CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20639

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Review of NDA **20-639**

Date: April 3, 1997

Reviewer: Lois M. Freed, Ph.D.

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Sponsor: Zeneca Limited-Macclesfield, England
US Agent-Zeneca Pharmaceuticals
1800 Concord Pike, PO Box 15437
Wilmington, DE 19850-5437

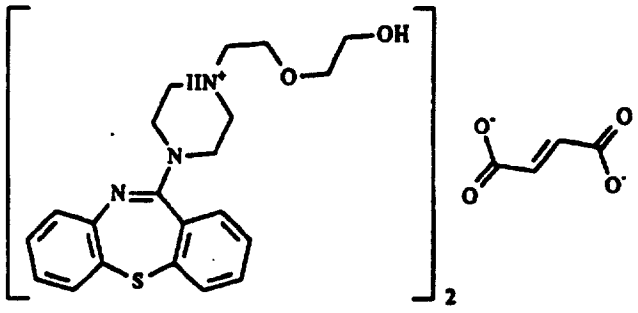
Drug: quetiapine fumarate (Seroquel)

Code Name: ICI 204,636, ZD5077, ZM 204,636

Pharmacologic Category:

Indication: management of the manifestations of psychotic disorders

Structure:



Molecular weight: 883.1 (fumarate salt)

Chemical Name: bis[2-(2-[4-(dibenzo[b,f][1,4]-thiazepin-11yl)piperazin-1-yl]ethoxy)ethanol] fumarate (IUPAC)

Drug Formulation: 25, 100, 200 mg oral tablets

Related IND: IND

Previously Reviewed Studies
Pharmacodynamics
Pharmacokinetics and ADME
Liquid chromatographic method for ICI 204,636 and its metabolites,

2-month gavage study (with 1 month withdrawal) to evaluate potential physiological and pathological effects on the rat thyroid [(TKR/1804) (8/LJ/014882)]

The review of data relating to the possible thyroid toxicity of ICI 204,636 by Dr. Charles Capen (Ohio State University).

The results of the histopathological studies (using the light microscope) of the rat thyroid.

A review of the findings from the ophthalmologic examinations in dogs receiving ICI 204,636 by Dr. Gustavo Aguirre (Diplomate, American College of Veterinary Ophthalmologists).

Report on the brain sections from cynomolgus monkeys dosed with Compound ICI 204,636 over the course of one year (Dr. J.B. Cavanaugh, Department of Neurology, Institute of Psychiatry, London)

Dependence study on ICI 204,636 in rhesus monkeys and rats [(TKN/163) (10/IE/US/1020233)]

Carcinogenicity

Ninety day sighting oncogenicity study in mice: dietary administration [(THM/599) (12/IA/017433)]

Three month sighting oncogenicity study in rats: oral administration [(THR/2047) (11/ID/1011620)]

Reproductive Studies

Fertility and General Reproductive Performance Study in Female Rats. Oral administration. [(TGR/1711) (12/LJ/015472)]

Fertility and General Reproductive Performance Study in Male Rats. Oral administration. [(TGR/1617) (12/HI/013991)].

Teratology Study in Rats: Oral Administration. [(TTR/1537) (3/HI/012670)]

Teratology Study in Rabbits: Oral Administration. [(TTB/402) (3/HI/012577)]

Mutagenicity Studies

Ames Test: bacterial mutagenicity study using selected strains of Salmonella Typhimurium: standard method. [(TMV/257) (1/HH/010686)]

Ames Test: bacterial mutagenicity study using selected strains of Salmonella Typhimurium: standard method. [(TMV/258) (1/HH/010685)]

Ames Test (Bacteria Mutation Assay to Assess the Potential Mutagenic Effect) [(TMV/369) (8/HI/013366)]

Ames test: bacterial mutagenicity study using selected strains of Salmonella Typhimurium: standard method. [(TMV/384) (3/HI/012653), Vol. 1.59]

Ames test: bacterial mutagenicity study using selected strains of Salmonella Typhimurium: standard method. [(TMV/385) (4/HI/012778), Vol. 1.59]

Bacterial mutagenicity study using a selected strain of escherichia coli: standard method, [(TMV/261) (1/LJ/014204)]

Micronucleus Test in the Rat: Oral Administration [(TQR/1535) (7/HI/013245)]

Previous Reports:

10/25/88	Original Review and Evaluation of Pharmacology and Toxicology Data (R. Hollenbeck, Ph.D.)
8/28/89	Review and Evaluation of Pharmacology and Toxicology Data (J.J. DeGeorge, Ph.D.)
12/15/89	Review and Evaluation of Pharmacology and Toxicology Data, Supplement II (J.J. DeGeorge, Ph.D.)
7/13/90	Review and Evaluation of Pharmacology and Toxicology Data, Supplement III (J.J. DeGeorge, Ph.D.)
4/9/92	Review and Evaluation of Pharmacology and Toxicology Data, Consultation (Kumar D. Mainigi, Ph.D.)
6/25/92	Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D.)

6/29/92	Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D.)
7/31/92	Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D.)
2/5/93	Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D)
3/9/94	Review and Evaluation of Pharmacology and Toxicology Data (L.M. Freed, Ph.D)

Studies Reviewed in the NDA submission:

Pharmacology (the sponsor's summary, Vol. 1.14)

ADME (Vol. 1.60-1.68; studies relevant to the i.m. formulation were not reviewed)

Toxicology

- Acute studies:
  - Acute toxicity (limit) in mice: oral administration [ZM 213,841] [(TLM/919) (11/IE/1018108, Vol. 1.51]
  - Acute toxicity (limit) in mice: oral administration [ZM 236,303] [(TLM/921) (11/IE/1017298, Vol. 1.51]
  - Acute toxicity (limit) in mice: oral administration [ZM 214,227] [(TLM/920) (11/IE/1017297), Vol. 1.51]
- Subchronic studies:
  - Oral (gavage) maximum tolerated daily dose (MTDD) toxicity study in the cynomolgus monkey [(TAP/83) (4/IE/1014013), Vol. 1.31]
- Chronic studies:
  - Twelve month oral toxicity study in rats [(TFR/1626) (9/ID/1010581); Vol. 1.21]
  - Twelve month oral toxicity study in dogs [(TFD/501) (6/ID/1009118), Vol. 1.28-1.29]
  - 56 week oral (gavage) chronic toxicity study in the cynomolgus monkey [(TFP/84) (11/IE/1017802); Vol 1.32-1.33]
- Special studies:
  - Examination of thyroid glands from studies THR/2047 and TKR/1924 when stained by Schmorl's and the Masson-Fontana methods (12/IC/1006118; Vol. 1.24)
  - Comparison of thyroid pigmentation in five studies in the rat (TKR/1924, TAR/1621, THR/2047, TPR/1616) (1/ID/1006212, Vol. 1.24)
  - Topical tolerance assessment: in vitro assessment of cytotoxicity and irritant hazard [(TVN/108) (2/HH/010902), Vol. 1.50]
  - Assessment of iodine uptake and organification in primary rat thyrocytes exposed to ICI 204,636 [(TKN/175) (6/ID/1009251), Vol. 1.50]
  - Assessment of iodine uptake and organification in primary rat thyrocytes exposed to ICI 214,227, ICI 213,841, and ICI 236,303 [(TKN/197) (7/IE/1015141), Vol 1.50]
  - Assessment of in vitro inhibition of cholesterol synthesis in cultured dog lenses exposed to ICI 204,636 [(TVN/202 (9/IE/1016424), Vol. 1.50]
  - Assessment of in vitro cataract formation in cultured dog lenses exposed to ICI 204,636 [(TVN/198) (9/IE/1016371), Vol. 1.50]
  - Investigative study to determine the in vitro inhibition of cholesterol synthesis in HEPG2 cells by ICI 204,636 [(TVN/199) (9/IE/1016408), Vol. 1.50]
  - Assessment of thyroid function in mice [(TKM/913) (10/IE/1017274, Vol. 1.50)]
  - Assessment of thyroid function in rats [(TKR/2271) (9/IE/1016207), Vol.

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PHARMACOLOGY

NOTE: the following review is based on a summary provided by the sponsor and not on review of the original reports.

PRIMARY

***In vitro* studies**

Receptor binding: The receptor binding profile for ICI 204,636 is summarized in the following sponsor's Tables 15 -17:

TABLE 15 Affinities of ICI 204,636 and clozapine for various receptors in brain

Receptor	Ligand*	ICI 204,636	Clozapine
		(IC_{50}#, nM)	
D2	^{3}H-Spiperone	329	132
D1	^{3}H-SCH 23390	1268	322
5-HT_2	^{3}H-Ketanserin	148+	20
5-HT_{1A}	^{3}H-8-OH DPAT	717	316
α_1	^{3}H Prazosin	94	50
α_2	^{3}H-Rauwolscine	271	28
Muscarinic	^{3}H-QNB	>10000	287
Benzodiazepine	^{3}H-Flunitrazepam	>5000	>5000

TABLE 16 PanLab's receptor profile for ICI 204,636.

Binding Site	Ligand	ICI 204,636 IC_{50}(nM)	Ki(nM)
Adenosine A1	[^{3}H]DPCPX	>10000	
Adenosine A2	[^{3}H]CGS-21680	>10000	
α_1 adrenoceptor	[^{3}H]Prazosin	50	13
α_2 adrenoceptor	[^{3}H]Rauwolscine	880	782
Androgen	[^{3}H]Mibolerone	>10000	
β_1-Adrenoceptor	[^{3}H]CGP-12177	>10000	
β_2-Adrenoceptor	[^{3}H]CGP-12177	>10000	
Bradykinin BK2	[^{3}H]Bradykinin	>10000	
Calcium (L)	[^{3}H]Nitrendipine	>10000	
Dopamine D1	[^{3}H]SCH-23390	920	558
Dopamine D2	[^{3}H]Raclopride	690	531
GABAA	[^{3}H]Muscimol	>10000	
Glutamate	[^{3}H]L-Glutamate	>10000	
Glycine (Strychnine Sensitive)	[^{3}H]Strychnine	>10000	
Insulin	[^{3}H]Insulin	>10000	
Histamine H1	[^{3}H]Pyrilamine	30	10
Muscarinic M2	[^{3}H]NMS	5740	2150
Muscarinic M3	[^{3}H]NMS	>10000	
NMDA	[^{3}H]CGS-19755	>10000	
Opiate Delta	[^{3}H]DPDPE	>10000	
Opiate Kappa	[^{3}H]U-69593	>10000	
Opiate Mu	[^{3}H]DAMGO	4570	
PCP	[^{3}H]TCP	>10000	
Phorbal Ester	[^{3}H]PDBu	>10000	
Potassium (Ik,ATP)	[^{3}H]Glyburide	>10000	
Progestin	[^{3}H]R5020	>10000	
Serotonin 5-HT_1	[^{3}H]5-HT	1297	557
Serotonin 5-HT_2	[^{3}H]Ketanserin	460	288
Sigma	[^{3}H]DTG	90	87

TABLE 17 Cloned (human recombinant) dopamine receptor binding (Ki nM) for ICI 204,636 and clozapine

Drug	Ki (nM) D1	D2	D3	D4
ICI 204,636	293	358	469	>10000
Clozapine	73	274	449	59

In Table 15, the sponsor noted that a repeat assay for $5HT_2$ receptor binding, an IC_{50} of 254 nM was obtained for ICI 204,636. One additional receptor site was assayed (but not listed in the tables), $5HT_6$. Both ICI 204,636 and clozapine were found to have high affinity for this receptor subtype (K_i of 33 and 9.5 nM, respectively).

In vivo studies

Animal models of antipsychotic activity: the results of behavioral tests of antipsychotic activity are provided in the following table (drugs were administered p.o., except for the PPI study and the multiple-dosing study which involved s.c. and i.p. dosing, respectively):

SPECIES/ STRAIN	PARADIGM	EFFECT	ED# (mg/kg)	COMPARATORS (ED_{50})
ACUTE DOSING				
squirrel monkey	conditioned avoidance	disruption of avoidance response	10.5*	CLOZ: 23.8 HAL: 0.298 CPZ: 2.92
	apo-induced blinking	antagonism	2.1*	CLOZ: 7.7 CPZ: 1.7
cat	apo-induced visual searching		2.5**	CLOZ: 5 CPZ: 0.62
mouse	apo-induced climbing		80**	CLOZ: 40
	apo-induced disruption of swimming		40**	CLOZ: 40
	amp-induced disruption of swimming		40**	CLOZ: 5 CPZ: 2.5
rat	amp-induced hyperactivity		20**	CLOZ: ≤10 FLU: ≤10
	apo-induced disruption of PPI		5**	CLOZ: 12
MULTIPLE DOSING (7-day)				
mouse	amp-induced disruption of swimming	antagonized at 40 mg/kg, similar to acute	n/a	CLOZ: antagonized at 40 mg/kg, similar to acute

#effective dose, expressed either as *ED_{50} or **MED (minimal effective dose)

In the CA paradigm, all four compounds also increased escape failures. Clozapine was the only one of the drug tested that was more potent in disrupting the CA response relative to the escape response.

Animal models of extrapyramidal side-effect liability: the results of behavioral tests of eps liability are summarized in the following table:

SPECIES/ STRAIN	PARADIGM	EFFECT	COMPARATORS
Sprague-Dawley rat	behavioral supersensitivity to apo-induced biting	no supersensitivity	CLOZ: none HAL: supersensitivity, 1 mg/kg
Cebus monkey	haloperidol-sensitized dyskinesia	dyskinesia at 5 (1/13), 10 (1/13), 20 (2/13) mg/kg p.o.	CLOZ: none HAL: dyskinesia at 0.125 (3/12), 0.25 (6/6), 0.5 (2/2), 1.0 (13/13) mg/kg p.o. THOR: dyskinesia at 2/5 (4/13), 5.0 (7/7), 10.0 (11/13) mg/kg p.o.
	ICI 204,636-induced sensitized dyskinesia	"weak" dyskinesia in 7/13 at 20 mg/kg p.o.	CLOZ: no dyskinesia in 4 HAL: "weak to strong" dyskinesia in 24/26
rat	catalepsy	induced catalepsy at all doses tested (20-80 mg/kg i.p.)	CLOZ: induced catalepsy at doses of 20-80 mg/kg i.p., but not at 10 mg/kg i.p. HAL: induced catalepsy at doses of 0.5-4 mg/kg i.p., but not at 0.25 mg/kg i.p.

In sensitization studies in monkeys, there were 2 unscheduled deaths, 1/5 at 40 mg/kg p.o. (single dose) and 1/14 at 20 mg/kg p.o. (2nd wk of dosing). Haloperidol produced a more rapid onset of sensitization than ICI 204,636, as well as a greater percentage of affected animals.

In the catalepsy studies in rat, the magnitude of the effect (i.e., seconds of immobility) was similar with ICI 204,636 at 80 mg/kg i.p. and with haloperidol at 4 mg/kg (i.e., ≈38-39 sec, respectively). At the highest dose tested, clozapine only induced ≈9.5 sec of immobility.

Electrophysiology: ICI 204,636 was tested in two paradigms: (1) d-amphetamine-induced inhibition of dopamine (DA) cell firing in the A9 (striatum) and A10 (ventral tegmental) regions and (2) spontaneous DA cell firing in A9 and A10. Both studies were conducted in male Sprague-Dawley rats. ICI 204,636 reversed the inhibitory effect of d-amphetamine in both A9 and A10 regions; however, ICI 204,636 was almost 3-fold more potent in the A10 region (ED_{50} = 2.63 and 0.95 mg/kg i.v. in A9 and A10, respectively). In contrast, thioridazine and, interestingly enough, clozapine were equipotent on A9 and A10 neurons (ED_{50} = 1.95 and 1.79 mg/kg i.v. on A9 and A10 , respectively, for thioridazine, and 1.32 and 1.40 mg/kg i.v. on A9 and A10, respectively, for clozapine. Haloperidol was 10 times more potent in the A9 region (ED_{50} = 0.007 and 0.074 mg/kg i.v. for A9 and A10, respectively).

The effects of ICI 204,636 on the number of spontaneously firing A9 and A10 neurons were tested following acute (5-20 mg/kg p.o.) and multiple (28-day, 20 mg/kg p.o.) dosing. Acute doses of ICI 204,636 produced dose-related increases (11-90%, statistically significant only at the HD) in the number of spontaneous active A10 neurons, but had no effect on A9 neurons. Following multiple dosing with ICI 204,636, there was a decrease (62%) in the number of active A10 neurons, with only a slight effect on A9 neurons (19%, n.s.). Clozapine and haloperidol were also tested in these paradigms for comparison. Clozapine acutely increased the number of spontaneous firing neurons in the A10 region (29-75% at 10-20 mg/kg p.o.), and, with multiple dosing (20 mg/kg p.o.), the number in the A10 region was reduced to an extent similar to that obtained with ICI 204,636 at the same dose. Neither ICI 204,636 nor clozapine had an significant effects in the A9 region with multiple dosing. Haloperidol, in contrast, caused changes in the number of spontaneously firing DA neurons in both the A9 and A10 regions after acute (0.5-1 mg/kg p.o.) and multiple dosing (1 mg/kg p.o.). With acute dosing, the number of spontaneously firing A9 and A10 neurons were increased (20-54 and 36-89%, respectively). With multiple dosing, the number of spontaneously firing A9 and A10 neurons were reduced (62-65%).

Plasma prolactin: The effects of ICI 204,636 (20 mg/kg p.o., i.p.), clozapine (20 mg/kg p.o., i.p.), and haloperidol (0.25 mg/kg i.p.) on plasma prolactin levels were compared in rats (strain not specified). When administered i.p., ICI 204,636 and haloperidol increased plasma prolactin levels significantly, with peak levels occurring more rapidly with ICI 204,636 (≈15 min vs 30

min for haloperidol). By 120 min postdosing, prolactin was still elevated with both drugs. When administered p.o., ICI 204,636 produced increases in prolactin, but to a lesser extent than when given i.p.; the time course was similar between the two routes. Clozapine, given p.o., resulted in a significant decrease in plasma prolactin (at 45-60 min postdosing).

Apomorphine antagonized the increases in plasma prolactin produced by acute doses of both ICI 204,636 and clozapine (given i.p.).

In rats treated for either 1 dose or 3-wks of once daily dosing, ICI 204,636 (20 mg/kg i.p.) produced similar increases in plasma prolactin.

Ex vivo studies

Neurochemistry: The effects of ICI 204,636 (p.o., i.p.) on brain levels of dopamine and dopamine metabolites were tested in male Sprague-Dawley rats. Striatal levels of dopamine, DOPAC, and HVA were significantly elevated (27, 100, and 52%, respectively) following a single oral dose of ICI 204,636 (20 mg/kg). Clozapine (20 mg/kg) also increased brain levels of these compounds in the striatum (22, 80, and 46%, respectively). Neither ICI 204,636 or clozapine significantly changes the levels of dopamine or metabolites in the olfactory tubercle or in whole brain. The time course of the oral effect in the striatum was similar for ICI 204,636 and clozapine; peak levels were detected at ≈1 hr postdosing for both drugs. When administered i.p., ICI 204,636 and clozapine increased HVA and DOPAC levels in the striatum at doses of 10-40 mg/kg; however, at 20 and 40 mg/kg, increases were significantly lower with ICI 204,636 as compared to clozapine. Therefore, ICI 204,636 (i.p.) was less efficacious than clozapine in the striatum.

Striatal levels of another dopamine metabolite, 3-MT (3-methoxytyramine), were measure following administration of MAO inhibitor, pargyline (75 mg/kg i.p.). ICI 204,636 and clozapine both increased 3-MT levels at doses of 10 and 20 mg/kg, however, clozapine produced slightly higher levels at each dose than did ICI 204,636.

In animals pretreated with apomorphine, neither ICI 204,636 or clozapine (20 mg/kg i.p.) reversed the dopamine agonist-induced decreases in striatal DOPAC and HVA.

The effects of ICI 204,636 (20 mg/kg i.p.) on striatal DOPAC and HVA levels following acute and multiple (3-wks) dosing were tested in rat. Similar increases in levels of the two metabolites were obtained with the two dosing regimens; therefore, no tolerance to ICI 204,636 was demonstrated.

In rats pretreated with SKF 525A (a microsomal enzyme inducer), ICI 204,636 still significantly increased striatal DOPAC and HVA levels to a similar extent. The sponsor concluded that this demonstrated that increases in drug metabolism did not significantly affect ICI 204,636's response. Increases in hepatic enzyme activity were not, however, documented; therefore, the results are not definitive.

Receptor occupancy: The occupancy of D_2 and $5HT_2$ receptors by ICI 204,636 (40 mg/kg i.p.), clozapine (40 mg/kg i.p.), and haloperidol (1 mg/kg i.p., D_2 only) was estimated using N-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ). [The number of D_2 and $5HT_2$ receptors were determined using ^{3}H-ketanserin and ^{3}H-spiperone, respectively.] The sponsor pointed out that this paradigm provides an underestimation of actual occupancy (i.e., % protection depends on competition of drug with EEDQ for the specific receptor type).

ICI 204,636 and clozapine significantly protected $5HT_2$ and D_2 receptors from EEDQ-inactivation, with the effects being greater for clozapine (39-31% for ICI 204,636 vs 68-60% for clozapine) . The degree of protection were similar for the two receptors for both drugs; however,

the time course was somewhat different between drugs and between receptors. Haloperidol protected against EEDQ-induced D_2 inactivation at all times tested (57-66% from 30-240 min postdosing).

The sponsor concluded that ICI 204,636 demonstrated greater occupancy of $5HT_2$ receptors relative to D_2. The table values for % protection, however, were similar for both receptors (39 and 31% for $5HT_2$ and D_2, respectively) and there was no indication that this small difference was statistically significant.

Other studies

The ability of "typical" and "atypical" antipsychotic drugs to induce c-fos (or fos-like immunoreactivity, FLI) in several brain regions [dorsolateral striatum and limbic structures (nucleus accumbens, lateral septum, prefrontal cortex)] was compared. (The species studied was not specified.) According to the abstract, "Clozapine, ICI 204,636, fluperlapine, risperidone, and others either failed to increase or produced minor elevations in FLI in the dorsolateral striatum.....All antipsychotic agents [including chlorpromazine, haloperidol, loxapine, metoclopramide, molindone, fluphenazine] elevated FLI in the nucleus accumbens and medial striatum..." ICI 204,636 and clozapine did not elevate c-fos in the dorsolateral striatum.

In the stimulus discrimination paradigm, conducted in squirrel monkey, ICI 204,636 fully substituted for clozapine (as did perlapine and "...several experimental dibenzazepines..."). The sponsor noted that risperidone failed to substitute for clozapine in this paradigm.

The amp-induced social isolation paradigm, conducted in Java monkeys, was used to assess the potential for ICI 204,636 to affect positive and negative symptoms of schizophrenia. ICI 204,636 (1-10 mg/kg i.m.) reversed the following amp-induced behaviors: (1) decreases in social behavior (as noted by increases in passive grooming, active and passive proximity) and (2) increases in submissive behavior (considered an animal model of paranoid delusions).

The differential effects of ICI 204,636 and clozapine on fore- and hind-limb retraction were compared in the paw test in rats, a model considered to be an alternative to the catalepsy test for assessing eps liability. ICI 204,636 increased forelimb retraction time, considered a measure of eps liability, only at the highest dose tested (i.e., 100 mg/kg i.p.). In contrast, ICI 204,636 produced a dose-related increase in hindlimb retraction (MED = 25 mg/kg i.p.), considered a measure of antipsychotic potential. Therefore, there was a 4-fold difference in the two effects. This is compared to a ratio of >5 for clozapine and of 1 for haloperidol and chlorpromazine.

SECONDARY

CNS studies

ICI 204,636 was tested in a variety of assays/paradigms in order to assess CNS effect unrelated to therapeutic potential.

The propensity for ICI 204,636 to induce motor incoordination was assessed in male Wistar rats using the rotorod. Results were compared to those for clozapine and haloperidol. The ED_{50}'s for impairment performance on the rotorod were >80, 49.8, and 1.1 mg/kg p.o. for ICI 204,636, clozapine, and haloperidol, respectively.

The effect of ICI 204,636 on spontaneous motor activity (considered a measure of the sedative potential of a compound) was tested in male Sprague-Dawley rats. The MED_{50} (i.e., the minimum dose producing at least a 50% decrease in SMA) was 40.0, 10.0, and 1.0 mg/kg p.o. for ICI 204,636, clozapine, and haloperidol, respectively.

The anxiolytic potential of ICI 204,636 was tested in the shock-induced suppression of drinking paradigm in male Wistar rats. ICI 204,636 was found to have no effect at the doses tested (5-40 mg/kg p.o., 1.25-40 mg/kg i.p.).

In vivo activity at the cholinergic receptor was assessed in mice (strain not specified) using the physostigmine-induced lethality paradigm. The effects of clozapine and ICI 204,636 were compared to the atropine (a standard anticholinergic agent). ICI 204,636 was inactive in this paradigm at the doses tested (3-60 mg/kg i.p.). The MED_{50} for clozapine and atropine were 30.0 and 1.7 mg/kg i.p., respectively.

The seizure liability of ICI 204,636 was tested in two paradigms, i.e., potentiation of metrazole-induced seizures and ecs-induced convulsions. The effects of ICI 204,636, clozapine, haloperidol, and risperidone on the number of animals exhibiting seizures following a 50 mg/kg i.p. injection of metrazole were tested in male Swiss-Webster mice. ICI 204,636 did not produce a statistically significant increase in the % affected animals; however, the % of affected animals increased with dose (0-10, 10, 20, and 30% at doses of 0, 20, 40, and 80 mg/kg p.o., respectively. Clozapine produced a dose-related increase in the % affected at doses of 30-90 mg/kg; at the HD, 100% of animals exhibited seizures. Haloperidol did not significantly increase the % affected animals. Risperidone significantly increased the % affected at the highest dose tested, 30 mg/kg p.o. All compounds tested increased the potential for ecs-induced tonic convulsions (at 3.5 mA). Although not statistically significant, the % of animals exhibiting tonic convulsions with ICI 204,636 (at 80 mg/kg p.o.) was 50% compared to control values of 0-15%. Clozapine, haloperidol, and risperidone produced statistically significant increases in the % affected at doses of 30-90, 16-32, and 3.8-30 mg/kg p.o., respectively. All three of these compounds resulted in tonic convulsions in 80% of the animals tested.

Non-CNS studies

Functional receptor assays: The effects of ICI 204,636 and clozapine were compared in a number of functional receptor assays. Studies were conducted in selected tissues from rat, rabbit, and guinea pig: α_1 receptor (rat spiral aortic strips), α_2 receptor (rat vas deferens), β_1 and β_2 (spontaneously beating guinea pig right atria), H_1 and H_2 receptor (spiral strips of rabbit aorta, spontaneously beating guinea pig right atria), muscarinic receptor (spiral strips of guinea pig aorta), $5HT_2$ receptor (rabbit spiral aortic strips), Ca channel (rabbit spiral aortic strips), prostanoid receptor (rabbit spiral aortic strips). The results of these experiments are summarized in the following sponsor's Tables 26-27:

TABLE 26 Agonist responses in isolated tissues in the absence (control) and presence (treated) of ICI 204,636

Receptor	Agonist	n*	Conc.(M)	EC_{50}, nM(95% CL#)		pK_B
			ICI 204,636	Control	Treated	
α_1	Phenylephrine	7	1×10^{-6}	12(4.3-19.5)	206(85.7-327)	6.2 ± 0.1
α_2	Clonidine	5	1×10^{-6}	4.8 (2.4-7.2)	8.0 (2.8-13.3)	<5
β_1	Norepinephrine	5	1×10^{-6}	35 (20-50)	36 (23-47)	<5
β_2	Salbutamol	3	1×10^{-6}	33 (15-52)	13 (NC-27)	<5
H1	Histamine	4	1×10^{-7}	3180 (2230-4130)	1,101,000 (188,000-2,026,000)	9.5 ± 0.1
H2	Histamine	5	1×10^{-6}	816 (746-886)	1380 (864-1904)	<5
$5\text{-}HT_2$	Serotonin	3	1×10^{-6}	311 (157-465)	14,950 (10,200-19,700)	6.7 ± 0.02
Muscarinic	Carbachol	5	1×10^{-6}	94 (49-140)	368 (159-576)	5.4 ± 0.2
Thromboxane	U46619	4	1×10^{-6}	55 (38-72)	106 (NC-232)	<5
Ca^{++}channel	K^+ (80 mM)	5	1×10^{-6}			6.2 ± 0.7% +

* n = Number of independent assays.
\# CL = Confidence limit, NC = not calculable
\+ % inhibition of 80 mM K^+ contraction at 10^{-6} M.

TABLE 27 Agonist responses in isolated tissues in the absence (control) and presence (treated) of clozapine

Receptor	Agonist	n*	Conc.(M) Clozapine	EC_{50}, nM (95% CL#) Control	EC_{50}, nM (95% CL#) Treated	pK_B
α_1	Phenylephrine	3	1×10^{-7}	15 (NC-40)	64 (NC-148)	7.5 ± 0.2
α_2	Clonidine	3	1×10^{-6}	6.5 (5.4-7.5)	58 (NC-130)	5.9 ± 0.1
β_1	Norepinephrine	5	1×10^{-6}	31 (24-38)	71 (41-100)	5.0 ± 0.2
β_2	Salbutamol	3	1×10^{-6}	37 (16-57)	36 (11-60)	<5
H1	Histamine	5	1×10^{-7}	3870 (2830-4920)	2,000,000 (1,100,000-2,900,000)	9.7 ±0.1
H2	Histamine	5	1×10^{-5}	951 (401-1500)	1450 (905-2000)	<5
5-HT_2	Serotonin	3	1×10^{-7}	380 (307-453)	7560 (5650-9460)	8.3 ±0.1
Muscarinic	Carbachol	3	1×10^{-6}	90 (9.4-171)	1280 (694-1870)	7.1 ±0.1
Thromboxane (Vascular)	U46619	5	1×10^{-6}	38 (10-67)	67 (28-107)	<5
Ca^{++} channel	K^+ (80mM)	5	1×10^{-6}			15 ± 3.9%+

* n = Number of independent assays.
\# CL = Confidence limit, NC = not calculable
\+ % inhibition of 80 mM K^+ contraction at 10^{-6} M.

In the following Table 28 (from NDA submission), the sponsor compared the potency of ICI 204,636 and clozapine for selected receptors:

TABLE 28 Potency differences between ICI 204,636 and clozapine on various receptors

Receptor Assay	pK_B Clozapine	pK_B ICI 204,636	Potency Difference (ICI 204,636 vs. clozapine)
α_1	7.5	6.2	20 times less potent
α_2	5.9	< 5.0	>8 times less potent
H1	9.7	9.5	1.6 times less potent
5-HT_2	8.3	6.7	40 times less potent
Muscarinic	7.1	5.4	50 times less potent

Cardiovascular studies: ICI 204,636 was tested in a number of assays in order to assess potential cardiovascular/autonomic effects.

Antagonism of phenylephrine-induced vasopressor effects was tested in the pithed male Sprague-Dawley rat (4/dose). The effects of ICI 204,636 (1-10 mg/kg i.v.) were compared to those of clozapine (1-10 mg/kg i.v.) and prazosin (an α_1 antagonist, 0.03 mg/kg i.v.). At the lowest doses tested, ICI 204,636, clozapine, and prazosin produced 2-, 6-, and 24-fold shifts in the (indirect blood pressure) dose-response curve for phenylephrine. Higher doses (3 mg/kg i.v.) of ICI 204,636 and clozapine produced proportionately greater effects (6- and 15-fold shifts, respectively).

Antagonism of phenylephrine-induced vasopressor effects in ganglionic-blocked, atropinized, ß-blocked dogs (3/drug) was assessed for ICI 204,636 and clozapine. Both drugs were administered i.v. (bolus) in increasing doses of 0.3, 1, 3, and 10 mg/kg, with ≈40-70 min between injections. ICI 204,636 and clozapine both increased the dose of phenylephrine (i.v.) required to produce a 50 mm Hg increase in diastolic blood pressure; however, clozapine was more potent than ICI 204,636. The required dose of phenylephrine was increased 4-70 and 3-19 fold by clozapine and ICI 204,636, respectively.

In the normotensive rat (male Sprague-Dawley, 3/grp), both ICI 204,636 and clozapine produced decreases in systolic and diastolic blood pressure and heart rate at doses of 1, 3, and 10 mg/kg i.v. Reductions in heart rate were dose-related for both drugs (4-16%). The effects on

blood pressure (reductions of 14-35%) were not dose-related for either ICI 204,636 or clozapine, although the smallest effects were generally observed at the LD.

The propensity for ICI 204,636 to produce orthostatic hypotension (head tilt procedure) was tested in normotensive Beagle dogs (3/drug) at increasing doses of 0.3, 1, and 3 mg/kg (total dose = 4.3 mg/kg) i.v. In the resting state, ICI 204,636 and clozapine reduced diastolic and systolic blood pressure and heart rate in a fairly dose-related manner. Clozapine had a slightly greater effect on blood pressure than ICI 204,636 (SAP: 16-27 and 27-41%, respectively; DAP: 20-35 and 40-52%, respectively); the effect on heart rate was fairly similar for the two drugs (9-15%). Head tilt data were provided only in graph form. Both ICI 204,636 and clozapine exhibited vasodepressor effects following head tilt, delaying or preventing compensatory mechanisms (i.e., returning of blood pressure to baseline values). Clozapine's effect appeared slightly more potent than that of ICI 204,636; however, at 3 mg/kg, there was no evidence of compensation with either drug by 60 min postdosing.

The cardiovascular effects of ICI 204,636 and clozapine were tested in normotensive female Beagle dogs (3/drug). Measurements included arterial blood pressure (SAP, DAP), heart rate, and Lead II ECG. ICI 204,636 and clozapine were injected i.v. at increasing doses of 0.3, 1, and 3 mg/kg. Each dose was given over 1 min, with 60-90 min between doses. According to the sponsor, heart rate "...changes were slight and variable..." and no significant changes were detected in Lead II ECG parameters (no data presented in abstract). Both ICI 204,636 and clozapine produced decreases in SAP and DAP (8-25%).

Pulmonary study: one study was conducted in anesthetized male Beagle dogs (n = 3). Body temperature was maintained at 37° C. ICI 204,636 was administered i.v. in rising doses of 0.3, 1, and 3.0 mg/kg. Each dose was infused over 5 min, and there was a 10 min interval between doses. Animals were observed during the infusion periods and for 30 min after the last dose. The data were provided (in the abstract) only in graph form. According to the sponsor, there was a drug-related "...a rise in resistance and tidal volume and a decline in respiratory rate..."; however, this effect ("...generally less than 30% of baseline...") was not clearly dose-related. From the graph, it is clear that the SEMs for respiratory rate and pulmonary resistance were larger for drug-treated than control animals; therefore, the drug-effect may have been greater in individual animals. The graph for dynamic lung compliance suggests that ICI 204,636 may have had a slight inhibitory effect on this parameter at the HD.

General pharmacology

ICI 204,636 was tested at 1-2 doses in a number of paradigms (a general screen) to assess the secondary pharmacological effects of this drug. The assays and results are summarized below:

(1) ICI 204,636 significantly antagonized the effects of ACh (i.e., contractions) in the isolated guinea pig ileum at 10 but not 0.1 μM. ICI 204,636 had no agonist effects in this assay.

(2) ICI 204,636 significantly antagonized the effects of histamine (i.e., contractions) in the isolated guinea pig ileum at 0.1 and 10 μM (indicative of H_1 activity); no agonist effects were noted at either concentration.

(3) ICI 204,636 "weakly" antagonized the effects of histamine (i.e., beats) in the isolated guinea pig right atrium at 10 μM, but had no effect at 0.1 μM; no agonist effects were noted at either concentration.

(4) ICI 204,636 exhibited no agonist or antagonist effects at the β_2 adrenoreceptor when tested in isolated guinea pig trachea rings (i.e., isoprenaline-induced relaxation) at concentrations of 0.1 and 10 μM.

(5) ICI 204,636 did not antagonize clonidine-induced inhibition of contraction in isolated mouse vas deferens at concentrations of 0.1 or 10 μM; at 10 μM, however, ICI 204,636 exhibited agonist effects in this preparation (i.e., α_2 receptor).

(6) ICI 204,636 antagonized agonist (phenylephrine)-induced contractions in isolated mouse vas deferens at 10, but not 0.1 μM.; no agonist effects of ICI 204,636 were noted at either concentration.

(7) ICI 204,636 inhibited 5HT-induced contractions in spirally cut rat caudal artery at 0.1 and 10 μM, in a concentration-related manner; no agonist activity was noted at either concentration.

(8) ICI 204,636 "weakly" inhibited 5HT-induced contractions in isolated rat fundic strips at 10 μM; no antagonist activity was noted at 0.1 μM, nor were agonist effects noted at either concentration.

(9) CNS effects were tested in a variety of paradigms: general observation, ecs (tonic convulsion threshold), sleeping time, hot-plate, and local anesthesia in Swiss-Webster mice, and rotorod performance in Wistar rats. N's per grp ranged from 5-10. The results were as follows:

(a) ICI 204,636 was tested at 25 and 50 mg/kg p.o. (single dose) in a general observation battery. No effects were noted at 25 mg/kg; at 50 mg/kg, sedation, decreased spontaneous motor activity, and reductions in body temperature were noted.

(b) ICI 204,636 did not affect the ecs-induced seizure threshold at 25 mg/kg p.o.

(c) ICI 204,636 had no effect on rotorod performance following a single 25 mg/kg p.o. dose.

(d) ICI 204,636 had no sedative potentiating effects in subthreshold barbital-treated animals following a single 25 mg/kg dose.

(e) ICI 204,636 displayed no analgesic effects in the mouse hot-plate paradigm at a single dose of 25 mg/kg p.o.

(f) When injected into the popliteal space (i.e., the area of the sciatic nerve), ICI 204,636 produced hindlimb impairment, as noted by an inability to walk, at acute oral doses of 12.5 (5/10 mice) and 25 (9/10 mice) mg/kg. dose; the effect last for the entire 20 min observation period. According to the sponsor, a dose of 6.25 mg/kg had no effect; however, according to the data table, 1 mouse (of 10) also exhibited an inability to walk which lasted for 10 min postdosing following the 6.25 mg/kg dose. No control animal exhibited impaired behavior. Therefore, no NOEL was established for this effect

(10) Cardiovascular effects of ICI 204,636 were tested in several assays: antihypertension in SHR, blood pressure and blood flow effects in Beagle dogs. The results are as follows:

(a) ICI 204,636 reduced blood pressure in female SHR (n not specified) following 3 daily doses of 50 mg/kg p.o. The effect was noted at 2 and 5 hr, but not at 24 hr, postdosing. Sedation was observed in these animals at 2 and 5 hr, but not at 24 hr, and may have contributed to the blood pressure effects. No changes

in heart rate were observed.

(b) In the conscious dog (5/grp), ICI 204,636 produced a slight, but statistically significant decrease in systolic blood pressure (10%) at 25 mg/kg p.o.; no significant effects were noted on diastolic blood pressure or on heart rate. The only ECG effect noted was a shortening of the PR-interval (at 25 mg/kg p.o.); the mechanism underlying this effect is unknown.

(c) Cardiovascular effects of ICI 204,636 i.v. were tested in anesthetized Beagle dogs (n not specified). At 2.5 mg/kg i.v., ICI 204,636 produced small (n.s.)decreases in blood pressure (MAP) and heart rate, but had no effects on vascular resistance, or on PR or QT-intervals.

(d) These data were conducted during the pulmonary evaluation [described in (11)] in the same animals. According to the sponsor (i.e., data not provided), there were no effects on heart rate or blood pressure at 2.5 mg/kg i.v. At 10 mg/kg i.v., there was an increase in heart rate (27%, n.s.) and decreases in SAP and DAP (≈50%, statistically significant for both parameters).

(11) Pulmonary effects of ICI 204,636 were tested in anesthetized female Beagle dog (3/grp) at doses of 2.5 and 10 mg/kg i.v. Assessment of pulmonary resistance (indicative of airway narrowing/dilation) and dynamic compliance (indicative of elastic properties of lung) was conducted during dosing and for 30 min postdosing. There were no statistically significant changes in either of the parameters measured at either dose of ICI 204,636. The % increase in pulmonary resistance from baseline was almost 3-fold higher following 10 mg/kg i.v. than in the C grp; however, the maximum response was similar between grps (4.3-4.4 cm $H_20/L/s$).

(12) The effects of ICI 204,636 on GI function were tested in 2 studies. In male Beagle dog (n = 3) with Heidenhain pouches, ICI 204,636 was found to reduce histamine-gastric acid secretion at a dose of 50 mg/kg p.o. No data were provided. The sponsor stated that the effect was "slight", and possibly attributable to the marked sedation noted in these dogs.

In male mice (10/grp), ICI 204,636 inhibited gastric motility (32%), i.e., retarded movement of charcoal through the GI tract. By comparison, carbachol increased and morphine decreased transit by 41 and 22%, respectively. The sponsor attributed the effect of ICI 204,636 to sedation.

(13) The effects of ICI 204,636 on renal function was tested in rats (n not specified). According to the sponsor (i.e., no data provided) there were no significant changes in "...diuresis, saluresis or kaliuresis during a 6 hour period in rats dosed po with ICI 204,636 (50 mg/kg)..."

PHARMACOLOGY OF METABOLITES

According to the sponsor, ICI 204,636 represents only a "...small proportion..." of drug-related material following administration of ^{14}C-ICI 204,636 in rat, dog, cynomolgus monkey, and human. As stated by the sponsor, "In all species, the compound is extensively metabolized and appears to undergo significant pre-systemic metabolism". Therefore, the pharmacological activity of 3 metabolites, ICI 214,227 (7-OH), M 236,303 (7-OH, N-dealkyl), and ICI 213,841 (sulfoxide), was assessed. According to the sponsor, ICI 213,841 is the major plasma metabolite in humans, whereas, ICI 214,227 and M 236,303 are the major metabolites in the animal species. These metabolites were synthesized for analysis.

ICI 214,227: this metabolite was tested at 1-2 doses in a number of paradigms (a general screen) to assess the secondary pharmacological effects of this drug. The assays and results are summarized below:

(1) ICI 214,227 exhibited no agonist effects in a isolated guinea pig ileum preparation at concentrations of 0.1 and 10 μM. ICI 214,227 did antagonize agonist (i.e., ACh, histamine, barium chloride)-induced contractions at both concentrations; at 0.1 μM, decreases of 19, 57, and 21%, respectively, were obtained. In addition, ICI 214,227 antagonized histamine effects at 0.01 μM. The sponsor concluded that ICI 214,227 functioned as a non-competitive antagonist at the H_1 and muscarinic receptors in this preparation.

(2) ICI 214, 227 had no effects on agonist [isoprenaline ($ß_1$), histamine (H_2)]-induced positive chronotropic effects on guinea pig atria or on the electrically-stimulated atria, nor did it exhibit any agonist effects at concentrations of 0.1 or 10 μM.

(3) ICI 214,227 had no effect on isoprenaline-induced relaxation or on basal tone of isolated guinea pig tracheal spiral rings at concentrations of 0.1 and 10 μM.

(4) ICI 214,227 antagonized 5HT-induced contraction of the isolated rat aorta at concentrations of 0.1 and 10 μM; no agonist effect was noted.

(5) CNS effects were assessed in a number of paradigms: observational battery, sleeping time, hot-plate, local anesthesia in mice and rotorod performance in rats. There were 8-10 animals/grp in these studies. The results are as follows:

(a) Following a single 25 mg/kg p.o. dose, ICI 214,227 produced "...marked ataxia and ptosis...", and decreases in spontaneous motor activity (46% at the end of the 2-hr observation period) and body temperature (0.9 ° C).

(b) ICI 214,227 had no effect on rotorod performance following a single dose of 25 mg/kg.

(c) ICI 214,227 (25 mg/kg p.o.) did not potentiate sleeping in animals treated with a subthreshold dose of sodium barbital. This dose did, however, produce ataxia, tremors, and ptosis in these animals.

(d) ICI 214,227 exhibited no analgesic effects in the mouse hot-plate paradigm at a dose of 25 mg/kg p.o.

(e) ICI 214,227 exhibited local anesthetic effects when injected into the area of the sciatic nerve. At a dose of 25 mg/kg, 6/10 mice were unable to walk on a wire grid. At 12.5 mg/kg i.m., 2/10 mice were affected; however, at 6.25 mg/kg, no effect was observed.

(6) Cardiovascular effects of ICI 214,227 were tested several assays: male SHR (n = 3), blood pressure and flow in Beagle dogs (n = 4/grp/parameter). The results were as follows:

(a) There was a slight increase in heart rate (36%) and blood pressure (DAP,SAP, 7-10%) in male SHR following a single 25 mg/kg p.o. dose of ICI 214,227. The sponsor indicated that these changes were correlated with increases in spontaneous motor activity, and could be artifactual.

(b) ICI 214,227 had no statistically significant effect on blood pressure or heart rate

in conscious dogs at 25 mg/kg p.o. for up to 20 hr postdosing. There was, however, a shortening of the PR-interval (data not provided).

(c) ICI 214,227 produced slight decreases (n.s.) in heart rate and blood pressure in anesthetized dogs; however, P-R and Q-T intervals and vascular resistance were not affected up to 90 min postdosing. [Numerical data were not provided in the abstract; data were presented only in graph form.]

(7) ICI 214,227 had no significant effect on pulmonary parameters (i.e., pulmonary resistance, dynamic compliance) when administered to anesthetized Beagle dogs (n = 3) at 2.5 mg/kg i.v. Measurements were recorded up to 30 min postdosing.

(8) The effects of ICI 214,227 on gastric motility were tested in male albino mice (10/grp) using charcoal as a marker for transit. ICI 214,227 (25 mg/kg p.o.) slightly prolonged transit time (11%); however, the effect was not statistically significant. By comparison, carbachol significantly decreased transit time by 16% and morphine significantly increased transit time by 46%.

(9) The effects of ICI 214,227 on the renal system were tested in male Wistar rats at 25 mg/kg p.o. ICI 214,227 had no effect on any parameter measured (i.e., urinary volume, Na, K, and Cl excretion, or on urinary osmolality).

M 236,303

The same general pharmacology parameters were tested for M 236,303 as were conducted on ICI 214,227. The results are summarized below:

(1) In isolated guinea pig ileum, M 236,303 inhibited histamine-induced contractions at concentrations of 0.1 and 10 μM (non-competitive inhibition), and ACh-induced contractions at 10 μM (competitive inhibition). M 236,303 also inhibited the maximum response to barium chloride at 10 μM. No agonist activity was noted.

(2) M 236,303 had no effect on the beating rate of isolated guinea pig right atria, nor did it inhibit agonist (isoprenaline, histamine)-induced changes.

(3) M 236,303 had no effect on resting tone of isolated guinea pig trachea, nor did it inhibit agonist (isoprenaline)-induced changes.

(4) M 236,303 had no agonist effects on rat aorta; however, M 236,303 did inhibit (79%) 5HT-induced contractions at 10 μM. It was unclear whether or not M 236,303 had an affect at 0.1 μM; this concentration resulted in a dose-ratio of 26.5.

(5) CNS effects included the following:

(a) M 236,303 (25 mg/kg p.o.) reduced body temperature slightly (maximum of ≈0.9° C at 30 min postdosing), but had no effect on spontaneous motor activity or behavior.

(b) M 236,303 (25 mg/kg p.o.) had no effect on rotorod performance.

(c) M 236,303 (25 mg/kg p.o.) did not potentiate sleeping time in animals treated with a subthreshold dose of sodium barbital.

(d) M 236,303 (25 mg/kg p.o.) had no analgesic effects in the mouse hot-plate paradigm.

(e) M 236,303 had local anesthetic effects at all doses tested. At 25 mg/kg p.o., 8/10 mice were unable to walk on a wire grid following an i.m. injection of M 236,303 into the area of the sciatic nerve. At 12.5 mg/kg, 5/10 animals were affect, and at 6/25, 1/10 was affected. All C animals were successful in walking the grid.

(6) Cardiovascular effects included the following:

(a) M 236,303 produced increases in heart rate (22%) and blood pressure (9-11%) in SHR following a 25 mg/kg p.o. dose. Maximum effects were noted 2 hr postdosing.

(b) M 236,303 had no effect on heart rate or blood pressure in the conscious dog at a dose of 25 mg/kg p.o., but did produce a slight shortening of the P-R interval (15%).

(c) M 236,303 increased vascular resistance in anesthetized dog following a 2.5 mg/kg i.v. dose. No notable effects were noted on QT or PR interval or on heart rate or blood pressure up to 90 min postdosing.

(7) M 236,303 had no effect on either pulmonary resistance or pulmonary compliance in anesthetized dogs following a 2.5 mg/kg i.v. dose.

(8) M 236,303 produced a slight increase in transit time (7%) in charcoal-treated mice. By comparison, carbachol decreased transit time by 24% and morphine prolonged transit time by 41%.

(9) M 236,303 had no effect on renal parameters, e.g., urinary volume, Na, K, and Cl excretion, or on urinary osmolality, following a 25 mg/kg p.o. dose.

ICI 213,841

The same general pharmacology parameters were tested for ICI 213,841 as were conducted on ICI 214,227 and M 236,303. The results are summarized below:

(1) ICI 213,841 had no agonist effects on isolated guinea pig ileum; however, this metabolite did inhibit histamine, barium chloride, and ACh-induced contractions at concentrations of 0.1 and 10 μM. ICI 213,841 reduced the maximum response to barium chloride (25%) and histamine (87%) at 10 μM, and the maximum response to barium chloride (20%), histamine (20%), and ACh (21%) at 0.1 μM. Therefore, ICI 213,841 exhibited significant antagonist effects at the histamine and ACh receptors.

(2) ICI 213,841 had no effect on basal activity of isolated guinea pig right atria, isolated guinea pig trachea, or isolated rat aorta; nor did this metabolite inhibit agonist responses in these tissues (i.e., isoprenaline and histamine in right atria, isoprenaline in trachea, or 5HT in aorta).

(3) ICI 213,841 was without CNS effects at a dose of 25 mg/kg p.o., except this metabolite did produce a local anesthetic effect when injected i.m. into the area of the sciatic nerve. At 25 mg/kg, 8/10 injected mice could not walk on a wire grid and at 12.5 mg/kg i.m., 1/10 was affected. A 6.25 mg/kg dose was without effect.

(4) The cardiovascular effects of ICI 213,841 were as follows:

(a) ICI 213,841 (25 mg/kg p.o.) had no effect on blood pressure or heart rate in SHR; however, high baseline values for heart rate, DAP, and SAP (35-52%

higher than C baseline values) made the data somewhat difficult to interpret.

(b) ICI 213,841(25 mg/kg p.o.) increased heart rate (23%) and shortened the P-R interval (14%), but had only slight (n.s.) effects (i.e., reduction) on blood pressure, in conscious dog. The effect on the P-R interval was still evident at 6 hr postdosing.

(c) In the anesthetized dog, ICI 213,841 (2.5 gm/kg i.v.) caused slight decreases in heart rate and MAP, and an increase in vascular resistance by 75 min. postdosing. [Numerical data were not provided; data were presented in graph form only.]

(5) ICI 213,841 had no effect on pulmonary resistance or compliance at a dose of 2.5 mg/kg i.v. in anesthetized dog.

(6) ICI 213,841 increased transit time (=17%) in charcoal-fed mice at a dose of 25 mg/kg p.o. By comparison, carbachol decreased transit time by =19% and morphine increased transit time by 56%.

(7) ICI 213,841 had no effect on renal parameters (i.e., urinary volume and osmolality, and excretion of Na, K, and Cl) at a dose of 25 mg/kg p.o.

A comparison of the binding profile for selected receptors was provided in the following sponsor's table (the plasma data were collected in monkey):

TABLE 67 Pharmacology profile (in vitro receptor binding data) for ICI 204,636 and active metabolites

Compound description	Receptor binding (Ki nM)			Plasma concentration (ng/ml)*	
	D1	D2	5-HT_2	Oral	Intramuscular
ICI 204,636 (parent)	1116	192	80	72.4	341
ICI 214,227 (7-hydroxy)	251	49 (~3.9X)#	51 (~1.5X)	80.3	66.3
M 236,303 (7-hydroxy,N-dealkylated)	288	35 (~5.5X)	6.5 (~12X)	47.4	12.1
M 234,676 (O-dealkylated)	477	100	59	NA+	NA
M 211,803 (N-dealkylated)	1489	234	27	NA	NA

* Mean plasma concentration at 1 hour postdose (po) and 1.5 hours postdose (im)
# Values in parenthesis represent relative potency compared to parent compound.
+ NA - Plasma concentration not analyzed.

Interestingly, ICI 204,636 was either inactive or had lower potency when administered i.m. as compared to p.o. The results of the comparative tests were provided in the following sponsor's table:

TABLE 68 Summary of in vivo animal pharmacology data following intramuscular and oral administration in monkeys

Dose route	Conditioned Avoidance (Cebus monkey)	Apomorphine blinking (Squirrel monkey)	Negative symptoms (Java monkey)
Intramuscular	Inactive (5 mg/kg)	Active (ED_{50} - 13 mg/kg)	Active (1-10 mg/kg)
Oral	Active (ED_{50} - 5 mg/kg)	Active (ED_{50} - 4.2 mg/kg)	Not Tested

Others

In vitro receptor binding profiles (for D_1, D_2, and $5HT_2$ receptors only) were determined for a number of other plasma metabolites. ICI 213,841(sulfoxide), M 235,101 (8-OH), M 276,660 (O-dealkyl acid), M 282,445 (O-dealkyl sulfoxide acid), M 289,663 (parent acid), and M 289,886 (sulfoxide acid) had little or no affinity for any of the receptors tested (i.e., $K_i \leq 1.0$ μM). M 235,101, M 276,660, and M289,886 had weak affinity for the $5HT_2$ receptor (i.e., K_i = 1274-4870 nM) and M 235,101 also had weak affinity for the D_2 receptor (K_i = 1565 nM).

METHODS

1. **The synthesis of 2-(2-(4-([11-14C]dibenzo[b,f][1,4]thiazepin-11yl) piperazin-1-yl]ethyxy)ethanol hydrogen fumarate** (Study no. 204636 KML 002/01, 1/ID/1006384, Vol 1.60).

2. **The synthesis of [14C]-ICI 204,636** (Study no. 204636DDL004/01, 9/HH/011723, Vol. 1.60).

3. **Development and validation of a liquid chromotographic method for the determination of ICI 204,636 in plasma RAB #1** (U.S. Study no. DDM24027004, 8/HH/011522, Vol 1.60).

4. **Development and validation of a liquid chromatographic method for the determination of ICI 204,636 and its metabolites, ICI 213,227 and ICI 213,841 in plasma** (Study no. DDM24027009, 11/HH/012053, Vol 1.60).

5. **Determination of ICI 204636 in dog plasma by solid phase extraction and high performance liquid chromotography with uv detection. Validation of method 31-VIII (16P-05)** (Study no. 204636DBQ030/01, 9/ID/1011556, Vol 1.60).

6. **Determination of ICI 204636, ICI 214227 and ICI 213841 in rat plasma by high performance liquid chromatography. Validation of method number 16-09** (Study no. 5077DBQ040, 4/ID/1008054, Vol 1.60).

7. **Determination of ICI 204636, ICI 214227 and ICI 213841 in dog plasma by high performance liquid chromatography. Validation of method number 16-09** (Study no. 5077DBQ046, 4/ID/1008700, Vol 1.60).

8. **Determination of ICI 204,636 and metabolites in monkey and mouse plasma by HPLC validation of method number 16-11.** (Study no. 5077DBQ047, 7/ID/1009578, Vol 1.60).

These studies were conducted in order to develop sample preparation and quantitation techniques for ICI 204,636 and metabolites in rat, dog, monkey, and mouse plasma. The final assay developed involved an initial 3-step liquid-liquid extraction, followed by quantitation using reversed phase HPLC with uv detection (225 nM). The extraction procedure consisted of the following steps: (1) extraction in basic ethyl acetate, (2) back-extraction into acidic ethyl acetate, (3) extraction into basic ethyl acetate.

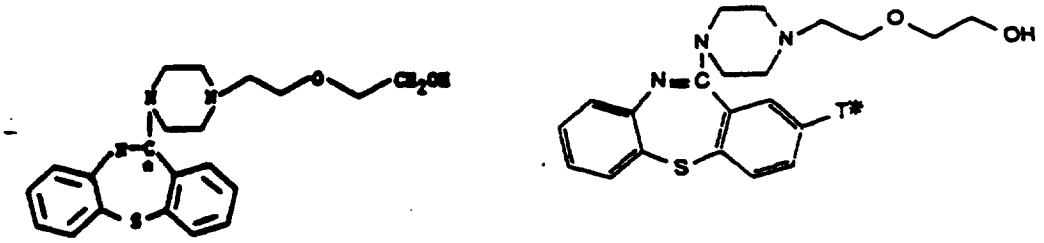
In rat plasma, the LLOQ was 10 ng/mL for ICI 204,636, and 20 ng/mL for metabolites ICI 214,227 and ICI 213,841. Recovery was ≈85-90% for the three compounds. Stability of the parent compound and metabolites was demonstrated when plasma was stored at -20° C for up to 259 days.

In dog plasma, the initial addition of 0.5 mL of water to the plasma sample was necessary to prevent extraction of interfering substances not present in the plasma of other species. The LLOQ was 10 ng/mL for ICI 204,636 and ICI 214,227, and 30 ng/mL for ICI 213,841. Recovery was ≈65-70% for the three compounds. Stability of ICI 204,636, ICI 214,227, and ICI 213,841 in plasma was demonstrated when plasma was stored at -20° C for 106 days.

In monkey plasma, the LLOQ was 5 ng/mL for ICI 204,636, ICI 214,227, and ICI 236,303, and 10 ng/mL for ICI 213,841. Recovery ranged from 41% for ICI 213,841 to 52% for ICI 204,636. The stability through 4 freeze-thaw cycles was established for the four compounds; however, documentation of stability during storage was ongoing at the time of the report.

In mouse plasma, the LLOQ was 20 ng/mL for ICI 204,636, ICI 213,841, and ICI 214,227. Recovery was ≈45-50% for the three compounds. The stability through 4 freeze-thaw cycles was established for the four compounds; however, documentation of stability during storage was ongoing at the time of the report.

9. **Determination of the mtabolic stability of tritium-labeled ICI 204,636 in the rat** (Study no. 5077DMR042, 1/ID/1006503, GLP, Vol. 1.61: 5.I.17).



[^{3}H]-ICI 204,636

This study was performed in male Sprague-Dawley rats. The metabolic stability of ^{3}H-ICI 204,636 was compared to that of ^{14}C-ICI 204,636 (lot no. W3595B1, unlabeled). Three rats received a single dose of ^{14}C-ICI 204,636 (25 mg/kg p.o.) followed 1 wk later with a single dose of ^{3}H-ICI 204,636 (25 mg/kg p.o.). Animals received food and water ad lib throughout the study. After both doses, urine was collected for 24 hrs postdosing.

^{3}H-ICI 204,636 and ^{14}C-ICI 204,636 were administered to additional rats (6/isotope) at a dose of 25 mg/kg p.o. For each isotope, 3 rats were sacrificed at 0.5 hr postdosing and the remaining 3 rats were sacficied 2.0 hr postdosing. Blood samples were collected at sacrifice.

Plasma radioactivity (mean ± SEM) following administration of ^{14}C- and ^{3}H-labeled ICI 204,636 were 2.43 ± 0.62 and 3.58 ± 0.45 µg-eq/g, respectively, at 0.5 hr postdosing, and 1.31 ± 0.19 and 1.39 ± 0.02 µg-eq/g, respectively, at 2 hr postdosing. The % extractable radioactivity was 60.3-62.7% for ^{14}C-ICI 204,636 and 74.9-77.7% for ^{3}H-ICI 204,636. The sponsor attributed the difference between isotopes at 0.5 hr postdosing to interanimal variation and not inter-isotope differences.

Urinary radioactivity was determined on samples collected at 0-6 and 6-24 hr intervals, as well as for overall urinary radioactivity (i.e., 0-24 hr). For ^{14}C-ICI 204,636, the % of dose (mean ± SEM) excreted were 4.1 ± 0.6, 4.5 ± 0.1, and 8.5 ± 0.7% at 0-6, 6-24, and 0-24 hr intervals, respectively. For ^{3}H-ICI 204,636, the % of dose excreted were 2.8 ± 0.7, 3.9 ± 0.5, and 6.7 ± 0.2%, at 0-6, 6-24, and 0-24 hr intervals, respectively. The % extractable radioactivity in the 0-6 hr samples were 42.2-44.5% for both isotopes. The data indicated that the metabolic stability of the ^{3}H-labeled compound is fairly similar to that of the ^{14}C-labeled compound, i.e., that not a notable amount of tritiated water was formed following dosing with the tritiated compound.

BALANCE STUDIES

Mouse

1. **ICI 204,636: Absorption and elimination in the mouse following administration of a single oral dose of ^{14}C-ICI 204,636** (Study No. 204636DMM019, Report No. SP2510/B, Vol 1.61: 5.I.9).

This study was conducted in order to support the 2-yr carcinogenicity in mice. ^{14}C-ICI 204,636 (lot no. U24027F7 for unlabeled substance) was administered orally as a single 20-mg dose to mice (alpk C57B1; 20/sex). Animals were "partially" fasted (i.e., given 6 food pellets) for ≈16 hr prior to dosing. During sample collection, mice were housed 5/cage; therefore, data are based on pooled samples. Urine and feces were collected up to 120 hrs postdosing at the following intervals: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hrs for urine; 0-24, 24-48, 48-72, 72-96, and 96-120 hr for feces. Cage washings were collected at the end of the study. The results are summarized in the following sponsor's Table 1:

Table 1: Elimination of radioactivity [Mean(SEM)]

	Male	Female	p
Urine	33.4 (1.4)	42.9 (1.4)	0.0027
Feces	60.2 (1.1)	49.6 (0.6)	0.00014
Cage Wash	2.1 (0.5)	3.5 (1.1)	
Recovery	95.6 (0.7)	95.9 (0.7)	

Rat

1. **Absorption, metabolism and elimination following administration of a single oral or intravenous dose of ^{14}C-ICI 204,636 to male and female rats** (Study no. 204636DMR033, 12/IB/019258, Vol. 1.61: 5.I.10).

Methods:. ICI 204,636 (lot no. 45103187, cold substance) was administered orally (gavage, 25 mg/kg) or i.v. (5 mg/kg) as single doses to Sprague-Dawley rats (3/sex/route). Animals were "partially" fasted (i.e., given 6 food pellets) for ≈16 hr prior to dosing. Urine and fecal samples were collected in individual animals at the following intervals: 0-6, 6-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hrs for urine; 0-24, 24-48, 48-72, 72-96, and 96-120 hr for feces. Cage washings were collected at the end of the study.

2. **Absorption, metabolism and elimination in bile duct-cannulated male rats** (U.S. Study no. DDM24027002, 5/HH/011180, Vol 1.61: 5.I.11).

Methods: Male Sprague-Dawley rats (6/route) were administered ICI 204,636 (lot no. 24027F2) as either a single p.o. or i.v. dose (5 mg/kg for both routes) following bile-duct cannulation. Animals were given "...a limited number of food pellets..." the day prior to dosing, and, if not properly hydrated, 5 mL of Prosobee solution by gavage 1 hr prior to dosing. Urine, bile, and fecal samples were collected for 48 hr after dosing, and cage washings were collected at the end of the study. Samples were analyzed untreated and after conjugate hydrolysis (with glusulase, a type H-1 glucuronidase).

3. **Absorption, distribution and elimination of ^{14}C-ICI 204,636 in bile duct-cannulated male rats after a 25 mg/kg oral dose** (Study no. DDM24027010, 4/HI/012818, Vol 1.61: 5.I.12).

Methods: same as for #2, except that this study was conducted in 5 male Sprague-Dawley rats, each receiving a single 25 mg/kg oral dose.

4. **Absorption, distribution and elimination in bile duct-cannulated female rats after 5**

and 25 mg/kg administration of ^{14}C-ICI 204,636 (Study no. DDM24027011, 8/HI/013338, Vol 1.61: 5.I.13).

Methods: same as for #2, except that this study was conducted in female Sprague-Dawley rats (5/grp), each receiving a single dose of ICI 204,636 (lot no. 24027F2) at either 5 mg/kg i.v. or p.o. or 25 mg/kg p.o.

5. **Metabolism and elimination in male and female rats after multiple doses of 14C-ICI 204,636** (Study no. 5077DMR037, 12/IC/1005826, Vol 1.61: 5.I.15).

Methods: ICI 204,636 [lot no. ADM44040/92 (W3595B1), cold] was administered to Sprague-Dawley rats (3/sex) daily for 7 days at a dose of 25 mg/kg/day. Animals received food and water ad lib throughout the study. Urine and fecal samples were collected continuously from the initial dose to 192 hr after the final dose. Cage washings were obtained after the final sample collection. Fecal samples collected at 6-24 hr after each dose were analyzed for metabolites by HPLC following extracton.

The data from these studies are summarized in the attached Tables 1- 3.

6. **Pharmacokinetics in male and female rats after 5 and 25 mg/kg doses** (US Study no. DDM24027003, 5//HH/011179, Vol 1.61: 5.I.14)

Methods: This study was conducted in Sprague-Dawley rats (3-4/sex/grp). ^{14}C-ICI 204,636 (lot no. 31078A1, unlabeled) was administered at single doses of 5 mg/kg i.v., p.o., and 25 mg/kg p.o. Animals were fed "...only a few pellets..." the night prior to dosing. Blood samples were collected at intervals from 5 to 24 hr postdosing for (HPLC) quantitation of the parent compound and metabolites, ICI 214,227 (2-OH), ICI 213,841 (sulfoxide), and U31744 (dealkylated 2-OH).

7. **Pharmacokinetics in male and female rats after single and multiple doses of [^{14}C]ICI 204,636** (Study no. 5077DMR038, 7/ID/1009722, GLP, Vol 1.61: 5.I.16)

Methods: In this study, ^{14}C-ICI 204,636 (lot no. ADM44040/92, unlabeled) was administered as single doses of 25 and 125 mg/kg p.o. and 10 mg/kg i.v., and as 7 consecutive daily doses at 25 mg/kg p.o. Animals received food and water ad lib throughout the study. Blood samples were collected at 0, 5 (i.v.), 10 (p.o.), 15 (i.v.), 20 (p.o.), 30, and 45 min after single doses, and at 0, 1, 2, 4, 6, 8, 12, and 24 hr after the 7th dose in multiply-dosed animals. Plasma was used to quantitate the parent compound and metabolites, ICI 214,227 (7-OH) and ICI 213,841 (sulfoxide).

The data for reports # 6 and 7 are summarized, in part, in the attached Table 4.

Dog

1. **Absorption, excretion and pharmacokinetics in male dogs** (Study no. DDM24027001, 9/HH/011721, ICI Pharmaceuticals, Vol 1.62: 5.I.19)

Methods: ^{14}C-ICI 204,636 (lot no. 24027F2, unlabeled) was administered at single doses of 1 mg/kg i.v., p.o., 10 and 25 mg/kg p.o. to 4 male Beagle dogs (fasted overnight) in the following manner. ICI 204,636 was made up in physiologic saline (1 mg/kg p.o., i.v.) or 0.5% HPMC/0.1% TWEEN 80 (10 mg/kg p.o.). On Day 1, 2 dogs received single doses of 1 mg/kg i.v. and the remaining 2 dogs received single doses of 1 mg/kg p.o. Two weeks later, those dogs that had been dosed with 1 mg/kg i.v. on Day 1 received the same dose, but p.o., and vice versa. Two wks

TABLE 1: ELIMINATION OF RADIOACTIVITY IN SPRAGUE-DAWLEY RATS AFTER SINGLE AND MULTIPLE DOSES OF ICI 204,636

STUDY NO.	DOSE/ROUTE	DURATION	SEX	DAY	ELIMINATION (% of dose radioactivity)			
					URINE	BILE	FECES	TOTAL RECOVERY
204636DMR033	25 mg/kg p.o.	single dose	M		9.4 ±0.5*		88.7 ± 1.1	98.5 ± 0.8
			F		10.7 ± 2.0		87.8 ± 1.5	99.0 ± 3.7
	5 mg/kg i.v.		M		11.0 ± 1.7		80.5 ± 1.7	91.5 ± 3.1
			F		5.8 ± 0.7		84.9 ± 1.7	90.8 ± 1.1
DDM24027002	5 mg/kg p.o.	single dose	M		9.76 ± 5.36*	77.28 ± 11.51	2.13 ± 0.87	89.17 ± 9.50
	5 mg/kg i.v.				7.30 ± 3.43	75.85 ± 12.31	3.60 ± 1.49	86.75 ± 11.32
DDM24027010	25 mg/kg p.o.	single dose	M		7.46 ± 1.56**	87.4 ± 2.20	2.94 ± 1.24	98.4 ± 2.05
DDM24027011	5 mg/kg p.o.	single dose	F		9.10 ± 2.24**	79.6 ± 5.60	1.46 ± 0.63	91.0 ± 2.23
	5 mg/kg i.v.				12.9 ±6.48	70.5 ± 8.48	0.93 ± 0.30	86.3 ± 5.75
	25 mg/kg p.o.				12.0 ± 4.56	77.0 ± 5.86	2.40 ± 0.52	93.3 ± 0.99
5077DMR037#	25 mg/kg p.o.	7 days	M + F	1	6.8 ± 0.8**		76.6 ± 4.4	83.4 ± 4.3
				7	7.5 ±0.7		87.6 ± 1.6	95.1 ± 1.4

*mean ± SD; **mean ± SEM; #data are expressed as total elimination as % of total dose

TABLE 2: METABOLIC PROFILE IN BILE / URINE COLLECTED FROM SPRAGUE-DAWLEY RATS TREATED WITH SINGLE DOSES OF ICI 204,636

STUDY NO.	DOSE (mg/kg) /ROUTE	SEX	BILE*					URINE*				
			PC	2-OH	S	Dealkyl 2-OH	Dealkyl	PC	2-OH	S	Dealkyl 2-OH	Dealkyl
DDM24027002	5 p.o.	M	0.11 ± 0.09** (0.38 ± 0.13)	0.20 ± 0.09 (5.61 ± 4.64)	0.80 ± 0.73 (1.28 ± 0.88)	0.89 ± 0.73 (7.33 ± 4.4)		0.27 ± 0.17 (0.11 ± 0.04)	2.88 ± 1.29 (0.64 ± 0.17)	0.55 ± 0.41 (0.31 ± 0.16	7.13 ± 2.77 (2.28 ± 1.90)	
	5 i.v.		0.06 ± 0.04 (0.19 ± 0.07)	0.14 ± 0.07 (6.20 ± 4.51	0.89 ± 0.7 (0.75 ± 0.7)	0.39 ± 0.15 (4.06 ± 2.68)		0.41 ± 0.28 (0.12 ± 0.03)	2.79 ± 1.41 (0.55 ± 0.12	0.58 ± 0.33 (0.27 ± 0.16)	3.76 ± 1.51 (1.28 ± 0.71)	
DDM24027010	25 p.o.	M	0.05 ± 0.01 (0.36 ± 0.01	1.17 ± 0.37 (7.99 ± 0.65)	<LLOQ (0.60 ± 0.11)	0.10 ± 0.02 (4.25 ± 0.30)		0.16 ± 0.01 (0.15 ± 0.02)	2.71 ± 0.25 (0.54 ± 0.18)	1.98 ± 0.43 (0.40 ± 0.11)	7.57 ± 0.91 (2.60 ± 0.32)	
DDM24027011	5 p.o.	F	0.06 ± 0.01 (0.45 ± 0.09)	0.21 ± 0.03 (13.8 ± 1.31)	0.14 ± 0.04 (0.42 ± 0.11)	0.37 ± 0.10 (3.62 ± 0.26)	0.08 ± 0.02 (0.26 ± 0.11)	1.95 ± 0.94 (0.34 ± 0.10)	9.32 ± 2.00 (2.59 ± 0.34)	0.88 ± 0.04 (0.38 ± 0.05)	6.29 ± 1.38 (3.01 ± 0.54)	17.0 ± 5.39 (2.11 ± 0.64)
	5 i.v.		0.08 ± 0.02 (0.33 ± 0.09)	0.10 ± 0.04 (12.2 ± 2.49)	0.08 ± 0.02 (0.28 ± 0.12)	0.22 ± 0.10 (3.65 ± 0.38)	0.10 ± 0.05 (0.23 ± 0.13)	1.43 ± 0.65 (0.59 ± 0.11)	8.61 ± 3.43 (5.03 ± 2.17)	0.98 ± 0.35 (0.53 ± 0.18)	3.85 ± 1.40 (3.15 ± 0.86)	21.1 ± 9.86 (2.62 ± 1.21)
	25 p.o.		0.12 ± 0.08 (0.54 ± 0.25)	0.15 ± 0.03 (15.0 ± 1.55	0.14 ± 0.05 (0.71 ± 0.18	0.34 ± 0.05 (6.19 ± 0.21)	0.27 ± 0.12 (0.59 ± 0.26)	5.34 ± 3.16 (0.42 ± 0.10)	6.26 ± 2.89 (3.93 ± 1.40)	0.76 ± 0.18 (0.66 ± 0.25)	4.05 ± 1.47 (3.60 ± 0.92)	19.6 ± 9.75 (2.78 ± 0.86)

*% of bile or urine radioactivity. **data for the free compound are expressed as mean ± SD (data for the conjugated compound presented in parentheses)

TABLE 3: PLASMA AUC'S FOR ICI 104,636 AND METABOLITES FOLLOWING SINGLE AND MULTIPLE DOSING IN SPRAGUE-DAWLEY RAT

STUDY	DOSE/ROUTE	SEX	TOT RAD (ng-eq/g)	ICI 204,636*	2-OH	S	Dealkyl 2-OH	F (%)
DDM24027003	5 mg/kg i.v.	M	2871 ± 264	563.6 ± 134.4	131.4 ± 42.35	29.4 ± 20.56	181.9 ± 28.80	
		F	2684 ± 253	878.4 ± 311.7	335.2 ± 81.25	69.47 ± 22.04	60.40 ± 20.16	
	5 mg/kg p.o.	M	2177 ± 180	<LLOQ	95.46 ± 53.32	25.96 ± 28.11	183.0 ± 52.43	n.c.**
		F	2845 ± 1882	46.25 ± 50.55	239.8 ± 78.02	44.39 ± 30.03	62.16 ± 22.67	5.39 ± 6.01
	25 mg/kg p.o.	M	19870 ± 14150	145.5 ± 46.93	694.6 ± 457.3	<LLOQ	935.2 ± 22.15	5.33 ± 1.88
		F	16550 ± 6560	379.0 ± 358.6	1308 ± 344.4	124.1 ± 140.7	417.6 ± 49.82	8.95 ± 8.21
5077DMR037	10 mg/kg i.v.	M		1310	298			
		F		1380	883			
	25 mg/kg p.o.	M		n.c.	978			
		F		n.c.	3040			
	125 mg/kg p.o.	M		2130	20800			
		F		7510	14900			
	25 mg/kg p.o. (7 days)	M		n.c.	2460			
		F		707	2580			

*data for parent compound and metabolites are expressed as AUC (ng•hr/g), ** not calculable

TABLE 4: PHARMACOKINETIC PARAMETERS FOR ICI 24,636 AND METABOLITES IN PLASMA OF SPRAGUE-DAWLEY RATS (STUDY NO. 5077/DMR038)

DOSE (mg/kg)	SEX	T_{max} (hr)	C_{max} (ng/mL)	$t_{1/2}$ (hr)	Cl (mL/min/kg)	V_{ss} (L/kg)
ICI 204,636						
10 i.v.	M	--	--	0.44	127	2.72
	F	--	--	0.27	120	2.65
25 p.o.	M	0.5	91.8	0.42	n.c.**	n.c.
	F	0.33	312	0.49	n.c.	n.c.
125 p.o.	M	1.0	428	3.51	n.c.	n.c.
	F	1.0	1430	3.9	n.c.	n.c.
25 p.o.*	M	1.0	76.1	0.51	n.c.	n.c.
	F	0.33	914	1.71	n.c.	n.c.
ICI 214,227						
10 i.v.	M	0.5	298	0.46		
	F	0.25	681	2.64		
25 p.o.	M	0.75	410	1.84		
	F	0.75	855	9.53		
125 p.o.	M	1.0	3180	5.8		
	F	1.0	2650	6.27		
25 p.o.*	M	1.0	1320	2.47		
	F	0.33	1230	4.9		
ICI 213,841						
10 i.v.	M	0.25	63			
	F	0.08	45.7			
25 p.o.	M	0.5	227			
	F	0.33	146			
125 p.o.	M	0.75	794			
	F	0.5	536			
25 p.o.*	M	0.75	120			
	F	0.33	326			

*multiple dose, **not calculated

after the second dose, all dogs received a single 25 mg/kg p.o. dose. Blood, urine, and fecal samples were collected up to 48 (blood) or 120 (urine, feces) hr after each dose. Radioactivity was quantitated using LSC. Parent compound and metabolites were quantitated using HPLC. Conjugated and unconjugated compounds were quantitated following separation by extraction and centrifugation.

2. **Balance and pharmacokinetics study in female dogs after oral and intravenous administration of ^{14}C-ICI 204,636** (Study no. 204636DMD021/01, Report no. SP2364/B, 4/IB/017990, ICI Pharmaceuticals, Vol 1.62: 5.I.20)

Methods: same as those follows for Study no. DDM24027001 in male dogs.

3. **Absorption, distribution and elimination in a bile duct-cannulated male dog** (Study no. DDM24027005, 3/HI/012580, ICI Pharmaceuticals, Vol 1.62: 5.I.21)

Methods: this study was conducted in 1 male Beagle dog (fasted overnight) that had undergone bile-duct cannulation. ^{14}C-CI 204,636 (lot no. U24027-F2, unlabeled) was administered i.v. and p.o. as single doses (1 mg/kg) one wk apart. The final drug formulation contained 25% ethanol (v/v). Bile samples were collected continuous for 48 hr postdosing (3-hr intervals up to 12 hr, then 12 hr intervals up to 48 hr). Blood samples were collected at the same time bile samples were collected, i.e., at 3, 6, 9, 12, and 48 hr postdosing. Urine and fecal samples were collected at 12, 24, 48, 72, and 96 hr postdosing. Sample radioactivity was determined by LSC; ICI 204, 636 and metabolites, ICI 214,227 (2-OH), ICI 213,841 (sulfoxide), and U31744 (dealkylated 2-OH), were quantitated by HPLC.

The data for reports # 1, 2, and 3 in dog are summarized in the attached Tables 5-8.

Monkey

1. **Balance and pharmacokinetics study in cynomolgus monkeys after oral and intravenous administration of 14C-ICI 204.636** (Study no. 204636DBPO22/01, 10/IA/017185, [illegible], ICI Pharmaceuticals Grp, Vol 1.62: 5.I.23)

Methods: ^{14}C-ICI 204,636 (lot no. U31078B1) was administered to 3-4 cynomolgus monkeys as single doses of 0.5 mg/kg i.v. (n = 4) and 10 mg/kg p.o. (n = 3). Vehicles were physiological saline for the i.v. dose and 0.5% HPMC/0.1% TWEEN 80 for the p.o. dose. (Feeding schedule prior to dosing was not described.) Blood samples were collected for up to 48 hr postdosing. Urine and fecal samples were collected up to 120 hr postdosing. Radioactivity was quantitated using LSC, and parent compound and metabolites, U31744 (dealkylated 7-OH), ICI 214,227 (7-OH), and ICI 213,841 (sulfoxide), were quantitated using HPLC. Quantitation of free and conjugated compounds was performed following extraction, and hydrolysis of conjugates using glusulase (type H-1 glucuronidase).

The balance data are summarized in the following sponsor's table:

TABLE 5: ELIMINATION OF RADIOACTIVITY IN BEAGLE DOGS AFTER SINGLE DOSES OF ICI 204,636

STUDY NO.	DOSE/ROUTE	SEX	ELIMINATION (% of dose radioactivity)			
			URINE	BILE	FECES	TOTAL RECOVERY
DDM24027001	1 mg/kg p.o.	M	24.76 ± 4.46		73.88 ± 4.79	98.64 ± 2.61
	1 mg/kg i.v..		20.65 ± 6.79		78.29 ± 6.38	98.93 ± 2.90
	10 mg/kg p.o.		23.16 ± 8.19		78.89 ± 12.02	102.1 ± 10.43
204636DMD021/01	1 mg/kg p.o.	F	25.0 ± 1.6		68.1 ± 3.5	97.4 ± 2.4
	1 mg/kg i.v.		28.3 ± 3.1		72.2 ± 0.9	99.0 ± 2.3
	10 mg/kg p.o.		25.9 ± 1.5		69.1 ± 0.8	96.3 ± 0.7
DDM/24027005	1 mg/kg p.o.	M	16.85	73.0	5.10	94.95
	1 mg/kg i.v.		12.31	85.5	1.48	99.29

*mean ± SD; **mean ± SEM; #data are expressed as total elimination as % of total dose

TABLE 6: METABOLIC PROFILE IN BILE AND URINE COLLECTED FROM BEAGLE DOGS TREATED WITH SINGLE DOSES OF ICI 204,636

STUDY NO.	DOSE /ROUTE	SEX	BILE*				URINE**			
			PC	2-OH	S	Dealkyl 2-OH	PC	2-OH	S	Dealkyl 2-OH
DDM24027001	1 mg/kg p.o.	M					22.75 ± 17.58 (34.92 ± 19.13)	47.04 ± 8.87 (282.5 ± 160.1)	49.36 ± 6.37 (179.0 ± 71.28)	
	1 mg/kg i.v.						12.75 ± 6.02 (30.87 ± 19.55)	19.80 ± 3.61 (245.2 ± 147.0)	28.13 ± 5.18 (123.1 ± 46.85)	
	10 mg/kg p.o.						289.3 ± 142.2 (430.5 ± 309.6)	580.1 ± 148.7 (2315.0 ± 1322.0)	608.8 ± 219.4 (1876.0 ± 1091.0)	
204636DMD02 1/01	1 mg/kg p.o.	F					<LLOQ (11.1 ± 2.6)	120 ± 47.9 (223 ± 43.9)	56.7 ± 17.9 (94.1 ± 36.6)	
	1 mg/kg i.v.						<LLOQ (9.7 ± 1.9)	36.5 ± 2.3 (245 ± 40.0)	38.1 ± 9.0 (86.3 ± 7.9)	
	10 mg/kg p.o.						58.7 ± 12.3 (236 ± 52.0)	1090 ± 176 (2650 ± 471)	454 ± 74.1 (498 ± 43.7)	
DDM/24027005	1 mg/kg p.o.	M	0.02#	3.67	<LLOQ	0.21	0.15##	0.91	1.08	1.43
	1 mg/kg i.v.		<LLOQ	1.58	<LLOQ	0.47	0.29	0.45	1.40	1.19

*% of sample radioactivity, #0-3 hr samples, ##12-24 hr sample, **data for the free compound are expressed as mean ± SD of total sample in µg (data for the conjugated compound presented in parentheses)

TABLE 7: PLASMA AUC'S FOR ICI 104,636 AND METABOLITES FOLLOWING SINGLE DOSING IN BEAGLE DOGS

STUDY	DOSE/ROUTE	SEX	TOT RAD (ng-eq/g)	ICI 204,636*	2-OH	S	F (%)
DDM24027001	1 mg/kg p.o.	M	3342.0 ± 857.9	41.9 ± 22.4 (57.2 ± 18.7)	37.7 ± 11.8 (52.1 ± 48.9)	122.6 ± 29.4 (56.2 ± 26.6)	7.84 ± 4.51
	1 mg/kg i.v.		2933.0 ± 614.0	525.1 ± 63.9 (75.5 ± 33.1)	54.1 ± 16.8 (54.4 ± 34.2)	61.6 ± 11.4 (63.2 ± 31.4)	
	10 mg/kg p.o.		29820 ± 6590	990.0 ± 419.7 (629.0 ± 303.1)	641.9 ± 328.7 (347.3 ± 192.8)	1475.0 ± 495.9 (573.3 ± 306.6)	17.82 ± 5.15
204636DMD021/01	1 mg/kg p.o.	F	2730 ± 290	<LLOQ (<LLOQ)	40.8 ± 12.4 (179 ± 24.0)	96.7 ± 7.5 (55.3 ± 20.0)	n.c.***
	1 mg/kg i.v.		3830 ± 674	357 ± 34.1 (74.5 ± 57.5)	31.8 ± 7.9 (277 ± 98.4)	27.6 ± 9.9 (73.8 ± 40.6)	
	10 mg/kg p.o.		315000 ± 4160	1030 ± 124 (509 ± 147)	1120 ± 235 (1780 ± 476)	1400 ± 151 (572 ± 61.2)	29

*data for parent compound and metabolites are expressed as ng•hr/g, mean ± SD, $AUC_{0\text{-}tf}$, (data for conjugated compound presented in parentheses), ***not calculable

TABLE 8: PLASMA C_{max}'s FOR ICI 204,636 AND METABOLITES FOLLOWING SINGLE DOSING IN BEAGLE DOGS

STUDY	DOSE/ROUTE	SEX	ICI 204,636*	2-OH	S
DDM24027001	1 mg/kg p.o.	M	30.1 ± 7.5 (59.9 ± 20.9)	36.1 ± 15.5 (47.9 ± 43.1)	63.0 ± 9.6 (40.3 ± 18.7)
	1 mg/kg i.v.		372.3 ± 130.9 (35.9 ± 29.9)	17.5 ± 5.2 (24.4 ± 16.3)	19.8 ± 3.9 (18.2 ± 8.8)
	10mg/kg p.o.		722.4 ± 234.4 (520.7 ± 286.5)	438.5 ± 209.7 (290.0 ± 173.0)	754.4 ± 182.2 (242.7 ± 146.0)
204636DMD021/01	1 mg/kg p.o.	F	25.7 ± 9.9 (69.6 ± 26.8)	49.5 ± 5.9 (105 ± 13.3)	62.4 ± 10.7 (56.0 ± 16.0)
	1 mg/kg i.v.		371 (43.0 ± 14.9)	34.4 ± 5.4 (70.0 ± 17.3)	27.9 ± 6.1 (25.5 ± 5.1)
	10 mg/kg p.o.		891 ± 56.3 (519 ± 45.4)	865 ± 138 (712 ± 181)	890 ± 15.0 (297 ± 42.9)

*data for parent compound and metabolites are expressed as ng/mL, mean ± SD, (data for conjugated compound presented in parentheses)

Table 1: Summary of Excretion of Radioactivity (% Dose) over 120 Hours After Administration of a 0.5 mg/kg Intravenous Dose or a 10 mg/kg Oral Dose of ^{14}C-ICI 204,636 to Cynomolgus Monkeys

Matrix	IV Dose 0.5 mg/kg n=4	Oral Dose* 10 mg/kg n=3
Urine	43.6 ± 3.4	46.3 ± 2.1
Feces	37.9 ± 5.9	43.4 ± 1.7
Cage Wash	1.1 ± 0.3	0.9 ± 0.2
%Recovery	82.5 ± 5.1	90.6 ± 3.8

* Monkey 3 vomited dose, therefore data was not included.

Quantitation of metabolites in urine, feces, and plasma are summarized in attached Table 9.

Rabbit

1. **ICI 204,636: Metabolism and elimination following single oral doses of [^{14}C]-ICI 204,636 to female rabbits at 25 mg/kg** (Study no. 204636 KMB 003, ICI Pharmaceuticals, GLP, Vol 1.62: 5.I.18)

Methods: ^{14}C-ICI 204,636 (lot no. ADM 56074/86, unlabeled) was administered to 4 female Dutch Belted rabbits at the LD used in the rabbit teratology study (i.e., 25 mg/kg p.o.). Urine and fecal samples were collected for up to 120 hr postdosing.

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

TABLE 9: ELIMINATION OF ICI 204,636 AND METABOLITES AFTER SINGLE DOSES OF ICI 204,636 IN MONKEY

DOSE	URINE					FECES				
	ICI 204,636	7-OH	S	Dealkyl 7-OH	UNK	ICI 204,636	7-OH	S	Dealkyl 7-OH	UNK
0.5 mg/kg i.v.	1.0 ± 0.2 (0.3 ± 0.1)	0.2 ± 0.1 (1.9 ± 0.6)	0.4 ± 0.1 (0.2 ± 0.0)	2.2 ± 0.7 (4.9 ± 0.5)	32.5	0.4 ± 0.1	4.7 ± 0.9	1.5 ± 0.2	8.6 ± 2.0	22.7
10 mg/kg p.o.	1.6 ± 1.2 (1.2 ± 0.8)	0.3 ± 0.2 (3.2 ± 0.7)	0.7 ± 0.2 (0.4 ± 0.0)	2.0 ± 0.3 (9.4 ± 2.1)	27.5	0.4 ± 0.1	8.3 ± 4.7	2.4 ± 0.1	8.9 ± 1.8	23.4

TABLE 10: PLASMA AUC'S FOR ICI 204,636 AND METABOLITES FOLLOWING SINGLE DOSING IN MONKEYS

DOSE	ICI 204,636	7-OH	S	Dealkyl 7-OH	UNK **	F (%)
0.5 mg/kg i.v.	509* (47.6)	62.6 (193)	49.1 (NC)	NC (500)	52.0	
10 mg/kg p.o.	460 (NC)	1340 (4480)	1140 (NC)	NC (20800)	51.8	5.7 ±4.1

*AUC_{0-tf} (ng•hr/mL) NC = not calculated, **% of total circulating radioactivity

Table 1 : 204636 KMB 003 : Recovery of the dose following oral administration of [^{14}C]-ICI 204,636 to female rabbits.

Sample	Time (hours)	Percentage recovery				
		Rabbit 1	Rabbit 2	Rabbit 3	Rabbit 4	Mean ± SE (n = 4)
Urine	0 - 24	48.2	52.2	58.6	45.6	51.1 ± 2.8
	24 - 48	3.4	5.6	0.9	10.6	5.1 ± 2.1
	48 - 72	0.6	2.1	0.5	1.2	1.1 ± 0.4
	72 - 96	0.5	1.0	0.4	0.4	0.6 ± 0.1
	96 - 120	0.1	0.4	0.4	0.3	0.3 ± 0.1
	0 - 120	52.8	61.3	60.8	58.1	58.3 ± 1.9
Cagewash	0 - 120	3.9	11.2	6.3	5.1	6.6 ± 1.6
Faeces	0 - 24	32.6	9.9	25.7	25.4	23.4 ± 4.8
	24 - 48	3.3	2.8	1.1	2.8	2.5 ± 0.5
	48 - 72	0.5	2.0	0.9	1.4	1.2 ± 0.3
	72 - 96	0.3	1.4	0.2	0.4	0.6 ± 0.3
	96 - 120	0.1	0.5	0.1	0.1	0.2 ± 0.1
	0 - 120	36.8	16.7	27.9	30.1	27.9 ± 4.2
Total	0 - 120	93.5	89.1	95.1	93.3	92.8 ± 1.3

TLC of sample extracts indicated (1) the presence of at least 4 metabolites, (2) no detectable parent compound in urine extracts, (3) the presence of at least 3 metabolites, with ≈60% of applied radioactivity remaining at the origin, and (4) parent compound accounted for ≈10% of sample radioactivity in feces.

TISSUE DISTRIBUTION

Rat

1. **The distribution of radioactivity in hooded and albino male rats as determined by whole body autoradiography following single oral doses of [^{14}C]-ICI 204,636** (Study no. 204636 KMR 001, 7/HI/013244, ICI Pharmaceuticals, GLP, Vol 1.63: 5.I.34)

In this study, [^{14}C]ICI 2,40636 (lot no. ADM 56074/86, unlabeled compound) was administered orally as a single 25 mg/kg dose (vehicle: 0.5% hydroxypropyl methylcellulose, 0.1% polysorbate 80) to 6 male Wistar albino and 3 male LISFAP/alpk hooded rats (initial body weights: 193-212 g). The radiochemical purity of the isotope was verified by TLC prior to dosing. Food and water were provided ad lib. Albino rats were killed at 30 min, 1, 2, 6, 24, and 168 hr postdosing (n = 1/time point); hooded rats were killed at 6, 24, and 168 hr postdosing (n = 1/time point). Blood samples were collected just prior to sacrifice. Tissue radioactivity was determined qualitatively by whole body autoradiography.

Since tissue exposure was qualitatively estimated, there were no data provided. The sponsor summarized the results as follows.

In albino animals,

(1) at 30 min postdosing, radioactivity was detected in the GI tract (esophagus, stomach, duodenum), with "...large amounts of radioactivity..." noted in stomach and duodenum. Excluding the GI tract, the highest levels appeared in

liver. "Trace levels..." were detected in kidney and lung, and urine and bile were labeled.

(2) at 1 hr postdosing, liver contained the highest levels of radioactivity (lower than the 30-min level). Radioactivity was detected in kidney and lung (higher levels than at 30 min in both organs),"...bone marrow, pituitary, Harderian, salivary and preputial glands", and the GI (i.e., esophagus, stomach, duodenum, bile, urine).

(3) at 2 hr postdosing, radioactivity was detected in liver (highest levels, similar to 1-hr), kidney, and GI (stomach through cecum, bile, urine).

(4) at 6 hr postdosing, radioactivity was detected throughout the GI tract (from stomach on), liver (lower than at 2 hr), inner cortex of kidney, and lung (trace).

(5) at 24 hr postdosing, radioactiity was detected in GI tract, liver, and inner cortex of kidney.

(6) at 168 hr postdosing, radioactivity was detectable only in GI.

In hooded animals,

(1) at 6 hr postdosing, the highest levels of radioactivity were detected in GI (i.e., stomach, duodenum, large intestine, cecum, urine, bile), choroid of eye, and liver (in that order), followed by kidney, then lung and bone marrow. Low levels were detected in pituitary, Harderian gland, thyroid, salivary glands, spleen, and skin. Trace levels were detected in blood.

(2) at 24 hr postdosing, levels of radioactivity were in general lower than at 6 hr. Radioactivity was detected in GI (only trace in stomach), liver, kidney, and skin (trace amounts).

(3) at 168 hrs postdosing, radioactivity was still detectable in eye (at lower levels than at 24 hrs) and GI (trace).

2. **Quantitative tissue distribution study in male albino and hooded rats after a single oral dose of 25 mg/kg** (US Study no. 204636DMR010, 6/HI/013054, ICI Pharmaceuticals, Vol 1.63: 5.I.35)

Tissue distribution of radioactivity following single oral doses of ^{14}C-ICI 204,636 (lot no. 24027F2, unlabeled compound) was assessed in male Sprague-Dawley and male Long-Evans hooded rats. Purity of the isotope was determined to be 99%. Animals were fasted overnight prior to dosing. Albino rats were sacrificed at 1, 2, 6, 24, 72, and 168 hr postdosing (n = 3/time point). Hooded animals were sacrificed at 1, 24, 72, and 168 hr postdosing and only blood, plasma, and eyes were examined (n = 3/time point). Radioactivity was determined using LSC. Dosimetry calculations were provided to aid extrapolation of animal data to human (assuming 70 kg man receiving a 40 µCi dose).

Tissue levels peaked at 1-2 hr postdosing. At all time points sampled, the highest levels of radioactivity were detected in GI (contents). Excluding GI, the highest levels at 1 hr postdosing were detected in liver (i.e., 47.9 µg-eq/g; >kidney>lung>salivary glands>bladder>thyroid=spleen); heart, fat, thymus, msucle, eyes, and brain contained ≤2.46 µg-eq/g. The lowest tissue levels were detected in brain, and only up to 2 hr postdosing. Eye radioactivity was markedly higher (≈640 fold based on AUC) in pigmented animals. At 168 hr postdosing, radioactivity was only detectable in GI (mostly contents), thyroid, liver, and spleen in albino rats, and radioactivity was still detectable in eye of hooded rats (≈40% of peak levels).

The tissue AUC data are summarized in the following sponsor's Table:

Table 9

ICI 204,636: Quantitative Tissue Distribution Study in Male Albino and Hooded Rats After a Single Oral Dose of 25 mg/kg
Study Number 204636DM010

Tissue Exposure Calculations

Tissue	AUC Rat (µg-equiv.day/g)	Half-Life Rat (day)	Actual Dosimetry Rat (mSv)	Projected Dosimetry Human (mSv)
Liver	37.4	1.65	25.8	0.024
Sm. Intest.	27.9	0.11	19.3	0.018
Thyroid	19.7	4.38	13.6	0.012
Stomach	11.4	1.76	7.87	0.0072
Lg. Intest.	7.63	0.95	5.26	0.0005
Spleen	5.21	3.29	3.59	0.0033
Kidneys	4.45	0.89	3.07	0.0028
Bladder	3.46	0.37	2.39	0.0022
Thymus	2.29	0.53	1.58	0.0014
Lung	1.70	0.15	1.17	0.0011
Heart	0.52	0.21	0.36	0.0003
Muscle	0.41	0.21	0.28	0.0003
Fat	0.39	0.15	0.27	0.0002
Eyes(albino)	0.16	0.24	0.11	0.0001
Brain	0.05	0.07	0.03	0.0001
Eyes (pigmented)	103	11.6	71.1	0.065

3. **The tissue distribution of total radioactivity in the rat following a single or multiple oral administration of [^{14}C]-ICI 204636** (IRI Project No. 153269, Study No. 5077DMR034, 6/ID/1009227, GLP, Vol 1.64: 5.I.36)

The tissue distribution of radioactivity was determined after single and multiple (9 consecutive days) oral dosing (vehicle: 0.5% HPMC/0.1% TWEEN 80) with [^{14}C]-ICI 204,636 (lot no. ADM 4005/91; 25 mg/kg) in Sprague-Dawley rats (152-242 g body weight). Animals received food and water ad lib throughout the experiment. Following single dose administration, animals were sacrificed at 0.5, 1, 4, 12, 24, 72, and 168 hr postdosing (n = 3/sex/time point). Following multiple dosing, animals were sacrificed on Day 9 (same time points as with single dosing; n = 3/sex/time point). In addition, 3/sex were dosed orally with [^{14}C]-ICI 204,636 for 7 consecutive days; these animals were sacrificed 24 hr after the last dose. Radioactivity was determined on blood/plasma and tissue homogenates using LSC.

The data are summarized in the following sponsor's tables (#1, 2, 3, 4, 7, 8, 9, 1013, 14):

TABLE 1

Mean Levels of Total Radioactivity in Tissues Following Single Oral Administration of [^{14}C]-ICI-204,636 to Male Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as µg equiv.g^{-1} or ml^{-1}

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Adrenals	6.44	9.73	2.64	0.61	0.32	0.26	0.05
Blood	1.66	2.29	0.60	0.22	0.11	0.02	0.02
Bone mineral	1.42	1.29	0.50	0.25	0.13	0.25	0.02
Bone marrow	2.78	4.91	1.57	1.43	1.33	0.57	0.07
Brain	0.38	0.66	0.16	0.06	0.03	0.01	0.00
Eyes	0.57	0.91	0.51	0.09	0.05	0.10	0.00
Fat - brown	1.67	2.93	0.59	0.25	0.11	0.15	0.02
Fat - white	0.74	1.58	0.33	0.09	0.04	0.47	0.03
Harderian gland	3.39	11.55	2.43	0.58	0.27	0.17	0.02
Heart	1.71	3.12	0.57	0.17	0.08	0.04	0.01
Kidneys	10.29	13.98	4.82	2.26	1.32	0.54	0.25
Large intestine	2.02	7.39	124.32	79.96	4.28	1.69	0.87
Liver	27.49	45.42	17.47	9.72	6.52	2.29	0.76
Lungs	5.60	9.78	3.03	0.50	0.16	0.07	0.03
Lymph nodes (mesenteric)	1.80	4.25	3.38	0.41	0.16	0.14	0.05
Muscle	1.22	2.01	0.43	0.14	0.06	0.30	0.01
Pancreas	4.31	5.85	1.39	0.26	0.07	0.09	0.01
Pituitary	6.25	7.98	3.29	0.48	0.15	0.00	0.04
Plasma (ml^{-1})	2.45	2.69	0.79	0.21	0.08	0.02	0.04
Preputial Gland	2.48	5.08	1.52	0.83	0.44	0.41	0.06
Prostate	1.58	4.20	4.15	0.39	0.08	0.04	0.01
Rectum	21.00	2.26	0.96	74.91	3.34	0.80	0.49
Skin	1.81	2.26	0.95	0.18	0.06	1.36	0.02
Small intestine	98.40	265.80	135.86	7.68	3.29	0.69	1.72
Spinal cord	0.65	0.86	0.53	0.10	0.03	0.02	0.01
Spleen	3.77	5.99	1.29	0.53	0.32	0.42	0.41
Stomach	438.87	217.79	111.68	0.44	1.39	0.18	0.73
Sub-mandibular gland	4.47	7.60	2.24	0.43	0.08	0.11	0.02
Testes	0.42	1.05	0.54	0.33	0.17	0.26	0.02
Thymus	1.32	2.57	0.80	0.20	0.06	0.03	0.01
Thyroid	2.91	4.43	2.75	2.17	1.61	1.31	1.05
Urinary bladder	2.24	23.31	26.95	5.11	1.38	0.64	0.09

TABLE 2

Mean Levels of Total Radioactivity in Tissues Following Single Oral Administration of [^{14}C]-ICI-204,636 to Female Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as μg equiv.g^{-1} or ml^{-1}

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Adrenals	18.27	5.14	1.55	1.18	0.22	0.07	0.06
Blood	2.83	1.13	0.40	0.22	0.03	0.02	0.01
Bone mineral	1.86	0.91	0.58	0.28	0.37	0.25	0.05
Bone marrow	6.78	3.00	2.28	2.43	1.58	1.06	0.31
Brain	0.93	0.39	0.13	0.04	0.01	0.01	0.00
Eyes	1.16	0.58	0.24	0.37	0.02	0.03	0.01
Fat - brown	3.23	1.68	0.54	0.25	0.05	0.04	0.01
Fat - white	2.31	1.22	0.65	0.15	0.03	0.16	0.00
Harderian gland	11.35	7.09	4.09	1.17	0.11	0.07	0.00
Heart	4.44	1.85	0.62	0.25	0.03	0.02	0.01
Kidneys	14.50	5.33	1.80	0.89	0.20	0.13	0.07
Large intestine	5.08	4.82	198.98	129.24	4.00	0.23	0.06
Liver	51.46	31.05	12.97	5.98	1.17	0.45	0.19
Lungs	11.78	9.92	2.08	0.68	0.09	0.05	0.01
Lymph nodes (mesenteric)	4.96	1.66	1.57	0.45	0.06	0.07	0.01
Mammary Glands	2.61	2.23	1.01	0.37	0.05	1.22	0.02
Muscle	2.80	1.25	0.59	0.16	0.01	0.04	0.01
Ovaries	6.52	3.27	1.55	0.36	0.02	0.05	0.02
Pancreas	9.07	15.59	1.41	0.37	0.02	0.09	0.00
Pituitary	8.01	11.35	1.54	0.45	0.10	0.10	0.01
Plasma (ml^{-1})	3.39	1.39	0.59	0.29	0.03	0.01	0.02
Preputial Gland	2.01	3.80	12.52	1.25	0.46	0.21	0.04
Rectum	3.09	1.65	1.57	124.80	6.17	0.15	0.01
Skin	2.49	1.09	0.74	0.48	0.03	0.34	0.01
Small intestine	171.47	177.60	161.98	22.90	1.67	0.17	0.03
Spinal cord	1.09	0.89	0.45	0.11	0.01	0.01	0.00
Spleen	8.61	4.69	1.33	0.61	0.31	0.38	0.53
Stomach	409.54	868.04	77.33	5.98	5.96	0.11	0.01
Sub-mandibular gland	8.42	4.35	2.01	0.78	0.05	0.01	0.00
Thymus	3.52	1.75	0.68	0.23	0.04	0.03	0.00
Thyroid	6.55	2.85	2.65	0.80	1.43	1.26	1.69
Uterus	4.29	1.85	1.62	0.28	0.05	0.07	0.02
Urinary bladder	7.21	2.57	5.49	1.53	0.10	0.02	0.00

TABLE 3

Mean Levels of Total Radioactivity in Gastrointestinal Tract Contents Following Single Oral Administration of [^{14}C]-ICI-204,636 to Male Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as total µg equiv.

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Large intestine contents	8.02	28.88	1876.13	1503.34	100.19	29.91	23.75
Small intestine contents	871.00	2383.25	1194.78	117.99	94.54	3.65	26.82
Stomach contents	2319.79	797.30	372.89	0.64	19.60	1.31	17.69
Rectal contents	2.61	0.68	0.20	262.19	11.28	1.81	2.69

TABLE 4

Mean Levels of Total Radioactivity in Gastrointestinal Tract Contents Following Single Oral Administration of [^{14}C]-ICI-204,636 to Female Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as total µg equiv.

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Large intestine contents	20.64	13.03	2793.89	2455.19	67.59	5.09	0.62
Small intestine contents	1187.16	668.48	886.09	200.04	17.11	1.19	0.07
Stomach contents	2265.23	1652.53	319.42	6.71	38.84	1.52	0.05
Rectal contents	0.70	0.16	0.25	501.52	14.13	0.40	0.01

In males, peak levels occurred at 1 hr postdosing in most tissues examined. Highest tissue levels were detected in GI, then liver>urinary bladder>kidney>Harderian gland>lung=adrenals. Levels in bone mineral, brain, eyes, spinal cord, and testes were lower than those in plasma/blood. The tissue:plasma ratio for peak brain levels was ≈0.3. At 168 hr, radioactivity was low, but detectable in most tissues; highest levels were noted in GI and thyroid (≈24% of peak levels).

In females, peak tissue levels occurred in most tissues at 0.5 hr postdosing. Highest peak levels of radioactivity were detected in GI, followed by liver>adrenals>kidney>Harderian gland= lungs. Lowest levels occurred in brain, eyes, and spinal cord. The tissue:plasma ratio for peak brain levels was ≈0.3. At 168 hr postdosing, radioactivity was low, but detectable in many tissue; highest levels were noted in thyroid (≈25% of peak levels).

Peak tissue levels were, in general, higher in females than in males (up to ≈3 fold), with the greatest difference noted in adrenals (1.9 fold), pancreas (2.7 fold), , preputial gland (3.3 fold). Exceptions were Harderian gland and urinary bladder (0.3 to 1 fold). The sponsor indicated that the $t_{1/2}$ was similar in males and females for tissues except for liver and kidney, which had ≈2-fold longer $t_{1/2}$'s in males. Peak blood/plasma levels were ≈25% higher in females than males, however, the GI contents was similar at 0.5 hr postdosing, suggesting that differences in dose did not account for the differences in tissue exposure.

TABLE 7

Mean Levels of Total Radioactivity in Tissues Following the Last of 9 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Male Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as μg equiv.g^{-1} or ml^{-1}

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Adrenals	5.64	8.42	3.09	1.50	0.83	0.46	0.38
Blood	1.35	1.90	0.79	0.47	0.21	0.09	0.06
Bone mineral	1.05	1.90	1.07	0.97	0.67	0.47	0.18
Bone marrow	7.61	8.59	6.59	4.98	4.16	2.06	0.66
Brain	0.39	0.43	0.25	0.12	0.06	0.03	0.01
Eyes	0.60	0.87	0.36	0.21	0.10	0.05	0.06
Fat - brown	1.84	2.69	1.27	0.70	0.29	0.22	0.13
Fat - white	0.75	1.06	0.70	0.30	0.13	0.06	0.04
Harderian gland	4.24	7.78	4.63	1.66	0.47	0.18	0.08
Heart	1.87	2.81	0.89	0.40	0.18	0.10	0.05
Kidneys	12.04	15.04	7.35	5.15	3.22	1.82	1.05
Large intestine	14.95	24.28	181.74	184.12	17.84	0.35	0.18
Liver	44.09	64.14	31.24	26.83	14.74	7.09	2.41
Lungs	5.35	8.23	3.41	1.37	0.46	0.26	0.16
Lymph nodes (mesenteric)	2.64	4.32	2.72	0.65	0.71	0.24	0.19
Muscle	1.07	1.71	0.59	0.30	0.13	0.06	0.15
Pancreas	4.66	4.73	1.74	0.63	0.16	0.09	0.09
Pituitary	4.18	7.38	2.66	1.79	0.67	0.15	0.17
Plasma (ml^{-1})	1.94	2.43	0.91	0.43	0.12	0.04	0.01
Preputial Gland	4.00	5.16	3.27	2.99	1.55	0.81	0.44
Prostate	1.58	2.82	1.40	0.81	0.22	0.08	0.04
Rectum	6.70	11.48	24.42	78.95	7.34	0.23	0.20
Skin	1.13	1.95	0.92	0.50	0.21	0.12	0.23
Small intestine	95.31	178.21	154.87	33.45	4.04	0.41	0.23
Spinal cord	0.60	0.58	0.67	0.29	0.05	0.03	0.01
Spleen	9.31	9.65	5.45	4.84	3.69	3.14	2.76
Stomach	201.38	704.89	97.42	3.22	3.22	0.12	0.11
Sub-mandibular gland	4.18	8.32	4.69	1.65	0.27	0.08	0.07
Testes	0.78	1.16	1.11	0.82	0.36	0.20	0.17
Thymus	1.42	2.44	0.95	0.53	0.21	0.12	0.08
Thyroid	14.21	17.87	20.76	21.58	14.44	11.40	12.48
Urinary bladder	5.07	18.05	13.62	12.36	3.13	0.67	0.61

TABLE 8

Mean Levels of Total Radioactivity in Tissues Following the Last of 9 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Female Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as μg equiv.g^{-1} or ml^{-1}

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Adrenals	7.96	8.29	3.23	1.01	0.38	0.20	0.14
Blood	2.11	2.45	0.41	0.22	0.10	0.05	0.04
Bone mineral	2.34	1.85	1.45	1.31	0.81	0.65	0.38
Bone marrow	9.93	9.03	8.90	10.27	5.15	3.41	2.00
Brain	1.00	1.61	0.13	0.05	0.01	0.00	0.00
Eyes	0.73	1.43	0.26	0.12	0.03	0.02	0.05
Fat - brown	3.46	4.25	0.79	0.35	0.15	0.19	0.12
Fat - white	1.53	2.44	0.39	0.20	0.05	0.04	0.08
Harderian gland	7.25	17.14	4.22	1.30	0.29	0.05	0.05
Heart	3.21	3.96	0.61	0.29	0.11	0.06	0.05
Kidneys	12.33	16.87	2.66	1.54	0.84	0.53	0.53
Large intestine	37.57	19.55	219.00	135.53	14.52	0.16	0.15
Liver	49.95	54.20	15.68	7.53	3.32	1.64	0.78
Lungs	8.11	12.08	2.19	0.93	0.32	0.16	0.08
Lymph nodes (mesenteric)	3.72	9.16	2.67	0.66	0.21	0.08	0.12
Mammary Glands	1.94	4.03	1.12	0.70	0.09	0.19	0.75
Muscle	1.55	2.47	0.38	0.16	0.04	0.02	0.05
Ovaries	3.75	5.23	1.09	0.21	0.13	0.04	0.07
Pancreas	9.01	8.98	1.78	0.35	0.08	0.04	0.05
Pituitary	5.07	6.41	1.69	0.31	1.32	0.00	0.00
Plasma (ml^{-1})	3.02	3.71	0.53	0.21	0.06	0.01	0.00
Preputial Gland	4.15	8.95	3.27	1.85	0.91	0.57	0.11
Rectum	19.31	13.28	8.05	80.97	5.78	0.18	0.06
Skin	1.59	2.88	0.65	0.44	0.14	0.03	0.40
Small intestine	129.84	164.98	157.83	29.64	5.00	0.20	0.20
Spinal cord	1.01	1.82	0.65	0.10	0.02	0.00	0.01
Spleen	11.10	11.22	5.70	5.27	3.68	5.21	3.07
Stomach	870.31	1179.31	137.30	1.94	2.88	0.07	0.07
Sub-mandibular gland	6.84	9.06	2.19	1.06	0.13	0.03	0.03
Thymus	2.45	4.77	0.69	0.37	0.18	0.10	0.06
Thyroid	14.51	12.67	9.57	7.31	6.02	6.88	6.93
Uterus	2.39	3.40	0.71	0.31	0.14	0.15	0.17
Urinary bladder	4.74	7.46	2.40	0.83	0.23	0.05	0.06

TABLE 9

Mean Levels of Total Radioactivity in Gastrointestinal Tract Contents Following the Last of 9 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Male Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as total µg equiv

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Large intestine contents	354.26	497.60	3737.41	2833.91	507.87	7.84	3.91
Small intestine contents	982.13	3166.90	1789.84	268.78	51.60	3.76	1.48
Stomach Contents	5677.42	2230.31	1528.87	2.90	48.50	0.24	0.60
Rectal Contents	44.32	40.94	106.57	520.20	67.42	0.83	0.25

TABLE 10

Mean Levels of Total Radioactivity in Gastrointestinal Tract Contents Following the Last of 9 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Female Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as total µg equiv

Sample	Sacrifice Time						
	0.5 h	1 h	4 h	12 h	24 h	72 h	168 h
Large intestine contents	698.24	319.28	3191.79	2283.24	381.37	1.88	2.48
Small intestine contents	715.71	1083.84	1252.33	188.38	67.99	1.03	0.74
Stomach Contents	2954.13	2332.84	507.03	4.03	18.90	0.09	0.18
Rectal Contents	55.57	24.28	42.98	914.36	46.26	0.25	0.18

With multiple dosing, the pattern of tissue distribution of radioactivity was similar to that following acute dosing. In males, peak levels were attained, in general, at 1 hr postdosing. Highest levels were detected in GI, then liver, followed by somewhat lower levels in thyroid>urinary bladder>kidney. Lowest levels were noted in brain, spinal cord, and eyes. The brain to plasma or blood ratios were 0.18-0.23. In females, peak levels were detected at 1 hr. Highest peak levels were obtained in GI, followed by liver, then by Harderian gland>kidney>thyroid>lung>spleen. Lowest levels were detected in brain, eyes, and spinal cord. Brain to plasma/blood ratios were 0.38-0.58.

In general, peak tissue levels of radioactivity were higher in females than males, as was noted after acute dosing (up to 3.3 fold). The greatest differences were noted in brain and spinal cord (≈3 fold), white fat, Harderian gland, mesenteric lymph nodes, pancreas, and thymus (all ≈2-fold). Notable exceptions were liver, pituitary, thyroid, and urinary bladder, which were 15-18% or 50-140% (thyroid, urinary bladder) higher in males.

Comparisons of tissue levels after acute and multiple dosing are discussed below.

TABLE 13

Mean Levels of Total Radioactivity in Tissues at 24 h Following the Last of 7 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Rats. Target Dose Level 25 mg.kg^{-1}

Results expressed as μg equiv.g^{-1} or ml^{-1}

Sample	Male	Female
Adrenals	0.70	0.38
Blood	0.17	0.09
Bone mineral	0.68	0.98
Bone marrow	5.67	6.35
Brain	0.18	0.01
Eyes	0.10	0.05
Fat - brown	0.32	0.28
Fat - white	0.10	0.07
Harderian gland	0.49	0.32
Heart	0.17	0.11
Kidneys	2.73	0.85
Large intestine	13.55	17.12
Liver	12.97	3.32
Lungs	0.40	0.28
Lymph nodes (mesenteric)	0.40	0.19
Mammary Glands	-	0.49
Muscle	0.11	0.06
Ovaries	-	0.11
Pancreas	0.16	0.12
Pituitary	0.70	0.41
Plasma (ml^{-1})	0.13	0.06
Preputial Gland	1.36	1.01
Prostate	0.30	-
Rectum	15.02	9.21
Skin	0.19	0.18
Small intestine	6.44	4.83
Spinal cord	0.04	0.03
Spleen	2.81	3.62
Stomach	3.03	1.50
Sub-mandibular gland	0.24	0.15
Testes	0.38	-
Thymus	0.17	0.22
Thyroid	24.72	9.31
Uterus	-	0.14
Urinary bladder	2.38	0.19

TABLE 14

Mean Levels of Total Radioactivity in Gastrointestinal Tract Contents at 24 h Following the Last of 7 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Rats. Target Dose Level 25 mg.kg^{-1}

Sample	Male	Female
Large intestine contents	586.31	391.72
Small intestine contents	146.47	54.83
Stomach contents	136.62	41.86
Rectal contents	41.72	18.44

Comparison of acute and 9-day multiple dosing data indicated that tissue levels were 2-3 fold higher after dosing for 9 days. Therefore, grps of animals were treated for 7 consecutive days in an attempt to determine time to reach steady state tissue levels.

In the following table, the sponsor has compared 24-hr postdosing levels in selected tissues after 1, 7, and 9 doses:

Table 6

Total Radioactivity at 24 h Following the Last of 1, 7 or 9 Daily Oral Administrations of [^{14}C]-ICI-204,636 to Male and Female Rats

Sample	Mean μg equiv.g^{-1} (ml^{-1})					
	Dose 1		Dose 7		Dose 9	
	♂	♀	♂	♀	♂	♀
Adrenals	0.32	0.22	0.70	0.38	0.83	0.38
Bone Marrow	1.33	1.58	5.67	6.35	4.16	5.15
Brain	0.05	0.01	0.18	0.01	0.06	0.01
Harderian Gland	0.27	0.11	0.49	0.32	0.47	0.29
Kidneys	1.32	0.28	2.73	0.85	3.22	0.84
Liver	6.32	1.17	12.97	3.32	14.74	3.32
Pituitary	0.15	0.10	0.78	0.41	0.67	1.32
Spleen	0.32	0.31	2.81	3.62	3.69	3.68
Plasma	0.08	0.05	0.13	0.06	0.12	0.06
Thyroid	1.61	1.43	26.72	9.31	14.44	6.02

These data indicate that steady-state tissue levels were achieved by 7 days postdosing. Tissue levels after 7 days of dosing were up to 15 times higher as compared to acute dosing, with little or no further accumulation from 7 to 9 doses. The greatest accumulation occurred in thyroid in males.

4. **Secretion of radiolabeled material in rat milk following a single oral dose of [^{14}C]-ICI 204636** (Study no. 204636DBR025/01, 6/IA/1017008, ICI Pharmaceuticals, GLP, Vol 1.64: 5.I.37)

Lactating Wistar rats received ^{14}C-ICI 204,636 (lot no. U31078A4, unlabeled compound) orally (vehicle: 0.5% HPMC/0.1%TWEEN 80) as a single dose of 25 mg/kg at Day 10-15 postpartum. Milk and blood samples were collected at 0.5 and 4.0 hr postdosing in one grp, at 1.0 and 6 hr postdosing in another grp, and at 2 and 24 hr in a third grp. There were 3 to 4 females per grp. Pups were removed from dams for at least 1.5 hr prior to milk collection. Oxytocin was administered to females 5-10 min prior to milking. Approximately 0.5 mL of milk was

collected at each sampling time. Radioactivity was quantitated using LSC. In addition, parent compound and metabolites (dealkylated 7-OH, 7-OH, and sulfoxide) were separated and quantitated using HPLC followed by LSC.

At all time points sampled, the milk-to-blood ratio was > 1 (1.1-2.0), indicating accumulation in milk. Peak levels occurred at 0.5 hr (2950 and 4750 ng-eq/mL in blood and milk, respectively). On 2-3% of peak radioactivity was detected in milk and blood at 24 hr postdosing. The sponsor indicated that only 28-39% of radioactivity was recovered by the first extraction. Following hydrolysis with a glucuronidase/sulfatase enzyme mixture, an additional 10-20% of radioactivity was extracted. Therefore, 40-60% of total milk radioactivity was not extractable.

In the extractable material, the parent compound and three metabolites were identified. Peak levels of radioactivity were as follows: 107 ng/mL for parent, and 135, 54.8, and 22.3 ng/mL for ICI 214,227, ICI 213,841, and U 31744, respectively. All of these compounds were detected in the free form; no conjugates were noted. Each of the metabolites accounted for <5% of total radioactivity. The majority of drug-related material in milk (up to 4 hr postdosing) was unidentified.

Dog

1. **An investigative study into the disposition of [^{14}C]-ICI 204636 in selected tissues after single and multiple dosing in male dogs** (Study no. 5077DMD051, 2/IE/1013497, Report no. SP2891/B, ICI Pharmaceuticals, GLP, Vol 1.64: 5.I.38)

The primary impetus for this study, according to the sponsor, was the finding of cataracts in dogs in oral toxicity studies. In this study, distribution of ^{14}C-ICI 204,636 (lot no. ADM28005/94, unlabeled compound) into eye and other selected tissues was evaluated in Beagle dogs receiving doses of 25 or 100 mg/kg p.o. (vehicle: 0.5% HPMC/0.1% TWEEN 80). Animals received either single doses (n = 1/dose) or 7 daily doses (n = 4, 2/dose). Blood samples were collected at 0.5 (Day 7) or 24 (Day 1, 7) hr postdosing. Urine and fecal samples were collected over a 24 hr period after dosing on Day 1 (all 6 dogs) and on Day 7 to the 2 dogs (1/dose) sacrificed at 24 hr postdosing. The following tissues were collected either at 24 hr [after single and multiple (in 1/dose) dosing] or at 0.5 hr postdosing (in 1/dose): liver, eye (aqueous humor, cornea, iris, lachrymal gland, lens, vitreous humor, remainder), salivary gland, thyroids, adrenals, bone marrow, kidneys, pancreas, brain, pituitary, spleen, and bile. Total radioactivity was quantitated using LSC. In addition, levels of parent compound and metabolites, ICI 214,227 (7-OH) and ICI 213,841 (sulfoxide), were quantitated using HPLC followed by LSC. The LLOQ were 50, 10, and 30 ng/mL, respectively. [The sponsor pointed out that due to only 1 animal/data point, these data should be considered preliminary and suggestive only of trends.]

There were no unscheduled deaths during the study. Clinical signs noted in dogs (as summarized by the sponsor) receiving 25 mg/kg were as follows: ptosis, third eyelid relaxed, red eyes, ataxia, salivation, and "anal relaxation". In dogs receiving 100 mg/kg, the following were noted: signs as noted at 25 mg/kg, and moderate sleep, agressive behavior, vocalization, miosis, shivers (whole body), and muscle tremors (limbs only). Plasma levels of total radioactivity at 24-hr postdosing were higher on Day 7 than on Day 1 (55-155%), consistent with previous data in rats. Plasma levels of 6.41 and 23.4 µg-eq/mL were determined at 25 and 100 mg/kg, respectively at 0.5 hr postdosing.

Parent compound was <LLOQ at 24 hr postdosing at both doses on Day 1. On Day 7, levels were 479 and 2310 ng/mL at 25 and 100 mg/kg, respectively at 0.5 hr postdosing, and <LLOQ and 51.8 ng/mL, respectively, at 24 hr postdosing. ICI 213,841 was the more abundant metabolite, with levels of 428 and 1540 ng/mL at 0.5 hr postdosing on Day 7. ICI 214,227 was present at levels of 180 and 421 ng/mL at 0.5 hr postdosing on Day 7...Except for ICI 213,841 at 24 hr postdosing on

Day 1, the metabolites were quantifiable in plasma at all sampling times.

Analysis of the eye, indicated the following:

(1) the highest concentrations of radioactivity were detected in the iris, i.e., 282 µg-eq/mL at 0.5 hr postdosing on Day 7 at 25 mg/kg, and 1140 µg-eq/mL at 24 hr on Day 7 at 100 mg/kg.

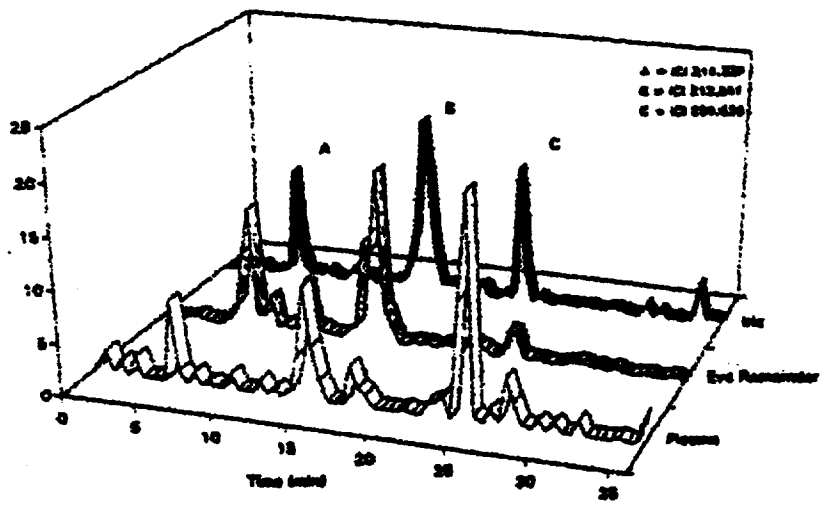
(2) appreciable amounts of radioactivity was also detected in the "remainder" areas (i.e., retina, choroid, and uveal tract). At 25 and 100 mg/kg, the highest levels were detected at 24 hr postdosing on Day 7 (33.6 and 138 ng/mL, respectively).

(3) in both eye areas, there was accumulation of radioactivity with multiple dosing, i.e., 3-4 fold in "remainder", and 9-14 fold in iris at 24 hr postdosing.

(4) radioactivity was detectable in all eye areas, including lachrymal gland, examined (i.e., aqueous humor, cornea, lens, vitreous humor, in addition to iris and eye remainder).

The sponsor compared these result with those previously published for chlorpromazine.

Chromatograms of extracted drug-related material from eye remainder and iris (accounting for 66 and 77% of drug-related material, respectively) indicated the presence of parent compound and metabolites, ICI 214,227 and ICI 213,841. Graphic representation of the chromatograms were presented in the following sponsor's figure:

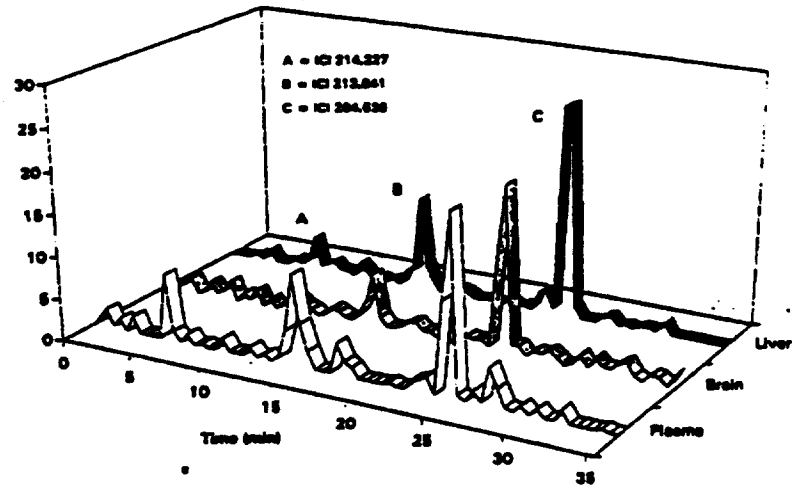


The metabolic profile was qualitatively similar in aqueous humor; however, levels of all three compounds were substantially lower than in iris, eye remainder, and plasma.

Radioactivity was detected in all other tissues examined (not including GI tract). Highest levels were obtained in liver (56.1 and 290 µg/g at 0.5 hr after 25 and 100 mg/kg, respectively, for 7 days). Substantial, but lower levels, were also detected in kidney>adrenals. As in rat, there was accumulation of radioactivity in the majority of tissues from Day 1 to Day 7 (up to 6-fold). [It appears that the Day 1 and/or 7, 24-hr values given for pituitary may be in error.]

Parent compound, ICI 214,227, and ICI 213,841 were detected in liver, brain, and plasma

extracts. In all three of these tissues/fluids, the parent compound appeared to be the major drug-related compound. Graphic representation of the chromatograms as provided by the sponsor follows:



METABOLISM

Mouse

1. **The effects of ICI 204,636 on the hepatic microsomal mixed function oxidase enzymes of the female mouse** (Study no. 204636 KMM 005, 5/IB/018210, ICI Pharmaceuticals, GLP, Vol 1.64: 5.I.39)

In previous toxicity studies in mice, liver enlargement was observed. Since this finding may be associated with enzyme induction, this study was conducted in order to determine the effect of ICI 204,636 on hepatic microsomal enzyme activity/content.

ICI 204,636 (lot no. ADM 45026/89) was administered to female CD-1 mice (16/grp) at doses of 0 (2 grps), 10, 25, 50, 200, and 400 mg/kg in the diet for 1-3 mo. Homogeneity and stability of the drug in the diet are to be documented in a separate report. According to the sponsor, the drug was homogeneously mixed in the diet, and was stable in diet for 6 wks when stored "...under normal conditions...". Animals were housed "...multiply..." according to dose. According to the sponsor, achieved doses were, for the most part, within 10% of intended. At sacrifice, livers were removed and microsomal fractions prepared; analyses were performed on pooled samples (4/data point).

The HD grp was sacrificed first, and found to have only a mean increase in liver weight of 11%. Since this was substantially less than previously observed at a similar dose (i.e., 28%), the lower dose grps were continued on drug for an additional 2 months. At the end of 3 mo of dosing, the relative liver weight was increased 8-10% in the lower dose grps; the differences were not dose-related, nor statistically significant. The data are summarized in the following sponsor's table and figure:

Table 4 : 204636 KMM 005 : The effects of ICI 204,636 on the hepatic microsomal mixed function oxidase enzymes of the female mouse

Group	Nominal dose level (mg/kg/day)	P450 (nmol/mg protein)	NADPH-cytochrome c reductase (nmol/mg/min)	Aldrin epoxidase (nmol/mg/min)	Ethoxycoumarin O-deethylase (nmol/mg/min)	Ethoxyresorufin O-deethylase (pmol/mg/min)	Pentoxyresorufin O-dealkylase (pmol/mg/min)
I	0	0.70 ± 0.03	194 ± 5	1.13 ± 0.10	0.50 ± 0.08	29 ± 2	13 ± 5
VII	400	1.39 ± 0.07***	290 ± 16**	7.48 ± 1.56**	1.65 ± 0.08***	44 ± 1**	57 ± 6**
II	0	0.65 ± 0.04	148 ± 9	1.60 ± 0.40	0.72 ± 0.03	31 ± 2	12 ± 3
III	10	0.63 ± 0.03	148 ± 14	1.49 ± 0.16	0.71 ± 0.04	28 ± 1	14 ± 0
IV	25	0.74 ± 0.03	156 ± 9	2.05 ± 0.19	0.88 ± 0.01***	33 ± 1	24 ± 1**
V	50	0.81 ± 0.02*	152 ± 4	2.41 ± 0.13	0.94 ± 0.04**	35 ± 2	28 ± 1**
VI	200	0.99 ± 0.03***	156 ± 6	3.81 ± 0.24**	1.56 ± 0.07***	42 ± 2*	38 ± 1***

Results show the mean ± SE obtained from 4 samples per group. Groups I and VII were dosed for one month and the other groups were dosed for three months.

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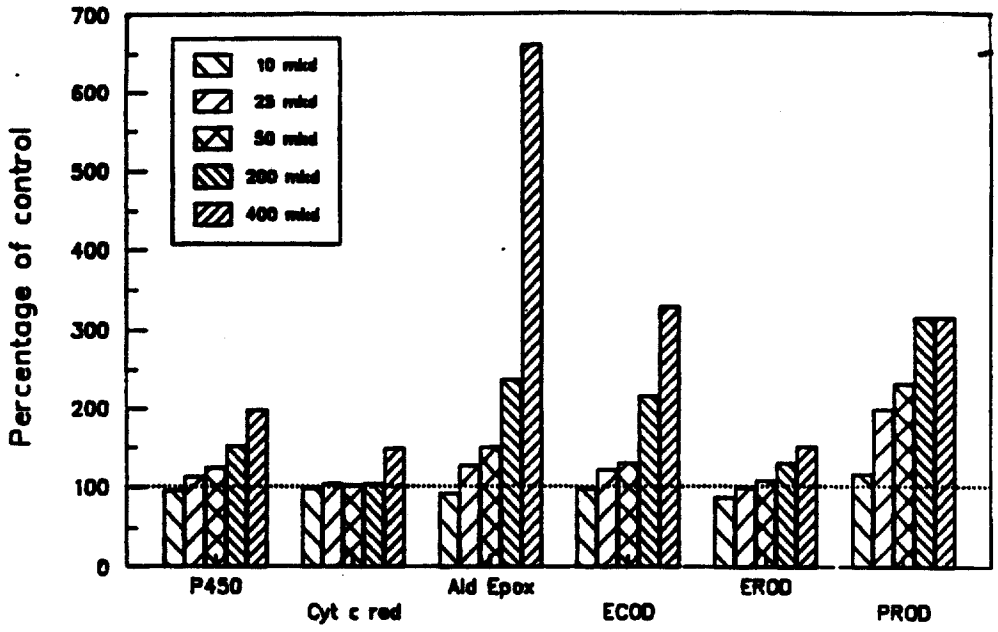
Figure 2 : 204636 KMM 005 : The effect of ICI 204,636 on the hepatic microsomal mixed function oxidase enzymes of the female mouse



Results show the mean ± SE obtained from 4 samples per group. The 400 mkd group was dosed for one month, all others were dosed for three months.

At the HD (administered for 1 mo), there was an increase in all microsomal enzymes assayed, as well as P450 content (1.5-6.6 fold). At the lower doses, there were dose-related increases in P450 content, and ethoxycoumarin O-deethylase and pentoxyresorufin activity. Aldrin epoxidase and ethoxyresorufin-deethylase activities were elevated at 200 mg/kg. No enzyme induction was observed at the LD.

Rat

1. **Determination of the potential inhibitory effect on hepatic microsomal enzymes after single oral administration to rats** (Study no. 204636DMR017/01, 9/HI/013468, SP Report no. SP1859/B, ICI Pharmaceuticals, GLP, Vol 1.64: 5.1.40)

In this study, ICI 204,636 (lot no. ADM 45103/87) was administered to Sprague-Dawley rats at single oral doses of 150 mg/kg (the HD in a 4-wk oral toxicity study; vehicle: 0.5% HPMC/0.1% TWEEN 80). For evaluation of antipyrine clearance and plasma $t_{1/2}$, 3/sex/grp were pretreated with either vehicle, ICI 204,636, or metyrapone (positive control, 100 mg/kg p.o.). One to 1.5 hr later, animals were injected with antipyrine (50 mg/kg i.v.). Blood samples were collected at 0, 60, 90, 120, 150, and 240 min after antipyrine administration. Four rats/sex (untreated) were used for preparation of liver microsomes

Both ICI 204,636 and metyrapone resulted in decreases in Cl (≈40-50 and 70%, respectively) and increases in $t_{1/2}$ (≈1.4-2 and 3-fold, respectively) for antipyrine in both males and females.

Addition of ICI 204,636 (0-0.1 mM) to liver microsomes from untreated animals, produced no

binding spectrum with cytP450. Therefore, a metabolite is implicated in the inhibitory effect of ICI 204,636 on antipyrine metabolism.

2. **Determination of the effect on hepatic microsomal enzymes after oral administration to rats for 14 days** (US Study no 24027XAR42, 9/HH/011722, ICI Pharmaceuticals, GLP, Vol 1.64: 5.I.41)

In this study, ICI 204,636 (lot no. ADM 56074/86; PDRM W1978) was administered to Sprague-Dawley rats (6/sex/grp) at doses of 0, 25, 50, and 150 mg/kg p.o. (vehicle: 0.5% HPMC/0.1% TWEEN 80) for 14 days. At sacrifice, hepatic microsomes were prepared and assayed for MFO activity. [Two males (LD, MD) died during the study due, according to the sponsor, to dosing accidents.]

Relative liver weight was elevated at all doses in females (14, 13, 19% at LD, MD, HD). In males, cytP450 content and aminopyrine N-demethylase activity were reduced at the MD and HD (11-26%), but not in a dose-related manner; ethoxyresorufin O-deethylase activity was elevated at the HD (30%).

3. **Metabolism in the rat** (Study no. 204636DMR020/01, Report no. SP2328/B, 4/IB/017976, ICI Pharmaceuticals, Vol 1.65: 5.I.44)

This study was conducted using urine collected from female rats in metabolism study DDM24027011 (Report no. SP1881/B) and in bile collected from male Sprague-Dawley rats in this study. ^{14}C-ICI 204,636 (lot no U24027F7, unlabeled compound) was administered orally (vehicle: 0.5% HPMC/0.1% TWEEN 80) to males at a dose of 25 mg/kg given in 4 doses over a 30 hr period, i.e., 0, 6, 12, and 24 hr. Bile samples were collected at 0-6, 6-12, 12-24, 24-30, and 30-48 hr. Bile samples were analyzed for parent compound and metabolites with and without prior hydrolysis (using glusulase). Parent compound and metabolites were identified following extraction using HPLC/MS.

Ten metabolites and the parent compound were detected in bile samples, with 4 metabolites being identified using authentic standard (and confirmed by MS): dealkylated 7-OH (MRB-A1), 7-OH (MRB-A3), 8-hydroxide (MRB-F3), and ethoxy metabolite (U26342, MRB-H). The parent compound corresponded to peak MRB-I. Although drug-related compounds in bile were identified in rats receiving multiple doses, quantitation of parent compound and metabolites in bile was performed on bile collected from other male rats (Study no. DDM24037010) which had received a single 25 mg/kg p.o. dose. These data are summarized in the following sponsor's table; "ND" indicates that, following a single 25 mg/kg dose, the compound was <LLOQ.

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Table 2: Quantitation of Identified Metabolites in Male Rat Bile After a 25 mg/kg Oral dose of ^{14}C-ICI 204,636

Metabolite	% Radioactivity in Bile	% Dose
MRB-A1 (U31477)	6.3	5.1
MRB-A2	1.6	1.3
MRB-A3 (ICI 214,227)	14.2	11.6
MRB-B	2.5	1.9
MRB-C	1.4	1.2
MRB-F1	5.9	4.7
MRB-F3 (U29441)	1.6	1.3
MRB-H (U26342)	ND	ND
MRB-I (ICI 204,636)	0.4	0.4
MRB-L	34.7	28.1
MRB-N	2.1	1.7
TOTAL	70.7	57.3

The major biliary metabolite was identified as an hydroxy acid (MRB-L). It was identified in the non-extractable fraction, indicating that it is not a conjugate.

The situation in female rat urine is slightly unclear. In one part of the report, it is stated that "one additional" metabolite was identified in urine. However, in a different section, it was indicated that "a single metabolite" was isolated from female rat urine. It is unclear whether or not the single metabolite was, in fact, the only metabolite isolated, simply the only one that was not detected in male rat bile. Nonetheless, this single metabolite was designated FRU-1 and was identified as an acid (carboxylated) metabolite of ICI 204,636. It was detected in urine from only 5 of the 15 females sampled. In those females in which FRU-1 was detected, urinary radioactivity accounted for 26% of dose radioactivity, whereas in those in which it was not detected, urinary radioactivity accounted for only 6% of dose radioactivity. The metabolic scheme in rats is presented in the sponsor's figure (attached).

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Human

1. **The inhibitory effects of ICI 204636 and metabolites on human cytochrome P450 activities in vitro** (Study no. 5077DMX053, 3/IE/1013647, Zeneca Pharmaceuticals, GLP, Vol 1.64: 5.I.42)

This study was conducted in microsomes prepared from human (fresh, snap frozen) liver tissue purchased from the International Institute for the Advancement of Medicine. ICI 204,636 and a number of metabolites were incubated with the human microsomal preparation (at concentrations of 30 and/or 150 µM) in order to determine the effects of these compounds on P450 enzymes.

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

Table 1: Inhibitory potential of ICI 204,636 on specific cytochrome P450 activities in human liver microsomes.

		Percent Inhibition at 150 / 30 µM				
Compound	Description	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
ICI 204,636	parent compound	17*	47	40	62/30	40
ICI 213,841	sulfoxide	14	47	*	59/18	56/33
M 211,803	N-dealkylated	43	37	50	76/37	87/52
ICI 214,227	7-hydroxy	37	50	68/47	87/43	9
M 236,303	N-dealkylated, 7-hydroxy	51/33	63/30	68/52	61/50	89/16
M 289,663	parent acid	12	47	3	6	19
M 234,676	O-dealkylated	21	38	*	65/41	53/8
M 276,660	O-dealkylated acid	37	72/38	76/54	50	11
M 282,445	sulfoxide-O-dealkylated acid	20	40	*	53/37	31
M 289,886	sulfoxide acid	20	42	24	49	13

* The test compounds were not incubated at 30 µM when less than 50 percent inhibition of a P450 activity was observed at the 150 µM concentration.
* The test compound coeluted with 4-hydroxy-mephenytoin preventing the calculation of percent inhibition.

The data indicate that the parent compound and a number of metabolites had inhibitory effects on one or more P450 enzymes.

2. **Determination of the human cytochrome P450 enzymes involved in forming metabolites of ICI 204636 in vitro** (Study no. 5077DMX058, 10/IE/1017427, Zeneca Pharmaceuticals, GLP except for MS analyses, Vol 1.65: 5.I.43)

Human liver microsomes were prepared from fresh or snap frozen livers obtained from the

International Institute for the Advancement of Medicine and from human lymphoblastoid cell lines expressing cyp1A2, cyp2C9, cyp2C19, cyp2D6, cyp2E1, and cyp3A4 purchased from Gentest. [^{14}C]ICI 204,636 was used to quantitate metabolism by the human microsomal fractions in the presence and absence of specific enzyme inhibitors (as follows).

Table 2 : Concentrations of the CYP inhibitors

Inhibitor Name	CYP enzyme inhibited	'Selective' concentrations (μM)		
		Low	Mid	High
furafylline	1A2	1	5	25
sulfaphenazole	2C9	5	25	100
quinidine	2D6	0.2	1	5
DDC	2E1	5	25	100
ketoconazole	3A4	0.02	0.1	1

Parent compound and metabolites were quantitated using HPLC and HPLC/MS.

Metabolism of ^{14}C-ICI 204,636 by human microsomes produced 5 peaks. Four of these were identified through the use of authentic standards as the 7-hydroxylated (ICI 214,227), sulfoxide (ICI 213,841), O-dealkylated (ICI 211,803), N-dealkylated (ICI 234,676). A fifth minor peak was tentatively identified as an O-dealkylated, sulfoxide metabolite; however, an authentic standard was not available for positive identification.

The sponsor indicated that additional metabolites detected in vivo in humans were not produced by human microsomes in vitro (e.g., ICI 289,663 and ICI 276,660). Other secondary metabolites detected in vivo were also not formed in vitro.

In the kinetic studies, the V_{max} for formation of the four major in vitro metabolites was not achieved at substrate concentrations up to 100 μM. Estimates of K_m were 160 ± 63 (7-OH), 110 ± 15 (sulfoxide), 100 ± 5 (N-desalkyl), and 170 ± 18 (O-desalkyl) μM. The high K_m values would suggest that ICI 204,636 is a low-affinity substrate for the P450 enzymes involved in its metabolism.

Inhibition studies were conducted at a substrate concentration of 15 μM (which, according to the sponsor, is ≈5 times the plasma level in humans following 375 mg bid dosing for 18 days). Inhibitors of cyp2C9 (sulfaphenazole), cyp2D6 (quinidine), and cyp3A4 (ketoconazole) inhibited formation of one or more metabolites of ICI 204,636, indicating the role of these P450 enzymes in the metabolism of ICI 204,636.

Using recombinant human cytP450 enzymes, the following were observed:

(1) no metabolites form when ICI 204,636 was incubated with cell expressing human cyp1A2, cyp2C9, cyp2C19, or cyp2E1.

(2) all five metabolites were detected following incubation of ICI 204,636 with human cyp3A4.

(3) formation of the 7-OH metabolite following incubation of ICI 204,636 with human cyp2D6.

Interspecies comparisons

1. **Metabolism of ICI 204,636 by rat, dog, cebus monkey and man** (Study no. 204636DMN002/01, 8/HH/011521, ICI Americas, Inc., Vol 1.65: 5.I.45)

The metabolic profile of ICI 204,636 was assessed in rat, dog, and monkey plasma. The rat (n = 1) was dosed with 80 mg/kg p.o. of ICI 204,636 (Lot F4) as a single dose. Blood was collected at 1 hr postdosing. Dog and monkey plasma was obtained in a separate study or studies in which they were dosed with 80 mg/kg p.o.; blood was collected at 1 hr postdosing. In addition to studies in plasma, rat and human liver homogenates were incubated with ICI 204,636 for 0.5 and 2.0 hr. Drug-related material was extracted with ethyl acetate, and metabolites were detected using reversed phase HPLC with uv monitoring (219 nm). Peaks were compared to authentic standards of parent compound and several metabolites, U27130 (sulfoxide), U28521 (2-OH), U31172 (dealkylated), and U29441 (3-OH). Extracts of rat liver homogenates following incubation with ICI 204,636 were also analyzed using MS. No quantitative assessment was conducted.

The metabolic profile data (qualitative) are summarized in the following sponsor's Table 1:

TABLE I

Summary of Metabolic Profiles Obtained for Rat, Dog, and Cebus Monkey Plasma and Rat and Human Liver Homogenate

Drug or Metabolite	Rat Plasma	Dog Plasma	Cebus Plasma	Rat Liver	Human Liver
ICI 204,636	Trace	+++	+++	-	+
U27130	Trace	+++	+++	+++	+++
U28521	++	+++	+++	+	+
U31172	-	-	Trace	-	Trace
U29441	-	-	-	-	-
Other (I)[a]	+++	-	+	+++	-
Other (II)[b]	Trace	-	-	++	+

[a]The mass spectral data for this metabolite indicates the 2-OH of U31172
[b]The mass spectral data for this metabolite indicates the sulfoxide of U31172.

2. **The comparative disposition in mouse rat rabbit dog cynomolgus monkey and man** [Study no. 5077DMN031, 7/IE/1015184, Zeneca Pharmaceuticals, GLP (except for MS analyses), Vol 1.65: 5.I.46]

This report is, for the most part, a summary of a number of previous ADME/PK studies. Data included in the report came from the following studies: DDM24027003 (rat PK), DDM24027002, DDM24027010, DDM24027011, 204636DMR020, 204636DMR033 (rat balance), 204636DMM019 (mouse balance), DDM24027001, DDM24027005, 204636DMD021, 5077DMD051 (dog balance/PK), 204636KMB003 (rabbit balance), 204636DBP022 (monkey balance/PK), 5077US/0002 (human balance/PK). In addition to these data, new samples were collected from rat (n = 2) and dog (n = 1) at doses of 25 and 10 mg/kg p.o., respectively (using ^{14}C-ICI 204,636, lot no. U24027F7, unlabeled compound). Blood (0.5 hr postdosing) was collected in 1 rat. In the other rat, urine (0-6, 6-12, 12-24, 24-48 hr postdosing) and feces (0-24, 24-48 hr postdosing) were collected at the time points indicated. Analyses were performed on the 0.5 hr blood sample, the 0-6 hr urine sample, and the 0-24 hr fecal sample since, according to the

sponsor, these samples contained the most radioactivity. In the 1 dog, blood, urine, and fecal samples were collected at the same time points used in the 1 rat.

Samples in the previous studies were obtained from animals dosed as follows: cynomolgus monkey (n = 1, 10 mg/kg p.o.; 2 hr plasma, 0-6 hr urine, 0-24 hr feces), human (n = 1, 150 mg p.o., steady state; 1 hr plasma, 2-4 hr urine, 0-12 hr feces; and for polar fraction, 1.5 hr samples from 6 Ss were pooled for analysis), dog (n = 3, 25 mg/kg p.o., for analysis of polar fraction, 0.5 hr plasma samples from 3 dogs were pooled).

For analysis, samples were processed as follows: (1) made basic and extracted with ethyl acetate, i.e., extractable fraction, (2) aqueous fractions were incubated with a mixture of glucuronidase and sulfatase, made basic, and back-extracted into ethyl acetate (conjugated fraction), and (3) in those cases where "sufficient" radioactivity remained in the aqueous phase following back-extraction, samples were loaded onto an XAD-7 resin column and eluted with methanol (polar fraction). Peaks were identified by HPLC, and identification was made by HPLC/MS by comparison to authentic standards.

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

Table 2: Recovery of total radioactivity

Species	Dose	Recovery of total 14C (% Dose) (a)				
(number of animals/sex)	(mg/kg) Route	Bile	Urine	Feces	Absorption (b)	Study reference
Rat (5 M)	5 iv	77.3	9.8	2.1	87.1	DDM24047002
	5 po	75.9	7.3	3.6	83.2	DDM24027002
	25 po	87.4	7.5	2.9	94.9	DDM24027010
Rat (4 M)	5 iv	c	11.0	80.5	11.0	DMR033
	25 po	c	9.4	88.7	9.4	DMR033
Rat (5 F)	5 iv	70.5	12.9	0.9	83.4	DDM24027011
	5 po	79.6	9.1	1.5	88.7	DDM24027011
	25 po	77.0	12.0	2.4	89.0	DDM24027011
Rat (4 F)	5 iv	c	5.8	84.9	5.8	DMR033
	25 po	c	10.7	87.8	10.7	DMR033
Mouse (20M)(e)	20 po	c	33.4	60.2	33.4	DMM019
Mouse (20F) (e)	20 po	c	42.9	49.6	42.9	DMM019
Dog (1 M)	1 iv	85.5	12.3	2.5	97.8	DDM24027005
	1 po	73.3	16.9	5.1	89.9	DDM24027005
Dog (4 M)	10 po	c	23.2	76.9	23.2	DDM24027001
Dog (3 F)	10 po	c	25.9	69.1	25.9	204636DMD0211
Rabbit (4F)	25 po	c	58.3	27.9	58.2	KMB 003
Monkey(4 M)	0.5 iv	c	43.6	37.9	43.6	DBP022/01
(3 M)	10 po	c	46.3	43.4	46.3	DBP022/01
Human (6 M)	150 po (d)	c	72.9	21.0	72.9	5077US/0002

(a) Collections were made over 2 (rat & dog bile), 5 (mouse, rat, rabbit, dog & monkey) and 7(human) days.
(b) Absorption calculated as the sum of the total ^{14}C recovery in bile and urine (rat and dog bile studies) or urine alone (all species) and is considered the minimum value.
(c) Bile duct-cannulated animals not studied at the dose level/route.
(d) Administered dose was 150 mg oral at steady state (~2 mg/kg).
(e) 5 mice were pooled in groups of 5 of the same sex.

Table 3: Summary of plasma total radioactivity and ICI 204,636 concentration data (mean ± SEM)

Species	No./Sex	Dose (mg/kg)	Analyte	Conc (ng/mL)	Time (hr)	% ICI 204,636 Circulating (c)	Study Reference
Rat	3 M	5	Total 14C	197 ± 94	0.5		DDM24027003
			ICI 204,636	11 (a)	0.5	5.6	DDM24027003
	4 F	5	Total 14C	532 ± 288	0.5		DDM24027003
			ICI 204,636	19 ± 24	0.5	3.6	DDM24027003
	3 M	25	Total 14C	2802 ±1788	0.5		DDM24027003
			ICI 204,636	86 ± 67	0.5	3.4	DDM24027003
	3 F	25	Total 14C	2966 ± 1306	0.5		DDM24027003
			ICI 204,636	116 ± 112	0.5	3.9	DDM24027003
Dog	4 M	1	Total 14C	749 ± 190	0.5		DDM24027001
			ICI 204,636	20 ± 8	0.5	2.7	DDM24027001
	4 M	10	Total 14C	6811 ± 1584	0.5		DDM24027001
			ICI 204,636	600± 148	0.5	8.8	DDM24027001
Monkey	3 M	10	Total 14C	6180± 1430	2.0		DBP022/01
			ICI 204,636	102 ± 37	2.0	1.7	DBP022/01
Human	6 M	150 (b)	Total 14C	1880 ± 155	2.0		5077US/0002
			ICI 204,636	399 ± 65	2.0	21.1	5077US/0002

(a) single determination only
(b) 150 mg oral dose
(c) (Conc ICI 204,636/Conc total ^{14}C) x 100

Table 5: % Circulating at maximum plasma concentration of total radioactivity

Number	Name	Species			
		Rat	Dog	Monkey	Man
ICI 204,636	parent	1.5	8.4	2.6	23.2
	conjugate	0.5	12.4	0.5	–
M1	N-dealkylated-7-hydroxy	28.7	–	0.9	–
	conjugate	1.5	1.0	33.7	1.4
M2	7-hydroxy	13.5	6.7	8.5	–
	conjugate	0.5	9.1	9.8	2.5
M4	sulfoxide	4.1	8.0	6.9	15.1
	conjugate	0.4	4.2	0.9	–
M7	O-dealkylated sulfoxide acid	1.6	1.8	2.0	<0.5
M8 *	sulfoxide acid	8.6	2.7	2.8	0.9
M9 *	O-dealkylated acid	2.4	2.7	4.7	3.4
M10 *	parent acid	11.0	13.8	2.1	14.7
Sub-total		74.3	72.6	75.4	61.5
	Unknowns (% circulating/ # of peaks)	19.9 / 19	23.7 / 24	21.6 / 23	32.2 / 32
Total		94.2	96.5	97.0	93.7

* Small amounts of these metabolites appear in the *extractable* and *conjugate* fractions but are not noted in the tables in Appendix B

Table 6: Summary of metabolite quantitation (as % dose) excreted in urine and feces

Metabolite Number	Name	Species							
		Rat		Dog		Monkey		Man	
		Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
ICI 204,636	parent	ND	ND	<0.5	23.8	<0.5	<0.5	<0.5	0.5
	conjugate	ND	<0.5	<0.5	4.6	0.6	<0.5	1.4	<0.5
M1	N-dealkylated 7-hydroxy	3.0	15.3	0.5	4.6	1.2	13.3	0.6	3.4
	conjugate	0.6	0.8	0.7	1.0	7.5	1.4	0.2	<0.5
M2	7-hydroxy	ND	3.5	<0.5	18.1	0.7	4.1	<0.5	0.5
	conjugate	<0.5	6.7	1.5	3.8	3.3	2.3	<0.5	1.0
M4	sulfoxide	0.6	4.0	0.5	2.3	0.7	ND	4.4	1.5
	conjugate	<0.5	1.6	<0.5	0.5	0.6	<0.5	0.5	<0.5
M7	O-dealkylated sulfoxide acid	1.2	ND	0.9	ND	4.1	ND	2.3	ND
M8	sulfoxide acid	<0.5	13.5	2.8	8.0	5.1	3.1	24.1	3.6
M9	O-dealkylated acid	<0.5	2.0	1.8	1.6	2.9	0.8	2.9	0.8
M10	parent acid	<0.5	8.7	2.9	3.0	3.7	1.0	27.4	1.6
Sub-total		5.7	58.2	12.3	69.1	30.6	26.8	64.4	13.7
	Unknown (% Dose/ # of peaks)	3.1 / 15	23.1 / 10	10.1 / 13	5.1 / 6	14.6 / 14	12.3 / 10	4.4 / 7	4.3 / 11
Total		8.8	79.4	22.4	74.1	45.2	39.1	68.8	18.0
Recovery		88.2		96.5		84.3		86.8	

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PLASMA PROTEIN BINDING/DISTRIBUTION

1. **Plasma protein binding study** (US Study no. DDM24027006, 5/HH/011182, ICI Pharmaceuticals, Vol 1.63: 5.I.30)

The extent of plasma protein binding of ^{14}C-ICI 204,636 (lot no. 31078A1, unlabeled compound) was determined by equilibrium dialysis in fresh plasma collected from rats, dogs, (strains not specified) and humans. At plasma concentrations of 0.2 to 3.0 µg/mL, protein binding of ^{14}C-ICI 204,636 was 62.8 ± 3.0 (mean ± SD), 68.6 ± 1.7, and 83.0 ± 1.1% in rat, dog, and human plasma, respectively. [The % recovery of radioactivity was not specified.]

2. **The binding of ^{14}C-ICI 204,636 to mouse and rabbit plasma** (US Study no. 204636DMJ016/02, 6/HI/013163, ICI Pharmaceuticals, Vol 1.63: 5.I.31)

The extent of ^{14}C-ICI 204,636 (lot no. U31078A3, unlabeled compound) binding to plasma proteins was determined in plasma purchased from Protein binding was determined using equilbrium dialysis. At plasma concentrations of ^{14}C-ICI 204,636, protein binding was 64.9 ± 1.0 (mean ± SEM) and 70.9 ± 0.5% in mouse and rabbit plasma. [The % recovery of radioactivity was not specified.]

3. **ICI 204,636: Distribution in blood components** (Study no. 204636DMN018/01, Report no. SP1973/B, 4/IB/017978, ICI Pharmaceuticals, Vol 1.63: 5.I.32)

The distribution of ^{14}C-ICI 204,636 (lot no. U31078A3, unlabeled compound) in blood was assessed in fresh rat blood (strain not specified) using final concentrations of 0.25 to 5 µg/mL. Plasma was separated from rbc after incubation at 37 °C for 1 hr.

Following centrifugation, 72.2 ± 6.5% of initial radioactivity was recovered in plasma overall. Distribution into plasma ranged from 72.1 to 82.9% at concentrations of 0.22 to 1.79 µg/mL, and was slightly lower at the highest concentrations (i.e., 2.51 and 4.79 µg/mL)

4. **Study of the competitive binding of ICI 204,636, warfarin and diazepam to human serum albumin** (Study no. 5077DMJ036, 3/ID/1007650, Zeneca Pharmaceuticals, GLP, Vol 1.63: 5.I.33)

The binding of ^{14}C-ICI 204,636 (lot nos. U24027F7, W3595B1 for unlabeled compound) to HSA was determined in the presence of warfarin and diazepam at ICI 204,636 concentrations of 0.025 to 5.0 µg/mL. Protein binding was assessed using equilibrium dialysis. The only significant effect obtained was a slight (4.6%) increase in binding of diazepam at 0.025 µg/mL (but not at 1.0 µg/mL) in the presence of ICI 204,636 (concentration not specified); this finding was attributed to a low % binding in the absence of competitor.

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