

## Results

There were no drug-related effects on mortality or clinical signs of treatment. There was a decrease in body weight gain (12% difference from control at termination) which became significant from week 62 onwards in the high dose treatment group. There was no effect on food consumption.

Mean hormonal levels among control male rats varied with time as follows. LH levels declined from a high of approximately 1.0 ng/ml at 3 weeks progressively to a plateau of approximately 0.3 ng/ml beginning at week 37 until the end of the study. FSH levels declined from an initial high at 9.0 ng/ml to a plateau of approximately 4.0 ng/ml which was maintained from weeks 12 through 52. It then declined gradually to approximately 2.0 ng/ml by week 78 and afterwards continually increased until the end of the study. Serum testosterone declined from its high (ca. 2.5 ng/ml) at week 3 to its low (ca. 0.3 ng/ml) at week 26. It fluctuated between 0.3 and 0.9 ng/ml from week 12 to study termination. Serum prolactin fluctuated in the 10-25 ng/ml range for the first 26 weeks of the study and steadily increased to a peak of 160 ng/ml by study termination. Growth hormone reached its highest level at week 3 and mean values randomly fluctuated over the course of the study with high standard deviations.

In the treated groups, mean serum LH was significantly higher in the high dose group than the concurrent control from week 52 onwards. At earlier time points the levels of LH were generally lower than control but significance was reached only at week 12. Mean serum FSH of the high dose group was significantly higher than control at weeks 19, 66, 78, and 86. At other points there was no consistent difference observed. Mean testosterone levels were significantly lower in the high dose group on weeks 78, 86, and 104. Earlier time points showed no dose or drug-related trends in testosterone levels. Mean prolactin levels were depressed in the high dose group in the latter weeks of the study (weeks 66, 78, and 94 were significant) and to a lesser degree in the low dose group (weeks 78 and 94 were significant). The variability in the growth hormone levels precluded any meaningful interpretation of the data.

Among biochemical and receptor analyses, there were no drug-related changes in pituitary concentrations of LH or FSH measured at week 10. Mean testicular testosterone concentrations were significantly reduced in the low dose group relative to control but not at the high dose (probably due to one high value). The concentration of LH and GnRH receptors in the testes was not affected by drug treatment during week 10. In terminally and moribund sacrificed animals, the only significant difference relative to control was a decrease (35%) in testicular LH receptor content.

Upon microscopic evaluation, there was an increase in Leydig cell tumors and a decrease in pituitary adenomas among animals treated with the high dose of PN 200-110. The numbers of Leydig cell tumors among 65 naturally dying or terminally sacrificed animals were 1, 3, and 11 in the control, low dose, and high dose treatment groups, respectively. Hyperplasia of the Leydig cells was also increased with treatment (0, 1, and 4 in the control, low dose, and high dose groups). These results virtually replicate the findings of the first carcinogenicity study.

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Effects of Chronic (4- and 13- Week) Oral Administration of  
PN 200-110 on Leydig Cell LH Receptors, In Adult Male  
Wistar and Sprague-Dawley Rats

Methods

PN 200-110 was administered in the feed at doses of 0, 0.625, 6.25, or 62.5 mg/kg/day to male 8-week old Sprague-Dawley (Charles River) or Wistar (Madorin) rats (6/group) for either 4 or 13 weeks. Testicular LH receptor content was measured upon sacrifice by 125 I-HCG binding.

Results

In Sprague-Dawley rats, the HCG binding was significantly reduced in testes from rats of the high dose group at both 4 and 13 weeks. No trend or significant differences were observed at the lower dose levels. In Wistar rats there was also a reduction in binding at the highest dose but significance was achieved only at 13 weeks.

Ten-Week Oral (Diet) Testicular Mechanism Study  
in the Rat on 200-110

Methods

PN 200-110 was administered in the diet at doses of 0, 6.25, or 62.5 mg/kg to male 5-week-old Sprague-Dawley (Charles River) rats (25/group) for a period of 10 weeks. Clinical observations, mortality, body weight, and food consumption were monitored. Serum LH and testosterone determinations were made during weeks 1, 3, and 10. At termination, the testes were analyzed for testosterone content and LH and GnRH receptor content.

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Results

There were no drug-related effects on in-life observations including the hormone level measurements. The terminal testicular content and concentration of testosterone was increased in the high dose group. There was no measurable effect on GnRH receptors with treatment, but the LH receptor content was reduced by approximately 20% in the high dose group. The latter difference was not significant.

A Clinical Study Evaluating the Endocrine Effects of  
Chronic Treatment with PN 200-110

A multi-center clinical study (no. 351) evaluated the hormonal effects of chronic (1 year) treatment with PN 200-110 (2.5 or 10 mg b i.d.) in hypertensive patients (23) relative to placebo treated patients (21) under double-blind conditions. Serum FSH, LH, prolactin, and testosterone were measured weekly during the placebo baseline period and monthly during the double-blind treatment phase. Results were expressed as mean changes from baseline for each 3-month interval of the study.

Mean serum FSH level was increased significantly over baseline value in three of the four quarterly periods in the PN 200-110 treatment group and was decreased non-significantly in all four intervals in the placebo group. The intergroup comparison was significantly different in the first quarter only. Similarly, mean serum LH levels were increased in all four quarters in the treated group (2/4 significant) and decreased in all four quarters in the placebo group (none significant) relative to baseline levels. The intergroup difference was statistically significant only for the first quarter. Because the mean baseline levels of FSH and LH in the placebo group were higher than the treated group the net effect of these changes brought the absolute mean levels closer to each other in the final analysis. For example, in the first 3-month interval FSH significantly increased from 10.53 U/ml to 11.70 U/ml in the PN 200-110 group and decreased from 13.34 U/ml to 12.60 U/ml in the placebo group. While the intergroup comparison of change was significant, the absolute values of FSH were still in the normal range.

The mean serum levels of testosterone were uniformly decreased from baseline for all intervals in both treatment groups. Most of these differences were significant. However the degree of change was comparable in both groups and there were no significant intergroup differences.

In general, mean serum prolactin levels increased slightly from baseline in both groups. None of these changes were significant and the degree of change was comparable in both groups.

Acute and Subchronic Exploratory Intravenous Toxicity Study  
in the Rat on PN 200-110

Methods

Study Facility/Dates: Sandoz Res. Inst. E. Hanover, NJ (March 1983)

Strain/Source: Taconic Sprague-Dawley (TAC: N[SD] Taconic Farms  
(Germantown, NY)

Duration: Acute (single dose) sacrifice on day 15  
~~Subchronic (5 cons. daily doses) sacrifice on day 15~~

Route: intravenous (tail vein)

Dose Levels: 0.1, 0.3, 1.0, 1.5, 2.0, and 3.0 mg/kg/day (acute);  
0.0, 0.5, 1.0 mg/kg (subchronic)

No. of Animals/Sex/Dose: 5 males (acute); 4 males (subchronic)

Age at Study Initiation: 4 weeks

Weight Range: Males: 144-199 g. (acute); 185-214 (subchronic)

Parameters Measured:

Clinical Observations:

- \*Mortality
- \*Clinical signs of toxicity
- \*Body weight

Results

In the acute study, the lowest doses, 0.1 and 0.3 mg/kg, of PN 200-110 were well tolerated. At 1.0 mg/kg, clinical signs of toxicity included decreased motor activity, flattened body position, loss of righting reflex and pale eyes. No animals died at this dose. At 1.5 mg/kg all of the animals displayed labored breathing; one became cyanotic and died. At the highest dose (2.0 and 3.0 mg/kg) all animals exhibited the above signs and died on day 1.

In the subchronic study, all of the animals receiving 0.5 mg/kg exhibited signs of labored breathing. At 1.0 mg/kg, apnea and cyanosis occurred in 3/4 animals and one animal died.

Two Week Intravenous Toxicity Study in the Rat on PN 20: 110

Methods

Study Facility/Dates Sandoz Res. Inst. E. Hanover, NJ (April 1983)

Strain/Source: Charles River - CD (Sprague Dawley Kingston, NY)

Duration: 2 weeks

Route: Intravenous

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Dose Levels: 0, 0.04, 0.12, 0.40 mg/kg/day (mean)

No. of Animals/Sex/Dose: 10

Age at Study Initiation: 6 weeks

Parameters Measured:

Clinical Observations: (daily body weight twice weekly, food consumption weekly)

- \*Mortality
- \*Clinical signs of toxicity
- \*Body weight
- \*Food consumption

Ophthalmoscopy: (pretest and week 2)

- \*direct

Hematology: (week 2)

- |   |                                 |
|---|---------------------------------|
| *Red blood cell count                             | *Reticulocyte count             |
| *Hemoglobin                                       | *Thrombocyte (platelet) count   |
| *Hematocrit                                       | *Prothrombin time               |
| *Mean corpuscular volume (MCV)                    | *White blood cell count         |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH)                | *Erythrocyte sedimentation      |
| *Erythrocyte morphology                           | *Clotting time                  |

Clinical Biochem'istry: (week 2)

*Blood urea nitrogen (BUN	*Total protein
*Serum glutamic oxaloacetic transaminase (SGOT)	*Sodium
*Serum glutamic pyruvic transaminase (SGPT)	*Potassium
*Alkaline phosphatase (AP)	*Chloride
*Glucose	*Calcium
	*Cholesterol
	*Total bilirubin

Urinalysis: (week 2)

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*pH	*Proteins
*Glucose	*Occult blood
*Ketones bodies	*Specific gravity
*Sediment (microscopic analysis)	*

Results

All animals survived the study; body weight gain and food consumption were unimpaired by treatment. Clinical signs of toxicity were only observed at the high dose and included: ataxia, loss of righting reflex, pale eyes, labored breathing, apnea, and a decrease in locomotor activity. There were no drug-related alterations in ophthalmology, hematology, biochemistry or urinalysis.

PN 200-110/A 4-Week Oral Toxicity Study in Dogs

Methods

Study Facility/Dates: Sandoz Ltd. R&D Basle, Switzerland  
(July-October 1981)

Strain/Source: Beagles; Marshall (North Rose, NY)

Duration: 4 weeks (one animal/sex/dose treated as a recovery animal  
for four additional weeks)

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Route: oral (capsules)

Dose Levels: 0, 2, 6, and 20 mg/kg/day; high dose<sup>f</sup> reduced to 15 mg/kg  
on day 5

No. of Animals/Sex/Dose: 3

Age at Study Initiation: 8-9.5 mos.

Weight Range: Males: 8.8-12.6 kg  
Females: 8.0-13.5 kg

Parameters Measured:

Clinical Observations:

- \*Mortality
- \*Clinical signs of toxicity
- \*Body weight
- \*Food consumption
- \*Behavior/motor effects

Ophthalmoscopy: (pretest and week 4)

Electrocardiograms: (pretest and weeks 2 and 4)

Hematology: (pretest and week 4)

- |   |                                 |
|---|---------------------------------|
| *Red blood cell count                             | *Reticulocyte count             |
| *Hemoglobin                                       | *Thrombocyte (platelet) count   |
| *Hematocrit                                       | *Prothrombin time               |
| *Mean corpuscular volume (MCV)                    | *White blood cell count         |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH)                | *Erythrocyte sedimentation      |
| *Erythrocyte morphology                           | *Clotting time                  |

Clinical Biochemistry: (pretest and week 4)

*Serum glutamic oxaloacetic transaminase (SGOT)	*Total protein
*Serum glutamic pyruvic transaminase (SGPT)	*Sodium
*Alkaline phosphatase (AP)	*Potassium
*Glucose	*Cholesterol
*Creatinine	*Total Bilirubin
	*Triglycerides

Urinalysis: (week 4)

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*pH	*Proteins
*Glucose	*Occult blood
*Ketones bodies	*Specific gravity
*Sediment (microscopic analysis)	

Macroscopic and Microscopic Examination.

Complete

Results

Three animals died the first week of treatment at the high dose (20 mg/kg/day). The dose was subsequently reduced to 15 mg/kg/day for the remainder of the study. Weight gain and food consumption were slightly inhibited at the mid dose. Animals at the high dose which died displayed a weight gain despite inhibited food intake; others of this group had slightly inhibited weight gain.

Clinical signs clearly associated with treatment included prolapse of the nictating membrane even after one day at the low dose. During the first week sedation occurred in two animals at the mid-dose and in 5/6 at the high doses. Also, sporadic emesis was frequently observed at the high dose. These signs reversed on the first day following discontinuation of treatment.

Erythrocyte values were distinctly reduced in two high dose dogs (normochromic anemia) and slightly in several other dogs of the mid and high dose group. This was clearly a dose-limiting toxicity of the drug.

Electrocardiographic findings were expected for a calcium antagonist and probably related to an exaggerated pharmacodynamic response. Reflex tachycardia probably due to reduced blood pressure was seen at two hours after treatment. Sinus bradycardia with prolongations of the QT interval was a dose-dependent observation 24 hours after treatment, suggesting a direct effect of the drug on the sinus node.

These effects reverted upon discontinuation of treatment.

Definite treatment related histologic changes included hemorrhaging of the GI tract and lymph nodes; marked congestion in the CNS, myocardium kidney, liver, and spleen; vacuolization and necrosis of hepatocytes; and reduced spermatogenesis and mild atrophy of the prostate. The latter finding, was evident even after one week of treatment in both male dogs which died at the high dose. All three animals which died (2M, 1F) showed a severe loss of colloid in the thyroid, a finding which was not evident in any other animal on study. Doses of 2 and 6 mg/kg/day were well tolerated and the latter represents a no effect level in the dog.

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PN 200-110/A 26-Week Oral Toxicity Study in Dogs

Methods

Study Facility/Dates: Sandoz Ltd. R&D Basle, Switzerland (10/81-6/82)

Strain/Source: Beagles; Marshall (North Rose, NY)

Duration: 26 weeks (one animal/sex/dose retained for recovery phase for four weeks)

Route: oral (capsules)

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Dose Levels: 0, 1, 3.5, and 12 mg/kg/day

No. of Animals/Sex/Dose: 3

Age at Study Initiation: 7.5-9.5 mos.

Weight Range: Males: 9-11.5 kg  
Females: 6-9 kg

Parameters Measured:

Clinical Observations: (daily; body weight and food consumption weekly)

- \*Mortality
- \*Clinical signs of toxicity
- \*Body weight
- \*Food consumption
- \*Behavior/motor effects

Ophthalmoscopy: (pretest and week 26)

- \*Slit lamp exam and fundoscopy

Electrocardiograms: (pretest and weeks 13 and 26)

Hematology: (pretest and weeks 3, 6, 13, and 26)

- |   |                                 |
|---|---------------------------------|
| *Red blood cell count                             | *Reticulocyte count             |
| *Hemoglobin                                       | *Thrombocyte (platelet) count   |
| *Hematocrit                                       | *Prothrombin time               |
| *Mean corpuscular volume (MCV)                    | *White blood cell count         |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH)                | *Erythrocyte sedimentation      |
|   | *Coagulation time               |

Clinical Biochemistry: (pretest and weeks 3, 6, 13 and 26)

*Serum glutamic oxaloacetic transaminase (SGOT)	*Total protein
*Serum glutamic pyruvic transaminase (SGPT)	*Albumin
*Alkaline phosphatase (AP)	*Sodium
*Glucose	*Potassium
*Urea	*Cholesterol
*Creatinine	*Total bilirubin
	*Triglycerides

Urinalysis: (week 26)

*pH	*Proteins
*Glucose	*Occult blood
*Ketone bodies	*Specific gravity
*Sediment (microscopic analysis)	

Macroscopic and Microscopic Examination:

Complete

Results

One animal in each of the mid and high dose groups failed to survive treatment. The mid dose female died during week 25; the high dose female died on the third day of the study. Food intake was sporadically inhibited at the mid dose; food intake and weight gain were more consistently impaired at the highest dose.

Clinical signs of toxicity were observed only in one animal at the highest dose and included: severe sedation, lateral position and forced emesis just before death.

A slight anemia and neutropenia was observed in individual animals of the mid and high dose groups. All values returned to pre-test levels during the recovery phase.

Urinalysis revealed no findings which could be related to treatment.

Electrocardiographic changes included sinus bradycardia at all doses which progressed to AV block in some dogs of the mid dose group. All changes were reversible upon cessation of treatment.

Microscopic observations included congestion of several organs for the two animals which died. This was similar to that observed in the 4-week study.

The histologic changes at the mid dose were only observed in the one animal which died. Kidney and heart deterioration could be attributed to hypoxia and cardiac failure or partial autolysis. The same could be reported for the single animal of the high dose group which died during the study. No other notable microscopic changes were observed.

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One Year Oral Toxicity Study in Dogs on PN 200-110

Methods

Study Facility/Dates: Sandoz Res. Inst., E. Hanover, NJ (10/83-11/84)

Strain/Source: Beagle (White Eagle, PA)

Duration: 1 year

Route: oral (capsules)

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Dose Levels: 0, 1, 3.5, and 12 mg/kg/day

No. of Animals/Sex/Dose: 4

Weight Range: Males: 9.4-10.6 kg  
Females: 7.4- 8.8 kg

Parameters Measured:

Clinical Observations:

- \*Mortality
- \*Clinical signs of toxicity (twice daily)
- \*Body weight (weekly)
- \*Food consumption (daily)
- \*Behavior/motor effects (pretest and weeks 1, 2, 6, 12, 20, 28, 36, 44 and 52)

Ophthalmoscopy:

- \*Indirect ophthalmoscopy exam (twice pretest and weeks 4, 8, 14, 19, 26, 38 and 52)

Electrocardiograms: (pretest and weeks 3, 7, 13, 18, 24, 37 and 51)

Hematology: (twice pretest and weeks 4, 8, 14, 19, 26, 38 and 52)

- |   |                                 |
|---|---------------------------------|
| *Red blood cell count                             | *Reticulocyte count             |
| *Hemoglobin                                       | *Thrombocyte (platelet) count   |
| *Hematocrit                                       | *Prothrombin time               |
| *Mean corpuscular volume (MCV)                    | *White blood cell count         |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH)                | *Erythrocyte sedimentation      |
|   | *Clotting time                  |

Clinical Biochemistry: (twice pretest and weeks 4, 8, 14, 19, 26, 38 and 52)

*Blood urea nitrogen (BUN)	*Total protein
*Serum glutamic oxaloacetic transaminase (SGOT)	*Sodium
*Serum glutamic pyruvic transaminase (SGPT)	*Potassium
*Alkaline phosphatase (AP)	*Chloride
*Glucose	*Calcium
	*Cholesterol
	*Total bilirubin

Urinalysis: (twice pretest and weeks 4, 8, 14, 19, 26, 38 and 52)

*pH	*Proteins
*Glucose	*Occult blood
*Ketones bodies	*Specific gravity
*Sediment (microscopic analysis)	

Macroscopic and Microscopic Examination:

Complete

### Results

One animal in the high dose group had to be sacrificed early in the study. Body weight loss was observed at the high dose and impaired weight gain was apparent at the mid dose.

Clinical signs of toxicity included a redness and swelling of the gums, mammary enlargement and injection of sclera at all doses.

Electrocardiographic changes included the detection of AV blocks in 4 high dose animals. As in shorter term studies, tachycardia was apparent 2 hours post-dose and bradycardia emerged 24 hours post-dose.

As in shorter term studies decreases in hematocrit and hemoglobin concentrations occurred with treatment at the mid and high doses. Anemia was not present.

Clinical chemistry, urinalysis, and ophthalmoscopic examinations were unremarkable.

Among animals dying during the course of the study, only centrilobular vacuolization and focal necrosis of the liver were observed histologically. The cause of death for these animals could not be ascertained.

In animals surviving to study termination, the relative heart weight was increased in all treatment groups and absolute weight was elevated at the two higher doses. Absolute adrenal weights were also increased at the two higher doses. Gingival hyperplasia was also evident in all groups, displaying a dose relationship to treatment.

Microscopically, the gingival hyperplasia resembled that of a focal subacute hyperplasia. Definite hyperplasia was also evident in the adrenal cortex of mid and high dose animals.

The sponsor felt that the cardiac changes may have been an indication of .. prolonged compensatory mechanism, whereas the adrenal changes were the product of stress.

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Two-Week Intravenous Toxicity Study of PN 200-110 in the Dog

Method:

Study Facility/Dates: Sandoz R&D, E. Hanover, NJ (3/83-4/83)

Strain/Source: Beagle (White Eagle, PA)

Duration: 2 weeks

Route: intravenous

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Dose Levels: 0, 0.01, 0.03, and 0.10 mg/kg/day

No. of Animals/Sex/Dose: 3

Age at Study Initiation: ca. 9 mos.

Weight Range: Males: 6.4-10.9 kg  
Females: 5.9-10.2 kg

Parameters Measured:

Clinical Observations:

- \*Mortality
- \*Clinical signs of toxicity
- \*Body weight
- \*Food consumption
- \*Behavior/motor effects
- \*Temperature

Ophthalmoscopy: (pretest and week 2)

Electrocardiograms: (pretest and week 2)

Hematology: (pretest and week 2)

- |   |                                 |
|---|---------------------------------|
| *Red blood cell count                             | *Reticulocyte count             |
| *Hemoglobin                                       | *Thrombocyte (platelet) count   |
| *Hematocrit                                       | *Prothrombin time               |
| *Mean corpuscular volume (MCV)                    | *White blood cell count         |
| *Mean corpuscular hemoglobin concentration (MCHC) | *Differential white blood count |
| *Mean corpuscular hemoglobin (MCH)                | *Clotting time                  |

Clinical Biochemistry: (pretest and week 2)

*Blood urea nitrogen (BUN)	*Total protein
*Serum glutamic oxaloacetic transaminase (SGOT)	*Sodium
*Serum glutamic pyruvic transaminase (SGPT)	*Potassium
*Alkaline phosphatase (AP)	*Chloride
*Glucose	*Calcium
	*Cholesterol
	*Total bilirubin
	*Triglycerides

Urinalysis: (pretest and week 2)

*pH	*Proteins
*Glucose	*Occult blood
*Ketones bodies	*Specific gravity
*Sediment (microscopic analysis)	

Macroscopic and Microscopic Examination:

Complete

Results

All dogs survived the study and there were no treatment related effects on body weight or food consumption. Clinical signs of toxicity included decreases in locomotor activity in some animals of all treatment groups shortly after injection. The effect was most pronounced on day 1 and included loss of bladder and bowel control. One high dose animal exhibited a tonic convulsion of day 1. Reddening of the conjunctiva, sclera, and gums occurred within 8 minutes of dosing in all treatment groups.

Ophthalmologic examinations and electrocardiographic recordings were unremarkable. Similarly, the hematologic, blood chemistry, and urinalysis findings were uneventful.

No statistically significant differences from control were noted in the absolute or relative organ weights of the treated groups. Macroscopic and microscopic examination of tissues revealed no changes which could be related to treatment. All lesions reported were common to beagle dogs and were randomly scattered among control and treated animals.

SYNOPTIC TABULATION TABLE E.2-2

MULTIDOSE TOXICITY STUDIES (Subchronic, Chronic, Carcinogenicity)

Species	Strain	Initial #/Sex Group	Mode of Administration	Group Avg. Doses (mg/kg/day)	Study Duration (weeks)	Interim Sacrifice (weeks)	Laboratory	Comments
a. mouse	CR-CD-1	40 all males	diet	2.5 10 40-60-80	13	none	Sandoz Inc. U.S.A.	no toxic-effect level greater than 80 mg/kg/day
b. mouse	CR-CD-1	70	diet	2.6 15.4 81.8	105	none		no evidence of oncogenicity or carcinogenicity
c. rat	KFM-Wist (Füllinsdorf CH)	15	diet	4.3 13 42	4	none	Sandoz Ltd. Basel Switz.	no-toxic-effect level between 13 and 42 mg/kg/day no target organ, only effect on feed intake
d. rat	"	15	diet	2.5 10 41	26			slight hemoconcentration and lowered glucose at two higher levels no toxic-effect level is 41 mg/kg/day

SYNOPTIC TABULATION TABLE E.2-2 (Continued)

MULTIDOSE TOXICITY STUDIES

Species	Strain	Initial #/Sex Group	Mode of Administration	Group Avg. Doses (mg/kg/day)	Study Duration (weeks)	Interim Sacrifice (weeks)	Laboratory	Comments
e. rat	CR-CD SD	75	dlet	2.5 12.5 62.5	104	none	Sandoz Inc. U.S.A.	62.5 mg approximates maximum tolerated dose; drug-induced increased incidence of interstitial (Leydig) cell benign tumors in high-dose male group
f. rat	Taconic SD	5 males only	i.v.	0.5 1.0	2	none	Sandoz Inc. U.S.A.	1 of 4 deaths at 1.0 mg/kg dose level up to 0.5 mg/kg/day tolerated
g. rat	CR-CD SD	10	i.v.	0.04 0.12 0.40	2	none	Sandoz Inc. U.S.A.	reactive hyperplasia of sublumbar lymph node; questionable mild periportal hepatocyte lipidosis

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SYNOPTIC TABULATION TABLE E.2-2 (Continued)

MULTIDOSE TOXICITY STUDIES

Species	Strain	Initial #/Sex Group	Mode of Administration	Group Avg. Doses (mg/kg/day)	Study Duration (weeks)	Interim Sacrifice (weeks)	Laboratory	Comments
h. dog	beagle	3	oral	2 6 20-15	4	none	Sandoz Ltd. Switzerland	n.o.-toxic-effect lrv: 2-6 mg/kg/day 20 mg/kg lethal: ECG changes anemia and reduced spermatogenesis at r dose only
i. dog	beagle	3	oral	1 3.5 12	26	none		ECG changes death at 12 mg/kg 1 mg/kg/day non-toxic
j. dog	beagle	4	oral	1 3.5 12	52	none	Sandoz Inc. U.S.A.	gingival hyperplasia ECG changes, increased heart weights: mortality at high dose, mammary gland swelling
k. dog	beagle	3	i.v.	0.01 0.03 0.10	2	none	Sandoz Inc. U.S.A.	all survived, signs less severe after first day

4) Mutagenicity Studies

Four short term mutagenicity tests were conducted with PN 200-110. These included: Ames test with and without metabolic activation; micro nucleus test in mice; mammalian cell (279 Chinese hamster) transformation; and, unscheduled DNA synthesis in rat hepatocytes.

PN 200-110 was tested in the classical Ames test at concentrations ranging from 30-3000 ug/plate with and without metabolic activation by an S9 rat liver homogenate. No increase in the number of revertants from any of the tester strains (TA 1535, 1537, 1538, 98, and 100). The positive controls (2-amino anthracene, 6-amino anthracene, and benzo(a)pyrene) all tested positive in the appropriate strains.

PN 200-110 was orally administered twice to CD-1 mice (2/sex/dose) at doses of 25, 80, and 250 mg/kg along with an appropriate control group. The mean numbers of micronucleated polychromatic erythrocytes were comparable between groups and within the normal historical control range. The positive control tested positive.

PN 200-110 (up to 75 ug/ml; higher concentrations cytotoxic) had no effect on mammalian transformation of chinese hamster V79 cells whether or not it was tested in the presence of S9 rat liver homogenate. The activity of the positive control validated the assay.

Using rat hepatocytes, the incorporation of radiolabeled thymidine was not affected by the inclusion of PN 200-110 up to concentrations of 300 ug/ml, which proved to be toxic. The positive controls, 2-acetylaminofluorene and aflatoxin B<sub>1</sub>, both were active.

## SYNOPTIC TABULATION TABLE E.2-3

## MUTAGENICITY STUDIES

Species	Strain	Initial #/Sex Group	Mode of Administration	Group Avy. Doses (mg/kg/day)	Study Duration (weeks)	Interim Sacrifice (weeks)	Laboratory	Comments
a. Salmonella (Ames)							Sandoz Ltd. Basel Switzerland	not mutagenic
b. Mouse (micronucleus)	CD-1	4	oral	25 80 250	2	NA	Sandoz Ltd. Basel Switzerland	not mutagenic
c. V79 Chinese Hamster Cells								not mutagenic
d. DNA Repair Synthesis Rat Hepatocytes								not mutagenic

5) Special Studies (Sandoz Ltd., Switzerland)

Special studies with PN 200-110 included an assessment of hemolytic effects on human erythrocytes, and local intravenous tolerance in the rabbit.

PN 200-110 was dissolved and diluted in a cremophor vehicle and admixed with 1 ml samples of fresh EDTA human blood at concentrations up to 1 mg/ml for 30 minutes. No hemolytic effects were observed.

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Solutions of PN 200-110 (0.001% and 0.0003%) in a cremophor vehicle were perfused into the ears of chinchilla rabbits at 4 sites. Slight inflammation at the injection sites was observed in all animals, including controls, 24-48 hours after injection. There was no detectable inflammation one week later. A second perfusion (4 ml at 0.5 ml/min) with a higher concentration (0.05%) of PN 200-110 revealed the same pattern with no drug-related inflammation greater than control.

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## 6) REPRODUCTION STUDIES

PN 200-110: Fertility and General Reproductive Performance  
Study in Male and Female Rats (Oral)

### Methods

Study Facility/Dates: Sandoz Ltd., R&D Basle, Switzerland (1/82-5/83)

Study Type:            Segment: I

Strain: Kfm: WIST (Kleintierfarm, Switzerland)

Route: Oral by gavage in 2% gelatin solution

Dose Levels: 0, 6, 20, or 60 mg/kg/day

Number of Animals/Dose: 15 males; 30 females

Weight Range: Males: 252-256 g (mean)  
                  Females: 198-201 g (mean)

### Mating

Males were treated for 9 weeks and females 2 weeks prior to mating through sacrifice. Equal sized groups of treated females were randomly allocated to the prenatal or postnatal study. One from each study was mated with a treated male. The prenatal study females were sacrificed 14 - 15 days post coitus and were examined macroscopically as well as for uterine contents. The postnatal study females were allowed to litter, sacrificed at weaning (21 days post partum), and macroscopically examined. The males were sacrificed after all dams had given birth (generally 101 - 108 days after treatment initiation) and examined macroscopically. F<sub>1</sub> embryos of the prenatal part were examined for gross aberrations. The F<sub>1</sub> pups selected from the postnatal study for continuation underwent tests to assess: physical development; functional and behavioral development; startle reflex; homing response; swimming; papillary reflex, and learning ability. Finally, reproductive ability was assessed for selected pups of each group.

### Results

Among F<sub>0</sub> males, one high dose male was sacrificed because of extreme weight loss; another high dose male died of unknown causes, and a low dose male was killed through an intubation error. Body weight was slightly lower among treatment groups but not significantly. Macroscopic tissue findings were unremarkable.

Among F<sub>0</sub> females, 4 mid dose and 2 high dose animals failed to survive. Body weight gain was unaffected by treatment; the copulation and pregnancy rates were comparable. Pregnancy length was prolonged dose-dependently, but not significantly at any dose.

Mean numbers of corpora lutea, implantation sites and viable fetuses were not significantly different between dams of the prenatal part. Embryos were not morphologically affected.

The pups derived from dams of the high dose group in the postnatal part of the study were fewer in number and underwent significant postnatal mortality during the first week post partum. Body weight was always lower among offspring of the high dose group. The sex distribution of live pups was acceptable and morphological findings were not remarkable. Morphological, functional and behavioral development was retarded for some parameters (ear opening, vaginal opening, homing response, and testes descensus) in all the treatment groups but these were not statistically significantly different from control. Mating and reproduction of the F<sub>1</sub> generation was uneventful.

The sponsor provided a tabulation of these results and these are presented on the attached pages.

Summary (cont.)

Results, F<sub>0</sub> Males

Dose (mg/kg)	No. of males		weight gain (%) before pairing
	total	died	
Controls	15	0	52
6	15	1	48
20	15	0	49
60	15	1	48

Results, F<sub>0</sub> Females\*

Dose (mg/kg)	No. of females						Copulation rate (%)		Pregnancy rate (%)	
	total		died		paired					
Controls	15	15	0	0	15	15	100	100	87	93
6	15	15	0	0	15	15	100	100	93	80
20	15	15	3	1	14	14	100	100	71	86
60	15	15	1	1	13	15	100	100	92	80

Dose (mg/kg)	Precocial interval (days)		Pregnancy length (days)		Weight gain (%)					
					before pairing		during pregnancy		during lactation	
Controls	4.1	2.9	-	22.1	8	8	19	44	-	16
6	2.7	3.5	-	22.3	9	8	24	38	-	10
20	3.4	3.1	-	22.4	8	9	18	44	-	14
60	2.7	2.9	-	22.5	7	7	21	40	-	15

\* left columns: prenatal part / right columns: postnatal part.

Summary (cont.)

Litter Data of Prenatal Part

Dose (mg/kg)	Corpora lutea	Implan-tations	Litter size	Preimpl. loss (%)	Postimpl. loss (%)	abnormal embryos
Controls	13.2	11.0	9.8	21.0	24.1	0
6	12.6	11.6	9.9	10.1	14.7	0
20	12.7	11.6	10.6	7.7	8.6	0
60	11.7	9.0	7.7	24.1	15.0	0

Litter Data of Postnatal Part

Dose (mg/kg)	Implan-tations	Litter size (day 0 pp)	Pre- + perina-tal loss (%)	Post-natal loss (%)	Body* weight day 21 pp (g)	Abnor-mal pups (%)	Behavior affected pups (%)
Controls	12.0	11.4	5.3	0.9	47.0	0.8	31.3
6	10.0	9.1	11.5	2.7	47.5	3.9	45.4
20	11.5	9.4	17.6	0.0	46.5	1.9	45.8
60	10.4	8.5	19.1	22.0	43.1	1.2	37.5

Results of F<sub>1</sub> Fertility Study

Dose (mg/kg)	Copulation rate (%)	Pregnancy rate (%)	Pre- and perinatal loss (%)	Postnatal loss (%)	Abnormal F <sub>2</sub> pups (%)
Controls	100	93	5.3	0.8	4.1
6	91	100	12.6	5.3	6.8
20	100	78	2.3	1.4	0.0
60	100	88	6.2	1.0	2.0

\* = combined results of males and females

\*\* = p < 0.05

Embryotoxicity Study in Rats (Oral Administration)

Methods

Study Facility/Dates: Sandoz Ltd., R&D Basle, Switzerland (12/81-1/83)

Study Type: Segment: II  
Treatment Schedule: Days 6-15 of gestation  
Sacrifice: Day 21

Strain: KFM-WIST

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Route: Oral by gavage (in 2% gelatin)

Dose Levels: 6, 20, and 60 mg/kg/day

No. of Animals/Sex/Dose: 25 mated females

Age Range: 11 weeks

Parameters Measured.

Dams: Clinical signs of toxicity  
Body weight  
Mortality  
Corpora lutea  
Implantation sites  
Resorptions

Fetuses: Live/dead fetuses  
Body weight  
Sex ratio  
Fetuses examined for: external (gross)  
abnormalities, skeletal abnormalities, and soft  
tissue abnormalities

Statistical Methods

Fisher's t-test for frequency comparison; Kruskal-Wallis for mean comparisons.

## Results:

Two females of the mid dose group and one in the high dose group died suddenly. In life observations included bleeding from the vaginas in two mid-dose animals. These were not the animals which died. Necropsy of the dams revealed nothing remarkable. Body weight gain was significantly lower among females of the 60 mg/kg group during treatment relative to control, but weights were comparable by the end of the study.

Pregnancy rates, number of corpora lutea, implantations, and pre- and post-implantation losses were comparable among groups.

---

Fetal body weights were slightly but not significantly lower in the treatment groups. No dose dependency was observed. No dead fetuses were recorded in any treatment group and the sex ratios of fetuses were comparable.

The morphological exams recorded growth retardations, minor anomalies, and major anomalies. Retardation was comparable between groups. Minor anomalies were observed in two fetuses of the high dose group only: asymmetrical sternbrae in one and kinked tail in the other. The only major anomaly occurred in one fetus of the high dose group: multiple lumbar vertebral defect.

A pilot study of 200 mg/kg/day 200-110 produced a significant impairment in body weight gain (controls, +17.9%; 200 mg/kg/day, +8.2% during treatment) and a significant increase in post-implantation loss (controls, 5.2%; 200 mg/kg/day, 33.1%). Thus it is concluded the compound was embryo-lethal only at doses which produced significant maternal toxicity and was not teratogenic at lower doses.

A tabulation of results is provided in the following table.

## SUMMARY

4) DAYS	FEMALES (ATED)	LITTERS	MORTALITY	ABORTIONS	PERINATALITY RATE %	BODY + WEIGHT GAIN %
CONTROLS	25	21	0	0	100.0	13.7
6.0 MG/KG	25	19	0	0	92.0	11.4
20.0 MG/KG	25	21	2/25	0	92.0	12.1
60.0 MG/KG	25	23	1/25	0	100.0	8.7*

4) LITTERS (MEANS)	CORPORA LUTEA	IMPLANTATIONS	LITTER SIZE	PRE-IMPLANTATION LOSS %	POST-IMPLANTATION LOSS %
				1)	2)
CONTROLS	11.0	10.1	9.4	8.4	14.0
6.0 MG/KG	10.6	4.3	7.8	12.2	16.6
20.0 MG/KG	10.4	9.7	9.1	11.4	15.7
60.0 MG/KG	11.4	9.7	8.4	15.0	13.8

C) FETUSES	BODY WEIGHT G	PLACENTA WEIGHT G	SEX M/F	FET. WITH RETARD.	MONOP. LIMBS	FLUID-FILLING ADJUN
CONTROLS	5.9	.73	1.07	37/175	0/127	0/127
6.0 MG/KG	4.7	.71	.95	30/145	0/154	0/154
20.0 MG/KG	4.8	.71	.94	32/171	0/165	0/165
60.0 MG/KG	4.7	.69	1.02	51/194	2/196	1/196

- \* DIFFERENCE TO CONTROL GROUP VALUE SIGNIFICANT AT 5.0% LEVEL  
 1) % OF CORPORA LUTEA  
 2) % OF IMPLANTATIONS  
 \* DURING TREATMENT

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PN 200-110: Embryotoxicity Study in Rabbits  
(Oral Administration)

Methods

Study Facility/Dates: Sandoz Ltd., R&D Basle, Switzerland (6/82-1/83)

Study Type:                    Segment: 1!  
Treatment Schedule: Days 6-18 of gestation  
Sacrifice: Day 29

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Strain: KFM-RUS

Route: Oral by gavage (in 2% gelatin)

Dose Levels: 1, 3, and 10 mg/kg/day

No. of Animals/Sex/Dose: 16 mated females

Age Range: 6-7 months

Parameters Measured:

Dams:                    Clinical signs of toxicity  
                              Body weight  
                              Mortality  
                              Corpora lutea  
                              Implantation sites  
                              Resorptions

Fetuses:                Live/dead/fetuses  
                              Body weight  
                              Sex ratio  
                              Fetuses examined for: external (gross)  
                              abnormalities, skeletal abnormalities, and soft  
                              tissue abnormalities

Statistical Methods

Fisher's t-test for frequency comparison; Kruskal-Wallis for mean comparisons.

## Results

Body weight gain was dose-dependently and significantly decreased at the two higher doses. Individual does in the low dose group also displayed impaired weight gain but the group mean was similar to control. Two does of the mid-dose and one of the high-dose treatment groups died while on study. In all three cases pleuritis was judged to be the cause of death and is a common finding in this rabbit strain.

In-life observations did not reveal any drug related effects. Doe 10 of the high dose group exhibited vaginal bleeding. This was not the doe which died. Post mortem evaluation showed a high incidence of lung lesions ranging from gross discoloration to pneumonia equally distributed between groups.

Two does in the low dose treatment group spontaneously aborted. The mean numbers of corpora lutea and implantation sites were comparable between groups. No dead fetuses were recorded. However, the mean number of viable fetuses was lower than control at the two higher doses. The dose-dependent significantly higher rate of embryonic resorptions in these two groups accounted for all of the difference.

Fetal body weights and sex ratios were not affected by treatment. Retarded fetal growth was distributed equally between groups. Likewise, minor anomalies which included fused sternbrae and a constriction of the gall bladder showed no relationship to treatment. No major anomalies were recorded.

Doses of PN 200-110 above 1 mg/kg produced decrements in body weight gain and were maternotoxic. Increased resorptions were recorded at these higher doses and this increased embryotoxicity was seemingly related to the maternal toxicity. There was no evidence of teratogenicity at any dose administered.

The sponsor tabulation of results is presented on the following page.

A) DAMS	FEMALES MATED	LITTERS	MORTALITY	ABOR-TIONS	PREGNANCY RATE %	BODY WEIGHT GAIN %
CONTROLS	16	14	0	0	87.5	5.4
1.0 MG/KG	16	12	0	2	93.8	3.7
3.0 MG/KG	16	12	2/16	0	87.5	1.3
10.0 MG/KG	16	8	1/14	0	71.4	-2.6*

B) LITTERS (MEANS)	CORPORA LUTEA	IMPLAN-TATIONS	LITTER SIZE	PRE-IMPLAN-TATION LOSS % 1)	POST-IMPLAN-TATION LOSS % 2)
CONTROLS	6.6	4.5	3.8	34.1	25.4
1.0 MG/KG	7.3	5.3	5.2	26.6	2.1
3.0 MG/KG	7.0	5.3	3.3	22.8	43.1
10.0 MG/KG	6.0	4.7	1.5	21.0	75.0

C) FETUSES	BODY WEIGHT G	PLACENTA WEIGHT G	SEX M/F	FET. WITH RETARD.	MORPH. MINOR	FINDINGS MAJOR	FETAL LOSS 24H %
CONTROLS	41.4	7.07	.96	4/53	1/53	0/53	1.8
1.0 MG/KG	41.0	7.16	.91	1/63	4/63	0/63	2.4
3.0 MG/KG	40.4	7.10	.60	1/40	1/40	0/40	2.5
10.0 MG/KG	41.9	7.44	.50	0/12	0/12	0/12	.0

\* DIFFERENCE TO CONTROL GROUP VALUE SIGNIFICANT AT 5.0% LEVEL

1) % OF CORPORA LUTEA

2) % OF IMPLANTATIONS

\* DURING TREATMENT

PN 200-110: Peri- and Postnatal Study in Rats  
(Oral Administration)

Methods

Study Facility/Dates: Sandoz Ltd., R&D Basle, Switzerland (2/84-1/85)

Study Type: Segment: III  
Treatment Schedule: Day 15 p.c.-day 21 p.p.  
Sacrifice: Day 21 p.p.

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Strain: KFM-WIST

Route: Oral by gavage (in 2% gelatin)

Dose Levels: 6, 20, and 60 mg/kg/day

No. of Animals/Sex/Dose: 24 mated females

Age Range: 12-13 weeks

Parameters Measured:

Dams: Clinical signs of toxicity  
Body weight  
Duration of gestation  
Mortality  
Histopathology where indicated

Pups: Body weight  
Litter size  
Survival  
Developmental Effects - ear and eye opening,  
vaginal opening and testes decensus, functional  
and behavioral development, auditory response,  
homing response, swimming, pupillary reflex, and  
learning ability, fertility.

Histopathology - major organs

Statistical Methods

Fisher's t-test for frequency comparison; Kruskal-Wallis for mean comparisons.

## Results

Two mid-dose females died during pregnancy and one high-dose female died during lactation. All were sudden deaths without any pre-mortem signs. Body weight gain was significantly reduced at the higher doses during the latter phase of gestation but weight gain was increased in these groups during the lactation period. Pregnancy length was not unduly prolonged by treatment although the sponsors claim that the control's duration was longer than in any previous study. Some animals in the treated groups (C-1, M-2, H-1) had to be sacrificed due to dystocia and two high-dose dams delivered on day 24.

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Postnatal survival was lower in pups from dams of the high dose group. Body weight at birth was significantly lower at the the two higher doses and in addition, at weaning the pup body weight was lower in all 3 groups relative to control. Sex distribution was normal. Autopsy findings of pups revealed nothing remarkable.

Behavioral development of pups from treated groups was impaired. In low dose group the percentage of pups failing to meet the criterion for body weight development, ear opening, eye opening, vaginal opening, homing response, and swimming were outside the control range. In the mid dose swimming was affected. In the high dose group body weight development, ear opening, homing response, swimming and learning were affected. As a consequence, the sum total of scores of affected pups for these development parameters was dose-dependently higher (control 24.9%; low dose, 48.6%; mid dose, 42.7%; high dose, 69.3%). These were not significantly different. The distribution of affected parameters among individual pups within litters also suggests that these developmental differences were independent of individual reductions in body weight gain. A no effect level was not determined in this study. Although development was retarded, no permanent effects were noted.

The fertility of the  $F_1$  pups was normal with some significant decreases in the number of implantations and numbers of live pups observed among the high dose offspring.

Development of the  $F_2$  generation was uneventful.

A tabulation of the results is provided on the following page.

Results, F<sub>0</sub>

Dose (mg/kg)	No. of Females		Weight gain (%) during treatment		Pregnancy rate (%)	Pregnancy length (days)
	inseminated	died	pregnancy*	lactation		
Controls	24	0	19	10	83	22.4
6	24	0	16	10	83	22.2
20	24	2	13***	16	100	22.4
60	24	1	8***	20***	83	22.5

Litter Data

Dose (mg/kg)	Implantations	Litter size (day 0 pp)	Pre- and perinatal loss (%)	Postnat. loss (%)	Body-weight day 21 pp (g)	Abnormal pups (%)	Behav. affected pups (%)
Controls	11.3	10.6	7.9	0.6	46.0	5.2	24.9
6	10.5	9.3	12.0	2.0	42.1	0.7	48.6
20	11.8	9.7	19.2***	12.2	39.6***	2.0	42.7
60	10.6	8.3	24.1	12.2	37.9***	0.0	69.3

Results of F<sub>1</sub> Fertility Study

Dose (mg/kg)	Copulation rate (%)	Pregnancy rate (%)	Pre- and perinatal loss (%)	Postnatal loss (%)	Abnormal F <sub>2</sub> pups (%)
Controls	100	100	5.9	1.7	0.4
6	100	95	4.7	6.5	1.4
20	100	100	8.4	0.7	0.5
60	100	88	10.5	0.8	0

\* days 15-20 p.c.

\*\* combined results of males and females

\*\*\* P < .05

## SYNOPSIS REPRODUCTION TABLE E.2-5

## REPRODUCTION STUDIES

Species	Strain	Initial #/Sex Group	Mode of Administration	Group Avg. Doses (mg/kg/day)	Study Duration (weeks)	Interim Sacrifice (weeks)	Laboratory	Comments
a. Fertility rat	KFM/Wist	15	oral	6 20 60	9 pre-breed males  2 pre-breed females	day 14 p.c. end of Fl	Sandoz Ltd. Basel Switzerland	reduced wt. gain and increased post natal mortality at mid and high dose
b. Teratology rat	KFM/Wist	25	oral	6 20 60	days 6-15 post coitus	day 21 term	Sandoz Ltd. Basel Switzerland	no evidence of teratogenicity
c. Peri-post natal rat	KFM/Wist	24	oral	6 20 60	day 15 p.c. to day 21 p.p.	-		maternal tox at 60 mg/kg; increased pup mortality mid and high dose
d. Teratology rabbit	KFM/Rus	16	oral	1 3 10	6-18 p.c.	term. day 29 p.c.		weight loss of dams and embryo lethality at 10 mg/kg; not teratogenic

## C) Pharmacokinetics

### Mouse

Blood levels, tissue distribution and excretion patterns were studied in the Charles River CD-1 mouse after oral or intravenous doses of  $^{14}\text{C}$ -labelled PN 200-110. In the oral study, the drug was admixed in the diet (0.01% or 17 mg/kg/24 hours) and administered to male mice (n=7). The intravenous study utilized a bolus injection (1 mg/kg) to male mice (n=3). The majority of the radioactivity was excreted in the first 24 hours (>70%). The amount of PN 200-110 absorbed was judged to approximate 60% of the oral dose. Appreciable blood levels were obtained within the first two hours following presentation of the food-drug mixture suggesting that the absorption was rapid.

Similarly designed distribution studies showed that peak blood levels of radioactivity were achieved within 15 minutes (2nd timepoint) after injection and around 15 hours after offering the oral diet-drug mixture. Circulating radioactivity had been reduced to 2% of peak 12 hours after the intravenous dose. With both the oral and intravenous route, the distribution was greatest to the liver and kidneys, tissue levels were comparable to 2-fold greater than blood eight hours after drug administration. The elimination half-life for radioactivity was calculated to be 30 hours and there was virtually no retained radioactivity 96 hours after treatment.

### Rat

Male and female Han Wistar rats received 0.5 mg/kg  $^{14}\text{C}$ -labelled PN 200-110 either orally by gavage or intravenously. Some animals were cannulated for collection of bile. Biliary excretion accounted for the bulk of drug elimination; approximately 20% was recovered in the urine independent of the route of administration. Excretion of radioactivity was complete within 48 hours. No enterohepatic cycling of drug seemed evident. Based on the results, oral absorption of PN 200-110 was judged to be near complete in the rat and relatively rapid.

Further studies documented peak plasma levels occurring within 1.5 hours of a single oral dose. Terminal half-lives for the excretion of radioactivity were 24 and 29 hours after single oral and intravenous doses, respectively. Distribution was greatest to the liver, kidney, fat, and lung. For up to 24 hours only the concentration in the liver was higher than the plasma level of PN 200-110. After 96 hours independent of the route of administration, appreciable levels of radioactivity were evident only in the liver, especially of females.

## Rabbit

Female New Zealand White rabbits received either a single intravenous dose (0.3 mg/kg) or oral dose (3.0 mg/kg) of radiolabelled PN 200-110 and urine and feces collected over a period of 96 hours. The oral absorption of PN 200-110 was rapid with significant levels appearing in the blood within one-half hour. Absorption was nearly complete and fecal excretion accounted for only 10% of the administered dose utilizing either route. Approximately 85% of the dose was eliminated in the first 48 hours.

In oral distribution studies, peak plasma levels of radioactivity were observed within one hour and declined monoexponentially with a half-life of 9.6 hours. After intravenous dosing by slow injection, peak plasma levels occurred at 15 minutes (2nd timepoint) and the primary and terminal half-lives for plasma decay were calculated to be 2.6 and 26.1 hours, respectively.

In pregnant rabbits, multiple daily oral doses (3.0 mg/kg) of <sup>14</sup>C-PN200-110 were administered. Two hours after the 5th daily dose, concentrations of radioactivity in the liver and kidney were 2 to 5 times greater than blood; the other tissues, including amniotic fluid, fetus, placenta, ovaries, corpora lutea, and uterus, had concentrations ranging from 60%-90% of the blood level. On the 13th day of dosing, the same trends appeared except that the fetal tissue and amniotic fluid concentrations were much lower. These results indicate that fetal transfer of drug in the rabbit is limited.

## Dog

All pharmacokinetic studies with PN 200-110 were conducted in the same three beagle dogs (2M, 1F). Single oral doses of 1.0 mg/kg and intravenous doses of 0.5 mg/kg were administered. After the oral dose, peak levels were reached in 4-8 hours. Plasma levels were 2-3 times higher than blood levels indicating an uneven distribution between cells and plasma. The terminal radioactive half-life was estimated to be between 37 and 46 hours.

After an initial 5 minute (earliest measurement) plasma drug level peak, a second peak occurred 2 hours after intravenous dosing. The terminal half-life by this route was similar to that obtained with oral dosing.

Excretion of administered drug was approximately one-third urinary and two-thirds fecal following nearly complete (90%) oral absorption.

### Comparative Pharmacokinetics

The following table summarizes the comparative pharmacokinetic data after oral dosing:

	<u>% Abs.</u>	<u>t<sub>max</sub></u>	<u>t<sub>1/2</sub> (hr)</u>	<u>Excretion</u>	
				<u>Urine (%)</u>	<u>Fecal (%)</u>
Mouse	60	2	37	24.1%	75.9%
Rat	95	1.5	23	19.3%	80.7%
Rabbit	100	1	10	89.4%	10.6%
Dog	90	4	38	30.3%	69.7%
Man	90-95	3	28	70.0%	30.0%

For most parameters, there is good agreement between species. However only the rabbit and man eliminate the drug and its metabolites predominantly in the urine. This would suggest that there may be significant interspecies differences in the biotransformation of the drug.

Another difference is the relative blood level of drug following a single dose. The results are presented as F values which corrects for dosage differences. The peak F for man is 1.234 @ 2 hours; the other species reach only fractions of these levels (mouse, 0.172; rat, 0.532; rabbit, 0.221; dog, 0.687). This suggests that man has a smaller volume of distribution because absorption is comparable for the most part.

### Comparative Metabolism

PN 200-110 is extensively metabolized. The metabolic pathways and extent of biodegradation vary by species. The sites and type of metabolism are depicted on the attached map and include:

- 1) hydrolysis of the isopropyl or methylester
- 2) oxidation of the dihydropyridine ring.
- 3) oxidation of the isopropyl group and subsequent conjugation with glucuronic acid.
- 4) conjugation of the mono acid with glucuronic acid.
- 5) oxidation of the 2-methyl group to an alcohol with formation of the cyclic lactone.

- 6) oxidation conjugation of the phenyl ring with conjugation to glutathione
- 7) oxidation of the 6-methyl to an acid followed by conjugation with glucuronic acid.

Based on studies with radiolabeled material, the relative amounts of parent and metabolites in the blood and plasma are tabulated below:

TABLE C-28

QUANTITATIVE COMPARISON OF THE METABOLITES OF PN 200-110  
IN THE BLOOD AND PLASMA OF MAN AND SEVERAL ANIMAL SPECIES

Metabolite Number	% of Radioactivity Quantitated			
	Man 0.17 mg/kg	Mouse 14.3 mg/kg	Rat 1.8 mg/kg	Dog 1 mg/kg
0	4.2	1.2	2.4	2.5
2		32.3	68.9	50.7
4		18.4	5.7	
11	43.4	15.9	11.9	6.8
14	{47.0	{ 6.1		20.1
16	{	{		
18				4.4
Total	94.6	73.9	88.9	84.5

{ Means the two adjacent fractions are combined in one total figure, eg. the combination of metabolites 14 and 16 in man constitute 47.0% of the administered dose.

A similar tabulation of urinary products follows:

TABLE C-29

QUANTITATION AND COMPARISON OF THE METABOLITES OF PN 200-110  
IN THE URINE OF MAN AND SEVERAL ANIMAL SPECIES

Metabolite Number	% of Radioactivity Quantitated			
	Man 0.17 mg/kg	Rat 3.1 mg/kg	Rabbit 0.75 mg/kg	Dog 1 mg/kg
4	9.4	2.3	8.5	
7	{ 5.2		16.8	
10	{			
11	19.1	47.9	14.9	11
14	{30.7		49.9	
16	{	21.1		41.9
18				8.0
TOTAL	64.4	71.3	90.1	60.9

Biliary metabolites were only examined in the rat. The results from three rats administered 3.1 mg/kg (2) or 166.7 mg/kg (1) PN 200-110 are given below. There were no significant differences in the metabolic pattern between the two dose levels.

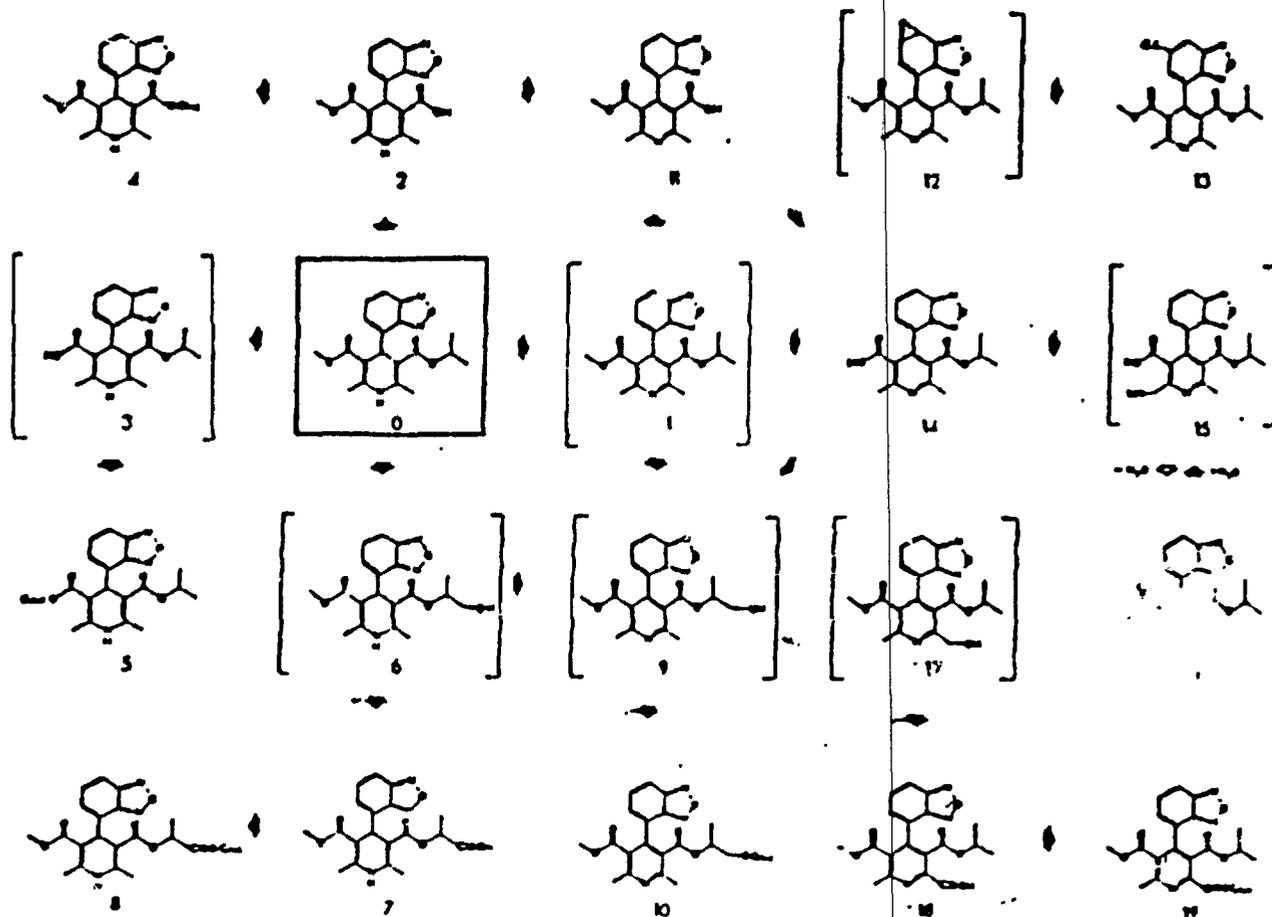
Metabolite	<u>4</u>	<u>5</u>	<u>10</u>	<u>11</u>	<u>16</u>	<u>18</u>	<u>13 &amp; 19</u>
Mean (%)	10.3	5.2	16.4	6.0	15.9	5.5	19.2

Considering the metabolic data, there seems to be enough information to conclude that exposure in rats is similar enough to man to validate the use of this species for the long-term carcinogenicity studies. ~~The rabbit does not form the cyclic lactone (16) following methyl ester hydrolysis (14).~~ Data on the dog is less good considering that 70% of the drug is eliminated in the feces but the plasma data suggests that most major routes in man are operative in the dog and exposure is sufficient. The lack of either urinary or fecal metabolite data in the mouse is more problematic. The blood data shows that the hydrolysis of the isopropyl ester and glucuronide conjugation is a primary route. There is some evidence of pyridine ring oxidation (followed by methyl ester hydrolysis (14) and subsequent formation of the cyclic lactone (16) but these are minor metabolites in the blood (6.1%). In the rat, plasma levels of metabolites 14 and 16 were undetectable, yet urinary excretion of metabolite 16 was relatively high (21.1%) and the metabolite did appear in the bile suggesting that the low blood levels may not be indicative that this is a minor route. A conservative estimate based on blood level data would be that metabolites of this pathway are only 10% of those achieved in man administered a comparable dose.

There were no studies which adequately addressed metabolic induction.

SCHEME E.3-1

BIOTRANSFORMATION PATHWAYS OF PN 200-110 IN ANIMALS AND MAN



### Additional Studies

Two metabolites (1 and 3) and the related analogs of PN 200-110 were tested for cardiovascular activity in the cat. Metabolite 3, the methyl ester hydrolysis product, displayed minimal and transient effects on anesthetized, open chest cats instrumented to measure HR, CO, BP, and peak acceleration. The relative potency to PN 200-110 on these cardiovascular indices was 1/100. This is not a major metabolite in animals or man. The other products tested showed no activity.

In vitro distribution studies with PN 200-110 in human blood revealed 91% of the drug was bound to plasma proteins, 2% in plasma water, and 7% in blood cells. In vivo studies in rats and dogs showed that 96% was protein bound with residual amounts in plasma water and negligible amounts in cells. The differences were accounted for by metabolism in vivo. The distribution to erythrocytes was linearly dependent over a range of PN 200-110 concentrations in a separate experiment. The primary human plasma binding protein for PN 200-110 proved to be  $\alpha_1$ -AGP ( $k = 1.2 \times 10^5 M^{-1}$ ) and this was saturable. The binding to albumin and lipoproteins was secondary to this and was not saturable over a range of concentrations. The critical break in the near complete binding of PN 200-110 by  $\alpha_1$ -AGP came at concentrations greater than 1 ug/ml ( $\alpha_1$ -AGP conc. = 0.9 g/l). This concentration is approximately 100 times the therapeutic blood level of drug. No species differences were observed in the binding and distribution characteristics of the drug.

There were no studies examining the binding of metabolites or displacement of highly protein bound drugs by PN 200-110 or its metabolites.

### Evaluation

The in vitro and in vivo pharmacology of PN 200-110 definitely establish it as a calcium channel blocker with an activity profile similar to other members of its class (e.g., verapamil, diltiazem, and nifedipine). In comparative studies with vascular ring preparations from rabbits and dogs, PN 200-110 proved more potent than verapamil, nifedipine, nimodipine, and nisoldipine in blocking calcium induced contractions. Other agonists were non-competitively antagonized only at much higher doses. There was also some evidence that PN 200-110 displayed a preferential selectivity for the cerebral vasculature over peripheral vessels. In guinea pig and rabbit heart preparations, PN 200-110 showed a preferential inhibition of heart rate over contractile

force and was devoid of effects on the refractory period, in contrast to verapamil.

In cats, dogs, and rabbits, PN 200-110 produced the expected hemodynamic changes. Blood pressure and heart rate were reduced in all species. In the rabbit, the reflex tachycardia to the drop in pressure was offset by the bradycardia produced by the drug. This was the only parameter measured where PN 200-110 differed qualitatively from nifedipine, the comparative drug in all three experiments. Interestingly, the effects on heart rate (and right atrial pressure) in the cat reversed more quickly than the effect on blood pressure and other hemodynamic parameters.

In spontaneously hypertensive rats, PN 200-110 produced significant decreases in blood pressure and at the lower doses without the usual reflex tachycardia.

In ancillary pharmacology studies, PN 200-110 inhibited histamine and acetylcholine induced bronchospasm in guinea pigs at 10 mg/kg, iv. PN 200-110 also produced a marked reduction in serum triglycerides.

The LD<sub>50</sub> values after oral administration of PN 200-110 were 216, >3000, and 58 mg/kg in the mouse, rat, and rabbit, respectively. The clinical signs and symptoms of acute toxicity were consistent with an exaggerated drop in blood pressure, the pharmacologic activity of the compound. The clinical therapeutic dose of 20 mg (0.4 mg/kg/day), when considered in relation to acutely toxic doses in animals, suggests an acceptable margin of safety for the drug.

The multidose studies examining the subchronic and chronic toxicity of PN 200-110 revealed the following:

In the mouse, there was no remarkable toxicity recorded in a 13-week study with oral doses of PN 200-110 up to 80 mg/kg/day.

The same dose levels were utilized in the carcinogenicity study. The survival among male mice was marginally impaired. Although it did not reach significance (p=0.053) at the highest dose by the sponsor's analysis, there was a positive trend with dose which was significant (p=0.038). These results indicate that a maximum tolerated dose had been administered in this study.

Considering potentially drug-related pathology, an increase in carcinoma of the adrenal cortex was noted among males of the mid-dose group but the incidence at the high dose was lower than control and it can be concluded from the pattern of results that

this was not a drug-related finding. There was an increased incidence of inflammation and hyperplasia of the stomach mucosa of treated mice of both sexes. These increases reached statistical significance for the mid and high dose males. - There was no increase in the incidence of gastric neoplasia observed in any treatment group.

The sponsor notes that these changes occurred during the last six months of the study and attributes them possibly to an outbreak of murine colonic bacteria, specifically C. freundii. The sponsor cannot explain why the outbreak was confined to animals of treatment groups even though they were randomly housed. Given the pattern of events, a more probable explanation would be that aging mice of both sexes were more prone to a slight inflammatory action of the drug or that the drug interacted with C. Freundii to enhance its pathologic activity. If some inflammatory response in the gastrointestinal tract was noted in the clinical trials with PN 200-110 then some mention of this finding in the mouse should be included in the labeling.

An increase in the incidence of hepatocellular carcinoma was significant among high-dose males ( $p < 0.05$ ). The trend with dose was also significant. Given the marginally impaired survival among males of the high dose treatment group, it was suggested that the sponsor supply computer tapes or suitable tables for the analysis of these data by the Peto method. The analyses by CDER's Biometrics Division showed a positive dose-response relationship for both hepatocellular carcinoma ( $p = 0.004$ ) and for carcinoma pooled with nodular proliferation ( $p = 0.011$ ).

Although the sponsor saw statistical significance in some of the intergroup comparisons for these neoplastic findings, considered alone or together, they did not feel that these findings were of biological significance, pointing to variability between the control groups.

Nonetheless, there was a highly significant dose-response relationship in the incidence hepatocellular carcinoma among male mice. Although significance was strongly influenced by the increased incidence of tumors and impaired survival in the high dose treatment group, it must for that reason alone be considered a drug-related finding.

The metabolic studies of PN 200-110 in the mouse are of limited utility. Bloc' level data suggest that the primary metabolic pathway operative in the mouse may differ from man. There are indications, however, that the metabolites occurring in man are present in the mouse and the amounts may still be in excess of

that occurring in man because of the large multiple of the therapeutic dose employed.

Drugs producing mouse liver tumors have been approved in the past (e.g., clofibrate). The approved  $Ca^{++}$  influx inhibitors (verapamil, nifedipine, and diltiazem) have shown no evidence of carcinogenicity in chronic rodent studies. There have been no studies into the mechanism of PN 200-110 related increases in hepatocellular carcinomas.

There was nothing observed in the subchronic rat study to give any indication of drug related histopathology with PN 200-110. The metabolism of PN 200-110 in the rat is similar enough to man to warrant its use in this species for an assessment of human toxicity and carcinogenicity. In the chronic study body weight reductions (>10%) were produced by treatment in the high dose animals of both sexes.

Grossly, the absolute and relative (brain) weights of the liver and thyroid were increased but there was no evidence of non-neoplastic or neoplastic pathology in these tissues.

Chronic progressive nephrosis was observed more frequently among males of the high dose treatment group dying on study.

The most important finding was an increased incidence of Leydig cell hyperplasia and tumors among male rats of the high dose group relative to control. Cox's trend test gave a p-value less than 0.01 for an increase as a function of dose. A Peto analysis performed by CDER's Biometrics Division confirmed that the dose-relationship was highly significant.

An amendment submitted on 3/31/88 provided an overall evaluation of the Leydig cell tumor findings in the rat carcinogenicity study. The evaluation considered several studies which had been previously submitted in the NDA but also several others for which full reports had not been received. The full reports were requested by Dr. Resnick on 4/8/88 and submitted in a subsequent amendment on 7/6/88.

These studies included a repeat two-year study in male Sprague-Dawley rats administered the low and high doses of the original study and incorporating several hormonal analyses throughout. The significant increases in Leydig cell tumors and hyperplasia with the high dose of PN 200-110 were confirmed. Non-significant increases were observed in the low dose group. Measurement of serum and testicular hormone levels showed a dose dependent elevation of LH and FSH during the latter half of the

study. Testosterone levels were lower in the high dose group at weeks 78, 86, and 104. Prolactin levels were reduced in both groups relative to control throughout most of the study. Testicular LH receptor levels were decreased in the treated groups at termination and GnRH receptor levels were increased.

The results are consistent with a drug-related down regulation of LH receptors, possibly due to the decreased circulating prolactin. Consequently, testosterone production is decreased, its feed back to the pituitary diminished, and LH and FSH serum levels increased. Although this is a viable interpretation of the hormone and receptor changes and the relationship between testicular maturation and elevated gonadotropin levels is well documented, there was no correlation between levels of gonadotropins and tumor appearance in those animals which developed tumors. However the weight of evidence does suggest that PN 200-110 does influence the sex hormone levels in the male rat at toxic doses but does not affect hormonal levels of male hypertensives when taken at therapeutic doses. Therefore, it can be extrapolated that the risk for developing testicular tumors would be less for humans receiving PN 200-110 than that predicted by the results of these rat studies. Other mechanistic studies of this phenomenon seem to support this conclusion.

Three studies (4, 26, and 52 weeks) of orally administered PN 200-110 were conducted in beagle dogs. In all studies food intake and weight gain were reduced at the higher doses. Animals which died on study showed marked congestion of several organs suggestive of an exaggerated hypotensive effect. ECGs provided evidence of sinus bradycardia and AV block in some dogs, which may have contributed to their demise. Much of the pathology, including cardiac, adrenal, and thyroid changes, was judged to be due to compensatory mechanisms or induced by stress.

The gingival hyperplasia evident with other calcium antagonists was also a property of PN 200-110 in dogs. Normochromic anemia, which reversed upon cessation of treatment, was also a consistent finding and may have been drug-related at the higher doses.

There was no evidence of mutagenic potential with PN 200-110. Studies include the following: Ames test; unscheduled DNA synthesis; mammalian transformation; and micronucleus test.

In the reproductive toxicity studies, there was no effect on fertility among male or female rats. Body weight, development and survival of pups was adversely affected, particularly at the high dose (60 mg/kg).

No consistent teratogenic or embryo-lethal events were observed in rats. In rabbits, doses of PN 200-110 above 1 mg/kg inhibited body weight gain and produced maternal toxicity which resulted in an increased number of resorptions. There was no evidence of a teratogenic effect of the drug.

In the peri/post-natal study in rats, maternal body weight gain was reduced. Postnatal survival was lower at the high dose (60 mg/kg) and pup body weight was reduced at birth at the two higher doses and was lower in all dose groups at weaning. Development of offspring was retarded but no permanent effects were observed.

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#### Recommendations

This NDA can be considered approvable only if the therapeutic benefit outweighs the potential risk of neoplasia suggested by the increased incidence of hepatocellular carcinoma among male mice and of Leydig cell tumors in male rats in the oncogenicity studies conducted with PN 200-110.

Assuming that the drug is approvable, the draft labeling submitted by the sponsor has been revised (see below) to reflect acceptable wording for the nonclinical sections.

## Labeling

The labeling for Dynacirc (isradipine) was reviewed for nonclinical statements of fact, appropriateness of inclusion, format, and consistency of style.

It is recommended that the section Clinical Pharmacology - Nonclinical Effects be deleted entirely from the package insert. While the statements included herein are for the most part accurate representations of animal study findings, they implicitly contain superiority claims over other drugs and other desirable actions in animal models which might be extrapolated to mean that isradipine is useful for other indications. No balanced presentation of any toxic effects of the drug observed in animals is included in this section.

The section on the Mechanism of Action is based largely on animal results but it is considered acceptable because it gives an accurate description of the results obtained in experiments which would be unethical in patients. The consistency between the responses observed at the molecular and tissue level and the clinical response suggest that the same or similar mechanisms may be operative in man.

The sections on Carcinogenesis, Mutagenesis, Impairment of Fertility and Pregnancy have been rewritten to conform with current style and the review of the submitted studies. The differences in the revised version made the following rewrites seem more appropriate than simple editing of these sections.

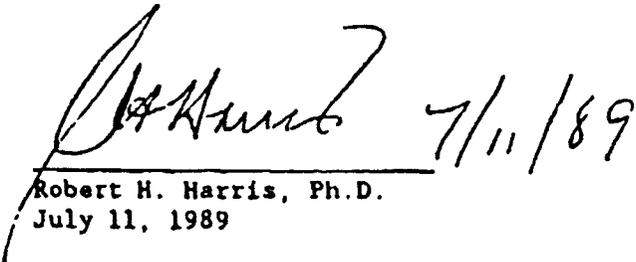
### Carcinogenesis, Mutagenesis, Impairment of Fertility

Treatment of male rats for 2 years with 2.5, 12.5, or 62.5 mg/kg/day isradipine admixed with the diet (equivalent to multiples of approximately 6, 31, and 156 times the maximum recommended daily dose based on a 50 kg man) produced dose dependent increases in the incidence of benign Leydig cell tumors and testicular hyperplasia relative to untreated control animals. These changes may have been indirectly related to an effect of isradipine on circulating gonadotropin levels in the rats; a comparable endocrine effect is not evident in male patients receiving therapeutic doses of the drug on a chronic basis. Treatment of mice for two years with 2.5, 15, or 80 mg/kg/day isradipine in the diet (equivalent to multiples of approximately 6, 38, and 200 times the maximum recommended daily dose based on a 50 kg man) produced an increase in the incidence of hepatocellular carcinoma appearing among male mice treated with the highest dose of isradipine relative to untreated control

animals. No increase in the incidence of hepatocellular carcinoma was observed among female mice treated with comparable doses of isradipine. There was no evidence of mutagenic potential based on the results of a battery of mutagenicity tests. No intrinsic effect on fertility was observed in male and female rats treated with up to 60 mg/kg/day isradipine.

#### Pregnancy

CATEGORY C. Isradipine was administered orally to rats and rabbits during organogenesis. Treatment of pregnant rats with doses up to 60 mg/kg/day (equivalent to 150 times the maximum recommended dose) produced a significant reduction in maternal weight gain during treatment with the highest dose but no lasting effects on the mother or the offspring. Treatment of pregnant rabbits with oral doses of 1, 3, or 10 mg/kg/day isradipine (equivalent to 2.5, 7.5, and 25 times the maximum recommended therapeutic dose) produced decrements in maternal body weight gain and increased fetal resorptions at the two higher doses. There was no evidence of embryotoxicity at doses which were not maternotoxic and no evidence of teratogenicity at any dose tested. In a peri/postnatal administration study in rats, reduced maternal body weight gain during late pregnancy at 20 and 60 mg isradipine/kg/day was associated with reduced birth weights and decreased peri and postnatal pup survival.

  
Robert H. Harris, Ph.D.  
July 11, 1989

cc:  
Orig. NDA  
HFD-345/GJames  
HFD-502/JWeissinger  
HFD-110  
HFD-110/CSO  
HFD-110/RHarris

R/D init: CResnick/7/18/89