TOXICOLOGY

Acute studies

1. **Acute toxicity (limit) study in mice oral administration** (Study no. TLM/919, 11/IE/1018108, Zeneca Pharmaceuticals, study dates: 6/95, GLP, Vol 1.51)

2. **Acute toxicity (limit) study in mice oral administration** (Study no. TLM/921, 11/IE/1017298, Zeneca Pharmaceuticals, study dates: 6/95, GLP, Vol 1.51)

3. **Acute toxicity (limit) study in mice oral administration** (Study no. TLM/920, 11/IE/10172, Zeneca Pharmaceuticals, study dates: 6/95, GLP, Vol 1.51)

The purpose of these studies was to assess the acute toxicity of 3 plasma metabolites of ICI 204,636, i.e., ICI 213,841, ICI 236,303, and ICI 214,227. They were conducted in Alpk:AP$_f$CD-1 (AP) mice (5/sex/grp; no controls). The metabolites were administered by gavage [vehicle: 0.5% (w/v) HPMC/0.1% (w/v) aqueous polysorbate 80] at single doses of 500 mg/kg. Observations consisted of clinical signs, body weight, necropsy, and limited histopathology (in animals sacrificed moribund).

ICI 213,841: there were no deaths. Drug-related clinical signs consisted of subdued behavior and ptosis. All animals gained weight during the 14-day observation period. There was no gross pathology.

ICI 236,303: 1 M was sacrificed moribund on Day 1, 6-7 hr postdosing. Clinical signs consisted of subdued behavior and ptosis. All survivors gained weight during the study. At necropsy, firmness of the heart and discoloration of multiple organs were noted; microscopic changes in liver consisted of loss of glycogen and increased fat vacuolation.

ICI 214,227: 1 M and 1 F were sacrificed moribund (Day 1 and 2, respectively). Drug-related clinical signs consisted of subdued behavior, hunched posture, ptosis, abnormal breathing, and hollowed abdomen. These signs continued into Day 2 in females. Weight loss was noted in 1 animal. Histopathological analysis in 4 animals (including the 2 sacrificed moribund) revealed enlarged intestinal Peyer's patches and discoloration of liver, heart, and lungs; none of these were considered directly related to drug.

Subchronic studies

1. **Oral (gavage) maximum tolerated daily dose (MTDD) toxicity study in the cynomolgus mokey** (Study no. TAP/83, 4/IE/1014013, [redacted] study dates: 1/94-5-94, GLP, Vol 1.31)

Methods: In order to determine an MTD, ICI 204,636 was administered to cynomolgus monkeys (3/sex, "purpose-bred") at increasing doses (6-350 mg/kg t.i.d.). Each dose was given for 7 days before raising to the next dose level (total of 14 doses). The total duration of the study was 98 days. Batch numbers for ICI 204,636 were: ADM44005/91, ADM44076/91, ADM33076/91, ADM44005/91. The vehicle was 0.5% hydroxypropyl methylcellulose in 0.1% aqueous polysorbate 80. Observations included clinical signs, body weight, food consumption, ophthalmology, hematology (hgb, MCV, rbc, MCH, MCHC, pcv, wbc (total, differential), platelet count, PT, APTT), clinical chemistry (AST, ALT, alkaline phosphatase, LDH, Na, Cl, P_i, urea, creatinine, albumin, total cholesterol, K, Ca, glucose, bilirubin, protein, A/G ratio), TK, terminal studies [gross pathology, histopathology (adrenal, aorta, bone marrow smear, brain/brainstem, cecum, colon, duodenum, epididymides, eyes/optic nerve, femur,

gallbladder, heart, ileum, jejunum, kidneys, lacrimal glands, liver, lungs/mainstem bronchi, lymph nodes, esophagus, ovaries, pancreas, pituitary, prostate, rectum/anus, salivary glands/submandibular, sciatic nerves, seminal vesicles, skeletal muscle/quadriceps, skin/mammary gland, spinal cord, spleen, sternum/bone marrow, stomach, testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, vagina/cervix, gross lesions), Masson-Fontana silver impregnation staining of thyroid].

Results: the TK data are summarized in the following sponsor's table:

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Text table: **Mean pharmacokinetic parameters for Days 13, 27, 41, 55, 69, 83, 90 and 97**

Component	Day	Dose mg/kg/day	C_{max} ng/mL	T_{max} h	$AUC_{0,7}$ ng.h/mL	Half life h	C_{min} ng/mL
ICI 204 636	13	12	95.7	1.33	274	2.37	10.9
	27	36	499	0.833	1410	2.79	80.0
	41	60	620	1.00	1790	2.25	88.6
	55	108	1600	0.75	5280	2.58	310
	69	150	1820	0.833	5470	3.05	307
	83	225	1170	1.75	4120	3.24	322
	90	285	2460	1.00	9280	3.18	578
	97	350	3000	2.67	13700	3.86	1200
ICI 213 841	13	12	189	1.17	648	2.06	26.6
	27	36	584	0.833	2140	3.98	174
	41	60	727	1.25	3000	3.15	231
	55	108	1340	1.08	5910	4.43	532
	69	150	1630	0.833	6880	3.93	480
	83	225	1440	2.08	5520	3.66	508
	90	285	2650	1.17	12300	4.76	1080
	97	350	4120	1.83	20500	3.51	1800
ICI 214 227	13	12	126	1.67	413	2.16	19.5
	27	36	571	1.50	2300	4.78	213
	41	60	1210	1.25	4360	4.02	334
	55	108	2560	0.750	10300	4.66	941
	69	150	2240	0.667	7340	3.11	440
	83	225	2080	1.75	8370	5.22	833
	90	285	3200	0.583	10700	4.66	783
	97	350	2800	1.83	12900	3.84	1270
M 236 303	13	12	NC	NC	NC	NC	NC
	27	36	NC	NC	NC	NC	NC
	41	60	NC	NC	NC	NC	NC
	55	108	48.6	0.60	151	4.19	10.5
	69	150	46.2	1.33	171	4.49	13.2
	83	225	40.4	2.50	174	5.77	18.1
	90	285	105	1.33	386	3.84	28.7
	97	350	191	1.50	782	NC	78.6

NC = not calculable

For the most part, levels of ICI 213,841 and ICI 214,227 were higher than those of the parent compound, except that levels of ICI 214,227 were similar to those of ICI 204,636 at the later time points. Metabolite M236,303 was not detected until the Day 55 (108 mg/kg) measurement. From then on, however, levels increased throughout the

remainder of the dosing period.

There was only one unschedule death during the study: in 1 M died prior to the first dose on Day 99. Subdued behavior was noted in this animal prior to death. No cause of death could be determined. Drug-related clinical signs consisted of subdued behavior (incidence and severity were dose-related), hunched posture (≥24 mg/kg), tremor (≥36 mg/kg), salivation (≥48 mg/kg), impaired mobility (≥132 mg/kg), staggering gait (≥150 mg/kg), prostration, slowed respiration, convulsion (1 F) (≥285 mg/kg), and lack of muscular tone (350 mg/kg). Due to severe clinical signs, dosing was withheld in 1 M and 1 F (at 285 mg/kg, for 5 sessions. Dosing was stopped after the third HD due to death of the 1 M at that dose. Animals were necropsied on Day 100. Body weight was affected at doses ≥150 mg/kg, with weight loss noted in some animals at the lower doses and in 5/6 monkeys at the HD. Food consumption was reduced at doses ≥285 mg/kg. No drug-related effect were detected upon ophthalmology examination or on hematology parameters. Clinical chemistry findings consisted of decreases in alkaline phosphatase (M, F at ≥285 mg/kg), total cholesterol (20-40 and 80% at 285 and 350 mg/kg, respectively), total bilirubin (40-75% at ≥285 mg/kg).

There were no drug-related findings at necropsy or upon microscopic examination, according to the sponsor. Without controls, however, drug-related effects could not be adequately evaluated. No thyroid changes (pigment deposition, hypertrophy) were noted.

Chronic studies

1. **Twelve month oral toxicity study in rats** (Study no. TFR/1626, 9/ID/1010581, Zeneca Pharmaceuticals, study dates: 8/92-9/93, GLP, Vol 1.21-1.23)

Animals: CrkL(WI)BR Wistar rats
initial age: 43 days
initial body weight: 172-247 gm for males, 125-189 gm for females
housing: 5/cage
food/water: *ad lib*
n = 30/sex for C and HD grps, 20/sex for other grps (i.e., LD, MD-1, MD-2). Of the C and HD animals, 10/sex/grp were followed for a 5-wk recovery period.

Drug: ICI 204,636 (analytical reference numbers: ADM 45026/89, ADM45029/89, ADM440005/91)
stability/impurities: Certificates of Analysis were provided.
vehicle: 0.5% HPMC in 0.1% Tween 80
formulation: suspension, 2.3-57.50 mg/mL (drug expressed as fumarate salt); actual concentrations were shown to be ≈96-105% of intended.
route: oral (gavage)
doses: 0, 10, 25, 75, 250 mg/kg (i.e., C, LD, MD-1, MD-2, and HD)
duration: daily for 52 wks; recovery animals were followed for an additional 5 wks

Observations

Clinical signs: all animals were observed at least twice per day. Detailed physical examinations were performed weekly.

dosing. Body weight: body weights were recorded prior to the start of dosing, on Day 1 of weekly for the first 12 wks, and the monthly thereafter.

Food consumption: food consumption was recorded for 1 wk prior to the start of dosing, weekly for the first 12 wks of dosing, and the for 1 wk per month thereafter.

Ophthalmology: ophthalmology examinations were performed once prior to the start of dosing in all animals, and at Wks 14, 27, 40, and 52 of dosing in C and HD grps. Examinations were performed following application of 1% tropicamide to dilate the pupils using a direct ophthalmoscope.

Hematology: blood samples were collected from 10/sex/grp during Wks 13, 26, and 51 of dosing, and at Wk 56 (for recovery animals) for analysis of the following parameters: hgb, rbc, hct, MCV, MCH, MCHC, red blood cell distribution width, wbc (total, differential), cell morphology, and platelet count. Blood films were prepared and examined when determined to be necessary. Reticulocyte films were also prepared, but not examined.

Clinical chemistry: blood samples were collected from 10/sex/grp during Wks 13, 26, and 51 of dosing, and at Wk 56 (for recovery animals) for analysis of the following parameters: glucose, urea, total protein, albumin, A/G ratio, bilirubin, ALT, AST, alkaline phosphatase, Na, K, Cl, Ca, P_i, cholesterol, TG, and creatinine.

Additional blood samples were collected for measurement of serum prolactin [5/sex/grp during Wks 5, 20, 52, and 57 (recovery animals)], and TSH and thyroxine [5/sex/grp during Wks 52 and 57 (recovery animals)].

Urinalysis: urine samples (overnight, 16-hrs) were collected from 10/sex/grp during Wks 14, 27, and 52 of dosing, and during Wk 56 (recovery animals) for analysis of the following parameters: volume, specific gravity, color, appearance, glucose, protein, ketones, bilirubin, blood, pH, microscopic examination of sediment.

TK: blood samples were collected from 3/sex/grp (except for C) at 0.5, 1, and 24 hr postdosing during Wks 5, 13, 26, and 51. Plasma levels of ICI 204,636, ICI 213,841, and ICI 214,227 were quantitated using SOP 35.H(S)39, 41, and 42 (p2); however, these methods were not acceptable for quantitating ICI 214,227 and those data were not reported.

A new analytical method was used to quantitate the plasma levels of all three compounds in blood samples collected during Wks 26 and 51. The new method involved liquid-liquid extraction, followed by reversed phase HPLC with uv detection. According to the sponsor, these new methods provided more reliable quantitation of both ICI 204,636 and ICI 214,227.

With the new method, if ≤50% of individual values were <LLOQ, the mean was recorded as the LLOQ; if >50% of individual values were <LLOQ, no mean was recorded and NC (not calculated) was recorded.

Terminal studies

Gross pathology: a complete necropsy was performed on all animals, including those that died or were sacrificed moribund.

Organ/tissue weights: weights of the following organs/tissues were recorded, apparently in survivors and in recovery animals (details were not given in the

methods section of the report): liver, heart, ovary, prostate, uterus, adrenal, spleen, brain, kidney, pituitary, testes/epididymis, and lungs.

Histopathology: the following tissues were examined microscopically in all C and HD animals sacrificed at the end of the dosing period, and in all animals which died or were sacrificed moribund:

Adrenal gland, heart, liver, lungs, mammary glands, pancreas, thyroid, and femur were also examined microscopically in the LD, MD-1, and MD-2 grps at the end of the dosing period, and all animals sacrificed at the end of the recovery period.

Additional examinations consisted of the following: (1) sections of thyroid gland were taken from each animal and stained using the Masson Fontana technique, (2) immunohistochemical staining of pituitary gland was conducted in 5/sex in C and HD grps for analysis of FSH, LH, ACTH, prolactin, and TSH, (3) immunohistochemical staining of pancreas was conducted in 3/sex C and HD animals for analysis of glucagon and insulin, (4) Oil red O staining was conducted on liver slices for 3/sex C and HD animals for analysis of lipid, and (5) immunohistochemical staining of liver slices was conducted in 2/sex C and HD animals for analysis of cytochrome P450.

Results

Mortality: there were 31 unscheduled deaths. These are summarized in the following table. According to the sponsor, there was "No single cause of death...identified for these animals...", and none were considered drug-related. Clearly, in females, there were no dose-related increases in either the number of animals found dead or sacrificed moribund. In males, however, the incidence of death (i.e., found dead) was dose-related.

DEATH	C		LD		MD-1		MD-2		HD	
	M	F	M	F	M	F	M	F	M	F
found dead	0	1	1	0	2	1	2	3	5	1
moribund sacrifice	3	2	3	1	1	0	0	1	3	1

According to the sponsor, the increased deaths in HDM were due dosing or sampling accidents in 5 HDM.

Clinical signs: the primary clinical signs, noted in all dosed animals, were transient subdued behavior, ptosis, and drowsiness which were evident throughout the dosing period. According to the sponsor, these signs were not evident in HD recovery animals by Wk 56. In addition, transient, excessive salivation (MD-2, HD animals) and urine staining (HD animals, particularly HDF; Wk 20-54) were observed. Convulsions were noted in 1 CF, 1 HDM, and 1 HDF. None of these signs were evident in recovery animals.

Ophthalmology: there were no drug-related findings (comparing C and HD grps).

Body weight: in males, median body weight was reduced at MD-2 and HD (compared to CM) throughout the dosing period (from Wk 1-3 on; 12 and 20%,

respectively, at Wk 52). In terms of body weight gain, the median body weight gain (cumulative) was reduced by 18 and 26% at the MD-2 and HD, respectively, at Wk 52. In HD recovery animals, median body weight was still reduced compared to C at Wk 56 (13%).

In females, median body weight was increased (compared to CF) at all doses starting at Wk 1-3. At the LD and MD-1, body weight remained elevated throughout the dosing period (22% at both doses at Wk 52). At MD-2, the maximum increase in body weight noted at Wks 10-32 (12-16%). From Wk 36 on, body weight was still higher in MD-2F than in CF; however, by Wk 52, the increase was only 4%. At the HD, body weight was elevated (compared to CF) primarily during Wks 1-12 (6-10%), but by Wk 36 was similar to CF, and during Wks 40-52 was lower than CF (3-5%). In HD recovery animals, median body weight was even lower than CF at Wk 56 (14%).

Food consumption: in males, median food consumption was elevated in MD-1M fairly consistently throughout the dosing period (5-19%), and in LDM but only sporadically (4-8%). In recovery animals, food consumption was similar between grps.

In females, food consumption was elevated at all doses during the first 1-3 wks of dosing (11-20%). At the HD, food intake was reduced from Wk 32 to the end of dosing (5-12%). Food intake in C and HD recovery animals was similar.

Hematology: the primary drug-related findings were increases in wbc, neutrophils, and lymphocytes. The median wbc count was increased in MD-2M (12 and 22% at Wks 13 and 51), HDM (30-6% at Wks 13-51), and HDF (30% at Wk 13). Median absolute neutrophil count was increased in MD-2F at Wk 26 (32%) and at all doses in F at Wk 51 (35, 24, 34, and 81% at LD, MD-1, MD-2, and HD, respectively). Median absolute lymphocyte count was increased in HDM (41 and 35% at Wk 13 and 26, respectively) and HDF (44-28% at Wks 13-51).

In recovery animals, the median absolute wbc, neutrophil, and lymphocyte counts were still slightly (not statistically significant) elevated at Wk 56 (30, 35, and 11%, respectively).

Clinical chemistry: the following were of note: (1) slight reductions in glucose in females at the HD during Wks 13 (17%), and at all doses during Wk 51 (9-11%, not dose-related), (2) decrease in urea in HDF (20-14% at Wks 26 and 51), (3) decrease in creatinine in F at all doses (2-8%) at Wk 51, (4) decreases in alkaline phosphatase at all doses in M throughout the dosing period (5-38, 9-40, 12-49, and 17-58 at LD, MD-1, MD-2, and HD, respectively) and in HDF (23-46% at Wks 13 and 26), (5) decreases in cholesterol in HDM (17-40% at Wks 26,51) and HDF (16-53% at Wks 13-51), (6) decreases in TG in HDM (41-45%) and HDF (38-61%) throughout the dosing period, (7) an increase in T_4 in MD-2M (39%) and HDM (22%) when measured at Wk 52, (8) an increase in TSH at the MD-1, MD-2, and HD in males (32, 58, 160%, respectively) and females (25, 81, 53%, respectively) when measured at Wk 52. In recovery animals, alkaline phosphatase was still reduced in HDM (42%) and cholesterol was still reduced in HDM (25%) and HDF (34%).

Increases in median prolactin levels were evident in males and females; however, the values were quite variable which made comparisons among grps somewhat difficult. In males, serum prolactin was elevated throughout the dosing period (from Wk 5 on) at the MD-1, MD-2, and HD; however, the increases

(3-32 fold) were not necessarily dose-related. At the LD, prolactin was elevated only at Wk 52 (12-fold). In females, serum prolactin was elevated at all doses during Wks 20 and 52 (6-13 fold), although, as with males, the response was not necessarily dose-related. Prolactin levels were also elevated during Wks 1 and 5; however, the median values were within the C median range. There were no differences between grps in prolactin levels in recovery animals.

There were slight changes in a number of other parameters; however, the median values tended to be within the C range.

Urinalysis: there were a number of significant findings; however, they were either not dose-related, were within normal variation (compared to C values), or were transient.

TK: the data collected using the new methods are summarized in the following tables. The sponsor did provide data for Wks 5 and 13; however, these were collected using older quantitation methods which were either less sensitive or less reliable for ICI 204,636 and ICI 214,227.

ICI 204,636 (ng/mL)

WK	SEX	TIME*	LD	MD-1	MD-2	HD
26	M	0.5	n.c.	76.6 ± 30.7	957 ± 93.5	1060 ± 80.9
		1	37.8 ± 8.90	82.4 ± 31.8	651 ± 325	900 ± 160
		24	n.q.	n.q.	n.q.	n.c.
	F	0.5	80.0 ± 32.3	137 ± 38.7	1600 ± 258	1360 ± 221
		1	31.0 ± 2.26	211 ± 22.6	768 ± 56.9	1120 ± 215
		24	n.q.	n.q.	n.q.	38.6 ± 9.32
51	M	0.5	89.2 ± 47.8	131 ± 13.0	585 ± 177	700 ± 28.0
		1	33.6 ± 0.200	59.3 ± 3.58	358 ± 131	634 ± 239
		24	n.q.	n.q.	n.q.	n.q.
	F	0.5	49.5 ± 16.4	273 ± 121	1580 ± 345	1530 ± 65.0
		1	49.2 ± 20.9	220 ± 28.9	611 ± 136	1550 ± 158
		24	n.q.	n.q.	n.c.	43.1 ± 12.0

*time (hr) postdosing

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ICI 214,227 (ng/mL)

WK	SEX	TIME*	LD	MD-1	MD-2	HD

26	M	0.5	537 ± 196	848 ± 374	1990 ± 0	2000
		1	201 ± 42.7	1010 ± 252	1940	n.c.
		24	n.q.	43.3 ± 3.30	136 ± 27.7	595 ± 69.7
	F	0.5	1100 ± 163	1330 ± 306	1790	1300 ± 104
		1	670 ± 2.33	1640 ± 161	1830 ± 107	1590 ± 252
		24	n.q.	64.3 ± 24.3	150 ± 32.5	745 ± 118
51	M	0.5	647 ± 267	1260 ± 38.4	1470 ± 90.0	1580
		1	274 ± 68.3	946 ± 114	1590 ± 125	1880 ± 90.0
		24	n.q.	n.q.	56.3 ± 8.28	384
	F	0.5	765 ± 138	1330 ± 254	1860 ± 60.0	1110 ± 15.0
		1	756 ± 45.2	1550 ± 187	1640 ± 76.9	1550 ± 6.67
		24	n.q.	n.q.	77.7 ± 16.7	806 ± 220

ICI 213,841 (ng/mL)

WK	SEX	TIME*	LD	MD-1	MD-2	HD
26	M	0.5	n.c.	n.c.	195 ± 29.3	314 ± 40.1
		1	n.q.	n.c.	157 ± 76.9	240 ± 32.0
		24	n.q.	n.q.	n.q.	n.q.
	F	0.5	n.c.	52.4 ± 6.48	381 ± 51.4	478 ± 64.0
		1	n.q.	55.3 ± 12.2	158 ± 4.63	360 ± 90.1
		24	n.q.	n.q.	n.q.	n.q.
51	M	0.5	n.c.	97.7 ± 5.35	144 ± 36.5	242
		1	n.q.	43.7 ± 3.70	117 ± 11.5	183 ± 51.5
		24	n.q.	46.6 ± 6.55	n.q.	n.q.
	F	0.5	n.q.	113 ± 42.1	301 ± 61.0	570 ± 10.5
		1	n.q.	80.5 ± 9.58	123 ± 18.4	422 ± 82.0
		24	n.q.	n.q.	n.q.	n.q.

*time (hr) postdosing

Terminal studies

Gross pathology: selected findings were summarized by the sponsor in the following table:

LESIONS	GROUP	INCIDENCE OF LESIONS(ANIMAL NOS)									
		MALES					FEMALES				
		I 0 mg/kg /day	II 10 mg/kg /day	III 25 mg/kg /day	IV 75 mg/kg /day	V 250 mg/kg /day	I 0 mg/kg /day	II 10 mg/kg /day	III 25 mg/kg /day	IV 75 mg/kg /day	V 250 mg/kg /day
ADRENAL GLANDS:		(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Discolouration		2	2	3		1	1	8	2	7	6
LIVER:		(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Enlarged		1	1	5	2	6					1
MAMMARY GLANDS:		(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Increased quantity							2	6	13	13	16
THYROID GLAND:		(20)	(19)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Enlarged				3		2					1
Discolouration		5	8	7	19	16		1	1	7	17
UTERUS:							(20)	(20)	(20)	(20)	(20)
Thin										2	5

Figures in brackets represent the number of animals from which that organ was examined at necropsy
The absence of a numeral indicates that the lesion specified was not identified.

Organ/tissue weights: the following findings were of noted: (1) increased liver absolute median weight in MD-1M (17%), HDM (17%), and at all doses in females (14, 20, 8, and 18% at LD, MD-1, MD-2, and HD, respectively); relative weight was increased in MD-2M (5%), HDM (35%), and HDF (19%), (2) decreased prostate weight (absolute-relative) in HDM 9-15%), (3) reduced uterus weight (absolute-relative) at all doses (34-38, 39-43, 42-46, 41% at LD, MD-1, MD-2, HD, respectively), (4) increased adrenal weight (absolute-relative) in MD-1M (25-14%), MD-2M (31-36%), and HDM (33-42%), (5) increased pituitary weight (absolute-relative) in MD-1M (21-14%), MD-2M (43%), HDM (21-36%), and HDF (16%), and (6) testes/epididymis weight was reduced slightly at the HD (8%). Relative weights of lung, and kidney and spleen were elevated in HDM (lung, 15%) or MD-2 and HDM [kidney (8-6%), spleen (16-25%)].

In recovery animals, the following were noted in HD animals: (1) a decrease in prostate weight (26-19%), (2) a decrease in uterus weight (54%), (3) an increase in adrenal weight in M (9-36%), (4) a decrease in spleen weight in F (14%), and (5) increases in pituitary weight in M and F (27-19 and 33%, respectively).

Histopathology: selected data are summarized in the attached table (incidences taken from sponsor's Table 17).

2. **ICI 204,636: Twelve month oral toxicity study in dogs** (Study no. TFD/501, 6/ID/1009118, Zeneca Pharmaceuticals, study dates: 3/92-6/93, GLP, Vol 1.28-1.29)

Animals: Beagle dogs
initial age: 53-63 wks
initial body weight: 14.9-21.3 kg for males, 11.7-18.7 kg for females
feed/water: all dogs received ≈400 g of food in morning prior to dosing. Water was available *ad lib.*
n = 4/sex/grp for main study, an additional 3/sex for C and HD grps to

assess recovery.

Drug: ICI 204,636 (analytical reference numbers: ADM 44003/91, ADM 440005/91, ADM 45026/89, ADM 45027/89, ADM 45028/89, and ADM 45103/87).
identity/purity: Certificates of Analysis were provided
route: p.o.
formulation: 25, 100, 200, mg coated tablets inserted into gelatin capsules as appropriate for dose. Tablets contained povidone USP, dicalcium phosphate dihydrate USP, microcrystalline cellulose NF, sodium starch glycolate NF, lactose NF ., magnesium stearate NF.

mg/kg doses: 0, 2.5, 6.25, 12.5, and 25.0 mg/kg during Wk 1, 0, 5.0, 12.5, 25.0, and 50.0 during Wk 2, and 0, 10, 25, 50, and 100 mg/kg during Wks 3-54. The dose grps will be referred to as C, LD, MD-1, MD-2, and HD, respectively.
duration: 54 wks for main study animals; recovery animals were followed for an additional 8 wks.

Observations

Clinical signs: all animals were observed at twice per day. Observations were conducted up to 3-6 hr postdosing (daily) during Wks 1-6, twice a week during Wks 7-12, and weekly during the rest of the dosing period. It is unclear whether these "timed" observations were conducted in addition to or as part of the twice daily general observations.

Veterinary examination: all animals were examined prior to the start of dosing, and during Wks 15, 28, 41, and 54 in all animals, and at Wk 62 in recovery animals. The exact nature of the examination was not specified.

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1-yr rat toxicity study, histopathology findings:

FINDING	MALES					FEMALES				
	C	LD	MD-1	MD-2	HD	C	LD	MD-1	MD-2	HD
Adrenal gland										
cortical vacuolation										
minimal	1/20	1/20	1/20	4/20	6/18	0/20	1/20	2/20	2/20	12/20
mild	4/20	2/20	2/20	6/20	7/18	0/20	0/20	1/20	8/20	7/20
moderate	1/20	0/20	0/20	0/20	0/18	0/20	0/20	0/20	0/20	0/20
zona fasciculata										
cellular hypertrophy (mild)	0/20	0/20	0/20	0/20	1/18	0/20	0/20	1/20*	0/20	7/20
Heart										
myocardial fibrosis										
minimal	1/20	2/20	6/20	3/20	4/18	0/20	0/20	1/20	2/20	3/20
mild	4/20	7/20	8/20	7/20	5/18	0/20	1/20	0/20	2/20	2/20
moderate	0/20	0/20	1/20	0/20	0/18	0/20	0/20	0/20	0/20	0/20
severe	0/20	0/20	0/20	0/20	0/18	0/20	0/20	0/20	0/20	0/20
Liver										
midzonal fat vacuolation										
minimal	0/20	2/20	0/20	0/20	2/20	0/20	0/20	0/20	1/20	5/20
mild	0/20	0/20	0/20	3/20	3/20	0/20	0/20	1/20	0/20	8/20
moderate	0/20	0/20	0/20	0/20	3/20	0/20	0/20	0/20	0/20	1/20
centrilobular fat vacuolation										
minimal	0/20	5/20	0/20	1/20	0/20	0/20	0/20	0/20	0/20	0/20
mild	0/20	4/20	6/20	11/20	2/20	0/20	0/20	0/20	1/20	0/20
moderate	0/20	0/20	0/20	0/20	3/20	0/20	0/20	0/20	0/20	1/20
reduced glycogen vacuolation										
mild	0/20	1/20	0/20	2/20	3/20	3/20	3/20	4/20	6/20	8/20
moderate	1/20	0/20	2/20	2/20	0/20	0/20	1/20	1/20	4/20	8/20
centrilobular hepatocyte hypertrophy										
mild	0/20	0/20	0/20	0/20	4/20	0/20	0/20	0/20	4/20	12/20
moderate	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	6/20
Lung										
alveolar macrophage										
minimal	0/20	0/20	1/20	1/20#	1/20#	0/20	1/20	1/20	2/20	1/20
mild	6/20	3/20	4/20	7/20	8/20	3/20	4/20	2/20	8/20	12/20
moderate	0/20	0/20	0/20	0/20	2/20	0/20	0/20	2/20	0/20	1/20
severe	0/20	0/20	0/20	1/20	2/20	0/20	0/20	0/20	0/20	0/20
Mammary gland										
glandular hyperplasia										
minimal	0/14	0/13	0/12	4/18	3/15	1/20	5/20	2/19	1/20	0/20
mild	2/14	0/13	1/12	8/18	5/15	2/20	11/20	13/19	11/20	9/20
moderate	0/14	0/13	0/12	0/18	1/15	1/20	0/20	2/19	6/20	11/20
severe	0/14	0/13	0/12	0/18	0/15	0/20	0/20	1/19	0/20	0/20

*minimal, #degree not stated, apparently <minimal

1-yr rat toxicity study, histopathology findings (con't):

FINDING	MALES					FEMALES				
	C	LD	MD-1	MD-2	HD	C	LD	MD-1	MD-2	HD
Pancreas										
islet cell hypertrophy	0/20	1/20	1/20	4/20	12/20	0/20	0/20	1/20	4/20	11/20
Thyroid gland										
hypertrophic follicular epithelium										
minimal	0/20	0/19	0/20	0/20	2/20	0/20	0/20	0/20	1/20	1/20
mild	1/20	1/19	2/20	0/20	5/20	0/20	0/20	0/20	1/20	3/20
moderate	0/20	0/19	0/20	1/20	0/18	0/20	0/20	0/20	0/20	0/20
pigment deposition										
minimal	6/20	11/19	2/20	0/20	1/20	1/20	9/20	7/20	5/20	0/20
mild	5/20	0/19	12/20	15/20	4/20	0/20	3/20	4/20	14/20	14/20
moderate	0/20	0/19	5/20	5/20	14/20	0/20	0/20	0/20	1/20	6/20
marked	0/20	0/19	0/20	0/20	1/20	0/20	0/20	0/20	0/20	0/20
Bone marrow										
fat replacement										
minimal	1/20	8/20	2/20	2/20	1/20	4/20	2/20	0/20	2/20	3/20
mild	7/20	4/20	9/20	3/20	18/20	11/20	16/20	12/20	13/20	14/20
moderate	9/20	6/20	7/20	14/20	0/20	0/20	0/20	7/20	3/20	2/20
severe	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20

APPEARS THIS WAY
ON ORIGINAL

Ophthalmology: all animals were examined prior to the start of dosing, and at Wks 15 and 28 of dosing. At Wks 42/43 and 51/52 for all animals, and Wk 61/62 for recovery animals, examinations were performed both by the study veterinarian and by a consultant veterinarian, Dr. G. D. Auirre. At Wk 42/43, "some" animals were examined by two in-house veterinarians (one being the study veterinarian). For all examinations, mydriasis was induced using 1% tropicamide. In some treated animals, the 1% tropicamide was inadequate; therefore, application of 1% tropicamide was followed 30 min later by phenylephrine HCl drops (10%) from Wk 15 on.

Through Wk 28, examinations were performed using a binocular indirect ophthalmoscope. A Kowa SL-5 hand held slit lamp biomicroscope was also used to examine "some" animals after Wk 28, and at Wk 42/43 both instruments were used to examine all animals. Photographs were taken of some findings (e.g., lens opacities, abnormalities) and of the fundus in all animals [prior to dosing, at Wk 54, and Wk 62(recovery animals)].

Body weight: body weights were recorded for all animals prior to the start of dosing, and weekly throughout the rest of the study.

Food consumption: food intake was recorded for all animals prior to the start of dosing, and daily throughout the rest of the study.

Physiological measurements: ECG, direct arterial blood pressure, and rectal temperature were recorded for all dogs prior to the start of dosing, and during Wks 3, 15, 28, 42, and 53, and at Wk 60 in recovery animals. Heart rate was recorded for all dogs during Wks 1, 6, and 10. During the dosing period, these parameters were measured prior to dosing, and at 3 hr postdosing.

Hematology: blood samples were collected from all animals prior to the start of dosing, and during Wks 28 and 53, at Wk 4 and 8 of the recovery period, and at additional times in selected grps (i.e., Day 127 in CM 101, Day 200 in MD-2F 123, and Day 260 in HDM 107) for analysis of the following parameters: hgb, rbc, hct, MCV, MCH, MCHC, red cell distribution width, wbc (count, differential), rbc and wbc morphology, platelet count, prothrombin time, partial thromboplastin time with kaolin. Blood films and reticulocyte films were prepared and read as necessary, or stored unexamined.

Clinical chemistry: blood samples were collected from all animals prior to the start of dosing, and during Wks 28 and 53, at Wk 4 and 8 of the recovery period, and at additional times in selected grps (i.e., Day 127 in CM 101, Day 200 in MD-2F 123, and Day 260 in HDM 107) for analysis of the following parameters: glucose, urea, total protein, albumin, A/G ratio, bilirubin, ALT, AST, alkaline phosphatase, Na, K, Cl, Ca, P_i, cholesterol, TG, creatinine, tri-iodothyronine, thyroxine, TSH.

Urinalysis: urine samples were collected from all animals ("...following a 1% body weight water load...")prior to start of the dosing period, and during Wks 28 and 53 of dosing for analysis of the following parameters: specific gravity, color, appearance, glucose, protein, ketones, bilirubin, blood, pH, Na, K, creatinine, and microscopic analysis of sediment.

TK: blood samples were collected from all treated animals during Wk 15 and 28 (at 0.5, 2, and 24 hr postdosing), and at the "penultimate dose" during Wk 54 (at 0.5, 1, 2,

4, 8, 12, and 24 hr postdosing). In addition, a blood sample was collected at 1 hr postdose from a CM (101) after exhibiting ataxia and subdued behavior.

Plasma levels of ICI 204,636, ICI 213,841, and ICI 214,227 were quantitated by HPLC (uv detection) following liquid-liquid extraction. The initial quantitation (Wk 15 and 28) was performed using SOP 35H(S)39, 41&42(P.2). Blood samples collected during Wk 54 were analyzed using an improved method, Method 16-09. This method, according to the sponsor, resulted in greater reliability for quantitating ICI 214,227, and increased sensitivity for ICI 213,841 and ICI 204,636.

Terminal studies

Gross pathology: a complete necropsy was performed on all animals. Bone marrow smears were prepared, but not examined.

Organ/tissue weights: the following organs/tissues were weighed in all animals, with certain exceptions: adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, testes, thyroid glands, prostate gland, spleen and uterus. Heart and thyroid were not weighed in CF 149, nor was epididymis and testes in CM 101.

Histopathology: the following tissues were examined microscopically in all main study animals: adrenal glands, aorta (arch), gallbladder, urinary bladder, bone and marrow (rib), bone marrow smear, brain, bronchus, cervix, epididymides, eyes with lids, heart (atria, ventricles, papillary muscle, intestine (duodenum, jejunum, ileum, ileo-cecal-colic junction, colon), kidney, lacrimal glands, liver (central, lateral lobes), lungs (diaphragmatic lobes), lymph nodes (bronchial, cervical, mesenteric), mammary gland, abdominal skin, skeletal muscle, sciatic nerve, esophagus, ovaries, pancreas, parathyroid glands, pituitary gland, prostate, salivary gland (parotid, submaxillary, sublingual), spinal cord (lumbar), spleen, stomach (fundic, pyloric), testes, thymus, thyroid gland, tongue, trachea, uterus, vagina, macroscopic abnormalities.

In addition, eyes, lacrimal glands, liver, and thyroid were examined in recovery animals.

Special stains were used in the following cases: (1) Von Kossa (mineral/bone/osteoid), Fouchet (bile pigments), Prussian blue (hemosiderin), Masson-Fontana (melanin/lipofuscin/argentaffin), Schmorl's (lipofuscin/melanin), Ziehl-Neelson (lipofuscin), PAS (periodate-reactive carbohydrates) were used on lacrimal glands from 10 dogs (CM 138, CF 121, MD-1M 144, MD-2M 105, HDM 133, LDF 237, MD-1F 103, HDF 113, HDF 150, HDF 258), on kidneys from CM 155, MD-1M 82, MD-2M 154, and HDM 152, and on liver from CF 92, CF 136, LDM 88, and HDM 139, (2) thyroid gland from all animals were examined using Masson-Fontana stain. Unstained samples of lacrimal gland were examined under uv light using fluorescent microscopy in HDM 133, HDF 150, HDF 258, and HDF 113.

EM: sections of lacrimal gland from CF 92 and HDF 113 were processed for transmission EM.

Results

Mortality: there were no unscheduled deaths during the study.

Clinical signs: the drug-related findings are summarized in the following sponsor's tables:

For Wks 1-10 (left), Wks 11-54 (right):

Observation	Group/ Dose ICI 204,636 * mg/kg/day				
	I	II	III	IV	V
	0	10	25	50	100
Ataxia	0/14	0/8	5/8	7/8	12/14
Shivers	0/14	0/8	1/8	1/8	7/14
Subdued	0/14	1/8	7/8	8/8	12/14
Sleep - slight	0/14	0/8	3/8	3/8	10/14
Sleep - moderate	0/14	0/8	1/8	1/8	5/14
Sleep - extreme	0/14	0/8	0/8	1/8	0/14
Drooping Eyelid	0/14	0/8	3/8	4/8	10/14
Third Eyelid Relaxed	0/14	0/8	1/8	0/8	2/14
Miosis	0/14	1/8	8/8	8/8	14/14
Anal Relaxation	0/14	3/8	8/8	8/8	14/14
Muscle Tremors	0/14	0/8	0/8	2/8	6/14

Observation	Group/ Dose ICI 204,636 * mg/kg/day				
	I	II	III	IV	V
	0	10	25	50	100
Ataxia	1/14	0/8	1/8	3/8	3/14
Shivers	0/14	0/8	0/8	0/8	4/14
Subdued	1/14	0/8	4/8	5/8	7/14
Sleep - slight	0/14	0/8	1/8	1/8	2/14
Sleep - moderate	0/14	0/8	0/8	0/8	2/14
Sleep - extreme	0/14	0/8	0/8	0/8	1/14
Drooping Eyelid	0/14	0/8	1/8	3/8	3/14
Miosis	0/14	1/8	8/8	8/8	14/14
Anal Relaxation	0/14	1/8	8/8	8/8	13/14
Muscle Tremors	0/14	0/8	0/8	0/8	3/14

Drug-related signs were noted primarily at doses >LD; however, a few LD animals were affected. TK analysis conducted on blood samples collected from CM 101 indicated detectable levels of ICI 204,636.

Ophthalmology: findings are summarized in the following sponsor's table:

Group/sex	Cat	Pre-study	Week 15	Week 28	Week 42/43	Week 51/52	Week 61/62 (Withdrawal)
I M	O	7	7	5	4	4	1
	N			2	3	3	2
n = 7 (3w)	P						
I F	O	7	7	7	4	5	3
	N				3	2	
n = 7 (3w)	P						
II M	O	3	3	3	1	1	
	N	1	1	1	3	3	
n = 4	P						
II F	O	2	3	3	1	1	
	N	2	1	1	3	3	
n = 4	P						
III M	O	3	3	2	3	2	
	N	1	1	2	1	2	
n = 4	P						
III F	O	3	4	3	1	2	
	N	1		1	3	2	
n = 4	P						
IV M	O	3	4	2		1	
	N	1		2	4	3	
n = 4	P						
IV F	O	4	3	3	1		
	N		1	1	3	4	
n = 4	P						
V M	O	7	5				
	N		2	2	2	2	1
n = 7 (3w)	P			5	5	5	2
V F	O	6	3				
	N	1	2	3	1	1	1
n = 7 (3w)	P		2	4	6	6	2

Key: Cat = Category of lens finding
O = No findings
N = Normal variations
P = Lens pathology
[For details of categories see consultant veterinary ophthalmologists report - Appendix 4]
n = Number of animals in group
w = Withdrawal animals

Although findings were noted at all doses (and in C grps), those detected at doses <HD were considered (by the consult veterinarian; cf. Appendix A) to be normal variations. At the HD, however, there was clear evidence of drug-related pathology. The data in the table above were expressed as the number of animals in which some pathology was detected (i.e., Grades ≥5; Grades 1-4 were considered normal variation, or normal, age-related progression). Pathology was noted first in HDM, but was more severe at Wks 42/43 and 51/52 in HDF. In HD recovery animals, pathology was noted in 2/3 HDM and 2/3 HDF.

The individual severity grades were summarized in the following sponsor's tables:

Group/ sex	Animal No	Pre-study B	L	R	Week 15 B	L	R	Week 28 B	L	R	Week 42/43 B	L	R	Week 51/52 B	L	R	Comments
I M	157	0			0			0			0			0			
	138	0			0			0			0			4			
	155	0			0			0			2			0			
	134	0			0			0			0			0			
I F	92	0			0			0			0			0			
	136	0			0			0			1			4			
	T31	0			0			0			1			0			
	121	0			0			0			1			0			
II M	88	0			0			0			0			0			
	76	0			0			0				0	2		0	2	* - other lens findings - not compound/dose related - not scored
	158	1			0			0				2	0		4	0	
	135	0			2*			2*				1	3	1			
II F	242	0			0			0				0	2	0			
	199	2			2			2			2			2			
	237	0			0			0			0			4			
	757	1			0			0				0	2	2			
III M	144	3			3			3			3			4			
	75	0			0			0			0			0			* - other lens findings - not compound/dose related - not scored
	82	0			0*				0*	1*	[0*]			0			
	126	0			0			0			0			4			
III F	112	0			0			2			3			4			
	239	0			0			0			1			4			
	90		1	0	0			0			4			0			
	103	0			0			0			0			0			

Group/ sex	Animal No	Pre-study B	L	R	Week 15 B	L	R	Week 28 B	L	R	Week 42/43 B	L	R	Week 51/52 B	L	R	Comments
IV M	109	1			0			1			3			4			
	159	0			0			0				0	4	0			
	154	0			0			0			4			4			
	105	0			0			2			2			4			
IV F	256	0			0			0			4			2			
	102	0			0			0			4			4			
	123	0			2			2			3			4			
	202	0			0			0			0			1			
V M	139	0			0			3			3			4			
	124	0			0			6			8			8			
	152	0			0			8*			8			8			
	133	0			0			5			[5]			5			* - Early stage
V F	113	0			5			6			7			7			
	258	1			0			2			6			6			* - Limited extension of opacity into anterior cortex
	150	0				0	2	5			6			6			
	241	0			6			6			6*			6*			

Group/ sex	Animal No	Pre-study B	L	R	Week 15 B	L	R	Week 28 B	L	R	Week 42/43 B	L	R	Week 51/52 B	L	R	Week 61/62 (W)	Comments
I M	146	0			0				0	1	1			1			1	
	145	0			0			2			2			4			4	
(W)	101	0			0			0			0			0			0	
I F	243	0			0			0			0			0			0	
	210	0			0			0			0			0			0	
(W)	149	0			0			0			0				0	4	0	
V M	107	0			0			8*			8			8			8+	* - Early stage
	116	0			2			5			7			7			6"	+ - Pathology considered to have progressed during withdrawal
(W)	74	0			2			2			2			2			4	" Considered to have improved during withdrawal
V F	174	0			1			1			4			4			4	* - Considered to have progressed slightly during withdrawal
	238	0			0			1			5			5			6*	@ - Clustering of opaque granules in the axial posterior cortex.
(W)	89	0			0			5				3	5	0			0	

(W) = Withdrawal

Body weight: there were no drug-related effects.

Food consumption: there were no drug-related effects.

Physiological measurements: there were no clear drug-related effects. Noted changes were either inconsistent (i.e., transitory or in opposite directions) or within C or baseline ranges.

Hematology: according to the sponsor, there were no drug-related findings. Of note, however, were increases in median platelet counts in MD-2M (Wk 53, 38%), HDM (Wk 28, 53: 36, 69%), MD-2F (Wk 28, 53: 14, 38%), and HDF (Wk 28,53: 40,34%).

Clinical chemistry: the primary drug-related effects (routine parameters) were noted as changes in alkaline phosphatase [increased in MD-2M (19-32%), HDM (18-55%), and HDF (23-56%)], cholesterol [decreased in MD-2M (6-25%), HDM (6-22%), and at all doses in F (19-27%)], and triglycerides ([decreases in MD-1M (6-27%), MD-2M (28-45%), HDM (28-45%), and at all doses in F (25-64%)]. In recovery animals, alkaline phosphatase was still slightly elevated in HDM (18-7%) and HDF (23-43%), and triglycerides were still reduced in HDF (31-20%).

On thyroid hormone parameters, the only finding was a decrease in T_4 in MD-2M (21-64% at Wks 15-42) and HDM (42-66% from Wk 7 on); in females, only sporadic decreases were noted in HDF (12-58% at Wks 7, 15, and 53). In recovery HDM, T_4 levels were still slightly reduced (29-20%).

Serum prolactin levels were below the LLOQ (i.e., 2 ng/mL) in almost all cases.

Urinalysis: there were no apparent drug-related findings.

Terminal studies

Gross pathology: according to the sponsor, there were no drug-related findings.

Organ/tissue weights: there were no apparent drug-related findings, except, perhaps, for slight increases in liver weight (A-R) in HDM (38-35%).

Histopathology: drug-related findings were limited to the eye. The findings were summarized in the attached sponsor's Table 18. The pigment in lacrimal gland of drug-treated animals (all doses) was located in epithelial cell cytoplasm and characterized as "Fine dark brown granules...". The pigment reacted positively with Masson-Fontana (black) and Schmorl's (blue/brown) stains, and appeared fluorescent (cream/yellow) under uv light. According to the sponsor, this would be suggestive of lipofuscin.

EM: ultrastructural examination of lacrimal tissue was conducted in order to further investigate the pigment detected with light microscopy. Upon EM, the pigment was localized to secretory droplets or remnants of secretory droplets within individual cells. The sponsor indicated that the pigment appeared "...ultrastructurally distinct from the lipofuscin-like pigment granules in lacrimal glands of beta-blocker treated dogs..." These pigments were detected in drug-treated recovery animals.

LESIONS	GROUP	INCIDENCE OF LESIONS									
		MALES					FEMALES				
		I 0 mg	II 10 mg	III 25 mg	IV 50 mg	V 100 mg	I 0 mg	II 10 mg	III 25 mg	IV 50 mg	V 100 mg
EYES:		(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Minimal fibre swelling posterior cortex		8	8	8	2		8	8	8	6	1
Mild fibre swelling posterior cortex					6	4				2	4
Moderate fibre swelling posterior cortex						4					3
Minimal globule formation posterior cortex											1
Mild globule formation posterior cortex						1					3
Minimal liquefaction posterior cortex											1
Mild liquefaction posterior cortex						1					2
More prominent swelling fibres posterior suture			1		2		2	4	1	1	
Mild swelling fibres posterior suture		1	2	2			1	3		5	3
Moderate swelling fibres posterior suture						4					4
Mild globule formation posterior suture						3					1

LESIONS	GROUP	INCIDENCE OF LESIONS									
		MALES					FEMALES				
		I 0 mg	II 10 mg	III 25 mg	IV 50 mg	V 100 mg	I 0 mg	II 10 mg	III 25 mg	IV 50 mg	V 100 mg
EYES:		(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Mild liquefaction posterior suture						3					
Minimal fibre swelling equator											1
Mild globule formation anterior cortex											1
Minimal fibre swelling anterior suture										1	
Minimal nuclear retention											1
Mild nuclear retention						4					3
LACRIMAL GLAND:		(3)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Adenitis minimal		1	2	1	1	1		2	1		
mild				1	2	2	1	1	2	3	2
moderate				2					1		1
severe					1						1
Acinar epithelial brown pigment											
minimal			3	2	1	1		2	3	3	1
mild				1	2	2		1	1	1	3

LESIONS	GROUP	INCIDENCE LESIONS			
		MALE		FEMALE	
		I 0 mg	IV 100 mg	I 0 mg	IV 100 mg
EYES:		(6)	(6)	(6)	(6)
Minimal fibre swelling posterior cortex		6	3	6	6
Mild fibre swelling posterior cortex			3		
Mild globule formation posterior cortex			1		
Mild liquefaction posterior cortex			2		
More prominent swelling fibres posterior cortex			2	1	1
Mild swelling fibres posterior suture		3	1	1	3
Moderate swelling fibres posterior suture			2		
Minimal globule formation posterior suture					1
Mild globule formation posterior suture			1		
Moderate globule formation posterior suture			2		
Mild liquefaction posterior suture			2		

LESIONS	GROUP	INCIDENCE LESIONS			
		MALE		FEMALE	
		I 0 mg	IV 100 mg	I 0 mg	IV 100 mg
EYES:		(6)	(6)	(6)	(6)
Mild fibre swelling anterior cortex			2		
Mild fibre swelling anterior suture			2		1
Nuclear retention			3		1
LACRIMAL GLAND:					
Adenitis minimal		2			2
mild			2		1
Acinar epithelial brown pigment mild			3		2

TK: the data are summarized in the following table (data were pooled across sex):

ICI 204,636

WK	TIME*	LD	MD-1	MD-2	HD
PLASMA LEVELS (ng/mL ± SEM)					
15	0.5	124 ± 47.3	559 ± 331	239 ± 125	108 ± 58.6
	2	223 ± 37.4	731 ± 165	1820 ± 786	598 ± 323
	24	n.c.##	n.c.	n.c.	64.9 ± 11.2
28	0.5	144 ± 60.2	789 ± 399	705 ± 312	108 ± 22.8
	2	153 ± 48	1020 ± 184	1220 ± 461	1010 ± 501
	24	n.c.	n.c.	n.c.	57.5 ± 18.8
54#	0.5	130 ± 55.9	618 ± 322	786 ± 501	630 ± 319
	2	189 ± 44.2	776 ± 130	1040 ± 402	1030 ± 364
	24	n.c.	n.c.	20.9 ± 4.47	70.7 ± 13.9
AUC (ng•hr/mL ± SEM)					
54	0-T	949 ± 130	3930 ± 669	8980 ± 766	20500 ± 2220
Cl/F (L/h/kg ± SEM)					
54		13.9 ± 3.92	7.71 ± 1.25	5.86 ± 0.51	5.54 ± 0.52

*hr postdose, #different method from Wk 15, 28, ## not calculable.

ICI 213,841

WK	TIME*	LD	MD-1	MD-2	HD
PLASMA LEVELS (ng/mL ± SEM)					
15	0.5	133 ± 55.1	357 ± 149	320 ± 133	253 ± 49.5
	2	295 ± 45.8	704 ± 145	1300 ± 509	565 ± 178
	24	n.c.##	n.c.	n.c.	288 ± 39.7
28	0.5	152 ± 61.7	424 ± 187	389 ± 110	317 ± 66.1
	2	182 ± 44.8	855 ± 129	817 ± 274	822 ± 244
	24	n.c.	32.5 ± 7.20	136 ± 51.4	287 ± 73.9
54#	0.5	205 ± 73.7	450 ± 185	372 ± 166	455 ± 122
	2	299 ± 69.1	862 ± 105	1210 ± 351	869 ± 236
	24	n.c.	53 ± 12.8	95.3 ± 17.6	236 ± 39.7
AUC (ng•hr/mL ± SEM)					
54	0-T	2500 ± 416	9470 ± 2430	11400 ± 902	23600 ± 1280

*hr postdose, #different method from Wk 15, 28, ## not calculable.

WK	TIME*	LD	MD-1	MD-2	HD
PLASMA LEVELS (ng/mL ± SEM)					
54	0.5	111 ± 31.8	159 ± 49.7	122 ± 50.0	107 ± 23.0
	2	113 ± 35.5	219 ± 35.8	198 ± 59.2	147 ± 34.9
	24	47.6 ± 13.4	48.7 ± 11.8	33.8 ± 5.59	69.5 ± 10.4
AUC (ng•hr/mL ± SEM)					
54	0-T	1420 ± 227	2946 ± 424	3270 ± 232	4110 ± 221

*hr postdose, #different method from Wk 15, 28, ## not calculable.

At Wk 54, additional time points were examined (i.e., 0.5, 1, 2, 7, 8, 12, and 24 hr). Only the 0.5, 2, and 24 hr data were included in the tables since it is only at these sampling times that compariosons can be made over wks of dosing. However, at Wk 54, the C_{max} did not always occur at 0.5 or 2 hr postdosing; therefore, C_{max} (ng/mL ± SEM) for Wk 54 for ICI 204,636 and metabolites were as follows:

COMPOUND	LD	MD-1	MD-2	HD
ICI 204,636	251 ± 67.9*	824 ± 262	1150 ± 454	1570 ± 317
ICI 213,841	375 ± 72.8	862 ± 105	1210 ± 351	1650 ± 223
ICI 214,227	182 ± 40.9	219 ± 35.8	221 ± 54.4	262 ± 34.7

*these values are based on the highest means ± SEM provided by the sponsor; the C_{max} values as reported by the sponsor differ somewhat from these values. According to the methods section, the sponsor determined C_{max} "...using individual animal data..."

3. **ICI 204,636: 56 week oral (gavage) chronic toxicity study in the cynomolgus monkey** (Study no. TFP/84, 11/IE/1017802, drug analysis/TK data: Zeneca Pharmaceuticals, conduct of study: study dates: 8/94-8/95, GLP, Vol 1.32-1.33).

Animals: cynomolgus monkeys (*Macaca fascicularis*)

initial age: 2-3 yrs old
initial body weight: 2.3-3.0 kg for males, 2.1-2.7 kg for females
diet/water: in general, animals were given 100 g of primate diet at ≈0700 hr, a Bonio biscuit at ≈0800 hr, and fruit/corn at ≈1400 hr; water was available *ad lib.*
n = 4/sex/grp for main study, an additional 3/sex for C and HD grps for the recovery period

Drug: ICI 204,636 (batch no. 54055/93)
identity/purity: a Certificate of Analysis was provided
vehicle: 0.5% HPMC in 0.1% Tween 80
formulation: suspension; analyses indicated that achieved concentrations were ≈94-

108% of intended.

doses: final doses: 0, 25, 100, and 225 mg/kg. Prior to initiation of the 56-wk study, doses were increased over a 29-day period according to the following schedule: C: vehicle daily for 28-days, LD: vehicle on Days 1-24, 15 mg/kg/day on Day 25-28, MD: vehicle on Day 1-12, 15, 30, 45, 60, and 80 mg/kg/day on Days 13-15, 16-18, 19-21, 22-24, and 25-28, respectively; HD: 15, 30, 45, 60, 80, 100, 120, 140, 160, 190 mg/kg/day on Days 1-2, 3-4, 5-6, 7-9, 10-12, 13-15, 16-18, 19-21, 22-24, and 25-28. All doses were given as 3 divided doses (i.e., t.i.d.)

dosing frequency: t.i.d. (0800, 1400, 2100 hr), with some exceptions relating primarily to withholding of doses for ophthalmology examination.

duration: 28-day dose escalation period + 52-wk main study + 4 wk recovery period (C, HD)

route: p.o. (gavage)

Observations

Clinical signs: all animals were observed daily throughout the dosing period, prior to dosing, immediately, 1, and 3 hr after the 1st and 2nd daily doses, and immediately and 1 hr after the 3rd daily dose. In addition, complete physical examinations were performed weekly throughout the study.

Body weight: body weights were recorded weekly throughout the study.

Food consumption: food consumption was estimated daily (0700-1100 hr) throughout the study.

Rectal temperatures: rectal temperatures were measured during Wks 12, 25, 38, and 51 at ~2 hr after the 2nd daily dose, and during Wk 60 in recovery animals.

Ophthalmology: ophthalmology examinations were performed on all animals prior to the start of dosing, and during Wks 14, 27, 39, and 50 of the dosing period, and at Wk 60 in recovery animals. Additional examinations were performed by Dr. Aguirre, the sponsor's ophthalmology consultant, during Wk 55.

A few animals were re-examined during Wk 20 (1 HDM, 1 LDF, 1 MDF, 1 HDF) or Wk 53 (1 LDF, 1 MDF) either because of inadequate mydriasis in a previous examination, or to photograph lesions seen previously (Wk 53).

Examinations were conducted using a indirect ophthalmoscope, following application of mydriatic agent (1% tropicamide and/or 10% phenylephrine).

ECG/blood pressure: ECG recordings were obtained from 3/sex from C and HD grps, prior to the start of dosing, and during Wks 1, 25/28, and 51 of dosing. Heart rate, RR, PR, QRS, QT, and QT_c were taken from lead II.

Blood pressure measurements were taken from 3/sex C and HD animals, prior to the start of dosing, and during Wks 1, 25/28, and 51, at the same time ECG measurements were recorded.

Hematology: blood samples were collected during Wks 13, 26, 31, 39, and 52 of dosing, and at Wk 60 in recovery animals for analysis of the following parameters: hgb, MCV, rbc count, MCH, MCHC, pcv, wbc (total, differential), platelet count, reticulocyte count, prothrombin time, APTT, Factors VIII, IX, XI, and XII (Wk 42 only).

Clinical chemistry: blood samples were collected during Wks 13, 26, 39, and 52 of dosing, and at Wk 60 in recovery animals for analysis of the following parameters: AST, ALT, alkaline phosphatase, LDH, Na, Cl, P_i, urea, creatinine, albumin, total cholesterol, K, Ca, glucose, total bilirubin, total protein, A/G ratio, triglyceride (Wks 26, 39, and 52 only), and hormones (TSH, T_3, T_4, and prolactin).

Additional blood samples were collected from all animals during Wk 49 for analysis of "...sterol profile analysis by the sponsor".

TK: blood samples were collected from all animals during Wks 1, 26, and 52, prior to the second daily dose and at 0.5, 1, 2, 3, 5, and 7 hr after the second daily dose. Plasma was analyzed for ICI 204,636, ICI 214,227, ICI 213,841, and ICI 236,303 (aka M 236,303). Analyses were conducted by the sponsor.

Urinalysis: urine samples were collected prior to the start of dosing and during Wks 13, 26, and 52 for analysis of the following parameters: volume, specific gravity, protein, ketones, blood reducing substances, Cl, K, color, pH, glucose, total bilirubin, urobilinogen, microscopic analysis of sediment, Na, creatinine.

Terminal studies

Gross pathology: a complete necropsy was performed on all animals.

Organ/tissue weights: the weights of the following organ/tissues were recorded in all animals: adrenals, brain (including brainstem), heart, ovaries, pituitary, prostate, kidneys, liver, lung, spleen, testes/epididymides, thyroids, uterus. Paired organs were weighed separately, but were reported as combined weights.

Histopathology: the following tissues were examined microscopically in all animals: adrenals, aorta, bone marrow smear (femur), brain (including brain stem), cecum, colon, duodenum, epididymides, eyes (with optic nerve), femur (including articular surface), gallbladder, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs (with mainstem bronchi), lymph nodes (mandibular, mesenteric), esophagus, ovaries, pancreas, peripheral nerves (sciatic), pituitary, prostate, rectum (with anus), salivary glands (submandibular), seminal vesicles, skeletal muscles (quadriceps), skin and mammary gland, spinal cord (lumbar, cervical, thoracic), spleen, sternum (with bone marrow), stomach, testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus (corpus, cervix), vagina, all gross lesions.

The brain was examined as follows (quoted verbatim from sponsor):

"Level 1: sample through the anterior forebrain at the level of the olfactory lobes.

Level 2: sample through the posterior forebrain at the level of the optic chiasma and the underlying hypothalamus.

Level 3: sample through the cerebrum and the underlying brain stem at the level of the mammillary bodies.

Level 4: sample through the mid-brain at the level of the optic lobes and crura cerebri.

Level 5: sample through the hind-brain to include the cerebellum and medulla oblongata.

Level 6: sample through the caudal region of the cerebrum in the region of the post-lateral gyri.

Eyes were sectioned as follows: (1) for most animals, "...five sections were taken at 50 µm intervals starting at the optic disc/papilla", (2) for the remaining animals (MDM 815, LDF 833, HDF 838, HDF 839), "...50 sections were taken at 100 µm intervals through the middle 5 mm of the lens".

Additional sections of the thyroid were stained using the Masson-Fontana technique. Whether or not this was done for each animal was not clearly stated.

Sections were examined by the sponsor.

Results

Mortality: there were no unscheduled deaths during the study.

Clinical signs: drug-related clinical signs were noted at all doses, with incidence and severity being dose-related. Hunched posture and subdued behavior were noted at all doses. At the MD and HD, additional signs consisted of rapid eye movements, salivation, vomiting, tremors, and circling behavior. Prostration was observed only at the HD.

According to the sponsor, no clinical signs were noted during the recovery period.

Body weight: in males, body weight was lower in drug-treated grps; however, the effect was not dose related (Wk 52: 14,9, and 11% at LD, MD, and HD, respectively). Overall body weight gain was reduced by 26, 14, and 31% at LD, MD, and HD, respectively.

In females, body weight was reduced (compared to CF) by 5, 6, and 10% at Wk 52 in LDF, MDF, and HDF, respectively. Overall body weight gain was reduced in MDF and HDF (13 and 40%, respectively).

In recovery animals, body weight in HD animals remained lower than C animals (7-21% for HDM, 8-13% for HDF).

Food consumption: in males, food consumption was reduced at all doses (overall: 10, 6, and 14% at LD, MD, and HD, respectively) throughout the dosing period; however, the effect was significant only at the HD. In females, overall food consumption was reduced at the HD (15%).

In recovery animals, food consumption in HD animals remained lower than Cs (4-14% for HDM, 2-16% for HDF).

Rectal temperatures: there were no drug-related effects in males. In females, rectal temperature was slightly, but significantly, reduced at the HD during most of the dosing period (2-4%, or 0.7-1.4° C). In HD recovery females, rectal temperature was 1° C lower than in CF (recovery); however, rectal temperature increased in both grps as compared to measurements taken during the dosing period.

Ophthalmology: the only drug-related effect, according to the sponsor, was the

detection of a striated appearance of the anterior lens surface in 2 HDF. The incidences of other findings, e.g., changes in posterior lens sutures, posterior cortical opacities, macular depigmentation), would suggest that they were not drug-related.

According to the sponsor's ophthalmology consultant, Gustavo D. Aguirre, V.D.M., (cf. Appendix B) the striated appearance of the anterior lens surface "...is likely...associated with the administration of the test compound." However, Dr. Aguirre concluded that since this finding was not associated with changes in lens transparency, "...it may not reflect actual lens pathology, and may be the result of a transient and/or reversible alteration in the most superficial anterior cortical lens fibers." Therefore, Dr. Aguirre considered the HD to be the no-effect dose for ophthalmology effects. Dr. Aguirre did note in his report (8/22/95) that "The structural correlates of this clinical observation [i.e., striated appearance] must await histopathologic evaluation of the lens."

ECG/blood pressure: there were no clear drug-related effects.

Hematology: drug-related findings were as follows: (1) decreases in rbc parameters were noted in both males and females, primarily at the HD, throughout the dosing period: hgb (8-12%), rbc (7-10%), pcv (9-12%); hgb and pcv also tended to be lower at the LD and MD (4-10%), depending upon the measurement time. (2) changes in wbc parameters consisted of (a) decreases in wbc count in LDM and HDM (27-51%, not dose-related, Wks 13-39) and at all doses in females (9-13, 22-29%, and 19-33% at LD, MD, and HD, respectively, Wks 13-52), (b) increases in neutrophil count in HDM (17-48%) and HDF (13-44%), (c) decreases in lymphocyte count in LDM (36-46%), HDM (31-46%), and HDF (38-48%); decreases were also noted in MDM (0-31%), LDF (11-37%), and MDF (18-27%), but the differences did not reach statistical significance. (d) monocyte count was reduced at all doses in females (20-100% at Wks 31-52).

In terms of coagulation parameters, the following were noted: (1) an increase in APTT in HDF (14-50%) throughout the dosing period, with increases up to 2-fold in individual animals; there was also a small increase in APTT in HDM at Wk 42 (22%) and (2) increases in Factor VIII (4 and 16% in HDM and HDF, respectively, not significant), Factor IX (24 and 37% in HDM and HDF, respectively), and Factor XII (16 and 27% in HDM and HDF, respectively); at Wk 42, only C and HD animals were examined.

In recovery animals, there were no significant differences between C and HD grps; APTT, however, was still elevated in 1 HDF (#842, 55% above mean CF value).

Clinical chemistry: drug-related findings were as follows: (1) decreases in alkaline phosphatase in males (all doses: 13-18, 14-18, 30-44% at LD, MD, and HD, respectively) and HDF (31-41%) throughout the dosing period, (2) decrease in P_i in HDM (18-21) at Wks 13-39, (3) transient/sporadic increase in glucose in males (17, 17, and 27% at LD, MD, and HD, respectively, Wk 13) and MDF (39-6%, Wk 13, 52) and HDF (16-11%, Wk 13,52), (4) decreases in total bilirubin in males (all doses: 32-62, 35-59, and 41-56% at LD, MD, and HD, respectively) and females (all doses: 37-46%, 33-43, 44-61% at LD, MD, and HD, respectively), (5) increased creatinine in MDM (10-12%), HDM (10-20%), and HDF (10-20%) throughout the dosing period, (6) decreases in total cholesterol in HDM (30-52%) and HDF (51-61%) throughout the dosing period; this parameter was reduced by 20-77% (compared to baseline levels) in the 2 HDF in which lens findings were observed,

(7) decreases in T_3 in MDM (19-30%), HDM (30-56%), MDF (10-25%), and HDF (33-36%) at Wks 13-52 in males and through Wk 39 in females, (8) increased serum prolactin in males only (MD: 2.2-1.6 fold, HD: 3-4 fold) at Wks 13 and 26; at Wk 52, serum prolactin was decreased in females at all doses (72, 70, and 32% at LD, MD, and HD, respectively), (9) transient increase in urea in HDF (31% at Wk 13), (10) an increase in TSH in MDF and HDF (67 and 58%, respectively) at Wk 39, (11) decrease in albumin (7% in MDM and HDM, 12% in HDF) and A/G ratio (19% in HDM) at Wk 52, and (12) an increase in triglycerides in males at Wk 52 (14, 26, and 45% in LDM, MDM, and HDM, respectively).

In recovery animals, the only finding was a decrease in total bilirubin in HDM (26%) and HDF (51%).

Urinalysis: there were no consistent drug-related effects. The following, however, were noted: (1) an increase in volume in MDM (68%) and HDM (32%) at Wk 13, in MDF throughout the dosing period (160-110%) and in HDF at Wks 13 and 26 (100-110%), (2) increases in total Na in HDM (140-120%) at Wks 13 and 52, and in females (LD: 70% at Wk 13, MD: 120-10% at Wk 13 and 26; HD: 180 and 90% at WK 13 and 26), (3) increases in total K in MDF and HDF (≈100%) at Wks 13 and 26, and (4) decreases in creatinine at all doses in males at Wk 13 (32, 23, 12% at LD, MD, and HD, respectively) and at all doses in females at Wk 13 and 52 (LD: 40-50%, MD: 37-49%, HD: 57-53%).

TK: the data are summarized in the following tables (data taken from sponsor's Table T1):

DOSE (mg/kg)	WK	$t_{1/2}$ (h)	$AUC_{(0-7\ h)}$ (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	T_{max} (h)
ICI 204,636						
25	1	1.74 ± 0.100	1090 ± 541	386 ± 176	26.6 ± 11.3	1.04 ± 0.221
	26	3.33 ± 0.577	610 ± 153	169 ± 64.3	41.1 ± 9.36	1.35 ± 0.265
	52	1.99 ± 0.180	540 ± 68.7	136 ± 18.8	19.6 ± 2.53	1.18 ± 0.224
100	1	2.95 ± 0.127	6020 ± 1920	1360 ± 336	403 ± 154	1.38 ± 0.181
	26	2.92 ± 0.566	2900 ± 410	853 ± 126	152 ± 29.5	1.10 ± 0.136
	52	3.13 ± 0.262	3460 ± 797	738 ± 144	239 ± 67.5	1.40 ± 0.344
225	1	4.06 ± 0.619	9260 ± 878	1930 ± 185	721 ± 103	1.76 ± 0.364
	26	3.23 ± 0.228	9180 ± 789	2060 ± 167	659 ± 61.4	1.50 ± 0.230
	52	4.14 ± 0.599	8190 ± 837	1980 ± 264	558 ± 85.6	1.48 ± 0.266

DOSE (mg/kg)	WK	$t_{1/2}$	$AUC_{(0-7\ h)}$ (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	T_{max} (h)
ICI 213,841						
25	1	2.19 ± 0.229	1640 ± 426	447 ± 79.6	75.8 ± 30.2	1.14 ± 0.211

	26	6.23 ± 1.780	1300 ± 177	294 ± 58.2	111 ± 17.6	1.50 ± 0.254
	52	2.31 ± 0.191	1030 ± 66.2	225 ± 14.4	50.1 ± 5.83	1.53 ± 0.226
100	1	3.90 ± 0.253	6960 ± 1100	1470 ± 175	577 ± 133	1.73 ± 0.269
	26	3.94 ± 0.830	4530 ± 366	1070 ± 107	335 ± 52.1	1.34 ± 0.274
	52	4.64 ± 0.387	4900 ± 615	902 ± 97.2	446 ± 75.9	2.03 ± 0.564
225	1	5.80 ± 0.670	14700 ± 918	2830 ± 294	1310 ± 103	1.76 ± 0.287
	26	4.70 ± 0.501	14800 ± 697	2810 ± 128	1380 ± 88.1	2.01 ± 0.244
	52	5.21 ± 0.591	12500 ± 692	2420 ± 166	1100 ± 102	1.71 ± 0.27

DOSE (mg/kg)	WK	$t_{1/2}$	$AUC_{(0\text{-}7\ h)}$ (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	T_{max} (h)
ICI 214,227						
25	1	3.38 ± 0.302	2620 ± 320	749 ± 97.8	173 ± 31.6	1.24 ± 0.193
	26	6.85 ± 1.730	2060 ± 161	453 ± 52.7	199 ± 31.0	1.62 ± 0.248
	52	6.00 ± 1.780	2350 ± 203	460 ± 34.4	217 ± 34.6	1.56 ± 0.340
100	1	8.76 ± 3.230	10400 ± 1800	2080 ± 415	1060 ± 204	1.66 ± 0.343
	26	4.50 ± 0.667	6670 ± 1360	1670 ± 332	543 ± 138	1.05 ± 0.151
	52	5.33 ± 0.937	10900 ± 2090	2180 ± 398	842 ± 181	1.54 ± 0.557
225	1	5.85 ± 1.000	14900 ± 1430	3080 ± 283	1310 ± 154	1.62 ± 0.262
	26	4.84 ± 0.865	11400 ± 1380	2280 ± 240	991 ± 155	1.71 ± 0.242
	52	5.17 ± 0.990	14400 ± 1850	3100 ± 443	1180 ± 170	1.55 ± 0.328

DOSE (mg/kg)	WK	$t_{1/2}$	$AUC_{(0\text{-}7\ h)}$ (ng·h/mL)	C_{max} (ng/mL)	C_{min} (ng/mL)	T_{max} (h)
M 236,303						
25	1	n.c.	n.c.	n.c.	n.c.	n.c.
	26	n.c.	n.c.	8.2 ± 0.838	n.c.	4.42 ± 0.840

	52	13.20 ± 6.870	118 ± 13.7	20.9 ± 1.84	12.7 ± 2.19	1.77 ± 0.334
100	1	n.c.	n.c.	51.8 ± 5.18	n.c.	1.72 ± 0.261
	26	3.27 ± 0.490	156 ± 17.8	39.6 ± 4.01	13.8 ± 3.51	1.20 ± 0.162
	52	7.34 ± 1.640	428 ± 46.8	86.5 ± 11.4	42.1 ± 7.05	1.28 ± 0.327
225	1	5.18 ± 0.721	898 ± 203	199 ± 50	76.9 ± 22.2	1.74 ± 0.275
	26	4.66 ± 0.557	838 ± 187	174 ± 32.8	76.9 ± 16	1.99 ± 0.255
	52	5.17 ± 0.757	912 ± 97.3	196 ± 17.8	78.6 ± 12.4	1.75 ± 0.354

Terminal studies

Gross pathology: the only drug-related findings at necropsy were (1) enlarged liver (2/7 HDM) and (2) dark discoloration of the thyroid in 3/7 HDM (#816, 818, 822) and 4/7 HDF (#839, 841, 842, 844); of these affected animals, 1 HDM and 2 HDF were recovery animals.

Organ/tissue weights: the primary drug-related findings were as follows: (1) an increase in liver wt in HDM (A-R: 34-28%), MDF (A-R: 24-18%), and HDF (A-R: 24-18%), (2) a decrease in heart wt in males at all doses (LD: 18-26%, MD: 12-17%, HD: 15-25%).

In recovery animals, thyroid wt was elevated in HDM (68-100%) and HDF (100%); an examination of individual data indicated that 2 HDF (#842, 844) that had discolored thyroid also had increases in thyroid weight (136, 68% compared to mean of CF).

Bone marrow smears: according to the sponsor, there were no drug-related effects; the data were not provided in the study report.

Histopathology: drug-related findings were detected primarily in liver, mammary gland, and thyroid gland. In liver, findings were as follows: (1) centrilobular hepatocyte hypertrophy was detected only in 4/7 HDM (2 minimal, 2 mild; all main study animals), (2) hepatocyte vacuolation was detected in 1/7 HDM and 2/7 HDF (all main study animals). Glandular hyperplasia of the mammary gland was seen at all doses, including the C grp in females; in males, this finding was detected only in 2/4 MDM (minimal) and 4/7 HDM (mild; 2 main study, 2 recovery). Thyroid gland follicular cell hypertrophy was detected only in 3/4 MDM and 5/7 HDM (4 main study, 1 recovery).

There were no histopathological correlates of the anterior lens surface findings during ophthalmology examination or of the necropsy findings in thyroid gland. Tyroid gland follicular cell hypertrophy (mild) was detected in 3 HDM in which dark discoloration of the thyroid gland was evident at necropsy, but also in 2 HDM in which dark discoloration was not noted and none of the 4/7 HDF females in which dark discoloration was noted. According to the sponsor, the thyroid pigment detected in all grps, including Cs, was identified as lipofuscin by Masson-Fontana stain. There was no relationship between the presence of Masson-Fontana positive pigment and dark thyroid findings. The incidence of mineral deposition in brain was either not clearly dose-related (main study animals), or was similar between C and HD grps (recovery animals).

Special studies

Thyroid

1. **Examination of thyroid glands from Studies THR/2047 and TKR/1924 when stained by Schmorl's and the Masson-Fontana methods** (Study no. 12/IC/1006118, Zeneca Pharmaceuticals, report date: 11-12/93, Vol 1.24)

The purpose of the study was to further examine sections of thyroid from two previous studies in rat, i.e., a 1-mo (TKR/1924) and a 3-mo (THR/2047) toxicity study conducted by the sponsor at Alderley Park, UK. Thyroid sections from all animals in these studies were stained using Masson-Fontana and Schmorl's methods. Results were compared between studies and with the results of another previous study, TKR/1804 (56-day, rat study, ICI US). Different strains of rats were used in each study: TKR/1804 (Sprague-Dawley), THR/2047 (Charles River Wistar), TKR/1924 (Alderley Park, Wistar-derived).

The data are summarized in the following tables (data taken from sponsor's Tables 1, 2, and 3):

Pigment identified by Masson-Fontana stain (doses expressed in mg/kg):

STUDY NO.	DAY	FINDING	MALE					FEMALE				
			0	25	50	150	300	0	25	50	150	300
THR/1924	-30	minimal*	0/9 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)		0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	
THR/2047	-90	minimal	1/4 (25%)	2/5 (40%)	2/5 (40%)	2/5 (40%)	2/5 (40%)	1/5 (20%)	0/5 (0%)	2/5 (40%)	2/5 (40%)	0/4 (0%)
		mild	0/4 (0%)	0/5 (0%)	2/5 (40%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	1/5 (20%)	1/4 (25%)
		moderate	0/4 (0%)	0/5 (0%)	1/5 (20%)	2/5 (40%)	3/5 (60%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	3/4 (75%)
		TOTAL	1/4 (25%)	2/5 (40%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	1/5 (20%)	0/5 (0%)	2/5 (40%)	3/5 (60%)	4/4 (100%)
TKR/1804	1	minimal*	4/9 (44%)			6/10 (60%)						
	3	minimal*	2/10 (20%)			7/10 (70%)						
	7	minimal	6/10 (60%)			9/10 (90%)						
		moderate	0/10 (0%)			1/10 (10%)						
		TOTAL	6/10 (60%)			10/10 (100%)						
	14	minimal	7/9 (78%)			6/10 (60%)						
		moderate	0/9 (0%)			4/10 (40%)						
		TOTAL	7/9 (78%)			10/10 (100%)						
	28	minimal	3/10 (30%)			9/10 (90%)						
		moderate	0/10 (0%)			1/10 (10%)						
		TOTAL	3/10 (30%)			10/10 (100%)						
	56	minimal	6/10 (60%)			3/10 (30%)						
		moderate	0/10 (0%)			7/10 (70%)						
		TOTAL	6/10 (60%)			10/10 (100%)						
	56+28R	minimal	8/10 (80%)			1/9 (11%)						
		moderate	0/10 (0%)			8/9 (89%)						
		TOTAL	8/10 (80%)			9/9 (100%)						

*all findings were minimal, so incidences (%) reflect total.

Pigment identified by Schmorl's stain (doses expressed in mg/kg):

STUDY NO.	DAY	FINDING	MALE					FEMALE				
			0	25	50	150	300	0	25	50	150	300
THR/1924	-30	mini-mal*	0/9 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	[illegible]	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	
THR/2047	-90	minimal	0/5 (0%)	1/5 (20%)	3/5 (60%)	2/5 (40%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/4 (0%)
		mild	1/5 (20%)	0/5 (0%)	2/5 (40%)	2/5 (40%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	2/5 (40%)	1/4 (25%)
		moder-ate	0/5 (0%)	0/5 (0%)	0/5 (20%)	1/5 (20%)	5/5 (100%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	3/4 (75%)
		TOTAL	1/5 (20%)	1/5 (20%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	2/5 (40%)	4/4 (100%)

Characterization of the thyroid pigment was attempted using a variety of different stains. The data are summarized in the following sponsor's Table 7; results are compared to pigments produced by other agents [minocycline (a tetracycline-derived antibiotic shown to produce black thyroid in several animal species and human), 2,4-DAAS (2,4-diaminoasole, a hair dye), paracetamol (aniline analgesic)].

	Minocycline	2,4-DAAS	Paracetamol	204636
Masson-Fontana	-	+	nd	+
Lipofuscin	+	nd	+	+/-
Acid fast lipofuscin	-	-	+	-
Iron	-	-	+/-	+/-
PAS	+	-	+	+/-
Fluorescence	-	nd	+	nd
Birefringence	-	nd	nd	nd
Calcium	nd*	nd	nd	-
Alkaline phosphatase	nd	nd	+	nd

* - elemental spectra peaked for calcium and sulphur

2. **Comparison of thyroid pigmentation in five studies in the rat** (TKR/1924, TAR/1621, TKR/1804, THR/2047, TPR/1616; Report 1/ID/1006212, Zeneca Pharmaceuticals, report date: 9/11/93, Vol 1.24)

The sponsor compared the results of 5 different toxicity studies in rat in terms of thyroid findings. At least one thyroid gland slide was examined by an independent pathologist (i.e., not involved in the listed studies). According to the sponsor, the findings of the independent pathologist did not differ in any way that would change the conclusions of the studies.

3. **Assessment of iodine uptake and organification in primary rat thyrocytes exposed to ICI 204,636** (Study no. TKN/175, 6/ID/1009251, Zeneca Pharmaceuticals, study dates: 10/93-10/94, GLP, Vol 1.50)

The purpose of the study was to examine the effects of ICI 204,636 on iodine uptake and thyroperoxidase activity in primary rat thyrocytes; results were compared to a known goitrogenic agent, propylthiouracil. Propylthiouracil acts directly on the thyroid by binding to and inactivating thyroid peroxidase.

Methods: Primary thyrocytes were prepared from the thyroid glands of 40 male Alpk:AP$_f$SD (Wistar-derived) rats. Total cellular iodine and iodine incorporated into thyroglobulin (a measure of thyroperoxidase activity) were quantitated using ^{125}I. ICI 204,636 (ADM 440005/91) was tested at concentrations of 0, 1, 10, and 100 µg/mL; ICI 204,636 was found to be cytotoxic at 500 µg/mL. Propylthiouracil was tested at a concentration of 10 µg/mL.

Result: At concentrations of 10 and 100 µg/mL, ICI 204,636 decreased the total iodine content of the thyrocyte cultures (13 and 53%, respectively). ICI 204,636 had no effect on thyroperoxidase activity at the concentrations tested. In contrast, propylthiouracil decreased the mean %^{125}I-incorporation into thyroglobulin by 64%.

4. **ICI 204636, ICI 214227, ICI 213841, ICI 236303: Assessment of iodine uptake and organification in primary rat thyrocytes exposed to ICI 214,227, ICI 213,841, and ICI 236,303** (Study no. TKN/196, 7/IE/1015141, Zeneca Pharmaceuticals, study dates: 1/95-2/95, GLP, Vol 1.50).

The purpose of the study was to examine the effects of three plasma metabolites of ICI 204,636 (ICI 214,227, ICI 213,841, and ICI 236,303) on iodine uptake and thyroperoxidase activity in primary thyrocytes; results were compared to propylthiouracil.

Methods: Methods used were similar to those in Study no. TKN/175. The concentrations of the metabolites used to assess effects on iodine uptake and enzyme activity were as follows: 0, 10, 100, 300, and 500 µg/mL for ICI 214,227, 0, 10, 100, 200, 300, 400, and 500 µg/mL for ICI 213,841 and ICI 236,303. Propylthiouracil was tested at a concentration of 5 µg/mL.

Results: All three metabolites reduced ^{125}I-uptake and thyroperoxidase activity (i.e., ^{125}I-org). ICI 214,227 significantly reduced ^{125}I-uptake at concentrations of 300 and 500 µg/mL (48 and 77%, respectively) and % ^{125}I-org at concentrations of 100, 300, and 500 µg/mL (19, 49, and 39%, respectively). ICI 213,841 significantly reduced ^{125}I-uptake at the HC (34%) and ^{125}I-org at concentrations of 300, 400, and 500 µg/mL (25, 26, and 19%, respectively). ICI 236,303 significantly reduced ^{125}I-uptake at concentrations of 300, 400, and 500 µg/mL (38, 91, and 94%, respectively) and ^{125}I-org at concentrations of 100, 200, and 300 µg/mL (70, 78, and 71%, respectively); at higher concentrations (400 and 500 µg/mL) only minimal reductions were noted (10-20%). In the same assays, propylthiouracil reduced ^{125}I-uptake by 43-52% and ^{125}I-org by 65-83%.

The sponsor indicated that, although all three metabolites reduced ^{125}I-incorporation into thyroglobulin, they did so (by 50%) at concentrations 15-150 fold higher than predicted plasma levels in humans. Therefore, they would not be expected to have similar effects *in vivo* at plasma levels associated with a therapeutic response.

5. **Assessment of thyroid function in mice** (Study no. TKM/913, 10/IE/1017274, Zeneca Pharmaceuticals, performed liver enzyme assays, study dates: 6/95-7-95, GLP, Vol 1.50).

Animals: male C57BL/10J$_f$CD-1/Alpk (C57BL/10) mice (Alderley Park)
initial age: 107 days
initial body weight: 25.2-34.2 gm
diet: powdered diet up to Day 3-7, then pelleted diet from Day 3-7 on. Food and water were available *ad libitum*
n = 10/grp for Grps I and II, 50/grp for Grps III and IV

Drug: ICI 204,636 (ADM 44039/92)
identity/purity: Certificate of Analysis provided
doses: Grps I and III: Control throughout the study
Grp II and IV: 400, 500, 600, and 750 mg/kg during Wk 1, 2, 3, and 4, respectively.
Grps I and II were used to study pathology and liver enzymes. Grps II and IV were used to study thyroxine clearance.

According to the sponsor's analyses, the actual doses were 192-201, 117-119, 114-120, and 94-97% of intended doses or 767-802, 595-586, 682-723, and 731-705 mg/kg (Grp II-IV).
route/formulation: p.o. in the diet. Diet admixture on powdered-diet days, and pelleted on other days. To form pellets, water was added as necessary; moist pellets were dried prior to feeding. The sponsor indicated that the stability and concentration of ICI 204,636 in the pellets was assayed was confirmed for six weeks when stored. According to the sponsor, the powdered diet contained 91-103% of the intended concentration; whereas the pelleted diet contained 78-87% of the intended concentration.
duration: 4 wks

Observations

Clinical signs: all mice were observed twice daily. A more complete physical examination was performed weekly.

Body weight: body weights were recorded in all animals prior to the start of dosing, on Day 1, and weekly thereafter.

Food consumption: food consumption was assessed prior to the start of dosing, and weekly during the dosing period.

Terminal studies

Hormone clearance: thyroxine blood levels were quantitated at 1, 6, 12, 24, and 30 hr after injection of ^{125}I-thyroxine. [Animals also received i.p. injections of sodium iodide immediately after and at 12 and 30 hr after injection of the radiolabel. Blood samples were collected from 10/grp/time point at sacrifice. Radiolabeled thyroxine was quantitated using RIA.

Liver enzyme assay: liver samples were collected from all animals in Grps I and II for quantitation of thyroxine uridine diphosphatase glucuronyltransferase (T_4UDPGT), total protein, and total cytochrome P450.

Gross pathology: a complete necropsy was performed on all animals in Grps I and II, including those that were found dead (with the exception of 1 M and 1 F in Grp II).

Organ/tissue weights: only liver weight was recorded, and only in survivors at necropsy.

Histopathology: sections of liver, pituitary, thyroid gland, and macroscopic abnormalities were examined microscopically in all animals in Grps I and II. Pituitary glands were preserved in Bouin's fixative. "All other tissues were preserved in 10% buffered formalin." All tissues were stained with H & E for analysis; additional sections of thyroid were stained with Masson-Fontana stain.

Results

Mortality: the sponsor indicated that ICI 204,636 was irritating to the skin, and mice responded to introduction of the drug-in-diet by self-mutilating and food wastage. Apparently, spreading of the diet admixture resulted in control animals (Grp I, III) in adjacent cages being affected. On Day 2, 2 Grp II and 1 Grp IV animals were sacrificed with wounds to skin and/or genital areas. Following introduction of the pelleted diet, additional animals were affected and were either sacrificed or died (1 Grp III animal on Day 3, 2 Grp I animals on Day 10, and 1 Grp I, 2 Grp III, and 4 Grp IV animals on Day 11. [Apparently, only 1 Grp I animal was found dead (Wk 2); all others were sacrificed moribund.]

Clinical signs: according to the sponsor, there were no drug-related signs except for the skin irritation. It may be that the skin irritation and associated behaviors may have masked other drug-related findings.

Body weight: body weight loss was noted in both drug-treated grps (0.8-1.0 gm). In contrast, C grps gained 2.2-3.1 gm. Body weights at the end of the dosing period were reduced by 17 and 9% in Grps II and IV, respectively.

Food consumption: food consumption could not be accurately assessed during the first 3-7 due to food wastage in drug-treated grps. After introduction of the pelleted diet, there were no clear differences among grps.

Terminal studies

Hormone clearance: plasma T_4 was reduced in Grp IV compared to control Grp III at all times measure. At the first measurement time, 1 hr after injection of radiolabel, the mean T_4 level in Grp IV was ≈30% lower than that of Grp III. By the last measurement time (30 hr post injection), the mean T_4 level in Grp IV was reduced by 66% compared to that in Grp III. The plasma T_4 clearance was increased 2-fold in Grp IV (compared to Grp III). Half-life data for T_4 were calculated based on grp means and, therefore, could not be analyzed statistically; however, the estimated $t_{1/2}$ was 30% lower in Grp IV than in Grp III (11.45 vs 7.87%).

Liver enzyme data: protein and cytochrome P450 content of liver were increased in Grp II animals (1.5 and 3-fold, respectively) as compared to Grp I. Although there was a slight increase in the activity of T_4 UDPGT in Grp II (15-28%), the effect was not statistically significant.

Gross pathology: the cause of death in 3 control animals was determined to be due to ulceration of the skin with/without scab formation. This was considered to be due to intercage contamination with powdered diet-drug admixture. The only other drug-related finding was in liver. Livers in 3/8 Grp II animals were

enlarged, and discoloration (red mottling) of all lobes was detected in 2/8 Grp II animals.

Organ/tissue weights: Absolute liver weight was similar in Grps I and II; however, relative liver weight was increased 33% in Grp II animals. (Body weight in Grp II was 16% lower than in Grp I.

Histopathology: the only drug-related finding (except for signs of dermal irritation, e.g., ulceration, cellulitis, scab formation) was diffuse centrilobular hepatocyte hypertrophy (moderate degree) in 8/8 Grp II animals (no Grp I animal was affected). It is interesting that skin effects were noted only in C animals. No drug-related pituitary or thyroid changes were detected.

6. **Assessment of thyroid function in rats** (Study no. TKR/2271, Zeneca Pharmaceuticals, study dates: 1/95-2-95, GLP except for T_4-UDPGT assay, Vol 1.51)

Animals: Wistar rats
initial age: 71-77 days for males, 108-117 days for females
initial body weight: 276-371 gm for males, 248-302 gm for females
n = 10/sex/grp for Grps I and II, 20/sex/grp for Grps III and IV
diet/water: food and water were provided *ad libitum*

Drug: ICI 204,636 (ADM 44039/92)
identity/purity: Certificate of Analysis was provided
vehicle/formulation: suspension in 0.5% HPMC in 0.1% Tween 80
concentration: 0, 34.5 mg/mL; analysis confirmed that the actual concentration was 103.3% of intended.
doses: 0 (Grps I and III) and 300 (Grps II and IV) mg/kg
route: p.o. (gavage)
duration: 14 days for Grps I and II, 18 days for Grps III and IV

Observations

Clinical signs: all animals were observed twice a day, and a more detailed physical examination was performed weekly.

Body weight: body weights were recorded in all animals, prior to the start of dosing, on Day 1, and weekly thereafter. In addition, selected animals were weighed daily to document weight changes due to sedation.

Thyroid hormones: blood samples were collected prior to the start of dosing and on Day 15 from all animals in Grps I and II for measurement of TSH, T_4, and free T_3.

Terminal studies

Hormone clearance: In Grp III and IV animals, ^{125}I-thyroxine was injected i.v. on Day 17. Immediately following injection of the radiolabel, and at 12 and 24 hr post injection, all surviving animals received an i.p. injection of NaI (1 mg/kg). [NaI was given to prevent accumulation of ^{125}I in thyroid.] Blood samples were collected at 30 min, 1, 2, 4, 8, 12, 24, and 30 hr after injection of ^{125}I-thyroxine. The final collection was terminal.

Plasma levels of radiolabelled thyroxine were quantitated using RIA.

Liver enzyme assay: liver samples were collected from Grp I and II animals at necropsy on Day 15 (24 hr after last dose of ICI 204, 636) and microsomal fractions were prepared. The following were analyzed: thyroxine uridine diphosphate glucuronyl transferase (T_4-UDPGT) activity, total protein, total cytochrome P450, cyp1A1/2 [7-thoxyresorufin O-deethylation (EROD)], cyp2B1/2 [7-pentoxyresorufin O-dealkylation (PROD)], cyp3A [N-demethylation of erythromycin], cyp3A1/2 [metabolism of [4-^{14}C]androstenedione].

Gross pathology: necropsy was performed on all animals in Grps I and II.

Organ weights: liver weight was recorded in all animals necropsied.

Histopathology: in animals necropsied, the following tissues were examined microscopically: liver (left, right lateral and left, right median lobes), pituitary gland, thyroid gland.

Liver and thyroid were preserved in 10% buffered formalin, while Bouin's fixative was used to preserve pituitary gland. Tissues were stained with H & E for examination. Sections of thyroid and liver were also examined using Masson-Fontana and Oil-Red O, respectively.

Results

Mortality: there were no unscheduled deaths.

Clinical signs: data were not provided. According to the sponsor, sedation was noted throughout the dosing period in drug-treated animals, and "Coat staining, loss of skin tone, nasal and lachrymal deposits, urine staining and general hair loss were also noted in a small number of animals...".

Body weight: body weight gain was reduced in males, particularly during the first wk of dosing (45-56%, 17-15% at Wk 2); however, body weight was not significantly affected. In females, body weight and body weight gain were elevated in drug-treated animals (8-13%; body weight gain: 0-11 gm in C grps vs 28-32 gm in DT grps).

Terminal studies

Hormone levels: the only drug-related effect was a 3-fold increase in TSH in drug-treated females; the magnitude of this effect was due, in part, to a small increase in CF [as compared to the increase noted in males (C and DT)]. TSH was not affected in drug-treated males, and T_4 and free T_4 levels were similar in treated and control grps.

Thyroxine clearance: Plasma thyroxine (T_4) clearance was increased (32 and 52% for males and females, respectively) and the $t_{1/2}$ was shorter (≈30%) in Grp IV animals.

Liver enzyme assays: the data are summarized in the following sponsor's Table 1 and Table 2:

Dose level	Cytochrome P450 (nmol/mg protein)	Ethoxyresorufin O-deethylase (pmol/mg/min)	Pentoxyresorufin O-dealkylase (pmol/mg/min)	Erythromycin N-demethylase (nmol/mg/min)	Androstenedione 6β-hydroxylase (nmol/mg/min)
Males 0 mg/kg/day	0.86 ± 0.04	36 ± 3	< 10	1.51 ± 0.60	1.61 ± 0.11
Males 300 mg/kg/day	0.96 ± 0.08	103 ± 12***	369 ± 54***	2.50 ± 0.44	0.80 ± 0.13***
Females 0 mg/kg/day	0.53 ± 0.03	51 ± 5	< 10	1.28 ± 0.42	0.13 ± 0.01
Females 300 mg/kg/day	0.78 ± 0.04***	78 ± 10*	114 ± 30***	0.99 ± 0.48	0.20 ± 0.02**

Statistically different from control, * p<0.05, ** p<0.01, *** p<0.001
Values show the mean ± SE obtained from ten animals in each group.

Increases in cyt P450 content, and activity of ethoxyresorufin O-deethylase and pentooxyresorufin O-dealkylase was increased in Grp II males and females. The effect of ICI 204,636 on androstenedione 6ß-hydroxylase activity was not consistent in Grp II males and females.

T_4-UDPGT activity was increased in Grp II males and females whether expressed as per gm liver, or per whole liver; when activity was expressed as per mg protein, the increase was significant only in Grp II females.

	Units of T4 UDPGT Activity		
Dose level	pmol/hr/ mg protein	pmol/hr/ g liver	pmol/hr/ whole liver
Males 0 mg/kg/day	53 ± 3	509 ± 20	8559 ± 328
Males 300 mg/kg/day	60 ± 4	616 ± 25 **	10747 ± 622**
Females 0 mg/kg/day	40 ± 4	325 ± 23	3051 ± 253
Females 300 mg/kg/day	57 ± 3 **	603 ± 49***	7846 ± 686***

Statistically different from control, * p<0.05, ** p<0.01, *** p<0.001
Values show the mean ± SE obtained from ten animals in each group.

The microsomal content of cyp1A1/2, cyp2B1/2, and cyp3A1/2 protein levels was assessed qualitatively using Western immunoblot analysis. No effect was noted on cyp1A1/2 bands, whereas cyp2B1 appeared more abundant in Grp II males and females, and cyp3A1 appeared to be increased in Grp II females.

Gross pathology: at necropsy, an increase in the quantity of mammary gland was noted in 3/10 Grp II females, and thyroid discoloration was detected in 5/10 Grp II males.

Organ weight: absolute and relative liver weight was increased in Grp II females (43-33%), but was unaffected in Grp II males.

Histopathology: drug-related findings were as follows: (1) fat vacuolation in liver in 6/10 Grp II males and 9/10 Grp II females; none was noted in Grp I animals, (2) thyroid findings consisted of (a) pigment deposits as identified using Masson-Fontana stain in 4/10 Grp II males and 2/10 Grp II females, (b) an increase in the average follicular epithelial height (Grade 3: 10/10 Grp I M, 3/10 Grp II M, 1/9 Grp I F, and 8/10 Grp II F; Grade 4: 7/10 Grp II M, 2/10 Grp II F). No drug-related effects on the pituitary were detected.

7. **Investigative study to assess localisation of ICI 204,636 in the rat thyroid gland** (Study no. TKR/2332, 3/IE/1013852, Zeneca Pharmaceuticals, study date: 3/94-6/94, GLP, Vol 1.51).

Methods: This study was conducted in male Alpk:AP(SD)(AF) (Wistar-derived) rats. ICI 204,636 (ADM44076/91, ADM44005/91, ADM44039/92; Certificate of Analysis provided) was administered at a dose of 250 mg/kg. The drug formulation was a suspension in 0.5% HPMC in 0.1% Tween 80. Dosing was orally by gavage. ICI 204,636 was first given in combination with [^{3}H]-ICI 204,636 (1000 µCi) for 28 days (n = 5), and then alone for an additional 3 days (wash-out period in 3/5 M). The 3/5 "wash-out" animals were killed following the 3-day wash-out period. The remaining 2 M were killed after an 8-wk withdrawal period. Observations consisted of the following: clinical signs, body weight (data not reported), food consumption (all 5 caged together; data not reported), terminal studies [histopathology of thyroid and liver, special studies consisted of the following: (1) staining of sections of thyroid with toluidine blue for light microscopy, (2) transmission EM of ultrathin sections of thyroid stained with heavy metal salts (enhance tissue contrast) for measuring the organelle area fraction; performed on 15 different follicular epithelial cells per animal, (3) autoradiography of thyroid and liver; ultrastructural autoradiography performed only on ultrathin sections of thyroid.]

Results: there were no unscheduled deaths during the study. Drug-related clinical signs consisted of sedation, salivation, ptosis, pilo-erection, and staining of fur. Pigment deposition was detected in thyroid follicular epithelial cells, and occasionally in large colloid droplets, affecting ≈1-1.6% of the total cell area. 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 by EM. Radioactivity was detected over pigment and colloid. Pigment was detected in thyroid up to 8 wks after the last dose. There was no association between presence of lipofuscin and ICI 204,636 radioactivity.

Eye

1. **Assessment of in vitro inhibition of cholesterol synthesis in cultured dog lenses exposed to ICI 204,636** (Study no. TVN/202, 9/IE/1016424, Zeneca Pharmaceuticals, study dates: 3/95-5/95, GLP, Vol 1.50)

Methods: In this study, the effects of ICI 204,636 on cholesterol synthesis in lens was tested in an *in vitro* assay using lens obtained from 9 female Beagle dogs. Lens cultures (4 replicates/concentration) were incubated with 0, 1, 10, and 100 µg/mL of ICI 204,636 (ADM44039/92) for 25 hr. Cholesterol biosynthesis was tracked by incubating control and treated lens cultures with a radiolabeled substrate, [1,2-^{14}C]acetate. Radiolabeled lipids were analyzed, following extraction, by HPLC with uv/radiochemical detection. Peak identification was accomplished using GC/MS.

Results: drug-related findings were as follows: (1) there was a decrease in the cholesterol/lathosterol fraction at 10 and 100 µM, expressed either as mean peak area (20 and

40%, respectively) or % of total peaks (12 and 70%, respectively), (2) there was an increase in the desmosterol fraction at all concentrations (1, 10, and 100 μM), expressed either as mean peak area (45, 27, 300%, respectively), or as % total peaks (23, 50, 100%, respectively), (3) there was an increase in two unidentified peaks at 100 μM, and (4) there was an increase in total counts at 100 μM (64%). [Cholesterol and lathosterol, which the sponsor noted differ only by 2 H atoms, could not be separated by the methodology used.]

According to the sponsor's report, the 1 and 10 μM concentrations approximate the serum levels of ICI 204,636 obtained in the 1-yr study in dogs at 100 mg/kg (the dose associated with cataracts).

2. **Assessment of in vitro cataract formation in cultured dog lenses exposed to ICI 204636** (Study no. TVN/198, 9/IE/1016371, Zeneca Pharmaceuticals, study dates: 5/95, GLP, Vol 1.50)

Methods: The potential for ICI 204,636 (ADM 44039/92) to induce cataracts was tested in culture lenses (from Beagle dogs, 4 lenses/concentration) at concentrations of 0, 1, 10, and 100 μM. Lenses were incubated with ICI 204,636 for 9 days. At the end of the incubation period, lenses were examined using digital photography and histologically after fixation in Davidson's solution.

Results: No changes in lens optical clarity or microscopic findings were observed at any concentration of ICI 204,636. The sponsor did state that in a previous study, chlorpromazine (100 μM) produced "severe cataracts" after 9 days incubation under similar experimental conditions.

3. **Investigative study to determine the in vitro inhibition of cholesterol synthesis in HepG2 cells by ICI 204,636** (Study no. TVN/199, 9/IE/1016408, Zeneca Pharmaceuticals, study dates:.2/95-3/95, GLP, Vol 1.50)

Methods: The potential for ICI 204,636 (ADM 44039/92) to affect cholesterol synthesis was tested in a human hepatoma cell line, HepG2, using lipoprotein-deficient medium. Cells were incubated for 1 or 24 hrs with ICI 204,636 at concentrations of 0.1, 1, 10, and 100 μM. 25-hydroxy cholesterol was used as a positive control. Cholesterol synthesis was followed by incubating cells with radiolabeled precursor, ^{14}C-acetate. At the end of incubation, cells were washed, extracted, and lipids were analyzed by HPLC with uv/radiochemical detection. Lipids were identified by GC/MS.

Results: the data were presented as chromatograms; no quantitative data were provided (for sterol peaks). At the 1-hr incubation, ICI 204,636 appeared to produce relative decreases in the cholesterol peak and increases in the desmosterol peak at all concentrations. In addition, there were increases in unidentified peaks, one immediately following desmosterol on the chromatogram. The positive control, 25-hydroxy cholesterol, decreased the cholesterol relative to the desmosterol peak (which appeared increased).

At the 24-hr incubation, ICI 204,636 similar findings were noted; however, the sponsor noted that the total number of counts on the radiochromatogram was reduced at 100 μM.

The sponsor concluded that ICI 204,636 appeared to inhibit cholesterol synthesis, with the block occurring after the synthesis of lanosterol; however, the exact location of the block could not be determined from the data.

4. **Investigation of cholesterol synthesis in the dog** (Study no. TKD/812, 10/IE/1016979, Zeneca Pharmaceuticals, study dates: 11/94-6/95, GLP, Vol 1.52)

The purpose of this study (2 separate experiments) was to assess the effects of ICI 204,636 on cholesterol synthesis in liver of Beagle dogs.

Methods: In Exp. I and II, ICI 204,636 (ADM 44063/92, ADM 54038/93, ADM 54083/93) was administered to male Beagle dogs (3/grp) as a single dose of 100 mg/kg p.o. (CM received lactose). Five hr after dosing with ICI 204,636 or lactose, all animals received a single dose of 5-^{3}H-mevalonolactone (a cholesterol precursor) i.v. Animals were sacrificed at either 15 (Exp I) or 60 (Exp. II) min after injection of the radiolabel. At sacrifice, lens and liver samples (Exp I) or just liver samples (Exp II) were collected from each animal. Lipids were extracted from the tissues, and separated and measured using HPLC with uv/radiochemical detection. Sterol fractions were identified by GC/MS.

Results: It should be noted that with the techniques used, cholesterol and lathosterol could not be separated using radiochemical detection, and with uv detection, both cholesterol and β-sitosterol peaks interfered with detection of lathosterol.

According to the sponsor, ICI 204,636 appeared to reduce the cholesterol fraction relative to the lathosterol fraction, and vice versa at 15 and 60 min postlabelling as visualized by radiochemical detection. [These changes were not easy to see. In the control sample, the cholesterol/lathosterol fractions eluted at 9.30 and 9.80, respectively. In the "treated" chromatograms, peaks corresponding to these elution times were not detected. Instead, peaks were noted at 11.20-12.8.] No drug-related changes were noted in lens sterols with either radiochemical or uv detection; in fact, no radioactivity was detected in lens samples.

The sponsor concluded that ICI 204,636 blocks the conversion of lathosterol to cholesterol in liver. The failure to note any changes in lens was due to the inability of the radiolabel to penetrate the lens blood-aqueous barrier.

5. **Investigation of cataracts in dogs** (Study no. TKD/827, 1/IF/1018552, Zeneca Pharmaceuticals, study dates: 1/95-9/95, GLP except for analysis of sterols, Vo. 1.53)

Animals: female Beagle dogs
initial age: 42-55 wks
initial body weight: 9.0-14.9 kg
diet/water: 400 gm food/day in morning prior to dosing, water *ad lib*
n = 2 C (Wk 7), 8 C (Wk 35/36), 4 DT (Wk 7), 8 DT (Wk 35/36)

Drug: ICI 204,636 (analytical ref no. ADM 44039/92, ADM 54038/93)
identity/purity: Certificate of Analysis provided
formulation: 25 and 100 mg coated tablets in gelatin capsules; controls received either an empty gelatin capsule or were undosed.
route: p.o.
doses: rising doses of 25 mg/kg (Wk 1), 50 mg/kg (Wk 2), 100 mg/kg (Wk 3-35/36)
duration: 7 or 35/36 wks

Observations

Clinical signs: all animals were observed twice daily, with a more detailed physical examination being performed weekly except for Wk 33.

Veterinary ophthalmology: ophthalmology was performed in all survivors prior to the start of dosing, and once during Wks 6, 14, 18, 10 (#73 only), 21, 23, 25, 27, 31, 34, and 35. Four additional C animals were examined twice prior to the start of dosing, and once during Wks 5, 8, 10, 12, 14, 19, 21, and 22.

Prior to examination, animals received eye drops to dilate pupils (1% tropicamide, 10% phenylephrine). Examinations were performed using a

"..binocular, indirect ophthalmoscope and a hand-held slit-lamp biomicroscope..." Photographs were taken of lenses in several dogs during Wks 19, 21, 23, and 25.

Body weight: animals were weighed prior to start of dosing (original animals), and weekly during the dosing period (all dogs).

Food consumption: food was weighed for all original dogs prior to the start of dosing, and during the dosing period (all dogs, intervals not specified).

Clinical chemistry: blood samples were collected prior to the start of dosing and during Wks 6 (except for 4 C dogs), 15, 23, and 35. Aqueous humor samples were collected at necropsy (Wk 7) from 2 C and 4 DT for analysis of glucose and osmolality. The sponsor noted that the standard method for quantitating cholesterol detects double bonds at the Δ^4 or Δ^5 positions (since the assay measures the activity of cholesterol oxidase); therefore, the assay is not specific for cholesterol.

Some plasma samples were extracted and analyzed by GC/MS in order to identify sterol components.

Terminal studies

Gross pathology: at the end of the dosing period (either Wk 7 or Wk 35-36), animals were sacrificed and the following tissues were collected: lens (left eye) for biochemical analysis (Wk 7: radiolabeling; Wk 35/36: sterol analysis), lend (right eye) for histopathology and ultrastructural examination, aqueous humor (from right eye, Wk 7 only) for clinical chemistry, aqueous humor (from left eye, Wk 7 only) for PK analysis (not done), vitreous humor (from right eye, Wk 7)-not analyzed, lacrimal gland (left and right) for histopathology, and liver (Wk 35/36) for sterol analysis-not examined.

Histopathology: lenses were removed from eye, immersed in either Karnovsky's fixative (Wk 7), or 2.5% glutaraldehyde/0.1 M cacodylate buffer with subsequent fixation in aqueous 1% osmium tetroxide solution (Wk 35/36). As described by the sponsor, "Lens were ... dehydrated in graded alcohols and 1,2 epoxypropane and divided into eight....pieces prior to infiltration with and embedding in araldite resin. Following polymerisation, sections (1 µm thick) of plastic-embedded lens were stained with toluidine blue and examined by light microscopy..."

The lacrimal glands were examined by light microscopy following placement in 10% buffered formalin, embedding in paraffin wax, and staining with H & E.

Ultrastructural examination: for examination by transmission EM, ultrathin sections of lens were stained with uranyl acetate and lead citrate solutions.

Ex vivo lens radiolabelling: in animals sacrificed at Wk 7, eyes were removed and lenses excised. Lenses were then cultured and incubated with [1,2-^{14}C]acetic acid for 24 hrs. Lens lipids were then saponified, extracted (non-saponifiable), and separated by HPLC with uv/radiochemical detection. Radiochromatographs were compared to control chromatograms (of liver sterols) prepared in a previous study. The assumption was made that there were no unique sterols in lens. Peak data were expressed as % of total counts on radiochromatogram.

Lens dissolution/sterol analysis: in animals sacrificed at Wk 35/36, the left eyeball was removed and the lens collected. Lenses were then placed in phosphate buffered saline for dissolution according to the method of Cenedella (Inv Ophth Vis Sci 34:2186-2194, 1993).

Following dissolution, samples of dissolution fractions and lens "remainder homogenate" were analyzed for sterol content using techniques similar to those used for detecting plasma sterols (HPLC, GC/MS). When possible, area under the peaks for cholesterol, Δ^8-cholestanol, and lathosterol were integrated and normalized against the internal standard, epicoprostanol. Corrected values were expressed as cholesterol equivalents. This method assumes that the detector response to all sterols is the same as that for cholesterol.

Results

Clinical signs: drug-related clinical signs consisted of subdued behavior, unsteady gait, difficulty in rousing, and vocalization.

Body weight: according to the sponsor, there were no drug-related effects. (The data were not summarized.)

Food consumption: according to the sponsor, there were no drug-related effects. (The data were not summarized.)

Veterinary ophthalmology: lenses were graded from 0-8, with Grades 0-4 considered by the sponsor to be "Normal variations" and Grades 5-8 considered "Pathological changes". Very few findings were noted in C animals. A grade of "0", "No abnormalities detected. Lens appears optically clear and homogenous on direct retroillumination and biomicroscopic examination" as defined by the sponsor, was noted throughout the study (Wk 22-35) in 3/8 animals. At the final observation time, i.e., Wk 22 or 35, a grade of "0" was noted for 7/8 C animal; one C animal received a grade of "2" (defined by the sponsor as "Slight opacity of short sections of the posterior Y suture: opacities are usually located a the Y suture tips").

In contrast, a grade of "5" (defined by the sponsor as "Broadening of the posterior Y suture arms with slight accumulation of fine granular opacities along the suture arms") was noted in 2/8 treated animals at Wk 14-15. A grade of "8" (defined by the sponsor as "Posterior axial triangular cataract. The posterior Y suture arms and/or tips may be opacified.") was noted in 1/8 treated animals at Wk 18. At the end of dosing (Wk 35), a grade of "4" (defined by the sponsor as "Broadening of the posterior Y suture arms") was noted for 4/8 treated animals, a grade of "5" was noted for 3/8 treated animals, and a grade of "8" was still noted in the 1/8 animals that developed cataracts (#73). In #73, cataracts ["..bilateral subcapsular posterior axial triangular lens opacities (cataracts)..."] developed in one eye between Wks 14 and 18; by Wk 19, both eyes were affected and remained so during the rest of the dosing period.

Clinical chemistry: plasma cholesterol was significantly reduced in drug-treated animals at Wks 23 and 35 (14 and 26%, respectively). In female #73, plasma cholesterol levels were reduced 18-26% below baseline (CF values ranged from 2.9-5.5 at Day 240). Of interest was CF #61. In this animal, plasma cholesterol

was similar to that in #73 (i.e., 2.2-2.9 vs 2.5 for #73 at Day 240), however, no lens changes were noted (i.e., Grade 0). One difference between #61 and #73 was that for #73, cholesterol levels were reduced below baseline whereas in #61, they were not.

No drug-related changes were noted in glucose levels or osmolality of aqueous humor samples (at Wk 7).

Terminal studies

Gross pathology: no drug-related changes were detected.

Histopathology (lens, lacrimal gland): findings are summarized in the following table (observations taken from sponsor's Tables 3 and 4).

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FINDINGS	Wk 7		Wk 35/36	
	0*	100	0	100

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ON ORIGINAL

Eye				
no abnormalities detected			5/8	0/8
anterior cortical fiber polychromasia				
minimal	0/2	1/4	0/8	1/8
mild	0/2	0/4	0/8	1/8
moderate	0/2	0/4	0/8	2/8
anterior cortical fiber swelling				
minimal	0/2	1/4	1/8	2/8
mild	0/2	0/4	0/8	2/8
posterior cortical fiber swelling				
minimal	2/2	0/4	0/8	2/8
mild	0/2	3/4	0/8	1/8
moderate	0/2	0/4	0/8	2/8
posterior cortical fiber polychromasia				
minimal	0/2	0/4	1/8	2/8
mild	0/2	0/4	0/8	2/8
equatorial cortical polychromasia (mild)	0/2	0/4	0/8	1/8
EM: superficial swollen anterior cortical fibers	0/2	1/4	0/8	0/8
EM: electron lucent swollen posterior cortical fibers	1/2	3/4	0/8	0/8
EM: anterior epithelial cells-cytoplasmic vesicular inclusions	0/2	0/4	0/8	1/8
EM: cortical fiber polychromasia	0/2	0/4	0/8	2/8
EM: anterior cortical fiber swelling	0/2	0/4	0/8	2/8
EM: posterior cortical fiber swelling	0/2	0/4	0/8	1/8
EM: posterior cortical fiber polychromasia	0/2	0/4	0/8	1/8
EM: superficial posterior cortical fibers-cytoplasmic vesicular inclusions	0/2	0/4	1/8	1/8
Lacrimal gland				
acinar brown pigmentation				
minimal	0/2	3/4	0/8	2/8
mild	0/2	1/4	0/8	5/8
moderate	0/2	0/4	0/8	1/8
chronic dacryoadenitis				
minimal	2/2	0/4	3/8	2/8
mild	0/2	2/4	1/8	3/8
moderate	0/2	0/4	0/8	1/8

*doses in mg/kg

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ON ORIGINAL

Histopathology and ophthalmology findings are compared in the following sponsor's tables:

Table 5 : Study number TKD/827. Histopathological and ophthalmological findings - interim kill (week 7).

Group (Animal no)	Ophthalmology grade	Histopathology (LM)
I (10)	0	Posterior cortical fibre swelling - minimal
I (60)	0	Posterior cortical fibre swelling - minimal
II (18)	0	Anterior cortical fibre swelling - minimal Posterior cortical fibre swelling - mild
II (22)	0	Anterior cortical fibre polychromasia - minimal Posterior cortical fibre swelling - mild
II (34)	0	NSAD
II (39)	0	Posterior cortical fibre swelling - mild

NSAD - No salient abnormalities detected

Ophthalmology grade

0 - No abnormalities detected. Lens appears optically clear and homogeneous on direct retroillumination and biomicroscopic examination.

LM = Light microscopy

Table 6 : Study number TKD/827. Histopathological and ophthalmological findings - terminal kill (weeks 35/36).

Group (Animal no)	Ophthalmology grade	Histopathology (LM)
I (24)	0	Posterior cortical fibre polychromasia - minimal
I (32)	2	NSAD
I (47)	0	Anterior cortical fibre swelling - minimal
I (61)	0	NSAD
I (370)	0	NSAD
I (399)	0	NSAD
I (535)	0	NSAD
I (543)	0	NSAD
II (12)	4	Posterior cortical fibre swelling - minimal
II (13)	5	Posterior cortical fibre swelling - minimal
II (44)	5 (slight)	Anterior cortical fibre polychromasia - mild Anterior cortical fibre swelling - minimal Equatorial cortical polychromasia - mild Posterior cortical fibre polychromasia - minimal
II (46)	4	Anterior cortical fibre swelling - mild Posterior cortical fibre polychromasia - minimal
II (59)	5 (faint)	Anterior cortical fibre polychromasia - moderate Anterior cortical fibre swelling - minimal Posterior cortical fibre polychromasia - mild Posterior cortical fibre swelling - moderate

LM = Light microscopy NSAD = No salient abnormalities detected

Continued ...

Group (Animal No)	Ophthalmology grade	Histopathology (LM)
II (67)	4	Posterior cortical fibre polychromasia - mild
II (68)	4	Anterior cortical fibre polychromasia - minimal Posterior cortical fibre swelling - moderate
II (73)	8	Anterior cortical fibre polychromasia - moderate Anterior cortical fibre swelling - mild Posterior cortical fibre swelling - mild

In one drug-treated animal, #59, the thickness of polychromatic (i.e., electron-dense and electron-lucent) lenticular fibers were compared to normochromatic lenticular fibers from 2 CF. The mean fiber thickness of polychromatic fibers were 18-47% less than that of normochromatic fibers.

Ex vivo lens radiolabelling: incorporation of radiolabel into cholesterol was reduced by 58% in lenses from drug-treated animals. Incorporation of radiolabel into lathosterol was not affected. Radiolabelling of an unknown sterol (retention time of 9.6-9.9 min) was increased in drug-treated animals (6-23% for DTF vs 0-5% of total for CF). No differences between grps were detected using uv detection.

Plasma cholesterol analysis: as measured by GC, there was an ≈20% decrease in plasma cholesterol (at Wk 23) in DTF (range for CF: 1.024-1.635 mg/mL; range for DTF: 0.951-1.358). There was one peak in the chromatogram from DTF that was not detected in any from CF. Preliminary analysis (using MS) suggested that this peak represented Δ^8-cholestanol (i.e., 5α-cholest-8-en-3ß-ol), an immediate precursor of lathosterol (sponsor's Fig 6 is presented below).

*"steroid-8-ene isomerase, catalyzes isomerization of Δ^8- to Δ^7 sterols

In DTF, the mean plasma level of Δ^8-cholestanol, expressed as cholesterol equivalents, was 0.075 mg/mL (none was detected in plasma from CF).

Comparison of plasma cholesterol data analyzed by the enzymatic (standard) and GC methods indicated that, for CF, the data were in good agreement (1.393 vs 1.336 mg/mL). For DTF, however, the GC method resulted in levels ≈11% lower than the enzymatic method.

Lens dissolution/sterol analysis: the cholesterol concentration of 4 different fractions of lens was analyzed. The only difference between DTF and CF was a lower concentration of cholesterol in fraction 1 (the outermost layer) in DTF (26%). Two sterols, tentatively identified as Δ^8-cholestanol and lathosterol, were detected in all DTF, but only in 1 CF. The concentrations of these 2 sterols were greatest in fraction 1, and the concentration of Δ^8-cholestanol was

>4-fold greater than that of lathosterol [0.692 and 0.156 cholesterol-eq (μg/% total lens wt), respectively].

The sponsor concluded that the data suggest that ICI 204,636 inhibits the reaction catalyzed by steroid-8-ene isomerase (i.e., conversion of lanosterol to cholesterol), but that more than one metabolic site may be affected.

Dermal irritation

1 **Topical tolerance assessment: in vitro assessment of cytotoxicity and irritant hazard** (Study no. TVN/108, 2/HH/010902, ICI Pharmaceuticals, England, study date: 2/88, GLP, Vol 1.50)

Methods: This study was conducted in two established cell lines, the 3T3 Swiss mouse fibroblast and XB-2 mouse teratomal keratinocyte cell lines. The cells were incubated with ICI 204,636 (ADM 56074/86, for 2-hr to 6 days) at concentrations of 0.1, 1, 10, 100, 200, and 500 μg/mL in DMSO. The following endpoints were measured: (1) LDH release in 3T3 cells, (2) hexosaminidase release in 3T3 cells, (3) Neutral red assay of 3T3 cells (cell survival), (4) Rhodamine B assay of keratin formation in XB-2 cells, (5) Nile blue stain (colony area) of XB-2 cells (cell survival), (6) XB-2 cell number.

Results: The MTC (maximum tolerated concentration) was similar for the 3T3 and XB-2 cells (i.e., 1.0 μg/mL), indicating no differential sensitivity to ICI 204,636. The IC_{50} for cell survival was, however, somewhat lower for XB-2 than for 3T3 cells (34.2 and 50.3 μg/mL, respectively). According to the sponsor, the fact that the IC_{50} for XB-2 is not 50% lower than that for 3T3 cells indicates that "... ICI 204,636 does not score positive in this criterion of specific toxicity to XB-2 cells..."

The IC_{50} for reduced keratin production (54.3 μg/mL) in XB-2 cells was higher than that for cell survival. Therefore, ICI 204,636 did not affect keratin production at relatively non-cytotoxic concentrations.

The IC_{50} for colony area in XB-2 cells was 10-times that for absolute cell number (7.6 and 0.78 μg/mL, respectively). Therefore, absolute cell number is reduced at concentrations which have no effect on colony area. The sponsor concluded that IC 204,636 met the criterion for specific effects on XB-2 stratification.

The IC_{50}'s for enzyme leakage were 100 and 200μg/mL for LDH and hexosaminidase, respectively. Compared to the maximum tolerated concentration of 200 μg/mL of 200 μg/mL, it is clear that, for 3T3 cells, enzyme leakage occurred at non-cytotoxic (or "subcytolethal") concentrations.

Based on these data, the statistical probability of irritant potential was calculated (using logistic regression model) for ICI 204,636, using "0" or "1" for either not meeting or meeting criterion for each of 4 endpoints (i.e., specific XB-2 toxicity, inhibition of keratinization, effect on stratification, and subcytolethal leakage). For example, 4 "0" would give an irritant probability of 0.064, whereas 4 "1" would give an irritant potential of 0.968. The calculated irritant potential ("p") for ICI 204,636 was 0.761. According to the sponsor, this "...derived logistic regression probability....classifies ICI 204,636 as having "...high irritant potential and would be predicted to be a topical irritant in vivo".

2. **Contact sensitisation study in the guinea pig** (Study no. TDG/106, ICI Pharmaceuticals, England, study dates: 9/87-1/88, GLP, Vol 1.52)

The Guinea Pig Maximization assay was used to assess the potential for ICI 204,636 to induce contact sensitization. ICI 204,636 (ADM 56074/86) was administered to male Dunkin Hartley guinea pigs intradermally (0.1-0.2% hydroxypropylmethyl cellulose in 0.1% aqueous polysorbate 80 suspension) and topically (25% suspension in same vehicle). Freunds complete adjuvant was used as a promoting agent. The design of the study and dosing schedule were summarized in the following sponsor's Table 1:

Table 1 : ICI 204,636 : Contact sensitisation study in the guinea pig. Study number TDG/106. Experimental design and dosing schedule.

Group	Induction Intradermal injection	Promoting agent Topical application	Induction Topical application	Challenge Topical application
	Day 1	Day 7	Day 8	Day 22
I	1) Freund's complete adjuvant/distilled water 2) monothioglycerol 3) Freund's complete adjuvant/ monothioglycerol	sodium dodecyl sulphate	monothio-glycerol	monothio-glycerol
II	1) Freund's complete adjuvant/distilled water 2) ICI 204,636 3) Freund's complete adjuvant/ ICI 204,636	sodium dodecyl sulphate	ICI 204,636	ICI 204,636
III	1) Freund's complete adjuvant/distilled water 2) 0.5% w/v HPMC in 0.1% w/v aqueous polysorbate 80 3) Freund's complete adjuvant/0.5% w/v HPMC in 0.1% w/v aqueous polysor-bate 80	sodium dodecyl sulphate	0.5% w/v HPMC in 0.1% w/v aqueous polysorbate 80	ICI 204,636 and monothio-glycerol

1), 2), and 3) refer to pairs of intradermal injections which were administered in two row, one on each side. Animals were shaved prior to dosing on Days 1, 7 and 8. On Day 7, 10% sodium dodecyl sulphate was applied dermally to the area of the intradermal injections. SDS is used to enhance the sensitivity of the assay by producing a "...mild inflammatory reaction". Vehicle or drug application on Day 8 involved occlusion of the application site with a patch made of Whatman filter paper covered by adhesive tape. Patches remained in place to Day 10.

For challenge, skin was shaved and areas treated as noted in the table. Drug or positive control was applied to one side and the vehicle was applied to the other. Treatments were covered and remained so for 24 hrs. Sites were examined on Day 23, and at 24 and 48 hr after patch removal. Sites were graded according to a I-V scale (weak to extreme). Clinical signs and body weights were recorded.

No drug-related clinical signs were noted. One Grp II animal was sacrificed moribund due to rectal prolapse (Day 10). Body weights appeared unaffected. No evidence of contact sensitization was obtained with ICI 204,636. In contrast, 50% of positive control animals exhibited a reaction (Grade III), classifying monothioglycerol as a "moderate" sensitizer.

3. **Topical tolerance assessment. Dermal tolerance study in rabbits** (Study no. TIB/398, 11/HH/010675, ICI Pharmaceuticals, England, study dates: 8/87-11/87, GLP, Vol 1.52)

The dermal irritation potential of ICI 204,636 (ADM 56074/86) was tested in New Zealand White rabbits (3/sex/grp, Sodium dodecyl sulphate (SDS) was used as a positive control. ICI 204,636 (500 mg) or SDS were applied to clipped skin at two sites per animal. The areas were occluded for 6 hr per day. SDS was applied only on Day 1. ICI 204,636 was applied daily on Days 1-5 and 8-12 for a total of 10 applications. Application sites were examined ≈1 and 24 hrs after patch removal. Reactions were scored from 0-4 (no reaction to severe erythema to slight eschar formation) for erythema/eschar formation and from 0-4 (no reaction to severe edema, i.e., >1 mm raised surface extending beyond area of exposure). The two scores for each site were added, then divided by 2 for a maximum total score of 8.

The mean irritation score for ICI 204,636 was 0.47, as calculated by the sponsor. This value included scores at 1 and 24 hr post patch removal. If only 1-hr scores were averaged, the mean irritation score for ICI 204,636 was 0.55 (range: 0.33-0.83). The mean irritation score for 24-hr data was 0.27 (range: 0.17-0.42). The sponsor described the reaction to ICI 204,636 as "...very slight to well defined erythema..." SDS produced reactions characterized by the sponsor as "...very slight to well defined erythema at all twelve application sites", with a moderate-to-severe reaction at one site and no evidence of edema. The sponsor concluded that ICI 204,636 has "...slight irritation potential".

4. **Topical tolerance assessment. Ocular tolerance study in rabbits.** (Study no. TIB/399, 1/HH/010670, ICI Pharmaceuticals, study dates: 9/87-11/87, GLP, Vol 1.52)

The potential for ocular irritation with ICI 204,636 was tested in New Zealand White rabbits . In a preliminary application, ICI 204,636 (ADM 56074/86) was applied to the conjunctival sac of one eye in 1 rabbit. [It was noted that the entire dose (10 mg) was not applied since drug was seen to adhere to the gelatin capsule.] The eye was held closed for 5-10 sec. The eyes were examined 4 time within the first 1 hr after dosing. Ocular lesions were scored using the Draize scale, with the scores for cornea, iris, and conjunctiva added together.

Irritation, characterized as "...redness of the conjunctiva..." was noted with in 2 min of application of ICI 204,636. Chemosis and conjunctival discharge were noted by 31 min postdosing, and slight opacity of the cornea which "....involved a large area..." was detected at 56 min postdosing (as detected using fluorescein dye). The sponsor noted that "A large area of the cornea was noted to retain a faint colour of fluorescein".

Due to the reaction obtained in this one eye, the treated animal was sacrificed and dosing of additional animals was not initiated. The sponsor concluded that ICI 204,636 "..would be a moderate to severe irritant".

CARCINOGENICITY

MOUSE

1. **ICI 204,636: Thirty day pilot (dietary palatability) study in mice** [Study no. TSM/598, 6/ID/1008909, ICI Pharmaceuticals, study dates: 4/89-5/89, **ICI GLP** (not FDA), Vol 1.35].

ICI 204,636 (lot no. not specified) was administered to C57BL/10jfCD-1/Alpk mice (9/sex/grp; 43-45 days old, 13.7-23.3 gm) at doses of 0, 25, 50, 100, and 200 mg/kg in the diet for "at least" 30 days. Animals were housed 3/sex/cage. Concentrations of ICI 204,636 in the diet were adjusted weekly; analysis of diets indicated that the actual mean concentrations were slightly higher than intended (≈105-108%). Observations included the following: clinical signs, body weight, food consumption, and TK (ICI 204,636, ICI 214,227, and ICI 213,841).

There were no unscheduled deaths during the study. Although the sponsor did not consider there to be an effect on body weight, body weight gain was reduced in males at doses ≥ 50 mg/kg (12, 22 and 20% at 50, 100, and 200 mg/kg, respectively) and at 100 and 200 mg/kg in females (33 and 43%, respectively). Food consumption at doses up to 100 mg/kg was fairly similar to C grps, but tended to be slightly higher in HD grps (8-14%). No useful TK data were produced. According to the sponsor, the methods used to quantitate ICI 204,636 were inadequate. The sponsor reported that levels of ICI 204,636 and ICI 213,841 were <LLOQ in all samples, and that ICI 214,227 was detected in most samples, but could be quantitated in only one plasma sample.

2. **ICI 204,636: Ninety day sighting oncogenicity study in mice: dietary administration** (Study no. THM/599, ICI Pharmaceuticals, study dates: 8/90-11/90, GLP, Vol 1.35).

Animals: C57BL/10JfCD-1/Alpk mice
initial age: 44-47 days
initial body weight 12.8-25 g
housing: 5/sex/cage
n = 25/sex/grp

Drug: ICI 204,636 (lot no. not specified)
doses: 0, 50, 100, 200, 300, and 400 mg/kg/day
route: p.o. in diet
formulation: drug concentration adjusted weekly for changes in body weight.
analysis: assays performed to verify drug concentrations. Overall achieved concentrations were 98-102% of intended. During Wk 1 in males, however, achieved concentrations were only 82-83% of intended. In females, achieved concentrations in the 300 mg/kg grp was 89 and 113% of intended at Wk 1 and 3, respectively, and 111% of intended in the 400 mg/kg grp at Wk 8. [These are based on cage intake, not individual.]

Observations

Clinical signs: animals were observed at least twice daily. Full physical examinations were conducted weekly.

Body weight: body weights were recorded prior to dosing, on Day 1, and weekly thereafter.

Food consumption: food consumption was recorded for each cage of animals prior to dosing, and weekly throughout the study.

Water consumption: water consumption was recorded during Wk 11.

Hematology: blood samples were collected on Day 92 from animals not used for TK analysis (n = 8-10/grp) for analysis of the following parameters: hgb, rbc, hct, MCV, MCH, MCHC, platelets, wbc (total, differential).

Clinical chemistry: blood samples were collected on Day 92 from animals not used for TK analysis (n - 8-10/grp) for analysis of the following parameters: glucose, urea, creatinine, total protein, albumin, A/G ratio, alkaline phosphatase, total bilirubin, ALT, AST, Na, K, Cl, Ca, P_i, triglycerides, cholesterol.

TK: blood samples were collected from 3/sex/grp (except C, samples pooled) at each of the following time points: 0600, 0900, 1200, 1800, and 2100 hrs on Day 92. Plasma levels of ICI 204,636, and two metabolites, ICI 213,841 and ICI 214,227 were quantitated using HPLC.

Terminal studies

Gross pathology: a complete necropsy was performed on all animals dying or sacrificed prematurely (when possible) and all survivors, except for those used for TK analysis.

Organ/tissue weights: the following organs/tissues were weighed at necropsy: adrenal glands, brain, heart, kidneys, liver, ovaries, prostate gland, testes/epididymides), uterus.

Histopathology: the following tissues were examined in all C and HD animals: adrenal glands, aorta (arch), bone and marrow (sternum), brain, cervix, eyes, femur, gall bladder, Harderian glands, heart, intestine (jejunum, ileum, colon, cecum), kidneys, liver (left lateral lobe, right median lobe), lung/bronchus, lymph node (cervical/mandibular, mesenteric), mammary gland (inguinal) with overlying skin, muscle (skeletal), nerve (sciatic), esophagus/thyroid/parathyroid/trachea, ovaries, pancreas, pituitary gland, prostate gland (ventral), salivary gland (parotid, submaxillary/sublingual), seminal vesicles, spinal cord (lumbar), spleen, stomach/duodenum, testes/epididymides, thymus, tongue, urinary bladder, uterus, vagina, abnormal tissue.

In addition, liver (left lateral and right median lobes) and testes/epididymides were examined in all dosed grps.

In an addendum (#1), the results of urinary bladder examination (for hyaline droplets and pigment deposits) were provided. This additional examination were conducted (in animals in the 100-400 mg/kg grps) because hyaline droplets and pigment deposits within the epithelium of the urinary bladder were detected in mice dosed (in a separate study) at 800 and 1100 mg/kg.

Results

Mortality: there were 4 unscheduled deaths during the study: 1 F at 50 mg/kg (Day 35, found dead), 1 F at 100 mg/kg (Day 19, found dead), 1 CM (Day 7, unscheduled sacrifice), 1 M at 100 mg/kg (Day 71, unscheduled sacrifice for loss

of function, swelling of hind limbs).

Clinical signs: according to the sponsor, there were no drug-related findings.

Body weight: body weight was not affected at the LD. Body weight gain was reduced, however, at doses >LD. In males, the effect was noted throughout the dosing period, whereas in females, the effect was transient.

In males, final body weights were 3-5% lower at doses ≥100 mg/kg as compared to CM. Overall body weight gain was reduced by 10, 12, 16, and 9% at 100, 200, 300, and 400 mg/kg, respectively.

In females, body weight was lower at 100, 300, and 400 mg/kg (8-5% maximum) than in CF mostly during Wks 1-4. Overall body weight gain was similar among grps.

Food consumption: food consumption (based on cage consumption) tended to be higher in dosed grps than in Cs for both males and females; however, the effects were not necessarily dose-related and in males only sporadically observed.

Water consumption: in males, water consumption was fairly similar among grps. In females, water consumption tended to be higher in dosed grps, with a significant (35%) increase over CF occurring at the HD.

Hematology: the only notable effect was an increase in platelets at all doses; however, the increases were not dose-related (34, 18, 27, 45, and 25% at 50, 100, 200, 300, and 400 mg/kg, respectively).

Clinical chemistry: drug-related effects were noted at all doses as follows: (1) reduced total protein (8, 10, 9, 11, and 12% at 50, 100, 200, 300, and 400 mg/kg, respectively), (2) decreased albumin (4, 6, 5, 8, and 7% at 50, 100, 200, 300, and 400 mg/kg, respectively), (3) increased A/G (8, 9, 8, and 11% at 100, 200, 300, and 400 mg/kg, respectively), (4) increased alkaline phosphatase (7, 11, 14, 16, and 30% at 50, 100, 200, 300, and 400 mg/kg, respectively), (5) increased Na (3-5% at all doses), (6) reduced K at all doses (not dose-related, 30-45%), (7) reduced triglycerides (14, 20, 28, 29, and 45% at 50, 100, 200, 300, and 400 mg/kg, respectively) and cholesterol (30, 38, 52, 65, and 74% at 50, 100, 200, 300, and 400 mg/kg, respectively).

TK: for the parent compound, the highest (pooled) plasma levels were obtained at 2100 hr. In males, the C_{max} was 0.070, 0.080, 0.113, 0.119, and 0.150 µg/mL at 50, 100, 200, 300, and 400 mg/kg, respectively. In females, the peak levels (at 2100 hr) were 0.038, 0.056, 0.087, 0.138, and 0.134 µg/mL, respectively.

For ICI 214,227, the highest plasma levels were also detected at 2100 hr, except in HDF. C_{max} was 0.061, 0.066, 0.083, 0.104, and 0.118 µg/mL in males at 50, 100, 200, 300, and 400 mg/kg, respectively. In females, peak levels were 0.071, 0.079, 0.093, 0.116, and 0.114 µg/mL at 50, 100, 200, 300, and 400 mg/kg, respectively; the C_{max} at the HD occurred at 0600 hr.

For ICI 213,841, limited data were available because of "...unacceptable quality control data". In males, the highest plasma levels were obtained at 2100 hr at 300 and 400 mg/kg (0.256 and 0.276 µg/mL, respectively). In females, peak values were 0.297 µg/mL (0600 hr) at 300 mg/kg and 0.302 µg/mL (2100 hr) at

400 mg/kg.

Note: AUC data were not provided in the original report, but were included in the sponsor's PK/ADME summary in the NDA. The data are summarized in the following sponsor's Table 5-2:

Table 5-2 Mean $AUC_{(0-t)}$ values for ICI 204,636, ICI 214,227 and ICI 213,841 following 91 days of continuous dietary administration of ICI 204,636 to mouse (THM699[1])

DOSE (mg/kg/day)	SEX	ICI 204,636 $AUC_{(0-t)}$ (ng.h/mL)	ICI 214,227 $AUC_{(0-t)}$ (ng.h/mL)	ICI 213,841 $AUC_{(0-t)}$ (ng.h/mL)
50	M	NC	650	NC
	F	NC	780	NC
100	M	670	730	NC
	F	890	910	NC
200	M	1130	920	NC
	F	920	1190	NC
300	M	1180	1080	2700
	F	1210	1280	3230
400	M	1360	1300	3330
	F	1180	1260	3300

1 – Parameter values are not provided in the report, but were subsequently calculated from plasma concentration time data in report.
NC – Not calculated. Plasma concentrations not obtained due to assay difficulty.

Terminal studies

Gross pathology: according to the sponsor, there were no drug-related findings at necropsy.

Organ/tissue weights: in males, reductions were noted in weight of testes and heart. All doses were affected, however, the changes were not dose-related (absolute-relative): testes: 0-4, 9-6, 5-4, 11-4, and 14-11% at 50, 100, 200, 300, and 400 mg/kg, respectively; heart: 7-14, 13-9, 7-8, 11-10, and 15-14% at 50, 100, 200, 300, and 400 mg/kg, respectively. Liver weight was increased in a dose-related manner (absolute-relative): 14-15, 16-14, and 26-24% at 200, 300, and 400 mg/kg, respectively.

In females, the following were noted: (1) reduced adrenal weight at the HD (13-20%), (2) reduced heart weight (absolute-relative): 11-13, 4-11, 10-16, 9-10, and 11-16% at 50, 100, 200, 300, and 400 mg/kg, respectively, (3) increased liver weight (absolute-relative): 8-10, 11-19, 14-20, 22-22, 28-35%, respectively, and (4) reduced relative brain weight at all but the LD (7, 7, 3, and 8% at 100, 200, 300, and 400 mg/kg, respectively.

Histopathology: the primary findings summarized by the sponsor were (1) centrilobular hepatocyte enlargement in males and females at doses ≥200 mg/kg and (2) testicular atrophy (multifocal bilateral tubular) in males, with an increase in severity, but not the total incidence, of the effect being dose-related. The data are summarized in the following sponsor's Table 10:

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC)											
		MALES						FEMALES					
		I 0 mg/kg /day	II 50 mg/kg /day	III 100 mg/kg /day	IV 200 mg/kg /day	V 300 mg/kg /day	VI 400 mg/kg /day	I 0 mg/kg /day	II 50 mg/kg /day	III 100 mg/kg /day	IV 200 mg/kg /day	V 300 mg/kg /day	VI 400 mg/kg /day
LIVER:		(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
Centrilobular hepatocyte enlargement					7	9	10				4	7	10
TESTES:		(10)	(10)	(10)	(10)	(10)	(10)						
Multifocal bilateral tubular atrophy													
mild		8	6	3	4	1							
moderate		1	4	7	6	9	10						
Total		9	10	10	10	10	10						

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified.

Individual histopathology findings were not provided by the sponsor. According to the report, there were histopathological correlates of changes in weight of heart, adrenal gland, or brain.

In the addendum, there was a dose-related increase in epithelial hyaline droplets in males and females (both total incidence and severity). The data are summarized in the following sponsor's Table 1:

Table 1 : Study number THM/599. Incidence of hyaline droplets and pigment in urinary bladder.

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC)											
		MALES						FEMALES					
		I 0 mg/kg /day	II 50 mg/kg /day	III 100 mg/kg /day	IV 200 mg/kg /day	V 300 mg/kg /day	VI 400 mg/kg /day	I 0 mg/kg /day	II 50 mg/kg /day	III 100 mg/kg /day	IV 200 mg/kg /day	V 300 mg/kg /day	VI 400 mg/kg /day
URINARY BLADDER:		(10)		(10)	(10)	(10)	(10)	(10)		(10)	(10)	(10)	(10)
Epithelial hyaline droplets													
minimal					1	4	8	4		2	3	4	3
mild							2					5	6
moderate												1	1
Total incidence for score expanded finding					1	4	10	4		2	3	10	10
Epithelial pigment deposits											1		

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified.

3. **ICI 204,636: Ninety day rising dose sighting study in mice-dietary administration** (Study no. THM/810, ICI Pharmaceuticals, GLP, **but not audited by ICI Quality Assurance**, Vol 1.35)

Animals: mice (strain, supplier not specified)

initial age: 42-43 days
initial body weight: 19-25 g for males, 15.1-19.5 g for females
housing: 5/cage according to sex
n = 15/sex/grp

Drug: ICI 204,636 (lot no. not specified)
doses: 0, 300-800, 400-1100 mg/kg. For the LD grp, doses were increased weekly by 100 mg/kg, until 800 mg/kg, then maintained at 800 mg/kg for the remainder of the study. For the HD grp, doses were increased weekly by 100 mg/kg until Wk 4, then weekly by 200 mg/kg until Wk 6, then maintained at 1100 for the remainder of the study.
route: p.o. (dietary)
analysis: samples of all batches of diet containing drug were taken for verification of drug concentration.

Observation

Clinical signs: animals were observed at least twice daily and a detailed physical examination was conducted weekly.

Body weight: body weights were recorded prior to dosing, on Day 1, and then weekly thereafter.

Food consumption: food consumption was recorded prior to dosing, and weekly during the dosing period.

TK: blood samples were collected from the heart at necropsy (3-4 hr after the start of lights on). At this time, doses were 0, 800, and 1100 mg/kg. Plasma was separated for analysis of IC 204,636; quantitation was performed using HPLC with uv detection.

Terminal studies

Gross pathology: a complete necropsy was conducted on all animals.

Organ/tissue weights: weights of the following organ/tissues were recorded in all animals: adrenal, brain, heart, kidneys, liver, ovaries, prostate gland, spleen, testes/epididymides, thymus, uterus.

Histopathology: the following tissues were examined microscopically in all animals: adrenal, aorta (thoracic), gallbladder, urinary bladder, bone and marrow (sternum), bone marrow (smear), brian, bronchus, cervix, epididymides, eyes, femur, Harderian glands, heart (all chambers), intestine (duodenum, jejunum, ileum, colon, cecum), kidneys, liver (left lateral, right median lobes), lungs, lymph node (mandibular, mesenteric), mammary gland (inguinal), muscle (skeletal), nerve (sciatic), esophagus, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, salivary gland (parotid, submaxillary, sublingual), seminal vesicle, skin (abdominal), spinal cord (lumbar), spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, uterus, vagina, abnormalities.

Results

Mortality: there were no unscheduled deaths.

Clinical signs: according to the sponsor, there were no drug-related clinical signs except for a reduction in hair loss in HDF.

Body weight: body weight was reduced (compared to Cs) in both dose grps. In males, final body weight was 21 and 22% lower than CM at the LD and HD, respectively, and total body weight gain was reduced by 57 and 88%, respectively. In females, body weight was reduced (compared to CF) in the HD grp (11%), and overall body weight gain was reduced in both dose-grps (11 and 42% in LDF and HDF, respectively).

Food consumption: food consumption was reduced in males in both dose grps (maximum of 16-18%) and in HDF (up to 25%).

TK: in females, the plasma levels of ICI 204,636 were <LLOQ at both dose levels. In males, plasma levels were 193 and 94 ng/mL for the 800 and 1100 mg/kg grps, respectively.

Terminal studies

Gross pathology: according to the sponsor, drug-related findings consisted of (1) enlargement of the liver (3/30 at 800 mg/kg and 3/30 at 1100 mg/kg), (2) small ovaries in 2/15 females at 1100 mg/kg, and (3) "thin" uterus in 1/15 at 800 mg/kg and 6/15 at 1100 mg/kg.

Organ/tissue weights: in males, the following were noted: (1) increased liver weight (absolute-relative) in both dose-grps (26-55 and 30-72% at 800 and 1100 mg/kg, respectively), (2) decreased heart weight (26-9 and 26-10% at 800 and 1100 mg/kg, respectively), (3) reduced spleen weight (30-14 and 36-21% at 800 and 1100 mg/kg, respectively), (4) reduced thymus weight at the HD (31-11%), and (5) reduced testes/epididymides weight (27-11 and 33-12% at 800 and 1100 mg/kg, respectively).

In females, the following were noted: (1) increased liver weight (absolute-relative) in both dose-grps (40-21 and 41-63% at 800 and 1100 mg/kg, respectively), (2) reduced heart rate at the HD (14-10%), (3) reduced ovary weight (18-13 and 45-29% at 800 and 1100 mg/kg, respectively), (4) reduced uterus weight (14-17 and 47-42% at 800 and 1100 mg/kg, respectively), (5) reduced spleen weight at the HD (17-11%), and (6) reduced lung weight at the HD (26-14%).

Absolute brain weight was slightly reduced in both dose-grps in males (4%) and in HDF (6%).

Histopathology: individual data were not provided. The sponsor summarized the findings in the following tables (from the sponsor's submission):

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC) MALES I 0 mg/kg /day	MALES II 800 mg/kg /day	MALES III 1100 mg/kg /day	FEMALES I 0 mg/kg /day	FEMALES II 800 mg/kg /day	FEMALES III 1100 mg/kg /day
LIVER:		(15)	(15)	(15)	(15)	(15)	(15)
Diffuse hepatocyte hypertrophy			15	15		15	15
OVARIES:					(14)	(15)	(15)
Reduced corpora lutea						1	11
SALIVARY GLANDS - PAROTID:		(15)	(15)	(15)	(15)	(15)	(15)
Increased basophilia			7	8		4	12
TESTES:		(15)	(15)	(15)			
Seminiferous tubular atrophy							
mild		12					
moderate		2	9	10			
severe		1	6	5			
Total incidence for score expanded finding		15	15	15			

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC) MALES I 0 mg/kg /day	MALES II 800 mg/kg /day	MALES III 1100 mg/kg /day	FEMALES I 0 mg/kg /day	FEMALES II 800 mg/kg /day	FEMALES III 1100 mg/kg /day
URINARY BLADDER:		(15)	(15)	(15)	(15)	(15)	(15)
Multifocal epithelial hyaline droplets							
minimal			5	8			10
mild			3	5		7	4
moderate			3			8	
Total incidence for score expanded finding			11	13		15	14
Epithelial pigment deposits							
minimal			2				
mild			1				1
moderate				1			
Total incidence for score expanded finding			3	1			1

Figures in brackets represent the number of animals from which this tissue was examined microscopically. The absence of a numeral indicates that the lesion specified was not identified.

4. **ICI 204,636: Two year oncogenicity study in mice: dietary administration**
(Study no. TCM/600, Zeneca Pharmaceuticals, study dates: 10/92-10/94, GLP, Vol 1.36-1.41)

Animals: C57BL/10J$_f$CD01/Alpk mice
initial age: 43-48 days
initial body weight: 14.8-24.7 g for males, 13.3-20.4 g for females
housing: 5/cage according to sex
n = for main study, 100/sex for C grps, 50/sex/grp for dosed grps; for PK study, 20/sex/grp

Drug: ICI 204,636 (analytical reference no. ADM44039/92, batch 17E)
doses: 0, 20, 75, 250, 250-750 mg/kg (Grps I, II, II, IV, and V, respectively). In the rising dose grp, the dose was increased by 50 mg/kg each week up to 750 mg/kg (considered the MTD based on body weight). Achieved doses were calculated weekly for the first 13 weeks and every 4 wks thereafter; calculations were based on group mean body weight and group mean food consumption.
formulation: diet-drug admixtures were adjusted for "predicted" body weight and food consumption changes weekly for the first 13 wk, then every 4 wks throughout

the remainder of the study.

route: p.o. (in diet)

analysis: the homogeneity of the dietary admixture was assessed in a separate study. According to the sponsor, the drug was homogeneously distributed into the pelleted diet and was stable for 6 wks.

The sponsor did indicate that the "...early batches..." of drug-diet mixtures were stored for up to 2 yrs prior to analysis, and subsequent analysis indicated drug concentrations on 50-60% of intended. It was determined that the drug was not stable in the diet for this period of time. Therefore, the actual drug concentration of the diet was determined "...by modelling the actual data and generating an appropriate correction factor for each result". Using this technique, the sponsor was convinced that "...these batches contained the required concentration of test article".

Using these analyses, the achieved doses were calculated to be 83-120% of intended throughout the dosing period (from Wk 11 on for the rising dose grp).

duration: 104 wks

Observations (note: satellite PK animals were used only for TK analysis)

Clinical signs: animals were observed "...at least twice daily...". In addition, a detailed physical examination (including palpation for masses) was conducted weekly on all main study animals.

Ophthalmology: ophthalmology examinations were performed on 20/sex/grp during Wk 3, and in Grp I, IV, and V survivors during Wks 21-22, 46, 73, and 93. Examinations were conducted using a direct ophthalmoscope and following dilation with 1% tropicamide (MYDRIACYL).

Body weight: body weights were recorded prior to dosing, on Day 1, weekly for the first 17 wks, and then every 4 wks for the rest of the dosing period.

Food consumption: food consumption was recorded daily for the first 13 wks, then daily for 1 wk in 4 for the rest of the dosing period.

Water consumption: water consumption was recorded daily for 1 wk for 20 C cages and 10 cages/grp for DT animals during Wks 63, 75, and 91.

Hematology: blood samples were collected at sacrificed (scheduled and moribund) for analysis of the following parameters: rbc and total wbc. Blood films were also prepared and examined if considered necessary.

TK: blood samples were collected from 3/sex/grp (for each sampling day) in drug-treated animals, 3-4 hr after the start of the light phase of the cycle during Wks 5, 26, 51, 76, and 103; samples were pooled for Day 32 due to insufficient plasma volume. Plasma levels of ICI 204,636, ICI 213,841, and ICI 214,227 were quantitated using LL extraction, and HPLC with uv detection.

Terminal studies

Gross pathology: a complete necropsy was performed on all main study animals, including those found dead.

Histopathology: the following tissues were examined microscopically in all animals: adrenal glands, aorta, urinary bladder, bone and marrow (sternum),

brain, bronchus, cervix, epididymides, eyes, femur, gall bladder, Harderian glands, heart (all chambers), intestine (duodenum, jejunum, ileum, colon, cecum), kidneys, liver (left lateral, right median lobes), lungs, lymph nodes (mandibular and mesenteric), mammary gland (inguinal), muscle (skeletal), nerve (sciatic), esophagus, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, salivary gland (parotid, sublingual, submaxillary), seminal vesicle, skin (abdominal), spinal cord (lumbar), spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, uterus, vagina, macroscopic abnormalities.

Tissue were fixed in 10% buffered formalin, except for eyes and Harderian glands which were fixed in Davidson's fixative. Sections were stained with H & E for microscopic examination. In addition, sections of thyroid were stained with Masson-Fontana stain.

Statistics: for statistical analysis, lymphoma, histiocytic sarcoma, malignant lymphoma lymphoblastic, angiosarcoma, and angiosarcoma/angioma were combined across tissues.

Results

Mortality: the cumulative survival rates are summarized in the following sponsor's table:

Males Weeks	Number of animals per group 100	50	50	50	50
	Group I 0 mg/kg/day	Group II 20 mg/kg/day	Group III 75 mg/kg/day	Group IV 250 mg/kg/day	Group V 750 mg/kg/day
1-26	0	0	0	0	1
27-52	2	1	2	1	2
53-65	3	1	2	2	3
66-78	9	7	5	3	6
79-91	17	12	15	11	10
92-104	52	27	26	20	16
105-108*	58	29	27	22	16
survival % to necropsy	48%	46%	48%	60%	68%
Females					
1-26	1	0	0	0	0
27-52	2	0	3	0	6
53-65	5	3	4	2	8
66-78	10	6	8	5	12
79-91	24	11	11	10	16
92-104	48	20	23	23	23
105-108*	50	21	25	26	27
survival % to necropsy	52%	60%	54%	54%	54%

* Necropsy phase

In males, survival rates were highest in Grp IV and V. In females, survival rates were fairly similar among grps, with the highest survival rate being in Grp II (LDF).

Clinical signs: according to the sponsor, there were no drug-related clinical signs, nor were there any drug-related findings upon veterinary examination.

Palpable masses: the data are summarized in the following sponsor's table:

Weeks	Number of animals per group: 100	50	50	50	50
	Group I 0 mg/kg/day	Group II 20 mg/kg/day	Group III 75 mg/kg/day	Group IV 250 mg/kg/day	Group V 750 mg/kg/day
Males					
1-26	0	0	0	0	0
27-52	0	0	0	0	0
53-65	2	4	0	1	0
66-78	11	10	6	4	3
79-91	20	18	14	15	7
92-104	29	22	18	19	9
% Total	29	44	36	38	18
Females					
1-26	0	0	0	0	0
27-52	0	0	0	2	0
53-65	4	2	1	5	2
66-78	13	8	4	8	3
79-91	26	13	12	13	7
92-104	40	20	20	22	14
% Total	40	40	40	44	28

There were no consistent trends in the incidences, except that the lowest incidences were in Grp V males and females.

Ophthalmology: there were no drug-related findings, according to the sponsor. [Findings were not summarized.]

Body weight: the data are presented in the sponsor's Figs. 1-4 (attached).

In males, body weight was reduced (compared to CM) at all doses, however, only consistently in Grps IV and V. In Grp II, body weight was reduced by 2-8% during Wks 56-96. In Grp II, a similar reduction in body weight was noted at Wks 9-96. In Grps IV and V, reductions (compared to CM) were noted throughout the dosing period (2-9 and 4-18%, respectively). Final body weights were 7 and 12% lower than CM in Grps IV and V, respectively. Overall body weight gain was reduced in all dose grps: 6, 6, 12, and 33% in Grps II, III, IV, and V, respectively.

In females, body weight was reduced (compared to CF) consistently on in Grp V (3-9%); in Grp IV, body weight was reduced primary during Wks 2-11 and Wks 64-92 (2-6%). Final body weights were reduced (compared to CF) only in Grp V (9%). Overall body weight gain was reduced in Grps III, IV, and V (9, 9, and 22%, respectively).

Food consumption: food consumption (based on intake/cage) was not consistently affected at any dose in either males or females. In Grp V males, food consumption was reduced (up to 14%) primarily during Wks 3-44 and in Grp V females, food consumption was reduced (up to 15%) primarily during Wks 3-32.

Water consumption: in males, there was a dose-related increase in water consumption at Wks 63 and 75 (8-12, 12-21, 16-38, and 40-88% in Grp II, III, IV, and V, respectively). At Wk 91, however, water consumption was only increased in Grp V (68%).

In females, water consumption elevated in dosed grps, but not in a dose-

dependent manner. The increase was highest (66-78%) and consistently observed only in Grp V.

Hematology: rbc count was reduced in Grp IV and V males (7 and 11%, respectively). Rbc counts in females were fairly similar among grps, as were wbc counts in both males and females

TK: with only one exception (Wk 5, ICI 213,841), plasma levels of ICI 204,636, ICI 213,841, and ICI 214,227 were <LLOQ (i.e., 20, 50, and 30 ng/mL, respectively) in Grps II and III.

In Grps IV and V, only sporadic data are available. The data were summarized in the following sponsor's table:

Compound	Week	Dose Level							
		20 mg/kg/day		75 mg/kg/day		250 mg/kg/day		750 mg/kg/day	
	Sex	M	F	M	F	M	F	M	F
ICI 204,636	5	NC	NC	NC	NC	NC	NC	106±NC	71.0±NC
	51	NQ	NQ	NQ	NQ	NQ	NQ	107±NC	33.2±NC
	76	NQ	NQ	NQ	NQ	NQ	NC	26.9±NC	NC
	103	NQ	NQ	NC	NQ	24.0±3.6	NC	NQ	20.5±0.5
ICI 213,841	Sex	M	F	M	F	M	F	M	F
	5	NC	NC	49.2±NC	67.9±NC	70.9±NC	130±NC	217±NC	121±NC
	51	NQ	NQ	NQ	NQ	NQ	55.3±5.3	83.5±33.5	NQ
	76	NQ	NQ	NQ	NQ	NQ	149±NC	NC	124±NC
	103	NQ	NQ	NQ	NQ	NC	NQ	NQ	74.6±24.6
ICI 214,227	Sex	M	F	M	F	M	F	M	F
	5	NC	NC	NC	NC	NC	NC	NC	NC
	51	NQ	NQ	NQ	NQ	NQ	38.2±0.2	42.0±NC	54.7±21.2
	76	NQ	NC	NC	NC	NC	88.6±NC	NC	67.4±NC
	103	NQ	NQ	NC	NQ	33.7±2.1	NC	42.6±8.1	48.7±17.5

Note: Week 5 animals for Group V were dosed at 450 mg/kg/day.
NC = Not calculable.
NQ = Not quantifiable; less than quantitation limit of 20.0, 30.0 or 50.0 ng/ml for ICI 204,636, ICI 214,227 or ICI 213,841, respectively.

Terminal studies

Cause of death: overall summary incidence tables were not provided. According to the sponsor, there were no differences among grps in terms of cause of death (neoplastic or non-neoplastic).

Gross pathology: the incidences of discoloration of the kidney and bilateral firmness of the kidney were increased in Grp IV and Grp V males (discoloration:

7/100, 10/50, 7/50, 11/50, and 17/50 in Grp I, II, III, IV, and V, respectively; firmness: 0/100, 0/50, 0/50, 1/50,and 6/10 in Grp I, II, III, IV, and V, respectively). In females, the incidence of thin uterus was dose-related (2/100, 0/50, 3/50, 5/50, and 9/50 in Grp I, II, III, IV, and V, respectively).

Histopathology: the total tumor incidence is summarized in the following sponsor's table:

TUMOUR TABLE

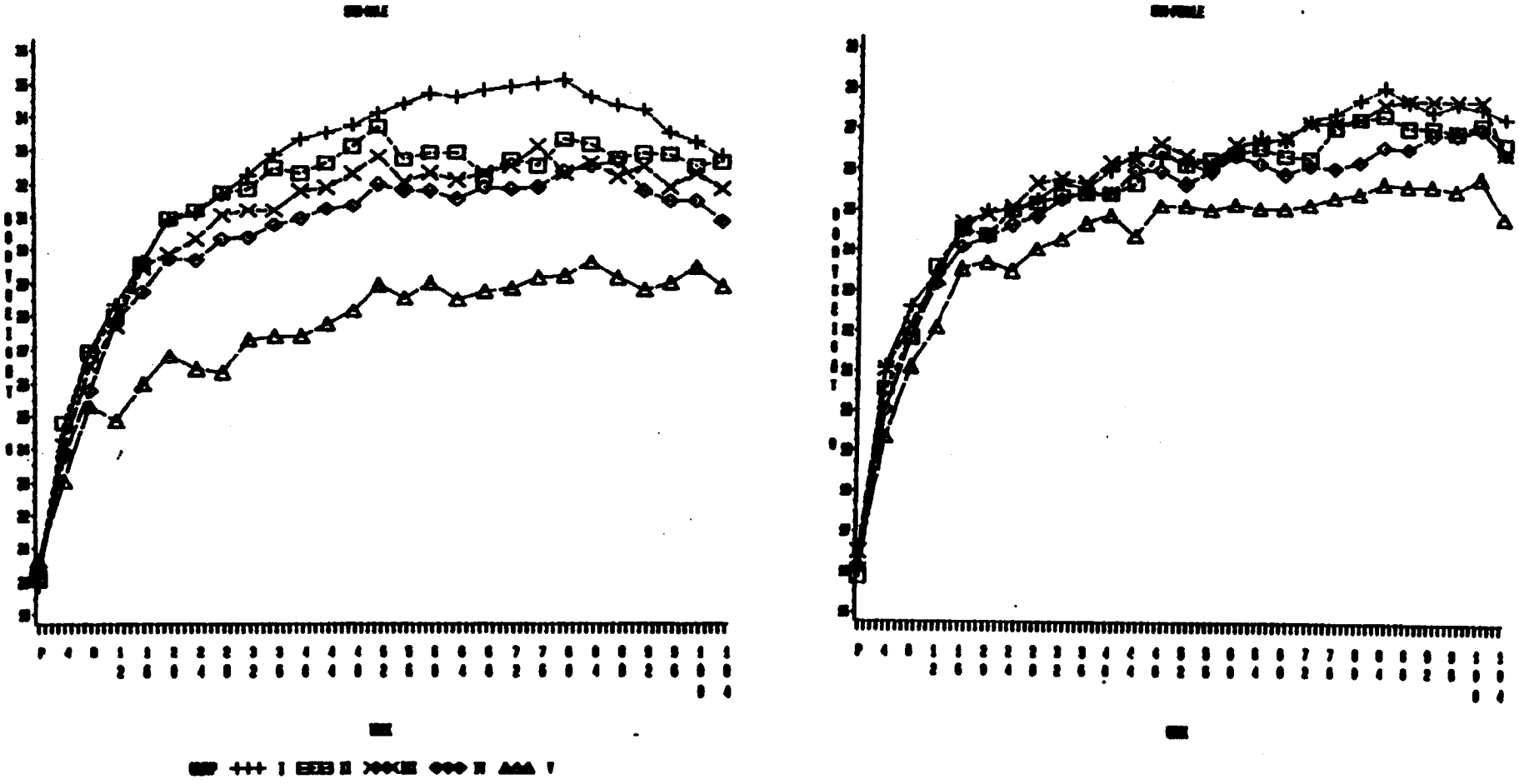
GROUP	MALES I 0 mg/kg /day	II 20 mg/kg /day	III 75 mg/kg /day	IV 250 mg/kg /day	V 750 mg/kg /day	FEMALES I 0 mg/kg /day	II 20 mg/kg /day	III 75 mg/kg /day	IV 250 mg/kg /day	V 750 mg/kg /day
NUMBER OF ANIMALS:	100	50	50	50	50	100	50	50	50	50
NO. OF ANIMALS WITH TUMOURS	80	40	38	41	44	93	46	44	43	41
NO. OF ANIMALS WITH SINGLE TUMOURS	61	28	28	33	18	57	24	26	26	27
NO. OF ANIMALS WITH MULTIPLE TUMOURS	19	12	10	8	26	36	22	18	17	14
NO. OF ANIMALS WITH BENIGN TUMOURS	15	14	7	8	31	32	18	11	11	9
NO. OF ANIMALS WITH MALIGNANT TUMOURS	78	36	36	39	39	90	46	44	41	40
TOTAL NUMBER OF TUMOURS	108	55	48	53	74	141	78	67	65	55
TOTAL NUMBER OF BENIGN TUMOURS	22	15	8	10	35	41	25	14	14	9
TOTAL NUMBER OF MALIGNANT TUMOURS	86	40	40	43	39	100	53	53	51	46
% ANIMALS WITH TUMOURS	80	80	76	82	88	93	92	88	86	82
% ANIMALS WITH SINGLE TUMOUR	61	56	56	66	36	57	48	52	52	54
% ANIMALS WITH MULTIPLE TUMOURS	19	24	20	16	52	36	44	36	34	28
% ANIMALS WITH BENIGN TUMOURS	15	28	14	16	62	32	36	22	22	18
% ANIMALS WITH MALIGNANT TUMOURS	78	72	72	78	78	90	92	88	82	80

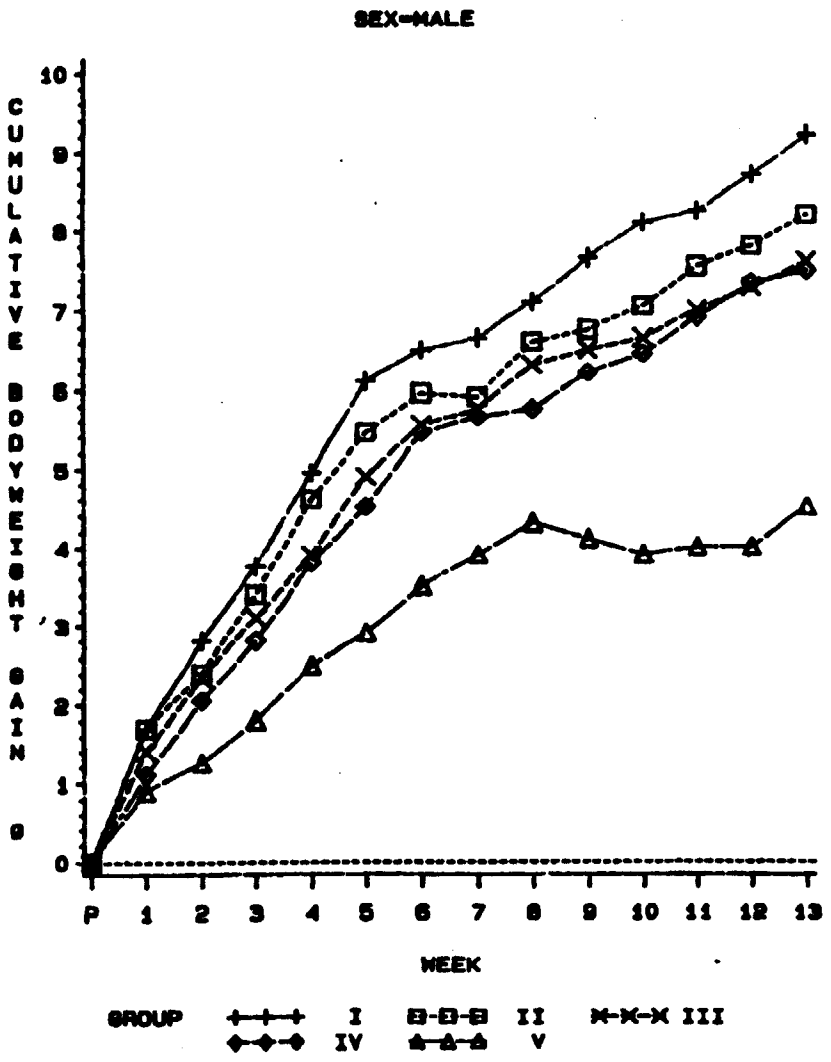
The incidences of selected non-neoplastic and neoplastic findings are summarized in the attached table.

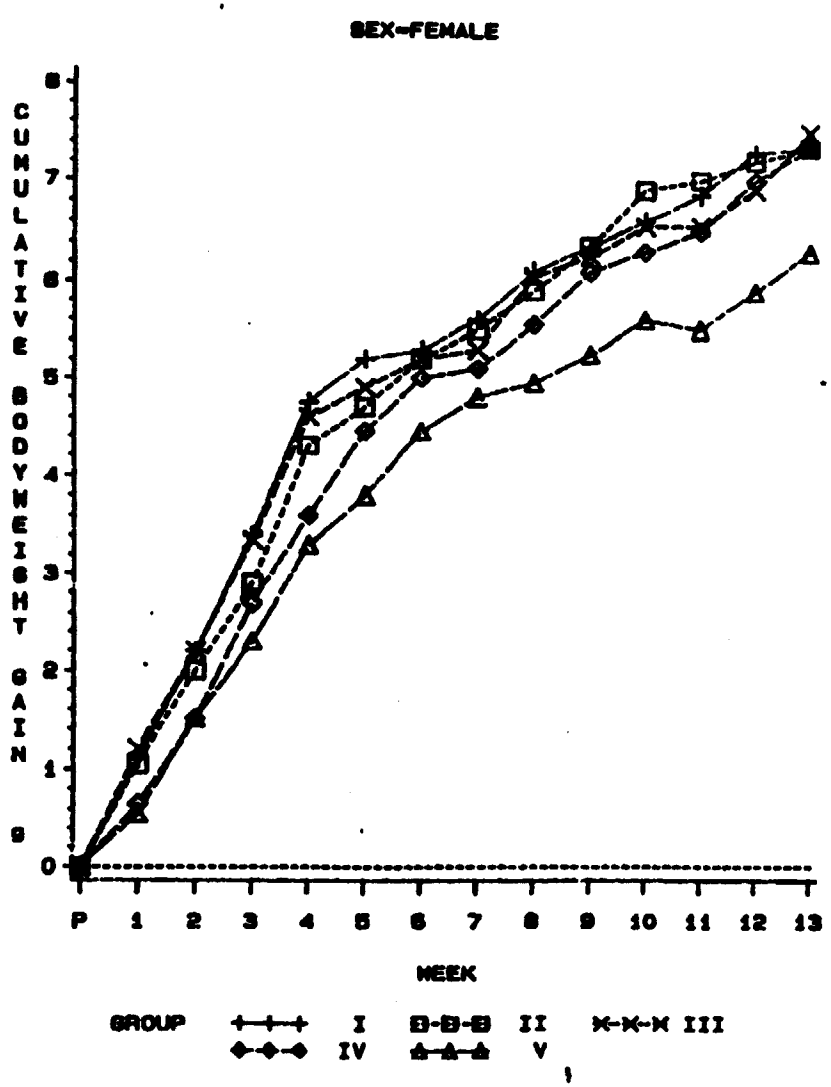
For the one neoplastic finding (in males) considered to be drug-related, the majority of thyroid follicular adenomas were detected in male survivors (3/30 Grp IV, 27/34 Grp V) rather than in male premature decedents (1/20 Grp IV, 2/10 Grp V).

No mammary gland neoplasms were reported in any animal examined. The incidences of non-neoplastic mammary gland changes (glandular cystic hyperplasia and glandular hyperplasia) were not clearly related to drug (none were detected in males):

FINDING	GRP 1F	GRP IIF	GRP IIIF	GRP IVF	GRP VF
Mammary gland					
glandular cystic hyperplasia	9/100	4/50	6/50	2/50	1/50
glandular hyperplasia	52/100	31/50	32/50	37/50	28/50







RAT

1. **ICI 204,636: Two year oncogenicity study in rats oral administration** (Study no. TCR/1624, Zeneca Pharmaceuticals, study dates: 7/92-7/94, GLP, Vol 1.43-1.49)

Animals: Wistar rats
initial age: 36-42 days
initial body weight: 141-298 gm for males, 146-218 gm for females
housing: 5/cage by sex
diet: *ad lib* except during urine collection periods
water: *ad lib*
n = 100/sex for C grps, 50/sex/grp for drug-treated grps

Drug: ICI 204,636 (ADM44005/91, ADM44039/92)
analysis: the drug substance was analyzed for identity, strength, and purity. Stability of drug suspension was demonstrated for 42 days at 4° C. Samples were taken during Wks 1, 13, 27, 52, and 104 for analysis of actual concentration.
drug formulation: suspension in 0.5 w/v hydroxypropyl methylcellulose in 0.1% w/v aqueous polysorbate 80 at concentrations of 0, 4.6, 17.25, and 57.50 mg/mL.
dosing volume: 0.5 mL/100 gm
doses: 0, 20, 75, and 250 mg/kg
route: p.o. (gavage)
duration: 729 consecutive days, 1 dose/day

Observations

Clinical signs: animals were observed at least twice per day, and, in addition, were examined by a veterinary surgeon as considered necessary.

Ophthalmology: all animals were examined prior to the start of dosing. All survivors in Grps I and IV were also examined during Wks 83 and 102 of dosing. For examination, pupils were dilated with tropicamide (MYDRIACYL, Alcon).

Body weight: body weights were recorded in all animals prior to the start of dosing, and in survivors on Day 1, weekly for the first 12 wks, then once every 4 wks during the rest of the dosing period.

Food consumption: food consumption was recorded daily for the first 12 wks of dosing, and then once every 4 wks for the rest of the dosing period.

Hematology: blood samples were collected (prior to the daily dose) from 10/sex/grp (except during Wk 104) for analysis of the following parameters: hgb, rbc, hct, MCV, MCHC, MCH, rbc distribution width, wbc (total, differential), rbc and wbc morphology, platelet count. In addition blood films were prepared and examined if considered necessary.

In animals sacrificed moribund, blood samples were collected at sacrifice for total rbc and wbc counts.

Clinical chemistry: blood samples were collected from 10/sex/grp (except during Wk 104-105) for analysis of the following parameters: visual inspection for hemolysis, glucose, urea, total protein, albumin, A/G ratio, bilirubin, ALT, AST, alkaline phosphatase, Na, K, Cl, Ca, P_i, cholesterol, triglycerides, and creatinine.

Urinalysis: urine samples were collected overnight (over 16 hrs) from 10/sex/grp (when possible) during Wk 104 for analysis of the following parameters: volume, specific gravity, color, appearance, glucose, protein, ketones, bilirubin, blood, pH, Na, K, creatinine, and microscopic examination of sediment.

TK: blood samples were collected from 3/sex in Grps II, III, and IV at 30 min postdosing on Day 1 and during Wk 26, 52, 78, and 104 for quantitation of ICI 204,636, ICI 213,841, and ICI 214,227.

Data for the parent compound, collected on Day 1 and Wk 26, were considered unacceptable [using SOP 35.H(S)39,41&42(P.2)]. A new method (Method 16-09) was developed and used to quantitate the plasma concentration of all three compounds in blood samples collected during Wks 52, 78, and 104.

Terminal studies

Gross pathology: a complete necropsy was conducted in all animals. A bone marrow smear (from femoral) was prepared, but not examined.

Organ/tissue weights: the weights of the following organs/tissues were recorded in 10/sex/grp (survivors only): adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes/epididymides, and uterus. Tissue containing obvious masses was not weighed.

Histopathology: the following tissues were examined microscopically in all animals: adrenal glands, aorta, urinary bladder, bone and marrow (sternum), bone marrow (smear), brain, bronchus, cervix, epididymides, eyes, femur, Harderian glands, heart (all chambers), intestine (duodenum, jejunum, ileum, colon, cecum), kidneys, liver (left lateral, right median lobes), lungs, lymph node (mandibular, mesenteric), mammary gland (inguinal), muscle (skeletal), nerve (sciatic), esophagus, ovaries, pancreas, parathyroid glands, pituitary gland, prostate gland, salivary gland (parotid, submaxillary, sublingual), seminal vesicle, skin (abdominal), spinal cord (lumbar), spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, uterus, vagina, all macroscopic abnormalities.

All tissues were preserved in 10% buffered formalin, except for eyes and Harderian glands (Davidson's fixative), and stained with H & E for examination.

Special procedures: the following additional analyses were conducted:

1. thyroid glands: sections stained using the Masson Fontana technique and the average "height" of follicular epithelial cells were graded subjectively (scale: grade 1-5). According to this scale, grades of 1, 2, 3, 4, and 5 were given when the average cell height thickness was equal to 0.5, 1, 1.5, 2, and 2.5 nuclei, respectively.

2. pituitary glands: in 3 M and 4 F per grp (Grps I-IV), sections of pituitary were examined using immunocytochemistry for detection of FSH, LH, ACTH, prolactin, GH, and TSH.

3. adrenal glands: "A number of adrenal glands..." were stained using the Masson Fontana technique and by immunocytochemistry for neuron-specific enolase (NSE), according to the sponsor, "...to aid diagnosis of cortical and medullary tumours/foci."

2-yr mouse carcinogenicity study, non-neoplastic and neoplastic findings

TISSUE	FINDING	MALES					FEMALES				
		0	20	75	250	750	0	20	75	250	750
NON-NEOPLASTIC											
Kidney	cortical tubular basophilia										
	minimal	28/100	11/50	10/50	4/50	4/50	5/100	1/50	4/50	5/50	10/49
	mild	30/100	15/50	17/50	23/50	31/50	2/100	1/50	1/50	1/50	6/49
	cortical microlithiasis										
	minimal	5/100	0/50	2/50	1/50	2/50	10/100	13/50	20/50	30/50	35/49
	mild	94/100	0/50	48/50	48/50	46/50	0/100	0/50	0/50	3/50	8/49
Salivary gland	acinar basophilia										
	mild	1/96	0/49	2/49	7/50	27/44	0/99	0/49	3/49	9/50	24/47
	moderate	0/96	0/49	1/49	0/50	11/44	0/99	0/49	0/49	1/50	8/47
Thyroid gland	hypertrophic follicular epithelium										
	minimal	1/97	0/49	0/50	1/50	1/44	1/100	0/50	1/50	1/50	1/47
	mild	1/97	0/49	3/50	4/50	24/44	2/100	0/50	0/50	1/50	4/47
	moderate	0/97	0/49	0/50	0/50	4/44	0/100	0/50	0/50	0/50	0/47
	follicular cell hyperplasia										
	mild	0/97	0/49	0/50	4/50	13/44	3/100	0/50	1/50	0/50	0/47
	moderate	0/97	0/49	0/50	1/50	16/44	0/100	0/50	0/50	1/50	0/47
	follicular cell fine pigment in Masson-Fontana stain										
	minimal	24/97	14/49	14/50	20/50	7/44	15/100	7/50	11/50	9/50	13/47
	mild	3/97	1/49	6/50	20/50	27/44	1/100	2/50	5/50	8/50	22/47
	moderate	0/97	0/49	1/50	0/50	0/44	0/100	0/50	0/50	0/50	1/47
	follicular cysts	4/97	7/49	3/50	12/50	11/44	19/100	14/50	10/50	15/50	12/47
	basophilic follicular colloid	0/47	0/49	0/50	0/50	5/44	0/100	0/50	0/50	0/50	0/47
Urinary bladder	transitional epithelial hyaline droplets										
	minimal	0/100	1/50	0/49	1/50	25/48	9/100	1/48	4/48	26/50	29/48
	mild	0/100	0/50	0/49	0/50	3/48	0/100	0/48	0/48	1/50	6/48
Uterus	atrophy										
	mild						1/100	0/50	1/50	3/50	2/50
	moderate						0/100	0/50	0/50	0/50	4/50
NEOPLASTIC											
Thyroid gland	follicular adenoma	0/97	0/49	0/50	4/50**	29/44***	1/100	2/50	0/50	1/50	2/47

*p<0.05, **p<0.01, ***p<0.001

Results

Mortality: the mortality data are summarized in the following sponsor's table:

Week	Group and sex			
	I M Control 0 mg/kg/day (100)	II M 20 mg/kg/day (50)	III M 75 mg/kg/day (50)	IV M 250 mg/kg/day (50)
1-26	0	1	4	3
27-39	0	3	6	5
40-52	11	3	11	11
53-65	18	4	14	11
66-78	31	10	18	13
79-91	47	20	23	19
92-104	66	34	33	32
Surviving to scheduled necropsy	34	16	17	18

Week	Group and sex			
	I F Control 0 mg/kg/day (100)	II F 20 mg/kg/day (50)	III F 75 mg/kg/day (50)	IV F 250 mg/kg/day (50)
1-26	0	1	5	6
27-39	1	2	6	6
40-52	4	2	8	6
53-65	8	5	9	8
66-78	20	11	12	10
79-91	36	16	21	20
92-104	58	23	32	29
Surviving to scheduled necropsy	42	27	18	21

( ) Number of animals in group

In terms of % survival, survival in males was similar among grps (34, 32, 34, and 36% in CM, LDM, MDM, and HDM, respectively). In females, the % survival was slightly higher in LDF(54%) and slightly lower in MDF (36%) than in either CF or HDF (42% in both grps). That is, in neither males or females was there a dose-related effect on mortality.

Clinical signs: according to the sponsor, sedation (subdued/drowsy) and excessive salivation were observed in all dosed grps throughout the dosing period. The severity of sedation was dose-related and persisted for several hours after dosing. Urinary incontinency was observed from Wk 23 on in HDM and HDF; urogenital staining was evident after dosing, but not by the next morning (pre-dosing).

Selected findings (taken from the sponsor's summary table; data expressed in "animal days") are as follows (note:...100/sex/grp for C grps, 50/sex/grp for DT

grps):

FINDING	DAYS	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MD	HD
urine staining	1-365	9	2	9	122	6	26	40	375
	366-742	50	70	21	100	78	89	364	1037
malocclusion of teeth	1-365	55	32	111	122	25	1	0	4
	366-742	22	110	112	83	111	42	2	8
stains around muzzle	1-365	1	4	0	8	1	0	2	0
	366-742	11	3	3	4	19	8	1	35
dark deposits on tail	1-365	0	0	72	261	0	0	0	0
	366-742	90	44	486	1107	0	0	0	0
subdued	1-365	1	0	1	3	4	0	0	0
	366-742	33	5	5	20	22	8	11	45
salivation	1-365	0	1	0	0	0	0	0	0
	366-742	0	0	0	1	0	0	0	0
corneal opacity-both eyes	1-365	0	0	0	0	0	0	0	0
	366-742	14	7	3	21	0	0	0	0
noisy breathing	1-365	1	0	2	0	1	0	1	1
	366-742	1	1	2	2	1	4	3	22

The reason for the discrepancy between the narrative and the incidence tables for sedation and salivation is unknown.

Palpable masses: the data are summarized in the following sponsor's tables:

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Group and sex	Number of animals developing their first palpable mass* in weeks				Total number of animals with masses
	1-26**	27-52	53-79	80-106	
I M	2/48	11/48	20/48	15/48	48/100 (48%)
II M	1/25	6/25	8/25	10/25	25/50 (50%)
III M	0/23	10/23	4/23	9/23	23/50 (46%)
IV M	3/23	12/23	5/23	3/23	23/50 (46%)
I F	0/61	9/61	27/61	25/61	61/100 (61%)
II F	0/34	3/34	11/34	20/34	34/50 (68%)
III F	1/32	3/32	10/32	18/32	32/50 (64%)
IV F	0/30	7/30	9/30	14/30	30/50 (60%)

* Expressed as a fraction of the number of animals developing masses.
** Time (weeks) of first recording of the palpable masses.

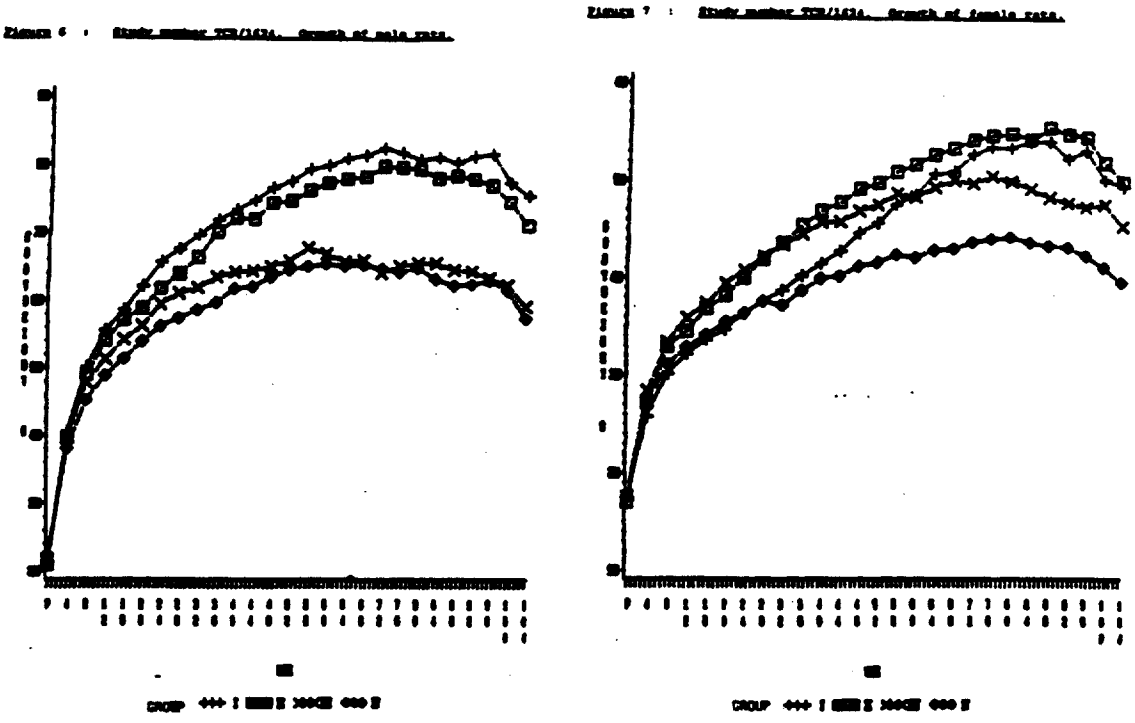
Group and sex	Number of palpable masses per animal*					
	0	1	2	3	4	>4
I M	52/100	36/48	6/48	5/48	0/48	1/48
II M	25/50	14/25	7/25	3/25	1/25	0/25
III M	27/50	9/23	11/23	2/23	0/23	1/23
IV M	27/50	10/23	7/23	4/23	2/23	0/23
I F	39/100	32/61	19/61	9/61	1/61	0/61
II F	16/50	11/34	15/34	3/34	4/34	1/34
III F	18/50	16/32	4/32	8/32	3/32	1/32
IV F	20/50	20/30	6/30	1/30	3/30	0/30

* Expressed as a fraction of the number of animals with masses, except for animals with no masses, which are expressed as a fraction of the number of animals per group.

There was no significant difference among grps in terms of either the % of animals with palpable masses or in the no. of masses/animal/grp.

Ophthalmology: according to the sponsor, there were no drug-related findings.

Body weight: the data are presented in the following sponsor's figs:



In males, body weight was reduced (compared to CM) at all doses. At the MD and HD, lower body weight was consistently observed from Wk 2-5 on. At the LD, the effect was smaller (2-6%) and more sporadic. Overall body weight gain was reduced at all doses, but significantly only at the MD and HD (5, 30, and 34% at LD, MD, and HD, respectively).

In females, body weight was reduced (compared to CF) at the MD and HD. At the MD, body weight was comparable to CF values until Wk 84, whereas at the HD, lower body weight was evident by Wk 44. Reductions in overall body weight gain were 13 and 29% at the MD and HD, respectively.

Food consumption: food consumption was similar among grps in males. In females, food consumption was increased at all doses (non-dose related, 3-17%) during the first 4 wks of the dosing period. During the rest of the dosing period, food consumption was reduced only sporadically at the HD.

Hematology: small changes (2-12%) were noted in a number of rbc parameters: (1) decreased hgb and rbc in HDF, (2) reduced rbc width at all doses in females (not dose-related), (3) increases in MCV in males and females at all doses (not dose-related), and (4) increased MCH at all doses in males and at the MD and HD in females.

In wbc parameters, the following were noted: (1) reduced total wbc count at all doses in females (12, 27, and 26% at LD, MD, and HD, respectively), (2) decreased lymphocyte count in MDM and HDM (39 and 17%, respectively), (3) reduced monocyte count at all doses in males (11, 27, and 27% at LD, MD, and HD, respectively), and (4) a decrease un large unnucleated cells in MD and HD males (49% at both doses).

Clinical chemistry: the primary findings were decreases in cholesterol and triglycerides in HDM, MDF, and HDF (cholesterol: 50, 19, and 46%, respectively; triglycerides: 32, 23, and 61%, respectively). Also, small increases (1-5%) were noted in total protein in males and females at all doses (dose-related).

Urinalysis: the following were noted: (1) increased urinary volume in males at all doses (5, 26, and 47% at LD, MD, and HD, respectively), (2) increased Na in HDF (2.3-fold), (3) increased K at all doses in females (31, 49, and 73% at LD, MD, and HD, respectively), and (4) increased creatinine in MDF and HDF (63 and 55%, respectively).

TK: the data are summarized in the following sponsor's table (data expressed means ± SEM, in units of ng/mL); NC indicates the lack of sufficient sample to assay):

COMPOUND	DAY/ WEEK	DOSE LEVEL		
		20 mg/kg/day	75 mg/kg/day	250 mg/kg/day
ICI 204,636	Day 1	149 ± 60.6	339 ± 124	754 ± 303
	Week 26	779 ± 203	1360 ± 323	1630 ± 630
	Week 52	150 ± 45.9	1240 ± 197	1210 ± 136
	Week 78	107 ± 20.7	973 ± 168	1140 ± 303
	Week 104	199 ± 65.0	1270 ± 361	1880 ± 208
ICI 214,227	Day 1	NC	NC	NC
	Week 26	NC	NC	NC
	Week 52	1160 ± 177	2100 ± 153	1670 ± 99.1
	Week 78	1130 ± 146	1930 ± 192	1550 ± 116
	Week 104	1000 ± 173	1790 ± 326	1760 ± 222
ICI 213,841	Day 1	NC	NC	352 ± 42.8
	Week 26	NC	330 ± 54.7	556 ± 104
	Week 52	NC	293 ± 40.0	413 ± 42.0
	Week 78	NC	222 ± 28.9	383 ± 92.0
	Week 104	78.1 ± 17.0	211 ± 53.6	372 ± 61.8

NC = Not calculable.

According to the sponsor, these plasma levels (collected at 30 min postdosing) approximate C_{max}. Plasma levels of the parent compound did not increase linearly with dose; dose-corrected exposure was highest at the MD and slightly less at the HD than at the LD.

Plasma levels of ICI 214,227 increased in a less-than dose proportionate manner, as did those of ICI 213,841 (at the MD and HD). Plasma levels of ICI 204,636 and ICI 214,227 were fairly similar at the HD, whereas at the lower doses, levels of ICI 214,227 were higher than that of the parent compound (almost 10-fold at the LD). Plasma levels of ICI 213,841 were lower (4-6 fold at the MD and HD) than those of ICI 204,636 and ICI 214,227 at all doses.

Terminal studies

Cause of death: the "principle" causes of death (as selected by the sponsor) are summarized in the following sponsor's table:

CAUSE OF DEATH / Group	MALES				FEMALES			
	I	II	III	IV	I	II	III	IV
N=	100	50	50	50	100	50	50	50
Dosing accident	17	7	11	10	3	2	10	7
Chronic renal disease	10	2	4	2	3	0	1	0
Mammary gland neoplasm	1	1	0	0	13	8	13	10
Pituitary gland neoplasm	9	4	4	4	14	8	2	7
Skin neoplasm	7	3	1	3	3	1	0	1
Haematopoietic neoplasm	5	1	1	4	3	1	0	1

N = number of animals in group.

None of these causes of death were clearly dose-related, except, perhaps, for the incidence of hematopoietic neoplasms (increased in HDM). The incidence of fatal mammary gland neoplasms was increased in all dosed grps, but the increases were not dose-related. Dosing accident was determined by the presence of "...eosinophilic material in alveoli, subpleural staining changes and bronchial and tracheal epithelial sloughing, necrosis and haemorrhage".

Although there was no dose-related incidence of total mammary gland tumors, the sponsor did note that when the incidences were determined according to benign and malignant tumors, there was a dose-related increase in adenocarcinomas (1, 6, 10, and 12% in CF, LDF, MDF, and HDF, respectively).

Gross pathology: a summary of "noteworthy" findings was provided by the sponsor. These findings (except for findings in Harderian gland which tended to decrease with dose) are presented in the following table and are based on the sponsor's table; however, the incidences are expressed as "% of total no. of animals".

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FINDING	MALES				FEMALES			
	C	LD	MD	HD	C	LD	MD	HD
Adrenal gland								
enlarged	6	4	20	16	2	0	4	10
discolored	35	18	12	26	56	32	20	30
Liver								
enlarged	9	8	16	28	5	6	4	10
Lungs								
irregular surface	22	22	18	50	18	10	22	56
Mammary gland								
increased amount	2	2	4	2	41	78	64	72
Pancreas								
discolored	3	10	10	14	3	2	10	20
Pituitary gland								
enlarged	2	6	14	6	5	14	32	34
Seminal vesicles								
enlarged	2	4	14	24				
discolored	3	6	10	20				
Thyroid gland								
discolored	8	26	56	84	2	4	26	72

Organ/tissue weights: in males, the following were noted: (1) increased liver weight (A-R) at the MD (6-25%) and HD (34-53%), (2) increased adrenal weight (A-R) at the MD (24-50%) and HD (19-42%), and (3) increased pituitary weight (A-R) at all doses (LD: 24-64%, MD: 41-77%, HD: 53-91%).

In females, the following were noted: (1) a slight decrease in ovary weight (A-R) at the HD (14%), (2) increased adrenal weight (A-R) at the MD (6-38%) and HD (2-44%), and (3) increased pituitary weight (A-R) at all doses (LD: 48-32%, MD: 26-23%, HD: 44-65%).

Histopathology: The incidences of selected non-neoplastic and neoplastic findings are summarized in the attached tables.

APPEARS THIS WAY
ON ORIGINAL

2-yr rat carcinogenicity study, non-neoplastic findings

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MC	HD
Adrenal	cortical hemocysts								
	minimal	6/100	9/50	4/50	5/50	9/100	8/50	6/50	8/50
	mild	2/100	6/50	10/50	9/50	52/100	27/50	16/50	14/50
	moderate	0/100	0/50	0/50	2/50	24/100	7/50	4/50	6/50
	severe	0/100	0/50	0/50	0/50	1/100	0/50	0/50	1/50
	medullary cortical ectopia								
	minimal	80/100	15/50	13/50	19/50	58/100	32/50	36/50	32/50
	mild	4/100	27/50	25/50	18/50	28/100	8/50	8/50	5/50
	moderate	0/100	3/50	5/50	3/50	2/100	1/50	0/50	1/50
	severe	0/100	0/50	0/50	0/50	1/100	0/50	0/50	0/50
	cortical nodular hyperplasia								
	minimal	13/100	8/50	5/50	11/50	19/100	7/50	3/50	11/50
	mild	46/100	4/50	7/50	4/50	37/100	6/50	3/50	2/50
	moderate	3/100	0/50	2/50	0/50	1/100	0/50	0/50	0/50
	medullary hyperplasia	17/100	11/50	11/50	12/50	6/100	7/50	6/50	3/50
Bronchus	epithelial sloughing	2/100	0/50	2/50	5/50	0/98	1/50	2/50	0/50
Cervix	mucification								
	mild					31/100	15/50	24/50	18/50
	moderate					7/100	12/50	7/50	12/50
Epididymides	exoliated seminiferous epithelial cells	10/100	3/50	10/50	9/50				
Harderian gland	dacryoadenitis	27/99	12/50	14/50	21/50				
Heart	myocardial fibrosis								
	minimal	14/100	17/50	7/50	1/50	16/100	10/50	10/50	12/50
	mild	46/100	21/50	20/50	33/50	20/100	7/50	7/50	16/50
	moderate	4/100	0/50	2/50	2/50	0/100	0/50	0/50	1/50
Kidneys	urolithiasis	21/100	10/50	17/50	15/50	65/100	32/50	31/50	20/50
	microlithiasis	45/100	29/50	28/50	32/50	81/100	44/50	3/50	40/50
	tubular dilatation/cyst	7/100	4/50	6/50	6/50	8/100	2/50	5/50	7/50

2-yr rat carcinogenicity study, non-neoplastic findings

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MC	HD
Liver	basophilic foci								
	minimal	0/100	0/50	1/50	2/50	7/100	9/50	8/50	6/50
	mild	1/100	1/50	0/50	0/50	7/100	7/50	5/50	4/50
	eosinophilic foci								
	minimal	4/100	0/50	1/50	3/50	0/100	3/50	0/50	1/50
	mild	11/100	11/50	6/50	16/50	10/100	6/50	10/50	7/50
	moderate	3/100	1/50	1/50	0/50	4/100	1/50	0/50	1/50
	hepatocyte fat vacuolation								
	minimal	28/100	15/50	8/50	11/50	13/100	11/50	5/50	10/50
	mild	17/100	5/50	5/50	13/50	10/100	2/50	5/50	21/50
	moderate	0/100	2/50	2/50	11/50	2/100	2/50	0/50	5/50
	severe	0/100	0/50	0/50	1/50	0/100	0/50	0/50	0/50
	centrilobular hypertrophy	0/100	0/50	0/50	9/50	0/100	0/50	0/50	30/50
Lungs	foamy alveolar macrophages								
	minimal	27/100	21/50	17/50	3/50	35/100	17/50	20/50	7/50
	mild	44/100	10/50	14/50	33/50	15/100	9/50	12/50	23/50
	moderate	0/100	1/50	1/50	9/50	2/100	0/50	0/50	15/50
	severe	4/100	0/50	1/50	1/50	2/100	0/50	0/50	0/50
	eosinophilic material present	14/100	9/50	11/50	12/50	6/100	1/50	9/50	8/50
Lymph node (mesenteric)	giant cells								
	minimal	33/100	19/50	11/50	7/49	17/100	9/50	13/50	3/50
	mild	60/100	28/50	34/50	34/49	81/100	40/50	34/50	39/50
	moderate	0/100	0/50	0/50	4/49	0/100	0/50	0/50	3/50
Mammary gland	hyperplasia								
	mild	16/51	13/29	14/32	7/28	38/99	16/50	19/50	20/50
	moderate	6/51	1/29	0/32	0/28	30/99	28/50	26/50	28/50
	severe	0/51	0/29	0/32	0/28	8/99	4/50	4/50	1/50
Pancreas	reduced exocrine acinar granulation	8/100	5/50	5/50	14/49	16/100	14/50	7/50	8/50
Pituitary gland	diffuse pars distalis hyperplasia								
	mild	0/100	0/50	0/50	0/50	1/100	5/50[1]	8/50	12/50
	moderate	0/100	0/50	0/50	0/50	1/100	3/50	6/50	7/50
	pars distalis degeneration	0/100	0/50	0/50	0/50	0/100	0/50	0/50	2/50

2-yr rat carcinogenicity study, non-neoplastic findings

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MC	HD
Prostate gland	prostatitis								
	mild	3/100	7/50	8/50	11/50				
	moderate	5/100	1/50	6/50	9/50				
	severe	1/100	1/50	1/50	1/50				
	mineral deposits	1/100	0/50	1/50	2/50				
	glandular epithelial hyperplasia	1/100	1/50	1/50	3/50				
	glandular ectasia	0/100	1/50	2/50	3/50				
Salivary gland (parotid)	diffuse acinar basophilia								
	mild	18/99	12/49	20/49	28/50	10/100	8/50	21/49	33/50
	moderate	0/99	1/49	2/49	9/50	3/100	1/50	2/49	5/50
	acinar vacuolation	2/99	4/49	3/49	4/50	11/100	3/50	4/49	8/50
Salivary gland (sublingual)	acinar degeneration/atrophy	0/99	1/49	1/49	4/50	0/100	3/50	0/49	3/50
Seminal vesicles	cystic dilatation								
	moderate	3/100	1/50	6/50	11/50				
	severe	1/100	0/50	0/50	1/50				
	vesiculitis								
	minimal	1/100	2/50	2/50	1/50				
	mild	10/100	8/50	13/50	20/50				
	moderate	4/100	2/50	6/50	7/50				
	secretion coagulation	16/100	9/50	23/50	33/50				
Thymus	cyst	0/95	0/45	0/49	0/47	0/94	0/50	0/48	3/49
Uterus	proestrus					2/100	4/50	8/50	8/50
	estrus					9/100	1/50	1/50	2/50
	metoestrus					9/100	3/50	4/50	7/50

2-yr rat carcinogenicity study, non-neoplasic findings

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MC	HD
Vagina	mucification								
	mild					35/100	15/50	23/50	13/50
	moderate					19/100	19/50	13/50	19/50
Thyroid gland	follicular epithelial brown pigmentation								
	minimal	0/100	5/49	1/49	0/50	0/100	2/50	1/49	3/50
	mild	0/100	8/49	35/49	6/50	0/100	1/50	41/49	17/50
	moderate	0/100	0/49	10/49	42/50	0/100	0/50	1/49	28/50
	parafollicular cell hyperplasia								
	minimal	7/100	3/49	1/49	4/50	7/100	3/50	4/49	2/50
	mild	3/100	6/49	5/49	14/50	8/100	6/50	5/49	15/50
	basophilic colloid								
	minimal	45/100	17/49	13/49	1/50	86/100	39/50	24/49	5/50
	mild	31/100	22/49	22/49	25/50	8/100	8/50	18/49	35/50
	moderate	6/100	0/49	3/49	19/50	0/100	0/50	0/49	6/50
	follicular cell desquamation								
	minimal	59/100	18/49	27/49	13/50	82/100	38/50	30/49	11/50
	mild	21/100	20/49	10/49	29/50	13/100	9/50	15/49	34/50
	black granules recorded-Masson Fontana stain								
	none	83/100	17/49	3/49	1/50	96/100	16/50	5/49	4/50
	mild	16/100	30/49	14/49	6/50	4/100	33/50	40/49	11/50
	moderate	0/100	2/49	31/49	43/50	0/100	1/50	4/49	35/50
	follicular epithelial height								
	grade 1	0/100	0/49	0/49	0/50	4/5100	0/50	0/49	0/50
	grade 2	17/100	5/49	4/49	1/50	56/100	31/50	17/49	8/50
	grade 3	53/100	30/49	25/49	15/50	35/100	16/50	29/49	25/50
	grade 4	10/100	4/49	8/49	25/50	1/100	1/50	1/49	14/50
	grade 5	4/100	0/49	0/49	1/50	0/100	0/50	1/49	0/50
	follicular cyst	0/100	0/49	1/49	2/50	0/100	0/50	0/49	0/50
	parafollicular cell hypertrophy	0/100	0/49	0/49	0/50	0/100	0/50	0/49	1/50
	follicular hyperplasia	3/100	0/49	3/49	3/50	0/100	0/50	1/49	2/50
	brown pigment	4/100	2/49	3/49	2/50	1/100	1/50	0/49	0/50
	arteritis	0/100	0/49	0/49	2/50	1/100	0/50	0/49	0/50
	reduced colloid	1/100	0/49	0/49	2/50	0/100	0/50	0/49	1/50

2-yr rat carcinogenicity study, neoplastic findings

TISSUE	FINDING	MALES				FEMALES			
		C	LD	MD	HD	C	LD	MC	HD
Adrenal gland	cortical carcinoma cortical adenoma	0/100 5/100	0/50 4/50	0/50 3/50	2/50* 3/50	1/99 5/99	0/50 4/50	0/50 2/50	0/50 1/50
Mammary gland	fibrosarcoma fibroma fibroadenoma adenoma adenocarcinoma	0/51 2/51 0/51 0/51 0/51	0/29 0/29 3/29 0/29 0/29	0/32 1/32 1/32 0/32 0/32	0/28 0/28 3/28 0/28 0/28	0/99 5/99 53/99 0/99 10/99	0/50 2/50 30/50 0/50 13/50	1/50 5/50 25/50 0/50 11/50	0/50 1/50 16/50 1/50 16 /50***
Pancreas	islet cell adenoma islet cell carcinoma	3/100 1/100	4/50 4/50	5/50 2/50	2/49 3/49	4/100 1/98	1/50 2/50	1/50 2/50	0/50 2/50
Pituitary gland	carcinoma adenoma	0/100 32/100	0/50 23/50	0/50 13/50	0/50 24/50	1/100 47/100	1/50 30/50	2/50 14/50	0/50 24/50
Thyroid gland	follicular adenoma	6/100	3/49	0/49	16/50**	0/100	0/50	0/50	1/50
Thymus	benign mixed thymoma malignant thymic lymphoma (lymphoblastic)	3/95 0/95	0/45 0/45	2/49 0/49	3/47 1/47	5/94 0/94	4/50 0/50	2/48 0/48	3/49 0/49

*p<0.043 (trend test), **p<0.0001 (trend, and step-down trendfor HD), ***p<0.005 (trend), step-down trends: p<0.0082 (LD), p<0.011 (MD), p<0.005 (HD)