NTP
National Toxicology Program

Vinpocetine

NTP Technical Report on the Prenatal Developmental Toxicity Studies of Vinpocetine in Sprague Dawley (Hsd: Sprague Dawley SD) Rats and New Zealand White (Hra:NZW SPF) Rabbits (Gavage Studies)

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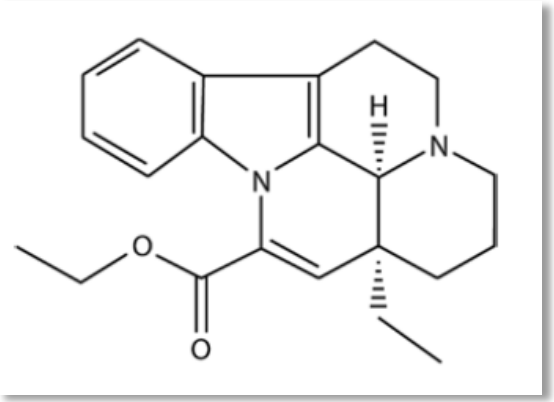
Vinpocetine



- Semi-synthetic / synthetic agent
- Dietary supplement (U.S.) for cognitive enhancement
  - Some products are specifically marketed towards students as brain supplements for increasing cognitive performance
- Pharmaceutical agent for treatment of cerebrovascular and cognitive disorders
  - Primarily for use in the elderly for Alzheimer's, dementia, and ischemic stroke
- **Study Rationale**: Concerns for consumer exposure through dietary supplement use, signals of developmental toxicity in the literature, and lack of adequate toxicity data

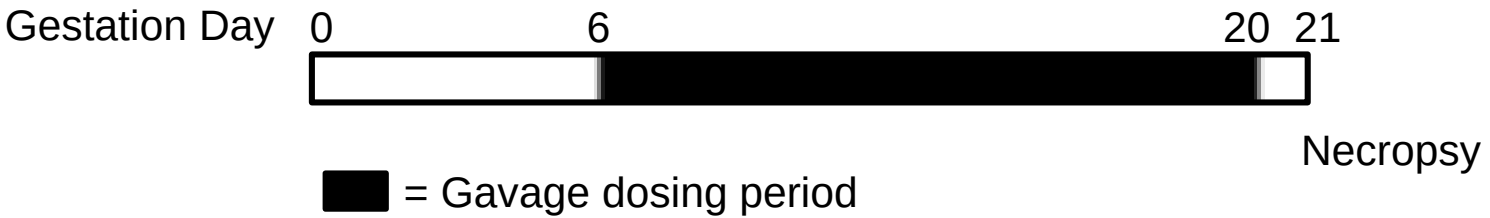
Background

- In the United States, vinpocetine products are available
  - Typical manufacturer recommendation: 5 to 10 mg taken 1 to 3 times daily

- NTP conducted a literature review comparing internal dose in rodents and humans following exposure to vinpocetine
  - Exposures in rats following repeated dosing with 5 mg/kg is similar to that following a single 10 mg human dose

Waidyanatha et al., Toxicol Appl Pharmacol 2018

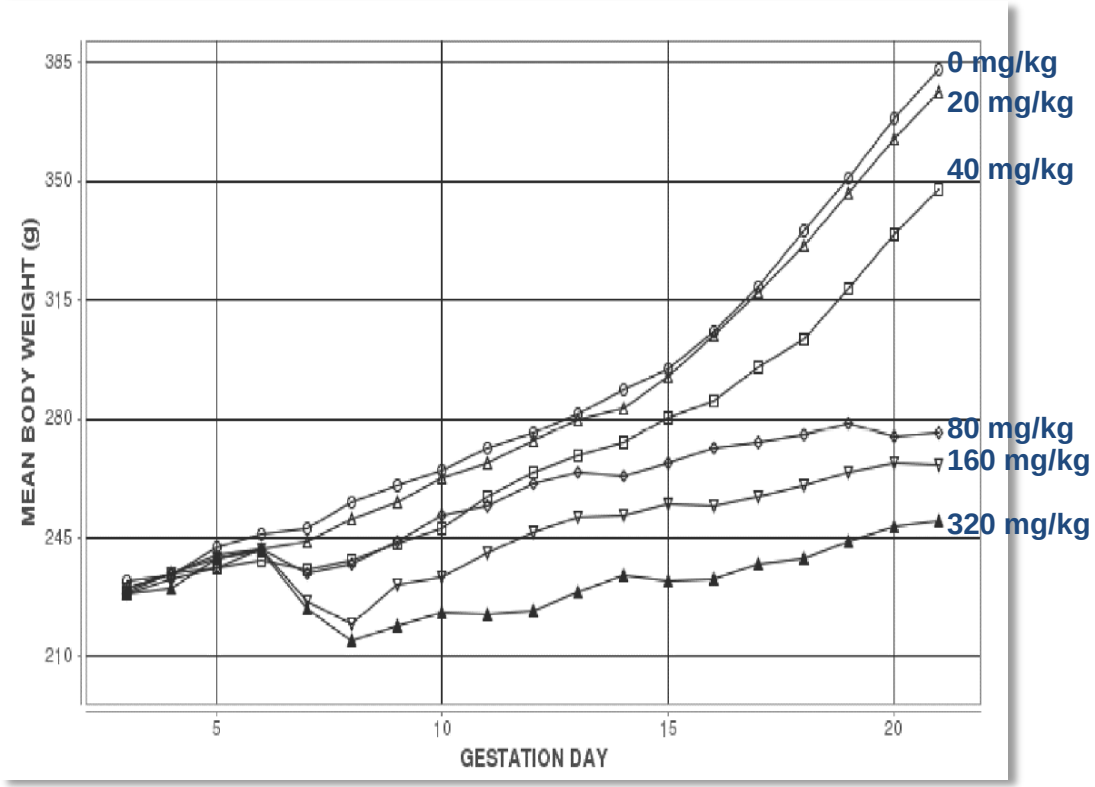
Rat Dose Range-Finding Study Design



- **Doses: 0, 20, 40, 80, 160, or 320 mg/kg/day (gavage)**
- N=10 time-mated, female rats per group
- Maternal endpoints: Clinical observations, body weights, feed consumption, and uterine parameters
- Fetal endpoints: fetal weight, external examination, and litter parameters including number of live/dead fetuses, and sex ratio

Rat Range-Finding Study: Maternal Findings

- No dams removed due to moribundity/mortality
- Clinical observations
  - Dose-related increase in incidence of vaginal discharge
  - Discoloration of the nares in groups ≥ 40 mg/kg
  - Piloerection in groups ≥ 160 mg/kg
- Maternal body weight:
  - Dose-related decreases body weight gains and body weight ≥ 40 mg/kg (9, 28, 31, and 35%)



Rat Range-Finding Study: Uterine and Litter Parameters

Endpoint	0 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg
Maternal Terminal Body Weight (g)	383.2 ± 4.7**	376.2 ± 10.7	**347.5 ± 13.9****	**276.1 ± 3.4****	**266.3 ± 5.4****	**250.2 ± 5.3****
Gravid Uterine Weight (g)	96.41 ± 3.2**	83.23 ± 11.8	**71.35 ± 12.4***	**2.35 ± 0.2****	**10.78 ± 8.2****	**3.09 ± 0.6****
No. Litters	8	10	8	**0**	**1**	**0**
No. Live Fetuses	109	115	81	**0**	**12**	**0**
No. Live Fetuses per Litter	13.6 ± 0.53**	11.5 ± 1.78	10.1 ± 1.85	**0****	**1.2 ± 1.2****	**0****
No. Resorptions (Early + Late)	6	26	31	**134**	**133**	**131**
No. Whole Litter Resorptions	0**	1	1	**10****	**9****	**9****
Post-implantation Loss	5.3 ± 1.8%**	18.4 ± 11.7%	27.6 ± 12.4%	**100%****	**90.8 ± 9.2%****	**100%****
Fetal Weight per Litter (g)	5.18 ± 0.07	5.26 ± 0.16	5.06 ± 0.16	**-**	4.98	**-**

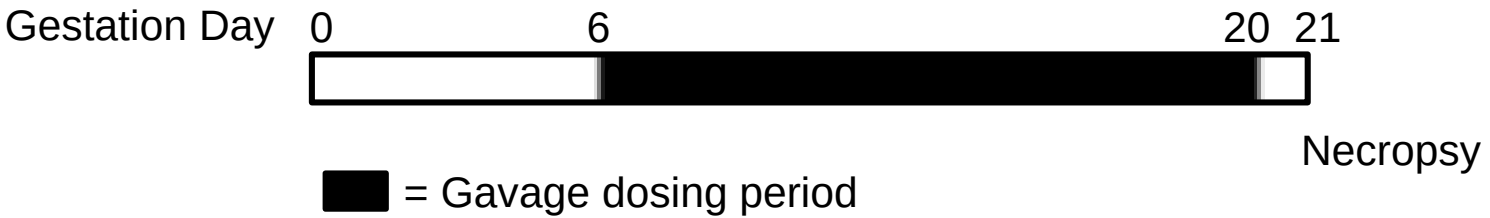
Values are reported as counts or mean ± standard error; (g)= grams
Statistically significant trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); *P≤0.05, ** P≤0.01

- Decreased maternal terminal body weight and gravid uterine weight
- Fewer number of litters and live fetuses for evaluation
- Significant increase in post-implantation loss

Rat Dose Range-Finding Summary

- Increase incidences of vaginal discharge
- Maternal body weight
  - Dose-related decreases in maternal body weight and body weight gains at 40 mg/kg or greater
- Treatment-related increase in post-implantation loss
  - 28% at 40 mg/kg and 90-100 percent at ≥ 80 mg/kg
- Based on these findings, doses of **0, 5, 20, and 60 mg/kg/day** were selected for the main study.

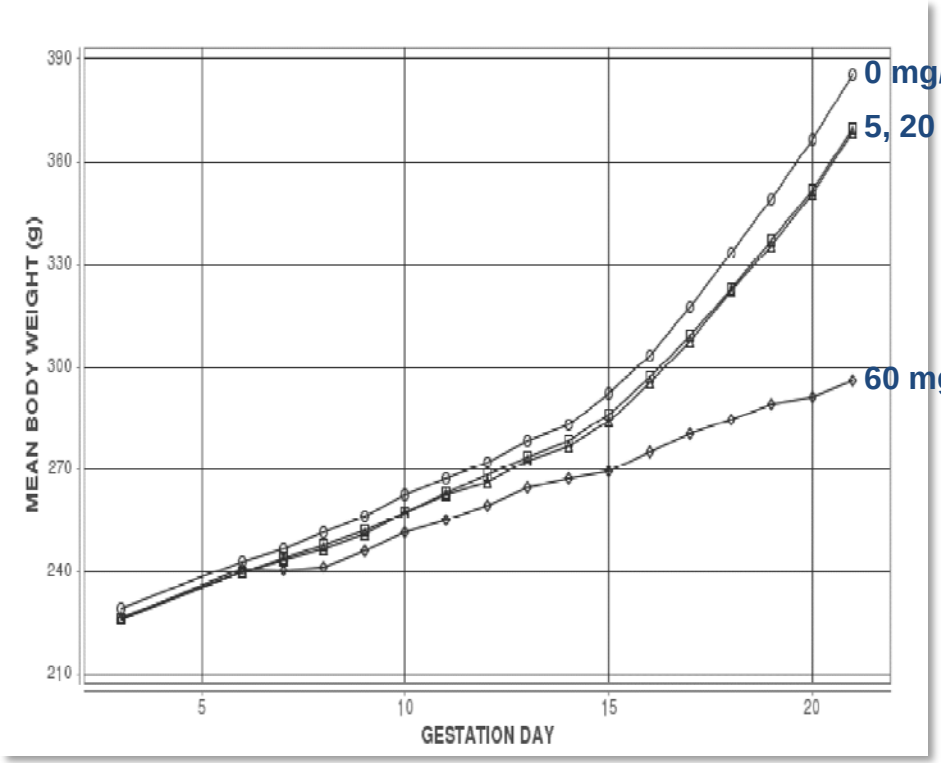
Main Study Design



- **Doses: 0, 5, 20, 60 mg/kg/day (gavage)**
- N=25 time-mated, female rats per group
- Additional endpoints (in addition to those assessed in the Dose Range-Finding Study):
  - Fetal: Visceral, head, and skeletal examinations

Main Study: Maternal Findings

- No dams removed due to moribundity/mortality
- Clinical observations
  - Dose-related increase in the incidence of vaginal discharge
- Maternal body weight
  - Significant decrease in maternal body weight on GD21 (23%) at 60 mg/kg (associated with an 83% post-implantation loss)



Main Study: Uterine and Litter Parameters

Endpoint	0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg
Maternal Terminal Body Weight (g)	385.7 ± 4.2**	368.5 ± 8.2	370.0 ± 5.5	396.1 ± 8.2
Gravid Uterine Weight (g)	97.79 ± 3.1**	83.89 ± 6.6	85.07 ± 5.3	**19.52 ± 6.5**
No. Litters	21	19	21	**8**
No. Live Fetuses	293	239	261	**51**
No. Live Fetuses per Litter	13.9 ± 0.6**	11.9 ± 1.0	11.9 ± 0.9	**2.6 ± 1.0****
No. Resorptions (Early + Late)	8	12	21	**208**
No. Whole Litter Resorptions	0	1	1	**12**
Post-implantation Loss	3.29 ± 1.3**	10.67 ± 5.3	11.13 ± 4.7	**83.13 ± 6.5****
Fetal Weight per Litter (g)	5.15 ± 0.07	5.29 ± 0.16	5.21 ± 0.12	5.11 ± 0.10

Values are reported as counts or mean ± standard error; (g)= grams
Statistically significant trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); *P≤0.05, ** P≤0.01

- Decreased gravid uterine weight, number of litters and number of live fetuses
- Treatment-related increases in post-implantation loss at 60 mg/kg

Main Study: Fetal Visceral Findings

Endpoint		0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg	Historical Controls
No. fetuses examined		293	239	261	51	1,326
No. litters examined		21	19	21	8	104
Ventricular septal defect (Malformation)	Fetuses	0*	3 (1.3%)	8 (3.1%)	**2 (3.9%)**	**0 – 0.48%**
	Litters	0*	3 (15.8%)	7 (33.3%)	**2 (25.0%)**	**0 – 5.26%**

* Statistically significant trend (Cochran-Armitage), $p<0.05$

- Positive trend in percent of fetuses with ventral septal defect
  - At 60 mg/kg there were a limited number of fetuses for evaluation

Main Study: Fetal Skeletal Findings

Endpoint		0 mg/kg	5 mg/kg	20 mg/kg	60 mg/kg	Historical Controls
No. fetuses examined		293	239	261	51	1,324
No. litters examined		21	19	21	8	104
Incomplete ossification of the thoracic centrum, total (V)	Fetuses	1 (0.3%)**##	1 (0.4%)	**6 (2.3%)*#**	**8 (17.0%)**##**	0% - 0.8%
	Litters	1 (4.8%)**	1 (5.3%)	**5 (23.8%)**	**3 (42.9%)***	0% - 11.1%
Full thoracolumbar SNR, total (M)	Fetuses	1 (0.3%)**##	5 (2.1%)	12 (4.6%)**	**12 (25.5%)**##**	0.3% - 3.4%
	Litters	1 (4.7%)*	3 (15.8%)	4 (19.1%)	**3 (42.9%)***	4.8% - 31.6%

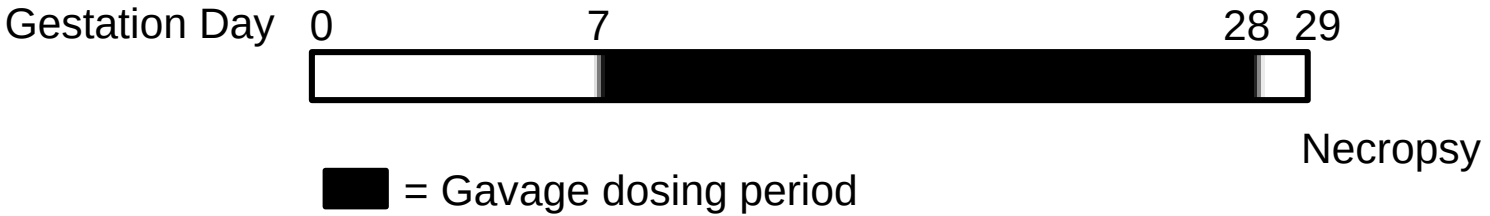
(SNR) = supernumerary ribs
Statistically significant (P<0.05) according to mixed effects logistic regression. ##P<0.01.
* Statistically significant (P<0.5) according to Cochran-Armitage (trend) or Fisher exact (pairwise) test. **P<0.01

- Increased incidences of incomplete ossification of the thoracic centra and full thoracolumbar ribs

Rat Main Study Summary

- Clinical observations
  - Dose-related increase in the incidence of vaginal discharge (20 and 60 mg/kg)
- Decreased maternal body weight
  - Significant decrease in maternal body weight on GD21 (23%) at 60 mg/kg
- Treatment-related increase in post-implantation loss
  - 83% at 60 mg/kg
- Fetal findings
  - Increase incidences in the percent of fetuses with ventral septal defect (malformation)
  - Increased incidences of incomplete ossification of the thoracic centra (variation) and full thoracolumbar ribs (malformation)

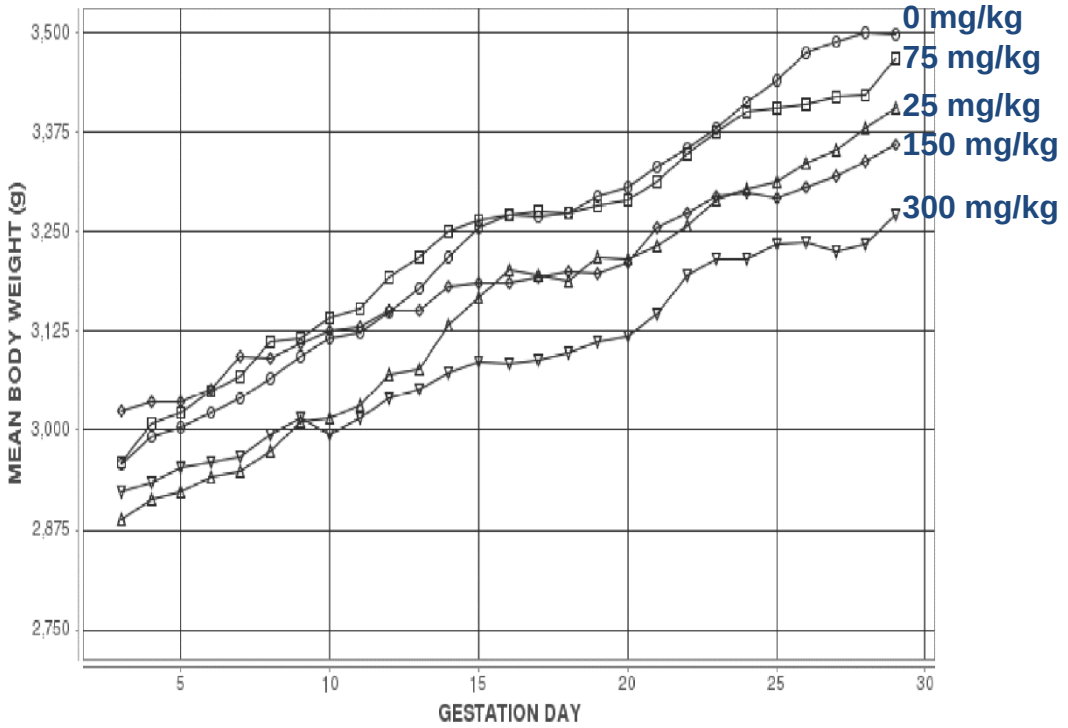
Rabbit Dose Range-Finding Study



- **Doses: 0, 25, 75, 150, or 300 mg/kg/day (gavage)**
  - Based on results from the Dose Range-Finding study in the rat and available toxicokinetic data from the literature
- N=8 time-mated, female rabbits per group
- Maternal endpoints: Clinical observations, body weights, feed consumption, and uterine parameters
- Fetal endpoints: fetal weight, external examination, and litter parameters including number of live/dead fetuses, and sex ratio

Rabbit Range-Finding Study: Maternal Findings

- No does were removed due to morbidity/mortality
  - One female in the 150 mg/kg group removed (abortion)
- No clinical signs of toxicity were noted in any dose group
- Maternal body weight
  - Decreases in maternal body weight gains at 150 and 300 mg/kg (44% and 34%)



Rabbit Range-Finding Study: Uterine and Litter Parameters

Endpoint	0 mg/kg	25 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Maternal Terminal Body Weight	3449.4 ± 64.6*	3406.5 ± 58.0	3467.7 ± 95.0	3358.4 ± 105.6	3271.4 ± 34.2
Gravid Uterine Weight	515.3 ± 14.7**	470.1 ± 20.7	483.9 ± 32.2	**421.9 ± 39.3***	**340.9 ± 27.7****
No. Litters	8	7	8	7	8
No. Live Fetuses	73	54	72	53	52
No. Live Fetuses per Litter	9.13 ± 0.4*	7.71 ± 0.4	9.0 ± 0.5	7.57 ± 0.8	**6.50 ± 0.7***
No. Resorptions (Early + Late)	1	1	2	1	**15**
No. Whole Litter Resorptions	0	0	0	0	0
Post-implantation Loss	1.4 ± 1.4%	3.37 ± 2.2%	2.53 ± 1.7%	3.57 ± 3.6%	**20.42 ± 9.1%**
Fetal Weight per Litter (g)	39.72 ± 1.3**	41.47 ± 0.9	37.53 ± 0.9	39.36 ± 1.7	**35.78 ± 1.2**
	--	--	-5%	--	**-10%**

Values are reported as counts or mean ± standard error; (g)= grams
Statistically significant trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column); *P≤0.05, ** P≤0.01

- Decrease in gravid uterine weight at 150 and 300 mg/kg
- Decrease in the number of live fetuses per litter and fetal weight per litter
- Increased post-implantation loss

Rabbit Range-Finding Summary

- Decrease in maternal body weight gains at 150 and 300 mg/kg
- Exposure-related effect on embryo-fetal survival at 300 mg/kg:
  - Significant decrease in the number of live fetuses per litter which was associated with an increase in early resorptions per litter
  - Increase in percent post-implantation loss at 300 mg/kg (20.4%)
- Data from this rabbit dose range-finding study support findings observed in the rat dose range-finding study and rat prenatal developmental toxicity studies (e.g., increase in post-implantation loss)
  - Differences in sensitivity and magnitude of response likely attributable to a species difference in metabolism (Catlin et al., Birth Defects Res 2018)

Vinpocetine

Under the conditions of the rat prenatal study:

- ***Clear evidence*** of developmental toxicity of Vinpocetine in Harlan Sprague Dawley rats at all groups based on findings of:
  - Increased post-implantation loss
  - Increased incidences of ventricular septal defects
  - Increased thoracolumbar ribs (full)
  - Increased incidences of incomplete ossification of the thoracic centrum

- These findings occurred in the absence of overt maternal toxicity

Questions?