In conclusion, WAL 801 CL did not showed mutagenic potential under the present testing conditions. Results of this study were in contrast to the weakly positive findings with 14 different batches of WAL 801 CL synthesized between 1982 and 1988. The impurities [redacted] showed a similar mutagenic pattern. The sponsor indicated that negative results of this study were "most probably attributable to optimization processes in the route of synthesis ultimately leading to a drug substance of higher purity compared with previous materials".

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

Key study findings: The most recently synthesized batches (1005138 and 1005146) of WAL 801 did not showed mutagenic potential in this Ames test.

Document #: U03-1137
Study Nº: 02B203
Conducting laboratory and location: Department of Non-Clinical Drug Safety, Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach/Riss, Germany
Date of study initiation/completion: November 21, 2002/December 13, 2002
GLP compliance: Yes
QA report: Yes
Purpose: Fourteen batches of epinastine HCl synthesized in the 1980s showed positive results in TA 1538. Newly synthesized batches showed negative results. In this study, two newly synthesized batches (1005138 and 1005146) were tested to confirm epinastine's genotoxic potential.
Drug: WAL 801 CL (Batches 1005138 and 1005146)
Method:

Cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA1938, TA98, TA100, and TA102
Dose selection:
Basis of dose selection: Cytotoxicity
Test agent stability: Sufficiently stable under conditions relevant for this test
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Distilled water
Negative control: Distilled water
Positive control: With S9: 2-aminoanthracene (AA)
Without S9: 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: 30 to 2000 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. Cytotoxicity was assessed by examining bacterial lawn density and numbers of spontaneous revertants per plate.

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 an expected positive effect. The study was acceptable.

Solubility and cytotoxicity: No precipitation was observed up to the highest concentration. Bacteriotoxicity was observed at the concentration of $\geq$ 100 µg/plate with S9, and $\geq$ 300 µg/plate without S9.

Mutagenicity: Treatment with WAL 801 CL at concentrations up to 2000 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.

In conclusion, WAL 801 CL did not showed mutagenic potential under the present testing conditions. Results of this study were in contrast to the weakly positive findings with 14 different batches of WAL 801 CL synthesized between 1982 and 1988. The sponsor indicated that the negative results of this study were "a consequence of using standardized genotoxicity assay methodology and use of purer solvents and reagents during the manufacturing process."

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

Key study findings: WAL 801 CL (Batch 1003564) was not clastogenic in this study.

Document #: U02-1311
Study Nº: 02B032
Conducting laboratory and location: Department of Non-Clinical Drug Safety, Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach/Riss, Germany
Date of study initiation/completion: February 11, 2002/May 8, 2002
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL containing 0.1% [redacted] and 0.2% [redacted] Batch 1003564)
Method:

Target cells: Lymphocytes from the peripheral circulation of healthy human volunteers
Dose selection:
Basis of dose selection: Cytotoxicity (based on the previous study data)
Range finding studies: No
Test agent stability: Not provided
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Medium 1A
Negative control: Medium 1A
Positive control: Adriamycin (ADR), 0.05 µg/ml without S9, Cyclophosphamide (CP), 7 µg/ml with S9.

Exposure conditions:
Incubation and sampling times and doses used in definitive study:
Toxicity experiments: Not performed.
Experiment 1 without S9: 25, 50, 75, 100, 200, and 400 µg/ml
Experiment 2 without S9 (24 hr exposure): 25, 50, 75, 100, 150, and 200 µg/ml
Experiments 2 without S9 (delayed harvest): 75, 100, 150, and 200 µg/ml
Experiments 3 with S9: 25, 50, 75, 100, 200, and 400 µg/ml

Summary of treatment conditions

Treatment	Hours	Experiment	S9	Duration of treatment (hr)	Harvest time (hr after start of treatment)
Pulse	4+20	1	-	4	24
Continuous	24+0	2	-	24	24
Pulse	24+24	2D	-	24	48
Pulse	4+20	1	+	4	24

Study design: The clastogenic potential was evaluated by an increase in the percentage of cells showing structural chromosomal aberrations with or without S9 activation.

Analysis:

No. of slides analyzed: 2/culture

Counting method: The slides were analyzed using a light microscope. One hundred metaphases from each slide were analyzed and aberrations were recorded. Cells with more than 5 aberrations were recorded as multiple aberrant cells.

Statistical methods: Chi square test

Criteria for positive results: A reproducible and concentration-dependent increase in the aberration frequency in the exposed cultures

Criteria for negative Results: If no reproducible statistically significant increase in the number of aberrant cells above concurrent control frequencies were observed at any dose level.

Historical negative control data: 0.72% aberrant cells (0-4%, n=61) in the medium control and 0.62% abnormal cells (0-3%, n=57) in the S9 control.

Results:

Cytotoxicity test: A decrease in mitotic index by greater than 50% was only noted in Experiment 2 without S9 activation. The mitotic index was not affected in Experiment 1 without S9 (87% of control), and in Experiment 1 with S9 activation (100% of control). This did not meet the recommendation by the ICH guidance (≥ 50% inhibition). The sponsor indicated in the highest concentrations, the samples were not evaluable because there were not sufficient metaphases.

Chromosomal aberration test: Results are summarized in the table below. WAL 801 CL did not induce a statistically significant increase in the frequency of chromosomal aberrations after either 4 hr or 24 hr treatment with or without S9 activation. For the experiment with 24 hr treatment followed by 24-hr incubation without S9 activation (Experiment 2, delayed harvest), the aberration rate (4.8%) was slightly higher than the historical range (0-4%) at the highest analyzable concentration (100 µg/ml).

Chromosomal aberration test in human peripheral blood lymphocytes without S9 activation (%)

	Experiment 1 w/o s9		Experiment 2 w/o s9		Experiment 2D w/o s9		Experiment 1 w/ s9	
Concentration (µg/ml)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)
Historical (-)		**0-4**		**0-4**		**0-4**		**0-3**
Vehicle	**100**	**1.0**	**100**	**1.0**	**100**	**2.0**	**100**	**0.5**
25	90		103				124	
50	83	0.5	130	0.5			115	1.5
75	93	NA	111	NA	88	1.0	94	
100	93	2.0	131	1.5	56	**4.8**	112	2.0
150	ND	ND	95	1.3	37	NE		
200	107	1.5	10	NE	32	NE	124	2.0
400	87	NE	ND	ND		ND	105	NE
Positive C.	**106**	**16.5**	**77**	**15.5**	**105**	**23.5**	**93**	

ND not done; NA: not analyzed; NE: not evaluable [few cells, normal number of metaphases showing poor quality (pyknosis)]

In summary, no sufficient cytotoxicity was observed in Experiment 1. However, the sponsor indicated that at higher concentrations, the samples were not evaluable because of insufficient metaphases. No statistically significant increase in the chromosomal aberrations was

noted in this study. However, in Experiment 2 with delayed harvest, the percentage of cells with aberration (4.8%) slightly exceeded the historical control range (0-4%) at the highest evaluable concentration (100 µg/ml). In conclusion, WAL 801 CL (Batch 1003564) was not clastogenic in this study.

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

Key study findings: WAL 801 CL (Batches 1005138 and 1005146) was not clastogenic in this study.

Document #: U03-1176
Study N^{o}: 02B202
Conducting laboratory and location: Department of Non-Clinical Drug Safety, Boehringer Ingelheim Pharma GmbH & Co. KG, 88397 Biberach/Riss, Germany
Date of study initiation/completion: November 13, 2002/February 7, 2003
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batches 1005138 and 1005146)
Purpose: To confirm the genotoxic potential of two new batches of epinastine HCl in chromosomal aberration assay.
Method:

Target cells: Lymphocytes from the peripheral circulation of healthy human volunteers

Dose selection:

Basis of dose selection: Cytotoxicity (based on the previous study data)
Range finding studies: No

Test agent stability: "The stability of the test article solutions can be ensured."
Metabolic activation system: Rat liver S9-mix
Control:

Vehicle: Medium 1A
Negative control: Medium 1A
Positive control: Adriamycin (ADR), 0.05 µg/ml without S9, Cyclophosphamide (CP), 7 µg/ml with S9.

Exposure conditions:

Incubation and sampling times:

Summary of treatment conditions

Treatment	Hours	Experiment	S9	Duration of treatment (hr)	Harvest time (hr after start of treatment)
Pulse	4+20	1	-	4	24
Pulse	4+20	1	+	4	24
Delayed	24+24	2	-	24	48
Continuous	24+0	2	-	24	24

Doses used in definitive study: 10 to 400 µg/ml

Study design: The clastogenic potential was evaluated by an increase in the percentage of cells showing structural chromosomal aberrations with or without S9 activation.

Analysis:

No. of slides analyzed: 2/culture

Counting method: The slides were analyzed using a light microscope. One hundred metaphases from each culture (200 cells/concentration) were analyzed and aberrations were recorded. Cells with more than 5 aberrations were recorded as multiple aberrant cells.

Statistical methods: Fisher's Exact Test

Criteria for positive results: A reproducible and concentration-dependent increase in the aberration frequency in the exposed cultures

Historical negative control data: 0.76% aberrant cells (0-4%, n=64) without S9 and 0.61% abnormal cells (0-3%, n=58) with S9.

Results:

The vehicle control data were within historical ranges and positive controls showed significant increases in the cells with chromosomal aberrations. The study is acceptable.

Cytotoxicity: A decrease in mitotic index by greater than 50% was noted in all experiments at concentrations $\geq$ 150 µg/ml with or without S9 activation.

Chromosomal aberration test: Results are summarized in the table below. WAL 801 CL did not induce a statistically significant increase in the frequency of chromosomal aberrations after either 4 hr or 24 hr treatment with or without S9 activation. All data were within the historical ranges with an exception of Batch 1005146 in Experiment 2 with delayed harvest, where the percentage of aberrant cells (4.5%) slightly exceeded the historical control range (0-4%) at the only evaluable concentration (50 µg/ml).

Chromosomal aberration test in human peripheral blood lymphocytes without S9 activation (%)

	Experiment 1 w/o s9		Experiment 2 w/o s9		Experiment 2D w/o s9		Experiment 1 w/ s9	
Concentration (µg/ml)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)
Historical (-)		0-4		0-4		0-4		0-3
Batch 1005138								
Vehicle	100	0.5	100	0.5	100	1.0	100	0
10			97	1.5		ND		
25	98	1.0	86	1.5		ND	96	0.5
50	87	2.5	99	1.5	88	1.5	109	1.5
100	95	2.5	56	NE	61	NE	73	1.0
150	ND	ND	No metaphase, NE		No cells	NE		NE
200	Few cells	NE	ND	ND		ND	No cells	
400	No cells		ND	ND			No cells	
Positive C.	88	16.0	52	17.9			80	32.5
Batch 1005146								
Vehicle	100	0.5	100	0.5	100	1.0	100	
10		ND	64	2.5		ND		
25	117	1.0	108	1.5		ND	111	
50	8484	1.0	59	2.3	85	4.5	100	
100	84	1.5	38	NE	67	NE	79	
150	ND	ND	5	NE	18	NE		
200	199	3.6		ND		ND	102	
300	No cells						No cells	
400	No cells						No cells	
Positive C.	88	16.0	52	17.9			80	

ND not done; NA: not analyzed; NE: not evaluable

In summary, no statistically significant increase in the chromosomal aberrations was noted in this study. WAL 801 CL (Batches 1005138 and 1005146) was not clastogenic in this study. Results of this study were in contrast to the positive findings with different batches of WAL 801 CL synthesized in the 1980s. The sponsor indicated that the negative results of this study were "a consequence of using standardized genotoxicity assay methodology and use of purer solvents and reagents during the manufacturing process."

U82-0062: Point mutagenic activity of WAL 801 CL in *Salmonella typhimurium*. Vol. 20, Page 189

Key study findings: WAL 801 CL showed a mutagenic effect on TA1538 with and without S9 activation.

Document #: U82-0062
Test Nº: MUT 0024
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein, Germany
Date of study initiation/completion: September 14, 1982/December 6, 1982
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: II) dissolved in saline buffer
Method:

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

Dose selection:

Basis of dose selection: Solubility and precipitation
Test agent stability: No data were provided
Metabolic activation system: Rat liver S9-mix
Control:

Vehicle: Saline buffer
Negative control: Saline buffer
Positive control: 2-aminoanthracene (AA), Benzo(a)pyrene (BP), 1-methyl-3-nitro-1-nitrosuguanidine (MNN), 9-aminoacridine (9A), 2-nitrofluorene (NF), 4-nitroquinoline-1-oxide (NO)

Treatment protocol of positive control

Bacteria	Strain	dose µg/plate (w/S9)	dose µg/plate (w/o S9)
Salmonella typhimurium	TA1535	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 2.5 µg, BP 5 µg	AA 2.5 µg, BP 5 µg
	TA1537	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 2.5 µg, BP 5 µg	AA 2.5 µg, BP 5 µg
	TA1538	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg
	TA98	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg
	TA100	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg

Exposure conditions:

Incubation and sampling times: 2 days
Doses used in definitive study: 10 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: Not indicated.
Genetic toxicity endpoints/results: A clear increase in the colony numbers
Statistical methods: Not indicated

Criteria for positive results: At least a doubling of the mutation frequency for *S. typhimurium* strains TA1535, TA1537, TA1538, and TA98 at two successive test points. An increase of the mutation frequency over the control value by a factor of 1.5 for *S. typhimurium* strain TA100.

Results:

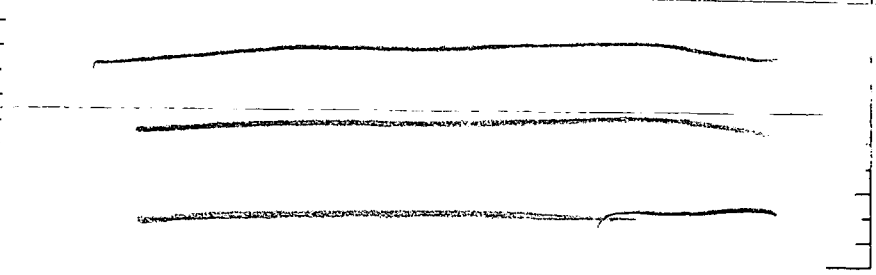
Study validity: The solvent control data were within the "spontaneous range known for the particular test strain." The positive control chemicals induced a positive effect. The study was valid. [**Reviewer's Comments**: No historical range data were provided.]

Study outcome: Bacteriotoxicity was observed at the concentration of ≥ 1000 µg/plate (with S9) and ≥ 3000 µg/plate (without S9). At 5000 µg/plate, no cell growth could be found in most cases. Treatment with WAL 801 CL at the concentrations up to 5000 µg/plate did not increase the numbers of revertant colonies in *S. typhimurium* strains TA1535, TA1537, TA100,

and TA98 above the control with or without S9 activation. WAL 801 CL showed a mutagenic effect on TA1538 with and without S9 activation (see table below).

Mutagenic effect of WAL 801 CL on *S. typhimurium* strain TA1538

Treatment	Concentration	Test 1 (w/o S9)	Test 2 (w/o S9)	Test 1 (with S9)	Test 2 (with S9)	Test 3 (with S9)
Control						
WAL 801 CL	10					
	100					
	500					
	1000					
	3000					
	5000					
NF	10					
NO	1					
AA	0.5					
BP	5					



In conclusion, WAL 801 CL did not showed mutagenic potential under the present testing conditions in *S. typhimurium* strains TA1535, TA1537, TA100, and TA98. However, WAL 801 CL showed a mutagenic effect on TA1538 with and without S9 activation.

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

Key study findings: WAL 801 CL showed a mutagenic effect on TA1538 with and without S9 activation. No batch variations were seen.

Document #: U82-0597
Test Nº: MUT 0153
Conducting laboratory and location: Laboratories of the Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, Ingelheim, Germany
Date of study initiation/completion: February 16, 1988/May 11, 1988
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #s: D, E, II-XIII) dissolved in distilled water
Purpose: To evaluate mutagenic activity of 14 batches of WAL 801 CL.
Method:

Cell line: *Salmonella typhimurium* strain TA1538
Dose selection:
Basis of dose selection: Not indicated.
Test agent stability: No data were provided
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Distilled Water
Negative control: Distilled Water
Positive control: 2-aminoanthracene (AA), 0.5 µg/plate with S9, 4-nitroquinoline-1-oxide (NO), 1 µg/plate, without S9

Exposure conditions:

Incubation and sampling times: 2 days
Doses used in definitive study: 1000-3000 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: Manually
Genetic toxicity endpoints/results: A clear increase in the colony numbers
Statistical methods: Not indicated

Criteria for positive results: At least a doubling of the mutation frequency for *S. typhimurium* strain TA1538 at two successive test points.

Results:

Study validity: The solvent control data were within the "spontaneous range known for the particular test strain." The positive control chemicals induced a positive effect. The study was valid.

Study outcome: Bacteriotoxicity was observed at the concentration of ≥ 2500 µg/plate without S9. Treatment with all batches of WAL 801 CL slightly increased the number of revertant colonies in *S. typhimurium* strain TA1538 up to about double of the negative control levels with and without S9 activation. Preincubation of WAL 801 CL (Batch XIII) in aqueous solution at 37 °C up to 24 hr prior to mutagenic test did not influence the mutagenic activity of WAL 801 CL, suggesting that mutagenic activity of WAL 801 CL was not changed during this pre-experimental period.

In conclusion, WAL 801 CL showed a mutagenic effect on TA1538 with and without S9 activation. No batch variations were seen.

U83-0048: Micronucleus test of WAL 801 CL in the bone marrow of the mouse. Vol. 20, Page 264

Key study findings: WAL 801 CL was not clastogenic under the present testing conditions.

Document #: U83-0048
Test Nº: MUT 0030, A0000954
Conducting laboratory and location: Laboratories of the Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, Ingelheim, Germany
Date of study initiation/completion: February 22, 1983/May 10, 1983
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: II)
Method:

Animals: Chbi:NMRI (SPF) mice, 10 weeks old, 26-43 g, 5/sex/dose group
Dose selection:
Basis of dose selection: LD_{50} data
Range finding studies: No
Test agent stability: Not indicated
Target cells: Polychromatic erythrocytes in bone marrow
Control:
Vehicle: Distilled water
Negative control: Distilled water
Positive control: Cyclophosphamide (CP), 50 mg/kg, po, single dose

Exposure conditions:
Incubation and sampling times: 30 hr after administration
Doses used in definitive study: 10, 50, and 250 mg/10 ml/kg, po, Single dose
Study design: The mutagenic potential of WAL 801 CL was assessed by testing the effect of the test substance on inducing micronuclei in polychromatic erythrocytes in the bone marrow of mice.
Analysis:
No. of slides analyzed: 2 per animal
Counting method: For each mouse, at least 1000 polychromatic erythrocytes (PE) of 2 slides were analyzed and the number of micronucleated polychromatic erythrocytes (MPE) was recorded.
Genetic toxicity endpoints/results: The incidence of MPE
Statistical methods: The tables of Kastenbaum and Boewman
Criteria for positive results:
--statistically significant increase in the micronucleus frequency above the control level

Results:

Toxicity findings: Animals showed no significant changes during the 30-hr period after treatment.

Micronucleus assay: Mice treated orally with WAL 801 CL showed no changes in the ratio of polychromatic and normochromatic erythrocytes, indicating no bone marrow toxicity. The frequency of micronucleated normochromatic erythrocytes was "in the usual low range" in all treated groups. There was no increase in the frequency of micronucleated polychromatic erythrocytes compared to the vehicle control (see table below). Therefore, WAL 801 CL was not clastogenic under the present testing conditions.

Results of micronucleus assay (mean ± SD)

Compound	Dosage (mg/kg)	micronucleated polychromatic erythrocyte 1/1000)	
		Males	Females
Vehicle	0	2.4±0.5	3.0±2.5
WAL 801 CL	10	3.6±2.5	2.8±1.8
WAL 801 CL	50	1.6±1.7	2.2±1.1
WAL 801 CL	250	1.6±0.9	1.8±1.8
Cyclophosphamide (Positive control)	50	42.5±9.2	25.6±9.0

U88-0706: WAL 801 CL: Micronucleus test in mice (Project # MUT 0156). Vol. 25, Page 446

Key study findings: WAL 801 CL was not clastogenic under the present testing conditions.

Document #: U88-0706
Test Nº: MUT 0156
Conducting laboratory and location: Laboratories of the Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, Ingelheim, Germany
Date of study initiation/completion: March 21, 1988/March 24, 1988
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: XIII, purity ——, dissolved in distilled water
Method:

Animals: Chbi:NMRI (SPF) mice, 10 weeks old, 29-47 g, 5/sex/group
Dose selection:
Basis of dose selection: Toxicity
Range finding studies: Yes
Test agent stability: Not indicated
Target cells: Polychromatic erythrocytes in bone marrow
Control:
Vehicle: Distilled water
Negative control: Distilled water
Positive control: Cyclophosphamide (CP), 50 mg/kg po, single dose

Exposure conditions:
Incubation and sampling times: Control groups: 24 hr after administration; treated groups: 24, 48, and 72 hr after dosing
Doses used in definitive study: 250 mg/10 ml/kg, po (by gavage), single dose
Study design: The mutagenic potential of WAL 801 CL was assessed by testing the effect of the test substance on inducing micronuclei in polychromatic erythrocytes in the bone marrow of mice.
Analysis:
No. of slides analyzed: 2 per animal
Counting method: For each mouse, at least 1000 polychromatic erythrocytes (PE) of 2 slides were analyzed and the number of micronucleated polychromatic erythrocytes (MPE) was recorded. The ratio of polychromatic to normochromatic erythrocytes was also determined.
Genetic toxicity endpoints/results: The incidence of MPE
Statistical methods: The tables of Kastenbaum and Bowman
Criteria for positive results:
--statistically significant increase in the micronucleus frequency above the control level

Results:

Dose-finding study:

A dose finding test was conducted. Mice (4/sex/group) were treated with a single dose of WAL 801 CL at 250, 300, 350 and 400 mg/kg. Mortality occurred within 24 hr of dosing in one female at 300 mg/kg, three females at 350 mg/kg, and two males at 400 mg/kg. The LD_{50} dose was determined as 519 mg/kg. Clinical signs including piloerection, peripheral hyperemia, strong sedation, and ptosis were seen at 300 mg/kg and/or higher doses. Ptosis was also seen at 250 mg/kg. Based on the study results, 250 mg/kg was selected in the micronucleus test.

Micronucleus test:

Toxicity findings: In animals treated with WAL 801 CL, one male and two females died within the first hr of dosing. Another female animal died several hr later. Piloerection, ptosis and strong sedation were noted in treated animals.

Micronucleus assay: Mice treated orally with WAL 801 CL showed no changes in the ratio of polychromatic and normochromatic erythrocytes. The frequency of micronucleated normochromatic erythrocytes was "in the usual low level" in all treated groups. There was no increase in the frequency of micronucleated polychromatic erythrocytes in any WAL 801 CL groups compared to the vehicle control (see table below). Therefore, WAL 801 CL was not clastogenic under the present testing conditions.

Results of micronucleus assay (mean ± SD)

Compound	Dosage (mg/kg)	Sampling time (hr)	micronucleated polychromatic erythrocyte x 1/1000)	
			Males	Females
Vehicle	0	24	3.0±2.5	2.8±2.9
WAL 801 CL	250	24	2.0±2.5	1.0±1.0
WAL 801 CL	250	48	1.2±1.6	2.8±1.5
WAL 801 CL	250	72	2.4±1.5	2.6±1.1
Cyclophosphamide	50	24	24.2±7.7	20.7±8.0

U84-0288: WAL 801 CL: Cytogenetic study in Chinese hamsters. Vol. 20, Page 368

Key study findings: WAL 801 CL was not clastogenic in Chinese hamsters in this *in vivo* study.

Document #: U84-0288
Project Nº: 731183
Conducting laboratory and location: [redacted]

Date of study initiation/completion: July 7, 1983/October 16, 1983
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: IV)
Method:

Animals: Chinese hamster, 6-8 weeks old, 16-27 g, 5/sex/dose group for cytogenetic and toxicity tests, and 1/sex/dose for dose finding test

Dose selection:

Basis of dose selection: Mortality
Range finding studies: Yes

Test agent stability: Not indicated

Target cells: Bone marrow cells
Control:
Vehicle: Distilled water
Negative control: Distilled water
Positive control: Cyclophosphamide (CP), 30 mg/kg/day x 5 days, po

Exposure conditions:
Incubation and sampling times: Animals were dosed for 5 consecutive days. Bone marrow samples were collected 6 hr after the last dose.
Doses used in definitive study: 35, 113, and 352 mg/10 ml/kg, po, qd x 5 days
Study design: The clastogenic potential was evaluated by an increase in the number of cells showing structural chromosomal aberration.
Analysis:
No. of slides analyzed: 5 per animal
Counting method: For each animal, 50 metaphases with a minimum of 20 well spread chromosomes were examined using microscopes and scored.
Statistical methods: Chi-square and Fisher exact tests
Criteria for positive results: A significant increase in the aberration frequency

Results:

Dose finding test: Animals (1/sex/dose) were treated with WAL 801 CL at 50 to 1000 mg/kg/day for 5 days. Only one HD male died on day 5. Clinical signs observed in this animal included hypothemia, coma, and soiled coat.

Main toxicity test: Animals (5/sex/dose) were treated with WAL 801 CL at 750, 1000, and 1250 mg/kg/day for 5 days. All MD and HD animals, and 2 male and 3 female LD animals died. Clinical signs observed in this test included hypokinesia, sedation, prostration, hypothemia, coma, and soiled coat.

Chromosomal aberration test: Animals (5/sex/dose) were treated with WAL 801 CL at 35, 113 and 352 mg/kg/day for 5 days. No abnormal findings were noted during the treatment period. WAL 801 CL did not induce an increase in the frequency of chromosomal aberrations in Chinese hamsters after 5-day treatment. In the animals treated with CP at 30 mg/kg/day, a significant increase ($P < 0.001$) in the chromosomal aberrations was noted. In conclusion, WAL 801 CL was not clastogenic in this *in vivo* study.

U85-0296: Mutagenicity of WAL 801 CL in the V79 (HGPRT) forward mutation test. Vol. 22, Page 313

Key study findings: WAL 801 CL did not showed mutagenic potential under the present testing conditions. However, with S9 activation, the cell growth was inhibited by only 60% at the highest concentration used by the sponsor (160 µg/ml). This part of the study was not valid.

Document #: U85-0296
Study Nº: Not indicated

Conducting laboratory and location: [redacted]

Date of study initiation/completion: February 6, 1985/March 28, 1985
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch V)
Method:

Cell line: V79 cells of the Chinese hamster
Dose selection:
Basis of dose selection: Cytotoxicity
Range finding studies: Yes
Test agent stability: No data were available
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Distilled water
Negative control: Distilled water
Positive control: Without S9: Ethylmethane sulfonate (EMS), 500 µg/ml; with S9: 7, 12-dimethylbenz(a)anthracene (DMBA), 10 µg/ml

Exposure conditions:
Doses used in definitive study: -S9: 25 to 500 µg/ml; +S9: 20-160 µg/ml
Incubation and sampling times: V79 cells were exposed to the drug with or without S9 activation for 2 hr. After multiple subcultivation (5 and 8 days), mutation was induced by incubation with 6-thioguanine (6-TG, 10 µg/ml) for 7 days. [**Reviewer's comments**: The exposure time appeared to be too short. According to ICH Guidance, it should be either 4 hr or 24 hr.]
Study design: 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: 5/concentration
Counting method: "Clones with more than 50 individual cells were counted visually."
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 HGPRT-deficient mutants is indicative of genotoxic activity.

Results:

Study validity: No historical data were provided. The positive control chemicals induced a positive effect. The +S9 part of the study was not valid because the cytotoxicity of 20% recommended by ICH Guidance was not reached at the highest concentration used by the sponsor (160 µg/ml). The sponsor should increase the drug concentrations.

Cytotoxicity: Bacteriotoxicity was observed at the concentration of ≥ 40 µg/ml. The cell survival rate was reduced to 17% or 8% at 100 µg/ml without S9 activation. However, with S9 activation, the cell growth was inhibited by only 60% at the highest concentration used by the sponsor (160 µg/ml). This part of the study was not valid since the sponsor did not increase the concentrations.

Mutagenicity: Treatment with WAL 801 CL at the concentrations up to 500 µg/ml without S9 and 160 µg/ml with S9 activation did not concentration-dependently increase the numbers of revertant colonies above the control. WAL 801 CL was not mutagenic under the present testing conditions.

In conclusion, WAL 801 CL did not showed mutagenic potential under the present testing conditions. However, with S9 activation, the cell growth was inhibited by only 60% at the highest concentration used by the sponsor (160 µg/ml). This part of the study was not valid. In addition, the exposure time appeared to be too short. According to ICH Guidance, it should be either 4 hr or 24 hr.

U86-0725: Cell transformation assay with Syrian hamster embryo (SHE) cells. Vol. 23, Page 301

Key study findings: WAL 801 CL did not induce cell transformation in SHE cell cultures under the experiment conditions in this study.

Document #: U02-1056
Study Nº: 217
Conducting laboratory and location: [redacted]

Date of study initiation/completion: June 2, 1986/July 4, 1986
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: VIII) dissolved in nutrient medium DMEM : Ham's F12 medium (1:1)
Method:

Cell line: Syrian hamster embryo (SHE) cells (prepared from Syrian golden hamsters (Strain: HAN:AURA)
Dose selection:
Basis of dose selection: Cytotoxicity
Range finding studies: Yes
Test agent stability: Not indicated
Metabolic activation system: Rat liver S9-mix
Control:
Negative control: Untreated cultures and cultures treated with solvent
Positive control: Without S9: N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 0.5 µg/ml; with S9: Benzo (a) pyrene (BP), 5 µg/ml

Exposure conditions:

Incubation and sampling times: 6 hr with S9; 6 and 48 hr without S9
Doses used in definitive study: With S9: 10, 25, 50, and 100 µg/ml; without S9: 5, 10, 25, and 50 µg/ml

Study design: Four to seven days after drug treatment (6 hr with S9; 6 and 48 hr without S9), the colonies were counted and scored for transformation.

Analysis:

No. colonies analyzed: 1000/concentration
Counting method:

Genetic toxicity endpoints/results: The occurrence of morphologically altered cells

Statistical methods: Not performed since the results were unequivocal.

Criteria for positive results: A colony is defined as being transformed when the cells pile up three-dimentionally mainly in the center and when they show crisscross growth in the marginal zone of the colony. The sponsor did not provide clear positive criteria. The sponsor indicated that the occurrence of more than one transformed colony in a cell sample could indicate a carcinogenic potential. However, in such a case, the sponsor also suggested that a repeated test should be conducted to see if it was reproducible.

Criteria for negative results: No transformation was induced in samples of each 100 colonies from 10 parallel flasks, both with and without S9 activation.

Results:

Dose range finding toxicity study: Data from the toxicity study showed that the treatment in the presence of S9 could not be extended over 6 hr because S9 mix proved to be toxic. The drug also showed cytotoxic property (see table below). Based on these results, the dose-range was determined for the definitive study: SHE cells were treated for 6 hr with S9 at 10, 25, 50 and 100 µg/ml, and 6 and 48 hr without S9 at 5, 10, 25 and 50 µg/ml.

Cytotoxicity of dose range finding study

WAL 801 CL concentration (µg/ml)	Relative cell survival (%) without S9	Relative cell survival (%) with S9
0 (medium)	100	100
1.0	109.2	86.4
3.3	110.0	68.8
10.0	106.0	70.6
33.3	79.9	54.8
100.0	5.4	36.0
333.3	0	12.9
1000.0	0	0
3333.3	0	0
5000.0	0	0

Definitive study: No transformed colonies were noted in negative control groups. Positive controls developed positive responses. In only one group, WAL 801 caused a single transformed colony in a sample of 1000 randomly selected colonies (see table below). The sponsor indicated that a single transformed colony was also occasionally found in the negative group. Therefore, this single transformed colony was not considered as a positive response.

Results of SHE cell transformation assay

		6 hr treatment with S9		6 hr treatment without S9		48 hr treatment without S9	
WAL 801	N of colonies	Transformed	% transformed	Transformed	% transformed	Transformed	% transformed

CL (µg/ml)	scored	colonies	colonies	colonies	colonies	colonies	colonies
Vehicle	1000		0		0		0
5	1000				0		0
10	1000		0		0		0
25	1000		0		0		0
50	1000		0		0.1		0
100	1000		0				
BP 5 µg/ml	1000		1.9				
MNN 0.5 µg/ml	1000				1.7		1.9

In conclusion, WAL 801 CL did not induce cell transformation in SHE cell cultures under the experiment conditions in this study.

U86-0368: Point mutagenicity study in *Salmonella typhimurium* of [redacted] and [redacted] Vol. 23, Page 270

Key study findings: [redacted] showed a mutagenic effect on TA1538, TA1537, and TA98 with S9 activation [redacted] showed a mutagenic effect on TA1538 with and without S9 activation.

Document #: U86-0368
Project N°: MUT 0092
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim Pharma KG, D-6507 Ingelheim am Rhein, Germany
Date of study initiation/completion: October 8, 1985/December 7, 1985
GLP compliance: Yes
QA report: Yes
Purpose: To evaluate the potential mutagenic activity of [redacted] oxidization products of WAL 801 CL [redacted], in the Ames test.
Drug: [redacted] (Batch #: E) dissolved in distilled water
[redacted] (Batch #: A) dissolved in distilled water
Method:

Cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100
Dose selection:
Basis of dose selection: Solubility and precipitation
Test agent stability: No data were provided
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Distilled water
Negative control: Untreated
Positive control: 2-aminoanthracene (AA), Benzo(a)pyrene (BP), Emodin, 1-methyl-3-nitro-1-nitrosuguanidine (MNN), 9-aminoacridine (9A), 2-nitrofluorene (NF), 4-nitroquinoline-1-oxide (NO)

Treatment protocol of positive control

Bacteria	Strain	dose µg/plate (w/oS9)	dose µg/plate (w/ S9)
Salmonella typhimurium	TA1535	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 2.5 µg, BP 5 µg	AA 2.5 µg, BP 5 µg, emodin 10 µg
	TA1537	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 2.5 µg, BP 5 µg	AA 2.5 µg, BP 5 µg, emodin 10 µg
	TA1538	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg, emodin 10 µg
	TA98	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg, emodin 10 µg
	TA100	NF 10 µg , 9A 100 µg, NO 1 µg, MNN 2.5 µg, AA 0.5 µg, BP 5 µg	AA 0.5 µg, BP 5 µg, emodin 10 µg

Exposure conditions:

Incubation and sampling times: 2 days

Doses used in definitive study: [redacted] 10, 50, 100, 500, 1000, 3000 (S9 only), and 5000 (s9 only) µg/plate; [redacted] 10, 50, 100, 500, 1000, 3000, and 5000 µg/plate, and 6000-10000 µg/plate for TA1538 only

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 compounds to induce gene mutations was determined.

Analysis:

No. plates analyzed: 3

Counting method: Manually.

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

Statistical methods: Not indicated

Criteria for positive results: Not indicated.

Results:

Study validity: The solvent and negative control data were within the spontaneous range known for the tester strains with the exception of one experiment with TA1537, in which the negative and vehicle controls caused a high mutation frequency. This part of the experiment was considered as invalid. The positive control chemicals induced a positive effect. The whole study was valid.

[redacted]. Bacteriotoxicity was observed at the concentration of ≥ 500 µg/plate (with S9) and ≥ 1000 µg/plate (without S9). Precipitation was noted at ≥ 500 µg/plate. Treatment with [redacted] at concentrations up to 5000 µg/plate did not increase the numbers of revertant colonies in *S. typhimurium* strains TA1535, TA1537, TA1538, TA100, and TA98 above the control without S9 activation. In the presence of S9 activation, [redacted] showed a mutagenic effect on TA1537, TA1538 and TA98 (see table below). However, the sponsor considered that [redacted] was not mutagenic with TA1537.

Mutagenic effect of [redacted] on *S. typhimurium* strains TA98, TA1537, and TA1538 with S9 activation

		TA98		TA1537		TA1538	
Treatment	Concentration	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Untreated		[redacted]					
Vehicle							
WAL 1097 CL	10						
	50						

	100
	500
	1000
AA	0.5
AA	2.5

[redacted] was not cytotoxic to the indicator cells. Treatment with [redacted] did not increase the numbers of revertant colonies in *S. typhimurium* strains TA1535, TA1537, TA100, and TA98 above the control with and without S9 activation. With TA1538, [redacted] showed a mutagenic effect with and without S9 activation (see table below).

Mutagenic effect of [redacted] on *S. typhimurium* strains TA1538 with and without S9 activation

		Test 1		Test 1 (Count 2)		Test 2		Test 3	
Treatment	µg/plate	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Untreated		[redacted]							
Vehicle									
WAL 1725 CL	10								
	100								
	500								
	1000								
	3000								
	5000								
	6000								
	7000								
	10000								
AA	0.5								
MNN	2.5								
NF	10								
NO	1								
9A									
BP	5								
Emodin	10								

In conclusion, both [redacted] showed mutagenic potential under the present testing conditions. In the case of [redacted], S9 activation was required.

U90-0078: Point mutagenicity study in *Salmonella typhimurium* of [redacted] and [redacted] Vol. 27, Page 410

Key study findings: [redacted] and [redacted] possible impurities of WAL 801 CL, did not show mutagenic potential under the present testing conditions.

Document #: U90-0078
Project Nº: MUT 0184
Conducting laboratory and location: Laboratories of the Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, Ingelheim, Germany
Date of study initiation/completion: August 23, 1989/December 13, 1989
GLP compliance: Yes
QA report: Yes
Purpose: To evaluate the potential mutagenic activity of [redacted] possible impurities, [redacted] and [redacted] in the Ames test.
Drug: [redacted] (Batch #: CI/4 UK II, purity: [redacted], dissolved in methanol

[redacted] (Batch #: Br/7 UK, purity: [redacted], dissolved in DMSO

Method:

Cell line: *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100

Dose selection:

Basis of dose selection: Bacteriotoxicity and precipitation

Test agent stability: No data were provided

Metabolic activation system: Rat liver S9-mix

Control:

Vehicle: Methanol for [redacted], and DMSO for [redacted]

Positive control: 2-aminoanthracene (AA), 0.5 and 2.5 µg/plate; 2-nitrofluorene (NF), 10 µg/plate; 4-nitroquinoline-1-oxide (NO), 2 µg/plate

Exposure conditions:

Incubation and sampling times: 2 days

Doses used in definitive study: 1 to 5000 µg/plate without S9; 10-500 µg/plate with S9

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 compounds to induce gene mutations was determined.

Analysis:

No. plates analyzed: 3

Counting method: Not indicated.

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

Statistical methods: Not indicated

Criteria for positive results: Not indicated.

Results:

Study validity: The solvent control data were within the spontaneous range known for the tester strains. The positive control chemicals induced a positive effect. The study was acceptable.

Treatment with [redacted] or [redacted] Precipitation was observed at concentrations ≥ 200 µg/plate without S9 and ≥ 500 µg/plate with S9. Bacteriotoxicity was observed at the concentration of ≥ 200 µg/plate (with S9) and ≥ 50 µg/plate (without S9). Treatment with WAL 1092 CL did not increase the numbers of revertant colonies in *S. typhimurium* strains TA1535, TA1537, TA1538, TA100, and TA98 above the control with or without S9 activation. For [redacted] increased revertant colonies in *S. typhimurium* strains TA1535 (+S9) and TA1537 (-S9) were noted (see table below). However, this increase was within the historical control range (Data found in Study U02-1056). Hence, this increase was not considered as a positive response.

Mutagenic effect of [redacted] on *S. typhimurium* strains TA1535 and TA 1537

		Experiment 1	Experiment 2	Experiment 1	Experiment 2
		TA1535		TA1537	
Treatment	µg/plate	+S9	+S9	-S9	-S9

Vehicle		
[redacted]	1	
	10	
	50	
	100	
	200	
	300	
	400	
	500	
AA	0.5	
Historical control		

In conclusion, both [redacted] and [redacted], possible impurities of WAL 801 CL, did not show mutagenic potential under the present testing conditions.

U87-0605: WAL 801 CL: Chromosomal aberrations in cells of Chinese hamster cell line V79. Vol. 24, Page 224

Key study findings: At 180 µg/ml, WAL 801 CL induce a clear increase in the frequency of chromosomal aberrations 18 hr after treatment with S9 activation.

Document #: U87-0605
Study N°: [redacted] 276, A0000946
Conducting laboratory and location: [redacted]

Date of study initiation/completion: December 15, 1986/February 2, 1987
GLP compliance: Yes
QA report: Yes
Method:

Target cells: Chinese hamster cell line V79
Test agent: WAL 801 CL (Batch #: VIII)
Dose selection:
Basis of dose selection: Cytotoxicity
Range finding studies: Yes
Test agent stability: Not provided
Metabolic activation system: Rat liver S9-mix
Control:
Vehicle: Nutrient medium without fetal calf serum (solvent)
Negative control: Solvent
Positive control: Ethylmethanesulfonate (EMS), 0.97 mg/ml without S9
Cyclophosphamide (CP), 1.40 µg/ml with S9

Exposure conditions:
Incubation and sampling times: Treatment time: 4 hr; sampling time: 7 (high concentrations only), 18, or 28 (high concentrations only) hr after the start of the treatment
Concentrations used in definitive study: 0.3, 15, and 60 µg/ml without S9, 8, 40, and 180 µg/ml with S9
Toxicity experiments: Yes

Study design: The clastogenic potential was evaluated by an increase in the percentage of cells showing structural chromosomal aberrations with or without S9 activation.

Analysis:

No. of slides analyzed: 4

Counting method: The slides were analyzed using [redacted] and [redacted] photomicroscopes with attached equipment.

Statistical methods: Not performed.

Criteria for positive results: 1. A clearly increased aberration rate compared with the negative control with one of the concentrations tested, and 2. A clear concentration-dependent increase in the aberration rats

A result was defined to be negative if 1 and 2 did not apply.

Historical negative control data: Not provided.

Results:

Pre-experiment toxicity assay: A decrease in mitotic index by greater than 50% was noted at 60 µg/ml without S9 activation (23.8%) and at 150 mg/ml with S9 activation (43.3%). Based on the pretest data, concentrations of 60 µg/ml and 180 µg/ml were selected as the highest concentrations with and without S9 activation, respectively.

Chromosomal aberration test: Results are summarized in the table below. At 180 µg/ml, WAL 801 CL induce a clear increase in the frequency of chromosomal aberrations 18 hr after the treatment with S9 activation. [**Reviewer's comments**: In the experiments without S9, cytotoxicity of 50% was not reached.]

Chromosomal aberration test in V79 cells with and without S9 activation

Concentration (µg/ml)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)	Mitotic index (% of control)	Aberrant cells (%)
Without S9	**7 hr after start of treatment**		**18 hr after start of treatment**		**28 hr after start of treatment**	
Vehicle	**100**	**1.5**	**100**	**1.25**	**100**	**2.0**
0.3			86.2	1.0		
15			141.4	3.50		
60	58.3	1.33	127.6	2.0	94.1	1.75
EMS 0.94 mg/ml			**69.0**	**31.5**		
With S9	**7 hr after start of treatment**		**18 hr after start of treatment**		**28 hr after start of treatment**	
Vehicle	**100**	**2.25**	**100**	**1.5**	**100**	**1.25**
8			91.2	2.0		
40			100	2.75		
180	51.6	**5.0**	50	**10.25**	78	1.0
CP 1.4 µg/ml			**70.6**	**22.0**		

U87-0606: WAL 801 CL: Chromosomal aberrations assay with human lymphocytes *in vitro*. Vol. 24, Page 277

Key study findings: WAL 801 CL was clastogenic in this study.

Document #: U87-0606

Project Nº: 733044, A0000947

Conducting laboratory and location: [redacted]

Date of study initiation/completion: March 8, 1985/June 27, 1986
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch V)
Method:

Target cells: Lymphocytes from the peripheral circulation of healthy human volunteers

Dose selection:

Basis of dose selection: Cytotoxicity
Range finding studies: Yes

Test agent stability: Not provided
Metabolic activation system: Rat liver S9-mix
Control:

Vehicle: Distilled water
Negative control: Untreated control
Positive control: Methyl methanesulfonate (MMS), 20 µg/ml and mitomycin C (MC) 0.1-0.25 µg/ml without S9 Cyclophosphamide (CP), 20 µg/ml and procarbazine (PC) 100 µg/ml with S9

Exposure conditions:

Incubation and sampling times and doses used in definitive study: Treatment time: 5 hr; sampling time: 49 hr after the start of the treatment
Toxicity experiments: Not performed.
Initial study without S9: 25, 50, 100 and 200 µg/ml
Initial study with S9: 50, 100, 200 and 400 µg/ml
Extension study without S9: 12.5, 25, 50, and 100 µg/ml
Extension study with S9: 12.5, 25, 50, and 100 µg/ml
(The initial study included 3 same tests, and the extension study included 2 same tests.)

Study design: The clastogenic potential was evaluated by an increase in the percentage of cells showing structural chromosomal aberrations with or without S9 activation.

Analysis:

No. of slides analyzed: At least 2
Counting method: The slides were analyzed in a random order using a [redacted] binocular microscope. Whenever possible, 100 cells with 44 or more chromosomes from 2 or more slides from each culture were analyzed and aberrations were recorded.
Statistical methods: Not performed.

Criteria for positive results: Whenever aberration incidence in the exposed culture exceeded the criteria for a negative or suspicious effect. The dose-related and reproducible increase in the aberration rate was also important.
A result was defined to be negative if aberration yields in both negative control and drug-treated cultures fell within normal historical ranges.

A suspicious response was recorded if the aberration frequency in the test culture exceeded the normal negative control range but did not attain the value for a true positive response.
For each cell culture, the numbers of cells showing true numerical chromosomal alterations (i.e., hyperdiploidy or polyploidy) were also recorded.
Historical negative control data:

		Aberration index (mean±SD)	% aberrant cells (mean ± SD)
	S9	Lesions/cell	Excluding gaps
Vehicle	+	0.03±0.02	0.3±0.7
	-	0.02±0.01	0.3±0.5
Untreated	+	0.03±0.01	0.4±0.5
	-	0.04±0.04	1.2±1.4
Negative response		0-0.06	0-2
Suspicious response		0.07-0.08	3
Positive response		>0.08	>3

Results:

Pre-experiment toxicity assay: In the preliminary toxicity test, cell cultures were exposed to WAL 801 CL at 50-5000 µg/ml. At 167 µg/ml with or without S9 activation, cell growth was inhibited by 25 to 50%. At 500 mg/ml, cell growth was inhibited by 50 to 75%. Based on this pretest, concentrations of 400 µg/ml and 200 µg/ml were selected as the highest concentrations with and without S9 activation, respectively.

Chromosomal aberration test: In the initial study, negative control groups showed negative results within the historical range, and the positive groups showed positive results. With S9, an aberration rate of 4% was noted at 50 µg/ml in Test B, and at 200 µg/ml in Test A. At 50 mg/ml in Test A, an aberration rate of 3% was noted. Without S9, Aberration rates higher than 3 were noted at 3 concentrations in all tests (see table below). WAL 801 CL was positive in the initial study.

Chromosomal aberration test in human peripheral blood lymphocytes without S9 activation

	Test A		Test B		Test C	
Concentration (µg/ml)	Lesions/cell	Aberrant cells (%)	Lesions/cell	Aberrant cells (%)	Lesions/cell	Aberrant cells (%)
Historical (-)	[illegible]	0-2	[illegible]	0-2	[illegible]	0-2
Vehicle	[illegible]	1	[illegible]	2	[illegible]	3
Untreated control	[illegible]	2	[illegible]	1	NA	NA
25	[illegible]	2	[illegible]	5	[illegible]	2
50	[illegible]	4	[illegible]	2	[illegible]	4
100	NA	NA	[illegible]	8	NA	NA
200	NA	NA	NA	NA	NA	NA
MMS	[illegible]	6	[illegible]	14	[illegible]	14
MC	NA	NA	NA	NA	NA	NA

NA: No analyzable metaphases

In the extension study, negative controls showed negative results, and positive controls showed positive results. WAL 801 CL induced no aberration results in the presence of S9 activation. Without S9, a reproducible increase in one of the aberration parameters (lesions/cell) was noted at 50 µg/ml. The response in one test was due to a single cell with multiple aberration, while the other showed both a multiple aberrant ([illegible]esions) cell and a moderately damaged [illegible] lesions) cell.

Chromosomal aberration test in extension study without S9 activation

	Test A		Test B	
Concentration (µg/ml)	Lesions/cell	Aberrant cells (%)	Lesions/cell	Aberrant cells (%)
Historical (-)		0-2		0-2
Vehicle		0		0
Untreated control		0		0
12.5		1		0
25		0		0
50		1		2
100	NA	NA		0
MC		11		5

In summary, in the initial study, WAL 801 CL induced an increase in the aberrant cells in the presence and absence of S9. In the extension study, the drug induced an increase in lesions/cell at 50 µg/ml in the absence of S9. In conclusion, WAL 801 CL was clastogenic in this study.

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

Key study findings: WAL 801 CL was negative in this UDS assay under the present conditions.

Document #: U89-0051
Study #: 10542-0-494
Conducting laboratory and location:

Date of study initiation/completion: August 18, 1988/October 19, 1988
Purpose: To detect DNA damage caused by WAL 801 CL or its metabolite, by measuring unscheduled DNA synthesis (UDS) induced *in vivo* in rat hepatocytes
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: XIII) dissolved sterile deionized water
Method:

Animals: Male Fischer 344 rats, 150-300 g
Dose: 300 mg/kg, single dose
Route: Oral (by gavage)
Indicator cells: Hepatocytes from adult male Fischer 344 rats, 3/group/time point
Control:

Vehicle: Deionized water
Negative control: Deionized water
Positive control: Dimethylnitrosamine (DMN), 10 mg/kg, po

Exposure conditions:

Male rats were treated with a single dose of WAL 801 CL or control material. About 4 and 15 hr after dosing, the liver samples of rats were collected and hepatocyte cultures were prepared. The hepatocyte cultures were incubated with 5 mCi/ml ^{3}H-thymidine (^{3}HTdr, 20 Ci/mMole) for 4 hr, then washed and incubated with unlabeled thymidine (0.25 mM) for about 18 hr. The cells were then analyzed for nuclear grains.

Doses used in definitive study: 300 mg/5 ml/kg, po (by gavage)

Group	Dosage (mg/5ml/kg)	N (liver sampling at 4 hr after dosing)	N (liver sampling at 15 hr after dosing)
Control	0	3	3
WAL 801 CL	300	3	3
DMN	10	3	3

The DNA damage was inferred from an increase in grain counts associated with the nucleus of cultured hepatocytes compared to untreated rats.

Analysis:

No. of slides analyzed: 3/treatment condition

Counting method: The cells were examined microscopically. The field was displayed on a video screen of an automatic counter. UDS was measured by counting nuclear grains and subtracting the average number of grains to background count. The net nuclear grain count was determined fro 50 cells on each slide.

Statistical methods: Not indicated.

Assay acceptance criteria:

1. The viability of the hepatocytes collected from perfusion process normally exceeds 70%.
2. The viability of the monolayer cell cultures used for the assay treatments must be 70% or greater.
3. For test materials causing weak or no UDS activity, the average response to the positive control treatment must exceed both criteria used to indicate UDS.
4. Grain count data obtained per animal are acceptable as part of the evaluation if obtained from two replicate cultures and at least 50 nuclei per culture.

Criteria for positive results:

An increase in the mean net nuclear grain count to at least 6 grains per nucleus after subtraction of the concurrent negative control value, and/or an increase in the percent of nuclei having 6 or more net grains to at least 10% of the analyzed population after subtraction of the concurrent negative control value.

Results:

For hepatocytes sampled at both 4 hr and 15 hr time points, the viability of the total cells collected, the attachment efficiencies, and the viability of attached cells were all within the range of historical data. Treatment with WAL 801 CL at 300 mg/kg did not cause nuclear labeling that was significantly different from the vehicle control. No elevation in net nuclear grains or percent nuclei with ≥ 6 grains was seen. On the other hand, the DMN treatment induced large increases in nuclear grains. In conclusion, WAL 801 CL was negative in this UDS assay under the present conditions.

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

Key study findings: WAL 801 CL was negative in this UDS assay in the hepatocytes collected 4 hr after dosing.

Document #: U89-0101
Study #: 10541-0-494
Conducting laboratory and location: [redacted]

Date of study initiation/completion: August 18, 1988/November 3, 1988
Purpose: To detect DNA damage caused by WAL 801 CL or its metabolite, by measuring unscheduled DNA synthesis (UDS) induced *in vivo* in rat hepatocytes
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch #: E) dissolved sterile deionized water
Method:

Animals: Male Fischer 344 rats, 150-300 g
Dose: 300 mg/kg, single dose
Route: Oral (by gavage)
Indicator cells: Hepatocytes from adult male Fischer 344 rats, 3/group/time point
Control:

Vehicle: Deionized water
Negative control: Deionized water
Positive control: Dimethylnitrosamine (DMN), 10 mg/kg, po

Exposure conditions:

Male rats were treated with WAL 801 CL or control material. About 4 and 15 hr after dosing, the liver samples of rats were collected and hepatocyte cultures were prepared. The hepatocyte cultures were incubated with 5 mCi/ml ^{3}H-thymidine (^{3}HTdr, 20 Ci/mMole) for 4 hr, then washed and incubated with unlabeled thymidine (0.25 mM) for about 18 hr. The cells were then analyzed for nuclear grains.
Doses used in definitive study: 300 mg/5 ml/kg, po (by gavage)

Group	Dosage (mg/5ml/kg)	N (liver sampling at 4 hr after dosing)	N (liver sampling at 15 hr after dosing)
Control	0	3	3
WAL 801 CL	300	3	3
DMN	10	3	3

The DNA damage was inferred from an increase in grain counts associated with the nucleus of cultured hepatocytes compared to untreated rats.

Analysis:

No. of slides analyzed: 3/treatment condition
Counting method: The cells were examined microscopically. The field was displayed on a video screen of an automatic counter. UDS was measured by counting nuclear grains and subtracting the average number of grains to background count. The net nuclear grain count was determined fro 50 cells on each slide.
Statistical methods: Not indicated.

Assay acceptance criteria:

1. The viability of the hepatocytes collected from perfusion process normally exceeds 70%.
2. The viability of the monolayer cell cultures used for the assay treatments must be 70% or greater.
3. For test materials causing weak or no UDS activity, the average response to the positive control treatment must exceed both criteria used to indicate UDS.
4. Grain count data obtained per animal is acceptable as part of the evaluation if obtained from two replicate cultures and at least 50 nuclei per culture.

Criteria for positive results:

An increase in the mean net nuclear grain count to at least 6 grains per nucleus after subtraction of the concurrent negative control value, and/or an increase in the percent of nuclei having 6 or more net grains to at least 10% of the analyzed population after subtraction of the concurrent negative control value.

Results:

For the hepatocytes sampled at 4 hr time points, the viability of the total cells collected, the attachment efficiencies, and the viability of the attached cells were all within the range of historical data. Treatment with WAL 801 CL at 300 mg/kg did not cause nuclear labeling that was significantly different from the vehicle control. No elevation in net nuclear grains or percent nuclei with ≥ 6 grains was seen. On the other hand, the DMN treatment induced large increases in nuclear grains. For hepatocytes sampled at 15 hr time points, the viability of the total cells collected and the viability of the attached cells were all within the range of historical data. However, the attachment efficiencies (38.3-88.2%) were much lower than those at 4 hr (62.0-89.2%). This was especially apparent in WAL 801 CL treated group (38.3%, 41.2%, and 66.3% for 3 slides, respectively), indicating the presence of toxicity. UDS analysis with microscopic examination was impossible in these samples. In conclusion, under the present conditions, WAL 801 CL was negative in the UDS assay in the hepatocytes collected 4 hr after dosing. The hepatocytes collected at 15 hr time point could not be analyzed due to toxicity.

U89-0664: WAL 801 CL: Testing for point-mutagenic activity with *Escherichia coli* (Project No. MUT 0183). Vol. 27, Page 395

Key study findings: WAL 801 CL did not showed mutagenic activities in *E. coli* strain WP2uvrA.

Document #: U89-0664
Study Nº: MUT 0183
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim, Germany
Date of study initiation/completion: September 4, 1989/September 28, 1989
GLP compliance: Yes
QA report: Yes
Drug: WAL 801 CL (Batch XIV, purity: [redacted] dissolved in distilled water
Method:

Cell line: *Escherichia coli* WP2uvrA

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: Distilled water
Negative control: Distilled water
Positive control: 2-aminoanthracene (AA), 5 μg/plate with S9; 1-ethyl-3-nitro-1-nitrosoguanidine (ENNG), 2.5 μg/plate without S9

Exposure conditions:
Incubation and sampling times: 2 days
Doses used in definitive study: 10 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: Not indicated.
Genetic toxicity endpoints/results: A clear increase in the colony numbers
Statistical methods: Not indicated.
Criteria for positive results: Not indicated.

Results:

Two same experiments were conducted. At the end of 2 days incubation, precipitation was seen at 5000 μg/plate. Cytotoxicity was noted at concentrations ≥ 2000 μg/plate in the absence of S9 activation. The mutation frequencies in the negative control group were all within the spontaneous range known for the tester strain. The positive control group showed obvious positive results. WAL 801 CL did not increase the spontaneous mutation frequency in the tester strain with and without S9 activation. In conclusion, under the present conditions, WAL 801 CL did not showed mutagenic activities in *E. coli* strain WP2uvrA.

Genetic toxicology summary and conclusion:

Seventeen genotoxicity studies were submitted in the original NDA submission and two more in a supplement. Epinastine HCl was negative in *in vivo* clastogenicity studies, including the mouse micronucleus assays and chromosome aberration assay in Chinese hamster ovary cells. Epinastine was also negative in a cell transformation assay using Syrian hamster embryo cells, a point mutation assay in V79/HGPRT mammalian cells, and an *in vivo/in vitro* unscheduled DNA synthesis assay using rat primary hepatocytes. Epinastine was negative in Ames tests with *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, and with *E. coli* strain WP2uvrA. Epinastine in old batches was weakly positive (2-3 folds of vehicle control) in *S. typhimurium* strain TA1538 in two studies conducted in 1980s. TA1538 is not used anymore. It is identical to TA98 except that it lacks the plasmid which gives TA98 error prone repair. Usually a tripling of background was considered as TA1538 positive. In light of negative

results in subsequent studies with new batches, the number of revertants of tester strain TA1538 was considered as negative. Positive results were seen in two *in vitro* chromosomal aberration studies conducted in 1980s with human peripheral lymphocytes and with V79 cells, respectively. In two Ames tests and two *in vitro* chromosomal aberration assays using human lymphocytes conducted in 2001 to 2003, newly synthesized batches of epinastine did not induce a genotoxic response. The sponsor indicated that "the reason for the negative results in recent batches is presumed to be a consequence of using standardized genotoxicity assay methodology (especially the cytogenetic assay) and use of purer solvents and reagents during the manufacturing process." The reviewer has asked the sponsor to clarify what are the differences between the old and new batches, and what are the differences in the assay methodology.

Label recommendations:
Epinastine in newly synthesized batches was negative for mutagenicity in the Ames/*Salmonella* assay and *in vitro* chromosome aberration assay using human lymphocytes. Positive results were seen with early batches of epinastine in two *in vitro* chromosomal aberration studies conducted in 1980s with human peripheral lymphocytes and with V79 cells, respectively. Epinastine was negative in the *in vivo* clastogenicity studies, including the mouse micronucleus assay and chromosome aberration assay in Chinese hamsters. Epinastine was also negative in the cell transformation assay using Syrian hamster embryo cells, V79/HGPRT mammalian cell point mutation assay, and *in vivo/in vitro* unscheduled DNA synthesis assay using rat primary hepatocytes.

VI. CARCINOGENICITY:

Studies reviewed:

U87-0064: WAL 801 CL: Carcinogenicity range finding study on mice administered perorally in feed. Vol. 23, Page 320
U91-0559: WAL 801 CL: 18-month carcinogenicity study in the mouse (administered in the diet). Vol. 29, Page 001
U91-0625: WAL 801 CL: 2-year carcinogenicity study in the rat (administered in the diet). Vol. 32, Page 001
U91-0017: WAL 801 CL: Plasma concentrations of WAL 801 CL in parallel to a carcinogenicity study over 18 months in mice (administered in the diet). Vol. 28, Page 052

U87-0064: WAL 801 CL: Carcinogenicity range finding study on mice administered perorally in feed. Vol. 23, Page 320

Document #: U87-0064
Report Nº: A0000969
Volume Number: 1-10
Study Purpose: To characterize the drug-related effects and the maximum tolerated dose in mice after oral administration in the long-term toxicological study.
Study Site: Boehringer Ingelheim KG
D-6507 Ingelheim/Rhein

Germany
Study Date: November 1985 to April 1986
GLP Compliance: Not indicated
Animal: Chbi:NMRI mice, 45 days old, males: 30 g, females: 24 g, 10/sex/group
Compound: WAL 801 CL (Batch Nº: VII)
Dose Level: 0, 2, 42.5 (200), 85 (260) and 170 mg/kg/day x 22 weeks
Route: Oral (in feed),
Group:

Group	Dose (mg/kg)	N/sex
0	Control	10
1	2	10
2	42.5 and 200*	10
3	85 and 260*	10
4	170	10

* Dosage increased at the start of Week 12 of the study. The study was planned for 12 weeks and was prolonged for 10 more weeks because the body weight course did not allow for any conclusions concerning the drug effects in Groups 1-4.

Observations and times: (The first day of dosing was designated as Day 1.)

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Water consumption: Weeks 11/12
TK: In the 6th week and at the end of the study
Gross pathology: At the end of the study

Results:

Mortality: Eight animals died while anesthetized for blood sampling in Week 6.

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

Body weight change: Body weight changes are summarized in the table below. It was not determined if the drug had any effects on body weight changes.

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

	Males					Females				
Group	Wt at start	Wt at end	Total Wt gain	Gain Wk1-11	Gain Wk 12-22	Wt at start	Wt at end	Total Wt gain	Gain Wk1-11	Gain Wk 12-22
0	29.8	40.8	11.0	7.3	3.7	24.3	32.6	8.1	5.0	3.1
1	30.3	42.8	12.5	8.4	4.1	23.8	30.3	6.5	5.4	1.1
2	31.0	39.5	8.5	6.5	2.0	23.6	31.5	7.9	5.9	2.0
3	30.3	41.3	11.0	8.4	2.6	24.2	31.0	6.8	4.1	2.7
4	30.0	40.8	10.8	7.5	3.3	24.1	32.6	8.5	5.6	2.9

Food and water consumption: No significant differences were noted. The drug consumption is summarized in the table below.

Average group drug consumption in mice

	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg)	2	42.5 and 200	85 and 260	170	2	42.5 and 200	85 and 260	170
Week 1-11	1.97	42.16	83.99	168.61	2.02	42.55	84.29	168.46
Week 12-22	1.99	201.99	263.55	173.29	2.04	201.98	263.72	171.85

Gross pathology: No treatment-related changes were noted. No target organs were determined. [**Reviewer's comments**: No detailed data were submitted.]

TK: The sponsor indicated that dose-related plasma concentrations were detected in all groups except for the low dose group. [**Reviewer's comments**: No detailed data were submitted.]

Summary: Mice (Chbi:NMRI) were treated orally (by feed) with WAL 801 CL at 0, 2, 42.5, 85 and 170 mg/kg for 22 weeks. At the start of the 12th week, the dosage of 42.5 mg/kg was changed to 200 mg/kg, and 85 mg/kg was changed to 260 mg/kg. Eight animals died during blood sampling. No drug-related abnormal findings were observed. Dose-related plasma drug concentrations were detected at doses ≥ 42.5 mg/kg.

U91-0559: WAL 801 CL: 18-month carcinogenicity study in the mouse (administered in the diet). Vol. 29, Page 001

Key study findings: Adenomas in the Harderian glands in HD M, but not considered toxicologically significant.
Doc. number: U91-0559
Study number: F 81
Study Purpose: To investigate whether WAL 801 CL possess a carcinogenic effect following oral administration to the mouse.
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Bochringer Ingelheim KG, D-6507 Ingelheim, Germany
Study Date: June 1986 to April 1988
GLP Compliance: Yes
QA Report: Yes
Drug, lot#, and % purity: WAL 801 CL, Batch Nº: IX
CAC concurrence: No
Study type 1.5 yr bioassay
Species/strain: Chbi:NMRI mice
Number/sex/group; age at start of study: 50/sex/group, 42±2 days old, males: 30.7 g, females: 26.4 g

Group	Dose (mg/kg)	N/sex
0	Control A	50
1	2	50
2	10	50
3	40	50
4	Control B	50

Drug stability/homogeneity: Adequate
Methods:
Doses: 2, 10 and 40 mg/kg/day
Basis of dose selection: 200 times the proposed therapeutic dose for humans
Restriction paradigm for dietary restriction studies: N/A
Route of administration: Dietary
Frequency of drug administration: N/A, diet

Dual controls employed: Yes
Interim sacrifices: No
Satellite PK or special study groups: 90 mice/sex at 40 mg/kg only (PK)
Observations and times:
Clinical signs: At least once daily
Body weights: Weekly
Food consumption: Weekly
Water consumption: Weeks 13, 26, 39, 52, 65 and 78
Effective dose: Weekly for the 1st 26 weeks, and monthly thereafter
Hematology: At termination, surviving animals and in animals sacrificed moribund
Clinical chemistry: Not performed
Organ weights: Not performed
Gross pathology: At termination
Histopathology: At termination, the following tissues in all groups were collected and determined: trachea, lungs, thyroid/parathyroid gland, thymus, aorta, sternum, heart, tongue, salivary glands, cervical lymph nodes, esophagus, stomach, duodenum/jejunum/ileum, cecum/colon/rectum, mesenteric lymph node, liver/gall bladder, pancreas, spleen, adrenal glands, kidneys, urinary bladder, gonads, prostate, seminal vesicles, uterus, cervix vagina, mammary tissue, skin, bone/bone marrow (femur), skeletal muscle, brain, pituitary gland, spinal cord, sciatic nerve, eyes, optic nerve, Harderian glands, all gross lesion.
Toxicokinetics: Blood samples were collected from 15 animals each time at 10 am in weeks 1, 24, 48, 64 and 78.

Results:

Mortality: Forty-five premature decedents (15 males and 30 females) occurred in this study. Based on the distribution and post-mortem examination data (see tables below), it seemed that the deaths were not drug-related. There was no significant difference in the time of mortality occurrence between treated and control mice.

Mortality data of mice in the 18-month carcinogenicity study

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Died	2	4	0	3	1	3	1	1	0	3
Sacrificed	2	3	0	5	2	2	2	4	5	2
Total	4	7	0	8	3	5	3	5	5	5
%	8	14	0	16	6	10	6	10	10	10

Causes of death and intercurrent sacrifice

Group	0 (Control A)		4 (Control B)	1		2		3	
Sex	♂	♀	♀	♂	♀	♂	♀	♂	♀
Total	4	7	8	3	5	3	5	5	5
Pyelonephritis	1								
Nonspecific lymphoma	1								
Not evident	2	2	2	1	1	1			
Chronic nephropathy		1	2		2				
Mast cell tumor		1						1	
Thymic lymphoma		2	1	1			1	2	4
Liver necrosis		1							
Pancreatitis				1					
Chronic nephritis					1				
Skin carcinoma					1				
Squamous cell carcinoma (stomach)						1			
Ulcerative dermatitis (skin)			1			1	1	2	

Gastroenteritis							1		
Fibrosarcoma (skin)							1		1
Abscesses/peritonitis							1		
Dermatitis			1						

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

Body weight change: No toxicologically significant body weight changes were noted. At the end of treatment period, mean body weights of the treated groups (Groups 1, 2 and 3) differed from that of the controls by 2.2% (+ 1.0 g), 2.1% (+ 0.9 g) and 0.8% (+ 0.4 g) in males, and by –2.6% (- 1.1 g), -5.5% (- 2.3 g) and 0.2% (+ 0.1 g) in females, respectively.

Food consumption: Group 3 animals seemed to have slightly less food consumption than the controls in several weeks (by up to 3.5 g/week in males, beginning at Week 10, and 6.3 g/week in females, beginning at Week 6). The toxicological relevance of the reduced food consumption was not clear. The reduction could be related to the taste change by the drug.

Effective dose: The effective dose is summarized in the table below. It seemed that the dose was generally conformed closely to the intended dose in all groups.

Effective drug consumption (mg/kg)

	Males			Females		
Group	1	2	3	1	2	3
Intended dose	2	10	40	2	10	40
Effective dose	2	10	40	2.1	10.4	41.4
% intended dose (%)	101	100	100	104	104	104
Range						

Water consumption: No treatment-related differences were noted.

Hematology: No treatment-related changes were noted.

Gross pathology: No treatment-related changes were noted.

Histopathology: No drug-related toxic effects were observed. No drug-related neoplastic lesions were noted. A number of neoplastic lesions were diagnosed (see tables below). The type and incidence, as well as the organ distribution, were considered to be similar in both treated and control mice. The sponsor indicated that all non-neoplastic and neoplastic changes observed were considered incidental findings that were commonly diagnosed in mice of this strain and age. The incidence of adenomas in the Harderian glands in Group 3 males (14%) showed a positive trend with statistical significance ($P < 0.0026$). The incidence, slightly higher than Group 1 (10.2%), was within historical control range (0-14%), and was not considered toxicologically significant.

Number of animals with benign neoplasms by organ/group/sex

	Males					Females				
Group	**0**	**4**	**1**	**2**	**3**	**0**	**4**	**1**	**2**	**3**
N	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Lungs										
Adenoma	10	8	13	15	9	2	5	3	6	8

Liver										
Hepatocyte adenoma	3	0	2	2	0	0	0	0	1	1
Hemangioma	0	1	0	1	0					
Pancreas										
Islet-cell adenoma	0	0	0	0/49	1					
Testes										
Leydig cell tumor	2	3	1	2	2					
Thyroid gland										
Follicular adenoma	0/49	0/48	0	0	1/49	1	0/48	0/49	0	0/49
Adrenal cortex										
A-cell adenoma	0/48	0/49	1/49	0/49	0					
Harderian gland										
Adenoma	5/49	0/49	0	0	7	0/49	0	1	1	1
Body cavities										
Lipoma	2/3	2/2		0/1	2/3	1/1	1/1	1/1	2/2	
Hibernoma	0/3	0/2		1/1	0/3					
Ovaries										
Granulosa cell tumor						3	1	0/49	1	0
Theca cell tumor						0	0	1/49	0	0
Sertoli cell tumor						0	0	0/49	1	0
Luteoma						1	0	1/49	0	0
Tubular adenoma						0	1	0/49	0	0
Teratoma						0	1	0/49	0	0
Hemangioma						0	0	0/49	1	0
Uterus										
Granular cell tumor						1/48	0/48	0	0	0
Leiomyoma						0/48	2/48	1	0	1
Hemangioma						0/48	0/48	0	1	0
Cervix										
Leiomyoma						0/42	0/49	0	0/47	1/44
Granular cell tumor						0/42	0/49	1	0/47	0/44
Mesent. Lymph node										
Hemangioma						0/47	0/46	0/47	1/48	0/45
Skin (abdominal)										
Keratoacanthoma						1	0	0	0	0

Number of animals with malignant neoplasms by organ/group/sex

	Males					Females				
Group	**0**	**4**	**1**	**2**	**3**	**0**	**4**	**1**	**2**	**3**
N	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Lungs										
Carcinoma	1	2	1	2	1	2	1	0	0	1
Stomach										
Squamous carcinoma	0	0	0	1/49	0					
Liver										
Hepatocyte carcinoma	2	0	2	0	2					
Hemangiosarcoma	0	0	1	1	0					
Hemolymphoreticular Sys.										
Thymic lymphoma	0	4	5	3	3	12	20	23	23	23
Non-thymic lymphoma	1	0	4	3	1	0	1	1	1	1
Nonspecific lymphoma	1	0	0	0	0					
Mast cell tumor	0	0	1	0	2	1	0	0	1	1
Harderian gland										
Adenocarcinoma	0/49	0/49	1	1	0	1/49	0	0	0	0
Salivary glands										
Carcinoma	1	0	0	0	0					
Uterus										
Leiomyosarcoma						0/48	0/48	1	0	1
Skin (abdominal)										
Fibrosarcoma						0	0	0	1	1
Hemangiosarcoma						0	0	1	0	0
Carcinoma						0	0	1	0	0

Number of animals with neoplasms

	Males	Females

Group	0	4	1	2	3	0	4	1	2	3
N examined	50	50	50	50	50	50	50	50	50	50
N affected	21	18	28	25	25	24	27	28	32	33
%	42.0	36.0	56.0	50.0	50.0	48.0	54.0	56.0	64.0	66.0

Number of animals with more than one neoplasm

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
N examined	50	50	50	50	50	50	50	50	50	50
N affected	6	2	4	7	6	2	5	7	8	6
%	12.0	4.0	8.0	14.0	12.0	4.0	10.0	14.0	16.0	12.0

Number of animals with metastases

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
N examined	50	50	50	50	50	50	50	50	50	50
N affected	0	0	0	0	0	1	0	0	0	0
%	0	0	0	0	0	2.0	0	0	0	0

Number of primary, benign, malignant neoplasms/group/sex

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
Primary tumor	27	20	33	32	31	26	33	36	41	41
Benign	22	14	18	21	22	10	11	9	15	12
Malignant	5	6	15	11	9	16	22	27	26	29

TK: Only plasma concentrations were measured. The data are summarized in the table below. No accumulation was observed. It seemed that the concentrations in the males were higher than those in the females.

Mean plasma WAL 801 CL concentrations in mice treated the drug at 40 mg/kg (ng/ml ± SD)

Week	1	24	48	64	78	Mean
Males	4.72±1.32	4.36±1.42	4.76±1.16	3.93±0.96	5.92±2.64	**4.74±1.62**
Females	3.24±1.46	2.86±1.04	3.36±0.39	2.84±0.65	3.52±1.69	**3.16±1.04**

Summary: Mice (Chbi:NMRI) were treated orally (by feed) with WAL 801 CL at 0, 2, 10 and 40 mg/kg for 18 months. Deaths occurred in 45 mice during the study period, and were not considered drug-related. No treatment-related abnormal changes in body weights, hematology and gross necropsy were observed. Slightly reduced food consumption was noted in high dose males and females. In histopathologic examinations, all non-neoplastic and neoplastic changes observed were considered incidental findings that were commonly diagnosed in mice of this strain and age.

There are several deficiencies in this study. The treatment duration is only 18 months, not 2 years that are recommended by ICH guideline. The dose selection was not concurred by the Agency. It is clear from the review that the dose of 40 mg/kg is not an MTD as defined in ICH guideline S1C. The high dose was selected based on a high multiple of the intended human therapeutic dose. The drug is positive in Ames test and in *in vitro* cytogenetic assay with human peripheral blood lymphocytes. According ICH guideline S1C, pharmacokinetic endpoints for dose selection can only be used for non-genotoxic pharmaceuticals. The dose selection for this study is not valid.

U91-0017: WAL 801 CL: Plasma concentrations of WAL 801 CL in parallel to a carcinogenicity study over 18 months in mice (administered in the diet). Vol. 28, Page 052

Key study findings: The plasma drug concentrations did not change significantly within the period of study. Higher concentrations were noted in males.

Document #: U91-0017
Study #: F81
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim, Germany
Date of study initiation: June 8, 1986
GLP compliance: No
QA report: Yes ( ) No (X)
Species/strain: Mice/Chbi:NMRI, SPF
N: 90/sex
Age/weight: 42±2 days old, males: 30.7 g, females: 26.4 g
Route: Oral (by feed)
Dosage: 40 mg/kg/day x 52 weeks
Drug: WAL 801 CL (Batch #s: IX)
Methods: [redacted]

This study was conducted in parallel to the 18-month mouse carcinogenicity study (Study F81). The purpose of this TK assay in the satellite group was to determine the absorption of the drug and the steady state values during the 18-month carcinogenicity study period. Blood samples were collected from 15 animals/sex by heart puncture at 10 am (4 hr after the start of the light phase) in Weeks 1, 24, 48, 64, and 78.

Results:

The results, summarized in the table below, showed the exposure of the animals to WAL 801 CL during the study period. The plasma concentrations in males were higher than those in female mice in all testing weeks. No accumulation was seen between Week 1 and later weeks, indicating the attainment of the steady state of plasma concentrations within Week 1.

Mean plasma concentrations of WAL 801 CL in mice (ng/ml, mean ± SD)

Weeks					
1	24	48	64	78	Mean
Males					
4.72±1.32	4.36±1.42	4.76±3.93	3.93±0.96	5.92±2.64	4.74±1.62
Females					
3.24±1.46	2.86±1.04	3.36±0.39	2.84±0.65	3.52±1.69	3.16±1.04

In conclusion, WAL 801 CL was absorbed systemically after oral administration in mice. The plasma drug concentrations did not change significantly within the period of study. Higher values were noted in male rats.

U91-0625: WAL 801 CL: 2-year carcinogenicity study in the rat (administered in the diet). Vol. 32, Page 001

Key study findings:
Hemangiomas in mesenteric lymph nodes in HD F, not treatment-related.
Adenocarcinomas in mammary glands in HD F.
Study number: F87
Doc. number: U91-0625
Study Purpose: To determine whether WAL 801 CL possess a carcinogenic effect following oral administration to the rat for 2 years.
Conducting laboratory and location: Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, D-6507 Ingelheim
Study Date: September 1986 to October 1988
GLP Compliance: Yes
QA Report: Yes
Drug, lot#, and % purity: WAL 801 CL, Batch N^{o}: X
CAC concurrence: No
Study type 2 yr bioassay
Species/strain: Rats/Chbb:THOM
Number/sex/group; age at start of study: 50/sex/group, 42±2 days old, males: 175.2 g, females: 149.1 g

Group	Dose (mg/kg)	N/sex
0	Control A	50
1	2	50
2	10	50
3	40	50
4	Control B	50

Drug stability/homogeneity: Adequate
Methods:
Doses: 2, 10 and 40 mg/kg/day
Basis of dose selection: A high multiple (100-200 times) of the intended human therapeutic dose
Restriction paradigm for dietary restriction studies: N/A
Route of administration: Dietary
Frequency of drug administration: N/A, diet
Dual controls employed: Yes
Interim sacrifices: No
Satellite PK or special study groups: 20 rats/sex at 40 mg/kg only (PK)
Observations and times:
Clinical signs: At least once daily
Body weights: Weekly
Food consumption: Weekly
Water consumption: Weeks 13, 26, 40, 51, 65, 78, 91 and 104
Effective dose: Weekly for the 1st 26 weeks, and monthly thereafter
Hematology: At termination, surviving animals and in animals sacrificed moribund
Clinical chemistry: Not performed
Organ weights: Not performed
Gross pathology: At termination
Histopathology: At termination, the following tissues in all groups were collected and determined: trachea, lungs, thyroid/parathyroid gland, thymus, aorta, heart, tongue, salivary glands, cervical lymph nodes, esophagus, stomach, duodenum/jejunum/ileum,

cecum/colon/rectum, mesenteric lymph node, liver, pancreas, spleen, adrenal glands, kidneys, urinary bladder, gonads, prostate, seminal vesicles, uterus, cervix, vagina, mammary tissue, skin, sternum, bone/bone marrow (femur), skeletal muscle, brain, pituitary gland, spinal cord, sciatic nerve, eyes, optic nerve, Harderian glands, all gross lesion.
Toxicokinetics: Blood samples were collected from 12 animals/sex each time at 7 am and 1 pm in weeks 1, 24, 48 and 64.

Results:

Mortality: 115 premature decedents (43 males and 72 females) occurred in this study. Based on the distribution, severity and post-mortem examination data (see tables below), it seemed that the deaths were not drug-related. There were no significant differences in the time of mortality occurrence between treated and control rats.

Mortality data of rats in the 2-year carcinogenicity study

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Died	2	3	3	2	1	2	0	0	2	4
Sacrificed	6	13	5	10	14	16	4	12	6	10
Total	8	16	8	12	15	18	4	12	8	14
%	16	32	16	24	30	36	8	24	16	28

Cumulative mortality data of rats in the 2-year carcinogenicity study (%)

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Week 2	0	0	0	0	0	0	0	0	0	2
Week 7	0	0	0	0	0	0	0	0	2	2
Week 12	0	0	2	0	2	2	2	0	2	4
Week 17	4	2	4	2	10	4	2	2	4	8
Week 20	10	12	12	6	20	16	6	12	8	12
Week 24	16	32	16	24	30	36	8	24	16	28

Causes of death and intercurrent sacrifice

Group	**0 (Control A)**		**4 (Control B)**		**1**		**2**		**3**	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total	**8**	**16**	**8**	**12**	**15**	**18**	**4**	**12**	**8**	**14**
Spinal cord/brain schwannoma	1				1					
Adrenal cortex adenocarcinoma	1									
Adrenal medulla tumor (malignant)	1	1							1	
Liver carcinoma	1		1							
Hind limb paraplegia	1									
Pituitary gland adenoma		9		5	1	9	1	7	1	8
Pyelonephritis		1								
Bronchopneumonia		1			1					
Brain oligodendroglioma/malignant			1		1					
Not evident	2	1	1							
Chronic nephropathy		1								
Uterus hemorrhage		1								1
Mammary gland fibroadenoma		1		1		2		2		1
Mammary gland squamous carcinoma						1				1
Histiocytic sarcoma					1				1	
Malignant fib. Histiocytoma								1		
Skin histiocytoma/benign					1					
Pulmonary granulomas					1					
Skin fibroma					3					
Heart circulatory failure					1					
Heart atrial thrombus					1					
Liver cholangiocarcinoma					1					1
Thymic lymphoma	1		1	2	1		1		1	

Nonthymic lymphoma						1			1	
Liver necrosis						1				
Uterus stromal cell sarcoma						1				
Uterus adenocarcinoma				1						
Uterus fibroma				1						
Pneumonia							1			
Brain granular cell tumor			1					1		
Stomach leiomyosarcoma								1		
Kidney hemangiosarcoma									1	
Kidney chronic nephropathy				1						
Mast cell tumor									1	
Skin sarcoma			1		1	1	1		1	
Bone osteochondroma						1				
Oral cavity ulcerative stomatitis						1				
Oral cavity ameloblastoma			1							
Lungs edema										1
Body cavity sarcoma										1
Skeletal muscle hemorrhagic necrosis			1							
Brain reticulosis				1						

Clinical signs: No treatment-related clinical signs were noted. In the examinations for nodules and masses in the mammary gland, high dose females showed a higher incidence (8/50) relative to the controls (4-5/50).

Body weight change: At the end of the treatment period, mean body weights of the treated groups (Groups 1, 2 and 3) differed from that of the controls by -3.2% (-19.7 g), -2.1% (-13.1 g) and -2.2% (-13.3 g) in males, and by 3.2% (10.7 g), 0.4% (1.5 g) and –0.7% (-2.5 g) in females, respectively. All treated male groups showed decreased body weight gain relative to the controls. However, the decreases were so slight (4.8%, 3.3% and 2.5% for Groups 1, 2 and 3, respectively) that they might not be toxicologically significant.

Food consumption: Group 3 male animals seemed to have slightly less food consumption than the controls in most weeks (by up to 17.32 g/week or 10.6%). In females, there were no toxicologically significant changes in food consumption.

Effective dose: The effective dose is summarized in the table below. It seemed that the dose was generally corresponded closely to the intended dose in all groups.

Effective drug consumption (mg/kg)

	Males			Females		
Group	1	2	3	1	2	3
Intended dose	2	10	40	2	10	40
Effective dose	2.0	9.9	40.0	2.0	10.1	40.0
% intended dose (%)	104	99	100	101	101	100
Range						

Water consumption: No treatment-related differences were noted.

Hematology: No treatment-related changes were noted based on the information the sponsor provided. (**Reviewer's Comments:** No detailed data were submitted.)

Gross pathology: The gross changes in the pituitary (enlargement and hemorrhage) were noted with the higher incidence in the female treated groups (see table below). These changes were considered spontaneous because no dose-dependence was found in the

treated groups, and no differences in histopathology examinations were noted. No other treatment-related changes were noted.

Gross changes in the pituitary (enlargement and hemorrhage)

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N	50	50	50	50	50	50	50	50	50	50
%	2	44	10	47	6	66	7	66	5	58

Histopathology: No non-neoplastic lesions were considered drug-related. Regarding neoplastic findings, a number of neoplastic lesions were observed (see tables below). Except for adenocarcinomas of the mammary gland and hemangiomas of the mesenteric lymph nodes in females, the type and incidence as well as the organ distribution were not distinguishable between control and treated animals. The number of primary neoplasms, the number of rats with primary neoplasm, the number of rats with metastases, and the number of combined benign and combined malignant neoplasms per dose group and sex were similar in both treated and control rats.

Number of animals with benign neoplasms by organ/group/sex

	Males					Females				
Group	**0**	**4**	**1**	**2**	**3**	**0**	**4**	**1**	**2**	**3**
N	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Brain										
Oligodendroglioma	0	1	0	0	0					
Ependymoma	0	0	1	0	0					
Oral cavity										
Ameloblastoma		1/1			0/1					
Liver										
Hepatocyte adenoma	0	0	1	2	2	0	0	0	0	1
Cholangioma	0	0	1	0	0	0	1	1	0	1
Pancreas										
Acinar adenoma	0	0	0	0	1					
Islet-cell adenoma	1	3/49	0	0	0	0	1/48	0	0	0
Testes										
Leydig cell tumor	11	13/49	7	8	10					
Pituitary gland										
Adenoma	3/49	4	5	6	6	26	26	28	33	32
Thyroid gland										
C-cell adenoma	6	6/49	3	1	3	0	5	3	2	2
Follicular adenoma	2	0	0	1	1	0	0	0	0	1
Parathyroid gland										
Adenoma	3/49	2/49	0	0	0	0	0	1	0	0
Adrenal cortex										
Adenoma	1	1	0	1	1	0	0	1	0	0
Adrenal medulla										
Medulla tumor	5	4	1	4	2/49	1	2	2	3	0
Ganglioneuroma	0	0	0	1	1/49					
Mammary gland										
Fibroadenoma						8/49	7/45	3/48	5/49	5/47
Adenoma						1/49	0/45	1/48	0/49	0/47
Bone										
Osteochondroma								1/1		
Ovaries										
Granulosa cell tumor						1	2	0	1	2
Theca/granul. cell tumor						2	2	0	6	4
Uterus										
Fibroleiomyoma						0	0	1	0	0
Leiomyoma						1	3	0	2	0
Fibroma						0	1	0	0	1
Mesent. Lymph node										
Hemangioma	2	5	1	7/49	2/49	2/49	0	0	2	4
Skin (abdominal)										
Keratoacanthoma	4	1	3	2	5					
Lipoma	0	1	0	0	0					
Fibroma	1	2	4	0	0	1	0	1	0	0
Histiocytoma	0	0	1	0	1	0	0	0	1	0
Sebaceous adenoma	1	0	0	0	0					
Trichoepithelioma	0	1	0	0	0					

Number of animals with malignant neoplasms by organ/group/sex

	Males					Females				
Group	**0**	**4**	**1**	**2**	**3**	**0**	**4**	**1**	**2**	**3**
N	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Brain										
Oligodendroglioma	0	0	1	0	0					
Schwannoma	0	0	1	0	0					
Focal reticulosis						0	1	0	0	0
Granular cell tumor	0	1	1	0	0	0	0	0	1	0
Spinal cord										
Schwannoma	1	0	0	0	0					
Heart										
Endocardial sarcoma	5	1	1	0	0	1	2	2	1	1
Oral cavity										
Hemangiosarcoma		0/1			1/1					

Jejunum										
Hemangiosarcoma	0/49	0/48	1	0	0					
Ileum										
Stromal sarcoma						1/47	0/48	0/49	0/49	0/49
Adenosarcoma	1/48	0/48	0	0	0					
Pancreas										
Carcinoma/islet cell	0	0/49	1/49	0	0					
Kidneys										
Lipomatous tumor						0	0	1	0	0
Hemangiosarcoma	0	0	0	0	1					
Urinary bladder										
Transit cell carcinoma	1	0	0	1	0					
Adrenal cortex										
Adenocarcinoma	1	0	0	0	0					
Adrenal medulla										
Medulla tumor	2	1	2	3	1/49	1	0	0	0	0
Spleen										
Hemangiosarcoma	0	0	1	0	0					
Body cavity										
Sarcoma			¼	0/1	0/3	0/1	0/2	0/6	0/4	1/3
Undiff. Cyst. Sarcoma						0/1	0/2	1/6	0/4	1/3
Mesothelioma			¼	0/1	0/3					
Stomach										
Leiomyosarcoma						0	0	0	1	0
Liver										
Hemangiosarcoma						0	1	0	0	0
Hepatocyte carcinoma	3	1	1	0	1					
Cholangiosarcoma	0	0	1	0	0					
Systemic neoplasms										
Thymic lymphoma	1	1	4	1	2	0	3	3	0	1
Non-thymic lymphoma	0	0	0	0	1	0	0	2	1	0
Mal. Fib. Histiocytoma						0	0	0	1	0
Histiocytic sarcoma	0	0	1	0	1	0	0	1	1	0
Mast cell tumor	0	0	0	0	1	0	0	0	1	0
Mammary gland										
Adenocarcinoma						0/49	0/45	1/48	2/49	6/47
Squamous carcinoma						1/49	0/45	1/48	0/49	0/47
Comedocarcinoma	0/20	0/16	0/29	0/16	1/19					
Cecum										
Adenocarcinoma						1/48	0	0	0	0
Uterus										
Granular cell tumor						0	1	0	0	0
Adenocarcinoma						0	1	0	0	1
Squamous carcinoma						0	0	1	0	0
Stromal cell sarcoma						0	0	2	0	1
Skin										
Squamous carcinoma	0	0	0	1	1	1	0	2	0	0
Sebaceous carcinoma	0	0	1	0	0					
Hemangiosarcoma	0	1	0	0	0					
Basalioma						0	0	0	1	0
Ossifying sarcoma	0	0	1	0	0					
Sarcoma	0	0	0	0	1	0	1	0	0	0
Thyroid gland										
C-cell carcinoma						2/49	0	0	0	0
Parathyroid glands										
Carcinoma						0/49	0/49	0	1/49	0

Number of animals with neoplasms

	Males					Females				
Group	**0**	**4**	**1**	**2**	**3**	**0**	**4**	**1**	**2**	**3**
N examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
N affected	36	36	35	30	34	33	38	39	42	40
%	72	72	72	62	68	66	76	78	84	80

Number of animals with more than one neoplasm

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
N examined	50	50	50	50	50	50	50	50	50	50
N affected	15	12	13	7	13	14	16	15	18	18
%	30	24	26	14	26	28	32	30	36	36

Number of animals with metastases

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
N examined	50	50	50	50	50	50	50	50	50	50
N affected	2	0	1	1	2	1	1	1	0	0
%	4	0	2	2	4	2	2	2	0	0

Number of primary, benign, malignant neoplasms/group/sex

	Males					Females				
Group	0	4	1	2	3	0	4	1	2	3
Primary tumor	57	54	52	40	49	51	60	62	66	65
Benign	42	48	32	34	37	43	50	45	55	53
Malignant	15	6	20	6	12	8	10	17	11	12

The incidence of hemangiomas in the mesenteric lymph nodes was 4, 10, 2, 14.3 and 4.1% in male Groups 0, 4, 1, 2 and 3, respectively. In females, the incidence was 4.1, 0, 0, 4 and 8% in Groups 0, 4, 1, 2 and 3, respectively. When tested for a positive trend with respect to dose rates, hemangiomas in females yielded a one-tailed $p = 0.0048$. However, the incidence in Group 3 females was lower than that in Group 4 males, which indicated that these changes might not be treatment-related.

The number of adenocarcinomas in the female mammary gland were 0, 0, 1, 2 and 6 in Groups 0, 4, 1, 2 and 3, respectively (0, 0, 2.1, 4.1 and 12.8%). When tested for a positive trend with respective to dose rates, adenocarcinomas in females yielded a one-tailed $p < 0.005$. The sponsor considered that the malignant mammary tumors were a random event and unrelated to treatment because of the following points:

1. Historical control data showed that the incidence of adenocarcinoma in female mammary gland was 0-10%.
2. The mammary adenocarcinomas did not appear earlier, did not show multiple occurrence, and displayed a heterogeneous histopathological growth pattern.
3. The non-neoplastic lesions in the mammary gland did not indicate any growth stimulation.
4. Combing benign and malignant mammary tumors for statistical assessment, there is no significant trend and no significant difference between treated and control rats.

Histopathological findings in mammary glands

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N	20	49	16	45	29	48	16	49	19	47
Lacteal cyst	0	9	0	3	0	3	0	3	0	6
Mineralization	0	0	0	0	0	0	0	1	0	0
Focal hyperplasia	0	2	0	1	0	0	0	2	0	0
Adenoma	0	1	0	0	0	1	0	0	0	0
Fibroadenoma	0	8	0	7	0	3	0	5	0	5
Adenocarcinoma	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**2**	**0**	**6**
Squamous carcinoma	0	1	0	0	0	1	0	0	0	0
Comedocarcinoma	0	0	0	0	0	0	0	0	1	0
Total tumors	0	10	0	7	0	6	0	7	1	11

TK: Only plasma concentrations were measured. The data are summarized in the table below.

Mean plasma WAL 801 CL concentrations in rats treated the drug at 40 mg/kg (ng/ml)

Week	1	24	48	64
7 AM				
Males	3.52	16.42	11.45	5.68
Females	4.18	12.12	10.05	20.71
1 PM				
Males	2.32	12.32	10.21	3.92
Females	2.93	6.87	7.16	15.43

Summary: Rats (Chbb:THOM) were treated orally (by feed) with WAL 801 CL at 0, 2, 10 and 40 mg/kg for 2 years. Deaths occurred in 115 rats during the study period, and were not considered drug-related. No treatment-related, toxicologically significant abnormal changes in body weights, hematology and gross necropsy were observed. Slight reduced food consumption was noted in high dose males. In histopathological examinations, no drug-related non-neoplastic changes were observed. In the examinations on female mammary glands, treated animals showed an increased incidence of adenocarcinomas, which could be treatment-related although the sponsor claimed that this change was random and not treatment-related.

The dose selection was not concurred by the Agency. It is clear from the review that the dose of 40 mg/kg is not an MTD defined in ICH guideline S1C. The high dose was selected based on "a high multiple of the intended human therapeutic dose". The sponsor indicated that the high dose selected here "fulfills the conditions of the directive of being at least 100-times the human therapeutic dose". However, the drug is positive in *in vitro* cytogenetic assay with human peripheral blood lymphocytes. According ICH guideline S1C, pharmacokinetic endpoints for dose selection can only be used for non-genotoxic pharmaceuticals. Therefore, the dose selection for this study is not valid.

SUMMARY OF CARCINOGENICITY:

Carcinogenicity studies were conducted in mice and rats because epinastine HCl was originally developed as an oral tablet for chronic administration. The dose selection was not concurred by the agency. The high dose of 40 mg/kg/day was selected by the sponsor because it was 200 fold the anticipated human oral dose, which was one of the acceptable criteria for dose selection according to European guidelines when studies were conducted (1986-1988).

In both rat and mouse carcinogenicity studies, a dose of 40 mg/kg was chosen as the high dose. It is clear from the review that the dose of 40 mg/kg is not an MTD as defined in ICH guideline S1C. In both studies, the high dose was selected based on "a high multiple of the intended human therapeutic dose". The sponsor indicated that the high dose selected here "fulfills the conditions of the directive of being at least 100-times the human therapeutic dose". However, the drug is positive in *in vitro* cytogenetic assay with human peripheral blood lymphocytes. According ICH guideline S1C, pharmacokinetic endpoints for dose selection can only be used for non-genotoxic pharmaceuticals. Therefore, the dose selection for these 2 carcinogenicity studies is not valid, and the studies are not acceptable. Furthermore, the guidance available in the United States may be somewhat different than those in other regions (i.e., 100 times of human dose)

Mouse study: No treatment-related neoplastic findings were observed.

Rat study: In Group 3 females, the incidence of hemangiomas in the mesenteric lymph node and adenocarcinomas in the mammary glands was higher relative to the controls.

The incidence of hemangiomas in the mesenteric lymph nodes was 4, 10, 2, 14.3 and 4.1% in male Groups 0, 4, 1, 2 and 3, respectively. In females, the incidence was 4.1, 0, 0, 4 and 8% in Groups 0, 4, 1, 2 and 3, respectively. When tested for a positive trend with respect to dose rates, hemangiomas in females yielded a one-tailed $p = 0.0048$. However, the incidence in Group 3 females was lower than that in Group 4 males, which indicated that these changes might not be treatment-related.

The number of adenocarcinomas in the female mammary gland were 0, 0, 1, 2 and 6 in Groups 0, 4, 1, 2 and 3, respectively (0, 0, 2.1, 4.1 and 12.8%). When tested for a positive trend with respective to dose rates, adenocarcinomas in females yielded a one-tailed $p < 0.005$. The sponsor considered that the malignant mammary tumors were a random event and unrelated to treatment. Because of the following points:

1. Historical control data showed that the incidence of adenocarcinoma in female mammary gland was 0-10%.
2. The mammary adenocarcinomas did not appear earlier, did not show multiple occurrence, and displayed a heterogeneous histopathological pattern.
3. The non-neoplastic lesions in the mammary gland did not indicate any growth stimulation.
4. Combing benign and malignant mammary tumors for statistical assessment, there is no significant trend and no significant difference between treated and control rats (see table below).

Histopathological findings in mammary glands

Group	0 (Control A)		4 (Control B)		1		2		3	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
N	20	49	16	45	29	48	16	49	19	47
Lacteal cyst	0	9	0	3	0	3	0	3	0	6
Mineralization	0	0	0	0	0	0	0	1	0	0
Focal hyperplasia	0	2	0	1	0	0	0	2	0	0
Adenoma	0	1	0	0	0	1	0	0	0	0
Fibroadenoma	0	8	0	7	0	3	0	5	0	5
Adenocarcinoma	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**2**	**0**	**6**
Squamous carcinoma	0	1	0	0	0	1	0	0	0	0
Comedocarcinoma	0	0	0	0	0	0	0	0	1	0
Total tumors	**0**	**10**	**0**	**7**	**0**	**6**	**0**	**7**	**1**	**11**

The reviewing pharmacologist is not convinced by the sponsor that the malignant tumors were a random event and unrelated to treatment. If the doses were higher, a higher incidence of adenocarcinomas might have occurred. The current control rats showed no adenocarcinomas. These results will be presented to ECAC to determine the final disposition and potential future requirements regarding this study.

ECAC Meeting Minutes

Executive CAC
September 18, 2001
Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Al DeFelice, Ph.D., HFD-110, Alternate Member
Robert Osterberg, Ph.D., Team Leader
Zhou Chen, Ph.D., Presenting Reviewer
Author of Minutes: Zhou Chen
The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.
IND 61,025
Drug Name: Epinastine
Sponsor: Allergan
Background:
Epinastine is a histamine H_1 receptor antagonist. The sponsor is seeking to develop epinastine HCl ophthalmic solution for the prevention of allergic conjunctivitis. In the original IND submission, 2 carcinogenicity study reports from Boehringer-Ingelheim GmbH were included. The sponsor requested prompt response from FDA regarding the reports.

Mouse Carcinogenicity Study:
MOUSE STUDY DURATION: 18 months
MOUSE STRAIN: Chbi/NMRI
ROUTE: Oral (in feed)
DOSING COMMENTS: No.
NUMBER OF MICE:
- Control-1 (C1): 50/sex
- Control-2 (C2): 50/sex
- Low Dose (LD): 50/sex

- Middle Dose (MD): 50/sex
- High Dose (HD): 50/sex
MOUSE DOSE LEVELS (mg/kg/day):
- Low Dose: 2
- Middle Dose: 10
- High Dose: 40
(Dose adjusted during study) No.
BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): Dose selection was based on a high multiple of the intended human therapeutic dose.
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No.
MOUSE CARCINOGENICITY (conclusion: negative;positive;MF;M;F): Negative.
MOUSE TUMOR FINDINGS (details):
A number of neoplastic lesions were diagnosed. The type and incidence, as well as the organ distribution, were considered to be similar in both treated and control mice. The incidence of adenomas in the Harderian glands in Group 3 males (14%) showed a positive trend with statistical significance ($P < 0.0026$). The incidence, slightly higher than Group 1 (10.2%), was within historical control range (0-14%), and was not considered toxicologically significant.
MOUSE STUDY COMMENTS:
The dose selection was not concurred by the Agency. It is clear from the review that the dose of 40 mg/kg is not an MTD defined in ICH guideline S1C. The high dose was selected based on a high multiple of the intended human therapeutic dose. The drug is positive in Ames test and in two *in vitro* cytogenetic assays. According ICH guideline S1C, pharmacokinetic endpoints for dose selection can only be used for non-genotoxic pharmaceuticals. The dose selection for this study is not valid.

Rat Carcinogenicity Study:

RAT STUDY DURATION (weeks): 104 weeks
RAT STRAIN: Chbb:THOM
ROUTE: Oral (in feed)
DOSING COMMENTS: No.
NUMBER OF RATS:
- Control-1 (C1): 50/sex
- Control-2 (C2): 50/sex
- Low Dose (LD): 50/sex
- Middle Dose (MD): 50/sex
- High Dose (HD): 50/sex
RAT DOSE LEVELS (mg/kg/day):
- Low Dose: 2
- Middle Dose: 10
- High Dose: 40
(Dose adjusted during study): No
BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): Dose selection was based on a high multiple of the intended human therapeutic dose.
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): No.
RAT CARCINOGENICITY (conclusion: negative;positive;MF;M;F):
Adenocarcinomas in mammary glands in HD F.
RAT TUMOR FINDINGS (details):

A number of neoplastic lesions were observed. Except for adenocarcinomas of the mammary gland and hemangiomas of the mesenteric lymph nodes in females, the type and incidence as well as the organ distribution were not distinguishable between control and treated animals. The number of primary neoplasms, the number of rats with primary neoplasm, the number of rats with metastases, and the number of combined benign and combined malignant neoplasms per dose group and sex were similar in both treated and control rats.

The incidence of hemangiomas in the mesenteric lymph nodes was 4, 10, 2, 14.3 and 4.1% in male Groups 0, 4, 1, 2 and 3, respectively. In females, the incidence was 4.1, 0, 0, 4 and 8% in Groups 0, 4, 1, 2 and 3, respectively. The incidence in high dose females was lower than that in Control 2 (Group 4) males, which indicated that these changes might not be treatment related.

The number of adenocarcinomas in the female mammary gland was 0, 0, 1, 2 and 6 in Groups 0, 4, 1, 2 and 3, respectively (0, 0, 2.1, 4.1 and 12.8%). When tested for a positive trend with respective to dose rates, adenocarcinomas in females yielded a one-tailed $p < 0.005$. Similar findings were not observed in mice. The sponsor considered that the malignant mammary tumors were a random event and unrelated to treatment because of the following points:

1. Historical control data showed that the incidence of adenocarcinoma in female mammary gland was 0-10%.
2. The mammary adenocarcinomas did not appear earlier, did not show multiple occurrence, and displayed a heterogeneous histopathological growth pattern.
3. The non-neoplastic lesions in the mammary gland did not indicate any growth stimulation.
4. Combing benign and malignant mammary tumors for statistical assessment, there is no significant trend and no significant difference between treated and control rats (see table below).

study group	0		4		1		2		3	
no. animals	50 m	50 f	50 m	50 f	50 m	50 f	50 m	50 f	50 m	50 f
dose (oral, diet)	control A		control B		2 mg/kg		10 mg/kg		40 mg/kg	
no. of mammary glands evaluated	20	49	16	45	29	48	16	49	19	47
lacteal cyst	-	9	-	3	-	3	-	3	-	4
mineralization	-	-	-	-	-	-	-	1	-	-
focal hyperplasia	-	2	-	1	-	-	-	2	-	-
adenoma	-	1	-	-	-	1	-	-	-	-
fibroadenoma	-	8	-	7	-	3	-	5	-	5
adenocarcinoma	-	-	-	-	-	1	-	2	-	6
squamous carcinoma	-	1	-	-	-	1	-	-	-	-
carcinosarcinoma	-	-	-	-	-	-	-	-	1	-
total tumours	-	10	-	7	-	6	-	7	1	11

In "Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies" (McConnell *et al.*, 1986), it is indicated that various types of carcinomas can be combined for evaluation, and sometimes benign and malignant epithelial neoplasms can be combined for evaluation. In that case, the incidence of epithelial neoplasms in the female mammary gland was 10, 7, 6, 7 and 11 in Groups 0, 4, 1, 2 and 3, respectively (20.4, 15.6, 12.5, 14.3 and 23.4%). The incidence rate was within the historical control range, and there was no statistical significance.

RAT STUDY COMMENTS:

The dose selection is not valid.