

Study title: Twelve-month oral toxicity study of iloperidone in dogs (Study 0992-0220)

Key study findings:

Iloperidone was administered orally to beagle dogs (4/sex/dose) at 6, 12, and 24 mg/kg/d for 12 months. There was no drug-related mortality. Drug-related clinical signs were noted at all dosages and included decreased spontaneous activity, tremors, bizarre behaviors, labored breathing, scleral infection, ptosis; slow response times and/or lack of pupillary reflex were revealed at neurological examination. Most of the clinical signs were noted in all treated animals and showed little dose dependence. Drug-related findings in the mid- and high-dose groups that were not seen in the low dose group included ataxia, loss of righting and toe pinch reflex (in single animals), emaciation. Body weight losses occurred in LD and HD groups; mean body weight decreases of 7.3% and 9.2% vs. control were registered over the treatment period at LD and HD, respectively. Hematology and clinical chemistry changes were induced at MD and HD, i.e., dose-dependent decreases in mean erythrocyte count and in hemoglobin and hematocrit levels in males and females; lower cholesterol and triglyceride levels in the females throughout the dosing period, and increase in alanin aminotransferase in HD males. No abnormalities were found in any dose group on ECG and auditory examination.

Drug-related gross and microscopic pathology changes were not found in LD and MD groups; in the HD group, higher mean absolute and relative liver weights and hepatocellular hypertrophy resulting from proliferation of the endoplasmic reticulum were found in the males, probably secondary to liver enzyme induction .

The toxicokinetic analysis showed that mean plasma concentrations of iloperidone at week 49 were 5.0, 4.3, and 29.7 ng/ml for males, and BLQ, 29.7 ng/ml, and 119.8 ng/ml for females, at 6, 12 and 24 mg/kg/d, respectively, pointing to an over dose-proportional increase in plasma concentrations in the females. The principal metabolite was P89 9430, which was detected at higher levels than iloperidone, while P89 9124 and P88 8991 were below the limits of quantification.

The MTD in this study is 6 mg/kg/d in view of severe clinical signs and emaciation induced at and above the next higher dose of 12 mg/kg/d.

A NOAEL was not reached in this study as the lowest dose induced decreased body weight and neurological clinical signs.

Study no: 0992-0220

Volume # and page #: N.A. (electronic submission)

Conducting laboratory and location: Drug Safety, Hoechst-Roussel Pharmaceuticals Inc, Somerville, NJ

Date of study initiation: June 16, 1992

GLP compliance: Yes

QA report: yes

Drug, lot #, radiolabel, and % purity: Batch/Lot RC 4840, Purity 99.8%;
Batch RC 5634, Purity >99.1 %

Formulation/vehicle: gelatin capsules

Methods: Iloperidone was administered orally in gelatin capsules to male and female beagle dogs at 6, 12, and 24 mg/kg/d (4/sex/dose) for 12 months. A control group received capsules containing microcrystalline cellulose.

Dosing:

Species/strain: Dog/Beagle

#/sex/group or time point (main study): 4

Satellite groups used for toxicokinetics or recovery: TK analysis performed on all main study dogs; recovery not studied.

Age: 13-36 months

Weight:

Doses in administered units: 6, 12, and 24 mg/kg/d

Route, form, volume, and infusion rate: orally in gelatin capsules

Observations and times:

Parameters evaluated for adverse effects included mortality, general condition and clinical signs, body weight, food consumption, neurological (righting reflex, placing reflex and pinch toe reflex), auditory and ophthalmoscopic examination, electrocardiography (ECG tracings of Lead II in conscious dogs from all groups recorded at baseline and at weeks 13, 26, and 53), hematology, serum chemistry, urinalysis, organ weights, gross and microscopic pathology, and electron microscopic examination of livers (high dose group only). TK analysis was performed on blood samples collected from all dogs at 4 hours post-dose on the first day of dosing and again during the last month of dosing (Week 49).

Results: No spontaneous mortality occurred in this study.

Clinical signs: Drug-related clinical signs noted at all dosages included decreased spontaneous activity and/or crouching posture, intermittent tremors, starting during the 1st month of treatment, with a dose-related incidence, highest at HD, bizarre behaviors (sniffing, chewing, pawing, nose-jamming, circling, jumping at cage walls, jerking head about, aggressive barking when disturbed, stereotypy) in 2, 2 and 6 animals at LD, MD and HD, respectively; labored breathing (in 2, 2, and 4 animals at LD, MD and HD, respectively), scleral infection, eyelid ptosis (all dose groups), prolapsed nictitating membranes, and glassy eyes. Most of these signs were noted in all treated animals and showed little dose dependence. Drug-related findings in the mid- and high-dose groups that were not seen in the low dose group included ataxia and or lethargy (1 MDF, 1 HDM and 1 HDF), loss of righting and toe pinch reflex (in single MD and HD animals)

Body weights: Body weight losses occurred primarily during the latter half of the study (weeks 28 through 39) in LD and HD groups; mean body weight decreases of 7.3% and 9.2% in males vs. control were registered at LD and HD, respectively (see sponsor's table on the next page). Emaciation was noted after the 2nd month of dosing in 2 MD animals (1M, 1 F) and in 4 HD animals (3M, 1F).

Food consumption: The relative mean food consumption (as shown in sponsor's table following the body weight table) was decreased in LD (males) at weeks 4 and 29; and in HD (females) at weeks 0 through 6. During the rest of the study, the food consumption values were comparable to control.

Mean body weights (kg)
 TWELVE-MONTH ORAL TOXICITY STUDY OF HP 873 IN DOGS
 HOECHST-ROUSSEL PHARMACEUTICALS INC. STUDY NO. 0922 - 0220

TEST WEEK	MALES: DAILY DOSE IN MG/KG				FEMALES: DAILY DOSE IN MG/KG			
	0	6	12	24	0	6	12	24
PRE	10.9	14.0	10.7	11.3	11.0	9.2	9.3	9.9
0	10.5	13.7	10.4	10.9	10.8	9.0	9.0	9.5
1	10.4	13.5 *	10.2	10.6	10.7	8.7	8.9	9.1
2	10.6	13.7 *	10.2	10.7	11.0	9.0	9.2	9.3
3	10.4	13.4 *	9.9	10.4	10.9	8.9	8.8	9.0
4	10.7	13.1	10.1	10.4	10.9	9.1	8.8	9.2
5	10.9	13.4	10.1	10.6	11.0	9.1	9.0	9.2
6	10.9	13.3	10.2	10.7	11.1	9.2	9.1	9.1
7	11.1	13.6	10.5	10.9	11.2	9.4	9.4	9.4
8	11.2	13.7	10.7	10.9	11.4	9.5	9.5	9.4
9	11.2	13.7	10.7	11.1	11.3	9.4	9.3	9.4
10	11.5	13.4	10.7	10.9	11.4	9.5	9.4	9.5
11	11.4	13.5	10.6	11.0	11.4	9.7	9.4	9.3
12	11.6	13.8	10.8	11.2	11.6	10.0	9.6	9.7
13	11.4	13.4	10.7	11.0	11.6	9.7	9.4	9.5
14	11.3	13.6	10.7	11.0	11.6	9.6	9.4	9.5
15	10.9	13.2	10.6	10.9	11.4	9.5	9.2	9.7
16	11.2	13.5	10.6	11.0	11.3	9.7	9.5	9.9
17	11.1	13.6	10.8	11.0	11.3	9.5	9.5	9.7
18	11.3	13.6	10.8	11.0	11.6	9.6	9.7	10.1
19	11.2	13.5	10.8	10.7	11.4	9.6	9.6	10.0
20	11.3	13.5	10.9	10.8	11.4	9.7	9.7	10.0
21	11.2	13.7	10.9	10.7	11.4	9.7	9.6	9.9
22	11.5	13.8	11.0	10.9	11.6	9.7	9.7	10.0
23	11.5	13.7	11.0	10.8	11.6	10.0	9.8	10.0
24	11.7	13.8	11.0	10.8	11.6	9.7	9.6	10.0
25	11.5	13.7	11.1	10.8	11.6	9.8	9.7	10.0
26	11.3	13.6	11.1	10.7	11.7	9.7	9.8	9.9
27	11.3	13.4	10.7	10.4	11.6	9.8	9.8	9.8
28	11.6	13.6	10.9	10.2	11.8	9.9	9.9	9.9
29	11.4	13.1	10.7	10.3	11.5	9.6	9.9	9.8
30	11.4	13.1	10.8	10.6	11.5	9.8	10.0	10.1
31	11.3	13.1	10.8	10.2	11.5	9.7	9.6	9.7
32	11.3	13.2	10.9	10.4	11.4	9.6	9.7	9.6
33	11.6	13.1	10.6	10.0	11.6	9.7	9.8	9.8
34	11.5	13.2	10.5	10.4	11.6	9.5	9.3	9.8
35	11.4	13.2	10.3	10.4	11.5	9.6	9.0	9.6
36	11.6	13.5	10.6	10.3	11.6	9.6	8.9	9.3
37	11.5	13.1	10.5	10.0	11.9	9.6	9.3	9.4
38	11.4	13.1	10.6	10.0	11.8	9.5	9.3	9.7
39	11.5	13.0	10.6	9.5	11.8	9.5	9.0	9.4
40	11.7	13.3	10.8	9.7	12.0	9.6	9.1	9.4
41	11.7	13.3	10.8	9.7	12.1	9.8	9.0	9.4
42	11.8	13.1	10.7	9.5	12.3	9.8	9.0	9.4
43	12.1	13.5	10.7	9.7	12.5	9.9	9.1	9.8
44	12.0	13.0	10.9	9.6	12.3	9.8	9.3	9.6
45	12.1	13.4	10.9	9.7	12.4	9.6	9.5	9.4
46	12.1	13.6	11.2	9.8	12.3	9.9	9.6	9.9
47	12.1	13.2	10.9	9.8	12.2	9.8	9.5	9.6
48	12.0	13.4	11.1	10.1	12.4	10.0	9.6	9.5
49	12.2	12.8	10.6	9.9	12.6	10.2	9.5	9.5
50	12.0	12.9	10.7	10.0	12.4	10.1	9.1	9.3
51	12.1	13.2	10.9	9.9	12.5	10.1	9.0	9.6
52	11.9	12.7	10.7	9.9	12.6	10.0	9.3	9.6

N = 4 VALUES OR LESS PER MEAN AS INDICATED IN THE INDIVIDUAL DATA
 * = SIGNIFICANTLY DIFFERENT FROM CONTROL (P<0.05)

Weight Changes (kg)

WEIGHT CHANGES (WEEKS)	MALES: DAILY DOSE IN MG/KG				FEMALES: DAILY DOSE IN MG/KG			
	0	6	12	24	0	6	12	24
0 - 26	0.8	-0.1	0.7	-0.2	0.9	0.7	0.8	0.4
0 - 52	1.4	-1.0	0.3	-1.0	1.8	1.0	0.3	0.1

MEAN FOOD CONSUMPTION VALUES - UPTAKE(G)/BODY WEIGHT(KG)/ANIMAL DAY
 TWELVE-MONTH ORAL TOXICITY STUDY OF HP 873 IN DOGS

TEST WEEK	MALES: DAILY DOSE IN MG/KG				FEMALES: DAILY DOSE IN MG/KG			
	0	4	12	24	0	4	12	24
0	18.6	18.9	22.5 *	21.0	19.0	17.0	20.8	13.8
1	24.3	17.1	24.1	22.5	23.3	18.9	24.8	12.8
2	24.8	18.7	23.5	22.8	25.4	23.9	24.7	28.8
3	24.9	17.1	24.5	21.3	25.0	23.0	24.7	19.0
4	26.3	15.1 *	27.3	21.9	23.1	22.5	23.4	19.9
5	27.1	19.7	28.8	25.9	24.5	22.3	24.9	21.4
6	26.4	19.1	28.2	22.5	25.2	21.7	23.8	19.1
7	25.8	20.3	29.5	27.9	24.7	25.7	24.7	22.8
8	25.7	21.0	30.5	26.3	24.6	24.2	27.6	22.8
9	24.6	19.1	26.9	25.6	23.5	24.0	23.3	23.1
10	24.7	16.4	25.8	22.1	21.2	23.9	22.1	22.5
11	22.1	19.5	28.7	24.9	20.0	25.4	23.1	22.8
12	24.6	20.5	27.6	21.6	22.4	23.2	24.2	27.1
13	21.9	17.6	29.4 *	22.3	22.4	22.9	24.2	27.1
14	20.7	19.8	24.6	27.2	21.7	18.2	23.1	24.5
15	18.3	15.3	22.3	22.4	19.4	16.7	19.6	22.5
16	25.5	19.3	29.0	22.8	23.0	22.3	20.9	28.5
17	28.5	20.7	29.8	26.8	21.4	18.9	23.1	21.3
18	24.9	19.1	26.4	25.5	25.6	21.2	24.9	27.1
19	25.4	19.5	30.7	21.0	21.4	22.8	24.4	24.0
20	24.2	18.8	26.9	22.4	22.6	23.0	25.5	22.4
21	23.7	20.0	28.5	22.4	21.8	22.3	24.2	20.4
22	26.2	21.7	24.8	24.8	24.4	22.8	24.8	24.5
23	24.7	20.1	28.7	23.1	23.2	25.3	25.3	24.4
24	22.2	19.4	27.9	24.8	22.0	20.6	18.4	22.4
25	22.7	19.4	27.5	23.2	23.0	23.6	24.4	21.0
26	22.8	19.2	28.4	23.8	22.6	21.4	24.9	19.9
27	22.7	18.4	24.3	21.8	22.4	22.4	23.2	20.7
28	25.1	18.5	24.8	19.4	24.0	21.7	23.2	22.4
29	23.6	13.9 *	28.7	24.8	29.3	19.4	22.5	23.7
30	24.1	18.4	29.5	28.5	22.4	22.8	24.7	26.3
31	22.1	14.8	27.7	24.8	22.7	19.4	18.5	17.5
32	22.1	21.7	30.2	31.2	23.5	24.6	24.3	21.2
33	22.1	20.2	25.4	27.4	21.1	19.6	22.2	24.4
34	22.5	21.3	24.2	27.0	21.0	20.1	16.5	17.8
35	21.9	19.1	26.4	27.2 *	19.4	20.9	14.8	19.5
36	25.3	23.3	30.2	25.9	24.7	21.2	25.4	14.8
37	25.1	18.4	30.4	19.4	24.7	23.8	28.3	24.4
38	24.9	19.3	31.2	29.0	19.7	20.9	27.7	25.1
39	24.3	17.3	27.7	24.4	23.0	21.8	23.4	21.0
40	24.1	21.5	31.5	28.2	27.0	23.3	29.4	21.3
41	25.6	22.2	29.5	29.1	24.3	27.5	33.4	24.7
42	22.3	18.4	25.5	23.2	24.4	23.8	28.8	24.5
43	27.2	21.8	30.4	30.3	27.0	27.8	34.8	27.2
44	23.8	17.9	31.4	30.1	22.3	21.7	29.2	27.0
45	24.1	23.3	27.4	27.9	24.3	21.3	33.0	22.5
46	22.3	24.1	30.3 *	29.2	19.1	20.9	24.4	23.4
47	21.7	18.2	30.7	29.1	20.5	23.4	24.4	21.4
48	21.0	23.1	29.0 *	31.2 *	23.4	24.7	28.5	24.3
49	21.1	14.3	29.4	25.5	24.7	19.4	23.2	22.0
50	22.9	17.7	31.5 *	29.0	20.1	21.1	23.9	21.1
51	21.1	21.0	29.1	25.1	20.4	20.3	26.4	23.3
52	19.7	17.0	23.8	27.2	21.0	21.9	33.5	24.5

n = 4 VALUES OR LESS PER MEAN AS INDICATED IN THE INDIVIDUAL DATA
 * = SIGNIFICANTLY DIFFERENT FROM CONTROL (P<0.05)

Neurology and auditory examination: Pupillary light responses were slow or non-apparent in all treated males and females; this finding occurred earlier at HD (month 1 as compared to month 3 for LD and MD) and the frequency of occurrence was highest at the HD. The righting reflex was slow or absent in single animals from LD and MD groups, and the placement reflex and the toe-pinch reflex were absent in single females from MD and HD groups. Auditory tests were not remarkable.

Ophthalmoscopy: On indirect ophthalmoscopy no ocular abnormalities were found.

Electrocardiography: Electrocardiographic analysis of ECG tracings by a veterinary cardiologist did not show drug-related changes in the parameters evaluated,

i.e., average heart rate, rhythm, P-QRS wave amplitude and duration, P-R interval, Q-T interval, ST segment and T-wave.

Hematology: Mean erythrocyte count, hemoglobin and hematocrit were decreased dose-dependently in both genders at MD and HD (significantly different only for erythrocyte count in HDM).

MEAN HEMATOLOGY VALUES
TWELVE-MONTH ORAL TOXICITY STUDY OF HP 073 IN DOGS

DETERMINATION (UNITS)	TEST WEEK	MALES: DAILY DOSE 10 MG/KG				FEMALES: DAILY DOSE 10 MG/KG			
		0	4	12	24	0	4	12	24
ERYTHROCYTE COUNT (MILLION/CUBIC MM)	PRE	7.94	7.60	7.46	7.73	8.05	7.82	8.02	6.92
	4	7.55	7.54	7.40	7.39	7.54	7.37	7.27	6.38
	14	7.33	7.08	7.40	6.96	7.21	7.33	7.40	6.42
	26	7.23	6.93	7.43	6.89	7.43	7.28	4.93	6.58
	52	7.58	7.25	7.13	6.40*	7.30	7.38	6.35	6.60
HEMATOCRIT (PER CENT)	PRE	54	53	50	53	53	54	53	49
	4	51	53	49	51	52	51	50	45
	14	50	49	49	48	51	53	52	45
	26	49	47	49	45	50	49	47	43
	52	49	48	46	42	48	49	42	43
HEMOGLOBIN (GRAMS/DECI LITER)	PRE	17.0	16.6	15.9	16.0	17.6	16.9	17.3	15.2
	4	16.1	16.3	15.3	15.8	16.3	15.7	15.6	14.2
	14	17.2	16.7	17.1	16.2	17.4	17.7	17.8	15.3
	26	16.7	16.4	17.0	15.8	17.2	16.8	16.6	15.7
	52	17.3	16.7	16.2	14.7	17.1	17.0	14.7	15.6
MEAN CORPUSCULAR VOLUME (CUBIC MICRONS)	PRE	68	70	66	69	69	69	69	71
	4	68	70	66	69	70	70	69	71
	14	68	70	67	69	71	71	70	71
	26	67	68	67	67	66	67	68	69
	52	64	66	64	63	66	65	65	66
MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (PER CENT)	PRE	32	32	32	32	32	32	32	31
	4	31	31	32	31	31	31	31	32
	14	35	34	35	34	34	34	34	34
	26	34	35	35	35	35	35	35	35
	52	36	35	36	35	36	36	36	36
MEAN CORPUSCULAR HEMOGLOBIN (PICO GRAMS)	PRE	22	22	21	22	22	22	22	22
	4	22	22	21	21	22	22	22	22
	14	23	24	23	23	24	24	24	24
	26	23	24	23	23	23	23	24	24
	52	23	23	23	23	23	23	23	24

n = 4 VALUES OR LESS PER MEAN AS INDICATED IN THE INDIVIDUAL DATA
* = SIGNIFICANTLY DIFFERENT FROM CONTROL (P<0.05)

Clinical chemistry and Urinalysis:

Drug-related differences in clinical chemistry (as shown in the sponsor's table below) included cholesterol and triglyceride levels in females, that were lower than control values throughout the study (weeks 4, 14, 26, and 52), statistically significant at MD and HD. Other differences in serum chemistry included sporadically higher mean alanine aminotransferase values in all groups (statistically significant at MD (M and F) at week 26 and at HD (M) at week 52) and lower alkaline phosphatase values in LD and HD males at week 14. These differences were variable, not dose-related and did not have histological correlates. Iron values were moderately increased in LDM and HDF at week 52; no dose-related trends were seen.

There were no drug-related findings in the urinalysis data.

MEAN SERUM CHEMISTRY VALUES

DETERMINATION (UNITS)	TEST WEEK	MALES: DAILY DOSE IN MG/KG				FEMALES: DAILY DOSE IN MG/KG			
		0	6	12	24	0	6	12	24
CHOLESTEROL (MG/DECILITER)	PRE	119	119	121	125	154	197	115	172
	4	124	143	131	129	224	193	150	174
	14	125	141	121	121	245	177	124	150
	26	115	129	117	116	240	201	144	141
	52	136	126	115	120	243	191	123*	144*
TRIGLYCERIDES (MG/DECILITER)	PRE	23	35	42	28	40	38	32	36
	4	41	72	41	39	67	54	59	59
	14	49	47	58	42	74	56	48*	47*
	26	45	55	57	38	91	62	62	65
	52	52	52	49	37	140	59	40*	39*
ALANINE AMINOTRANSFERASE (IU/LITER)	PRE	60	40	57	64	50	41	51	35*
	4	44	72	116	131	29	45	57	27
	14	64	36	40	90	42	72	55	30
	26	65	121	68	170	40	62	72*	39
	52	64	69	485	234	41	258	791	72
ASPARTATE AMINOTRANSFERASE (IU/LITER)	PRE	24	26	33	43	29	26	31	19
	4	28	39	42	38	31	31	40	20
	14	30	25	39	30	32	27	36	18
	26	26	32	41*	29	24	23	24	15
	52	24	25	39	46	26	23	36	25
ALKALINE PHOSPHATASE (IU/LITER)	PRE	35	21	40	32	32	36	28	29
	4	39	18*	38	28	37	34	23	28
	14	57	19*	45	30*	52	40	27	47
	26	45	20	33	33	35	36	37	42
	52	35	14	42	35	33	35	59	41
IRON (MICROGRAMS/DECILITER)	PRE	164	219	103	168	258	265	100	284
	4	352	242	261	309	268	448	401	349
	14	213	292	249	231	220	266	204	248
	26	297	267	299	297	357	333	455	359
	52	460	737	452	488	402	394	226	729

n = 4 VALUES OR LESS PER MEAN AS INDICATED IN THE INDIVIDUAL DATA
 * = SIGNIFICANTLY DIFFERENT FROM CONTROL (P<0.05)

Organ weights: No statistically significant differences in mean organ weights were registered. Higher mean absolute and relative liver weights were seen in HD males.

MEAN ORGAN WEIGHT DATA
 Week 53 Necropsy

DETERMINATION (UNITS)	MALES: DAILY DOSE IN MG/KG				FEMALES: DAILY DOSE IN MG/KG			
	0	6	12	24	0	6	12	24
ORGAN WEIGHTS (GMS)								
LIVER	341.625	426.025	395.175	383.200	385.900	362.350	382.185	320.325
KIDNEYS	68.400	74.000	68.625	65.150	55.600	48.050	48.475	49.650
HEART	103.050	102.600	95.475	84.025	84.825	71.925	69.400	72.950
BRAIN	76.225	78.325	78.700	78.900	72.200	72.000	68.300	72.025
COVARS	10.939	13.806	14.325	12.760	1.562	1.106	1.086	1.157
PROS/UTS	12.661	14.725	12.650	10.154	11.086	9.619	9.132	8.663
ADRENALS	1.373	1.322	1.230	1.277	1.582	1.240	1.492	1.308
THYROID	0.955	0.939	0.881	1.048	0.729	0.728	0.850	0.779
PITUITARY	0.060	0.059	0.061	0.055	0.058	0.068	0.063	0.063
TERMINAL BODY WEIGHT (GMS)								
	12129	13212	10978	10372	12595	10367	9713	9612
ORGAN/BOUY WT. RATIOS (GMS)								
LIVER	2.855	3.203	3.091	3.761	3.092	3.584	3.583	3.261
KIDNEYS	0.548	0.563	0.638	0.624	0.445	0.475	0.522	0.526
HEART	0.866	0.785	0.790	0.810	0.681	0.701	0.719	0.763
BRAIN	0.642	0.574	0.749	0.800	0.594	0.713	0.729	0.766
COVARS	0.093	0.122	0.135	0.126	0.013	0.012	0.012	0.012
PROS/UTS	0.106	0.112	0.117	0.096	0.095	0.103	0.096	0.076
ADRENALS	0.011	0.010	0.012	0.013	0.013	0.012	0.016	0.014
THYROID	0.008	0.007	0.008	0.010	0.006	0.007	0.009	0.008
PITUITARY	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

n = 4 VALUES OR LESS PER MEAN AS INDICATED IN THE INDIVIDUAL DATA
 * = SIGNIFICANT DIFFERENCE FROM CONTROL (P<0.05)

Pathomorphological examination: All specified organs and tissues from the control and HD groups were stained with H&E and examined microscopically. These tissues included:

the liver, spleen, kidneys, aorta, lung, trachea, esophagus, heart, adrenal glands, thyroid and parathyroid glands, thymus gland, pancreas, lacrimal gland, salivary gland, mesenteric lymph node, peripheral nerve, pituitary gland, stomach, duodenum, jejunum, ileum, colon, testes or ovaries, epididymides and prostate or uterus, urinary bladder, gall bladder, skeletal muscle (diaphragm), tongue, brain (cerebrum and cerebellum), spinal cord, skin, mammary gland, eyes (with optic nerve), bone (rib), and tissues with gross lesions.

The microscopic examination of H&E stained tissues of LD and MD groups was limited to the liver, heart, adrenal glands, bone marrow, and tissues with gross lesions. In addition, liver, kidney and spleen sections stained with the periodic acid-Schiff (PAS) technique, and liver sections stained with oil-red-O (ORO) were processed and examined from all animals in all groups. Electron microscopic evaluation was performed on liver samples from the control and HD groups.

Gross pathology: Compound-related gross alterations were not observed.

Histopathology: "Minimal to mild" enlargement of hepatocytes was found in all HD males, but the special staining did not demonstrate an increase in neutral fat or glycogen. These findings were "consistent with hepatocellular hypertrophy induced in HD males presumably resulting from a proliferation of cellular organelles, i.e., the endoplasmic reticulum". This was confirmed by the ultrastructural examination of liver which indicated moderate to marked proliferation of the smooth endoplasmic reticulum in HD males. The amount of the smooth endoplasmic reticulum in hepatocytes of HD males was increased 2-fold vs. controls, and "in many regions it appeared to supplant other cellular organelles". No such changes were observed in the HD females. The hepatocellular hypertrophy and smooth endoplasmic reticular proliferation may be an indication of metabolic enzyme induction in the HD males. No neoplastic lesions were found.

Toxicokinetics:

PLASMA CONCENTRATIONS OF HP 873 AND ITS METABOLITES				
Dosage (mg/kg/day)	Plasma Concentration (ng/ml) ^a			
	P89 9430	P89 9124	P88 8991	HP 873
Males				
1st Dose				
0	BLQ ^b	BLQ	BLQ	BLQ
6	20.6 ± 21.1	BLQ	BLQ	0.5 ± 1.0
12	48.9 ± 85.2	BLQ	BLQ	0.9 ± 1.9
24	4.3 ± 6.8	BLQ	BLQ	BLQ
Week 49				
0	BLQ	BLQ	BLQ	BLQ
6	32.8 ± 73.8	BLQ	BLQ	3.0 ± 7.6
12	12.1 ± 24.3	BLQ	BLQ	4.3 ± 8.6
24	168.3 ± 278.4	BLQ	BLQ	29.7 ± 57.1
Females				
1st Dose				
0	BLQ	BLQ	BLQ	BLQ
6	11.7 ± 12.8	BLQ	BLQ	BLQ
12	1.4 ± 2.6	BLQ	BLQ	BLQ
24	13.5 ± 16.6	BLQ	BLQ	BLQ
Week 49				
0	BLQ	BLQ	BLQ	BLQ
6	10.3 ± 18.7	BLQ	BLQ	BLQ
12	250.5 ± 458.8	BLQ	BLQ	29.7 ± 48.9
24	310.7 ± 321.2	3.5 ^c	3.2 ^d	119.5 ± 180.2

^a Blood was collected from animals (n = 4) at 4 hours after the 1st dose and 49 weeks repeated dosing.
^b BLQ = below limit of quantification (BLQ for HP 873, P89 9124 and P88 8991 was 2.0 ng/ml; P89 9430 was 4.0 ng/ml)
^c Average of the two values reported (4.2 and 3.3 ng/ml), two other values were BLQ
^d One value reported (3.2 ng/ml), three other values were BLQ

The TK analysis showed that the mean plasma concentrations of iloperidone at week 49 were 5.0, 4.3, and 29.7 ng/ml for males, and BLQ, 29.7 ng/ml, and 119.8 ng/ml for females, at 6, 12 and 24 mg/kg/d, respectively. Plasma concentrations in the females increased over dose-proportionally. The principal metabolite was P89 9430, which was detected at higher levels than iloperidone, while P89 9124 and P88 8991 were generally below the limits of quantification.

Conclusion: The MTD in this study is 6 mg/kg/d in view of the severe clinical signs (ataxia, emaciation) at the next higher dose of 12 mg/kg/d. NOAEL was not reached as the lowest dose induced decreased body weight and neurological clinical signs.

Summary of individual study findings: A summary of the findings of this study is presented the following sponsor's table.

Repeat-dose toxicity (12-month oral study in dogs) (pivotal study)

Daily Dose (mg/kg)	0 (Control)		6		12		24	
No. of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Toxicokinetics: Mean plasma concentrations at Week 49 (ng/mL)	Ilo: BLQ P899430: BLQ P899124: BLQ P888991: BLQ	Ilo: BLQ P899430: BLQ P899124: BLQ P888991: BLQ	Ilo: 5.0 P899430: 52.6 P899124: BLQ P888991: BLQ	Ilo: BLQ P899430: 10.3 P899124: BLQ P888991: BLQ	Ilo: 4.3 P899430: 12.1 P899124: BLQ P888991: BLQ	Ilo: 29.7 P899430: 250.8 P899124: BLQ P888991: BLQ	Ilo: 29.7 P899430: 156.3 P899124: BLQ P888991: BLQ	Ilo: 119.8 P899430: 310.7 P899124: 3.8 P888991: 3.2
Noteworthy Findings:	--	--	--	--	--	--	--	--
Died or Sacrificed Moribund (kg)	0	0	0	0	0	0	0	0
Mean Body Weight Change Weeks 0-52 (kg)	1.4	1.8	-1.0	1.0	0.3	0.3	-1.0	0.1
Mean Food Consumption Week 52 (g/kg/animal day)	19.7	21.0	17.0	21.9	23.8	33.5	27.2	26.5
Clinical Observations	--	--	Noted in every dosage group (most noted in every animal): Decreased spontaneous activity and/or crouching posture, tremors, bizarre behavior, labored breathing, scleral injection, ptosis of eyelids, prolapsed nictitating membranes, glassy eyes, changes in appearance of feces.					
Ophthalmoscopy	No ocular abnormalities associated with administration of Ilo seen.							
Electrocardiography	No ECG abnormalities of toxicological significance observed.							
Hematology (↓ or ↑ relative to control at Week 52)	--	--	--	None	↑RBC	↑RBC, ↓Hct	↑RBC, ↓Hct, ↓Hb	↑RBC, ↓Hct
Serum Chemistry (↓ or ↑ relative to control at Week 52)	--	--	None	None	↓cholesterol, ↑triglycerides	None	None	↓cholesterol, ↑triglycerides
Urinalysis (↓ or ↑ relative to control at Week 52)	--	--	None	None	None	None	None	None
Mean Organ/ Terminal Body Weight at Week-53 Necropsy (g)	12129	12595	13212	10307	10878	9713	10372	9613
Gross Pathology at Week-53 Necropsy	Definitive compound-related alterations not observed.							
Histopathology at Week-53 Necropsy	--	--	None	None	None	None	Liver: Minimal to mild enlargement of hepatocytes (all animals)	None
Additional Examinations:								
Neurologic Examination:								
	--	--	Slow papillary light response					
	--	--	Slow or absent righting reflex					
	--	--	--	--	--	Placement reflex absent (1 animal)	--	Toe pinch reflex absent (1 animal)
Auditory Examination	No abnormalities of toxicological significance observed.							
Electron Microscopy at Necropsy	--	--	--	--	--	--	Moderate to marked proliferation of smooth endoplasmic reticulum	--
Postdose Evaluation No. Evaluated:	ND	ND	ND	ND	ND	ND	ND	ND

-- No findings of note.

* Significantly different from control ($P < 0.05$ by Dunnett's 2-sided multiple comparisons test).

a No statistically significant differences from control in mean individual organ weights.

ND = not done; RBC = red blood cell count; Hct = hematocrit; AST = aspartate aminotransferase.

Repeat-dose studies conducted with iloperidone metabolite P95

Summary: The repeat oral dose toxicity of iloperidone metabolite P95 was evaluated in 4 studies: a 4-week study in mice (Study 0170087), a 13-week study with 4-week recovery (Study 007008) in Wistar rats, a 13-week dose range-finding study in Sprague-Dawley (CD) rats, and a 26-week study with 4-week recovery study in Wistar rats (Study 017013). The design and noteworthy findings of these studies are summarized in the sponsor's table below. Among these studies, the pivotal one is the 26-week study in rats.

Summary of P95 repeat-dose toxicity studies

Study No.	Species/ Strain	Route	Gender and No./ group	Duration of treatment	Range of doses (mg/kg)	MTD or NOEL	Noteworthy findings
0170087	Mouse B6.129- Twp55 ^{tm1} N3, Wild Type	p.o.	M, F 50 (M, F), 250 (M), 250→125 (F), 750→500 (M), 750 (F), 1500 (M, F) 10/group	4 weeks	0 to 1500	MTD: M: 50 F: 125 mg/kg/d	Mortality: All animals at 1500, all F at 750, all M at 750→500, 6 M at 250, 5 F at 250. Drug-related clinical signs: Sedation, cold to touch, dehydration, ↓ locomotor activity, loss of/impaired righting reflex, recumbency, muscle tremors, hunched posture, labored/ shallow respiration, absent/reduced feces, ptosis, dry perineal staining, relaxed vaginal opening/scrotum, ↓ body wt surviving M at 250; ↑ wt F at 125. Pathology: Macroscopic discoloration of kidney, liver, pancreas; dilatation of vagina, ↓ thymus size; microscopic changes in kidney, bladder, testes, female reproductive tract. Laboratory findings (F only at 750): ↑ BUN, creatinine, phosphorus, ALT, and/or AST; ↓ albumin. TK: All mice exposed to P95; exposure tended to be dose- proportional, no sex-related differences; data incomplete due to mortalities.
007008	Rat/ IGS Wistar Han	p.o.	M, F 10/group	13 weeks with 4- week recovery	0 to 500	MTD <500 mg/kg	Mortality: 1 F at 500. Drug-related clinical signs at all doses: Ptosis, reddened/relaxed scrotums/vaginal opening, slight ↓ locomotor activity, relaxed anal opening, ↓ body weight (M); increased body weight (F). Pathology: Liver weight decreased (M) or increased (F), increased adrenal weight (M), increased thyroid weight (M, F); induction of mammary gland secretion (M, F) (reversible); no hepatocellular proliferation. Laboratory findings: ↑ prolactin levels (M), reversible. TK: P95 rapidly absorbed with T _{max} 1-2 hours; all animals were exposed with exposure over- proportional with dose; no sex- related differences or consistent differences between Weeks 1 and 12.

Summary of P95 repeat-dose toxicity studies (continued)

Study No.	Species/ Strain	Route	Gender and No./ group	Duration of treatment	Range of doses (mg/kg)	MTD or NOEL	Noteworthy findings
TAJ0006	Rat/CD	p.o.	M, F 40	13 weeks	0 to 500 mg/kg	MTD 200 mg/kg	<p>Mortality: 2 main, 3 TK M; 3 main, 5 TK F at 500.</p> <p>Clinical signs: Ptosis, ↓ locomotor activity, reddened scrotums. ↑ body wt F.</p> <p>Decedents: ↑ blood chemistry parameters related to biliary obstruction (biliary hyperplasia, bile duct degenerative changes); ↑ urine volume, ↓ specific gravity, ↑ phosphorus related to kidney degenerative changes; birefringent crystals seen in bile ducts and kidney. Systemic (metastatic) mineralization in cardiovascular system, other tissues. Terminal sacrifice and decedents: Adrenal cortical changes, alteration of mammary tissue (males) and hyperplasia (females), with secretion, prolonged CIs, vaginal mucification.</p> <p>TK: P95 measurable in plasma up to 24 hours post dose in most animals. T_{max} of P95 = 0.5 to 2 hours post dose. Thereafter, plasma concentrations declined with t_{1/2} of approximately 3 hours. Systemic exposure appeared to increase in a greater than dose-proportional manner. There was appreciable (1.30 to 2.84-fold) accumulation of plasma P95 over the dosing period. Kinetics were non-linear with time. Systemic exposure in females was 2.0- to 3.1-fold greater than in males.</p>
017013	Rat/ IGS Wistar Han	p.o.	M, F 40	26 weeks with 4- week recovery	0 to 500 mg/kg	MTD <500 mg/kg (M)	<p>Mortality: 1 M at 500.</p> <p>Drug-related clinical signs at both doses: Ptosis, muscle flaccidity, ↓ locomotor activity, relaxed anal and vaginal opening/relaxed scrotum; ↓ body weight M, ↑ body wt/↑ food consumption F.</p> <p>Pathology: Increased weight in adrenal (M, F), pituitary (M), liver (F), heart (F), ↓ wt uterus; microscopic findings in adrenal, pituitary, mammary gland, ovary; ↑ cell proliferation in mammary (M, F), pancreas (M, F), pituitary (M) with BrdU.</p> <p>TK: P95 exposure ↑ over-proportionally with dose; no-sex-related differences; exposure ↑ over time.</p>

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen;

The predominant clinical signs associated with P95 were similar to those seen with iloperidone, and included ptosis, relaxed scrotum, relaxed vaginal opening, and decreased spontaneous motor activity. An MTD of P95 in the 26-week study in Wistar rats was not reached in females; in males it was less than 500 mg/kg/d (due to body weight loss and mortality at 500 mg/kg/d). A NOAEL was not reached, as morphological changes in both genders were induced at the lowest P95 dose tested. The target tissue effects were in the mammary gland, uterus/vagina, adrenals, and thyroid; some of these effects were likely to be pharmacologically mediated. Drug-related histopathology changes, demonstrable by routine histology and/or immunohistochemical method (BrdU labeling), were induced in endocrine glands (pituitary and adrenals in males, thyroid in females; and pancreas in both genders), mammary gland (both genders) and reproductive organs (ovary, uterus, testes, prostate). Most of these histopathology deviations (with the exception of the pancreatic, adrenal, and testicular pathology) were induced dose-dependently at both tested dose levels of P95, corresponding at LD to plasma exposure (AUC 0-24) approximately 2 to 3x the human exposure at MRHD of 12 mg twice daily. In the 26-week P95 study, histological evidence of hyperplasia and/or proliferation as assessed by BrdU labeling was found in 5 tissues: mammary gland, pituitary, endocrine pancreas, thyroid, and ovary. As BrdU labeling was not performed in any of iloperidone repeat-dose toxicity studies, no comparison of these findings can be made to iloperidone. The morphologic pathology findings for iloperidone (by routine histology) were limited to mammary gland epithelial vacuolization in both genders at all dose levels with dose-related incidence and severity, dose-related reduced size of testicles and testicular degeneration/atrophy (in single animals) and higher incidence of prostate inflammation at HD.

An additional 13-week study was performed in Sprague-Dawley (CD) rats to assess strain differences in preparation for a carcinogenicity study, as all of the general toxicity studies in rats with the parent iloperidone were conducted with Sprague-Dawley rats. In this study, at all doses, effects on potential pharmacological targets (mammary gland, reproductive organs, adrenal) were similar to those seen with Wistar rats. However, at the top dose of 500 mg/kg/d, mortalities and toxic effects on the biliary system (biliary obstruction) and the kidney (tubular degeneration) with secondary systemic mineralization due to electrolyte imbalances were seen. The overall pathogenesis was compatible with incompetent metabolism and excretion of P95 by the hepatobiliary system with development of local toxicity, increased renal excretion with toxic effects on tubules and other renal components, and systemic deposition of mineral secondary to electrolyte imbalances. Sprague-Dawley rats appeared to be more susceptible to these effects than Wistar rats, as the exposures achieved in Sprague-Dawley rats at comparable doses were lower, yet associated with a significant increase in toxicity.

The pivotal 26-week study in Wistar rats was previously reviewed by this reviewer under IND 36827, SN 207 of 8/23/2002, review dated 07/14/2007. Relevant parts of the review are reproduced on the next pages.

Study title: Iloperidone metabolite P95 12113 26-week oral (gavage) toxicity study in rats with a 4-week recovery period

Key study findings: Administration of iloperidone metabolite P95 for 6 months to Wistar rats at oral (gavage) doses of 50 and 500 mg/kg/day (yielding P95 plasma exposures of about 2 to 3x and 150 to 400x, respectively, the human P95AUC at iloperidone MRHD of 24 mg/d), induced dose-dependent clinical signs at both dose levels throughout the entire treatment period, indicative of CNS depression and relaxation (ptosis, decreased motor activity, relaxation of the scrotum, anus, vaginal opening) that are attributable to exaggerated pharmacological effect. At HD, there was one possibly treatment-related death (1 HDM) on day 123 (the cause of death could not be determined). Body weight and weight gain decrease (not due to reduced food intake) was induced at HD in males only (by 11% and 26%, respectively vs. control by the end of treatment); the decrease was not compensated during the recovery period. There were no drug-related abnormal findings in hematology, clinical chemistry (including prolactin plasma levels), or urine analysis. Drug-related histopathology changes, demonstrable by routine histology and/or immunohistochemical method (BrdU labeling) were induced in endocrine glands (pituitary and adrenals in males, thyroid in females, and pancreas in both genders), mammary gland (both genders) and reproductive organs (ovary, uterus, testes, prostate). In females, dose-dependent cycle prolongation occurred at both LD and HD, consistent with the finding of vaginal epithelium mucification and decreased uterine weight in the treated groups. In the ovaries, a dose-related interstitial cell hyperplasia was observed at LD and HD that was likely associated with the reduced estrus cycle activity. After the recovery period the prolongation of estrus cycle did not fully normalize, and increased corpora lutea were observed in the ovaries of HDF, concordant with increased ovarian weight. In the thyroid, diffuse follicular hyperplasia was induced in both LDF and HDF; this finding did not normalize after the recovery period. In males, pituitary changes were induced at both tested doses, while adrenal, testicular and secondary sex organ pathology was seen at HD only. Pituitary histopathology (decreased eosinophilia of the acidophil cells of the distal part of the pituitary) was noted in both LDM and HDM with a dose-related incidence. These morphological findings were supported by an increase in the cell proliferation index in male pituitary at all time points, consistent with the increased pituitary weight. Adrenal morphological changes as well as testicular and secondary sex organ pathology occurred in HD males only. In the adrenals, an increased incidence of diffuse cortical hypertrophy and reduced cortical cytoplasmic vacuolation was seen in HDM; this finding was concordant with the increased absolute and relative adrenal weight and was reversible after the recovery period. Atrophy of testicular seminiferous tubule epithelium (in 2 animals) and an increased incidence of mixed cell inflammation of prostate gland with associated degenerative changes were found at HD. Prostate changes were reversible after the recovery period. In both genders, increased cellular proliferation in the mammary gland (alveolar hyperplasia, increased secretion and dilatation of mammary ducts) occurred with dose-related severity at LD and HD during treatment and even after recovery period. Statistically significant, treatment-related increase in cell proliferation (increased proportion of cells in S phase of the cell cycle, as assessed by BrdU labeling) was found in pituitary (LDM and HDM), mammary gland (duct and alveoli) in both genders (LDM, HDM, HDF), and the endocrine pancreas in both genders (HDM, HDF). Most of these histopathology deviations (with the exception of the adrenal, testicular and secondary sex organ pathology in males) were induced in a dose-dependent manner at both tested dose levels, corresponding at LD to plasma exposure (AUC 0-24) approximately 2 to 3x the human exposure at MRHD of 12 mg twice daily.

An NOAEL was not reached in either male or female rats in view of the presence of pathomorphological changes in multiple organs/tissues at the lowest tested dose of 50 mg/kg/day. The MTD was <500 mg/kg/day for males in view of mortality and body weight decrease at 500 mg/kg/day; for females, an MTD was not reached.

Study no: 017013

Conducting laboratory and location: Novartis Pharmaceuticals Corporation, East Hanover, NJ

Date of study initiation: Feb 16, 2001

GLP compliance: Yes; **QA report:** yes

Drug, lot #, radiolabel, and % purity: Iloperidone metabolite P95 12113, batch #011983, purity 99.8%

Formulation/vehicle: 0.5% carboxymethylcellulose

Methods (unique aspects): Immunohistochemical staining for assessment of cell proliferation in selected tissues (endocrine pancreas, liver, pituitary, and mammary gland)

Dosing:

Species/strain: IGS Wistar Hannover rats

#/sex/group or time point (main study): 40

Satellite groups used for toxicokinetics or recovery: 10 sex/group

Age: 9 weeks

Weight: Males: 247-304 g; Females: 171-201 g.

Doses in administered units: 0, 50 and 500 mg/kg/day

Route, form, volume, and infusion rate: oral gavage, suspension in 0.5% carboxymethylcellulose, dosing volume 5 ml/kg

The dose selection was based upon the results of a 13-week oral (gavage) toxicity study in rats with a 4-week recovery period (study 007008) that employed P95 12113 doses of 50, 200 and 500 mg/kg/day. In the 13-week study, P95 12113 produced "microscopic alterations in the mammary gland consistent with induction of secretion in both sexes" at doses ≥ 200 mg/kg/day, as well as adrenal cortical hypertrophy and increase in plasma prolactin in males at 500 mg/kg/day (prolactin increase and microscopic changes were reversible).

Observations and times:

- Mortality and clinical signs (twice daily)
- Body weight and food consumption (weekly during dosing wks 1-14 and recovery; every 2 wks during dosing weeks 16-26)
- Ophthalmology (pre-test: all groups; Wk 12 and 24: HD and control animals)
- Clinical chemistry – routine hematology and urine analysis: wks 14 and 25 (all groups); end of recovery period (control, HD)
- Hormone analysis – plasma prolactin on wks 14, 25 and end of recovery (all groups)
- Pathology. The tissues listed in sponsor's table below were collected from all animals at the 26-wk necropsy. The control and HD group, as well as all unscheduled deaths and macroscopic lesions were processed to hematoxylin/eosin-stained tissue sections. Organ weights from the complete listing were recorded at the 26-wk and recovery necropsies; liver, pituitary and brain weight were recorded during interim necropsies after 1 and 13 weeks of dosing.
- Microscopic examination (as described by the sponsor):

Microscopic examinations were performed on a standard set of tissues from the control and high dose nonrecovery groups at the 26-week necropsy and for all unscheduled deaths/sacrifices. All macroscopic lesions, liver, mammary gland, testes, prostate, epididymides, ovaries, cervix, vagina, uterus, adrenal (males only), pituitary (males only), thyroid (females only), and brain (females only) were processed from the low and recovery 26-week necropsy dose groups. All assessments were peer-reviewed.

- Immunohistochemical staining and assessment of cell proliferation in selected tissues [endocrine pancreas, liver, pituitary (pars distalis), and mammary gland (ducts and secretory units)]: Seven days prior to the scheduled necropsy, osmotic pumps containing 20 mg/ml 5-bromo-2'-deoxyuridine (BrdU, a nucleotide analog that is incorporated in the DNA of cells during DNA synthesis) were surgically implanted subcutaneously to 10 animals/sex/group. Cell proliferation was assessed in selected tissues (upon immunohistochemical staining) by "determining the fraction of cells that passed through the S-phase of cell cycle from the time of initiation of systemic delivery of BrdU". BrdU immunoreactivity was demonstrated in formalin-fixed paraffin-embedded tissue sections, using a monoclonal antibody to BrdU (systemic delivery of BrdU was confirmed for each animal by the presence of immunoreactivity in the villous epithelium of duodenum, a tissue with high proliferation rate). Cell proliferation was estimated using an image analysis system; the acceptance criterion for morphometric evaluation was set at 1000 nuclei (2000 for the liver) counted for each tissue from 2 step sections of any given block (all counts were performed by the same technician to avoid inter-operator variations). Treatment effects were assessed by statistical evaluation of the ratio of stained to total nuclei.

- TK: Blood samples for TK analysis were taken at 1, 2, 4, 6 and 24 h post-dose from 2 animals per sex/group at each time point. Urine samples for exploratory investigation of P95 12113 and metabolites were collected from all groups on day 180 (as explained by the sponsor, these exploratory investigations were intended to "aid in determining the possible cause of measurable plasma levels of P95 12113 in control animals").

Results:

- Mortality: A drug-related mortality occurred in 1/20 HDM; the animal displayed clinical signs consistent with P95 exposure daily between days 1-122 and was found dead on dosing day 123. The case of death could not be determined from the histological examination. The sponsor stated that "a relationship to treatment could not be discounted". Two other cases of mortality (1 HDF and 1 control male) were due to dosing accidents, as confirmed by necropsy and histopathology.

- Clinical signs: Ptosis, decreased activity, muscle relaxation were observed in both genders at and above 50 mg/kg/day, throughout the dosing period (daily, at about 2 h. post-dose). The signs abated upon cessation of drug administration and all animals were asymptomatic by day 8 of the recovery period.

- Ophthalmoscopy: No drug-related findings

- Body weight (see sponsor's tables on next page): Drug-related, sex-specific body weight changes occurred at ≥ 50 mg/kg/day in F (body weight increase) and at 500 mg/kg/day in M (body weight decrease). In males, statistically significant b. wt. decreases were observed at HD (500 mg/kg/day) from day 15 through day 176; the mean b.wt. by the end of the dosing period was about 11% lower than control. Similarly, statistically significant decreases in absolute body wt gain were observed in HDM at each assessment interval between from day 8 through 176; the mean absolute b.wt. gain by the end of dosing period was about 26% lower than control. The decrease was not compensated during the recovery period: by the end of the 4-week recovery period, the mean body wt. and the mean absolute body wt gain in HDM were decreased by about 12% and 22%, respectively, as compared to control. In females, a statistically significant increase in b. wt. and absolute b.wt. gain were observed at 50 mg/kg/day between days 8 and 176; by the end of the dosing period, the mean female b.wt. and b.wt gain at 50 mg/kg/day were about 7% and 18%, respectively, above the concurrent control levels. The weight increase in the females was reversible after discontinuation of treatment (no

differences from control were seen in HD females by the end of the 4-week recovery period, as shown in sponsor's tables below).

Mean body weight (treatment day 85 through day 176 and recovery period)

Table 1.2 Novartis Pharmaceuticals Corporation Toxicology Department Rat/Wistar-Kyoto MND		Mean Animal Body Weights in (g) Study number: 617613 Study start dates: 26-Feb-01												Printed: 13 Page: 2
Group(s)		85	92	106	120	134	148	162	176	4*	11	18	25	
1	(N)	40	40	30	30	30	30	30	30	30	30	30	30	
	Means	434.7	440.4	445.8	452.7	462.3	464.5	470.3	472.3	479.9	484.4	482.2	482.6	
	Stdevs	39.28	39.38	41.39	42.94	45.74	47.82	47.88	50.54	56.77	57.48	58.23	57.73	
2	(N)	30	30	20	20	20	20	20	20	20	20	20	20	
	Means	432.2	437.6	436.3	445.9	443.3	454.8	463.4	465.3	465.3	465.3	465.3	465.3	
	Stdevs	41.97	42.31	42.83	42.44	44.34	46.39	49.47	50.06	50.06	50.06	50.06	50.06	
3	(N)	40	40	30	30	30	30	30	30	30	30	30	30	
	Means	387.2*	388.6*	397.3*	403.2*	411.0*	418.2*	419.3*	421.8*	429.6*	444.8*	455.6*	463.0*	
	Stdevs	34.33	34.84	37.05	36.88	35.71	35.60	37.82	38.49	37.87	44.8*	48.85*	44.0*	
1	(N)	40	40	30	30	30	30	30	30	30	30	30	30	
	Means	238.3	241.3	240.3	244.6	249.0	248.4	250.5	251.5	252.3	253.1	256.9	258.7	
	Stdevs	18.31	17.67	18.48	19.16	20.38	18.79	20.31	20.92	21.18	21.05	21.35	20.14	
2	(N)	30	30	20	20	20	20	20	20	20	20	20	20	
	Means	248.4	251.8	255.5	254.4	260.6	262.3	265.8*	268.4*	268.4*	268.4*	268.4*	268.4*	
	Stdevs	24.66	27.48	30.48	28.41	32.13	34.97	34.82	33.82	33.82	33.82	33.82	33.82	
3	(N)	30	30	20	20	20	20	20	20	20	20	20	20	
	Means	235.4	244.3	244.0	246.4	251.6	251.6	256.7	258.9	271.1	283.3	271.6	289.3	
	Stdevs	28.68	31.09	22.23	23.81	33.38	33.13	33.84	33.25	34.81	28.3	37.36	28.58	

Notes: 1 = treatment phase; * = recovery phase
 () = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
 () = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

Mean absolute body weight gain (treatment day 85 through day 176 and recovery period)

Table 1.3 Novartis Pharmaceuticals Corporation Toxicology Department Rat/Wistar-Kyoto MND		Mean Animal Absolute Weight Gains in (g) Study number: 617613 Absolute weight gains referenced to treatment phase (Day 1) Study start dates: 26-Feb-01												Printed: 1 Page: 2
Group(s)		92	106	120	134	148	162	176	4*	11	18	25		
1	(N)	40	30	30	30	30	30	30	30	30	30	30		
	Means	159.9	171.5	178.4	188.0	192.0	196.0	201.7	208.5	234.9	239.9	242.1		
	Stdevs	38.11	29.81	31.33	34.05	36.27	35.74	38.60	43.87	43.82	46.77	45.91		
2	(N)	30	20	20	20	20	20	20	20	20	20	20		
	Means	193.2	165.6	175.2	183.2	188.1	193.7	195.1	195.1	195.1	195.1	195.1		
	Stdevs	31.76	32.72	32.63	34.82	34.71	39.89	40.49	40.49	40.49	40.49	40.49		
3	(N)	40	30	30	30	30	30	30	30	30	30	30		
	Means	119.1*	124.7*	130.1*	137.6*	141.8*	145.9*	148.4*	156.4*	172.6*	183.3*	189.8*		
	Stdevs	18.43	29.78	29.83	29.33	28.83	31.27	31.91	49.88	39.34	36.75	38.25		
1	(N)	40	30	30	30	30	30	30	30	30	30	30		
	Means	53.8	55.4	59.1	62.4	62.9	65.1	66.0	67.9	70.4	72.4	74.1		
	Stdevs	11.10	12.97	13.20	14.71	13.61	14.33	14.84	15.15	16.86	16.62	15.59		
2	(N)	30	20	20	20	20	20	20	20	20	20	20		
	Means	60.9	66.3	67.1	71.4	72.7	80.4*	80.1	80.1	80.1	80.1	80.1		
	Stdevs	12.87	26.37	24.35	28.40	30.89	23.57	19.34	19.34	19.34	19.34	19.34		
3	(N)	30	30	20	20	20	20	20	20	20	20	20		
	Means	35.9	34.7	39.3	44.3	45.8	49.4	48.7	48.4	78.4	60.3	79.2		
	Stdevs	15.19	16.97	16.30	16.30	18.24	18.01	18.41	18.92	16.86	16.85	15.41		

Notes: 1 = treatment phase; * = recovery phase
 () = mean value of group was significantly different from control at P = 0.05(0.01) with Dunnett's test of significance
 () = mean value of group was significantly different from control at P = 0.05(0.01) with Modified T test of significance

- Food consumption: The observed decrease in body weight & weight gain in HD males was not due to a decrease in food consumption; on the contrary, a transient increase in mean food consumption occurred in LDM and HDM during the first 2 weeks of treatment, and no differences from control were observed thereafter. In the females, the body weight increases at LD and HD were paralleled by increases in food consumption between days 8 and 92 (LDF) and day 8 through 176 (HDF). During the recovery period, concordant with the normalization in female body weight, a dose-related decrease in the mean food consumption was observed in the female dose groups, statistically significant at HD (by approximately 11% vs. control) (see the following sponsor's table).

Prolactin in Plasma (Individual values)

Males (Group 1: Control; Group 2: 50 mg/kg/day; Group 3: 500 mg/kg/day)

Table 4.2
Novartis Pharmaceuticals Corporation
Toxicology Department
Rat/Mistar-Hannover NSDD

Data Listings by Animal for SPECIAL TESTS
Study number: 017013

Study start date: 26-Feb-01

Animal Number	Sex	Group/ Subgrp	Day/week of Phase	Seas #	Entry Type	PROL	Animal Number	Sex	Group/ Subgrp	Day/week of Phase	Seas #	Entry Type	PROL
1041	M	1/2	93/141	S 1	Key	47	2038	M	2/2	93/141	S 1	Key	35
			170/25	S 1	Key	49				170/25	S 1	Key	37
			26/4*	S 1	Key	37	2039	M	2/2	93/141	S 1	Key	19
1042	M	1/2	93/141	S 1	Key	52				170/25	S 1	Key	74
			170/25	S 1	Key	53	2040	M	2/2	93/141	S 1	Key	30
			26/4*	S 1	Key	49				170/25	S 1	Key	77
1043	M	1/2	93/141	S 1	Key	51	3041	M	3/2	93/141	S 1	Key	6
			170/25	S 1	Key	132				170/25	S 1	Key	110
			26/4*	S 1	Key	137				26/4*	S 1	Key	20
1044	M	1/2	93/141	S 1	Key	16	3042	M	3/2	93/141	S 1	Key	7
			170/25	S 1	Key	34				170/25	S 1	Key	21
			26/4*	S 1	Key	73				26/4*	S 1	Key	26
1045	M	1/2	93/141	S 1	Key	47	3043	M	3/2	93/141	S 1	Key	46
			170/25	S 1	Key	25				170/25	S 1	Key	227
			26/4*	S 1	Key	55				26/4*	S 1	Key	34
1046	M	1/2	93/141	S 1	Key	33	3044	M	3/2	93/141	S 1	Key	28
			170/25	S 1	Key	27				170/25	S 1	Key	80
			26/4*	S 1	Key	65				26/4*	S 1	Key	68
1047	M	1/2	93/141	S 1	Key	12	3045	M	3/2	93/141	S 1	Key	227
			170/25	S 1	Key	26				170/25	S 1	Key	13
			26/4*	S 1	Key	10				26/4*	S 1	Key	36
1048	M	1/2	93/141	S 1	Key	31	3046	M	3/2	93/141	S 1	Key	39
			170/25	S 1	Key	61				170/25	S 1	Key	135
			26/4*	S 1	Key	59				26/4*	S 1	Key	38
1049	M	1/2	93/141	S 1	Key	42	3047	M	3/2	93/141	S 1	Key	19
			170/25	S 1	Key	21				170/25	S 1	Key	60
			26/4*	S 1	Key	72				26/4*	S 1	Key	40
1050	M	1/2	93/141	S 1	Key	8	3048	M	3/2	93/141	S 1	Key	3
			170/25	S 1	Key	7				93/141	S 1	Key	35
			26/4*	S 1	Key	6	3049	M	3/2	170/25	S 1	Key	48
2031	M	2/2	93/141	S 1	Key	11				170/25	S 1	Key	63
			170/25	S 1	Key	27				93/141	S 1	Key	10
2032	M	2/2	93/141	S 1	Key	26				170/25	S 1	Key	26
			170/25	S 1	Key	59				26/4*	S 1	Key	37
2033	M	2/2	93/141	S 1	Key	5							
			170/25	S 1	Key	35							
2034	M	2/2	93/141	S 1	Key	14							
			170/25	S 1	Key	18							
2035	M	2/2	93/141	S 1	Key	19							
			170/25	S 1	Key	102							
2036	M	2/2	93/141	S 1	Key	11							
			170/25	S 1	Key	63							
2037	M	2/2	93/141	S 1	Key	11							
			170/25	S 1	Key	25							

Note: 1 = treatment phase; * = recovery phase

Females (Group 1: Control; Group 2: 50 mg/kg/day; Group 3: 500 mg/kg/day)

Animal Number	Sex	Group/ Subgrp	Day/week of Phase	Seas #	Entry Type	PROL	Animal Number	Sex	Group/ Subgrp	Day/week of Phase	Seas #	Entry Type	PROL
1541	F	1/2	93/141	S 1	Key	8	2538	F	2/2	93/141	S 1	Key	25
			170/25	S 1	Key	171				170/25	S 1	Key	13
			26/4*	S 1	Key	15	2539	F	2/2	93/141	S 1	Key	6
1542	F	1/2	93/141	S 1	Key	119				170/25	S 1	Key	6
			170/25	S 1	Key	18	2540	F	2/2	93/141	S 1	Key	16
			26/4*	S 1	Key	89				170/25	S 1	Key	55
1543	F	1/2	93/141	S 1	Key	107	3541	F	3/2	93/141	S 1	Key	3
			170/25	S 1	Key	177				170/25	S 1	Key	30
1544	F	1/2	93/141	S 1	Key	69				26/4*	S 1	Key	10
			170/25	S 1	Key	124	3542	F	3/2	93/141	S 1	Key	315
			26/4*	S 1	Key	240				170/25	S 1	Key	34
1545	F	1/2	93/141	S 1	Key	18				26/4*	S 1	Key	196
			170/25	S 1	Key	178	3543	F	3/2	93/141	S 1	Key	191
			26/4*	S 1	Key	145				170/25	S 1	Key	44
1546	F	1/2	93/141	S 1	Key	11	3544	F	3/2	26/4*	S 1	Key	213
			170/25	S 1	Key	153				93/141	S 1	Key	130
			26/4*	S 1	Key	66				170/25	S 1	Key	0
1547	F	1/2	93/141	S 1	Key	54	3545	F	3/2	26/4*	S 1	Key	2
			170/25	S 1	Key	190				93/141	S 1	Key	18
			26/4*	S 1	Key	37				170/25	S 1	Key	48
1548	F	1/2	93/141	S 1	Key	7	3546	F	3/2	26/4*	S 1	Key	52
			170/25	S 1	Key	166				93/141	S 1	Key	24
			26/4*	S 1	Key	73				170/25	S 1	Key	87
1549	F	1/2	93/141	S 1	Key	59	3547	F	3/2	93/141	S 1	Key	95
			170/25	S 1	Key	94				26/4*	S 1	Key	3
			26/4*	S 1	Key	86				170/25	S 1	Key	47
1550	F	1/2	93/141	S 1	Key	4				26/4*	S 1	Key	63
			170/25	S 1	Key	57	3548	F	3/2	93/141	S 1	Key	23
			26/4*	S 1	Key	428				170/25	S 1	Key	38
2531	F	2/2	93/141	S 1	Key	58				26/4*	S 1	Key	55
			170/25	S 1	Key	18	3549	F	3/2	93/141	S 1	Key	310
2532	F	2/2	93/141	S 1	Key	4				170/25	S 1	Key	5
			170/25	S 1	Key	29				26/4*	S 1	Key	15
2533	F	2/2	93/141	S 1	Key	61	3550	F	3/2	93/141	S 1	Key	8
			170/25	S 1	Key	28				170/25	S 1	Key	4
2534	F	2/2	93/141	S 1	Key	62				26/4*	S 1	Key	8
			170/25	S 1	Key	198							
2535	F	2/2	93/141	S 1	Key	35							
			170/25	S 1	Key	19							
2536	F	2/2	93/141	S 1	Key	135							
			170/25	S 1	Key	122							
2537	F	2/2	93/141	S 1	Key	42							
			170/25	S 1	Key	11							

Note: 1 = treatment phase; * = recovery phase

- Organ weights: The principal organ weight changes upon 26 weeks of treatment are summarized in the sponsor's table below. Individual and mean values of organ weights by gender (absolute and relative to body and brain weight) are shown in excerpts from sponsor's tables on the next pages. The most marked treatment-related effect was a decrease in uterine weight (absolute and relative by up to 37%) that was found in both LD and HD females. In the males, testicular weights were decreased in two HD animals with testicular atrophy. At HD, there were increases in liver weight in females (by 13% absolute and 16% relative to body wt) and heart weight in females (by 11% absolute and 14% relative to body wt); in the males, several organ weights were increased relative to body weight (liver, heart, brain, kidney, spleen, and prostate) without changes in these organs' absolute weights; these changes were not associated with deviations in clinical chemistry or microscopic changes and were likely associated with the body weight changes observed in these groups. Increases in mean absolute and relative weights of adrenals (in HDM and HDF, by up to 34% and 14%, respectively), pituitary (HDM, by up to 33%), and thymus (HDM, absolute wt increase by 24%) were found at 500 mg/kg/day; these effects were attributed to stress. After the 4-week recovery period, persisting changes were found in female reproductive organ weights [increased ovarian absolute (by 63%) and relative weights, and decreased uterine absolute (by 32%) and relative weights].

Dose (mg/kg/day)	Males						Females					
	% Absolute		% Relative to body weight		% Relative to brain weight		% Absolute		% Relative to body weight		% Relative to brain weight	
	50	500	50	500	50	500	50	500	50	500	50	500
Adrenal	-	↑ 20	-	↑ 34	-	↑ 17	-	↑ 12	-	↑ 14	-	↑ 12
Pituitary	-	↑ 18	-	↑ 33	-	↑ 21	-	-	-	-	-	-
Uterus	N/A	N/A	N/A	N/A	N/A	N/A	↓ 33	↓ 37	↓ 36	↓ 36	↓ 35	↓ 37
Liver	-	-	-	-	-	-	-	↑ 13	-	↑ 16	-	↑ 13
Heart	-	-	-	-	-	-	↑ 13	↑ 11	↑ 7	↑ 14	↑ 10	↑ 11

- Histopathology: Drug-related microscopic changes were found in endocrine glands (pituitary and adrenals in males, and the thyroid in females), mammary gland (both genders) and reproductive organs (ovary, uterus, testes, prostate). The incidence and type of microscopic findings in these tissues are shown in sponsor's tables on the next page. In females, dose-dependent disturbances in the estrus cycle (cycle prolongation) were found at both LD and HD, as demonstrated by the increased proportion of animals in the diestrus and metestrus phase of the cycle (i.e., 15/20 and 19/20 for LD and HD, respectively, as compared to 10/20 for control). This was consistent with the finding of vaginal epithelium mucification in the treated groups. In the ovaries, a dose-related interstitial cell hyperplasia was observed in HDF (11/20 vs. 3/20 in control), that was likely associated with the reduced estrus cycle activity. Endometrial gland atrophy was present in single animals at both LD and HD, consistent with the reduction of mean uterine weight noted in both these groups (see organ weights table on the previous page).

After the recovery period, i.e, 4 wks after the discontinuation of treatment, the prolongation of estrous cycle diminished but did not fully normalize, and increased corpora lutea were observed in the ovaries of HDF (4/10 animals vs. 0/10 in the control), concordant with increased ovarian weight. In the mammary gland, alveolar hyperplasia, increased secretion and dilatation of mammary ducts occurred with dose-related severity in LDF and HDF during treatment; similar, although milder mammary changes persisted after the recovery period. In the thyroid, diffuse follicular hyperplasia was induced in both LDF and HDF; this finding did not normalize after the recovery period.

In males, mammary gland and pituitary changes were induced at both tested doses, while testicular and secondary sex organ pathology and adrenal morphological changes were seen at the high dose only. The mammary changes had different morphological characteristics at the different dose levels. At LD, there was a "minimal" alveolar hyperplasia with increased secretion and no proliferation of mammary ducts. At HD, increased number of ducts, decreased number of alveoli, and increased mammary secretion with ductular dilatation was found (persistent, though less severe after the recovery period). The sponsor speculates that these changes "might represent either independent atrophy of alveoli and proliferation of ducts or conversion of alveoli to ducts in a test article-induced phenotypic change".

Pituitary histopathology (decreased eosinophilia of the acidophil cells of the distal part of the pituitary) was noted in both LDM and HDM with a dose-related incidence (4/20 and 9/21 animals, respectively).

Testicular and secondary sex organ pathology and adrenal morphological changes occurred in the high dose males only. Atrophy of testicular seminiferous tubule epithelium was observed in 2/21 HDM; the sponsor proposes that this might be a secondary effect of trauma or local disruption to the blood supply (as suggested by the variable severity of atrophy between the different tubular cross-section in one of the animals); a possible predisposing factor to testicular trauma, according to the sponsor, was the clinically observed scrotal "relaxation" (however, this clinical sign was present at both tested dose levels, while testicular atrophy was not found in the LD group).

Drug-related prostate changes (an increased incidence of mixed cell inflammation of prostate gland) were found at HD; associated degenerative changes were found in some of the more severe cases. In one case, inflammatory changes were also found in the seminal vesicles of HDM. The sponsor attributed prostate inflammatory changes as secondary to the drug-induced decrease of muscle tone in the urogenital area that could have "allowed urinary reflux into the prostatic duct with secondary infection of the gland". Prostate changes were not found after the recovery period.

Some increase in the incidence of adrenal microscopic changes was seen in HDM (diffuse or focal cortical hypertrophy in 4/21 HDM vs. 1/20 in control, and reduced cortical cytoplasmic vacuolation in 7/21 HDM vs. 3/20 in control); this finding was concordant with the increased absolute and relative adrenal weight. Adrenal microscopic changes were not observed after the recovery period.

Microscopic changes in endocrine glands, reproductive organs and mammary gland (week 26)

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS					
		Males			Females		
		0 mg/kg/day	50 mg/kg/day	500 mg/kg/day	0 mg/kg/day	50 mg/kg/day	500 mg/kg/day
ENDOCRINE SYSTEM							
ADRENAL		(20)	(20)	(21)	(20)	(2)	(20)
Within normal limits		13	15	7	6	2	8
Vacuolation, cortex, focal.		2	1				
Pigment, cortex.		1	2	2	13		10
Inflammation, lymphocyte, cortex, focal, chronic.				1			
Infiltrate, lymphocyte, corticomedullary junction.					1		1
Hypertrophy, cortex, diffuse.		1		3	1		2
Hypertrophy, cortex, focal.				1	2		2
Fibrosis, capsule, focal.		1					
Congestion.				1			
Vacuolation, decreased, cortex.		3	3	7			
PITUITARY		(20)	(20)	(21)	(20)		(20)
Within normal limits		10	12	8	14		19
Vacuolation, increased, pars distalis.		3		1			
Hyperplasia, pars distalis, focal.		1	1	1	1		
Eosinophilia, decreased, pars distalis.			4	3			
Cyst, pars intermedia.		8	3	9	5		1
Cyst, pars distalis.			1				
THYROID		(20)		(21)	(20)	(20)	(20)
Within normal limits		15		17	18	15	13
Thinned, epithelium, follicle.		2		1			
Infiltrate, lymphocyte, ganglion.							1
Hyperplasia, follicular, diffuse.		2		2	1	5	6
Hyperplasia, follicular.				1			
Ectopic thymus.					1		
Cyst.		1					1
GENITOURINARY SYSTEM							
CERVIX					(19)	(20)	(20)
Within normal limits					19	20	20
EPIDIDYMIDES		(20)	(20)	(21)			
Within normal limits		17	20	14			
Spermatocela.				1			
Oligospermia.				2			
Inflammation, interstitium, focal, chronic.		2		4			
Granuloma, spermatic, unilateral.		1		1			
Debris, lumen.				2			
OVARY					(20)	(20)	(20)
Hyperplasia, interstitial cell.					3	4	11
Within normal limits					16	16	9
Corpora lutea, decreased.					1	1	
PROSTATE		(20)	(20)	(21)			
Within normal limits		15	17	10			
Mineralization, lumen.			2	1			
Inflammation, mixed cell, focal.		1	1	9			
Inflammation, mixed cell.				2			
Inflammation, focal, chronic.		4		1			
Degeneration, focal.				3			
SEMINAL VESICLE		(20)		(21)			
Within normal limits		20		19			
Secretion reduced.				1			
Inflammation, mixed cell.				1			
TESTIS		(20)	(20)	(21)			
Within normal limits		19	20	19			
Fibrosis, tunica albuginea, focal.		1					
Atrophy, seminiferous tubule.				2			

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

Microscopic changes in endocrine glands, reproductive organs and mammary (continued)

ORGAN/TISSUE Finding	TREATMENT	INCIDENCE OF MICROSCOPIC FINDINGS							
		Males			Females				
		0 mg/kg/day	50 mg/kg/day	500 mg/kg/day	0 mg/kg/day	50 mg/kg/day	500 mg/kg/day		
UTERUS							(20)	(20)	(20)
Within normal limits					18	19	16		
Infiltrate, lymphocyte, myometrium, focal.							1		
Hyperplasia, endometrium.					1		1		
Dilatation, gland, endometrium.							1		
Dilatation.					1				
Atrophy, glandular.						1	1		
VAGINA							(19)	(20)	(20)
Within normal limits					18	12	10		
Mucification.					1	6	6		
Necrosis, single cell, epithelium.						2	1		
MAMMARY GLAND							(20)	(20)	(20)
Within normal limits		17	9		20	10			
Concretion.		1	2	2					
Increase, duct.		1		18					
Decrease, alveolus.		1		14					
Secretion, increased.			4	10		2	11		
Mineralization.				3			1		
Inflammation, focal, chronic.							1		
Hyperplasia, alveolus.			6			10	20		
Fibrosis, focal.							1		
Dilatation, duct, focal.		1							
Dilatation, duct.				3		1	7		

[M] Malignant tumor

Figures in () represent the number of animals from which this organ/tissue was examined microscopically

-Immunohistochemical assessment of cell proliferation:

Statistically significant, treatment-related increase in cell proliferation (increased proportion of cells in S phase of the cell cycle, as reflected by the labeling index) was found in pituitary (LDM and HDM), mammary gland (duct and alveoli) in both genders (LDM, HDM, HDF), and the endocrine pancreas in both genders (HDM, HDF). The findings are summarized in sponsor's tables below and on the next page.

Summary of treatment-related changes in cell proliferation

Dose (mg/kg/day)	Males		Females	
	50	500	50	500
Week 1				
Pituitary	-	↑	-	-
Mammary				
duct	-	-	-	↑
alveoli	-	T	-	↑
Week 13				
Pituitary	-	↑	-	-
Mammary				
duct	-	↑	-	-
alveoli	↑	↑	-	-
Pancreas	-	T	-	↑
Week 26				
Pituitary	↑	↑	-	-
Mammary				
duct	-	-	-	-
alveoli	-	↑	-	-
Recovery				
Pituitary	NA	-	NA	↑
Mammary	NA	-	NA	-
duct	-	-	-	↓
alveoli	-	↑	-	↓

↑ or ↓: statistically significant increase or decrease shown by tests for heterogeneity

T: Statistical significance shown by trend-test

Cell proliferation assessment: Summary of statistically significant findings

Sex	Organ	Interval	Heterogeneity	Trend
Male	Mammary Alveolar	Week 1		+*
Female	Mammary Alveolar	Week 1	3+**	+**
Male	Mammary Alveolar	Week 13	2+*, 3+**	+**
Male	Mammary Alveolar	Week 26	3+**	+**
Male	Mammary Alveolar	Recovery	3+**	
Female	Mammary Alveolar	Recovery	3-*	
Female	Mammary Ducts	Week 1	3+**	+**
Male	Mammary Ducts	Week 13	3+*	+*
Female	Mammary Ducts	Recovery	3-*	
Male	Pancreas	Week 13		+*
Female	Pancreas	Week 13	3+**	+**
Male	Pituitary	Week 1	3+**	+**
Male	Pituitary	Week 13	3+**	+**
Male	Pituitary	Week 26	2+*, 3+**	+**
Female	Pituitary	Recovery	3+**	

Explanations: + = Increased direction - = Decreased direction
 (R) = Rank transformed data * = Significant at p<0.05 ** = Significant at p<0.01

- Toxicokinetics

A summary of TK parameters for P95 in plasma is presented in the sponsor's table below.

Toxicokinetic parameters for P9512113 metabolite in rat plasma

Dose	Week	Gender	AUC (ng-h/mL)	±	SE	Cmax (ng/mL)	Tmax (h)	Dose normalized AUC (ng-h/mL) / (mg/kg/day)	Dose normalized Cmax (ng/mL) / (mg/kg/day)
50	1	male	740	±	107	945	1	14.8	18.9
		female	866	±	173	326	4	17.3	6.52
	13	male	2120	±	123	454	1	42.4	9.08
		female	2780	±	563	726	1	55.6	14.5
	26	male	3890	±	1500	709	2	77.8	14.2
		female	1790	±	297	1320	1	35.8	26.4
500	1	male	115000	±	63200	13400	1	230	26.8
		female	67900	±	16300	15800	1	136	31.6
	13	male	40700	±	6600	19900	1	81.4	39.8
		female	nc	±	nc	nc	nc	nc	nc
	26	male	169000	±	81800	24000	2	338	48
		female	463000	±	126000	36700	6	926	73.4

nc = not calculated due to insufficient data

Exposure to P95 (as assessed by Cmax and AUC) increased over dose-proportionally with increasing dose (about 3x and >4x over the dose-proportional levels for Cmax and AUC, respectively). Exposure also increased with the increased duration of treatment, suggestive of drug accumulation. There were no consistent gender differences in exposure parameters throughout the treatment period, as assessed on weeks 1, 13 and 26 (note: HDF exposure on wk 13 was not determined due to insufficient data).

Comparison with human exposure

P95 exposure data from 26-week Wistar rat study (at Week 26) compared to human exposure at steady state

Dose (mg/kg/d)	Exposure	Rat		Human (12 mg BID) ^a	Rat/Human Exposure Margin
		M	F		
50	C _{max} (ng/mL)	709	1320	58.4	12x, 23x ^b
	AUC ₍₀₋₂₄₎ (ng·h/mL)	3890	1790	1138	3.4x, 1.6x ^b
500	C _{max} (ng/mL)	24,000	36,700	58.4	411x, 628x ^b
	AUC ₍₀₋₂₄₎ (ng·h/mL)	169,000	463,000	1138	149x, 407x ^b

^a Data taken from Clinical Study CILO522A0112. Actual AUC(0-12) value (568.9 ng·h/mL) is doubled to correlate with rodent AUC(0-24) and human kinetics (Module 2.7.2).

^b Male, female

The P95 plasma exposure value (AUC) for 24-h exposure in humans at iloperidone MRHD of 24 mg/day is 1138 ng·h/ml. In comparison, the corresponding AUC values of P95 exposure in rats on wk 26 are 2 to 3x and 150 to 400x the human value at the LD and HD, respectively.

Comments

The 26-week rat study of P95 oral toxicity at doses of 50 and 500 mg/kg/day shows that non-neoplastic proliferative changes (detected by either routine histology and/or by immunohistochemical staining for cell proliferation) occurred in the mammary gland, ovary, anterior pituitary, thyroid gland and endocrine pancreas. The sponsor attributes these changes largely to a “reduction of dopamine-mediated inhibition of prolactin secretion by the pituitary, leading to raised serum prolactin”, but this contention is not supported by the results of the 26-week P95 rat study that failed to find prolactin increase (as determined twice in the course of treatment – at wks 14 and 26). Neither is it supported by P95 pharmacological characteristics, i.e., if P95 dopaminergic activity is much lower than that of parent drug and P95 “does not contribute to the primary pharmacological activity of iloperidone” (as stated by sponsor), the proliferative changes observed in the 26-week study are not likely to be secondary to P95 dopaminergic activity. Moreover, similar proliferative changes were not observed in 26-week toxicity study with the parent compound (iloperidone) in the same species, despite of the parent’s much higher dopaminergic activity. In the 6-month iloperidone toxicity study in rat [at oral (gavage) doses of 12, 24, and 48 mg/kg/day], the morphologic pathology findings for iloperidone (by routine histology) were limited to mammary gland epithelial vacuolization in both genders at all dose levels with dose-related incidence and severity, dose-related reduced size of testicles and testicular degeneration/atrophy (in single animals) and higher incidence of prostate inflammation at HD. As BrdU labeling was not performed in any of iloperidone repeat-dose toxicity studies, no comparison of these findings can be made between P95 and iloperidone. However, proliferative changes in multiple organs/tissues (i.e., pituitary, adrenals, thyroid, mammary gland) were found by routine histology methods in the 6-month P95 study. Effects on thyroid, and mammary gland persisted following the 4-week recovery period. Therefore, there is a substantial

toxicological difference between the parent compound and P95 metabolite with regard to P95 cellular proliferation capacity that is not seen with the parent drug.

Conclusion: Chronic (26-week) administration of iloperidone metabolite P95 to Wistar rats at oral (gavage) doses of 50 and 500 mg/kg/day, [corresponding to plasma exposure (AUC 0-24) of about 2 to 3x and 150 to 400x the human AUC at MRHD at the LD and HD, respectively] produced non-neoplastic proliferative changes (detected by either routine histology or by immunohistochemical staining for cell proliferation) in the mammary gland, ovaries, anterior pituitary, adrenals, thyroid gland and endocrine pancreas. These changes were induced by both tested doses in a dose-dependent manner (with the exception of the pancreas and the ovaries that were affected at the HD level only). Effects on estrous cycle, thyroid, and mammary gland alterations persisted following the 4-week recovery period. An NOAEL was not reached in either male or female rats in view of the presence of pathomorphological changes in multiple organs/tissues at the lowest tested dose of 50 mg/kg/day. The MTD was <500 mg/kg/day for males in view of mortality and body weight decrease at 500 mg/kg/day; for females, an MTD was not reached.

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2.6.6.4 Genetic toxicology

Iloperidone:

In vitro:

Non-mammalian cell assays: Ames test conducted in *Salmonella typhimurium* (Study 12048-0-401R); *Escherichia coli*/mammalian-microsome reverse mutation assay (Study 14476-0-402R).

Mammalian cell assays: A chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells (Study 14476-0-437); a test of induction of forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in CHO cells (Study 14476-0-435); two additional chromosomal aberration assays in CHO cells evaluated the mutagenicity of iloperidone and micronized iloperidone (Study 1463/63-D5140 and Study 1463/70, respectively).

In vivo: Genotoxicity potential of iloperidone was evaluated in 4 in vivo studies. These included 3 bone marrow micronucleus assays conducted in mice, the first of which was a dose-finding assay of bone marrow cytotoxicity (Studies 14476-0-459PO, 14476-0-455, and 998068) and 1 hepatocyte micronucleus assay conducted in rats (Study 1463/71-D5140, micronized iloperidone).

The specifications of the drug substances used in each of these studies are listed in the following sponsor's table.

Batch/Lot No.	Drug Substance		Test Article: Iloperidone		
	Purity*	Specified Impurities (%)		Study No.	Type of Study
PROPOSED SPECIFICATION:		Each	Total		
Lot RC 4094	Not stated	Not stated	Not stated	12048-0-401R	<i>Salmonella</i> /mammalian-microsome reverse mutation assay (Ames test)
Lot RC 4537	Not stated	Not stated	Not stated	14476-0-437	Chromosomal aberration assay in CHO cells
Lot RC 4537	Not stated	Not stated	Not stated	14476-0-435	CHO/HGPRT forward mutation assay
Batch 98908	99.4%			1463/63-D5140	Chromosomal aberration assay in CHO cells
Batch 9929011	99.5%			1463/70	Chromosomal aberration assay in CHO cells
Lot RC 4537	Not stated	Not stated	Not stated	14476-0-402R	<i>E. coli</i> mammalian-microsome reverse mutation assay
Lot RC 4537	Not stated	Not stated	Not stated	14476-0-459PO	Dose-ranging in vivo murine micronucleus assay
Lot RC 4537	Not stated	Not stated	Not stated	14476-0-455	In vivo mammalian micronucleus assay
Batch 98908	99.4%			998068	In vivo mouse micronucleus assay
Batch 9929011	99.5%			1463/71-D5140	Induction of micronuclei of rat liver in vivo

b(4)

2.6.6.4.1. In vitro non-mammalian cell system

Mutagenicity test on HP 873 in the salmonella/mammalian-microsome reverse mutation assay (Ames test) with confirmatory assay (Study 12048-0-401R)

Study design: A GLP-compliant bacterial reverse mutation test was performed to investigate the possible genotoxicity of iloperidone. The test was carried out using 5 *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA1538. On the basis of the results of a previous dose-finding experiment, iloperidone (Lot RC 4094) was tested at 6 dose levels from 3,330 to 66.7 µg per plate in the presence of S9 and from 1,000 to 33.3 µg per plate in the absence of S9. Each strain was tested with or without a metabolic activation system, an S9 mixture prepared from the liver of a rat given Aroclor™. Positive control substances included 2-aminoanthracene, 2-nitrofluorene, sodium azide, and ICR-191.

The following validity criteria were employed:

/ Page(s) Withheld

~~X~~ Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Report Title: Mutagenicity test on HP 873 in the salmonella/mammalian-microsome reverse mutation assay (Ames test) with confirmatory assay			Test Article: Iloperidone				
Test for Induction of: reverse mutations at histidine locus		No. of Independent Assays: 2			Study No. 12048-0-401R		
Strains: <i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538		No. of Replicate Cultures: 3 plates/dose level					
Metabolizing System: Aroclor-induced rat liver S9		No. of Cells Analyzed/Culture: NA					
Vehicles:	For Test Article: DMF	For Positive Controls: Not stated (commercially obtained)			GLP Compliance: Yes		
Treatment: 3,330-66.7 µg/plate with S9; 1,000-33.3 µg/plate without S9 (6 dose levels each).					Date of Treatment: February 8, 1990		
Cytotoxic Effects (in dose range-finding assay): ≥1000 µg/plate with S9; ≥667 µg/plate without S9.							
Genotoxic Effects: None in the presence or absence of S9 activation.							
Metabolic Activation	Test Article	Concentration (µg/plate)	TA98	TA100	TA1535	TA1537	TA1538
Without Activation	Iloperidone	33.3	22 ± 4	100 ± 13	15 ± 3	5 ± 3	18 ± 6
		66.7	25 ± 9	96 ± 5	12 ± 2	5 ± 3	22 ± 2
		100	22 ± 11	99 ± 9	16 ± 2	4 ± 2	20 ± 4
		333	28 ± 3	98 ± 17	13 ± 4	8 ± 3	31 ± 1
		667	28 ± 10	89 ± 19	8 ± 3	6 ± 2	17 ± 3
		1000	12 ± 3	18 ± 11	7 ± 1	2 ± 3	0 ± 0
Vehicle control	0	23 ± 9	98 ± 13	15 ± 4	7 ± 5	9 ± 5	
Positive control ^a	2-nitrofluorine: 1.0 sodium azide: 2.0 ICR-191: 2.0	153 ± 9	587 ± 48	491 ± 61	350 ± 50	243 ± 16	

Both in an initial and confirmatory assay, iloperidone did not cause a positive increase in the number of histidine revertants per plate of any of the tester strains either in the presence or absence of microsomal enzymes prepared from Aroclor-induced rat liver.

Mutagenicity test of HP 873 in the *Escherichia coli* WP2uvrA-/mammalian microsome reverse mutation assay with a confirmatory assay (Study 14476-0-402R)

Study design: A GLP-compliant bacterial reverse mutation test was performed to investigate the potential of iloperidone to cause point mutations (base pair substitutions). The test was carried out using the *Escherichia coli* strain, WP2uvrA. On the basis of the results of a previous dose-finding experiment, iloperidone (Lot RC 4537) dissolved in dimethylformamide, was used at 6 dose levels ranging from 3330 to 66.7 µg per plate. Each dose was tested with or without S9. The following criteria for a valid assay were employed:

b(4)

b(4)

The following criteria for positive response were employed:

b(4)

Results: A summary of the design and findings of this study is presented in the following sponsor's table.

Report Title: Mutagenicity test on HP 873 in the <i>Escherichia coli</i> WP2uvrA/mammalian-microsome reverse mutation assay with a confirmatory assay			Test Article: loperidone		
Test for induction of: reverse mutation in bacterial cells		No. of Independent Assays: 3 (Assay 1 repeated in Assay 3 due to dilution error in Assay 1)		Study No. 144776-0-402R	
Strains: <i>E. coli</i>		No. of Replicate Cultures: 3		Location in CTID: Vol. Page	
Metabolizing System: Aroclor-induced rat liver S9		No. of Cells Analyzed/Culture: -			
Vehicles:	For Test Article: DMF	For Positive Controls: Not stated (commercially obtained)		GLP Compliance: Yes	
Treatment: Plate incorporation for 48 hours.				Date of Treatment: April 3, 1991 (Assay 1), April 9, 1991 (Assay 2), April 18, 1991 (Assay 3)	
Cytotoxic Effects: Lawn extremely reduced at 3330 µg, none at all other doses.					
Genotoxic Effects: None.					
Metabolic Activation	Test Article	Dose per Plate (µg)	Mean Revertants per Plate (±SD)		
			Assay 1	Assay 2 (confirmatory)	Assay 3 (repeat of 1) ^a
Without Activation	Vehicle control	0	17 ± 6	12 ± 3	21 ± 3
	Negative control	0	23 ± 5	15 ± 3	23 ± 3
	loperidone	10	24 ± 1	ND	ND
		66.7	ND	9 ± 2	20 ± 5
		100	17 ± 3	14 ± 6	17 ± 5
		100	19 ± 2	ND	ND
		333	15 ± 2	19 ± 5	18 ± 5
		667	ND	14 ± 4	17 ± 5
		1000	15 ± 4	10 ± 3	14 ± 3
		3330 ^b	0 ± 0	0 ± 0	1 ± 1

	4-nitroquinoline-N-oxide	10	450 ± 41	709 ± 34	455 ± 49
With Activation	Vehicle control	0	27 ± 8	16 ± 2	22 ± 1
	Negative control	0	24 ± 3	17 ± 3	25 ± 7
	Iloperidone	10	16 ± 3	ND	ND
		66.7	ND	17 ± 1	17 ± 5
		100	25 ± 3	19 ± 6	19 ± 3
		100	20 ± 8	ND	ND
		333	19 ± 5	16 ± 5	26 ± 8
		667	ND	13 ± 2	28 ± 5
		1000	18 ± 1	13 ± 3	17 ± 4
		3330	0 ± 0	0 ± 0	1 ± 1
	Z-aminocanthracene	25	175 ± 29	422 ± 25	244 ± 17

^a Assay 3 conducted to repeat Assay 1 because of a dilution error made in Assay 1 that led to testing 2 doses which had not been selected.

^b Lawn extremely reduced at this dose in each assay.

Conclusion: Under the conditions of this study, in both an initial and confirmatory assay, iloperidone did not cause a positive increase in the numbers of histidine revertant per plate either in the presence or absence of metabolic activation.

2.6.6.4.2. In vitro mammalian cell system

Genotoxicity test on iloperidone measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells: multiple harvests under conditions of metabolic activation with a confirmatory assay (Study 14476-0-437)

Study design and methods: The possible genotoxicity of iloperidone (Lot 4537) was evaluated in vitro in a chromosome aberration study conducted in a CHO cell line. The study was GLP-compliant

The assay is designed to examine cells in the first mitosis after chemical exposure; this design “limits loss of aberrant cells during the division process or conversion into complex derivatives during the subsequent cell cycles”. Because a test compound can cause a delay of progression through the cell cycle, the assay is designed to detect this delay (in a preliminary range-finding assay) and allow for slower growth of damaged cells by adjustment in the time between treatment and cell fixation.

In the range-finding assay, the cultures were incubated for 25-26 h with 5-bromo-2'-deoxyuridine (BrdUrd); this “enabled the majority of cells to progress through two cell divisions, thus providing assessment of cell cycle kinetics”. The aberrations assay was conducted at the 10 hour harvest time if there was no cell cycle delay and at 20 h harvest time if there was a cell cycle delay. In the range-finding assay, iloperidone concentration range of 0.0334 µg/ml to 1000 µg/ml was tested, with and without metabolic activation. The test article was suspended in ethanol at a target stock concentration of 100 mg/ml.

In the aberration assay, cells were cultured in growth medium containing 100 to 1000 µg/ml of iloperidone (initially dissolved in ethanol) in the presence or absence of a metabolic activation mixture. The dose range was chosen based on the preliminary range-finding test. Mitomycin C and cyclophosphamide were used as positive control substances for the nonactivation and S9 metabolic activation series, respectively. Replicate cultures were used at each dose level; single cultures were used for the negative control, solvent control, and the positive controls. In the non-activation assay, 20 h. harvest was conducted; in the S9 activation assay, 10- and 20 h. harvests were performed. Chromosomal aberrations were analyzed for the cultures treated “at the four highest

doses from which results could be obtained and from only one of the positive control doses".

Results:

In the range-finding assay, precipitate was observed after dosing at 1000 µg/ml, and a slight precipitate at 334µg/ml; cellular debris and reductions in the cell monolayer confluence (-30% and -15%, respectively) were observed at both these concentrations. Slight reductions in numbers of visible mitotic cells were observed at 100, 334, and 1000 µg/ml. Severe cell cycle delay was evident in the cultures dosed with 33.4, 100, 334, and 1000 µg/ml in the range-finding study without activation, and no cell cycle delay was evident in the range-finding assay with activation. No reduction in mitotic index was observed in the test cultures when compared to the solvent control. Based on these results, replicate cultures of CHO cells were incubated with concentrations of 100 to 1000 µg/ml of the test compound in a 20-hour aberration assay without metabolic activation, and in 10 and 20-hour aberration assay with metabolic activation.

RANGE-FINDING ASSAY FOR ASSESSING DELAY OF CELL CYCLE PROGRESSION						
Compound: HP 873			Assay Number: 14475			
Metabolic Activation: Without			Lab No: CY3191		Trial No.: 1	
Treatment	µg/ml (µl/ml)	X Cells ^a			Confluence ^b % Solvent Control	Z Mitotic Index
		M ₁	M ₁₊	≥M ₂		
Negative Control McCoy's 5a		1	8	91	100	10.5
Solvent Control Ethanol (11.0)			6	94	100	5.0
Positive Control MMC	0.250	97	3		100	*
HP 873	33.4	66	34		100	14.9
	100	92	8		100	3.4
	334	100			86	4.3
	1000	88	12		71	5.4

Metabolic Activation: With			Lab No: CY3191		Trial No.: 1	
Treatment	µg/ml (µl/ml)	X Cells ^a			Confluence ^b % Solvent Control	Z Mitotic Index
		M ₁	M ₁₊	≥M ₂		
Negative Control McCoy's 5a			7	93	100	10.9
Solvent Control Ethanol (11.0)			3	97	100	13.9
Positive Control CP	2.00		37	63	100	*
HP 873	33.4		18	82	100	13.0
	100		26	74	100	14.8
	334		29	71	100	12.5
	1000		35	65	100	11.7

^aX cells that have completed one (M₁), between one and two (M₁₊) or two or more (≥M₂) cycles in BrdUrd.

^bThis endpoint is based upon visual observations which are made prior to the harvest of the metaphase cells. Actual cell counts are not taken and any hypertrophy of the attached cells cannot be evaluated. At the time of the confluence observation the flasks are also evaluated for the appearance of floating mitotic cells and dead cells.

*Positive control not used for the set of cultures used for mitotic index analysis.

In the aberration assay, no significant increases in cells with chromosomal aberrations were observed at the concentrations analyzed either with or without metabolic activation. These results were confirmed in the repeat assays. The sensitivity of the cell culture for induction of chromosomal aberrations was shown by the increased frequency of aberrations in the cells exposed to the positive control agents. Therefore, iloperidone was

negative for inducing chromosomal aberrations in CHO cells under both non-activation and activation conditions.

A summary of the design and findings of this study is presented in the following sponsor's table.

Report Title: Mutagenicity test on HP 873 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells: multiple harvests under conditions of metabolic activation with a confirmatory assay				Test Article: Iloperidone					
Test for Induction of: Chromosomal aberrations		No. of Independent Assays: 2		Study No. 14476-0-437					
Strain: Chinese Hamster Ovary cell line		No. of Replicate Cultures: 2							
Metabolizing System: Aroclor-induced rat liver S9		No. of Cells Analyzed/Culture: 200							
Vehicles:		For Test Article: Ethanol		For Positive Controls: Not stated		GLP Compliance: Yes			
Treatment: Cells incubated with target concentrations of 100-1000 µg/mL in 20-hour nonactivation or 10- and 20-hour activation assay				Date of Treatment: March 27, 1991					
Cytotoxic Effects: Without metabolic activation, reduction in cell number at 994 µg/mL, 15% reduction in monolayer confluence at ≥249 µg/mL, slight reductions in visible mitotic cells at ≥99.4 µg/mL; with metabolic activation, no cytotoxicity observed in first 10-hour assay, slight reduction in mitotic cell number at 984 µg/mL in second 10-hour assay, slight reduction in numbers of mitotic cells, 25% reduction in monolayer confluence, and floating debris seen at 994 µg/mL, 15% reduction in monolayer confluence at ≥99.4 µg/mL, cellular debris noted at 1020 µg/mL in confirmatory 20-hour assay.									
Genotoxic Effects: None									
Metabolic Activation	Test Article	Concentration or Dose Level (µg/mL)	Cells Fixed at 20 Hours After Treatment (Trial 1)			Cells Fixed at 10 Hours After Treatment			
			No. of Aberrations per Cell	% Cells with Aberrations	% Cells with >1 Aberrations	No. of Aberrations per Cell	% Cells with Aberrations	% Cells with >1 Aberrations	
Without Activation	Vehicle	0	0.01	1.0	0.0	ND	ND		
		Iloperidone	249	0.01	0.5	0.0	ND	ND	
			497	0.01	0.5	0.0	ND	ND	
			746	0.00	0.0	0.0	ND	ND	
			994	0.02	2.0	0.0	ND	ND	
	Mitomycin C	0.040	0.36	28.0*	3.0*	ND	ND		
With activation	Vehicle	0	0.00	0.0	0.0	0.00	0.0	0.0	
		Iloperidone	250	0.02	2.0	0.0	0.02	2.0	0.0
			500	0.00	0.0	0.0	0.00	0.0	0.0
			749	0.00	0.0	0.0	0.00	0.0	0.0
			999	0.00	0.0	0.0	0.00	0.0	0.0
	Cyclophosphamide	25.0	0.22	18.0*	4.0*	0.22	18.0*	4.0*	

* Significantly greater than pooled negative and solvent controls, P < 0.01 by Fisher's exact test with adjustment for multiple comparisons.

Note: The results of Trial 1 are presented in this table. The results of Trial 2 confirmed these results.

Summary and conclusions: Iloperidone was tested in a genotoxicity test on chromosomal aberrations in Chinese Hamster Ovary (CHO) cells in vitro, with and without metabolic activation. In the range-finding study employing a concentration range of 0.0334 - 1000 µg/ml, severe cell cycle delay was evident in the cultures dosed with 33.4, 100, 334, and 1000 µg/ml without activation; no cell cycle delay was evident with activation. Based on these results, replicate cultures of CHO cells were incubated with concentrations of 100 to 1000 µg/ml of the test compound in a 20-hour aberration assay without metabolic activation, and in 10 and 20-hour aberration assay with metabolic activation. No significant increases in cells with chromosomal aberrations were observed at the concentrations analyzed. These results were confirmed in the repeat assays. The sensitivity of the cell culture for induction of chromosomal aberrations was shown by the increased frequency of aberrations in the cells exposed to the positive control agents. Therefore, iloperidone was negative for inducing chromosomal aberrations in CHO cells under the conditions of this assay.

Mutagenicity test on HP 873 in the CHO-HGPRT forward mutation assay with an independent repeat (Study 14476-0-435)

Study design:

This in vitro study tested the ability of iloperidone (Lot RC 4537) to induce forward mutations at the HGPRT (hypoxanthine-guanine phosphoribosyl transferase) locus in the CHO-K1-BH Chinese hamster ovary (CHO) cell line as assessed by colony growth in the presence of 6-thioguanine with and without metabolic activation. The study was GLP-compliant. The rationale is adequately described sponsor's paragraph reproduced below:

Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) is a cellular enzyme that allows cells to salvage hypoxanthine and guanine for use in DNA synthesis. The HGPRT enzyme utilizes the substrates 5-phosphoribosyl-1-pyrophosphate and hypoxanthine or guanine to catalyze the formation of inosine- or guanosine monophosphate. If a purine analog such as 6-thioguanine (T6) is included in the growth medium, the analog will be phosphorylated via the HGPRT pathway and incorporated into nucleic acids, eventually resulting in cellular death. The HGPRT locus is located on the X chromosome. Since only one of the two X chromosomes is functional in the female CHO cells, a single-step forward mutation from HGPRT+ to HGPRT- in the functional X chromosome will render the cell unable to utilize hypoxanthine, guanine, or T6 supplied in the culture medium. Such mutants are as viable as wild-type cells in normal medium because DNA synthesis may still proceed by *de novo* synthetic pathways that do not involve hypoxanthine or guanine as intermediates. The basis for the selection of HGPRT- mutants is the loss of their ability to utilize toxic purine analogs (e.g., T6), which enables only the HGPRT- mutants to grow in the presence of T6. Cells which grow to form colonies in the presence of T6 are assumed to have mutated, either spontaneously or by the action of the test article, to the HGPRT- genotype.

Two independent assays were performed using both non-activation and S9 metabolic activation. Both trials used 6 doses ranging from 100 µg/mL to 1000 µg/mL under both non-activation and S9 metabolic activation conditions.

Test article: Primary 100% stocks of the test material were prepared using absolute ethanol as the diluent; the primary test material stocks were then diluted 1:100 into culture medium resulting in test material concentrations that contained 1% absolute ethanol.

Control articles:

- Negative (media) controls were performed for each portion of the study by carrying cells unexposed to test material through all of the assay operations. Triplicate cultures were used in the cytotoxicity assays. Negative controls were not used in the mutation assays.
- Vehicle controls: Concurrent vehicle controls were performed for each portion of the study by exposing cells to 1% absolute ethanol in culture medium for 4 hours. In the activation portion of the assay, the vehicle controls were also exposed to S9. Triplicate cultures were used in the cytotoxicity assays and duplicate controls were used in the mutation assays.
- Positive controls: 5-Bromo-2'-deoxyuridine (BrDU) 50 µg/ml (for non-activation mutation assays). BrDU is highly and reproducibly mutagenic to CHO cells without S9 metabolic activation. 3-Methylcholantrene (3-MCA) 5µg/ml (for mutation assays with S9 activation). 3-MCA requires metabolic activation by microsomal enzymes to become mutagenic to CHO cells.

Range-finding cytotoxicity testing: Ten iloperidone concentrations (ranging from 1.95 to 1000 µg/ml) were tested for cytotoxicity with and without S9. One negative control and one vehicle control were used in each cytotoxicity assay. Cytotoxicity was expressed as a % of colony counts in treated vs. control cultures. The preliminary cytotoxicity information was used to select 6 doses for the mutation assay that covered a range from

approximately 0% to 90% reduction in colony-forming ability, corresponding to iloperidone concentration range from 100 to 1000 ug/ml.

Mutation assays: Two non-activation and 2 activation assays were performed, each with its own set of vehicle and positive controls. The procedures were identical, except for the addition of the S9 fraction of rat liver homogenate and necessary cofactors during a 4-h treatment period (NADP sodium salt, glucose-6-phosphate, Ca chloride, potassium chloride, Mg chloride in a pH 7.8 sodium phosphate buffer).

Parameters: The following parameters were calculated, as described by the sponsor:

1. Relative Survival to Treatment

This parameter gives the clonal cytotoxicity of each treatment by showing what percentage of the cells were able to form colonies after the treatment period in both the range-finding cytotoxicity assays and the mutation assays relative to the concurrent vehicle controls. The average number of colonies in three dishes (seeded at 200 cells each) was determined for each treatment condition.

Relative Survival (%) = $\frac{\text{Average no. of colonies per treated culture}}{\text{Average no. of colonies per vehicle control dish}} \times 100\%$

2. Relative Population Growth

This parameter shows the cumulative growth of the treated cell population, relative to the vehicle control growth, over the entire expression period and prior to mutant selection. In general, highly toxic treatments will reduce the growth rate as well as the survival.

Values less than 100% indicate growth inhibition. For example, 50% and 25% relative growth values would indicate treated cell populations that were one and two population doublings behind the vehicle control cultures. Treated populations that are more than 2 or 3 doublings behind the control might not achieve maximum expression of the TB-resistant phenotype. The relative population growth is calculated from cell count data not presented in this report and is intended to provide only an approximate indication of growth during the expression period, since cells are easily lost or not completely released by trypsin during the subculture procedures.

Relative Population Growth (%) = $\frac{\text{Treated culture population increase over the expression period}}{\text{vehicle control population increase over the expression period}} \times 100\%$

3. Absolute Cloning Efficiency

The ability of the cells to form colonies at the time of mutant selection is measured by the absolute cloning efficiency (CE). This parameter is used as the best estimate of the cloning efficiency of the mutant cells in the selection dishes. Thus, the observed number of mutant colonies can be converted to the frequency of mutant cells in the treated population.

Absolute CE (%) = $\frac{\text{Average no. of viable colonies per dish}}{200} \times 100\%$

4. Mutant Frequency

The mutant frequency is the endpoint of the assay. It is calculated as the ratio of colonies found in thioguanine-selection medium to the total number of cells seeded, adjusted by the absolute C.E. The frequency is expressed in units of 10^6 , e.g., the number of mutants per one million cells.

Mutant Frequency = $\frac{\text{Total mutant clones}}{\text{no. of dishes} \times 2 \times 10^6 \times \text{abs. C.E.}}$

Assay acceptance criteria (as cited from the sponsor's report):

b(4)

b(4)

Assay evaluation criteria:

Range-finding cytotoxicity study: Cell cultures should be exposed to about 6 to 8 concentrations of the test article that are expected to span a range of cellular responses from no observed toxicity to about 10% survival.

Mutagenicity study: Five dose levels selected on the basis of the range-finding study should cover a range of toxicities with emphasis placed on the most toxic doses. A 95% confidence level of the difference in mutant frequency between each tested concentration and negative control. In addition, the mutant frequency must meet or exceed 15×10^{-6} in order "to compensate for random fluctuations in the 0 to 10×10^{-6} background mutant frequencies that are typical for this assay". Observation of a mutant frequency that meets the minimum criteria for a positive response in a single treated culture within a range of assayed concentrations is not sufficient evidence to evaluate a test article as a mutagen. The following test results must be obtained to reach this conclusion (as cited from the sponsor's report):

- A dose-related or toxicity-related increase in mutant frequency should be observed. It is desirable to obtain this relation for at least three doses. However, this depends on the concentration steps chosen for the assay and the toxicity at which mutagenic activity appears.
- A mutagenic dose-response in one mutation assay should be confirmed in the second mutation assay. While it is desirable to confirm significant mutagenesis in both trials, it may not always be possible due to normal assay variation, especially under toxic or insoluble treatment conditions, and also due to the possible use of different dose levels in the two trials.

- If an increase in mutant frequency is observed in one trial for a single dose near the highest testable toxicity, as defined previously, and the number of mutant colonies is more than twice the value needed to indicate a significant response, the test article generally will be considered mutagenic. Smaller increases at a single dose near the highest testable toxicity will require confirmation by a repeat assay. Lack of confirmation in the independent trial will result in a negative evaluation under the conditions of testing.
- For some test articles, the correlation between toxicity and applied concentration is poor. The proportion of the applied article that effectively interacts with the cells to cause genetic alterations is not always repeatable or readily controlled. Conversely, measurable changes in the frequency of induced mutants may occur with concentration changes that cause only small changes in observable toxicity. Therefore, either parameter, applied concentration or toxicity (percent survival), can be used to establish whether the mutagenic activity is related to an increase in effective treatment.
- Treatments that reduce relative clonal survival to less than five percent may be included in the assay but will not be used as sufficient evidence for mutagenicity as it relates to risk assessment.

Results: The preliminary range-finding cytotoxicity assay showed that all of the tested 10 dose levels (ranging from 1.95 to 1000 ug/ml) were non-toxic to CHO cells in culture both with and without metabolic activation (see sponsor's table below)

CYTOTOXICITY ASSAY WITH HP 873				
SAMPLE IDENTITY: HP 873		CELL TYPE: CHO-K1-BH		
ASSAY NUMBER: 14476		CELLS SEEDED PER DISH: 200		
TEST DATE: April 16, 1991		pH ALTERATIONS: NONE		
SOLVENT: ABSOLUTE ETHANOL		COMMENTS ON TREATMENT: FOUR-HOUR EXPOSURE		
APPLIED CONCENTRATION UG/ML	WITHOUT METABOLIC ACTIVATION AVERAGE COUNT	% RELATIVE SURVIVAL ^a	WITH METABOLIC ACTIVATION AVERAGE COUNT	% RELATIVE SURVIVAL ^a
Ncb	173.0	102.5	138.3	103.4
Vcc	168.7	100.0	133.7	100.0
1.95	164.3	97.4	125.3	93.7
3.91	175.7	104.1	150.0	112.2
7.81	177.0	104.9	132.7	99.3
15.6	178.3	105.7	148.7	111.2
31.3	171.7	101.8	146.0	109.2
62.5	177.7	105.3	149.3	111.7
125	188.0	111.4	171.0	127.9
250	174.3	103.3	167.7	125.4
500	180.7	107.1	164.7	123.2
1000	170.3	100.9	161.7	120.9

^aRelative to vehicle control cloning efficiency for all treatments.
^bNc = Negative (media) control.
^cVc = Vehicle control, 1% absolute ethanol.

Thus, the range-finding cytotoxicity study did not meet the following evaluation criterion: "cell cultures should be exposed to concentrations of the test article that are expected to span a range of cellular responses from no observed toxicity to about 10% survival."

In the mutagenicity assays conducted, the mutant frequencies seen in treated cultures varied randomly with the dose within the range acceptable for background mutation frequency. A single culture in the second non-activation mutation assay (400ug/ml) had a significantly elevated mutant frequency which was determined to be "a result of normal assay variation". In the first activation mutation assay cultures treated with 600 and 800ug/ml had mutant frequencies significantly elevated over the concurrent negative control. However, these values did not exceed the acceptable range for background mutant frequency variation (15×10^{-6}) and were not replicated in the 2nd activation mutation assay. The mutant frequency of each vehicle and positive control was acceptable and within the historical range for the conducting laboratory. A detailed

summary of the design and findings of this study is presented in the following sponsor's table:

Report Title: Mutagenicity test on HP 873 in the CHO/HGPRT forward mutation assay with an independent repeat						Test Article: Iloperidone		
Test for Induction of: forward mutations in the HGPRT locus in CHO cells				No. of Independent Assays: 2		Study No. 14476-4-435		
Strains: Chinese Hamster Ovary cell line				No. of Replicate Cultures: Cytotoxicity-3 Mutagenicity-2				
Metabolizing System: Aroclor-induced rat liver S9				No. of Cells Analyzed/Culture: NA				
Vehicles:		For Test Article: Absolute ethanol		For Positive Controls: Not stated		GLP Compliance: Yes		
Treatment: Iloperidone from 100 to 1000 µg/mL for 4 hours, followed by a 7-day expression period.						Date of Treatment: April 26, 1991		
Cytotoxic Effects: None.								
Genotoxic Effects: None.								
Metabolic Activation	Test Article	Concentration (µg/mL)	Survival to treatment		Relative Population Growth (% of control)	Total Mutant Colonies	Absolute CE (±SD)	Mutant Frequency in 10 ⁶ Units*
			Mean Colony No. (±SD)	Percent Vehicle Control				
Without Activation	Vehicle control	0	231.3 ± 7.4	104.3	103.4	19	112.5 ± 1.5	7.0
	Vehicle control	0	212.3 ± 19.1	95.7	96.6	11	95.2 ± 7.2	4.8
	Iloperidone	100	207.0 ± 18.3	93.3	118.3	11	108.0 ± 1.3	4.2
		200	189.3 ± 13.3	85.3	112.1	9	122.8 ± 8.6	3.1
		400	189.3 ± 13.3	85.3	117.0	18	123.0 ± 4.4	6.1
		600	194.7 ± 17.0	87.8	122.7	12	114.0 ± 3.8	4.4
		800	183.3 ± 9.8	82.6	138.6	1	103.9 ± 7.1	0.4
		1000	214.3 ± 11.0	96.6	111.8	20	122.2 ± 8.2	6.8
BrdU	50	187.3 ± 6.8	84.4	52.5	358	110.9 ± 5.1	134.5*	
With Activation	Vehicle control	0	274.3 ± 16.5	109.4	103.3	14	118.9 ± 8.9	4.9
	Vehicle control	0	227.0 ± 4.6	90.6	96.7	5	118.7 ± 2.5	1.8
	Iloperidone	100	200.7 ± 15.9	80.1	104.3	9	115.5 ± 3.5	3.2
		200	213.7 ± 11.2	85.3	104.5	17	120.7 ± 4.2	5.9
		400	192.0 ± 10.6	76.6	122.2	16	115.5 ± 10.1	5.8
		600	200.0 ± 15.0	79.8	97.6	18	119.7 ± 15.8	6.3**
		800	237.7 ± 24.5	94.8	117.9	24	106.2 ± 6.7	9.4***
		1000	206.7 ± 11.7	82.5	109.9	12	122.2 ± 6.8	4.1
3-MCA	5	223.3 ± 16.4	89.1	65.8	1184	114.3 ± 5.9	431.6*	

* Mutant frequency = total mutant colonies/(no. of dishes × 2 × 10⁵ × absolute CE).

* P < 0.01 by Kastenbaum Bowman test and mutant frequency ≥ 15 × 10⁻⁶.

** P < 0.05 by Kastenbaum Bowman test but mutant frequency is within acceptable background range (<15 × 10⁻⁶).

*** P < 0.01 by Kastenbaum Bowman test but mutant frequency is within acceptable background range (<15 × 10⁻⁶).

Note: The results of Trial 1 are presented. The results of Trial 2 confirmed the results of Trial 1.

3-MCA = 3-methylcholanthrene; BrdU = bromodeoxyuridine; CE = cloning efficiency; NA = not applicable; SD = standard deviation.

Based on these findings, the sponsor considered iloperidone as negative for mutagenicity in CHO cells under both non-activation and activation conditions in this study. However, in the absence of mutagenic activity, the study did not meet the necessary acceptance criteria, i.e.,

- For test articles with little or no mutagenic activity, an acceptable assay should include applied concentrations that reduce the clonal survival to approximately 10% to 15% of the average of the vehicle controls, reach the maximum applied concentrations given in the evaluation criteria, reach a concentration that is approximately twice the solubility limit of the test article in culture medium or include a high concentration that is at least 75% of an excessively toxic concentration. There is no maximum toxicity requirement for test articles which clearly show mutagenic activity.

(as cited from the sponsor).

Conclusion: The test did not provide positive results for induction of forward mutations at the HGPRT locus in Chinese hamster ovary cells with or without metabolic activation. However, all of the tested concentration levels were non-toxic to CHO cells in culture either with or without metabolic activation. For test articles with little or no mutagenic activity, an acceptable assay should include applied concentrations that reduce the clonal survival to approximately 10% to 15% of the average of the vehicle control. Since none of the tested concentration levels were toxic to CHO cells, the study acceptance criteria have not been met and the study is not considered valid.

Induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells (Study 1463/63-D5140)

Study design: The possible genotoxicity of iloperidone (Batch 98908) was evaluated in a chromosomal aberration study conducted in CHO cells. The study was GLP-compliant. Three independent experiments were conducted. In experiment 1, cells were cultured for 3 hours in growth medium containing up to 64.57 µg/mL in the absence of S9 and up to 88.57 µg/mL in the presence of S9, followed by a 17-hour recovery period prior to harvest. In experiment 2, cells were cultured for 20 hours in the absence of S9 only, up to a highest iloperidone dose of 31.06 µg/mL. In experiment 3, cells were cultured for 20 hours in the presence of S9 only, up to a highest iloperidone dose of 98.41 µg/mL. Positive control: 4-nitroquinolone 1-oxide and cyclophosphamide in the absence and presence of S9, respectively.

Results: The results of this study are summarized in the sponsor's table on the next page. The results were previously reviewed by Lois Freed, Ph.D. under IND 36827 (Pharmacology/Toxicology Memorandum of 5/13/1999 re Amendment N-123, dated 4/27/99). Excerpts from Dr. Freed's review are reproduced below:

For the current *in vitro* chromosomal aberration assay, the data in the absence of metabolic activation (S9) are summarized in the following sponsor's tables below. For the 3-hr treatment, there was a 9-fold increase in the total number of cells with aberrations at the HC in the absence of S9. The HC was associated with a 65% decrease in cell count. At the 20-hr treatment, there was only a tendency for the total number of cells with aberrations to be increased. At the HC used, there was a 50% decrease in mean cell count. [Individual replicate data for the positive control excluding gaps were 20-15 (total = 35) and 44-37 (total = 81) cells for the 3- and 20-hr treatment times, respectively.] As noted, the sponsor considered ILO522 to be negative in the absence of S9; however, at least at the 3-hr treatment time, the number of cells with aberrations was increased. Although not a strong signal, this increase cannot be dismissed.

Clear increases in cells with aberrations were observed in the presence of S9 at 88.57 µg/mL in the presence of S9 in Exp. 1 and 3 (3-hr treatment), and at 98.42 µg/mL (7-fold) in Exp 3. [Individual replicate data excluding gaps were as follows: Exp 1, +S9: 11 and 11 (total = 22) cells at 88.57 µg/mL; Exp 3, +S9: 50 and 13 (total = 63) cells at 88.57 µg/mL and 60 and 53 (total = 113) cells at 98.42 µg/mL. Comparable data for the positive control: 33 and 34 (total 67) cells in Exp 1, 75 and 51 (total = 126) cells in Exp 3.] These increases were associated with reductions in mean cell count of 48-60 and 50%, respectively. The positive effects observed at the 3-hr treatment time (with ILO522; Exp 3) increased with concentration, but the increase far exceeded in magnitude the small (11%) increase in concentration.

The results indicate that IL0522 was equivocally positive in the absence of metabolic activation and reproducibly positive in the presence of metabolic activation. The sponsor emphasized that these positive effects were observed at "...strongly cytotoxic concentrations..." First, the concentrations associated with clastogenic effects were not "strongly cytotoxic". According to the OECD guidelines (1994), a > 50% decrease in mitotic index, cell confluency, or cell count should be observed at the HC. Only the HCs used in Exp 1 met this level of "...significant reduction..." (OECD guidelines, 1994). Regardless, the degree of cytotoxicity cannot be used in this assay to mitigate a positive response.

(End citation)

Report Title: Induction of chromosomal aberrations in cultured Chinese Hamster Ovary (CHO) cells				Test Article: Iloperidone	
Test for Induction of: Chromosomal aberrations		No. of Independent Assays: 3		Study No. 1463/63-DS140	
Strains: Chinese Hamster Ovary cell line		No. of Replicate Cultures: 2			
Metabolizing System: Aroclor-induced rat liver S9		No. of Cells Analyzed/Culture: 100, 200 total			
Vehicles:		For Test Article: Acetone	For Positive Controls: DMSO	GLP Compliance: Yes	
Treatment: Experiment 1: cells treated with iloperidone with and without activation for 3 hours and sampled at 20 hours; Experiment 2: cells treated with iloperidone without activation only for 20 hours; Experiment 3: cells treated with iloperidone with activation only for 20 hours.				Date of Treatment: Not stated	
Cytotoxic Effects: Experiment 1: 61% and 55% reduction in cell number seen at highest dose without and with activation, respectively; Experiment 2: 45% reduction in cell number seen at highest dose; Experiment 3: 57% reduction in cell number at highest dose.					
Genotoxic Effects: None under nonactivation conditions; iloperidone induced chromosome aberrations when tested at high, strongly cytotoxic concentrations.					
Experiment (Exp)	Test Article	Concentration (µg/mL)	Cells with Aberrations Including Gaps (per 200 cells scored)	Cells with Aberrations Excluding Gaps (per 200 cells scored)	Mean Cell No. × 10 ⁶
Expt 1: 3-hour treatment, 17-hour recovery without activation	Vehicle	0	2	1	4.0
	Iloperidone	34.32	5	3	3.2
		47.07	5	4	2.5
		64.57	13	9	1.4
	4-nitroquinoline 1-oxide	0.125	39*	35*	NR
Expt 1: 3-hour treatment, 17-hour recovery with activation	Vehicle	0	2	2	3.4
	Iloperidone	47.07	2	2	3.7
		64.57	5	2	2.4
		88.57	32*	22*	1.6
	Cyclophosphamide	12.5	85*	67*	NR
Expt 2: 20-hour treatment without activation	Vehicle	0	3	2	3.2
	Iloperidone	19.07	5	4	2.1
		26.4	3	3	1.5
		31.06	5	5	1.6
	4-nitroquinoline 1-oxide	0.25	82*	81*	NR
Expt 3: 20-hour treatment with activation	Vehicle	0	22	16	2.0
	Untreated	0	8	6	3.1
	Iloperidone	71.74	4	3	2.8
		88.57	65*	63*	1.1
		98.41	113*	113*	1.0
Cyclophosphamide	25	126*	126*	NR	

* $P \leq 0.001$ versus historical controls by Fisher's exact test.

NR = not reported.

Induction of chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells by micronized iloperidone (Study 1463/70)

Study design: The possible genotoxicity of micronized iloperidone (Batch 9929011) was evaluated in this GLP chromosomal aberration study conducted in CHO cells. Cells were cultured for 3 hours in growth medium containing up to 64.12µg/mL in the absence of S9 and up to 88.74µg/mL in the presence of S9, followed by a 17-hour recovery period prior to harvest. Positive control chemicals were 4-nitroquinolone 1-oxide and cyclophosphamide in the absence and presence of S9, respectively.

Results: Micronized iloperidone induced chromosomal aberrations in cultured CHO cells following treatment in both the presence and absence of S9. The effects were observed over a dose range of 46.3 to 64.1µg/ml and 75.4 to 88.7µg/ml in the absence and presence of S9, respectively. The maximal reduction of cell number at the highest concentrations of micronized iloperidone employed in this study was 63% and 68%, in the absence and presence of metabolic activation, respectively. Having in mind that according to ICH S2A guidelines, the highest concentration in mammalian cell mutation tests should produce at least 80% toxicity (no more than 20% survival); this reviewer does not agree with the sponsor that the positive results in the chromosomal aberration assays in CHO cells should be ascribed to excessive cytotoxicity. More importantly, the mean cell survival at the lowest dose positive for chromosomal aberrations was 40-44%.

A summary of the study results is presented in the following sponsor's table:

Report Title: Induction of chromosomal aberrations in cultured Chinese Hamster Ovary (CHO) cells				Test Article: Iloperidone (micronized)	
Test for Induction of Chromosomal aberrations		No. of Independent Assays: 1		Study No. 1463/70	
Strains: Chinese Hamster Ovary cell line		No. of Replicate Cultures: 2			
Metabolizing System: Aroclor-induced rat liver S9		No. of Cells Analyzed/Culture: 100, 200 total			
Vehicles:		For Test Article: Acetone	For Positive Controls: DMSO	GLP Compliance: Yes	
Treatment: Cells treated with iloperidone with and without activation for 3 hours and sampled at 20 hours				Date of Treatment: Not stated	
Cytotoxic Effects: 63% and 66% reduction in cell number at highest dose under nonactivation and activation conditions, respectively.					
Genotoxic Effects: Micronized iloperidone induced chromosome aberrations at cytotoxic concentrations.					
Treatment	Test Article	Concentration (µg/mL)	Cells with Aberrations Including Gaps (per 200 cells scored)	Cells with Aberrations Excluding Gaps (per 200 cells scored)	Mean Cell No. x 10 ⁶
3-hour treatment, 17-hour recovery without activation	Vehicle	0	1	0	2.7
	Iloperidone	28.45	1	0	2.0
		46.32	16*	15*	1.5
		64.12	37*	36*	1.0
3-hour treatment, 17-hour recovery with activation	4-nitroquinoline 1-oxide	0.25	63*	61*	NR
	Vehicle	0	0	0	2.5
	Iloperidone	54.50	1	1	2.0
		75.43	15*	15*	1.5
88.74		72*	68*	0.8	
	Cyclophosphamide	12.5	93*	91*	NR

* P ≤ 0.001 versus historical controls by Fisher's exact test.
NR = not reported.

2.6.6.4.3. In vivo mammalian system

Mutagenicity test on HP 873 in vivo mammalian micronucleus assay (Study 14476-0-455)

Study design: A micronucleus test on iloperidone (lot RC 4537) was performed in ICR mice to investigate the potential to induce micronuclei in bone marrow polychromatic erythrocytes (PCEs). A total of 130 animals were treated (5 per sex per group). Iloperidone was suspended in corn oil and administered by oral gavage at 5, 16.7, and 50 mg/kg. The dose selection was based

on the results of a preliminary dose range-finding study (Study 14476-0-459PO) in the same species and strain at doses 10, 55, 100, 150, and 200 mg/kg administered by the same route; severe toxicity was observed at 55 mg/kg. Vehicle and positive control (cyclophosphamide) groups were euthanized approximately 24 hours after dosing. Animals treated with iloperidone were euthanized approximately 24, 48, and 72 hours after dosing for extraction of the bone marrow. One thousand PCE per animal were scored. The frequency of micronucleated cells (MNC) was expressed as %MNC based on the total PCE in the scored optic field. The study was GLP-compliant. The criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a statistically significant dose response, nor a statistically significant and reproducible increase at one dose level was considered negative.

Results: Signs of toxicity were present at all tested doses in a dose-dependent manner. Iloperidone did not induce a significant increase in micronucleated polychromatic erythrocytes over the concurrent vehicle control levels in either sex or any of the harvest times, except in the males from the HD group at 72 h. harvest. This was attributed to the low vehicle control values for the males. Compared to the historical control values for males (i.e., 0.08%±0.04%), the 72-h. values at HD were not significantly increased. None of the animals in the HD group exceeded the normal frequency of micronuclei in this mouse strain (0.0-0.4%). The positive control (cyclophosphamide) induced significant increases in micronucleated PCE in both sexes, with mean values of 1.62% and 1.08% for males and females, respectively. The results are summarized in the sponsor's table below.

Report Title: Mutagenicity test on HP 873 - In vivo mammalian micronucleus assay		Test Article: Iloperidone					
Test for Induction of: Bone-marrow micronuclei		Treatment Schedule: Acute dose			Study No. 14476-0-455		
Species/Strain: ICR mice		Sampling Time: 24, 48, and 72 hours after dose			Location in CTD: Vol. Page		
Age: 8 weeks		Method of Administration: Oral gavage					
Cells Evaluated: Polychromatic erythrocytes		Vehicle/Formulation: Corn oil suspension			GLP Compliance: Yes		
No. of Cells Analyzed/Animal: 1000/animal		Date of Dosing: April 16, 1991					
Special Features: None.							
Toxic/Cytotoxic Effects: All doses, clinical signs (anquid or prostrate); remaining animals appeared normal by 48 hours and remained healthy until harvest.							
Genotoxic Effects: None.							
Evidence of Exposure: Clinical signs at all doses.							
Test Article	Dose (5/sex/dose) (mg/kg)	Harvest Time (hours)	Mean % MN PCEs (±SE)			Ratio PCE:NCE (±SE)	
			Males	Females	Total	Males	Females
Vehicle	0	24	0.04 ± 0.02	0.08 ± 0.04	0.06 ± 0.02	0.42 ± 0.06	0.67 ± 0.17
Iloperidone	5	24	0.04 ± 0.04	0.10 ± 0.05	0.07 ± 0.03	0.33 ± 0.08	0.95 ± 0.24
		48	0.12 ± 0.05	0.10 ± 0.05	0.11 ± 0.03	0.43 ± 0.11	0.92 ± 0.21
		72	0.10 ± 0.03	0.08 ± 0.02	0.09 ± 0.02	0.55 ± 0.03	0.95 ± 0.14
		16.7	24	0.06 ± 0.02	0.08 ± 0.06	0.07 ± 0.03	0.34 ± 0.07
		48	0.10 ± 0.04	0.06 ± 0.02	0.08 ± 0.02	0.43 ± 0.07	0.56 ± 0.17
		72	0.06 ± 0.04	0.04 ± 0.02	0.05 ± 0.02	0.53 ± 0.13	0.78 ± 0.13
		50	24	0.04 ± 0.04	0.02 ± 0.02	0.03 ± 0.02	0.42 ± 0.07
		48	0.08 ± 0.04	0.06 ± 0.02	0.07 ± 0.02	0.40 ± 0.04	0.86 ± 0.18
		72	0.22 ± 0.05**	0.10 ± 0.03	0.16 ± 0.03	0.52 ± 0.14	0.43 ± 0.09
Cyclo-phosphamide	80	24	1.62 ± 0.34*	1.08 ± 0.12*	1.35 ± 0.19*	0.37 ± 0.13	0.68 ± 0.11

* Significantly greater than the corresponding vehicle control, P < 0.05 by Tukey's studentized range test.

** Significance due to low vehicle control values for males. Historical vehicle control data for males are 0.08 ± 0.04.

Conclusion: Iloperidone did not induce a significant increase in micronuclei in bone marrow PCEs in a valid mouse bone marrow micronucleus test and was considered negative under the conditions of this assay.

In vivo mouse micronucleus assay (Study 998068)

Study design: This GLP study was designed to evaluate iloperidone (Batch 98908) for in vivo clastogenic activity by detecting micronuclei in polychromatic erythrocyte cells in the bone marrow of male ICR mice. Iloperidone was suspended in corn oil and administered by oral gavage at 5, 16.7, and 50 mg/kg to 12 animals per dose level. Twelve vehicle control and 6 positive control (cyclophosphamide 80 mg/kg) animals were treated. Animals treated with iloperidone were euthanized approximately 24 and 48 hours after dosing for extraction of the bone marrow. At least 2000 PCEs per animal were analyzed for the frequency of micronuclei. Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 1000 erythrocytes tested for each animal. Plasma concentrations of iloperidone were determined in pooled control and dosed samples, collected 1.h post dose from 3 mice per dose (0 and 50 mg/kg) and pooled for each dose group.

Results: The test compound induced clinical signs of toxicity (prostration, ptosis, hypoactivity, hunched posture) at all dose levels in a dose-dependent manner; polypnea was noted at the HD. Iloperidone plasma concentration in the pooled dose group (50 mg/kg) sample was 1290 ng/ml, demonstrating that the animals were systemically exposed to the test compound. There was no statistically significant decrease in the polychromatic/normochromatic erythrocyte ratio, demonstrating that the test article was not cytotoxic to the bone marrow. Iloperidone did not induce a statistically significant increase in the frequency of micronucleated PCEs. Therefore, iloperidone was negative in the mouse bone marrow micronucleus assay under the testing conditions employed.

A summary of the design and findings of this study is presented in the following sponsor's table.

Report Title: In vivo mouse micronucleus assay		Test Article: Iloperidone		
Test for Induction of: Bone-marrow micronuclei	Treatment Schedule: Acute dose	Study No. 998068		
Species/Strain: ICR mice (males)	Sampling Time: 24 and 48 hours after dose	Location in CTD: Vol. Page		
Age: 8 weeks	Method of Administration: Oral gavage			
Cells Evaluated: Polychromatic erythrocytes	Vehicle/Formulation: Corn oil suspension	GLP Compliance: Yes		
No. of Cells Analyzed/Animal: 2000	Date of Dosing: May 19, 1999			
Special Features: None.				
Toxic/Cytotoxic Effects: Prostration and squinted eyes at all iloperidone dose levels; polypnea in all high-dose animals by 1-hour timepoint, slight hypoactivity in majority of low-dose animals on Day 1, hypoactivity and hunched posture sporadically in low- and high-dose animals and majority of mid-dose animals.				
Genotoxic Effects: None.				
Evidence of Exposure: Toxic signs seen in high-dose animals.				
Test Article	Dose (5 animals/group) (mg/kg)	Harvest Time (hr)	% MN PCEs (±SE)	Ratio PCE:NCE
Vehicle	0	24	0.06 ± 0.02	0.67 ± 0.07
	0	48	0.07 ± 0.05	1.20 ± 0.20
Iloperidone	5	24	0.06 ± 0.03	0.55 ± 0.03
		48	0.06 ± 0.04	0.84 ± 0.09
	16.7	24	0.06 ± 0.03	0.57 ± 0.05
		48	0.12 ± 0.05	1.15 ± 0.22
	50	24	0.09 ± 0.06	0.69 ± 0.07
		48	0.05 ± 0.02	0.76 ± 0.10
Cyclophosphamide	80	24	2.71 ± 0.33*	0.74 ± 0.06

*Significantly greater than the corresponding vehicle control, $P < 0.01$ by Dunnett's t-test.

MN = micronucleated; NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte; SE = standard error.

Conclusion: Iloperidone was negative in a valid mouse bone marrow micronucleus assay under the testing conditions.

Induction of micronuclei in rat liver in vivo (Study 1463/71-D5140)

Study design: A GLP micronucleus test was performed in male Wistar rats to investigate the potential of micronized iloperidone (Batch N 9929011) to induce micronuclei in rat liver in vivo. In a preliminary dose-finding study, iloperidone suspended in corn oil at doses of 10, 25, and 50 mg/kg was administered to rats 1 day prior and 1 day following partial hepatectomy; animals were observed for a 2-day period following the second administration. Based on the results, 10 mg/kg was identified as the maximum acceptable dose. For the micronucleus test, vehicle control (corn oil), positive control (dimethylnitrosamine), or micronized iloperidone at 5 and 10 mg/kg was administered by oral gavage to rats at 0 h. (dose 1) and 48 h. (dose 2). A total of 21 and 26 rats were treated (7 per iloperidone and vehicle administration; 5 per positive control administration) and subsequently euthanized at 72 and 96 hours after first administration. Partial hepatectomy was done at 24 hours after the first dose.

Results: A summary of the design and findings of this study is presented in the following sponsor's table.

Report Title: Induction of micronuclei in rat liver in vivo			Test Article: Iloperidone (micronized)		
Test for Induction of: Hepatocyte micronuclei		Treatment Schedule: 2 doses, 1 before and 1 after partial hepatectomy		Study No. 1463/71-D5140	
Species/Strain: Wistar rats (out-bred), males		Sampling Time: 72 and 96 hours after dose		Location in CID: Vol. Page	
Age: 7-8 weeks		Method of Administration: Oral gavage			
Cells Evaluated: Hepatocytes		Vehicle/Formulation: Corn oil suspension		GLP Compliance: Yes	
No. of Cells Analyzed/Animal: 2000			Date of Dosing: May 26, 1999		
Special Features: None.					
Toxic/Cytotoxic Effects: Clinical signs observed at both doses included lethargy, eye closure, prostration, hunched posture, piloerection, eye secretion; low body temperature observed at the 5-mg dose in the animals sampled at 96 hours; red staining on head seen at the 10-mg dose.					
Genotoxic Effects: None.					
Evidence of Exposure: Overt toxicity at both doses.					
Test Article	Dose (mg/kg)	No. of Animals	Micronucleated Hepatocytes	Micronucleated Cells/1000 Scored	Mitotic Index (mean)
72-hour sampling time					
Vehicle	0	5	17	1.70	0.91
Iloperidone (micronized)	5	5	9	0.90	0.84
	10	5	8	0.80	1.08
96-hour sampling time					
Vehicle	0	5	23	2.30	0.16
Iloperidone (micronized)	5	5	23	2.30	0.13
	10	5	15	1.50	0.05
DMN	10 ^a	3	104	17.33*	0.10

^a Single administration.

* $P \leq 0.001$ by chi-square test.

DMN = dimethylnitrosamine.

Slides from 5 animals were analyzed from each of the iloperidone-dosed groups and from the negative (vehicle) control group; slides from 3 animals were analyzed from the

positive control group. Negative control rats exhibited acceptable group mean frequencies of micronucleated hepatocytes that were within historical negative control ranges. Positive control animals exhibited increased numbers of micronucleated hepatocytes and the mean micronucleus frequency was significantly greater vs. concurrent control. In rats treated with iloperidone, the group mean frequencies of micronucleated hepatocytes were similar to and not significantly different from those seen in the concurrent vehicle controls at both the 72 h. and 96 h. sampling times. The frequencies of micronucleated hepatocytes in all treated groups were within the normal range. Micronized iloperidone did not induce micronuclei in the hepatocytes of male rats treated up to 10 mg/kg (2 doses), at which clinical signs of toxicity (prostration, ptosis, hunched posture) were observed.

Conclusion

This GLP study evaluated the ability of micronized iloperidone to induce micronuclei in rat hepatocytes in vivo upon oral (gavage) administration to male Wistar rats. The test compound did not induce micronuclei in the hepatocytes of male rats treated up to 10 mg/kg, at which clinical signs of toxicity were observed. Iloperidone was negative in a valid rat hepatocyte micronucleus assay under the testing conditions.

2.6.6.4.4. Genotoxicity studies with iloperidone metabolite P95

The potential genotoxicity of iloperidone metabolite P95 was evaluated in 3 studies: an Ames tested conducted in *S. typhimurium* (Study 991801), a chromosomal aberration test conducted in V79 CHO cells (Study 991831), and a bone marrow micronucleus test in rats administered orally up to 2000 mg/kg (Study 001887). P95 was found to be negative in all the three tests.

The specifications of the drug substance are listed in the sponsor's table below.

P95 Genotoxicity Studies

Batch/Lot No.	Drug Substance		Test Article: Iloperidone	
	Purity ^a	Specified Impurities (%)	Study No.	Type of Study
PROPOSED SPECIFICATION:		Each Total		
Batch 0038001	99.6%	Not stated	001887	Oral bone marrow micronucleus test in rats
Batch Versuch 5	99.9%	Not stated	991801	Mutagenicity test using <i>Salmonella typhimurium</i>
Batch Versuch 5	99.9%	Not stated	991831	Chromosomal aberration test in V79 Chinese hamster cells

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The studies are summarized in the following sponsor's table.

Test article Species/Strain	Method of Administration	Duration of Dosing	Doses	Test Article: Iloperidone metabolite P95			
				Gender and No. per Group	MTD or NOEL	Noteworthy findings	Study No.
<i>S. Typhimurium</i>	NA	NA	80-50,000 µg/mL	NA	NA	P95 showed no mutagenic potential in bacterial cells in the presence or absence of metabolic activation.	991801
CHO cell line	NA	NA	64.6-300 µg/mL	NA	NA	P95 did not induce chromosomal aberrations in CHO cells in the presence or absence of metabolic activation.	991831
Rat/ WI(G LX/BRL/HAN) IGS BR	p.o.	Acute (2 doses)	200, 630, 2000	M, F 5	NA	P95 showed no potential to cause chromosomal damage or to affect the spindle apparatus of rat bone marrow cells in vivo.	001887

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P95 Ames test (Study 991801)

Design: The tests were conducted with *S. typhimurium* strains TA 100, TA 1535, TA97a, TA 98, and TA 102. The experiments were carried out in the absence and presence of an S9-metabolizing system derived from rat liver homogenate. The test substances were suspended in DMSO. Negative controls included solvent and untreated cells and positive controls included sodium azide, 9-aminoacridine, 2-nitrofluorene or benzo(a)pyrene, 2-aminoanthracene and mitomycin C. P95 was tested at a dose range of 80 to 50,000 µg in a first experiment and 1875 to 30000 µg per plate in a second experiment.

Results: Treatment of cells with P95 did not result in relevant increases in the number of revertant colonies in either the presence or absence of metabolic activation. Therefore, this test article was not considered to be mutagenic in this bacterial test system.

P95 Chromosomal aberration test in V79 Chinese hamster cells (Study 991831)

Study design: P95 clastogenic potential was tested in vitro by determining its effects on chromosomal aberrations in V79 Chinese hamster cells in the presence and absence of rat liver S9. P95 concentrations tested ranged from 300 to 64.6 µg/ml with and/or without metabolic activation. Two independent experiments were conducted with a 3-hour treatment and 17-hour recovery and 1 experiment used a 20-hour continuous treatment with no recovery. Positive controls included cyclophosphamide and ethyl methanesulphonate with and without S9, respectively.

Results: P95 did not induce a concentration-dependent decrease in the mitotic index. In the presence of metabolic activation, no statistically significant increase in structural chromosomal aberrations was found. In the absence of metabolic activation after 3 hours of treatment, a statistically significant increase in structural chromosomal aberrations was seen at concentrations of 139.2 and 300µg/mL. However, the values (1.6% and 2%) were within the historical negative control range and the corresponding negative control had 0% aberrant cells. Thus, this effect did not indicate clastogenicity and was not biologically significant. After 20 hours of treatment without S9, an increase in structural chromosomal aberrations was found at concentrations of 191.2 and 146.2 µg/mL, where 5% aberrant cells were found, which was above the historical control range. However, the effect was not statistically significant and not reproducible, and was therefore concluded to be not biologically significant. No relevant increase in polyploidy cells was seen with or without metabolic activation.

In conclusion, P95 was not found to be clastogenic in the chromosomal aberration test with V79 Chinese hamster cells under the conditions of this study.

P95 Oral bone marrow micronucleus test in rats (Study 001887)

Study design: The potential of iloperidone metabolite P95 to induce micronuclei in polychromatic erythrocytes (PCEs) in the bone marrow was evaluated in — WI(GLX/BRL/HAN) IGS BR rats treated orally by gavage twice with an interval of 24 hours, and bone marrow was sampled 48 hours after the first application. P 95 doses of 200, 630, and 2000 mg/kg were chosen for the micronucleus test on the basis of a dose-finding experiment, in which treatment with 2000 mg/kg/d p.o. induced signs of toxicity such as ataxia, sedation, ptosis, piloerection, crouching, and labored respiration. Animals

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treated with 1% CMC served as negative controls; positive control animals were treated orally with cyclophosphamide.

Results: The percentages of PCE were 52.8%, 46.3%, 52.6%, and 45.8% in the vehicle control and 200, 630, and 2000 mg/kg groups, respectively, and the corresponding mean percentages of micronucleated PCE were 0.16%, 0.17%, 0.22%, and 0.16%, respectively. The positive control group had a mean percentage of micronucleated PCE of 3.42%. There were no statistically significant differences between the treated and negative control groups. Based on these findings, P95 administered orally to rats up to a dose of 2000 mg/kg was found to be negative in a valid bone marrow micronucleus mutagenicity test. This indicates that P95 has no apparent potential to cause chromosomal damage or to affect the spindle apparatus in rat bone marrow.

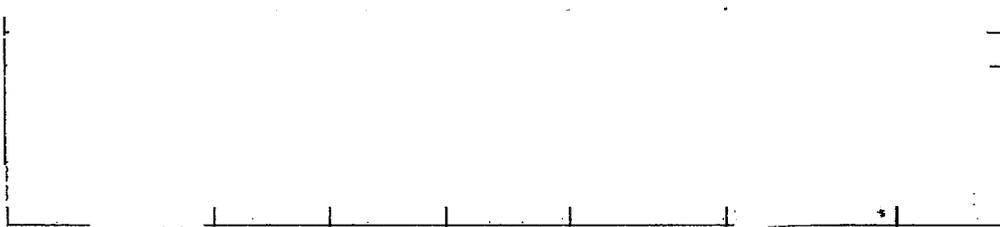
2.6.6.4.5. Genotoxic impurities

Among iloperidone impurities (as shown in sponsor's table below), — compounds had structures associated with potential genotoxicity: _____

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Codes	Chemical Name	Structure	Classification	Control Method and Limit of Detection
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Genetic toxicology summary and conclusions

Iloperidone tested negative for genotoxic potential in the following valid in vitro assays in both the presence and absence of metabolic activation: the salmonella/mammalian-microsome reverse mutation assay (Ames test) conducted in *Salmonella typhimurium* (Study 12048-0-401R), the *Escherichia coli*/mammalian-microsome reverse mutation assay (Study 14476-0-402R), and a chromosomal aberration assay conducted in Chinese Hamster Ovary (CHO) cells (Study 14476-0-437). Two additional chromosomal aberration assays in CHO cells evaluated the clastogenicity of iloperidone and micronized iloperidone (Study 1463/63-D5140 and Study 1463/70, respectively). Iloperidone was found to be equivocally positive in inducing chromosomal aberrations in CHO cells in the absence of metabolic activation and reproducibly positive in the presence of metabolic activation; micronized iloperidone was found to induce chromosomal aberrations in CHO cells under both metabolic activation and non-activation conditions; the effect was seen at concentrations claimed to be "excessively cytotoxic" by the sponsor. However, the concentrations associated with clastogenic effects were not "excessively cytotoxic". According to the OECD guidelines, a > 50% decrease in mitotic index, cell confluency, or cell count should be observed at the high concentration. The degree of cytotoxicity cannot be used in this assay to mitigate a positive response.

The maximal reduction of cell number attained at the highest employed iloperidone concentrations was 53% survival (in the presence of metabolic activation); the corresponding survival values attained at the highest concentrations of micronized iloperidone were 63% and 68%, in the absence or presence of metabolic activation, respectively. Having in mind that according to ICH S2A guidelines, the highest concentration in mammalian cell mutation tests should produce at least 80% toxicity (no more than 20% survival), we do not agree with the sponsor that the positive results in the chromosomal aberration assays in CHO cells should be ascribed to excessive cytotoxicity. More importantly, the mean cell survival at the lowest dose of iloperidone positive for chromosomal aberrations was 40-45%.

In vivo genotoxicity potential of iloperidone was evaluated in 4 studies. These included 3 bone marrow micronucleus assays conducted in mice, the first of which was a dose-finding assay of bone marrow cytotoxicity (Studies 14476-0-459PO, 14476-0-455, and 998068) and 1 hepatocyte micronucleus assay conducted in rats (Study 1463/71-D5140, micronized iloperidone). The results of these assays indicated that iloperidone did not induce a significant increase in bone marrow micronuclei in mice, nor did micronized iloperidone induce a significant increase in hepatocyte micronuclei in rats.

Iloperidone metabolite P95 was evaluated for potential genotoxicity and was found to be negative in a battery of 3 tests: an Ames tested conducted in *S. typhimurium* (Study 991801), a chromosomal aberration test conducted in V79 CHO cells (Study 991831), and a bone marrow micronucleus test in rats.

Among iloperidone impurities — compounds had structures associated with potential

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In conclusion, the results of in vitro and in vivo genotoxicity studies indicate that iloperidone was clastogenic in one in vitro test (chromosomal aberration assay in CHO cells) but was not clastogenic in vivo under the assay conditions used. It is likely that the positive results obtained in chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells in vitro are of little biological relevance, having in mind the negative results obtained in the in vivo micronucleus assays in rat hepatocytes and mouse bone marrow. Iloperidone metabolite P95 was negative for potential genotoxicity in a battery of 3 tests: an Ames, a chromosomal aberration test in CHO cells, and a bone marrow micronucleus test in rats. For iloperidone genotoxic and potentially genotoxic impurities (

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2.6.6.5 Carcinogenicity

The protocols of the 2-year carcinogenicity studies of iloperidone in rats and mice were approved by the Executive CAC and the doses were selected in accordance with the Executive CAC recommendations (Pharm/Tox Memorandum by Dr. L. Freed of 9/14/1994).

Study title: A 24-month oral oncogenicity study of ILO 522 in mice (Study No. 988053)

Key findings:

Iloperidone administration to mice at doses of 2.5, 5, and 10 mg/kg/d caused a decreased survival at HD (both genders) and in females from all treated groups. For this reason, the HD groups (M and F) were terminated at study week 82 when the survival was approximately 33% for both genders; all the remaining females were discontinued at study week 90 when the survival was 33% and 32% for LDF and MDF, respectively, vs. 63% and 65% in the control female groups. For the remaining males, the duration of dosing was 104 weeks, and the survival rate at study week 103 was 42% and 38% for LDM and MDM, respectively, vs. 50% and 48% in the control male groups. Mean body weight was slightly decreased in all dosed male groups (the average difference vs. control was -4% and the maximal difference was -7.4%) and increased in all dosed female groups (the largest difference vs. control was +13%). Mean food consumption was increased in all treated groups of both genders throughout the study.

An increased incidence of malignant mammary tumors was seen in females at the lowest dose only (23% vs. 0%, 2% in Control 1 and 2; 3% in MD and 2% in HD groups); the incidence seen at the low dose group was higher than the historical control range for this species and strain. On an mg/m² basis, there is no safety margin between the lowest dose employed in the mouse carcinogenicity study (2.5 mg/kg/day) and the maximal recommended dose in humans (24 mg/day). However, iloperidone administration showed no dose relationship to mammary tumorigenesis. Mammary tumor incidences were not increased in the mid- and high-dose groups, although the duration of treatment was the same in the mid-dose and low dose groups. Increased incidence of proliferative mammary changes (duct ectasia/ galactocele and glandular hyperplasia) was seen in females from all dose groups without dose dependence.

According to the statistical reviewer and in agreement with the sponsor, "there was not a single statistically significant tumor trend among either gender whether all treatment groups were used and censored at the time the high dose was terminated or whether the high dose was excluded from the analyses and the remaining groups were censored at their later terminal sacrifice".

The sponsor attributed the mammary gland changes, including the increased incidence of tumors at the low dose, to endogenous hormonal imbalances (increased prolactin secondary to pharmacological inhibition of the dopamine receptor). Elevated prolactin and increased incidence of non-neoplastic proliferative mammary changes (duct ectasia/ galactocele and glandular hyperplasia) as well as pharmacological clinical signs were seen in females from all dose groups without dose dependence. Prolactin levels were increased in both genders; a dose-response was apparent for males but not for females. Iloperidone administration showed no dose relationship to mammary tumorigenesis in the mouse.

Drug-related non-neoplastic lesions were observed in all treated groups and were noted in female mammary gland (proliferative mammary changes, i.e., glandular hyperplasia and

duct ectasia/galactocele), uterus (uterine adenomyosis), in the heart (cardiomyopathy and/or atrial thrombosis) and in the lung (chronic interstitial inflammation/fibrosis and alveolar macrophages). Generally, the incidences of these findings did not increase dose-dependently; this was considered to be due to the decreased survival in the high dose group, resulting in less time for development of the lesions.

The MTD in the mouse study was exceeded in the females based on the decreased survival in all iloperidone-treated female groups as compared to the control group. For the males, the MTD was 5 mg/kg/day based on a significant mortality increase at the next tested dose level of 10 mg/kg/day. Since a decreased survival was induced even at the lowest tested dose, an NOAEL for iloperidone administration was not reached in this study, having in mind that the lowest tested dose (2.5 mg/kg/d) induced increased mortality (in females) and pathological cardiac and pulmonary non-neoplastic lesions in both genders.

**APPEARS THIS WAY
ON ORIGINAL**

Study title: A 24-month oral oncogenicity study of ILO 522 in mice (Study No. 988053)

Volume and page: electronic submission

Conducting laboratory and location: _____

Date of study initiation: January 17, 1995

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Test article: Iloperidone (ILO522, HP 873)

Lot number: RC 5842

Purity: 99.4-99.8%

Vehicle control: 2% aqueous starch

Methods

Doses: 0, 0, 2.5, 5 and 10 mg/kg/day (males); 0, 0, 2.5, 5 and 10 mg/kg/day (females).

Basis of dose selection: The selection of doses for the 2-year study was based on the 13-week repeated-dose study and it was in accordance with the Executive CAC recommendations (IND 36827, Pharm/Tox Memorandum by Dr. L. Freed of 9/14/1994)

Species/strain: Mouse — CD-1[®](ICR)BR

Number/sex/group (main study): 60/sex/dose group

TK study: 24 sex/dose group

Prolactin determination: 10 sex/dose group

Route, formulation, volume: oral gavage, suspension in vehicle (2% aqueous starch), dose volume: 2.5, 5 and 10 ml/kg (for iloperidone LD, MD and HD, respectively) and 10 ml/kg for control groups

Frequency of dosing: Daily

Treatment duration: 104 weeks (male)/ 90 weeks (female); HD terminated at 82 weeks (M, F)

Satellite groups used for toxicokinetics: 24 animals/sex/group (blood samples collected from 3- 4 animals/sex/group at each time point)

Age: 6 weeks at dosing initiation

Animal housing: 3 animals per sex per cage in wire-mesh suspended cages in a controlled environment.

Restriction paradigm for dietary restriction studies: non applicable

Drug stability/homogeneity: Dose form: suspension; Stable under the storage conditions used in these studies; Homogeneous under the conditions used in these studies

Dual controls employed: Two control groups 60 mice/sex each

Interim sacrifices: At study week 82 for HD group (M and F) and at study week 90 for the remaining female groups

Deviations from original study protocol: Interim sacrifices due to decreased survival. These changes did not adversely affect the study integrity.

Observation times:

Live Phase

Observations: Daily for mortality and morbidity; weekly for detailed physical examinations and palpable masses.

Body weights: Weekly

Food consumption: Weekly for the first 14 weeks and biweekly thereafter

Clinical Pathology

Hematology: Blood samples taken from all animals euthanized at extremis, from all surviving HD animals at week 82 interim necropsy, and from 10 mice/sex/group at week 90 interim (female) necropsy and week 104 terminal (male) necropsy.

Prolactin determination: At study week 4, 10 animals/sex from all dosed groups and control.

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Morphologic Pathology:

Macroscopic examination: A complete necropsy was performed on all animals in the main study (60/sex/group). The following tissues and organs were collected and preserved in 10% neutral buffered formalin:

Tissues collected for histopathology evaluation

Adrenals (2)	Mammary gland (females only)
Aorta	Ovaries with oviducts (2)
Bone with marrow (femur*)	Pancreas
Brain (forebrain, midbrain, hindbrain)	Peripheral nerve (sciatic)
Eyes with optic nerve (2)	Pituitary
Gallbladder	Prostate
Gastrointestinal tract	Salivary glands [submandibular (2)]
Esophagus	Seminal vesicles (2)
Stomach	Skeletal muscle (vastus medialis)
Duodenum	Skin
Jejunum	Spinal cord (thoracic)
Ileum	Spleen
Cecum	Testes with epididymides(2)
Colon	Thymus (if present)
Rectum	Thyroid [with parathyroids if present (2)]
Heart	Trachea
Kidneys (2)	Urinary bladder
Liver (sections of two lobes)	Uterus with vagina
Lungs (including bronchi, fixed by inflation with fixative)	All gross lesions
Lymph nodes	
Mandibular	
Mesenteric	

* = Bone marrow smears were collected from 10 mice/sex/group at the scheduled necropsies (study weeks 82, 90 and 104). Slides were prepared but not placed in formalin. Evaluation of myeloid:erythroid ratios from bone marrow smears (200 cell count) were performed by _____

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Histopathology: Conducted on a full list of tissues from all main study animals from the control and treated groups and all unscheduled deaths. Tissues were evaluated by light microscopy. Pathology peer review was conducted.

Toxicokinetics: Blood collection prior to dosing and at 0.5, 1, 2, 4, and 8 hours post-dose on study day 185 from 3 to 4 mice/sex from LD, MD and HD TK groups

Results

Mortality:

Sponsor's analysis

Drug-related decreased survival, more pronounced in the females, was noted in all treated groups. For this reason, the HD groups (M and F) were terminated at study week 82 when the survival was approximately 33% for both genders; all the remaining females were discontinued at study week 90 when the survival was 33% for LDF and MDF, vs. 63% and 65% in the control female groups. For the remaining males, the duration of dosing was 104 weeks, and the survival rate at study week 103 was 42% and 38% for LDM and MDM, respectively, vs. 50% and 48% in the control male groups. The cause of death was not determined for the majority of cases. Some early deaths were ascribed to intubation trauma. Atrial thrombosis was considered to be the cause of death for 2/31, 2/33, 7/35, 10/37 and 9/39 males and 0/22, 1/21, 3/40, 8/41 and 8/40 females in the 0, 0, 2.5, 5, and 10 mg/kg/day groups, respectively. The sponsor considered this increase in

the incidence of death caused by atrial thrombosis as test-article related. Survival at study weeks 51, 77, 81, 89 and 103 is presented in the following sponsor's table.

Survival at study weeks 51, 77, 81, 89 and 103
Number and percentage of animals surviving (N=60/group)

GROUP	MALES					FEMALES				
	1	2	3	4	5	1	2	3	4	5
STUDY WEEK										
51	50 83%	52 87%	49 82%	48 80%	43 72%	52 87%	55 92%	51 85%	50 83%	40 67%
77	48 80%	44 73%	47 78%	39 65%	26 43%	44 73%	48 80%	38 63%	33 55%	22 37%
81	47 78%	42 70%	46 77%	37 62%	21bd 35%	43 72%	46 77%	35 58%	30ad 50%	21bd 35%
89	44 73%	40 67%	37 62%	32 53%	NA	38 63%	39 65%	20bd 33%	20bd 33%	NA
103	30 50%	29 48%	25 42%	23 38%	NA	NA	NA	NA	NA	NA

a = Significantly different from control group 1 at 0.05 using the RXC Chi-square test
b = Significantly different from control group 1 at 0.01 using the RXC Chi-square test
d = Significantly different from control group 2 at 0.01 using the RXC Chi-square test

Statistical reviewer's analysis (as cited from the review of Roswitha Kelly):

Due to the early termination of the high dose animals but not of one of the control groups, the reviewer performed two sets of analyses: one (per gender) where all animals were used but all were censored at the time of the terminal sacrifice of the high dose animals, and one (again per gender) where the high dose was excluded and the remaining animals analyzed using their terminal sacrifice time.

The reviewer used the sponsor's SAS transport file for mice to analyze the mortality and tumor data of the female gender. Compared to the sponsor's Table 1 in their Final Report, she observed identical numbers of animals surviving to various study weeks and until the early terminal sacrifice and very similar numbers for the animals living to the late sacrifice. In female mice, the tests for increased mortality with dose were highly statistically significant when all animals were censored at the time when the high dose was terminated (as shown in the reviewer's table on the next page). When the high dose is excluded, there still remained a highly statistically significant negative effect on survival of the low and mid-dose animals, which confirms the sponsor's statement that 'Test article-related reductions in survival were noted in all treated groups and were more pronounced in the female groups.' In male mice, when the high-dose animals were included and all animals censored at time of their (the high-dose's) termination, the trend tests in mortality were highly statistically significant ($p=0.0000$) (as shown in the reviewer's table on the next page). When the high dose male animals were excluded from the mortality analyses, the trend tests were statistically significant only at $\alpha=0.05$.

(Therefore), when all animals were used, the increase in mortality with dose was highly statistically significant for both the male and female mice. When the high dose animals were excluded from the analyses, the trends for increase in mortality among the male mice were now statistically significant at only $\alpha=0.05$ whereas for the females the high level of significance did essentially not change.

The sponsor concluded that the MTD was exceeded based on the decreased survival in all treated groups compared to the control groups. The statistical reviewer agreed with this statement with respect to the female mice.

In the P/T reviewer's opinion, an MTD in male mice was reached at the dose level of 5 mg/kg/day based on the highly statistically significant increase in mortality at the next tested dose level (10 mg/kg/day).

FDA Statistical Reviewer's Mortality Table

Female Mice

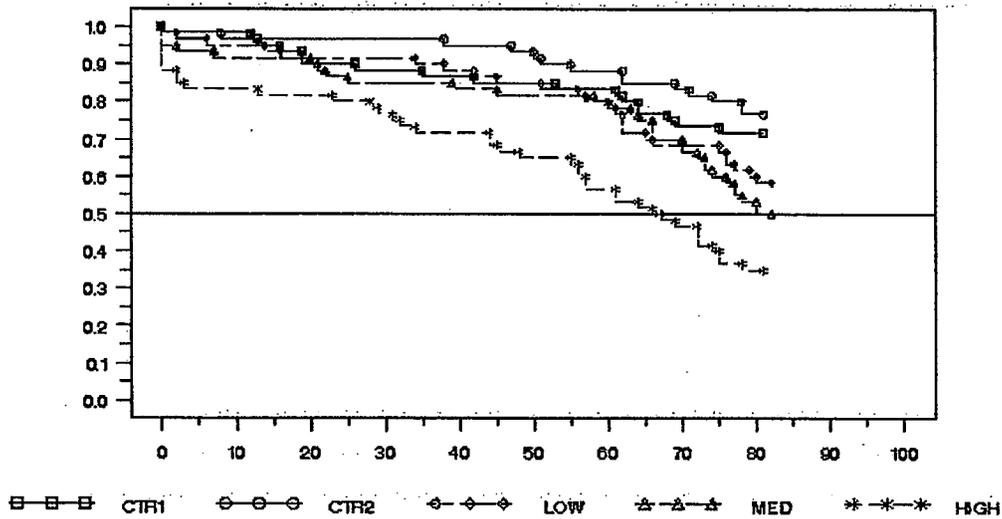
	Analysis of Mortality	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
	0-52	60	8	52	86.7	13.3
CTR1	53-78	52	8	44	73.3	26.7
	79-82	44	1	43	71.7	28.3
	FINALKILL 83-91	43	0	43		
	0-52	60	5	55	91.7	8.3
CTR2	53-78	55	7	48	80.0	20.0
	79-82	48	2	46	76.7	23.3
	FINALKILL 83-91	46	0	46		
	0-52	60	9	51	85.0	15.0
LOW	53-78	51	13	38	63.3	36.7
	79-82	38	3	35	58.3	41.7
	FINALKILL 83-91	35	0	35		
	0-52	60	10	50	83.3	16.7
MED	53-78	50	17	33	55.0	45.0
	79-82	33	3	30	50.0	50.0
	FINALKILL 83-91	30	0	30		
	0-52	60	20	40	66.7	33.3
HIGH	53-78	40	18	22	36.7	63.3
	79-82	22	1	21	35.0	65.0
	FINALKILL 83-91	21	0	21		

Male Mice

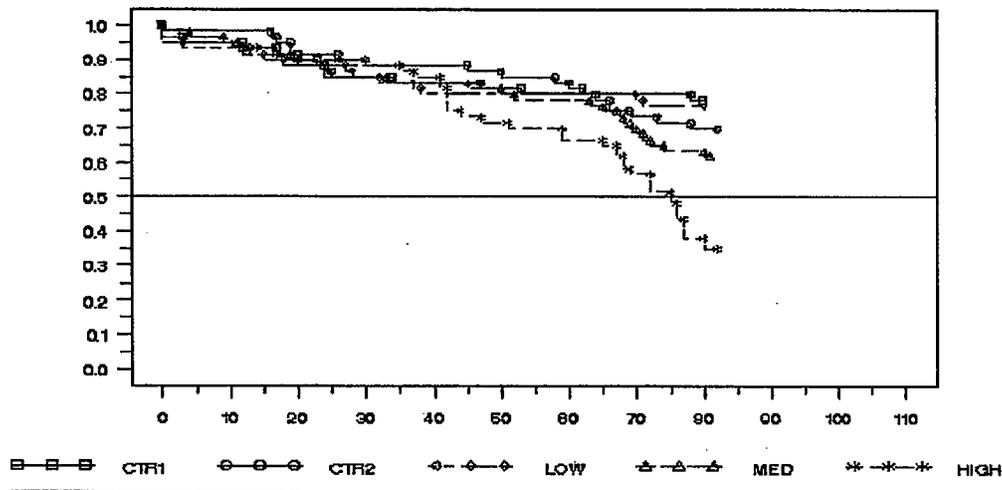
	Analysis of Mortality	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
	0-52	60	10	50	83.3	16.7
CTR1	53-78	50	2	48	80.0	20.0
	79-82	48	1	47	78.3	21.7
	FINALKILL 83-105	47	0	47		
	0-52	60	8	52	86.7	13.3
CTR2	53-78	52	9	43	71.7	28.3
	79-82	43	1	42	70.0	30.0
	FINALKILL 83-105	42	0	42		
	0-52	60	11	49	81.7	18.3
LOW	53-78	49	2	47	78.3	21.7
	79-82	47	1	46	76.7	23.3
	FINALKILL 83-105	46	0	46		
	0-52	60	12	48	80.0	20.0
MED	53-78	48	9	39	65.0	35.0
	79-82	39	2	37	61.7	38.3
	FINALKILL 83-105	37	0	37		
	0-52	60	17	43	71.7	28.3
HIGH	53-78	43	17	26	43.3	56.7
	79-82	26	5	21	35.0	65.0
	FINALKILL 83-105	21	0	21		

Kaplan-Meier Survival Curves

Female Mice



Male Mice



Clinical findings: The predominant clinical findings were mainly pharmacological in nature (e.g., ptosis, lacrimation, relaxed scrotum/vaginal opening, hypoactivity), were noted one hour following dosing and were observed in all treated groups during the first 6-12 months of study, without a clear dose-dependence.

Body weight: Mean body weight was decreased in all dosed male groups (the average difference vs. control was -4% and the maximal difference was -7.4%) and increased in all dosed female groups (the largest difference vs. control was +13%). In males, the mean body weights were decreased at the HD vs. control during study weeks 1-3 and from week 22 through week 82 interim necropsy; the MD and LD male groups had sporadic decreases in mean body weight vs. control during study weeks 22-60. In females, the mean body weights were increased in all dosed groups from study week 2 through week 78.

Mean body weight gains in the treated male groups were reduced vs. control values during the 1st week and in HDM also during study weeks 13 – 15, and similar to control from study week 15 to the end of the study; in females, after initial transient decrease in HDF during the 1st week of study, body weight gains were increased in all treated female groups from week 2 through 4, and similar to control thereafter.

The mean body weight changes from interval 0 (the initiation of dosing) in the treated groups compared to control group 1 are shown in the following sponsor's table:

Body weight changes from interval 0 (the initiation of dosing) in the treated groups compared to control group 1

Weeks	Dosage Level (mg/kg/day)					
	Males			Females		
	2.5	5.0	10.0	2.5	5.0	10.0
13	-6%	-6%	-2%	+51%	+40%	+53%
26	-11%	-11%	-16%	+32%	+29%	+36%
52	-12%	-13%	-17%	+25%	+19%	+22%
78	+1%	+0%	-7%	+21%	+20%	+13%
82	+0%	-2%	-9%	+22%	+18%	+20%
90	-5%	-4%	NA	+17%	+16%	NA
104	+0%	+7%	NA	NA	NA	NA

Mean food consumption tended to be increased in all treated groups (both genders) vs. control (as illustrated by the following sponsor's table). A transient significant decrease occurred in HDM during the 1st week, correlating with the body wt loss; however, significant increases in food consumption vs. control became apparent at study week 9-10 in males and week 8-9 in females, continuing throughout the study.

PROJECT NO. 3
SPONSORING PARTY
SPONSOR NO. US 288883

A 24-MONTH ORAL TOXICOLOGIC STUDY OF ILORES IN MICE
WEEKLY FOOD CONSUMPTION (GRAMS/ANIMAL/DAY) - SUMMARY OF MEANS

PM

GROUP:	M A L E					
	0 MG/KB/DAY	0 MG/KB/DAY	2.5 MG/KB/DAY	5.0 MG/KB/DAY	10.0 MG/KB/DAY	
WEEK 36 TO 38						
MEAN	4.7	4.7	4.9	5.1	NA	
S.D.	0.62	0.78	0.71	0.58		
N	40	35	33	28		
36 TO 37						
MEAN	4.7	4.6	4.8	5.2b,d	NA	
S.D.	0.69	0.56	0.78	0.69		
N	39	34	33	26		
38 TO 39						
MEAN	4.7	4.7	4.9	5.1a	NA	
S.D.	0.71	0.63	0.87	0.63		
N	37	34	31	28		
100 TO 101						
MEAN	4.7	4.5	4.9	5.2nd	NA	
S.D.	0.62	0.52	0.81	0.76		
N	33	31	30	25		
102 TO 103						
MEAN	4.6	4.7	5.1a	5.1ac	NA	
S.D.	0.76	0.45	0.67	0.49		
N	32	31	26	23		

For statistical analyses, control group 1 was compared to groups 2, 3, 4 and 5;
control group 2 was compared to groups 1, 3, 4 and 5.
a = Significantly different from control group 1 at 0.05 using Dunnett's test
b = Significantly different from control group 1 at 0.01 using Dunnett's test
c = Significantly different from control group 2 at 0.05 using Dunnett's test
d = Significantly different from control group 2 at 0.01 using Dunnett's test
NA = NOT APPLICABLE

b(4)

GROUP	----- P R E K A L D -----				
	0 MG/KG/DAY	0.5 MG/KG/DAY	2.5 MG/KG/DAY	5.0 MG/KG/DAY	10.0 MG/KG/DAY
86 TO 88					
MEAN	4.8	4.8	4.9	5.2	NA
S.D.	0.88	0.83	1.10	0.90	
N	41	42	28	22	
86 TO 87					
MEAN	4.7	4.8	5.2	5.3a	NA
S.D.	0.89	0.94	0.81	1.04	
N	41	41	25	21	
88 TO 89					
MEAN	4.6	4.7	5.1	5.0	NA
S.D.	0.86	0.85	1.00	0.88	
N	41	40	22	19	
89 TO 91					
MEAN	4.5	4.5	5.0	5.0	NA
S.D.	0.86	0.88	0.92	1.20	
N	37	38	17	18	

For statistical analysis, control group 1 was compared to groups 2, 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
 a - Significantly different from control group 1 at 0.05 using Dunnett's test
 NA - NOT APPLICABLE

Neoplastic findings:

Sponsor's data

An increased incidence of malignant mammary tumors was seen in females at the lowest dose only (23% vs. 0-2% in Control, 3% in MD and 2% in HD groups), as shown in the sponsor's table below). However, Peto analysis failed to identify a dose-related trend either for mammary gland adenocarcinoma alone or for combined incidences of adenoma/adenocanthoma/adenocarcinoma).

Incidence of mammary tumors in females

	Group									
	1		2		3		4		5	
Number Examined	51		60		60		59		58	
Incidence/Percentage	N	%	N	%	N	%	N	%	N	%
Adenoma	0	0	0	0	1	2	0	0	0	0
Adenocarcinoma	0	0	1	2	12	20	2	3	1	2
Adenocanthoma, malignant	0	0	0	0	1	2	0	0	0	0
Mice with Mammary Tumors	0	0	1	2	13	23	2	3	1	2

The sponsor attributed the increased number of mammary neoplasms in the LD group to endogenous hormonal imbalances induced by pharmacological activities of the test drug. It is of note that elevated prolactin and increased incidence of non-neoplastic proliferative mammary changes (duct ectasia/ galactocele and glandular hyperplasia) was seen in females from all dose groups without dose dependence (see sponsor's table below).

Incidence of non-neoplastic proliferative mammary changes

GROUP	MALES					FEMALES				
	1	2	3	4	5	1	2	3	4	5
Mammary Gland (No. Examined)	3	NA	1	NA	NA	51	60	60	59	58
Glandular Hyperplasia	NA	NA	NA	NA	NA	2	4	8	10	8
Ectasia, Ducts/Galactocele	3	NA	1	NA	NA	6	6	27	18	16

In males, according to the sponsor's description,

A slight increase in the incidence of alveolar-bronchiolar adenomas was observed in the 2.5 mg/kg/day males when compared to the control groups. These tumors were analyzed statistically by the method of Peto^{7,A}. A significance level of $p < 0.05$ was shown in the incidences of bronchiolar/alveolar adenomas alone; none of the other analyses (bronchiolar/alveolar carcinomas, or combined incidences of bronchiolar/alveolar adenomas and carcinomas) attained a level of significance ($p < 0.05$). However, bronchiolar/alveolar tumors are considered common tumors (spontaneous incidence rates of $>1\%$) in the strain and age of mice used in this study^{21, 22}. Because the significance level for testing positive linear trends in the incidence rate for common tumors is considered to be 0.005 by the FDA²², the significance level of $p < 0.05$ for the incidences of bronchiolar/alveolar adenomas was not considered indicative of a test article-related effect. Furthermore, there was no other evidence (such as multiplicity of tumors, increased incidence of preneoplastic changes or increased incidence of lung tumors in female mice) to suggest a relationship to treatment. Therefore, the slight increase in lung bronchiolar/alveolar tumors is considered incidental and unrelated to the administration of ILO522.

(End citation)

FDA statistical reviewer's analysis:

Females:

The reviewer obtained identical to the sponsor's incidences for each of the tumors per treatment group. In the reviewer's analyses, consistently with the sponsor's report, "none of the trend tests for increase in tumor incidences with increasing dose approached statistical significance when all dose groups were used or when the high dose group was excluded from the analyses". The results are shown in the statistical reviewer's table reproduced on the next page.

Males:

The sponsor reported a statistically significant finding at the 0.05 level for alveolar-bronchiolar adenomas by the method of Peto. In the reviewer's analysis, alveolar-bronchiolar carcinomas or the combined tumor types did not attain such a level of significance. The reviewer was "not clear how the sponsor reached a p-value of <0.05 for the observed incidences of 7, 8, 12, 7, 2 (control 1, control 2, low, medium, and high dose groups respectively)". To the reviewer, "it seemed unusual that a sequence of such numbers could result in a minimally statistically significant linear trend". In the reviewer's analysis, "the exact permutation trend test with all groups (and censored at the time of the terminal sacrifice of the high dose) produced a p-value of 0.8415 which was corroborated by the normal approximation test with a p-value of 0.8319. When the high dose was excluded and the terminal sacrifice was after week 103, the respective p-values were 0.3252 and 0.3028."

However, more importantly, the sponsor's and the reviewer's conclusions are "consistent in that these findings do not approach the level of statistical significance necessary for common tumors".

The reviewer also agreed with the sponsor that "neither alveolar bronchiolar carcinomas in the lung nor any other tumor finding approached statistical significance when all dose groups were used or when the high dose was excluded".

The results of the statistical reviewer's analysis are shown in the tables reproduced below and on the next page.

Tumor trends for female mice (statistical reviewer's analysis)

AD	ADRENAL GLANDS	HP001006	#B PHEOCHROMOCYTOMA	0	0	1	0	0	0.4885	0.5850
KI	KIDNEYS	HP020022	#M CARCINOMA, RENAL CELL	0	0	1	0	0	0.4041	0.3892
LI	LIVER	HP021004	#B ADENOMA, HEPATOCELLULAR	1	2	1	1	0	0.8777	0.8626
LU	LUNGS	HP026003	#B ADENOMA, BRONCHIOLAR/ALVEOL	6	4	4	1	2	0.8504	0.8468
LU	LUNGS	HP026005	#M CARCINOMA, BRONCHIOLAR/ALVE	2	0	0	1	0	0.8370	0.8125
MG	MAMMARY GLAND	HP027004	#M ADENOCARCINOMA	0	1	12	2	1	0.3835	0.3541
MG	MAMMARY GLAND	HP027009	#B ADENOMA	0	0	1	0	0	0.5059	0.5752
MG	MAMMARY GLAND	HP027010	#M ADENOACANTHOMA	0	0	1	0	0	0.5059	0.5752
OV	OVARIES	HP033006	#B LUTEOMA	1	0	0	0	0	1.0000	0.8220
OV	OVARIES	HP033016	#B CYSTADENOMA	0	1	0	0	0	1.0000	0.8967
PA	PANCREAS	HP034017	#M CARCINOMA, ISLET CELL	0	1	0	0	0	1.0000	0.8217
PI	PITUITARY	HP040004	#B ADENOMA, PARS DISTALIS	1	0	0	1	0	0.5497	0.5621
PI	PITUITARY	HP040008	#B ADENOMA, PARS INTERMEDIA	0	0	0	0	1	0.4043	0.1623
SK	SKIN	HP046011	#B TRICHOEPITHELIOMA	0	0	0	1	0	0.2914	0.2790
SK	SKIN	HP046015	#B ADENOMA, SEBACEOUS	0	0	0	1	0	0.2914	0.2790
SY	SYSTEMIC TUMORS	HP015001	#M LYMPHOMA, MALIGNANT	10	11	11	3	3	0.9244	0.9145
SY	SYSTEMIC TUMORS	HP015002	#M SARCOMA, HISTIOCYTIC	2	0	3	1	0	0.5112	0.5055
SY	SYSTEMIC TUMORS	HP015003	#M HEMANGIOSARCOMA	2	2	1	2	1	0.4968	0.4923
SY	SYSTEMIC TUMORS	HP016004	#B HEMANGIOMA	0	0	1	2	0	0.4246	0.4006
TG	THYROID GLANDS	HP053007	#B ADENOMA, FOLLICULAR CELL	0	0	0	0	1	0.2903	0.1062
UB	URINARY BLADDER	HP059015	#M LEIOMYOSARCOMA	0	0	0	0	1	0.1221	0.0187
UT	UTERUS	HP060004	#B POLYP, ENDOMETRIAL STROMAL	8	2	1	1	1	0.9583	0.9415
UT	UTERUS	HP060005	#M SARCOMA, STROMAL CELL	4	1	0	0	0	1.0000	0.9612
UT	UTERUS	HP060016	#B LEIOMYOMA	1	0	0	1	0	0.7263	0.7097
XX	HARDERIAN GLANDS	HP081003	#B ADENOMA	1	0	2	0	0	0.7145	0.7107

Tumor trends for male mice (statistical reviewer's analysis)

AD	ADRENAL GLANDS	HP001002	#B ADENOMA, CORTICAL	1	0	0	1	0	0.5032	0.5032
AD	ADRENAL GLANDS	HP001006	#B PHEOCHROMOCYTOMA	1	0	0	0	0	1.0000	0.8375
DU	DUODENUM	HP010004	#M SARCOMA, UNDIFFERENTIATED	0	0	1	0	0	0.6157	0.6655
GB	GALLBLADDER	HP016011	#M SARCOMA, UNDIFFERENTIATED	0	0	0	1	0	0.3098	0.3023
GB	GALLBLADDER	HP016012	#B ADENOMA, PAPILLARY	0	0	1	0	0	0.5673	0.5075
KI	KIDNEYS	HP020022	#M CARCINOMA, RENAL CELL	0	0	1	0	0	0.5389	0.5798
KI	KIDNEYS	HP020024	#B ADENOMA, RENAL CELL	0	0	0	1	0	0.3005	0.2802
LI	LIVER	HP021004	#B ADENOMA, HEPATOCELLULAR	5	5	3	0	1	0.9818	0.9698
LI	LIVER	HP021009	#M CARCINOMA, HEPATOCELLULAR	3	2	0	3	1	0.6040	0.5019
LU	LUNGS	HP026003	#B ADENOMA, BRONCHIOLAR/ALVEOL	7	8	12	7	2	0.8415	0.8319
LU	LUNGS	HP026005	#M CARCINOMA, BRONCHIOLAR/ALVE	5	3	4	2	0	0.9448	0.9270
PI	PITUITARY	HP040004	#B ADENOMA, PARS DISTALIS	0	0	0	1	0	0.3060	0.2796
PI	PITUITARY	HP040008	#B ADENOMA, PARS INTERMEDIA	0	0	0	1	0	0.3060	0.2796
SG	SALIVARY GLANDS	HP043013	#B SCHWANNOMA	0	0	0	1	0	0.3021	0.2808
SG	SALIVARY GLANDS	HP043014	#M ADENOCARCINOMA	0	0	1	0	0	0.4507	0.4277
ST	STOMACH	HP049014	#M ADENOCARCINOMA	0	0	1	0	0	0.4375	0.4222
ST	STOMACH	HP049016	#M SARCOMA, UNDIFFERENTIATED	0	0	1	0	0	0.6842	0.7268
SY	SYSTEMIC TUMORS	HP015001	#M LYMPHOMA, MALIGNANT	3	4	1	3	2	0.3597	0.3479
SY	SYSTEMIC TUMORS	HP015002	#M SARCOMA, HISTIOCYTIC	2	1	0	0	0	1.0000	0.9397
SY	SYSTEMIC TUMORS	HP015003	#M HEMANGIOSARCOMA	1	3	5	1	0	0.7714	0.7568
SY	SYSTEMIC TUMORS	HP015004	#B HEMANGIOMA	1	0	1	1	0	0.7813	0.7726
TE	TESTES	HP051009	#B ADENOMA, INTERSTITIAL CELL	1	0	2	1	0	0.5728	0.5671
TG	THYROID GLANDS	HP053010	#M CARCINOMA, FOLLICULAR CELL	0	0	1	0	0	0.5340	0.5720
TH	THYMUS GLAND	HP052021	#B THYMOMA	0	0	1	0	0	0.5815	0.6191
XX	EXTERNAL SURFACE	HP076004	#B PAPILOMA, SKIN	0	0	0	1	0	0.3005	0.2802
XX	EXTERNAL SURFACE	HP076014	#B NERVE SHEATH TUMOR	0	0	1	0	0	0.5388	0.5798
XX	SUBCUTIS	HP088004	#B LIPOMA	0	0	1	0	0	0.6842	0.7229

The sponsor attributed the mammary gland changes, including the increased incidence of tumors at the low dose, to endogenous hormonal imbalances (increased prolactin secondary to pharmacological inhibition of the dopamine receptor). Elevated prolactin and increased incidence of non-neoplastic proliferative mammary changes (duct ectasia/ galactocele and glandular hyperplasia), as well as pharmacological clinical signs were seen in females from all dose groups without dose dependence. As shown in the sponsor's table below, prolactin levels were increased in both genders; a dose-response was apparent for males but not for females.

Despite the elevation of prolactin and the development of hyperplastic changes in mammary gland, iloperidone administration showed no dose relationship to mammary tumorigenesis in the mouse.

Prolactin determination

PROJECT NO. 49		A 14-WEEK ORAL CHRONICITY STUDY OF ILOPERIDONE IN MICE					PAGE
SPONSOR: NOVARTIS		SPECIAL CHEMISTRY VALUES -- SUMMARY OF MEANS					10
STUDY NO. 100 00000							
		M A L E					
ANALYSIS GROUP:		0 MG/28/DAY	0 MG/28/DAY	2.5 MG/28/DAY	5.0 MG/28/DAY	10.0 MG/28/DAY	
PROLACTIN (ng/ml)							
MEAN		5.70	NA	31.81b	57.96b	85.49b	
S.D.		3.834		13.915	16.452	14.774	
N		10		10	10	10	
ng/ml = NANMOGRAMS/MILLILITER							
		F E M A L E					
ANALYSIS GROUP:		0 MG/28/DAY	0 MG/28/DAY	2.5 MG/28/DAY	5.0 MG/28/DAY	10.0 MG/28/DAY	
PROLACTIN (ng/ml)							
MEAN		84.68	NA	236.81b	362.66b	334.66b	
S.D.		91.429		111.939	193.923	79.948	
N		10		10	10	10	
ng/ml = NANMOGRAMS/MILLILITER							

For statistical analysis, control group 1 was compared to groups 2, 4 and 5.
 b = significantly different from control group 1 at 0.01 using Dunnett's test

There were no notable drug-related changes in other clinical pathology parameters.

Non-neoplastic findings

Drug-related non-neoplastic lesions were observed in all treated groups and were noted in female mammary gland (proliferative mammary changes, i.e., glandular hyperplasia and duct ectasia/galactocele), uterus (uterine adenomyosis), in the heart (cardiomyopathy and/or atrial thrombosis) and in the lung (chronic interstitial inflammation/fibrosis and alveolar macrophages). The incidence of non-neoplastic findings is shown in the sponsor's table on the next page. The following sponsor's narrative describes adequately the non-neoplastic histopathology findings:

Increased incidences of duct ectasia/galactoceles and glandular hyperplasia were observed in the mammary gland in all treated group females and were considered test article-related effects (Text Table 3). Hyperplasia was characterized by proliferation of the alveolar and/or ductal epithelium, while duct ectasia consisted of dilated ductular structures that frequently contained eosinophilic secretion. Both of these changes, ductal proliferation and increased secretions, have been associated with prolactin¹⁷, and in this study, these effects may be secondary to the hyperprolactinemia observed in the treated groups.

b(4)

Incidence of non-neoplastic drug-related findings in males and females

GROUP	MALES					FEMALES				
	1	2	3	4	5	1	2	3	4	5
Mammary Gland (No. Examined)	3	NA	1	NA	NA	51	60	60	59	58
Glandular Hyperplasia	NA	NA	NA	NA	NA	2	4	8	10	8
Ectasia, Ducts/Galactocoele	3	NA	1	NA	NA	6	6	27	18	16
Uterus (No. Examined)	NA	NA	NA	NA	NA	60	60	60	60	60
Adenomyosis	NA	NA	NA	NA	NA	2	1	30	21	20
Heart (No. Examined)	60	60	60	60	60	60	60	60	60	60
Atrial Thrombosis	7	3	9	12	12	0	1	4	10	15
Mild	0	1	2	1	2	0	0	1	4	5
Moderate	3	0	4	7	6	0	0	2	0	9
Severe	4	2	3	4	4	0	1	1	6	1
Cardiomyopathy	32	35	49	49	47	19	28	49	43	43
Minimal	18	15	17	11	16	15	24	30	9	10
Mild	10	17	21	22	26	4	4	11	19	24
Moderate	4	3	11	15	5	0	0	8	15	9
Severe	0	0	0	1	0	0	0	0	0	0
Lung (No. Examined)	60	60	60	60	60	60	60	60	60	60
Chronic Inflammation/Fibrosis	15	16	16	20	17	8	9	13	16	21
Minimal	8	6	5	9	6	4	5	7	4	4
Mild	6	10	8	7	4	3	3	3	9	11
Moderate	1	0	3	4	7	1	0	3	3	6
Severe	0	0	0	0	0	0	1	0	0	0
Macrophages, Alveolar	10	19	21	25	16	4	16	14	21	21
Minimal	1	5	5	8	2	1	11	3	6	4
Mild	6	10	7	9	2	3	4	4	4	3
Moderate	3	4	9	8	12	0	1	7	11	14

Uterine adenomyosis consists of the presence of well-differentiated endometrial glands and stroma in the myometrium; the glands sometimes extend through the muscular wall just beneath the serosal surface. The incidence of adenomyosis was increased in all treated group females (Text Table 3), with the highest incidence in the 2.5 mg/kg/day group. Adenomyosis may have resulted from the increased serum prolactin levels in the treated females, since prolactin causes hyperplasia of endometrium and endometrial glands¹⁸, and treatment of mice with prolactin or compounds known to be associated with hyperprolactinemia, such as diethylstilbestrol or dopamine antagonists, has been associated with high incidences of adenomyosis in mice¹⁹.

Two test article-related effects were observed in the heart - cardiomyopathy and atrial thrombosis. Cardiomyopathy was characterized by myocardial degeneration and fibrosis and mononuclear cell infiltrates. This is a common, spontaneous lesion in aging laboratory mice²⁰, but the increase in incidence and severity in all treated groups of males and females suggests that the test article exacerbated the spontaneous lesion in the treated animals. In addition, the incidence of atrial thrombosis was increased in the 5.0 and 10.0 mg/kg/day group females; a slight increase in the incidence of atrial thrombosis was also observed in the 5.0 and 10.0 mg/kg/day males. As mentioned previously, atrial thrombosis was considered to be the cause of death more frequently in treated animals than in controls.

Test article related findings in the lung consisted of chronic interstitial inflammation/fibrosis and alveolar macrophages. Both of these findings were observed with increased severity in all treated groups of males and females. Because of the increased incidences of cardiomyopathy and atrial thrombosis in these same groups, the lung changes are considered secondary to the cardiac changes, and not direct effects of the test article.

(End citation)

The findings in the lungs were considered to be secondary to the cardiac changes, and the findings in the uterus and the mammary glands were likely related to the increased serum prolactin levels in the dosed groups. Generally, the incidences of these findings did not increase dose-dependently; this was considered to be due to the decreased survival in the high dose group, resulting in less time for development of the lesions.

Toxicokinetics

Iloperidone systemic exposure generally increased in a dose-proportional manner in both males and females, but due to low iloperidone plasma levels, reliable pharmacokinetic results could only be determined at the HD. The results indicated similar exposure in males and females, with AUC₀₋₈ values of 658.8 and 617.8 ng/ml·h, respectively. Considerable levels of the P89 9124 metabolite were observed in both males and females.

Adequacy of the carcinogenicity study and appropriateness of the test model

The study was conducted according to standard procedures. The species and strain was selected based on recommendations of applicable guidelines and the available background data. Mice were treated with iloperidone by oral gavage for up to 104 weeks at dosages accepted by the Executive CAC. The oral route was chosen because it is the intended route for therapeutic use in humans. The treatment produced evidence of toxicity based on reductions in survival, significant in all treated female groups, as well as in the high-dose male group. Therefore, the MTD was achieved in this study. Because of decreased survival (approximately 33%), the HD mice of both genders were euthanized at Week 82. The females from the LD, MD and control groups were euthanized at Week 90, when survival rates were 63%, 65%, 33%, and 32% in both control groups and the 2.5- and 5-mg/kg groups, respectively. The males from the LD, MD and control

groups were euthanized at Week 104. Iloperidone systemic exposure generally increased in a dose-proportional manner in both males and females, but due to low iloperidone plasma levels, reliable pharmacokinetic results could only be determined at the HD. The results indicated similar exposure in males and females, with AUC₀₋₈ values of 658.8 and 617.8 ng/mL•h, respectively.

There was not a single statistically significant tumor trend among either gender whether all treatment groups were used and censored at the time the high dose was terminated or whether the high dose was excluded from the analyses and the remaining groups were censored at their later terminal sacrifice. A carcinogenicity study is considered valid despite the absence of significant tumor findings if the following two criteria are met:

- If sufficient numbers of animals were exposed long enough to allow for late developing tumors;
- If the high dose provided a sufficient tumor challenge.

The number of animals is generally considered adequate if 20-30 animals survive through weeks 80-90. Though the high dose was terminated early for both genders at week 83, there were still 21 male and female mice alive before that early sacrifice. The control and other treatment groups had at least 30 animals left at that time point. Hence there were sufficient numbers of animals exposed long enough to allow for late-developing tumors.

In determining whether the high dose provided an adequate tumor challenge, the MTD in the mouse study was exceeded in the females based on the decreased survival in all iloperidone-treated female groups as compared to the control group. For the males, the MTD was 5 mg/kg/day based on a significant mortality increase at the next tested dose level of 10 mg/kg/day. The sponsor's Peer Reviewer and the FDA statistical reviewer concurred with the findings and conclusions of the study.

It is concluded that this is a valid carcinogenicity study.

Evaluation of tumor findings:

Sponsor's findings:

Females: An increased incidence of malignant mammary tumors was seen in females at the lowest dose only (23% vs. 0%, 2% in Control 1 and 2; 3% in MD and 2% in HD groups). However, Peto analysis failed to identify a dose-related trend either for mammary gland adenocarcinoma alone or for combined incidences of adenoma/adenocanthoma/adenocarcinoma). **Males:** A slight increase in the incidence of alveolar-bronchiolar adenomas was observed at the low dose vs. control groups. A significance level of $p < 0.05$ was shown by the method of Peto in the incidences of alveolar-bronchiolar adenomas alone; none of the other analyses (alveolar-bronchiolar carcinomas, or combined incidences of alveolar-bronchiolar adenomas and carcinomas) attained a level of significance. Since alveolar-bronchiolar tumors are considered common tumors (spontaneous incidence rates of $> 1\%$) in the strain and age of mice used in this study, and the significance level for testing positive linear trends in the incidence rate of common tumors is considered to be 0.005 by the FDA, the significance level of $p < 0.05 > 0.01$ was not considered indicative of a test-article related effect. Furthermore, there was no other evidence (such as multiplicity of tumors, increased incidence of pre-neoplastic changes or increased incidence of lung tumors in the females) to suggest a relationship to treatment. Therefore, the slight increase in lung bronchiolar/alveolar tumors is considered incidental and unrelated to the administration of the test drug.

Statistical reviewer analysis: Statistical review and evaluation of the results of this study was independently conducted by the statistical reviewer Roswitha Kelly.

Females:

The reviewer obtained identical to the sponsor's incidences for each of the tumors per treatment group. In the reviewer's analyses, consistently with the sponsor's report, "none of the trend tests for increase in tumor incidences with increasing dose approached statistical significance when all dose groups were used or when the high dose group was excluded from the analyses".

Males:

In the reviewer's analysis, alveolar-bronchiolar carcinomas or the combined tumor types did not attain the level of significance reported by the sponsor. The reviewer was "not clear how the sponsor reached a p-value of <0.05 for the observed incidences of 7, 8, 12, 7, 2 (control 1, control 2, low, medium, and high dose groups respectively)". To the reviewer, "it seemed unusual that a sequence of such numbers could result in a minimally statistically significant linear trend". In the reviewer's analysis, "the exact permutation trend test with all groups (and censored at the time of the terminal sacrifice of the high dose) produced a p-value of 0.8415 which was corroborated by the normal approximation test with a p-value of 0.8319. When the high dose was excluded and the terminal sacrifice was after week 103, the respective p-values were 0.3252 and 0.3028."

However, more importantly, the sponsor's and the reviewer's conclusions are "consistent in that these findings do not approach the level of statistical significance necessary for common tumors". The reviewer also agreed with the sponsor that "neither alveolar bronchiolar carcinomas in the lung nor any other tumor finding approached statistical significance when all dose groups were used or when the high dose was excluded".

In conclusion, a 2-year iloperidone administration to male and female D-1 (ICR) BR mice at oral doses of 2.5, 5, and 10 mg/kg/d caused an increased mortality in females at all dose levels and in males at HD. In males, there was no carcinogenic effect attributable to the test article based on the lack of a dose-response relationship or statistical significance level of the difference in tumor incidence in any of the observed tumor types. In females, the incidence of malignant mammary tumors was significantly increased above the concurrent and historical control range in the low dose group only. On an mg/m² basis, there is no safety margin between the low dose employed in the study (2.5 mg/kg/day) and the maximal recommended dose in humans (24 mg/day). However, mammary tumor incidences were not increased in the mid- and high-dose groups, although the duration of treatment was the same in the mid-dose and low dose groups. It is not clear why similar increases in mammary tumor incidences were not seen at higher doses.

b(4)

CAC concurrence:

- The Committee agreed that the study would be accepted, noting prior agreement on doses by the Exec CAC. The Committee noted that termination of some groups may have been conducted prematurely in this case, reducing the power of the test to detect neoplasms.
- The incidence of mammary tumors in the low dose females was significantly increased, was notably above the historical range, and appeared to be drug related. The Exec CAC believes this should be included in the labeling. It was not clear why similar increases were not seen at higher doses.

Study title: A 24-month oral oncogenicity study of ILO 522 in rats (Study No. 988054)

Key findings:

In the rat carcinogenicity study conducted at doses of 4, 8, and 16 mg/kg/d for 24 months, the treatment did not affect survival, but induced a dose-related, significant decrease in mean body weights in the dosed groups vs. control, by 13%, 22% and 28% in males and by 10%, 17% and 21% in females at LD, MD and HD, respectively, as well as a decrease in food consumption and pharmacological clinical signs that subsided during the course of the study. Body weight gains in the treated groups normalized after the first 3-4 months of study. However, due to the earlier substantial decreases in body weight, the average body weight values for the entire period of the study were significantly lower than the control.

There were no signs of systemic toxicity in either clinical pathology or histopathology parameters. Signs of pharmacological effect were present at all dose levels, manifested in clinical signs and dose- and time-dependent pronounced increase in serum prolactin in both genders, but much more pronounced in females, with associated non-neoplastic proliferative mammary changes in females (glandular hyperplasia and galactocele, all dose levels).

In males, there was no significant increase in neoplastic incidence or dose-dependence for any of the observed tumor types or combinations. In females, the combined incidences for pancreatic islet cell adenomas and islet cell carcinomas were increased (2, 2, 0, 3, 7 for the two controls, LD, MD and HD, respectively). The incidence value at HD was within historical control range for this species and strain; the dose-response trend analysis showed a p-value of 0.0051 that approached but did not reach the level of statistical significance required for common tumors ($\alpha=0.005$). There was no increase in the incidence of other tumors or tumor combinations of any type, including mammary tumors, although non-neoplastic proliferative mammary changes were increased in all dosed female groups. An MTD was achieved or exceeded in this study, based on decreases in mean body weight of over 10% in all treated groups.

In summary, iloperidone administration to rats for 2 years was not tumorigenic in males; in females, it produced an increased incidence of combined pancreatic benign and malignant islet cell tumors (islet cell adenomas and carcinomas) at a p-value of 0.0051, that approached the statistical significance level for common tumors ($p < 0.005$).

Study title: A 24-month oral oncogenicity study of ILO 522 in rats (Study No. 988054)

Volume and page: electronic submission

Conducting laboratory and location: _____

Date of study initiation: August 27, 1993

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity:

Test article: Iloperidone (ILO522, HP 873)

Lot number: RC 5842

Purity: 99.4-99.8%

Vehicle control: 2% aqueous starch

Methods

Doses: 0, 0, 4, 8 and 16 mg/kg/day (males); 0, 0, 4, 8 and 16 mg/kg/day (females).

Basis of dose selection: The selection of doses for the 2-year study was based on the 13-week and 6-month repeated-dose studies and it was in accordance with the Executive CAC recommendations (IND 36827, Interoffice Memorandum of 7/20/1993)

Species/strain: Rat - CD[®](SD)BR

Number/sex/group (main study): 60/sex/dose group

Route, formulation, volume: oral gavage, suspension in vehicle (2% aqueous starch), dose volume: 0.4, 0.8 and 1.6 ml/kg (for LD, MD and HD, respectively) and 1.6 ml/kg for control

Frequency of dosing: Daily

Treatment duration: 104 weeks

Satellite groups used for toxicokinetics: none

Age: 7 weeks at dosing initiation

Animal housing: 3 animals per sex per cage (upon arrival for a minimum of 3 days) and individually thereafter in wire-mesh suspended cages in a controlled environment.

Restriction paradigm for dietary restriction studies: non applicable

Drug stability/homogeneity: Dose form: suspension; Stable under the storage conditions used in these studies; Homogeneous under the conditions used in these studies

Dual controls employed: Two control groups 60 rats/sex each

Interim sacrifices: none

Deviations from original study protocol: Deviations from the specified dosing regimen did not affect the data validity and study integrity.

Observation times:

Live Phase

Observations: Daily for mortality and morbidity; weekly for detailed physical examinations and palpable masses.

Body weights: Weekly

Food consumption: Weekly for the first 13 weeks and biweekly thereafter

Clinical Pathology:

- Leucocyte differential counts: Blood samples taken from 10 animals/sex/group at 12, 18 and 24 months and from all animals euthanized at extremis.
- Prolactin determination: Blood samples collected from 10 animals/sex/group at 1 and 12 months (study weeks 4 and 51), approximately 2 hours after dosing.

Ophthalmic examination: Indirect ophthalmoscopy conducted on all rats prior to initiation of dosing and during study week 103.

b(4)

b(4)

Morphologic Pathology:

Macroscopic examination: A complete necropsy was performed on all animals (60/sex/group). The following tissues and organs were collected and preserved in 10% neutral buffered formalin:

Tissues collected for histopathology evaluation

Adrenals (2)	Lymph node - mesenteric
Aorta	Mammary gland (female)
Bone with marrow*	Ovaries with oviducts (2)
Brain (forebrain, midbrain, hindbrain)	Pancreas
Eyes with optic nerve (2)	Peripheral nerve (sciatic)
Gastrointestinal tract	Pituitary
Esophagus	Prostate
Stomach	Salivary glands [submaxillary (2)]
Duodenum	Seminal vesicles (2)
Jejunum	Skeletal muscle (vastus medialis)
Ileum	Skin
Cecum	Spinal cord (thoracic)
Colon	Spleen
Rectum	Testes with epididymides (2)
Heart	Thymus (if present)
Kidneys (2)	Thyroids [both lobes with parathyroids if present (2)]
Lachrymal gland	Trachea
Liver (sections of two lobes)	Urinary bladder
Lungs [including bronchi, fixed by inflation with fixative (2)]	Uterus with cervix and vagina
Lymph node - mandibular	All gross lesions

* - Not placed in formalin

Histopathology: Conducted on a full list of tissues from all main study animals from the control and treated groups and all unscheduled deaths. Tissues were evaluated by light microscopy. Pathology peer review was conducted.

Toxicokinetics: not performed

Results

Mortality:

Sponsor's analysis:

There was no drug-related effect on survival. The 24-month survival numbers in Control group 1, Control group 2, LD, MD and HD were 30, 25, 25, 20 and 30 for males and 22, 21, 28, 27 and 20 for females, respectively. This represents a survival rate of 50%, 42%, 42%, 33% and 50% for males and 37%, 35%, 47%, 45% and 33%, respectively.

Statistical reviewer's analysis (as cited from the review of Roswitha Kelly):

The reviewer used the sponsor's SAS transport file for mice to analyze the mortality. She obtained almost identical numbers of animals surviving till the terminal sacrifice and agreed with the sponsor's conclusions, that survival was not affected by the test article in either males or females (as shown in the following tables and figures reproduced from the reviewer's report).

FDA Statistical Reviewer's Rat Mortality Table

Female Rats

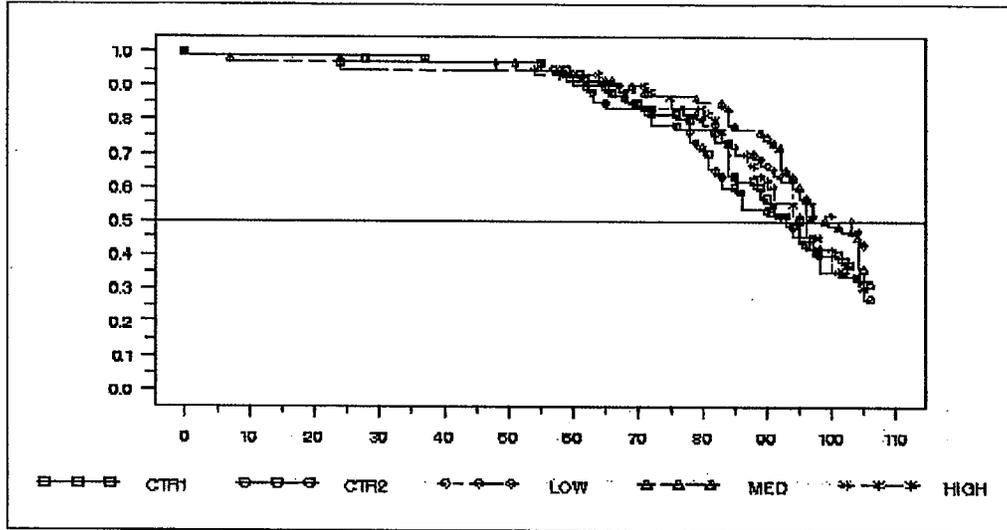
	Analysis of Mortality	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mort
	0-52	60	1	59	98.3	1.7
	53-78	59	11	48	80.0	20.0
CTR1	79-91	48	15	33	55.0	45.0
	92-103	33	11	22	36.7	63.3
	FINALKILL104-106	22	22	0		
	0-52	60	2	58	96.7	3.3
	53-78	58	12	46	76.7	23.3
CTR2	79-91	46	14	32	53.3	46.7
	92-103	32	11	21	35.0	65.0
	FINALKILL104-106	21	21	0	0.0	100.0
	0-52	60	2	58	96.7	3.3
	53-78	58	8	50	83.3	16.7
LOW	79-91	50	11	39	65.0	35.0
	92-103	39	9	30	50.0	50.0
	FINALKILL104-106	30	30	0		
	0-52	60	2	58	96.7	3.3
	53-78	58	5	53	88.3	11.7
MED	79-91	53	9	44	73.3	26.7
	92-103	44	16	28	46.7	53.3
	FINALKILL104-106	28	28	0		
	0-52	60	1	59	98.3	1.7
	53-78	59	7	52	86.7	13.3
HIGH	79-91	52	16	36	60.0	40.0
	92-103	36	15	21	35.0	65.0
	FINALKILL104-106	21	21	0		

Male Rats

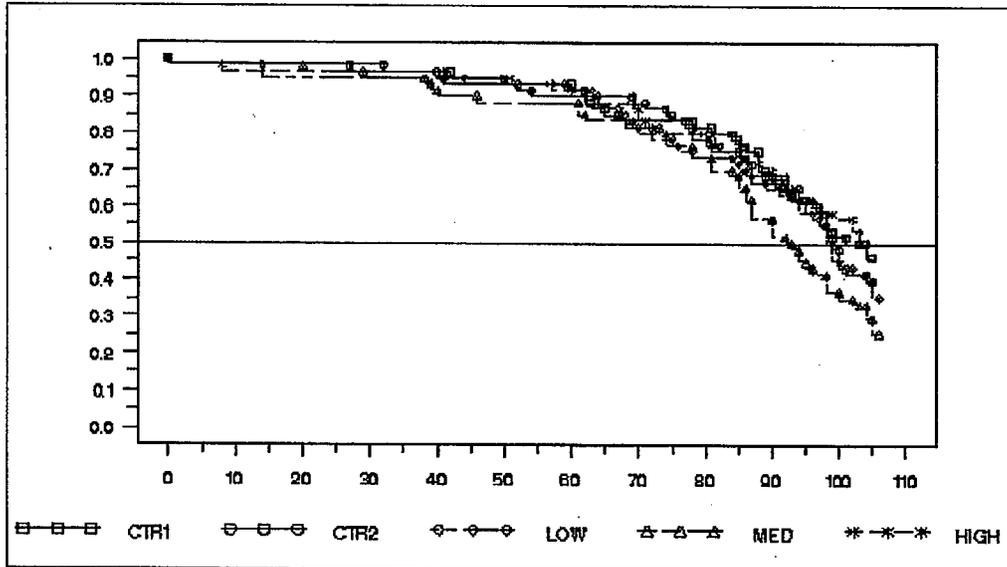
	Analysis of Mortality	No. Risk	No. Died	No. Alive	Pct Survival	Pct Mort
	0-52	60	3	57	95.0	5.0
	53-78	57	7	50	83.3	16.7
CTR1	79-91	50	10	40	66.7	33.3
	92-103	40	10	30	50.0	50.0
	FINALKILL104-106	30	30	0		
	0-52	60	4	56	93.3	6.7
	53-78	56	7	49	81.7	18.3
CTR2	79-91	49	8	41	68.3	31.7
	92-103	41	15	26	43.3	56.7
	FINALKILL104-106	26	26	0		
	0-52	60	3	57	95.0	5.0
	53-78	57	12	45	75.0	25.0
LOW	79-91	45	6	39	65.0	35.0
	92-103	39	13	26	43.3	56.7
	FINALKILL104-106	26	26	0		
	0-52	60	6	54	90.0	10.0
	53-78	54	8	46	76.7	23.3
MED	79-91	46	12	34	56.7	43.3
	92-103	34	14	20	33.3	66.7
	FINALKILL104-106	20	20	0	0.0	100.0
	0-52	60	3	57	95.0	5.0
	53-78	57	8	49	81.7	18.3
HIGH	79-91	49	7	42	70.0	30.0
	92-103	42	10	32	53.3	46.7
	FINALKILL104-106	32	32	0		

Kaplan-Meier Survival Curves

Female Rats



Male Rats



Clinical findings: Drug-related clinical signs were observed in all treated groups primarily 1 hour post dosing. The most prominent of these clinical signs were pharmacological in nature (e.g., ptosis, lacrimation, relaxed scrotum/vaginal opening), were noted one hour following dosing and were observed in all treated groups in a dose-related manner. There were no treatment-related increases in incidence of hypoactivity or ataxia. The incidence of ptosis decreased during the last year of the study and the incidence of relaxed scrotum/vaginal opening decreased during the last 6 months of study, suggestive of a tolerance development. Similarly, increased incidences of rectal

mucoïd exudate were observed during the first 18 months of the study but subsided thereafter.

Body weight:

A statistically significant reduction of body weight gains in the treated vs. control groups occurred during the first 3 to 4 months of the study. Thereafter, the mean weekly body weight gains in the treated groups were comparable or slightly lower than the control values, suggestive of a tolerance development (see sponsor's tables appended in Attachment 2 of this review). In males, the mean weekly body weight gains were reduced from study week 1 through 17 (at HD), week 1 through 12 (at MD) and during study weeks 1 through 2 (at LD). In females, due to an initial increase in food consumption, the decrease in body weight gain started later than in the males. The mean weekly body weight gains in treated females were reduced from study week 3 through 16 (at HD), 5 through 16 (at MD) and week 9 through 12 (at LD). Other intermittent fluctuations in body weight gain registered in the treated groups were small, not typically repeated during consecutive weeks and without an apparent trend.

The mean body weight was decreased in comparison to control in all dose groups, dose-dependently in both genders, but more expressed in the males (see sponsor's tables appended in Attachment 2 of this review). At the end of the study (week 104), the mean body weights at LD, MD and HD were lower than the mean control values by 13%, 22% and 28% for males and by 10%, 17% and 21% for females, respectively. In the male groups, the mean body weights were decreased throughout the study; in females, decreased mean body weights were registered from study week 8 onwards (at HD) and from week 15 onwards (at LD and MD).

Food consumption

The reduction of body weight gain in the treated groups was associated with a reduction in food consumption. A statistically significant reduction of food consumption vs. control was registered in all dosed female groups and in the MD and HD male groups, in parallel to the reduction in body weight. In comparison to control group 1, the mean food consumption was reduced by up to 11% and up to 18% in males at MD and HD, and by up to 13%, 17% and 14% in females at LD, MD and HD, respectively. The decrease took place during the first 12-18 months of the study, in parallel to the duration of the most prominent pharmacological clinical signs. In males, the mean food consumption values were reduced from study week 2 through 88 (at HD) and during week 6 through 78 (at MD). In females, after an initial rise in food consumption during study weeks 0 through 3-4, the mean food consumption was reduced from study week 7 through 64 and again from week 83 to 88 (at HD), and from week 11 through 76 (at MD and LD).

Clinical pathology: Serum prolactin, determined at study week 4 and 51, was significantly elevated vs. control in all male and female treated groups, more pronounced in females. The increase was dose- and time-dependent, so that within the same dose group, the values measured at week 51 were approximately 1.5x and 3x higher than those measured at week 4 in males and females, respectively (see the following sponsor's table).

No changes in the other studied clinical pathology parameters were registered.

Serum Prolactin Determination

ANALYSIS	GROUP	----- M A L E -----					
		CONTROL 1	CONTROL 2	4 MG/RS/DAY	8 MG/RS/DAY	16 MG/RS/DAY	
P R O L A C T I N (ug/ml)							
WEEK 4	MEAN	45.81	NA	69.93a	81.55b	86.73b	
	S.D.	16.581		15.76f	16.72g	12.187	
	N	10		10	10	10	
WEEK 51	MEAN	38.55	NA	96.66b	144.82b	186.76b	
	S.D.	12.658		33.811	49.324	32.409	
	N	10		10	10	10	

ANALYSIS	GROUP	----- F E M A L E -----					
		CONTROL 1	CONTROL 2	4 MG/RS/DAY	8 MG/RS/DAY	16 MG/RS/DAY	
P R O L A C T I N (ug/ml)							
WEEK 4	MEAN	84.78	NA	129.46	178.65b	179.56b	
	S.D.	71.792		64.923	32.621	19.939	
	N	10		10	10	10	
WEEK 51	MEAN	100.56	NA	313.08a	311.01a	489.60b	
	S.D.	76.667		239.885	142.686	182.749	
	N	10		10	10	10	

For statistical analyses, control group 1 was compared to groups 2, 3, 4 and 5; control group 2 was compared to groups 1, 3, 4 and 5.
a = significantly different from control group 1 at 0.05 using Dunnett's test
b = significantly different from control group 1 at 0.01 using Dunnett's test
NA = NOT APPLICABLE

Macroscopic pathology: Open sores (primarily on hind paws) were observed with drug-related increased incidence (2-fold over control in MD and HD females and in LD and HD males – affecting about 70% of HD animals vs. 30% in control). The increased incidence of sores (morphologically characterized as ulcerative pododermatitis) was “an expected finding with housing of older rats in wire mesh cages” and was attributed by the sponsor to “potentially decreased activity (not of sufficient magnitude to be noted clinically), consistent with the pharmacological activity of a neuroleptic agent”.

Neoplastic findings:
Sponsor's data

According to the sponsor, there were “no significant drug-related increases in tumor incidences of any type, including mammary tumors of the fibroadenoma and adenocarcinoma classification”, although non-neoplastic proliferative mammary changes were increased in all dosed groups (see sponsor's table below).

Incidence of mammary tumors and proliferative mammary changes in females

	Group									
	1		2		3		4		5	
Number Examined	59		58		60		58		60	
Incidence/Percentage	N	%	N	%	N	%	N	%	N	%
Galactocele	31	53	24	41	25	42	32	55	18	30
Hyperplasia, Glandular	5	8	1	2	13	22	22	38	13	22
Adenoma	1	2	4	7	0	0	2	3	1	2
Fibroadenoma	34	58	39	67	26	43	21	36	21	35
Adenocarcinoma	10	17	21	36	15	25	20	34	12	20

“Analysis of pancreatic islet cell tumors (adenomas and carcinomas) using the method of Peto showed a significance level of p<0.05 in the incidences of adenomas (both genders),

carcinomas (females) and combined adenomas and carcinomas (females). The incidence of islet cell adenomas in the high-dose female group was higher than the combined incidences of adenomas in the 2 control female groups ($p < 0.05$, Fisher's exact test)."

Incidence of pancreatic islet cell tumors and proliferative lesions in the pancreas

Group	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Number Examined	59	60	60	60	60	60	60	60	60	60
Hyperplasia, Islet Cell	3	6	11	6	7	9	15	8	17	14
Adenoma, Islet Cell	3	2	3	8	6	2	2	0	2	5
Carcinoma, Islet Cell	0	2	0	1	0	0	0	0	1	2
Total	6	10	14	15	13	11	17	8	20	21

The incidences of islet cell tumors in this study were within reported historical control ranges for this strain, as evaluated by the sponsor with reference to **Lang, P.L. (1992) Spontaneous neoplastic lesions and selected non-neoplastic lesions in the Cr:CD BR rat. Charles River Laboratories, Inc.**

As stated by the sponsor, "because pancreatic islet cell tumors (adenomas and carcinomas) are considered common tumors (spontaneous incidence rate of $>1\%$) in rats of the strain and age used in this study, and the significance level for testing positive linear trend in the incidence rate of common tumors is considered to be 0.005 by the FDA", the sponsor "did not consider the significance level of 0.05 for islet cell tumors to be indicative of a drug-related effect". In addition, "there was no other evidence (such as multiplicity of tumors, increased incidence of preneoplastic changes, clear dose-response relationship or increased mortality attributable to this tumor type) to indicate a relationship to treatment".

There was "a slight increase in the incidence of thyroid C-cell adenomas in the low dose males", but there was "no statistical significance when the incidences of thyroid C-cell adenomas alone, carcinomas alone, or adenomas and carcinomas combined were analyzed". Since these thyroid tumors lacked statistical significance and a dose-response trend, a treatment-related effect was not suggested.

Incidence of thyroid C-cell adenomas

----- MALES -----					
GROUP:	1	2	3	4	5
NUMBER OF ANIMALS IN DOSE GROUP	60	60	60	60	60
NUMBER OF ANIMALS (ALL TYPES OF DEATHS COMBINED)	60	60	60	60	60
THYROID GLANDS - CONTINUED					
-IS ADENOMA, FOLLICULAR					
PRESENT	1	3	2	0	3
ABSENT	1	3	2	NONE	2
-IS ADENOMA, C-CELL					
PRESENT	7	7	11	3	6
ABSENT	7	7	11	3	6
-HYPERPLASIA, C-CELL					
PRESENT	18	16	10	7	8
ABSENT	6	10	4	2	6
1- CONTROL 1	2- CONTROL 2	3- 4 MG/KG/DAY	4- 8 MG/KG/DAY	5- 16 MG/KG/DAY	

0 = NEOPLASIA, B = BENIGN, M = MALIGNANT

FDA statistical reviewer's analysis:

Females:

The reviewer used the sponsor's SAS transport file for rats to analyze the tumor data.

For males, none of the tumor findings increased significantly with dose.

In females, the combined incidences for pancreatic islet cell adenomas and islet cell carcinomas were increased (2, 2, 0, 3, and 7 for the two controls, LD, MD and HD,

respectively). The incidence value at HD was within historical control range for this species and strain; the dose-response trend analysis showed a p-value of 0.0051 that approached but did not reach the level of statistical significance required for common tumors (alpha=0.005).

The sponsor provided incidence tables for several mammary and pituitary tumors among the females. The reviewer obtained identical incidences for mammary and pituitary tumors among the females per treatment group. As the tumor/tissue combinations were recorded in the data set, none reached statistical significance.

The statistical reviewer's tables for tumor trends in males and females are reproduced below and on the next page.

Tumor trends for female rats (statistical reviewer's analysis)

AD	ADRENAL GLANDS	HP001002	#B PHEOCHROMOCYTOMA	2	1	2	2	2	0.4453	0.4243
AD	ADRENAL GLANDS	HP001015	#M CARCINOMA, CORTICAL	1	0	1	0	0	0.8749	0.8478
AD	ADRENAL GLANDS	HP001019	#B ADENOMA, CORTICAL	0	4	2	1	0	0.9720	0.9536
AD	ADRENAL GLANDS	HP001024	#M PHEOCHROMOCYTOMA	0	1	0	1	0	0.7029	0.6774
BR	BRAIN	HP007008	#B ASTROCYTOMA	0	0	0	0	2	0.0391	0.0086
CX	CERVIX	HP083001	#M LEIOMYOSARCOMA	1	0	0	0	0	1.0000	0.8586
CX	CERVIX	HP083002	#B LEIOMYOMA	2	0	0	0	0	1.0000	0.8200
CX	CERVIX	HP083011	#M SCHWANNOMA MALIGNANT	2	0	0	0	0	1.0000	0.8276
DU	DUODENUM	HP010002	#B LEIOMYOMA	1	0	0	0	0	1.0000	0.8569
EY	EYES/OPTIC N.	HP014012	#M CARCINOMA, SQUAMOUS CELL	0	0	0	1	0	0.4050	0.3673
HE	HEART	HP017013	#M CHONDROSARCOMA	0	0	0	1	0	0.4000	0.3755
JE	JEJUNUM	HP019004	#M LEIOMYOSARCOMA	0	0	0	0	1	0.1765	0.0404
LJ	LIVER	HP021011	#B ADENOMA, HEPATOCELLULAR	2	3	0	1	1	0.8835	0.8617
LJ	LIVER	HP021021	#M CARCINOMA, HEPATOCELLULAR	1	0	0	0	1	0.3953	0.2888
LU	LUNGS	HP026008	#B ADENOMA, BRONCHIOLAR/ALVEOL	1	0	0	0	0	1.0000	0.8320
MG	MAMMARY GLAND	HP027002	#B FIBROADENOMA	34	39	28	21	21	1.0000	0.9999
MG	MAMMARY GLAND	HP027003	#M ADENOCARCINOMA	10	21	15	20	12	0.7718	0.7638
MG	MAMMARY GLAND	HP027007	#B ADENOMA	1	4	0	2	1	0.8320	0.8134

Continued on next page

Tumor trends for female rats (statistical reviewer's analysis) - Continued

OV	OVARIES	HP033004	#B GRANULOSA CELL TUMOR	1	0	1	0	0	0.8777	0.8320
OV	OVARIES	HP033005	#M GRANULOSA CELL TUMOR	0	1	0	0	0	1.0000	0.8900
PA	PANCREAS	HP034007	#B ADENOMA, ISLET CELL	2	2	0	2	5	0.0561	0.0427
PA	PANCREAS	HP034014	#M CARCINOMA, ISLET CELL	0	0	0	1	2	0.0335	0.0149
PI	PITUITARY	HP040001	#B ADENOMA, PARS DISTALIS	52	51	32	20	29	1.0000	1.0000
PI	PITUITARY	HP040002	#B ADENOMA, PARS INTERMEDIA	0	0	0	1	0	0.4016	0.3960
PI	PITUITARY	HP040008	#M CARCINOMA, PARS DISTALIS	1	0	1	1	0	0.7085	0.6993
PT	PARATHYROID	HP035002	#B ADENOMA	1	0	0	0	1	0.4190	0.3337
SK	SKIN	HP046001	#B KERATOACANTHOMA	0	0	1	1	1	0.1630	0.1322
SK	SKIN	HP046014	#B ADENOMA, BASAL CELL	0	0	1	0	0	0.5714	0.5783
SY	SYSTEMIC TUMORS	HP025001	#M SARCOMA, HISTIOCYTIC	3	2	3	0	0	0.9935	0.9923
SY	SYSTEMIC TUMORS	HP025002	#M LYMPHOMA, MALIGNANT	1	0	1	0	0	0.8433	0.8214
SY	SYSTEMIC TUMORS	HP025003	#M HEMANGIOSARCOMA	3	1	0	0	0	1.0000	0.9734
SY	SYSTEMIC TUMORS	HP025004	#B HEMANGIOMA	1	0	0	0	0	1.0000	0.8320
TG	THYROID GLANDS	HP053002	#B ADENOMA, FOLLICULAR	2	1	0	0	1	0.7980	0.7689
TG	THYROID GLANDS	HP053003	#B ADENOMA, C-CELL	8	3	5	7	6	0.5190	0.5080
TG	THYROID GLANDS	HP053008	#M CARCINOMA, C-CELL	0	2	0	0	0	1.0000	0.9884
TG	THYROID GLANDS	HP053009	#M CARCINOMA, FOLLICULAR CELL	0	1	1	0	0	0.8777	0.8320
TH	THYMUS GLAND	HP052003	#M THYMOMA, MALIGNANT	0	0	0	2	0	0.3074	0.2375
TH	THYMUS GLAND	HP052004	#B THYMOMA	1	1	0	1	0	0.8189	0.7927
UT	UTERUS	HP060006	#B POLYP	3	3	5	2	4	0.4468	0.4322
VA	VAGINA	HP061010	#B SCHWANNOMA	0	0	0	1	0	0.5000	0.4388
XX	EXTERNAL SURFACE	HP075003	#B PAPILOMA	0	1	0	0	0	1.0000	0.8600
XX	MASS(ES)	HP084002	#B FIBROMA	0	0	1	3	0	0.3568	0.3318
XX	MASS(ES)	HP084009	#B NEOPLASM, NEURAL CREST	1	0	0	0	0	1.0000	0.8600
XX	MASS(ES)	HP084011	#M SCHWANNOMA, MALIGNANT	0	1	0	0	0	1.0000	0.8502
XX	MASS(ES)	HP084012	#M RHABDOMYOSARCOMA	1	0	0	0	0	1.0000	0.8577
XX	MASS(ES)	HP084013	#M FIBROSARCOMA	0	1	0	1	1	0.3019	0.2556
XX	MASS(ES)	HP084025	#M SARCOMA, UNDIFFERENTIATED	0	1	0	0	0	1.0000	0.8480
XX	MASS(ES)	HP084029	#M CARCINOMA, ZYMBAL'S GLAND	1	0	0	3	0	0.5377	0.5056
XX	ABSCCESS(ES)	HP094002	#M CARCINOMA, ZYMBAL'S GLAND	0	0	0	1	0	0.4286	0.3967
XX	ADIPOSE TISSUE	HP065003	#B HIBERNOMA	0	0	0	0	1	0.1721	0.0388

* Tumor incidences are shown for each control group but were combined for the trend tests.

Tumor trends for male rats (statistical reviewer's analysis)

AD	ADRENAL GLANDS	HP001002	#B PHEOCHROMOCYTOMA	3	11	0	7	0	0.3405	0.3288
AD	ADRENAL GLANDS	HP001010	#B ADENOMA, CORTICAL	0	3	0	1	1	0.6320	0.6070
AO	AORTA	HP003005	#B CHONDROMA	0	0	1	0	0	0.5821	0.6410
BR	BRAIN	HP007008	#B ASTROCYTOMA	0	3	1	2	1	0.6003	0.5940
BR	BRAIN	HP007014	#B OLIGODENDROGLIOMA	0	0	0	0	1	0.1078	0.0478
CE	CECUM	HP008006	#B LEIOMYOMA	1	0	0	0	1	0.4397	0.3253
EY	EYES/OPTIC N.	HP014004	#M MELANOMA, MALIGNANT-AMELANO	1	0	0	0	0	1.0000	0.8408
EY	EYES/OPTIC N.	HP014008	#B ADENOMA, MEIBOMIAN GLAND	0	1	0	0	0	1.0000	0.8408
HE	HEART	HP017006	#B MESOTHELIOMA, ATRIOCAVAL	0	0	2	2	0	0.4070	0.4602
JE	JEJUNUM	HP019003	#M ADENOCARCINOMA	0	0	0	0	1	0.2326	0.0502
KI	KIDNEYS	HP020004	#B LIPOMA	1	0	0	1	0	0.6855	0.6670
KI	KIDNEYS	HP020010	#B ADENOMA, RENAL CELL	0	1	0	0	0	1.0000	0.8451
KI	KIDNEYS	HP020025	#M LIPOSARCOMA	0	0	0	0	1	0.1013	0.0342
LI	LIVER	HP021011	#B ADENOMA, HEPATOCELLULAR	1	1	0	0	1	0.6036	0.5654
LI	LIVER	HP021015	#B CHOLANGIOMA	1	0	0	0	1	0.4122	0.3202
LI	LIVER	HP021021	#M CARCINOMA, HEPATOCELLULAR	2	3	5	0	2	0.7797	0.7645
LM	LYMPH NODE,MES	HP024012	#B LYMPHANGIOMA	0	1	0	0	0	1.0000	0.8504
LU	LUNGS	HP026008	#B ADENOMA, BRONCHIOLAR/ALVEOL	0	1	0	0	0	1.0000	0.8383
LU	LUNGS	HP026010	#M CARCINOMA, BRONCHIOLAR/ALVE	1	0	0	0	0	1.0000	0.8378
PA	PANCREAS	HP034007	#B ADENOMA, ISLET CELL	3	2	3	8	8	0.0511	0.0423
PA	PANCREAS	HP034014	#M CARCINOMA, ISLET CELL	0	2	0	1	0	0.8272	0.8173
PI	PITUITARY	HP040001	#B ADENOMA, PARS DISTALIS	37	33	38	29	30	0.9732	0.9708
PI	PITUITARY	HP040002	#B ADENOMA, PARS INTERMEDIA	1	0	0	1	0	0.6692	0.6800
PI	PITUITARY	HP040006	#M CARCINOMA, PARS DISTALIS	0	0	1	0	0	0.5071	0.6543
PT	PARATHYROID	HP035002	#B ADENOMA	0	3	0	3	1	0.4818	0.4386
PW	PATELLA	HP022001	#M FIBROSARCOMA	0	1	0	0	0	1.0000	0.8393
SK	SKIN	HP046001	#B KERATOACANTHOMA	3	5	1	1	2	0.8844	0.8682
SK	SKIN	HP046013	#B ADENOMA, SEBACEOUS GLAND	0	0	0	1	0	0.4288	0.3536
SK	SKIN	HP046015	#M BASAL CELL CARCINOMA	0	0	0	0	1	0.2388	0.0630
SV	SEMINAL VESICLES	HP044008	#B ADENOMA	0	0	0	1	1	0.1134	0.0687
SY	SYSTEMIC TUMORS	HP025001	#M SARCOMA, HISTIOCYTIC	2	6	2	4	1	0.9061	0.8820
SY	SYSTEMIC TUMORS	HP025002	#M LYMPHOMA, MALIGNANT	1	0	1	4	0	0.4633	0.4442
SY	SYSTEMIC TUMORS	HP025003	#M HEMANGIOSARCOMA	0	3	2	4	0	0.7483	0.7324
SY	SYSTEMIC TUMORS	HP025004	#B HEMANGIOMA	2	0	3	0	1	0.6894	0.6750
TE	TESTES	HP051005	#B ADENOMA, INTERSTITIAL CELL	2	2	2	2	0	0.9082	0.8882
TG	THYROID GLANDS	HP053002	#B ADENOMA, FOLLICULAR	1	3	2	0	2	0.6550	0.6378
TG	THYROID GLANDS	HP053003	#B ADENOMA, C-CELL	7	7	11	3	6	0.8390	0.8286
TG	THYROID GLANDS	HP053008	#M CARCINOMA, C-CELL	1	0	0	0	0	1.0000	0.8393
TH	THYMUS GLAND	HP052003	#M THYMOMA, MALIGNANT	1	0	1	0	0	0.8092	0.8014
TH	THYMUS GLAND	HP052004	#B THYMOMA	1	0	0	1	0	0.6787	0.6885

Tumor trends for male rats (statistical reviewer's analysis) - Continued

XX	EXTERNAL SURFACE	HP075003	#B PAPILOMA	2	0	2	0	0	0.0153	0.8941
XX	CRANIAL CAVITY	HP077001	#M SCHWANNOMA	0	1	0	0	0	1.0000	0.8445
XX	MASS(ES)	HP084002	#B FIBROMA	3	6	2	3	4	0.6216	0.6089
XX	MASS(ES)	HP084003	#B LIPOMA	2	3	0	1	2	0.6471	0.6300
XX	MASS(ES)	HP084007	#M MELANOMA, MALIGNANT-AMELANO	1	0	0	0	0	1.0000	0.8393
XX	MASS(ES)	HP084009	#B NEOPLASM, NEURAL CREST	0	0	0	1	0	0.4419	0.3563
XX	MASS(ES)	HP084011	#M SCHWANNOMA, MALIGNANT	0	0	0	1	0	0.3913	0.3708
XX	MASS(ES)	HP084013	#M FIBROSARCOMA	0	1	0	0	0	1.0000	0.8378
XX	MASS(ES)	HP084014	#B MYXOMA	1	0	0	0	0	1.0000	0.8393
XX	MASS(ES)	HP084016	#B PAPILOMA	0	1	0	0	0	1.0000	0.8393
XX	MASS(ES)	HP084023	#M MALIGNANT FIBROUS HISTIOCYT	0	0	0	0	1	0.2085	0.0510
XX	MASS(ES)	HP084025	#M SARCOMA, UNDIFFERENTIATED	0	0	1	0	1	0.2038	0.1685
XX	MASS(ES)	HP084029	#M CARCINOMA, ZYMBAL'S GLAND	0	0	1	2	1	0.1709	0.1396
XX	MAMMARY GLAND	HP090005	#B ADENOMA	0	0	0	1	0	0.3871	0.3433
XX	MAMMARY GLAND	HP090006	#B FIBROADENOMA	2	0	5	0	1	0.7394	0.7230
XX	MAMMARY GLAND	HP090007	#M ADENOCARCINOMA	0	0	0	0	2	0.0557	0.0129
XX	ADIPOSE TISSUE	HP095002	#B LIPOMA	0	0	0	0	1	0.1613	0.0342
XX	DIAPHRAGM	HP096002	#M LIPOSARCOMA	0	0	0	1	0	0.3871	0.3433

- Tumor incidences are shown per control group but were combined for the trend tests.

Non-neoplastic findings

Drug-related non-neoplastic lesions were observed in female mammary gland (proliferative mammary changes, i.e., glandular hyperplasia and duct ectasia/galactocele), at all dose levels without apparent dose dependence.

The only non-neoplastic finding that was a common cause of death was advanced nephropathy (see sponsor's table below), interpreted as "a common old-age lesion of this strain of rat" (Bolton et al, 1976, Owen, Heywood, 1986, as referenced by the sponsor)

GROUP:	SEX			
	1	2	3	4
NUMBER OF ANIMALS IN DOSE GROUP	60	60	60	60
NUMBER OF ANIMALS (ALL TYPES OF DEATH COMBINED)	60	60	60	60
KIDNEYS				
TOTAL NUMBER EXAMINED	60	60	60	60
EXAMINED, UNREMARKABLE	0	1	3	6
-NEPHROPATHY				
MINIMAL	8	6	18	16
MILD	17	23	17	14
MODERATE	22	18	14	13
SEVERE	11	12	5	3

A slight increase in alveolar histiocytosis in the lungs of treated rats was attributed to "local pulmonary irritation due to potential aspiration of the test article solution following gavage dosing".

Comments: Though mean body weight in treated groups was markedly lower than control, body weight gains in treated groups normalized after the first 3-4 months of study. Body weight reduction was associated with reduced food consumption which, along with clinical signs of sedation, was most expressed in the beginning of the study. A tolerance to these effects developed during the study duration, so that after the 1st year food consumption was no longer significantly different from control and the drug-related clinical signs diminished. Body weight gains in the treated groups normalized after the first 3-4 months of study. However, due to the earlier substantial decreases in body weight, the average body weight values for the entire period of the study were significantly lower than the control. There were no signs of systemic toxicity in either clinical pathology or histopathology parameters. A sign of pharmacological effect was the dose- and time-dependent pronounced increase in serum prolactin in all dose groups, more expressed in females with associated proliferative mammary changes (glandular hyperplasia and galactocele, in all female dose groups).

An MTD was achieved in this study based on the significant mean body weight reductions vs. control. This reviewer is not of the opinion that the MTD was largely exceeded, despite of the greater than 10% reduction in the mean body weight of the treated groups vs. control because body weight gains normalized after 4 months of study, and food consumption after the first year of study was not significantly different from control, as well as because there were no signs of systemic toxicity on the background of well manifested pharmacological activity at all tested dose levels.

Adequacy of the carcinogenicity study and appropriateness of the test model:

The study was conducted according to standard procedures to assess the carcinogenic potential of the test article. The CD (SD) strain was selected based on recommendations of applicable guidelines and the available background data for this species. Rats were treated with iloperidone by oral gavage for 104 weeks at dosages accepted by the Executive CAC. The oral route was chosen because it is the intended route for therapeutic use in man. The treatment produced evidence of toxicity without affecting survival, based on dose-related, significantly decreased mean body weights in the dosed groups vs. control, by 13%, 22% and 28% in males and by 10%, 17% and 21% in females at LD, MD and HD, respectively, by study week 104. Since survival was not affected by the treatment, there were sufficient numbers of animals exposed sufficiently long to allow for late developing tumors. In determining whether the high dose presented a sufficient tumor challenge, an MTD in the rat study was achieved in both genders based on the dose-dependent decrease in mean body weight of $\geq 10\%$ in all iloperidone-treated groups as compared to the control group. This reviewer is not of the opinion that the MTD was largely exceeded, despite of the $>10\%$ mean body weight reduction because body weight gains normalized after 4 months of study, and food consumption during after the first year of study was not significantly different from control, as well as because there were no signs of systemic toxicity on the background of well manifested pharmacological activity at all tested dose levels. It is concluded that this is a valid carcinogenicity study.

Evaluation of tumor findings:

Sponsor's findings: "Analysis of pancreatic islet cell tumors (adenomas and carcinomas) using the method of Peto showed a significance level of $p < 0.05$ in the incidences of adenomas (both genders), carcinomas (females) and combined adenomas and carcinomas (females). The incidence of islet cell adenomas in the high-dose female group was higher than the combined incidences of adenomas in the 2 control female groups ($p < 0.05$, Fisher's exact test)." The incidences of islet cell tumors in this study were within reported historical control ranges for this strain, as referenced by the sponsor.

As stated by the sponsor, "because pancreatic islet cell tumors (adenomas and carcinomas) are considered common tumors (spontaneous incidence rate of $>1\%$) in rats of the strain and age used in this study, and the significance level for testing positive linear trend in the incidence rate of common tumors is considered to be 0.005 by the FDA", the sponsor "did not consider the significance level of 0.05 for islet cell tumors to be indicative of a drug-related effect". In addition, "there was no other evidence (such as multiplicity of tumors, increased incidence of preneoplastic changes, clear dose-response relationship or increased mortality attributable to this tumor type) to indicate a relationship to treatment".

Statistical reviewer analysis: Statistical review and evaluation of the results of this study was independently conducted by the statistical reviewer Roswitha Kelly.

Individual tumor/tissue combinations did not approach statistical significance. However, the incidence of combined pancreatic islet cell adenomas and islet cell carcinomas was increased in females (2, 2, 0, 3, 7 for the two controls, LD, MD and HD, respectively), at a p-value of 0.0051 that approached the statistical significance level for common tumors ($p < 0.005$).

There was no increase in the incidence of other tumors or tumor combinations of any type, including mammary tumors. For males, none of the tumor findings increased significantly with dose.

In conclusion, based on the lack of a dose-response relationship or statistical significance in tumor incidence in any of the observed tumor types in a valid carcinogenicity study, there was no carcinogenic effect in the male rats attributable to the test article. In females, the combined incidences for pancreatic islet cell adenomas and islet cell carcinomas were increased at HD (2, 2, 0, 3, and 7 for the two controls, LD, MD and HD, respectively). The incidence value at HD was within historical control range for this species and strain; the dose-response trend analysis showed a p-value of 0.0051 that approached but did not reach the level of statistical significance required for common tumors ($\alpha = 0.005$). Having in mind that the incidences of pancreatic islet cell tumors in this study were within the reported historical control range for this species and strain and that there was no other evidence indicating a treatment-related effect (such as multiplicity of tumors, increased incidence of pre-neoplastic findings), it is concluded there was no carcinogenic effect in the female rats attributable to the test article.

CAC concurrence:

- The Committee agreed that the study was adequate, noting prior agreement by the Exec CAC
- The Committee found that there were no statistically significant tumors attributable to the test article.