REPRODUCTION

1. **Pilot study in non-pregnant rats. Oral administration** (Study no. TRR/1629, 11/ID/1011613, Report date: 9/21/87, non-GLP; **summary only**, Vol 1.56).

Female rats (strain not specified; 3/grp) were dosed with ICI 204,636 at doses of 0, 25, 150, 300, and 450 mg/kg p.o. (suspension in HPMC, Tween 80) for 10 days. Observations consisted of body weights, clinical signs, and gross pathology (in survivors).

All HD animals were sacrificed moribund on Day 2. Clinical signs in these animals consisted of "...laboured breathing, abnormal respiratory noise, facial staining and urine staining". At 300 mg/kg, clinical signs included urine staining, noisy respiration, and piloerection; body weight was reduced by 6%. At 150 mg/kg, urine staining and noisy respiratory were observed, but body weight was not affected. The sponsor stated that hypoactivity and unsteady were observed at all doses, with the incidence being dose-related. The LD was considered a NOTEL.

2. **Sighting teratology study in rats. Oral administration** (Study no. TRR/1536, 11/ID/1011615, ICI Pharmaceuticals, report date: 4/5/88, non-GLP, **summary report; no individual data provided**, Vol 1.56).

ICI 204, 636 (lot not specified) was administered to pregnant rats (10/grp; 2 additional C and HD animals were used for TK analysis) at doses of 0, 25, 75, 150, and 250 mg/kg p.o. on Days 6 through 15 of gestation. Pregnant dams were sacrificed on Day 20 of pregnancy and fetuses were examined macroscopically (no visceral or skeletal examinations were performed).

Three HD animals were sacrificed moribund (2 main study, 1 TK-satellite, Day 10, 12, 13 of gestation). In 1 female at 150 mg/kg, there was evidence of total resorption of litter. Clinical signs which were noted at all doses included hypoactivity, unsteady gait, abnormal/noisy breathing, and respiratory difficulty. Urine staining was noted at all but the LD, and piloerection and facial/forepaw staining were evident at 150 and 250 mg/kg. Body weight gain was reduced at 150 and 250 mg/kg (28 and 64%, respectively, during the dosing period). No drug-related effects were noted on the following parameters: no. of live fetuses per litter, mean pre-implantation loss, mean post-implantation loss, "...fetal body weight, placental weight, placental index, amniotic fluid weight per fetus or male proportion per litter...", or external fetal or placental anomalies. At the HD, plasma level of ICI 204,636 was 1.05 µg/mL and the embryonic tissue level was 0.86 µg/g. The sponsor concluded that there was no evidence of embryotoxicity or teratogenicity (gross) at any of the doses used in this study.

3. **A preliminary study of reproductive and developmental toxicity study in rats administered ICI 204.636 orally during fetal organogenesis (Seg II)** (Study no. TTR/2180, 8/IE/1017026, , report date: 12/11/92, translated from final report, translation date: 8/25/95, non-GLP, Vol 1.56)

ICI 204,636 (lot no. 45028/89; vehicle: HPMC/Tween 80) was administered to female Wistar rats (n = 7/grp, 10-11 wks old, 218-246 gm) at doses of 0, 100, 150, and 200 mg/kg p.o. (by gavage) from Day 6 to 15 of gestation. Observations consisted of the following: clinical signs, body weight, food intake, duration of gestation (all dams allowed to deliver naturally), terminal studies (implantation sites counted), F_1 observations (no. of live and dead pups, external anomalies, sex, body weight, clinical signs).

There were no unscheduled deaths during the study. There were 7 pregnant dams per grp which delivered. Clinical signs in dams included ptosis, and "...irregular respiration, decreased movement, ptosis and prone position..." throughout the dosing period at all doses. There were no drug-related effects on body weight or food consumption in dams. There were no drug-related effects on the following parameters: duration of gestation, no. of dams with live offspring, no. of implantation sites, no. of offspring/litter, and no. of live newborns with external anomalies. There were dose-related effects on the number of pup deaths (i.e., immediately after birth; 0, 0, 0, and 2 at C, LD, MD, and HD, respectively) and stillborns (1, 0, 5, and 15 at C, LD, MD, and HD, respectively). In addition 2 HD pups were cannibalized; therefore, the total number of dead newborns were 1, 0, 5, and 19 at C, LD, MD, and HD, respectively. The number of male live newborns (in %) was slightly higher in dosed grps, particularly at the HD (47.2, 51.8, 50.5, and 57.4% at C, LD, MD, and HD, respectively). There were no differences among grps in pup wt by Day 4 postpartum.

4. **Study of rats orally administered during the period of fetal organogenesis** (Study no. TTR/2257, 7/IE/US/1020232, study dates: 8/92-6-95, non-GLP, study conducted according to "the Standards in Conducting Safety Studies of Drugs", Vol 1.57)

[Note: due to the inadequacy of the study and the lack of time, individual data were not examined.]

Animals: Wistar rats
initial age: 11-12 wks
initial body weight: 235-268 gm
n = 13/grp

Drug: ICI 204,636 (lot no. 45028/89)
stability: reported to be confirmed (Report No. 3/IA/015697, ICI Pharmaceuticals; report not provided)
vehicle: 0.5% HPMC in 0.1% polyoxyethylene (20) Sorbitan Monooleate
formulation: suspension, concentrations (0.5, 1.0, 4.0 % w/v) confirmed at time of preparation
doses: 0, 25, 50, 200 mg/kg
dosing volume: 5 mL/kg
route: p.o. (gavage)
duration: Day 6 to Day 15 of gestation

Observations

F_0 [note: **all dams were allowed to deliver naturally**]

Clinical signs: all dams were observed during gestation and lactation: 3 times a day during the dosing period, twice a day after Day 15 through parturition, and once daily on other days.

Body weight: body weights were recorded on Days 0 and 3 of gestation, daily from Day 6 until parturition, and on Days 0, 4, 7, 11, 14, 18, and 22 of lactation.

Food consumption: food consumption was recorded on Days 0-3, 3-6, of gestation, daily from Day 6 until the day prior to parturition, and on Days 0-4, 4-7, 7-11, 11-14, 14-18, and 18-22 of lactation.

Reproduction: the following parameters were assessed: length of gestation, abnormalities at parturition, pup mortality, lactation performance.

Terminal studies

Gross pathology: those dams who had not delivered by Day 25 of gestation, were sacrificed on Day 25 and necropsied. Those dams that delivered live pups were sacrificed on Day 22 of lactation and necropsied; implantation sites were counted.

F_1

Procedure: all pups were observed after birth until sexual maturity. At weaning, 2/sex were selected from each litter and assessed for reproductive performance. An additional 1/sex/litter were followed for behavioral assessment. On Day 4 postpartum, litters were culled to 4/sex. Necropsies were not performed on the culled pups.

Observations at birth: the following parameters were assessed: no. of live and dead pups, mortality, external anomalies (including oral cavity), body weight.

Observations during the lactation period: the following parameters were assessed: viability index (Day 4 postpartum), weaning index, body weight (Days 4, 7, 11, 14, 18, and 22 postpartum), clinical signs (daily), physical differentiation [all pups; separation of ear auricle (Day 4 postpartum), '...growth of abdominal hair and eruption of upper incisor..." (Day 11, 14 postpartum), separation of eyelid (Day 11, 18 postpartum)], behavior [all pups on Day 15 postpartum; '...negative geotaxis, pivoting, straight walking, back righting, cliff drop aversion, grasp reflex, bar holding, front placing, auditory startle, mass reaction; cf. McClearn *et al*, 1970].

Observations following weaning (selected pups): the following parameters were assessed: clinical signs (daily up to Day 70 postpartum), sensory functioning [at 5 wks postpartum; "...visual placing response, Preyer's reflex, pupillary reflex, corneal reflex, pain response"], behavior [in 1/sex/litter; open field test (at 6 wks), shuttle box conditioned avoidance (at 7-8 wks)], and reproductive performance [testicular descent (28, 35 days postpartum), development of U-type penis (49, 56 days postpartum), vaginal opening (42, 49 days postpartum), estrous cycle (from 8-9 wks of age to day of successful mating), mating (at 10-11 wks of age, for 14 days; if unsuccessful, mated for a second time with different partner for 7 days).

Reproductive performance: body weights were recorded on Days 0, 4, 7, 11, 14, 17, and 20 of gestation. F_1 dams were sacrificed on Day 20 of gestation and the following parameters were assessed: no. of corpora lutea, no. of implants, dead fetuses (categorized as implantations sites, early death, late death, macerated fetus), live fetuses. Live fetuses were examined for external anomalies including "...those in the oral cavity, sex, placental form and properties of amniotic fluid), body weight, and placental wts were recorded.

Terminal studies

Gross pathology: all but selected pups (i.e., the 3/sex/litter used for reproduction and further behavioral assessment) were sacrificed on Day 22 postpartum and examined macroscopically.

Pups selected for assessment of post-weaning behavior were sacrificed at 10 wks of age and necropsied.

Male pups allowed to mate were sacrificed 5 days following cesarean delivery of dams, and necropsies performed.

Females mated unsuccessfully were sacrificed 11 days after mating and necropsied.

Females mated successfully were sacrificed on Day 20 of gestation (cf Reproductive performance).

Organ/tissue weights: weights of the following organs were recorded in the pups sacrificed on Day 22 postpartum: brain, thymus, lungs, heart, liver, spleen, kidneys.

In male pups allowed to mate, weights of testes, epididymides, seminal vesicles, and prostate were recorded.

Results

F_0

Mortality: there were no unscheduled deaths.

Clinical signs: ptosis was observed in all treated dams during the entire dosing period. Decreased activity, prone posture, and irregular respiration were noted at all doses (in all HDF), with the incidences being dose-related. No clinical signs were noted during the lactation period.

Body weight: there were no differences among grps in terms of body weight.

Food consumption: there were drug-related effects.

Reproductive performance: there were 12, 12,13, and 12 pregnant dams in the C, LD, MD, and HD, respectively. There were no drug-related effects on the following parameters: duration of gestation, no. of implantation sites, no. of newborns, delivery index (i.e., no. of newborns/no. of implantation sites), no. of live newborns, birth index (i.e., no. of live newborns/no. of implantation sites), pup body weight, sex ratio, no of dead newborns. There were no live pups with external anomalies.

Lactation performance: according to the sponsor, there was no evidence of nursing abnormalities in any grp.

Terminal studies

Gross pathology: Two dams (1 C, 1 HD) exhibited no signs of pregnancy and were sacrificed on Day 25. In the HDF, a dead fetus with micrognathia was detected. The CF also had a dead fetus; however, there was no evidence of fetal abnormality.

In dams sacrificed on Day 22 of lactation, there were no drug-related macroscopic findings.

F_1

Viability: there were no drug-related effects on viability on Day 4 of lactation (at which time pups were culled), or on weaning index (i.e., no. of live pups on Day 22/no. of live pups after culling on Day 4).

Clinical signs: there did not appear to be any drug-related signs.

Body weight: there were no drug-related effects up to Day 70 postpartum.

Physical differentiation: there were no drug-related findings on early physical indices.

Sensory/behavioral assessment: there were no differences among grps in terms of development of sensory functions (at 5 wks of age); all animals in all grps displayed normal function. There were also no differences among grps on performance in the open field test, spontaneous motility, or the shuttlebox conditioned avoidance paradigm.

Reproductive performance: testis descent and "development of U-type penis" (assumed to mean preputial separation) were observed in all pups in all grps on Days 28 and 35 postpartum. In female pups, vaginal opening was noted in all pups in all grps at 42 and 49 days postpartum. In terms of estrus cycle, the following were noted: (1) a slight increase in the length of the estrus cycle at the HD; 3 HDF (vs 0-1 in other grps) had a cycle 5 days in length and 1 HDF (none in the other grps) had a cycle of 5-6 days in length, (2) no difference in the mean number of cycles.

There were no clear differences among grps in the number of female pups that successfully mated or became pregnant. There was, however, a decrease in the number of male pups that successfully mated (both mating periods; 95.7 vs 100% in other grps); the number of males that successfully impregnated partners (expressed as a percent that mated successfully) were fairly similar among grps.

There were no differences among grps in body weight during gestation.

On litter parameters, the following were noted: (1) no drug-related effects on no. of corpora lutea, no. of implants, no. of live fetuses, no. of dead fetuses (i.e., implantation sites, early deaths, late deaths, macerated fetuses), the no. of live fetuses with external anomalies, or with abnormal placenta, (2) a decrease in implantation index (i.e., no. of implants/no. of corpora lutea; 3 and 5% at MD and HD, respectively), (3) an increase in the % of males (6, 6, and 9% at the LD, MD, and HD, respectively), (4) an increase in the body weight of live fetuses (3, 4, and 6% at LD, MD, and HD, respectively), and (5) an increase in the placental weight for live fetuses (3, 5, and 8% at LD, MD, and HD, respectively)

Terminal studies

Gross pathology: there were no drug-related findings in male or female pups sacrificed on Day 22 of lactation.

In 10-wk old pups, there were no drug-related macroscopic findings.

In male pups sacrificed at 16-17 wks postpartum, there were no drug-related macroscopic findings.

Organ/tissue weights: there were no clear drug-related findings in pups sacrificed on Day 22 of lactation.

There were no drug-related findings in males sacrificed at 16-17 wks postpartum, or in females at cesarean delivery (Day 20 of gestation).

5. **Pilot study in non-pregnant rabbits oral administration** (Study no. TRB/400, 11/ID/1011614, report date: 10/87, non-GLP, **only a brief abstract provided**, Vol 1.58)

Non-pregnant rabbits (strain not specified; "n" not specified) were dosed with ICI 204,636 (lot no. not specified) at doses of 50, 100, and 200 mg/kg p.o. for 13 days. According to the report, the following were observed: (1) a transient reduction in mean body weight at 100 mg/kg (4% by Day 6, recovery by Day 13), (2) hypoactivity and unsteadiness of gait were observed at the MD and HD. The sponsor concluded that ICI 204,636 was "severely toxic" when given for up to 10 days to non-pregnant rabbits at 200 mg/kg, "mild toxicity" was noted at 100 mg/kg (for up to 13 days), and the LD could be considered a NOEL.

6. **ICI 204,636: Sighting teratology study in rabbits: oral administration** (Study no. TRB/401, 5/HH/1013346, Zeneca Pharmaceuticals, report date: 5/88, non-GLP, **only summary and summary data provided**, Vol 1.58)

ICI 204,636 (lot no. not specified) was administered to pregnant rabbits (n = 8/grp for main study) strain not specified) at doses of 0, 25, 75, 100, and 150 mg/kg p.o. from Day 6 through Day 18 of gestation. An additional 6 rabbits dosed at 100 mg/kg and 3 additional C rabbits were used for TK analysis; these animals were sacrificed 2 hr after the last dose (Day 18). The main study animals were sacrificed on Day 28 of gestation and fetuses examined macroscopically.

There were 8 unscheduled deaths (main study: 1, 1, and 3 at 25, 100, and 150 mg/kg, respectively; satellite-TK: 3 at 150 mg/kg); these animals were either sacrificed moribund or were found dead. The death at 25 mg/kg was considered unrelated to drug. Due to mortality, the lack of litters (total resorption or implantation sites only in 2 dams at 25 mg/kg, 1 dam at 75 mg/kg, 1 dam at 100 mg/kg) or pregnancy (1 CF, 1 female at 100 mg/kg), there were only 6, 5, 7, 4, and 5 pregnant dams at 0, 25, 75, 100, and 150 mg/kg, respectively.

Drug-related clinical signs consisted of "impaired" defecation, hypoactivity, and unsteadiness (noted at all doses), and alopecia and respiratory problems at 100 and 150 mg/kg. Maternal body weight loss was noted at 75 and 150 mg/kg (7 and 46 gm, respectively) during the dosing period. Overall (i.e., Day 0-28), body weight gain was reduced at doses ≥75 mg/kg, and body weight loss was noted at the HD (72.2 gm). There were no drug-related effects noted on the following parameters: mean empty uterus weight, mean no. of live fetuses per litter, mean pre- or post-implantation losses per litter, mean placental index per litter, or mean amniotic fluid weight/fetus/litter. Mean placental weight per litter was slightly reduced in dosed grps (7, 9, 12, and 11% at 25, 75, 100, and 150 mg/kg). Mean fetal body weight per litter was significantly reduced at the HD (15%). Mean proportion of males per litter was lower in dosed grps; although the effect was not dose-related, the effect was greatest at the HD (16, 7, 17, and 22% at 25, 75, 100, and 150 mg/kg, respectively). There were no drug-related effects on fetal gross abnormalities/anomalies. Plasma levels of ICI 204,636 ranged from 0.58 to 4.97 in the 3 dams dosed at 100 mg/kg. In embryonic tissue, ICI 204,636 could only be analyzed in only 1 of the 3 dams due the lack of pregnancy in 1 dam and total resorptions in another. In the 1 pregnant dam, embryonic tissue contained 6.89 µg of ICI 204,636 per gm of tissue. The

embryonic tissue to maternal plasma ratio in this one dam was 1.39 to 1.

7. **Sighting peri and post natal study in rats oral administration** (Study no. TWR/1709, 11/ID/1011617, ICI Pharmaceuticals Inc., report date: 2/1/89, United Kingdom GLP, Vol 1.58)

Animals: Alpk:APfSD
initial age: 12 wks
initial body weight: not specified
n = 12/grp

Drug: ICI 204,636 (analytical reference no. ADM 45103/87)
identity/strength/purity: Certificate of Analysis provided, 98.2% pure
stability: not documented, statement indicating that "test article was used within its predicted shelf-life. Suspensions stored at 4° C, in dark.
vehicle: 0.5% w/v HPC in 0.1% Tween 80
formulation: suspension, 5.75 and 34.50 mg/mL; samples collected for analysis of actual concentrations immediately upon formulation. Actual concentrations were determined to be 97-102% of intended.
dosing volume: 0.5 mL/100 gm
doses: 0, 25, and 150 mg/kg
route: p.o. (by gavage)
duration: from Day 16 of gestation through Day 8 of lactation

Observations

Clinical signs: all dams were observed at least twice per day.

Body weight: body weights were recorded on Day 1 (day of sperm-positive vaginal smear), 16, and 21 of gestation, and on Days 1, 5, and 8 of lactation.

Parturition/lactation: all dams were allowed to deliver naturally. Observations consisted of: duration of gestation, parturition abnormalities, no. of dead pups, no. of live pups, pup sex, pup body weight (Day 1, 5, and 8 of lactation).

Terminal studies

Gross pathology: dams and pups were sacrificed on Day 8 of lactation and necropsied. Dead pups were examined for external abnormalities and discarded.

Results

Mortality: there were 2 unscheduled deaths (1 LDF, 1 HDF). The death at the LD was determined to be a dosing accident; the HDF was sacrificed moribund due to poor condition. In addition, 2 LDF and 5 HDF were killed because of death of the entire litters.

Clinical signs: the primary drug-related findings were (1) increases in the incidence of vaginal bleeding during parturition (2/12 CF, 6/12 LDF, 6/12 HDF), (2) poor mammary gland development (0/12 CF, 2/12 LDF, 3/12 HDF), and presumed agalactia (0/12 CF, 2/12 LDF, 5/12 HDF).

Body weight: body weight was reduced (compared to CF) at the HD (8% on Day 21 of

gestation). Body weight gain during Days 16-21 of gestation was reduced by 40-43% at the HD.

Body weight was reduced (compared to CF) throughout the lactation period at the HD (5-15%). Body weight gain was reduced at the LD (59%), and body weight loss/no gain was noted at the HD.

Parturition/lactation: there was a slight, but significant, increase in gestation length at the HD; however, the values were within the concurrent C range. Litter survival data were summarized in the following sponsor's table. The total no. of pups, and pup survival during the lactation period studied were reduced at both doses, as well as the number of entire litters lost.

Index	Day p.p.	Group I Control		Group II 25 mg/kg			Group III 150 mg/kg		
		No Dams	Total	No Dams	Total	p	No Dams	Total	p
Total No. of pups	Day 1	12	158	12	142		11	126	*
No. of pups alive (percentage of total no. of pups)	Day 1	12	155 (98.1%)	12 +	139 (97.9%)	-	11	124 (98.4%)	-
	Day 5	12	141 (89.2%)	11	91 (67.9%)	*	11	43 (34.1%)	***
	Day 8	12	140 (88.6%)	11	87 (64.9%)	*	11	37 (29.4%)	***
Viability index (percentage of live pups day 1)	Day 8	12	140 (90.3%)	11	87 (66.4%)	*	11	37 (29.8%)	***
No. of whole litters lost	Day 8		0		2			5	*
No. of litters with at least one dead pup	Day 1		3		3			2	
	Day 8		9		9			10	

Notes to table:

p = statistical significance of difference from control group
Significance levels - * p<.05 *** p<.001
(one-sided to test for decrease in survival due to dosing)
A dash (-) under p indicates unsuitable data for analysis
(+) Dam 4 Group II was lung dosed day 1; 8 live pups killed day 1; litter excluded from analysis for days 5 and 8

APPEARS THIS WAY
ON ORIGINAL

Additional litter indices are summarized in the following sponsor's table:

Index	Time	Group I Control		Group II 25 mg/kg			Group III 150 mg/kg			LSD
		No	Mean	No	Mean	p	No	Mean	p	
Litter size	Day 1	12	12.9	12	11.6		11	11.3		—
	Day 5	12	11.8	10	9.1		8	5.4		—
	Day 8	12	11.7	9	9.7		6	6.2		—
Litter mean weight (g)	Day 1	12	5.449	11	5.950	*	9	5.580		0.384
	Day 5	12	7.869	10	7.889		8	6.400	*	1.349
	Day 8	12	12.120	9	12.921		6	11.333		1.719
Male proportion	Day 1	12	0.545	11	0.526		11	0.412	*	0.126
	Day 5	12	0.569	10	0.440		8	0.444		0.220
	Day 8	12	0.552	9	0.470		6	0.550		0.204

Notes to table:

p = statistical significance of difference from control mean
Significance levels - * p<.05
The litter size is presented to aid interpretation of the other indices - no analysis has been done
LSD is approximate Least Significant Difference from control mean (except male proportion for day 1)

Litter size was reduced at both doses (17 and 47% by Day 8 at LD and HD, respectively), as was the proportion of male pups per litter (Day 1: 4 and 24% at LD and HD, respectively; Day 8: 15% at LD). Mean litter weight was reduced at the HD, but slightly elevated (Day 1 only) at the LD.

Terminal studies

Gross pathology: there were no drug-related findings at necropsy in either dams or pups.

8. **Peri and post natal study in rats: oral administration** (Study no. TWR/1625, 10/HI/013739, ICI Pharmaceuticals, study dates: 6/88-8-88, United Kingdom GLP, Vol 1.58)

Animals: Alpk:APfSD
initial age: ≈11 wks
initial body weight: 224-306 gm
n = 22/grp

Drug: ICI 204,636 (analytical reference no. ADM 45103/87)
identity, strength, purity: Certificate of Analysis provided, purity was 98.2%
stability: previously established, according to sponsor, up to 42 days as suspension (no documentation provided, nor was report containing data identified).
vehicle: 0.5% w/v HPMC in 0.1% Tween 80
formulation: suspension, concentrations of 0.23, 2.3, and 4.6 mg/mL; actual concentrations were confirmed to be 98-104% of intended on sample collected immediately upon formulation.
storage: 4° C, dark

dosing volume: 0.5 mL/100 gm
doses: 1, 10, 20 mg/kg
route: p.o. (by gavage)
duration: from Day 16 of gestation to Day 21 of lactation.

Observations

F_0 generation

Clinical signs: all dams were observed at least twice per day.

Body weights: all dams were weighed on Day 1 (i.e., day of sperm-positive vaginal smear), 7, 11, 16, and 21 of gestation, and on Days 1, 5, 8, 15, and 22 of lactation.

Parturition/lactation: all dams were allowed to deliver naturally. The following litter parameters were assessed: duration of gestation, parturition abnormalities, no. of dead pups, no. of live pups, pup sex, pup weights. Dead pups were examined for external abnormalities prior to discarding.

Terminal studies

Gross pathology: dams which did not deliver by Day 25 of gestation were killed and uterus was examined for presence of implantation sites.

All dams delivering live litters were killed on Days 22-24 of lactation and necropsied. If tissues were taken at necropsy, "...they were fixed in 10% buffered formalin, except for eyes which were fixed in Davidson's fixative".

F_1 generation

Body weights: litters were weighed on Days 1, 5, 8, 15, and 22 of lactation.

Postnatal development: the following parameters were assessed: (1) pinna detachment, adult hair growth pattern, crawling, adult locomotion, open eyes (for these parameters, the day on which ≥50% of each litter achieved the endpoint was recorded), (2) startle response (tested on Day 22 of lactation).

Terminal studies

Gross pathology: all pups were sacrificed on Day 22-24 of lactation. Eyes were taken from 5/sex/grp for microscopic examination.

Pups found dead were examined for external abnormalities and discarded.

Results

F_0 generation

Mortality: there were no animals found dead or sacrificed moribund during the study. Six dams were sacrificed due to the loss of, or failure to litter (2 CF, 1 LDF, 2 MDF, 1 HDF).

Clinical signs: according to the sponsor, there were no drug-related clinical signs in the dams.

Body weight: there were no differences in mean body weight or mean body weight gain during lactation among grps. During lactation, body weights were fairly similar among grps; however, cumulative body weight gain was reduced throughout the lactation period at the HD (20-26%).

Parturition/lactation: there were no drug-related effects on the following parameters: gestation length, no. of live pups, viability index (Day 5), lactation index (Day 22), no. of whole litters lost (Day 22), no. of litters with ≥1 dead pup, litter size, mean litter weight, proportion of male per litter.

Terminal studies

Gross pathology: there were no drug-related findings.

F_1 generation

Clinical signs: according to the sponsor, there were no drug-related clinical signs in the pups.

Postnatal development: the data were summarized in the following sponsor's table. There were no differences among grps in any of the parameters assessed.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

Index	Group I		Group II		Group III		Group IV		LSD
	Control		1 mg/kg		10 mg/kg		20 mg/kg		
	No	Mean	No	Mean p	No	Mean p	No	Mean p	
Pinnae of 50% of litter unfolded	21	4.2	21	4.3	20	4.2	21	4.2	0.4
Crawling of 50% of litter	20	4.8	21	4.8	20	5.1	21	4.5	0.6
Adult locomotion of 50% of litter	20	11.2	21	11.3	20	11.0	21	10.8	0.6
Adult hair growth of 50% of litter	20	15.1	21	15.3	20	14.9	21	14.6	0.6
Eyes opening of 50% of litter	20	20.0	21	20.0	20	19.8	21	19.9	0.4
Startle response (% of pups/litter)	20	100.0	21	100.0 -	20	100.0 -	21	100.0 -	

p = Statistical significance of difference from control mean (none was significant, $p > 0.05$).
A dash (-) under p indicates unsuitable data for significance test.
LSD is Least Significant Difference from control mean.

Terminal studies

Gross pathology: there were no drug-related findings.

Histopathology: only eyes were examined microscopically. The tabulation of the data (individual tables only) was unclear. When findings were listed, there was no indication how many and which pups (per dam) were affected. According to the sponsor, there were no drug-related findings.

9. **ICI 204,636: Teratology study in rabbits oral administration** (Study no. TTB/402, 3/HI/012577, ICI Pharmaceuticals, study dates: 11/87-12/87, GLP, Vol 1.58).

Note: this study was reviewed previously (P/T Review, J.J. DeGeorge, Ph.D., 12/15/89); however, only a portion of that review was found in the division file. Therefore, the study is reviewed here.

Animals: Dutch Belted rabbits
initial age: sexually mature
initial body weight: 1667-2607 gm
diet/water: *ad lib* throughout the study
n = 20/grp for main study, and an additional 6 C and HD animals for TK analysis

Drug: ICI 204,636 (analytical reference no. ADM 56074/86)
vehicle: 0.5% w/v HPMC in 0.1% Tween 80
formulation/concentration: suspension, at 0, 28.75, 57.5, 115.0 mg/mL. Analysis of suspensions indicated that 96.3-102.2% of intended concentrations were achieved.
identity/purity: a Certificate of Analysis was provided. Total impurities = 1.1%.

doses: 0, 25, 50, 100 mg/kg
route: p.o. (gavage)
duration: Days 6 to 18 of pregnancy

Observations

Clinical signs: all animals were examined daily.

Body weight: body weights were recorded on Days 0, 6, 10, 14, 18, 21, and 28 of pregnancy in main study animals.

Terminal studies

TK: TK animals were sacrificed on Day 18 of pregnancy and blood samples were collected from dams (n = 4) and embryos (from the 4 dams) for analysis of ICI 204,636 levels.

Gross pathology: all surviving animals were killed on Day 28 of pregnancy and complete necropsies were performed. Necropsies were also performed on animals that died or were sacrificed moribund.

Reproductive/Litter parameters: at necropsy, the following parameters were analyzed: weight in intact uterus and contents, no. and position of viable, dead and resorbed fetuses, fetal body weights, fetal sex, placental weights, empty uterus weight, collective wt of resorptions, no. of corpora lutea.

Fetal examination: fetuses (and placenta) were examined macroscopically and all surviving fetuses were further examined for visceral and skeletal (x-ray) findings. The method used for examination was not specified.

Results

Mortality: the status of animals at the end of the study was summarized in the following sponsor's Table 3:

Group	Number pregnant at necropsy	Number not pregnant at necropsy	Number with resorptions/ implantation site only	Number killed prior to day 28
I: Control	18	0	2	6*
II: ICI 204,636 25 mg/kg/day	19	1	0	0
III: ICI 204,636 50 mg/kg/day	16	1	1	2
IV: ICI 204,636 100 mg/kg/day	15	1	3	1 + 6*

* Six rabbits from Group IV and six undosed controls were allocated for pharmacokinetics; one of the Group IV females was killed for humane reasons on day 12 of pregnancy and a further Group IV female had only resorptions at necropsy.

There were 4 unscheduled deaths: 1 MDF and 1 HDF were sacrificed due to complete loss of litters, 1 MDF was sacrificed on Day 10 (reason not specified),

and 1 HDF-TK was sacrificed ("...for humane reasons...") on Day 12.

Clinical signs: drug-related clinical signs were evident at all doses, with the no. of observations and incidence (i.e., no. of animals) were dose-related. The primary signs included reduced/ceased defecation, unsteady mobility/gait, hypoactivity, noisy breathing/respiratory difficulties, and nasal discharge. Unsteady mobility/gait and hypoactivity were noted in all HD animals (including satellite-TK animals).

Body weight: body weight loss was noted at all doses (-3.5, -1.0, and -119 gm at LD, MD, and HD) during the dosing period; in comparison, during the same period, CF gained a mean of 90 gm.

Terminal studies

TK: the data were summarized in the following sponsor's table:

Concentration of ICI 204,636			
Animal number	Maternal plasma (µg/ml)	Embryonic tissue (µg/g)	Ratio of concentrations: Embryonic tissue : Maternal plasma
2797	1.32	1.70	1.29
2798	6.45	NS	-
2801	5.17	2.80	0.54
2802	1.75	1.63	0.93
2807	1.69	1.57	0.93
Mean ± SE	3.28 ± 1.06	1.93 ± 0.29	0.92 ± 0.15

Limit of quantification for maternal plasma = 0.2 µg/ml.
Limit of quantification for embryonic tissue = 0.5 µg/g.
NS = No sample, resorption only.

Gross pathology: in the HDF sacrificed on Day 12, findings consisted of friable liver and white areas of liver (covering ≈25% of total area).

There were no clear drug-related findings. Enlarged gallbladder was detected in 2 HDF only.

Reproductive/Litter parameters: mean empty uterus weight was slightly lower in all dosed grps (9-13%); however, the effect was not significant nor clearly dose-related. There were no clear drug-related effects on the following parameters: no. of live fetuses/litter, total implants, pre-implantation or post-implantation losses, placental wt/litter, amniotic fluid wt/fetus/litter, no. of corpora lutea.

The data are summarized in the following sponsor's tables:

Index	Group I		Group II		Group III		Group IV		LSR
	Control		25 mg/kg		50 mg/kg		100 mg/kg		
	No	Mean	No	Mean p	No	Mean p	No	Mean p	
Number of live fetuses	18	7.0	19	5.9	16	6.2	15	7.1	1.3
Total implants	18	7.2	19	6.1	16	6.7	15	7.3	1.3
Number of corpora lutea	18	8.1	19	7.3	16	7.5	15	8.9	1.3
Pre-implantation loss	18	0.9	19	1.2	16	0.9	15	1.6	2.0
Post-implantation loss	18	0.2	19	0.3	16	0.6	15	0.2	4.0

Notes to table:
p = statistical significance of the control group ratio (none were significant p > 0.05)
The adjusted means are quoted
LSR is the approximate Least Significant Ratio - if the ratio of a dosed group mean to that of the control (or its reciprocal) exceeds the quoted figure, then statistical significance is achieved

Index	Group I		Group II		Group III		Group IV		LSD
	Control		25 mg/kg		50 mg/kg		100 mg/kg		
	No	Mean	No	Mean p	No	Mean p	No	Mean p	
Fetal bodyweight (g)	17	28.58	19	29.16	16	28.82	15	26.01 *	2.02
Placental weight (g)	17	3.684	19	4.099 *	16	3.811	15	3.758	0.404
Placental index	17	13.00	19	14.06	16	13.35	15	14.50 *	1.22
Amniotic fluid per fetus (g)	17	2.719	19	3.297 *	16	2.956	15	2.931	0.479
Male proportion	16	0.576	19	0.624	16	0.523	15	0.452 *	0.115

Notes to table:
p = statistical significance of difference from control mean
Significance levels - * p<.05
LSD is approximate Least Significant Difference from control mean
The least squares means are quoted

Mean fetal body weight and the ratio of male-to-female were reduced at the HD (9 and 22%, respectively). [The M/F ratio was notably lower in 2 HDF: 0.14 vs 0.25-0.75 in CF.] Placental index (i.e., total placental wt of litter/total fetal wt of litter) was increased at the HD (11%).

Fetal examination: a total of 127, 112, 100, and 111 fetuses were examined in

C, LD, MD, and HD grps, respectively.

The external and placental findings were summarized in the following sponsor's table. The sponsor considered there to be no drug-related findings. However, there was a dose-related incidence (affected fetuses and litters) of carpal/tarsal flexture(s): (fetuses: 0, 0, 1, 5; litters: 0, 0, 1, 4 in C, LD, MD, and HD, respectively).

Group	Number of fetuses examined	Fetal and placental examination results
I: Control	127	5 Reduced/vestigial tail (two litters) 1 Displaced carotid arteries 1 Oedematous; reduced nares; distended aorta; absent pulmonary artery 1 Placental anomaly Remaining fetuses and placentas NAD
II: ICI 204,636 25 mg/kg/day	112	1 Spina bifida; displaced testes 1 Blood in testes 1 Blood in stomach; pale lungs 2 Enlarged cardiac vessels (one litter) 2 Pale lungs (one litter) 3 Transparent retinae (one litter) 1 Cleft palate Remaining fetuses and placentas NAD
III: ICI 204,636 50 mg/kg/day	100	2 Vestigial/absent tail (one litter) 1 Carpal flexure 1 Pale lungs 1 Bilobed gall bladder 1 Blood in testis 1 Placental anomaly Remaining fetuses and placentas NAD
IV: ICI 204,636 100 mg/kg/day	111	2 Vestigial/reduced tail (two litters) 5 Carpal/tarsal flexure(s) (four litters) 2 Pale spleen (one litter) 1 Transparent retina 1 Cleft palate 1 Reduced nares 1 Ablepharia; abdominal oedema; enlarged atria(um) and cardiac vessels; thickened septum 1 Enlarged heart; abdominal oedema 1 Umbilical hernia; nares reduced 3 Placental anomalies Remaining fetuses and placentas NAD

NAD = No abnormality detected.

Skeletal findings were summarized in the following sponsor's table. The data were expressed as the no. (and percentage) of affected fetuses, but not in terms of no. (%) of affected litters. The only statistically significant finding was an

increase in the incidence of incomplete ossification of hyoid. There was also an increased incidence of this finding when expressed as no. (%) of affected litters [litters (%): 7 (39%), 8 (42%), 6 (38%), 11 (73%) at C, LD, MD, and HD, respectively]. Although not statistically significant, the following findings were notable: (1) a dose-related increase in abnormal sternebral ossification, expressed as the % of affected litters (33, 42, 44, and 47% in C, LD, MD, and HD grps, respectively), (2) an increase in incomplete sternebral ossification at the MD and HD, expressed as % of affected litters (22, 26, 44, and 40% in C, LD, MD, and HD, respectively), (3) incomplete ossification of inter-parietals was noted only in dosed grps, and the % affected fetuses and litters was highest at HD.

Anomaly	Group I	Group II		Group III		Group IV	
	Control	25 mg/kg		50 mg/kg		100 mg/kg	
	No (%)	No (%)	p	No (%)	p	No (%)	p
Incomplete cranial ossification	35 (27.8%)	27 (24.1%)		38 (38.0%)		44 (39.6%)	
Incomplete ossification of parietals	24 (19.0%)	11 (9.8%)		21 (21.0%)		25 (22.5%)	
Incomplete ossification of inter-parietals	0 (0.0%)	2 (1.8%)	–	1 (1.0%)	–	3 (2.7%)	–
Incomplete ossification of hyoid	10 (7.9%)	13 (11.6%)		16 (16.0%)		27 (24.3%)	**
Widened anterior fontanelle	3 (2.4%)	7 (6.3%)		3 (3.0%)		4 (3.6%)	
Abnormal sternebral ossification	9 (7.1%)	14 (12.5%)		14 (14.0%)		13 (11.7%)	
Bifid sternebrae	3 (2.4%)	1 (0.9%)	–	0 (0.0%)	–	1 (0.9%)	–
Asymmetric sternebrae	2 (1.6%)	5 (4.5%)	–	0 (0.0%)	–	2 (1.8%)	–
Incomplete sternebral ossification	5 (4.0%)	9 (8.0%)		14 (14.0%)		11 (9.9%)	
Vestigial ribs	37 (29.4%)	33 (29.5%)		35 (35.0%)		24 (21.6%)	
Number of dams	18	19		16		15	
Number of fetuses	126	112		100		111	

Notes to table:
p = statistical significance increase from control group incidence
Approximate significance levels – * p<.05 ** p<.01
A dash (–) under p indicates unsuitable or insufficient data for significance test

APPEARS THIS WAY
ON ORIGINAL

MUTAGENICITY

1. **In vitro mammalian cell gene mutation assay in Chinese Hamster Ovary cells** (Study No. TMV/259, ICI Pharmaceuticals, Study dates: 7/13/89-10/31/89, Report date: 7/11/90, UK GLP).

The mutagenic potential of ICI 204,636 (lot no. ADM 45103/87) was tested in CHO cells (HGPRT locus) both in the absence and presence of metabolic activation (rat S9, Aroclor-induced). The data were expressed in terms of mutation frequency, defined as the number of mutant colonies per 10^6 viable cells. [This number of cells is acceptable since it is greater than 10 times the inverse of the spontaneous mutation frequency, i.e., <20 per 106 cells.] Positive controls were EMS (-S9) and 20-MC (+S9). The criteria for a positive response were as follows: (1) "...a statistically significant dose-related increase in mutant frequency at concentrations of test compound which result in greater than 10% cell survival", (2) the effect must be reproducible, and (3) an increase in mean mutant frequency in treated cultures should be greater than historical control and the spontaneous levels.

In a preliminary assay to test for cytotoxicity, ICI 204,636 was tested at concentrations up to 750 µg/mL (i.e., 652.2 µg/mL base, the limit of solubility). According to the sponsor (i.e., no data provided), ICI 204,636 was cytotoxic at concentrations of ≥250 and ≥500 µg/mL in the absence and presence of S9, respectively.

Two separate tests were conducted. In the first main study, ICI 204,636 was tested at concentrations of 0, 15.6, 31.3, 62.5, 125, 200, and 250 µg/mL in the absence of S9, and at concentrations of 0, 31.3, 62.5, 125, 250, and 375 µg/mL in the presence of S9. A second test was conducted due to excessive cytotoxicity in the first test. In the second main study, ICI 204,636 was tested at concentrations of 0, 15.6, 31.3, 62.5, 125, and 160 µg/mL in the absence of S9, and concentrations of 0, 31.3, 62.5, 125, 250, and 300 µg/mL in the presence of S9.

In the first study, there was 0% relative cell survival at the highest concentrations tested both in the absence and presence of S9 (i.e., 200 and 375 µg/mL, respectively). At the lower concentrations (i.e., ≤125 and ≤250 µg/mL with and without S9, respectively), relative cell survival ranged from 74 to 98% (-S9) and 79-107% (+S9). There was no increase in mutation frequency either with or without S9. In the second study, relative cell survival at the highest concentrations tested were 82-118% (-S9) and 60-68% (+S9). There were no significant increases in mean mutation frequency either in the absence or presence of S9. Positive controls produced large increases in mutation frequency in both studies.

2. **In vitro cytogenetic study using cultured human lymphocytes** (Study No. TYX/25, ICI Pharmaceuticals Inc., study dates: 9/7/87-2/8/88, report date: 2/24/88, GLP).

The clastogenic potential of ICI 204,636 (lot no. ADM 56074/86) was tested in cultured human lymphocytes at concentrations of 0, 12.5, 25, 50, 125, 250, and 500 µg/mL, both in the absence and presence of metabolic activation (rat S9, Aroclor 1254-induced). Methylmethane sulphonate (-S9) and cyclophosphamide (+S9) were used as positive controls. Cytotoxicity was expressed as reductions in MI (no. of cells in division per 1000 scored cells). Two hundred (2 x 100) metaphases were examined for aberrations at each concentration, and 400 (4 x 100) metaphases were examined for vehicle.

ICI 204,636 was cytotoxic at 500 µg/mL, resulting in an MI of 0% at this concentration; therefore, cultures exposed to 0, 25, 125, and 250 µg/mL of ICI 204,636 were scored for chromosomal aberrations both in the absence and presence of S9. There was no increase in

structural chromosomal aberrations either with or without S9. There was a statistically significant increase in polyploidy at 125 µg/mL in the presence of S9. The frequency of polyploidy at this dose was 3 out of 200 metaphases, or 1.5%; this is within the historical control frequency range (provided by the sponsor, 0-3.3%).

Summary of previously reviewed mutagenicity studies

Ames tests: Studies TMV/257, TMV/258, TMV/369, TMV/384, TMV/385, and TMV/261 have been previously reviewed; therefore, they will be summarized here. All of these studies were conducted under GLP. All studies but TMV/369 were conducted by the sponsor (ICI Pharmaceuticals); TMV 369 was performed by Huntingdon Research Center. In Studies TMV/257, TMV/258, and TMV/369, the lot no. of ICI 204,636 was ADM 56074/86 (purity = 98.5%). In Studies TMV/384, TMV/385, and TMV/261, the lot no. was ADM 45103/87 (purity = 98.2%).

All but Study TMV/361 were conducted in Salmonella Typhimurium strains TA 1535, TA 1537, TA 1538, TA 100, and TA 98. ICI 204,636 was tested at the concentrations noted in the following summary table. According to the sponsor, the HC was selected based on concentration-limiting toxicity; however, in only one study (TMV/369) were preliminary toxicity data provided, and this study was not the initial study conducted. ICI 204,636 was tested in the five strains both in the absence and presence of metabolic activation (rat S9, Aroclor 1254-induced). In none of the studies was there an increase in revertants detected with tester strains TA 1535, TA 1537, TA 100, or TA 98 either with or without metabolic activation, or in TA 1538 in the absence of S9. In Studies TMV/384 and TMV/385, there were concentration-related increases in revertants with tester strain TA 1538 in the presence, but not in the absence of S9. The data (expressed as no. of revertant colonies) for TA 1538 (+S9) are summarized in the following table:

STUDY NO.	LOT NO.	CONCENTRATION (µg/plate)	INDIVIDUAL COUNTS	MEANS
TMV/257	ADM 56074/86	control	7, 6, 7	6.7 ± 0.6
		50	5, 3, 3	3.7 ± 1.2
		200	7, 7, 5	6.3 ± 1.2
		400	4, 1, 2	2.3 ± 1.5
		600	3, 4, 6	4.3 ± 1.5
		800	6, 11, 6	7.7 ± 2.9
TMV/258	ADM 56074/86	control	3, 3, 2	2.7 ± 0.6
		50	4, 0, 8	4.0 ± 4.0
		200	4, 2, 5	3.7 ± 1.5
		400	2, 1, 2	1.7 ± 0.6
		600	3, 2, 1	2.0 ± 1.0
		800	3, 1, 2	2.0 ± 1.0
TMV/369	ADM 56074/86	control	11, 14, 16	14.2 ± 2.5
		30	8, 10, 11	10 ± 1.5
		100	11, 10, 7	9 ± 2.1
		300	8, 15, 12	12 ± 3.5
		1000	14, 11, 7	11 ± 3.5
		3000	7, 9, 7	8 ± 1.2
		control	14, 16, 15	15 ± 1.0
		30	13, 14, 13	13 ± 0.6
		100	14, 18, 11	14 ± 3.5
		300	12, 11, 10	11 ± 1.0
		1000	15, 14, 8	12 ± 3.8
		3000	11, 7, 10	9 ± 2.1
TMV/384	ADM 45103/87	control	7, 11, 15	11.0 ± 4.0
		50	8, 9, 14	10.3 ± 3.2
		200	14, 8, 13	11.7 ± 3.2
		400	13, 13, 12	12.7 ± 0.6
		600	16, 24, 15	18.3 ± 4.9
		800	23, 13, 16	17.3 ± 5.1
		control	12, 17, 11	13.3 ± 3.2
		50	8, 11, 6	8.3 ± 2.5
		200	9, 11, 9	9.7 ± 1.2
		400	5, 19, 16	13.3 ± 7.4
		600	24, 11, 14	16.3 ± 6.8
		800	7, 24, 14	15.0 ± 8.5
TMV/385	ADM 45103/87	control	4, 12, 4	6.7 ± 4.6
		50	7, 4, 7	6.0 ± 1.7
		200	8, 3, 14	8.3 ± 5.5
		400	9, 9, 15	11.0 ± 3.5
		600	22, 18, 17	19.0 ± 2.6
		800	9, 25, 15	16.3 ± 8.1
		control	7, 12, 13	10.7 ± 3.2
		50	9, 15, 8	10.7 ± 3.8
		200	14, 13, 7	11.3 ± 3.8
		400	8, 14, 15	12.3 ± 3.8
		600	30, 24, 25	26.3 ± 3.2
		800	16, 24, 36	25.3 ± 10.1

In addition to these main study data, additional experiments were conducted in Study TMV/385; although not specifically stated, it is assumed that the same lot no. of ICI 204, 636

was used as in the main assays in this study. The data from these are presented in the following sponsor's Tables A4/1 and A4/2. In Table A4/1, the sponsor has provided individual and summary data for TA 1538, using two different stocks of cells (A, B). With Stock A, there was an ≈2-fold increase in revertants at 1000 µg/plate, and a smaller increase (≈1.5 fold) in revertants at 750 and 1500 µg/plate. With Stock B, there were increases in revertants at 750 and 1000 µg/plate (1.8 and 1.6 fold, respectively). Cytotoxicity was evident at 2000 µg/plate as evidenced by a notable decrease in revertants with both Stock A and B at this dose (and perhaps at 1500 µg/plate with Stock B).

Table A4/1 : ICI 204,636 : Ames test : Bacterial mutagenicity study using selected strains of Salmonella typhimurium : standard method. Study number TMV/385. Ames test using two stocks of strain TA 1538.

Strain	Compound	Amount µg per plate	Revertant colony counts	+ S-9 mix
				Mean ± SD
TA 1538 Stock A	DMSO	100 µl	10, 10, 14	11.3 ± 2.3
	ICI 204,636	250	6, 18, 17	13.7 ± 6.7
		500	8, 15, 12	11.7 ± 3.5
		750	25, 11, 15	17.0 ± 7.2
		1000	30, 20, 14	21.3 ± 8.1
		1250	20, 13, 16	16.3 ± 3.5
		1500	14, 7, 9	10.0 ± 3.6
		2000	4, 6, 0	Toxicity
	Untreated	0	10, 8, C	9.0 ± 1.4
	2AAF	50	1511, 1266, 1676	1484.3 ± 206.3
TA 1538 Stock B	DMSO	100 µl	11, 12, 11	11.3 ± 0.6
	ICI 204,636	250	13, 11, 12	12.0 ± 1.0
		500	12, 15, 16	14.3 ± 2.1
		750	19, 13, 28	20.0 ± 7.5
		1000	17, 26, 13	18.7 ± 6.7
		1250	18, 13, 11	14.0 ± 3.6
		1500	11, 6‡, 7	8.0 ± 2.6
		2000	3, 7, 0	Toxicity
	Untreated	0	10, 11, 14	11.7 ± 2.1
	2AAF	50	1678, 1189, 1806	1557.7 ± 325.6

C = Contaminated.
SD = Standard deviation.
DMSO = Dimethyl sulphoxide.
2AAF = 2-Acetylaminofluorene.
Stock A was from liquid nitrogen, frozen November 1987.
Stock B was in current use.
‡ Reduced bacterial lawn.

In Table A4/2, the sponsor has summarized the data on TA 1538 using the preincubation method, which is presumed to provide a more sensitive assessment of both mutagenicity and cytotoxicity. ICI 204,636 was clearly cytotoxic at 700 µg/plate, as evidence by the decrease in revertants at this dose. Increases in revertants were noted at 300 and 400 (particular in one culture) µg/plate (≈2 and 1.5-fold, respectively).

Table A4/2 : ICI 204,636 : Ames test : Bacterial mutagenicity study using selected strains of Salmonella typhimurium : standard method. Study number TMV/385. Preincubation method.

Compound	Amount µg per plate	Revertant colony counts	Mean revertant colony counts ± SD
DMSO	100 µl	14, 14, 18	15.3 ± 2.3
ICI 204,636	100	25, 19, 20	21.3 ± 3.2
	200	22, 17, 25	21.3 ± 4.0
	300	30, 33, 27	30.0 ± 3.0
	400	30, 21, 17	22.7 ± 6.7
	500	12, 16, 20	16.0 ± 4.0
	600	17, 9, 13	13.0 ± 4.0‡
	700	9, 7, 5	7.0 ± 2.0‡
Untreated	0	18, 25, 24	22.3 ± 3.8
2AAF	50	1362, 1113, 1805	1426.7 ± 350.5

SD = Standard deviation.
DMSO = Dimethyl sulphoxide.
2AAF = 2-Acetylaminofluorene.
‡ Reduced bacterial lawn.

In terms of the overall acceptability of the Ames test, it must be noted that there was no evidence of cytotoxicity provided for the majority of assays conducted. No mention of cytotoxicity was found for all tester strains in the presence of S9 in Studies TMV/257, TMV/258, TMV/384, and TMV/385. In the absence of S9, no cytotoxicity was noted as follows: TMV/257: TA 1535, TA 98; TMV/258: TA 1535, TA 1538, TA 98; TMV/384: TA 1535, TA 98, TA 100; and TMV/385: TA 1535, TA 98, TA 100. In Study TMV/469, where higher concentrations were used, the sponsor indicated that cytotoxicity was observed with all tester strains in the absence of S9, and with all but TA 1538 or TA 1537 and TA 1537 (with replicate) in the presence of S9; in these cases, however, there was evidence of cytotoxicity (i.e., decrease no. of revertants) at the HC.

Study TMV/261 was conducted in *E. coli* strain WP_2 uvrA pKM101 at concentrations of ICI 204,636 (ADM 45103/87) of 25, 50, 100, 250, 500, and 1000 µg/plate, both in the absence and presence of metabolic activation. As with the Ames assays using *S. typhimurium*, cytotoxicity at the HC was not demonstrated. There were no increases in revertants either with or without metabolic activation in the *E. coli* strain.

Micronucleus Test in the Rat: This study (TQR/1535) was previously reviewed (J.J. DeGeorge, Ph.D., 12/15/89), and will, therefore, only be summarized here. This *in vivo* test was conducted in Alpk:APfSD rats (10/sex) at an oral dose of 500 mg/kg; each animal received only one dose. Positive and negative control grps were included (10/sex/grp). Cyclophosphamide (20 mg/kg) was used as the positive control. Animals were sacrificed and bone marrow smears were prepared at 24 and 48 hr postdosing (5/sex/time point/grp). Two thousand polychromatic erythrocytes (PCEs) were examined in each animal for micronuclei. The dose of ICI 204,636 was considered to be an MTD based on data from a previous limit dose study.

Drug-related clinical signs were evident in both males and females, and consisted of subdued behavior, ptosis, and hunched posture. There were no increases in micronuclei in males at either 24 or 48 hrs. In females, however, there was a significant increase in micronuclei at 48 hr (6.4 ± 6.0 vs 2.4 ± 2.3 for CF). This increase was due to a high value in 1 F (16 vs 0-8 in other CF and DT at 48 hr). In order to check the "reproducibility" of the effect was tested by the sponsor by examining an additional 2000 PCEs from the same animals (C and DT). No significant differences were noted following the second examination.

These data do not suggest a clastogenic potential for ICI 204,636. The significant effect in treated females at 48 hr should not be considered biologically significant because the increase in micronuclei was due to a high value in only 1 DTF, not because of the second analysis. The method used cannot be considered an assessment of "reproducibility".

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

SUMMARY AND EVALUATION

PHARMACOLOGY

ICI 204,636 was tested in a number of *in vitro* and *in vivo* assays in order to provide a mechanistic basis for both primary (i.e., those related to therapeutic effects and to adverse effects at the same receptors) and secondary (i.e., those related to side effects unrelated to therapeutic effects) pharmacological effects.

Effects related to therapeutic efficacy: In *in vitro* receptor binding studies, ICI 204,636 demonstrated fairly high affinity only for the α_1 adreno-, H_1, and $5HT_6$ receptors (K_i = 13, 10, and 33 nM, respectively). Affinity for the D_2 and $5HT_2$ receptors was weak (IC_{50} = 329-690 and 148-288 nM, respectively; K_i = 531 and 288 nM, respectively). ICI 204,636 exhibited 1.3-2 fold greater potency at the $5HT_2$ than at the D_2 receptor. In comparison, clozapine was found to have an almost 7-fold greater potency at the $5HT_2$ receptor. Clozapine has greater affinity for the D_1, D_2, D_4, $5HT_{1A}$, $5HT_2$, α_1, α_2, and muscarinic receptors than does ICI 204,636 when compared in the same assays. ICI 204,636 had no affinity for either the muscarinic or D_4 receptors. ICI 204,636 and clozapine had similar (weak) binding affinity for the D_3 receptor.

In electrophysiology studies, ICI 204,636 (p.o.) was shown to preferentially affect ventral tegmental (A10) over striatal (A9) dopamine neurons. When given acutely, ICI 204,636 increased spontaneous firing in A10, but not A9, dopamine neurons, whereas when given chronically, ICI 204,636 reduced spontaneous firing in A10, but not A9, neurons. These effects were similar to those produced by clozapine. In contrast, haloperidol produced effects in both the A9 and A10 regions.

In contrast to the electrophysiology studies, ICI 204,636 (p.o., i.p.) increased dopamine turnover (as measured by increases in dopamine metabolites, HVA, DOPAC, and 3-MT) in the striatum, but not olfactory tubercle or whole brain in rat.

In vivo occupancy of D_2 and $5HT_2$ receptors was assessed *in vivo* using protection from N-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)-induced inactivation as a measure. ICI 204,636 was protective at both receptors, as was clozapine. From these data, it was estimated that, at a dose of 40 mg/kg i.p., there was ≈30-40% occupancy of the $5HT_2$ and D_2 receptors with ICI 204,636, and ≈60-70% occupancy for clozapine. For comparison, haloperidol, at 1 mg/kg i.p., resulted in ≈60-70% "occupancy" of the D_2 receptor. The sponsor pointed out that this assay probably provides an underestimate of receptor occupancy since it is based on competition of drug with EEDQ for the receptor.

ICI 204,636 was active in a variety of animal models considered to be predictive of antipsychotic activity in humans. ICI 204,636 disrupted conditioned avoidance responding in monkey, and apomorphine- and amphetamine-induced behaviors in monkey, cat, mouse, and rat at ED_{50}'s ranging from 2.1-80 mg/kg p.o. When compared directly, clozapine was shown to have less, greater, and similar potency than ICI 204,636 depending upon the particular assay involved. In monkey, ICI 204,636 was 2-4 fold more potent than clozapine in disrupting the conditioned avoidance response and in antagonizing apomorphine-induced blinking in squirrel monkey.

Tolerance to the effects of ICI 204,636 upon multiple dosing was not observed when assessed by measurement of serum prolactin (elevations) or dopamine metabolites in rat brain.

ICI 204,636 demonstrated some side-effect liability when administered p.o. in *in vivo* paradigms

of eps potential. ICI 204,636 induced dyskinesia in haloperidol-sensitized and in naive animals at 5-20 and 20 mg/kg p.o., respectively, and catalepsy in rats at all doses tested (20-80 mg/kg i.p.). In comparison to ICI 204,636, clozapine was, in general, less effective, whereas haloperidol was markedly more effective in these models. In the paw test in rats, ICI 204,636 and clozapine preferentially prolonged hindlimb (compared to forelimb) retraction time, suggesting reduced eps liability. In contrast, haloperidol was equally effective on fore- and hindlimbs.

ICI 204,636 fully substituted for clozapine in the stimulus discrimination paradigm, whereas risperidone did not.

In an animal model proposed to predict clinical efficacy against negative symptoms, i.e., social isolation in monkey, ICI 204,626 antagonized amphetamine-induced reductions in social behavior (negative signs) and increases in submissive (positive signs) behavior.

Secondary pharmacology: in *in vivo* functional studies of receptor activity, ICI 204,636 exhibited no activity at the α_2, $ß_1$, $ß_2$, H_2, or thromboxane receptors, fairly weak antagonist activity at the α_1, $5HT_2$, and muscarinic receptors, and high activity at the H_1 receptor. Clozapine exhibited similar activity at the H_1 receptor, but was >8 to 50 times more potent than ICI 204,636 at the α_1, α_2, $5HT_2$, and the muscarinic receptors. ICI 204,636 also antagonized α_1-agonist-induced cardiovascular parameters *in vivo*.

ICI 204,636 produced decreases in blood pressure in normal (1-10 mg/kg i.v.) and SH rats (50 mg/kg p.o.) and dogs (0.3-3 mg/kg i.v.) and in heart rate in rat (1-10 mg/kg i.v.). ICI 204,636 also inhibited compensatory mechanisms following head tilt in dogs. The only ECG effect documented was a shortening of the P-R interval in conscious dogs at 25 mg/kg p.o. The cardiovascular effects (i.e., blood pressure, heart rate, P-R interval) of ICI 204,636 in dog tended to be somewhat inconsistent among studies. None of the parameters were markedly affected by ICI 204,636.

ICI 204,636 exhibited no anxiolytic or analgesic activity in rodents, but did slightly increase (i.e., neither effect was statistically significant) the potential for metrazole-induced seizures and ecs-induced tonic convulsions in rats. ICI 204,636 produced local anesthetic effects in mice when injected into the area of the sciatic nerve at doses of 6.25-25 mg/kg i.m. ICI 204,636 did not potentiate sleeping time in animals treated with a subthreshold dose of sodium barbital. GI effects of ICI 204,636 included slight decreases in gastric acid secretion (50 mg/kg p.o.) in dog and a decrease in gastric motility in mice. No renal effects were observed.

Pharmacology of metabolites

Three metabolites, ICI 214,227, M 236,303, and ICI 213,841, were tested in the general pharmacology battery used to assess ICI 204,636. For the most part, these metabolites had effects similar to those of ICI 204,636. All had functional (antagonist) effects on muscarinic and H_1 receptors in isolated tissue preparations, all produced some cardiovascular effects (M 236,303 and ICI 213,841 shortened the P-R interval), local anesthetic effects, and reduced gastric motility. M 236, 303 and ICI 213,841 increased vascular resistance. None had any effect on renal parameters. It should be noted, however, that these studies were conducted *in vivo* and primarily using the oral route; there was no evaluation of the oral absorption or bioavailability of these metabolites.

The *in vitro* binding affinity of ICI 204,636 and 4 metabolites (including ICI 214,227 and M 236,303) for the D_1, D_2, and $5HT_2$ receptors was quantitated. Both metabolites had higher affinity for all three receptors than did ICI 204,636. In particular, M 236,303 had 12 times higher affinity for the $5HT_2$ receptor as compared to the parent compound (K_i = 6.5 and 80 nM,

respectively). Two other metabolites, M 234,676 (7-OH, N-dealkylated) and M 211,803 (N-dealkylated), also exhibited affinity (moderate to weak) for the D_2 and $5HT_2$ receptor. M 234,676 also had weak affinity for the D_1, whereas M 211,803 exhibited little affinity (i.e., K_i > 1 μM) for this receptor.

PK/ADME

The PK/ADME of ICI 204,636 was studied primarily in rat, dog, and monkey; however, limited study was conducted in mouse and rabbit to support toxicity studies.

Mouse: only two ADME studies were conducted in mouse. These studies, a balance study and an hepatic microsomal enzyme induction study, were conducted in male and female Alpk C57Bl and female CD-1 mice, respectively. There was a statistically significant sex difference in terms of route of elimination of radioactivity (single oral dose, 20 mg). In males, the primary route was via the feces (60.2% of radioactivity vs 33% for urinary); in females, elimination via the urine and feces were fairly similar (43 and 50% for urine and feces, respectively). At 120 min postdosing, 96% of radioactivity was recovered. Since only oral dosing was tested in mice, no estimate of oral bioavailability can be made; however, the extent of urinary elimination of radioactivity would suggest that, at a minimum, 30-40% of the oral dose was absorbed in the mouse.

The effect of ICI 204,636 on hepatic microsomal enzymes was tested at doses of 10-400 mg/kg given for 1 (HD) to 3-mo (drug-diet admixture). Liver weight was increased slightly after 1-mo at 400 mg/kg; however, since the magnitude of this effect was less than observed previously (i.e., 28 vs 11%), the lower doses were allowed to continue for an additional 2 mo of dosing. At 3-mo, liver weight was not significantly elevated at any of the lower doses. Enzyme induction was, however, observed at all but the LD (i.e., 10 mg/kg). At 400 mg/kg, increases were noted in P450 content and in the activity of all enzymes measured (i.e., NADPH-cytochrome c reductase, aldrin epoxidase, ethoxycoumarin O-deethylase, ethoxyresorufin O-deethylase, and pentoxyresorufin O-dealkylase). At doses of 50 and 200 mg/kg, increases were noted in P450 content (also slightly elevated at 25 mg/kg). Ethoxycoumarin O-deethylase and pentoxyresourfin O-dealkylase were elevated at doses ≥25 mg/kg. Aldrin epoxidase and ethoxyresorufin O-deethylase were also significantly elevated at 200 mg/kg. NADPH-cytochrome c reductase was only elevated at 400 mg/kg.

The metabolic profile of ICI 204,636 was not assessed in mouse.

Rat: the PK/ADME of ICI 204,636 was studied primarily in Sprague-Dawley rats (pigmented strains were used for evaluation of tissue distribution). In male and female rats, the primary route of elimination of radioactivity was via the feces (>80% of dose, after 1 and 7-daily doses), with biliary secretion of radioactivity accounting for a majority of fecal radioactivity. In bile-cannulated male and female rats, <5% of dose radioactivity (single doses of 5 mg/kg i.v., p.o., 25 mg/kg p.o.) was excreted in the feces, whereas 70-80% of dose radioactivity was secreted in bile.

Pharmacokinetic parameters were calculated for ICI 204,636 and two plasma metabolites, ICI 214,227 (7-OH, also sometimes referred to as 2-OH) and ICI 213,841 (sulfoxide) after single and multiple dosing (7-day) with ICI 204,636 at doses of 25 (multiple dose) and 125 mg/kg p.o. and 5 mg/kg i.v. (single doses). The rate of absorption tended to be faster in females at the lower oral dose, but was similar between sexes at the higher oral dose. Also, peak levels of the metabolites, particularly ICI 213,841, were reached faster in females than in males. For ICI 204,636, $t_{1/2}$'s were similar in males and females after i.v. and p.o. dosing, except after multiple dosing; with multiple dosing, there was an increase in the $t_{1/2}$ in females, but not in males. There was also an increase in $t_{1/2}$ with increase in oral dose (3.5-4 hr vs <0.5 hr) in both males and females. Clearance rates in males and females were similar, and were >2-fold

higher than hepatic blood flow in this species, suggesting extra-hepatic clearance. The V_{ss} was also similar in males and females, and was consistent with distribution into tissues. Plasma AUCs were similar between sexes after i.v. dosing; however, after oral dosing, the AUC was markedly higher in females at 125 mg/kg and at 25 mg/kg (for 7-days). After a single 25 mg/kg p.o. dose, an AUC for ICI 204,636 was not calculable in either males or females. For ICI 214,227 (a major plasma metabolite), peak plasma levels were achieved at 0.25-0.5 hr after i.v. dosing and at 0.33-1.0 hr after p.o. dosing depending on sex and dose (increased with dose). The $t_{1/2}$ was longer in females after p.o. and i.v. dosing. There was no consistent difference in peak plasma levels between males and females. In both males and females, plasma exposure (C_{max}, AUC) to ICI 214,227 was greater than that to ICI 204,636; this was particularly notable for males. For ICI 213,841, peak levels were greater than those of ICI 204, 636 in males, but not females (AUC was not calculated for ICI 213,841) after oral dosing.

Absolute oral bioavailability was <10% after single oral doses of 5 and 25 mg/kg (compared to a 5 mg/kg i.v. dose).

Tissue distribution of radioactivity was assessed in three separate studies. Tissue distribution following a single oral dose (^{14}C-ICI 204,636, 5 mg/kg) was tested in male albino (Wistar, Sprague-Dawley) and pigmented (LISFAP/alpk hooded, Long-Evans hooded) rats. Data were either qualitative, or expressed as µg-equiv•day/gm tissue. Qualitatively, the highest tissue levels were detected in GI, liver, and kidney. At the end of the sampling period (168 hr postdosing), detectable levels were only noted in GI in albino rats. In pigmented rats, notable radioactivity was detected in eye (choroid), even at 168 hr postdosing. Quantitatively, levels of radioactivity (AUCs) were highest in eye (pigmented rats only), liver, GI (particularly small intestine), and thyroid. Lower levels (i.e., AUC) were noted in spleen, kidney, bladder, and thymus. The $t_{1/2}$ of tissue radioactivity was longest in eye (of pigmented animals, i.e., 11.6 days), thyroid (4.4 days), spleen (3.3 days), and stomach (1.8 days); in other tissues, the $t_{1/2}$ was < 1 day. [It should be noted that peak levels were not always highest in those tissues with the greatest exposure based on AUC. Highest peak levels were detected in eye (pigmented rats only), liver, kidney, lung, salivary glands, urinary bladder, thyroid and spleen. The lowest tissue levels were detected in brain.]

Tissue levels of radioactivity following acute and multiple oral dosing (in male and female Sprague-Dawley, 25 mg/kg for 7-9 days) were compared in one study. In this study, peak tissue levels following single dosing occurred earlier and were generally higher in females than in males (0.5 vs 1 hr), with the distribution being slightly different between the two sexes. Although the highest levels of radioactivity were detected in the GI tract in both species, levels in adrenal and pancreas were relatively higher in females, whereas levels in urinary bladder were markedly higher in males. Tissue distribution after multiple dosing was similar to that after single dosing; however, levels in thyroid, stomach, and spleen were higher in both males and females after multiple dosing, consistent with the longer $t_{1/2}$ in these tissues. When measured 24 hr following the last of 7 daily doses, tissue levels of radioactivity were still detectable in most tissues; however, levels in thyroid gland were higher than peak levels obtained in a number of other tissues (both male and female). A comparison of 24-hr radioactivity levels in selected tissues after 1, 7, and 9 daily doses indicated that steady-state tissue levels were achieved by 7 days.

The metabolic profile of ICI 204,636 following oral dosing was tested in male (bile) and female (urine) Sprague-Dawley rats following a dose of 25 mg/kg (given in 4 doses over a 30-hr period). The major biliary metabolites were MRB-L (unidentified, but characterized as a non-conjugated hydroxy acid metabolite) and ICI 214,227; ICI 204,636 accounted for <1% of radioactivity in bile. The situation in urine was somewhat unclear. Either a single metabolite or a metabolite that was not detected in bile was detected in female urine. This metabolite, designated FRU-1 and characterized as an acid metabolite of ICI 204,636, was detected in the

urine of only 5/15 females. When present, however, it accounted for the majority of urinary radioactivity. As determined in other PK/ADME studies, ICI 214,227, ICI 213,841, dealkylated ICI 214,227 and dealkylated ICI 204,636 were detected in bile and urine of males and females. In plasma, ICI 214,227, ICI 213,841, and dealkylated ICI 214,227 were detected following doses of 5 mg/kg p.o., i.v., and 25 mg/kg p.o. Following i.v. dosing, the parent compound was the major drug-related compound in plasma (based on AUC), whereas after oral dosing, levels of ICI 214,227 and the dealkylated ICI 214,227 were higher than either ICI 204,636 or ICI 213,841.

The effect of ICI 204,636 on the activity of hepatic microsomal enzymes was tested in Sprague-Dawley rats after single (150 mg/kg) and multiple oral doses (14-days, 25, 50, 150 mg/kg). After a single dose, antipyrine clearance was decreased in both males and females, whereas after addition of ICI 204,636 to liver microsomes from untreated animals, there was no binding of ICI 204,636 to cyt P450. Therefore, the data suggest that a metabolite of ICI 204,636 inhibits antipyrine metabolism (and cyt P450 enzymes). Following multiple dosing, decreases in activity of aminopyrine N-demethylase and cyt P450 content were noted in males at 50 and 150 mg/kg (not dose-related), whereas ethoxyresorufin O-deethylase activity was increased at 150 mg/kg. In females, liver weight was increased, but no effects were noted on hepatic enzyme activity.

Dog: The major route of elimination of radioactivity in Beagle dog following single i.v. (1 mg/kg) and p.o. (1, 10 mg/kg) doses was via the feces (70-80% of dose radioactivity), with the majority of fecal radioactivity secreted in bile. In bile-cannulated dogs, biliary and fecal radioactivity accounted for 70-85% and 1-5% of dose radioactivity, respectively. Absolute oral bioavailability was ≈8-30% at doses of 1 and 10 mg/kg (compared to 1 mg/kg i.v.), and was somewhat higher at 10 mg/kg. Pharmacokinetic parameters were calculated following single i.v. and oral doses. The $t_{1/2}$ was 1.4-1.8 hr for ICI 204,636 and ≈15 hr for total radioactivity. The clearance rate was calculated to be 30 mL/min/kg following a single 20 mg/kg i.v. dose. T_{max} was similar after single and multiple (4-wk) dosing at 25, 50, and 100 mg/kg, but increased with dose (0.96-1.29 hr to 4.09-4.37 hr). Plasma levels of ICI 204,636, ICI 214,227, and ICI 213, 841 were measured in plasma following single oral (1, 10 mg/kg) and i.v. (1 mg/kg) doses. After i.v. dosing, plasma levels (AUC, C_{max}) of ICI 204,636 were higher than those of the metabolites. After p.o. dosing, the metabolic pattern was somewhat different at the two doses tested, and differed slight between males and females (or between studies). At 1 mg/kg, plasma levels of ICI 213,814 (C_{max}, AUC) were higher than those of parent compound or ICI 214,227 in both males and females. Plasma levels of ICI 204,636 and ICI 214, 227 were either similar or those of ICI 204,636 were slightly lower than those of ICI 214,227. At 10 mg/kg, the AUC for ICI 213,841 was higher than AUCs for ICI 204,636 and ICI 214,227 in both males and females; however, in males, levels of ICI 214,227 were lower than those of ICI 204,636, whereas in females, levels of ICI 204,636 were slightly lower than those of ICI 214,227. For C_{max} (at 10 mg/kg), levels of all three compounds were similar in females; in males, levels of ICI 204,636 and ICI 214,227 were similar, but both higher than those of ICI 214,227. Distribution of radioactivity into selected tissues was assessed after single and multiple doses (25, 100 mg/kg p.o.). In eye, the highest concentrations of radioactivity were detected in the iris; however, appreciable levels were also detected in the other areas, (i.e., retina, choroid, and uveal tract). There was an accumulation of radioactivity with multiple dosing in a major of tissues examined (including eye). Tissue levels (except for eye) were highest in GI tract and liver; substantial, but lower levels, were detected in kidney and adrenals. In liver, brain, and plasma, ICI 204,636 appeared to be the major drug-related substance; however, ICI 214,227 and ICI 213,741 were detected in eye, liver, brain, and plasma.

The metabolic profile of ICI 204,636 in dog was tested in urine, bile, and plasma (some discussed previously). In bile, only ICI 214,227 and dealkylated ICI 214,227 were detectable after single doses of 1 mg/kg p.o. and i.v at 12-24 hr postdosing. In urine, ICI 204,636, ICI 214,227 (and dealkylated form at 12-24 hr postdosing), and ICI 213,841 were detected

following single doses of 1 (p.o., i.v.) and 10 (p.o.) mg/kg. Levels of ICI 204,636 were the lowest. In males, levels of ICI 214,227 and ICI 213,841 were similar in urine, whereas in females, levels of ICI 214,227 tended to be higher after p.o. dosing. At 12-24 hr postdosing, all compounds were detectable, however, lowest levels were associated with the parent compound. In dog, additional analyses indicated that ICI 204,636 and 7 metabolites were detected in plasma. M10 (parent acid) accounted for the majority of dose radioactivity, whereas ICI 204,636, ICI 214, 227, and ICI 213,841 all accounted for between 8 and 9% of dose radioactivity. [The total % of unidentified material was ≈24%.]

Monkey: one PK/ADME study was conducted in cynomolgus monkeys at single doses of 0.5 mg/kg and 10 mg/kg p.o. In terms of excretion, fecal and urinary radioactivity accounted for fairly equal percentages of dose radioactivity, and were similar after i.v. and p.o. dosing. The absolute oral bioavailability of the 10 mg/kg dose was ≈6%. That urinary radioactivity accounted for ≈45% of dose would suggest that the low oral bioavailability is due to an extensive first-pass effect.

The metabolic profile of ICI 204,636 was assessed in urine, fecal, and plasma samples. ICI 204,636, ICI 214,227, dealkylated ICI 214,227 (not in plasma), and ICI 213,841 were detected urine, feces, and plasma. In urine, the major drug-related compound was the dealkylated ICI 214,227 after both i.v. and p.o. dosing. In feces, the dealkylated ICI 214,227 was the highest, followed by ICI 214,227, after i.v. dosing; however, after p.o. dosing, levels of these two compounds were similar and higher than those of ICI 204,636 or ICI 213,841. In plasma, the parent compound was the major drug-related compound (based on AUC) following i.v. dosing; with p.o. dosing, however, levels of ICI 214,227 and ICI 213,841 were similar and higher than those of ICI 204,636. Twenty to 30% of dose radioactivity was unidentified in urine and feces, whereas, in plasma, ≈50% of dose radioactivity was unidentified. With subsequent analysis of plasma radioactivity, the major drug-related material in plasma was identified as the conjugate of N-dealkylated ICI 214,227. ICI 214,227 (free and conjugated) and ICI 213,841 both accounted for a greater % of circulating plasma radioactivity than did ICI 204,636.

Rabbit: ICI 204,636 was administered to Dutch Belted female rabbits at 25 mg/kg p.o., the LD used in the teratology study. The major route of elimination was via the urine. Urinary radioactivity accounted for ≈60% of dose, whereas fecal radioactivity accounted for ≈30% of dose. Analysis of sample extracts indicated the presence of at least 4 metabolite, with detectable levels of ICI 204,636 only in feces (≈10% of fecal radioactivity).

Conclusion: the PK/ADME of ICI 204,636 was assessed primarily in rat, dog, and monkey, and to a lesser extent in mouse and rabbit. Absolute oral bioavailability was <10% in rat and monkey, and slightly higher (particularly at higher oral doses) in dog (8-30%). The major route of elimination was fecal in male mouse, rat, and dog, with bile accounting for the majority of fecal drug-related material. In monkey, urinary and fecal elimination were fairly similar, whereas in rabbit, the primary route of elimination was via the urine.

Clearance and V_d estimates in rat indicated clearance rates greater than hepatic blood flow (suggestive of extra-hepatic clearance) and V_d consistent with tissue distribution of drug-related material. Studies in rat demonstrated highest distribution of drug-related radioactivity into organs of elimination (GI, liver, kidney) and eye (in pigmented animals). The $t_{1/2}$ of radioactivity was longest in eye (pigmented), thyroid, stomach, and spleen; therefore, there is the possibility that drug will accumulate in these tissues with chronic dosing.

The metabolic profile of ICI 204,636 was assessed in rat, dog, monkey, and human. In these species, identified metabolites and parent compound account for 65-80% of circulating drug-related material. Therefore, 20-35% of drug-related material in plasma of these species is unknown. Metabolism of ICI 204,636 involves sulfoxidation, oxidation to the acid form, hydroxylation of the dibenzothiazepine ring, O- and N-dealkylation, and subsequent

glucuronidation. The major plasma drug-related compound in rat, monkey, and human are N-dealkylated-7-hydroxy, n-dealkylated-7-hydroxy conjugate, and the parent compound, respectively. In dog, the parent acid is most abundant; however, slightly lower levels of parent, 7-hydroxy, and the sulfoxide were also detected.

TOXICOLOGY

Acute

Acute toxicity studies were conducted in Swiss-Webster mice (p.o., i.p.), Sprague-Dawley rat (p.o., i.p.), and Beagle dog (p.o.). These studies were reviewed by Robert Hollenbeck, Ph.D. (10/28/88), and the following summary is based on Dr. Hollenbeck's review (quotes are from the review).

In mouse, ICI 204,636 was administered at doses of 250 and 500 mg/kg p.o. and 100 mg/kg i.p. The LD_{50}s were calculated to be >250 mg/kg and >100 mg/kg, for p.o. and i.p. dosing, respectively. Clinical signs at doses of 500 mg/kg p.o. and 100 mg/kg i.p. consisted of "...decreased motor activity, ptosis, loss of righting reflex, fluid around the mouth, tremors and convulsions". No gross pathology or histopathology findings were identified in mouse following acute dosing.

In rat, ICI 204,636 was administered at doses of 500 mg/kg p.o. and 100 mg/kg i.p. The LD_{50}s were calculated to be >500 and >100 mg/kg for p.o. and i.p. dosing, respectively. Clinical signs associated with ICI 204,636 were similar to those observed in mouse. Liver enlargement and hepatocyte vacuolation was detected some drug-treated animals following a single 100 mg/kg i.p. dose.

In dog, ICI 204,636 was administered at doses of 0, 10, 20, 30, 40, 50, and 75 mg/kg p.o. LD_{50}s were calculated to be >75 and >50 mg/kg in males and females, respectively. Clinical signs associated with ICI 204,636 consisted of decreases in spontaneous motor activity, ataxia, prostration, and tremors at doses >20 mg/kg.

Metabolites

Limited acute toxicity studies on 3 plasma metabolites, ICI 213,841, ICI 236,303, and ICI 214,227, were conducted in mice [Alpk:AP$_f$CD-1 (AP)] at one dose level, 500 mg/kg. There were no spontaneous deaths in these studies. One M dosed with ICI 236,303 and 1/sex treated with ICI 214,227 were sacrificed moribund. Clinical signs consisted primarily of subdued behavior and ptosis. Hunched posture, abnormal breathing, and hollowed abdomen were also noted following ICI 214,227. Clinical signs were more prolonged in females than in males with ICI 214,227. No gross or histopathology (limited assessment) changes directly related to drug were noted.

The sponsor stated that a comparison of the results of acute studies with ICI 204,636 and metabolites indicated that ICI 213,841 and ICI 236,303 were less toxic than ICI 204,636, whereas ICI 214,227 had similar toxicity. However, since these drugs were tested in separate studies, definitive comparisons of toxicity cannot be made.

Subchronic/Chronic

Definitive subchronic toxicity studies were conducted in Sprague-Dawley rat (4-wk + 4-wk recovery) and Beagle dog (4-wk + 4-wk recovery, 6-mo + 8-wk recovery). Additional subchronic toxicity studies were conducted in Alderley Park (Wistar-derived; 1-mo, bridging, non-GLP) and Sprague-Dawley (1 day-2 mo special) rat and Beagle dog (1-mo bridging, non-GLP).

Definitive chronic toxicity studies were conducted in rat (6-mo + 4-wk recovery in Sprague-Dawley; 1-yr + 5-wk recovery in Wistar), Beagle dog (1-yr + 8-wk recovery), and cynomolgus monkey (1-yr). An additional 1-yr study in cynomolgus monkey was performed (one-dose level).

Rat. In rat, the definitive 4-wk and 6-mo studies were conducted at doses of 0, 25, 50, and 150 mg/kg orally (by gavage). In the 1-yr study, a wider dose range was used (0, 10, 25, 75, and 250 mg/kg (by gavage). No unscheduled deaths occurred in the 4-wk study. In the 6-mo and 1-yr studies, there were 26 and 31 unscheduled deaths, respectively; however, none was attributed to ICI 204,636. The primary drug-related clinical sign, observed at all doses in all three studies, was ptosis. Decreased motor activity was also noted at 150 mg/kg in the 4-wk study and at 50 and 150 mg/kg in males and females (females more affected) in the 6-mo study. In the 1-yr study, excessive salivation (≥75 mg/kg) and urine staining (250 mg/kg) were also observed. Body weight gain was reduced in males (4-wk: HD; 6-mo, 1-yr:≥50 mg/kg). In females, body weight gain was reduced at 150 mg/kg in the 6-mo study; however, in the 1-yr study, body weight was elevated at all doses compared to CF; only at 250 mg/kg was there a slight reduction in body weight gain and only toward the end of the dosing period. In the 6-mo and 1-yr studies, the reduced body weight (compared to C) in males at ≥150 mg/kg was still evident in recovery animals. No drug-related effects were noted on food consumption except in the 1-yr study and only in females. In females, changes in food consumption were fairly consistent with those noted in body weight. No drug-related effects were noted upon ophthalmology or bone marrow examination, or on urinalysis parameters. On hematology parameters, drug-related effects were observed in the 6-mo and 1-yr studies. Wbc and lymphocytes were increased both studies. In the 6-mo study, increases were noted only in males at 150 mg/kg. In the 1-yr study, increases in wbc were evident in males at doses ≥75 mg/kg and in females at 250 mg/kg and increases in lymphocytes were noted in males and females at 250 mg/kg. In the 1-yr study, neutrophil count was elevated at all doses in females, but particularly at 250 mg/kg. These findings were also evident (although to a lesser extent) in recovery animals in both the 6-mo and 1-yr studies. There were no consistent changes in clinical chemistry parameters. In the 6-mo study, the only finding was an apparently transient increase in serum cholesterol in males at 150 mg/kg. In the 1-yr study, the primary findings were decreases in serum cholesterol and triglycerides in males and females at 250 mg/kg. Changes were noted in alkaline phosphatase in both males and females; however, this parameter was decreased in males (10-250 mg/kg) and increased (transiently) in females at 250 mg/kg. Reductions in alkaline phosphatase and cholesterol were still evident in recovery males and in recovery males and females, respectively, at 250 mg/kg. Thyroid hormone status was evaluated only in the 6-mo (TSH, T_3, T_4) and 1-yr (TSH, T_4) studies. TSH was elevated in males and females at 150 mg/kg in the 6-mo study, but at doses ≥25 mg/kg in the 1-yr study. T_4 was unchanged in the 6-mo study, but increased in males at doses ≥75 mg/kg in the 1-yr study. T_3 was slightly reduced in females at all doses in the 6-mo study (this parameter was not measured in the 1-yr study). All changes were still evident in recovery animals (150, 250 mg/kg). Serum prolactin was measured only in the 1-yr study. Levels were elevated throughout the dosing period in males at doses ≥25 mg/kg and in females at all doses (i.e., 10-250 mg/kg), and at 10 mg/kg in males at the last measurement time (Wk 52). Although elevated, the magnitude of the effect was not clearly dose-related, probably due, in part, to the marked variability noted in this parameter. Serum prolactin was not elevated in recovery animals. TK analysis was performed in all studies. The plasma exposure data for ICI 204,636, ICI 214,227, and ICI 213,841 are summarized in the attached tables. Plasma levels of ICI 204,636 and ICI 213,841 (to a lesser extent) were consistently higher in females than males, whereas levels of ICI 214,227 were fairly similar in males and females. In general, plasma exposure was highest to metabolite ICI 214,227, with ICI 204,636 be less, and plasma exposure to ICI 213,841 being the lowest. Plasma levels of ICI 204,636 tended to increase in a greater-than dose-proportionate manner up to doses of 150, but in a less-than dose-proportionate manner in the chronic studies between doses of 75 and 225 mg/kg. Plasma levels of ICI 213,841 increased in a greater-than dose-proportionate manner

in the subchronic study, but in a less-than dose-proportionate manner in the chronic studies. Plasma levels of ICI 214,227 tended to decrease with increasing dose. Whether these findings indicate actual changes in the kinetics of ICI 204,363 or are artifacts of interanimal variability is unclear. AUC data were collected only in the subchronic study and the time intervals used for calculation of AUCs differed among doses, sexes, and sampling times.

Organ weight changes were observed in all three studies. Increases in liver weight and decreases in uterine weight were consistently observed. Liver weight was increased at all doses (25-150 mg/kg) in females in the 4-wk and 6-mo studies, but primarily at 250 mg/kg in the 1-yr study. In males, liver weight was increased at 150 mg/kg in the 4-wk study and at doses ≥75 mg/kg in the 1-yr study. In the 1-yr study, increases in liver weight were still evident in recovery males at 250 mg/kg. Uterine weight was reduced at all doses in all studies (i.e., 10-250 mg/kg); in the 1-yr study, the effect was still evident in recovery animals (at 250 mg/kg). Increases in adrenal gland wt was noted at all doses in females in the 6-mo study, but in males at doses ≥25 mg/kg in the 1-yr study. Adrenal gland was still elevated in recovery males in the 1-yr study. Effects on pituitary gland wt occurred in all studies; however, decreases were noted in the 4-wk and 6-mo studies in females (25-150 mg/kg) and increases were noted in the 1-yr study (at doses ≥25 mg/kg in males and at 250 mg/kg in females); recovery animals were still affected (at 250 mg/kg). Additional findings noted in the 1-yr study were decreases in testis and prostate weight at 250 mg/kg in main study and recovery (prostate only) animals. Thyroid gland was not weighed in any of the studies.

Macroscopic and microscopic findings were observed only in the 6-mo and 1-yr studies. Thyroid gland pigment deposition was the only macroscopic finding noted in both studies. In the 6-mo study, thyroid discoloration was detected in 8/15 males and 1/17 females at 150 mg/kg; 5/8 recovery males were also affected (at 150 mg/kg). In the 1-yr study, the incidence of thyroid discoloration was increased at all doses in males and females, including recovery animals. Additional macroscopic findings in the 1-yr study consisted of adrenal gland discoloration (in females at ≥75 mg/kg), enlarged liver [males at 25 and 250 mg/kg, and recovery males (250 mg/kg)], increased quantity of mammary gland (all doses in females, i.e., 10-250 mg/kg), and thin uterus (≥75 mg/kg). There were microscopic changes in a number of tissues in both chronic studies. Thyroid, liver, and mammary gland were target organs in both studies. Mammary gland changes consisted of hyperplasia in females at all doses in both studies (i.e., ≥10 mg/kg). In males, hyperplasia was detected at 75 and 250 mg/kg in the 1-yr study; however, in the 6-mo study drug-induced changes in males consisted of atrophy accompanied by cellular vacuolation at doses of 50 and 150 mg/kg. Thyroid gland changes consisted of pigment deposition, hypertrophy, reduced colloid, and/or increases in globular/clumped colloid. Pigment deposition was evident at all doses in males and females in both studies (i.e., ≥10 mg/kg), including recovery animals (150 and 250 mg/kg). Follicular cell hypertrophy was detected in males at doses of 50 and 150 mg/kg in the 6-mo study, and at 250 mg/kg in the 1-yr study; in females, follicular cell hypertrophy was detected at doses ≥75 mg/kg. Reduced colloid and increases in globular/clumped colloid were observed in females in the 6-mo study at doses of 50 and 150 mg/kg. Liver changes consisted of hepatocyte hypertrophy and vacuolation (fat). In males, hypertrophy was detected at 150 and 250 mg/kg, whereas vacuolation was detected at dose of 50 and 150 mg/kg in the 6-mo study and at all doses (i.e., ≥10 mg/kg) in the 1-yr study. Liver findings were not detected in females in the 6-mo study, except, perhaps, for a slight increase in vacuolation at 150 mg/kg (if recovery animals are included). In the 1-yr study, hepatocyte hypertrophy was detected at doses ≥ 75 mg/kg and vacuolation at 250 mg/kg. Additional microscopic findings in the 6-mo study consisted of an increase in corpora lutea with single cell necrosis at doses of 50 and 150 mg/kg, atrophy/mucification of the cervix/vaginal mucosa at all doses (25-150 mg/kg), and prostatitis at 150 mg/kg. In the 1-yr study, additional findings consisted of (1) cortical vacuolation of the adrenal gland at 75 and 250 mg/kg in males and females, (2) cellular hypertrophy of the zona fasciculata (adrenal gland) in females at 250 mg/kg, (3) myocardial fibrosis at doses ≥25 mg/kg in females, and at all doses in males (i.e., ≥10 mg/kg); according to the sponsor's report, "There was an increase in the incidence of myocardial fibrosis, of

minimal/mild degree, in all males and the females in the 75 and 250 mg/kg/day groups." (4) alveolar macrophages in lung at doses of 75 and 250 mg/kg in males and females, and (5) pancreatic islet cell hypertrophy at 75 and 250 mg/kg in males and females. In recovery animals, changes in thyroid and lung were still evident.

Dog. Two definitive subchronic (4-wk + 4-wk recovery, 6-mo + 8 wk recovery) and one chronic (1-yr + 8-wk recovery) studies were performed in Beagle dog. In the 4-wk study, there were 3/sex/grp for the main study and an additional 3/sex for C and HD during the recovery period. In the 6-mo study, main study animals consisted of 3-5/sex/grp, with an additional 2-4/sex for C, MD, and HD recovery grps. In the 1-yr study, there were 4/sex/grp for main study, and an additional 3/sex for C and HD grps for the recovery period. Doses in the 4-wk and 6-mo studies were 0, 25, 50, and 100 mg/kg; in the 1-yr study, doses of 0, 10, 25, 50, and 100 mg/kg were used. All dosing was oral (tablet).

There were no unscheduled deaths in any of the studies. Drug-related clinical signs were noted at doses ≥25 mg/kg in all three studies, and consisted of sedation/somnolence, ataxia, and miosis. In the 6-mo study, emesis was also noted at all doses and twitching/jerking and convulsions (1 F) were observed at 100 mg/kg in females. In the 1-yr study, additional signs consisted of anal relaxation (all doses, i.e., ≥10 mg/kg), shivers (primarily at 100 mg/kg), muscle tremors (50 and 100 mg/kg), and miosis (at ≥10 mg/kg). Drug-related effects on body weight were not consistent among studies. In the 4-wk study, body weight gain was reduced at 50 and 100 mg/kg in males. In the 6-mo study, body weight gain was reduced significantly in males only at 50 mg/kg, and at all doses in females (significant only at 100 mg/kg). In these studies, body weight tended to normalized during the recovery period. In the 1-yr study, no drug-related effects were noted on body weight. Food consumption did not appear to be affected in any of the studies. Cardiovascular parameters were monitored in all studies. No drug-related effects were noted on ECG. In both the 4-wk and 6-mo studies, heart-rate was elevated in drug-treated animals (50 and 100 mg/kg); in the 6-mo study, the effect diminished over the course of the dosing period.

Drug-related ophthalmology findings were observed in the 6-mo and 1-yr studies; findings in both studies were evaluated (for the sponsor) by an independent veterinary ophthalmologist (reports appended). In the 6-mo study, findings consisted of (1) posterior triangular cataracts (both eyes, 3 females at 100 mg/kg), (2) granular opacification of the axial posterior cortex in the same animals that later developed cataracts, (3) focal opacities of the posterior Y suture arms, with the posterior Y sutures being dense and granular (1 male at 100 mg/kg), and (4) prominent posterior Y sutures (dose-related, all doses affected, i.e., 25-100 mg/kg). Cataracts were detected in 2 females at Wk 13, and in a third female at Wk 26. In the 1 female in which cataracts were not observed until Wk 26, granular opacification of the axial posterior cortex was noted at Wk 13. Cataracts and granular opacity of the posterior Y suture remained unchanged in affected animals followed during the recovery period. There did seem to be some regression in prominent posterior Y sutures during the recovery period (in 2 C animals as well). In the 1-yr study, ophthalmology findings were graded 1-8, with grades 1-4 being defined as "normal variations" and grades 5-8 being defined as "pathologic changes resulting from administration of ICI 204,636". Using this scoring system, the following findings were noted: (1) an increase in lens grades at all doses; however, in all but the HD (100 mg/kg), the findings were considered normal variation, (2) definite lens pathology ranging, according to the consult's report, from "dense granular deposits of the posterior Y-suture arms to posterior cortical triangular cataracts" in 3/4 males, 4/4 females, 2/3 recovery males, and 3/3 recovery females at 100 mg/kg. Posterior axial triangular cataracts were detected in 2/4 females and 1/3 recovery males. In 1 recovery male and 1 recovery female, there was evidence of progression of lens pathology during the recovery period; there was no evidence of regression of "definite lens pathology" during the recovery period. It is unfortunate that the data for individual animals were presented as either "normal variation" or "lens pathology" and not as actual findings. Use of the grade system also resulted in what appeared to be discrepancies between the 6-mo and 1-yr report in terms of drug-related pathology. The best example of

this is that in the 6-mo report, the consultant concluded that there was a drug-related increase in prominent posterior Y sutures (with no NOEL); in the 1-yr report, however, this finding was "lumped" in with other "normal variations" and not considered drug-related. It was also not clear that designating a specific grade, e.g., Grade 4: broadening of the posterior Y suture arms, implied that the affected individual also exhibited the findings labeled at Grades 1 through 3. According to the consult's report, "In general, the severity of lens pathology increases with the higher lens grade assigned; however, it has not been possible to observe consistently in each individual animal the transition from the lower to the higher lens grades as lens pathology progresses." Taken together, the 2 long-term studies in dogs indicate that increases in normal lens variations (e.g., those associated with aging) were increased at all doses tested (i.e., ≥10 mg/kg); however, cataracts (including findings identified as pathological opacities) were detected only at 100 mg/kg in both studies.

TK data (summarized in the attached tables) were collected in the 4-wk (ICI 204,636 only), 6-mo, and 1-yr studies. Plasma levels of ICI 204,636, ICI 214,227, and ICI 213,841 were quantitated. Plasma exposure to ICI 204,636 increased in a fairly dose-proportionate (C_{max}) or slightly greater than dose-proportionate (AUC) manner depending upon the parameter. ICI 213,841 increased with multiple dosing, but tended to stabilize by Day 92 (based on AUC). Plasma levels of ICI 214,227 decreased with multiple dosing and in a less-than dose-proportionate manner (C_{max}, AUC). In the 1-yr study, C_{max} remained stable from 25 to 100 mg/kg. As in rat, it was not possible to determine whether these trends reflected true changes in kinetics or were artifacts of wide interanimal variability. Total plasma exposure (AUC) was lowest for ICI 214,227, whereas AUCs for ICI 204,636 and ICI 213,841 were fairly similar (or slightly higher for ICI 213,841).

There were no clear drug-related effects on hematology parameters; in the 1-yr study, platelet counts tended to be elevated at 50 and 100 mg/kg in males and females. Drug-related findings on clinical chemistry parameters were observed in the 6-mo and 1-yr studies. Decreases in serum cholesterol and increases in alkaline phosphatase were noted in both studies (cholesterol: 6-mo: 100 mg/kg in males and females; 1-yr: ≥50 mg/kg in males, ≥10 mg/kg in females; alkaline phosphatase: 6-mo: 100 mg/kg in males and females, 1-yr: ≥50 mg/kg in males, 100 mg/kg in females). A decrease in triglycerides was noted only in the 1-yr study (≥25 mg/kg in males, ≥10 mg/kg in females). Effects on alkaline phosphatase (male and females) and triglycerides (female) were still evident in recovery animals (100 mg/kg). Thyroid hormones (T_3, T_4, TSH) were assayed only in the 1-yr study. The only drug-related effect was a decrease in T_4 in males at doses of 50 and 100 mg/kg and at 100 mg/kg in females. The effect was sporadic in females. T_4 was still slightly reduced in males during the recovery period. Urinalysis parameters were not affected, nor were there any drug-related macroscopic findings at necropsy. There were no consistent effects on organ/tissue weights. Of note, however, were increases in parathyroid/thyroid weight (corrected for body weight) in males at doses of 50 and 100 mg/kg, and at all doses in females (i.e., ≥25 mg/kg) in the 6-mo (but not 1-yr) study; this effect was still evident in recovery males and females (100 mg/kg). Histopathology findings were limited to the eye (6-mo and 1-yr studies). [An increase in coronary artery arteritis was observed in males at 100 mg/kg in the 6-mo study; however, this finding was not noted in the 1-yr study.] Drug-related effects on the eye were extensive and were observed in both studies. In the 6-mo study, cataracts were confirmed in 2 of the 3 affected females (100 mg/kg) and there was a dose-related (all doses) increase in the incidence and severity of swelling of the posterior lens fiber tips. In recovery animals, the later finding tended to normalize. In the 1-yr study, the following drug-related microscopic findings were observed in eye: (1) an increase in swelling of the posterior cortex and/or sutures (50, 100 mg/kg), (2) globule formation in anterior cortex, posterior cortex, and/or posterior suture (50, 100 mg/kg), (3) liquefaction of the posterior cortex (100 mg/kg), (4) liquefaction of posterior cortex and/or suture (100 mg/kg), and (5) nuclear retention (100 mg/kg). At 50 mg/kg, the primary drug-related findings (all animals affected) were increases in fiber swelling of the posterior subcapsular cortex and posterior suture; fiber swelling in these

areas as well as in the equator region was detected in 1 female at this dose. At 100 mg/kg, fiber swelling was more severe and widespread, and more often (all females, 2/4 males) associated with globule formation, liquefaction, and nuclear retention. These findings were also observed in recovery animals (100 mg/kg), although the severity of fiber swelling in the posterior cortex was somewhat less. One additional microscopic finding in the 1-yr study was pigment deposition (characterized as acinar epithelial brown pigment) at all doses (i.e., ≥10 mg/kg; dose-related severity) in both males and females (including recovery animals).

Monkey: two chronic oral (gavage) toxicity studies were conducted in cynomolgus monkey. These studies were conducted in order to further assess the thyroid and eye findings observed in oral toxicity studies in rat and dog, respectively.

The first was an investigative study using a single dose level (with C grps,.i.e., 0 and 50 mg/kg); doses were initiated in a rising-dose manner over 7 days prior to the 1-yr dosing period. The second was a conventional (definitive) study at final doses of 25, 100, and 225 mg/kg; in this study, doses were administered in equal doses t.i.d., and were initiated in a rising-dose manner over 29 days. Final doses were administered for 52 wks. Selected animals were followed for a 4-wk recovery period.

There were no drug-related unscheduled deaths in either study. Drug-related clinical signs were observed at all final doses and consisted of subdued behavior and hunched posture. In the investigative study, additional signs included hypoactivity, a lack of response to external stimuli, ptosis, and unusual behavior (2 DT animals). In the definitive study, REM, salivation, vomiting, tremors, and circling behavior were noted at 100 and 225 mg/kg; prostration was noted only at the HD. None of these was observed in recovery animals, nor were clinical signs noted at 5 mg/kg during the rising-dose portion of the investigative study. No effects on body weight or food consumption were observed in the investigative study. In the definitive study, however, body weight was reduced in a dose-dependent manner at all doses in females. In males, body weight was lower in all dosed grps; however, the effect was not dose-related. Food consumption was reduced at all doses in males, and at the HD in females. In recovery animals, body weight and food consumption remained reduced in HD animals. Rectal temperature was studied only in the definitive study, and was reduced at 225 mg/kg in females only. No ophthalmology findings were observed in the investigative study. In the definitive study, however, a striated appearance of the anterior lens surface was detected in 2/7 HDF (225 mg/kg). In the opinion of the sponsor's ophthalmology consult (Dr. G.D. Aguirre), this was possibly related to drug. However, since this finding was not associated with reductions in lens transparency, Dr. Aguirre did not consider it a pathological change. No histopathological correlated of the finding was detected upon microscopic analysis. ECG/blood pressure were measured only in the definitive study; no drug-related effects were noted. No effects on hematology or routine clinical chemistry were noted in the investigative study. In the definitive study, the following hematological findings were noted: (1) decreases in rbc parameters (hgb, rbc, pcv) at 225 mg/kg (male, female), (2) decreases in wbc count at all doses in females, (3) increases in neutrophil count at 225 mg/kg, (4) decreases in lymphocyte count primarily at 225 mg/kg but also at the lower doses, (5) decreases in monocyte count at all doses in females. On coagulation parameters, increases were noted in APTT at 225 mg/kg in females (and in 1 female recovery animal at 225 mg/kg) and increases in Factor VIII and Factor XII at 225 mg/kg in males and females; effects on Factors VIII and XII were not noted in recovery animals. There were also a number of changes in clinical chemistry parameters. These consisted primarily of the following: (1) decreases in alkaline phosphatase at all doses in males and at 225 mg/kg in females, (2) decreases in total bilirubin at all doses in males and females, (3) increases in creatinine at 100 and 225 mg/kg in males and at 225 mg/kg in females, and (4) decreases in total cholesterol at 225 mg/kg in males and females throughout the dosing period; in the 2 HDF in which lens changes were observed, serum cholesterol remained lower than at baseline throughout the dosing period (20-77%). Serum prolactin was measured in both studies. In the investigative study, serum prolactin was reduced in drug-treated animals (50 mg/kg). In the definitive study, serum prolactin was increased in

males at 100 and 225 mg/kg, but was reduced in females at all doses (not dose-related). Thyroid hormones were measured in both the investigative (T_3, FT_3, T_4, FT_4, TSH) and definitive studies (TSH, T_3, T_4). No drug-related effects were noted in the investigative study; however, methodological problems with the TSH assay precluded a valid assessment. In the definitive study, the following were noted: (1) decreases in T_3 at 100 and 225 mg/kg in both males and females, (2) a transient increase in TSH at 100 and 225 mg/kg in females. In recovery animals, the only finding noted was a decrease in bilirubin in males and females (225 mg/kg).

TK data were collected in both 1-yr studies (data are summarized in the attached tables). ICI 204,636, ICI 213,841, ICI 214,227, and M 236,303 were quantitated in plasma samples. Little or no evidence of accumulation was noted for parent compound or any of the metabolites measured. For ICI 204,636, plasma exposure increased in a fairly dose-proportionate manner. For ICI 214,227, plasma exposure increased in a less-than dose proportionate manner from 100 to 225 mg/kg. For ICI 213,841, increases in plasma exposure were fairly linear with dose; levels (C_{max}, AUC) tended to be highest on Day 1. Plasma levels of M 236,303 increased linearly with dose. In terms of relative exposure, it must be kept in mind that the AUC values for the 1-yr investigative study (TKP/56) represent exposure over 24 hr, whereas, for the definitive study (TFP/84), the AUC represents only part of the total daily exposure (i.e., 0-7 hr; dosing was t.i.d.). Therefore, data between the studies are not comparable. For the investigative study, plasma levels of ICI 214,227 were higher than those of ICI 204,636 (data for ICI 213,841 are not available for this study). For the definitive 1-yr study, plasma exposure to ICI 214,227 was highest, and levels of ICI 213,841 were higher than those of ICI 204,626. The lowest plasma exposure was to M 236,303.

No consistent drug-related effects were noted on urinalysis parameters in either study. No drug-related changes in any terminal study was noted in the investigative study, including detailed microscopic examination of selected tissues (e.g., eye, thyroid), except for increases in relative weights of adrenal (males) and spleen (females) at 50 mg/kg. In the definitive study, the following were noted at sacrifice: (1) increases in liver wt at 225 mg/kg in males and at 100 and 225 mg/kg in females, and a decrease in heart wt in males at all doses; in recovery animals, thyroid wt was elevated in males and females (225 mg/kg) ((2) gross pathology findings consisted of enlarged liver in males at 225 mg/kg, dark discoloration of the thyroid at 225 mg/kg in males and females (including 3 recovery animals, 2 of which also had discolored thyroid), (3) no drug-related effects on bone marrow smears, and (4) microscopic changes primarily in liver, mammary gland, and thyroid gland. Liver findings consisted of centrilobular hepatocyte hypertrophy in males at 225 mg/kg and hepatocyte vacuolation in 1/7 males and 2/7 females at 225 mg/kg. Glandular hyperplasia of the mammary gland was noted at all doses, including CF; in males, this finding was detected only at 100 and 225 mg/kg. Thyroid gland follicular cell hypertrophy was detected only males and at 100 and 225 mg/kg. In the definitive study, sections of thyroid gland were stained with Masson-Fontana stain in order to characterized the dark discoloration noted at necropsy. According to the sponsor, the pigment was identified as lipofuscin, and the presence of pigment deposition was not related to the incidences of dark discoloration.

Special Toxicity Studies

Special toxicity studies were conducted in rat/mice and dog in order to further evaluate drug-induced changes in thyroid and eye, respectively. Also, dermal irritation was assessed *in vitro* in two mouse cell lines and *in vivo* in guinea pigs. Ocular irritation was assessed *in vivo* in 1 rabbit.

Thyroid. Two of the special toxicity reports were re-evaluations and/or re-examinations of tissues/data from previous toxicity studies. Mechanistic studies to assess drug effects on thyroid consisted of the following: assessment of iodine uptake and incorporation into

PLASMA EXPOSURE TO ICI 204,636 IN RAT

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)		AUC (µg•h/mL)	
				M	F	M	F
TAR/1621	Sprague-Dawley	25	1	not calculated	184	n.c.	225[a]
			28	79 ± 36	89 ± 48	n.c.	166[a]
		50	1	99 ± 76	553	n.c.	844[c]
			28	189	392 ± 16	438[b]	885[c]
		125	1	486	1359 ± 622	2115[c]	5046[c]
			28	856 ± 302	1661 ± 17	3714[c]	5688[c]
TPR/1616	Sprague-Dawley	25	1	39	94	126[d]	643
			96	87 ± 55	405 ± 198	474	1260
			181	61	340	421	983
		50	1	87 ± 26	163	144	984
			96	297	859 ± 236	2040	2070
			181	118	432	724	2100
		150	1	270	829 ± 82	1530	4030
			96	1390 ± 190	2270 ± 1190	5320	8430
			181	1140 ± 100	2410	4550	11400
TFR/1626	Wistar	10	182	37.8 ± 8.90	80.0 ± 32.3		
			357	89.2 ± 47.8	49.5 ± 20.9		
		25	182	82.4 ± 31.8	211 ± 22.6		
			357	131 ± 13.0	273 ± 28.9		
		75	182	957 ± 325	1600 ± 258		
			357	585 ± 177	1580 ± 345		
		250	182	1060 ± 80.9	1360 ± 215		
			357	700 ± 28.0	1550 ± 158		
TCR/1624 (data were pooled across sex)	Wistar	20	1	149 ± 60.6			
			182	779 ± 203			
			364	150 ± 45.9			
			728	199 ± 65.0			
		75	1	339 ± 124			
			182	1360 ± 323			
			364	1240 ± 197			
			728	1270 ± 361			
		250	1	754 ± 303			
			182	1630 ± 630			
			364	1210 ± 136			
			728	1880 ± 208			

[a]$AUC_{(0\text{-}4\ h)}$, [b]$AUC_{(0\text{-}8\ h)}$, [c]$AUC_{(0\text{-}12\ h)}$, [d]$AUC_{(0\text{-}24\ h)}$ for all values in 6-mo study

PLASMA EXPOSURE TO ICI 214,227 IN RAT

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)		$AUC_{(0-24\ h)}$ (µg•h/mL)	
				M	F	M	F
TPR/1616	Sprague-Dawley	25	1	241 ± 17	487	775	1840
			96	966 ± 143	668 ± 165	2920	2560
			181	853 ± 734	711 ± 69	3870	3970
		50	1	960 ± 217	999	2020	3900
			96	2140	1320 ± 280	10900	6700
			181	1240	1190	9010	5680
		150	1	1450	1900 ± 80	11600	14400
			96	2300 ± 110	1350	15500	15300
			181	2260 ± 270	1750	23700	19700
TFR/1626	Wistar	10	182	537 ± 196	1100 ± 163		
			357	647 ± 267	765 ± 138		
		25	182	1010 ± 252	1640 ± 161		
			357	1260 ± 114	1550 ± 187		
		75	182	1990 ± 0	1830 ± 107		
			357	1590 ± 195	1860 ± 60.0		
		250	182	2000	1590 ± 252		
			357	1880 ± 90.0	1550 ± 6.7		
TCR/1624 (data were pooled across sex)	Wistar	20	1	n.c.			
			182	n.c.			
			364	1160 ± 177			
			728	1000 ± 173			
		75	1	n.c.			
			182	n.c.			
			364	2100 ± 153			
			728	1790 ± 326			
		250	1	n.c.			
			182	n.c.			
			364	1670 ± 99.1			
			728	1760 ± 222			

PLASMA EXPOSURE TO ICI 213,841 IN RAT

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)		$AUC_{(0-24\ h)}$ (µg•h/mL)	
				M	F	M	F
TPR/1616	Sprague-Dawley	25	1	–	36	n.c.	394
			96	55	129 ± 52	n.c.	484
			181	64	83	n.c.	434
		50	1	–	120	n.c.	517
			96	113	313 ± 73	343	664
			181	61	162	n.c.	341
		100	1	205	343 ± 34	1200	11400
			96	617 ± 215	475	1930	2040
			181	512	731	1820	2880
TFR/1626	Wistar	10	182	n.c.	n.c.		
			357	n.c.	n.c.		
		25	182	n.c.	55.3 ± 12.2		
			357	97.7 ± 5.35	113 ± 42.1		
		75	182	195 ± 29.3	381 ± 51.4		
			357	144 ± 36.5	301 ± 61.0		
		250	182	314 ± 40.1	478 ± 64.0		
			357	242	570 ± 10.5		
TCR/1624 (data were pooled across sex)	Wistar	20	1	n.c.			
			182	n.c.			
			364	n.c.			
			728	78.1 ± 17.0			
		75	1	n.c.			
			182	330 ± 54.7			
			364	293 ± 40.0			
			728	211 ± 53.6			
		250	1	352 ± 42.8			
			182	556 ± 104			
			364	413 ± 42.0			
			728	372 ± 61.8			

PLASMA EXPOSURE TO ICI 204,636 IN DOG

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)
TAD/500	Beagle	25	1	960 ± 270	2300 ± 460
			29	1290 ± 330	3220 ± 730
		50	1	2630 ± 2290	5940 ± 2730
			29	1720 ± 620	5830 ± 1560
		100	1	4090 ± 1940	15900 ± 6170
			29	4370 ± 1620	17800 ± 6580
TPD/497	Beagle	25	1	1670 ± 420	3070 ± 240
			92	2350 ± 330	5160 ± 600
			183	3550 ± 260	6630 ± 340
		50	1	4040 ± 960	7480 ± 440
			92	4650 ± 840	11500 ± 1670
			183	3900 ± 490	11100 ± 2100
		100	1	2570 ± 660	18200 ± 2040
			92	7850 ± 111	29100 ± 3310
			183	7150 ± 1030	30000 ± 2820
TFD/501	Beagle	10	105	223 ± 37.4	
			196	153 ± 48	
			378	251 ± 67.9	949 ± 130
		25	105	731 ± 165	
			196	1020 ± 184	
			378	824 ± 262	3930 ± 669
		50	105	1820 ± 786	
			196	1220 ± 461	
			378	1150 ± 454	8980 ± 766
		100	105	598 ± 323	
			196	1010 ± 501	
			378	1570 ± 317	20500 ± 2220

PLASMA EXPOSURE TO ICI 213,841 IN DOG

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)
TPD/497	Beagle	25	1	1160 ± 140	3950 ± 320
			92	1320 ± 110	8190 ± 760
			183	1720 ± 140	8330 ± 660
		50	1	1840 ± 200	7660 ± 650
			92	2280 ± 250	13600 ± 2730
			183	2310 ± 270	13100 ± 2380
		100	1	1770 ± 160	19000 ± 1410
			92	4210 ± 400	33200 ± 3350
			183	3630 ± 360	31400 ± 3230
TFD/501	Beagle	10	105	295 ± 45.8	
			196	182 ± 44.8	
			378	375 ± 72.8	2500 ± 416
		25	105	704 ± 145	
			196	855 ± 129	
			378	862 ± 105	9470 ± 2430
		50	105	1300 ± 509	
			196	817 ± 274	
			378	1210 ± 351	11400 ± 902
		100	105	565 ± 178	
			196	822 ± 244	
			378	1650 ± 223	23600 ± 1280

PLASMA EXPOSURE TO ICI 214,227 IN DOG

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (ng•hr/mL)
TPD/497	Beagle	25	1	870 ± 110	2000 ± 220
			92	433 ± 42	1920 ± 100
			183	466 ± 85	1420 ± 250
		50	1	1260 ± 170	3240 ± 540
			92	623 ± 56	2670 ± 530
			183	419 ± 47	1980 ± 360
		100	1	826 ± 109	4780 ± 410
			92	724 ± 60	4200 ± 540
			183	459 ± 35	3330 ± 530
TFD/501	Beagle	10	105	--*	–
			196	–	–
			378	182 ± 40.9	1420 ± 227
		25	105	–	–
			196	–	–
			378	219 ± 35.8	2946 ± 424
		50	105	–	–
			196	–	–
			378	221 ± 54.4	3270 ± 232
		100	105	–	–
			196	–	–
			378	262 ± 34.7	4110 ± 221

*there were no data for this metabolite for Days 105 and 196 due to methodological problems.

PLASMA EXPOSURE TO ICI 204,636 IN MONKEY

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)		AUC (µg•h/mL)	
				M	F	M	F
ICI 204,636							
TKP/56	cynomolgus	50	1	1770 ± 200	580 ± 15	8260 ± 3080[a]	2520 ± 130
			91	952 ± 82	480 ± 82	3960 ± 760	1880 ± 290
			182	915 ± 161	1100 ± 640	3970 ± 2190	4530 ± 2190
			364	1130 ± 310	538 ± 209	5590 ± 1080	2450 ± 380
TFP/84 (data are pooled across sex)	cynomolgus	25	1	386 ± 176		1090 ± 541[b]	
			182	169 ± 64.3		610 ± 153	
			364	136 ± 18.8		540 ± 68.7	
		100	1	1360 ± 336		6020 ± 1920	
			182	853 ± 126		2900 ± 410	
			364	738 ± 144		3460 ± 797	
		225	1	1930 ± 185		9260 ± 878	
			182	2060 ± 167		9180 ± 789	
			364	1980 ± 264		8190 ± 837	

[a]$AUC_{(0\text{-}24\ h)}$, [b]$AUC_{(0\text{-}7\ h)}$

PLASMA EXPOSURE TO ICI 214,227 IN MONKEY

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)		AUC (µg•h/mL)	
				M	F	M	F
TKP/56	cynomolgus	50	1	3410 ± 500	1620 ± 290	18300 ± 340[a]	7510 ± 840
			91	2000 ± 610	2150 ± 170	11200 ± 2820	8690 ± 500
			182	2640 ± 390	2020 ± 130	17000 ± 280	10300 ± 600
			364	3110 ± 680	2140 ± 220	20000 ± 3700	11300 ± 1100
TFP/84 (data are pooled across sex)	cynomolgus	25	1	749 ± 97.8		2620 ± 320[b]	
			182	453 ± 42.7		2060 ± 161	
			364	460 ± 34.4		2350 ± 203	
		100	1	2080 ± 415		10400 ± 1800	
			182	1670 ± 332		6670 ± 1360	
			364	2180 ± 398		10900 ± 2090	
		225	1	3080 ± 283		14900 ± 1430	
			182	2280 ± 240		11400 ± 1380	
			364	3100 ± 443		14400 ± 1850	

PLASMA EXPOSURE TO ICI 213,841[a] AND M 236,303 (data are pooled across sex)

STUDY	STRAIN	DOSE (mg/kg)	DURATION (days)	C_{max} (ng/mL)	AUC (μg·h/mL)
ICI 213,841					
TFP/84	cynomolgus	25	1	447 ± 79.6	1640 ± 426[b]
			182	294 ± 58.2	1300 ± 177
			364	225 ± 14.4	1030 ± 66
		100	1	1470 ± 175	6960 ± 1100
			182	1070 ± 107	4530 ± 366
			364	902 ± 97.2	4900 ± 615
		225	1	2830 ± 294	14700 ± 918
			182	2810 ± 128	14800 ± 697
			364	2420 ± 166	12500 ± 692
M 236,303					
TFP/84	cynomolgus	25	1	n.c.	n.c.
			182	8.2 ± 0.383	n.c.
			364	20.9 ± 1.84	118 ± 13.7[b]
		100	1	51.8 ± 5.18	n.c.
			182	39.6 ± 4.01	156 ± 17.8
			364	86.5 ± 11.4	428 ± 46.8
		225	1	199 ± 50	898 ± 203
			182	174 ± 32.8	838 ± 187
			364	196 ± 17.8	912 ± 97.3

thyroglobulin (indicative of thyroperoxidase activity) in primary rat thyrocytes), general thyroid function studies in mouse and rat, and assessment of localization of ICI 204,636 in rat thyroid gland.

In one of the re-evaluation reports, the thyroid gland slides from 5 different toxicity studies were re-examined by an independent pathologist. No findings sufficient to draw additional or different conclusions resulted. In the second re-evaluation study, samples of thyroid were examined using a number of stains in an attempt to identify or characterize the pigment; the results were compared to a similar analysis of thyroid samples from animals treated with other compounds known to produce pigment deposition. The thyroid pigment detected in ICI 204,636-treated rats could not be identified, nor did it share similar staining characteristics with pigment produced by other compounds (e.g., minocycline, 2,4-diaminoanisol).

Uptake of radiolabeled iodine into thyroid gland and incorporation into thyroglobulin was tested in primary (Wistar-derived) rat thyrocytes incubated with ICI 204,636 (1-100 µg/mL) and metabolites, ICI 213,227 (10-500 µg/mL), ICI 213, 841 (10-500 µg/mL), and ICI 236,303 (10-500 µg/mL). [Propylthiouracil was used as a positive control.] ICI 204,636 decreased total uptake of iodine (10-100 µg/mL), but had no effect on incorporation of iodine into thyroglobulin (i.e., thyroperoxidase activity). The three metabolites, on the other hand, decreased both the total uptake of iodine and incorporation of iodine into thyroglobulin. Effective concentrations of metabolite were as follows. For uptake of iodine by thyroid, concentrations of 300-500 µg/mL (ICI 21,227, ICI 236,303) and 500 µg/mL (ICI 213,841) were effective. For iodine incorporation, concentrations of 100-500 (ICI 214,227), 300-500 (ICI 213,841), and 100-300 (ICI 236,303) µg/mL were effective; for ICI 236,303, minimal effects on iodine incorporation were noted at higher concentrations (400-500 µg/mL).

Special thyroid toxicity studies were conducted in mouse (C57BL) and rat (Wistar). In mouse, ICI 204,636 was administered in the diet at doses of 400, 500, 600, and 750 mg/kg, with each dose being given (to each treated animal) for 1 wk starting at the LD. Interestingly, the sponsor indicated that ICI 204,636 was irritating to the skin, with the introduction of the moistened powdered diet accompanied by signs of self-mutilation and food wastage in both drug-treated and control animals. Several animals (2 C, 1 DT) were sacrificed with wounds to the skin and/or genital areas. Even with introduction of a pelleted diet, additional animals were sacrificed (5 C, 4 DT) or died spontaneous (1 C) during Wk 1-2. Interestingly, similar problems were apparently not encountered in other dietary studies in mouse (e.g., dose-range finding, 2-yr carcinogenicity). Except for those associated with skin irritation, there were no drug-related clinical signs. Body weight was reduced by the end of the dosing period in both DT grps. Food consumption could not be evaluated when the powdered diet was used because of food wastage; however, food intake of pellets was not affected by drug. In terminal studies, one C and DT grp were used for pathology and liver enzyme study, whereas the other C and DT grp were used to assess thyroxine clearance. Plasma thyroxine (T_4) levels were quantitated following injections of ^{125}I-thyroxine and sodium iodide (given immediately after, 12, and 30 hr after injection of radiolabel, to prevent accumulation of ^{125}I in thyroid). The plasma clearance rate of T_4 was increased 2-fold, with plasma levels reduced by ≈66% and the $t_{1/2}$ estimated to be ≈30% shorter, in DT animals. Measurement of liver enzymes indicated that protein and cytochrome P450 content of liver were increased in DT animals (1.3 and 3-fold, respectively). Although there was a slight increase in T_4UDPGT activity (i.e., thyroxine uridine diphosphate glucuronyl transferase) in DT animals (15-28%), the effect was not statistically significant. Gross findings at necropsy consisted of wound associated with skin irritation (e.g., ulceration with/without scab formation; noted in 3 C animals) and changes in liver (enlarged, discoloration). Liver weight was increased relative to body weight in DT animals; there were no differences between grps in terms absolute liver wts. Upon microscopic analysis, the only drug-related finding was diffuse centrilobular hepatocyte hypertrophy in all DT animals examined. Interestingly, microscopic correlates of skin findings (e.g., ulceration, cellulitis, scab formation) were observed only in C animals. No drug-related

effects were noted in pituitary or thyroid.

In the rat study, ICI 204,636 was administered by gavage at one dose level (300 mg/kg) for 14-18 days. There were two C and two DT grps. One set (C, DT) was used for assessing thyroid hormones, liver enzymes, and gross and histopathology. The other set was used to assess thyroxine clearance. Observations were similar to those assessed in the mouse study, except that plasma hormone levels (TSH, T_4, FT_4) and additional liver enzymes were measured in this study. There were no unscheduled deaths in this study. Drug-related clinical signs (as described by the sponsor) consisted of sedation (noted throughout the dosing period in DT animals), poor grooming (coat staining, nasal and lachrymal deposits), and general hair loss. Body weight was slight (not statistically significant) reduced in DT males relative to CM, whereas in DT females, body weight was elevated relative to CF. Plasma TSH levels were increased 3-fold in DT females (compared to CF, ≈60% compared to CM and DTM), but unaffected by drug treatment in males. No differences between groups were noted in plasma levels of T_4 or T_4 in either males or females. Plasma thyroxine clearance was increased in DT males and females (32-52%), with the $t_{1/2}$ being ≈30% shorter. In terms of liver enzymes, the cytochrome P450 content was significantly increased only in females, whereas ethoxyresorufin O-deethylase and pentoxyresorufin O-dealkylase activities were increased in both males and females. No changes were noted erythromycin N-demethylase activity, and consistent changes were not observed in androstenedione 6ß-hydroxylase activity (i.e., significantly increased in females, and decreased in males). T_4 UDPGT activity was increased in both males and females when expressed as per g liver or per whole liver, but only in females when expressed as per mg protein. The microsomal content of cyp1A1/2, cyp2B1/2, and cyp3A1/2 levels were assessed qualitatively using Western immunoblot analysis. Only cyp2B1 appeared more abundant in DT males and females; cyp3A1 appeared to be increased in DTF only. At necropsy, thyroid discoloration was detected in 5/10 DTM. Liver wt was increased in DTF, but was unaffected in DTM. Findings at histopathology consisted of the following: (1) fat vacuolation of liver in DT animals (6/10 M, 9/10 F), (2) pigment deposition (detected using Masson-Fontana stain) in DT animals (4/10 M, 2/10 F), (3) increased average follicular epithelial height in DT animals (7/10 M, 2/10 F). No drug-related microscopic findings were noted in pituitary gland.

An analysis of individual data for the rat study indicated that plasma TSH was elevated in 10/10 DTF and liver wt (absolute and relative) was increased in 7/10 DTF.

One final special study was conducted (in male Wistar-derived rats) in order to localize the distribution of ICI 204,636 in thyroid gland. ICI 204,636 was administered by gavage in combination with [^{3}H]ICI 204,636 at a dose of 250 mg/kg for 28 days, and alone (i.e., without radiolabel) for an additional 3 days. Animals were sacrificed either at the end of this period, or after an 8-wk withdrawal period. There were no unscheduled deaths in this study. Drug-related clinical signs were noted (e.g., sedation, salivation, poor grooming). Pigment deposition was detected in thyroid follicular epithelial cells. The pigment was characterized as "...finely granular, moderately electron-dense and...membrane-delimited". ICI 204,636 radioactivity was localized to the epithelial cell layer by light microscopy, and to the follicular epithelial cells (over pigment and colloid) by EM. Pigment was still detectable after the 8-wk recovery period. There was no association between the presence of lipofuscin and ICI 204,636 radioactivity.

Eye. The sponsor conducted 5 special studies (3 *in vitro*, 2 *in vivo*) in an attempt to clarify the effects of ICI 204,636 on eye.

In vitro studies were conducted in cultured dog lenses (2 studies) and in a human hepatoma cell line (HepG2). The effect of ICI 204,636 on cholesterol synthesis was tested in both systems. Incubation of lenses or HepG2 cells with ICI 204,636 and radiolabeled precursor for 1-25 hrs resulted in decreases in the cholesterol/lathosterol fraction and increases in the

desmosterol fraction at concentrations of 1-100 μM. An increase in unidentified peaks were also observed in both systems. These data would suggest that ICI 204,636 may inhibit cholesterol synthesis between desmosterol and cholesterol.

ICI 204,636 did not induce cataracts when was tested *in vitro* in cultured dog lenses at concentrations of 1-100 μM for 9 days. No positive control was used in the study; however, the sponsor stated that in a previous study, chlorpromazine produced severe cataracts when incubated with dog lenses for 9 days at 100 μM.

In vivo studies were conducted in Beagle dogs. In one study, ICI 204,636 was administered to 3 male dogs as an acute dose of 100 mg/kg p.o. Five hrs after dosing, all animals (including Cs) received a single dose of radiolabeled mevalonolactone (a cholesterol precursor) i.v. At sacrifice (15-60 min later), lens and liver samples were analyzed for sterol content (qualitative analysis). According to the sponsor, ICI 204,636 appeared to reduce the cholesterol fraction relative to the lathosterol fraction in liver samples; however, the sponsor also noted that cholesterol and lathosterol (and ß-sitosterol presumably from the diet) could not be separated using either radiochemical or uv detection. No radioactivity was detected in lens samples, indicating that the radiolabel was not taken up into the lens.

In the second study, ICI 204,636 was administered to female Beagle dogs (2-8 C, 4-8 CT) at rising doses of 25 (Wk 1), 50 (Wk 2), and 100 mg/kg (Wk 3-35) p.o. Animals were sacrificed after 7 or 35/36 wks of dosing. Observations consisted of clinical signs, body weight, food consumption, ophthalmology examination, clinical chemistry (plasma cholesterol; aqueous humor for glucose, osmolality), and terminal studies [gross pathology, histopathology (lens, lacrimal gland), EM (lens), *ex vivo* radiolabeling of lens, lipid analysis of lens]. Drug-related clinical signs consisted of subdued behavior, unsteady gait, difficulty in rousing, and vocalization. Body weight and food consumption were not affected by ICI 204,636. Upon ophthalmology examination, a clear increase in "lens grade" (0-4 = "normal variation", 5-8 = "Pathological changes") in DT animals was noted by Wk 14-15. At this time, broadening of the posterior Y suture arms with slight focal areas of opacity noted along the suture arms was detected. Cataracts (grade 8) was noted in 1/8 DT animals at Wk 18. The affected dog developed cataracts in one eye by Wk 14-18, and in the other eye by Wk 19, which then remained for the rest of the observation period. By the end of Wk 35/36, lens pathology was detected in 4/8 DT animals. There was also an increase in the number of animals receiving a grade of 4 (no C animal received a grade higher than 2; 7/8 C animals were graded as "0"). Plasma cholesterol was reduced in DT animals as measured enzymatically. When analyzed by GC/MS, one peal tentatively identified as Δ^8-cholestanol (an immediate precursor of lathosterol). This would suggest drug-induced inhibition at this enzymatic step (steroid-8-ene isomerase). In the 1 dog developing cataracts, plasma cholesterol was reduced 18-26% below baseline levels. No drug-related changes were noted in glucose levels or osmolality of aqueous humor samples, or on gross pathology. Microscopic analysis of the lens and lacrimal gland revealed drug-induced changes in anterior, posterior, and equatorial cortical areas consisting primarily of fiber swelling and/or polychromasia. These findings were confirmed upon EM analysis, with additional observations including cytoplasmic vesicular inclusions in anterior epithelial cells and superficial posterior cortical fibers. These findings increased with duration of dosing. At Wk 7 only minimal anterior cortical fiber polychromasia/swelling and mild posterior cortical fiber swelling were noted. At Wk 35/36 these increased in incidence and/or severity and additional findings were observed. Drug-induced changes in lacrimal gland consisted of acinar brown pigment deposition, and chronic dacryoadenitis (i.e., inflammation). Incorporation of radiolabeled precursor into cholesterol in lens was reduced in DT animals; however, the incorporation into lathosterol was not affected. There was an increase in radiolabeling of an unidentified sterol in DT animals. No differences in sterol composition were noted using uv detection. Upon analysis of different fractions of lens, the only drug-related effect was a decrease in the cholesterol fraction in the outermost lens layer. Two sterols, tentatively identified as Δ^8-cholestanol and lathosterol, were detected in all DT,

but only 1 C animal, with the level of Δ^8-cholestanol >4-fold higher than that of lathosterol.

Dermal irritation: ICI 204,636 was classified as having high irritant potential (p = 0.761) based on *in vitro* studies in two mouse (3T3 Swiss mouse fibroblast and XB-2 mouse teratomal keratinocyte) cell lines. In *in vivo* studies, ICI 204,636 (500 mg) was found to produce slight contact sensitivity in guinea pigs, and moderate-to-severe ocular irritation (with production of corneal opacities) when administered (10 mg) to 1 eye in 1 rabbit (plans to dose additional animals was suspended).

Interestingly, ICI 204,636 was shown to be highly irritating in one dietary dose-range finding study in mice, whereas similar effects were not observed in the other dietary studies, including the 2-yr dietary carcinogenicity study in mice.

Conclusions: ICI 204,636 produced the following major findings in general oral toxicity studies in rat, dog, and monkey: In addition, CNS signs (i.e., behavioral changes) were observed in all species tested except mouse.

1. the major toxicities in rat consisted of drug-related changes in thyroid, liver, and mammary gland. No overall NOEL was established in rat.

   b. mammary gland hyperplasia was noted at all doses tested in females (≥10 mg/kg). In males, hyperplasia was detected at 75 and 250 mg/kg in the 1-yr study; however, mammary gland atrophy accompanied by cellular vacuolation was observed at 50 and 150 mg/kg in the 6-mo study.

   b. a number of microscopic changes were noted in thyroid gland. The only change in thyroid gland consistently noted in the chronic toxicity studies was deposition of pigment in thyroid follicular epithelial cells. Pigment deposition occurred at all doses in both males and females. Follicular cell hypertrophy was noted in females at doses ≥75 mg/kg. In males, the finding was not as consistent. In the 6-mo study, hypertrophy was noted at doses of 50 and 150 mg/kg, whereas in the 1-yr study, hypertrophy was observed only at 250 mg/kg. Reduced colloid and increased globular/clumped colloid was reported only in females and only in the 6-mo study (doses of 50 and 150 mg/kg).

   There was no overall NOEL for thyroid gland toxicity in rat. If the pigment deposition and the changes potentially related to thyroid gland stimulation (e.g., hypertrophy, reduced colloid) are considered separately, a NOEL was still not established for pigment deposition. For thyroid gland stimulation, however, doses of 25 and 50 mg/kg could be considered NOELs in males and females, respectively. These doses are 0.3 and 0.6 times the maximum recommended human dose on a mg/m^2 basis. Plasma exposure (based on all available data, range of means) at these doses are as follows:

   ICI 204,636
   - C_{max}: 60-130 and 400-800 ng/mL at 25 and 50 mg/kg
   - AUC: 400-500 and 1000-2000 ng/mL at 25 and 50 mg/kg

   ICI 214,227
   - C_{max}: 100-1300 and 1200-1300 ng/mL at 25 and 50 mg/kg
   - AUC: 3000-4000 and 6000-7000 ng/mL at 25 and 50 mg/kg

   ICI 213,841
   - C_{max}: 50-100 and 160-300 ng/mL at 25 and 50 mg/kg
   - AUC: 300-700 ng/mg at 50 mg/kg (data not calculable at 25 mg/kg)

The proposed mechanisms underlying these toxicities (and those in liver), as well as supportive evidence, are discussed in a separate section.

c. microscopic findings in liver consisted of centrilobular hypertrophy and fat vacuolation. In males, hypertrophy was noted at doses of 150 and 250 mg/kg and fat vacuolation was detected at all doses (≥10 mg/kg). In females, hypertrophy was evident at doses ≥75 mg/kg and vacuolation at 250 mg/kg.

There was no NOEL for liver findings in males. In females, the NOEL was 50 mg/kg. This dose is 0.6 times the maximum recommended human dose on a mg/m^2 basis.

d. Microscopic findings observed only in the 1-yr study consisted of (1) cortical vacuolation and cellular hypertrophy of the zona fasciculata of the adrenal gland were observed at doses of 75 and 250 mg/kg in males and at 250 mg/kg in females, respectively, (2) myocardial fibrosis at all doses in males, and at doses ≥25 mg/kg in females, (3) alveolar macrophages in lung at 75 and 250 mg/kg in males and females, and (4) pancreatic islet cell hypertrophy at 75 and 250 mg/kg in males and females. Recovery animals exhibited changes in lung.

In the 2-yr carcinogenicity study in rat, none of these findings were clearly related to drug treatment. The severity of foamy alveolar macrophages was increased in drug-treated males and females (i.e., increased incidences of mild and moderate cases); however, the incidence of severe cases was highest in CM and CF.

2. the major toxicity in **dog** consisted of drug-related changes in the eye, with the most serious being formation of cataracts. In ophthalmology examinations, drug-related effects consisted of the following: (1) miosis and difficulty in inducing mydriasis (all doses, ≥10 mg/kg) and (2) posterior triangular cataracts (100 mg/kg). Additional findings in the 6-mo study consisted of (1) granular opacification of the axial posterior cortex and posterior Y suture (100 mg/kg) and (2) prominent posterior Y sutures (all doses, 25-100 mg/kg). In the 1-yr study, the data were expressed as grades of 1 to 8, with 1-4 being considered "normal variation" and 5-8 being considered "lens pathology". There was an increase in lens grades at all doses (10-100 mg/kg); however, in all but the 100 mg/kg grp, the findings were considered "normal variation". At 100 mg/kg (the HD), lens pathology was evident and consisted of cataracts, posterior subcapsular cortex/suture fiber swelling associated with globule formation, liquefaction, nuclear retention. One finding noted only in the 1-yr study was brown pigment deposition (in acinar epithelial cells) at all doses (≥10 mg/kg, also in recovery animals). Although some of the more minor findings tended to normalize during the recovery period, the more serious changes, particularly cataracts, were not reversible.

There was no NOEL for eye findings in either males or females (and, therefore, no overall NOEL). Cataracts occurred only at 100 mg/kg. This dose is 4 times the maximum human dose on a mg/m^2 basis. Plasma levels at this doses were as follows (range of means from all available data):

ICI 204,636
C_{max} = 1000-8000 ng/mL; AUC = 20500-30000 ng•hr/mL
ICI 214,227
C_{max} = 250-700 ng/mL; AUC = 4000-5000 ng•hr/mL
ICI 213,841
C_{max} = 850-4000 ng/mL; AUC = 24000-33000 ng•hr/mL

3. In **monkey**, the dose-limiting toxicity involved the CNS. Drug-related clinical signs were observed at all doses (25-225 mg/kg), including subdued behavior and hunched posture. Signs noted at higher doses included tremor, vomiting, and unusual behavior (including circling behavior). Other target organs included thyroid, liver, mammary gland, and possibly eye.

   (a) mammary gland hyperplasia occurred at all doses in females and at 100 and 225 mg/kg in males. The NOEL (50 mg/kg) for this finding in males is 1.2 times the maximum human dose on a mg/m^2 basis.

   (b) liver findings consisted of increases in liver wt (at 225 mg/kg in males, 100 and 225 mg/kg in females), centrilobular hepatocyte hypertrophy (at 225 mg/kg in males), and fat vacuolation of hepatocyte (at 225 mg/kg in males and females). The NOEL (50 mg/kg) for this finding in females is 1.2 times the maximum recommended human dose on a mg/m^2 basis. In males, the NOEL (100 mg/kg) is 5.5 times the maximum recommended human dose on a mg/m^2 basis.

   (c) thyroid gland follicular cell hypertrophy (at 100 and 225 mg/kg in males). Pigment deposition was noted in males and females at 225 mg/kg; however, the pigment was identified as lipofuscin and, therefore, not considered to be related to that noted in rat. The NOEL (50 mg/kg) for hypertrophy in males is 1.2 times the maximum recommended human dose on a mg/m^2 basis.

   In the 1-yr study, a striated appearance of the anterior lens surface was detected in 2/7 HDF (at 225 mg/kg). In the opinion of the sponsor's ophthalmology consult, this finding was possibly related to drug; however, since it was not associated with reductions in lens transparency, this finding was not considered a pathological change. No histopathological correlates were detected upon examination of eye. Although the change was not considered "pathology" as such, it is consistent with the increased incidence and severity of lens changes not considered "lens pathology" at all doses in rat.

   No overall NOEL was established in monkey, based on clinical signs and changes in mammary gland at all doses.

One final comment is in regard to the irritancy (dermal, ocular) potential of ICI 204,636 as noted in mouse, guinea pig, and rabbit. In a special toxicity (thyroid) study in mouse, the irritation produced by both a powdered and pelleted drug-diet admixture resulted in death and/or moribund sacrifice of both drug-treated and control animals. In some cases, the control animals were more affected than the drug-treated animals. These results are consistent with the dermal/ocular irritation studies (particularly the *in vitro* irritancy studies). However, the same irritancy potential was not observed in dose-range finding and 2-yr carcinogenicity studies in mouse in which dosing was achieved in the diet. How the apparently severe irritancy problem was overcome in these later studies is unclear.

CARCINOGENICITY

Two-year carcinogenicity studies were conducted in C57BL mice and Wistar rats. The sponsor requested the Division's review of their dose-selection for both studies. The data in support of the doses selected were reviewed by the Division; however, no concurrence was given. It was the opinion of this reviewer that the available data were insufficient to determine appropriate doses (P/T review, 6/29/92).

Mouse. Dose-selection for the 2-yr carcinogenicity study was based on the results of a number of dose-range finding studies. In those studies reviewed here, doses up to 1100 mg/kg/day were administered to mice (C57BL; strain in one study not specified) for up to 90 days. Reductions in body weight (as compared to controls) of ≈20% were noted at doses of 800 and 1100 mg/kg/day. Microscopic findings consisted of (1) hepatocyte hypertrophy at doses ≥200 mg/kg, (1) tubular atrophy of the testes at all doses (i.e., ≥50 mg/kg/day), and (3) epithelial hyaline droplets at doses ≥100-200 mg/kg.

In the 2-yr study, ICI 204,636 was administered in the diet to mice (100/sex for C grp, 50/sex for dosed grps, 20/sex/grp for satellite-TK) at doses of 0, 20, 75, 250, and 250-750 (Grps I, II, III, IV, and V, referred to here as LD, MD-1, MD-2, and HD). In the HD grp, the dose was increased by 50 mg/kg weekly up to 750 mg/kg. This dose was defined as the MTD based on body weight data. Animals were housed 5/cage by sex. This is unfortunate since individual food consumption, and, therefore, drug doses could not be determined or controlled. This problem was, in part, ameliorated by determination of plasma drug levels in individual animals (with certain exceptions in which blood samples had to be pooled). Observations consisted of the following: clinical signs, ophthalmology, body weight, food consumption (based on cage intake), water consumption, hematology (rbc, wbc), TK (ICI 204,636, and metabolites, ICI 213,841, and ICI 214,227), and terminal studies (gross pathology, histopathology).

There were no drug-related increases in mortality rate. In females, the % survival was similar among groups, whereas in males, the % survival was highest in MD-2M and HDM. Also, no drug-related clinical signs were noted either upon daily observations or during veterinary examinations, or on ophthalmology examination. The number of palpable masses were not increased by drug-treated; the overall incidence was lowest in HDM and HDF. Body weight was reduced (compared to CM) at all doses, but consistently only in MD-2M (2-8%) and HDM (4-18%). There were no consistent effects on food consumption, although food consumption was reduced in HDM and HDF from Wk 3 to Wks 44 or 32, respectively. Water consumption was increased at all doses in males (dose-related) and females (not dose-related). On hematology parameters (only rbc and wbc counts), the only drug-related finding was a decrease in rbc in MD-2M and HDM. Upon macroscopic analysis, the following were noted: (1) increases in kidney discoloration and bilateral firmness in MD-2M and HDM and (2) a dose-related increase in the incidence of thin uterus at doses >LD. Upon microscopic analysis, there were a number of drug-related findings. In terms of **non-neoplastic changes**, the following were noted: (1) an increase in cortical tubular basophilia in MD-2M, HDM, and HDF, (2) an increase in cortical microlithiasis in females at all doses, (3) acinar basophilia in salivary gland in MD-2M, HDM, MD-1F, MD-2F, and HDF, (4) transitional epithelial hyaline droplets (urinary bladder) in HDM, MD-2F, and HDF, (5) atrophy of the uterus in MD-2F and HDF, and (6) changes in thyroid gland consisting of increases in (a) hypertrophic follicular epithelium (>LD in males, HDF), (b) follicular cell hyperplasia (MD-2M, HDM), pigment deposition (>LD in males and females, and (c) basophilic follicular colloid in HDM. Interestingly, there were no clear drug-related changes in mammary gland in either males or females. In terms of **neoplastic findings**, there was an increase in the % of males with multiple and with benign tumors at the HD. However, the only drug-related finding a dose-related increase (at MD-2, HD) in thyroid gland follicular adenoma in males. In females, the incidence of thyroid gland follicular adenoma was highest at the LD and HD; there was no positive linear trend in females.

At the doses associated with thyroid gland follicular adenomas in males, **plasma levels** were as follows:

ICI 204,636:	not quantifiable (nq) at MD-2, nq-107 ng/mL at HD
ICI 213,841:	nq-79 ng/mL at MD-2, nq-217 ng/mL at HD
ICI 214,227:	nq-33.7 ng/mL at MD-2, 42-43 ng/mL at HD

Statistical Evaluation: According to the sponsor's statistical analysis, the only significant neoplastic finding was an increase in follicular adenomas in MD-2M and HDM ($p<0.01$ and

p<0.001, respectively). Independent statistical analysis of the data was conducted by Sue-Jane Wang, Ph.D. (Statistician, HFD-710); Dr. Wang concurred with the sponsor's findings (cf. Appendix C).

Conclusion: the only drug-related neoplastic finding was an increase in thyroid gland follicular cell adenoma in males at doses of 250 and 750 mg/kg. These doses are 1.5 and 4.5 times the maximum human therapeutic dose (800 mg/day) on a mg/m^2 basis. Sufficiently high doses were used based on reductions in final body weight (as compared to C grps) of 9-12% at the HD. Of concern, however, were the low plasma levels of drug-related compounds. At the lower doses (20 and 75 mg/kg), plasma levels of ICI 204,636, ICI 213,841, and ICI 214,227 were <LLOQ, and at the higher doses (250 and 750 mg/kg) data were only sporadically available. The plasma data from the 2-yr study are compared to those obtained in two dose-range finding studies in the following table:

STUDY	DOSES (mg/kg/day)										
	20	50	75	100	200	250	300	400	750	800	1100
ICI 204,636 (µg/mL)											
THM/599		0.070 0.038		0.080 0.056	0.113 0.087		0.119 0.138	0.150 0.134			
THM/810										193 <LLOQ	94 <LLOQ
TCM/600	<LLOQ <LLOQ		<LLOQ <LLOQ			<LLOQ-0.024 <LLOQ			0.027-0.107 0.021-0.071		
ICI 213,841 (µg/mL)											
THM/599		n/a*		n/a	n/a		0.256 0.297	0.276 0.302			
THM/810											
TCM/600	<LLOQ <LLOQ		<LLOQ-0.049 <LLOQ-0.068			<LLOQ-0.079 <LLOQ-0.169			<LLOQ-0.217 <LLOQ-0.124		
ICI 214,227 (µg/mL)											
THM/599		0.061 0.071		0.066 0.079	0.083 0.093		0.104 0.116	0.118 0.114			
THM/810											
TCM/600	<LLOQ <LLOQ		<LLOQ <LLOQ			<LLOQ-0.034 0.030-0.081			0.042-0.043 0.049-0.067		

*data not available

Clearly, in none of the studies did the plasma levels of the parent compound or either metabolite increase proportionately with dose. There are a number of reasons for this finding, such as poor methodology and/or sampling techniques, delayed absorption with increasing dose (if sampling times were inadequate to define the C_{max}), non-linear kinetics (suggesting formation of unmeasured metabolites), reduced absorption with increasing dose. [There would also appear to be fairly significant differences in plasma levels among studies.] The concern for this study is that the dose, expressed either as mg/kg or mg/m^2 may not be a good basis for calculating safety margins between mice and humans.

The results of this study were reviewed by the Exe-CAC (cf. Appendix D).

Rat. Dose-selection for the 2-yr study was based, according to the sponsor, primarily on data from a previous 3-mo dose-range finding study (THR/2047). Based on the data from that study, the HD selected for the 2-yr study was predicted to produce reductions in body weight gain, and microscopic changes in thyroid and liver.

ICI 204,636 was administered by gavage (1 dose/day) to rats (n = 100/sex for C grps, 50/sex for drug-treated grps) at doses of 0, 20, 75, and 250 mg/kg. Observations consisted of the following: clinical signs, ophthalmology, body weight, food consumption, hematology (10/sex/grp), clinical chemistry (10/sex/grp), urinalysis (10/sex/grp), TK (for quantitation of ICI 204,636, ICI 213,841, and ICI 213,227), and terminal studies (gross pathology, organ/tissue wts, and histopathology). Special analyses were conducted on sections of thyroid gland (Masson Fontana stain, grading of height of follicular epithelial cells), pituitary gland (3-4/sex/grp, immunohistochemistry for FSH, LH, ACTH, prolactin, GH, and TSH), and adrenal gland (Masson Fontana technique, immunocytochemistry for neuron-specific enolase).

There were no drug-related effects on mortality rate. Drug-related clinical signs were evident at all doses and included urine staining, malocclusion of the teeth, and, according to the sponsor, sedation and excessive salivation [however, the summary incidence tables did not reflect these findings]. Other findings included dark deposits on tail (MDM, HDM), noisy breathing (MDM, HDM; all doses in females), and corneal opacities (both eyes, HDM). There were no differences among groups in the incidence of palpable masses, expressed either as % of animals, or the no. of masses/animals/grp, or on ophthalmology examination. Body weight was reduced in males at all doses (compared to CM), and in females at the MD and HD. Food consumption was increased at all doses (non-dose related) in females during the first 4 wks of dosing, but was not consistently affected at any dose during the rest of the dosing period; in males, food consumption was similar among grps. Small, mostly non dose-related, changes in some hematology parameters were noted in both males (increases in MCV, MCH; decreases lymphocytes, monocytes, large unnucleated cells) and females (decreases in hgb, rbc, rbc width; increases in MCV, MCH; decreases in wbc count). On clinical chemistry parameters, the primary findings were dose-related decreases in cholesterol and triglycerides in HDM, MDF, and HDF. Urinalysis findings consisted primarily of increases in urinary volume (dose-related in males, all doses), increased Na in HDF, and increased K in females (dose-related, all doses). TK data indicated the following: (1) plasma levels of ICI 204,636 were 107-779, 339-1360, and 754-1880 ng/mL at the LD, MD, and HD, respectively; dose-corrected exposure was highest at the MD and slightly less at the HD than at the LD, (2) plasma levels of ICI 214,227 were below the LLOQ during the first 26 wks of dosing; at Wk 52-104, levels were 1000-1160, 1790-2100, and 1550-1760 ng/mL at the LD, MD, and HD, respectively, (3) plasma levels of ICI 213,841 throughout the dosing period only at the HD. At the LD, this metabolite was detectable only at Wk 104, and at the MD, it was detectable from Wk 26 on. Levels were 78.1, 211-330, and 352-556 ng/mL at the LD, MD, and HD, respectively. By the end of the dosing period, levels of ICI 204,636 and ICI 214,227 were similar, and those of ICI 213,841 were ≈80% lower. Plasma levels of all three compounds were fairly stable from Wk 26 on (except, of course, for ICI 213,841 at the LD).

There were no clear drug-related causes of death when overall findings were considered. Of note, however, was (1) an increase in fatal hematopoietic neoplasms in HDM and (2) a non-dose related increase in fatal mammary gland neoplasms at all doses in females. When the mammary gland data were separately analyzed in terms of benign and malignant tumors, however, there was a dose-related increase in fatal adenocarcinomas in females (all doses affected). At necropsy, a number of dose-related findings were noted. In particular, an increase in "discolored" pancreas (dose-related, all doses in males; MD and HD females), seminal vesicles (all doses, dose-related), and thyroid gland (all doses, dose-related in males; MD and HD females) was reported. Other findings included the following: (1) enlarged adrenal gland (MDM, HDM, HDF), (2) enlarged liver (MDM, HDM, HDF), (3) irregular lung surface (HDM,

HDF), (4) increased amount of mammary gland (all doses females, not dose-related), (5) enlarged pituitary (all doses, dose-related in females), (6) enlarged seminal vesicles (MDM, HDM). Increases in weights of liver, adrenal, and pituitary gland confirmed macroscopic findings in these organs (i.e., enlarged), and extended certain findings to MDF (i.e., increased adrenal gland wt) and all doses in males (i.e., increased pituitary gland wt). In addition, there was a small decrease in ovary weight in HDF.

Drug-related **non-neoplastic** findings were detected in a number of organs. In liver, the primary findings consisted of increases in the incidence of fatty vacuolation in HDM, MDF, and HDF and centrilobular hypertrophy in HDM, HDF. Examination of the individual data indicated that all 3 HDM and all 5 HDF that had decreases in serum cholesterol and triglycerides also had fatty vacuolation of the liver. Fatty vacuolation was not noted in MDF that had reduced serum cholesterol and/or triglycerides; however, serum lipids were not as low in this grp as in the HD grps. In thyroid gland, the following were observed: (1) presence of brown pigment in follicular cells at all doses, with the severity being dose-related in both males and females, (2) an increase in the severity of parafollicular cell hyperplasia at all doses in males and females (not dose-related), (3) increases in the severity of basophilic colloid at all doses in males and females (dose-related), (4) an increase in the severity of follicular cell desquamation in HDM, and at all doses in females (dose-related), (5) presence of black granules at all doses, with the severity being dose-related in both males and females, (6) a tendency for follicular epithelial height to be greater in HDM, MDF, and HDF, (7) an increase in follicular hyperplasia in MD and HD males and females, (8) parafollicular cell hypertrophy was noted only in 1 HDF. Non-neoplastic findings in other organs were as follows: (1) lung findings consisted of foamy alveolar macrophages and presence of eosinophilic material. For foamy alveolar macrophages, the incidence of mild and/or moderate severity were increased in HDM, and at all doses (dose-related) in females; however, the incidence of severe effects was not increased. The incidence of eosinophilic material was increased at all doses in males (dose-related), and in MDF and HDF. (3) an increase in the severity of giant cells in lymph node was noted in HDM and HDF. (4) the incidences of reduced exocrine acinar granulation were increased in HDM, (5) the incidences of diffuse hyperplasia of the pituitary gland (pars distalis) was increased at all doses (dose-related) in females, and degeneration of the pars distalis was detected only in 2 HDF. (6) the incidences and/or severity of prostate gland findings (i.e., prostatitis, glandular epithelial hyperplasia, and glandular ectasia) were increased in MDM and HDM; the incidence of mild prostatitis was also increased at the LD. (7) the incidences (or severity) of diffuse acinar basophilia of the parotid salivary gland and acinar degeneration/atrophy of the sublingual salivary gland were increased in males at all doses, and in females (all doses; not always dose-related) (8) the incidence/severity of cystic dilatation, vesiculitis, and/or secretion coagulation of the seminal vesicles were increased at all doses, (9) thymic cysts were detected only in 3 HDF. (10) increases in proestrus and decreases in estrus were evident at all doses (dose-related). (11) an increase in the severity of mucification of the vagina was increased at all doses (not dose-related).

Drug-related **neoplastic** findings were detected primarily in mammary gland and thyroid gland. The incidence of adenocarcinoma of the mammary gland was increased in females at all doses. The incidence of thyroid gland follicular adenomas was increased in HDM. Other findings of noted were as follows: (1) adrenal gland cortical carcinomas were noted in only 2/50 HDM and 1/99 CF. When the incidence of cortical carcinomas and adenomas were combined, the total was increased at all doses in males (5/100, 4/50, 3/50, and 5/50 in CM, LDM, MDM, and HDM, respectively). (2) the incidence of pancreatic islet cell carcinoma was increased at all doses (not dose-related) in males and females; the combined incidence of islet cell adenoma and carcinoma was elevated only in males (all doses, not dose-related), (3) an increase in pituitary gland adenoma in LDM and HDM, (4) an increase in benign mixed thymoma in HDM, and detection of malignant thymic lymphoma (lymphoblastic) only in 1/47 HDM.

At the doses associated with mammary gland adenocarcinomas, **plasma levels** were as

follows:

ICI 204,636: 107-779 ng/mL at LD, 339-1360 ng/mL at MD, 754-1880 ng/mL at HD
ICI 213,841: 78 ng/mL at LD, 211-330 ng/mL at MD, 352-556 ng/mL at HD
ICI 214,227: 1000-1160 ng/mL at LD,1790-2100 ng/mL at MD, 1550-1760 ng/mL at HD

At the doses associated with thyroid gland follicular adenoma, plasma levels were as follows:

ICI 204,636: 754-1880 ng/mL
ICI 213,841: 352-556 ng/mL
ICI 214,227: 1550-1760 ng/mL

Statistical Evaluation: According to the sponsor's statistical analysis, the following neoplastic findings were statistically significant: (1) adenocarcinoma of the mammary gland in females at all doses, (2) follicular adenoma of the thyroid gland in HDM, and (3) adrenal gland cortical carcinoma in HDM. Independent statistical analysis of the data was conducted by Sue-Jane Wang, Ph.D. (Statistician, HFD-710). Dr. Wang concurred with the sponsor's analysis of mammary and thyroid gland findings (cf. Appendix C), however, according to Dr. Wang's analyses, the trend test for the adrenal cortical carcinomas in males was not statistically significant. The sponsor did not consider this latter finding a biologically significant effect.

Conclusion: the drug-related neoplastic findings in the 2-yr study consisted of (1) adenocarcinoma of the mammary gland in females at all doses tested (20, 75, 250 mg/kg, or 0.3, 0.9, and 3.0 times the maximum human therapeutic dose on a mg/m^2 basis) and (2) thyroid gland follicular cell adenoma in males at 250 mg/kg (or 3.0 times the maximum human therapeutic dose on a mg/m^2 basis). The sponsor reported a statistically significant increase in adrenal gland cortical carcinomas in males at 250 mg/kg; however, re-analysis by Dr. Wang did not reveal a significant effect. In addition, no drug-related preneoplastic changes were evident in adrenal gland.

The results of this study were reviewed by the Exe-CAC (cf. Appendix D).

MECHANISMS UNDERLYING THYROID, EYE, AND MAMMARY GLAND FINDINGS

Thyroid: the sponsor has proposed two different mechanisms, one to explain the deposition of black pigment in thyroid gland of rat and mouse and another to explain the thyroid gland hyperplasia and follicular cell adenomas observed in rat and mouse. [Thyroid findings are summarized in the attached table.]

Thyroid gland pigment: the sponsor has proposed that the dark pigment detected in rodent thyroid is due to oxidation of ICI 204,636 (or metabolite) by thyroidal peroxidase (thyroperoxidase), an enzyme catalyzing the oxidation of iodide and subsequent incorporation of iodine into thyroglobulin. In the process, ICI 204,636 (or metabolite) is proposed to form a pigmented compound which is then deposited in thyroid. Special toxicity studies were conducted in rat and mouse to provide support for this mechanism. These studies demonstrated the following:

(1) the pigment could not be identified, but was found to have characteristics different from those of pigment produced by other compounds, including minocycline (a tetracycline derivative).

(2) radioactivity detected in thyroid gland following oral administration of radiolabeled ICI 204,636 was localized to the follicular cell, spatially associated with pigment and colloid.

(3) in primary rat thyrocyte cultures, ICI 204,636 decreased the total uptake of iodine into

thyroid, but had no effect on incorporation of iodine into thyroglobulin. Plasma metabolites, ICI 214,227, ICI 213,841, and ICI 230,303, decreased the total uptake of iodine into thyroid and incorporation of iodine into thyroglobulin; the effect of ICI 236,303 on iodine incorporation was not concentration-related.

It is the sponsor's opinion that the deposition of pigment in thyroid, shown to be potentially irreversible, has no effect on thyroid function. [Thyroid function was not directly tested, e.g., by administration of exogenous thyroid hormones, etc.] There was no clear evidence of hypothyroidism in rats in the subchronic and chronic oral toxicity studies aside from changes in thyroid hormones. However, thyroid gland follicular cell hyperplasia and follicular cell adenomas were observed in rat and mouse. The sponsor has proposed a separate mechanism to explain these morphological changes.

The sponsor provided literature references on minocycline-induced black thyroid in laboratory animals and humans. Minocycline is thought to be a competitive inhibitor of a thyroidal peroxidase. Oxidization of minocycline results in formation of an insoluble black compound which is presumed to account for the black pigment deposition. It is a similar mechanism that the sponsor has proposed for ICI 204,636. Experiments with minocycline have indicated that, although the presence of pigment did not reduce the thyroid's response to TSH in rat, pigment deposition was associated with morphological evidence of thyroid stimulation (i.e., hyperplasia, increased follicular cell height, reduced colloid) in rat and dog. In human, black pigment deposition (following prolonged treatment with minocycline) was associated with slight interstitial fibrosis and pyknotic nuclei (suggesting epithelial damage).

The sponsor has provided data which suggest that ICI 204,636 (and/or metabolites) may have antithyroid effects. The three metabolites studies were shown to inhibit uptake of iodine into thyroid (as did ICI 204,636) and the incorporation of iodine into thyroglobulin, an obligatory step in thyroid hormone synthesis. The effective concentrations ranged from 10-500 µg/mL, levels that would not likely be achieved in plasma clinically, although it is possible that tissue levels may approach effective concentrations. In rat, radioactivity following administration of radiolabeled ICI 204,636 was shown to accumulate in thyroid gland, and the $t_{1/2}$ in thyroid was longer than for all other tissues examined (4.4 hr vs 0.07-3.3 hr). It should be noted the effective concentrations in these *in vitro* studies are markedly higher than the plasma levels (of ICI 204,636) associated with thyroid gland pigment deposition in the oral toxicity studies in rat.

It is not possible, based on the data provided, to conclusively determine the mechanism underlying pigment deposition or to rule out that pigment deposition has no morphological consequences. Follicular cell hypertrophy was observed in the oral toxicity studies. The results of studies on minocycline suggest that pigment deposition may be associated with such changes.

Thyroid gland hyperplasia/neoplasia: the sponsor has proposed that the thyroid gland stimulation observed in the oral toxicity studies in rat and mouse result from hepatic enzyme induction, with increased metabolism of thyroid hormones and subsequent increases in TSH. The particular enzyme of interest was T_4 UDPGT, the enzyme involved in glucuronidation of thyroxine (a major pathway in metabolism/elimination of thyroid hormones). In addition to the findings in the oral toxicity studies, special studies were conducted in rat and mouse to determine the effects of orally administered ICI 204,636 on liver enzymes and plasma thyroxine clearance. The results have been summarized elsewhere (also, see attached table). Increases in TSH were evident in the chronic toxicity studies in rat, as were microscopic findings indicative enzyme induction in liver (e.g., hypertrophy). However, hepatocellular hypertrophy and thyroid gland follicular cell hypertrophy/hyperplasia were not always noted at the same doses. Most notably, in the 2-yr mouse carcinogenicity study, follicular cell hypertrophy/hyperplasia and adenomas were observed, whereas no microscopic findings were

detected in liver.

It is interesting to note that TSH was shown to be elevated in male and female rats in certain subchronic studies, however, the incidence of follicular cell adenomas was significantly increased only in males in the 2-yr carcinogenicity study in rat.

In a special toxicity study in mouse, evidence of hepatic enzyme induction was obtained (e.g., increased liver wt, increased protein and cytochrome P450 content). However,the activity of T_4 UDPGT was not significantly elevated. This would suggest that, in mouse, hepatic enzyme induction may not be the mechanism underlying the thyroid findings.

In a special toxicity study in rat, the activity of T_4 UDPGT (as well as other hepatic enzymes) was elevated in drug-treated animals, but at a dose higher than those used in any of the definitive oral toxicity studies.

As with thyroid pigmentation, the data do not convincingly support a specific mechanism. That is, it is not possible to conclude that ICI 204,636 exerts toxic effects on thyroid through an indirect mechanism. Also, the sponsor concluded that the thyroid findings were a rodent-specific effect. However, similar changes in thyroid gland, thyroid hormones, and liver were observed in monkeys in the definitive 1-yr oral toxicity study. As in rat, there was no clear relationship among the findings. For example, TSH was elevated in females at 100 and 225 mg/kg, whereas, follicular cell hypertrophy was increased only in males (at the same doses).

Eye: the sponsor has proposed that ICI 204,636 produces lens pathology (including cataracts) in dog indirectly through inhibition of cholesterol synthesis. Lens pathology (summarized in an attached table) was evident in the 6-mo and 1-yr oral toxicity studies, but not in the 4-wk studies at similar doses. Although serum cholesterol was also reduced in these studies (as well as in one of the 4-wk studies), there was no clear relationship between lens pathology and serum cholesterol levels in individual animals.

The sponsor conducted *in vitro* and *in vivo* studies to further investigate the lens changes produced by ICI 204,636 and the possible relationship to effects on cholesterol biosynthesis. The sponsor also provided several literature references on the relationship between cholesterol biosynthesis and cataracts. Together, these studies document the association between hypolipidemic agents (late-stage inhibitors of cholesterol synthesis) and cataract formation.

The relationship between cholesterol biosynthesis and cataract formation is based on the fact that the lens fiber cell membrane is enriched in cholesterol, with the lens epithelial cell having a fairly high rate of cholesterol synthesis (particularly in the outer cortex and subcapsular areas). As in all cells, changes in lipid content of the membrane may have significant effects on cell membrane integrity and fluidity. In the lens, cholesterol is thought to be involved in stabilization of the lens intercellular junctions (Kuszak JR *et al. Invest Ophthal & Visual Sci* Vol 29(2):261-267, 1988). It has been hypothesized that inhibition of cholesterol biosynthesis in the lens may result in cataracts through, perhaps, a variety of mechanisms including the loss of cell membrane integrity or destabilization of lens intercellular junctions. According to Kuszak *et al.* (1988), the latter effect might ... "result in a loss of the uniform packing arrangement of lens fiber cells thought to be necessary for the lens to be transparent". There does, however, appear to be differences in the development and characteristics of cataract formation depending upon the type of drug-induced inhibition. Two classes of cholesterol inhibitors which have been studied are (1) the "late-stage" inhibitors, of which the most notable example is triparanol, and (2) HMG CoA reductase inhibitors, e.g., lovastatin, simvastatin. One major difference between these classes of inhibitors is that with late-stage inhibitors, there is buildup of abnormal or intermediate lipids. With HMG CoA reductase inhibitors, this is not the case. This is a potentially

STUDY	SPECIES STRAIN	DOSES (mg/kg)	FINDINGS		
			THYROID	LIVER	THYROID HORMONES
4-wk toxicity (TAR/1621)	Sprague-Dawley rat	25, 50, 150	none	wt: ↑ (HDM, LDF, MDF, HDF) macroscopic: none microscopic: none	not measured
4-wk bridging, non -GLP (TKR/1924)	Wistar-derived rat	25, 50, 150	wt: not measured macroscopic: none microscopic: pigment in follicular epithelial cells (HDM, HDF) hypertrophy (HDM, HDF)	wt: ↓ (HDM), ↑ (LDF, MDF, HDF) macroscopic: none microscopic: vacuolation (HDF)	TSH: none T_4: slight ↑ (LD, MD, HD) T_3: none
3-mo dose-range (THR/2047)	Wistar rat	25, 50, 150, 300	wt: not measured macroscopic: dark red/brown color (MD-2M, HDM, HDF) microscopic: follicular cell hypertrophy (MD-2M, HDM, HDF) ↓ colloid (HD) pigment in follicular epithelial cells (MD-2M, HD)	wt: ↑ (HDF) macroscopic: none microscopic: fat vacuolation (MD-2M, HD)	TSH: ↑ (HD)
2-mo special (THR/1084)	male Sprague-Dawley rat	150	wt: none macroscopic: brown discoloration (1 rat) microscopic: brown pigment hypertrophy (↑ severity) colloid depletion (↑ severity)	wt: not measured macroscopic: none microscopic: not measured	TSH: none T_4: ↑ (transient) FT_4: ↑ (transient) T_3:↑ FT_3: none rT_3: ↑ (transient)
6-mo toxicity (TPR/1616)	Sprague-Dawley rat	25, 50, 150	wt: not measured macroscopic: thyroid discoloration (HDH, HDF) microscopic: pigment in follicular epithelial cells (all) hypertrophy (MDM, HDM, HDF) decreased colloid (MDF, HDF) globular/clumped colloid (MDF, HDF)	wt: ↑ (LDF, MDF HDF) macroscopic: none microscopic: hypertrophy (HDM) vacuolation (fat) (MDM, HDM)	TSH: ↑ (HDM, HDF) T_4: no change T_3: slight ↑ (LDF, MDF, HDF)
1-yr toxicity (TFR/1626)	Wistar rat	10, 25, 75, 250	wt: not measured macroscopic: thyroid discoloration (all) microscopic: pigment in follicular epithelial cells (all) hypertrophy (HDM, MD-2F, HDF)	wt: ↑ (MDM, HDM, HDF) macroscopic: enlarged (MD-1M, HDM) microscopic: hypertrophy (HDM, MDF, HDF) vacuolation (fat) (all M, HDF)	TSH: ↑ (all but LD, M,F) T_4: ↑ (MD-2M, HDM) T_3: not measured
2-yr car. (TCR/1624)	Wistar rat	20, 75, 250	wt: not measured macroscopic: discolored thyroid (LDM, MDM, HDM, MDF, HDF) microscopic: brown pigment (all) parafoll. hyperplasia (all, not dose-related) basophilic colloid (all) follicular cell desquamation (HDM, all F) pigment in follicular epithelial cells (all) follicular hyperplasia (MDM, HDM, MDF, HDF) ↑ follicular cell height (HDM, MDF, HDF) follicular adenoma (HDM)	wt: ↑ (MDM, HDM, HDF) macroscopic: enlarged (MDM, HDM, HDF) microscopic: hypertrophy (HDM, HDF) fatty vacuolation (HDM, MDF, HDF)	not measured

STUDY	SPECIESS TRAIN	DOSES (mg/kg)	FINDINGS		
			THYROID	LIVER	THYROID HORMONES
14-18 day special toxicity (TKR /2271)	Wistar-derived rat	300	wt: not measured macroscopic: discoloration microscopic: pigment deposition (DTM, DTF) ↑ average follicular epithelial height (DTM, DTF)	wt:↑ (DTF) enzymes: ↑ P450 content (DTM, DTF) ↑ EROD (DTM, DTF) ↑ PROD (DTM, DTF) ↑ T_4 UDPGT (DTM, DTF) macroscopic: none microscopic: fat vacuolation (DTM, DTF)	TSH: ↑ (DTF) T_4: none FT_4: none T_4 clearance: ↑ (DTM, DTF)
4-wk special toxicity (TKM/913)	C57BL mouse	400,500, 600,750*	wt: not measured macroscopic: none microscopic: none	wt: ↓ corrected for body weight (DT) enzymes: ↑ P450 content slight ↑ (n.s.) in T_4UDPGT (DT) macroscopic: enlarged (DT) microscopic: centrilobular hepatocyte hypertrophy (DT)	T_4: ↓ (DT) T_4 clearance: ↑ (DT)
3-mo dose-ranging (THM/599)	C57BL mouse	50, 100, 200, 300, 400	wt: not measured macroscopic: none microscopic: none	wt: ↑ (all) macroscopic: none microscopic: centrilobular hepatocyte hypertrophy (≥200 mg/kg)	not measured
3-mo dose-ranging (THM/810)	(?) mouse	300-800, 400-1100	wt: not measured macroscopic: none microscopic: none	wt: ↑ (all) macroscopic: enlarged liver (all) microscopic: none	not measured
2-yr car (TCM/600)	C57BL mouse	20, 75, 250, 750	wt: not measured macroscopic: none microscopic: follicular epithelium hypertrophy (MD-2M, HDM, HDF) follicular cell hyperplasia (MD-2M, HDM) pigment deposition (MD-1M, MD-2M, HDM, MD-2F, HDF) basophilic follicular colloid (HDM) follicular adenoma (MD-2M, HDM)	wt: not measured macroscopic: none microscopic: none	not measured
12-mo toxicity (TFP/84)	cynomolgus monkey	25, 100, 225	wt: ↑ (HD recovery) macroscopic: discolored thyroid (lipofuscin, HD) microscopic: follicular cell hypertrophy (MDM, HDM)	wt: ↑ (HDM, MDF, HDF) macroscopic: enlarged (HDM) microscopic: centrilobular hypatocyte hypertrophy (HDM) vacuolation (HD)	TSH: ↑ (MDF, HDF) T_4: none T_3: ↓ (MD, HD)

*doses increased from 400 to 750 mg/kg, with each dose being given for 1 wk.

important distinction since accumulation of one or more of these abnormal lipids may influence the course of cataract development. The cataracts produced by HMG CoA reductase inhibitors, lovastatin and simvastatin, are similar to those observed in ICI 204,636-treated dogs. As described by Gerson RJ *et al.* (Exp Eye Res 50:65-78, 1990), the "Cataracts observed in dogs [with lovastatin, simvastatin] were always subcapsular in nature, occurring posteriorly and occasionally in the anterior lenticular region. These lesions were initially characterized by an increased prominence of the lenticular suture lines". Interesting, these authors could not demonstrate a correlation between serum cholesterol and the incidence of cataracts in dogs. [This was also the case with ICI 204,636.] There was, however, a strong positive correlation between plasma levels of the HMG CoA reductase inhibitors and the incidence of cataracts. In discussing the relevance of the effects of these drug to humans in clinical use, the authors concluded that it was unlikely that humans would be affected, mainly because there was a large margin of safety, based either on dose or plasma level comparisons. The authors pointed out that this is "...in sharp contrast to triparanol which produced cataracts in animals at clinical dosages. In addition, the biochemical mechanism of action of HMG-CoA reductase inhibitors is unique and does not lead to an accumulation of abnormal sterols as demonstrated with triparanol".

Triparanol is a Δ^{24} reductase inhibitor; this enzyme catalyzes the formation of cholesterol from desmosterol. Triparanol was removed from the market by the manufacturer in April of 1962, following reports of drug-induced cataracts in humans. One particularly compelling observation was the young age of some of the affected individuals (i.e., 6, 23, and 37 yrs). The clinical and preclinical observations with triparanol were reviewed by Kirby in a 1967 publication (Kirby TJ Tr Am Ophth Soc 65:493-543, 1967). The cataracts produced by triparanol involved primarily the posterior and anterior subcapsular area, and, in some cases, continued to develop even after drug-withdrawal (postdosing interval: 0-12 mo). The overall incidence of cataracts in patients taking triparanol was apparently fairly low; however, the fact that young patients were affected was troublesome, as well as was the lack of understanding of factors underlying individual sensitivity.

In the special studies conducted by the sponsor, ICI 204,636 appeared to inhibit cholesterol synthesis in cultured dog lens and in HepG2 cells. Inhibition of conversion of desmosterol to cholesterol (a final step) was suggested by reductions in the cholesterol fraction and increases in the desmosterol fraction. ICI 204,636 did not produce cataracts (or other signs of lens pathology) when incubated with dog lenses for 9 days. In special *in vivo* studies, ICI 204,636 was found to inhibit cholesterol biosynthesis in liver to some degree. Due to methodological problems effects on lens could not be assessed in the acute dosing study. In the 7-35 wk study, ICI 204,636 induced lens pathology, including cataracts in dogs at as early as Wk 14-18 of dosing. Analytical studies demonstrated that ICI 204,636 reduced plasma and lens cholesterol, while increasing levels of Δ^{8}-cholestanol. In lens, incorporation of radiolabeled precursor into cholesterol, but not lathosterol, was reduced.

These data suggest that ICI 204,636 has some inhibitory effects on cholesterol biosynthesis. The data on sterols profiles in these studies were contradictory in terms of which step(s) were inhibited by ICI 204,636, as determined by build-up of pathway intermediates. In some studies, desmosterol was increased, whereas in others Δ^{8}-cholestanol was increased. Also, changes in lathosterol were not consistent. Although the potential site of inhibition cannot be determined from these data, it is possible that ICI 204,636 (or a metabolite) inhibits a late stage of biosynthesis (as compared to, for example, HMG-CoA ligase inhibition) and results in increases in abnormal or intermediate sterols. How or if this relates to the formation of cataracts is uncertain. It has been documented in the literature that certain drugs which inhibit cholesterol biosythesis are associated with cataract formation. However, in some published studies and in the case of ICI 204,636, there was no relationship between plasma cholesterol levels and the incidence of cataracts in individual animals. Also, the data do not address whether it is ICI 204,636 or a metabolite, or both that may be involved since the

effects of the major plasma metabolites on cholesterol biosynthesis and cataract formation were not directly tested.

Mammary gland. With drug such as ICI 204,636, that are antagonists at the D_2 receptor, mammary gland effects are expected. The proposed mechanism is via increases in serum prolactin levels. ICI 204,636 produced mammary gland hyperplasia in rats (6-mo, 1-yr) and monkey (1-yr). Serum prolactin was measured in all 1-yr studies. In rats, serum prolactin was elevated in males and females at all doses (10-250 mg/kg) through most of the dosing period; the effects were not necessarily dose-related in either sex. The elevations in serum prolactin were observed throughout the dosing period in males, except at the LD (increased only at the last measurement period) and in females from Wk 20 on. In monkey, serum prolactin was transiently increased at doses of 100 and 225 mg/kg in males, and decreased in females at all doses (25-225 mg/kg) during the last week of dosing. In dogs, no mammary gland changes were noted and serum prolactin levels were <LLOQ in almost all cases.

In the 2-yr carcinogenicity in mouse, mammary gland hyperplasia was noted in all grps (including C) and the incidences were not dose-related. Serum prolactin was not quantitated. In the 2-yr carcinogenicity study in rat, there was an increase in mammary gland adenocarcinoma at all doses in females. Incidences of "moderate" mammary gland hyperplasia somewhat elevated in drug-treated animals. Serum prolactin was not quantitated in either study.

The data do confirm that, at least in rat, increases in serum prolactin levels may underlie the mammary gland changes. It is interesting, however, that, although elevations in serum prolactin were higher and more prolonged in male rats in the 1-yr study, mammary gland changes were evident only in females.

REPRODUCTION

Reproductive toxicity studies were conducted in Alpk:ApfSK (Wistar-derived) rats and Dutch Belted rabbits: Segment I studies in male and female rats in separate studies, Segment II studies in rat and rabbit, and a Segment III study in rat. These studies were all conducted according to GLP (UK). Only the Segment II study in rabbits and the Segment III study were reviewed in this review; the rest of the studies were reviewed previously (Segment II study in rat, P/T Review, J.DeGeorge, Ph.D., 12/15/89; Segment I studies, P/T Review, L.M. Freed, Ph.D., 6/25/92).

A second "teratology" study (non-GLP) was performed in rats at doses of 0, 25, 50, and 200 mg/kg p.o. on Days 6-15 of gestation; however, since no fetal examinations were conducted, this study was not considered in assessing the teratogenic potential of ICI 204,636. A sighting peri- and post-natal study (GLP) was also performed in rats; doses of 0, 25, and 150 mg/kg p.o. were administered from Day 16 of gestation through Day 8 of lactation. The results of this later study were considered.

Segment I studies: In the **male** study (n = 25/grp), ICI 204,636 was administered orally (by gavage) only to males at doses of 0, 25, 50, and 150 mg/kg for 11 wks prior to mating, and during the first pairing (each M with 2 F for up to 3 wks). CM and HDM were mated a second time (each M with 2 F), 2 wks after the end of dosing (i.e., at the end of the first pairing period) for 2 wks. Observations included the following: clinical signs, body weight, reproductive measurements (pregnancy, interval to pregnancy, interval to mating, uterine examination), gross pathology, histopathology (only performed in infertile males and was confined to reproductive tract). Females were sacrificed on Days 18-21 of pregnancy; LDM and MDM were sacrificed at Wk 17-18, and HDM were sacrificed at the end of the second pairing.

STUDY	SPECIES STRAIN	DOSES (mg/kg)	FINDINGS	
			EYE	SERUM CHOLESTEROL
4-wk toxicity (TAD/500)	Beagle	25, 50, 100	none	no changes
4-wk, non-GLP bridging toxicity (TKD/629)	Beagle	25, 50, 100	ophthalmology: difficulty inducing mydriasis (all) microscopic: none	↓ (MD, HD)
6-mo toxicity (TPD/497)	Beagle	25, 50, 100	ophthalmology: posterior triangular cataracts (HDF) granular opacification of axial posterior cortex (HDF) foacl opacities of posterior Y suture (sutures dense, granular) (HDM) prominent posterior Y sutures (all) microscopic:cataracts (HDF) swelling of posterior lens fiber tips (all)	↓ (HDM, HDF)
12-mo toxicity (TFD/501)	Beagle	10, 25, 50, 100	ophthamology: ↑ lens grade (all) lens pathology (defined as lens grade 5-8) (HDM, HDF) posterior axial triangular cataracts (HDM, HDF) ↑ in normal lens variation (all) microscopic: ↑ swelling of posterior cortex/sutures (MD-2, HD) globule formation in anterior cortex, posterior cortex, and/or posterior suture (MD-2, HD) liquifaction of posterior cortex (HD) liquifaction of posterior cortex and/or suture (HD) nuclear retention (HD)	↓ (MD-2M, HDM, all F)
12-mo toxicity (TFR/1626)	Wistar rat	10, 20, 75, 250	ophthalmology: none microscopic: none	↓ (HD)
1-2-mo toxicity (TFP/84)	cynomolgus monkey	25, 100, 225	ophthalmology: striated appearance of anterior lens surface (HDF) microscopic: none	↓ (HD)

There were 4 unscheduled deaths. Three of these deaths were attributed to dosing accident, but for 1 LDM the cause of death was unknown. Clinical signs were observed at all doses. Ptosis, hypoactivity, and staining of the mouth were evident at all doses. Salivation was noted only in MDM and HDM. Body weight was reduced at all doses by Wk 11 (6-7% at LD, MD and 14% at HD). Body weight normalized in LDM and MDM after the end of dosing, but was still reduced in HDM at sacrifice. Drug-related effects on mating and fertility were observed at the MD and HD. The no. of males with a female with a postcoital interval >5 days was increased at the HD during both mating periods, as was the no. of males requiring more than one mating to impregnate. The no. of males with a non-pregnant female was increased at the MD and HD (both matings at HD); however, each treated male successfully impregnated at least 1 female. The interval to pregnancy was increased at the HD, and only during the first pairing. Upon uterine examination, there were no apparent drug-related after the first mating. After the second mating (HD only), there was an increase in post-implantation loss at the HD; this effect, however, was primarily due to post-implantation loss in 1 "HD" female.

The sponsor concluded that there was no drug-related effect on fertility in males at the MD since all MDM successfully impregnated at least 1 of the 2 females and the interval to pregnancy was not increased; however, 2 MDM did not impregnate 1 of the females they were mated with. In order to conclusively show that the two non-pregnant females (at the MD) were infertile, they should have been paired with proven, non-treated males; this was not done. Considering the drug-related effect on fertility at the HD, an effect (although lesser) at the MD cannot be excluded. The LD, therefore, could be considered a NOEL for impaired fertility. Unfortunately, microscopic analysis of the reproductive tract or sperm analysis was not performed in a systematic manner, therefore, drug-related effects at LD cannot be entirely ruled out.

In the **female** study (n = 66/grp), ICI 204,636 was administered orally (by gavage) only to females at doses of 0, 1, 10, and 50 mg/kg beginning 2 wks prior to mating and continuing through Day 21 of pregnancy (33/grp) or throughout pregnancy and lactation (33/grp). For HDF who were assigned to deliver naturally, the dose was reduced to 1 mg/kg from Day 17 of pregnancy through Day 6 of lactation to minimize the pup death rate. Observations in F_0 and F_1 dams included the following: clinical signs, body weight, reproductive parameters (estrus cycles, precoital intervals, mating, fertility, uterine examination, litter survival), fetal examinations [F_1 and F_2 fetuses: external, visceral, skeletal (Alizarin red); however, data were provided only for skeletal findings], and terminal studies (histopathology). F_0 dams were sacrificed at the end of dosing (i.e., Day 21 of gestation or Day 22-24 of lactation). For evaluation, the F_1 generation consisted of 30-25 M/grp and 60-50 F/grp (the initial no. was later culled to make smaller grps). F_1 males were sacrificed at the end of the mating period, and F_1 females were sacrificed at either Day 21 of gestation (21-25/grp) or Day 22-24 of lactation (20-25/grp). F_1 generation pups were assessed for attainment of developmental milestones, and selected F_1 generation pups were followed after weaning and were mated to assess reproductive performance. In those F_1 pups sacrificed at the end of the weaning period (i.e., Day 22-24 postpartum), eyes from ≥5/sex/grp were examined microscopically. Those pups followed through mating, pregnancy, and lactation were sacrificed at the end of the lactation period. The F_2 generation pups followed through lactation were sacrificed at weaning (Day 22-24 of lactation). Observations in F_2 pups followed through lactation consisted of attainment of developmental milestones and histopathology eyes were examined in F_2 pups as for F_1 pups sacrificed on Day 22-24 postpartum.

One HDF died during the dosing period; necropsy findings consisted of congested lungs. In addition, 2 CF, 2 MDF, and 1 HDF were sacrificed moribund; none were directly attributable to drug. Drug-related clinical signs consisted of ptosis, scabs, and salivation. Ptosis and scabs were evident at all doses, with incidences being dose-related; salivation was observed only at

the HD. Body weight gain in HDF was higher (≈2-fold) prior to mating, but lower (9%) during pregnancy as compared to CF; during lactation, body weights were fairly similar among grps. There were dose-related increases in MDF and HDF with irregular estrus cycles, and the precoital interval was increased in HDF. Mating and fertility were impaired in HDF as indicated by a number of parameters (e.g., no. mated/no. paired, no. pregnant/no. mated, number pregnant/no. paired). There were no apparent drug-related effects on uterine parameters: no. of corpora lutea, no. of implants, no. of live fetuses, pre- and post-implantation loss, fetal body weight, placental weight, placental index, amniotic fluid per fetus, M/F ratio, empty uterus weight. There was also no effect on length of gestation, on fetal/litter survival, or on histological examination of dams or pups (F_1, F_2). In both F_1 and F_2 fetuses, there were dose-related (all doses) increases in extra vestigial or true ribs (seventh cervical, first lumbar centra). There were no drug-related effects on developmental landmarks in F_1 or F_2 pups, or on reproductive performance in F_1 pups. In terms of developmental landmarks, however, the way in which the data were collected made the measurements somewhat insensitive. The data were expressed as either the day on which "...at least half of each litter..." achieved each of the landmarks, or, as for the startle response, the response was tested only on Day 22 of lactation. Using this method, delays in physical maturation could be missed. This is particularly true for assessment of the startle response (conducted on Day 22 postpartum), a response which is generally attained around Day 12 postpartum.

Segment II studies: In the **rat** study (n = 22/grp for main study; an additional 4 CF and HDF for TK analysis), ICI 204,636 was administered orally (by gavage) at doses of 0, 25, 50, and 200 mg/kg on Days 6-15 of gestation. Observations included clinical signs, body weight,TK, fetal examination [external in all fetuses, visceral in 1/3 of fetuses, and skeletal (using Alizarin red) in 2/3 of fetuses], and terminal studies (necropsy). Dams were sacrificed on Day 20 of gestation.

There were 8 unscheduled deaths, all at the HD. Three HDF were found dead, and 5 were sacrificed moribund. Only one of these deaths was attributed to dosing accidents by the sponsor. Drug-related clinical signs were noted at the MD and HD, and consisted primary of sedation, piloerection, poor grooming, and unsteady gait; respiratory difficulty/noisy breathing and lacrimation were observed only at the HD. Body weight gain was reduced only at the HD. No drug-related effects were noted on the following parameters: no. of live fetuses/litter, pre- and post-implantation loss, M-to-F ratio. There was a small, but significant, decrease in fetal body weight at the HD. There were no drug-related effects evident upon external or visceral examination. [It should be noted that 1 dam (#4074) was included in the wrong grp for analysis of visceral findings, i.e., the MD instead of the LD grp. Also, the data for HDF 4109 was not included in the individual data table for visceral findings.] There were, however, drug-related increases in skeletal anomalies. The only statistically significant finding were increases in the incidences (expressed as no. and % affected fetuses) of dumb-bell centra in MDF and HDF. Although not statistically significant, there were also (1) dose-related increases in incomplete cranial ossification (all doses) and (2) incomplete ossification of sternebrae (HD). For dumb-bell centra and incomplete ossification of sternebrae, the % of affected litters was also increased at the MD and HD, and at the HD, respectively. These data suggest that ICI 204,636 may delay skeletal development. There was no evidence of a teratogenic effect at any of the doses tested. TK data were available only for 2 HDF. For these dams, plasma levels of ICI 204,636 were 0.39-0.56 µg/mL and embryonic tissue levels of ICI 204,636 were 1.95-1.98 µg/mL; these data suggest accumulation of the parent compound in fetal tissue.

In the **rabbit** study (n = 20/grp for main study, an additional 6 C and HD dams for TK analysis), ICI 204,636 was administered orally (by gavage) at doses of 0, 25, 50, and 100 mg/kg on Days 6 to 18 of gestation. Observations consisted of the following: clinical signs, body weight, TK, reproductive/litter parameters, fetal examinations (all fetuses examined for external, visceral, skeletal findings), and gross pathology.

There were 4 unscheduled deaths; 1 MDF and 1 HDF were sacrificed due to complete loss of litters, and 1 MDF and 1 HDF were sacrificed for "humane reasons" (Day 10 and 12, respectively; reasons not specified). Drug-related clinical signs were evident at all doses. The primary signs were reduced/ceased defecation, unsteady mobility/gait, hypoactivity, noisy breathing/respiratory difficulties, and nasal discharge. Unsteady mobility/gait and hypoactivity were noted in all HD (including TK) animals. Body weight loss occurred at all doses (greatest at the HD), in contrast CF gained a mean of 90 gm during the same period. There were no clear drug-related effects on the following parameters: no. of live fetuses/litter, total implants, pre- or post-implantation losses, placental wt/litter, amniotic fluid wt/fetus/litter, no. of corpora lutea, empty uterus wt. Mean fetal body weight and the male-to-female ratio were both reduced at the HD, whereas the placental index (i.e., total placental wt of litter/total fetal wt of litter) was increased at the HD. The sponsor considered there to be no drug-related effects on external or visceral findings. There was a dose-related (at MD, HD) increase in the incidence (% affected fetuses and litters) of carpal/tarsal flexure(s). Since these findings were not confirmed upon skeletal examination, they should be considered soft tissue findings. These data were discussed with Edward Fisher, Ph.D. (Pharmacologist, member of the Reproductive Toxicology Committee, HFD-120). According to Dr. Fisher, the increase in carpal/tarsal flexure (a minor visceral anomaly) (primarily) at the HD is consistent with a general embryotoxic effect as evidenced by a significant reduction in mean fetal body weight at the HD (cf. Palmer A.K. *Adv Exp Med Biol* 27:45-60, 1972) . The historical incidence of this finding in New Zealand white rabbits is 0.07-0.088% of fetuses and 0.56% of litters (cf. Clemens GR, *et al. Teratology* 49:388-9, 1994); the incidences in the MD and HD grps were 1 and 4.5% of fetuses, respectively, and 6 and 26% of litters, respectively. Upon skeletal examination, there was evidence of delayed development as evidenced by a statistically significant increase (% fetuses and litters) in the incidence of incomplete ossification of hyoid at the HD. Although not statistically significant, there were increases in incomplete sternebral ossification (MD, HD) and increases in abnormal sternebral ossification (similar effect at all doses) expressed as % of litters; also, incomplete ossification of inter-parietals was noted only in dosed grps (not dose-related).

[The sponsor was asked to provide historical control data for the incidence of carpal/tarsal flexure. According to the data provided (cf. Appendix E), the lab HC incidence of this finding ranged from 0-5 fetuses or a mean of 1.61 per study (total of 18 studies) and 0-3 litters or a mean of 0.94 litters per study. Therefore, the HC incidence of this finding was equal to that in the current study when expressed as percent of total fetuses, but was lower when the data were expressed as percent of total litters. In the HC database, the highest number of affected fetuses, or 5, occurred in 2 litters; in two of the HC studies, 4 affected fetuses were from 3 litters.]

Segment III: in this study, ICI 204,636 was administered to Wistar rats (n = 22/grp) at doses of 1, 10, and 20 mg/kg orally (by gavage) from Day 16 of gestation to Day 21 of lactation. Observations in the F_0 generation consisted of the following: clinical signs, body weights, reproductive/litter parameters, and terminal study (gross pathology). Observations in the F_1 generation consisted of the following: clinical signs, body weights (during lactation), postnatal development, and terminal study (gross pathology). The reproductive performance of the F_1 generation was not assessed.

There were no unscheduled deaths during the study; however, 6 dams (2/CF, 1 LDF, 2 MDF, 1 HDF) were sacrificed due to loss of or failure to litter. There were no drug-related clinical signs, nor were there any effects on body weight during lactation. There was, however, a reduction in body weight gain during lactation at the HD. The following parameters were unaffected by drug-treatment: length of gestation, no. of live pups, viability index (Day 5), lactation index (Day 22), no. of whole litters lost (Day 22), no. of litters with ≥ 1 dead pup, litter size, mean litter wt, or proportion of males per litter. No drug-related findings were noted in

dams at necropsy (no histopathology performed).

In the F_1 generation, no drug-related effects were observed on any of the parameters assessed. However, the assessment of postnatal development was conducted in a manner similar to that used in the Segment I study in female rats. That is, the data were expressed as either the day on which ≥50% of the litter achieved each landmark (for pinna detachment, adult hair growth pattern, crawling, adult locomotion, open eyes) or on a particular day (e.g., startle response was tested only on Day 22 of lactation). As noted previously, these types of assessment are somewhat insensitive (particularly for the startle response which is attained ≈Day 12 postpartum), and delays in physical maturation could be missed. There were no drug-related effects evident at necropsy, nor upon microscopic analysis of eyes (in F_1 pups).

In a sighting peri- and post-natal development study (conducted under GLP), Wistar rats (12/grp) were given ICI 204,636 at doses of 0, 25, or 150 mg/kg from Day 16 of gestation through Day 8 of lactation. One HDF was sacrificed moribund due to poor condition, and 2 LDF and 5 HDF were killed because of the death of entire litters. Body weight was reduced at the HD (compared to CF) during the dosing period; at the LD, body weight gain was reduced only during the lactation period. Drug-related effects consisted of the following: (1) dose-related decrease in the no. of pups alive at Day 5 and Day 8 postpartum, (2) dose-related increases in the no. of entire litters lost, (3) decrease in mean litter wt on Day 5 postpartum at the HD, and (4) a decrease in the male-to-female ratio on Days 1 and 5 postpartum at the HD (statistically significant only on Day 1). No drug-related findings were apparent in either dams or pups at necropsy.

Conclusions: ICI 204,636 were found to have the following effects on reproduction:

(1) impairment of mating and fertility in males at doses of 50 and 150 mg/kg p.o., as evidenced by adverse effects on a number of parameters, including increases in precoital interval and the number of males requiring more than one mating to impregnate a female. At these doses, body weight was reduced by 6-7 and 14% (compared to CM), respectively. These effects were evident in HDM in a second pairing after a 2-wk drug-free period and with no drug treatment during pairing. The NOEL for effects on mating and fertility was 25 mg/kg.

(2) impairment of mating and fertility in females at 50 mg/kg p.o. as evidenced by decreases in a number of parameters, including number mated per number paired, number pregnant per number mated, and number pregnant per number paired, and an increase in precoital interval. At this dose, body weight gain was higher than in control animals during the pre-mating period, but was reduced during pregnancy. The incidence of irregular estrus cycles was increased at 10 and 50 mg/kg. The NOEL for effects on mating and fertility in females was 1 mg/kg.

(3) delays in skeletal development in both rats and rabbits when ICI 204,636 was administered during pregnancy. In rats, effects observed included increases in the incidence of dumb-bell centra, incomplete cranial ossification, and incomplete ossification of sternebra. These findings were noted primarily at 50 and 200 mg/kg. Body weight in the treated dams was adversely affected only at 200 mg/kg, and was accompanied by decreases in fetal body weight. The fact that delays in ossification were noted at 50 mg/kg, a dose not associated with reductions in maternal or fetal body weight, would suggest a specific effect on bone ossification. The NOEL for this effect was 25 mg/kg. A finding observed in the Segment I (fertility) study in females, i.e., an increase in vestigial or true ribs, was not replicated at the higher doses used in the teratology study; therefore, this finding is of questionable biological significance.

In rabbits, effects included increased incidences of incomplete ossification of hyoid at

100 mg/kg and incomplete sternebral ossification at 50 and 100 mg/kg p.o.; other findings (i.e., increases in abnormal sternebral ossification and incomplete ossification of inter-parietals) were noted at all doses, but the incidences were not dose-related. A minor soft tissue anomaly, i.e., carpal/tarsal flexure, was noted only at 50 and 100 mg/kg (incidences were dose-related). This finding suggests a general embryotoxic effect, and is consistent with reductions in body weight noted at all doses in the dams (particularly marked at 100 mg/kg) and in fetal body weight noted at 100 mg/kg. The NOAEL for adverse skeletal effects was 25 mg/kg.

(4) ICI 204,636 did not appear to have any teratogenic effects at doses up to 200 mg/kg in rats and 100 mg/kg in rabbits, nor any adverse effect on the reproductive performance of the F_1 generation in the rat at doses up to 50 mg/kg. Although there were no adverse effects on neonatal development in rats at doses up to 50 mg/kg, the methods used for assessing attainment of developmental milestones were insensitive and may have missed differences among grps.

(5) ICI 204,636 had no adverse effects on peri- and post-natal development when administered late in gestation through the lactation period at doses up to 20 mg/kg p.o. However, at higher doses (i.e., 25 and 150 mg/kg p.o.) used in a preliminary "sighting" study (conducted under GLP), there were dose-related decreases in pup survival (up to Day 8 postpartum) and dose-related increases in entire litters lost. In addition, mean litter weight and the male-to-female ratio were decreased at the HD. There was evidence of maternal toxicity at the HD as evidenced by a reduction in body weight (compared to CF) throughout the dosing period; at the LD, body weight gain (but not body weight) was reduced during lactation.

GENOTOXICITY

The genotoxic potential of ICI 204,636 was tested in the following assays: *in vitro* bacterial gene mutation assay (6 studies; 5 in *S. typhimurium* strains and 1 in *E. coli*.), *in vitro* mammalian gene mutation assay in CHO cells, *in vitro* mammalian cytogenic study in cultured human lymphocytes, *in vivo* micronucleus assay in rats. SEROQUEL produced a reproducible increase in mutations in the *in vitro* bacterial gene mutation assay in one tester strain, TA 1538, in the presence of metabolic activation. No evidence of clastogenic potential was obtained in the *in vitro* assays in Chinese Hamster Ovary cell or in cultured human lymphocytes, or in the *in vivo* micronucleus assay in rats (at a dose of 500 mg/kg).

CONCLUSIONS

1. In in *vitro* receptor binding assays, ICI 204,636 was shown to have high affinity for the α_1, H_1, and $5HT_6$. Functional assays demonstrated *in vivo/ex vivo* effects at the dopamine, 5HT, histamine, and ACh muscarinic receptors.

   ICI 204,636 was active in a number of animal models considered to have predictive validity for antipsychotic potential in humans. ICI 204,636 did demonstrate some eps liability in animal models (e.g., catalepsy in rats, dyskinesia in monkeys), but, in general, at higher doses.

2. The PK/ADME of ICI 204,636 was studied primarily in rat, dog, and monkey. Following acute oral doses, the $t_{1/2}$ of ICI 204,636 in plasma was 0.4-3.9 (depending upon dose), 4, 5, and ≈4 hr in rat, dog, cynomolgus monkey, and human, respectively. The V_d was estimated in rat, and was consistent with significant tissue distribution in this species. Oral absorption was fairly rapid in all species; however the absolute oral bioavailability was low (<10%) in rat, dog (1 mg/kg), and monkey; in dog, oral bioavailability tended to increase with dose (30% at 10 mg/kg).

   Cl rate was similar to hepatic blood flow in dog, monkey, and human (≈30, 16.5, and ≈27 mL/min/kg, respectively); in rat, Cl rate was slightly higher (≈120-130 mL/min/kg) than hepatic blood flow.

   Plasma protein binding was fairly low in all species studied (≈63, 69, 83, 65, and 71% in rat, dog, human, mouse, and rabbit, respectively).

   The metabolic profile of ICI 204,636 was assessed in rat, dog, monkey, and human. In these species, identified metabolites and parent compound account for 65-80% of circulating drug-related material. Therefore, 20-35% of drug-related material in plasma of these species is unknown. Metabolism of ICI 204,636 involves sulfoxidation, oxidation to the acid form, hydroxylation of the dibenzothiazepine ring, O- and N-dealkylation, and subsequent glucuronidation. The major plasma drug-related compound in rat, monkey, and human were N-dealkylated-7-hydroxy (M 236,303), N-dealkylated-7-hydroxy conjugate, and the parent compound, respectively. In dog, the parent acid (M 289,663) was most abundant; however, slightly lower levels of parent, 7-hydroxy (ICI 214,227), and the sulfoxide (ICI 213,841) were also detected.

3. General oral toxicity study of ICI 204,636 was tested in Sprague-Dawley, Wistar, and Wistar-derived rat (10-250 mg/kg), Beagle dog (10-100 mg/kg), and cynomolgus monkey (25-225 mg/kg). Dosing was once daily by gavage in rat and dog. In cynomolgus monkey, the investigative 1-yr study was conducted using once-daily gavage dosing; however, in the definitive 1-yr study, dosing was t.i.d. (the intended human dosing regimen).

   (a) No overall NOEL was determined in rat. The primary target organs were thyroid, liver, and reproductive organs (e.g., mammary gland). Thyroid changes involved both pigment deposition (not reversible) in and hypertrophy/hyperplasia of follicular cells. Liver changes consisted of centrilobular hypertrophy and fatty vacuolation. Mammary gland changes consisted primarily of hyperplasia; however, in one study (6-mo) mammary gland atrophy accompanied by cellular vacuolation was noted in males.

   No NOEL was established for thyroid gland pigment deposition.

The NOELs for thyroid gland hypertrophy in male and female rats were 0.3 and 0.6 time the maximum recommended human dose (i.e., 800 mg/day) on a mg/m^2 basis.

A NOEL for liver changes could not be established in males. In females, the NOEL for this target organ was 0.6 times the maximum recommended human dose on a mg/m^2 basis.

(b) No overall NOEL was determined in dog. The primary target organ was the eye. Drug-induced changes consisted of increases in the "severity" of changes regarded as "normal variation" and in "lens pathology". Cataracts (not reversible) were observed only at 100 mg/kg. This dose is 4 times the maximum recommended human dose on a mg/m^2 basis.

(c) No overall NOEL was determined in monkey. The primary target organs were CNS, reproductive organs (e.g., mammary gland), thyroid, liver, and possibly eye. Drug-induced CNS signs consisted of subdued behavior (all doses tested) and, at higher doses, tremor, and unusual behavior. Mammary gland changes consisted of hyperplasia. Liver findings consisted of centrilobular hepatocyte hypertrophy and fatty vacuolation. Thyroid gland hypertrophy and pigment deposition were noted in monkey; however, the pigment in this case was identified as lipofuscin and, therefore, was not considered to be related to that noted in rat. Eye findings consisted of striated appearance of the anterior lens surface in 2 female monkey.

Except for mammary gland hyperplasia in females and CNS signs in both males and females, the NOEL for the findings listed is 1.2 times the maximum recommended human dose on a mg/m^2 basis.

4. 2-yr carcinogenicity studies were conducted in C57BL mice (20-750 mg/kg) and Wistar rats (20-250 mg/kg). Dosing was dietary in the mouse study, and by (once-daily) gavage in the rat study. The dietary study probably more closely approximates the proposed human regimen of t.i.d. dosing. Both studies were adequate; the lack of information on the metabolic profile of ICI 204,636 in mouse precludes an assessment of the relevance of this species to human. Plasma levels of parent compound and the two metabolites measured (ICI 214,227 and ICI 213,841) were low (<LLOQ at the two lower doses) and did not necessarily increase with dose. This would suggest that, in mouse, absorption from the diet-drug admixture was poor or that a major drug-related compound in plasma was not quantitated.

(a) in mice, the only drug-related neoplastic finding was an increase in thyroid gland follicular cell adenoma in males at doses 1.5 and 4.5 times the maximum recommended human dose on a mg/m^2 basis.

(b) in rats, drug-related neoplastic findings consisted of increases in adenocarcinoma of the mammary gland in females at all doses tested (0.3-3 times the maximum recommended human dose on a mg/m^2 basis) and thyroid gland follicular cell adenomas in males at 225 mg/kg (3 times the maximum recommended human dose on a mg/m^2 basis).

5. The sponsor proposed mechanisms for the thyroid gland (pigment deposition, hypertrophy/hyperplasia/neoplasia), eye (cataracts), and mammary gland (hyperplasia/neoplasia) findings.

(a) deposition of pigment in thyroid in rat was attributed to oxidation of ICI 204,636

(or metabolite) by thyroidal peroxidase (enzyme catalyzing the oxidation of iodide and incorporation of iodine into thyroglobulin) and subsequent deposition of oxidized ICI 204,636 (or metabolite) in thyroid follicular cells. According to the sponsor, pigment deposition should not be associated with functional/ morphological changes in the gland.

(b) thyroid gland hypertrophy/hyperplasia/neoplasia in rat was attributed to increased thyroid hormone degradation resulting from hepatic enzyme induction (particularly of T_4 UDPGT, an enzyme involved in glucuronidation of thyroxine). Increases in plasma TSH levels were proposed to mediate this effect.

(c) cataract formation in dogs was attributed to inhibition of cholesterol biosynthesis.

(d) mammary gland (and other reproductive) changes were attributed to elevations in serum prolactin.

The relationship between mammary gland changes and elevations in serum prolactin is one consistently noted with drugs that act as antagonists at the D_2 receptor. Therefore, it is not unreasonable to assume such a relationship with ICI 204,636, particularly since ICI 204,636 was shown to produce consistent elevations in serum/plasma prolactin in rats. It should be noted, however, that increases in serum/plasma prolactin were also noted in male rats, but no neoplasias were detected in males.

For the thyroid and eye findings, the mechanisms proposed were tested in special toxicity studies. Some evidence was obtained for both mechanisms; however, a consistent relationship between findings and changes associated with the proposed mechanisms could not be verified in individual animals. For example, there was no relationship between plasma cholesterol levels and the incidence of cataracts in individual dogs, even though mean plasma levels were reduced. Also, it is not possible to rule out the relevance of any of these toxicities to humans based on the available data.

(6) TK data were collected in a number of the toxicity studies, including the chronic and carcinogenicity studies. However, since major drug-related substances were not necessarily monitored either in the preclinical or human studies, comparisons among species were difficult to make. For example, the acid form of the parent compound (M 289,663), the major drug-related substance in dog plasma, was not monitor, nor was M 236,303 (except in one monkey study), a major plasma metabolite in rat, monkey, and human. The low levels of ICI 204,636 and 2 metabolites obtained in the mouse (2-yr carcinogenicity study) would suggest that major metabolites were also not measured in this species. In human, only "pharmacologically active" drug-related compounds were monitored in the clinical trials so, for example, ICI 213,841, which is a major plasma metabolite in human (as well as in rat, dog, and monkey), was not monitored routinely.

(7) Reproductive studies were conducted in rat (Segment I, II, III) and rabbit (Segment II).

(a) mating and fertility was impaired in males and females treated with ICI 204,636. The NOELs were 25 and 1 mg/kg, respectively. These doses are 0.3 and 0.1 times the maximum recommended human dose on a mg/m^2 basis.

(b) in embryofetal toxicity studies (Segment II), no teratogenic effects were noted in either rat or rabbit. Delays in skeletal development (i.e., bone ossification)

were noted in both rats and rabbits. In rats, this effect was observed at doses not associated with maternal toxicity (e.g., decreases in body weight). In rabbit, a minor soft tissue anomaly (i.e., carpal/tarsal flexure) was noted at doses at which maternal and fetal toxicity (i.e., reductions in body weight) was observed; therefore, this finding suggest a general embryotoxic effect.

The NOEL/NOAEL for skeletal effects was 25 mg/kg in both species. This dose was 0.3 (rat) or 0.6 (rabbit) times the maximum recommended human dose on a mg/m^2 basis.

(c) in offspring of treated dams, there did not appear to be any adverse effects on reproductive behavior or on neonatal development; however, the methods used for assessing attainment of developmental milestones were insensitive and may have missed drug-related effects.

(d) ICI 204,636 resulted in decreases in pup survival (up to Day 8 postpartum) and dose-related increases in loss of entire litters in rats when administered at doses 3 times the maximum recommended human dose on a mg/m^2 basis. The NOEL for this effect was 20 mg/kg, a dose 0.24 times the maximum recommended human dose on a mg/m^2 basis.

(8) The genotoxic potential of ICI 204,636 was tested in the following assays: *in vitro* bacterial gene mutation assay (6 studies; 5 in *S. typhimurium* strains and 1 in *E. coli.*), *in vitro* mammalian gene mutation assay in CHO cells, *in vitro* mammalian cytogenic study in cultured human lymphocytes, *in vivo* micronucleus assay in rats. SEROQUEL produced a reproducible increase in mutations in the *in vitro* bacterial gene mutation assay in one tester strain, TA 1538, in the presence of metabolic activation. No evidence of clastogenic potential was obtained in the *in vitro* assays in Chinese Hamster Ovary cell or in cultured human lymphocytes, or in the *in vivo* micronucleus assay in rats (at a dose of 500 mg/kg).

Recommended labelling (multiples are based on a maximum recommended human dose of 800 mg/day)

Pharmacodynamics

The mechanism of action of SEROQUEL, as with other antipsychotic drugs, is unknown. However, it has been proposed that antipsychotic activity is mediated through a combination of dopamine 2 (D_2) and serotonin type 2 ($5HT_2$) receptor antagonism. Antagonism at receptors other than D_2 and $5HT_2$ may also contribute to the pharmacologic effects of SEROQUEL.

SEROQUEL is an antipsychotic agent that interacts with multiple neurotransmitter receptors in the brain (Table 1). SEROQUEL has lower affinity for dopamine D_2 receptors than serotonin $5HT_2$ receptors. SEROQUEL has moderate-to-high affinity at histamine H_1 receptors and adrenergic α_1 receptors, with a lower affinity at adrenergic α_2 receptors), but no appreciable affinity at cholinergic muscarinic and benzodiazepine receptors (IC_{50s} > 5000 nM).

affinity (nM)	$5HT_{1A}$	$5HT_2$	D_1	D_2	H_1	α_1	α_2	muscarinic	benzodiazepine
IC_{50}	717	460	920	690	30	50	860	>10000	>5000
K_i	---*	288	558	531	10	13	782	---	---

*(not calculated

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity studies were conducted in C57BL mice and Wistar rats. SEROQUEL (quetiapine) was administered in the diet to mice at doses of 20, 75, 250, and 750 mg/kg and to rats by gavage at doses of 25, 75, and 250 mg/kg for two years. These doses are equivalent to 0.1, 0.5, 1.5, and 4.5 times the maximum human dose (800 mg/day) on a mg/m^2 basis (mice) or 0.3, 0.9, and 3.0 times the maximum human dose on a mg/m^2 basis (rats). There were statistically significant increases in thyroid gland follicular adenomas in male mice at doses of 250 and 750 mg/kg or 1.5 and 4.5 times the maximum human dose on a mg/m^2 basis and in male rats at a dose of 250 mg/kg or 3.0 times the maximum human dose on a mg/m^2 basis. In rats, there were statistically significant increases in mammary gland adenocarcinomas in females at all doses tested (25, 75, and 250 mg/kg or 0.3, 0.9, and 3.0 times the maximum recommended human dose on a mg/m^2 basis).

Thyroid follicular cell adenomas may have resulted from chronic stimulation of the thyroid gland by thyroid stimulating hormone (TSH) resulting from enhanced metabolism and clearance of thyroxine by rodent liver. Changes in TSH, thyroxine, and thyroxine clearance consistent with this mechanism were observed in subchronic toxicity studies in rat and mouse and in a 1-yr toxicity study in rat; however, the results of these studies were not definitive. The relevance of the increases in thyroid follicular cell adenomas to human risk, through whatever mechanism, is unknown.

Antipsychotic drugs have been shown to chronically elevate prolactin levels in rodents.

Serum measurements in a 1-yr toxicity study showed that SEROQUEL increased median serum prolactin levels a maximum of 32 to 13-fold in male and female rats, respectively. Increases in mammary neoplasms have been found in rodents after chronic administration of other antipsychotic drugs and are considered to be prolactin-mediated. The relevance of this increased incidence of prolactin-mediated mammary gland tumors in rats to human risk is unknown (see Hyperprolactinemia in **PRECAUTIONS, General**).

Mutagenesis

The mutagenic potential of SEROQUEL was tested in six *in vitro* bacterial gene mutation assays and in an *in vitro* mammalian gene mutation assay in Chinese Hamster Ovary cells. However, sufficiently high concentrations of SEROQUEL may not have been used for all tester strains. SEROQUEL did produce a reproducible increase in mutations in one *Salmonella typhimurium* tester strain in the presence of metabolic activation. No evidence of clastogenic potential was obtained in an *in vitro* chromosomal aberration assay in cultured human lymphocytes or in the *in vivo* micronucleus assay in rats.

Impairment of Fertility

SEROQUEL (quetiapine) decreased mating and fertility in male Sprague-Dawley rats at oral doses of 50 and 150 mg/kg or 0.6 and 1.8 times the maximum human dose on a mg/m^2 basis. Drug-related effects included increases in interval to mate and in the number of matings required for successful impregnation. These effects continued to be observed at 150 mg/kg, or 1.8 times the maximum human dose on a mg/m^2 basis, even after a two-week period without treatment. The no-effect dose for impaired mating and fertility in male rats was 25 mg/kg, or 0.3 times the maximum human dose on a mg/m^2 basis. SEROQUEL (quetiapine) adversely affected mating and fertility in female Sprague-Dawley rats at an oral dose of 50 mg/kg, or 0.6 times the maximum human dose on a mg/m^2 basis. Drug-related effects included decreases in matings and in matings resulting in pregnancy, and an increase in the interval to mate. An increase in irregular estrus cycles was observed at doses of 10 and 50 mg/kg, or 0.1 and 0.6 times the maximum human dose on a mg/m^2 basis. The no-effect dose in female rats was 1 mg/kg, or 0.01 times the maximum human dose on a mg/m^2 basis.

Pregnancy

Teratogenic Effects-Pregnancy Category C

The teratogenic potential of SEROQUEL (quetiapine) was studied in Wistar rats and Dutch Belted rabbits dosed during the period of organogenesis. No evidence of a teratogenic effect was detected in rats at doses of 25 to 200 mg/kg or 0.3 to 2.4 times the maximum human dose on a mg/m^2 basis or in rabbits at 25 to 100 mg/kg or 0.6 to 2.4 times the maximum human dose on a mg/m^2 basis. There was, however, evidence of embryotoxic effects on the fetus. Delays in skeletal ossification were detected in rat fetuses at doses of 50 and 200 mg/kg (0.6 and 2.4 times the maximum human dose on a mg/m^2 basis) and in rabbits at 50 and 100 mg/kg (1.2 and 2.4 times the maximum human dose on a mg/m^2 basis). A minor soft tissue anomaly (carpal/tarsal flexure) was detected in rabbit fetuses at doses of 50 and 100 mg/kg (1.2 and 2.4 times the maximum human dose on a mg/m^2 basis). Fetal body weight was reduced in rat fetuses at 200 mg/kg and rabbit fetuses at 100 mg/kg (2.4 times the maximum human dose on a mg/m^2 basis for both species). In a peri/postnatal reproductive study in rats, no drug-related effects were observed at doses of 1, 10, and 20 mg/kg or 0.01, 0.12, and 0.24 times the maximum human dose on a mg/m^2 basis. However, in a preliminary peri/postnatal study, there were increases in fetal and pup death, and decreases in mean litter weight at 150 mg/kg (or 3.0 times the maximum human dose on a mg/m^2 basis.

There are no adequate and well-controlled studies in pregnant women. SEROQUEL

should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Toxicology: SEROQUEL caused a dose-related increase in pigment deposition in thyroid gland in toxicity studies in rat and mouse at doses of 10-250 mg/kg in rats, 75-750 mg/kg in male mice, and 250-750 mg/kg in female mice; these doses are 0.1-3.0, 0.5-74.5, and 1.5-4.5 times the maximum recommended human dose (on a mg/m^2 basis), respectively. Pigment deposition was shown to be irreversible in rats. The identity of the pigment could not be determined, but was found to be co-localized with SEROQUEL in thyroid gland follicular epithelial cells. The functional effects and the relevance of this finding to human risk are unknown.

In dogs, SEROQUEL caused focal triangular cataracts located at the junction of posterior sutures in the outer cortex of the lens at a dose of 100 mg/kg or 4 times the maximum recommended human dose on a mg/m^2 basis. This finding may be due to inhibition of cholesterol biosynthesis by SEROQUEL. SEROQUEL caused a dose related reduction in plasma cholesterol levels in repeat-dose dog and monkey studies; however, there was no correlation between plasma cholesterol and the presence of cataracts in individual dogs. SEROQUEL did produce 25% reduction in cholesterol content of the outer cortex of the lens in dogs. Increases in certain intermediates (e.g., desmosterol, Δ^8-cholesterol) in *in vitro* and *in vivo* studies in dogs are consistent with inhibition of a late stage in cholesterol biosynthesis. Drug-related cataracts have not been seen in any other species; however, in a 1-yr study in monkeys, a striated appearance of the anterior lens surface was detected in 2/7 females at a dose of 225 mg/kg or 5.5 times the maximum recommended human dose on a mg/m^2 basis.

RECOMMENDATIONS

From a pharmacology/toxicology standpoint, there is no objection to the approval of this NDA.

The thyroid gland and eye toxicities observed primarily in rat and dog, respectively, are drug-related effects that may have significance to humans.

Lois M. Freed

Lois M. Freed, Ph.D.

cc
NDA orig (20-639)
div file
HFD-120 GJF 6/11/97
/G. Fitzgerald/L.M.Freed/S.Hardeman