sodium salicylate (Foulon *et al.*, 1999), bromoxynil phenol or bromoxynil octanoate (Rogers *et al.*, 1991), and valproic acid (Narotsky *et al.*, 1994). Additionally, increased incidences of SNR have previously been associated with maternal stress, although this effect appears to be species specific as it has been demonstrated mainly in mice (Beyer and Chernoff, 1986; Chernoff *et al.*, 1987). Aside from the maternal effects associated with significant embryo-fetal loss, the doses of vinpocetine administered in the current studies did not produce signs of maternal stress or toxicity, indicating that the increased incidences of full SNR in the current studies were likely related to vinpocetine exposure.

As incidences of full SNR are indicators of developmental changes in axial skeleton development, they are generally not isolated events. Their formation has been significantly correlated with other findings in mice, such as the presence of an additional pre-sacral vertebra (Chernoff and Rogers, 2010). An increase in the incidences of greater than 26 pre-sacral vertebrae was seen in the current studies in fetuses from dams exposed to 60 mg/kg vinpocetine. All of the fetuses with this variation also had incidences of full SNR (bilateral or on the left only).

Incidences of short SNR, or rudimentary ribs, were significantly increased in the fetuses of dams exposed to 20 and 60 mg/kg vinpocetine. However, these increased incidences of short SNR may or may not have biological significance as they are a common background variation in this strain of rat and their presence is transient and has been shown to diminish during the post-natal period in rats (Wickramaratne, 1988; Chernoff *et al.*, 1991, Foulon *et al.*, 2000). In contrast, full SNR have been shown to persist from birth into adulthood, as demonstrated by Foulon *et al.* (2000) who examined salicylate-induced full SNR over time through radiography. Incidences of full SNR in the lumbar region have also been reported in humans, where they have been associated with adverse outcomes such as pain in the lumbar region and increased incidences of L4 and L5 degeneration (Chernoff and Rogers, 2010).

Exposure to vinpocetine during gestation resulted in evidence of developmental toxicity, manifested as fetal growth retardation in the rats. This was demonstrated by significant increases in the percentage of fetuses with incomplete ossification of the thoracic centrum and decreased fetal weights. The thoracic centrum is the body, or centrum, of the thoracic vertebrae and is routinely ossified before birth. Aside from fetal weight, the degree of ossification of the main components of the axial skeleton and the extremities in the fetus are typical indicators of developmental status (Kehra, 1981). Cyclophosphamide is an example of another toxicant where exposure in the mouse and rat resulted in fetal resorptions, as well as growth retardation, delayed ossification, and skeletal malformations (Ujházy *et al.*, 1979; Jeyaseelan and Singh, 1984; Matalon *et al.*, 2004). Maternal stress and malnutrition, especially during the period of rapid fetal growth late in gestation, can also result in reduced fetal weight and incomplete skeletal ossification. However, this is likely not the case with vinpocetine exposure in these studies, as the reduced maternal body weights and feed consumption seen in this study were a result of fetal loss and not maternal toxicity.

A dose range-finding study was also performed in rabbits to determine if the effects observed in the rat would be observed in a second nonrodent species. In the dose range-finding study in rabbits, daily oral gavage exposure of 0, 25, 75, 150, or 300 mg/kg did not result in overt maternal toxicity. As was seen in the rats, the vinpocetine-exposed does displayed several effects related to embryo-fetal loss, including decreased body weights and feed consumption, and clinical observations of abnormal vaginal discharge. However, these effects were mainly limited to the does in the 300 mg/kg group. Information available in the literature on the effects of vinpocetine administration during gestation in rabbits was provided as a paragraph in the Cholnoky and Dömök (1976) publication and had limited details, but it was noted that a small significant reduction in body weight gain in the high dose group was observed (orally administered, 18 mg/kg) with no other maternal toxicity.

Similar to the rats, there was also an increase in percent post-implantation loss in the rabbits. The increase in post implantation loss in the rabbits was limited to the 300 mg/kg group, and the magnitude of the response was diminished compared to the rat, although significantly increased compared to the vehicle controls. Additionally, there was a decrease in fetal weight at 300 mg/kg observed in both males and females.

The developmental toxicity of vinpocetine was notable in that related findings, including embryo-fetal lethality and decreased fetal weights, occurred in two species in the absence of overt maternal toxicity. The doses where these effects were significant were 60 mg/kg in the rat and 300 mg/kg in the rabbit. In a toxicokinetics study in pregnant rats, significant fetal transfer of vinpocetine occurred following repeat administration of vinpocetine (5 and 20 mg/kg) from GD 6 to 18 (Waidyanatha *et al.*, 2018). In this study, pooled fetal C_{max} and AUC values were ≥ 55% of dam values, respectively. Additionally, this study identified the rapid metabolism of vinpocetine to its main metabolite, apovincaminic acid in the dam, with apovincaminic acid levels 2.7-fold higher, based on C_{max} and AUC, than vinpocetine in dam plasma. However, apovincaminic acid levels in the fetus were much lower than vinpocetine. Examination of the plasma levels of vinpocetine and apovincaminic acid in dosed rabbits (GD19) revealed that both compounds were increased in a less than dose-proportional manner. In the limited comparison between the two species, dose-normalized vinpocetine levels at 1 and 2 h, following the last dose administration, were found to be 7- to 15-fold higher in the rats (5 and 20 mg/kg) compared to the rabbits (25 mg/kg). In contrast, the dose normalized apovincaminic acid levels in rabbits were 19- to 75-fold higher than rats. These findings

indicate a species difference in metabolism, with higher vinpocetine levels in the rat and higher apovincaminic acid levels in the rabbit, and offer a plausible explanation for the species difference observed in fetal mortality (Catlin *et al.*, 2018).

The doses recommended by the Physicians' Desk Reference for Nutritional Supplements and the doses that are suggested on available product labels range from 5 to 60 mg/day (Hendler and Rorvik, 2001). A comparison of exposure in rats at 5 mg/kg to suggested doses in humans (single 10 mg dose), resulted in exposure multiples of ≤ 13.6 and ≤ 8.5 for C_{max} and AUC, respectively, based on blood levels between the two species (Waidyanatha *et al.*, 2018). These dose comparisons suggest that exposure to vinpocetine in rats following a repeated 5 mg/kg dose (as conducted in these studies) is similar to that following a single 10 mg dose in humans.

Exposure to vinpocetine during gestation in rats and rabbits resulted in evidence of developmental toxicity as exhibited by embryo-fetal death. Additional findings included reductions in fetal weight (rat and rabbit) and malformations and variations of the heart and skeleton of the rat.

CONCLUSIONS

Under the conditions of this prenatal study, there was *clear evidence* of developmental toxicity of vinpocetine in Hsd:Sprague Dawley SD rats based on increased post-implantation loss and increased incidences of ventricular septum defects, thoracolumbar ribs (full), and incomplete ossification of the thoracic centrum in the absence of overt maternal toxicity.

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* Explanation of Levels of Evidence of Prenatal Developmental Toxicity is on page 11.

REFERENCES

Armitage, P. (1955). Tests for linear trends in proportions and frequencies. *Biometrics* **11**, 375-386.

Avula, B., Chittiboyina, A.G., Satyanarayanaraju, S., Wang, Y.-H., Wang, M., Khan, I.A., and Cohen, P.A. (2015). Identification and quantification of vinpocetine and picamilon in dietary supplements sold in the United States. *Drug Test. Anal.* **8**, 334-343.

Bereczki, D., and Fekete, I. (2008). Vinpocetine for acute ischemic stroke. *Stroke* **39**, 2404-2405.

Beyer, P.E., and Chernoff, N. (1986). The induction of supernumerary ribs in rodents: Role of the maternal stress. *Teratog. Carcinog. Mutagen.* **6**, 419-429.

Branch, S., Rogers, J.M., Brownie, C.F., and Chernoff, N. (1996). Supernumerary lumbar rib: Manifestation of basic alteration in embryonic development of ribs. *J. Appl. Toxicol.* **16**, 115-119.

Catlin, N., Waidyanatha, S., Mylchreest, E., Miller-Pinsler, L., Cunny, H., Foster, P., Sutherland, V., and McIntyre, B. (2018). Embryo-fetal development studies with the dietary supplement vinpocetine in the rat and rabbit. *Birth Defects Res.* **110**, 883-896.

Chernoff, N., and Rogers, J.M. (2010). Hypoxia and the Edema Syndrome: Elucidation of a mechanism of teratogenesis. *Birth Defects Res. B Dev. Reprod. Toxicol.* **89**, 300-303.

Chernoff, N., Kavlock, R.J., Beyer, P.E., and Miller, D. (1987). The potential relationship of maternal toxicity, general stress, and fetal outcome. *Teratog. Carcinog. Mutagen.* **7**, 241-253.

Chernoff, N., Setzer, W., Miller, D.B., Rosen, M.B., and Rogers, J.M. (1990). Effects of chemically induced maternal toxicity on prenatal development in the rat. *Teratology* **42**, 651-658.

Chernoff, N., Rogers, J.M., Turner, C.I., and Francis, B.M. (1991). Significance of supernumerary ribs in rodent developmental toxicity studies: Postnatal persistence in rats and mice. *Fundam. Appl. Toxicol.* **17**, 448-453.

Cholnoky, E., and Dömök, L.I. (1976). Summary of safety tests of ethyl apovincaminate. *Arzneimittelforschung* **26**, 1938-1944.

Code of Federal Regulations (CFR) **21**, Part 58.

Crosby, M.G., and Bennett, R.M. (2004). Compositions and methods for enhancing or treating female sexual response. United States Patent 6,737,084 B2.

Crosby, M.G., and Bennett, R.M. (2012). Compositions and methods for enhancing and treating female sexual response. United States Patent 8,128,972 B2.

DeSesso, J.M. (2006). Comparative features of vertebrate embryology. In *Developmental and Reproductive Toxicology: A Practical Approach* (R.D. Hood, Ed.), pp. 147-197. CRC Press, Boca Raton, FL.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Du, Z.D., Roguin, N., and Wu, X.J. (1998). Spontaneous closure of muscular ventricular septal defect identified by echocardiography in neonates. *Cardiol. Young.* **8**, 500-505.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Ebi, O. (1985). Open-labeled phase III clinical trials with vinpocetine in Japan. *Ther. Hung.* **33**, 41-49.

Elbary, A.A., Foda, N., El-Gazayerly, O., and El Khatib, M. (2002). Reversed phase liquid chromatographic determination of vinpocetine in human plasma and its pharmacokinetic application. *Anal. Lett.* **35**, 1041-1054.

Federal Register (2016). Department of Health and Human Services, U.S. Food and Drug Administration, Request for Comment on the Status of Vinpocetine. Docket No. FDA-2016-N-2523. Federal Register Vol. 81, No. 173. Washington, D.C.

Feigin, V.L., Doronin, B.M., Popova, T.F., Gribatcheva, E.V., and Tchervov, D.V. (2001). Vinpocetine treatment in acute ischaemic stroke: A pilot single-blind randomized clinical trial. *Eur. J. Neurol.* **8**, 81-85.

Fleeman, T.L., Cappon, G.D., and Hurtt, M.E. (2004). Postnatal closure of membranous ventricular septal defects in Sprague-Dawley rat pups after maternal exposure with trimethadione. *Birth Defect Res. B Dev. Reprod. Toxicol.* **71**, 185-190.

Foulon, O., Girard, H., Pallen, C., Urtizberea, M., Repetto-Larsay, M., and Blacker, A.M. (1999). Induction of supernumerary ribs with sodium salicylate. *Reprod. Toxicol.* **13**, 369-374.

Foulon, O., Jaussely, C., Repetto, M., Urtizberea, M., and Blacker, A.M. (2000). Postnatal evolution of supernumerary ribs in rats after a single administration of sodium salicylate. *J. Appl. Toxicol.* **20**, 205-209.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Gedeon Richter, Ltd. (1984). Cavinton. In: *Chemical Works of Gedeon Richter Ltd.*, pp. 360-363. Gedeon Richter, Ltd., Budapest, Hungary.

Grandt, R., Beitinger, H., Schaltenbrand, R., and Braun, W. (1989). Vinpocetine pharmacokinetics in elderly subjects. *Arzneimittelforschung* **39**, 1599-1602.

Gulyás, B., Halldin, C., Sandell, J., Karlsson, P., Sóvágó, J., Kárpáti, E., Kiss, B., Vas, Á., Cselényi, Z., and Farde, L. (2002a). PET studies on the brain uptake and regional distribution of [^{11}C]vinpocetine in human subjects. *Acta Neurol. Scand.* **106**, 325-332.

Gulyás, B., Halldin, C., Sóvágó, J., Sandell, J., Cselényi, Z., Vas, Á., Kiss, B., Kárpáti, E., and Farde, L. (2002b). Drug distribution in man: A positron emission tomography study after oral administration of the labelled neuroprotective drug vinpocetine. *Eur. J. Nucl. Med.* **29**, 1031-1038.

Hayes, A.W., and Kruger, C.L. (2014). *Hayes' Principles and Methods of Toxicology*, 6th ed., pp. 1670-1672. CRC Press, Boca Raton, FL.

Hendler, S.S., and Rorvik, D. (2001). Vinpocetine. In: *PDR for Nutritional Supplements*, pp. 460. Thomson Healthcare, Montvale, NJ.

Hoffman, J.I., and Kaplan, S. (2002). The incidence of congenital heart disease. *J. Am. Coll. Cardiol.* **39**, 1890-1900.

Hsu, J.C. (1992). The factor analytic approach to simultaneous inference in the general linear model.. *J. Computat. Graphic. Stat.* **1**, 151-168.

Hummel Surfactants Library (2018). IR – Surfactants, Hummel – Wiley. Product Code – 465700, Spectra – 1,030. Bio-Rad Spectral Databases. <http://www.knowitall.com/literaure/english/databases/465700-Bio-Rad_IR_Surfactants_Hummel_Wilely_Spectral_Database_Specification_Sheet.pdf>. Accessed August 28, 2018.

Infrared Characteristic Group Frequencies (ICGF) (1994). 2nd ed. (George Socrates, Ed.). John Wiley & Sons, New York.

Jeyaseelan, N., and Singh, S. (1984). Forelimb malformation in rats caused by cyclophosphamide. *Acta Orthop. Scand.* **55**, 643-646.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Khera, K.S. (1981). Common fetal aberrations and their teratologic significance: A review. *Fundam. Appl. Toxicol.* **1**, 13-18.

Kuge, Y., Nakazawa, H., Kometani, T., Sugaya, T., Mochida, K., and Tomioka, S. (1994). A facile one-pot synthesis of vinpocetine. *Synth. Commun.* **24**, 750-766.

Ley, B.M. (2000). *Vinpocetine: Boost Your Brain Power with Periwinkle Extract*, p. 48. B.L. Publications, Detroit Lakes, MN.

Li, B., Lingsma, H.F., Steyerberg, E.W., and Lesaffre, E. (2011). Logistic random effects regression models: A comparison of statistical packages for binary and ordinal outcomes. *BMC Med. Res. Methodol.* **11**, 77.

Linnea SA (2017). Vinpocetine. Linnea SA, Riazzino, TI, Switzerland. <http://www.linnea.ch/en/products/botanical-ingredients/vinpocetine> Accessed July 10, 2017.

Lohmann, A., Dingler, E., Sommer, W., Schaffler, K., Wober, W., and Schmidt, W. (1992). Bioavailability of vinpocetine and interference of the time of application with food intake. *Arzneimittelforschung* **42**, 914-917.

Luciano, J. (2012). Dietary supplement and a method to enhance sleep and lucid dreaming. United States Patent 8,092,840 B2.

Makris, S.L., Solomon, H.M., Clark, R., Shiota, K., Barbellion, S., Buschmann, J., Ema, M., Fujiwara, M., Grote, K., Hazelden, K.P., Hew, K.W., Horimoto, M., Ooshima, Y., Parkinson, M., and Wise, D.L. (2009). Terminology of developmental abnormalities in common laboratory mammals (version 2). *Reprod. Toxicol.* **28**, 371-434.

Manconi, E., Binaghi, F., and Pitzus, F. (1986). A double-blind clinical trial of vinpocetine in the treatment of cerebral insufficiency of vascular and degenerative origin. *Curr. Ther. Res. Clin. Exp.* **40**, 702-709.

Marr, M.C., Price, C.J., Myers, C.B., and Morrissey, R.E. (1992). Developmental stages of the CD (Sprague-Dawley) rat skeleton after maternal exposure to ethylene glycol. *Teratology* **46**, 169-181.

Matalon, S.T., Ornoy, A., and Lishner, M. (2004). Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta). *Reprod. Toxicol.* **18**, 219-230.

The Merck Index (2006). 14th ed. (M.J. O'Neil, Ed.), p. 1719. Whitehouse Station, New Jersey.

Miskolczi, P., Vereczkey, L., Szalay, L., and Göndöcs, C. (1987). Effect of age on the pharmacokinetics of vinpocetine (cavinton) and apovincaminic acid. *Eur. J. Clin. Pharmacol.* **33**, 185-189.

Miskolczi, P., Kozma, K., Polgár, M., and Vereczkey, L. (1990). Pharmacokinetics of vinpocetine and its main metabolite apovincaminic acid before and after the chronic oral administration of vinpocetine to humans. *Eur. J. Drug Metab. Pharmacokinet.* **15**, 1-5.

Misra, A.R., Gandhi, N.I., Bajaj, M.R., Shah, B.B., Samant, R.S., and Rana, H. (2011). Intranasal delivery to improve the performance of children suffering from dyslexia. World Intellectual Property Organization, International Bureau. International Application Published Under the Patent Cooperation Treaty (PCT). International Publication Date May 12, 2011. International Publication Number WO 2011/055383 A2.

Mondelo, F.C. (1989). Process for the obtention of the ethyl ester of the apovincaminic acid. United States Patent 4,870,178.

Narotsky, M.G., Francis, E.Z., and Kavlock, R.J. (1994). Developmental toxicity and structure-activity relationships of aliphatic acids, including dose-response assessment of valproic acid in mice and rats. *Toxicol. Sci.* **22**, 251-265.

Nie, S., Fan, X., Peng, Y., Yang, X., Wang, C., and Pan, W. (2006). *In vitro* and *in vivo* studies on the complexes of vinpocetine with hydroxypropyl-β-cyclodextrin. *Arch. Pharm. Res.* **30**, 991-1001.

Organisation for Economic Co-operation and Development (OECD) (2001). OECD guideline for the testing of chemicals: Proposal for Updating Guideline 414: Prenatal Developmental Toxicity Study. OECD, Paris. <https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl414.pdf>

Paladini, D., Palmieri, S., Lamberti, A., Teodoro, A., Martinelli, P., and Nappi, C. (2000). Characterization and natural history of ventricular septal defects in the fetus. *Ultrasound Obstet. Gynecol.* **16**, 118-122.

Pálosi, É., and Szporny, L. (1976). Effects of ethyl apovincaminate on the central nervous system. *Arzneimittelforschung* **26**, 1926-1929.

Pendergast, J.F., Gange, S.J., and Lindstrom, M.J. (2005). Correlated binary data. In *Encyclopedia of Biostatistics* (P. Armitage and T. Colton, Eds.). John Wiley, Hoboken, NJ.

Peruzza, M., and DeJacobis, M. (1986). A double-blind placebo controlled evaluation of the efficacy and safety of vinpocetine in the treatment of patients with chronic vascular or degenerative senile cerebral dysfunction. *Adv. Ther.* **3**, 201-209.

Ribeiro, L.S.S., Falcão, A.C., Patrício, J.A.B., Ferreira, D.C., and Veiga, F.J.B. (2007). Cyclodextrin multicomponent complexation and controlled release delivery strategies to optimize the oral bioavailability of vinpocetine. *J. Pharm. Sci.* **96**, 2018-2028.

Rogers, J.M., Francis, B.M., Barbee, B.D., and Chernoff, N. (1991). Developmental toxicity of bromoxynil in mice and rats. *Fundam. Appl. Toxicol.* **17**, 442-447.

Roguin, N., Du, Z.D., Barak, M., Nasser, N., Hershkowitz, S., and Milgram, E. (1995). High prevalence of muscular ventricular septal defect in neonates. *J. Am. Coll. Cardiol.* **26**, 1545-1548.

Sadtler's KnowItAll IR Spectral Library (2014). Retrieved from Bio-Rad Informatics "KnowItAll" System V.9.5 (No. 61,553). Bio-Rad, Hercules, CA.

Salewski, E. (1964). Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs. *Arch. Exp. Pathol. Pharmakol.* **247**, 367.

Scott, W.J., Jr., Resnick, E., Hummler, H., Clozel, J.-P., and Bürgin, H. (1997). Cardiovascular alterations in rat fetuses exposed to calcium channel blockers. *Reprod. Toxicol.* **11**, 207-214.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Sitges, M., Aldana, B.I., and Reed, R.C. (2016). Effect of the anti-depressant sertraline, the novel anti-seizure drug vinpocetine and several conventional antiepileptic drugs on the epileptiform EEG activity induced by 4-aminopyridine. *Neurochem. Res.* **41**, 1365-1374.

Solomon, H.M., Wier, P.J., Fish, C.J., Hart, T.K., Johnson, C.M., Posobiec, L.M., Gowan, C.C., Maleeff, B.E., and Kerns, W.D. (1997). Spontaneous and induced alterations in the cardiac membranous ventricular septum of fetal, weanling, and adult rats. *Teratology* **55**, 185-194.

South, C. (2007). Clayton's Health Facts: Vinpocetine. Bodybuilding.com, LLC, Boise, ID. <https://www.bodybuilding.com/fun/southfacts_vin.htm> .

Sozański, T., Magdalan, J., Trocha, M., Szumny, A., Merwid-Ląd, A., Słupski, W., Karaźniewicz-Łada, M., Kiełbowicz, G., Ksiądzyna, D., and Szeląg, A. (2011). Omeprazole does not change the oral bioavailability or pharmacokinetics of vinpocetine in rats. *Pharmacol. Rep.* **63**, 1258-1263.

Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology* **9**, A37-A38.

Stuckhardt, J.L., and Poppe, S.M. (1984). Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratog. Carcinog. Mutagen.* **4**, 181-188.

Suckow, M.A., Weisbroth, S.H., and Franklin, C.L. (2006). *The Laboratory Rat*, 2nd ed., Elsevier, Amsterdam.

Suckow, M., Stevens, K., and Wilson, R. (2012). *The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents*. Academic Press, Elsevier, Amsterdam.

Szabó, L., Kalaus, G., and Szántay, C. (1983). A new synthetic route to (+)-vincaminic and (+)-apovincaminic esters. *Arch. Pharm.* **316**, 629-638.

Szakács, T., Veres, Z., and Vereczkey, L. (2001). *In vitro-in vivo* correlation of the pharmacokinetics of vinpocetine. *Pol. J. Pharmacol.* **53**, 623-628.

Szatmári, S., and Whitehouse, P. (2009). Vinpocetine for cognitive impairment and dementia. Cochrane Database of Systematic Reviews, Issue 3. Art No. CD003119. The Cochrane Collaboration®. John Wiley & Sons, Ltd. Hoboken, NJ.

Taiji, H., and Kanzaki, J. (1986). Clinical study of vinpocetine in the treatment of vertigo. *Pharmacology and Treatment* **14**, 577-589.

Thal, L.J., Salmon, D.P., Lasker, B., Bower, D., and Klauber, M.R. (1989). The safety and lack of efficacy of vinpocetine in Alzheimer's disease. *J. Am. Geriatr. Soc.* **37**, 515-520.

Thompson, R.F. (1967). Basic Neuroanatomy. In *Foundations of Physiological Psychology* (R.F. Thompson, Ed.), Chapter 4, pp. 79-82. Harper and Row Publishers, New York.

Thorne Research, Inc. (2002). Vinpocetine. *Alter. Med. Rev.* **7**, 240-243. Thorne Research, Inc., Dover, ID.

Truss, M.C., Stief, C.G., Ückert, S., Becker, A.J., Schultheiss, D., Machtens, S., and Jonas, U. (2000). Initial clinical experience with the selective phosphodiesterase-I isoenzyme inhibitor vinpocetine in the treatment of urge incontinence and low compliance bladder. *World J. Urol.* **18**, 439-443.

Tyl, R.W. (2012). Commentary on the role of maternal toxicity on developmental toxicity. *Birth Defects Res. B Dev. Reprod. Toxicol.* **95**, 262-266.

Tyl, R.W., and Marr, M.C. (2006). Developmental toxicity texting – methodology. In *Developmental and Reproductive Toxicology* (R.D. Hood, Ed.), 2nd ed., pp. 201-261. Taylor and Francis Group, New York.

Ujházy, E., Preinerová, M., and Jozefík, M. (1979). Effects of cyclophosphamide on the prenatal development of the Swiss strain mice. *Neoplasma* **26**, 529-537.

U.S. Environmental Protection Agency (USEPA) (1991). Guidelines for Developmental Toxicity Risk Assessment. EPA Document No. EPA/600/FR-91/001. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC.

Vereczkey, L. (1985). Pharmacokinetics and metabolism of vincamine and related compounds. *Eur. J. Drug Metab. Pharmacokinet.* **10**, 89-103.

Vereczkey, L., and Szporny, L. (1976). Metabolism of ethyl apovincaminate in the rat. *Arzneimittelforschung* **26**, 1933-1938.

Vereczkey, L., Szentirmay, Zs., and Szporny, L. (1979a). Kinetic metabolism of vinpocetine in the rat. *Arzneimittelforschung* **29**, 953-956.

Vereczkey, L., Czira, G., Tamás, J., Szentirmay, Zs., and Szporny, L. (1979b). Pharmacokinetics of vinpocetine in humans. *Arzneimittelforschung* **29**, 957-960.

Vlase, L., Bodiu, B., and Leucuta, S.E. (2005). Pharmacokinetics and comparative bioavailabilty of two vinpocetine tablet formulations in healthy volunteers by using the metabolite apovincaminic acid as pharmacokinetic parameter. *Arzneimittelforschung* **11**, 664-668.

Waidyanatha, S., Toy, H., South, N., Gibbs, S., Mutlu, E., Burback, B., McIntyre, B.S., and Catlin, N. (2018). Systemic exposure of vinpocetine in pregnant Sprague Dawley rats following repeated oral exposure: An investigation of fetal transfer. *Toxicol. Appl. Pharmacol.* **338**, 83-92.

Wickramaratne, G.A. (1988). The post-natal fate of supernumerary ribs in rat teratogenicity studies. *J. Appl. Toxicol.* **8**, 91-94.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

World of Chemicals (WOC) (2017). Vinpocetine. Kimberlite Softwares Pvt. Ltd., India. <http://www.worldofchemicals.com/chemicals/chemical-properties/vinpocetine.html>.

Xia, H.-M., Su, L.-N., Guo, J.-W., Liu, G.-M., Pang, Z.-Q., Jiang, X.-G., and Chen, J. (2010). Determination of vinpocetine and its primary metabolite, apovincaminic acid, in rat plasma by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **878**, 1959-1966.

Xu, H., He, L., Nie, S., Guan, J., Zhang, X., Yang, X., and Pan, W. (2009). Optimized preparation of vinpocetine proliposomes by a novel method and in vivo evaluation of its pharmacokinetics in New Zealand rabbits. *J. Control. Release* **140**, 61-68

Zorrilla, E.P. (1997). Multiparous species present problems (and possibilities) to developmentalists. *Dev. Psychobiol.* **30**, 141-150.

APPENDIX A
SUMMARY OF FINDINGS IN RATS IN THE DOSE RANGE-FINDING GAVAGE STUDY OF VINPOCETINE

TABLE A1 Summary of Clinical Observations for Rats in the Dose Range-Finding Gavage Study of Vinpocetine A-2
TABLE A2 Summary of Mean Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of Vinpocetine A-3
TABLE A3 Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine A-4
TABLE A4 Summary of Fetal External Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine A-6
TABLE A5 Summary of Total Fetal Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine A-7
TABLE A6 Fetal Findings Cross Reference of Dams and Fetuses in the Dose Range-Finding Gavage Study of Vinpocetine A-8

TABLE A1
Summary of Clinical Observations for Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Pregnant Rats						
n	8	10	8	10	10	9
Discharge, anus	0	0	0	0	0	3 (GD 8)
Discharge, vagina; brown	0	0	0	1 (GD 13)	0	2 (GD 13)
Discharge, vagina; clear	0	1 (GD 19)	0	1 (GD 20)	0	0
Discharge, vagina; red	4 (GD 14)	5 (GD 13)	7 (GD 13)	10 (GD 13)	10 (GD 13)	9 (GD 13)
Discolored, nose/snout; brown	0	0	1 (GD 10)	2 (GD 8)	8 (GD 7)	9 (GD 7)
Piloerection	0	0	0	0	10 (GD 7)	9 (GD 7)
Wet, urogenital	0	0	0	0	0	1 (GD 12)
Non-pregnant Rats						
n	2	0	2	0	0	1
Discolored, nose/snout; brown	0		0			1 (SD 4)
Piloerection	0		0			1 (SD 2)
Scab, tail	0		1 (SD 5)			0
Sore, tail	0		1 (SD 3)			0

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses)
n = number of animals; GD = gestation phase; SD = study phase for females that were not pregnant

TABLE A2
Summary of Mean Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg		20 mg/kg			40 mg/kg			80 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	232.3 ± 4.6	8	229.9 ± 3.8	99.0	10	229.1 ± 3.6	98.6	8	228.2 ± 4.3	98.3	10
GD 4	233.8 ± 5.8	8	234.7 ± 3.8	100.4	10	234.5 ± 4.0	100.3	8	232.9 ± 3.9	99.6	10
GD 5	242.3 ± 3.0	8	239.8 ± 3.9	98.9	10	236.2 ± 4.0	97.5	8	236.2 ± 5.1	97.5	10
GD 6	246.1 ± 2.9	8	241.7 ± 3.7	98.2	10	238.4 ± 2.9	96.9	8	241.8 ± 2.9	98.2	10
GD 7	247.8 ± 4.2**	8	244.1 ± 3.1	98.5	10	235.9 ± 2.9*	95.2	8	234.3 ± 3.4*	94.5	10
GD 8	255.6 ± 2.6**	8	250.3 ± 4.2	97.9	10	238.3 ± 4.1**	93.2	8	237.1 ± 2.8**	92.8	10
GD 9	260.7 ± 3.6**	8	255.6 ± 4.4	98.0	10	243.6 ± 5.4**	93.4	8	243.9 ± 2.3**	93.5	10
GD 10	264.7 ± 2.9**	8	262.5 ± 4.8	99.2	10	247.6 ± 6.6*	93.5	8	251.4 ± 3.2*	95.0	10
GD 11	271.6 ± 3.3**	8	267.3 ± 4.6	98.4	10	257.2 ± 3.9*	94.7	8	254.3 ± 2.1**	93.7	10
GD 12	275.7 ± 3.2**	8	273.6 ± 4.5	99.2	10	264.2 ± 3.2	95.8	8	260.8 ± 2.7**	94.6	10
GD 13	281.6 ± 3.4**	8	279.7 ± 4.8	99.3	10	269.2 ± 3.4	95.6	8	264.2 ± 2.9**	93.8	10
GD 14	288.5 ± 2.6**	8	283.2 ± 4.7	98.2	10	273.3 ± 3.7*	94.7	8	263.0 ± 2.9**	91.2	10
GD 15	294.7 ± 2.9**	8	292.4 ± 5.7	99.2	10	280.3 ± 3.7*	95.1	8	267.0 ± 3.3**	90.6	10
GD 16	306.0 ± 2.9**	8	304.6 ± 6.3	99.5	10	285.4 ± 5.2**	93.3	8	271.5 ± 3.0**	88.7	10
GD 17	318.9 ± 2.3**	8	317.3 ± 6.9	99.5	10	295.4 ± 6.9**	92.6	8	273.2 ± 3.1**	85.7	10
GD 18	335.8 ± 3.1**	8	331.0 ± 8.1	98.6	10	303.7 ± 7.6**	90.4	8	275.5 ± 2.3**	82.0	10
GD 19	350.8 ± 3.6**	8	346.6 ± 9.2	98.8	10	318.4 ± 9.7**	90.8	8	278.9 ± 3.2**	79.5	10
GD 20	368.6 ± 4.1**	8	362.4 ± 9.7	98.3	10	334.3 ± 12.0**	90.7	8	275.0 ± 3.1**	74.6	10
GD 21	383.2 ± 4.7**	8	376.2 ± 10.7	98.2	10	347.5 ± 13.9**	90.7	8	276.1 ± 3.4**	72.0	10

	160 mg/kg			320 mg/kg		
	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	229.9 ± 3.9	99.0	10	228.7 ± 3.6	98.4	9
GD 4	234.8 ± 3.8	100.4	10	229.9 ± 4.8	98.3	9
GD 5	238.9 ± 3.6	98.6	10	239.1 ± 3.6	98.7	9
GD 6	241.5 ± 3.6	98.1	10	240.9 ± 4.1	97.9	9
GD 7	226.1 ± 3.6**	91.2	10	224.1 ± 4.1**	90.4	9
GD 8	219.7 ± 4.0**	85.9	10	214.9 ± 4.5**	84.1	9
GD 9	231.3 ± 3.4**	88.7	10	219.3 ± 4.5**	84.1	9
GD 10	233.6 ± 4.6**	88.3	10	222.8 ± 5.8**	84.2	9
GD 11	240.6 ± 3.9**	88.6	10	222.3 ± 5.7**	81.9	9
GD 12	246.7 ± 3.2**	89.5	10	223.7 ± 4.8**	81.1	9
GD 13	251.3 ± 4.2**	89.2	10	229.0 ± 5.7**	81.3	9
GD 14	251.4 ± 4.0**	87.1	10	234.1 ± 5.9**	81.1	9
GD 15	254.7 ± 3.7**	86.4	10	232.1 ± 4.5**	78.7	9
GD 16	254.3 ± 5.2**	83.1	10	233.1 ± 4.6**	76.2	9
GD 17	257.0 ± 4.1**	80.6	10	237.1 ± 5.3**	74.4	9
GD 18	260.3 ± 4.3**	77.5	10	239.2 ± 4.0**	71.2	9
GD 19	264.6 ± 4.0**	75.4	10	243.9 ± 5.4**	69.5	9
GD 20	267.0 ± 4.8**	72.4	10	248.2 ± 5.3**	67.3	9
GD 21	266.3 ± 5.4**	69.5	10	250.2 ± 5.3**	65.3	9

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

** P<0.01

[a] Data are displayed as mean ± standard error by gestation day (GD).

[b] Number of surviving dams

TABLE A3
Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia (GD 21, SD 15 to 16)	10	10	10	10	10	10
Number of animals examined	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, large, cecum	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, large, colon	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, large, rectum	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, small, duodenum	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, small, ileum	(10)	(10)	(10)	(10)	(10)	(10)
Intestine, small, jejunum	(10)	(10)	(10)	(10)	(10)	(10)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Right lobe; lobe, diaphragmatic, right; mass			1			
Pancreas	(10)	(10)	(10)	(10)	(10)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Cardiovascular System						
Heart	(10)	(10)	(10)	(10)	(10)	(10)
Left; mass; white			1			
Endocrine System						
Adrenal gland	(10)	(10)	(10)	(10)	(10)	(10)
General Body System						
None						
Genital System						
Ovary	(10)	(10)	(10)	(10)	(10)	(10)
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Lumen; fluid; yellow		1				
Vagina	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic System						
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Thymus	(10)	(10)	(10)	(10)	(10)	(10)
Integumentary System						
Skin	(10)	(10)	(10)	(10)	(10)	(10)
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A3
Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Respiratory System						
Lung	(10)	(10)	(10)	(10)	(10)	(10)
Thorax	(0)	(0)	(1)	(0)	(0)	(0)
Alopecia			1			
Fluid; clear			1			
Trachea	(10)	(10)	(10)	(10)	(10)	(10)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Ureter	(10)	(10)	(10)	(10)	(10)	(10)
Urinary bladder	(10)	(10)	(10)	(10)	(10)	(10)

[a] Number of animals examined at the site (displayed in parentheses) and number of animals with observation

TABLE A4
Summary of Fetal External Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	20 mg/kg	40 mg/kg	160 mg/kg
Number of fetuses examined	109	115	81	12
External				
Number of fetuses examined	109	115	81	12
Number of litters examined	8	9	7	1
Body: General				
Body, subcutaneous hemorrhage — [GF]				
Fetuses	0 (0.00)	1 (0.87)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	1 (11.11)	0 (0.00)	0 (0.00)
Extremities				
Limb, hind, right, malrotated — [M]				
Fetuses	1 (0.92)	0 (0.00)	0 (0.00)	0 (0.0)
Litters	1 (12.50)	0 (0.00)	0 (0.00)	0 (0.00)

[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.
[GF] = Gross Finding
[M] = Malformation

TABLE A5
Summary of Total Fetal Findings in Rats in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg		20 mg/kg		40 mg/kg		160 mg/kg	
All Exams								
Number of fetuses	109		115		81		12	
Number of litters	8		9		7		1	
Malformation								
Affected fetuses	1	(0.92)	0	(0.00)	0	(0.00)	0	(0.00)
Affected litters	1	(12.50)	0	(0.00)	0	(0.00)	0	(0.00)
Gross Finding								
Affected fetuses	0	(0.00)	1	(0.87)	1	(1.23)	0	(0.00)
Affected litters	0	(0.00)	1	(11.11)	1	(14.29)	0	(0.00)
External								
Number of fetuses	109		115		81		12	
Number of litters	8		9		7		1	
Malformation								
Affected fetuses	1	(0.92)	0	(0.00)	0	(0.00)	0	(0.00)
Affected litters	1	(12.50)	0	(0.00)	0	(0.00)	0	(0.00)
Gross Finding								
Affected fetuses	0	(0.00)	1	(0.87)	0	(0.00)	0	(0.00)
Affected litters	0	(0.00)	1	(11.11)	0	(0.00)	0	(0.00)

[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.

TABLE A6
Fetal Findings Cross Reference of Dams and Fetuses in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Number of fetuses examined	109	115	81	0	12	0
Number of dams examined	8	9	7	0	1	0
Placental						
Number of fetuses examined	109	115	81	0	12	0
Number of dams examined	8	9	7	0	1	0
Placentae						
Placenta, large — [GF]			24 (8)			
External						
Number of fetuses examined	109	115	81	0	12	0
Number of dams examined	8	9	7	0	1	0
Body - General						
Body, subcutaneous hemorrhage — [GF]		20 (8)				
Extremities						
Limb, hind, right, malrotated — [M]	9 (10)					

Findings are reported by dam ID number and fetus ID number (displayed in parentheses).
[GF] = Gross Finding
[M] = Malformation

APPENDIX B
SUMMARY OF FINDINGS IN RATS
IN THE PRENATAL DEVELOPMENTAL TOXICITY
GAVAGE STUDY OF VINPOCETINE

TABLE B1 Summary of Clinical Observations for Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-2

TABLE B2 Summary of Mean Maternal Body Weights of Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-3

TABLE B3 Summary of Gross Pathology Findings in Rats in the in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-4

TABLE B4 Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-6

TABLE B5 Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-11

TABLE B6 Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine B-13

TABLE B1
Summary of Clinical Observations for Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Pregnant Rats								
n	21		20		22		20	
Alopecia, thorax	0		0		1	(GD 19)	0	
Discharge, vagina; brown	0		2	(GD 14)	7	(GD 15)	11	(GD 15)
Discharge, vagina; clear	0		2	(GD 17)	2	(GD 17)	1	(GD 19)
Discharge, vagina; red	6	(GD 13)	3	(GD 13)	8	(GD 13)	16	(GD 13)
Scab, neck	0		0		1	(GD 17)	0	
Scab, tail	0		0		1	(GD 10)	1	(GD 14)
Sore, tail	0		0		1	(GD 7)	0	
Non-pregnant Rats								
n	4		5		3		5	
Discharge, vagina; clear	0		0		0		2	(SD 13)

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses)
n = number of animals; GD = gestation phase; SD = study phase for females that were not pregnant

TABLE B2
Summary of Mean Maternal Body Weights of Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg		5 mg/kg			20 mg/kg			60 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	229.2 ± 1.9	21	226.7 ± 2.6	98.9	20	226.4 ± 2.3	98.8	22	226.4 ± 2.8	98.8	20
GD 6	242.8 ± 2.0	21	239.8 ± 2.6	98.8	20	240.0 ± 2.0	98.8	22	240.8 ± 1.8	99.2	20
GD 7	246.9 ± 2.2	21	243.5 ± 2.5	98.6	20	244.1 ± 1.7	98.8	22	240.5 ± 2.0	97.4	20
GD 8	251.2 ± 1.8*	21	246.9 ± 2.8	98.3	20	247.8 ± 2.0	98.6	22	241.6 ± 2.5*	96.2	20
GD 9	256.0 ± 2.0**	21	251.1 ± 2.7	98.1	20	252.2 ± 1.7	98.5	22	246.0 ± 2.0**	96.1	20
GD 10	262.3 ± 2.3**	21	257.1 ± 2.6	98.0	20	257.3 ± 1.8	98.1	22	251.2 ± 2.0**	95.8	20
GD 11	267.3 ± 2.2**	21	262.5 ± 2.9	98.2	20	263.2 ± 2.0	98.5	22	255.0 ± 2.2**	95.4	20
GD 12	272.0 ± 2.1**	21	266.4 ± 2.8	97.9	20	268.2 ± 2.1	98.6	22	259.4 ± 2.5**	95.3	20
GD 13	278.0 ± 2.3**	21	272.4 ± 3.0	98.0	20	273.4 ± 2.1	98.4	22	264.6 ± 2.7**	95.2	20
GD 14	282.9 ± 2.4**	21	276.9 ± 3.2	97.9	20	278.3 ± 2.0	98.4	22	267.0 ± 2.4**	94.4	20
GD 15	292.7 ± 2.4**	21	284.1 ± 3.5	97.1	20	286.0 ± 2.2	97.7	22	269.2 ± 2.9**	92.0	20
GD 16	303.3 ± 2.4**	21	295.5 ± 3.8	97.4	20	297.1 ± 2.5	98.0	22	275.0 ± 3.0**	90.7	20
GD 17	317.7 ± 2.8**	21	307.9 ± 4.2	96.9	20	309.1 ± 3.0	97.3	22	280.3 ± 3.7**	88.2	20
GD 18	333.5 ± 3.1**	21	322.2 ± 5.2	96.6	20	323.0 ± 3.6	96.8	22	284.6 ± 4.5**	85.3	20
GD 19	349.4 ± 3.7**	21	335.8 ± 6.3	96.1	20	337.0 ± 4.1	96.5	22	289.3 ± 5.4**	82.8	20
GD 20	366.6 ± 4.0**	21	350.7 ± 7.2	95.7	20	351.9 ± 4.7	96.0	22	291.2 ± 6.5**	79.4	20
GD 21	385.7 ± 4.2**	21	368.5 ± 8.2	95.5	20	370.0 ± 5.5	95.9	22	296.1 ± 8.2**	76.8	20

* Statistically significant ($P \leq 0.05$) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

** $P<0.01$

[a] Data are displayed as mean ± standard error by gestation day (GD).

[b] Number of surviving dams

TABLE B3
Summary of Gross Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal euthanasia (GD 21, SD 15 to 18)	25	25	25	25
Number of animals examined	25	25	25	25
Alimentary System				
Esophagus	(25)	(25)	(25)	(25)
Intestine, large, cecum	(25)	(25)	(25)	(25)
Intestine, large, colon	(25)	(25)	(25)	(25)
Intestine, large, rectum	(25)	(25)	(25)	(25)
Intestine, small, duodenum	(25)	(25)	(25)	(25)
Intestine, small, ileum	(25)	(25)	(25)	(25)
Intestine, small, jejunum	(25)	(25)	(25)	(25)
Liver	(25)	(25)	(25)	(25)
Pancreas	(25)	(25)	(25)	(25)
Stomach, forestomach	(25)	(25)	(25)	(25)
Stomach, glandular	(25)	(25)	(25)	(25)
Cardiovascular System				
Heart	(25)	(25)	(25)	(25)
Endocrine System				
Adrenal gland	(25)	(25)	(25)	(25)
General Body System				
None				
Genital System				
Ovary	(25)	(25)	(25)	(25)
Uterus	(25)	(25)	(25)	(25)
Fluid; black			1	1
Fluid; yellow			1	
Lumen; dilation		1	2	1
Lumen; fluid; clear		1	1	
Vagina	(25)	(25)	(25)	(25)
Hematopoietic System				
Lymph node, mesenteric	(25)	(25)	(25)	(25)
Spleen	(25)	(25)	(25)	(25)
Thymus	(25)	(25)	(25)	(25)
Integumentary System				
Skin	(25)	(25)	(25)	(25)
Head; alopecia			1	
Head; crust			1	
Musculoskeletal System				
None				
Nervous System				
None				

TABLE B3
Summary of Gross Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Respiratory System				
Lung	(25)	(25)	(25)	(25)
Trachea	(25)	(25)	(25)	(25)
Special Senses System				
None				
Urinary System				
Kidney	(25)	(25)	(25)	(25)
Ureter	(25)	(25)	(25)	(25)
Urinary bladder	(25)	(25)	(25)	(25)

[a] Number of animals examined at the site (displayed in parentheses) and number of animals with observation

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Number of fetuses examined	293		239		261		51	
External								
Number of fetuses examined	293		239		261		51	
Number of litters examined	21		19		21		8	
Body: General								
Body, generalized subcutaneous edema — [M]								
Fetuses	0	(0.0)	1	(0.42)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	1	(4.76)	0	(0.00)
Extremities								
Tail, bent — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Tail, Short — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Trunk								
General, omphalocele — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Visceral								
Number of fetuses examined	293		239		261		51	
Number of litters examined	21		19		21		8	
Abdominal viscera								
Diaphragm, hernia — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Diaphragm, thin — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Liver lobe, additional fissure — [V]								
Fetuses	1	(0.34)	1	(0.42)	3	(1.15)	0	(0.0)
Litters	1	(4.76)	1	(5.26)	2	(9.52)	0	(0.00)
Liver lobe, left lateral, additional fissure — [V]								
Fetuses	0	(0.0)	1	(0.42)	2	(0.77)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	1	(4.76)	0	(0.00)
Liver lobe, left medial, additional fissure — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Liver lobe, left medial, Misshapen — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Liver lobe, right medial, additional fissure — [V]								
Fetuses	1	(0.34)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(4.76)	0	(0.00)	0	(0.00)	0	(0.00)
Liver lobe, right medial, supernumerary — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Liver lobe, see comment, supernumerary — [V]								
Fetuses	2	(0.68)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	2	(9.52)	0	(0.00)	1	(4.76)	0	(0.00)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Visceral (continued)								
Number of fetuses examined	293		239		261		51	
Number of litters examined	21		19		21		8	
General								
General, fluid-filled abdomen — [GF]								
Fetuses	0	(0.0)	1	(0.42)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	1	(4.76)	0	(0.00)
Heart								
Aortic valve, misshapen — [M]								
Fetuses	19	(6.48)	14	(5.86)	17	(6.51)	0	(0.0)*
Litters	12	(57.14)**	11	(57.89)	10	(47.62)	0	(0.00)**
Atrium, right, large — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Ventricle, bilateral, ventricular septum defect — [M]								
Fetuses	0	(0.0)**	3	(1.26)	8	(3.07)**	2	(3.92)*
Litters	0	(0.00)	3	(15.79)	7	(33.33)**	2	(25.00)
Ventricle, left, thick wall — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Major Vessels								
Carotid artery, right, supernumerary — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.96)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(12.50)
Ductus arteriosus, patent — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Innominate artery, absent — [V]								
Fetuses	4	(1.37)	7	(2.93)	8	(3.07)	1	(1.96)
Litters	4	(19.05)	6	(31.58)	5	(23.81)	1	(12.50)
Innominate artery, short — [V]								
Fetuses	3	(1.02)	4	(1.67)	2	(0.77)	1	(1.96)
Litters	3	(14.29)	4	(21.05)	2	(9.52)	1	(12.50)
Thoracic Viscera								
Lung lobe, accessory, absent — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Lung lobe, right cranial, fused — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thymus, split — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Head								
Number of fetuses examined	150		125		134		29	
Number of litters examined	21		19		20		8	
Brain								
Ventricles, dilated — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.75)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Ventricles, hydrocephaly — [M]								
Fetuses	0	(0.0)	1	(0.8)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Skeletal: Body[b]								
Number of fetuses examined	293		239		260		47	
Number of litters examined	21		19		21		7	
Ribs								
Costal cartilage, 7th right, not fused to sternum — [M]								
Fetuses	1	(0.34)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(4.76)	0	(0.00)	0	(0.00)	0	(0.00)
Sternebrae								
Sternebra, 4th, misaligned — [V]								
Fetuses	1	(0.34)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(4.76)	0	(0.00)	0	(0.00)	0	(0.00)
Supernumerary rib								
Thoracolumbar, full, — [M]								
Fetuses	1	(0.34)**##	5	(2.09)	12	(4.62)**	12	(25.53)**##
Litters	1	(4.76)*	3	(15.79)	4	(19.05)	3	(42.86)*
Thoracolumbar, short, — [V]								
Fetuses	29	(9.9)**#	30	(12.55)	55	(21.15)**#	10	(21.28)*#
Litters	14	(66.67)	10	(52.63)	17	(80.95)	5	(71.43)
Thoracolumbar, bilateral, full — [M]								
Fetuses	0	(0.0)**	2	(0.84)	9	(3.46)**	8	(17.02)**
Litters	0	(0.00)	2	(10.53)	1	(4.76)	2	(28.57)
Thoracolumbar, bilateral, short — [V]								
Fetuses	6	(2.05)	9	(3.77)	17	(6.54)**#	1	(2.13)
Litters	4	(19.05)	5	(26.32)	11	(52.38)*	1	(14.29)
Thoracolumbar, left, full — [M]								
Fetuses	0	(0.0)**	1	(0.42)	2	(0.77)	3	(6.38)**
Litters	0	(0.00)	1	(5.26)	2	(9.52)	1	(14.29)
Thoracolumbar, left, short — [V]								
Fetuses	21	(7.17)*#	10	(4.18)	22	(8.46)	7	(14.89)
Litters	13	(61.90)	6	(31.58)	13	(61.90)	5	(71.43)
Thoracolumbar, right, full — [M]								
Fetuses	1	(0.34)	2	(0.84)	1	(0.38)	1	(2.13)
Litters	1	(4.76)	2	(10.53)	1	(4.76)	1	(14.29)
Thoracolumbar, right, short — [V]								
Fetuses	2	(0.68)*	11	(4.6)**#	16	(6.15)**##	2	(4.26)#
Litters	2	(9.52)	6	(31.58)	12	(57.14)**	2	(28.57)
Vertebrae								
Cervical arch, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Lumbar arch, 5th left, fused — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Lumbar centrum, 1st, incomplete ossification — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Lumbar centrum, 3rd, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Lumbar centrum, 5th, fused — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Presacral vertebrae, greater than 26 — [V]								
Fetuses	0	(0.0)**	0	(0.0)	0	(0.0)	4	(8.51)**
Litters	0	(0.00)**	0	(0.00)	0	(0.00)	2	(28.57)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Skeletal: Body (continued)								
Number of fetuses examined	293		239		260		47	
Number of litters examined	21		19		21		7	
Vertebrae (continued)								
Sacral centrum, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic arch, 6th right, misshapen — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic arch, multiple sites, misshapen — [M]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 10th, incomplete ossification — [V]								
Fetuses	1	(0.34)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	1	(4.76)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, 11th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	3	(1.15)	2	(4.26)*
Litters	0	(0.00)**	0	(0.00)	3	(14.29)	2	(28.57)
Thoracic centrum, 12th, hemicentric — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 12th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	1	(0.38)	3	(6.38)**
Litters	0	(0.00)**	0	(0.00)	1	(4.76)	2	(28.57)
Thoracic centrum, 13th, incomplete ossification — [V]								
Fetuses	0	(0.0)**	0	(0.0)	0	(0.0)	2	(4.26)*
Litters	0	(0.00)**	0	(0.00)	0	(0.00)	2	(28.57)
Thoracic centrum, 1st, hemicentric — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Thoracic centrum, 1st, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Thoracic centrum, 5th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Thoracic centrum, 6th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, 9th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(14.29)
Thoracic centrum, incomplete ossification — [V]								
Fetuses	1	(0.34)**##	1	(0.42)	6	(2.31)*#	8	(17.02)**##
Litters	1	(4.76)**	1	(5.26)	5	(23.81)	3	(42.86)*
Thoracic centrum, multiple sites, incomplete ossification — [V]								
Fetuses	0	(0.0)	1	(0.42)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Skeletal: Skull				
Number of fetuses examined	143	114	124	20
Number of litters examined	21	19	21	5
Skull				
General, isolated ossification site — [V]				
Fetuses	0 (0.0)	1 (0.88)	1 (0.81)	0 (0.0)
Litters	0 (0.00)	1 (5.26)	1 (4.76)	0 (0.00)
General, supernumerary site — [V]				
Fetuses	1 (0.7)	0 (0.0)	1 (0.81)	0 (0.0)
Litters	1 (4.76)	0 (0.00)	1 (4.76)	0 (0.00)
Interparietal, incomplete ossification — [V]				
Fetuses	0 (0.0)	0 (0.0)	1 (0.81)	0 (0.0)
Litters	0 (0.00)	0 (0.00)	1 (4.76)	0 (0.00)

* Statistically significant (P≤0.05) according to the Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
# Statistically significant (P≤0.05) according to mixed effects logistic regression models with litter-based adjustments. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
P≤0.01
[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
[b] Skeletal-body examination was not performed on one fetus in the 20 mg/kg group
[M] = Malformation
[V] = Variation
[GF] = Gross Finding
Skeletal-body examination was not performed on one fetus in the 20 mg/kg group

TABLE B5
Summary of Total Fetal Findings in Rats
in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine[a]

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
All Exams								
Number of fetuses	293		239		261		51	
Number of litters	21		19		21		8	
Malformation								
Affected fetuses	20	(6.83)**##	23	(9.62)	35	(13.41)**#	15	(29.41)**##
Affected litters	12	(57.14)	13	(68.42)	17	(80.95)	4	(50.00)
Variation								
Affected fetuses	42	(14.33)**##	44	(18.41)	78	(29.89)**##	20	(39.22)**##
Affected litters	18	(85.71)	17	(89.47)	18	(85.71)	6	(75.00)
Gross Finding								
Affected fetuses	0	(0.00)	1	(0.42)	1	(0.38)	0	(0.00)
Affected litters	0	(0.00)	1	(5.26)	1	(4.76)	0	(0.00)
External								
Number of fetuses	293		239		261		51	
Number of litters	21		19		21		8	
Malformation								
Affected fetuses	0	(0.00)	1	(0.42)	4	(1.53)*	0	(0.00)
Affected litters	0	(0.00)	1	(5.26)	4	(19.05)	0	(0.00)
Visceral								
Number of fetuses	293		239		261		51	
Number of litters	21		19		21		8	
Malformation								
Affected fetuses	19	(6.48)	18	(7.53)	23	(8.81)	3	(5.88)
Affected litters	12	(57.14)	12	(63.16)	13	(61.90)	3	(37.50)
Variation								
Affected fetuses	10	(3.41)	15	(6.28)	16	(6.13)	2	(3.92)
Affected litters	7	(33.33)	11	(57.89)	9	(42.86)	2	(25.00)
Gross Finding								
Affected fetuses	0	(0.00)	1	(0.42)	1	(0.38)	0	(0.00)
Affected litters	0	(0.00)	1	(5.26)	1	(4.76)	0	(0.00)
Head								
Number of fetuses	150		125		134		29	
Number of litters	21		19		20		8	
Malformation								
Affected fetuses	0	(0.00)	1	(0.80)	0	(0.00)	0	(0.00)
Affected litters	0	(0.00)	1	(5.26)	0	(0.00)	0	(0.00)
Variation								
Affected fetuses	0	(0.00)	0	(0.00)	1	(0.75)	0	(0.00)
Affected litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)

TABLE B5
Summary of Total Fetal Findings in Rats
in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg		5 mg/kg		20 mg/kg		60 mg/kg	
Skeletal: Body[b]								
Number of fetuses	293		239		260		47	
Number of litters	21		19		21		7	
Malformation								
Affected fetuses	2	(0.68)**##	6	(2.51)	12	(4.62)**	13	(27.66)**##
Affected litters	2	(9.52)	4	(21.05)	4	(19.05)	3	(42.86)
Variation								
Affected fetuses	31	(10.58)**##	31	(12.97)	61	(23.46)**##	19	(40.43)**##
Affected litters	15	(71.43)	10	(52.63)	17	(80.95)	5	(71.43)
Skeletal: Skull								
Number of fetuses	143		114		124		20	
Number of litters	21		19		21		5	
Variation								
Affected fetuses	1	(0.70)	1	(0.88)	3	(2.42)	0	(0.00)
Affected litters	1	(4.76)	1	(5.26)	3	(14.29)	0	(0.00)

* Statistically significant ($P \leq 0.05$) according to the Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** $P \leq 0.01$
[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
[b] Skeletal-body examination was not performed on one fetus in the 20 mg/kg group
[M] = Malformation
[V] = Variation
[GF] = Gross Finding

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Number of fetuses examined	293	239	261	51
Number of dams examined	21	19	21	8
Placental				
Number of fetuses examined	293	239	261	51
Number of dams examined	21	19	21	8
External				
Number of fetuses examined	293	239	261	51
Number of dams examined	21	19	21	8
Body: General				
Body, generalized subcutaneous edema — [M]		31 (3)	69 (11)	
Extremities				
Tail, bent — [M]			61 (7)	
Tail, short — [M]			55 (1)	
Trunk				
General, omphalocele — [M]			63 (1)	
Visceral				
Number of fetuses examined	293	239	261	51
Number of dams examined	21	19	21	8
Abdominal Viscera				
Diaphragm, hernia — [M]		31 (3)		
Diaphragm, thin — [M]		37 (1)		
Liver lobe, left lateral, additional fissure — [V]		46 (4)	72 (9,10)	
Liver lobe, left medial, additional fissure — [V]			74 (16)	
Liver lobe, left medial, misshapen — [V]		44 (5)		
Liver lobe, right medial, additional fissure — [V]	20 (10)			
Liver lobe, right medial, supernumerary — [V]		45 (6)		
Liver lobe, see comment, supernumerary — [V]	7 (6) 20 (11)		69 (1)	
General				
General, fluid-filled abdomen — [GF]		31 (3)	69 (11)	

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Visceral (continued)				
Number of fetuses examined	293	239	261	51
Number of dams examined	21	19	21	8
Heart				
Aortic valve, misshapen — [M]				
	1 (12)	26 (2)	51 (1)	
	3 (2,6)	37 (2)	52 (5)	
	5 (6)	38 (9)	60 (3,10,12)	
	9 (13)	39 (8,15)	62 (5,14)	
	12 (6)	40 (5)	63 (9)	
	15 (5,11)	42 (1,3,8)	64 (10)	
	16 (7,14)	43 (3)	68 (11,12)	
	17 (14)	44 (13)	69 (5,11,13)	
	20 (2,4,6,11,13)	45 (13)	72 (11)	
	22 (7)	46 (14)	74 (1,17)	
	24 (5)	47 (4)		
	25 (4)			
Atrium, right, large — [M]				
			72 (11)	
Ventricle, bilateral, ventricular septum defect — [M]				
		31 (3)	64 (11)	96 (10)
		44 (8)	65 (2,14)	98 (3)
		47 (8)	66 (3)	
			68 (11)	
			69 (11)	
			73 (7)	
			74 (9)	
Ventricle, left, thick wall — [M]				
	72 (11)			
Major Vessels				
Carotid artery, right, supernumerary — [M]				
				77 (4)
Ductus arteriosus, patent — [V]				
			72 (11)	
Innominate artery, absent — [V]				
	9 (5)	26 (9)	53 (1)	77 (1)
	20 (9)	29 (1)	56 (9)	
	24 (8)	37 (1)	65 (2,3,4,5)	
	25 (1)	47 (11)	66 (10)	
		49 (1,15)	72 (8)	
		50 (1)		
Innominate artery, short — [V]				
	5 (10)	31 (1)	62 (6)	96 (5)
	7 (12)	37 (4)	65 (14)	
	13 (15)	43 (4)		
		46 (1)		
Thoracic Viscera				
Lung lobe, accessory, absent — [M]				
		31 (3)		
Lung lobe, right cranial, fused — [V]				
		31 (3)		
Thymus, split — [V]				
			61 (1)	

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Head				
Number of fetuses examined	150	125	134	29
Number of dams examined	21	19	20	8
Brain				
Ventricles, dilated — [V]				
			69 (11)	
Ventricles, hydrocephaly — [M]				
		31 (3)		
Skeletal: Body				
Number of fetuses examined	293	239	261	47
Number of dams examined	21	19	21	7
Appendicular Skeleton				
General, not examined			66 (6)	
Ribs				
Costal cartilage, 7th right, not fused to sternum — [M]				
	15 (1)			
Sternebrae				
Sternebra, 4th, misaligned — [V]				
	12 (13)			
Supernumerary rib				
Thoracolumbar, bilateral, full — [M]				
		42 (13)	65 (1,2,4,5,6,7,8,12,14)	88 (3,11,13)
		44 (11)		96 (3,6,8,10,11)
Thoracolumbar, bilateral, short — [V]				
	5 (5,7,12)	38 (4)	53 (13)	96 (7)
	11 (9)	39 (9)	56 (6,7)	
	13 (3)	42 (9)	57 (2)	
	17 (11)	44 (2,5,8,9,14)	60 (11)	
		49 (5)	61 (5,9,13)	
			62 (11)	
			63 (9)	
			64 (2,4,6)	
			65 (9,11)	
			69 (6)	
			72 (9)	
Thoracolumbar, left, full — [M]				
		44 (15)	54 (11)	88 (2,5,10)
			61 (7)	

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Skeletal: Body (continued)				
Number of fetuses examined	293	239	261	47
Number of dams examined	21	19	21	7
Supernumerary rib (continued)				
Thoracolumbar, left, short — [V]				
	1 (4,7)	27 (1)	53 (7,9,10)	88 (6)
	2 (1,2,10)	28 (4,8,9,11)	54 (10)	90 (7)
	3 (4)	37 (11)	55 (5)	95 (4)
	7 (16)	42 (2,12)	56 (8,10)	96 (4,5)
	8 (9)	44 (7)	60 (5,6)	98 (1,2)
	11 (6,13)	49 (8)	61 (1,4,6,10)	
	12 (12)		64 (13)	
	13 (2,12)		65 (13)	
	16 (1,9)		66 (7)	
	17 (6)		68 (5)	
	20 (1,3,4)		69 (15)	
	22 (8)		71 (1)	
	23 (19)		72 (4,7,14)	
Thoracolumbar, right, full — [M]				
	24 (5)	28 (4)	56 (10)	98 (1)
		44 (7)		
Thoracolumbar, right, short — [V]				
	5 (6)	28 (6,7,12)	52 (10)	88 (8)
	13 (5)	31 (1)	53 (3)	90 (4)
		37 (16)	54 (11)	
		39 (7)	55 (9)	
		44 (1,3,13,15)	57 (3,8,13)	
		48 (3)	61 (2,3,7)	
			62 (14)	
			65 (10)	
			66 (14)	
			68 (7)	
			69 (1)	
			72 (13)	
Vertebrae				
Cervical arch, multiple sites, misshapen — [M]				
		31 (3)		
Lumbar arch, 5th left, fused — [M]				
				88 (5)
Lumbar centrum, 1st, incomplete ossification — [V]				
		31 (3)		
Lumbar centrum, 3rd, incomplete ossification — [V]				
			68 (11)	
Lumbar centrum, 5th, fused — [M]				
				88 (5)
Presacral vertebrae, greater than 26 — [V]				
				88 (3,5)
				96 (3,11)
Sacral centrum, multiple sites, misshapen — [M]				
		31 (3)		
Thoracic arch, 6th right, misshapen — [M]				
				96 (1)
Thoracic arch, multiple sites, misshapen — [M]				
	31 (3)			
Thoracic centrum, 10th, incomplete ossification — [V]				
	21 (13)			90 (5)

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Skeletal: Body (continued)				
Number of fetuses examined	293	239	261	47
Number of dams examined	21	19	21	7
Vertebrae (continued)				
Thoracic centrum, 11th, incomplete ossification — [V]				
			54 (12)	88 (5)
			62 (5)	90 (10)
			69 (8)	
Thoracic centrum, 12th, hemicentric — [V]				
		31 (3)		
Thoracic centrum, 12th, incomplete ossification — [V]				
			68 (7)	88 (5,7)
				90 (8)
Thoracic centrum, 13th, incomplete ossification — [V]				
				88 (5)
				96 (4)
Thoracic centrum, 1st, hemicentric — [V]				
		31 (3)		
Thoracic centrum, 1st, incomplete ossification — [V]				
			53 (11)	
Thoracic centrum, 5th, incomplete ossification — [V]				
			68 (10)	
Thoracic centrum, 6th, incomplete ossification — [V]				
				96 (1)
Thoracic centrum, 9th, incomplete ossification — [V]				
				96 (11)
Thoracic centrum, multiple sites, incomplete ossification — [V]				
		31 (3)		
Skeletal: Skull				
Number of fetuses examined	143	114	124	20
Number of dams examined	21	19	21	5
Skull				
General, isolated ossification site — [V]				
		43 (2)	64 (12)	
General, supernumerary site — [V]				
	24 (4)		63 (4)	
Interparietal, incomplete ossification — [V]				

Findings are reported by dam ID number and fetus ID number (displayed in parentheses).
[M] = Malformation
[V] = Variation
[GF] = Gross Finding

APPENDIX C
SUMMARY OF FINDINGS IN RABBITS IN THE DOSE RANGE-FINDING GAVAGE STUDY OF VINPOCETINE

TABLE C1 Summary of Clinical Observations for Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine C-2
TABLE C2 Summary of Mean Maternal Body Weights of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine C-3
TABLE C3 Summary of Gross Pathology Findings in Rabbits in the in the Dose Range-Finding Gavage Study of Vinpocetine C-5
TABLE C4 Summary of Fetal External Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine C-7
TABLE C5 Summary of Total Fetal Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine C-8
TABLE C6 Fetal Findings Cross Reference of Does and Fetuses in the Dose Range-Finding Gavage Study of Vinpocetine C-9

TABLE C1
Summary of Clinical Observations for Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Pregnant Rabbits					
n	8	7	8	8	8
Discharge, vagina; red	1 (GD 22)	0	0	1 (GD 21)	1 (GD 20)
Discolored, hind limb; red	0	0	0	1 (GD 23)	0
Discolored, tip of tail; red	0	0	0	1 (GD 24)	0
Discolored, vagina; red	1 (GD 22)	0	0	1 (GD 21)	1 (GD 20)
Discolored, vagina	0	0	0	0	1 (GD 20)
Red substance in cage pan	0	0	0	1 (GD 25)	1 (GD 20)
Wound, mouth	0	0	0	0	1 (GD 7)

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses)
n = number of animals; GD = gestation phase

TABLE C2
Summary of Mean Maternal Body Weights of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg		25 mg/kg			75 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	2,957.8 ± 69.5	8	2,886.7 ± 43.5	97.6	7	2,960.4 ± 89.5	100.1	8
GD 4	2,991.9 ± 67.3	8	2,912.1 ± 38.0	97.3	7	3,007.7 ± 90.2	100.5	8
GD 5	3,002.4 ± 68.4	8	2,923.2 ± 34.8	97.4	7	3,020.9 ± 87.1	100.6	8
GD 6	3,021.6 ± 68.8	8	2,940.3 ± 28.9	97.3	7	3,050.8 ± 95.2	101.0	8
GD 7	3,039.3 ± 68.8	8	2,948.3 ± 31.5	97.0	7	3,068.4 ± 89.7	101.0	8
GD 8	3,065.1 ± 67.7	8	2,974.1 ± 40.4	97.0	7	3,112.9 ± 88.3	101.6	8
GD 9	3,092.5 ± 68.6	8	3,009.9 ± 36.4	97.3	7	3,117.5 ± 84.0	100.8	8
GD 10	3,117.3 ± 65.2	8	3,015.8 ± 41.1	96.7	7	3,141.7 ± 85.7	100.8	8
GD 11	3,122.8 ± 68.6	8	3,031.9 ± 38.8	97.1	7	3,154.1 ± 89.6	101.0	8
GD 12	3,149.2 ± 55.7	8	3,070.7 ± 29.0	97.5	7	3,192.3 ± 91.8	101.4	8
GD 13	3,179.6 ± 57.7	8	3,077.0 ± 30.0	96.8	7	3,219.6 ± 93.8	101.3	8
GD 14	3,218.9 ± 57.1	8	3,133.2 ± 26.0	97.3	7	3,251.1 ± 92.1	101.0	8
GD 15	3,254.5 ± 57.8	8	3,168.4 ± 29.0	97.4	7	3,263.8 ± 88.7	100.3	8
GD 16	3,272.3 ± 56.7	8	3,201.2 ± 40.6	97.8	7	3,271.0 ± 87.0	100.0	8
GD 17	3,269.7 ± 51.3	8	3,196.3 ± 37.6	97.8	7	3,277.2 ± 86.6	100.2	8
GD 18	3,274.6 ± 57.6	8	3,187.8 ± 35.9	97.3	7	3,274.8 ± 80.7	100.0	8
GD 19	3,294.9 ± 55.3*	8	3,219.5 ± 36.3	97.7	7	3,283.2 ± 76.0	99.6	8
GD 20	3,307.4 ± 57.4*	8	3,215.4 ± 36.5	97.2	7	3,291.1 ± 76.3	99.5	8
GD 21	3,330.9 ± 53.8	8	3,231.6 ± 43.7	97.0	7	3,314.2 ± 76.4	99.5	8
GD 22	3,355.1 ± 52.1	8	3,257.7 ± 49.2	97.1	7	3,347.0 ± 77.2	99.8	8
GD 23	3,380.4 ± 52.1	8	3,289.8 ± 57.2	97.3	7	3,376.4 ± 79.4	99.9	8
GD 24	3,413.1 ± 52.9*	8	3,303.1 ± 59.3	96.8	7	3,402.2 ± 82.9	99.7	8
GD 25	3,441.5 ± 49.2*	8	3,313.7 ± 53.5	96.3	7	3,406.2 ± 77.7	99.0	8
GD 26	3,476.1 ± 51.8*	8	3,336.9 ± 55.8	96.0	7	3,411.0 ± 80.4	98.1	8
GD 27	3,489.1 ± 53.2*	8	3,351.5 ± 58.6	96.1	7	3,420.6 ± 91.5	98.0	8
GD 28	3,501.1 ± 58.7*	8	3,380.2 ± 60.9	96.5	7	3,421.1 ± 92.6	97.7	8
GD 29	3,499.4 ± 64.6*	8	3,406.5 ± 58.0	97.3	7	3,467.7 ± 95.0	99.1	8

TABLE C2
Summary of Mean Maternal Body Weights of Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	150 mg/kg			300 mg/kg		
	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	3,025.3 ± 110.2	102.3	8	2,922.1 ± 45.9	98.8	8
GD 4	3,036.1 ± 109.1	101.5	8	2,932.9 ± 46.5	98.0	8
GD 5	3,034.9 ± 104.7	101.1	8	2,951.5 ± 47.5	98.3	8
GD 6	3,051.1 ± 102.2	101.0	8	2,960.0 ± 43.6	98.0	8
GD 7	3,094.6 ± 100.5	101.8	8	2,967.3 ± 42.8	97.6	8
GD 8	3,092.1 ± 98.9	100.9	8	2,995.0 ± 34.0	97.7	8
GD 9	3,109.5 ± 96.4	100.6	8	3,014.6 ± 31.2	97.5	8
GD 10	3,126.2 ± 103.0	100.3	8	2,993.9 ± 28.2	96.0	8
GD 11	3,130.7 ± 104.1	100.3	8	3,015.0 ± 37.0	96.5	8
GD 12	3,150.4 ± 100.7	100.0	8	3,041.4 ± 31.9	96.6	8
GD 13	3,152.4 ± 97.6	99.1	8	3,052.8 ± 33.3	96.0	8
GD 14	3,180.9 ± 107.3	98.8	8	3,073.6 ± 31.9	95.5	8
GD 15	3,186.5 ± 107.3	97.9	8	3,087.3 ± 34.0	94.9	8
GD 16	3,186.1 ± 107.1	97.4	8	3,085.4 ± 44.3	94.3	8
GD 17	3,194.0 ± 112.4	97.7	8	3,088.1 ± 46.6	94.4	8
GD 18	3,199.0 ± 107.1	97.7	8	3,098.4 ± 40.1	94.6	8
GD 19	3,197.7 ± 102.5	97.1	8	3,111.6 ± 32.5	94.4	8
GD 20	3,212.6 ± 106.6	97.1	8	3,119.9 ± 27.8	94.3	8
GD 21	3,254.4 ± 102.5	97.7	8	3,145.8 ± 27.0	94.4	8
GD 22	3,274.6 ± 99.6	97.6	8	3,194.6 ± 23.8	95.2	8
GD 23	3,294.9 ± 99.0	97.5	8	3,215.2 ± 25.9	95.1	8
GD 24	3,298.4 ± 96.8	96.6	8	3,217.2 ± 27.6	94.3	8
GD 25	3,293.5 ± 97.7	95.7	8	3,235.6 ± 26.6	94.0	8
GD 26	3,305.3 ± 112.8	95.1	7	3,237.2 ± 22.1	93.1	8
GD 27	3,319.7 ± 110.6	95.1	7	3,224.5 ± 25.9*	92.4	8
GD 28	3,338.6 ± 106.1	95.4	7	3,235.5 ± 25.9*	92.4	8
GD 29	3,358.4 ± 105.6	96.0	7	3,271.4 ± 34.2	93.5	8

* Statistically significant ($P \leq 0.05$) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

[a] Data are displayed as mean ± standard error by gestation day (GD).

[b] Number of surviving does

TABLE C3
Summary of Gross Pathology Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary					
Animals initially in study	8	8	8	8	8
Early deaths					
Euthanized, moribund	1				
Survivors					
Scheduled sacrifice, terminal (GD 29, SD 22)	8	8	8	7	8
Number of animals examined	8	8	8	8	8
Alimentary System					
Esophagus	(8)	(8)	(8)	(8)	(8)
Gallbladder	(8)	(8)	(8)	(8)	(8)
Intestine, large, cecum	(8)	(8)	(8)	(8)	(8)
Intestine, large, colon	(8)	(8)	(8)	(8)	(8)
Intestine, large, rectum	(8)	(8)	(8)	(8)	(8)
Intestine, small, duodenum	(8)	(8)	(8)	(8)	(8)
Intestine, small, ileum	(8)	(8)	(8)	(8)	(8)
Intestine, small, jejunum	(8)	(8)	(8)	(8)	(8)
Liver	(8)	(8)	(8)	(8)	(8)
Pancreas	(8)	(8)	(8)	(8)	(8)
Pharynx	(2)	(2)	(2)	(2)	(2)
Stomach, glandular	(8)	(8)	(8)	(8)	(8)
Ulcer					1
Tongue	(2)	(2)	(2)	(2)	(2)
Cardiovascular System					
Heart	(8)	(8)	(8)	(8)	(8)
Dilation				1	
Endocrine System					
Adrenal gland	(8)	(8)	(8)	(8)	(8)
Pituitary gland	(2)	(2)	(2)	(2)	(2)
Thyroid/parathyroid glands	(2)	(2)	(2)	(2)	(2)
General Body System					
None					
Genital System					
Ovary	(8)	(8)	(8)	(8)	(8)
Uterus	(8)	(8)	(8)	(8)	(8)
Vagina	(8)	(8)	(8)	(8)	(8)
Hematopoietic System					
Lymph node, mandibular	(2)	(2)	(2)	(2)	(2)
Lymph node, mesenteric	(8)	(8)	(8)	(8)	(8)
Spleen	(8)	(8)	(8)	(8)	(8)
Thymus	(8)	(8)	(8)	(8)	(8)
Integumentary System					
Skin	(8)	(8)	(8)	(8)	(8)
Musculoskeletal System					
None					

[a] Number of tissues examined at the site (displayed in parentheses) and number of tissues with observation

TABLE C3
Summary of Gross Pathology Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Nervous System					
Brain	(2)	(2)	(2)	(2)	(2)
Respiratory System					
Lung	(8)	(8)	(8)	(7)	(8)
Trachea	(8)	(8)	(8)	(8)	(8)
Special Senses System					
Eye	(2)	(2)	(2)	(2)	(2)
Urinary System					
Kidney	(8)	(8)	(8)	(8)	(8)
Ureter	(8)	(8)	(8)	(8)	(8)
Urinary bladder	(8)	(8)	(8)	(8)	(8)
Contents; cloudy				1	

TABLE C4
Summary of Fetal External Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Number of fetuses examined	73	54	72	53	52
External					
Number of fetuses examined	73	54	72	53	52
Number of litters examined	8	7	8	7	8
Body: General					
Body, localized subcutaneous edema — [M]					
Fetuses	0 (0.0)	0 (0.0)	1 (1.39)	0 (0.0)	0 (0.0)
Litters	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Body, subcutaneous hemorrhage — [GF]					
Fetuses	1 (1.37)	1 (1.85)	0 (0.0)	1 (1.89)	0 (0.0)
Litters	1 (12.50)	1 (14.29)	0 (0.00)	1 (14.29)	0 (0.00)

[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.
[M] = Malformation
[GF] = Gross Finding

TABLE C5
Summary of Total Fetal Findings in Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine[a]

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Number of fetuses examined	73	54	72	53	52
All Exams					
Number of fetuses	73	54	72	53	52
Number of litters	8	7	8	7	8
Malformation					
Affected fetuses	0 (0.00)	0 (0.00)	1 (1.39)	0 (0.00)	0 (0.00)
Affected litters	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Gross Finding					
Affected fetuses	1 (1.37)	1 (1.85)	0 (0.00)	1 (1.89)	0 (0.00)
Affected litters	1 (12.50)	1 (14.29)	0 (0.00)	1 (14.29)	0 (0.00)
External					
Number of fetuses	73	54	72	53	52
Number of litters	8	7	8	7	8
Malformation					
Affected fetuses	0 (0.00)	0 (0.00)	1 (1.39)	0 (0.00)	0 (0.00)
Affected litters	0 (0.00)	0 (0.00)	1 (12.50)	0 (0.00)	0 (0.00)
Gross Finding					
Affected fetuses	1 (1.37)	1 (1.85)	0 (0.00)	1 (1.89)	0 (0.00)
Affected litters	1 (12.50)	1 (14.29)	0 (0.00)	1 (14.29)	0 (0.00)

[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.

TABLE C6
Fetal Findings Cross Reference of Does and Fetuses in the Dose Range-Finding Gavage Study of Vinpocetine

	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Number of fetuses examined	73	54	72	53	52
Number of dams examined	8	7	8	7	8
Placental					
Number of fetuses examined	73	54	72	53	52
Number of dams examined	8	7	8	7	8
External					
Number of fetuses examined	73	54	72	53	52
Number of dams examined	8	7	8	7	8
Body: General					
Body, localized subcutaneous edema — [M]			14498 (6)		
Body, subcutaneous hemorrhage — [GF]	14475 (5)	14479 (5)		14469 (4)	

Findings are reported by dam ID number and fetus ID number (displayed in parentheses).
[M] = Malformation
[GF] = Gross Finding

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION D-2
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS D-3
FIGURE D1 Fourier Transform Infrared Absorption Spectrum of Vinpocetine D-4
FIGURE D2 Proton Nuclear Magnetic Resonance Spectrum of Vinpocetine D-5
TABLE D1 Gas Chromatography Systems Used in the Gavage Studies of Vinpocetine D-6
TABLE D2 Preparation and Storage of Dose Formulations in the Gavage Studies of Vinpocetine D-6
TABLE D3 Results of Analyses of Dose Formulations Administered to Female Rats in the Dose Range-Finding Gavage Study of Vinpocetine D-7
TABLE D4 Results of Analyses of Dose Formulations Administered to Female Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine D-7
TABLE D5 Results of Analyses of Dose Formulations Administered to Female Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine D-8

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Vinpocetine

Vinpocetine was obtained from Maypro Industries, LLC (Purchase, NY) in one lot (VA201211001) that was used in the dose range-finding studies in rats and rabbits and the prenatal developmental toxicity gavage study in rats. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH) for the study laboratory at Southern Research (Birmingham, AL). Reports on analyses performed in support of the vinpocetine studies are on file at the National Institute of Environmental Health Sciences.

Lot VA201211001 of the chemical, a white crystalline powder, was identified as vinpocetine using Fourier transform infrared (FTIR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and gas chromatography (GC) with mass spectrometry detection. The optical activity analysis indicated an average rotation of +131.6°, which is consistent with the optical rotation of vinpocetine. FTIR spectra were consistent with a literature spectrum (Sadtler, 2014) and the structure of vinpocetine. The proton and carbon-13 NMR spectra were consistent with those expected for the proposed structure of vinpocetine and with the ACD-predicted spectra. Representative FTIR and proton NMR spectra are presented in Figures D1 and D2, respectively. The mass spectrum of the major peak from the gas chromatographic analysis was consistent with the identity of vinpocetine; a single impurity observed in this analysis was tentatively identified as apovincamine, a structurally similar compound. Optical activity analysis of the bulk chemical conducted by Exova (Santa Fe Springs, CA) indicated an average rotation of +131.6°, consistent with the rotation range specified by the manufacturer.

Karl Fischer titration and elemental analyses of lot VA201211001 were conducted by Galbraith Laboratories, Inc. (Knoxville, TN). Additional elemental analyses (72 elements; sodium through uranium) were conducted by Elemental Analysis, Inc. (Lexington, KY) using proton-induced X-ray emission (PIXE) spectroscopy. The purity of the test chemical was determined using melting point analysis conducted on a Perkin-Elmer (Shelton, CT) Diamond differential scanning calorimetry (DSC) instrument scanning from 140° C to 152° C at a rate of 1° C per minute. Purity profiles were obtained using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection and GC with flame ionization detection (FID) by system A (Table D1). The HPLC system included an Agilent 1100 instrument (Agilent, Santa Clara, CA), a C_{18}, 250 mm × 4.6 mm, 4 µm particle size column (Phenomenex, Torrance, CA), mobile phases A) 10:90 (v:v) methanol:0.05 M ammonium acetate (pH 8.0) and B) 90:10 (v:v) methanol:0.05 M ammonium acetate (pH 8.0), an isocratic gradient of 10% A:90% B, UV detection at 230 nm, and a flow rate of 0.75 mL/minute. Screening for selected volatiles in the test chemical was performed using standard addition with authentic standards of non-halogenated (hexane, benzene, diethyl ether, acetone, 1,4-dioxane, and toluene), and halogenated (chloroform, carbon tetrachloride, trichloroethylene, and methylene chloride) volatile compounds using headspace GC/FID by system B.

Karl Fischer titration indicated less than 0.07% water. For lot VA201211001, elemental analyses for carbon, hydrogen, nitrogen, and oxygen were in agreement with the theoretical values for vinpocetine; PIXE analyses indicated no inorganic impurities greater than 0.1%. Melting point analysis by DSC averaged 149.88° C, which is consistent with a literature reference range (147° C to 153° C; *Merck*, 2006) and differential scanning calorimetry indicated a purity of 99.9%. HPLC/UV indicated one major peak (99.5% of the total peak area) and two impurities greater than 0.1% of the total peak area (0.17% and 0.28%). By comparison to retention times of authentic standards of structurally similar compounds, the larger impurity peak was tentatively identified as apovincamine. GC/FID indicated one major peak (99.3% of the total peak area) and one reportable impurity (0.67% of the total peak area); retention time comparison indicated tentative identification of this impurity as apovincamine. Screening for volatiles indicated the presence of 0.018% methylene chloride. The overall purity of lot VA201211001 was determined to be greater than 99.3%.

Stability studies of the bulk chemical were performed using GC/FID by system A. These studies indicated that vinpocetine was stable as a bulk chemical for at least 14 days when stored in sealed amber glass vials at

temperatures up to 60° C. To ensure stability, the bulk chemical was stored by the analytical chemistry laboratory at room temperature in sealed double plastic bags in a plastic bucket. Reanalysis of the bulk chemical was performed twice by the analytical chemistry laboratory during the studies with GC/FID by system C and no degradation of the bulk chemical was detected.

Methylcellulose

Methylcellulose was obtained from Spectrum Chemical Manufacturing Corporation (Gardena, CA) in two lots (2CB0045 and 2DH0326); lot 2CB0045 was used in the dose range-finding study in rats, and lot 2DH0326 was used in the prenatal developmental toxicity study in rats and the dose range-finding study in rabbits. Lots 2DH0326 and 2CB0045 were identified by the analytical chemistry laboratory as methylcellulose using FTIR spectroscopy; sample spectra were in good agreement with the structure of methylcellulose and a literature reference (Hummel, 2018) and cited absorptions were consistent with the structure of methylcellulose (ICGF, 1994). The methoxy content of lots 2DH0326 and 2CB0045 were determined by Galbraith Laboratories, Inc.; the results of duplicate determinations for methoxy group content were within the acceptance limits of 26.0% to 33.0%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for each study by mixing vinpocetine with 0.5% aqueous methylcellulose solution to give the required concentrations (Table D2). The dose formulations were stored at room temperature in clear glass bottles with Teflon®-lined lids in sealed amber plastic bags for up to 38 days.

The analytical chemistry laboratory performed homogeneity studies of 0.1 and 200 mg/mL formulations, syringeability studies for 18- and 22-gauge gavage needles using the 200 mg/mL formulation, resuspendability studies of the 200 mg/mL formulation, and stability studies of the 0.1 mg/mL formulation; all of these analyses were conducted using GC/FID by system D (Table D1). Homogeneity, syringeability, and resuspendability were confirmed, and stability was confirmed for at least 42 days for dose formulations stored in clear glass bottles with Teflon®-lined lids packaged in sealed amber plastic bags at room temperature and for 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations of vinpocetine were conducted by the analytical chemistry laboratory using GC/FID by system D. During the dose range-finding study in rats, the dose formulations were analyzed once; all five dose formulations analyzed and used were within 10% of the target concentrations (Table D3). Animal room samples of these dose formulations were also analyzed; four of five were within 10% of the target concentrations. During the prenatal developmental toxicity study in rats, the dose formulations were analyzed once; animal room samples of these dose formulations were also analyzed (Table D4). All three dose formulations and all three animal room samples were within 10% of the target concentrations. During the dose range-finding study in rabbits, the dose formulations were analyzed once (Table D5). Of the dose formulations analyzed during the study, all eight were within 10% of the target concentrations; two of four animal room samples were within 10% of the target concentrations.

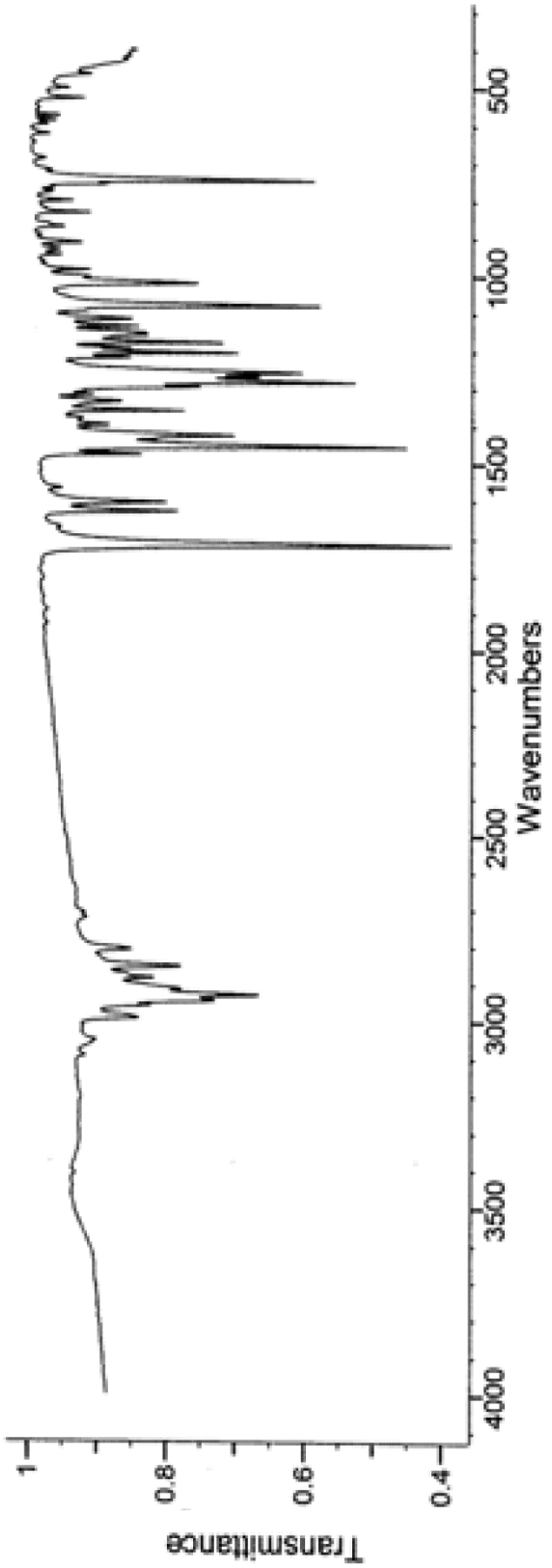

FIGURE D1
Fourier Transform Infrared Absorption Spectrum of Vinpocetine

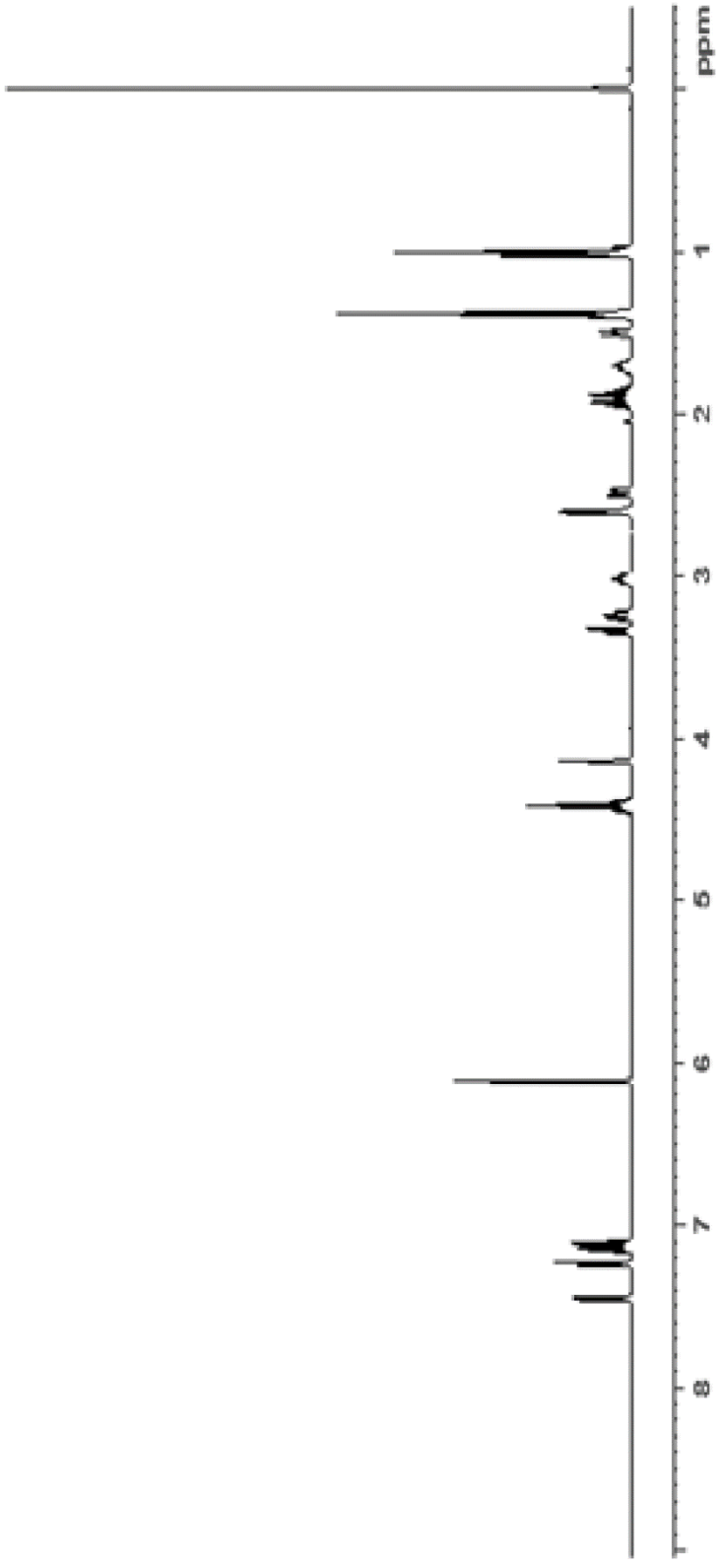

FIGURE D2
Proton Nuclear Magnetic Resonance Spectrum of Vinpocetine

TABLE D1
Gas Chromatography Systems Used in the Gavage Studies of Vinpocetine[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Flame ionization	Rtx®-5, 30 m × 0.32 mm, 0.5 µm film (Restek, Bellefonte, PA)	Helium at ~2 mL/minute	100° C to 150° C at 10° C/minute, then 15° C/minute to 300° C, held for 15 minutes
System B Flame ionization	Rtx®-624, 30 m × 0.53 mm, 3.0 µm film (Restek)	Helium at ~5 mL/minute	35° C for 14 minutes, then 15° C/minute to 40° C, held for 3 minutes, then 15° C/minute to 240° C, held for 2 minutes
System C Flame ionization	ZB-5, 30 m × 0.32 mm, 0.25 µm film (Phenomenex, Torrance, CA)	Helium at ~2 mL/minute	100° C to 150° C at 10° C/minute, then 15° C/minute to 300° C, held for 15 minutes
System D Flame ionization	Rtx®-5, 30 m × 0.32 mm, 0.5 µm film (Restek)	Helium at ~2 mL/minute	120° C to 150° C at 10° C/minute, then 15° C/minute to 300° C, held for 15 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

TABLE D2
Preparation and Storage of Dose Formulations in the Gavage Studies of Vinpocetine

Preparation
The dosing vehicle was prepared by mixing methylcellulose with heated, deionized water while stirring and then diluting with water to form a 0.5% solution, which was allowed to cool. The formulations were prepared by adding the appropriate amount of vinpocetine to a small amount of the vehicle in a mixing container and stirring manually to form a paste. The formulations were diluted to 90% of the final volume with vehicle and stirred with a Silverson mixer (Silverson Machines, Inc., East Longmeadow, MA) at approximately 4,500 rpm for approximately 10 minutes. Formulations were then diluted to final volume with vehicle and stirred with a stir bar on a stir plate with a vigorous vortex for approximately 2 minutes. The dose formulations were prepared once for each study.

Chemical Lot Number
VA201211001

Maximum Storage Time
Up to 42 days (dose range-finding study in rats)
Up to 42 days (prenatal developmental toxicity study in rats)
Up to 42 days (dose range-finding study in rabbits)

Storage Conditions
Stored in clear glass bottles with Teflon®-lined lids in sealed amber plastic bags at room temperature

Study Laboratory
Southern Research (Birmingham, AL)

TABLE D3
Results of Analyses of Dose Formulations Administered to Female Rats in the Dose Range-Finding Gavage Study of Vinpocetine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
February 10-14, 2014		4	4.22[b]	+6
		8	8.44	+6
		16	16.3	+2
		32	31.5[c]	–2
		64	67.1[b]	+5
	March 19, 2014[d]	4	4.29	+7
		8	7.59	–5
		16	15.2	–5
		32	32.8	+3
		64	55.2	–14

[a] Results of triplicate analyses except as noted. Dosing volume=5 mL/kg; 4 mg/mL=20 mg/kg, 8 mg/mL=40 mg/kg, 16 mg/mL=80 mg/kg, 32 mg/mL=160 mg/kg, 64 mg/mL=320 mg/kg.
[b] Nine replicates were analyzed.
[c] Five replicates were analyzed.
[d] Animal room samples

TABLE D4
Results of Analyses of Dose Formulations Administered to Female Rats in the Prenatal Developmental Toxicity Gavage Study of Vinpocetine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
January 5, 2015	January 8-9, 2015	1	0.962[b]	–4
		4	4.03	+1
		12	11.6[b]	–3
	February 5-6, 2015[c]	1	0.970	–3
		4	3.98	–1
		12	11.8	–2

[a] Results of triplicate analyses except as noted. Dosing volume=5 mL/kg; 1 mg/mL=5 mg/kg, 4 mg/mL=20 mg/kg, 12 mg/mL=60 mg/kg.
[b] Nine replicates were analyzed.
[c] Animal room samples

TABLE D5
Results of Analyses of Dose Formulations Administered to Female Rabbits in the Dose Range-Finding Gavage Study of Vinpocetine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
April 13, 2015	April 14-16, 2015	5	5.02[b]	0
		5	5.07	+1
		15	14.9	–1
		15	15.0	0
		30	30.2	+1
		30	30.3	+1
		60	57.4	–4
		60	61.2[b]	+2
	May 27-28, 2015[c]	5	5.84	+17[d]
		15	28.0	+87[d]
		30	29.9	0
		60	59.1	–2

[a] Results of triplicate analyses except as noted. Dosing volume=5 mL/kg; 5 mg/mL=25 mg/kg, 15 mg/mL=75 mg/kg, 30 mg/mL=150 mg/kg, 60 mg/mL=300 mg/kg.
[b] Nine replicates were analyzed.
[c] Animal room samples
[d] High results believed to be caused by an inability to aliquot a representative sample for analysis due to small volumes remaining.

APPENDIX E
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE E1 Ingredients of NIH-07 Rat and Mouse Ration E-2
TABLE E2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration E-2
TABLE E3 Nutrient Composition of NIH-07 Rat and Mouse Ration E-3
TABLE E4 Contaminant Levels in NIH-07 Rat and Mouse Ration E-4

TABLE E1
Ingredients of NIH-07 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground #2 yellow shelled corn	24.25
Ground hard winter wheat	23.00
Soybean meal (47% protein)	12.00
Fish meal (62% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil (without preservatives)	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Calcium phosphate, dibasic (USP)	1.25
Calcium carbonate (USP)	0.50
Sodium chloride	0.50
Premixes (vitamin)	0.25
Premixes (mineral)	0.15
Choline chloride (70% choline)	0.10

TABLE E2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration

	Amount	Source
Vitamins		
A	6,062 IU	Stabilized vitamin A palmitate or acetate
D	5,070 IU	D-activated animal sterol
K	3.09 mg	Menadione sodium bisulfite complex
E	22.0 IU	α-Tocopheryl acetate
Niacin	33.0 mg	
Folic acid	2.4 mg	
d-Pantothenic acid	19.8 mg	*d*-Calcium pantothenate
Riboflavin	3.8 mg	
Thiamin	11.0 mg	Thiamine mononitrate
B_{12}	50 µg	
Pyridoxine	6.5 mg	Pyridozine hydrochloride
Biotin	0.15 mg	*d*-Biotin
Minerals		
Iron	132 mg	Iron sulfate
Zinc	18 mg	Zinc oxide
Manganese	66 mg	Manganese oxide
Copper	4.4 mg	Copper sulfate
Iodine	1.5 mg	Calcium iodate
Cobalt	0.44 mg	Cobalt carbonate

TABLE E3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.6	NA	1
Crude fat (% by weight)	5.3	NA	1
Crude fiber (% by weight)	3.43	NA	1
Ash (% by weight)	6.33	NA	1
Amino Acids (% of total diet)			
Arginine	1.375 ± 0.065	1.30 – 1.43	8
Cystine	0.321 ± 0.035	0.274 – 3.72	8
Glycine	1.145 ± 0.077	1.06 – 1.131	8
Histidine	0.516 ± 0.023	0.497 – 0.553	8
Isoleucine	0.982 ± 0.025	0.952 – 1.03	8
Leucine	1.996 ± 0.054	1.93 – 2.08	8
Lysine	1.261 ± 0.032	1.22 – 1.32	8
Methionine	0.487 ± 0.015	0.468 – 0.515	8
Phenylalanine	1.091 ± 0.020	1.07 – 1.12	8
Threonine	0.919 ± 0.032	0.883 – 0.961	8
Tryptophan	0.280 ± 0.022	0.266 – 0.326	8
Tyrosine	0.855 ± 0.039	0.785 – 0.894	8
Valine	1.134 ± 0.0245	1.11 – 1.17	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.23 ± 0.211	2.04 – 2.59	8
Linolenic	0.25 ± 0.028	0.217 – 0.296	8
Vitamins			
Vitamin A (IU/kg)	3,910	NA	1
α-Tocopherol (ppm)	48.07 ± 4.38	40.3 – 52.73	8
Thiamine (ppm)[a]	13.4	NA	1
Riboflavin (ppm)	14.3 ± 3.58	10.0 – 19.8	8
Niacin (ppm)	99.4 ± 9.10	87 – 112	8
Pantothenic acid (ppm)	45.6 ± 3.13	40.4 – 51.1	8
Pyridoxine (ppm)[b]	12.33 ± 2.25	9.63 – 15.6	8
Folic acid (ppm)	2.47 ± 0.550	1.68 – 3.09	8
Biotin (ppm)	0.342 ± 0.125	0.25 – 0.64	8
Vitamin B_{12} (ppb)	50.21 ± 7.47	41.8 – 61.6	8
Choline (as chloride) (ppm)	1,776 ± 197	1,570 – 2,200	8
Minerals			
Calcium (%)	1.150	NA	1
Phosphorus (%)	0.919	NA	1
Potassium (%)	0.829 ± 0.036	0.77 – 0.88	8
Chloride (%)	0.625 ± 0.102	0.441 – 0.800	8
Sodium (%)	0.368 ± 0.047	0.318 – 0.469	8
Magnesium (%)	0.183 ± 0.009	0.170 – 0.194	8
Iron (ppm)	376.3 ± 52.5	276 – 455	8
Manganese (ppm)	91.03 ± 7.93	80.7 – 104.0	8
Zinc (ppm)	64.07 ± 11.32	52.4 – 89.2	8
Copper (ppm)	14.11 ± 2.91	11.9 – 21.1	8
Iodine (ppm)	1.71 ± 0.886	0.54 – 3.45	8
Chromium (ppm)	3.96 ± 0.033	3.91 – 4.00	8
Cobalt (ppm)	0.53 ± 0.293	0.01 – 0.963	8

[a] As hydrochloride (thiamine and pyridoxine)

TABLE E4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.436	NA	1
Cadmium (ppm)	0.085	NA	1
Lead (ppm)	0.117	NA	1
Mercury (ppm)	<0.012	NA	1
Selenium (ppm)	0.34	NA	1
Aflatoxins (ppb)	<5.00		1
Nitrate Nitrogen (ppm)[c]	9.65		1
Nitrite Nitrogen (ppm)[c]	<0.61		1
BHA (ppm)[d]	<1.0		1
BHT (ppm)[d]	<1.0		1
Aerobic Plate Count (CFU/gm)	<10		1
Coliform (MPN/gm	<3		1
Escherichia coli (MPN/gm)	<3		1
Salmonella (MPN/gm)	Negative		1
Total Nitrosamines (ppb)[e]	0	NA	1
N-Nitrosodimethylamine (ppb)[e]	0	NA	1
N-Nitrosopyrrolidine (ppb)[e]	0	NA	1
Pesticides (ppm)			
α-BHC	<0.01		1
β-BHC	<0.02		1
γ-BHC	<0.01		1
δ-BHC	<0.01		1
Heptachlor	<0.01		1
Aldrin	<0.01		1
Heptachlor epoxide	<0.01		1
DDE	<0.01		1
DDD	<0.01		1
DDT	<0.01		1
HCB	<0.01		1
Mirex	<0.01		1
Methoxychlor	<0.05		1
Dieldrin	<0.01		1
Endrin	<0.01		1
Telodrin	<0.01		1
Chlordane	<0.05		1
Toxaphene	<0.10		1
Estimated PCBs	<0.20		1
Ronnel	<0.01		1
Ethion	<0.02		1
Trithion	<0.05		1
Diazinon	<0.10		1
Methyl chlorpyrifos	0.045		1
Methyl parathion	<0.02		1
Ethyl parathion	<0.02		1
Malathion	0.033		1
Endosulfan I	<0.01		1
Endosulfan II	<0.01		1
Endosulfane sulfate	<0.03		1

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX F
SENTINEL ANIMAL PROGRAM

METHODS F-2
RESULTS F-2

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected and allowed to clot, and the serum was separated. All samples were processed appropriately and testing performed by IDEXX BioResearch [formerly Research Animal Diagnostic Laboratory (RADIL), University of Missouri (Columbia, MO)] for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the study are also listed.

Blood was collected from 10 female New Zealand White rabbits for testing.

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
CAR bacillus	Study termination
Clostridium piliform	Study termination
Encephalitozoon cuniculi	Study termination
Rotavirus	Study termination
Immunofluorescence Assay	Study termination
Treponema	Study termination

RESULTS

Antibodies to Rotavirus were detected in several samples. Rotavirus is a common virus in rabbits that was not considered to have impacted the study. All other test results were negative.