Phase B: Results are summarized in the table below. Very low radioactivity concentrations were noted in the untreated eye, suggesting that the drug reached the untreated eye through systemic circulation.

Ocular distribution of ^{14}C-epinastine radioactivity (1 hr after the unilateral administration)

	Cmax (ng-eq/g or ml)	
Tissue	Right eye (treated)	Left eye (untreated)
Eyelids		
Lower bulbar conjunctiva		
Upper bulbar conjunctiva		
Cornea		
Sclera		
Aqueous humor		
Iris		
Ciliary body		
Lens		
Choroid and retina		
Vitreous humor		
Optic nerve head		
Lacrimal glands		
Plasma		

BLQ: Below the limit of quantification

PK-02-008: ^{14}C-epinastine hydrochloride ocular tissue distribution studies in cynomolgus monkeys following repeated ophthalmic administration. Vol. 15, Page 307

Key study findings: Following repeated administration of bilateral ocular doses of ^{14}C-epinastine HCl to monkeys for 7 days (bid at a 12-hr interval), relatively high concentrations of radioactivity were measured in pigmented tissues (iris, ciliary body and choroid), the external ocular tissues (eyelids and conjunctiva) and in tears. The concentrations of radioactivity in pigmented tissues may be attributable to binding to melanin. After administration of repeated unilateral ocular doses (bid at a 12-hr interval x 7 days), low concentrations of radioactivity were measured in the choroid, ciliary body, eyelids and sclera of the untreated eye, indicating that radioactivity entered the untreated eye from systemic circulation.

Report Nº: PK-02-008
Study Nº: PK-01-P014
Conducting laboratory/location:

Study initiation: July 20, 2001
GLP: Yes
QA report: Yes (X) No ( )
Drug: ^{14}C-epinastine HCl (Batch #: NPE/ALG083/22, 55 mCi/mmol, 191 µCi/mg, radiochemical purity:
Epinastine HCl (Batch #: R12328, purity:
Methods: for radioactivity concentrations (LOQ:

Dosing
Species/strain: Male cynomolgus monkeys, 2 to 3 years old, 2.89-2.99 kg
N: 7
Frequency: Twice daily at 12-hr intervals for 7 days
Route: Ocular topical

Volume: 35 µl, 0.05% ^{14}C-Epinastine HCl, both eyes twice daily at 12-hr intervals for 7 days in 6 animals (Phase A) and one eye (right eye only) twice daily at 12-hr intervals for 7 days in 1 animal (Phase B)

Observations and times: Animals in Phase A were terminated at 1, 2, 6, 12, 24 and 48 hr after the last dose (1 animal/time point). The Phase B animal was terminated at 1 hr after the last dose. Ocular tissues and blood samples were collected at the termination. Blood samples were also collected from all animals prior to the 1st dosing, and from Phase A animals prior to the 2nd dosing, 1 and 2 hr after the 12th dosing, and immediately prior to the 14th dosing.

Results:

Blood and plasma radioactivity concentrations: Blood and plasma radioactivity parameters from Phase A animals are summarized in the table below. At 12 hr after the 1st dose, the mean plasma radioactivity concentration was 0.188 ng-eq/ml. At 12 hr after the 13th dose, the mean plasma concentration was 0.591 ng-eq/ml, which was about 3-fold greater than at 12 hours after the 1st dose.

Blood and plasma PK parameters in monkeys treated with ^{14}C-Epinastine (both eyes) bid for 7 days

	Cmax (ng-eq/ml)	Tmax (hr)	AUC_{0-12} hr (ng-eq•hr/ml)	AUC_{0-48} hr (ng-eq•hr/ml)	C (dose 1 + 12 hr) ng-eq/ml, mean ± SD)	C (dose 13 + 12 hr) ng-eq/ml, mean ± SD)
Plasma	1.651	2	9.102	23.16	0.188± 0.053	0.591± 0.215
Blood	1.281	2	7.121	13.61	BLQ	0.366± 0.3

Phase A: Results of ocular distribution are summarized in the table below. High concentrations of radioactivity were noted in the external ocular tissues (eyelids and conjunctiva) and in tears. However, the highest concentrations of radioactivity were measured in the iris.

Ocular distribution of ^{14}C-epinastine radioactivity (bilateral administration)

	Cmax (ng-eq/g or ml)			AUC_{0-48} hr (ng-eq•hr/ml)	Tmax (hr)	
Tissue	Left eye	Right eye	Mean (both eyes)	Mean (both eyes)	Left eye	Right eye
Eyelids	3982	7207	5595	117000	1	1
Lower bulbar conjunctiva	570.9	1109	840.0	11850	1	1
Upper bulbar conjunctiva	503.1	706.9	605.0	11230	12	1
Cornea	292.2	445.9	369.1	4503	2	2
Sclera	184.8	215.0	199.9	7430	2	12
Aqueous humor	5.412	5.733	5.573	55.02	2	2
Iris	15650	26350	21000	530400	2	2
Ciliary body	417.2	446.3	431.8	13630	2	2
Lens	4.183	5.052	4.618	158.0	2	1
Choroid	309.7	310.6	310.2	10670	48	12
Retina	4.046	4.084	4.065	102.4	2	2
Vitreous humor	0.921	1.441	1.181	18.06	2	6
Optic nerve head	24.05	5.525	14.79	125.5	6	6
Tears	2724	3863	3294	65550	1	6
Lacrimal glands	24.19	61.59	42.89	380.7	2	2
Plasma	1.651			0.591	2	
Whole blood	1.281			0.366	2	

Phase B: Results are summarized in the table below. Distribution and concentrations of radioactivity in the right (treated) eye were similar to those observed in the Phase A animal sacrificed at 1 hr after the final dose. Concentrations of radioactivity lower than plasma concentrations were measured in the untreated (left) eye in the choroid, ciliary body, eyelids, and sclera, suggesting that the drug reached the untreated eye through systemic circulation.

Ocular distribution of ^{14}C-epinastine radioactivity (1 hr after the unilateral administration)

	Cmax (ng-eq/g or ml)	
Tissue	Right eye (treated)	Left eye (untreated)
Eyelids		
Lower bulbar conjunctiva		
Upper bulbar conjunctiva		
Cornea		
Sclera		
Aqueous humor		
Iris		
Ciliary body		
Lens		
Choroid		
Retina		
Vitreous humor		
Optic nerve head		
Tears		
Lacrimal glands		
Plasma		
Whole blood		

BLQ: Below the limit of quantification

No half-life data were provided in this study. The sponsor indicated that the half-life could not be calculated because the concentrations of radioactivity tended to increase at later times in many tissues possibly due to the variability in the actual doses received by each eye and inter-individual variability.

U84-0606: Whole animal autoradiographic studies with [^{14}C]WAL 801 CL in rats. Vol. 16, Page 106

Key study findings: WAL 801 was uniformly distributed in the entire animal after iv or po administration. Higher radioactivity was detected mainly in the intestine contents, adrenal gland and kidneys. No passage across blood-brain barrier was noted.

Report #: U84-0606
Study #: Not indicated
Conducting laboratory and location: Department of Biochemistry, Research Division, Boehringer Ingelheim KG
Date of study initiation: May 1984
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Rats/Chbb:THOM
N: 4 males for iv group and 5/sex for po group
Age/weight: 150-170 g. No age data were provided.
Route: Intravenous and oral (by gavage)
Dosage: 10.3 mg/kg for iv and 38.9 mg/kg for oral, single dose
Drug: ^{14}C-WAL 801 CL (Batch #: I, 110 μCi/mg)
Methods:

The purpose of this study was to determine the distribution of WAL 801 CL and its metabolites following iv or oral administration in rats autoradiographically. Four male rats (one/time point) were sacrificed at 0.5, 2, 12 and 24 hr after iv dosing, five male rats (one/time

point) were sacrificed at 0.5, 2, 4, 12 and 24 hr after po dosing, and five female rats (one/time point) were sacrificed at 0.5, 3, 8, 12 and 24 hr after po dosing.

Results:

The distribution pattern was similar after either iv or po administration. WAL 801 was uniformly distributed in the entire animal. Higher radioactivity was detected mainly in the intestine contents, adrenal gland and kidneys. At 30 min after dosing, radioactivity concentration in the liver was higher in the po group than in iv group. Radioactivity was only detected in the intestine contents at 24 hr after dosing. No passage across blood-brain barrier was noted. [**Reviewer's Comments**: The data table was not translated into English.]

U86-0216: Placental passage of WAL 801 CL in rats. Vol. 16, Page 124

Key study findings: WAL 801 CL and its metabolites crossed the placental barrier after iv and oral administration.

Report #: U86-0216
Study #: Not indicated
Conducting laboratory and location: Department of Biochemistry, Research Division, Boehringer Ingelheim KG
Date of study initiation: Not indicated
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Pregnant rats/Chbb:THOM
N: 40 (3-8/group)
Age/weight: 230-270 g for pregnant day 14, and 235-290 g for pregnant day 19. No age data were provided.
Route: Intravenous and oral (by gavage)
Dosage: 8.03-10.03 mg/kg for iv and 33.35-37.45 mg/kg for oral, single dose
Drug: ^{14}C-WAL 801 CL (Batch #: I, 110 μCi/mg)
Methods: [redacted] and whole body autoradiography
Study design:

Examination	Days of pregnancy	N	Sampling time point (hr)
Intravenous route			
Whole-body autoradiography	14	3	0.5, 3, 8 (1/time point)
Blood and tissue radioactivity concentrations	14	6	0.5, 3, 8 (2/time point)
Whole-body autoradiography	19	3	0.5, 3, 8 (1/time point)
Blood and tissue radioactivity concentrations	19	8	0.5, 3, 8, 24 (2/time point)
Oral route			
Whole-body autoradiography	14	3	0.5, 3, 8 (1/time point)
Blood and tissue radioactivity concentrations	14	6	0.5, 3, 8 (2/time point)
Whole-body autoradiography	19	3	0.5, 3, 8 (1/time point)
Blood and tissue radioactivity concentrations	19	8	0.5, 3, 8, 24 (2/time point)

The purpose of this study was to determine the passage of WAL 801 CL and its metabolites through the placenta in rats. Whole-body autoradiography and radioactivity measurements were conducted in selected organs of dams and fetuses (blood, heart, liver, uterus,

placenta, and amniotic fluid). This study was conducted on gestation days 14 and 19 with iv and po administration routes.

Results:

The autoradiography showed that WAL 801 CL and its metabolites crossed the placental barrier after intravenous and oral administration. No differences were found between the two gestation days. In the iv group, 8 hr after dosing, radioactivity concentrations in fetuses were low and could not be visualized by autoradiography. Radioactivity concentration in the placenta was always higher than that in the fetuses.

Radioactivity concentrations after iv and oral administration are summarized in the table below. Radioactivity concentration in the placenta was higher than that in the dam blood and in the fetuses. A high concentration was noted in the fetal liver on gestation day 19. However, it was lower than that in the dam liver.

Radioactivity concentrations in Study U86-0216 (ng-eq/ml or g)

	14th day			19th day			
Time point	30 min	3 hr	8 hr	30 min	3 hr	8 hr	24 hr
Intravenous							
Blood of dam	1310	602	205	1863	744	200	51
Blood of fetus				330	247	94	55
Liver of dam				30516	13549	2343	994
Liver of fetus				1783	1162	448	188
Placenta	3691	3471	2295	4020	2433	1005	313
Fetus	397	283	133	802	493	172	70
Amniotic fluid				116.5	110	99	22
Oral							
Blood of dam	1256	1560	570	1122	2192	618	131
Blood of fetus				220	525	264	156
Liver of dam	48232	33834	9013	35294	41934	5353	1505
Liver of fetus				1358	2014	796	384
Placenta	2098	6025	4674	2327	5744	1500	401
Fetus	303	610	415	443	1095	443	208
Amniotic fluid				76	300	238	66.6

U90-0608: Concentrations of ^{14}C activity in blood, plasma and bone marrow of mice and Chinese hamsters after oral administration of single doses of 250 mg/kg and 350 mg/kg WAL 801 CL-^{14}C respectively. Vol. 16, Page 359

Key study findings: Radioactivity was detected in all blood, plasma and bone marrow samples 4 hr after dosing. Mean radioactivity concentration in the bone marrow was 2 to 3 times higher than in blood.

Report #: U90-0608
Study #: Not indicated
Conducting laboratory and location: Department of Biochemistry, Research Division, Boehringer Ingelheim KG
Date of study initiation: 6/21/1988
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Mice/Chbi:NMRI, 4-5 weeks old
Chinese hamsters/Han:CHIN, 10-12 weeks old

N: 5/sex/group
Route: Oral (by gavage)
Dosage: 250 mg/kg for mice and 350 mg/kg for Chinese hamsters, single dose
Drug: ^{14}C-WAL 801 CL (Batch 8, purity: [redacted], 192 µCi/mg) in distilled water
Methods: [redacted]

The purpose of this study was to determine the distribution of WAL 801 CL and its metabolites in the bone marrow of mice and Chinese hamsters that were used in genotoxicity studies. Blood and bone marrow samples were collected 4 hr after dosing.

Results:

Results are summarized in the table below. Radioactivity was detected in all samples. Mean radioactivity concentration in the bone marrow was 2 to 3 times higher than in the blood, suggesting high exposure of the bone marrow cells to the drug under the conditions of genotoxicity studies with WAL 801 CL.

Radioactivity concentrations in blood, plasma and bone marrow samples in mice and Chinese hamsters (µg-eq/ml or g, mean± SD)

Species	Blood	Plasma	Bone marrow	Plasma/blood	Bone marrow/blood
Mice	5.9± 1.5	5.4± 1.3	18.9± 5.8	0.93± 0.07	3.22± 0.34
Chinese hamster	4.8± 0.7	6.1± 1.0	10.0± 2.3	1.26± 0.12	2.08± 0.38

U95-0065: ^{14}C-WAL 801 CL (0.3%; w/v) ophthalmic solution: Distribution in the ocular structures after three instillations into the eyes of pigmented rabbits. Vol. 17, Page 294

Key study findings: After ocular administration of ^{14}C-epinastine HCl, high concentrations of radioactivity were observed in external ocular tissues and iris-ciliary body. Plasma concentrations were very low.

Document #: U95-0065
Study #: 06593
Conducting laboratory and location: [redacted]

Date of study initiation: May 10, 1994
GLP compliance: Yes
QA report: Yes (X) No ( )
Species/strain: Male rabbits/Fauve de Bourgogne
Age/weight: 4-month old, 2-2.5 kg
N: 15 (3/time point)
Route: Ocular, topical
Dosage: 50 µl, right eye only, three times (in 10 min intervals)
Drug: ^{14}C-WAL 801 CL (0.3%) eye drop solution (Batch #: Ch-B 40313, 24.27 µCI/ml)
Methods: [redacted]

The purpose of this study was to determine the distribution of epinastine hydrochloride in ocular tissues after three ocular topical administrations of ^{14}C-epinastine hydrochloride (0.3%) in

pigmented rabbit eyes. Blood and ocular tissue samples were collected before and at 0.5, 1, 2 and 4 hr after the instillation.

Results:

Radioactivity was detected in all samples after three instillations, but high concentrations were noted in external tissues. The table below summarizes Cmax values in different tissues. In most tissues, Tmax was 0.5 hr.

Cmax of radioactivity after ocular administration of ^{14}C-WAL 801 CL in rabbits

Tissues	Tmax (hr)	Cmax (ng-eq/g or ml)
Blood	0.5	14
Plasma	0.5	19
Tears	0.5	139543
Palpebral conjunctiva	0.5	30410
Bulbar conjunctiva	0.5	16185
Nictitating membrane	0.5	22124
Cornea	0.5	24256
Aqueous humor	1	336
Iris-ciliary body	4	5310
Lens	4	63
Vitreous body	0.5	11
Sclera	0.5	5838
Retina	0.5	83
Choroid	4	1136

In summary, after ocular administration of ^{14}C-epinastine HCl, high concentrations of radioactivity were observed in external ocular tissues and iris-ciliary body. Plasma concentrations were very low.

U96-0003: WAL 801 (0.3%; w/v) ophthalmic solution: Penetration into the aqueous humor after single instillation into the eye of pigmented rabbits. Vol. 17, Page 322

Key study findings: The contralateral passage and systemic absorption of WAL 801 after ocular administration in rabbits were very low.

Document #: U96-0003
Study #: 06493
Conducting laboratory and location: [redacted]

Date of study initiation: 10/11/1993
GLP compliance: Yes
QA report: Yes (X) No ( )
Species/strain: Rabbits/Fauve de Bourgogne
Age/weight: 4-month old, 2-2.5 kg
N: 27/sex (3/sex/time point)
Route: Ocular, topical
Dosage: 50 µl, right eye only, single dose (150 µg/animal)
Drug: WAL 801 CL (0.3%) eye drop solution (Batch #: F 3048)
Methods: [redacted]

The purpose of this study was to determine the ocular and systemic distribution of WAL 801 CL after a single instillation of WAL 801 ophthalmic solution (0.3%) into the right eye of pigmented rabbits. Three animals/sex each were sacrificed at 0, 5, 10 and 30 min and 1, 2, 4, 8 and 24 hr after dosing and blood and ocular samples were collected.

Results:

PK parameters in aqueous humor from the right eyes and plasma are summarized in the table below. In the untreated left eyes, WAL 801 levels were very low (ng/ml), indicating a very low contralateral passage. The systemic absorption of WAL 801 was also very low.

PK parameters in plasma and aqueous humor from the treated eyes of pigmented rabbits

	Tmax (min)	Cmax (ng/ml)	$AUC_{0-24\ hr}$ (ng-hr/ml)
Aqueous humor	60	68	27778
Plasma	5	5	2383

In summary, in rabbits treated topically with WAL 801 eye drops, the contralateral passage and systemic absorption of WAL 801 were very low.

Metabolism:

PK-02-048: Metabolite profiles in ocular tissues of cynomolgus monkey following repeated ocular administrations of ^{14}C-epinastine hydrochloride. Vol. 16, Page 001

Key study findings: Epinastine was not metabolized in monkey eyes following bilateral ocular doses of ^{14}C-epinastine hydrochloride, twice daily for 7 days.

Report #: PK-02-048
Study #: PK-01-P014
Conducting laboratory and location:

Date of study initiation: 7/20/2001
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Male cynomolgus monkeys
N: 7
Age/weight: 2-3 years old, 2.73-2.99 kg
Route: Ocular, topical (bilateral)
Dosage: 35 µl of 0.05% ^{14}C-epinastine hydrochloride solution, twice daily for 7 days
Drug: ^{14}C-epinastine hydrochloride (Lot #: CFQ12116, 55 mCi/mmol, 191 µCi/mg, radiochemical purity:
Methods:

The purpose of this study was to determine the metabolite profiles of epinastine in ocular tissues following repeated doses of ^{14}C-epinastine hydrochloride to male monkeys bid for 7 days. Animals (one/time point) were sacrificed at 1, 2, 6, 12, 24 and 48 hr after the final (14th) dose.

Conjunctiva, eyelids, lacrimal glands, vitreous, aqueous humor, retina, iris, cornea, ciliary body, lens, choroid, sclera and optic nerve head were collected and processed for metabolite profiling.

Results:

Results showed that epinastine was not metabolized in monkey eyes following repeated bilateral ocular dosing twice daily for 7 days

U92-0666: Plasma level of WAL 1097, a metabolite of WAL 801 in man and enantiomers of WAL 801 in rabbits. Vol. 17, Page 219

Key study findings: Plasma concentrations of WAL 1097 after oral administration of 20 mg epinastine HCl were not detectable in human plasma. In rabbit plasma, no differences were noted between plasma concentrations of two enantiomers of WAL 801.

Report #: U92-0666
Study #: Not indicated
Conducting laboratory and location: Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co. Ltd.
Date of study initiation: Not indicated
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Human
Rabbit/Himalayan, female, 2.2 kg
N: 1 rabbit. The number of humans was not given.
Age/weight: Not indicated
Route: Oral for human and iv for rabbit
Dosage: 20 mg for human and 10 mg/kg for rabbit
Drug: WAL 801 CL (Lot #: Not provided)
Methods:

The purpose of this study was to determine the plasma concentration of WAL 1097, a metabolite of WAL 801 after oral administration of 20 mg of WAL 801 CL in human, and to determine the difference between plasma levels of the enantiomers in rabbit plasma following an iv administration. Human blood samples were collected before administration and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24 and 36 hr after administration. Rabbit blood samples were collected at 15, 40, 60 and 120 min after iv administration.

Results:

Plasma concentrations of WAL 1097 after oral administration of 20 mg epinastine HCl to humans were not detectable in all samples (< 1g/ml).

In rabbit plasma, no differences were noted between plasma concentrations of two enantiomers of WAL 801 (see table below).

Rabbit plasma level of *d*- and *l*-WAL 801 (μg/ml)

	d-WAL 801	l-WAL 801
15 min		
40 min		

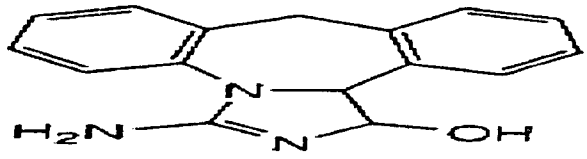
U98-0244: Structure elucidation of human hepatic microsomal metabolite of epinastine. Vol. 18, Page 034

Key study findings: The human metabolite M-1 was assumed to be generated by hydroxylation of the intact drug. The position of hydroxylation was possibly

Report #: U98-0244
Study #: NBIPK-9819
Conducting laboratory and location:

Date of study initiation: June 24, 1998
GLP compliance: No
QA report: Yes ( ) No (X)
Methods:

The purpose of this study was to elucidate the chemical structure of epinastine metabolite M-1 generated in human hepatic microsomes. ^{14}C-M-1 fraction obtained from the incubation mixture of human hepatic microsomes (1 mg protein/ml and 100 µM of ^{14}C-WAL 801 CL, incubated at 37°C for 4 hr) was analyzed with the The metabolite was assumed to be generated by hydroxylation of the intact drug (see table below) because a protonated molecular peak of the metabolite was higher than the intact drug. The position of hydroxylation was possibly because dehydration of the hydroxy group at formed a compound with a stable imidazole ring.



Excretion:

U84-0546: Biochemical investigations with WAL 801 CL-^{14}C in rats (absorption, distribution, metabolism, excretion). Vol. 16, Page 058

Key study findings: WAL 801 CL was absorbed after oral administration. The metabolism was very low (≤ 20%). The drug was eliminated from rats mainly through feces (60%). Biliary excretion was 47% after iv dosing. The binding of the drug to human serum albumin was 43.5%.

Report #: U84-0546
Study #: Not indicated
Conducting laboratory and location: Department of Biochemistry, Research Division, Boehringer Ingelheim KG
Date of study initiation: Not indicated

GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Rats/Chbb:THOM
N: 5/sex
Age/weight: Not provided
Route: Intravenous and oral (by gavage)
Dosage: 10 mg/kg for iv (2.5 mg/kg for biliary excretion assay) and 35 mg/kg for oral, single dose
Drug: ^{14}C-WAL 801 CL (Batch #: I, 110 µCi/mg, radiochemical purity: —)
Methods: —

The purpose of this study was to determine PK profiles of epinastine in rats following a single oral or iv dose of ^{14}C-epinastine hydrochloride. Blood samples were collected prior to dosing, and at 2, 5, 10, 15, and 30 min and 1, 1.5 2, 3, 4, 6, 8, 10, 12 and 24 hr (iv) or 15, 30, and 45 min and 1, 1.5 2, 3, 4, 6, 8, 10, 12 and 24 hr (po) after drug administration. Urine and feces samples were collected in 24-hr fractions for 96 hr. For biliary excretion assay in 5 rats, the bile duct was cannulated and bile excreted after iv administration was collected for 24 hr. The binding of the drug to plasma protein was determined by equilibrium dialysis. The metabolite pattern of the drug in urine, feces and serum was determined using — methods.

Results:

Excretion: After a single iv or oral dose of ^{14}C-epinastine hydrochloride in rats, the major portion of the radioactivity was excreted within 24 hr. About 60% of radioactivity was eliminated through feces (see table below).

Cumulative renal and fecal excretion of ^{14}C-WAL 801 CL in rats (median number of % dose)

	iv		po	
Time (hr)	Urine	Feces	Urine	Feces
0-24	25.5	51.8	36.3	47.3
0-48	36.8	59.9	37.6	56.1
0-72	37.3	60.2	37.9	57.1
0-96	37.5	60.4	38.0	57.4

Biliary excretion: The direct measurement of biliary excretion showed that within 10 hr after iv dosing (2.5 mg/kg), 40.9-52.5% (median: 44.9%) radioactivity dose was excreted with bile. At 24 hr, the cumulative values were 43.8-52.8% (median: 47%).

Blood PK parameters: Results are summarized in the table below. WAL 801 CL was still measurable 24 hr after the iv (0.03 µg-eq/ml) or po (0.1 µg-eq/ml) administration.

Blood PK parameters in rats treated with ^{14}C-WAL 801

	Cmax (µg-eq/ml)	Tmax (hr)	AUC (µg-eq hr/ml)	T1/2 (hr
IV (10 mg/kg)	13.78		8.91	3.2
PO (35 mg/kg)	2.49	6	29.45	

Protein binding: The binding of WAL 801 to rat serum and human serum albumin (HAS) is summarized in the table below.

Binding of WAL 801 to rat serum and HAS (%)

	Rat serum		Human serum albumin		Control
WAL 801 concentration (µg/ml)	0.25	0.5	0.25	0.5	0.25
Binding	60.17	56.2	43.5	41.1	3.6

Metabolism: The parent compound was the main component in rat serum, urine and bile. There were 3 to 4 small metabolite fractions that were ≤ 20% in urine and bile, and 30% in serum.

In summary, WAL 801 CL was absorbed after oral administration. The metabolism was very low. The drug was eliminated from the rat mainly through feces (60%). Biliary excretion was 47% after iv dosing. The binding of the drug to human serum albumin was 43.5%.

U86-1014: Total excretion and metabolic pattern in rats, mice, dogs, monkeys and pigs after peroral administration of WAL 801 CL-^{14}C. Vol. 16, Page 234

Key study findings: In all species, the total recovery rates were high (81-93%). In dogs, monkeys and pigs, about 50% of the radioactivity administered was excreted via urine, while 33-34% was recovered in feces. In rats and mice, 80-90% of the radioactivity administered was recovered in feces of these species. In all species, WAL 801 CL was the main component in the 0-24 hr urine. The main metabolite in urine was N-glucuronide of WAL 1097.

Report #: U86-1014
Study #: Not indicated
Conducting laboratory and location: Not indicated
Date of study initiation: Not indicated
GLP compliance: Not indicated
QA report: Yes ( ) No (X)
Species/strain: Mice/Chbi:NMRI, 10/sex, 16-20 g
Rats/Chbb:THOM, 3/sex, 197-218 g
Dogs/Chbi:beagle, 4 females, 12.2-13.5 kg
Monkeys/Rhesus, 2/sex, 16.6-19.3 kg
Pigs/Munich miniature, 2/sex, 4.9-13.2 kg
Age/weight: Not indicated
Route: Oral (by gavage)
Dosage: 8 mg/kg, single dose
Drug: ^{14}C-WAL 801 CL (Batch 3, radiochemical purity: [redacted]
Methods: [redacted]

The purpose of this study was to determine the total excretion of ^{14}C-radioactivity in urine and feces of mice, rats, dogs, monkeys and pigs after a single oral administration. Urine and feces samples were collected in 24-hr fractions for 96 hr or 144 hr (pigs and dogs). The metabolite pattern of the drug in urine (0-24 hr) was also determined.

Results:

Excretion: Results are summarized in the table below. In all species, the total recovery rates were high (81-93%). In dogs, monkeys and pigs, ^{14}C-radioactivity excreted in urine and feces was similar. About 50% of the radioactivity administered was excreted via urine, while 33-34%

was recovered in feces. In rats and mice, 80 - 90% of the radioactivity administered was recovered in feces of these species.

Mean cumulative renal and fecal excretion of ^{14}C-WAL 801 CL in five species (% of dose)

Species		0-24 hr	0-48 hr	0-72 hr	0-96 hr	Total
Rat	Urine	3.32	3.52	3.56	3.62	**92.25**
	Feces	85.93	88.37	88.53	88.63	
Mouse	Urine	7.98	9.74	10.42	11.94	**93.35**
	Feces	72.48	78.34	79.96	81.41	
Dog	Urine	39.18	50.64	52.36	55.00 (0-144 hr)	**89.21**
	Feces	7.14	27.37	32.03	34.14(0-144 hr)	
Monkey	Urine	45.95	49.95	51.25	51.98	**85.86**
	Feces	1.78	24.09	33.10	33.88	
Pig	Urine	38.71	44.79	46.58	47.95(0-144 hr)	**81.24**
	Feces	1.84	14.17	28.96	33.29(0-144 hr)	

Metabolism: Metabolic patterns in the 0-24 hr urine in all of the five species were similar. The [redacted] chromatograms all showed two distinct peaks and a broad zone comprising 3-5 smaller, polar metabolites. WAL 801 CL represented the largest peak. The main component of the second largest peak was N-glucuronide of WAL 1097.

PK/TK summary and conclusions:

Epinastine was rapidly absorbed following ocular administration to rabbits and monkeys with very low systemic exposure. High concentrations of radioactivity were measured in the surface tissues (conjunctiva, cornea and sclera) and in pigmented tissues (iris and ciliary body). In *in vitro* studies, epinastine reversibly bound to bovine ocular melanin. In studies conducted in rats and monkeys, the bioavailability of the drug after oral administration was low. Epinastine was distributed throughout peripheral body tissues, but did not cross the blood brain barrier. WAL 801 CL and its metabolites passed the placental barrier in rat studies. The radioactivity was also observed in the milk of lactational rats. The *in vitro* serum protein binding rate was about 60% in rats and 40% in humans. Conversion of epinastine to metabolites was similar across animal species tested. Epinastine and metabolites were excreted in urine and feces across animal species. Biliary excretion and entero-hepatic circulation were observed.

IV. GENERAL TOXICOLOGY:

Studies reviewed:

U82-0055: Acute oral toxicity of WAL 801 CL in beagles. Vol. 20, Page 131
U82-0056: Acute intravenous toxicity of WAL 801 CL in beagles. Vol. 20, Page 141
U82-0060: Acute intravenous toxicity of WAL 801 CL in Chbb:THOM (SPF) rats. Vol. 20, Page 153
U82-0061: Acute oral toxicity of WAL 801 CL in Chbb:THOM (SPF) rats. Vol. 20, Page 170
U91-0402: Epinastine (WAL 801 CL): Oral and intravenous single dose toxicity studies in rats. Vol. 28, Page 179
U84-0045: Acute intravenous toxicity of WAL 801 CL in Chbi:NMRI (SPF) mice. Vol. 20, Page 279
U84-0046: Acute oral toxicity of WAL 801 CL in Chbi:NMRI (SPF) mice. Vol. 20, Page 295
U84-0670: [redacted] Test for acute toxicity after intravenous administration in Chbi:NMRI (SPF) mice. Vol. 22, Page 293
U91-0550: [redacted]. Oral single dose toxicity study with [redacted] in rats. Vol. 28, Page 206

U85-0564: [redacted]. Acute toxicity trial after intravenous administration to Chbi:NMRI (SPF) mice. Vol. 22, Page 343
U90-0442: [redacted]degradation product of WAL 801 CL): Acute intravenous toxicity study in mice. Vol. 28, Page 001
U90-0443: [redacted]degradation product of WAL 801 CL): Acute intravenous toxicity study in mice. Vol. 28, Page 026
U87-0482: [redacted]st for acute toxicity after intravenous administration in Chbi:NMRI (SPF) mice. Vol. 24, Page 182
U85-0788: WAL 801 CL: Four-week subacute intravenous toxicity in rhesus monkeys. Vol. 23, Page 001
U84-0355: Subchronic (13-week) oral toxicity of WAL 801 CL in Rhesus monkeys. Vol. 21, Page 001
U84-0412: Three-month oral toxicity of WAL 801 CL in rats. Vol. 22, Page 001
U88-0232: WAL 801 CL: 52-week intragastric toxicity in rhesus monkeys. Vol. 25, Page 001
U88-0826: WAL 801 CL: Chronic toxicity study in the rat (administration with feed). Vol. 26, Page 001

U82-0055: Acute oral toxicity of WAL 801 CL in beagles. Vol. 20, Page 131

Key study findings: Drug-related effects including salivation and emesis at ≥ 25 mg/kg in all animals and in the male at 12.5 mg/kg. Blood was found in emesis and feces in both male and female dogs at 200 mg/kg. No NOAEL was determined in males. The dose of 12.5 mg/kg was considered as an NOAEL for females.

Document No: U82-0055
Research Subject No: 04 317
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: June 1, 1982
GLP compliance: Yes
QA report: Yes
Animal: Beagle dogs, 19-37 months old for male, 11.1-15.3 kg; 23-61 months old for females, 9.5-14.9 kg, 1/sex/group
Route: Oral (gavage)
Dosage: 12.5, 25, 50, 100, and 200 mg/kg
Drug: WAL 801 CL, Batch #: II in 0.5% Tylose solution
Dosing regimen: Single dose

The purpose of this study was to determine "the pattern of symptoms in the toxic dose range and the lethal dose". Dogs were observed for 2 weeks following a single oral dose.

Results:

No mortality occurred. Salivation was observed in males at all doses and in females at 25 mg/kg and higher from 1 min to 7.5 hr after dosing. Emesis was noted in all animals at doses of 25 mg/kg and higher from 18 min to 3.25 hr after dosing. At 200 mg/kg, blood was found in threw-ups (18 min to 1.5 hr after dosing) and feces (within 48 hr) in both male and female dogs.

In summary, beagle dogs were treated with a single oral dose of WAL 801 CL at 12.5, 25, 50, 100, and 200 mg/kg. Salivation was noted in all dose groups except the female dog at

12.5 mg/kg. Emesis was observed at ≥ 25 mg/kg in all animals. Blood was found in threw-ups and feces (within 48 hr) in both male and female dogs at 200 mg/kg.

U82-0056: Acute intravenous toxicity of WAL 801 CL in beagles. Vol. 20, Page 141

Key study findings: Mortality was observed at 50 mg/kg. Salivation and redness of skin and mucous membrane were noted in almost all animals. At higher doses (≥ 30 mg/kg), lateral position, ataxia, trembling, dyspnea, retching and cyanosis were noted.

Document No: U82-0056
Research Subject No: 04 317
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: July 8, 1982
GLP compliance: Yes
QA report: Yes
Animal: Beagle dogs, 16-25 months old for male, 11.5-14.2 kg; 17-30 months old for females, 9.9-13.3 kg, 1/sex/group
Route: Intravenous injection
Dosage: 10, 20, 30, 40, and 50 mg/kg
Drug: WAL 801 CL, Batch #: II dissolved at 3% in distilled water
Dosing regimen: Single dose

The purpose of this study was to determine "the pattern of symptoms in the toxic dose range, and the lethal dose". Dogs were observed for 2 weeks. Animals that died during the study were dissected immediately.

Results:

At 50 mg/kg, deaths occurred in the male dog (20 min after dosing) and female dog (3 min after dosing). Cyanosis, increased redness of the skin and mucous membrane, salivation, lateral position and dyspnea were observed in these two animals. Post-mortem examination in both dogs showed a thymus parenchymatous hemorrhage and hyperemia in the large parenchyma of the abdomen.

Clinical observations in other animals are summarized in the table below.

Clinical observations in dogs treated with WAL 801 CL

Dose (mg/kg)	10		20		30		40	
Sex	M	F	M	F	M	F	M	F
Salivation (duration)	90 min		55 min	1.5 hr	2.5 hr	3.5 hr	4.25 hr	6 hr
Redness of skin/mucous membrane (duration)	2.5 hr	2.5 hr	45 min	>12 hr	1 hr	5 hr	1.3 hr	5 hr
Emesis (start after dosing)			5 min	2-10 min	18 min	6-12 min	20-40 min	1.5 hr
Increased locomotion (duration)			1.5 hr					
Lateral position (duration)					1-3 min	1-5 min	7 min	20 min
Ataxia (duration)					3-23 min		3-15 min	20-70 min
Trembling (duration)					5-7 min			70 min
Dyspnea (duration)					4-7 min			1-18 min
Retching (duration)					5-6 min		6-7 min	
Cyanosis (duration)								2-4 min

In summary, beagle dogs were treated with a single iv dose of WAL 801 CL at 10, 20, 30, 40, and 50 mg/kg. Mortality was observed at 50 mg/kg in the male dog and female dog 20 and 3 min after dosing, respectively. Salivation and redness of skin and mucous membrane were noted in almost all animals. At the doses of 30 mg/kg and higher, emesis, lateral position, ataxia, trembling, dyspnea, retching and cyanosis were noted. No NOAEL was determined.

U82-0060: Acute intravenous toxicity of WAL 801 CL in Chbb:THOM (SPF) rats. Vol. 20, Page 153

Key study findings: The LD_{50} was 19.5 mg/kg for both males and females. Clinical signs including reduced motility, accelerated respiration, and prone or lateral position were observed in all surviving animal groups. No NOAEL was determined.

Document No: U82-0060
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: July 20, 1982
GLP compliance: Yes
QA report: Yes
Animal: Rats/Chbb:THOM (SPF), 71-75 days old, 296.9 g for males and 204.4 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 14, 18, 22, and 28 mg/kg (dosing volume: 10 ml/kg)
Drug: WAL 801 CL, Batch #: II dissolved in physiological saline solution
Dosing regimen: Single dose

The purpose of this study was to determine "the changes of behavior, toxicity symptoms, and possibly the target organs after single intravenous administration of toxic doses."

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: At the end of the study

Results:

Mortality: Twenty-one animals died at doses of 18 mg/kg and higher. All deaths occurred within 1 min after the dosing. The mortality rates between males and females were similar. The LD_{50} was 19.1 mg/kg for males, 19.9 mg/kg for females, and 19.5 mg/kg for both males and females.

Mortality data in rats treated intravenously with WAL 801 CL

Dose	14 mg/kg		18 mg/kg		22 mg/kg		28 mg/kg	
	♂	♀	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5	5	5
Death	0	0	3	2	3	3	5	5

Clinical observations: Clinical signs observed in the surviving animals included reduced motility, accelerated respiration, and prone or lateral position (see table below).

Clinical signs in rats treated intravenously with WAL 801 CL (number of animals)

Dose	14 mg/kg		18 mg/kg		22 mg/kg	
	♂	♀	♂	♀	♂	♀
N	5	5	2	3	2	2
Reduced motility	1	1	2	0	2	1
Prone position, lateral position	1	4	2	2	2	1
Accelerated respiration	5	5	2	3	2	2
Gasping breathing		1				
Coma			1			

Body weights: No treatment-related body weight changes were noted.

Necropsy: No abnormal findings were noted.

In summary, rats were treated with a single iv dose of WAL 801 CL at 14, 18, 22, and 28 mg/kg. Mortality was observed in both males and females within 1 min after the injection at 18 mg/kg and higher. Clinical signs including reduced motility, accelerated respiration, and prone or lateral position were observed in all surviving animal groups. No abnormal findings were noted in necropsy examination performed on surviving animals. No NOAEL was determined.

U82-0061: Acute oral toxicity of WAL 801 CL in Chbb:THOM (SPF) rats. Vol. 20, Page 170

Key study findings: The LD_{50} was 862.5 mg/kg for males and 593.8 mg/kg for females. Clinical signs including sedation and gasping breathing were observed in all surviving animal groups. No NOAEL was determined.

Document No: U82-0061
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: June 15, 1982
GLP compliance: Yes
QA report: Yes
Animal: Rats/Chbb:THOM (SPF), 57-63 days old, 232.8 g for males and 207.6 g for females, 5/sex/group

Route: Oral by gavage
Dosage: 400, 500, 630, 800, 1000, and 1260 mg/kg (dosing volume: 10 ml/kg). The highest dose (1260 mg/kg) was only used in males.
Drug: WAL 801 CL, Batch #: II dissolved in physiological saline solution (PSS) with 0.1% Tween 80 additive
Dosing regimen: Single dose

The purpose of this study was to determine "the changes of behavior, toxicity symptoms, and possibly the target organs after single intravenous administration of toxic doses."

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: At the end of the study, all surviving animals

Results:

Mortality: Mortality occurred at doses of 500 mg/kg and higher. All deaths occurred within 24 hr after dosing. The LD_{50} was 862.5 mg/kg for males and 593.8 mg/kg for females.

Mortality data in rats treated orally with WAL 801 CL

Dose (mg/kg)	400		500		630		800		1000		1260	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5	5	5	5	5	5	0
Death	0	0	1	1	1	4	2	4	2	5	5	

Clinical observations: Clinical signs are summarized in the table below. Sedation and gasping breathing were the main symptoms.

Clinical signs in rats treated orally with WAL 801 CL (number of animals)

Dose (mg/kg)	400		500		630		800		1000		1260
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
N	5	5	5	5	5	5	5	5	5	5	5
Sedation	5	5	5	5	4	5	5	5	3	3	2
Retching				2		1	1		1		
Gasping breathing	1		1	2	3	4	3	2	2	5	4
Clonic spasms					1	2		4	2	3	4

Body weights: No treatment-related effects on body weight changes were noted.

Necropsy: No abnormal findings were noted.

In summary, rats were treated with a single oral dose of WAL 801 CL at 400, 500, 630, 800, 1000, and 1260 mg/kg. Mortality was observed in both males and females within 24 hr after dosing at 500 mg/kg and higher. Clinical signs including sedation and gasping breathing were observed in all animal groups. No abnormal findings were noted in necropsy examination performed on surviving animals. No NOAEL was determined.

U91-0402: Epinastine (WAL-801 CL): Oral and intravenous single dose toxicity studies in rats. Vol. 28, Page 179

Key study findings: Mortality was observed in all dose groups. The LD_{50} was 314 mg/kg for males and 192 mg/kg for females in oral route, and 17 mg/kg for males and 22 mg/kg for females in iv route. Clinical signs included sedation, decreased activity, abdominal position, convulsion, and dyspnea. In the animals that died after dosing, necropsy and histopathologic examinations showed GI toxicity. No NOAEL was determined.

Document No: U91-0402
Study #: E9006, A0000914
Conducting laboratory and location: Nippon Boehringer Ingelheim Co., Ltd., Kawanishi Pharma Research Institute, 103 Takada, Yato, Kawanishi, Hyogo, 666-01 Japan
Date of study initiation: Not indicated.
GLP compliance: Yes
QA report: Yes
Purpose: To determine the acute toxicity of WAL 801 CL after a single oral or intravenous administration to rats.
Animal: Rats/Sprague-Dawley, 7-8 weeks old, 286-342 g for males and 180-234 g for females in po groups; 310-382 g for males and 204-262 g for females in iv groups, 5/sex/group
Route: Oral (by gavage) and intravenous injection
Dosage: 132, 278, 578, and 833 mg/kg for oral route (dosing volume: 10 ml/kg); 15, 19, 24, and 30 mg/kg for iv route (dosing volume: 1 ml/kg)
[**Reviewer's comments**: There was no vehicle control group.]
Drug: WAL 801 CL (Batch #: XII, purity: ———) dissolved in distilled water for po dosing and in physiological saline solution (PSS) for iv dosing. "The stability was confirmed in the dark at room temperature for 4 hr."
Dosing regimen: Single dose
Study design

Route	Dosage (mg/kg)	N/sex/dose	Dosing volume (ml/kg)
Oral (by gavage)	132, 278, 578, or 833	5	10
Intravenous injection	15, 19, 24, or 30 (females only)	5	1

Observations and times:

Clinical signs: Five, 15, and 30 min, and 1, 3, and 6 hr after dosing, and once daily thereafter for 14 days
Body weights: Daily
Necropsy: All animals
Histopathology: Organs with pathologic findings were examined histopathologically.

Results:

Mortality:

Oral groups: Mortality data are summarized in the table below. Most deaths occurred within 6 hr after dosing. The LD_{50} was 314 mg/kg for males and 192 mg/kg for females.

Mortality data in rats treated orally with WAL 801 CL

Dose (mg/kg)	132		278		578		833	
	♂	♀	♂	♀	♂	♀	♂	♀

N	5	5	5	5	5	5	5	5
Death	0	1	2	4	5	5	5	5

Intravenous injection groups: Mortality data are summarized in the table below. All of the deaths occurred during or immediately after dosing. The LD_{50} was 17 mg/kg for males and 22 mg/kg for females.

Mortality data in rats treated intravenously with WAL 801 CL

Dose (mg/kg)	15		19		24		30	
	♂	♀	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5	0	5
Death	1	0	4	2	5	3		5

Clinical observations:

Oral route: All rats showed sedation and abdominal position 5-15 min after dosing for more than 6 hr. At 278 mg/kg, decreased activity, respiration sounds, convulsion and abnormal respiration were noted. At 833 mg/kg, lacrimation or dyspnea was noted in all rats.

Intravenous injection route: General clinical signs included irregular abdominal breathing, bradypnea, convulsions followed by tachypnea and thereafter, abdominal position. Sedation and decreased activity were also noted.

Body weights: Since there was no vehicle control group, a clear conclusion cannot be made regarding the drug's effects on body weights. The data from different treatment groups were compared and showed that in oral route, body weight gain was affected at 208 mg/kg in the first few days after dosing. Body weight gain was not affected by the drug given by intravenous route.

Body weight gain of rats treated with WAL 801 CL (g, mean ± SD)

(mg/kg)	n	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14
Oral, males									
132	5	20±8	24±7	31±8	40±5	44±4	53±5	59±6	101±15
278	3	-3±16	5±19	19±13	24±12	32±9	41±11	47±6	100±11
Oral females									
132	4	9±3	12±3	20±3	27±5	29±3	30±6	37±9	61±11
278	1	-2	6	16	30	30	34	40	70
Intravenous route, males									
15	4	4±6	6±5	9±5	16±4	26±8	26±7	28±8	63±10
19	1	10	14	18	24	28	36	40	82
Intravenous route, females									
15	5	2±4	4±9	6±7	10±8	15±6	18±8	19±5	39±6
19	3	3±2	7±9	11±3	13±8	19±9	23±6	22±7	47±12
24	2	7±4	12±6	6±3	12±3	17±4	19±7	19±13	38±11
30	1	10	16	16	18	22	28	34	46

Necropsy:

Oral route: Retention of liquid in the gastro-intestinal tract was seen in all dead rats. Yellow-white changes and focal hyperemia were noted in gastric and duodenal mucosa. Congestion of lungs and liver, and pulmonary edema were also noted in some rats. In the surviving rats, a part of pyloric region was pale in one female at 278 mg/kg.

Intravenous route: Dilatation of GI tract and congestion of the liver were observed in all dead rats. No abnormal findings were seen in the surviving animals.

Histopathology: The sponsor provided histopathological examination data on 3 animals one each in 132, 278 and 578 mg/kg groups, respectively. Data are summarized in the table below.

Histopathologic findings in dead rats

	Female at 132 mg/kg	Female at 278 mg/kg	Male at 578 mg/kg
Stomach			
Mucosa:			
Degeneration, necrosis of epithelial cells	Slight	Marked	
Hemorrhage		Marked	
Adhesion		Slight	
Focal aggregation of mucus, and focal cystoid desquamation			Yes
Lamina propria			
Edema and hemorrhage		Slight	Yes, and necrosis
Submucosal layer			
Edema		Slight	marked
Muscular layer			
Atrophy of nucleus and vacuoles in muscular cells			Yes
Duodenum:			
Degeneration and necrosis of villi, slight hemorrhage	Yes		
Ileum			
Degeneration and necrosis of villi, slight hemorrhage	Yes		Yes
Jejunum:			
Degeneration and necrosis of villi, hemorrhage in lamina propria	Yes		Yes
Slight swelling of villi, edema of lamina propria		Yes	
Lungs			
Congestion, edema	Slight	Slight	Slight
Liver		Thrombus	Congestion
Spleen			Atrophy of red pulp

In summary, rats were treated orally (by gavage) or intravenously with a single dose of WAL 801 CL. Mortality was observed in all dose groups. The LD_{50} was 314 mg/kg for males and 192 mg/kg for females in oral route, and 17 mg/kg for males and 22 mg/kg for females in iv route. Clinical signs, similar in both routes, included sedation, decreased activity, abdominal position, convulsion, and dyspnea. In surviving animals, rats treated at 278 mg/kg group showed less body weight gain during the first few days after oral dosing compared to the LD animals. In the animals that died after dosing, necropsy and histopathologic examinations showed retention of liquid in the GI tract, yellow-white changes and focal hyperemia in gastric and duodenal mucosa, degeneration, necrosis, cystoid desquamation of the superficial mucosa, and perifocal edema. The sponsor indicated that these changes were considered as the direct action of epinastine. No NOAEL was determined.

U84-0045: Acute intravenous toxicity of WAL 801 CL in Chbi:NMRI (SPF) mice. Vol. 20, Page 279

Key study findings: The LD_{50} was 16.3 mg/kg for both males and females. Clinical signs including reduced motility, accelerated respiration, gasping breathing, clonic spasms, and prone position were observed in all surviving animal groups. No NOAEL was determined.

Document No: U84-0045

Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: June 28, 1983
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI (SPF), 43 days old, 24.9 g for males and 22.6 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 10, 14, 17, and 20 mg/kg (dosing volume: 10 ml/kg). Only females were dosed at 17 mg/kg.
Drug: WAL 801 CL, Batch #: II dissolved in distilled water
Dosing regimen: Single dose

The purpose of this study was to determine "the changes of behavior, toxicity symptoms, and possibly the target organs after single intravenous administration of toxic doses."

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: At the end of the study, all surviving animals

Results:

Mortality: Fourteen animals died at doses of 14 mg/kg and higher. All deaths occurred within 1 min after dosing. The LD_{50} was 16.8 mg/kg for males, 16.0 mg/kg for females, and 16.3 mg/kg for both males and females.

Mortality data in mice treated intravenously with WAL 801 CL

Dose (mg/kg)	10		14		17		20	
	♂	♀	♂	♀	♂	♀	♂	♀
N	5	5	5	5	0	5	5	5
Death	0	0	1	0		4	4	5

Clinical observations: Clinical signs are summarized in the table below. The most frequent symptoms were prone position, accelerated respiration, gasping breathing, and clonic spasms.

Clinical signs in mice treated intravenously with WAL 801 CL (number of animals)

Dose (mg/kg)	10		14		17		20	
	♂	♀	♂	♀	♂	♀	♂	♀
Reduced motility	0	2	0	1		1	1	0
Prone position	4	3	5	5		1	3	3
Accelerated respiration	5	5	3	5		1	1	0
Gasping breathing	3	1	3	2		3	2	1
Clonic spasms	1	0	1	2		3	1	3

Body weights: No treatment-related effects on body weight changes were noted.

Necropsy: No abnormal findings were noted.

In summary, mice were treated with a single iv dose of WAL 801 CL at 10, 14, 17 and 20 mg/kg. Mortality was observed within 1 min after the injection at 14 mg/kg and higher in males and at 17 mg/kg or higher in females. Clinical signs including reduced motility, accelerated respiration, gasping breathing, clonic spasms, and prone position were observed in all animal groups. No abnormal findings were noted in necropsy examination performed on the surviving animals. No NOAEL was determined.

U84-0046: Acute oral toxicity of WAL 801 CL in Chbi:NMRI (SPF) mice. Vol. 20, Page 295

Key study findings: Mortality occurred at doses of 250 mg/kg and higher for females and 400 mg/kg and higher for males. Necropsy examination in the LD female that died on day 7 showed pulmonary edema, hemorrhage in the lung parenchyma, and blood mucoenteritis of the small intestine. The most frequently observed clinical symptoms were reduced motility and accelerated respiration. No NOAEL was determined.

Document No: U84-0046
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: December 15, 1982
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI (SPF), 41-42 days old, 24.0 g for males and 19.8 g for females, 5/sex/group
Route: Oral by gavage
Dosage: 250, 400, 630, and 1000 mg/kg (dosing volume: 10 ml/kg)
Drug: WAL 801 CL, Batch #: II dissolved in PSS with 0.1% Tween 80 additive
Dosing regimen: Single dose

The purpose of this mouse study was to determine "the changes of behavior, toxicity symptoms, and possibly the target organs after single intravenous administration of toxic doses."

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: At the end of the study, all surviving animals and the animal died in day 7 of the study

Results:

Mortality: Nineteen animals died at doses of 250 mg/kg and higher for females and 400 mg/kg and higher for males (see table below). All deaths occurred within 24 hr after the dosing with the exception of one female that died on day 7 of the study. The LD_{50} was 546.8 mg/kg for males, 494.2 mg/kg for females, and 518.6 mg/kg for both males and females.

Mortality data in mice treated orally with WAL 801 CL

Dose (mg/kg)	250		400		630		1000	
	♂	♀	♂	♀	♂	♀	♂	♀

N	5	5	5	5	5	5	5	5
0-30 min			1	1	2	1	4	3
30-60 min					1	1		
2-4 hr								1
4-6 hr							1	1
6-24 hr				1				
7 days		1						
Total	0	1	1	2	3	2	5	5

Clinical observations: Clinical signs are summarized in the table below. The most frequently observed clinical symptoms were reduced motility and accelerated respiration.

Clinical signs in mice treated orally with WAL 801 CL (number of animals)

Dose (mg/kg)	250		400		630		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Reduced motility	1	0	2	1	2	2	3	1
Accelerated respiration	2	3	3	1	4	2	2	1
Gasping breathing						1		1
Clonic spasms	0	0	1	0	1	0	2	0
Erratic spasms	0	1	1	2	3	2	5	5

Body weights: No treatment-related effects on body weight changes were noted.

Autopsy: No abnormal findings were noted in the surviving animals. In the LD female that died on day 7, pulmonary edema, hemorrhage in the lung parenchyma, and blood mucoenteritis of the small intestine were observed.

In summary, mice were treated with a single oral dose of WAL 801 CL at 250, 400, 630, and 1000 mg/kg. Mortality was observed within 24 hr after dosing at 400 mg/kg and higher in males and females. One LD female died on day 7 of the study. Necropsy examination on this animal showed pulmonary edema, hemorrhage in the lung parenchyma, and blood mucoenteritis of the small intestine. The most frequent symptoms were reduced motility and accelerated respiration. No abnormal findings were noted in necropsy examination performed on the surviving animals. No NOAEL was determined.

U87-0482: [redacted] Test for acute toxicity after intravenous administration in Chbi:NMRI mice. Vol. 24, Page 182

Key study findings: Mortality was observed in two animals within 4 min after the injection at 100 mg/kg. The LD_{50} was determined as 140 mg/kg in both male and female animals. Clinical signs seen in HD animals included gasping breathing. The sponsor indicated that body weight gain was decreased in treated groups. The NOAEL was determined as 80 mg/kg in this study.

Document No: U87-0482
Study #: 37/87, A0000915
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: May 27, 1987
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI (SPF), 41 days old, 25.0 g for males and 20.9 g for females, 5/sex/group

Route: Intravenous injection
Dosage: 0, 80, and 100 mg/kg (dosing volume: 2 ml/kg)
Drug: [redacted] Batch #: A, dissolved in DMSO
Dosing regimen: Single dose, 0.1 ml/sec

[redacted] could occur as a contaminant in preparations of the active substance WAL 801 CL. The purpose of this study was to determine the potential toxicity of [redacted] after a single intravenous administration to mice. Toxicity was assessed as shown below.

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: All animals

Results:

Mortality: During the first 4 min after dosing, one male animal and one female animal at 100 mg/kg were dead. The LD_{50} was calculated as 140 mg/kg for both males and females.

Clinical observations: Clinical signs are summarized in the table below. Some clinical signs (e.g., hematuria) were observed in both control and treated animals and could be attributed to the solvent used.

Clinical signs in mice treated intravenously with [redacted] (number of animals)

Dose (mg/kg)	Control		80		100	
	♂	♀	♂	♀	♂	♀
Reduced motility	1	0	0	0	0	1
Lying on stomach or side	1	0	0	0	1	1
Gasping breathing	0	0	0	0	1	1
Hematuria	3	5	0	0	1	2

Body weights: The sponsor indicated that reduced body weight gain was seen at 80 and 100 mg/kg. No data were provided.

Necropsy: No abnormal findings were noted in any animals.

In summary, mice were treated with a single iv dose of [redacted] at 0, 80 or 100 mg/kg. Mortality was observed within 4 min after the injection at 100 mg/kg. The LD_{50} was determined as 140 mg/kg in both male and female animals. Clinical signs seen in HD animals included gasping breathing. Hematuria, reduced motility, and lying on stomach or side were noted in all groups and might be attributed to the solvent (dimethylsulfoxide) used in the study. The sponsor indicated that body weight gain was decreased in treated groups. No abnormal findings were noted in necropsy examination. The NOAEL was determined as 80 mg/kg.

U84-0670: [redacted]. Test for acute toxicity after intravenous administration in Chbi:NMRI (SPF) mice. Vol. 22, Page 293

Key study findings: The LD_{50}, 14.8 mg/kg for both males and females, was similar to that of WAL 801 CL (16.8 mg/kg). Clinical signs including reduced motility, sedation, gasping breathing, extension and twitch spasms, and salivation were observed in all animal groups. No NOAEL was determined.

Document No: U84-0670
Study #: 46/84, A0000917
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: April 18, 1984
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI (SPF), 44 days old, 25.8 g for males and 22.6 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 12.5, 16 and 20 mg/kg (dosing volume: 10 ml/kg). There was no vehicle control group.
Drug: [redacted] Batch #: D, dissolved in 0.9% NaCl solution
Dosing regimen: Single dose, 0.1 ml/sec

The purpose of this study was to determine the potential toxicity of [redacted], an impurity and oxidation product of WAL 801 CL, after a single intravenous administration to mice. Toxicity was assessed as shown below.

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: All animals

Results:

Mortality: During the first 30 min after dosing, 18 of 30 animals were dead. No subsequent mortality was recorded. The LD_{50} was 14.8 mg/kg for both males and females.

Mortality data in mice treated intravenously with WAL 1097 CL

Dose (mg/kg)	12.5		16		20	
	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5
Death	0	0	4	4	5	5

Clinical observations: Clinical signs are summarized in the table below. The most frequent symptoms were reduced motility and sedation that were mainly seen in LD animals. Salivation, extension and twitch spasms were mainly seen in MD and HD animals.

Clinical signs in mice treated intravenously with WAL 1097 CL (number of animals)

Dose (mg/kg)	12.5		16		20	
	♂	♀	♂	♀	♂	♀
Reduced motility	5	5	1	3	1	0
Sedation	5	5	1	1	0	0
Tremor	3	1	0	1	0	0
Rapid breathing	2	1	1	3	0	0
Gasping breathing	0	0	1	0	1	0
Stiffened tail0	1	1	2	1	0	
Vocalization	0	0	0	0	3	0
Salivation	2	1	1	4	5	2
Extension spasms	1	0	3	3	5	5
Twitch spasms	2	0	3	3	3	4
Death	0	0	4	4	5	5

Body weights: No treatment-related effects on body weight changes were noted in LD animals.

Necropsy: In the animals that died after dosing, only vascular congestion in the liver was noted. No abnormal findings were noted in the animals observed for 14 days.

In summary, mice were treated with a single iv dose of [redacted] at 12.5, 16, and 20 mg/kg. Mortality was observed within 30 min after the injection at 16 mg/kg (8 of 10) and at 20 mg/kg (10 of 10). The LD_{50} was determined as 14.8 mg/kg in both male and female animals. Clinical signs including reduced motility, sedation, salivation, extension and twitch spasms were noted in all groups. Vascular congestion in the liver was noted in the animals that died after dosing. No abnormal findings were noted in necropsy examination performed on the surviving animals. No NOAEL was determined.

U91-0550: [redacted] Oral single dose toxicity study with [redacted] CL in rats. Vol. 28, Page 206

Key study findings: Mortality was observed within 20 min after dosing at ≥ 30 mg/kg. The LD50 value of [redacted] was 42 mg/kg for both sexes. Clinical signs including sedation, abdominal position, and decreased activity were observed in animas at 12 and 30 mg/kg. Necropsy and histopathologic examinations on dead rats showed GI toxicity evidenced by mild white changes in gastric mucosa, superficial degeneration and desquamation of gastric mucosa and duodenal villi. No NOAEL was determined.

Document No: U91-0550
Study #: E9010
Conducting laboratory and location: Nippon Boehringer Ingelheim Co., Ltd., Kawanishi Pharma Research Institute, 103 Takada, Yato, Kawanishi, Hyogo, 666-01 Japan
Date of study initiation: Not indicated.
GLP compliance: Yes
QA report: Yes
Animal: Rats/Sprague-Dawley, 7-8 weeks old, 274-332 g for males and 182-228 g for females, 5/sex/group
Route: Oral by gavage
Dosage: 12, 30, 75, and 300 mg/kg (dosing volume: 10 ml/kg). There was no vehicle control group.

Drug: [redacted] (Batch #: E) dissolved in distilled water
Dosing regimen: Single dose

The purpose of this study was to determine the potential toxicity of [redacted] an impurity and oxidation product of WAL 801 CL, after a single oral administration to rats. The day of dosing was designated as day 1. Toxicity was assessed as shown below.

Observations and times:

Clinical signs:	Five, 15, and 30 min, 1, 3, and 6 hr after dosing, and once daily thereafter for 14 days
Body weights:	Daily
Necropsy:	All animals
Histopathology:	Organs with pathologic findings were examined histopathologically.

Results:

Mortality: Mortality occurred in males and females at doses of ≥ 30 mg/kg. All deaths occurred within 20 min after dosing. The LD_{50} was 42 mg/kg for both males and females.

Mortality data in rats treated orally with WAL 1097 CL

Dose (mg/kg)	12		30		75		300	
	♂	♀	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5	5	5
Death	0	0	1	1	5	5	5	5

Clinical observations: Clinical signs seen at 12 and 30 mg/kg included decreased activity, sedation, and abdominal position. Some animals showed convulsions. The clinical signs were seen from 5-15 min after dosing, and animals recovered after 6-24 hr. All animals at 75 and 300 mg/kg died 20 min after dosing. Clinical signs seen in these animals included decreased activity followed by severe clonic convulsions.

Body weights: For the first two days, the average body weight gain in females at 30 mg/kg was 6 and 14 g/day, which was lower than in animals at 12 mg/kg (17 and 22 g/day). The body weight gain was similar after day 3.

Necropsy: No abnormal findings were noted in the surviving animals. For animals that died after dosing, necropsy examination showed mild white changes in gastric mucosa, pulmonary emphysema, dark red lungs, and foam in trachea.

Histopathology: A superficial degeneration and desquamation of gastric mucosa and duodenal villi were noted in dead rats.

In summary, rats were treated with a single oral dose of [redacted] at 12, 30, 75 and 300 mg/kg. Mortality was observed in both males and females within 20 min after dosing at ≥ 30 mg/kg. The LD_{50} value of [redacted] was 42 mg/kg for both sexes. Clinical signs including sedation, abdominal position, and decreased activity were observed in animas at 12 and 30 mg/kg. Animals at 75 and 300 mg/kg showed decreased activity followed by severe clonic

convulsions. No toxicologically significant findings were noted in body weight examination. Necropsy and histopathologic examinations on dead rats showed GI toxicity evidenced by mild white changes in gastric mucosa, superficial degeneration and desquamation of gastric mucosa and duodenal villi. No NOAEL was determined.

U85-0564: [redacted] Acute toxicity trial after intravenous administration to Chbi:NMRI (SPF) mice. Vol. 22, Page 343

Key study findings: Mortality was noted at 40 mg/kg and higher. The LD_{50} was 44.8 mg/kg for both males and females. Clinical signs including reduced motility, prone/side position (MD and HD only), accelerated breathing, and clonic spasms were noted in all groups. Body weights seemed to be affected by the treatment. No NOAEL was determined.

Document No: U85-0564
Study #: 3/85, A0000916
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: January 15, 1985
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI (SPF), 43 days old, 29.3 g for males and 24.1 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 32, 40, and 50 mg/kg (dosing volume: 10 ml/kg). There was no vehicle control group.
Drug: [redacted] Batch #: A, dissolved in 0.9% NaCl solution
Dosing regimen: Single dose, 0.1 mg/sec

The purpose of this study was to determine the potential toxicity of [redacted], a possible decomposition product of WAL 801 CL, after a single intravenous administration to mice. Toxicity was assessed as shown below.

Observations and times:

Clinical signs: Twice daily (once daily on weekends) for 14 days
Body weights: Weekly
Necropsy: All animals

Results:

Mortality: During the first 7 min after dosing, 10 animals in MD and HD groups were dead (see table below). No subsequent mortality was recorded. The LD_{50} was 42.8 mg/kg for males, 47.4 mg/kg for females, and 44.8 mg/kg for both males and females.

Mortality data in mice treated intravenously with WAL 1725 CL

Dose (mg/kg)	32		40		50	
	♂	♀	♂	♀	♂	♀
N	5	5	5	5	5	5
Death	0	0	1	1	5	3

Clinical observations: Clinical signs are summarized in the table below. In LD animals, the most frequent symptom after dosing was accelerated breathing that lasted for 2.5 to 3 hr. Prone position/side position and clonic spasms were mainly seen in MD and HD animals.

Clinical signs in mice treated intravenously with WAL 1725 CL (number of animals)

Dose (mg/kg)	32		40		50	
	♂	♀	♂	♀	♂	♀
Reduced motility	2	1	3	3	0	2
Prone position/side position	0	0	5	4	4	5
Accelerated breathing	5	5	4	4	0	2
Gasping breathing	2	0	3	2	2	1
Clonic spasms	1	0	1	2	5	4
Death	0	0	1	1	5	3

Body weights: No detailed body weight data were provided. The sponsor indicated that MD males showed a lower body weight gain relative to the LD males. In MD females, a slight decrease in body weight was noted. [**Reviewer's comments**: Without a vehicle control group, it is hard to make a conclusion for treatment-related body weight changes.]

Necropsy: No drug-related abnormal findings were noted in animals that died after dosing and in animals after 14-day observations.

In summary, mice were treated with a single iv dose of [redacted] at 32, 40, and 50 mg/kg. Mortality was observed within 7 min after the injection at 40 mg/kg (2 of 10) and at 50 mg/kg (8 of 10). The LD_{50} was determined as 44.8 mg/kg for both male and female animals. Clinical signs including reduced motility, prone/side position (MD and HD only), accelerated breathing, and clonic spasms were noted in all groups. It seemed that body weights were affected by the treatment. No abnormal findings were noted in necropsy examination performed on dead and surviving animals. No NOAEL was determined.

U90-0442: [redacted] (degradation product of WAL 801 CL): Acute intravenous toxicity study in mice. Vol. 28, Page 001

Key study findings: Mortality was observed within 3 min after the injection at 40 mg/kg (5 of 10). The LD_{50} was determined as 37 mg/kg for male animals and 57 mg/kg for female animals. Clinical signs including prone or lateral position, reduced motility, sedation, dyspnea, and tachypnea were noted in both groups. No NOAEL was determined.

Document No: U90-0442
Study #: 61/89
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein, Germany
Date of study initiation: August 10, 1989
GLP compliance: Yes
QA report: Yes

Animal: Mice/Chbi:NMRI, 52 days old, 32.6±2.0 g for males and 24.0±1.7 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 32 and 40 mg/kg (dosing volume: 10 ml/kg). There was no vehicle control group.
Drug: [redacted] Batch # Br/7UK), dissolved in 20% polyethyleneglycol
Dosing regimen: Single dose, 0.1 ml/sec

The purpose of this study was to determine the potential toxicity of [redacted] a degradation product of WAL 801 CL, after a single intravenous administration to mice. The day of dosing was designated as day 1. Toxicity was assessed as shown below.

Observations and times:

Clinical signs: Twice daily (once daily on weekends and holidays) for 14 days
Body weights: Weekly
Necropsy: All animals

Results:

Mortality: During the first 3 min after dosing, 5 HD animals died (4 males and 1 female). No subsequent mortality was recorded. The LD_{50} was 37 mg/kg for males and 57 mg/kg for females.

Clinical observations: Clinical signs are summarized in the table below. The most frequent symptoms were reduced motility and sedation that were mainly seen in LD animals. Salivation, extension and twitch spasms were mainly seen in MD and HD animals.

Clinical signs in mice treated intravenously with WAL 1783 BR (number of animals/dose group)

Dose (mg/kg)	32		40		32		40	
	♂	Onset/duration (min/min)	♂	Onset/duration (min/min)	♀	Onset/duration (min/min)	♀	Onset/duration (min/min)
Prone or lateral position	1	1/31	4	1/1 to 5			2	imm*/1
Reduced motility	3	30/40	1	65/37	1	26/40		
Dyspnea	1	2/3	3	imm/1 to 3			2	imm/1 to 2
Tachypnea					1	1/1		
Sedation	1	17/10						
Exophthalmia	2	imm/1 to 2	4	imm/1 to 6			3	imm/1 to 2
Tonic convulsions	1	1/2	4	imm/0 to 3				
Saltatory convulsions			2	imm/1 to 2				

* imm: Immediately after dosing

Body weights: No treatment-related effects on body weight changes were noted.

Necropsy: No abnormal findings were noted.

In summary, mice were treated with a single iv dose of [redacted] at 32 and 40 mg/kg. Mortality was observed within 3 min after the injection at 40 mg/kg (5 of 10). The LD_{50} was determined as 37 mg/kg for male animals and 57 mg/kg for female animals. Clinical signs including prone or lateral position, reduced motility, sedation, dyspnea, and tachypnea were

noted in both groups. No abnormal findings were noted in necropsy examination performed on the dead and surviving animals. No NOAEL was determined.

U90-0443: [redacted] (degradation product of WAL 801 CL): Acute intravenous toxicity study in mice. Vol. 28, Page 026

Key study findings: Mortality was observed at 40 mg/kg (5 of 10) and 50 mg/kg (7 of 10). The LD_{50} was determined as 43.2 mg/kg for both males and females. Clinical signs including abdominal position, tonic convulsions, ataxia, dyspnea, and exophthalmia were noted in MD and HD groups. Tonic convulsions and reduced motility were also seen in LD males. No body weight gain was recorded in the surviving animals in the second week after dosing. No NOAEL was determined.

Document No: U90-0443
Study #: 62/89
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein, Germany
Date of study initiation: August 9, 1989
GLP compliance: Yes
QA report: Yes
Animal: Mice/Chbi:NMRI, 44 days old, 27.9±1.7 g for males and 23.9±1.4 g for females, 5/sex/group
Route: Intravenous injection
Dosage: 32, 40 and 50 mg/kg (dosing volume: 10 ml/kg). There was no vehicle control group.
Drug: [redacted] batch # Cl/4 UK II), dissolved in destilled water
Dosing regimen: Single dose, 0.1 ml/sec

The purpose of this study was to determine the potential toxicity of [redacted], a degradation product of WAL 801 CL, after a single intravenous administration to mice. The day of dosing was designated as day 1. Toxicity was assessed as shown below.

Observations and times:

Clinical signs: Twice daily (once daily on weekends and holidays) for 14 days
Body weights: Weekly
Necropsy: All animals

Results:

Mortality: During the first 6 min after dosing, 7 HD animals (4 males and 3 females) and 4 MD males died. One MD female died at 2.5 hr after dosing. The LD_{50} was 39.5 mg/kg for males, 47.4 mg/kg for females, and 43.2 mg/kg for both males and females.

Clinical observations: Clinical signs are summarized in the table below. All of the clinical signs started within 2 min after dosing and lasted from 1 min to 55 min.

Clinical signs in mice treated intravenously with WAL 1092 CL (number of animals/dose group)

Dose (mg/kg)	32		40		50	
Sex	♂	♀	♂	♀	♂	♀
Abdominal position			5	3	4	5
Reduced motility	1					
Dyspnea			2	1	3	2
Ataxia			1			
Exophthalmia			1		1	2
Tonic convulsions	1		4	1	3	4

Body weights: No data were provided. The sponsor indicated that no body weight gain was achieved in the second week of the study.

Necropsy: No abnormal findings were noted in the surviving animals and in the animals that died after dosing.

In summary, mice were treated with a single iv dose of [redacted], at 32, 40, and 50 mg/kg. Mortality was observed at 40 mg/kg (5 of 10) and 50 mg/kg (7 of 10). The LD_{50} was determined as 39.5 mg/kg for males, 47.4 mg/kg for females, and 43.2 mg/kg for both males and females. Clinical signs including abdominal position, tonic convulsions, ataxia, dyspnea, and exophthalmia were noted in MD and HD groups. Tonic convulsions and reduced motility were also seen in LD males. No body weight gain was recorded in the surviving animals in the second week after dosing. No abnormal findings were noted in necropsy examination performed on the dead and surviving animals. No NOAEL was determined.

U85-0788: WAL 801 CL: Four-week subacute intravenous toxicity in rhesus monkeys. Vol. 23, Page 001

Key study findings: Clinical signs (sedation, breakdown of the postural reflexes, ataxia, and prone position or sitting in the corner) were noted in HD (10 mg/kg) animals. All treated male groups and MD (1 mg/kg) and HD female groups showed a decrease in serum iron levels. An increase in urine amount was seen in HD animals. Postmortem examinations showed lesions at the injection site in all groups with a higher severity in HD animals. All abnormal findings were reversible in the recovery period with the exception of increased urine amount. An NOAEL was not determined in this study.

Document No: U85-0788
Study No: F 26
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: May 2, 1995
GLP compliance: Yes
QA report: Yes
Route: Intravenous injection
Dosage: 0.1, 1.0, and 10.0 mg/kg
Drug: WAL 801 CL, Batch #: V dissolved in 0.9% NaCl solution
Dosing regimen: qd x 4 weeks

Animal: Rhesus monkeys, 53-month old, 5.1 kg for males, and 52-month old, 4.7 kg for females

Dosing design:

Group	Main (sex/group)	Recovery (sex/group)	Total (sex/group)	Dose (mg/kg/day)	Dosing regimen	Concentration (%)	Dose volume (ml/kg)
0	3		3	0	Qd x 4 weeks	0	0.5
1	3		3	0.1	Qd x 4 weeks	0.02	0.5
2	3		3	1.0	Qd x 4 weeks	0.2	0.5
3	3	3	6	10.0	Qd x 4 weeks	2.0	0.5

The purpose of this study was to evaluate the toxicity potential of WAL 801 CL when administered by intravenous injection to monkeys for 4 weeks followed by an 8-week recovery period (HD animals only). Toxicity was assessed as shown below. [**Reviewer's comments**: No control animals were included in the recovery observations.]

Clinical observations: Once daily
Body weights: Weekly
Food consumption: No exact determination of food consumption was made, "since the animals sometimes scattered considerable amounts in the waste troughs."
ECG, heart rate and systolic blood pressure: Pretest and at 0.5, 2, and 24 hr after dosing in Weeks 1, 4, and 12
Ophthalmology: All animals, pretest and at Weeks 2 (HD group only), 4 and 12
Clinical pathology: All animals, pretest and Weeks 1, 4, and 12
Bone marrow study: All animals, at necropsy
Urinalysis: All animals, pretest and Weeks 1, 4, and 12
Necropsy: All animals
Organ weights: From all animals: heart, lungs, liver, kidneys, spleen, prostate, testes with epididymis, ovaries, adrenal glands, pituitary gland, thyroid gland, and brain
Histopathology: Please see Histopathology Inventory.

Results:

Mortality: No mortality occurred during the study period.

Clinical signs: No drug-related clinical signs were noted in LD and MD animals. In HD males and females, sedation, breakdown of the postural reflexes, and prone position or sitting in the corner were observed in most animals immediately after drug administration, followed by ataxia. The duration of these findings was less than 5 min.

Clinical signs in HD monkeys (number of animals affected)

Dosage (mg/kg)	10	
Gender	♂	♀
N	6	6
Prone position or sitting in the corner	4	6
Sedation	5	6
Ataxia	4	6
Loss of postural reflexes	4	6
Tremor	1	0

Body weights: The body weights and body weight gain between control and treated animals were similar. No toxicologically significant changes were noted.

Food consumption: Not performed.

ECG, heart rate and systolic blood pressure: No drug-related, biologically relevant abnormal findings were seen.

Ophthalmology: No treatment-related abnormal findings were noted with fundus camera examination.

Hematology and bone marrow assay: No toxicologically significant findings were observed.

Clinical chemistry: A decrease in serum iron concentrations was noted in all treated male groups and MD and HD female groups (see table below). With no changes in hematological and bone marrow examinations, the toxicological significance was not determined. Decreased iron levels were reversed in the recovery animals.

Serum iron levels in animals treated with WAL 801 CL (µmol/l)

	Males				Females			
Dose (mg/kg)	Control	0.1	1	10	Control	0.1	1	10
Pretreatment	30.37	27.27	23.73	32.05	32.57	32.37	26.07	29.92
Week 1	21.60	15.70	14.13	21.65	27.97	28.47	17.67	18.23
Week 4	27.50	16.27	15.87	16.68	27.50	22.10	14.00	16.10
Week 12				40.07				37.20

Urinalysis: The amounts of urine collected from all groups are summarized in the table below. High amount was noted in all treated groups. At the end of the recovery period, the amount of urine was still high. There were no other changes in urinalysis parameters. Without corresponding clinical chemistry and histopathologic changes, the increase in the amount of urine was not considered as toxicologically significant.

Urine excretion in monkeys treated with WAL 801 CL (ml)

	Males				Females			
Dose (mg/kg)	Control	0.1	1	10	Control	0.1	1	10
Week ½	440	563.3	766.7	696.7	291.7	231.7	398.3	662.5
Week 4	505.0	520.0	770.0	936.7	461.7	270.0	555.0	1040.0
Week 12				1020.0				1083.3

Gross pathology: Blood imbibitions were noted at the injection sites in all control, LD and MD animals. In 5 of 6 HD animals, hypodermis gelatinous and/or blood imbibitions were noted at the injection sites. No treatment-related abnormal findings were noted in recovery animals.

Organ weights: No toxicologically significant, drug-related findings were noted.

Histopathology: Lesions at the injection sites were noted in almost all animals in the control and treated groups that included fresh and/or old hemorrhage, edema, inflammatory cellular clearing reactions with/without granulation tissue formation, new formation of collagen fibers, or fibroses seen in or around the venous wall. These lesions were minimal to moderate in control, LD animals and in 5 of 6 MD animals. In 1 MD and 3 HD animals, these lesions were more severe. Also in these animals, unilateral or bilateral thrombosis in the stage of organization and recanalization was noted. The sponsor indicated that changes in the MD and HD animals

were due to intolerability and could be attributed to the 0.2% and 2.0% concentrations of the injection solutions. No other toxicologically significant, drug-related findings were noted.

In summary, monkeys were treated with WAL 801 CL by intravenous injection at doses of 0.1, 1.0, and 10.0 mg/kg for 4 weeks followed by an 8-week recovery period (HD animals only). No mortality occurred in the study. Clinical signs including sedation, breakdown of the postural reflexes, ataxia, and prone position or sitting in the corner were noted in HD animals. No drug-related abnormal findings in body weight changes, hematology, ECG, systolic blood pressure, and organ weights were observed. All treated male groups and MD and HD female groups showed a decrease in serum iron levels. An increase in urine amount was seen in HD animals. The toxicological significance was not determined without histopathological findings in kidneys. Postmortem examinations showed lesions at the injection site in all groups with a higher severity in HD animals. All of the abnormal findings were reversible in the recovery period with the exception of increased urine amount. An NOAEL was not determined in this study.

U84-0355: Subchronic (13-week) oral toxicity of WAL 801 CL in rhesus monkeys. Vol. 21, Page 001

Key study findings: High incidence of clinical signs including diarrhea, salivation, and emesis was noted in HD (60 mg/kg) animals. Slight increases in serum creatinine and BUN levels, and in urine amount were seen in HD animals. The dose of 8 mg/kg was considered as an NOAEL in this study.

Document No: U84-0355
Study No: E 69
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: November 22, 1982
GLP compliance: Yes
QA report: Yes
Route: Oral (by a nasal stomach tube)
Dosage: 1, 8, and 60 mg/kg
Drug: WAL 801 CL, Batch #: II dissolved at 3% in deionized water
Dosing regimen: qd x 13 weeks

Animal: Rhesus monkeys, 41-month old, 3.8 kg for males, and 42-month old, 4.0 kg for females

Dosing design:

Group	Main (sex/group)	Recovery (sex/group)	Total (sex/group)	Dose (mg/kg/day)	Dosing regimen	Dose volume (ml/kg)
0	3		3	0	Qd x 13 weeks	2
1	3		3	1	Qd x 13 weeks	2
2	3		3	8	Qd x 13 weeks	2
3	3	3	6	60	Qd x 13 weeks	2

The purpose of this study was to evaluate the toxicity potential of WAL 801 CL when administered orally (by a nasal stomach tube) to monkeys for 13 weeks followed by an 8-week

recovery period (HD animals only). Toxicity was assessed as shown below. [**Reviewer's comments**: No control animals were included in the recovery observations.]

Clinical observations: Daily
Body weights: Weekly
Food consumption: "No exact determination of food consumption was made, since the animals at times scattered substantial amounts in the excrement troughs."
Ophthalmology: All animals, slit lamp biomicroscopy and fundus camera, pretest, at the ends of treatment and recovery periods; HD animals, in Week 7
Clinical pathology: All animals, pretest and Weeks 1, 7, 13 and 21
Bone marrow study: All animals, at the ends of treatment and recovery periods
Urinalysis: All animals, at the ends of treatment and recovery periods
Necropsy: All animals
Organ weights: From all animals: heart, lungs, liver, kidneys, spleen, prostate, testes with epididymis, ovaries, adrenal glands, pituitary gland, thyroid gland, and brain
Histopathology: Please see Histopathology Inventory.

Results:

Mortality: No mortality occurred during the study period.

Clinical signs: Increased incidence of diarrhea and emesis were observed in HD animals (see table below). In addition, salivation was noted in 9 of 12 HD animals during the treatment period. In the recovery period, no monkey had diarrhea, and the incidence of emesis decreased to 8 times.

Clinical signs in monkeys (total incidence observed during the treatment period/animals affected)

Dosage (mg/kg)	Control		1		8		60	
Gender	♂	♀	♂	♀	♂	♀	♂	♀
N	3	3	3	3	3	3	6	6
Diarrhea (soft)	2/1	8/1	0	4/2	0	5/3	14/4	19/5
Diarrhea (liquid)	0	0	0	0	0	1/1	3/2	3/1
Emesis (slight)	0	0	0	0	0	2/1	15/3	3/3
Emesis (severe)	0	0	0	0	0	0	12/4	6/3

Body weights: Body weights and body weight gain between control and treated animals were similar. No toxicologically significant changes were noted.

Food consumption: The sponsor indicated that "a quantitative determination was impossible because of the rejection." The food consumption in LD and MD animals was similar to the control animals. However, less food consumption was noted in HD animals during the treatment and recovery periods.

Ophthalmology: No treatment-related abnormal findings were noted with indirect ophthalmoscopic examination.

Hematology: No toxicologically significant findings were observed.

Clinical chemistry: A slight increase in creatinine was noted in HD animals during the treatment period. BUN levels were very slightly increased. The increased creatinine and BUN levels were reversible during the recovery period. There were no abnormal renal findings in histopathological examination. The toxicity significance was not determined.

Serum creatinine and BUN levels in animals treated with WAL 801 CL (mean)

	Males				Females			
Dose (mg/kg)	Control	1	8	60	Control	1	8	60
Creatinine (µmol/l)								
Pretreatmen	73.1	69.2	78.7	76.0	79.9	77.5	73.4	68.4
Week 1	71.0	73.1	78.7	99.0	80.7	81.6	79.0	87.5
Week 7	67.5	68.4	72.2	97.4	71.3	76.0	75.7	81.2
Week 13	70.7	69.0	75.4	100.3	73.1	79.3	72.8	88.0
Week 21				84.9				82.2
BUN (mmol/l)								
Pretreatmen	7.715	8.546	8.072	7.419	7.241	7.478	7.834	8.309
Week 1	7.834	7.597	8.190	6.232	7.359	7.597	8.428	7.419
Week 7	8.546	8.546	8.190	8.962	8.190	7.478	8.072	9.081
Week 13	8.190	8.665	8.546	9.140	8.190	7.478	8.784	8.606
Week 21				6.766				6.172

Urinalysis: The amounts of urine collected at the end of the 13-week treatment period are summarized in the table below. High amount was noted in all dose groups. At the end of the recovery period, the amount of urine (130 ml for males and 140 ml for females) was similar to the control group. There were no other changes attributed to treatment in urinalysis parameters.

Urine excretion in monkeys treated with WAL 801 CL (ml)

	Males				Females			
Dose (mg/kg)	Control	1	8	60	Control	1	8	60
Week 13	103.3	135.0	178.3	308.3	141.7	218.3	220.0	248.3

Gross pathology: No toxicologically significant, drug-related findings were noted.

Organ weights: No toxicologically significant, drug-related findings were noted.

Histopathology: No toxicologically significant, drug-related findings were noted.

In summary, monkeys were treated with WAL 801 CL by a nasal tube at doses of 1, 8 and 60 mg/kg for 13 weeks followed by an 8-week recovery period (HD animals only). No mortality occurred in the study. High incidence of clinical signs including diarrhea, salivation, and emesis was noted in HD animals. HD animals also showed a decrease in food consumption. No drug-related abnormal findings in body weight changes, hematology, organ weights, necropsy and histopathology were observed. Slight increases in serum creatinine and BUN levels, and in urine amount were seen in HD animals. The toxicological significance was not determined without histopathological findings in kidneys. All abnormal findings in the HD animals were reversible following a recovery period of 8 weeks with the exception of decreased food consumption. The dose of 8 mg/kg was considered as the NOAEL in this study.

U84-0412: Three-month oral toxicity of WAL 801 CL in rats. Vol. 22, Page 001

Key study findings: Treatment-related toxicity was noted in animals at 35 and/or 200 mg/kg that included acute left ventricular failure and enlargement of salivary glands. The dose of 6 mg/kg was considered as the NOAEL in this study.

Document No: U84-0412
Study No: E 62, 0000933
Conducting laboratory and location: Research Division, Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein
Date of study initiation: September 9, 1982
GLP compliance: Yes
QA report: Yes
Route: Oral (by gavage)
Dosage: 6, 35 and 200 mg/kg
Drug: WAL 801 CL, Batch #: II dissolved in physiological saline solution
Dosing regimen: qd x 13 weeks

Animal: Rats/Chbb:THOM (SPF), 70±2 days old, 285.8 g for males, and 198.9 g for females

Dosing design:

Group	Main (sex/group)	Recovery (sex/group)	Total (sex/group)	Dose (mg/kg/day)	Dosing regimen	Dose volume (ml/kg)
0	12	12	24	0	Qd x 13 weeks	10
1	12		12	6	Qd x 13 weeks	10
2	12		12	35	Qd x 13 weeks	10
3	12	12*	24	200	Qd x 13 weeks	10

* Due to a high mortality rate in this group, only 3 males and 7 females were left as recovery animals.

The purpose of this study was to evaluate the toxicity potential of WAL 801 CL when administered orally (by gavave) to rats for 3 months followed by an 8-week recovery period (control and HD animals only). Toxicity was assessed as shown below.

Clinical observations: Twice daily on weekdays and once daily on weekends and holidays
Body weights: Weekly
Food consumption: Weekly
Water consumption: The last week of the treatment period
Clinical pathology: All animals, pretest and Weeks 1, 7, 13 and 21
Urinalysis: Main study animals of control and HD groups, Week 11
Necropsy: All animals
Organ weights: From all animals: heart, lungs, thyroid, thymus, salivary gland, liver, kidneys, spleen, prostate, testes with epididymis, ovaries, adrenal glands, pituitary gland, thyroid gland, and brain
Histopathology: All animals, please see Histopathology Inventory.

Results:

Mortality: Mortality data are summarized in the table below. The sponsor indicated that rats in LD and MD groups died during blood sampling. The deaths of the HD animals were drug-related, and were uniformly distributed during the 13-week treatment-period. One animal died in each of the first 4 weeks, and 2 to 5 animals died each week in the following weeks. The cause

of deaths was considered possibly as cardiovascular failure characterized by passive hyperemia of the lungs, liver, thymus, and kidneys.

Mortality in rats during the treatment period

Dosage (mg/kg)	Control		6		35		200	
Gender	♂	♀	♂	♀	♂	♀	♂	♀
N	24	24	12	12	12	12	24	24
Death	0	0	0	1	0	1	18	9
Moribund	0	0	0	0	0	0	0	1
Sacrificed at the end of the study	24	24	12	11	12	11	6	14

Clinical signs: No treatment-related clinical signs were noted, even in the animals that died during the treatment period.

Body weights: No toxicologically significant changes were noted in females and in LD and MD males. In HD males, body weights and body weight gain were lower relative to the control animals (see table below). At the end of the recovery period, body weights were similar between control and HD males.

Body weight changes in male rats treated with WAL 801 CL (g)

Treatment	Week 1	Week 13	% control	Gain (Wk1-Wk13)	% control	Week 21	Gain (Wk14-Wk21)
Control	309.6	459.4	100	149.8	100	495.5	38.5
6 mg/kg	294.4	431.3	93.9	136.9	91.4		
35 mg/kg	303.3	442.8	96.4	139.5	93.1		
200 mg/kg	397.8	383.1	83.4	85.3	56.9	452.3	71.3

Food consumption: HD animals consumed less food (males: [redacted] g/week, females: [redacted] g/week) than control animals (males: [redacted] g/week, females: [redacted] g/week). In general, food consumption in HD males was 12.9% less, and in females was 6.11% less than in control males and females, respectively. No toxicologically significant changes were noted between control and LD, MD animals, and between control and HD animals during the recovery period.

Water consumption: HD animals consumed more water than control animals (see table below).

Water consumption changes in rats treated with WAL 801 CL (ml)

	Males				Females			
Treatment	Control	6 mg/kg	35 mg/kg	200 mg/kg	Control	6 mg/kg	35 mg/kg	200 mg/kg
ml/week	160.3	164.35	159.18	209.51	142.94	145.43	153.56	181.39
% control	100	102.5	99.3	130.7	100	101.7	107.4	126.9

Hematology: No toxicologically significant findings were observed.

Clinical chemistry: No toxicologically significant, biologically relevant findings were observed.

Urinalysis: No toxicologically significant findings were observed.

Gross pathology: Positive results are summarized in the table below. In animals that died during the treatment period, circulatory changes, evidenced by hyperemia and hemorrhages in the lungs, kidneys, liver and thymus were observed. Salivary gland enlargement was noted in

HD animals and was considered as treatment-related. No drug-related abnormal findings were noted in recovery animals.

Positive necropsy findings in rats treated with WAL 801 CL

Animals died during the treatment period									
Organ	Changes	Control		6 mg/kg		35 mg/kg		200 mg/kg	
		Males	Females	Males	Females	Males	Females	Males	Females
		0	0	0	1	0	1	18	10
Lungs	Hyperemia, hemorrhages						1	16	8
	Enlarged, hepatized				1			2	1
	Distended, emphysematous								2
Heart muscle	Pale in color								2
Salivary glands	Enlarged and circulatory changes						1	6	2
Thymus	Hyperemia, hemorrhages						1	8	3
Liver	Hyperemia						1	11	5
	Enlarged							3	
	Rapture				1				
Kidney	Hyperemia						1	7	1
Small intestine	Catarrhal content							2	
Trachea, nose	With foam, discharge, reddened, swollen							3	2
Animals at terminal sacrifice									
Organ	Changes	Control		6 mg/kg		35 mg/kg		200 mg/kg	
		Males	Females	Males	Females	Males	Females	Males	Females
		12	12	12	11	12	11	3	7
Salivary glands	Enlarged and circulatory changes							3	3

Organ weights: The only drug-related finding was increased salivary gland weight in MD and HD animals. This finding was correlated with the necropsy observations. Following the recovery period, salivary gland weights were similar between control and HD animals.

Salivary gland weight changes in rats treated with WAL 801 CL (g, mean ± SD)

	Males				Females			
Treatment	Control	6 mg/kg	35 mg/kg	200 mg/kg	Control	6 mg/kg	35 mg/kg	200 mg/kg
Absolute	0.792±0.056	0.811±0.110	0.936±0.132	1.410±0.104	0.542±0.047	0.563±0.043	0.608±0.022	0.671±0.125
Relative %	0.170±0.018	0.188±0.022	0.213±0.038	0.360±0.015	0.202±0.013	0.214±0.020	0.230±0.017	0.250±0.037

Histopathology: Histopathologic findings were not completely translated into English. The reviewer cannot go through data smoothly. Based on the sponsor's description, no drug-related abnormal findings were noted in terminal sacrificed animals. Regarding the enlargement of salivary glands, "there was the impression with the 3 (surviving) male animals that the area fraction of the secretary acinic cells in the submaxillary gland were enlarged in comparison with that of the efferent ducts." However, "comparable changes in the way of parenchymal hypertrophy or hyperplasia were not found in the submaxillary glands" in the 18 HD males that died during the treatment, and in the HD females. Therefore, there were no convincing corresponding findings for the enlargement of the salivary gland.

For HD animals that died during the 13-week treatment period, the cause could be acute left ventricular failure. As a consequence of this acute insufficiency, there were moderate to severe pulmonary congestion, very slight to moderate pulmonary edema, and discrete pulmonary hemorrhage with lipoid release in the adrenal cortex (11 males, 7 females) and thymus hemorrhage (12 males, 7 females). The LD female died during the blood sampling. Capsule rapture of the liver and pronounced pulmonary atelectasis were seen in this animal. The MD

female showed severe pulmonary congestion with severe edema and hemorrhage (shock lung). The moribund HD female showed severe centrilobular hepatic necrosis.

In summary, rats were treated with WAL 801 CL by gavage at doses of 6, 35 and 200 mg/kg for 13 weeks followed by an 8-week recovery period (control and HD animals only). Drug-related mortality occurred in HD animals. The cause of deaths was considered as acute left ventricular failure. No drug-related clinical signs and abnormal clinical pathological findings were observed. Body weights and body weight gain were decreased in male HD animals. Decreased food consumption and increased water consumption were also seen in HD males. Necropsy examination showed hyperemia and hemorrhages in the lungs, kidneys, liver and thymus in the HD animals that died during the 13-week treatment period. Salivary gland enlargement was noted in both dead and surviving HD animals and was considered as treatment-related. Increased salivary gland weight was also noted in MD and HD animals. Histopathologic examination confirmed that the cause of the mortality was due to acute left ventricular failure. However, no other drug-related abnormal findings were noted. Hyperplasia and hypertrophy of the secretary part of salivary glands were only seen in 3 surviving HD males. In recovery animals, no abnormal findings were noted. In conclusion, treatment-related toxicity was noted in animals at 200 mg/kg evidenced by acute left ventricular failure and enlargement of salivary glands. The dose of 6 mg/kg was considered as the NOAEL in this study.

U88-0232: WAL 801 CL: 52-week intragastric toxicity in rhesus monkeys. Vol. 25, Page 001

Key study findings: High incidence of clinical signs including diarrhea, salivation, and emesis was noted in HD (60 mg/kg) animals. HD female animals also showed a decrease in body weight gain and food consumption. The dose of 8 mg/kg was considered as the NOAEL in this study in spite of a few clinical signs observed in this group.

Document No: U88-0232
Study No: F 60
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim, Germany
Date of study initiation: January 20, 1986
GLP compliance: Yes
QA report: Yes
Route: Oral (by gavage)
Dosage: 1, 8, and 60 mg/kg
Drug: WAL 801 CL, Batch #: VIII dissolved in demineralized water
Dosing regimen: qd x 52 weeks

Animal: Rhesus monkeys (*Macaca mulatta*), unknown age, 4.5 kg for males, 4.0 kg for females, 4/sex/group
Dosing design:

Group	Main (sex/group)	Recovery (sex/group)	Total (sex/group)	Dose (mg/kg/day)	Dosing regimen	Dose volume (ml/kg)
0	4	0	4	0	Qd x 52 weeks	2
1	4	0	4	1	Qd x 52 weeks	2
2	4	0	4	8	Qd x 52 weeks	2
3	4	0	4	60	Qd x 52 weeks	2

The purpose of this study was to evaluate the toxicity potential of WAL 801 CL when administered orally (by a nasal stomach tube) to monkeys for 52 weeks. Toxicity was assessed as shown below.

Clinical observations: Several times daily
Body weights: Weekly
Food consumption: "An exact determination of the feed consumption was not possible since, for part of the time, the monkeys threw away considerable quantities of feed. Thus, only a qualitative assessment could be made."
ECG and systolic blood pressure: During Weeks 2, 16, 32 and 48, at 3, 6, and 24 hr after dosing
Ophthalmology: All animals, fundus camera, pretest and Week 51; for HD animals, Weeks 15 and 28
Clinical pathology: All animals, pretest and Weeks 6/7, 13, 26, 39, and 52
Urinalysis: All animals, pretest and Weeks 5, 27, and 52
Necropsy: All animals
Organ weights: From all animals: heart, lungs, liver, kidneys, spleen, prostate, testes with epididymis, ovaries, adrenal glands, pituitary gland, thyroid gland, and brain
Histopathology: Please see Histopathology Inventory.

Results:

Mortality: Mortality occurred in one animal each from control, MD and HD groups (see table below). It was not drug-related.

Mortality data in monkeys treated with WAL 801 CL

Group	Number	Day of death	Cause
0	1♂	326	Progressive multifocal leukoencephalopathy
2	1♂	82	Failure of cardiovascular system possibly due to viral infection
3	1♂	49	Faulty administration

Clinical signs: Increased incidence of diarrhea and emesis were observed in HD animals (see table below). Diarrhea was also seen in one animal each in LD and MD groups, and vomiting was seen in one MD female.

Clinical signs in monkeys (total incidence observed during the treatment period/animals affected)

Dosage (mg/kg)	Control		1		8		60	
Gender	♂	♀	♂	♀	♂	♀	♂	♀
N	4	4	4	4	4	4	4	4
Diarrhea (soft)				4/1	9/1		14/2	12/2
Diarrhea (liquid)					2/1			
Salivation							61/3	35/2
Emesis (slight)						1/1	120/3	8/3

Body weights: Body weights and body weight gain between control and LD and MD animals were similar. HD females showed less body weight gain relative to the control animals.

Body weight changes in female monkeys treated with WAL 801 CL (g)

Treatment	Week 1	Week 52	% control	Gain (Wk1-Wk52)	% control
Control	4.27	5.02	100	0.75	100
1 mg/kg	3.90	4.87	97	0.97	100
8 mg/kg	4.87	5.67	100	1.05	100
60 mg/kg	4.62	4.65	92.6	0.63	84

Food consumption: No detailed data were provided. The sponsor indicated that food consumption in LD and MD animals was similar to that in the control animals. In HD group, three females "often consumed few pellets at the morning feed up to Week 36."

ECG and systolic blood pressure: No treatment-related differences in heart rate, ECG parameters, and systolic blood pressure were noted.

Ophthalmology: No treatment-related abnormal findings were noted.

Hematology and clinical chemistry: No toxicologically significant findings were observed.

Urinalysis: No biologically relevant findings were observed. There were no toxicologically significant differences in urine amounts.

Urine excretion in monkeys treated with WAL 801 CL (ml)

	Males				Females			
Dose (mg/kg)	Control	1	8	60	Control	1	8	60
Week 52	788.3	443.8	436.7	281.7	431.2	343.8	357.5	500.0

Gross pathology: No toxicologically significant, drug-related findings were noted.

Organ weights: No toxicologically significant, drug-related findings were noted.

Histopathology: No toxicologically significant, drug-related findings were noted.

In summary, monkeys were treated with WAL 801 CL by a nasal tube at 1, 8 and 60 mg/kg for 52 weeks. No treatment-related mortality occurred in the study. A high incidence of clinical signs including diarrhea, salivation, and emesis was noted in HD animals. HD female animals also showed a decrease in body weight gain and food consumption. No drug-related abnormal findings in ECG, systolic blood pressure, hematology, clinical chemistry, urinalysis, organ weights, necropsy and histopathology were observed. The dose of 8 mg/kg was considered as the NOAEL in this study in spite of a few clinical signs observed in this group.

U88-0826: WAL 801 CL: Chronic toxicity study in the rat (administration with feed). Vol. 26, Page 001

Key study findings: Treatment-related toxicity was noted in animals at 100 mg/kg that included decreased body weight gain and food consumption, and increased salivary gland weight. The dose of 10 mg/kg was considered as the NOAEL in this study.

Document No: U88-0286
Study No: F 33

Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim, Germany
Date of study initiation: August 1, 1985
GLP compliance: Yes
QA report: Yes
Route: Oral (by feed)
Dosage: 6, 35 and 200 mg/kg
Drug: WAL 801 CL, Batch #s: VI, VII, and VIII. The drug was stable in feed mixture over 13 weeks.
Dosing regimen: 2, 10 or 100 mg/kg/day in feed x 52 weeks
Animal: Rats/Chbb:THOM (SPF), 59 days old, 256.4 g for males, and 63 days old, 187.0 g for females, 20/sex/group

Dosing design:

Group	sex/group	Dose (mg/kg/day)
0	20	0
1	20	2
2	20	10
3	20	100

The purpose of this study was to evaluate the toxicity potential of WAL 801 CL when administered orally (by feed) to rats for 1 year. Toxicity was assessed as shown below.

Clinical observations: Twice daily on weekdays and once daily on weekends and holidays
Body weights: Weekly
Food consumption: Weekly
Water consumption: Pretest and Weeks 12, 27, 37, and 50
Chronic pharmacology: Locomotion: 5/sex/group, pretest and Weeks 3, 14, 24, 36, and 51
Ophthalmological examination: Slit lamp biomicroscope, Groups 0 and 3: pretest and Week 51; Groups 3: Weeks 14 and 24
Clinical pathology: All animals, pretest and Weeks 1, 6, 13, 26, 40, and 52
Urinalysis: 10/sex, control and HD groups, Weeks 2, 13, 26, 39, and 52
Necropsy: All animals
Organ weights: From all surviving animals: heart, lungs, thymus, salivary gland, liver, kidneys, spleen, prostate, testes with epididymis, ovaries, adrenal glands, pituitary gland, thyroid gland, and brain
Histopathology: All animals, please see Histopathology Inventory.

Results:

Drug intake: Deviation from intended drug intake is summarized in the table below. In most cases, the deviation was within positive or negative 10%.

Deviation from intended value (%)

Dose (mg/kg)	2		10		100	
Treatment week	♂	♀	♂	♀	♂	♀
9	-6.04	-3.87	-1.95	2.27	-2.45	-5.85
13	9.97	-7.24	9.17	11.26	5.26	-2.49
26	10.33	7.05	-8.16	5.69	-5.92	-5.01
37	0.67	9.28	-8.92	-9.50	-0.29	-4.67
50	10.07	-6.54	-0.75	5.23	-3.30	-3.26

Mortality: Mortality data are summarized in the table below. Three animals (one control male, one LD female, and one HD male) died during the anesthesia for blood sampling. Another control male died at 6 hr after blood sampling, and the cause of the death was unknown. One MD female died of an internal hemorrhage, which was sequela of a trauma. It seemed that the mortality was not treatment-related.

Mortality in rats during the treatment period

Dosage (mg/kg)	Control		2		10		100	
Gender	♂	♀	♂	♀	♂	♀	♂	♀
N	20	20	20	20	20	20	20	20
Death	2	0	0	1	0	1	1	0
Week of the death occurred	52/26	0	0	13	0	5	13	0

Clinical signs: No treatment-related clinical signs were noted.

Body weights: No toxicologically significant changes were noted in LD and MD animals. In HD animals, body weights and body weight gain were lower relative to the control animals (see table below), which was considered as drug-related effects.

Body weight changes in rats treated with WAL 801 CL (g, mean ± SD)

Treatment	Week -1	Week 52	% control	Gain (Wk-1-Wk52)	% control
Males					
Control	255.8±14.7	535.4±47.8	100	280.5	100
2 mg/kg	258.9±12.6	532.7±42.7	99.5	273.8	97.6
10 mg/kg	258.2±16.1	543.4±67.4	100	285.2	100
100 mg/kg	252.7±9.8	497.0±38.0	92.8	244.1	87.0
Females					
Control	186.8±9.6	271.4±22.2	100	84.6	100
2 mg/kg	189.2±7.1	278.2±17.4	100	89.6	100
10 mg/kg	187.0±14.1	283.8±24.4	100	98.1	100
100 mg/kg	185.1±9.4	261.1±17.4	96.2	76	90

Food consumption: Weekly food consumption is summarized in the table below. Every week during the treatment period, HD male animals consumed less food than control animals. In female HD animals, generally speaking, food consumption was similar to that in the control animals, but less food consumption was noted in a few weeks during the treatment period.

Weekly food consumption in rats treated with WAL 801 CL (g)

	Males				Females			
Dose (mg/kg)	Control	2	10	100	Control	2	10	100
Food consumption	141.9	138.7	139.7	133.0	98.1	97.0	99.8	94
% of food consumption in control group	100	97.7	98.4	93.7	100	98.9	100	97

Water consumption: HD animals consumed more water than control animals (see table below).

Water consumption changes in rats treated with WAL 801 CL (ml)

	Males				Females			
Treatment	Control	6 mg/kg	35 mg/kg	200 mg/kg	Control	6 mg/kg	35 mg/kg	200 mg/kg
ml/week	160.3	164.35	159.18	209.51	142.94	145.43	153.56	181.39
% control	100	102.5	99.3	130.7	100	101.7	107.4	126.9

Hematology: No toxicologically significant findings were observed.

Locomotion activity: No drug-related abnormal findings were observed.

Ophthalmology: No drug-related abnormal findings were noted.

Clinical pathology: No toxicologically significant, biologically relevant findings were observed.

Urinalysis: No toxicologically significant findings were observed.

Gross pathology: No drug-related abnormal findings were noted in the surviving animals.

Blood congestion in the thymus, lung and liver was noted in animals that died during or 6 hr after the anesthesia for blood taking. General anemia, as a consequence of internal hemorrhage, was seen in the MD female that died in Week 5.

Organ weights: An increase in the relative weight of salivary glands was noted in HD male animals (see table below). Similar finding was noted in the 3-month rat toxicity study (U84-0412). The sponsor indicated that increased salivatory gland weight was possibly due to a compensation of the pharmacologically demonstrated α-adrenolytic effect of WAL 801 CL. A decrease in spleen weight and an increase in thyroid weight were observed in female HD animals. Without corresponding histopathologic findings, the toxicological significance was not determined.

Several organ weight changes in rats treated with WAL 801 CL (g, mean ± SD)

	Males				Females			
Treatment	Control	2 mg/kg	10 mg/kg	100 mg/kg	Control	2 mg/kg	10 mg/kg	100 mg/kg
Salivary glands								
Absolute	0.848±0.123	0.801±0.080	0.788±0.108	0.903±0.105	0.551±0.050	0.596±0.057	0.562±0.074	0.551±0.049
Relative %	0.159±0.025	0.152±0.021	0.146±0.019	0.189±0.026	0.204±0.022	0.215±0.024	0.198±0.020	0.212±0.020
Spleen								
Absolute	0.964±0.162	0.904±0.149	1.002±0.268	0.893±0.234	0.658±0.145	0.563±0.086	0.625±0.115	0.531±0.069
Relative %	0.181±0.031	0.170±0.028	0.185±0.046	0.179±0.044	0.242±0.044	0.203±0.034	0.221±0.040	0.204±0.030
Thyroid								
Absolute	30.4±6.2	28.2±4.8	27.5±5.2	31.7±5.120.9	20.9±4.4	23.8±3.8	23.3±4.0	24.5±6.2
Relative %	0.057±0.011	0.053±0.010	0.050±0.008	0.064±0.010	0.078±0.017	0.086±0.014	0.082±0.011	0.094±0.023

Histopathology: No treatment-related histopathologic findings were noted. No specific findings related to the increased salivary gland weight were observed.

Three animals (one control male, one LD female, and one HD male) died from an acute cardiovascular failure during the anesthesia for blood taking. In addition to blood congestion in the parenchymatous organs, there was pronounced pulmonary edema in part with pulmonary hemorrhage. The MD female, died from the sequelae of a trauma, showed marked hematoma and severe purulent hemorrhagic pneumonia.

In summary, rats were treated with WAL 801 CL in feed at 2, 10, and 100 mg/kg for 52 weeks. No drug-related mortality occurred. No drug-related clinical signs and abnormal clinical pathological findings were observed. Body weights and body weight gain were decreased in HD animals. Slightly decreased food consumption was seen in HD males. Increased salivary gland weight (relative weight) was also noted in HD males. No drug-related abnormal findings were

noted in necropsy and histopathologic examinations. The dose of 10 mg/kg was considered as the NOAEL in this study.

Addendum: Histopathology inventory for NDA 21-565

Study N°	U84-0355	U84-0412	U85-0788	U88-0232	U88-0826
Duration	13 weeks	13 weeks	4 weeks	52 weeks	52 weeks
Species	Monkeys	Rats	Monkeys	monkeys	Rats
Adrenals	X	X	X	X	X
Aorta	X	X	X	X	X
Bone marrow smear	X		X	X	
Bone (femur/tibia)	X	X			X
Brain	X	X	X	X	X
Cecum		X		X	X
Cervix					X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X	X	X	X	X
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder	X		X	X	
Gross lesions				X	X
Harderian gland					X
Heart	X	X	X	X	X
Hypophysis					
Ileum	X	X	X	X	X
Injection site			X		
Jejunum	X	X	X	X	X
Kidneys	X	X	X	X	X
Lachrymal gland					
Larynx					X
Liver	X	X	X	X	X
Lungs	X	X	X	X	X
Lymph node, bronchial					
Lymph node, cervical	X		X	X	X
Lymph node, mandibular					
Lymph node, mesenteric	X	X	X	X	X
Mandibular gland	X		X		
Mammary gland	X	X	X	X	X
Nasal cavity					
Optic nerves	X	X	X	X	X
Ovaries	X	X	X	X	X
Pancreas	X	X	X	X	X
Parathyroids					
Peripheral nerve	X		X		
Pharynx					
Pituitary	X	X	X	X	X
Prostate	X	X	X	X	X
Rectum		X		X	X
Salivary gland		X		X	X
Sciatic nerve				X	X
Seminal vesicles					X
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X		X	X	X
Spleen	X	X	X	X	X
Sternum					X
Stomach	X	X	X	X	X
Testes	X	X	X	X	X
Thymus	X	X	X	X	X
Thyroid	X	X	X	X	X
Tongue	X		X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X	X	X	X	X

Vagina					X

Summary and conclusions:

The sponsor conducted several single dose systemic toxicity studies in mice, rats, and dogs, and repeated dose systemic toxicity studies with duration up to 1 year in rats and monkeys. Single dose of epinastine HCl in animals produced apparently stress-related symptoms of the central nervous system (salivation, sedation, emesis, ataxia, and dyspnea). The studies did not reveal any sex specific toxicity or indicate specific target organ of toxicity.

The major findings in repeated dose toxicity studies in rats and monkeys included mortality, decreased body weight gain and decreased food consumption, clinical signs including salivation, emesis, and diarrhea in monkeys, and increased salivary gland weight in rats. The increase in the salivatory gland weight in rats was possibly due to a compensation of the pharmacologically demonstrated α-adrenolytic effect of WAL 801 CL. The NOAELs were 8 mg/kg/day and 10 mg/kg/day in 1-year monkey study and 1-year rat study, respectively.

In both single dose and repeated dose toxicity studies, no specific target organ of toxicity was established. There was a great safety margin between the toxic doses and proposed human daily ocular dose, suggesting that toxicity observed in the systemic toxicity studies would not present a safety concern in clinical human application at the proposed daily ophthalmic dose (0.07 mg/day). The following table shows safety margins between the proposed human ophthalmic dose and the 1-year rat and monkey toxicity study data.

Key findings, systemic exposure and comparative dose of epinastine HCl in animal studies vs. human dose

Species/ treatment duration	Key findings	Dose (mg/kg/day)	Animal/ human ratio	Dose (mg/m^2/day)	Animal/ human ratio	Cmax (ng/ml)	Animal/ human ratio
Rat/1 year	No effect	10 (NOAEL)	7000	60	1160	2.36	56
	↓body weight gain, ↑salivary gland weight	100	70000	600	11600	104	2500
Monkey/1 year	No effect	8 (NOAEL)	5170	96	1850	114.14	2700
	↑salivation, emesis and diarrhea, ↓body weight gain and food consumption	60	42857	720	13900	1790	43000
Human		1.4 μg/kg/day		0.0518		0.042	

Human (50 kg) dose was based on 35 μl of 0.05% epinastine HCl instilled in both eyes twice daily.

Acute toxicity studies with impurities and degradation products of the drug specified in this submission showed that these impurities and degradation products did not alter the toxicological profile of epinastine HCl. The impurities and degradation products were not expected to pose a safety concern in humans.

V. GENETIC TOXICOLOGY:

Studies reviewed:

U02-1056: WAL 801 CL (Batch 1003564): Mutagenicity study using the *S. typhimurium*/mammalian-microsome assay (Ames test). Vol. 20, Page 068

U03-1137: Epinastine HCl (WAL 801 CL): Mutagenicity study (Ames retest) using the S. *typhimurium*/mammalian-microsome assay: Batch comparison Nos 1005138 and 1005146. Supplement, Page 001
U02-1311: Mutagenicity study (retest) for chromosomal aberration in human lymphocytes *in vitro* with epinastine HCl (WAL 801 CL): Batch No. 1003564. Vol. 20, Page 097
U03-1176: Epinastine HCl (WAL 801 CL): Mutagenicity study (retest) for chromosomal aberration in human lymphocytes *in vitro*: Batch comparison Nos 1005138 and 1005146. Supplement, Page 058
U82-0062: Point mutagenic activity of WAL 801 CL in *Salmonella typhimurium*. Vol. 20, Page 189
U88-0597: Studies on the point-mutagenic activity of several batches of the compound WAL 801 CL in *Salmonella typhimurium* strain 1538. Vol. 25, Page 423
U83-0048: Micronucleus test of WAL 801 CL in the bone marrow of the mouse. Vol. 20, Page 264
U88-0706: WAL 801 CL: Micronucleus test in mice (Project # MUT 0156). Vol. 25, Page 446
U84-0288: WAL 801 CL: Cytogenetic study in Chinese hamsters. Vol. 20, Page 368
U85-0296: Mutagenicity of WAL 801 CL in the V79 (HGPRT) forward mutation test. Vol. 22, Page 313
U86-0725: Cell transformation assay with Syrian hamster embryo (SHE) cells. Vol. 23, Page 301
U86-0368: Point mutagenicity study in *Salmonella typhimurium* of [redacted] and [redacted] Vol. 23, Page 270
U90-0078: Point mutagenicity study in *Salmonella typhimurium* of [redacted] and [redacted] Vol. 27, Page 410
U87-0605: WAL 801 CL: Chromosomal aberrations in cells of Chinese hamster cell line V79. Vol. 24, Page 224
U87-0606: WAL 801 CL: Chromosomal aberrations assay with human lymphocytes *in vitro*. Vol. 24, Page 277
U89-0051: Mutagenicity test on WAL 801 CL Batch XIII: In the *in vivo/in vitro* rat primary hepatocyte unscheduled DNA synthesis assay. Vol. 27, Page 343
U89-0101: Mutagenicity test on WAL 801 CL Batch E: In the *in vivo/in vitro* rat primary hepatocyte unscheduled DNA synthesis assay. Vol. 27, Page 370
U89-0664: WAL 801 CL: Testing for point-mutagenic activity with Escherichia coli (Project No. MUT 0183). Vol. 27, Page 395

Studies NOT reviewed:

[redacted]

U02-1056: WAL 801 CL (Batch 1003564): Mutagenicity study using the *S. typhimurium*/mammalian-microsome assay (Ames test). Vol. 20, Page 068

Key study findings: The most recently synthesized batch (1003564) of WAL 801 did not showed mutagenic potential in this Ames test.

Document #: U02-1056
Study Nº: 01B174
Conducting laboratory and location: Department of Non-Clinical Drug Safety, Boehringer Ingelheim Pharma KG, Biberach, Germany
Date of study initiation/completion: December 4, 2001/December 21, 2001
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch 1003564)

Method:

Cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA1938, TA98, TA100, and TA102

Dose selection:

Basis of dose selection: Solubility and precipitation

Test agent stability: Sufficiently stable under conditions relevant for this test

Metabolic activation system: Rat liver S9-mix

Control:

Vehicle: DMSO

Negative control: DMSO

Positive control: 2-aminoanthracene (AA), sodium azide, 9-aminoacridine (9A), 2-nitrofluorene (NF), mitomycin C

Treatment protocol of positive control

Bacteria	Strain	dose µg/plate (w/S9)	dose µg/plate (w/o S9)
Salmonella typhimurium	TA1535	AA 4 µg	Sodium azide 5 µg
	TA1537	AA 4 µg	9A 50 µg
	TA1538	AA 4 µg	NF 5 µg
	TA98	AA 4 µg	NF 10 µg
	TA100	AA 4 µg	Sodium azide 5 µg
	TA102	AA 10 µg	Mitomycin C 0.5 µg

Exposure conditions:

Incubation and sampling times: 2 days

Doses used in definitive study: 100 to 5000 µg/plate

Study design: After 2 days incubation, all colonies were counted. By comparing the number of colonies on solvent-treated control plates with those treated with the test compound, the potential of the compound to induce gene mutations was determined.

Analysis:

No. plates analyzed: 3

Counting method: Colony counting was done using an automatic colony counter

Genetic toxicity endpoints/results: A clear increase in the colony numbers

Statistical methods: Not performed since the results were unequivocal.

Criteria for positive results: A reproducible, concentration-dependent increase in the number of revertants of at least one test strain over the vehicle control value and/or outside the historical control range is indicative of genotoxic activity.

Results:

Study validity: The solvent control data were within the range of historical control data. The positive control chemicals induced a positive effect. The study was valid.

Study outcome: Bacteriotoxicity was observed at concentrations of ≥ 500 µg/plate. Treatment with WAL 801 CL at concentrations up to 5000 µg/plate did not increase the numbers of revertant colonies above the control with or without S9 activation. WAL 801 CL was not mutagenic under the present testing conditions.