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RESEARCH**

APPLICATION NUMBER:

21-775

PHARMACOLOGY REVIEW(S)

Comments on N21-775 Entereg
Abby Jacobs, AD OND/IO
May 6, 2008

I have read the Pharm/tox reviews and the proposed labeling.

I agree that there are no pharm/tox approvability issues for this NDA. The latest labeling proposed for the pharm/tox sections is reasonable.

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/s/

Abby Jacobs
5/6/2008 12:22:26 PM
PHARMACOLOGIST

osteoma/osteosarcomas in bones of female mice. The sponsor disagreed with the Agency's conclusion, and continued to support their previous interpretations in the study reports and other summary texts. The sponsor stated that the inter-group variation in tumor types noted in the mouse study, which achieved statistical significance in only some analyses, were fortuitous events and not associated with alvimopan treatment. The sponsor believed that results of genotoxicity and carcinogenicity studies identified no risk to patients for the intended post-operative ileus (POI) indication and dose-regime. Sponsor's conclusions were based on the following factors:

1. Analysis of the historic ranges for osteogenic and fibroblastic tumors in CD-1 mice, in comparison with control and treated mice in this study, suggest no treatment related effects.
2. There was no treatment related effect on time of onset, or first tumor observations.
3. There were no common sites of tumor formation for osteogenic and fibroblastic tumors.
4. The presence or degree of statistical significance is dependant on which control group (Water or Vehicle) is used in the comparison.
5. Alvimopan is not mutagenic or clastogenic in standard tests, suggesting no genotoxic carcinogenic risk.
6. There was no evidence for any treatment-related degenerative, inflammatory or proliferative changes, suggestive of non-genotoxic carcinogenesis, in this or previous studies.
7. There was no evidence of an increase in other mesenchymal tumors in alvimopan treated mice.
8. There are no remarkable changes in tumour incidence in rats at doses up to 500 mg/kg/day.

Reviewer's Comments: The Exec CAC met again on January 15, 2008 to discuss sponsor's interpretations and conclusions of the mouse carcinogenicity study. In that meeting, the Agency's statisticians confirmed that alvimopan caused a small, but statistically significant increase in the incidences of fibroma, fibrosarcoma and sarcoma in the skin/subcutis and osteoma/osteosarcoma in bones of female mice. The significance of these findings to humans is unknown. This type of finding, particularly for this type of indication for short-term use, would not generally preclude approval. The carcinogenicity data for alvimopan were evaluated using the Agency's normal criteria used for evaluation of the findings for other drugs. In the absence of historical control data for the vehicle (10% w/v/ aqueous acacia), the Agency considered that vehicle controls is the relevant control for tumor comparison. In addition, sponsor's comparison to the water control was not considered acceptable as per the Agency's criterion for comparison.

In addition, the historical data for the water control had a wide variation and were from a different laboratory. Based on these, we stand by our conclusions that alvimopan caused statistically significant increase in the incidences of fibroma, fibrosarcoma and sarcoma in the skin/subcutis and osteoma/osteosarcoma in bones of female mice.

This submission also responds to the Division facsimile dated December 17, 2007, in which clarification was requested by the Executive CAC for findings in the rat study. The Exec CAC requested the following clarification for the rat carcinogenicity study (from the Exec CAC meeting minutes dated December 4, 2007):

"The Committee tentatively concluded that there were no drug-related tumor findings in either sex. However, the Committee asked for a clarification of the discrepancy between the incidence of thymoma (epithelial) in the thymus of male rats in the tumor data set submitted for statistical review (Male: 3 of 57, 0 of 60, 0 of 60, 2 of 58 and 3 of 58 for Group 1, 2, 3, 4 and 5, respectively) and that presented in the study report for the pharmacology/toxicology review (Male: 0 of 57, 0 of 60, 0 of 60, 0 of 58, and 1 of 58 for Group 1, 2, 4, 5 and 6, respectively, from Table 7, page 161 of the study report)."

Sponsor's Clarification for the Rat Carcinogenicity Study as Requested by the Exec CAC:

In the rat carcinogenicity study, groups of 60 male and female rats were assigned to the following treatment groups: Group 1: Water control Group 2: Vehicle control Group 3: 30 mg/kg/day (note – females only) Group 4: alvimopan 100 mg/kg/day Group 5: alvimopan 200 mg/kg/day Group 6: alvimopan 500 mg/kg/day.

As listed in the final report [Table 7, page 169 (all animals)] the incidence of thymoma (epithelial) tumor in the thymus in males was 0 of 57; 0 of 60; 0 of 60; 0 of 58; 1 of 58 animals for Groups 1, 2, 4, 5 and 6, respectively. The thymus was missing from 3 rats from Group 1, 2 rats from Group 5 and 2 from Group 6, and that was why the total number of tissues examined was less than 60 for these dose groups.

As per the direction in the CDER Guidance for Industry (IT3: Providing Regulatory Submissions in Electronic Format; 1999), the missing tissues were included in the tumor dataset submitted for statistical review. In the Complete Response dated August 9, 2007, the numeric code '3' in the ORGANEXM column was used to identify when tissues were missing. The table below (from the sponsor's response) is a section of the tumor dataset extracted to the "Excel" program showing that for males, in addition to the single incidence of thymoma (epithelial), there were 7 occasions when thymus was missing with a group incidence detailed above.

Extract from Tumor Dataset for Thymus (males)

STUDYNUM	ANIMLNUM	DOSEGP	TUMORNAM	ORGANNAM	ORGANEXM
BVR389	2	1		THYMUS	3
BVR389	12	1		THYMUS	3
BVR389	15	1		THYMUS	3
BVR389	196	5		THYMUS	3
BVR389	201	5		THYMUS	3
BVR389	254	6	B-THYMOMA (EPITHELIAL)	THYMUS	1
BVR389	285	6		THYMUS	3
BVR389	292	6		THYMUS	3

The sponsor stated that it would appear that the group incidence for thymoma (epithelial) referred to in the Exec CAC's recommendation and conclusion may assume their occurrence in the missing tissues, resulting in a noted incidence of 3 of 57, 0 of 60, 0 of 60, 2 of 58 and 3 of 58 for Groups 1, 2, 4, 5 and 6, respectively. The sponsor examined their "Excel" spreadsheet derived from the SAS (statistical analysis system) file that had failed to find an explanation why the missing tissues appeared to have been associated with thymoma (epithelial) leading to the discrepancy noted by the Agency. The sponsor confirmed that the incidence of diagnosed thymoma (epithelial) tumors is as stated in the final report.

Reviewer's Comment: Sponsor's above clarification for the rat carcinogenicity data was communicated to the Exec CAC by the reviewer on February 1, 2008. Based on the sponsor's clarification, the statistical reviewer corrected the original tumor data in the rat database and reran the Peto trend test on the corrected data. The result from Peto's trend test shows that the dose response for the incidence of thymoma (epithelial) is not statistically significant ($p > 0.025$). The statistical reviewer concluded that there was no significant tumor finding in the rat carcinogenicity study (Statistical Review of NDA 21-775, Addendum dated February 8, 2008).

RECOMMENDATIONS:

1. We do not concur with sponsor's interpretation and conclusion of the mouse carcinogenicity study results.
2. We stand by our conclusion that alvimopan caused statistically significant increase in the incidences of fibroma, fibrosarcoma and sarcoma in the skin/subcutis and osteoma/osteosarcoma in bones of female mice.
3. Sponsor's clarification for the rat carcinogenicity study as requested by the Exec CAC appears to be adequate and acceptable and the rat study was concluded to be negative as per the Agency's reanalysis of the tumor data based on sponsor's clarification.

Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Date

Comment:

Sushanta K. Chakder, Ph.D.
Acting Team Leader, HFD-180

Date

cc:

HFD-180
HFD-181/CSO
HFD-180/Dr. Chakraborti
HFD-180/Dr. Chakder

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/s/

Tamal Chakraborti
2/11/2008 01:48:09 PM
PHARMACOLOGIST

Sushanta Chakder
2/11/2008 02:35:35 PM
PHARMACOLOGIST

**PHARMACOLOGIST'S REVIEW OF NDA 21-775
(Amendment Dated January 10, 2008)**

Sponsor & Address: Adolor Corporation
Exton, PA

Reviewer: Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

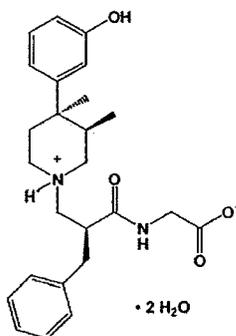
Date of Submission: January 10, 2008

Date of Receipt: January 10, 2008

Date of Review: January 18, 2008

Drug: Alvimopan Capsules (SB-767905)

Structure:



Category: Opioid μ receptor antagonist.

Indication: Alvimopan is indicated for the treatment of ~~_____~~

Submission Contents: The sponsor submitted the report of intravenous bone marrow micronucleus test in mice with ADL 08-0011, the active metabolite of alvimopan.

GENETIC TOXICOLOGY

In Vivo Mouse Micronucleus Assay with ADL 08-0011 Administration)

Key Findings: Negative

Study No.: ~~_____~~ Study No. AC07BM.123.BTL, Adolor Project No. 14TX025

Conducting Laboratory and Location: _____

Date of Study Initiation: September 27, 2007

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ()

Drug: ADL 08-0011, Lot No. AF04011, 99.7% pure

Vehicle: Sodium chloride for injection, 0.9% w/v, USP (saline), adjusted to pH 11, was used as the vehicle for ADL 08-0011 monohydrate.

Methods: The assay was performed in two phases. The first phase, the toxicity study (dose range finding study), was designed to assess the toxicity of the test article and to set dose levels for the definitive micronucleus study. The second phase, the definitive micronucleus study, was designed to evaluate the potential of the test article to increase the incidence of micronucleated polychromatic erythrocytes (MPE) in bone marrow of male and female ICR mice. In the definitive phase of the study, the vehicle was used as the negative control and cyclophosphamide monohydrate (CP), at an intravenous dose of 50 mg/kg, as the positive control. In both phases of the study, test or control articles were administered at a dose volume of 5 mL/kg body weight by a single intravenous injection (IV injection into the animal's lateral tail vein). Animals were observed for signs of toxicity during the course of each study.

For the toxicity study, the test article was administered at 1.25, 2.5, 5 and 10 mg/kg. A dose of 10 mg/kg was considered to be the maximum feasible dose (MFD) based on the limited solubility of the test article in the vehicle (a concentration of 2 mg/mL was the maximum soluble concentration) and maximum good practice dose volume, 5 mL/kg, for intravenous administration of solutions to mice. Five male and female mice were assigned to each dose. No mortality occurred at any dose level during the course of this study. Piloerection was noted in male and female mice in all dose groups following dose administration. Based upon these results, the high dose for the micronucleus test was set at 10 mg/kg, the maximum intravenous feasible dose (MFD).

The micronucleus study consisted of four groups, each containing 10 male and 10 female mice. Mice were treated intravenously (single administration) either with the vehicle control or with ADL 08-0011 monohydrate at 2.5, 5, or 10 mg/kg (5 mL/kg). Five male and five female mice were euthanized 24 hours after treatment and the remaining animals (5/sex) were euthanized 48 hours after treatment. In addition, five male and 5 female mice were assigned to the positive control group and were euthanized 24 hours after treatment. At the time of euthanasia, femoral bone marrow was collected and bone marrow smears (slides) were prepared and stained with May-Gruenwald-Giemsa stain. Two slides were prepared from each mouse. Two-thousand PCEs per mouse were screened (scored) for the presence of micronuclei resulting in evaluation of a total of 10,000 PCEs per each treatment group. The number of normochromatic erythrocytes (NCEs) and micronucleated NCEs (MNCEs) in the field of 1000 total erythrocytes

(ECs) was determined for each animal in order to determine the proportion of polychromatic erythrocytes to total erythrocytes (PCEs/ECs). The incidence of MNCEs per 2000 PCEs was enumerated for each animal. The incidence of micronucleated polychromatic erythrocytes per 2000 PCEs for each mouse and per 10,000 PCEs for each treatment group was determined. The incidence of micronucleated PCEs and the ratio of PCEs to total erythrocytes (PCEs/ECs ratio) served as indication of test article clastogenicity and cytotoxicity, respectively. The following table (from page 14 of sponsor's report) shows the study design.

Treatment (5 mL/kg)	Number of Mice/Sex Dosed	Number of Mice/Sex Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Vehicle Control: 0.9% w/v sodium chloride for injection, USP (saline) adjusted to pH 11	10+3*	5	5
Test Article: ADL 08-0011 monohydrate			
Low dose (2.5 mg/kg)	10+21*	5	5
Mid dose (5 mg/kg)	10+21*	5	5
High dose (10 mg/kg)	10+21*	5	5
Positive Control: CP (50 mg/kg)	5	5	0

*Satellite mice assigned for the toxicokinetic portion of the study.

Toxicokinetics: The definitive study consisted of a total of twenty-five toxicokinetic satellite groups of 3 male and 3 female mice each. In control, blood was collected from three mice at the predose. Blood was collected from 21 mice/dose group at 5 minutes, 15 minutes, 30 minutes, 1 hour, 4 hours, 24 hours or 48 hours post-dose. Mice in the remaining group were treated with the vehicle and were used for blood collection at 5 minutes post-dose. Blood was processed to plasma and plasma will be analyzed for concentrations of ADL 08-0011 free acid for confirmation of systemic exposure. The sponsor stated that the bioanalytical and toxicokinetic reports were not included in this report.

Results:

Study validity: The mean incidence of micronucleated PCEs did not exceed the historical vehicle control range in the vehicle control groups. The vehicle and positive controls were consistent with the historical control data, indicating that all criteria for a valid test were met as described in the protocol. No test article was detected in the vehicle control sample. The results of dosing solution analysis indicated accuracy of preparation and stability of the formulations used in this study.

Study outcome: The incidence of micronucleated PCEs per 10,000 PCEs in test article-treated groups was not statistically increased relative to their respective vehicle controls in either male or female mice, regardless of dose level or bone marrow collection time. Cyclophosphamide, the

positive control, caused a statistically significant increase in micronucleated PCEs in both male and female mice. The following Table (from page 25 of the study report) shows the results.

**Table 8.0-4: Summary of Bone Marrow Micronucleus Analysis
Following a Single Intravenous Dose of ADL 08-0011 monohydrate in ICR Mice**

Treatment (S mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Vehicle**	M	24	5	0.463 ± 0.07	---	0.1 ± 0.22	1 / 10000
	F	24	5	0.449 ± 0.06	---	0.4 ± 0.22	4 / 10000
ADL 08-0011 monohydrate							
2.5 mg/kg	M	24	5	0.482 ± 0.05	4	0.0 ± 0.00	0 / 10000
	F	24	5	0.534 ± 0.07	19	0.2 ± 0.27	2 / 10000
5 mg/kg	M	24	5	0.454 ± 0.03	-2	0.1 ± 0.22	1 / 10000
	F	24	5	0.442 ± 0.05	-2	0.0 ± 0.00	0 / 10000
10 mg/kg	M	24	5	0.436 ± 0.04	-6	0.3 ± 0.27	3 / 10000
	F	24	5	0.476 ± 0.04	6	0.2 ± 0.27	2 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.404 ± 0.07	-13	21.0 ± 3.22	*210 / 10000
	F	24	5	0.377 ± 0.10	-16	29.5 ± 8.23	*295 / 10000
Vehicle**	M	48	5	0.472 ± 0.06	---	0.1 ± 0.22	1 / 10000
	F	48	5	0.454 ± 0.04	---	0.2 ± 0.45	2 / 10000
ADL 08-0011 monohydrate							
2.5 mg/kg	M	48	5	0.467 ± 0.04	-1	0.1 ± 0.22	1 / 10000
	F	48	5	0.474 ± 0.06	4	0.2 ± 0.27	2 / 10000
5 mg/kg	M	48	5	0.512 ± 0.08	8	0.2 ± 0.45	2 / 10000
	F	48	5	0.424 ± 0.05	-7	0.3 ± 0.27	3 / 10000
10 mg/kg	M	48	5	0.424 ± 0.06	-10	0.3 ± 0.27	3 / 10000
	F	48	5	0.448 ± 0.05	-1	0.3 ± 0.27	3 / 10000

*p ≤ 0.05 (Kastenbaum-Bowman Tables)

**Vehicle = 0.9 w/v sodium chloride for injection, USP (saline) adjusted to pH 11

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Conclusions: Under the conditions of the assay, a single intravenous (IV) administration of ADL 08-0011 monohydrate at 2.5, 5 and 10 mg/kg did not cause a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow. Therefore, ADL 08-0011 monohydrate was concluded to be negative in the micronucleus test using male and female ICR mice. It is to be mentioned here that the sponsor did not submit the results of the toxicokinetic analysis in this submission.

SUMMARY AND EVALUATION:

This submission is in response to the Division's recommendation (Division letter dated 10 August, 2007) for a bone marrow micronucleus test with ADL 08-0011 to further explore the genotoxic potential of the metabolite of alvimopan.

Under the conditions of the assay, a single intravenous (IV) administration of ADL 08-0011 monohydrate at 2.5, 5 and 10 mg/kg did not cause a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow. Therefore, ADL 08-0011 monohydrate was concluded to be negative in the micronucleus test using male and female ICR mice. It is to be mentioned here that the sponsor did not submit the results of the toxicokinetic analysis in this submission. The sponsor should be asked to submit the toxicokinetic report for this study.

RECOMMENDATIONS: ADL08-0011, the active metabolite of alvimopan, was negative in the mouse micronucleus test under the conditions of the study.

Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Date

Comment:

Sushanta K. Chakder, Ph.D.
Acting Team Leader, HFD-180

Date

cc:

HFD-180
HFD-181/CSO
HFD-180/Dr. Chakraborti
HFD-180/Dr. Chakder

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/s/

Tamal Chakraborti
1/18/2008 02:15:40 PM
PHARMACOLOGIST

Sushanta Chakder
1/18/2008 02:20:37 PM
PHARMACOLOGIST

**PHARMACOLOGIST'S REVIEW OF NDA 21-775
(Sponsor's Communication Dated June 15, 2007)**

Sponsor & Address: Adolor Corporation
Exton, PA

Reviewer: Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

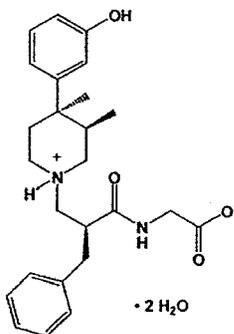
Date of Submission: June 15, 2007

Date of Receipt: June 15, 2007

Date of Review: June 18, 2007

Drug: Alvimopan Capsules (SB-767905)

Structure:



Category: Opioid μ receptor antagonist.

Indication: Alvimopan is indicated for the treatment of ~~_____~~

Submission Contents: Proposed Content of Complete Response (CR) to the Agency's letter dated November 6, 2006.

Background: The Division issued an approvable letter for NDA 22-175 dated November 6, 2006 asking the sponsor to submit the 12 month safety findings (including analyses of myocardial infarction, unstable angina, and other serious cardiovascular events) from Study SB767905/014 and to develop a risk management plan. In this letter, the sponsor submitted the proposed revised content of the CR to the Agency's approvable letter. Subsequent to the telephone conference on May 29, 2007, the sponsor revised the content of the CR to include the data and reports requested by the Division.

During the above telecon, Dr. Tamal Chakraborti, Pharmacologist, asked if the sponsor had tested the alvimopan metabolite (ADL 08-0011) for its genotoxic potential in the mouse micronucleus assay. In this letter, the sponsor stated: "Adolor conducted the preclinical tests defined for a major metabolite by the ICH S2A and S2B guidance documents, two *in vitro* genotoxicity screens to detect point mutations (Ames) and one to detect chromosomal aberrations. The guidance recommends a standard battery of genotoxicity studies if the results of the *in vitro* test are "equivocal and/or positive," and the results of the tests with ADL 08-0011 were negative." In addition, the sponsor stated that final reports of 2-year carcinogenicity studies in mice and rats will be included in the Complete Response.

SUMMARY AND EVALUATION: The sponsor did not conduct *in vivo* mouse micronucleus test with ADL 08-0011, the pharmacologically active metabolite of alvimopan. Sponsor's above statement could not be verified in ICH S2A or ICH S2B guidance documents. The ICH S2B guidance document (July 1997) states: "There are a small but significant number of genotoxic carcinogens that are reliably detected by the bone marrow tests for chromosomal damage that have yielded negative/weak/conflicting results in the pairs of *in vitro* tests outlined in the standard battery options, e.g., bacterial reverse mutation plus one of a selection of possible tests with cytogenetic evaluation of chromosomal damage or bacterial mutation plus the mouse lymphoma tk assay." ADL 08-0011 was negative in Ames test and chromosomal aberration test in CHO cells. As mentioned before, the sponsor did not test ADL 08-0011 in bone marrow test for chromosomal damage. We recommend that the sponsor conduct bone marrow micronucleus test with ADL 08-0011. This information is important from the perspective of future labeling. However, sponsor's proposal to include final reports of the 2-year carcinogenicity studies in mice and rats in their Complete Response to the Agency letter dated November 6, 2006 is acceptable.

RECOMMENDATIONS:

1. From a pharmacology/toxicology standpoint, sponsor's proposal to include final reports of the 2-year carcinogenicity studies in mice and rats in the Complete Response to the Agency letter dated November 6, 2006 appears to be acceptable.
2. The sponsor should be asked to conduct a bone marrow micronucleus test with ADL 08-0011 to further explore the genotoxic potential of the metabolite.

Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Date

Comment:

Jasti B. Choudary, B.V. Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:

HFD-180
HFD-181/CSO
HFD-180/Dr. Chakraborti
HFD-180/Dr. Chakder

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/s/

Tamal Chakraborti
6/19/2007 03:06:07 PM
PHARMACOLOGIST

Sushanta Chakder
6/19/2007 03:20:34 PM
PHARMACOLOGIST
Signed for Dr. Choudary

**PHARMACOLOGIST'S REVIEW OF NDA 21-775 [redacted] and IND 56,553
[NDA Amendment # 000 dated August 9, 2007; [redacted]
[redacted] IND 56,553 Amendment # 283 dated August 10, 2007]**

Sponsor & Address: Adolor Corporation
Exton, PA

Reviewer Name: Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Date of Submission: NDA 21-775 Amendment 000: August 9, 2007 (Adolor)
[redacted]
IND 56,553 Amendment 283: August 10, 2007 (Adolor)

Date of Receipt: NDA 21-775 Amendment 000: August 9, 2007 (Adolor)
[redacted]
IND 56,553 Amendment 283: August 13, 2007 (Adolor)

Date of Review: November 26, 2007

Drug: Alvimopan/Entereg® (ADL8-2698/LY246736/ SB-76905-KW)

Category: Opioid μ receptor antagonist.

Submission Contents:

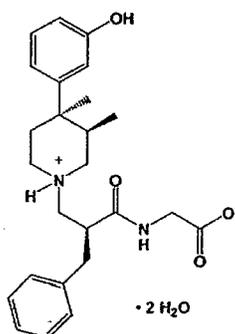
1. Complete Response to Approvable Letter dated November 3, 2006
2. 104-Week oral (gavage) carcinogenicity study in CD-1 Mice ([redacted] Study No. 1990/294; GSK Reference No. M24678)
3. 104-Week oral (gavage) carcinogenicity study in Sprague Dawley (SD) rats (Report No. R24679; [redacted] Study No. BVR/389)

Background: Alvimopan is a relatively selective, competitive, preferably peripherally acting, μ -opioid receptor antagonist that is being developed for the treatment of postoperative ileus (POI under the IND 56,553, Adolor Corporation) and for the treatment of chronic opioid-induced bowel dysfunction (OBD [redacted] GlaxoSmithKline). Results from the Study SB-767905/014, a long-term safety study of alvimopan for the treatment of OBD in chronic non-cancer patients, demonstrated an imbalance in reports of serious cardiovascular events (myocardial infarction/MI), an apparent increase in the incidence of benign and malignant neoplasms in the alvimopan group relative to the placebo group and an increase in the incidence of bone fractures when compared to the placebo. The Division asked the sponsor to submit the full reports of the carcinogenicity studies. In this submission, the sponsor submitted reports of carcinogenicity studies with alvimopan in mice and rats.

**CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND
FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET**
Review of Carcinogenicity Study Results

P/T REVIEWER(s): Tamal K. Chakraborti, Ph.D.
DATE:

NDA: 21-775
DRUG CODE#: ADL8-2698/LY246736/SB-76905-KW
CAS#: 170098-38-1
DIVISION: Division of Gastroenterology Products
DRUG NAME: Alvimopan/Entereg®
CHEMICAL STRUCTURE:



SPONSOR: Adolor Corporation, Exton, PA

LABORATORY: _____

CARCINOGENICITY STUDY REPORT DATE: June 19, 2007

THERAPEUTIC CATEGORY: Alvimopan is indicated to accelerate the time to recovery of gastrointestinal function following abdominal or pelvic surgery.

PHARMACOLOGICAL: Opioid μ receptor antagonist

MUTAGENIC/GENOTOXIC (y/n/equivocal/na; assay): No. Alvimopan was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y/TK^{+/+}) forward mutation test, the chromosome aberration test in Chinese Hamster Ovary (CHO) cells, and the oral mouse micronucleus test. The pharmacologically active metabolite ADL 08-0011 was also negative in both the Ames test and chromosome aberration test in CHO cells. Overall, alvimopan and its active metabolite, ADL 08-0011, do not appear to have genotoxic potential.

MOUSE CARCINOGENICITY STUDY:

STUDY DURATION (weeks): 104

STUDY STARTING DATE: October 29, 2003

STUDY ENDING DATE: June 19, 2007

MOUSE STRAIN: CD-1

ROUTE: Oral (gavage)

DOSING COMMENTS: The sponsor's used doses of 0 (purified water), 0 (vehicle), 100, 1000 or 4000 mg/kg/day were concurred by the Exec CAC (ExecCAC meeting minutes dated December 18, 2002).

NUMBER OF MICE:

- Control-1 (C1): 60/sex
- Control-2 (C2): 60/sex
- Low Dose (LD): 60/sex
- Middle Dose (MD): 60/sex
- High Dose-1 (HD1): 60/sex

MICE DOSE LEVELS* (mg/kg/day):

- Low Dose: 100 mg/kg/day
- Middle Dose: 1000 mg/kg/day
- High Dose-1: 4000 mg/kg/day

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): Maximum feasible dose (MFD)

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Yes. (ExecCAC meeting minutes dated December 18, 2002 attached)

MICE CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Positive in females.

MICE TUMOR FINDINGS (details): Peto's trend test showed statistically significant dose responses ("dose response" refers to the linear component of the effect of the treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases) in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor ($p = 0.0063$), and skin/appendage FIBROBLASTIC tumor ($p = 0.0003$) in the female mice. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical control incidence data (0 to 2.9%). The following table shows the significant tumor findings in the female mice (from the draft FDA statistical review).

Female Mice

Organ Name	Tumor Name	Vehicle	100 mg/kg/d	1000 mg/kg/d	4000 mg/kg/d	P-Value (Asymp.)
COMBINED	OSTEOGENIC TUMOR	0	0	1	4	0.0063
COMBINED	S/A FIBROBLASTIC TUMOR	0	0	0	5	0.0003
SKIN + SUBCUTIS	SARCOMA - NOS	0	0	0	3	0.0063

Besides the significant results for the tumor types found in trend tests, the statistical reviewer also performed pairwise comparisons (vehicle control group versus each treated group and versus water control group). The tumor types with a significant increase in the incidence over the vehicle control are summarized in the following table (from the draft FDA statistical review).

Specie	Sex	Comparison	Organ Name	Tumor Name	p-value
Mice	M	Vehicle vs 100 mg/kg/d	Liver	Hepatocellular carcinoma	0.0295
Mice	F	Vehicle vs 1000 mg/kg/d	Mammary gland	Adenocarcinoma	0.0313
Mice	F	Vehicle vs 1000 mg/kg/d	Ovary	Cystadenoma	0.0379
Mice	F	Vehicle vs 4000 mg/kg/d	Combined	Osteogenic tumor	0.0424
Mice	F	Vehicle vs 4000 mg/kg/d	Combined	S/A Fibroblastic tumor	0.0212

Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice.

MICE STUDY COMMENTS: In this study, groups of mice (60/sex/group) were administered 0 (purified water), 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia (10 mL/kg) by oral (gavage) administration once daily for up to 104 weeks. Survival in the female group at 100 mg/kg/day fell below 15 animals in Week 101, and all surviving females in this group were sacrificed in Week 101. Survival in the vehicle control female group fell below 15 in Week 102, and all remaining females from all groups were killed in Weeks 102/103. An additional 27 animals/sex were included at doses of 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW for toxicokinetic evaluation.

Peto's trend test showed statistically significant dose responses in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor crossed organs ($p = 0.0063$), and Skin/Appendage FIBROBLASTIC tumor ($p = 0.0003$) crossed organs in female mice. In addition, pairwise comparisons also revealed significantly higher incidences of S/A fibroblastic ($p = 0.0212$) tumor and osteogenic ($p = 0.0424$) tumors in females at high dose when compared to vehicle control. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at

4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was also higher compared to that of the historical control incidence data (0 to 2.9%).

Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice. The dose selection was per the ExecCAC recommendations (meeting minutes dated December 18, 2002). Overall, the conduct of the study appears to be adequate and acceptable.

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CARCINOGENICITY:

Study title: 104-Week Oral (Gavage) Carcinogenicity Study in CD-1 Mice

Key study findings: In this study, groups of mice (60/sex/group) were administered 0 (purified water), 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia (10 mL/kg) by oral (gavage) administration once daily for up to 104 weeks. Survival in the female group at 100 mg/kg/day fell below 15 animals in Week 101, and all surviving females in this group were sacrificed in Week 101. Survival in the vehicle control female group fell below 15 in Week 102, and all remaining females from all groups were killed in Weeks 102/103. An additional 27 animals/sex were included at doses of 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW for toxicokinetic evaluation.

The homogeneity tests for mortality data showed statistically significant differences in survivals for only male mice across treatment groups. P-values from both Cox and Kruskal-Wallis tests were approximately zero. From Kaplan-Meier Survival Functions for male mice, survival at 1000 mg/kg/day and 4000 mg/kg/day appeared to be much better than the vehicle control group. For comparisons of the two control groups, in males, the water control (Group 5) demonstrated significantly lower mortality than the vehicle control (Group 1) ($p = 0.032$). For females, there was no significant difference in mortality between the two control groups ($p = 0.05$).

There appeared to be no significant treatment-related effects on clinical signs, food consumption, body weight, hematology, and gross pathology. There was a higher incidence (up to 22% and 27% in males and females, respectively) of minimal to slight rhinitis in animals of both sexes at 4000 mg/kg/day, generally involving the anterior-most nasal cavity. This, however, fell within the historical control data for rhinitis (up to 32% and 38% in males and females respectively).

Peto's trend test showed statistically significant dose responses in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor crossed organs ($p = 0.0063$), and Skin/Appendage FIBROBLASTIC tumor ($p = 0.0003$) crossed organs in female mice. In addition, pairwise comparisons also revealed significantly higher incidences of S/A fibroblastic ($p = 0.0212$) tumor and osteogenic ($p = 0.0424$) tumors in females at high dose when compared to vehicle control. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical control incidence data (0 to 2.9%). The dose selection was per the ExecCAC recommendations (ExecCAC meeting minutes dated December 18, 2002 attached). The conduct of the study appears to be adequate and acceptable.

Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice.

Study number: Study No. 1990/294; Report No. M24678

Volume #, and page #: EDR dated August 9, 2007

Conducting laboratory and location: _____

Date of study initiation: October 29, 2003

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ()

Drug, lot #, and % purity: SB-767905. The batches and the purity data are shown in the following table (from 23 of the study report).

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GSK Reference Number M24678
Study Number 1990/294

Sponsor batch number	Purity as dihydrate (%)	Correction factor	Retest date
303015/1	98.9	1.011	_____
304016/1	98.7	1.013	
304016/2	98.7	1.014	
312021	98.9	1.012	
312021/2	98.9	1.011	
AD03735/1	99.1	1.009	
307020	98.8	1.013	
02-0233	98.9	1.012	
312021	98.8	1.012	
AD03736/2	99.6	1.004	
411023	99.0	1.010	

CAC concurrence: Yes. (ExecCAC meeting minutes dated December 18, 2002 attached)

Study Type: 2-year bioassay

Species/strain: Mice/CD-1

Number/sex/group; age at start of study: 60/sex/group; 6-7 weeks old

Animal housing: The mice were housed singly in individual cages. The air supply to the cages was filtered and provided a nominal 20 air changes/hour at negative pressure (-20 Pa).

Formulation/vehicle: Suspensions at concentrations of 0, 10, 100 and 400 mg/mL in 10% (w/v) aqueous acacia were dispensed weekly and were stable at room temperature (10 to 30°C) for 16 days.

Drug stability/homogeneity: Suspensions at concentrations of 0, 10, 100 and 400 mg/mL in 10% (w/v) aqueous acacia were dispensed weekly and were found to be stable at room temperature (10 to 30°C) for 16 days.

Methods:

Doses: 100, 1000 and 4000 mg/kg/day

Basis of dose selection: Maximum feasible dose as per the ExecCAC recommendations

Restriction paradigm for dietary restriction studies: None

Route of administration: Oral Gavage (10 mL/kg)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Study Design: The following table (from page 25 of the study report) shows the study design.

Group Number	Group Description	Dose ^a (mg/kg/day)	Dose Concentration (mg/mL)	Animal Numbers			
				Toxicology		Toxicokinetic	
				Male	Female	Male	Female
1	Vehicle control	0	0	1-60	301-360	601-627	709-735
2	Low	100	10	61-120	361-420	628-654	736-762
3	Intermediate	1000	100	121-180	421-480	655-681	763-789
4	High	4000	400	181-240	481-540	682-708	790-816
5	Water control	0	0	241-300	541-600		

a. Doses were expressed in terms of parent compound

Satellite PK or special study group(s): Blood samples were collected from toxicokinetic (TK) animals in Weeks 4 and 26 at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours time points.

Deviations from original study protocol: None mentioned

Statistical methods: Survival probability functions were estimated by using the Kaplan-Meier method. Permutational tests for both an increasing and a decreasing dose response in mortality were performed across the vehicle control and the treated groups (Groups 1 to 4). One directional pairwise tests of the treated groups against the vehicle control group were also performed. The vehicle and water controls (Group 1 and Group 5) were compared using two-sided tests.

Tumors of similar histogenic origin were combined. The following tables (from page 371 and 372 of the study report) show the tumor types analyzed. One directional pairwise tests of the treated groups against the vehicle control group were performed. Group 1 and Group 5 were compared using two-sided tests. Non-fatal tumors were analyzed using fixed intervals of 1 to 50 weeks, 51 to 80 weeks, 81 weeks to start of terminal kill and the terminal kill phase. Indication of a possible treatment effect was assessed on the basis of rare or common tumor type, in line with current FDA guidelines.

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NDA 21-775

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GSK Reference Number M24678
 Study Number 1990/294

Table 21 Tumour statistics

		Tumour types analysed	
Tissue type	Tumour type	Tissue	Finding
AD	B-SUBCAPSULAR CELL ADENOMA	ADRENAL	B-SUBCAPSULAR CELL ADENOMA
HE	M-HISTIOCYTIC SARCOMA	HAEMOLYMPHORETICULAR	M-HISTIOCYTIC SARCOMA
HE	M-GRANULOCYTIC LEUKAEMIA	HAEMOLYMPHORETICULAR	M-GRANULOCYTIC LEUKAEMIA
MA	M-ADENOCARCINOMA	MAMMARY GLAND	M-ADENOCARCINOMA
OV	B-CYSTADENOMA	OVARY	B-CYSTADENOMA
PA	B-ISLET CELL ADENOMA	PANCREAS	B-ISLET CELL ADENOMA
PI	B-ADENOMA	PITUITARY	B-ADENOMA
TE	B-INTERSTITIAL CELL ADENOMA	TESTIS	B-INTERSTITIAL CELL ADENOMA
HE	LIMPHOID TUMOUR	HAEMOLYMPHORETICULAR HAEMOLYMPHORETICULAR HAEMOLYMPHORETICULAR HAEMOLYMPHORETICULAR HAEMOLYMPHORETICULAR	M-MALIGNANT LYMPHOMA - LYMPHOCYTIC M-MALIGNANT LYMPHOMA - NOS M-MALIGNANT LYMPHOMA - PLASMACYTIC M-MALIGNANT LYMPHOMA - FLEOMORPHIC M-MALIGNANT LYMPHOMA-LYMPHOBLASTIC
HS	ADENOMA/CARCINOMA	HABERERIAN GLAND HABERERIAN GLAND	B-ADENOMA M-ADENOCARCINOMA
LI	HEPATOCELLULAR TUMOUR	LIVER LIVER	B-HEPATOCELLULAR ADENOMA M-HEPATOCELLULAR CARCINOMA
LU	ALVEOLAR EPITHELIAL TUMOUR	LUNG LUNG	B-BRONCHIOLO-ALVEOLAR ADENOMA M-BRONCHIOLO-ALVEOLAR CARCINOMA
OV	SEX CORD/STROMAL TUMOUR	OVARY OVARY	B-BENIGN LYMPHOMA B-BENIGN SEX CORD STROMAL TUMOUR
UT	SMOOTH MUSCLE TUMOUR	UTERUS UTERUS	B-LEIOMYOMA M-LEIOMYOSARCOMA
UT	SIRCHAL TUMOUR	UTERUS	B-STROMAL POLYP
UT	SIRCHAL TUMOUR	UTERUS	M-STROMAL SARCOMA
#	BLOOD VESSEL TUMOUR	ABDOMINAL CAVITY EAR FEMUR + MARROW LIVER LIVER LIMPH NOSE MESENTERIC LIMPH NOSE MUSCLE OVARY SPLEEN STERNUM + MARROW TESTIS	M-HAEMANGIOSARCOMA B-HAEMANGIOMA M-HAEMANGIOSARCOMA B-HAEMANGIOMA M-HAEMANGIOSARCOMA B-HAEMANGIOMA B-HAEMANGIOMA B-HAEMANGIOMA B-HAEMANGIOMA B-HAEMANGIOMA M-HAEMANGIOSARCOMA B-HAEMANGIOMA

= Merged tissues

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NDA 21-775

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GSK Reference Number M24678
 Study Number 1990/294

Table 21
 Tumour statistics

Tumour types analysed (continued)

Tissue type	Tumour type	Tissue	Finding
#	HISTIOCYTIC SARCOMA	CONNECTIVE TISSUE LIVER SKIN + SUBCUTIS UTERUS	M-HISTIOCYTIC SARCOMA M-HISTIOCYTIC SARCOMA M-HISTIOCYTIC SARCOMA M-HISTIOCYTIC SARCOMA
#	OSTEOGENIC TUMOUR	BONE BONE FEMUR + MARROW SPINAL CORD THORACIC CAVITY	B-OSTEOMA M-OSTEOSARCOMA B-OSTEOMA M-MALIGNANT OSTEOSARCOMA M-OSTEOSARCOMA
#	S/A FIBROBLASTIC TUMOUR	SKIN + SUBCUTIS SKIN + SUBCUTIS SKIN + SUBCUTIS TAIL	B-FIBROMA M-FIBROSARCOMA M-SARCOMA - NOS B-FIBROMA

= Merged tissues
 S/A = Skin/appendage

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Observations and times:

Mortality: Mortality was observed twice daily.

Clinical signs: Animals were observed for clinical signs on a daily basis.

Body weights: The animals were weighed twice during Week-1, before treatment on the first day of dosing, at weekly intervals for 16 weeks, once every four weeks thereafter and before necropsy.

Food consumption: The food consumption was determined in Week-1, weekly for the first 16 weeks and once in every four weeks thereafter.

Hematology: Blood samples were collected for hematology at necropsy.

Gross pathology: Gross pathology was conducted at necropsy.

Histopathology: The tissues marked "X" in the "Examine" column of the following table (from page 29 and 30 of the study report) were used for histopathological examinations from all main study animals.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Adrenals (x2)	X	Parathyroids	X
Animal identification site		Pituitary	X
Aorta (thoracic)	X	Preputial/clitoral glands	X
Brain (x3)	X	Prostate	X
Caecum	X	Rectum	
Colon	X	Salivary gland	
Duodenum	X	mandibular	X
Epididymides	X	sublingual	X

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CONFIDENTIAL

GSK Reference Number M24678

Study Number 199D/294

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Eyes/Optic nerves (x2)	X	parotid	X
Femur (Femoro-tibial joint)	X	Sciatic nerves (x2)	X
Gallbladder	X	Seminal vesicles (x2)	X
Gross lesions	X	Skin	X
Harderian glands	X	Spinal cord	
Head		cervical	
Heart	X	thoracic	
Ileum	X	lumbar	X
Jejunum	X	Spleen	X
Kidneys (x2)	X	Stemum with bone marrow	X
Larynx	X	Stomach	X
Liver (two lobes) (x2)	X	Testes	X
Lung (all lobes) (x2)	X	Thymus	X
Lymph node -		Thyroids	X
Mandibular	X	Tissue masses	X
Mesenteric	X	Tongue	X
Mammary gland (inguinal)	X	Trachea	X
Muscle (quadriceps)	X	Trachea bifurcation	
Nasal cavities and nasopharynx with skull ^a	X	Urinary bladder	X
Oesophagus	X	Uterus with cervix (x3)	X
Ovaries (x2)	X	Vagina	X
Pancreas	X		

a. Preserved with head in situ

Toxicokinetics: Blood samples were withdrawn from TK animals in Weeks 4 and 26 at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours time points.

Results:

Mortality: There were 379 decedents during the study (168 males and 211 females). The distribution and statistical analysis of these decedents are presented in the following table (page 33 of the study report).

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Sex	Type of death	Group					Results (P-values)				
		1 (C)	2 (L)	3 (I)	4 (H)	5 (W)	1-sided tests for increasing dose response				2-sided tests
							C v I, H	C v L	C v I	C v H	C v W
M	Accident	2	1	0	0	1	1.00	.425	1.00	1.00	.032*
	Dead or Moribund	42	41	25	24	32					
	Terminal Kill	16	18	35	36	27					
	Total	60	60	60	60	60					
F	Accident	1	0	2	1	0	0.972	0.460	0.637	0.959	0.118
	Dead or Moribund	45	46	42	36	38					
	Terminal Kill	14	14	16	23	22					
	Total	60	60	60	60	60					

C = Vehicle control, L = Low dose (100 mg/kg/day), I = Intermediate dose (1000 mg/kg/day), H = High dose (4000 mg/kg/day), W = Water control

* P<0.05

** P<0.01

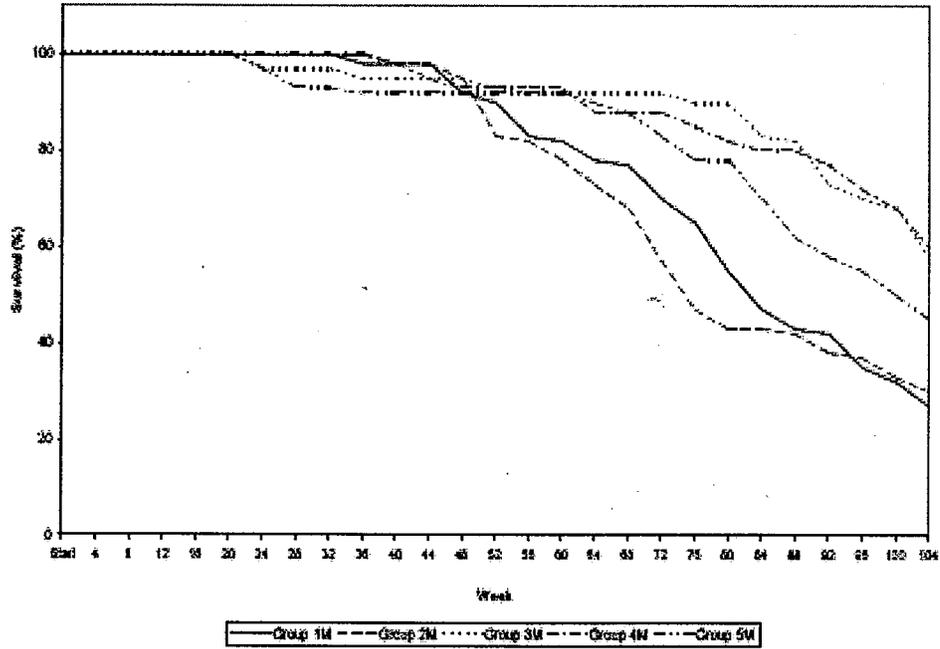
*** P<0.001

Per the FDA statistical analysis of mortality data, the homogeneity tests showed statistically significant differences in survivals for only male mice across treatment groups. P-values from both Cox and Kruskal-Wallis tests were approximately zero. From Kaplan-Meier Survival Functions for male mice, survival at 1000 mg/kg/day and 4000 mg/kg/day appeared to be higher than the vehicle control group. For comparisons of the two control groups, in males, the water control (Group 5) demonstrated significantly lower mortality than the vehicle control (Group 1) (P = 0.032). For females, there was no significant difference in mortality between the two control groups (P= 0.05). The following figures show the survival graphs (from page 44 and 45 of the study report).

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Figure 5 Group Survival – Males

Test Article	Vehicle Control		SE-767905		Water Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	100	1000	4000	0

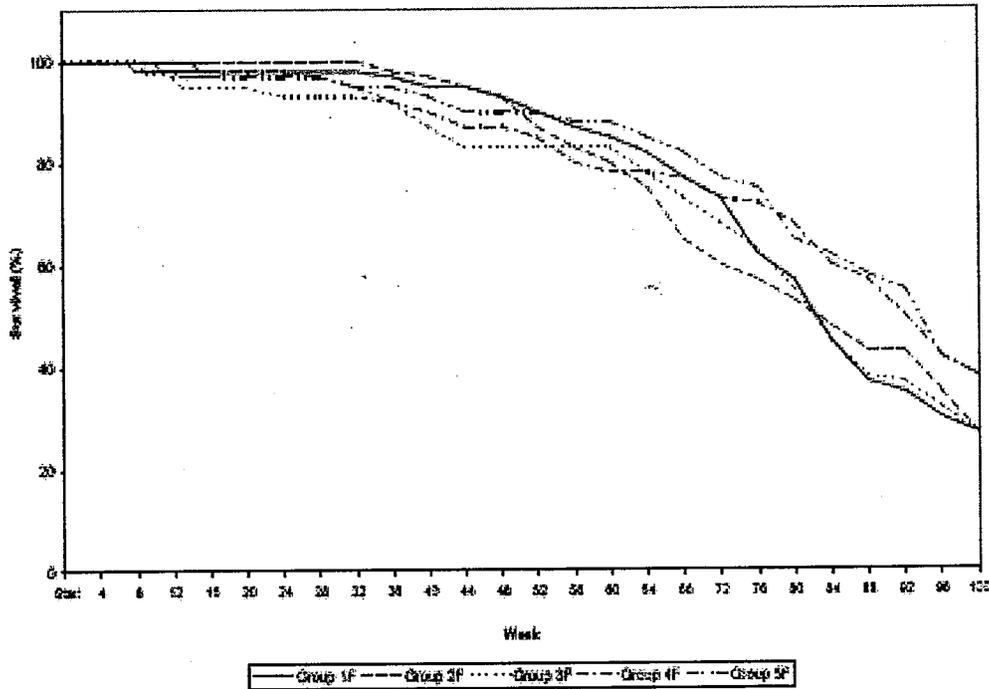


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Figure 6 Group Survival – Females

Test Article	Vehicle Control		SB-767905		Water Control	
Group	1	2	3	4	5	6
Level (mg/kg/day)	0	100	1000	4000	0	0



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Clinical signs: There were no significant clinical signs attributable to administration of SB-767905.

Body weights: The mean initial (Week -1) and final (Week 104) weights of control males were 30.9 and 44.9 g, respectively. The mean initial (Week -1) and final (Week 100) weights of control females were 23.8 and 40.9 g, respectively. Overall, there was no consistent pattern of variation in the body weight data to indicate an effect of treatment with SB-767905. The following table shows the absolute body weights (g) and body weight gains (g) for males and females.

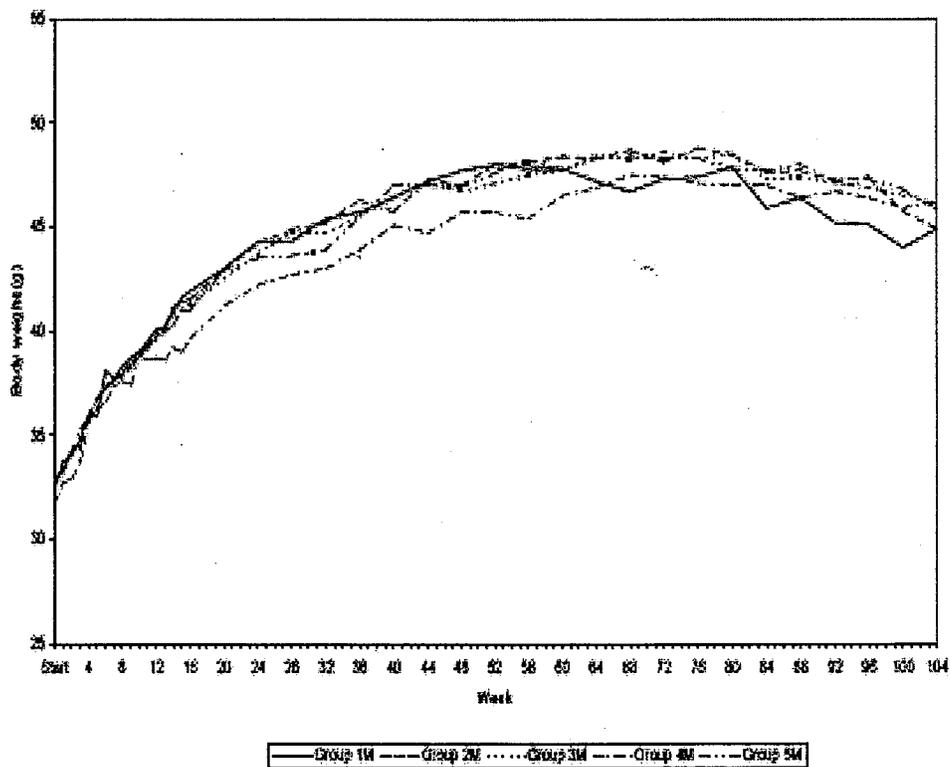
Males	Vehicle Control	100 mg/kg/day	1000 mg/kg/day	4000 mg/kg/day	Water Control
Group	1	2	3	4	5
Wk 0	32.5	31.7	32.9	32.7	33.0
Wk 24	44.3	44.2	43.7	42.2	43.5
% of Control, Wk 24	100.0	99.8	98.6	95.2	98.2
Δ Wk24-Wk0	11.8	12.5	10.8	9.5	10.5
BW Gain, % of Initial BW	36.3	39.4	32.8	29.0	31.8
BW Gain, % of Control	100.0	108.5	90.3	79.9	87.6
Wk 52	48	48	47.1	45.7	47.6
% of Control, Wk 52	100.0	100	98.1	95.2	99.2
Δ Wk52-Wk0	15.5	16.3	14.2	13	14.6
BW Gain, % of Initial BW	47.6	51.4	43.2	39.8	44.2
BW Gain, % of. Control	100.0	108.0	90.8	83.6	92.9
Wk 76	47.4	48.8	48.3	47.1	48.3
% of Control, Wk 76	100.0	102.9	101.9	99.4	101.9
Δ Wk76-Wk0	14.9	17.1	15.4	14.4	15.3
BW Gain, % of Initial BW	45.8	53.9	46.8	44.0	46.4
BW Gain, % of Control	100.0	117.7	102.2	96.0	101.3
Wk 104	44.9	44.9	46.1	46.2	45.8
% of Control, Wk 104	100.0	100	102.7	102.9	102.0
Δ Wk104-Wk0	12.4	13.2	13.2	13.5	12.8
BW Gain, % of Initial BW	38.2	41.6	40.1	41.3	38.8
BW Gain, % of Control	100.0	108.9	105.0	108.1	101.6

Females	Vehicle Control	100 mg/kg/day	1000 mg/kg/day	4000 mg/kg/day	Water Control
Group	1	2	3	4	5
Wk 0	24.9	25.3	25.6	25.6	26.0
Wk 24	35.2	36.4	36.0	35.7	35.9
% of Control, Wk 24	100.0	103.4	102.3	101.4	101.9
ΔWk24-Wk0	10.3	11.1	10.4	10.1	9.9
BW Gain, % of Initial BW	41.4	43.9	40.6	39.4	38.1
BW Gain, % of Control	100.0	106.0	98.1	95.2	92.0
Wk 52	38.9	41.1	39.1	39.1	40.1
% of Control, Wk 52	100.0	105.6	100.5	100.5	103.1
ΔWk52-Wk0	14	15.8	13.5	13.5	14.1
BW Gain, % of Initial BW	35.9	38.4	34.1	34.1	54.0
BW Gain, % of Control	100.0	106.9	94.9	94.9	150.4
Wk 76	40.3	42.2	41.1	39.9	42.0
% of Control, Wk 76	100.0	104.7	101.9	99.0	104.2
ΔWk76-Wk0	15.4	16.9	15.5	14.3	16
BW Gain, % of Initial BW	61.8	66.8	60.5	55.8	61.5
BW Gain, % of Control	100.0	108.1	97.9	90.3	99.5
	24.9	25.3	25.6	25.6	26.0
Wk 100	40.9	42.3	40.0	38.4	43.3
% of Control, Wk 100	100.0	103.4	97.8	93.9	105.9
ΔWk100-Wk0	16	17	14.4	12.8	17.3
BW Gain, % of Initial BW	64.2	67.2	56.2	50.0	66.5
BW Gain, % of Control	100.0	104.7	87.5	77.9	103.6

The following figures (from page 40 and 41 of the study report) show the growth curves in males and females.

Figure 1 Group Mean Body Weights - Males

Test Article	Vehicle Control		SB-767905		Water Control
Group	1	2	3	4	5
Level (mg/kg/day)	0	100	1000	4000	0

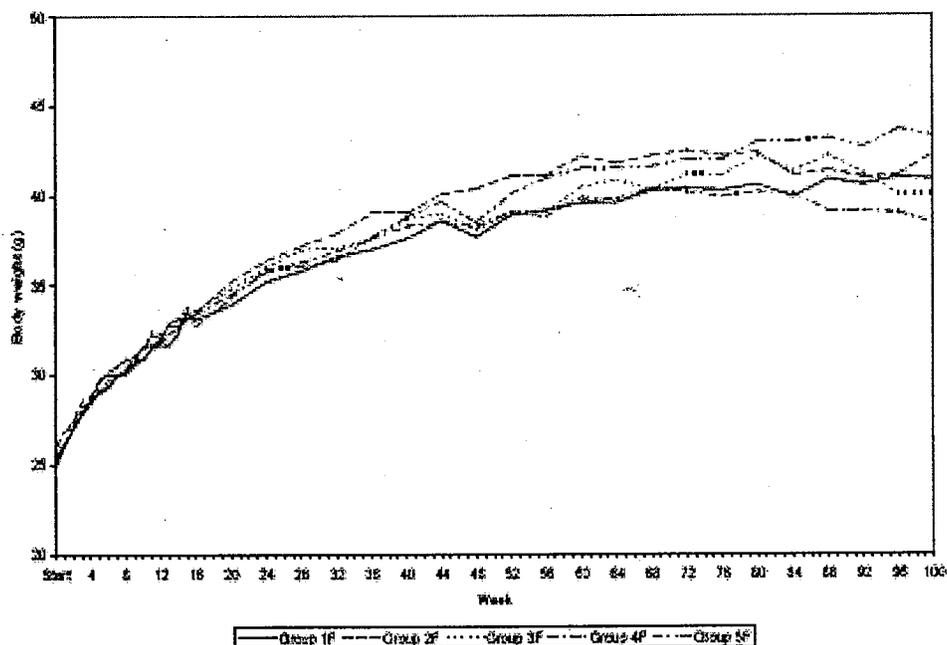


Pre-treatment body weights not presented

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Figure 2 Group Mean Body Weights – Females

Test Article	Vehicle Control		SB-767905		Water Control	
Group	1	2	3	4	5	
Level (mg/kg/day)	0	100	1000	4000	0	0



Pre-treatment body weights not presented

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Food consumption: The mean initial (week -1) and final (week 104) food consumption in control males was 6.06 and 4.91 g/animal/day, respectively. The mean initial (week -1) and final (Week 100) food consumption in control females was 5.3 and 5.17 g/animal/day, respectively. There were no significant treatment-related effects on food consumption.

Hematology: There were no significant treatment-related effects.

Gross pathology: There were no significant treatment-related effects.

Histopathology:

Non-neoplastic: There was a higher incidence (up to 22% and 27% in males and females, respectively) of minimal to slight rhinitis in animals of both sexes at 4000 mg/kg/day,

generally involving the anterior-most nasal cavity. This, however, fell within the historical control data for rhinitis (up to 32% and 38% in males and females respectively). The following table shows the findings in the nasal cavity.

FINDINGS	MALES					FEMALES				
	DOSE (MG/KG/DAY)					DOSE (MG/KG/DAY)				
	CON1	CON2	100	1000	4000	CON1	CON2	100	1000	4000
N	59	60	59	60	60	59	60	60	60	60
NASAL CAVITY 1										
RHINITIS	2 (3.4%)	2 (3.3%)	5 (8.5%)	13 (21.7%)	4 (6.7%)	1 (1.7%)	0 (0.0%)	1 (1.7%)	16 (26.7%)	2 (3.3%)
NASAL CAVITY 2										
RHINITIS	1 (1.7%)	3 (5.0%)	6 (10.2%)	11 (18.3%)	5 (8.3%)	3 (5.1%)	3 (5.0%)	1 (1.7%)	10 (16.7%)	2 (3.3%)
NASAL CAVITY 3										
RHINITIS	5 (8.5%)	2 (3.3%)	1 (1.7%)	6 (10.0%)	1 (1.7%)	3 (5.1%)	0 (0.0%)	0 (0.0%)	7 (11.7%)	0 (0.0%)
NASAL CAVITY 4										
RHINITIS	3 (5.1%)	2 (3.3%)	0 (0.0%)	4 (6.7%)	3 (5.0%)	2 (3.4%)	1 (1.7%)	0 (0.0%)	5 (8.3%)	0 (0.0%)

* P<0.05, ** P<0.01, *** P<0.001

Neoplastic: Peto's trend test showed statistically significant increase in the incidence of SARCOMA in the skin/subcutis (p = 0.0063), OSTEOGENIC tumor (p = 0.0063), and skin/appendage FIBROBLASTIC tumor (p = 0.0003) in the female mice. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical

control incidence data (0 to 2.9%). The following table shows the significant tumor findings in the female mice (from the draft FDA statistical review).

Female Mice

Organ Name	Tumor Name	Vehicle	100 mg/kg/d	1000 mg/kg/d	4000 mg/kg/d	P-Value (Asymp.)
COMBINED	OSTEOGENIC TUMOR	0	0	1	4	0.0063
COMBINED	S/A FIBROBLASTIC TUMOR	0	0	0	5	0.0003
SKIN + SUBCUTIS	SARCOMA - NOS	0	0	0	3	0.0063

Besides the significant results for the tumor types found in trend tests, the statistical reviewer also performed pairwise comparisons (vehicle control group versus each treated group and versus water control group). The tumor types with a significant increase in the incidence over the vehicle control are summarized in the following table (from the draft FDA statistical review).

Specie	Sex	Comparison	Organ Name	Tumor Name	p-value
Mice	M	Vehicle vs 100 mg/kg/d	Liver	Hepatocellular carcinoma	0.0295
Mice	F	Vehicle vs 1000 mg/kg/d	Mammary gland	Adenocarcinoma	0.0313
Mice	F	Vehicle vs 1000 mg/kg/d	Ovary	Cystadenoma	0.0379
Mice	F	Vehicle vs 4000 mg/kg/d	Combined	Osteogenic tumor	0.0424
Mice	F	Vehicle vs 4000 mg/kg/d	Combined	S/A Fibroblastic tumor	0.0212

Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice.

The following tables (from page 373-375 of the study report) show the summary of tumor incidences.

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GSK Reference Number M24678
Study Number 1990/294

Table 21
Tumour statistics

Tumour incidence in males
Numbers of tumour bearing animals & results of statistical tests

Tissue code	Tumour type		Group					P-values				
			1 (C)	2 (L)	3 (I)	4 (H)	5 (W)	1-sided tests (increasing dose response)		2-sided tests		
							C,L,I,H	CvL	CvI	CvH	CvW	
AD	B-SUBCAPSULAR CELL ADENOMA	Exam	58	60	59	60	60					
		NF	1	2	5	5	4	.178	.474	.278	.225	.404
HE	M-GRANULOCYTTIC LEUKAEMIA	Exam	59	60	59	60	60					
		F	1	0	1	0	2	.762	1.00	.762	1.00	1.00
TE	B-INTERSTITIAL CELL ADENOMA	Exam	59	60	59	60	60					
		NF	1	2	2	0	3	.932	.437	.696	1.00	.639
HE	LYMPHOID TUMOUR	Exam	59	60	59	60	60					
		F	3	4	6	2	7					
		NF	0	1	4	1	0					
		U	0	0	0	0	1					
		All (U1)	3	5	10	3	8	.943	.346	.300	.892	.371
		All (U2)	3	5	10	3	8	.943	.346	.300	.892	.367
HG	ADENOMA/CARCINOMA	Exam	59	60	59	60	60					
		F	2	0	0	1	0					
		NF	5	4	6	5	8					
		U	0	1	0	0	0					
		All (U1)	7	5	6	6	8	.792	.761	.948	.946	.782
		All (U2)	7	5	6	6	8	.760	.785	.948	.946	.782
LI	HEPATOCELLULAR TUMOUR	Exam	59	60	59	60	60					
		F	0	2	0	0	3					
		NF	9	12	19	15	12					
		All	9	14	19	15	15	.719	.121	.315	.390	.641
LU	ALVEOLAR EPITHELIAL TUMOUR	Exam	59	60	59	60	60					
		F	4	2	4	7	6					
		NF	15	6	20	11	13					
		U	0	1	0	0	0					
		All (U1)	19	9	24	18	19	.764	.980	.666	.942	.343
		All (U2)	19	9	24	18	19	.738	.983	.666	.942	.343
#	BLOOD VESSEL TUMOUR	Exam	59	60	59	60	60					
		F	1	1	2	1	0					
		NF	6	0	1	3	3					
		All	7	1	3	4	3	.688	.994	.981	.950	.108
#	S/A FIBROBLASTIC TUMOUR	Exam	59	60	59	60	60					
		F	0	2	1	0	0					
		NF	0	0	2	0	0					
		All	0	2	3	0	0	.855	.244	.208	1.00	1.00

C = Vehicle control, L = Low dose, I = Intermediate dose, H = High dose, W = Water control
F = Fatal, NF = Non-fatal, U = Uncertain

U1 = Uncertain considered as fatal
U2 = Uncertain considered as non-fatal
= Merged tissues
S/A = Skin/appendage

CONFIDENTIAL

GSK Reference Number M24678
 Study Number 1990/294

Table 21
 Tumour statistics

Tumour incidence in females
 Numbers of tumour bearing animals & results of statistical tests

Tissue code	Tumour type		Group					P-values				
			1 (C)	2 (L)	3 (I)	4 (H)	5 (W)	1-sided tests (increasing dose response)			2-sided tests	
							C,L,I,H	CvL	CvI	CvH	CvW	
HE	M-HISTIOCYTIC SARCOMA	Exam	60	60	60	60	60					
		F	1	1	2	0	0					
		NF	1	0	0	0	0					
		All	2	1	2	0	0	.904	.868	.692	1.00	.203
MA	M-ADENOCARCINOMA	Exam	58	60	57	59	60					
		F	0	0	1	1	0					
		NF	0	0	2	0	0					
		All	0	0	3	1	0	.315	1.00	.102	.574	1.00
OV	B-CYSTADENOMA	Exam	60	60	60	60	59					
		F	0	0	1	0	0					
		NF	0	0	2	3	0					
		All	0	0	3	3	0	.064	1.00	.122	.175	1.00
PA	B-ISLET CELL ADENOMA	Exam	59	60	60	60	60					
		NF	2	1	0	0	0	.985	.867	1.00	1.00	.487
PI	B-ADENOMA	Exam	59	58	58	60	60					
		NF	3	1	2	2	1	.675	.936	.833	.920	.301
HE	LYMPHOID TUMOUR	Exam	60	60	60	60	60					
		F	4	9	5	5	8					
		NF	1	1	3	4	2					
		U	0	1	0	0	0					
		All (U1)	5	11	8	9	10	.555	.107	.288	.371	.446
		All (U2)	5	11	8	9	10	.531	.107	.288	.371	.446
HE	ADENOMA/CARCINOMA	Exam	60	60	60	60	60					
		F	0	0	1	0	0					
		NF	4	3	4	6	3					
		All	4	3	5	6	3	.200	.793	.440	.288	1.00
LI	HEPATOCELLULAR TUMOUR	Exam	60	60	60	60	60					
		NF	1	1	0	1	4	.623	.734	1.00	.851	.363
LU	ALVEOLAR EPITHELIAL TUMOUR	Exam	60	60	60	60	60					
		F	2	2	3	0	1					
		NF	5	6	3	8	10					
		U	0	2	0	0	0					
		All (U1)	7	10	6	8	11	.618	.298	.669	.623	.455
		All (U2)	7	10	6	8	11	.591	.314	.669	.623	.455
OV	SEX CORD/STROMAL TUMOUR	Exam	60	60	60	60	59					
		NF	0	2	0	1	1	.572	.224	1.00	.605	.457

C = Vehicle control, L = Low dose, I = Intermediate dose, H = High dose, W = Water control
 F = Fatal, NF = Non-fatal, U = Uncertain
 U1 = Uncertain considered as fatal
 U2 = Uncertain considered as non-fatal

CONFIDENTIAL

GSK Reference Number M24678
Study Number 1990/294Table 21
Tumour statisticsTumour incidence in females
Numbers of tumour bearing animals & results of statistical tests

Tissue code	Tumour type		Group					P-values				
			1 (C)	2 (L)	3 (I)	4 (H)	5 (W)	I-sided tests (increasing dose response)		2-sided tests		
							C,L,I,H	CvL	CvI	CvH	CvW	
UT	SMOOTH MUSCLE TUMOUR	Exam	60	60	60	60	60					
		F	0	2	0	0	0					
		NF	1	2	2	3	1					
		All	1	4	2	3	1	.446	.186	.502	.380	1.00
UT	STROMAL TUMOUR	Exam	60	60	60	60	60					
		F	2	1	0	0	0					
		NF	3	5	2	0	2					
		All	5	6	2	0	2	.999	.518	.941	1.00	.241
#	BLOOD VESSEL TUMOUR	Exam	60	60	60	60	60					
		NF	2	0	0	1	4	.584	1.00	1.00	.875	.679
#	HISTIOCYTIC SARCOMA	Exam	60	60	60	60	60					
		F	1	0	1	1	2					
		NF	1	0	0	3	0					
		All	2	0	1	4	2	.062	1.00	.863	.338	1.00
#	OSTEOGENIC TUMOUR	Exam	60	60	60	60	60					
		F	0	0	1	1	1					
		NF	0	0	0	3	1	.035*	1.00	1.00	.210	.417
		All	0	0	1	4	2	.015*	1.00	.500	.119	.206
#	S/A FIBROELASTIC TUMOUR	Exam	60	60	60	60	60					
		F	0	0	0	4	1	.006**	1.00	1.00	.084	1.00
		NF	0	0	0	1	0					
		All	0	0	0	5	1	.002**	1.00	1.00	.051	1.00

C = Vehicle control, L = Low dose, I = Intermediate dose, H = High dose, W = Water control
F = Fatal, NF = Non-fatal

= Merged tissues

S/A = Skin/appendage

* P<0.05

** P<0.01

*** P<0.001

Toxicokinetics: Following 26 weeks of oral (gavage) administration of SB-767905-KW to male and female CD-1 mice, SB-767905 and SB-791399 (the amide hydrolysis metabolite of SB-767905) were quantifiable in the plasma of all animals up to at least 8 and 24 hours, respectively, after dose administration. The T_{max} occurred at approximately 0.5-8.0 and 1.0-24 hours after dosing for SB-767905 and SB-791399, respectively. Systemic exposure (AUC_{0-t}) to SB-767905 increased with increasing dose in a subproportional manner. Overall, for a 40-fold increase in dose from 100 to 4000 mg/kg/day, AUC_{0-t} increased about 7-fold in male mice and 5-fold in female mice. Systemic exposure (AUC_{0-t}) to SB-791399 and C_{max} was generally similar at Weeks 4 and 26, and did not increase with escalating dose or demonstrate any notable sex differences. Systemic exposure to SB-791399 was generally higher than the systemic exposure to SB-767905, in male and female animals. The following tables (page 2680 and 2681 of the study report) show the TK parameters for SB-767905 and SB-791399.

Table 1 Summary of the Composite Toxicokinetic Parameters for SB-767905 Derived from Plasma Concentration-Time Results from CD-1 Mice Following Oral Administration of SB-767905-KW for 26 Weeks

Sex	Dose-level (mg/kg/day)	Week	AUC _{0-t} (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
Male	100	4	9.31	1.92	5.99
		26	25.8	4.62	1.02
	1000	4	31.3	4.06	8.01
		26	136	30.8	0.50
	4000	4	63.0	5.55	4.00
		26	203	29.1	4.00
Female	100	4	32.4	15.3	0.50
		26	54.4	8.15	2.00
	1000	4	56.7	7.92	6.03
		26	238	35.1	6.00
	4000	4	127	30.8	7.99
		26	317	23.2	0.51

Table 2 Summary of the Composite Toxicokinetic Parameters for SB-791399 Derived from Plasma Concentration-Time Results from CD-1 Mice Following Oral Administration of SB-767905-KW for 26 Weeks

Sex	Dose-level (mg/kg/day)	Week	AUC ₀₋₂₄ (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
Male	100	4	269	38.8	12.01
		26	183	23.8	12.01
	1000	4	174	21.8	8.01
		26	224	17.5	8.01
	4000	4	229	33.1	1.00
		26	242	45.0	1.00
Female	100	4	87.1	8.77	6.01
		26	306	36.5	11.90
	1000	4	212	16.9	12.00
		26	407	32.0	24.02
	4000	4	130	14.9	7.99
		26	164	13.2	11.99

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This study appears to be adequate and acceptable. The dose selection based on MFD appears to be appropriate and was in concurrence with the ExecCAC recommendations. The selection of the test model also appears to be appropriate. Overall, the study was conducted in a valid manner.

Evaluation of tumor findings: Peto's trend test showed statistically significant increase in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor ($p = 0.0063$), and skin/appendage FIBROBLASTIC tumor ($p = 0.0003$) in the female mice. In addition, pairwise comparisons also revealed significantly higher incidences of S/A fibroblastic ($p = 0.0212$) tumor and osteogenic ($p = 0.0424$) tumors in females at high dose when compared to vehicle control. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical control incidence data (0 to 2.9%). The dose selection was per the ExecCAC recommendations (meeting minutes dated December 18, 2002). Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice.

Carcinogenicity summary: In this study, groups of mice (60/sex/group) were administered 0 (purified water), 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia (10 mL/kg) by oral (gavage) administration once daily for up to 104 weeks. Survival in the female group at 100 mg/kg/day fell below 15 animals in Week 101, and all surviving females in this group were sacrificed in Week 101. Survival in the vehicle control female group fell below 15 in Week 102, and all remaining females from all groups were killed in Weeks 102/103. An additional 27 animals/sex were included at doses of 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW for toxicokinetic evaluation. The dose selection was per the ExecCAC recommendations.

The homogeneity tests for mortality data showed statistically significant differences in survivals for only male mice across treatment groups. P-values from both Cox and Kruskal-Wallis tests were approximately zero. From Kaplan-Meier Survival Functions for male mice, survival at 1000 mg/kg/day and 4000 mg/kg/day appeared to be much better than the vehicle control group. For comparisons of the two control groups, in males, the water control (Group 5) demonstrated significantly lower mortality than the vehicle control (Group 1) ($P = 0.032$). For females, there was no significant difference in mortality between the two control groups ($P = 0.05$).

There appeared to be no significant treatment-related effects on clinical signs, food consumption, body weight, hematology, and gross pathology. There was a higher incidence (up to 22% and 27% in males and females, respectively) of minimal to slight rhinitis in animals of both sexes at 4000 mg/kg/day, generally involving the anterior-most nasal cavity. This, however, fell within the historical control data for rhinitis (up to 32% and 38% in males and females respectively).

Peto's trend test showed statistically significant increase in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor ($p = 0.0063$), and skin/appendage FIBROBLASTIC tumor ($p = 0.0003$) in the female mice. In addition, pairwise comparisons also revealed significantly higher incidences of S/A fibroblastic ($p = 0.0212$) tumor and osteogenic ($p = 0.0424$) tumors in females at high dose when compared to vehicle control. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical control incidence data (0 to 2.9%). The dose selection was per the ExecCAC recommendations (meeting minutes dated December 18, 2002). The conduct of the study appears to be adequate and acceptable.

Carcinogenicity conclusions: Alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis (trend test, $p = 0.0063$), OSTEOGENIC tumor (trend test, $p = 0.0063$), and skin/appendage FIBROBLASTIC tumor (trend test, $p = 0.0003$) in the female mice.

Recommendations for further analysis: None

RAT CARCINOGENICITY STUDY:

STUDY DURATION (weeks): 104
STUDY STARTING DATE: September 24, 2003
STUDY ENDING DATE: June 28, 2007
RAT STRAIN: Sprague Dawley (SD)
ROUTE: Oral (gavage)
DOSING COMMENTS: The sponsor's doses of 0 (water), 0 (vehicle), 100, 200 and 500 mg/kg/day were concurred by the ExecCAC (ExecCAC meeting minutes dated January 25, 2002 attached).

NUMBER OF RAT:

- Control-1 (C1): 60/sex
- Control-2 (C2): 60/sex
- Low Dose (LD): 60/sex
- Middle Dose (MD): 60/sex
- High Dose-1 (HD1): 60/sex

RAT DOSE LEVELS* (mg/kg/day):

- Low Dose: 100 mg/kg/day
- Middle Dose: 200 mg/kg/day
- High Dose-1: 500 mg/kg/day

*: A further group of 60 female rats was also treated at 30 mg/kg/day.

BASIS FOR DOSES SELECTED (MTD; AUC ratio; saturation; maximum feasible): Saturation of absorption

PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (y/n; Date): Yes (ExecCAC meeting minutes dated January 25, 2002 attached)

RAT CARCINOGENICITY (conclusion: negative; positive; MF; M; F): Positive in both sexes

RAT TUMOR FINDINGS (details): The Peto's trend tests showed statistically significant dose responses ("dose response" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases) in the incidence of THYMOMA (EPTIHELIAL, p = 0.014) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA (p = 0.0199) in thyroids in the female rats. The following tables (from the draft FDA statistical review) show the significant tumor findings.

Male Rats

Organ Name	Tumor Name	Vehicle	100 mg/kg/d	200 mg/kg/d	500 mg/kg/d	P-Value (Exact)
THYMUS	B-THYMOMA(EPIHELIAL)	0	0	2	3	0.0104

Female Rats

Organ Name	Tumor Name	Vehicle	30 mg/kg/d	100 mg/kg/d	200 mg/kg/d	500 mg/kg/d	P-Value (Exact)
THYROIDS	M-C-CELL CARCINOMA	0	1	0	0	3	0.0199

RAT STUDY COMMENTS: In this study, groups of rats (60/sex/group) were given 0 (water), 0 (vehicle), 100, 200 or 500 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia at a dose volume of 5 mL/kg by oral (gavage) administration once daily for 104 weeks. An additional group of 60 female rats was given 30 mg/kg/day, at a dose volume of 3 mL/kg, for the same period. The following evaluations were performed: in-life animal observations, palpation, body weight, food consumption, hematology, macroscopic observations and microscopic observations. Blood sampling for toxicokinetic (TK) evaluation was performed on Days 1 and 29 and in Weeks 13, 26 and 52. Treatment with SB-767905-KW had no effect on survival in either sex. There appears to be no significant in-life findings associated with treatment with SB-767905-KW. Macroscopic observations included statistically significant increased incidence of enlargement (27.3% versus 0% in controls) of the deep cervical lymph nodes in male decedent rats at 500 mg/kg/day. However, in terminal kill males at 500 mg/kg/day the incidence of deep cervical lymph node enlargement (3.7%) was similar to that in vehicle control males (3.3%). Compared with respective vehicle controls, a statistically significant increased incidence of enlargement (51.7% versus 28.3%) was noted in the lumbar lymph nodes of male rats (decedent and terminal combined) at 500 mg/kg/day. There were no significant treatment-related histopathology findings. The Peto's trend tests showed statistically significant dose responses in the incidence of THYMOMA (EPTIHELIAL, $p = 0.014$) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA ($p = 0.0199$) in thyroids in female rats. The dose selection was per the ExeCAC recommendations. Overall, the conduct of the study appeared to be adequate and acceptable.

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CARCINOGENICITY:

Study title: 104-Week Oral (Gavage) Carcinogenicity Study in Sprague Dawley Rats

Key study findings: In this study, groups of rats (60/sex/group) were given 0 (water), 0 (vehicle), 100, 200 or 500 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia at a dose volume of 5 mL/kg by oral (gavage) administration once daily for 104 weeks. An additional group of 60 female rats was given 30 mg/kg/day, at a dose volume of 3 mL/kg, for the same period.

Treatment with SB-767905-KW had no effect on survival in either sex. There was no significant in-life findings associated with treatment with SB-767905-KW. Macroscopic observations included statistically significant increased incidence of enlargement (27.3% versus 0% in controls) of the deep cervical lymph nodes in male decedent rats at 500 mg/kg/day. However, in terminal kill males at 500 mg/kg/day the incidence of deep cervical lymph node enlargement (3.7%) was similar to that in vehicle control males (3.3%). Compared with respective vehicle controls, a statistically significant increased incidence of enlargement (51.7% versus 28.3%) was noted in the lumbar lymph nodes of male rats (decedent and terminal combined) at 500 mg/kg/day. There were no significant treatment-related histopathology findings.

The Peto's trend tests showed statistically significant dose responses in the incidence of THYMOMA (EPTIHELIAL, $p = 0.014$) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA ($p = 0.0199$) in thyroids in female rats. Overall, the conduct of the study appeared to be adequate and acceptable.

Study number: Study No. BVR/389; Report No. R24679

Volume #, and page #: EDR submission dated August 9, 2007

Conducting laboratory and location:

Date of study initiation: September 24, 2003

GLP compliance: A statement of compliance was included.

QA report: yes (X) no ()

Drug, lot #, and % purity: SB-767905-KW. The details of the batches are shown below (from page 22 of the study report). The purity data not provided.

Batch/lot number	Expiry date	Conversion factor (g)	Weeks of administration	
			From	To
031001481 (02.0233)	31.08.04	1.011	1 16	14 16
031002575 (204007)	30.09.04	1.011	15 17	15 19
302013 (031015041)	31.01.05	1.011	20 56	29 56
312022 (041021383)	31.12.04	1.011	30	55
AD03735 (041047662)	31.03.05	1.009	57	66
02-0233/204007 (041052976)	30.04.05	1.009	67	73
AD03736 (051063816)	31.07.05	1.004	74	80
208009 (051072690)	31.03.06	1.013	81	85
501025 (051078392)	31.10.05	1.010	86	96
406001 (041040175)	31.01.06	1.011	97	98
411023 (051078391)	31.10.05	1.010	99	99
304016 (051092776)	31.06.06	1.013	100	100
303015 (051092775)	31.10.06	1.012	101	101
301011 (051092774)	31.07.06	1.015	102	105
501025	31.07.06	1.010	106	107

CAC concurrence: Yes (ExecCAC meeting minutes dated January 25, 2002 attached)

Study Type (2 yr bioassay, alternative model etc.): 2-year bioassay

Species/strain: Rats/Sprague Dawley

Number/sex/group; age at start of study: 60/sex/group, 5-6 weeks old. On Day 1 of treatment, rats were approximately 5 to 6 weeks old.

Animal housing: The rats were housed individually in individual ventilated cages. Each cage had its own filtered air supply to prevent unintended exposure of the animals to the test article. The internal cage atmosphere was maintained at positive pressure relative to that in the animal room. Following randomization, rats were acclimated to local housing conditions for

approximately 2 weeks. The environmental controls were set to maintain temperature within the range of 19 to 23°C and relative humidity within the range of 40 to 70%; with an approximate 12-hour light and 12-hour dark cycle.

Formulation/vehicle: Test article was suspended in 10% (w/v) aqueous acacia.

Drug stability/homogeneity: The mean concentrations of the formulations analyzed throughout the study were within $\pm 10\%$ of the nominal concentrations, indicating accuracy of formulation.

Methods:

Doses: 0, 0, 100, 200 and 500 mg/kg/day

Basis of dose selection: Saturation of absorption and was per the ExecCAC recommendations (meeting minutes dated January 25, 2002 attached)

Restriction paradigm for dietary restriction studies: None

Route of administration: Oral Gavage (5 mL/kg)

Frequency of drug administration: Once daily

Dual controls employed: Yes

Interim sacrifices: None

Study Design: The study design is shown in the table below (from page 24 of the study report).

Group details are summarised below:

Group Number	Dose ^a (mg/kg/day)	Dose Concentration (mg/mL)	Number/Sex
1	0 (water)	0	60M + 60F
2	0 (vehicle)	0	60M + 60F
3	30	10	60F
4	100	20	60M + 60F
5	200	40	60M + 60F
6	500	100	60M + 60F

a. Doses are expressed in terms of pure SB-767905-KW

Satellite PK or special study group(s): For the toxicokinetic (TK) study, two animals per sex were bled on Days 1 and 29 and during Weeks 13, 26 and 52 at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours time point.

Deviations from original study protocol: There were minor protocol deviations which did not seem to have any impact on the results and interpretations.

Statistical methods:

Mortality: The mortality data were analyzed by logrank tests for a trend across the groups. Males and females were analyzed separately (Mantel 1966, Peto 1974). The following statistical tests were carried out for groups 1 and 2: 1) a two-tailed pairwise comparison. The following statistical tests were carried out for groups 2, 4, 5 and 6 for males and groups 2, 3, 4, 5 and 6 for females: 1) a two-tailed test for a trend with dose level. 2) a two-tailed pairwise comparison test of each treatment group against the vehicle control group.

Tumor Data: The analyses were carried out for benign, malignant and benign and malignant tumors combined. The following (from 2725 and 2726 of the study report) tumors were analyzed for male and females separately. For incidental tumors (i.e. a tumor not contributing to the death), the following fixed time intervals were used to adjust for differential mortality between the treatment groups: 1-52, 53-78, 79-92, 93-104 and terminal sacrifice (FDA 2001). Log-rank methods were used to analyze the number of animals with tumors across treatment groups (Mantel 1966, Peto 1974, Peto et al. 1980). The following statistical test was carried out for groups 1 and 2 (water and vehicle control groups): 1) a two-tailed pairwise comparison. The following statistical tests were carried out for groups 2, 4, 5 and 6 for males and groups 2, 3, 4, 5 and 6 for females: a one-tailed test for a trend using nominal dose levels, with the vehicle control (group 2), a one-tailed pairwise comparison test of each treatment group against the vehicle control (group 2)

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Males

Adrenals - Benign phaeochromocytoma
Adrenals - Benign phaeochromocytoma and malignant phaeochromocytoma combined
Epididymides - Malignant mesothelioma
Liver - Benign hepatocellular adenoma
Pancreas - Benign Islet cell adenoma
Pancreas - Malignant Islet cell carcinoma
Pancreas - Benign Islet cell adenoma and malignant Islet cell carcinoma combined
Pituitary - pars distalis - Benign adenoma
Preputial glands - Benign adenoma
Skin - Benign squamous cell papilloma
Stomach - Benign squamous cell papilloma
Testes - Benign interstitial (Leydig) cell adenoma
Thyroids - Benign C-cell adenoma
Thyroids - Malignant C-cell carcinoma
Thyroids - Benign C-cell adenoma and malignant C-cell carcinoma combined
Thyroids - Benign follicular cell adenoma
Thyroids - Benign follicular cell adenoma and malignant follicular cell carcinoma combined
Haematopoietic Tumour - Malignant large granular cell lymphoma
Haematopoietic Tumour - Malignant histiocytic sarcoma

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R24679
BVR 389/033645**Females**

Adrenals - Benign pheochromocytoma
Adrenals - Benign cortical adenoma
Clitoral glands - Benign adenoma
Kidneys - Malignant renal liposarcoma
Kidneys - Benign tubular adenoma
Kidneys - Benign tubular adenoma and malignant tubular carcinoma combined
Liver - Benign hepatocellular adenoma
Mammary areas - Benign fibroadenoma
Mammary areas - Benign mammary adenoma
Mammary areas - Benign mammary fibroadenoma and benign mammary adenoma combined
Mammary areas - Malignant mammary adenocarcinoma
Mammary areas - Benign mammary fibroadenoma, benign mammary adenoma and malignant mammary adenocarcinoma combined
Pancreas - Benign Islet cell adenoma
Pituitary - pars distalis - Benign adenoma
Skin - Benign squamous cell papilloma
Skin - Benign keratoacanthoma
Thymus - Benign thymoma (epithelial)
Thyroids - Benign C-cell adenoma
Thyroids - Malignant C-cell carcinoma
Thyroids - Benign C-cell adenoma and malignant C-cell carcinoma combined
Thyroids - Benign follicular cell adenoma
Thyroids - Malignant follicular cell carcinoma
Thyroids - Benign follicular cell adenoma and malignant follicular cell carcinoma combined
Uterus - Benign endometrial polyp
Uterus - Malignant endometrial stromal sarcoma
Uterus - Malignant schwannoma
Uterus - Malignant adenocarcinoma
Uterus - Benign endometrial adenoma and malignant adenocarcinoma combined
Haematopoietic Tumour - Malignant large granular cell lymphoma
Haematopoietic Tumour - Malignant histiocytic sarcoma
Haematopoietic Tumour - Malignant mixed lymphoma

Observations and times:

Mortality: Animals were observed twice daily for mortality.

Clinical Signs: Animals were observed for clinical signs on a daily basis.

Body weights: The animals were weighed once in the week before commencement of treatment, on the day that treatment commenced, each week for the first 26 weeks of treatment and thereafter once every four weeks until Week 104 and again on the day of necropsy.

Food consumption: Food consumption was recorded during the week before treatment commenced, each week for the first 16 weeks of treatment and thereafter one week in every four weeks during the treatment period and in Week 104.

Hematology: Blood samples were collected for hematology at necropsy.

Gross pathology: Gross pathology was conducted at necropsy.

Histopathology: The tissues marked "X" in the "Examine" column of the table (from page 28 of the study report) below were used for histopathological examinations from all main study animals.

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Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Abnormalities	X	Pituitary	X
Adrenals	X	Preputial/clitoral glands	X
Aorta (thoracic)	X	Prostate	X
Brain	X	Rectum	
Caecum	X	Salivary glands -	
Colon	X	submandibular	X ¹
Duodenum	X	sublingual	X ¹
Epididymides	X	parotid	X ¹
Eyes	X	Sciatic nerve	X ¹
Femur (Femoro-tibial joint)	X	Seminal vesicles	X
Harderian glands	X	Skeletal muscle (thigh)	X ¹
Heart	X	Skin with mammary glands	X
Ileum	X	Spinal cord	X
Jejunum	X	Spleen ¹	X
Kidneys	X	Sternum with bone marrow	X
Larynx	X	Stomach	X
Liver (all lobes)	X	Testes	X
Lungs including bronchi	X	Thymus	X
Lymph node -		Thyroids with parathyroids	X
mandibular	X	Tongue	X
mesenteric	X	Trachea	X
regional to masses	X	Urinary bladder	X
Nasal cavities and skull		Uterus with cervix	X
Nasopharynx		Vagina	X
Oesophagus	X		
Optic nerves	X		
Ovaries	X		
Pancreas	X		

1. Only one examined

Toxicokinetics: For the toxicokinetic (TK) study, two animals per sex were bled on Days 1 and 29 and during Weeks 13, 26 and 52 at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours time point.

Results:

Mortality: The total number of unscheduled death is shown in the following table (from page 33 of the study report). There were no statistically significant differences in mortality between the two control groups or between vehicle control and treated groups.

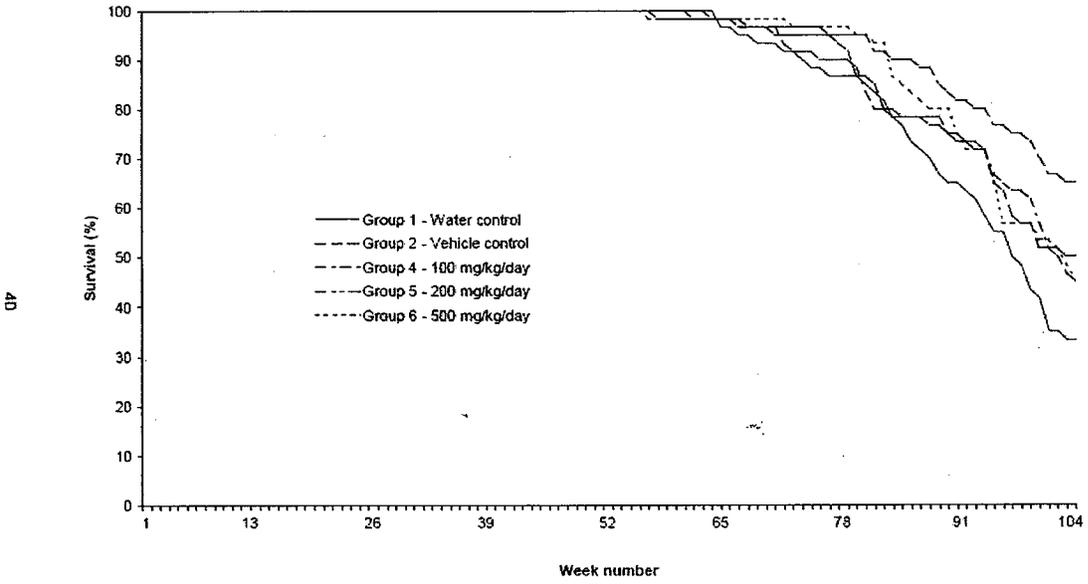
Sex	Type of Death	Weeks	Dose (mg/kg/day)					
			0 (W)	0 (V)	30	100	200	500
Male	Dead/Killed	1-52	0	0	-	0	0	0
		53-78	8	4	-	3	6	2
		79-92	14	12	-	8	10	15
		93-104	18	14	-	10	17	16
		Total	40	30	-	21	33	33
	Terminal kill	104	20	30	-	39	27	27
	Survival (%)	78	87	93	-	95	90	97
Survival (%)	104	33	50	-	65	45	45	
Female	Dead/Killed	1-52	0	0	0	0	0	0
		53-78	3	4	4	3	2	5
		79-92	6	8	5	9	7	5
		93-104	14	9	11	7	7	7
		Total	23	21	20	19	16	17
	Terminal kill	104	37	39	40	41	44	43
	Survival (%)	78	95	93	93	95	97	92
Survival (%)	104	62	65	67	68	73	72	

Key: W = water, V = vehicle

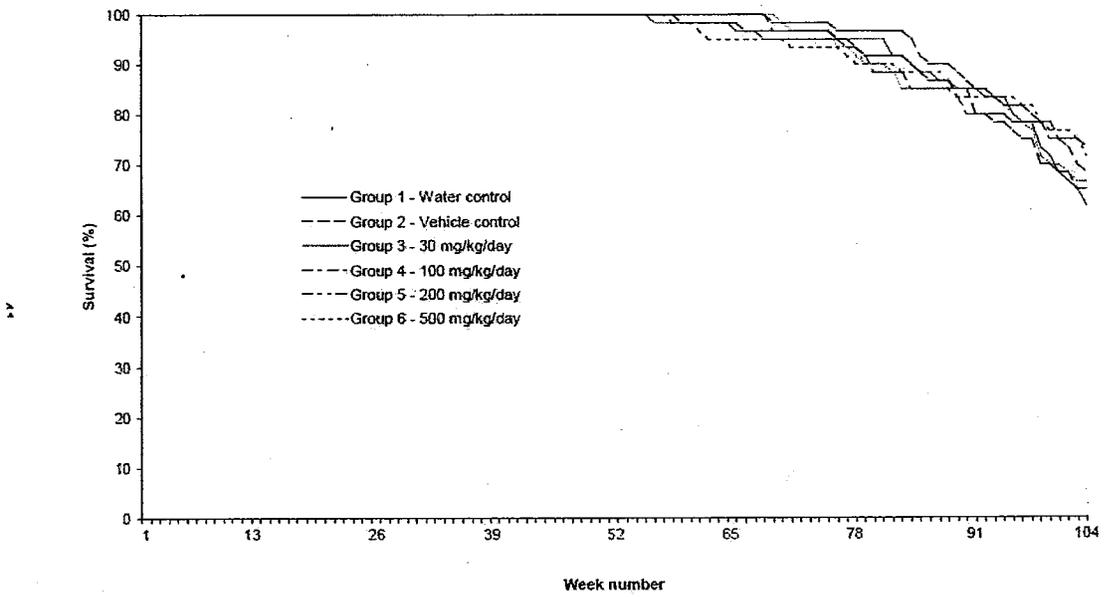
The following figures (from page 40 and 41 of the study report) show the survival curves.

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Survival - group mean values (%) for males



Survival - group mean values (%) for females



Clinical signs: No clinical signs considered to be related to treatment were observed.

Body weights: The mean initial (Week 0) and final (Week 104) weights of control (Group 1) males were 109 and 421 g, respectively. The mean initial and final weights of control (group 1) females were 95 and 295 g, respectively. There was no significant effect of treatment with SB-767905 on body weight. The following table shows the absolute body weights (g) and body weight gains (g) for males and females.

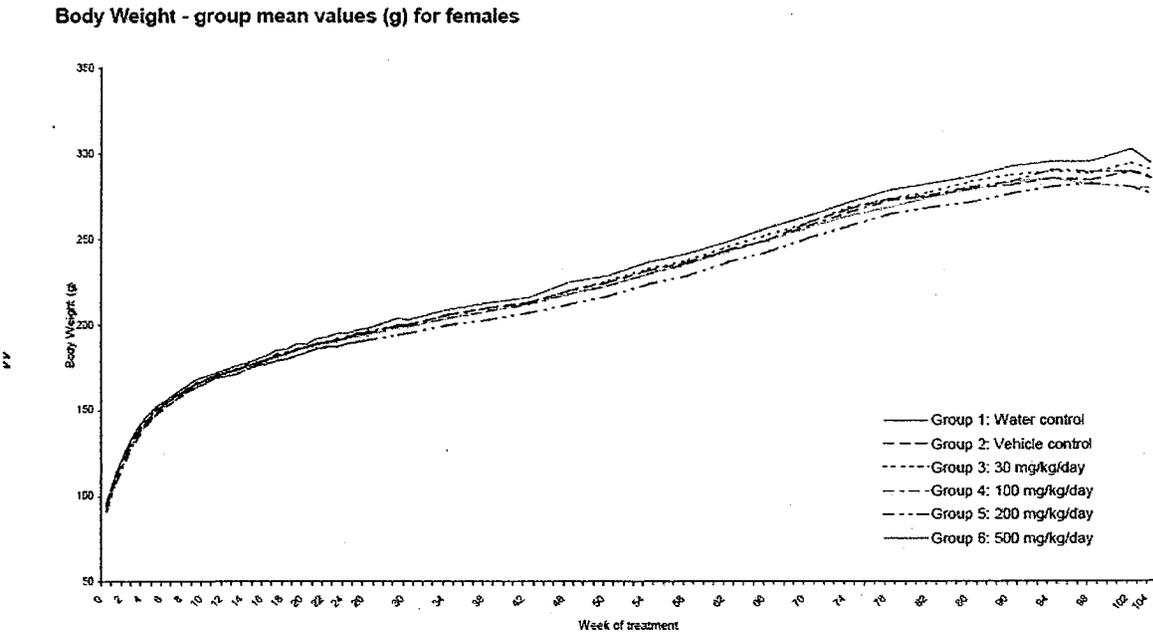
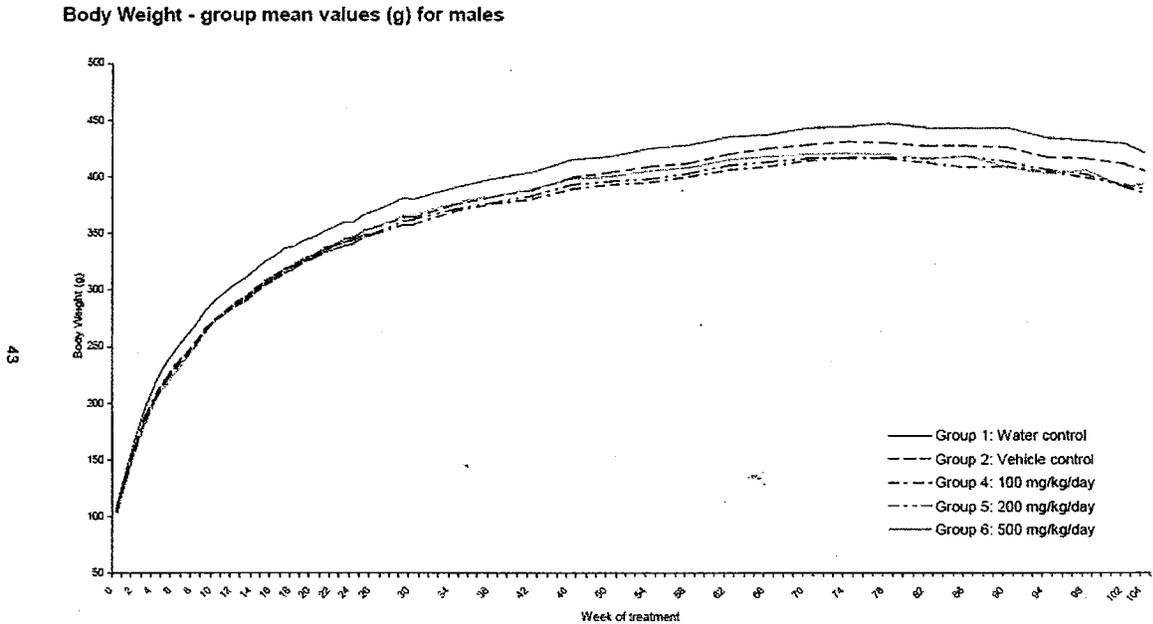
MALE	GROUP*				
Male	1	2	3	4	5
Wk 0	109	105	107	104	104
Wk 24	360	346	341	344	348
% of Control, Wk 24	100.00	96.11	94.72	95.56	96.67
ΔWk24-Wk0	251	241	234	240	244
BW Gain, % of Initial BW	230.28	229.52	218.69	230.77	234.62
BW Gain, % Of Control	100	99.67	94.97	100.21	101.88
Male	1	2	3	4	5
Wk 0	109	105	107	104	104
Wk 54	425	409	395	398	405
% of Control, Wk 24	100.00	96.24	92.94	93.65	95.29
ΔWk24-Wk0	316	304	288	294	301
BW Gain, % of Initial BW	289.91	289.52	269.16	282.69	289.42
BW Gain, % Of Control	100	99.87	92.84	97.51	99.83
Male	1	2	3	4	5
Wk 0	109	105	107	104	104
Wk 78	447	430	416	417	420
% of Control, Wk 24	100.00	96.20	93.06	93.29	93.96
ΔWk24-Wk0	338	325	309	313	316
BW Gain, % of Initial BW	310.09	309.52	288.79	300.96	303.85
BW Gain, % Of Control	100	99.82	93.13	97.06	97.99
Male	1	2	3	4	5
Wk 0	109	105	107	104	104
Wk 104	421	405	389	385	394
% of Control, Wk 24	100.00	96.20	92.40	91.45	93.59
ΔWk24-Wk0	312	300	282	281	290
BW Gain, % of Initial BW	286.24	285.71	263.55	270.19	278.85
BW Gain, % Of Control	100	99.82	92.07	94.39	97.42
FEMALE	GROUP*				
Female	1	2	3	4	5
Wk 0	95	95	92	91	92
Wk 24	195	192	192	189	192
% of Control, Wk 24	100.00	98.46	98.46	96.92	98.46
ΔWk24-Wk0	100	97	100	98	100

BW Gain, % of Initial BW	105.26	102.11	108.70	107.69	108.70
BW Gain, % Of Control	100	97.00	103.26	102.31	103.26
Female	1	2	3	4	5
Wk 0	95	95	92	91	92
Wk 54	237	232	230	224	230
% of Control, Wk 24	100.00	97.89	97.05	94.51	97.05
Δ Wk24-Wk0	142	137	138	133	138
BW Gain, % of Initial BW	149.47	144.21	150.00	146.15	150.00
BW Gain, % Of Control	100	96.48	100.35	97.78	100.35
Female	1	2	3	4	5
Wk 0	95	95	92	91	92
Wk 78	279	274	273	265	269
% of Control, Wk 24	100.00	98.21	97.85	94.98	96.42
Δ Wk24-Wk0	184	179	181	174	177
BW Gain, % of Initial BW	193.68	188.42	196.74	191.21	192.39
BW Gain, % Of Control	100	97.28	101.58	98.72	99.33
Female	1	2	3	4	5
Wk 0	95	95	92	91	92
Wk 104	295	286	287	277	280
% of Control, Wk 24	100.00	96.95	97.29	93.90	94.92
Δ Wk24-Wk0	200	191	195	186	188
BW Gain, % of Initial BW	210.53	201.05	211.96	204.40	204.35
BW Gain, % Of Control	100	95.50	100.68	97.09	97.07

*GROUP:

1. Control (Water)
2. Control (vehicle)
3. SB-767905: 100 mg/kg/day
4. SB-767905: 200 mg/kg/day
5. SB-767905: 500 mg/kg/day

The following figures (from page 43 and 44 of the study report) show the growth curves in males and females.



Food consumption: The mean initial (Week 1) and final (Week 104) food consumption in control (Group 1) males was 16.6 and 18.0 g/animal/day, respectively. The mean initial (Week 1) and final (Week 104) food consumption in control (Group 1) females was 13.0 and

13.14 g/animal/day, respectively. There was no significant effect of treatment on food consumption.

Hematology: Investigation of hematology parameters in Week 104 revealed no differences attributable to treatment.

Gross pathology: A statistically significant increase in the incidence of enlargement of the cervical lymph node was seen in male decedent rats at 500 mg/kg/day compared with male decedent vehicle controls as shown in the following table (from page 35 of the study report). However, in terminal kill males at 500 mg/kg/day the incidence of deep cervical lymph node enlargement (3.7%) was similar to that in vehicle control males (3.3%).

Dose (mg/kg/day)	Male (decedent)				
	0	0	100	200	500
Total	40	30	21	33	33
Deep cervical lymph node enlarged	5	0	0	3	9a

a p<0.05

A statistically significant increase incidence of enlargement of lumbar lymph nodes was noted in male rats (decedent and terminal combined) treated with 500 mg/kg/day compared with male vehicle controls. The following table (from page 35 of the study report) shows the lumbar lymph nodes findings.

Dose (mg/kg/day)	Male				
	0	0	100	200	500
Total	60	60	60	60	60
Lumbar lymph node enlarged	17	17	19	23	31b

b p<0.01

Histopathology:

Non-neoplastic: There were no significant non-neoplastic findings associated with treatment with SB-767905.

Neoplastic: The Peto's trend tests showed statistically significant dose responses in the incidence of THYMOMA (EPTIHELIAL, p = 0.014) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA (p = 0.0199) in thyroids in female rats. The following tables (from the draft statistical review) show the significant tumor findings.

Male Rats

Organ Name	Tumor Name	Vehicle	100 mg/kg/d	200 mg/kg/d	500 mg/kg/d	P-Value (Exact)
THYMUS	B-THYMOMA(EPITHELIAL)	0	0	2	3	0.0104

Female Rats

Organ Name	Tumor Name	Vehicle	30 mg/kg/d	100 mg/kg/d	200 mg/kg/d	500 mg/kg/d	P-Value (Exact)
THYROIDS	M-C-CELL CARCINOMA	0	1	0	0	3	0.0199

The following tables (from page 167-171) show the neoplastic findings for all animals.

Microscopic Pathology - group distribution of neoplastic findings for all animals

--- NUMBER OF ANIMALS AFFECTED ---

ORGAN AND FINDING DESCRIPTION	NUMBER	SEX: --- MALE ---						SEX: --- FEMALE ---					
		-1-		-2-		-4-		-1-		-2-		-3-	
		60	60	60	60	60	60	60	60	60	60	60	60
** TOP OF LIST **													
ADRENALS	NUMBER EXAMINED:	60	60	60	59	60	59	59	60	59	60	60	60
--M-MALIGNANT PHAEOCHROMOCYTOMA		1	0	0	0	1	0	0	0	0	0	0	0
--M-COMPLEX PHAEOCHROMOCYTOMA		1	0	0	0	0	0	0	0	0	0	0	0
--M-CORTICAL CARCINOMA		0	0	1	0	0	0	0	0	0	0	0	0
--B-PHAEOCHROMOCYTOMA		10	2	7	3	4	1	0	1	0	1	1	2
--B-CORTICAL ADENOMA		0	0	0	0	0	0	1	0	0	2	1	0
BRAIN	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--M-OLIGODENDROGLIOMA		0	0	0	0	0	0	1	0	1	0	0	0
--M-MENINGEAL SARCOMA		0	0	0	0	1	0	0	0	0	0	0	0
--M-ASTROCYTOMA		0	0	0	0	0	0	0	0	1	0	0	0
--M-MALIGNANT RETICULOSIS		0	0	0	0	1	0	0	0	0	0	0	0
--B-GRANULAR CELL TUMOUR		0	0	0	1	0	0	0	0	0	0	0	0
CARCUM	NUMBER EXAMINED:	59	59	60	59	60	60	59	60	58	60	60	60
--M-ADENOCARCINOMA		0	0	0	0	0	1	0	0	0	0	0	0
CLITORAL GLAND	NUMBER EXAMINED:	0	0	0	0	0	60	60	59	58	57	60	60
--B-ADENOMA		0	0	0	0	0	0	0	1	2	1	3	3
COLON	NUMBER EXAMINED:	59	60	60	58	60	60	60	60	60	60	60	60
--B-ADENOMA		1	0	0	0	0	0	0	0	0	0	0	0
EPIDIDYMIDES	NUMBER EXAMINED:	60	60	60	60	60	0	0	0	0	0	0	0
--M-MESOTHELIOA		6	0	1	3	0	0	0	0	0	0	0	0
EYES	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--B-SCHWANNOMA		0	1	0	0	0	0	0	0	0	0	0	0
HARDERIAN GLANDS	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--M-ADENOCARCINOMA		0	0	1	0	0	0	0	0	0	0	0	0

Microscopic Pathology - group distribution of neoplastic findings for all animals

		--- NUMBER OF ANIMALS AFFECTED ---											
		SEX: -----MALE-----						-----FEMALE-----					
		GROUP:	-1-	-2-	-4-	-5-	-6-	-1-	-2-	-3-	-4-	-5-	-6-
ORGAN AND FINDING DESCRIPTION	NUMBER:	60	60	60	60	60	60	60	60	60	60	60	60
JEJUNUM	NUMBER EXAMINED:	58	60	60	59	59	60	58	60	58	60	60	60
--M-ADENOCARCINOMA		0	1	0	0	0	0	0	1	0	0	0	0
KIDNEYS	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--M-RENAL LIPOSARCOMA		0	0	0	1	0	0	0	0	0	1	0	1
--M-RENAL MESENCHYMAL TUMOUR		0	0	0	0	0	0	1	0	0	0	0	0
--M-TUBULAR CARCINOMA		0	0	0	0	0	0	0	1	0	0	0	0
--B-TUBULAR ADENOMA		0	0	0	0	0	0	0	2	0	0	0	0
LIVER	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--B-HEPATOCELLULAR ADENOMA		1	3	5	4	3	5	3	1	2	5	4	
LUNGS & BRONCHI	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	60
--M-BRONCHIOLALVEOLAR ADENOCARCINOMA		2	0	0	0	0	0	0	0	0	0	0	0
MAMMARY	NUMBER EXAMINED:	59	60	60	60	60	60	60	60	60	60	60	60
--M-MAMMARY ADENOCARCINOMA		0	1	0	0	0	0	0	0	1	1	1	1
--M-DUCTULAR CARCINOMA		0	0	0	0	0	0	1	0	0	0	0	0
--B-MAMMARY FIBROADENOMA		0	0	0	0	0	10	9	14	16	12	8	
--B-MAMMARY ADENOMA		0	0	0	0	0	0	0	1	0	2		
OVARIES	NUMBER EXAMINED:	0	0	0	0	0	60	60	60	60	60	60	60
--B-GRANULOSA CELL TUMOUR		0	0	0	0	0	0	1	0	0	0	0	1
PANCREAS	NUMBER EXAMINED:	60	60	60	60	59	59	60	60	60	60	60	60
--M-ISLET CELL CARCINOMA		2	0	1	1	0	0	0	0	0	0	0	0
--B-ISLET CELL ADENOMA		5	6	5	8	8	1	2	0	1	1	1	
--B-MIXED CELL ADENOMA		1	2	0	0	0	0	0	0	0	0	0	0
PITUITARY	NUMBER EXAMINED:	60	60	59	59	60	60	60	59	60	60	59	59
--B-ADENOMA, PARS DISTALIS		20	12	13	17	11	20	20	22	21	21	21	27
--B-ADENOMA, PARS INTERMEDIA		1	0	0	0	0	1	0	0	0	0	0	0

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Microscopic Pathology - group distribution of neoplastic findings for all animals

ORGAN AND FINDING DESCRIPTION	--- NUMBER OF ANIMALS AFFECTED ---											
	SEX: MALE						SEX: FEMALE					
	GROUP: -1-	-2-	-4-	-5-	-6-	-1-	-2-	-3-	-4-	-5-	-6-	
	NUMBER:	60	60	60	60	60	60	60	60	60	60	60
PREPUTIAL GLANDS	NUMBER EXAMINED:	60	60	60	60	60	0	0	0	0	0	0
--B-ADENOMA		3	1	1	0	1	0	0	0	0	0	0
SALIVARY GLANDS	NUMBER EXAMINED:	60	60	60	60	60	60	59	60	60	60	60
--M-ADENOCARCINOMA		1	1	0	0	0	0	0	0	0	0	0
SEMINAL VESICLES	NUMBER EXAMINED:	60	59	58	60	60	0	0	0	0	0	0
--B-ADENOMA		0	1	0	0	0	0	0	0	0	0	0
SKELETAL MUSCLE	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60
--M-HEMANGIOSARCOMA		0	0	0	0	0	1	0	0	0	0	0
SKIN	NUMBER EXAMINED:	59	60	60	60	60	60	60	60	60	60	60
--M-BASAL CELL CARCINOMA		0	1	0	0	0	0	0	0	0	0	0
--B-SQUAMOUS CELL PAPILLOMA		1	1	1	1	0	0	0	1	0	1	0
--B-KERATOCANTHOMA		2	1	0	0	0	1	0	0	2	0	0
--B-LEIOMYOMA		1	0	0	0	0	0	0	0	0	0	0
SPLEEN	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60
--M-SARCOMA, UNDIFFERENTIATED		0	0	1	0	0	0	0	0	0	0	0
STOMACH	NUMBER EXAMINED:	60	60	59	60	60	60	60	60	60	60	60
--B-SQUAMOUS CELL PAPILLOMA		0	0	1	0	1	0	0	1	0	0	0
TESTES	NUMBER EXAMINED:	60	60	60	60	60	0	0	0	0	0	0
--B-INTERSTITIAL (LEYDIG) CELL ADENOMA		32	37	43	34	41	0	0	0	0	0	0
THYMUS	NUMBER EXAMINED:	57	60	60	58	58	57	59	60	60	59	60
--B-THYMOMA (EPITHELIAL)		0	0	0	0	1	0	0	1	0	0	1
THYROIDS	NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60
--M-C-CELL CARCINOMA		1	0	0	1	1	1	0	1	0	0	3

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Microscopic Pathology - group distribution of neoplastic findings for all animals

ORGAN AND FINDING DESCRIPTION	--- NUMBER OF ANIMALS AFFECTED ---											
	SEX: -----MALE-----						-----FEMALE-----					
	GROUP: -1-	-2-	-4-	-5-	-6-	-1-	-2-	-3-	-4-	-5-	-6-	
NUMBER EXAMINED:	60	60	60	60	60	60	60	60	60	60	60	
H'POIETIC TUMOUR	60	60	60	60	60	60	60	60	60	60	60	
--M-LARGE GRANULAR CELL LYMPHOMA	29	30	26	34	35	17	18	27	14	16	16	
--M-MALIGNANT LYMPHOMA	0	0	0	0	0	1	2	0	0	0	0	
--M-HISTIOCYTIC SARCOMA	1	0	0	2	1	0	0	1	0	0	1	
--M-MIXED LYMPHOMA	0	0	0	0	0	0	0	1	0	2	1	
HEAD	0	0	0	1	0	1	0	1	1	0	0	
--M-LEIOMYOSARCOMA	0	0	0	0	0	0	0	0	0	1	0	
--B-SQUAMOUS PAPILLOMA	0	0	0	1	0	1	0	0	0	0	0	
SUBCUTIS	12	6	12	7	7	1	0	2	0	1	4	
--M-FIBROSARCOMA	0	0	1	1	0	0	0	0	0	0	0	
--M-SCHWANNOMA	1	0	0	0	0	0	0	0	0	0	0	
--B-LIPOMA	0	0	2	0	1	0	0	0	0	0	0	
--B-FIBROMA	5	5	7	3	5	0	0	1	0	0	1	
TAIL	26	26	34	26	36	9	5	7	5	8	11	
--M-LEIOMYOSARCOMA	0	0	0	1	0	0	0	0	0	0	0	
THORAX	2	0	0	1	3	0	1	0	1	0	1	
--M-ANAPLASTIC CARCINOMA	0	0	0	0	1	0	0	0	0	0	0	

** END OF LIST **

Toxicokinetics: The levels of SB-767905 or its metabolite SB-791399 found in few control plasma samples were considered to be insignificant and therefore did not appear to have had any significant impact upon the integrity or interpretation of this study. The Tmax occurred between approximately 0.5 and 24 hours after dosing for SB-767905 and 1.0 and 24 hours after dosing for SB-791399. There was generally no clear relationship between dose and systemic exposure of SB-767905 in male and female animals. The systemic exposure to SB-767905 achieved at Week 52 (as measured by AUC_{0-t} and C_{max}) was generally similar to the systemic exposure achieved on Day 1 of the study, but in some cases varied quite considerably at the different time points. In general, there were no consistent notable sex related differences in the exposure to SB-767905.

Systemic exposure to SB-791399 (as measured by AUC_{0-t} and C_{max}) did not increase with escalating dose in male rats and increased in a less than proportional manner with escalating dose in female rats. Overall in female rats in Week 52, for an approximately 17-fold increase in dose from 30 to 500 mg/kg/day, AUC_{0-t} increased approximately 2.4-fold. The systemic exposure to SB-791399 achieved at Week 52 (as measured by AUC_{0-t} and C_{max}) was approximately 3-to 11-fold greater than that achieved on Day 1, at all doses and in both sexes. Systemic exposure values were generally similar at Weeks 13, 26 and 52 in males and females. Systemic exposure to SB-791399 (AUC_{0-t}), was consistently higher (approximately 1.2- to 4.6-fold) in female animals than that achieved in male animals. The systemic exposure (AUC_{0-t}) to SB-793199 was consistently higher (to approximately 1.4- to 86.4-fold) than the systemic exposure to SB-

767905, in both sexes. The following tables (page 2636-2639 of the study report) show the TK parameters for SB-767905 and SB-791399 in males and females.

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BVR 389/033645

Table 1 Summary of the Composite Toxicokinetic Parameters for SB-767905 Derived from Plasma Concentration-Time Results from Male F344 Rats Following Oral Administration of SB-767905-KW for 52 Weeks

Dose level (mg/kg/day)	Day/ Week	AUC ₀₋₁ (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
100	Day 1	86.3	62.1	0.50
	Day 29	6.76	2.14	1.00
	Week 13	28.4	3.57	2.01
	Week 26	20.0	6.85	0.50
	Week 52	57.7	5.46	0.50
200	Day 1	30.0	5.47	2.02
	Day 29	26.3	2.51	4.00
	Week 13	21.9	4.07	2.00
	Week 26	24.7	3.24	4.00
	Week 52	86.5	19.7	1.06
500	Day 1	92.4	8.90	5.99
	Day 29	43.6	4.52	4.00
	Week 13	60.2	5.66	6.00
	Week 26	43.7	4.74	2.00
	Week 52	119	9.61	1.08

Table 2 Summary of the Composite Toxicokinetic Parameters for SB-767905 Derived from Plasma Concentration-Time Results from Female F344 Rats Following Oral Administration of SB-767905-KW for 52 Weeks

Dose level (mg/kg/day)	Day/ Week	AUC ₀₋₁ (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
30	Day 1	99.5	42.1	12.03
	Day 29	4.41	1.40	4.00
	Week 13	29.5	3.94	4.00
	Week 26	48.5	5.11	1.00
	Week 52	146	116	1.01
100	Day 1	27.4	5.38	4.00
	Day 29	33.5	6.03	4.00
	Week 13	40.2	6.24	2.01
	Week 26	75.8	8.66	2.00
	Week 52	76.7	10.3	4.00
200	Day 1	29.5	6.53	2.03
	Day 29	22.9	4.49	1.07
	Week 13	59.1	4.17	24.00
	Week 26	84.0	8.47	2.00
	Week 52	131	20.8	0.50
500	Day 1	78.5	12.1	12.02
	Day 29	79.5	9.21	4.00
	Week 13	97.7	16.6	4.00
	Week 26	145	17.1	4.00
	Week 52	213	23.9	4.00

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**Table 3 Summary of the Composite Toxicokinetic Parameters for SB-791399
Derived from Plasma Concentration-Time Results from Male F344
Rats Following Oral Administration of SB-767905-KW for 52 Weeks**

Dose level (mg/kg/day)	Day/ Week	AUC _{0-t} (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
100	Day 1	120	12.9	8.00
	Day 29	361	19.8	24.00
	Week 13	522	30.3	4.07
	Week 26	458	24.7	12.00
	Week 52	517	38.3	8.00
200	Day 1	153	15.5	8.05
	Day 29	262	15.2	2.00
	Week 13	551	52.1	8.00
	Week 26	654	36.8	8.00
	Week 52	671	37.0	12.00
500	Day 1	246	24.9	11.99
	Day 29	314	18.1	6.00
	Week 13	815	42.8	1.00
	Week 26	675	36.4	8.00
	Week 52	835	47.5	12.00

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Table 4 Summary of the Composite Toxicokinetic Parameters for SB-791399 Derived from Plasma Concentration-Time Results from Female F344 Rats Following Oral Administration of SB-767905-KW for 52 Weeks

Dose level (mg/kg/day)	Day/ Week	AUC _{0-t} (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
30	Day 1	283	34.4	12.03
	Day 29	381	31.3	8.00
	Week 13	950	63.6	12.00
	Week 26	1000	76.7	8.00
	Week 52	1370	97.8	8.00
100	Day 1	198	17.0	6.00
	Day 29	867	45.1	4.00
	Week 13	1550	83.1	24.00
	Week 26	1660	104	12.00
	Week 52	1360	80.8	12.00
200	Day 1	308	32.9	12.00
	Day 29	920	45.9	12.00
	Week 13	1640	84.2	4.01
	Week 26	2600	161	12.00
	Week 52	2940	185	12.00
500	Day 1	301	35.5	12.02
	Day 29	824	70.2	6.00
	Week 13	2420	149	8.00
	Week 26	3110	162	24.00
	Week 52	3260	205	12.00

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: This study appears to be adequate and acceptable. The dose selection based on the saturation of absorption appears to be appropriate and was in concurrence with the ExecCAC recommendations. The selection of the test model also appears to be appropriate. Overall, the study was conducted in a valid manner.

Evaluation of tumor findings: The Peto's trend tests showed statistically significant dose responses in the incidence of THYMOMA (EPTIHELIAL, $p = 0.014$) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA ($p = 0.0199$) in thyroids in female rats. The sponsor did not provide historical control data.

Carcinogenicity summary: In this study, groups of rats (60/sex/group) were given 0 (water), 0 (vehicle), 100, 200 or 500 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia at a dose volume of 5 mL/kg by oral (gavage) administration once daily for 104 weeks. An additional group of 60 female rats was given 30 mg/kg/day, at a dose volume of 3 mL/kg, for the same period.

Treatment with SB-767905-KW had no effect on survival in either sex. There was no significant in-life findings associated with treatment with SB-767905-KW. Macroscopic observations included statistically significant increased incidence of enlargement (27.3% versus 0% in controls) of the deep cervical lymph nodes in male decedent rats at 500 mg/kg/day. However, in terminal kill males at 500 mg/kg/day the incidence of deep cervical lymph node enlargement (3.7%) was similar to that in vehicle control males (3.3%). Compared with male vehicle controls, a statistically significant increased incidence of enlargement (51.7% versus 28.3%) was noted in the lumbar lymph nodes of male rats (decedent and terminal combined) at 500 mg/kg/day. There were no significant treatment-related histopathology findings.

The Peto's trend tests showed statistically significant dose responses in the incidence of THYMOMA (EPTIHELIAL, $p = 0.014$) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA ($p = 0.0199$) in thyroids in female rats. Overall, the conduct of the study appeared to be adequate and acceptable.

Carcinogenicity conclusions: Alvimopan appears to have caused statistically significant increase in the incidence of THYMOMA (EPTIHELIAL, $p = 0.014$) in the thymus of male rats, and in the incidence of CELL CARCINOMA ($p = 0.0199$) in thyroids of female rats when compared to respective controls.

Recommendations for further analysis: None

Labeling Recommendations: The sponsor may be asked to modify the proposed label of Entereg® as suggested below.

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 Trade Secret / Confidential

 / Draft Labeling

 Deliberative Process

Withheld Track Number: Pharm/Tox- 8

SUMMARY AND EVALUATION:

Alvimopan is a relatively selective, peripherally active μ -opioid receptor antagonist intended for the management of postoperative ileus (POI), a serious condition following abdominal or pelvic surgery. The results from Study SB767905/014 (Study 014), a long-term study in non-cancer IBD patients, demonstrated an imbalance in the number of neoplasms, bone fractures and myocardial infarction compared to the placebo. The Division asked the sponsor to submit the full study reports of the carcinogenicity studies in mice and rats. In this submission, the sponsor presented the reports of 104-week oral carcinogenicity studies in mice and rats and proposed labeling for Carcinogenesis, Mutagenesis and Impairment of Fertility.

Mice

In a 104-week oral carcinogenicity study in CD-1 mice, groups of mice (60/sex/group) were administered 0 (purified water), 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia (10 mL/kg) by oral (gavage) administration once daily for up to 104 weeks. Survival in the female group at 100 mg/kg/day fell below 15 animals in Week 101, and all surviving females in this group were sacrificed in Week 101. Survival in the vehicle control female group fell below 15 in Week 102, and all remaining females from all groups were killed in Weeks 102/103. An additional 27 animals/sex were included at doses of 0 (vehicle), 100, 1000 or 4000 mg/kg/day SB-767905-KW for toxicokinetic evaluation.

The homogeneity tests for mortality data showed statistically significant differences in survivals for only male mice across treatment groups. P-values from both Cox and Kruskal-Wallis tests were approximately zero. From Kaplan-Meier Survival Functions for male mice, survival at 1000 mg/kg/day and 4000 mg/kg/day appeared to be much better than the vehicle control group. For comparisons of the two control groups, in males, the water control (Group 5) demonstrated significantly lower mortality than the vehicle control (Group 1) ($P = 0.032$). For females, there was no significant difference in mortality between the two control groups ($P = 0.05$).

There appeared to be no significant treatment-related effects on clinical signs, food consumption, body weight, hematology, and gross pathology. There was a higher incidence (up to 22% and 27% in males and females, respectively) of minimal to slight rhinitis in animals of both sexes at 4000 mg/kg/day, generally involving the anterior-most nasal cavity. This, however, fell within the historical control data for rhinitis (up to 32% and 38% in males and females respectively).

Peto's trend test showed statistically significant dose responses in the incidence of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor ($p = 0.0063$), and skin/appendage FIBROBLASTIC tumor ($p = 0.0003$) in the female mice. In addition, pairwise comparisons also revealed significantly higher incidences of S/A fibroblastic ($p = 0.0212$) tumor and osteogenic ($p = 0.0424$) tumors in females at high dose when compared to vehicle control. The sponsor stated that the higher incidence of fibroblastic tumors (8.3%) at 4000 mg/kg/day fell within the historical control range (0 to 9.8%) for this tumor in this mouse strain/sex in this laboratory. The sponsor did not submit the historical control data. Per the sponsor, the incidence (6.7%) of

osteogenic tumors in females at 4000 mg/kg/day was higher compared to that of the historical control incidence data (0 to 2.9%). The dose selection was per the ExecCAC recommendations (meeting minutes dated December 18, 2002). The conduct of the study appears to be adequate and acceptable.

Overall, it appears that alvimopan caused significant increase in the incidences of SARCOMA in the skin/subcutis ($p = 0.0063$), OSTEOGENIC tumor ($p = 0.0063$), and skin/appendage FIBROBLASTIC tumor ($p = 0.0003$) in the female mice.

Rats

In this study, groups of rats (60/sex/group) were given 0 (water), 0 (vehicle), 100, 200 or 500 mg/kg/day SB-767905-KW in 10% (w/v) aqueous acacia at a dose volume of 5 mL/kg by oral (gavage) administration once daily for 104 weeks. A further group of 60 female rats was given 30 mg/kg/day, at a dose volume of 3 mL/kg, for the same period.

Treatment with SB-767905-KW had no effect on survival in either sex. There was no significant in-life findings associated with treatment with SB-767905-KW. Macroscopic observations included statistically significant increased incidence of enlargement (27.3% versus 0% in controls) of the deep cervical lymph nodes in male decedent rats at 500 mg/kg/day. However, in terminal kill males at 500 mg/kg/day the incidence of deep cervical lymph node enlargement (3.7%) was similar to that in vehicle control males (3.3%). Compared with male vehicle controls, a statistically significant increased incidence of enlargement (51.7% versus 28.3%) was noted in the lumbar lymph nodes of male rats (decedent and terminal combined) at 500 mg/kg/day. There were no significant treatment-related histopathology findings.

Overall, the Peto's trend tests showed statistically significant increase in the incidence of THYMOMA (EPITHELIAL, $p = 0.014$) in the thymus in male rats, and in the incidence of C-CELL CARCINOMA ($p = 0.0199$) in thyroids in female rats. Overall, the conduct of the study appeared to be adequate and acceptable.

RECOMMENDATION: None

Labeling Recommendations: The sponsor may be asked to modify the proposed label of Entereg® as suggested in the text of this review.

Tamal K. Chakraborti, Ph.D. Date
Pharmacologist

Comment:

Sushanta K. Chakder, Ph.D. Date
Acting Team Leader

cc:

IND

HFD-180

HFD-181/CSO

HFD-180/Dr. Chakder

HFD-180/Dr. Chakraborti

APPENDIX-I: ExecCAC Meeting Minutes for the Mice Study, Page 59

APPENDIX-II: ExecCAC Meeting Minutes for the Rat Study, Page 62

IND 56,533

Executive CAC

Date of Meeting: December 10, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
Abby Jacobs, Ph.D., HFD-540, Alternate Member
Karen Davis Bruno, Ph.D., HFD-510, Alternate Member
Jasti Choudary, B.V.Sc, Ph.D., HFD-180, Supervisory Pharmacologist
Yash M. Chopra, M.D., Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Yash M. Chopra, M.D., Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 56,533

Drug Name: Alvimopan Capsules

Sponsor: Adolor Corporation, Exton, PA.

Alvimopan is a relatively selective peripheral μ -opioid receptor antagonist. The compound is currently under development for the treatment of opioid induced bowel dysfunction. The sponsor submitted a protocol and dose selection for a 2-year mouse carcinogenicity study, and reports of a 13-week oral toxicity study in mice and a special 5-day toxicokinetic study in mice. Sponsor also referred to an earlier submission (study # 808-007 in amendment #085 dated 12/19/2001) in which data relating to maximum feasible concentration (MFD) of the dosing solution was provided.

Mouse Carcinogenicity Study Protocol and Dose Selection:

In the 13-week toxicity study, mice were treated with oral doses of alvimopan at 100, 300, 600 and 1000 mg/kg/day. There were no treatment-related effects on body weights and food consumption of the animals. A small decrease in the absolute reticulocyte counts and slight increase in myeloid/erythroid ratio was seen males of 1000 mg/kg/day treatment group. Such effects were not seen in treated females. No specific target organs of the toxicity were identified. A maximum tolerated dose (MTD) could not be identified. The toxicokinetic data of the study indicated that the absorption of the compound was low and erratic. The data of the study were not useful for the dose

IND 56533
Page 2 of 2

selection of the 2-year mouse carcinogenicity study.

In a special 5-day toxicokinetic study, 5 groups of mice were treated with oral doses of 500, 1000, 2000 and 4000 mg/kg/day alvimopan. On day 1, alvimopan AUC values were 194, 468, 1013 and 1853 ng.hr/ml in males and 201, 728, 842 and 1185 ng.hr/ml in females of 500, 1000, 2000 and 4000 mg/kg/day treatment groups. On day 5, absorption of the compound was poor and erratic. The plasma concentrations of the metabolite, ADL 08-0011 were linear but not dose proportional on study day 1 and 5. The plasma AUC values of the parent compound or the metabolite were not dose related and not sufficiently high to provide the needed 25-fold ratio to the expected human plasma AUC at the projected clinical dose.

In the previous study to determine the maximum feasible concentration of alvimopan dosing solutions, the compound suspensions at the concentration from 150, 200, 300, 400 to 500 mg/ml in 5% gum acacia were examined for the uniform homogeneity and the ease of aspiration through a dosing camula. A suspension containing 500 mg/ml was more viscous and was difficult to aspirate. A suspension containing 400 mg/ml alvimopan was identified as a maximum feasible concentration in the study. This corresponds to maximum feasible dose (MFD) of 4000 mg/kg dose to be administered in 10 ml/kg. Based on the identified MFD, the dose of 4000 mg/kg/day was proposed by sponsor as the high dose. The proposed mid and low doses were 1000 and 100 mg/kg/day respectively. The proposed mid dose of 1000 mg/kg/day was the highest dose in the 13-week toxicity study. It did not exert any limiting toxic effect. The proposed low dose of 100 mg/kg/day alvimopan is expected to provide higher exposure than the expected exposure at the projected clinical dose of 3 mg/day. Sponsor proposed doses are acceptable.

In the proposed 2-year mouse carcinogenicity study, there will be 2 control groups (5% gum acacia vehicle and the other with distilled water) and 3 treatment groups. Additionally 4 groups of 15 animals/sex/group will be used to collect blood samples from 3 animals/sex/group prior to dosing, 1, 4, 8 and 24 hr post dosing at study week 13 and 26 to determine the toxicokinetic parameters of the alvimopan and its metabolite (ADL – 08-0011). Histopathological examination will be undertaken for tissues of animals of all the main study groups.

Executive CAC Recommendations and Conclusions:

1. The Committee concurs with the sponsor's proposed oral doses of 100, 1000 & 4000 mg/kg/day in males and females based on MFD.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

IND 56533
Page 3 of 3

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/Y. Chopra, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

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Executive CAC

January 22, 2002

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair
John Leighton, Ph.D., HFD-150, Alternate Member
Tim McGovern, Ph.D., HFD-170, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist
Tamal Chakraborti, Ph.D., HFD-180, Presenting Reviewer

Author of Draft: Tamal Chakraborti, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND #: 56, 553

Drug Name: ADL8-2698

Sponsor: Adolor Corporation

The sponsor has previously submitted dose selection/protocols in two separate occasions (Amendment # 047 dated April 3, 2001 and Amendment # 060 dated July 11, 2001). The Exec CAC did not concur with proposed doses of 50, 100, and 200 mg/kg/day (Amendment # 047 dated April 3, 2001) as 200 mg/kg/day did not appear to be the MTD. In the revised protocol (Amendment # 060 dated July 11, 2001) at 50, 100, 200, and 500 mg/kg/day, the Exec CAC concurred with the proposed high dose of 500 mg/kg/day in females based on saturation of absorption criterion; however, the committee did not concur with the proposed high dose of 500 mg/kg/day in males based on this criterion, which has not been demonstrated in males. In this submission, the sponsor presented another protocol at 100, 200, and 500 mg/kg/day and a pharmacokinetic study in rat in support of the dose selection.

Rat Carcinogenicity Dose Selection:

The sponsor submitted this revised protocol along with the report of a formulation and toxicokinetic study (Study No. 808-007) of ADL8-2698 and its metabolite ADL08-0011 in rat to support the basis of dose selection. In this study, formulations were prepared at 150, 200, 300, 400, and 500 mg/ml and assessments of homogeneity and ease of aspiration through a dosing tube were made. The maximum feasible concentration was determined to be 400 mg/ml in 10% w/v gum acacia suspension, as at 500 mg/ml, the suspension was not homogenous and was found to be difficult to aspirate through a rodent gavage tube and syringe. This maximum feasible concentration of 400 mg/ml corresponds to MFD of 4000 mg/kg at a dose volume of 10 ml/kg. In the toxicokinetic portion of this study, rats were treated (single, oral) at 500, 1000, 2000, or 4000 mg/kg (10 ml/kg) and serial plasma samples were collected for the determination of ADL8-2698 and its amide hydrolysis metabolite, ADL08-0011. There was no dose-related increase in

the exposure to the parent drug in either sex. In males, mean (\pm SD) AUC_{0-24h} values for the parent drug were 36.3 ± 7.8 , 37.5 ± 7.9 , 37.3 ± 9.8 , and 27.4 ± 9.7 ng.hr/ml at 500, 1000, 2000, and 4000 mg/kg/day, respectively. In females, mean AUC_{0-24h} values for the parent drug were 42.1 ± 7.2 , 44.0 ± 12.9 , 25.9 ± 3.0 , and 34.8 ± 7.5 ng.hr/ml at 500, 1000, 2000, and 4000 mg/kg/day, respectively. The plasma concentrations of ADL08-0011 (metabolite of ADL8-2698) increased over the 24-hr collection period in both sexes; however, there was no significant difference in the plasma concentrations of the metabolite either across sex or different dose groups. In males, the AUC_{0-24h} values for ADL8-0011 were calculated (by the reviewer using trapezoidal rule) to be 279.07, 258.29, 225.40, and 230.79 ng.h/ml at 500, 1000, 2000, and 4000 mg/kg, respectively, indicating no dose-related increase in exposure to the metabolite, which could be attributed to the saturation of absorption of the parent drug. In females, the AUC_{0-24h} values for ADL8-0011 were calculated (by the reviewer using trapezoidal rule) to be 791.43, 596.46, 360.68, and 481.44 ng.h/ml at 500, 1000, 2000, and 4000 mg/kg, respectively. Unlike the previous study in male rats, the results of the current study demonstrated an apparent saturation of absorption of ADL8-2698 in both males and females, as there was no dose-related increase in AUC values in either sex from 500 to 4000 mg/kg. There was no apparent sex-related difference in exposure to parent drug or metabolite either. It is to be mentioned here that the sampling time (up to 24 hr) in this study dose not appear to be adequate for a drug, which has a relatively long half-life (10 to 18 h) in rats. The sponsor should have extended the sampling time for at least up to 48 hours to achieve a steady state concentration of the drug. According to the toxicokinetic portion of the proposed carcinogenicity study protocol, plasma concentration of the test material will be determined at 1, 4, and 24 hours time point during Weeks 13, 26 and 52. The sponsor should include more time points for toxicokinetics portion of the proposed protocol and should extend the sampling time for at least up to 48 hours. Overall, based on the analysis of the AUC values in rats, the absorption of ADL8-2698 appears to be saturable at 500 mg/kg in both sexes following oral administration. It is to be mentioned here that in the proposed protocol, gastrointestinal tract was not included in the list of tissues for histopathology. The sponsor should include histopathological examination of the entire gastrointestinal tract (especially large intestine and cecum) that might reveal any site (of action)-specific toxicity.

Executive CAC Recommendations and Conclusions:

1. The committee concurred with the proposed doses of 100, 200, and 500 mg/kg/day based on the saturation of absorption (ADL8-2698) criterion.
2. The committee suggested that the sponsor should include histopathological examination of the entire gastrointestinal tract (especially large intestine and cecum) that might reveal any site (of action)-specific toxicity.
3. The committee also suggested that based on the relatively long half-life of the drug in rat, the sponsor should include more time points for toxicokinetics portion of the proposed protocol and should extend the sampling time for at least up to 48 hours.

Joseph Contrera, Ph.D.
Acting Chair, Executive CAC

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/JChoudary, HFD-180
/CSO/PM, HFD-181
/ASeifried, HFD-024

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Tamal Chakraborti
11/26/2007 09:49:23 AM
PHARMACOLOGIST

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283 dated August 10, 2007 (Adolor Corporation).

Sushanta Chakder
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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-775
SERIAL NUMBER:	Original (RUP 001): May 4, 2004 Amendment (RUP 001 BP): May 14, 2004
DATE RECEIVED BY CENTER:	Original (RUP 001): May 5, 2004 Amendment (RUP 001 BP): May 17, 2004
DRUG NAME:	Alvimopan (Entereg™ Capsules)
INDICATION:	To accelerate time to recovery of gastrointestinal function following abdominal or pelvic surgery
SPONSOR:	Adolor Corporation
DOCUMENTS REVIEWED:	EDR, CMA: Pilot I- RU for Item 5
REVIEW DIVISION:	Division of Gastrointestinal & Coagulation Drug Products (HFD-180)
PHARM/TOX REVIEWER:	Tamal K. Chakraborti, Ph.D.
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TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	5
2.6.1 INTRODUCTION AND DRUG HISTORY	5
PHARMACOLOGY	12
Brief Summary	12
Primary Pharmacodynamics	14
Secondary Pharmacodynamics	21
Safety Pharmacology	21
Pharmacodynamic Drug Interactions	25
PHARMACOKINETICS/TOXICOKINETICS	25
2.6.4.1 Brief Summary	26
2.6.4.2 Methods of Analysis	26
2.6.4.3 Absorption	27
2.6.4.4 Distribution	42
2.6.4.5 Metabolism	45
2.6.4.6 Excretion	56
2.6.4.7 Pharmacokinetic Drug Interactions	61
2.6.4.8 Other Pharmacokinetic Studies	62
2.6.4.9 Discussion and Conclusions	62
2.6.4.10 Tables and Figures to Include Comparative Toxicokinetic Summary	63
2.6.6 TOXICOLOGY	66
2.6.6.1 Overall Toxicology Summary	66
2.6.6.2 Single-Dose Toxicity	67
2.6.6.3 Repeat-Dose Toxicity	68
2.6.6.4 Genetic Toxicology	124
2.6.6.5 Carcinogenicity	137
2.6.6.6 Reproductive and Developmental Toxicology	138
2.6.6.7 Local Tolerance	164
2.6.6.8 Special Toxicology Studies	164
2.6.6.9 Discussion and Conclusions	171
2.6.6.10 Tables and Figures	172
OVERALL CONCLUSIONS AND RECOMMENDATIONS	174
APPENDIX/ATTACHMENTS	180

EXECUTIVE SUMMARY

I. Recommendations:

- A. Recommendation on Approvability: From a preclinical standpoint, this NDA may be approved.
- B. Recommendation for Nonclinical Studies: None.
- C. Recommendations on Labeling: Sponsor should be asked to change the proposed label of alvimopan as suggested in the text of the review.

II. Summary of Nonclinical Findings:

- A. Brief Overview of Nonclinical Findings: The systemic toxicity of alvimopan and its metabolite ADL 08-0011 was adequately evaluated following oral and intravenous (i.v.) administrations in mice, rats and dogs. Alvimopan has also been tested for its potential to cause genotoxicity in an adequate battery of genotoxicity tests. In addition, alvimopan has been evaluated for fertility and reproductive performance (Segment I) in rats, teratology (Segment II) in rats and rabbits and peri and postnatal development (Segment III) in rats. Adequate safety pharmacology studies were also conducted with alvimopan, which did not show any potential safety concern.

Alvimopan exhibited no significant target organs of toxicity when administered at sufficiently high oral doses up to 13 weeks in mice and up to 6 months in rats and dogs. Alvimopan was also tested intravenously at sufficiently high doses up to 2 weeks in rats and up to 1 month in dogs and produced no significant toxicity. After repeated oral administration of alvimopan to rats for 1 (200, 500 and 1000 mg/kg/day) or 6 months (50, 100 and 200 mg/kg/day), the no-observed-adverse-effect-levels (NOAELs) were 1000 and 200 mg/kg/day, respectively, the highest tested doses. After repeated oral doses of alvimopan to dogs for 1 (100, 250, 500 and 1000 mg/kg/day) or 6 months (10, 30 and 100 mg/kg/day), the NOAELs were 1000 and 100 mg/kg/day, respectively, the highest tested doses. In repeat dose i.v. toxicity studies in rats (1, 5 and 10 mg/kg/day) and dogs (0.05, 0.2 and 2.0 mg/kg/day), the NOAELs were 10 and 2 mg/kg/day, respectively.

ADL 08-0011, the pharmacologically active metabolite of alvimopan, was also tested intravenously up to 2 weeks in rats and dogs. ADL 08-0011 did not cause any significant toxicity in rats or dogs when administered up to 8 or 2 mg/kg/day, respectively. Exposure to ADL 08-0011 following multiple oral doses of alvimopan was species-dependent. Mice and rats administered multiple oral doses of alvimopan showed plasma concentrations of the metabolite, ADL 08-0011, in excess of the parent drug, while dogs showed minimal levels of ADL 08-0011 after alvimopan administration.

The proposed human oral dose for alvimopan is 12 mg b.i.d. or 24 mg/day or 0.48 mg/kg/day (based on 50 kg body weight), which is equivalent to 17.8 mg/m². The highest tested doses in rats (200 mg/kg/day) and dogs (100 mg/kg/day) in 6-month oral toxicity studies were approximately 67.4 and 112.3 times the proposed human dose (17.8 mg/m²), respectively, based on body surface area. Alvimopan and its active metabolite ADL 08-0011 did not show any potential for genotoxicity. In fertility and reproductive performance study in rats, alvimopan did not cause any adverse effect. It was not teratogenic in rats or rabbits.

In conclusion, the nonclinical studies conducted on alvimopan provide adequate assurance of safety for its proposed oral use as indicated in the draft labeling.

- B. Pharmacologic Activity: Alvimopan is a relatively selective and peripherally active μ -opioid receptor antagonist intended for the management of postoperative ileus (POI). Exogenous and endogenous opioids used to relieve post-operative pain have been implicated in the development of POI. Morphine and other μ -opioid receptor agonists can prolong the duration of POI through delayed gastric emptying, reduced GI motility, and disrupted colonic myoelectric activity. Alvimopan and its metabolite, ADL 08-0011, have been shown to be highly potent and relatively selective μ -opioid receptor antagonists in binding assays using cloned human (μ , δ , κ), rat whole brain (μ , δ), and guinea pig cortex (κ) opioid receptors. The K_i values for cloned human μ , δ and κ receptors are 0.44 nM, 10 nM and 100 nM, respectively. Alvimopan antagonized the peripheral effects of opioids on gastrointestinal motility and secretion by competitively binding to gastrointestinal tract μ -opioid receptors. Following oral administration in morphine or loperamide-treated mice, alvimopan antagonized the peripheral effects of opioids on gastrointestinal motility and secretion at 0.46-1.1 mg/kg doses. Alvimopan also (0.001 to 100 mg/kg, i.v.) dose-dependently produced diarrhea (ED-50 = 0.07 mg/kg) in morphine-dependent mice. Overall, various *in vitro* and *in vivo* pharmacology studies support its intended use as indicated in the draft labeling.
- C. Nonclinical Safety Issues Relevant to Clinical Use: None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA Number: 21-775

Review Number: 001

Sequence Number/Date/Type of submission:

Original (RUP 001): May 4, 2004

Amendment (RUP 001 BP): May 14, 2004

Information to Sponsor: Yes () No (X)

Sponsor: Adolor Corporation

Manufacturer for Drug Substance: _____

Reviewer Name: Tamal K. Chakraborti, Ph.D.

Division Name: Gastrointestinal & Coagulation Drug Products

HFD #: 180

Review Completion Date:

Drug:

Trade Name: Entereg

Generic Name: Alvimopan

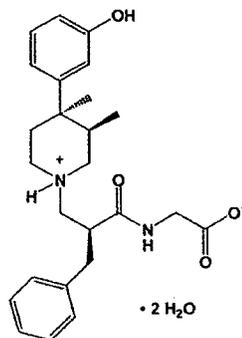
Code Name: ADL8-2698, LY246736

Chemical Name: [[2(S)-[[4(R)-(3-hydroxyphenyl)-3(R), 4- dimethyl-1-piperidiny]methyl]-1-oxo- 3-phenylpropyl] amino] acetic acid dihydrate

CAS Registry Number: 170098-38-1

Molecular Formula/Molecular Weight: C₂₅H₃₂N₂O₄• 2H₂O/460.6

Structure:



Relevant INDs/NDAs:

1. [REDACTED]
2. IND 56,553 (ADL8-2698, Adolor Corporation, HFD-180)
3. [REDACTED]
4. [REDACTED]

Drug Class: Opioid μ receptor antagonist.

Indication: Alvimopan is indicated to accelerate the time to recovery of gastrointestinal function following abdominal or pelvic surgery.

Clinical Formulation: The unit dose composition for alvimopan 6 mg capsules is shown in the following table (from Item 4, page 6 of sponsor’s submission).

1.2 Unit Dose Composition

Table 2 Unit Dose Composition for Alvimopan 6 mg (anhydrous) Capsules

Ingredient	mg/capsule
Alvimopan ¹	[REDACTED]
Polyethylene Glycol [REDACTED]	[REDACTED]
[REDACTED]	1 capsule
Total Fill weight	300.0

¹ equivalent to 6.0 mg alvimopan on an anhydrous basis

Route of Administration: Oral (capsules)

Proposed Use: Alvimopan is indicated to accelerate time to recovery of gastrointestinal function following abdominal or pelvic surgery.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies Reviewed Within This Submission: The following table shows the studies reviewed within this submission.

STUDY	REPORT/ STUDY NO.	TEST SITE	LOT NO.	REV. PAGE
PHARMACOLOGY*				12
ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION				25
ABSORPTION				27
Determination of portal and systemic plasma concentrations of radioactivity in rats dosed 10 mg/kg ¹⁴ C-alvimopan*	ADMERPT2/ 042R92	1	V86-CJS-005	27
Determination of plasma concentrations of alvimopan in Fischer 344 rats dosed with 200 mg/kg by oral gavage daily for one month*	ADMERPT5/ R02993	1	NA	28
Plasma concentrations of alvimopan in Beagle dogs dosed intravenously with 0.05 mg/kg, 0.2 mg/kg or 2.0 mg/kg alvimopan daily for 1 month*	ADMERPT3/ D04992	1	NA	30
Plasma concentrations of alvimopan in Beagle dogs dosed orally with 10 mg/kg, 30 mg/kg, or 100 mg/kg alvimopan Daily for 1 Month*	ADMERPT4/ D02793	1	NA	33
Blood level study in Fischer 344 rats given a single oral gavage dose of alvimopan	R05693	1	284-MH2	36
Pharmacokinetic study of alvimopan in Fisher rats*	808-005	3	R009340	36
Formulation and toxicokinetic study of alvimopan in Fischer rats*	808- 007	3	OR12098.N.0 1.D1.01	38
Calculation of alvimopan pharmacokinetic parameters following single intravenous doses of 1, 5 or 10 mg/kg to Fischer rats	14TK010	4	R009340	40
DISTRIBUTION				42
<i>In vitro</i> evaluation of binding of [³ H]alvimopan to mouse, rat, dog, and normal human plasma proteins	0833XA43.001	5	R009340	42
<i>In vitro</i> evaluation of binding of [³ H]alvimopan to rat, rabbit, and normal human plasma proteins	0833XA43.003	5	AC03434	42
<i>In vitro</i> evaluation of binding of [³ H]ADL 08-0011 to mouse, rat, rabbit, dog and normal human plasma proteins	0833XA43.004	5	AC03282	43
Red blood cell partitioning study with LC/MS/MS analysis of alvimopan in human, dog, rabbit, rat, and mouse	57- 0307	6	AC03282	43
<i>In vitro</i> evaluation of the distribution of [³ H]ADL 08-0011 in normal mouse, rat, rabbit, dog and normal human whole blood	0834XA43.002	5	01-STD-016	44
Whole-body autoradiographic distribution of ¹⁴ C-alvimopan in male Fischer 344 rats after a single oral 10 mg/kg dose*	ADME RPT 6	1	NA	44

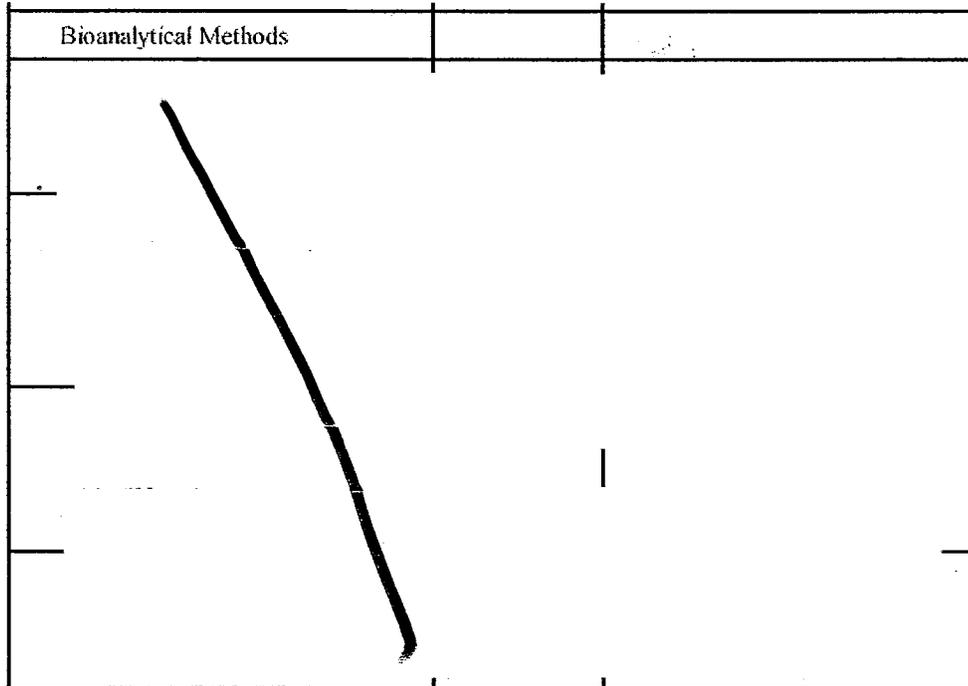
METABOLISM				45
Evaluation of the <i>in vitro</i> induction of CYP mRNA expression in rat hepatocytes by alvimopan using quantitative	DI01178	7	OR12098.N.0 0.03	45
Biotransformation of [¹⁴ C]alvimopan in intact and bile duct-cannulated rats*	PD- FR- 005-0501(13TX02)	4	NA	45
Biotransformation of [¹⁴ C]alvimopan in intact and bile duct-cannulated dogs*	PD- FR- 006-0501(13TX03)	4	NA	52
EXCRETION				56
Elimination of radioactivity in bile, urine and feces following intravenous or oral administration of [¹⁴ C]alvimopan to male rats*	7010-102	2	R002092	56
Elimination of radioactivity in bile, urine, and feces following intravenous or oral administration of [¹⁴ C]alvimopan to bile duct-cannulated male rats	7010-119	2	3463-001	59
Elimination of radioactivity in bile, urine and feces following intravenous or oral administration of [¹⁴ C]alvimopan to male dogs*	7010-103	2	284-MH2	59
TOXICOLOGY				66
Acute				67
Mice				67
Oral*	TOXRPT7/ M07293	1	284-MH2	67
Rat				67
Oral*	TOXRPT6/ R19693	1	284-MH2	67
Intravenous*	TOXRPT5/ R18893	1	284-MH2	67
Dog				67
Oral	D09392	1	284-MH2	67
Subacute/Subchronic/Chronic				68
Mice				68
5-Day, oral*	808-020	3	R009340	68
13-Week, oral*	808-002	3	A220201	72
Rat				77
2-Week, intravenous	808-021	3	R009340	77
2-Week, intravenous with ADL 08-0011	808-018	3	202002	83
1-Month, oral*	R02893 and R02993	1	284-MH2	88
4-week, oral	808-012	3	01-0088	91
6-Month, oral*	R04393	1	284-MH2	97
Dog				101
1-Month, oral*	TOXRPT9/ D02793	1	284-MH2	101
1-Month, intravenous*	TOXRPT8/ D04992	1	284-MH2	104
4-Week, oral	808-011	3	01-0088	107
2-week, intravenous with ADL 08-001-01	808-019	3	202002	113
6-Month, oral*	TOXRPT13/ D02393	1	284-MH2	119
GENOTOXICITY				124
Ames assay	93031AMS3684	1	284-MH2	124

Ames assay with ADL 08-0011	AA57TL.503.BTL	9	203004	125
Chromosome aberration assay in Chinese Hamster Ovary (CHO) cells*	15488-0-437	1	284-MH2	129
Chromosome aberration assay using CHO cells with ADL 08-0011	AA57TL.331.BTL	9	203004	130
Mouse lymphoma assay*	930421MLA3684	1	284-MH2	135
Mouse bone marrow micronucleus assay (oral)*	930331MNT3684	1	284-MH2	136
REPRODUCTIVE TOXICITY				138
Rat				138
Combined Segment I and II (oral)*	TOX RPT14 (R12693 and R12793)	1	284-MH2	138
Segment I (intravenous)	PD-FR-016-0701 (R10094, R0994)	1	284-MH2	145
Segment III (intravenous)	4401-001	8	01-0088	156
Rabbit				148
Segment II (intravenous)	4401-002	8	01-0088	148
SPECIAL TOXICITY STUDY				164
Primary eye irritation study in the rabbit	3130.117	10	284-MH2	164
Acute eye irritation study in the rabbit	808-013	3	R009340	165
Acute dermal toxicity/irritation study in the rabbit	3130.118	10	284-MH2	166
Acute dermal irritation study in the rabbit	808-014	3	R009340	167
Skin sensitization study (Buehler method) in the guinea pig	808-015	3	R009340	168

- 1.
- 2.
- 3.
4. Adolor Corporation, Malvern, PA
- 5.
- 6.
7. GlaxoSmithKline, Welwyn, UK
- 8.
- 9.
- 10.

* : Studies reviewed either under original submission of dated October 11, 1993 or Amendments of IND 56, 553. The reviews of these studies are incorporated in the appropriate sections of this review.

Studies Not Reviewed Within This Submission: The following study reports (from page 6, 7, and 8 of sponsor's pharmtoc.pdf) of analytical methods and validation were not reviewed, as these are reports of method development and validation:



1 Page(s) Withheld

 ✓ Trade Secret / Confidential

 Draft Labeling

 Deliberative Process

Adolor Corporation

ENTEREG™ (alvimopan)

NDA 21-775

Description	Paper Review Copy Volume Number	Electronic Archive Folder/File
		

PHARMACOLOGY

Brief Summary

In vitro and *in vivo* primary pharmacological studies were conducted to demonstrate the specificity of alvimopan as a peripherally selective μ opioid receptor antagonist. In addition, pharmacology studies also included characterization of ADL 08-0011, a pharmacologically active metabolite of alvimopan, as potent μ opioid receptor antagonist. Safety pharmacology studies examined the effects of alvimopan and ADL 08-0011 on the central nervous system (CNS), cardiovascular, respiratory, renal and gastrointestinal system.

Radioligand binding assays using cloned human (μ , δ , and κ), rat whole brain (μ and δ), and guinea pig cortex (κ) opioid receptors demonstrated that alvimopan is a potent, reversible, competitive and relatively selective μ receptor antagonist. The K_i values for cloned rat μ , δ , and κ receptors were 0.77, 4.4 and 40 nM, respectively. The K_i values for cloned human μ , δ , and κ receptors were 0.44, 10 and 100 nM, respectively. In multiple receptor screening assays () assays showed that alvimopan is a selective μ opioid receptor antagonist, as it did not bind to over 70 non-opioid receptors or enzymes at concentrations of 1 or 10 μ M.

ADL 08-0011, the amide hydrolysis metabolite (R3) formed in animals and humans following administration of alvimopan, was equipotent to alvimopan at the μ opioid receptor and was a more selective μ receptor antagonist than alvimopan. The K_i values for antagonism of [^3H]diprenorphine binding at the cloned human μ , δ , and κ receptors were 0.81, 110 and 290 nM, respectively. Therefore, it is likely that the μ receptor antagonism *in vivo* may be attributed to both the parent compound and the metabolite (ADL 08-0011). Like alvimopan, the selectivity of ADL 08-0011 was also demonstrated by the lack of binding to over 70 non-opioid receptors and enzymes at a concentration of 10 μM .

Functionally, alvimopan antagonized the agonist-induced stimulation of [^{35}S]GTP γ S binding to the μ receptor, with an IC-50 value of 1.7 nM. Alvimopan was less potent in inhibiting the decrease in cAMP levels produced by μ agonist in Chinese Hamster Ovary (CHO) cells, with an IC-50 of 24 nM. Alvimopan also antagonized morphine-induced inhibition of electrically stimulated contractions in the guinea pig ileum in a dose-related manner (ED-50 = 8×10^{-6} M). Alvimopan was less potent in antagonizing the stimulation mediated by δ or κ agonists, with IC-50 values of 50 and 53 nM, respectively. ADL 08-0011 was also a potent antagonist of the stimulation of [^{35}S]GTP γ S binding to the μ receptor produced by loperamide, with an IC-50 of 0.64 nM. Like alvimopan, ADL 08-0011 was less potent at inhibiting agonist-stimulated [^{35}S]GTP γ S binding through the δ and κ receptors, with IC-50 values of 110 and 59 nM, respectively.

Alvimopan was shown to be a potent antagonist of the effects of opioids on gastrointestinal transit in mice and rats when administered orally or intravenously. Alvimopan dose-dependently reversed morphine-induced inhibition of charcoal meal transit in the small intestine of mice (ED-50 = 1.1 mg/kg, p.o.). However, alvimopan did not alter charcoal meal transit in mice, suggesting that it does not have its effect on its own. Alvimopan also (0.001 to 100 mg/kg, i. v.) dose-dependently produced diarrhea (ED-50 = 0.07 mg/kg) in morphine-dependent mice and dose-dependently antagonized (ED-50 = 8.9 mg/kg) morphine-induced inhibition of acetic acid-induced writhing in mice, indicating a "central/peripheral" ratio of 127. This data suggested that alvimopan is more selective peripherally. Alvimopan (0.1-10 mg/kg p. o.) also produced a dose-related antagonism of loperamide (20 mg/kg p.o.)-induced gastrointestinal transit (GIT) using charcoal meal test. Following oral administration, alvimopan also antagonized morphine- and loperamide-induced inhibition of castor oil-induced diarrhea. The A50 (50% antagonism) values were 0.011 and 0.02 mg/kg for antagonism of morphine-induced inhibition of castor oil-induced diarrhea and for inhibition of loperamide-induced inhibition of castor oil-induced diarrhea, respectively. Since opioids like morphine and loperamide are considered to inhibit castor oil-induced diarrhea by inhibiting fluid secretion into the intestinal lumen, these data appear to suggest that alvimopan probably blocks the antisecretory component of opioid agonists in this model.

In safety pharmacology studies, alvimopan did not show any adverse effect on the cardiovascular, renal, pulmonary, gastrointestinal (GI) and CNS (behavioral). Alvimopan and ADL 08-0011 did not inhibit the cloned human cardiac potassium channel (hERG) at concentrations up to approximately 46 and 35 μM , respectively.

Neither alvimopan nor ADL 08-0011 produced any effect on action potential duration (APD) in the dog Purkinje fiber assay at concentrations up to 100 μ M. Alvimopan at intravenous doses of 0.05, 0.2 and 2.5 mg/kg showed no effect on the ECG or QTc in dogs.

Primary Pharmacodynamics

Drug Activity Related to Proposed Indication:

The Affinities and Selectivities of Alvimopan, [REDACTED] in the Synthesis of Alvimopan, and Potential Impurities of Alvimopan for the Opioid Receptors as Determined by Opioid Receptor Binding Assays

[REDACTED]

Table 10.A: Comparison of the Affinity and Selectivity of ADL 8-2698 with Those of the Centrally Acting Antagonist, Naloxone, and the Peripherally Selective Antagonist, Methylnaltrexone.

	K _i , nM			
	μ	δ	κ	ORL-1
ADL 8-2698 (ADL0102616)	0.44 (95%CI: 0.36-0.53) n=17	10 (95%CI: 7.2-15) n=11	99.6 (95%CI: 77-130) n=11	470 (95%CI: 1.5-140000) n=2
Methylnaltrexone (ADL0201517)	30 (95%CI: 22-42) n=21	870 (95%CI: 560-1300) n=14	101 (95%CI: 65-160) n=15	0% Inhibition at 10 μM (n = 1)
Naloxone (ADL0201251)	3.3 (95%CI: 2.5-4.4) n=14	33 (95%CI: 21-52) n=8	8.1 (95%CI: 4.9-13) n=14	0% Inhibition at 10 μM (n = 1)

Table 10.B Inhibition of [3H]Diprenorphine Binding to Cloned Human Opioid Receptors and of [3H]Nociceptin Binding to the Cloned Human ORL-1 Receptor by

μ	K _i = 45 nM (95% CI: 34-60, n=4)	1.4 nM (95% CI: 0.95-1.9, n=4)	0.81 nM (95% CI: 0.64-1.0, n=4)
δ	K _i = 390 nM (95% CI: 230-660, n=4)	89 nM (95% CI: 53-150, n=3)	110 nM (95% CI: 60-190, n=3)
κ	K _i = 230 nM (95% CI: 190-270, n=4)	27 nM (95% CI: 41-53, n=3)	290 nM (95% CI: 60-1400, n=3)
ORL-1	40% inhibition @ 10 μM	2300 nM (95% CI: 2000-2800, n=4)	25% inhibition @ 10 μM

In Vitro Antagonism of Opioid Receptor-Mediated [³⁵S]GTPγS Binding by Alvimopan, Methylnaltrexone, and Naloxone

Opioid receptors are coupled to G_i/G_o proteins to affect several different effectors, including inhibition of adenylyl cyclase, enhancement of K⁺ conductance, decrease in Ca⁺⁺ conductance and activation of p42/p44 mitogen-activated protein (MAP) kinases. The objective of this study was to characterize the ability of alvimopan, naloxone and methylnaltrexone to prevent opioid agonist-mediated [³⁵S]GTPγS binding to G proteins. The potencies of the antagonists were assessed by their ability to inhibit agonist-stimulated [³⁵S]GTPγS binding to membranes containing the cloned human μ, δ, and κ

opioid receptors in CHO cells. Alvimopan, naloxone and methylnaltrexone were competitive antagonists at all three (μ , δ , and κ) opioid receptors. Alvimopan inhibited agonist-stimulated [35 S]GTP γ S binding mediated by the μ opioid receptor with 5- to 118-fold greater potency than naloxone and methylnaltrexone. Similarly, alvimopan was more potent than naloxone and methylnaltrexone at inhibiting agonist-stimulated δ opioid receptor-mediated [35 S]GTP γ S binding. Alvimopan inhibited agonist-stimulated [35 S]GTP γ S binding mediated by the κ opioid receptor with similar potency to naloxone, but with greater potency than methylnaltrexone. The results are shown in the following table (from Table 10.1.A from page 8 of the sponsor's report: 14ph13.pdf).

Table 10.1.A: ADL 8-2698, methylnaltrexone and naloxone inhibition of agonist stimulation of [35 S]GTP γ S binding mediated by the cloned human μ , κ , and δ opioid receptors.

Compound	IC ₅₀ (nM)		
	μ	κ	δ
ADL 8-2698	1.7 (95% CI: 1.2-2.4) n=25	53 (95% CI: 37-76) n=13	50 (95% CI: 24-100) n=10
Methylnaltrexone	200 (95% CI: 130-300) n=11	1500 (95% CI: 810-2700) n=8	24000 (95% CI: 17000-35000) n=6
Naloxone	8.1 (95% CI: 5.7-11) n=20	45 (95% CI: 28-71) n=8	380 (95% CI: 250-580) n=8

Values shown in the above table 10.1.A are the means, 95% confidence intervals, and number of determinations. The agonists were loperamide for the μ receptor, U50,488H for the κ receptor and BW373U86 for the δ receptor.

Alvimopan-Mediated Antagonism of Morphine-Induced Inhibition of Forskolin-Stimulated cAMP Synthesis

In this study, Chinese Hamster Ovary (CHO) cells: [redacted] opioid receptor were preincubated for 15 min at 37°C with 500 μ M isobutylmethylxanthine (IBMX). Then antagonists (alvimopan, naloxone, naltrexone, and methylnaltrexone), morphine (1 μ M) and forskolin (25 μ M) were added simultaneously to reaction mixture. After incubation at 37°C for an additional 15 min, the reaction was terminated by the addition of 0.1 ml of luciferase cell culture lysis reagent. Concentrations of cAMP were determined in aliquots by using a [redacted] radioimmunoassay (RIA). Alvimopan antagonized morphine-induced inhibition of cAMP synthesis in a concentration-dependent manner, with an IC-50 of 24 nM. Similar results were obtained with naloxone and naltrexone, all of which had potencies similar to

that of alvimopan. Methylnaltrexone, on the other hand, was 25-fold less potent than alvimopan and inhibited the morphine effect with an IC₅₀ of 593 nM.

Antagonism of Opioid Receptor-Mediated [³⁵S]GTPγS Binding by [REDACTED] in the Synthesis of Alvimopan

The primary objective of this study was to examine the functional activity of selected [REDACTED] in the synthesis of alvimopan in μ, κ, and δ opioid receptor-mediated [³⁵S]GTPγS binding. All [REDACTED] were found to be most potent in inhibiting agonist-stimulated [³⁵S]GTPγS binding mediated by the μ opioid receptor and completely inhibited agonist-stimulated [³⁵S]GTPγS binding at all three opioid receptors. The rank order of potencies for the [REDACTED] in inhibiting agonist-stimulated [³⁵S]GTPγS binding mediated by the μ opioid receptor were alvimopan = [REDACTED]

[REDACTED]. The results are shown in the following table (Table 10.A from page 6 of the sponsor's report: 14ph05.pdf).

Table 10.A: ADL 8-2698, [REDACTED] inhibition of agonist stimulation of [³⁵S]GTPγS binding mediated by the cloned μ, κ, and δ opioid receptors.

Compound	IC ₅₀ (nM)		
	μ	κ	δ
ADL 8-2698	1.7, n=25 (95% CI: 1.2-2.4)	53, n=13 (95% CI: 37-76)	50, n=10 (95% CI: 24-100)
[REDACTED]	190, n=6 (95% CI: 140-270)	not determined	not determined
[REDACTED]	40, n=4 (95% CI: 34-47)	270, n=6 (95% CI: 195-390)	not determined
[REDACTED]	0.64, n=4 (95% CI: 0.3-1.3)	59, n=4 (95% CI: 27-130)	110, n=4 (95% CI: 46-260)
[REDACTED]	1.1, n=4 (95% CI: 0.46-2.5)	20, n=4 (95% CI: 10-38)	72, n=4 (95% CI: 25-210)
[REDACTED]	1.2, n=3 (95% CI: 0.97-1.6)	20, n=4 (95% CI: 5.5-73)	48, n=4 (95% CI: 9.5-240)

IC₅₀ values were determined as described in sections 7 and 9. Values shown are the means, 95% confidence intervals, and number of determinations. IC₅₀ values for [REDACTED] at the κ and δ receptors and [REDACTED] at the δ receptor were not determined because their K_i values in receptor binding assays were greater than 500 nM. (see study report 14PH15 entitled: 'The Affinities and Selectivities of ADL 8-2698, [REDACTED] in the Synthesis of ADL 8-2698, and Potential Impurities of ADL 8-2698 for the Opioid Receptors as Determined by Opioid Receptor Binding Assays').

Antagonism of Loperamide-Induced Inhibition of Gastrointestinal Transit (GIT) by Alvimopan

The objective of this study was to determine the potency of alvimopan to block the loperamide-induced inhibition of GIT in mice using charcoal meal test. The efficacy and potency of alvimopan were also compared to those of naloxone, a centrally acting antagonist, and N-methylnaltrexone (MNTX), a peripherally active analog of naltrexone with limited CNS access. Loperamide inhibited GIT in mice by 50-60 % after oral administration of doses ranging from 3-20 mg/kg. Alvimopan (0.1-10 mg/kg p. o.) produced a dose-related antagonism of loperamide-induced inhibition of GIT. Maximal antagonism (41%) was observed at 10 mg/kg when alvimopan was administered 6 hr prior to treatment with loperamide. MNTX produced only 44% antagonism of loperamide-induced inhibition of GIT at the highest tested dose of 300 mg/kg, p.o. Naloxone (0.1-10 mg/kg s.c.) produced a dose-related antagonism of loperamide-induced inhibition of GIT, with a 50% antagonism (A-50) at 0.65 mg/kg. Naloxone was found to be completely effective (97% antagonism) at 10 mg/kg, s.c. Overall, alvimopan was not fully effective in preventing loperamide-induced inhibition of GIT.

Effects of Alvimopan on Opioid-Induced Inhibition of Castor Oil-Induced Diarrhea in Mice

The goal of this study was to determine the efficacy and potency of alvimopan to block the constipation produced by two opioid agonists, morphine and loperamide, in mice. The efficacy and potency of alvimopan were also compared to those of naloxone and methylnaltrexone (MTNX). Following oral administration, alvimopan antagonized morphine- and loperamide-induced inhibition of castor oil-induced diarrhea. The A50 (50% antagonism) values were 0.011 and 0.02 mg/kg for antagonism of morphine-induced inhibition of castor oil-induced diarrhea and for inhibition of loperamide-induced inhibition of castor oil-induced diarrhea, respectively. After oral treatment with 1 mg/kg of alvimopan, antagonism of the morphine response was observed for at least 24 hour.

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PHARMACOLOGY:

The sponsor provided primary pharmacological studies to assess the specificity of LY246736 dihydrate as a μ opioid antagonist and as a peripherally versus a centrally (CNS) acting antagonist. Secondary pharmacology studies investigated the effects of LY246736 on neurotransmitter-induced activities, cardiovascular parameters, and serum and urine parameters.

Primary Pharmacology**1. In vitro Studies:**

The ability of LY246736 dihydrate to displace ^3H -naloxone (μ antagonist) and ^3H -DAGO (δ agonist) in rat brain homogenates and ^3H -ethylketocyclazocine (κ agonist) in guinea pig brain homogenates, was assessed. The K_i of LY246736 dihydrate in displacing ^3H -naloxone was 0.77 nM, in displacing ^3H -DAGO was 4.4 nM and in displacing ^3H -ethylketocyclazocine was 40 nM. These data suggest that LY246736 dihydrate is a moderately selective μ opioid ligand in that the K_i s indicate that LY246736 dihydrate has 5-6 times more affinity for μ sites than δ sites and about 50 times more affinity for μ sites than κ sites.

The ability of LY246736 dihydrate to antagonize morphine-induced inhibition of electrically stimulated contractions in the guinea pig ileum was also assessed. LY246736 dihydrate dose-dependently antagonized morphine-induced effects in the guinea pig ileum. The ED_{50} for morphine was approximately 8×10^{-8} M. In the presence of 1×10^{-8} M LY246736 dihydrate, the ED_{50} for morphine was shifted to approximately 8×10^{-6} M. In the presence of 1×10^{-6} M LY246736 dihydrate, the maximal effect of morphine was reduced by about 50%. Thus, LY246736 dihydrate functions as a μ opioid antagonist.

2. In vivo studies:

LY246736 dose-dependently (0, 0.3, 1.0 or 3.0 mg/kg by oral gavage) reversed morphine-induced inhibition (0, 0.7, 1.0, or 3.0 mg/kg, s.c.) of charcoal meal transit in the small intestine in mice. The oral ED_{50} for LY246736 dihydrate was 1.1 ± 0.14 mg/kg (mean \pm SE) and for naloxone was 2.1 ± 0.16 mg/kg. LY246736 dihydrate (3 mg/kg, p.o.) was effective in reversing morphine-induced inhibition (1 mg/kg, s.c.) of charcoal meal transit for up to 8 hours, but not at 24 hours. Thus, LY246736 dihydrate functions as an *in vivo* μ opioid antagonist.

In other studies, the acetic acid-induced writhing test in mice was used to assess any central effects of LY246736 dihydrate; on the other hand, any LY246736 dihydrate-induced diarrhea in morphine-dependent mice and rats was assessed to determine any peripheral effects of LY246736 dihydrate. LY246736 dihydrate

(0.001 to 100 mg/kg, i.v.) dose-dependently produced diarrhea in morphine-dependent mice and dose-dependently antagonized morphine-induced inhibition of acetic acid-induced writhing in mice; the ED₅₀ for producing diarrhea was 0.07 mg/kg, whereas the ED₅₀ for antagonizing morphine was 8.9 mg/kg. Thus, a "central/peripheral" ratio of 127 was evident. Furthermore, LY246736 dihydrate (0.01 to 100 mg/kg, p.o.) dose-dependently produced diarrhea in morphine-dependent mice (ED₅₀ of 0.64 mg/kg) and morphine-dependent rats (ED₅₀ of 0.31 mg/kg).

The "central/peripheral" ratios of the racemate (LY210274) of LY246736 dihydrate were also assessed. LY210274 (0.00001 to 100 mg/kg, i.v.) dose-dependently produced diarrhea in morphine-dependent mice and dose-dependently antagonized morphine-induced inhibition of acetic acid-induced writhing; the ED₅₀ for producing diarrhea was 0.0017 mg/kg, whereas the ED₅₀ for antagonizing morphine was >40.0 mg/kg. This results in a "central/peripheral" ratio of >1,081. Furthermore, LY210274 (0.001 to 10 mg/kg, s.c.), dose-dependently produced diarrhea in morphine-dependent mice, while the ED₅₀ for orally administered LY210274 to produce diarrhea within 30 minutes after administration was 2.6 mg/kg in mice.

Secondary Pharmacodynamics

Study for Alvimopan and ADL 08-0011

Alvimopan and its amide hydrolysis metabolite (ADL 08-0011) were evaluated across a broad *in vitro* selectivity panel that included adrenergic, muscarinic and nicotinic cholinergic, dopaminergic and serotonergic receptors, various ion channels and peptidergic receptors and their subtypes. Both alvimopan and ADL 08-0011 failed to demonstrate activity at over 70 non-opioid receptors and enzymes at a concentration up to 10 μ M.

Safety Pharmacology

The safety pharmacology studies have been reviewed (January 13, 1994) under original submission of [REDACTED]. The safety pharmacology reviews are incorporated in the appropriate sections below. Some of the safety pharmacology studies that were not reviewed previously are reviewed below.

Neurological Effects

The Acute Behavioral Profile of Alvimopan Following Oral Administration in CD-1 Mice

In a series of behavioral studies in mice, LY246736 dihydrate (0, 50, 100 and 200 mg/kg by gavage) did not have any effects on overt behavior, motor activity, electroshock-induced convulsions, pentylenetetrazol-induced convulsions, grip strength, or acetic acid-induced writhing. However, LY246736 dihydrate did reduce hexobarbital-induced sleep time (at a dose of 50 mg/kg only) and did decrease overall mean peak startle responses (at doses of 100 and 200 mg/kg). Moreover, LY246736 dihydrate (0, 50, 100 and 200 mg/kg by gavage) had no effect on gastrointestinal motility in mice. The overall negative nature of these findings are important because they suggest that LY246736 dihydrate possesses little or no opioid agonistic effects and little or no non-specific (non-opioid) effects, at the doses studied.

An Intravenous Neurobehavioral Study of ADL 08-0011 in Fischer Rats

This study was conducted to evaluate the potential behavioral neurotoxicity of ADL 08-0011 (metabolite of alvimopan) in Fischer Rats following intravenous administration. In this study, rats (n = 5/sex) received the vehicle or test article via intravenous bolus.

injection into the tail vein daily for three consecutive days at 0, 1, 2, 4, and 8 mg/kg (4 ml/kg) doses. All animals were observed twice daily for morbidity, mortality and any injury. In addition, clinical and functional observational battery (FOB) examinations and body weights were recorded during the course of the study. ADL 08-0011 was well tolerated when administered up to 8 mg/kg, i.v. for three days. Results of a FOB performed at 1 and 24 hours post-dosing were not statistically different between controls and treated animals. There were no treatment-related effects on FOB parameters.

Cardiovascular Effects

Effect of Alvimopan and ADL 08-0011 on Cloned hERG Channels Expressed in Mammalian Cells

In this study, *in vitro* effects of alvimopan and its metabolite, ADL 08-0011, on ionic currents was tested using voltage-clamped human embryonic kidney 293 (HEK-293) cells that stably expressed the human ether-a-go-go-related gene (hERG). Terfenadine was used as a concurrent positive control. Alvimopan and ADL 08-0011 were tested at 5, 15, and 50 μM concentrations. Alvimopan inhibited hERG current by 1.0% (n = 4), 0.7% (n = 3), and 1.8% (n = 3) at 5, 15, and 50 μM , respectively. ADL 08-0011 inhibited hERG current by 0.4% (n = 3), 0.5% (n = 4), and 0.5% (n = 3) at 5, 15, and 50 μM , respectively. The IC-50 for the inhibition of hERG current was not determined for either compound since neither produced greater than 50% inhibition of hERG current. Under identical experimental conditions, 60 nM terfenadine inhibited [76.5% (n = 2)] hERG current as expected indicating the validity of the experiment.

Effect of Alvimopan and ADL 08-0011-0 on Action Potential Parameters in Dog Isolated Purkinje Fibers

This study was conducted to examine the effects of alvimopan and ADL 08-0011-0 on the dog isolated Purkinje fiber action potential. Purkinje fibers were perfused with Tyrode's solution for baseline control and 10, 50 and 100 μM concentrations of alvimopan or ADL 08-0011-0. The following action potential parameters were measured: resting membrane potential (RMP), maximum rate of depolarization of the action potential upstroke (V_{max}), overshoot (OS) action potential amplitude (APA) and action potential duration at 30, 50 and 90% (APD_{30} , APD_{50} and APD_{90}) repolarization. Changes in these parameters were compared to the respective pretreatment group. Alvimopan and ADL 08-0011-0 did not show any significant effects on RMP, V_{max} , OS, APA, APD, APD_{30} , APD_{50} and APD_{90} . However, dl-sotalol (50 μM) used as a positive control, significantly prolonged APD_{50} and APD_{90} indicating the validity of the experiment. Alvimopan or its metabolite ADL08-0011-0 did not prolong action potential duration at any of the tested concentrations.

Cardiovascular Effects of Alvimopan Administered Orally to Conscious Male Sprague Dawley Rats

Alvimopan was examined for potential cardiovascular effects in conscious male Sprague Dawley rats (n = 4/group) at oral gavage doses of 50, 100, and 200 mg/kg (5 ml/kg). Four control animals received 10% acacia suspension by gavage (5 ml/kg). Blood pressure and heart rate were measured prior to dosing (time point 0) and at 10, 20, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 minutes postdose. Administration of 50, 100, or 200 mg/kg of alvimopan produced no biologically significant changes in any parameters tested (mean arterial pressure, systolic pressure, diastolic pressure, pulse pressure, or heart rate). Overall, alvimopan did not produce any biologically significant cardiovascular effects at the tested doses.

Cardiovascular Effects of Alvimopan in Dogs

In this study, six Beagle dogs (n = 3/sex) were administered a slow bolus intravenous injection (over a two-minute period) of alvimopan at 2 mg/kg. Blood pressure, heart rate, electrocardiogram (ECG) was recorded at 15, 30, 60, 120, and 240 minutes post dose. Blood samples were also collected immediately following recording of cardiovascular parameters. None of the animals displayed any abnormal behavior during the monitoring period of four hours. Alvimopan did not significantly alter blood pressure, heart rate and electrocardiograms recorded at any of the five-time points when compared to the base line values. Alvimopan showed no abnormal effects in the cardiovascular system of the beagle dog when tested at 2 mg/kg, i.v. dose.

Cardiovascular (Hemodynamic) and QTc Prolongation Evaluation of Alvimopan in Dogs

This study was conducted to examine the potential effects of intravenous infusion of alvimopan on blood pressure, heart rate and QT interval in anesthetized dogs. Four Beagle dogs (n = 2/sex) received alvimopan at 0.05, 0.2 and 2.5 mg/kg, i.v. dose (bolus). The following cardiovascular parameters were recorded: arterial blood pressure (systolic, diastolic and mean), heart rate, and electrocardiogram (ECG). Alvimopan had no effect on systolic, diastolic and mean arterial blood pressures or heart rate, ECG and QT at any of the tested doses. Acute intravenous administration of alvimopan showed no effect on systemic arterial blood pressure, average heart rate or QTc at any of the tested doses under the conditions of the experiment.

Pulmonary Effects

Pulmonary Assessment of Alvimopan in the Anesthetized Guinea Pig

In this respiratory function study, sixteen (n = 4/group) male guinea pigs were equipped with catheters in the esophagus to measure esophageal pressure, in the trachea to facilitate spontaneous breathing and in the jugular vein for drug administration. Each animal received an intravenous injection of vehicle (0.9% saline) or alvimopan at 0.054, 0.218 or 2.72 mg/kg (1 ml/kg). Changes in airway resistance (cm H₂O/ml/sec), dynamic

lung compliance (ml/cmH₂O), respiratory rate (breaths/min), tidal volume (ml) and minute volume (ml/min) were recorded every minute for the first 5 minutes and every 5 minutes thereafter for a total of 30 minutes following dose administration. The intravenous administration of alvimopan at 0.054, 0.218 or 2.72 mg/kg did not produce any statistically significant or biologically relevant changes in airway resistance, respiratory rate, tidal volume.

Renal Effects

Renal Pharmacology Study in Female Fisher 344 Rats Given a Single Gavage Dose of Alvimopan

3. In vivo effects on serum and urine parameters:

LY246736 dihydrate was orally administered by gavage at doses of 0, 50, 100 and 200 mg/kg to rats, followed by a hydrating dose by gavage of 25 ml/kg 0.9% Sodium Chloride Injection, USP. Urine was collected for 5 hours for the determination of volume, pH, sodium, potassium, chloride, creatinine, and osmolality. At the end of the urine collection period, blood samples were obtained for the determination of serum sodium, potassium, creatinine, and osmolality. Creatinine clearance, osmolal clearance, and fractional excretion of sodium were calculated. Urine chloride was significantly higher (17% and 21%) in the 100 and 200 mg/kg female groups; urine sodium was significantly higher (29%) in the 200 mg/kg female group. Serum potassium was significantly lower (4.4%) in the 200 mg/kg female group. Thus, the no effect oral dose of LY246736 dihydrate on serum and urine parameters is 50 mg/kg, while the ED₅₀ of oral LY246736 dihydrate for producing diarrhea in morphine-dependent rats was 0.31 mg/kg. In this case, therefore, the ratio of the no effect dose to the ED₅₀ pharmacological dose is approximately 160, suggesting a large safety margin.

Gastrointestinal Effects

In Vitro Studies of Alvimopan in the Hartley Albino Guinea Pig Ileum

1. In vitro effects on guinea pig ileum:

LY246736 dihydrate (1×10^{-9} M to 1×10^{-6} M) did not significantly inhibit or enhance the resting tension of the guinea pig ileum; however, at a concentration of 1×10^{-5} M, LY246736 dihydrate did significantly increase the resting tension. Thus, at relatively higher doses, LY246736 dihydrate may have either opioid agonistic or non-specific effects. LY246736 dihydrate (1×10^{-6} M to 1×10^{-5} M) did not significantly inhibit field-stimulated guinea pig ileum

contractions. LY246736 dihydrate (1×10^{-5} M) did not affect either acetylcholine-induced or histamine-induced contraction of the guinea pig ileum. However, LY246736 dihydrate (1×10^{-5} M) did inhibit angiotensin I-induced contraction of the guinea pig ileum. Thus, at relatively higher doses, LY246736 dihydrate may be an angiotensin I antagonist.

The Acute Effects of Alvimopan on Gastrointestinal Motility Following Oral Administration in Male CD-1 Mice

The acute gastrointestinal effects of alvimopan were evaluated in male CD-1 mice (n = 10/group) using charcoal meal transit test. Alvimopan was administered orally by gavage at single doses of 0, 50, 100, or 200 mg/kg. Groups of mice were treated 30 minutes before administration of charcoal meal (10% charcoal powder suspended in 5% aqueous acacia suspension). Twenty minutes following administration of the charcoal meal, animals were sacrificed and the peritoneal cavity was exposed. The intestinal tract from the pyloric sphincter to the cecum was excised. The distance (mm) the charcoal meal traveled and the total length (mm) of the intestine was recorded. The distance traveled by the charcoal meal was expressed as a percentage of the total length of the intestine. Alvimopan did not alter charcoal meal transit, suggesting that it does not affect gastrointestinal motility.

Pharmacodynamic Drug Interactions

No pharmacodynamic drug interaction study reports were included in this submission.

PHARMACOKINETICS/TOXICOKINETICS

Pharmacokinetic/toxicokinetic studies (absorption, distribution, metabolism and excretion) have been reviewed under ~~IND 56, 553~~ IND 56, 553. These reviews are incorporated in appropriate sections. New studies that were not reviewed previously have been reviewed here. Toxicokinetic studies are reviewed under specific toxicology study.

2.6.4.1 Brief Summary

Alvimopan was poorly absorbed after oral administration. It is to be mentioned here that in humans, the mean absolute bioavailability was 14 and 6% following administration of a 12 mg solution, or two 6 mg capsules, respectively. The estimated half-life in rats and dogs was approximately 2 and 0.2 hrs, respectively. The plasma half-life in humans after an i.v. dose of 12 mg was 5.3 hr. Plasma protein binding of alvimopan was different across species. Free fractions ranged from 20-30% for humans, 56% for rats, 67% for mice, and 72% for dogs. Distribution of total radioactivity in rats following single oral doses of alvimopan, as evidenced by quantitative whole body autoradiography, was generally limited to the gastrointestinal tract with little distribution to peripheral tissues. Distribution to the brain was negligible after oral or intravenous doses to rats. Alvimopan did not inhibit any major human cytochrome P450 (CYP450) isozymes in rat hepatocytes up to 100 μ M concentration. In intact or bile duct-cannulated rats and dogs, alvimopan was primarily excreted through bile and feces following oral or intravenous administration. In rats, approximately 70 and 30% of the administered dose was excreted via the bile and urine, respectively, after an intravenous dose. In dogs, approximately 65 and 26% of the dose was excreted via the bile and urine, respectively, after an intravenous dose. In rats and dogs, most of the fecal radioactivity excreted after oral doses (>89% for both species), was attributed to unabsorbed drug.

2.6.4.2 Methods of Analysis

Plasma Analysis of Alvimopan and ADL 08-0011 by LC/MS/MS:

Alvimopan and its primary metabolite ADL 08-0011 were quantified in the plasma using a validated electrospray liquid chromatography/mass spectrometric (LC/MS/MS) method with a lower limit of quantitation (LLQ) of 0.25 ng/ml for both alvimopan and ADL 08-0011. In this method, aliquots of mouse plasma containing the internal standard () were applied to chromatography columns and the analytes were eluted with methanol and dried under nitrogen. The dried residues were reconstituted in 0.1% acetic acid and injected onto the LC/MS/MS system. The following table 4.2.2.A. (from page 11 of the sponsor's report: pharmtox\pk\808-020pk.pdf) shows the assay range and limit of quantitation.

TABLE 4.2.2A. ASSAY RANGE AND LIMIT OF QUANTITATION	
Standard Curve	Plasma alvimopan
Linear Range (ng/mL)	0.25 ng/mL to 100 ng/mL
Lower Limit of Quantitation (ng/mL)	0.25 ng/mL
Standard Curve	Plasma ADL 08-0011
Linear Range (ng/mL)	0.25 ng/mL to 100 ng/mL
Lower Limit of Quantitation (ng/mL)	0.25 ng/mL

In Vitro Plasma Protein Binding Studies using Centrifugal Filtration Technique

Protein binding of [³H]alvimopan/alvimopan in the plasma samples was determined at five concentrations using the _____ filter units. Sample pH was adjusted to approximately 7.4 by CO₂. Triplicate one ml aliquots of each sample were placed in the filter units which were incubated for 15 minutes at 37°C within the centrifuge. Samples were then centrifuged at approximately 1000 x g at 37°C for 30 minutes. Duplicate aliquots of the original sample and filtrate were obtained and assayed for total radioactivity.

Whole Body Autoradiography

In this method, animals were treated with a single oral dose of radiolabeled alvimopan. Appropriate amounts of radiolabeled and unlabeled alvimopan were dissolved in ethanol and dried under nitrogen and dissolved in polyethylene glycol (PEG 600). Animals were sacrificed at 1, 4, 8, or 24 hours postdose and then rapidly frozen in dry ice and hexane, and processed for whole-body autoradiographic evaluation according to the method of Ullberg (Ullberg S. *Science Tool. The LKB Instrument Journal*, Special Issue, 2-29, 1977). Sagittal whole-body sections (40 µm) were exposed to X-ray film for 11 days. Autoradiograms were evaluated visually.

2.6.4.3 Absorption

Determination of Portal and Systemic Plasma Concentrations of Radioactivity in Rats Dosed 10 mg/kg ¹⁴C-Alvimopan (Study No. 042R92)

ABSORPTION, DISTRIBUTION, METABOLISM & EXCRETION (ADME):

1. Absorption:

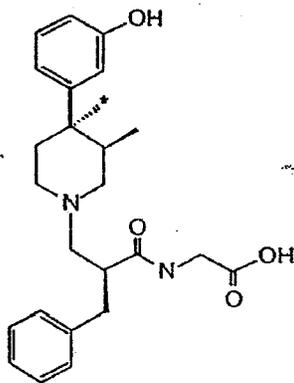
Rats:

Rats (n=4 per group) were orally dosed by gavage with 10 mg/kg (20 µCi/kg) ¹⁴C-LY246736 (see Figure 1) suspended in an aqueous 10% acacia solution. At specified times (0.5, 1, 3, 5, 7, 16 and 24 hours), successive groups of rats were anesthetized with Halothane and the abdominal cavity was opened to expose the inferior vena cava and the portal vein. Blood was withdrawn concomitantly from the inferior vena cava and the portal vein with heparinized syringes. Scintillation counting was done in triplicate. As indicated in Table 2 (these data were extracted from the sponsor's application, vol. 1.2, pg. 607), portal plasma

levels of ¹⁴C-LY246736 were relatively low. Moreover, systemic plasma concentrations of ¹⁴C-LY246736 were below the assay's 0.01 µg eq./ml limit of detection. Thus, orally administered LY246736 dihydrate is not well absorbed from the gastrointestinal tract and does not enter the hepatic system to any large degree.

Figure 1

The radiolabeled site of ¹⁴C-LY246736.



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¹⁴C-LY246736 (ACID) (* shows labeled site)

Table 2

Portal and systemic plasma concentrations (µg eq./ml) of ¹⁴C-LY246736 at various times after oral administration (10 mg/kg) by gavage in rats.

Portal plasma concentrations				Systemic plasma concentrations		
Hour	Mean	SEM	N	Hour	Mean	N
0.5	0.12	0.04	4	0.5	BQL	4
1	0.14	0.05	4	1	BQL	4
3	0.11	0.02	4	3	BQL	4
5	0.06	0.01	4	5	BQL	4
7	0.06	0.01	4	7	BQL	4
16	0.03	0.01	4	16	BQL	4
24	0.03	0.01	4	24	BQL	4

Determination of Plasma Concentrations of Alvimopan in Fischer 344 Rats Dosed with 200 mg/kg by Oral Gavage Daily for One Month (Study No. R02993)

In a second study, oral LY246736 dihydrate (200 mg/kg) was administered once daily by gavage to one group of 6 rats (3 M and

3 F) for 1 month. Blood samples were collected at 0:25, 0.5, 1, 2 and 4 hours after oral administration. Quantitative analysis of LY246736 was done by ~~HPLC~~. A data summary is shown in Table 3 for Day 0 and Day 29 (these data were extracted from the sponsor's application, vol. 2, pg. 654).

Table 3

Plasma levels (ng/ml) of LY246736 dihydrate at various times after oral administration in rats on Day 0 and Day 29 in a subacute study. Pharmacokinetic parameters (AUC, Cmax and Tmax) are also shown.

		Day 0 (ng/ml)						AUC (ng-hr/ml)	Cmax (ng/ml)	Tmax (hr)
	Sex	0.25 Hr	0.5 Hr	1 Hr	2 Hr	4 Hr				
MEAN	M	2.9	1.9	20.5	3.8	6.2	28.4	20.5	1.0	
SE	M	2.9	1.0	9.5	0.6	3.0				
N	M	3	3							
MEAN	F	7.1	5.6	3.3	11.5	2.9	25.6	11.5	2.0	
SE	F	4.0	2.3	0.6	4.5	3.0				
N	F	3	3							
MEAN	M+F	5.0	3.7	11.9	7.7	4.6	27.0	11.9		
SE	M+F	2.4	1.4	3.7	7.7	1.7				
N	M+F	6	6							
		Day 29 (ng/ml)						AUC (ng-hr/ml)	Cmax (ng/ml)	Tmax (hr)
	Sex	0.25 Hr	0.5 Hr	1 Hr	2 Hr	4 Hr				
MEAN	M	13.3	0.7	0.5	2.3	17.4	NC	NC	NC	
SE	M	8.2	0.7	0.8	2.3	9.7				
N	M	3	3	3						
MEAN	F	0.0	2.8	0.0	1.0	2.6	NC	NC	NC	
SE	F	0.0	2.8	0.0	1.0	1.5				
N	F	3	2							
MEAN	M+F	6.9	1.5	0.4	1.7	10.0	NC	NC	NC	
SE	M+F	4.8	1.1	0.4	1.1	5.5				
N	M+F	6	5	6	6	6				

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In general, plasma levels of orally administered LY246736 dihydrate were relatively low and did not increase over the 1

month of daily dosing

Plasma Concentrations of Alvimopan in Beagle Dogs Dose Intravenously with 0.05 mg/kg, 0.2 mg/kg or 2.0 mg/kg Alvimopan Daily for 1 Month (Study No. D04992)

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month of daily dosing

Dogs:

✓ I.v. LY246736 dihydrate (0.05, 0.2 or 2.0 mg/kg) was administered once daily to three groups of 6 dogs each (3 M and 3 F dogs in each group) for 1 month. Blood samples were collected at 5, 10, 20 and 40 minutes after i.v. administration. Quantitative analysis of LY246736 was done by HPLC. Data are summarized in Tables 4 (these data were extracted from the sponsor's application, vol. 2, pg. 628) and 5 (these data were extracted from the sponsor's application, vol. 2, pg. 629). On Day 0, values for AUC and C_{max} were dose-related and were not significantly different between males and females. Values for T_{max} were not dose-related; accurate estimates would require collection of blood samples at times less than 5 minutes after i.v. administration. Similar half-lives at each dose suggest zero-order metabolism of LY246736 dihydrate. On Day 29, results were generally similar to those found on Day 1.

Table 4 (Day 0)

Dose-related pharmacokinetic parameters of LY246736 dihydrate in dogs after oral administration on Day 0 in a subacute study.

		Sex	Treatment Group	AUC (ng-hr/ml)	AUC to Inf	Cmax (ng/ml)	Tmax (min)	Half-Life (min)
Treatment Group 01 0.05 mg/kg	Mean	M	1.0	2006.9	2113.4	115.0	5.0	11.2
	SE	M	1.0	325.4	311.1	19.9	0.0	0.7
	N	M	1.0	3.0	3.0	3.0	3.0	3.0
	Mean	F	1.0	2275.0	2373.8	131.7	5.0	10.8
	SE	F	1.0	519.2	538.5	30.6	0.0	0.3
	N	F	1.0	3.0	3.0	3.0	3.0	3.0
	Mean	M+F	1.0	2140.9	2243.6	123.3	5.0	11.0
	SE	M+F	1.0	280.5	284.1	16.7	0.0	0.4
	N	M+F	1.0	6.0	6.0	6.0	6.0	6.0
Treatment Group 02 0.2 mg/kg	Mean	M	2.0	7966.8	8359.2	475.3	5	10.5
	SE	M	2.0	482.9	452.4	32.9	0	0.4
	N	M	2.0	3.0	3.0	3.0	3.0	3.0
	Mean	F	2.0	6640.7	6885.8	388.0	5	9.7
	SE	F	2.0	632.6	653.1	39.4	0.0	0.3
	N	F	2.0	3.0	3.0	3.0	3.0	3.0
	Mean	M+F	2.0	7303.8	7622.6	431.7	5	10.1
	SE	M+F	2.0	463.3	484.6	30.1	0.0	0.3
	N	M+F	2.0	6.0	6.0	6.0	6.0	6.0
Treatment Group 03 2.0 mg/kg	Mean	M	3.0	53578.3	56000.6	3130.4	5	10.0
	SE	M	3.0	3671.6	3890.8	227.2	0.0	0.5
	N	M	3.0	3.0	3.0	3.0	3.0	3.0
	Mean	F	3.0	67193.0	70306.0	3881.1	5	9.4
	SE	F	3.0	14751.3	15503.6	871.3	0.0	0.7
	N	F	3.0	3.0	3.0	3.0	3.0	3.0
	Mean	M+F	3.0	60385.7	63153.3	3505.8	5	9.7
	SE	M+F	3.0	7448.8	7831.5	436.3	0.0	0.4
	N	M+F	3.0	6.0	6.0	6.0	6.0	6.0

Table 5 (Day 29)

Dose-related pharmacokinetic parameters of LY246736 dihydrate in dogs after oral administration on Day 29 in a subacute study.

		Sex	Treatment Group	AUC (ng-hr/ml)	AUC to Inf	Cmax (ng/ml)	Tmax (min)	Half-Life (min)
Treatment Group 01 0.05 mg/kg	Mean	M		1722.1	1865.7	97.1	5	12.5
	SE	M		88.4	79.4	5.0	0.0	1.8
	N	M		3.0	3.0	3.0	3.0	3.0
	Mean	F	1	2633.0	2732.1	142.6	3	10.0
	SE	F		704.7	715.1	44.8	0.0	0.5
	N	F		3.0	3.0	3.0	3.0	3.0
	Mean	M+F	1	2177.6	2298.9	119.8	5	11.1
	SE	M+F		377.3	375.6	22.6	0.0	1.0
	N	M+F		6.0	6.0	6.0	6.0	6.0
	Treatment Group 02 0.2 mg/kg	Mean	M	2	10944.1	11291.8	624.6	5
SE		M	2	1765.9	1750.4	105.5	0.0	0.5
N		M	2	3.0	3.0	3.0	3.0	3.0
Mean		F	2	7964.3	8310.3	418.7	5	10.0
SE		F	2	332.7	342.2	61.0	0.0	0.5
N		F	2	3.0	3.0	3.0	3.0	3.0
Mean		M+F	2	9454.2	9801.0	521.7	3	9.9
SE		M+F	2	1043.9	1039.6	71.3	0.0	0.3
N		M+F	2	6.0	6.0	6.0	6.0	6.0
Treatment Group 03 2.0 mg/kg		Mean	M	3	65316.5	67952.7	3759.2	5
	SE	M	3	4985.2	5162.1	260.1	0.0	1.3
	N	M	3	3.0	3.0	3.0	3.0	3.0
	Mean	F	3	75344.5	77588.3	4375.7	5	8.3
	SE	F	3	14575.3	14836.5	910.9	0.0	0.1
	N	F	3	3.0	3.0	3.0	3.0	3.0
	Mean	M+F	3	70330.5	72770.5	4067.4	5	8.7
	SE	M+F	3	7244.8	7356.3	445.5	0.0	0.7
	N	M+F	3	6.0	6.0	6.0	6.0	6.0

In general, plasma levels of orally administered LY246736 dihydrate were relatively low and did not increase over the 1 month of daily dosing.

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Plasma Concentrations of Alvimopan in Beagle Dogs Dosed Orally with 10 mg/kg, 30 mg/kg, or 100 mg/kg Alvimopan Daily for 1 Month (Study No. D02793)

✓ In a second study, oral LY246936 dihydrate (0, 10, 30 or 100 mg/kg) was administered once daily in gelatine capsules to four groups of 6 dogs each (3 M and 3 F dogs in each group) for 1 month. Blood samples were collected at 0.5, 1, 2, 4, 8, 12 and 24 hours after oral administration. Quantitative analysis of LY246736 was done by HPLC. On Day 0, values for AUC and C_{max} were dose-related; values were larger in females than in males. On day 29, values for AUC and C_{max} were dose-related; values were not different between Day 0 and Day 29 (note the large SEs).

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Table 6 (Day 0)

Dose-related pharmacokinetic parameters (AUC, ng·hr/ml; C_{max}, ng/ml) of LY246736 dihydrate in dogs after oral administration on Day 0 in a subacute study.

		Sex	Dose	AUC	C _{max}
Treatment Group 01	Mean	M	10 mg/kg	220.4	18.3
	SE	M	10 mg/kg	44.1	3.7
	N	M	10 mg/kg	3	3
	Mean	F	10 mg/kg	210.1	43.4
	SE	F	10 mg/kg	93.8	30.6
	N	F	10 mg/kg	3	3
	Mean	M+F	10 mg/kg	215.3	30.9
	SE	M+F	10 mg/kg	46.4	14.9
	N	M+F	10 mg/kg	6	6
Treatment Group 02	Mean	M	30 mg/kg	599.1	41.3
	SE	M	30 mg/kg	119.9	3.8
	N	M	30 mg/kg	3	3
	Mean	F	30 mg/kg	1304.3	108.0
	SE	F	30 mg/kg	314.8	9.2
	N	F	30 mg/kg	3	3
	Mean	M+F	30 mg/kg	951.7	74.6
	SE	M+F	30 mg/kg	211.6	15.6
	N	M+F	30 mg/kg	6	6
Treatment Group 03	Mean	M	100 mg/kg	920.5	82.1
	SE	M	100 mg/kg	373.0	11.1
	N	M	100 mg/kg	3	3
	Mean	F	100 mg/kg	1149.4	103.7
	SE	F	100 mg/kg	330.9	18.0
	N	F	100 mg/kg	3	3
	Mean	M+F	100 mg/kg	1034.9	92.9
	SE	M+F	100 mg/kg	228.8	10.6
	N	M+F	100 mg/kg	6	6

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Table 7 (Day 29)

Dose-related pharmacokinetic parameters (AUC, ng·hr/ml; Cmax, ng/ml) of LY246736 dihydrate in dogs after oral administration on Day 29 in a subacute study.

		Sex	Dose	AUC	Cmax
Treatment Group 01	Mean	M	10 mg/kg	293.4	30.7
	SE	M	10 mg/kg	84.4	9.4
	N	M	10 mg/kg	3	3
	Mean	F	10 mg/kg	258.3	23.3
	SE	F	10 mg/kg	51.4	7.1
	N	F	10 mg/kg	3	3
	Mean	M+F	10 mg/kg	275.8	27.0
	SE	M+F	10 mg/kg	44.9	5.5
	N	M+F	10 mg/kg	6	6
Treatment Group 02	Mean	M	30 mg/kg	669.6	81.7
	SE	M	30 mg/kg	113.0	16.9
	N	M	30 mg/kg	3	3
	Mean	F	30 mg/kg	980.0	102.4
	SE	F	30 mg/kg	120.3	3.5
	N	F	30 mg/kg	3	3
	Mean	M+F	30 mg/kg	824.8	92.1
	SE	M+F	30 mg/kg	101.3	9.0
	N	M+F	30 mg/kg	6	6
Treatment Group 03	Mean	M	100 mg/kg	793.4	80.0
	SE	M	100 mg/kg	237.6	5.3
	N	M	100 mg/kg	3	3
	Mean	F	100 mg/kg	2387.5	187.1
	SE	F	100 mg/kg	308.9	37.3
	N	F	100 mg/kg	3	3
	Mean	M+F	100 mg/kg	1590.4	133.5
	SE	M+F	100 mg/kg	396.8	29.3
	N	M+F	100 mg/kg	6	6

In general, the pharmacokinetic parameters that were studied suggest that plasma levels of orally administered LY246736 dihydrate were relatively low and did not increase over the 1 month of daily dosing.

Blood Level Study in Fischer 344 Rats Given a Single Gavage Dose of Alvimopan (Study No. R05693)

Methods: The objective of this exploratory study was to determine the plasma concentration of alvimopan after a single oral administration to Fischer rats at doses up to 400 mg/kg. In this study, three groups of 24 rats (n = 12/sex) were administered alvimopan at a dose of 100, 200 or 400 mg/kg by oral gavage (5 ml/kg). Blood samples were collected at 0.5, 1, 2 and 4 hours postdose.

Results: Peak plasma concentrations of alvimopan were reached within 1 hour after oral administration of 200 or 400 mg/kg. The following table (from page 5 of the sponsor's report: pharmtox\pk\r05693.pdf) shows the mean plasma concentrations of alvimopan in rats.

Table 1: Mean Plasma Concentration (ng/mL) of Alvimopan After a Single Oral Administration to Fischer 344 Rat.

Time-Point Postdosing	200 mg/kg		400 mg/kg	
	Male	Female	Male	Female
30 min	2.5	6.5	5.7	4.6
1 hr	1.2	4.6	0	6.2
2 hr	0	2.8	1.2	ND
4 hr	ND	3.4	4.6	ND

ND: No peak(s) detected

Peaks less than the limit of quantitation were considered "0".

Pharmacokinetic Study of Alvimopan in Fisher Rats (Study No. 808-005)

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1. Pharmacokinetic study with ADL8-2698 in the rat (Study No. 808-005)

Methods: In this study, male and female Fischer 344 rats (6/sex/group) were administered single oral (gavage) doses of ADL8-2698 at 200, 500 and 1000 mg/kg (dose volume = 10 ml/kg). The dosing solutions of the test article were prepared as a suspension in a 10% (w/v) suspension of Gum Acacia, NF in deionized water. Serial blood samples were obtained from each animal at the following time points: male, 15 min and 1, 2, 4, 8, and 24 h postdose; female, 15 min and 1, 4, and 24 h postdose. Plasma samples were analyzed for ADL8-2698 using a validated LC/MS/MS method.

Results: The mass spectral analysis did not reveal the presence of any metabolite related to ADL8-2698 and only the parent compound was detected in the plasma and hence, no metabolite concentrations were measured. Overall, the plasma concentrations of ADL8-2698 were found to be low (<10 ng/ml) at all dose levels in both sexes following oral administration, indicating poor absorption of ADL8-2698. However, plasma concentrations of > 10 ng/ml were also observed in some female rats at 500 mg/kg (maximum concentration = 14.16 ng/ml) and in one male rat at 1000 mg/kg (maximum concentration = 14.43 ng/ml). It is to be mentioned here that at 1000 mg/kg dose level, the drug was present (above limit of detection) in more sampling intervals compared to lower doses, which indicated some absorption at high dose albeit poor. There was no sex-related difference in the plasma concentrations of ADL8-2698. The mean plasma concentrations at 200, 500 and 1000 mg/kg were 1.32, 2.29 and 2.28 ng/ml, respectively. Overall, the data suggested poor absorption of ADL8-2698 from the gastrointestinal tract. The results of this study do not clearly demonstrate the saturation of absorption in the absence of significant dose-related increase in the plasma level of the drug followed by a plateau phase.

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IND 56, 553

9

The following table (from page 9 of sponsor's submission) shows the mean plasma concentrations of ADL8-2698 in Fischer 344 rats.

Table 4. Statistical Analysis for Combined Plasma Concentrations of ADL 8-2698 Following Single Oral Administration of 200, 500 and 1000 mg/kg to Male and Female Fischer 344 Rats.

Variable	200 mg/kg	500 mg/kg	1000 mg/kg
Number of values	60	60	60
Minimum (ng/mL)			
Maximum (ng/mL)			
Mean (ng/mL)	1.318	2.285	2.279
Std. Deviation	1.091	2.783	1.876
Std. Error	0.141	0.359	0.242
Lower 95% CI	1.036	1.566	1.795
Upper 95% CI	1.600	3.003	2.764

Formulation and Toxicokinetic Study of Alvimopan in Fischer Rats (Study No. 808-007)

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Formulation and toxicokinetic study of ADL8-2698 and its metabolite (ADL08-0011) in Fischer rats (Study No. 808-007)

Methods: This study was conducted to determine the maximum feasible concentration that can be delivered through a standard gavage tubing and to characterize the toxicokinetic profile of ADL8-2698 and its metabolite (ADL08-0011), following a single oral dose. In this study, male and female Fischer 344 rats (5/sex/group) were administered single oral (gavage) doses of ADL8-2698 at 500, 1000, 2000, and 4000 mg/kg (dose volume = 10 ml/kg). The dosing solutions of the test article were prepared as a suspension in a 10% (w/v) suspension of Gum Acacia, NF in deionized water. Formulations were prepared at 150, 200, 300, 400, and 500 mg/ml to determine the maximum feasible concentration. The formulations at 150, 200, and 400 (1 of 2 preparations) mg/ml were prepared using a tissue homogenizer; however, test material at 400 mg/ml was left in the grinding chamber of the homogenizer. Therefore, the formulations at 300, 400 (second preparation at this level), and 500 mg/ml concentrations were prepared using a tissue homogenizer in order to overcome the difficulty (increase in viscosity during the process due to lack of vehicle to rinse the equipment) in the preparation of formulation. Serial blood samples were obtained from each animal at the following time points: male, 15 min and 1, 2, 4, 8, and 24 h postdose; female, 15 min and 1, 4, and 24 h postdose. Plasma samples were analyzed for ADL8-2698 using a validated LC/MS/MS method. It appears that the sampling times are not adequate for a drug, which has a relatively long half-life (10 to 18 h) in rats.

Results: The formulations at different concentrations were evaluated for ease or difficulty to aspirate. All formulations were rated as easy to aspirate except 500 mg/ml, which was rated as difficult. Based on these observations, the maximum feasible concentration was determined as 400 mg/ml (MFD = 4000 mg/kg, 10 ml/kg). There was no significant difference in the mean C_{max} or AUC₀₋₁ values for the parent drug in males and females. Mean C_{max} values ranged from 2.4 to 2.9 ng/ml for males and 1.8 to 3.2 ng/ml for females. Mean AUC_{0-24h} values ranged from 27.4 to 37.5 ng.h/ml for males and 25.9 to 44.0 ng.h/ml for females. There was no dose-related increase in either parameter in males or females. In the previous study (Study No. 808-005), in case of males, the exposure of ADL8-2698 increased linearly with increase in dose (AUC_{0-24h} values were calculated by the reviewer and found to be 18.74, 28.31, and 58.44 ng.h/ml at 200, 500, and 1000 mg/kg, respectively). In the females, there was dose-related increase in exposure from 200 to 500 mg/kg; however, the exposure level tended to decrease from 500 to 1000 mg/kg (AUC_{0-24h} values were 28.00, 47.29, and 38.77 ng.h/ml at 200, 500, and 1000 mg/kg, respectively), indicating an apparent saturation of absorption. Although, the sponsor did not include doses lower than 500 mg/kg in this study; however, considering the results of the previous pharmacokinetic study, it appears that there is an apparent saturation of absorption of ADL8-2698 at 500 mg/kg, as the exposure did not increase from 500 to 4000 mg/kg.

The plasma concentrations of ADL08-0011 increased over the 24-h collection period (figure shown below from Vol. I, page 14 of Appendix-II of sponsor's submission); however, there was no significant difference in the plasma concentrations of the metabolite either across sex or

IND 56, 553

10

different dose groups. In males, the AUC_{0-24h} values for ADL8-0011 were calculated (by the reviewer using trapezoidal rule) to be 279.07, 258.29, 225.40, and 230.79 ng.h/ml at 500, 1000, 2000, and 4000 mg/kg, respectively, indicating no dose-related increase in exposure to the metabolite, which could be attributed to the saturation of absorption of the parent drug. In females, the AUC_{0-24h} values for ADL8-0011 were calculated (by the reviewer using trapezoidal rule) to be 791.43, 596.46, 360.68, and 481.44 ng.h/ml at 500, 1000, 2000, and 4000 mg/kg, respectively, indicating a somewhat dose-related decrease in exposure to the metabolite. It is to be mentioned here that no data is available regarding the pharmacological activity of ADL8-0011.

The mean (\pm SD) pharmacokinetic parameters of ADL8-2698 in male and female rats are shown in the following table.

MALE:

Parameter	Dose (mg/kg)			
	500	1000	2000	4000
AUC _{0-24h} (ng.h/ml)	36.3 \pm 7.8	37.5 \pm 7.9	37.3 \pm 9.8	27.4 \pm 9.7
Cmax (ng/ml)	2.8 \pm 1.0	2.9 \pm 1.2	2.4 \pm 0.7	2.7 \pm 0.8
Tmax (h)	3.2	2.5	4.4	3.1
T _{1/2} (h)	12.4	9.2	10.2	6.0

FEMALE:

Parameter	Dose (mg/kg)			
	500	1000	2000	4000
AUC _{0-24h} (ng.h/ml)	42.1 \pm 7.2	44.0 \pm 12.9	25.9 \pm 3.0	34.8 \pm 7.5
Cmax (ng/ml)	2.6 \pm 0.6	3.2 \pm 0.7	1.8 \pm 0.1	2.6 \pm 1.0
Tmax (h)	1.1	2.3	1.4	1.9
T _{1/2} (h)	18.9	10.1	10.4	11.3

Overall, the lack of increase in systemic absorption of ADL8-2698 or ADL8-0011 between 500 and 4000 mg/kg indicated an apparent saturation of absorption of ADL8-2698 at 500 mg/kg.

Calculation of Alvimopan Pharmacokinetic Parameters Following Single Intravenous Doses of 1, 5 or 10 mg/ kg to Fischer rats (Study No. 14TK010)

Methods: Alvimopan was administered as an intravenous bolus injection to 3 groups of Fischer 344 rats (16/sex/group) at 1, 5 or 10 mg/kg. Blood samples were collected at

predose and at 5 min, 15 min, 30 min, and 1, 2, 4, 8 and 24 hours postdose. Plasma samples were assayed by HPLC/MS/MS method.

Results: The elimination of alvimopan after single intravenous doses was biphasic, with a rapid distribution phase followed by a slower terminal elimination phase. Following intravenous administration, alvimopan was rapidly excreted at all three doses with plasma clearance values ranging from 2.85 to 5.42 L/hr/kg. Steady-state volume of distribution estimates ranged from 0.74 to 1.92 L/kg. The pharmacokinetic parameters are shown in the following table (from page 7 of the sponsor's report: 14tk010.pdf).

Alvimopan Pharmacokinetic Parameters Following Single Intravenous Bolus Injection to Male and Female Rats						
Nominal Dose (mg/kg)	Sex	Co (ng/mL)	AUC (ng·hr/mL)	CL (L/hr/kg)	V _{ss} (L/kg)	t _{1/2} (hr)
1	M	2087	316	2.85	1.11	2.0
	F	1297	185	4.86	1.37	2.5
5	M	11519	1279	3.52	0.76	1.9
	F	5486	831	5.42	1.92	1.8
10	M	19256	2198	4.10	0.74	1.8
	F	8356	1694	5.32	1.42	1.5

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2.6.4.4 Distribution

In Vitro Evaluation of Binding of [³H]Alvimopan to Mouse, Rat, Dog and Normal Human Plasma Proteins (Study No. 0833XA43.001)

Methods: This study was conducted to examine the *in vitro* binding of [³H]alvimopan to mouse, rat, dog and normal human plasma proteins. Protein binding was measured using centrifugal filtration technique as described before. Protein binding of [³H]alvimopan/alvimopan in these plasmas was assessed at the following nominal test article concentrations; mouse: 0.5, 5, 50, 250 and 500 ng/ml; rat: 0.5, 1, 5, 10 and 50 ng/ml; dog: 0.5, 5, 50, 100 and 250 ng/ml; human: 0.5, 5, 10, 50 and 100 ng/ml. The test article was prepared by adding a constant amount of [³H]alvimopan (28.0 Ci/mmol), and varying amounts of unlabeled alvimopan to the plasma matrix to yield the desired concentration level. Triplicate one ml aliquots of each preparation were placed in filters and incubated for 15 minutes at 37°C within the centrifuge. Samples were then centrifuged at approximately 1000 x g at 37°C for 30 minutes. Duplicate aliquots of each filtrate and the original sample were assayed for total radioactivity.

Results: There were species differences in protein binding of [³H]alvimopan. The highest degree of protein binding (approximately 70%) was observed in human plasma. Moderate binding was observed in mouse plasma (30 to 36%) and rat plasma (42 to 52%). The lowest amount of binding (approximately 25 to 32%) was observed in dog plasma. The mean free fraction (%) for each plasma species was as follows: mouse, 67%; rat, 56 %; dog, 72%; and human, 30%.

In Vitro Evaluation of Binding of [³H]Alvimopan to Rat, Rabbit and Normal Human Plasma Proteins (Study No. 0833XA43.003)

Methods: This study was conducted to examine the *in vitro* binding of [³H]alvimopan to rat, rabbit and normal human plasma proteins. Protein binding was determined using centrifugal filtration technique as mentioned before. Protein binding of [³H]alvimopan in these plasmas was evaluated at the following test article concentrations; rat: 10, 50, 500, 5000 and 20,000 ng/ml; rabbit: 5, 50, 500, 5000 and 30,000 ng/ml; human: 50, 100, 250, 500 and 1000 ng/ml. A constant amount of [³H]alvimopan (specific activity = 39.5 Ci/mmol) and varying amounts of unlabeled alvimopan was added to the plasma matrix to yield the desired concentration level. Triplicate one ml plasma aliquots were placed in the filter unit and incubated for 15 minutes at 37°C. Samples were then centrifuged at approximately 1000 x g at 37°C for 30 minutes. Duplicate aliquots of each filtrate and the original sample were assayed for total radioactivity.

Results: The extent of binding varied across different species. The greatest degree of binding, approximately 80%, was observed in human plasma. Binding to rabbit plasma proteins was approximately 65 to 75%. The lowest degree of binding, approximately 42 to 45%, was observed in Fischer 344 rat plasma. The mean free fraction (%) for each plasma species was: rat, 56%, rabbit, 29% and human, 20%.

In Vitro Evaluation of Binding of [³H]ADL 08-0011 to Mouse, Rat, Rabbit, Dog and Normal Human Plasma Proteins (Study No. 0833XA43.004)

Methods: This study was performed to examine the extent of protein binding of [³H]ADL 08-0011 (pharmacologically active amide hydrolysis metabolite of alvimopan) in the mouse, rat, rabbit, dog and normal human plasma. Protein binding was measured by ultrafiltration technique. Protein binding of [³H]ADL 08-0011/ADL 08-0011 in these plasmas was evaluated at the following test article concentrations: mouse: 0.5, 5, 50, 250 and 500 ng/ml; rat: 0.5, 5, 50, 500 and 5000 ng/ml; rabbit: 0.5, 5, 50, 250 and 500 ng/ml; dog: 0.5, 2.5, 25, 250 and 2500 ng/ml; and human: 0.5, 5, 10, 50, 100 and 1000 ng/ml. Each plasma preparation was spiked with the same amount of [³H]ADL 08-0011 (specific activity = 16.0 Ci/mmol) and varying amounts of unlabeled ADL 08-0011. Triplicate one ml plasma aliquots were placed in filter unit and incubated for 15 minutes at 37^oC. Samples were then centrifuged at approximately 1000 x g at 37^oC for 30 minutes. Duplicate aliquots of each filtrate and the original sample were assayed. Quantification of the total amount of test article in plasma and free fraction in the ultrafiltrate was determined by liquid scintillation counting of total [³H] radioactivity.

Results: The greatest degree of binding, approximately 95 to 96%, was observed in human plasma. Binding to rabbit plasma proteins was also high (91 to 94%). However, [³H]ADL 08-0011 was not highly protein bound in mouse, rat and dog plasmas. The lowest degree of binding was observed in mouse plasma (34 to 43%), followed by rat plasma (47 to 55%) and dog plasma (59 to 66%). The mean free fraction (%) for each plasma species was: mouse, 62.1%; rat, 50.3%; dog, 39.2%, rabbit, 6.7%; human, 4.5%.

Red Blood Cell Partitioning Study with LC-MS/MS Analysis of Alvimopan in Human, Dog, Rabbit, Rat, and Mouse (Study No. 57-0307)

Methods: The objective of this study was to determine the blood:plasma ratio and degree of *in vitro* red blood cell (RBC) uptake of alvimopan in human, dog, rabbit, rat, and mouse. In this study, alvimopan-spiked whole blood (50 and 500 ng/mL) samples from multiple species (human, dog, rabbit, rat, and mouse) were incubated in a 37^oC water bath for 30 minutes (alvimopan uptake reached equilibrium within 0.5 hours, which was established in a previous pilot study). At the end of incubation, an aliquot was taken from each sample and centrifuged to yield plasma, while the remaining whole blood was completely hemolyzed by repeated freezing and thawing. Alvimopan concentrations in plasma samples were quantitated using a validated LC-MS/MS assay.

Results: The distribution of alvimopan into RBC appeared to be concentration-independent. There was an apparent species difference in distribution of alvimopan in RBCs. The greatest distribution was observed in mouse RBCs (blood:plasma ratio 1.45 and 1.61 for 50 and 500 ng/mL, respectively), and the least distribution was observed in human (blood:plasma ratio 0.68 and 0.67 for 50 and 500 ng/mL, respectively). The blood:plasma ratios in rabbit were 0.90 and 0.99 for 50 and 500 ng/mL, respectively.

In Vitro Evaluation of the Distribution of [³H]ADL 08-0011 in Normal Mouse, Rat, Rabbit, Dog and Human Whole Blood (Study No. 0834XA43.002)

Methods: This study was conducted to examine the *in vitro* partitioning of [³H]ADL 08-0011 (a major pharmacologically active metabolite of alvimopan) to mouse, rat, rabbit, dog and human blood cells and to determine the blood to plasma ratios. In this study, whole blood sample was spiked with the same amount of [³H]ADL 08-0011 (specific activity = 16.0 Ci/mmol) and varying amounts of unlabeled ADL 08-0011. Triplicate aliquots of each concentration level of test article in whole blood were incubated at 37°C for specified time. Following incubation, the samples were centrifuged for 5 minutes at approximately 10,000 x g and samples were assayed for total radioactivity using liquid scintillation counter. An aliquot of the whole blood preparation after incubation was also assayed for total radioactivity.

Results: The extent of blood cell association was greatest in mouse ($K_p = 3.77$ to 2.57 , K_p = partition co-efficient), followed by rat ($K_p = 0.75$ to 1.78) and dog ($K_p = 0.99$ to 0.76) and was least in rabbit ($K_p < 0.07$) and human ($K_p = 0.10$ to 0.30). Overall, blood cell association in different species appeared to be concentration independent up to 500 ng/ml. The following table (from page 7 of the sponsor's report: pharmtox\pk\0834XA43002.pdf) summarizes the blood cell association of ADL 08-0011 in different species.

Results of the concentration dependency assessments are summarized below:

Species	Sex	Concentration Range	K_p Range	Blood:Plasma Ratio Range
CD-1 Mouse	Male	0.5 and 5 ng/ml; 50 to 500 ng/ml	3.77 and 3.25; 2.57 to 2.67	2.26 and 2.03; 1.70 to 1.76
Fischer 344 Rat	Male	0.5 to 500 ng/ml; 5000 ng/ml	0.75 to 0.96; 1.78	0.89 to 0.98; 1.34
NZW Rabbit	Female	0.5 to 250 ng/ml 500 ng/ml	NR 0.07	0.57 to 0.58 0.64
Beagle Dog	Male	0.5 to 250 ng/ml; 2500 ng/ml	0.86 to 0.99; 0.76	0.94 to 1.00; 0.89
Normal Human	Male	0.5 to 1000 ng/ml	0.10 to 0.30	0.60 to 0.69

K_p – partition coefficient; (ADL 08-0011 concn in blood cell fraction/ADL 08-0011 concn in plasma)
NR – not reported due to calculation artifact yielding values < 0

Whole-Body Autoradiographic Disposition of ¹⁴C-Alvimopan in Male Fischer 344 Rats After a Single Oral 10 mg/kg Dose (Study No. ADME RPT 6)

2. Distribution:

Four rats received an oral 10 mg/kg dose by gavage of ¹⁴C-LY246736 dihydrate (see Figure 1) in PEG 600. A single animal was euthanized with isoflurane at 1, 4, 8 or 24 hours after dosing. Animals were then rapidly frozen in dry ice and hexane, and processed for whole-body autoradiographic evaluation. At 1 hour postdose, highest concentrations of radioactivity appeared in the mucosal layer of the stomach and small intestine. Lower concentrations were seen in the esophagus, and stomach and intestinal contents. At 4 hours postdose, highest concentrations of radioactivity were found in the small intestine, and the contents of the stomach, intestine, cecum and feces. At 8 hours postdose, high levels of radioactivity were seen in intestinal and cecum contents and feces. At 24 hours postdose, cecum and feces contained moderate to high levels of radioactivity.

In summary, the localized and high radioactivity in the gastrointestinal tract and feces suggest that ¹⁴C-LY246736 dihydrate was not well absorbed.

2.6.4.5 Metabolism

Evaluation of the *In Vitro* Induction of CYP mRNA Expression in Rat Hepatocytes by SB-767905-KW (Alvimopan) Using [REDACTED] (Study No. DI01178)

Methods: The objective of the study was to establish the potential for alvimopan (SB-767905-KW) to induce cytochrome P450 (CYP) mRNA genes in cultured male rat hepatocytes using [REDACTED]. In this study, rat hepatocytes were incubated with alvimopan for 24, 48 or 72 hr at concentrations of 0, 1, 3, 10, 30 and 100 μM. Total RNA was isolated, quantified and subsequently synthesized into cDNA templates using [REDACTED]. The specific gene expression was quantitatively detected for the following genes: CYP 1A1, 1A2, 2B1, 2B2, 3A2, 3A9, 3A18, 3A23, 2E1, 4A1 and the housekeeping genes GAPDH and β-actin using [REDACTED].

Results: There was no evidence of significant induction of CYP450 mRNA levels in rat hepatocytes following 24, 48 or 72 h incubation with alvimopan. The results indicated that alvimopan has poor potential to cause induction of CYP450 enzymes.

Biotransformation of [¹⁴C] Alvimopan in Intact and Bile Duct-Cannulated Rats (Study No. 13TX02)

Biotransformation of [¹⁴C]ADL 8-2698 in Intact and Bile-duct Cannulated Rats (Study 13TX02)

Method: This study was previously submitted under Amendment # 035 dated December 21, 2000 and has been reviewed (pharmacology review dated February 27, 2001). In this submission, the sponsor presented the data regarding the metabolites of ADL 8-2698 found in the bile, urine and feces. In this study, rats were treated with a single 200 mg/kg oral or 20 mg/kg i.v. dose of [¹⁴C]ADL 8-2698 (71.0 μCi/mg). Urine, feces and bile samples were collected at

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IND 56, 553

2

selected intervals and were analyzed to determine the metabolite profile of [¹⁴C] ADL8-2698 using mass spectrometry (LC/MS/MS) and metabolites were identified in order of HPLC retention time. The following metabolites were characterized:

- R1: Sulfate conjugate of ADL 8-2698
- R2: Mono-oxygenated metabolite of ADL 8-2698
- R3: Amide hydrolysis metabolite of ADL 8-2698
- R4: Glucuronide metabolite of ADL 8-2698
- R5: Sulfate of the amide hydrolysis metabolite
- R6: A second glucuronide conjugate of ADL 8-2698
- R7: Glucuronide of the amide hydrolysis metabolite of ADL 8-2698

Results:

Rat Urine: Following oral administration of [¹⁴C] ADL8-2698 in intact or bile duct-cannulated (BDC) rats, the metabolite profile was not analyzed, as the total percent radioactivity excreted through urine was too low (< 1% of total radioactivity). However, after i.v. administration, approximately 20 to 23% of the radioactivity was recovered in the urine in intact and BDC animals. Unchanged ADL 8-2698 accounted for approximately 95% of the radioactivity and a minor amount (0.3 to 0.5% of administered dose) of sulfate conjugate (R1) was present in both intact and BDC rats. The urine from intact animals also contained small amount of mono-oxygenated metabolite (R2, 0.2% of the administered dose) and the amide hydrolysis metabolite (R3, ADL 08-0011, 1.0% of the administered dose). Overall, the metabolite profile in the urine of intact and BDC animals are comparable.

Rat Bile: Small amount of radioactivity (approximately 0.4% of the administered dose) was recovered in the bile after oral administration. The majority of the metabolite was found to be sulfate conjugate (R1, 0.3%). The other two minor metabolites detected were R6 (glucuronide conjugate of parent compound, <0.1%) and R7 (glucuronide of amide hydrolysis, <0.1%) in addition to parent compound (0.1%). After i.v. administration, approximately 79% of the radioactivity was recovered in the bile of BDC animals. Of the radioactivity recovered in the bile, the sulfate conjugate accounted for approximately 80% of the radioactivity (64.8% of the administered dose). The remaining radioactivity was characterized as mostly unchanged parent compound (13.2%) and small amount of glucuronide conjugate of ADL 8-2698 (R4, 0.3%) and the sulfate conjugate of amide hydrolysis product (R5, 0.7%).

Rat Feces: Following oral administration, the majority of the radioactivity was eliminated in the feces of both intact (89%) and BDC rats (88%), which was characterized mostly as unchanged ADL 8-2698. Only small amount of amide hydrolysis metabolite (R3, 1.6 to 4.0%) and a mono-oxidative metabolite (R2, 0.4 to 0.8%) were also detected. Following i.v. administration, small percentage (0.9% of the administered dose) of radioactivity was found in the BDC rats and about 74% of the administered radioactivity was found in the intact animals. The majority of the recovered radioactivity in the feces of intact animals was mostly unchanged parent compound (66%) and the remaining were characterized as R3 (7.3%) and R1 (1.3%).

IND 56, 553

3

Overall, no significant difference in metabolite profile was observed between BDC and intact animals following oral administration. The major metabolic pathway was characterized as unchanged ADL 8-2698 with minor mono-oxidative and conjugated pathways (sulfate and glucuronide). Similarly, the metabolite profile was comparable in BDC and intact animals following i.v. administration. The major metabolic pathways were unchanged ADL 8-2698 and its corresponding sulfate conjugate (R1). Minor biotransformation pathways included mono-oxidation, glucuronidation, and amide hydrolysis with subsequent sulfate conjugation. However, higher amounts of radioactivity was recovered in the bile of rats treated intravenously (79%) as compared to rats treated orally (0.4%). The following table summarizes the quantification of [¹⁴C] ADL 8-2698 and its metabolites after oral and intravenous administration (from page 6 of sponsor's submission) in rats.

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IND 56, 553

4

Table 2.7a Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in BDC Rats Following Intravenous (IV) Administration (20 mg/kg)

Metabolite Number	Structure Assignment	BDC IV	BDC IV	BDC IV
		Rat bile	Rat urine	Rat feces
		Mean % of dose	Mean % of dose	Mean % of dose
R4	glucuronide	0.3	ND	ND
R1	sulfate of parent	64.8	0.5	ND
R5	sulfate of amide hydrolysis	0.7	ND	ND
	Parent	13.2	21.8	0.7
R3	amide hydrolysis	ND	ND	0.2

Table 2.7b Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in BDC Rats Following Oral Administration (200 mg/kg).

Metabolite Number	Structure Assignment	BDC Oral	BDC Oral
		Rat bile	Rat feces
		Mean % of dose	Mean % of dose
R7	² glucuronide of amide hydrolysis	<0.1	ND
R1	sulfate of parent	0.3	ND
R2	mono-oxygenation	ND	0.8
R6	² glucuronide	<0.1	ND
	Parent	0.1	88.1
R3	amide hydrolysis	ND	1.6

ND = not detected

See Appendix 1 for the number of subjects used to determine the mean percent of dose value for each metabolite.

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IND 56, 553

5

Table 2.7c Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in Intact Rats Following Intravenous and Oral Administration (20 or 200 mg/kg).

Metabolite Number	Structure Assignment	Intact IV	Intact IV	Intact Oral
		Rat urine	Rat feces	Rat feces
		Mean % of dose	Mean % of dose	Mean % of dose
R1	sulfate of parent	0.3	1.3	ND
R2	mono-oxygenation	0.2	ND	0.4
	Parent	18.7	66.1	89.2
R3	amide hydrolysis	1.0	7.3	4.0

ND = not detected

See Appendix 1 for the number of subjects used to determine the mean percent of dose value for each metabolite.

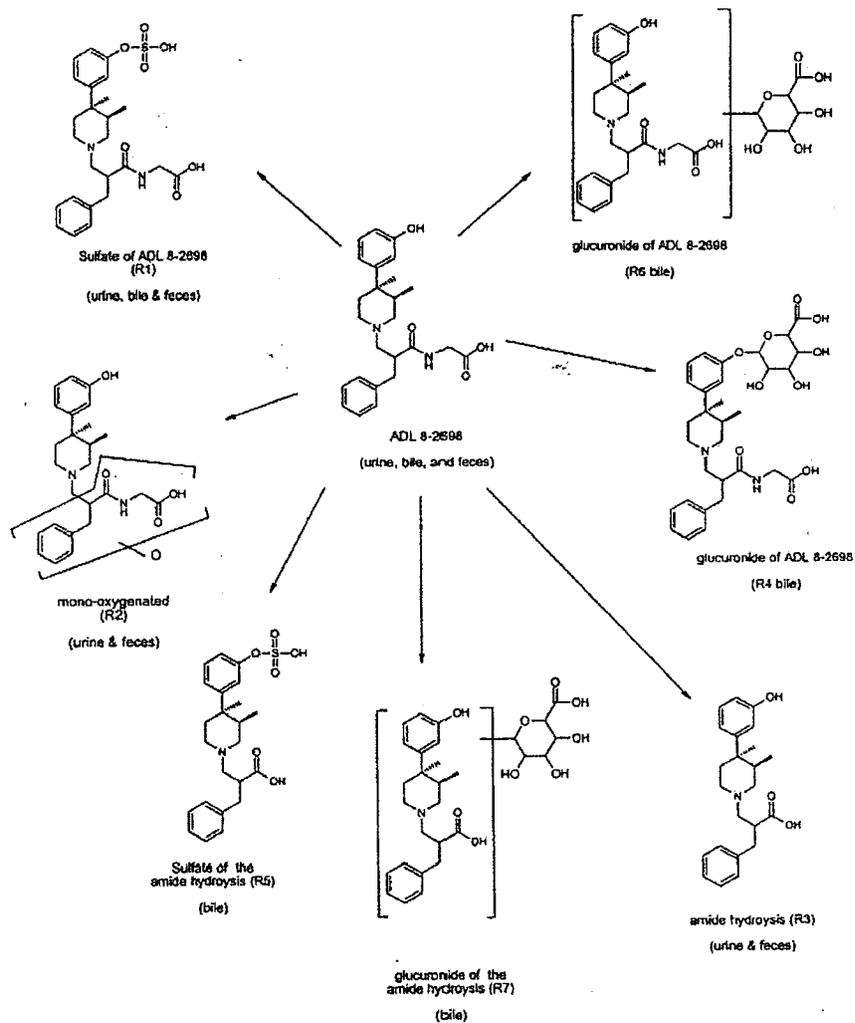
The proposed biotransformation pathways of [¹⁴C] ADL 8-2698 in rats are shown in the following diagram (from page 16 of sponsor's submission).

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IND 56, 553

6

5.4 Proposed Biotransformation Pathways of [¹⁴C] ADL 8-2698 in Rats



Biotransformation of [¹⁴C]Alvimopan in Intact and Bile Duct-Cannulated Dogs (Study No. 13TOX03)

IND 56, 553

7

Biotransformation of [¹⁴C]ADL 8-2698 in Intact and Bile Duct-Cannulated Dogs (Study 13TX03)

Method: This study was previously submitted under Amendment # 035 dated December 21, 2000 and has been reviewed before (pharmacology review dated February 27, 2001). In this submission, the sponsor presented the metabolite profiles of ADL 8-2698 in the bile, urine and feces. In this study, male beagle dogs were treated with a single 100 mg/kg oral or 2 mg/kg i.v. dose of [¹⁴C]ADL 8-2698 (71.0 µCi/mg). Urine, feces and bile samples were collected at selected intervals and were analyzed to determine the metabolite profile of [¹⁴C] ADL8-2698 using mass spectrometry (LC/MS/MS) and metabolites were identified in order of HPLC retention time. The following metabolites were characterized:

- D1: Sulfate conjugate of ADL 8-2698
- D4: Glucuronide metabolite of ADL 8-2698
- D8a & b: mono-oxygenated metabolites of ADL 8-2698
- D9: An oxidative metabolite of ADL 8-2698
- D10: An oxidative metabolite of ADL 8-2698

Results:

Dog Urine: Following oral administration of [¹⁴C] ADL8-2698 in intact dogs, the metabolite profile was not analyzed, as the total percent radioactivity excreted through urine was too low. However, about 2% of the radioactivity was found in the urine of BDC animals. After i.v. administration, approximately 22 to 25% of the radioactivity was recovered in the urine in intact and BDC animals. Unchanged ADL 8-2698 accounted for approximately 95% of the radioactivity (19.3 to 22.3% of administered dose) and a minor amount of sulfate (D1, 2 to 3%) and glucuronide (0.6 to 1%) conjugate were also present in both intact and BDC animals. Overall, the metabolite profile in the urine of intact and BDC animals are comparable.

Dog Bile: Small amount of radioactivity (approximately 5% of the administered dose) was recovered in the bile after oral administration. There were comparable amounts parent compound (1.7%), D1 (1.4%) and D4 (1.4%). After i.v. administration, approximately 65% of the radioactivity was recovered in the bile of BDC animals. Of the radioactivity recovered in the bile, there were comparable amounts of unchanged ADL 8-2698 (28.1%), the sulfate conjugate (D1, 12.7%) and the glucuronide conjugate (D4, 20.1%). In addition, there were two closely related mono-oxygenated metabolites (D8a & b) were also detected (0.9%).

Dog Feces: Following oral administration, the majority of the radioactivity was eliminated in the feces of both intact and BDC dogs (>90%), which was characterized mostly as unchanged ADL 8-2698. A small amount of oxidative (D9, 2.7%) metabolite was also detected in the feces. A very minor amount of D3 (amide hydrolysis metabolite) was detected by mass spectra; however, it was not quantifiable by radiochemical analysis. Following i.v. administration, the majority of radioactivity was eliminated through feces in BDC (2% of the recovered radioactivity) and intact animals (67% of the recovered radioactivity). The majority of the recovered radioactivity in the

IND 56, 553

8

feces of intact animals was mostly unchanged parent compound (55.6%) and the remaining were characterized as D1 (6.6%) and D10 (4.5%).

Overall, no significant difference in metabolite profile was observed between BDC and intact dogs following oral administration. The major metabolic pathway was characterized as unchanged ADL 8-2698 with minor mono-oxidative and conjugated pathways (sulfate and glucuronide). Similarly, the metabolite profile was comparable in BDC and intact animals following i.v. administration. The major metabolic pathways were unchanged ADL 8-2698 and its corresponding minor sulfate or glucuronide and mono- or di-oxidative metabolites. However, like rats, higher amount of radioactivity was recovered in the bile of dogs treated intravenously (65%) as compared to dogs treated orally (5%). The following table summarizes the quantification of [¹⁴C] ADL 8-2698 and its metabolites after oral and intravenous administration (from page 6 of study 13TX03 of sponsor's submission) in dogs.

**Appears This Way
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IND 56, 553

9

Table 2.7a Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in BDC Dogs Following Intravenous (IV) Administration (2 mg/kg)

		BDC IV	BDC IV	BDC IV
		Dog bile	Dog urine	Dog feces
Metabolite Number	Structure Assignment	Mean % of dose	Mean % of dose	Mean % of dose
D4	glucuronide	20.1	1.2	ND
D8a & b	mono-oxygenation	0.9	ND	ND
D1	sulfate of parent	12.7	2.5	ND
	Parent	28.1	20.3	1.1
D3	¹ amide hydrolysis	ND	ND	ND

Table 2.7b Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in BDC Dogs Following Oral Administration (100 mg/kg)

		BDC Oral	BDC Oral	BDC Oral
		Dog bile	Dog urine	Dog feces
Metabolite Number	Structure Assignment	Mean % of dose	Mean % of dose	Mean % of dose
D4	glucuronide	1.4	0.3	ND
D1	sulfate of parent	1.4	0.6	ND
D9	Mono-Oxygenated	ND	ND	2.7
	Parent	1.7	1.0	91.9
	¹ amide hydrolysis	ND	ND	ND

Table 2.7c Summary of Quantification of Metabolites of [¹⁴C] ADL 8-2698 in Intact Dogs Following Intravenous (IV) & Oral Administration (2 or 100 mg/kg)

		intact IV	intact IV	intact Oral
		Dog urine	Dog feces	Dog feces
Metabolite Number	Structure Assignment	Mean % of dose	Mean % of dose	Mean % of dose
D4	glucuronide	1.7	ND	ND
D1	sulfate of parent	2.0	6.6	ND
D10	+30 oxidative metabolite	ND	4.5	ND
	Parent	17.2	55.6	91.2
	¹ amide hydrolysis	ND	ND	ND

ND = not detected

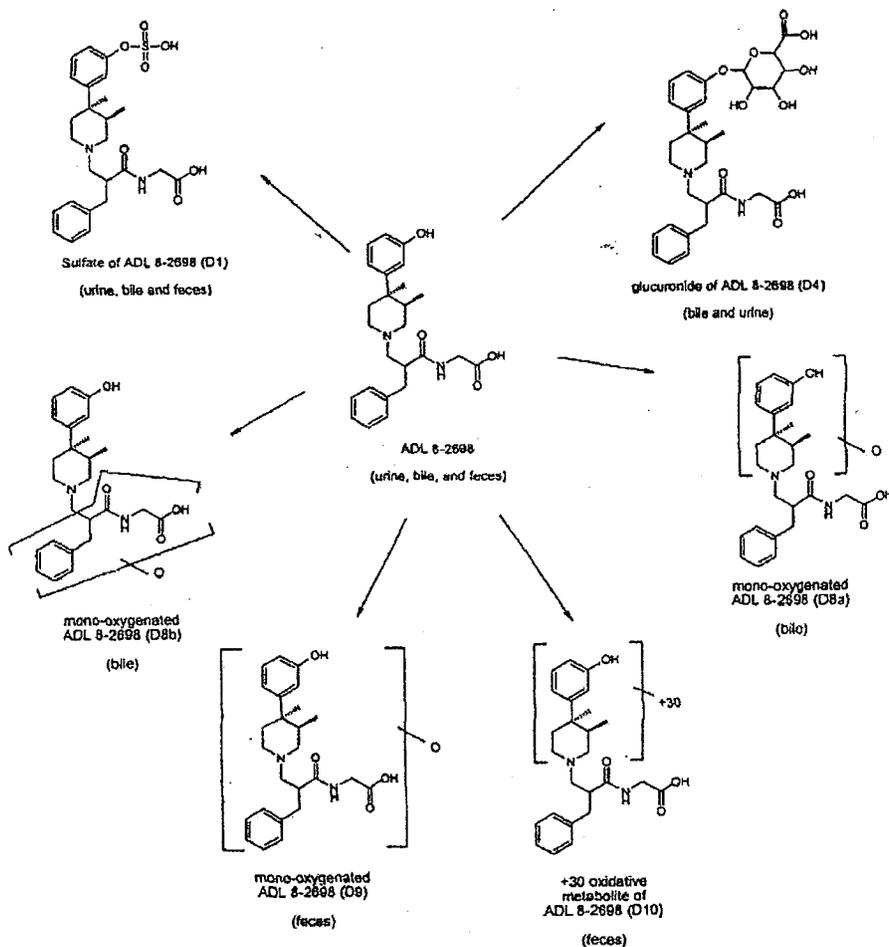
See Appendix 1 for the number of subjects used to determine the mean percent of dose value for each metabolite.

IND 56, 553

10

The proposed biotransformation pathways of ADL 8-2698 in dogs are shown in the following diagram (from page 15 of study I3TX03 of sponsor's submission).

5.4 Proposed Biotransformation Pathways of [¹⁴C] ADL 8-2698 in Dogs



2.6.4.6 Excretion

Elimination of Radioactivity in Bile, Urine and Feces Following Intravenous or Oral Administration of [¹⁴C] Alvimopan to male rats (Study No. 7010-102)

1. Elimination of Radioactivity in Bile, Urine and Feces Following Intravenous or Oral Administration of ¹⁴C-ADL 8-2698 to Male Rats (Study No. 7010-102)

Methods: This study was conducted to assess the extent of absorption, distribution, and elimination of ¹⁴C-ADL 8-2698 administered to rats by intravenous or oral route. Forty male rats (intact and bile duct-cannulated) were assigned to 7 groups and were treated with a single 200 mg/kg oral or 20 mg/kg i.v. dose of ¹⁴C-ADL 8-2698 (71.0 µCi/mg or 32.7 mCi/mmol). The oral dose was formulated as a suspension in 10% gum arabic at a concentration of 40 mg/ml. Radiolabeled (12.70 mg) ¹⁴C-ADL 8-2698 and non-radiolabeled ADL 8-2698 (1787.6 mg) were mixed with 45 ml of vehicle to prepare the suspension. The intravenous dose was prepared as a solution in sterile water (pH = 10.5 adjusted with 1 N NaOH) at a concentration of 2 mg/ml. Radiolabeled (8.5 mg) and non-radiolabeled (111.7 mg) ADL 8-2698 were mixed with 60 ml of vehicle to prepare the solution. The study design is shown below:

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IND 56, 553

2

Group*	Number of Males	Route	Dose (mg/kg)	Dose Volume (ml/kg)	Radioactive dose ($\mu\text{Ci}/\text{animal}$)	Collections
1	4	Oral	200	5	20	Urine and feces
2	4	IV	20	10	20	Urine and feces
3	4 BDC	Oral	200	5	20	Bile, urine, and feces
4	4 BDC	IV	20	10	20	Bile, urine, and feces
5	8	Oral	200	5	20	Carcass
6	8	IV	20	10	20	Carcass
7	8	Oral	200	5	20	Carcass

BDC: Bile duct-cannulated.

IV: Intravenous.

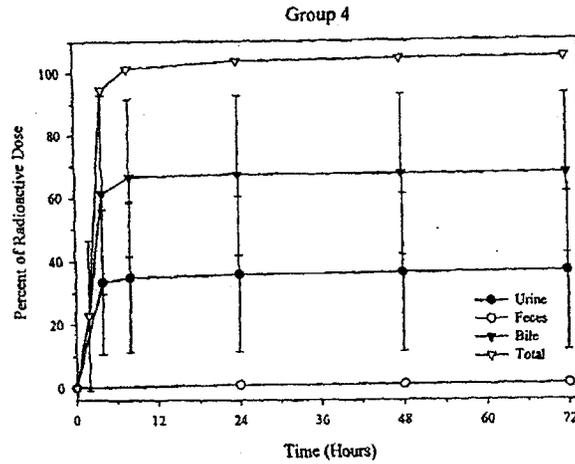
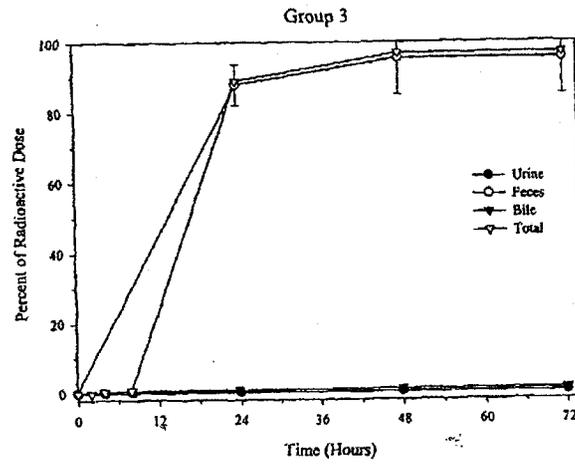
*: Animals in Groups 1 through 6 were Fischer 344 rats and animals in Group 7 were Long-Evans rats.

Bile, urine, and feces samples were collected at selected intervals (urine: 0-4, 4-8, 8-24, 24-48, and 48-72 hours postdose; feces: 0-24, 24-48, and 48-72 hours postdose; bile: 0-2, 2-4, 4-8, 8-24, 24-48, and 48-72 hours postdose) through 72 hours post-treatment from animals in Groups 1 through 4. Two animals per time point in groups 5 and 7 were sacrificed at 1, 4, 8, and 24 hours postdose and prepared for whole-body autoradiography (WBA). Two animals per time point in Group 6 were sacrificed at 5 and 30 minutes, and 2 and 8 hours postdose and prepared for whole-body autoradiography (WBA). Blood samples were collected from animals in Group 5 and 6 prior to sacrifice and centrifuged to obtain plasma. All samples were analyzed for radioactivity using liquid scintillation counter (LSC).

Results: Following i.v. administration, maximum concentration of radioactivity in plasma was obtained at 5 min after treatment, with an average value of 37.4 $\mu\text{g}/\text{g}$. Concentration declined over time steadily to 0.0265 $\mu\text{g}/\text{g}$ by 8 hours postdose. Following oral dose, maximum plasma concentrations (0.145 $\mu\text{g}/\text{g}$) in plasma were obtained at 1 hour postdose and declined rapidly to below the limit of quantitation at 8 hours postdose, which suggested minimal absorption of ^{14}C -ADL 8-2698 following oral administration. The main route of excretion of ^{14}C -ADL 8-2698 following i.v. dose was via feces in intact rats (75.4%). Most of the radioactivity was recovered (67.3%) in the bile in bile duct-cannulated rats following i.v. dose. The main route of excretion of ^{14}C -ADL 8-2698 following oral dose to both intact and bile duct-cannulated rats was via feces (92.3 and 95.4%, respectively). Tissue distribution of radioactivity after oral dose indicated little to no absorption. Nearly the entire radioactivity was confined in the gastrointestinal tract and contents at all time points. Low level of radioactivity was detected in the skin following oral administration. Tissue distribution of radioactivity after intravenous dose was rapid and widespread and maximum concentration was reached at 5 min after treatment in all tissue except intestinal tissues and contents. The highest level of radioactivity was found in kidney, liver, pancreas, and blood, with average values of 265, 139, 712, and 27.3 $\mu\text{g}/\text{g}$, respectively.

IND 56, 553

3



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The graph (above) shows the mean (\pm SD) cumulative percent of radioactive dose in urine, feces, and bile at specified intervals after administration of a single oral (Group 3, 200 mg/kg) or intravenous (Group 4, 20 mg/kg) dose of 14 C-ADL 8-2698 to male bile duct-cannulated rats.

Elimination of Radioactivity in Bile, Urine, and Feces Following Intravenous or Oral Administration of [¹⁴C]Alvimopan to Bile Duct-Cannulated Male Rats (Study No. 7010-119)

Methods: The objective of this study was to examine the absorption and elimination of [¹⁴C]alvimopan (specific activity = 56.98 mCi/mmol or 123.87 μCi/mg) following intravenous or oral administration to bile duct-cannulated male rats. In this study, eight bile duct-cannulated male Fischer 344 rats (n = 4/group) were used. Animals were treated with a single oral dose of [¹⁴C]alvimopan at 20 (Group 1) or intravenous dose of 2 (Group 2) mg/kg. Urine was collected at predose and at 0-4, 4-8, 8-24, 24-48, and 48-72 hours postdose. Feces were collected at predose and at 0-24, 24-48, and 48-72 hours postdose. Bile was collected at predose and at 0-2, 2-4, 4-8, 8-24, 24-48, and 48-72 hours postdose. All samples were analyzed for radioactivity using a liquid scintillation counter (LSC).

Results: Most of the radioactivity was recovered in the feces following an oral dose of [¹⁴C]alvimopan. A mean of 89.1% of the administered dose was recovered in the feces through 72 hours postdose. Urinary and biliary excretion accounted for only 1.66 and 5.13% of the dosed radioactivity, respectively, indicating an oral absorption of approximately 6.8%.

Following the intravenous dose of [¹⁴C]alvimopan, majority of the radioactivity was recovered in the bile. A mean of 73.5% of the administered dose was recovered in the bile through 72 hours postdose. Urinary and fecal excretion accounted for 24.0 and 0.81% of the administered radioactivity, respectively.

Elimination of Radioactivity in Bile, Urine and Feces Following Intravenous or Oral Administration of [¹⁴C]alvimopan to Male Dogs (Study No. 7010-103)

2. Elimination of Radioactivity in Bile, Urine and Feces Following Intravenous or Oral Administration of [¹⁴C]-ADL 8-2698 to Male Dogs (Study No. 7010-103)

Methods: The objective of this study was to examine the extent of absorption, distribution, and elimination of [¹⁴C]-ADL 8-2698 administered to dogs by intravenous or oral route. Male beagle

IND 56, 553

4

dogs (intact and bile duct-cannulated) were assigned to 2 groups in two phases for this study and were treated with a single 100 mg/kg oral or 2 mg/kg i.v. dose of ^{14}C -ADL 8-2698 (71.0 $\mu\text{Ci}/\text{mg}$ or 32.7 mCi/mmol). For the oral doses, the radiolabeled and nonradiolabeled test materials were formulated in a capsule with PEG 3350. The intravenous dose was prepared as a solution in sterile water (pH = 10.5 adjusted with 1 N NaOH) at a concentration of 2 mg/ml. In Phase 1, 3 dogs received a single oral dose of ^{14}C -ADL 8-2698 as a capsule (Group 1) and 3 dogs received a single i.v. dose of ^{14}C -ADL 8-2698 via a cephalic vein (Group 2). The animals were cannulated for the collection of bile in Phase 2. In Phase 2, the bile duct-cannulated dogs were treated with the same dosing regimen as in Phase 1. The group designations, number of animals, doses and dose volumes etc. are shown in the table below:

Group*	Number of Males	Route	Dose (mg/kg)	Dose Volume (ml/kg)	Radioactive dose ($\mu\text{Ci}/\text{animal}$)	Group Description
Phase 1 (Intact)						
1	3	Oral	100	1 capsule	30	Excretion/Mass Balance/Plasma
2	3	IV	10	1 ml/kg	30	Excretion/Mass Balance/Plasma
Phase 2 (Bile Duct-Cannulated)						
1	3	Oral	100	1 capsule	30	Excretion/Mass Balance/Bile
2	3	IV	10	1 ml/kg	30	Excretion/Mass Balance/Bile

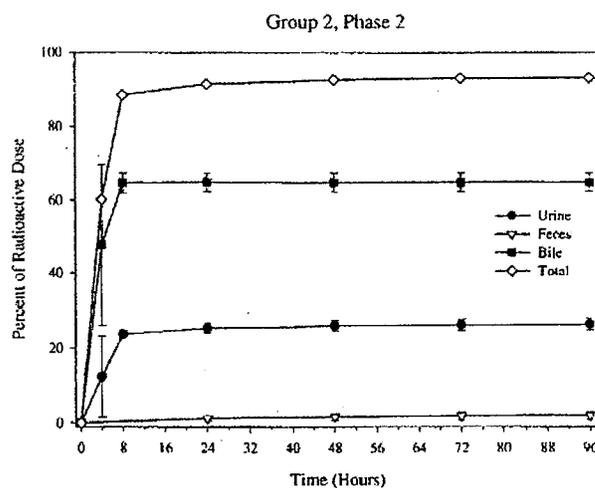
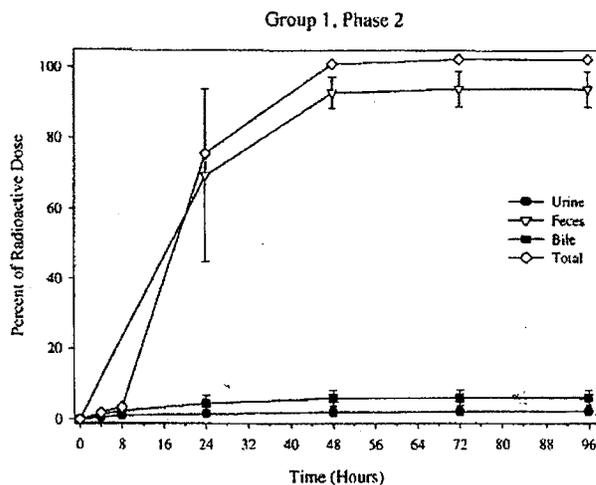
IV: Intravenous.

Blood samples were collected (Phase 1) after oral dose via a jugular vein at predose and at 15 and 30 minutes and at 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours postdose. Following i.v. dose, blood samples were collected predose and at 5, 15, and 30 minutes, and at 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours postdose. Bile (Phase 2), urine (Phase 1 and 2), and feces (Phase 1 and 2) samples were collected at selected intervals (urine: 0-4, 4-8, 8-24, 24-48, 48-72, and 72-96 hours postdose; feces: 0-24, 24-48, 48-72, and 72-96 hours postdose; bile: 0-4, 4-8, 8-24, 24-48, 48-72, and 72-96 hours postdose) through 96 hours post-treatment. All samples were analyzed for radioactivity using liquid scintillation counter (LSC).

Results: Following i.v. administration, maximum concentration of radioactivity was obtained at 5 min after treatment, with an average value of 4.96 $\mu\text{g}/\text{g}$. Concentration declined over time steadily to values below the limit of quantitation by 12 hours postdose. Following oral dose, all plasma concentrations were below the limit of quantitation, which suggested minimal absorption of ^{14}C -ADL 8-2698 following oral administration. The main route of excretion of ^{14}C -ADL 8-2698 following i.v. dose was via feces in intact dogs (67.5%), however, in bile duct-cannulated dogs, the majority of the radioactivity was found in the bile (64.9%). The main route of excretion of ^{14}C -ADL 8-2698 following oral dose was via feces in intact and bile duct-cannulated dogs (91.8 and 94%, respectively).

IND 56, 553

5



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The figure (above) shows the mean cumulative percent of radioactive dose in urine, feces, and bile at specified intervals after administration of a single oral (Group 1, 100 mg/kg) or intravenous (Group 2, 2 mg/kg) dose of ¹⁴C-ADL 8-2698 to male dogs in Phase 2.

2.6.4.7 Pharmacokinetic Drug Interactions

The sponsor did not include any pharmacokinetic drug interactions study reports in this submission.

2.6.4.8 Other Pharmacokinetic Studies

The sponsor did not include any other pharmacokinetic study reports in this submission.

2.6.4.9 Discussion and Conclusions

Systemic absorption of alvimopan after oral doses was low in animals, with less than 10% of the administered radiolabeled dose reaching systemic circulation. In humans given a 12 mg solution, or two 6 mg capsules orally, the mean absolute bioavailability for alvimopan was 14 and 6%, respectively. In humans, mean plasma AUC_{0-12h} and C_{max} for alvimopan after 12 mg oral b.i.d. dose for 4.5 days were approximately 40.2 ng.hr/ml and 10.98 ng/ml, respectively (data obtained from the study 14CL1-19). In repeated oral toxicity studies, the animal to human exposure ratios were approximately 4.0 ($AUC_{0-24h} = 150$ ng.hr/ml at 4000 mg/kg/day, p.o. in mice), 2.2 ($AUC_{0-24h} = 81$ ng.hr/ml at 1000 mg/kg/day, p.o. in rats) and 56.1 ($AUC_{0-24h} = 2100$ ng.hr/ml at 1000 mg/kg/day, p.o. in dogs) in mice, rats and dogs, respectively. Systemic plasma clearance of alvimopan in rats and dogs was rapid, ranging from 2.8 to 5.4 L/hr/kg in rats and 1.4 to 2.2 L/hr/kg in dogs given single intravenous doses. Systemic plasma clearance in humans was 0.34 L/hr/kg, was slower than for other species. The calculated volume of distribution at steady-state (V_{ss}) in rats ranged from 0.8 to 1.9 L/kg. The volume of distribution in dogs was low, 0.21 to 0.36 L/kg. The mean volume of distribution of alvimopan in humans was 0.43 L/kg. Plasma protein binding of alvimopan was modest or low and independent of concentration in all species. Free fractions ranged from 20-30% for humans, 56% for rats, 67% for mice, and 72% for dogs. Following single oral radioactive dose of alvimopan in rats, distribution of total radioactivity was generally limited to the gastrointestinal tract with little distribution to peripheral tissues. Alvimopan was also detected in milk on lactation Day 14 after daily intravenous bolus doses in rats. Alvimopan was primarily metabolized to ADL 08-0011 in humans, and to glucuronide and sulfate conjugates in rats and dogs. Alvimopan did not inhibit any of the 5 major human CYP isozymes in human liver microsomes or hepatocytes at concentrations up to 50 μ M. Alvimopan did not induce CYP isozymes in rats or dogs after 14 days of repeated dosing. Neither CYP 1A2 nor CYP 3A4 was induced in human hepatocytes incubated with alvimopan for 2 or 3 days. The estimated half-life in rats and dogs was approximately 2 and 0.2 hrs, respectively, reflecting the rapid clearance and tissue distribution. Alvimopan was demonstrated to be a substrate for the p-glycoprotein (Pgp) transporter. The plasma half-life in humans given an intravenous dose of 12 mg was 5.3 hr. Excretion of alvimopan occurred primarily via the bile and feces. In rats, approximately 70 and 30% of the administered dose was excreted via the bile and urine after an intravenous dose. In dogs, approximately 65 and 26% of the administered dose was excreted via the bile and urine after an intravenous dose. Most of the fecal radioactivity excreted after oral doses (>89% for both species) was attributed to unabsorbed drug.

The pharmacologically active metabolite, ADL 08-0011, was formed by all species following oral or intravenous administrations of alvimopan, including humans.

Generally, plasma concentrations of ADL 08-0011 after oral doses of alvimopan were greater than those of the parent compound in mice, rats, and human, but were less than those of the parent drug in dogs. Mean $AUC_{0-\infty}$ and C_{max} for ADL 08-0011 in humans following oral dose of 12 mg b.i.d. alvimopan for 4.5 days were 1642.5 ng.hr/ml and 35.73 ng/ml, respectively (data obtained from study 14CL119). Plasma protein binding of ADL 08-0011 was species dependent with free fractions ranging from 4.5% for humans to 62% for mice. In rats and mice, plasma protein binding of parent and metabolite was similar; while in humans, rabbits and Beagle dogs the binding of the metabolite was more extensive than binding of the parent drug. ADL 08-0011 was also detected in rat milk on lactation Day 14 after daily intravenous bolus doses. ADL 08-0011 was not formed by human hepatocytes incubated with alvimopan for up to 72 hours. ADL 08-0011 did not inhibit any of the 5 major human CYP isozymes in human liver microsomes or hepatocytes at concentrations up to 50 μ M. Repeated i. v. dosing of ADL 08-0011 for 14 days did not induce CYP isozymes in rats. ADL 08-0011 was also found to be a substrate for the p-glycoprotein (Pgp) transporter.

2.6.4.10 Tables and Figures to Include Comparative Toxicokinetic Summary

The following tables (from page 131 and 132 of the sponsor's report: pharmsum.pdf) show comparative pharmacokinetic data and systemic exposure to alvimopan and ADL 08-0011 following administration to mice, rats and dogs in comparison to human exposure.

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ENTEREG™ (alvimopan)

NDA 21-775

TX Table 3: Summary of Mean Systemic Exposure to Alvimopan After Repeated Administration of Alvimopan and Comparison to Mean Human Exposure.

Species	Study Duration	Dose (mg/kg/day) NOAEL	AUC Comparison ^f		C _{max} Comparison ^f		Reference Number
			AUC ₍₀₋₂₄₎ (ng•hr/mL)	Ratio ^a	C _{max} (ng/mL)	Ratio ^b	
Oral Administration							
Mouse	5 days ^c	4000	150 ^d	4.0	10	2.3	808-020
Rat	1 month	1000	54(M)	1.4	7.4(M)	1.7	808-012
			81(F)	2.2	8.0(F)	1.9	
Dog	5 days ^e						808-010
	capsule	100	394	10.5	53.8	12.5	
	suspension	1000	1371	36.7	106	24.7	
	1 month	1000 ^g	1500(M)	40.1	108(M)	25.1	808-011
		2100(F)	56.1	155(F)	36.0		
Intravenous Administration							
Rat	2 weeks	10	2621(M)	70.1	8387(M)	1950	808-021
			1915(F)	51.2	6246(F)	1453	
Dog	4 weeks	2	1128(M) ^h	30.2	3759(M)	874.2	TOX RPT 8 14TK011
			1295(F) ^h	34.6	4376(F)	1017.7	

a: Mean AUC_(0-24 hr) in human at oral dose of 6 mg b.i.d. was 37.4 ng•hr/mL, (twice the AUC₍₀₋₁₂₎)

b: Mean C_{max} in human at oral dose of 6 mg b.i.d. was 4.3 ng/mL.

c: Mean AUC and C_{max} values for males and females combined.

d: AUC from 0 to 48 hours

e: Alvimopan was administered twice a day (500 mg/kg/dose) at a total daily dose of 1000 mg/kg/day.

f: Comparisons to human data are based on end of study data from the applicable animal study

g: Kinetic results are from 14TK011; AUC = AUC₀₋₂₄

M: Male; F: Female

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TX Table 4: Summary of Mean Systemic Exposure to ADL 08-0011 After Repeated Administration of Alvimopan or ADL 08-0011 and Comparison to Mean Human Exposure.

Species	Study Duration	Dose (mg/kg/day) NOAEL	AUC Comparison ^f		C _{max} Comparison ^f		Reference Number
			AUC ₀₋₂₄ (ng•hr/mL)	Ratio ^a	C _{max} (ng/mL)	Ratio ^b	
Oral Administration of Alvimopan							
Mouse	5 days ^c	4000	1723 ^d	4.1	283	12.0	808-020
Rat	1 month	1000	358(M)	0.8	32.6(M)	1.4	808-012
			904(F)	2.1	58.3(F)	2.5	
Dog	5 days ^c						808-010
	capsule	100	191	0.5	12.8	0.5	
	suspension	1000	165	0.4	9.3	0.4	
	1 month	1000 ^e	58.3(M)	0.1	3.6(M)	0.2	808-011
		100(F)	0.2	5.3(F)	0.2		
Intravenous Administration of Alvimopan							
Rat	2 weeks	10	138(M)	0.3	19.4(M)	0.8	808-021
			367(F)	0.9	21.5(F)	0.9	
Intravenous Administration of ADL 08-0011							
Rat	2 weeks	8	3040(M)	7.2	1880(M)	79.6	808-018
			4140(F)	9.8	2850(F)	120.7	
Dog	2 weeks	2	3340(M)	7.9	1990(M)	84.3	808-019
			2930(F)	6.9	1890(F)	80.1	

a: Mean AUC_(0-24 hr) in human at oral dose of 6 mg b.i.d. was 422.5 ng•hr/mL.

b: Mean C_{max} in human at oral dose of 6 mg b.i.d. was 23.6 ng/mL.

c: Mean AUC and C_{max} values for males and females combined

d: AUC from 0 to 72 hours

e: Alvimopan was administered twice a day (500 mg/kg/dose) at a total daily dose of 1000 mg/kg/day.

f: Comparisons to human data are based on end of study data from the applicable animal study

M: Male; F: Female

2.6.6 TOXICOLOGY

2.6.6.1 Overall Toxicology Summary

Alvimopan and its active metabolite ADL 08-0011 were tested in mice, rats and dogs following intravenous and oral administration. Acute intravenous and oral toxicity studies were conducted in rats and mice. Alvimopan was non-lethal to rats at an i.v. dose of 20 mg/kg and an oral dose of 500 mg/kg. In mice, alvimopan was nonlethal at 500 mg/kg when administered orally. It is to be mentioned here that these are the only doses tested in acute toxicity studies in mice and rats. After a single oral administration of alvimopan to mice and rats, no mortality or toxic effects were observed at dosage levels up to 4000 mg/kg.

Alvimopan exhibited no significant target organs of toxicity when administered at sufficiently high oral doses up to 13 weeks in mice and up to 6 months in rats and dogs. Alvimopan was also tested intravenously at sufficiently high doses up to 2 weeks in rats and up to 1 month in dogs and produced no significant toxicity. After repeated oral administration of alvimopan to rats for 1 (200, 500 and 1000 mg/kg/day) or 6 months (50, 100 and 200 mg/kg/day), the no-observed-adverse-effect-levels (NOAELs) were 1000 and 200 mg/kg/day, respectively, the highest tested doses. After repeated oral doses of alvimopan to dogs for 1 (100, 250, 500 and 1000 mg/kg/day) or 6 months (10, 30 and 100 mg/kg/day), the NOAELs were 1000 and 100 mg/kg/day, respectively, the highest tested doses. In repeat dose i.v. toxicity studies in rats (1, 5 and 10 mg/kg/day) and dogs (0.05, 0.2 and 2.0 mg/kg/day), the NOAELs were 10 and 2 mg/kg/day, respectively.

ADL 08-0011, the pharmacologically active metabolite of alvimopan, was also tested intravenously up to 2 weeks in rats and dogs. ADL 08-0011 did not cause any significant toxicity in rats or dogs when administered up to 8 or 2 mg/kg/day, respectively.

The proposed human oral dose for alvimopan is 12 mg b.i.d. or 24 mg/day or 0.48 mg/kg/day (based on 50 kg body weight), which is equivalent to 17.8 mg/m². The highest tested doses in rats (200 mg/kg/day) and dogs (100 mg/kg/day) in 6-month oral toxicity studies were approximately 67.4 and 112.3 times the proposed human dose (17.8 mg/m²), respectively, based on body surface area. Alvimopan and its active metabolite ADL 08-0011 did not show any potential for genotoxicity. In fertility and reproductive performance study in rats, alvimopan did not cause any adverse effect. It was not teratogenic in rats or rabbits.

In conclusion, the nonclinical studies conducted on alvimopan provide adequate assurance of safety for its proposed oral use as indicated in the draft labeling.

2.6.6.2 Single-Dose Toxicity

The Acute Toxicity of Alvimopan Administered Orally to CD- 1 Mouse (TOX RPT 7/M07293)

The Acute Toxicity of Alvimopan Administered Orally to Fischer 344 Rats (TOX RPT 6/R19693)

The Acute Toxicity of Alvimopan Administered Intravenously to Fischer 344 Rats (TOX RPT 5/R18893)

TOXICOLOGY:

ACUTE TOXICITY:

An acute i.v. toxicity study in rats and acute oral toxicity studies in mice and rats did not provide any useful information. In each study, only one dose of LY246736 dihydrate was administered (an i.v. dose of 20 mg/kg in rats and an oral dose of 500 mg/kg in mice and rats) and none of the doses produced any lethality or clinical signs of toxicity. The sponsor should be advised to conduct multiple dose acute toxicity studies that include doses that produce lethality and clinical signs of toxicity.

A Blood Level Toxicity Study with Alvimopan Administered Orally as a Single Dose to Beagle Dogs (Study Number D09392)

Methods: This is a non-GLP study to support the dose selection of alvimopan for the subchronic study. In this study, six adult Beagle dogs (1/sex/group) were administered single oral (capsule) dose of alvimopan at 50, 100 or 200 mg/kg. All animals were observed daily for 14 days for mortality and clinical signs. Body weights were recorded during the pretest period. Food consumption was estimated daily. Blood samples were collected on Day 1 at 0.5, 1, 2 and 4 hours postdosing for determination of plasma concentration of alvimopan.

Results: The sponsor did not provide any data in this report. Only the summary was provided. There was no treatment-related mortality. The only clinical sign noted during the study was an episode of runny stools in one male given 100 mg/kg, 6 hours postdosing. Alvimopan was non-lethal up to 200 mg/kg, p.o.

2.6.6.3 Repeat-Dose Toxicity

Mice

Toxicokinetic Study of Alvimopan in CD-1 Mice Following Single and Repeat Oral Gavage Dosing for 5 Days (Study No. 808-020)

2. Single and Repeat Dose Toxicokinetic Evaluation of Orally Administered (Gavage) Alvimopan (ADL-8-2698) in CD-1 Mice: (Study #13TX007/ #808-020)

The purpose of the study was to determine toxicokinetics of the compound and its metabolite ADL 08-001 following single dose and repeat dosing for 5 days.

Conducting laboratory and location: _____

Date of study initiation & completion (Draft Report): April 22, 2002 and November 7, 2002

Drug lot # and % purity: Alvimopan (ADL 8-2698), R009340 JOR1208.N.00.01 and 93 -100% (As per sponsor, the homogeneity and stability was supported by study # 808-002 & 007)

Formulation/vehicle: 10% Suspension in gum acacia

Methods:

Dosing:

Species/strain: CD-1 mice

#/sex/group or time point (main study): Eight groups of 18 animals/sex

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IND 56,553
Page 11

Age: approximately 6 weeks

Mean Body Weight: 19.6 to 33.5 g (males) and 19.0 to 29.0g (females)

Doses in administered units: 500, 1000, 2000 and 4000 mg/kg/day

Route, form and volume: oral gavage, 10 ml/kg

The dose schedule and experimental design of the proposed study is shown in the following table: (vol 1.2, page 10):

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1 ^a	500	18	18
2 ^a	1000	18	18
3 ^a	2000	18	18
4 ^a	4000	18	18
5 ^b	500	18	18
6 ^b	1000	18	18
7 ^b	2000	18	18
8 ^b	4000	18	18

^aTest article was administered as a single dose on Day 1.
^bTest article was administered for five consecutive days.

Observations and times:

Clinical signs: The clinical examination for general observations of animals like salivation, motor activity, convulsions etc., mortality, injury and, available of food and water were made twice daily during the study.

Body weights: For main study groups animals - once prior to initial drug administration and daily during the study. These were not reported and maintained in testing laboratory records.

Plasma concentrations: The plasma concentrations of the compound and its metabolite ADL 08-0011 were determined on pooled 0.5 ml blood sample collected from 3 animals/sex/group per time period by cardiac puncture on study day 1 and day 5 at 0 (prior to treatment), 1, 4, 24, 48 and 72 hr post dose. The concentrations of compound was determined by LC/MS/MS method (limit of detection = 0.9 ng/ml). The animals were terminated without further evaluation. The samples were stored at -70°C for their

IND 56,553

Page 12

use at analytical laboratory for plasma concentration and TK parameter determinations (C_{max} and AUC_{0-∞ hr} values).

Results:

Toxicokinetics:

a. Alvimopan: On day 1, treatment related but non-dose proportional AUC_{0-∞} values of 176, 228, 138 and 4514 ng.hr/ml of the compound were seen in males after the administration of 500, 1000, 2000 and 4000 mg/kg alvimopan. On study day 5, a measurable concentration of the compound was seen at 0 hr (before the dosing) suggesting that the compound was not completely eliminated. On day 5, the AUC_{0-∞} values among males were 179, 902, 362 and 169 ng.hr/ml respectively, indicating that the compound had variable absorption. On day 1, non-dose proportional AUC_(0-∞ hr) values of 225, 1155 and 1696 ng.hr/ml of the compound were seen in females after the administration of 1000, 2000 and 4000 mg/kg alvimopan. The AUC value of the compound on day 1 at the dose of 500 mg/kg/day was below the limit of detection and was reported as NC (not calculated). On day 5, the AUC_{0-∞} values were 5436, 884, 264 and 130 ng.hr/ml respectively, in animals included in 500, 1000, 2000 and 4000 mg/kg alvimopan. There was poor and erratic absorption of the compound. The estimated half-lives of the compound on day 1 in males was 13.3, 7.9, 7.5 and 9.9 hr and, 10.0, 5.9, 8.4 and 13.2 hr in females. The half lives on day 5 were only slightly different than on day 1, i.e., 10.0, 5.9, 8.4 and 13.2 hr in males and, 6.0, 5.6, 6.7 and 7.0 hr in females included in 500, 1000, 2000 and 4000 mg/kg/day treatment groups (Data extracted from sponsor table in vol I.2, pp 417).

SUMMARY OF PLASMA ALVIMOPAN AUC_(0-∞ hr) VALUES AND T_{max} VALUES FOLLOWING ORAL ADMINISTRATION OF ALVIMOPAN (STUDY DAY 1 & 5) IN CD-1 MICE

DOSE	<u>MALES</u>		<u>FEMALES</u>	
	AUC _(0-∞ hr)	T _{max} (hr)	AUC _(0-∞ hr)	T _{max} (hr)
(DAY 1)				
500 mg/kg	176	1	NC	0
1000 mg/kg	208	1	225	4
2000 mg/kg	138	4	1155	24
4000 mg/kg	4514	24	1696	4
(DAY 5)				
500 mg/kg	179	0	5436	4
1000 mg/kg	902	1	884	1
2000 mg/kg	362	4	264	0
4000 mg/kg	169	1	130	4

b. ADL 08-0011: On day 1, linear but non-dose proportional AUC_(0-24 hr) values of 194, 468, 1013 and 1853 ng.hr/ml of metabolite in males and, 201, 728, 842 and 1185 ng.hr/ml in females, respectively were seen in 500, 1000, 2000 and 4000 mg/kg/day alvimopan treatment groups. AUC values of the metabolite on study day 5 in study animals are variable as shown in the following table. A small concentration of the metabolite (as seen with the parent compound) was seen at 0 hr (24 hr after the dosing) before the next dosing. The plasma peak concentration of the metabolite was usually

IND 56,553

Page 13

seen within 1 hr of the administration of the compound excepting in females included in 4000 mg/kg/day treatment group. The half-life of the metabolite ADL 08-0011 after 500, 1000, 2000 and 4000 mg/kg oral dose of the compound was 4.5, 6.7, 6.0 and 6.5 hr, respectively. The half-life of the metabolite on day 5 of the study were prolonged to 11.4, 12.4, 8.1 and 13.5 hr at 500, 1000, 2000 and 4000 mg/kg oral doses of the compound. The concentration of the metabolite in animals of 2000 and 4000 mg/kg/day treatment groups were lower than the concentrations seen in animals of low dose treatment group indicating limited absorption of the compound. The t_{max} values of the compound on day 5 and of metabolite ADL -08-0011 on day 1 and 5 are shown in the following table (data taken from sponsor table at vol 1.2, pp 417).

SUMMARY OF PLASMA ADL 08-0011 AND AUC VALUES FOLLOWING ORAL ADMINISTRATION OF ALVIMOPAN

DOSE	MALES		FEMALES	
	AUC _(0-∞ HR) (ng.hr/ml)	T(max) (hr)	AUC _(0-∞ HR) (ng.hr/ml)	T(max) (hr)
(DAY 1)				
500 mg/kg	194	1	201	1
1000 mg/kg	468	1	728	1
2000 mg/kg	1013	1	842	1
4000 mg/kg	1853	1	1185	1
(DAY 5)				
500 mg/kg	636	1	763	4
1000 mg/kg	460	1	509	1
2000 mg/kg	756	1	955	1
4000 mg/kg	1821	1	1550	1

Orally administered compound converted to metabolite ADL 08-0011 and it attained linear but non-dose proportional plasma concentration within 1 hr of administration of the compound. On day 5, the metabolite peak concentrations at these doses were seen after 1 to 4 hr of the administration of the compound and these were similar or only slightly increased than on day 1. Thus the continuous exposure of the compound after multiple doses provide reduced plasma levels of the compound and slight increased amount of the metabolite.

In summary, orally administered alvimopan from 500 to 4000 mg/kg/day for 5 days produced variable and non-dose proportional and erratic plasma concentrations. The plasma concentrations of the compound in animals included in 2000 and 4000 mg/kg/day treatment groups at this time were lower than the first day concentration. The orally administered alvimopan was absorbed in small amounts and the compound and metabolite ADL 08-0011 were seen after 1st hr of its administration. The half-life of metabolite was prolonged than alvimopan. There was no apparent sex related difference in the exposure levels of the compound in the mouse.

13-Week Oral Toxicity Study of Alvimopan in CD-1 Mice (Study No. 808-002)

IND 56,553
Page 5

TOXICOLOGY:

1. 13-Week Oral Toxicology study in Mice: (Study # ADL 8-2698; 808-002)

Conducting laboratory and location: _____

Date of study initiation: December 7, 2000

GLP compliance: Unsigned statement of compliance to GEP regulations was attached.

Drug lot # and % purity: Alvimopan (ADL 8-2698), R009340, MFG. Lot #A220201 and 93 -100%

Formulation/vehicle: 10% Suspension in gum acacia/water

Methods:

Dosing: Oral

Species/strain: CD-1 mice

#/sex/group or time point (main study): 10/sex/group

Satellite group animals used for toxicokinetics: 4 groups of 24 mice/sex

Age: approximately 4 weeks

Mean Body Weight: 20.86 to 21.88 g (females) and 28.14 to 28.83 g (males)

Doses in administered units: 0, 100, 300, 600 and 1000 mg/kg/day

Route, form, volume, and infusion rate: oral gavage, suspension, 10 ml/kg

The number of animals used in the main and satellite groups are shown in the following table (taken from sponsor's submission, vol I.1, pp 12):

IND 56,553

Page 6

Group Assignments			
Group Number	Dose Level (mg/kg/dose)	Number of Animals	
		Male	Female
Main Study			
1	0	10	10
2	100	10	10
3	300	10	10
4	600	10	10
5	1000	10	10
Toxicokinetic			
6	100	24	24
7	300	24	24
8	600	24	24
9	1000	24	24

Observations and times:

Clinical signs: The clinical examination was conducted once/week

Mortality: 2 times/day

Body weights: For main study groups animals - once prior to initial drug administration, once/week during the study. For TK groups animals: the body weights were taken but not recorded

Food consumption: recorded weekly in males and females animals

Ophthalmoscopy: was not done during the study

EKG: tracing was not obtained.

Hematology: On 0.7 ml/mouse blood samples (cardiac puncture) collected from all main study group overnight fasted animals at termination of the study. Bone marrow slides from males of 0 and 1000 mg/kg/day treatment groups were prepared.

Clinical chemistry: tests were performed on the blood samples collected for hematology parameters estimation.

Urinalysis: was not done

Plasma concentrations: 20 ml blood sample collected from 40/sex animals by cardiac puncture during the first week of acclimation period. Blood samples were collected at 1,

IND 56,553

Page 7

2, 6 and 24 hr post dose on day 1 and 90 from 3/sex/group from TK animals. The plasma concentrations of the compound were estimated by using LC/MS/MS method (limit of detection = 0.9 ng/ml).

Gross pathology: Each main study group animal was examined for external abnormalities, palpable masses and cavities.

Organs weighed: adrenal, brain, epididymides, heart, kidney, liver, pituitary, testes, thyroids, parathyroid, and uterus/cervix.

Histopathology: The histopathological examination of the tissues was performed for all the animals of control and 1000 mg/kg/day treatment groups and, for those animals of other groups which died spontaneously/killed during the study. The following tissues were examined: adrenal, kidney, lacrimal gland, exorbital, bone with marrow [femur and sternum], liver, lung, bone marrow smear, lymph nodes: mandibular and mesenteric, brain [cerebrum, midbrain, cerebellum, medulla/pons], mammary gland [females only], pancreas, eye including optic nerve (2), pituitary gland, gallbladder, prostate and seminal vesicle (2), gastrointestinal tract (stomach glandular and non-glandular), salivary glands (mandibular 2), duodenum, jejunum, ileum, cecum, colon, rectum, spleen, thyroid/parathyroid, tongue, trachea, urinary bladder, testes, with epididymides (2), uterus [both horns]/cervix, gross lesions, Harderian glands and heart. The microscopic examination of the lung of the animals belonging to 100, 300 and 600 mg/kg/day treatment groups was performed and bone marrow smears were collected at the scheduled sacrifice and held.

Results:

1. **Mortality:** One, 2, 1 and 1 males belonging to control, 100, 300 and 1000 mg/kg/day treatment groups died during the study. One, 2 and 1 females of 100, 600 and 1000 mg/kg/day treatment groups died during the study. All of these animals died due to gavage related errors.

2. **Clinical signs:** None of the dead animals showed any treatment or dose related adverse effects. Yellow hair/face coloration was seen in 1, 1 and 3 males of 300, 600 and 1000 mg/kg/day treatment group, respectively. None of the females showed any of these signs.

3. **Body weights:** On week 13, the body weights of males included in 100, 300, 600 and 1000 mg/kg/day treatment groups were 104.6, 100.6, 120.0 and 100% of the controls and, in females, these were 96.4, 94.0, 100.2, and 112% of the control females, respectively. The change in body weight gain among treatment groups males and females was neither dose nor treatment related.

IND 56,553

Page 8

Initial and Final Body weights of the animals of 13-Week
Toxicity Study in Mice

Dose(mg/kg/day)	Males		Females	
	Initial	Final	Initial	Final
Control	28.83	37.91	21.46	29.89
100	28.62	38.15	21.65	29.78
300	28.39	37.52	20.86	29.79
600	28.7	38.9	21.88	30.33
1000	28.14	37.91	21.41	30.86

4. **Food consumption:** The food consumption of the animals in treatment groups and control group was not affected during the study and no dose/treatment related effects were seen. The initial and final food consumption among control group animals were 6.5 and 7.3 g/day in males and, 5.1 and 6.2 g/day among females, respectively.

5. **Ophthalmoscopy:** was not done during the study

6. **Electrocardiography:** parameters were not evaluated during the study

7. **Hematology:** A slight decrease of 18.3% and 17.1% in the percent reticulocytes was seen in males and females of 1000 mg/kg/day treatment group. The myeloid/erythroid ratio was only slightly increased in males of 1000 mg/kg/day treatment group (control and treated males value were 2.01 and 1.57). Myeloid/erythroid ratios in study females were not provided.

8. **Clinical chemistry:** No significant or clinically important changes were reported in male and female animals included in treatment groups of the study.

9. **Urinalysis:** The urinalysis tests were not included in the study.

10. **Organ weights Changes:** There was no dose or treatment related changes in the organ weights of animals of the study.

11. **Gross pathology:** One out of 9 males of 1000 mg/kg/day treatment group had galactocoele of mild nature and, 1 out of 9 females in each of 100 and 1000 mg/kg/day treatment groups had ovarian cyst of mild intensity.

12. **Histopathological Changes:** Traces of hepatic lipidosis were found in 20 and 40% male in control and 1000 mg/kg/day treatment groups. Among females, 30 and 20% in each of control and 1000 mg/kg/day treatment groups had hepatic lipidosis. These fatty streaks were not of toxicological importance. Histiocytosis of mesenteric and mandibular lymph nodes was seen (see the table below). Thymus severe atrophy incidences in study animals sacrificed during the study were similar in control and treatment groups animals

IND 56,553

Page 9

as 1, 1 and 2 males belonging to control, 100 and 1000 mg/kg/day treatment groups showed the atrophy. One female of 1000 mg/kg/day sacrificed at the termination of the study also had thymus atrophy. Lymphoid necrosis of thymus gland was similar, i.e., 10, 10 and 20% males included in 0, 100 and 1000 mg/kg/day treatment groups (table below). The intensity of necrosis was in traces. These changes were claimed as not drug treatment related and incidental.

TABLE
Incidences of Histopathological Changes in Control and Treatment groups Mice

Histopathological Lesion	Treatment Groups (mg/kg/day, p.o.)				
	Control	100	300	600	1000
1. Liver: Lipidosis, Trace					
Male	2/9	-	-	-	4/9
Female	3/10	-	0/0	0/2	2/9
2. Lungs: Alveolar Adenoma					
Males	0	0	0	0	0
Females	0	0	0	0	1/9
3. Histiocytosis/Erythrocytosis Trace Mandibular Lymph Node:					
Males	0/8	1/1	-	-	1/9
Females	0/9	1/10	-	0/2	1/8
4. Histiocytosis Mesenteric Lymph Node					
Males	2/8	2/2	1/1	-	2/8
Females	2/9	0/1	0/1	1/1	2/8
5. Thymus Atrophy					
Males	0/8	1*/9	-	-	2*/9
Females	0	0	0	0	1*/8
6. Thymus Necrosis					
Males	1 ⁺ /9	2 ⁺ /2	1 ⁺ /1	0	2 (1 ⁺)/9
Females	1/9	1 ⁺ /1	0	0	2/9

* = Sacrificed during the study, * = Severe intensity

13. **Toxicokinetic:** On day 1, a treatment related and non-dose proportional plasma peak concentrations (Cmax) of 6.05, 8.97, 158 and 29.7 ng/ml were seen in males within 1 to 2 hr of the administration of the compound. The peak plasma concentration of 21.0, 25.3, 162 and 79.2 ng/ml were seen among the females within 1 to 6 hr. On day 90, the plasma concentration (Cmax) was not linear (see table below) and peak concentrations were seen in 1 to 6 hr in males and females. The plasma concentrations and Tmax values in males and in females included in the study are shown in the following table (prepared from the sponsor's table in vol 1.1, pp 236-39). Because of inconsistent absorption of the compound and the variable Cmax values in study animals, sponsor claimed that AUC values and t1/2 could not be calculated.

TABLE
Pharmacokinetic parameters on Day 1 and Day 90 in male and female CD-1 mice of 13-Week Toxicity Study with orally administered Alvimopan (ADL 8-2698)

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: Alvimopan, Lot No. R009340, 98.9%

Methods: In this study, four groups Fischer 344 rats (n = 10/sex/group) received the vehicle (0.9% NaCl) or test article via intravenous bolus injection into the tail vein daily for 14 days at dose levels of 0, 1, 5, and 10 mg/kg, respectively (dose volume = 4 ml/kg). Four additional groups of five male and five female rats designated for Functional Observational Battery (FOB) evaluations (behavioral tests: thermal response, forelimb grip strength, hindlimb grip strength, hindlimb splay) received the vehicle or test article daily for three days at identical dose levels and volume to the main study groups. After FOB evaluations on Day 3, these animals were euthanized and discarded without further evaluation. In addition, three toxicokinetic (TK) groups of 16 male and 16 female rats received the test article daily for 14 days at dose levels of 1, 5, and 10 mg/kg, respectively (dose volume = 4 ml/kg). Blood samples were collected from TK animals on Days 1 and 14, predose and at 5, 15, and 30 minutes, and 1, 2, 4, 8, and 24 hours postdose. After completion of the blood collection on Day 14, these animals were euthanized and discarded without further evaluation.

Doses: 0, 1, 5, and 10 mg/kg via i.v. bolus injection into the tail vein.

Basis of Dose Selection: The doses were selected based on the results of previous studies (details were not provided).

Species/Strain: Fischer 344 rats

Number/Sex/Group (Main Study): 10/sex/group

Route, Formulation, Volume: Intravenous (i.v.) bolus, solution in 0.9% NaCl, 4 ml/kg.

Satellite Groups for Toxicokinetics or Recovery: 16/sex/group

Age: 8 weeks

Study Design: The following table shows the study design (from page 14 of the study report).

Group Assignments			
Group Number	Dose Level (mg/kg)	Number of Animals	
		Male	Female
Main Study:			
1	0	10	10
2	1	10	10
3	5	10	10
4	10	10	10
FOB:			
5	0	5	5
6	1	5	5
7	5	5	5
8	10	5	5
Toxicokinetic:			
9	1	16	16
10	5	16	16
11	10	16	16

FOB – Functional Observational Battery

Observation and Times:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Functional Observational Battery (FOB): Behavioral tests (thermal response, forelimb grip strength, hindlimb grip strength, hindlimb splay) were conducted on all animals in Groups 5-8. FOB evaluations were conducted on Day 3, approximately, one hour postdose.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis for main study animals.

Hematology: Blood samples were taken at termination for hematology.

Clinical Chemistry: Blood samples were taken at termination for serum chemistry.

Urinalysis: Urine samples were taken at termination for urinalysis.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all main study animals at necropsy (from page 407 of the report):

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407

NDA 21-775

The following list constitutes the full complement of organs and tissues:

-
-
- | | |
|---|--|
| <ul style="list-style-type: none"> - Adrenal (2)* - Aorta - Bone with marrow [femur] - Bone with marrow [sternum] - Bone marrow smear* - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* - Eye including optic nerve (2) - Gastrointestinal tract: <ul style="list-style-type: none"> esophagus stomach [glandular and non-glandular] duodenum jejunum ileum cecum colon rectum - Gonads: <ul style="list-style-type: none"> ovary (2)* testis (2)* with epididymis (2) - Gross lesions - Harderian Gland (2) - Heart* - Injection site(s) | <ul style="list-style-type: none"> - Kidney (2)* - Lacrimal gland, exorbital (2) - Liver [3 sections collected, 2 sections examined]* - Lung (collected whole, 2 sections examined) - Lymph nodes: mandibular and mesenteric - Mammary gland [process females only] - Pancreas - Pituitary* @ - Prostate* and seminal vesicle (2)* - Salivary gland, mandibular (2) - Sciatic nerve - Skeletal muscle, biceps femoris - Skin - Spinal cord [cervical, thoracic, and lumbar] - Spleen* - Thymus* - Thyroid/parathyroid (2)* @ - Tongue - Trachea - Urinary bladder - Uterus [both horns]/Cervix* - Vagina |
|---|--|
-

*Bone marrow smears were collected at the scheduled sacrifice and held.

(2) Paired organ

* Weighed

@ Weighed following fixation

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Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (X), no ()

Histopathological examinations were conducted on the tissues listed above from control and high dose animals at the scheduled necropsies.

Toxicokinetics: Blood samples were collected from TK animals on Days 1 and 14, predose and at 5, 15, and 30 minutes, and 1, 2, 4, 8, and 24 hours postdose.

Tissue P450 Studies: The livers of all main study animals were frozen for P450 studies.

Results:

Mortality: There was no mortality.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial and final body weight of control males were 248.1 and 263.1 g, respectively. The mean initial and final body weight of control females were 157.1 and 168.6 g, respectively. There were no treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 16.50 and 17.97 g/animal/day; respectively. The mean initial and final food consumption in control females were 11.28 and 12.57 g/animal/day, respectively. There were no treatment-related changes.

Functional Observational Battery (FOB): There were no treatment-related changes.

Hematology: There were no treatment-related changes.

Clinical Chemistry: No treatment-related effects on clinical chemistry parameters were observed.

Urinalysis: No differences in urinalysis between control and treated groups were observed.

Gross Pathology: No test article-related macroscopic observations were noted in either sex.

Organ Weights: No treatment-related changes in organ weights were observed in either sex.

Histopathology: There were no significant treatment-related changes.

Tissue P450 Studies: Results were not provided in the report.

Toxicokinetics: Exposure to alvimopan and ADL 08-0011 increased in a dose-related manner. Exposure to ADL 08-0011 was generally greater (up to 1.8-fold) after repeated dosing and exposure in female rats was greater (up to 3.6-fold) than that in male rats over the course of the study. Animals were exposed to alvimopan to a greater extent than ADL 08-0011. The toxicokinetic parameters of alvimopan and ADL 08-0011 are shown in the following table (from page 329 and 330 of sponsor's submission).

Alvimopan Toxicokinetic Parameters Following Daily Intravenous Bolus Injection of Alvimopan to Male and Female Rats for 14 Days							
Dosage level (mg/kg/day)	Sex	Day	C _{max} ^a (ng/mL)	AUC(0-4 hr) (ng•hr/mL)	t _{1/2} ^b (hr)	AUC(tf) ^c (ng•hr/mL)	
1	M	1	986	264	8	267	(312)
	F	1	553	153	4	153	(184)
	M	14	867	260	4	260	(275)
	F	14	640	173	4	173	(194)
5	M	1	4211	966	8	970	(1273)
	F	1	2251	688	8	691	(825)
	M	14	5021	1228	8	1231	(1570)
	F	14	3900	851	8	855	(1182)
10	M	1	7365	1696	8	1701	(2195)
	F	1	5537	1551	8	1574	(1691)
	M	14	8387	2104	24	2122	(2621)
	F	14	6246	1541	24	1554	(1915)

a: C_{max} was observed at 5 minutes, the first blood collection time point after dosing
 b: Time of last quantifiable plasma concentration
 c: The AUC(tf) value calculated using an estimate of the expected plasma concentration immediately post dose (determined by back-extrapolation of the alvimopan plasma concentrations at 5 and 15 minutes post dose) is given in brackets.

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330

NDA 21-775

ADL 08-0011 Toxicokinetic Parameters Following Daily Intravenous Bolus Injection of Alvimopan to Male and Female Rats for 14 Days							
Dosage level (mg/kg/day)	Sex	Day	Peak 1		Peak 2		AUC(0-24 hr) ^a (ng•hr/mL)
			(ng/mL)	(hr)	(ng/mL)	(hr)	
1	M	1	1.46	0.083	0.894	8	12.8
	F	1	1.25	0.083	1.72	8	22.0
	M	14	1.77	0.083	0.775	8	13.3
	F	14	1.89	0.25	1.62	8	30.1
5	M	1	6.48	0.083	3.15	8	52.2
	F	1	5.65	0.083	10.0	8	169
	M	14	8.64	0.083	3.86	8	74.0
	F	14	9.88	0.083	10.8	8	184
10	M	1	12.5	0.083	4.96	24	78.0
	F	1	12.8	0.083	20.4	8	277
	M	14	19.4	0.083	6.44	8	138
	F	14	21.5	0.083	18.7	24	367

a: AUC(0-24 hr) values are likely to be underestimated since no blood samples were collected between 8 and 24 hours post dose

In a 2-week i.v. toxicity study in rats, animals received an i.v. bolus injection of alvimopan at 0, 1, 5, and 10 mg/kg/day. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL may be considered as 10 mg/kg/day.

Study Title: 2-Week Intravenous Toxicity Study of ADL 08-0011-0 (Amide Hydrolysis Metabolite of Alvimopan) in Fischer Rats

Key Study Findings: In a 2-week i.v. toxicity study in rats, Fischer 344 rats received an i.v. bolus injection of 2, 4, and 8 mg/kg/day of ADL 08-0011-0 for 2 weeks. ADL 08-0011 did not cause any significant organ toxicity at any of the tested doses. As a result, the target organ of toxicity could not be identified. The NOAEL was considered as the highest tested dose (8 mg/kg/day).

Study No.: 808-018

Volume # and Page #: EDR NDA 21-775: Pharmtox\tox\808-018.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: May 22, 2002

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: ADL 08-0011-0, Lot No. 202002, 99.9%

Methods: This study was conducted to evaluate the potential toxicity of ADL 08-0011-0 (pharmacologically active metabolite of alvimopan) following 2-weeks of intravenous bolus administration in Fischer 344 rats. Three main study treatment groups (10 rats/sex/group) received the test article at 2, 4, and 8 mg/kg/day (dose volume = 4 ml/kg). Additionally, three toxicokinetic treatment groups (12 rats/sex/group) received the test article at the same respective dose levels as the main study groups.

Doses: 0, 2, 4, and 8 mg/kg via i.v. bolus injection into the tail vein.

Basis of Dose Selection: The doses were selected based on the results of previous studies (details were not provided).

Species/Strain: Fischer 344 rats

Number/Sex/Group (Main Study): 10/sex/group

Route, Formulation, Volume: Intravenous (i.v.) bolus, solution in 0.9% NaCl, 4 ml/kg.

Satellite Groups for Toxicokinetics or Recovery: 12/sex/group

Age: 8 weeks

Study Design: The following table shows the study design (from page 12 of the study report).

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NDA 21-775

12

4.2.2 Assignment to Study and Maintenance

The animals were assigned to study as indicated in the following table.

Group Assignment				
Group	Dose Level (mg/kg/day)	Concentration (mg/mL)	Number of Animals	
			Male	Female
1	0	0	10	10
2	2	0.5	10	10
3	4	1.0	10	10
4	8	2.0	10	10
5	2	0.5	12	12
6	4	1.0	12	12
7	8	2.0	12	12

Observation and Times:**Mortality:** All animals were examined twice daily for mortality.**Clinical Signs:** All animals were examined once daily for clinical signs.**Body Weights:** Body weights were recorded on a weekly basis.**Food Consumption:** Food consumption was recorded on a weekly basis for main study animals.**Ophthalmoscopic Examinations:** A complete ophthalmologic examination was conducted on all animals prior to treatment and on all main study animals prior to study termination.**Hematology:** Blood samples were taken at termination for hematology from all main study animals.**Clinical Chemistry:** Blood samples were taken at termination for serum chemistry from all main study animals.**Urinalysis:** Urine samples were taken at termination for urinalysis from all main study animals.**Gross Pathology:** Gross pathology was conducted at necropsy on all main study animals.

Organ Weights: The following organs were weighed from all main study animals at necropsy (from page 246 of the report):

246

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ENTEREG™ (alvimopan)

NDA 21-775

The following list constitutes the full complement of organs and tissues:

-
- Adrenal (2) #
 - Aorta
 - Bone with marrow [femur]
 - Bone with marrow [sternum]
 - Bone marrow smear [2 collected]
 - Brain [cerebrum, midbrain, cerebellum, medulla/pons] #
 - Eye including optic nerve (2)
 - Gastrointestinal tract:
 - esophagus
 - stomach [glandular and nonglandular]
 - duodenum
 - jejunum
 - ileum
 - cecum
 - colon
 - rectum
 - Gonads:
 - ovary (2) #
 - testis (2) #
 - epididymis (2)
 - Gross lesions
 - Harderian gland (2)
 - Heart #
 - Injection site, tail
 - Lacrimal gland, exorbital (2)
 - Kidney (2) #
 - Liver [3 sections collected; 2 examined] #
 - Lung [2 sections examined]
 - Lymph node, mandibular [2 collected; 1 examined]
 - Lymph node, mesenteric
 - Mammary gland [process females only]
 - Pancreas
 - Pituitary #
 - Prostate #
 - Salivary gland, mandibular [2 collected; 1 examined]
 - Sciatic nerve
 - Seminal vesicle (2) #
 - Skeletal muscle, biceps femoris
 - Skin
 - Spinal cord [cervical, thoracic, and lumbar]
 - Spleen #
 - Thymus #
 - Thyroid/parathyroid (2) #
 - Tongue
 - Trachea
 - Urinary bladder
 - Uterus [both horns] with cervix #
 - Vagina
-

Organ weighed
 (2) Paired organ

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Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), no ()

Histopathological examinations were conducted on the tissues listed above from all main study animals at the scheduled necropsies.

Toxicokinetics: Blood samples were collected via the orbital sinus on Day 1 and 14 prior to dosing and at 0.25, 0.5, 1, 4, and 24 hours postdose.

Results:

Mortality: There was no mortality.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial and final body weight of control males were 246.0 and 262.5 g, respectively. The mean initial and final body weight of control females were 160.2 and 167.4 g, respectively. There were no treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 18.88 and 16.93 g/animal/day, respectively. The mean initial and final food consumption in control females were 12.50 and 11.96 g/animal/day, respectively. There were no treatment-related changes.

Ophthalmoscopy: There were no treatment-related changes.

Hematology: There were no treatment-related changes.

Clinical Chemistry: No treatment-related effects on clinical chemistry parameters were observed.

Urinalysis: No differences in urinalysis between control and treated groups were observed.

Gross Pathology: No test article-related macroscopic observations were noted in either sex.

Organ Weights: No significant treatment-related changes in organ weights were observed in either sex.

Histopathology: There were no significant treatment-related changes.

Toxicokinetics: Exposure to ADL 08-0011 increased in a dose-related manner. Females showed higher exposure than males. The toxicokinetic parameters of ADL 08-0011 are shown in the following table (from page 199 of sponsor's submission).

ADL 08-0011 Toxicokinetic Parameters Following Daily Intravenous Bolus Injection of ADL 08-0011-0 to Rats for 14 Days					
Dosage level (mg/kg/day)	Sex	Day	C _{max} ^a (ng/mL)	AUC(0-4 hr) (ng•hr/mL)	AUC(0-24 hr) (ng•hr/mL)
2	M	1	595	379	412
	F	1	827	590	642
	M	14	684	396	473
	F	14	761	695	795
4	M	1	1530	950	1020
	F	1	1700	1130	1250
	M	14	1120	1930	1970
	F	14	1440	2230	2300
8	M	1	2690	1940	2070
	F	1	2840	2490	2900
	M	14	1880	2930	3040
	F	14	2850	4010	4140

a: C_{max} was observed at 15 minutes, the first blood collection time point after dosing

808-018 FINAL

Page 8

In a 2-week i.v. toxicity study in rats, animals received an i.v. bolus injection of ADL 08-0011 at 2, 4, and 8 mg/kg/day. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL may be considered as 8 mg/kg/day. The mean C_{max} and AUC_{0-4h} value at 8 mg/kg/day at Day 14 was found to be approximately 2365 ng/ml and 3590 ng.hr/ml (approximately 2 times human AUC at the proposed dose), respectively (mean AUC_{0-α} and C_{max} for ADL 08-0011 in humans following oral dose of 12 mg b.i.d. dose for 4.5 days were 1642.5 ng.hr/ml and 35.73 ng/ml, respectively, from study 14CL119).

1-Month Subacute Oral Toxicity Study of Alvimopan in Fischer 344 Rats (Study Nos. R02893 and R02993)

SUBACUTE TOXICITY:

1. One month subacute oral toxicity of LY246736 dihydrate in Fischer 344 rats (Study Nos. 2893 and R02993).

Page 14

Testing Laboratory:

Sponsor's laboratory

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: January 13, 1993

Study Completed: August 27, 1993

Animals: Male (106.5 ± 4.0 g, mean ± SD) and female (88.5 ± 4.4 g) Fischer 344 rats, initial ages were 6 to 7 weeks.

Methods: LY246736 dihydrate was orally administered by gavage at doses of 0, 50, 100 and 200 mg/kg, once daily for 1 month, to four groups of 20 rats each. Each group consisted of 10 M and 10 F rats. Vehicle was 10% aqueous acacia solution.

Animals were examined daily for survival, general physical condition and behavior. A detailed examination was performed weekly.

Rats were weighed weekly and food consumption was determined weekly.

Hematologic parameters were determined from blood samples collected at termination of the study from the abdominal aorta for determination of activated partial thromboplastin time and prothrombin time and from the orbital plexus for all other hemotological parameters.

Blood chemistry parameters were determined from blood samples collected at termination of the study from the orbital plexus.

Urine was collected for approximately 16 hours near the end of the live-phase of the experiment. Urinalysis was performed and microscopic examinations were performed on any samples with an abnormal appearance or with results outside the established laboratory reference ranges for protein, occult blood, or bilirubin.

Organ weights were determined at termination of the study.

Gross pathological examinations and histopathological evaluations were done at termination of the study.

Blood was collected from 3 designated animals/sex/time point for

Page 15

determination of plasma concentrations of LY246736 on Day 0 and Day 29.

Results:

1. Observed Effects: No treatment-related clinical signs were observed during this study.
2. Mortality: None.
3. Body Weight: No treatment-related changes in body weight occurred during this study..
4. Food Consumption: No treatment-related changes in food consumption occurred during this study.
5. Hematology: The following hematological parameters were statistically different from control values and are expressed as % of control. In male rats, neutrophil count ($10^3/\text{mm}^3$), thrombocyte count ($10^3/\text{mm}^3$) and erythrocyte count ($10^3/\text{mm}^3$) were significantly lower (16%, 50% and 6%, respectively) in the 200 mg/kg group. In female rats, prothrombin time (sec) was significantly lower (4%) in the 200 mg/kg group.
6. Blood Chemistry: The following blood chemistry parameter was statistically different from the control value and is expressed as % of control. In female rats, triglyceride levels (mg/dl) were significantly lower (15%) in the 200 mg/kg group.
7. Urinalysis: No treatment-related changes in urinalysis parameters were seen in this study.
8. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No data were provided.
9. Organ Weights: No treatment-related changes in organ weights were seen in this study.
10. Gross Pathology : No treatment-related incidents of gross pathology were seen in this study.
11. Histopathology: No treatment-related histological changes were seen in this study.
12. Plasma Levels of the Drug: Blood samples were collected at 0.25, 0.5, 1, 2 and 4 hours on Days 0 and 29. The mean plasma levels of LY246736 dihydrate for males and females on Days 0 and 29 ranged from 2.8 to 20.5 ng/ml, and the overall average was 13 ng/ml.

In summary, the maximum no effect oral dose of LY246736 dihydrate

in rats was 100 mg/kg. A few hematological and blood chemistry parameters in the 200 mg/kg group were significantly different from control values, but associated quantitative differences were relatively moderate and may not reflect any biological significance. The doses of LY246736 dihydrate that were employed did not allow the identification of any target organs for toxicity. In order to elucidate clinical signs of toxicity and target organs for toxicity, the sponsor would need to repeat the study while using sufficiently higher doses of LY246736 dihydrate. Plasma levels of LY246736 dihydrate reflected poor absorption from the gastrointestinal tract.

Study Title: 4-Week Oral Toxicity Study of Alvimopan in Fischer Rats

Key Study Findings: In a 4-week oral gavage toxicity study in Fischer 344 rats, animals received oral doses of alvimopan at 100, 250 and 500 mg bid or 200, 500 and 1000 mg/kg/day for 4 weeks. There was no significant organ toxicity. The NOAEL was considered as 1000 mg/kg/day.

Study No.: 808-012/14TX006

Volume # and Page #: EDR NDA 21-775: Pharmtox\tox\808-012.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: March 15, 2002

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: Alvimopan, Lot No. R009340, 98.9%

Methods: Three groups of ten male and ten female rats (n = 10/sex/group) were administered the test article via oral gavage twice daily (approximately four hours apart) for 28 consecutive days at 0, 200, 500, and 1000 mg/kg/day (100, 250, and 500 mg/kg/dose). Control animals received the vehicle, 10% (w/v) suspension of Gum Acacia, NF. The dose volume was 10 ml/kg for all groups. In addition, three toxicokinetic (TK) groups of eight male and eight female rats were utilized to measure plasma levels of alvimopan at designated time points on Days 1 and 32.

Doses: 0, 200, 500 and 1000 mg/kg/day (100, 250 and 500 mg/kg bid) by oral gavage for 28 consecutive days

Basis of Dose Selection: The doses were selected based on the results of previous studies (details were not provided).

Species/Strain: Fischer 344 rats

Number/Sex/Group (Main Study): 10/sex/group

Route, Formulation, Volume: Oral (gavage), suspension in Gum Acacia, NF, 10 ml/kg

Satellite Groups for Toxicokinetics or Recovery: 8/sex/group

Age: 8 weeks

Study Design: The following table shows the study design (from page 13 of the study report).

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0	10	10
2	200	10	10
3	500	10	10
4	1000	10	10
Toxicokinetic			
5	200	8	8
6	500	8	8
7	1000	8	8

Observation and Times:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted during week -2 and 4.

Hematology: Blood samples were taken at termination for hematology from all main study animals.

Clinical Chemistry: Blood samples were taken at termination for serum chemistry from all main study animals.

Urinalysis: Urine samples were taken at termination for urinalysis from all main study animals.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all main study animals at necropsy (from page 216 of the report):

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ENTEREG™ (alvimopan)
216

NDA 21-775

The following list constitutes the full complement of organs and tissues:

-
-
- | | |
|--|--|
| - Adrenal (2) # | - Lacrimal gland, exorbital (2) |
| - Aorta | - Liver [3 sections collected; 2 examined] # |
| - Bone with marrow [femur] | - Lung [2 sections examined] |
| - Bone with marrow [sternum] | - Lymph node, mandibular [2 collected; 1 examined] |
| - Bone marrow smear * [2 collected] | - Lymph node, mesenteric |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons] # | - Mammary gland [females only] |
| - Eye including optic nerve (2) | - Pancreas |
| - Gastrointestinal tract: | - Pituitary # |
| esophagus | - Prostate # |
| stomach [glandular and nonglandular] | - Salivary gland, mandibular [2 collected; 1 examined] |
| duodenum | - Sciatic nerve |
| jejunum | - Seminal vesicle (2) # |
| ileum | - Skeletal muscle, biceps femoris |
| cecum | - Skin |
| colon | - Spinal cord [cervical, thoracic, and lumbar] |
| rectum | - Spleen # |
| - Gonads: | - Thymus # |
| ovary (2) # | - Thyroid/parathyroid (2) # |
| testis (2) # | - Tongue |
| epididymis (2) | - Trachea |
| - Gross lesions | - Urinary bladder |
| - Harderian gland (2) | - Uterus [both horns] with cervix # |
| - Heart # | - Vagina |
| - Kidney (2) # | |
-

* Bone marrow smears were collected at the scheduled sacrifice and held.

Organ weighed

(2) Paired organ

Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), no ()

Histopathological examinations were conducted on the tissues listed above from all main study animals in the control and high dose groups.

Toxicokinetics: Blood samples were collected prior to dosing and at 0.25, 1, 4, 24, 48, 72, and 96 hours postdose on Days 1 and 32.

Results:

Mortality: There was no mortality.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial and final body weight of control males were 263.3 and 273.9 g, respectively. The mean initial and final body weight of control females were 156.4 and 166.9 g, respectively. There were no treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 15.68 and 15.43 g/animal/day, respectively. The mean initial and final food consumption in control females were 10.55 and 10.66 g/animal/day, respectively. There were no treatment-related changes.

Ophthalmoscopy: There were no treatment-related changes.

Hematology: There were no treatment-related changes.

Clinical Chemistry: No treatment-related effects on clinical chemistry parameters were observed.

Urinalysis: No differences in urinalysis between control and treated groups were observed.

Gross Pathology: No test article-related macroscopic observations were noted in either sex.

Organ Weights: No treatment-related changes in organ weights were observed in either sex.

Histopathology: There were no significant treatment-related changes.

Toxicokinetics: Mean plasma concentrations of alvimopan were minimal. ADL 08-0011 was generally detectable in plasma up to 96 hours post dose. Animals were exposed to ADL 08-0011 to a greater extent than to alvimopan and exposure to ADL 08-0011 was

higher in females than in males. The toxicokinetic parameters of alvimopan and ADL 08-0011 are shown in the following table (from page 249 of sponsor's submission).

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202

NDA 21-775

Alvimopan Toxicokinetic Parameters Following Twice Daily Oral Administration of Alvimopan to Male and Female Rats for 28 Days				
Dosage level (mg/kg)	Sex	Cmax (ng/mL)	Tmax (hr)	AUC(0-24 hr) (ng•hr/mL)
100 bid	M	1.52	1	25.1
	F	5.16	1	54.1
250 bid	M	2.72 ^a	4	47.6
	F	4.80 ^a	1	46.7
500 bid	M	7.37	1	54.2
	F	8.01	0.25	81.3

Note: Rats were initially administered a single dose of alvimopan, then after 96 hour the rats were dosed twice daily (approximately 4 hours apart) for 28 days; parameters determined after the second daily dose

a: Cmax over the first 24 hours post dose

ADL 08-0011 Toxicokinetic Parameters Following Twice Daily Oral Administration of Alvimopan to Male and Female Rats for 28 Days				
Dosage level (mg/kg)	Sex	Cmax (ng/mL)	Tmax (hr)	AUC(0-24 hr) (ng•hr/mL)
100 bid	M	18.0	Predose	310
	F	86.1	Predose	1090
250 bid	M	23.3	24	427
	F	50.2	Predose	644
500 bid	M	32.6	Predose	358
	F	58.3	Predose	904

Note: Rats were initially administered a single dose of alvimopan, then after 96 hour the rats were dosed twice daily (approximately 4 hours apart) for 28 days; parameters determined after the second daily dose

In a 4-week oral gavage toxicity study in Fischer 344 rats, animals received oral doses of alvimopan at 100, 250 and 500 mg/kg bid or 200, 500 and 1000 mg/kg/day for 4 weeks. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL may be considered as 1000 mg/kg/day. The mean C_{max} and AUC_{0-4h} value at 1000 mg/kg/day was found to be approximately 7.7 ng/ml and 67.75 ng.hr/ml, respectively, which is about 1.7 times the human AUC at the proposed therapeutic oral dose (mean AUC_{0-12h} and C_{max} for alvimopan in humans at oral dose of 12 mg b.i.d. dose were 40.2 ng.hr/ml and 10.98 ng/ml, respectively. Data obtained from study 14CL119).

6-Month Oral Toxicity Study in Rats (Study No. R04393)

6-Month Oral Toxicity Study in Rats (Study No. R04393)

Testing Laboratory: _____

Date Started: June 17, 1993

Date Completed: December 17, 1993

GLP Compliance: A statement of compliance is included.

Animals: Fischer 344 rats (both sexes) were used in this study. Animals were approximately 6 to 7 weeks old and the initial mean body weights for males and females were 115.8 g and 94.8 g, respectively.

Drug Batch: ADL8-2698, Lot 284MH2.

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IND 56, 553

2

Methods: Fischer rats (16/sex/group) were administered orally by gavage at single daily doses of 50, 100 and 200 mg/kg for a 6-month period at a dose volume of 5-ml/kg. The control animals received 10% aqueous acacia vehicle. The doses were selected based on the results of a previous 1-month oral (gavage) toxicity study in Fischer rats at 0, 50, 100, or 200 mg/kg dose levels. There were no treatment-related changes in clinical signs, body weight, gross or histopathology. The doses selected for the current study were the same as those used in the 1-month oral toxicity study. In this study, the high dose of 200 mg/kg was selected because previous data indicated saturation of absorption at oral doses higher than 200-mg/kg. Animals were monitored daily for clinical signs of toxicity and moribundity/mortality. Body weight and food consumption was recorded weekly. Blood samples were collected from 10 rats/sex/treatment at approximately 3 months and from all surviving animals at necropsy for hematology and clinical chemistry. Urinalysis was also done according to the above schedule. Rats were sacrificed at the end of the treatment period and subjected to a gross necropsy (all rats). The following organs were weighed: kidneys, liver, heart, spleen, ovaries, testes, prostate, adrenals, thyroid with parathyroid, brain, uterus, and pituitary. Histopathological examinations were done on the following tissues collected from all animals: adrenals, aorta, bone (femur and sternum) and bone marrow, brain stem, cerebrum, cerebellum, epididymides, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, ileum, jejunum), kidneys, liver, lungs, lymph nodes, mammary glands, ovaries, pancreas, parathyroid, peripheral nerve (sciatic), pituitary gland, prostate, salivary gland, seminal vesicle, skeletal muscles, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus plus cervix, and vagina.

Results:

1. **Observed Effects:** No treatment-related effects were observed.
2. **Mortality:** One male at 100 mg/kg died due to gavage accident. There was no other mortality.
3. **Body Weight:** The mean initial (Day -1) and final (Day 180) body weights of control males were 116.2 g and 411.8 g, respectively. The mean initial (Day -1) and final (Day 180) body weights of control females were 95.8 g and 214.9 g, respectively. There were no treatment-related changes in body weight. The growth curves are shown below:

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IND 56, 553

3

Figure D-1.1. Mean Body Weight.

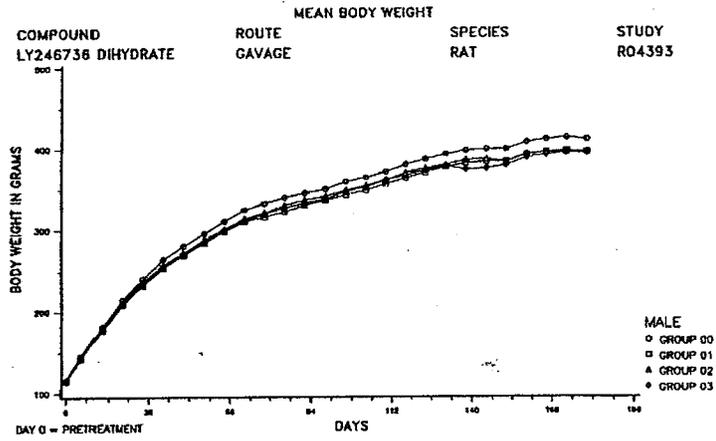
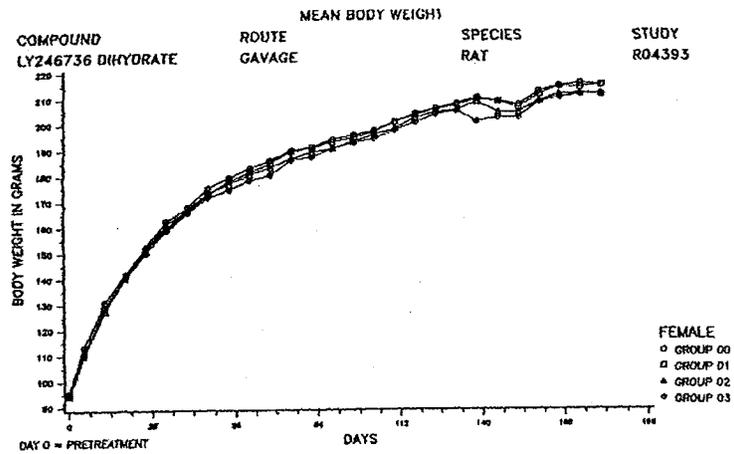


Figure D-1.2. Mean Body Weight.



IND 56, 553

4

4. **Food Consumption:** The mean initial (Day -1) and final (Day 180) food consumption of control males were 14.8 g/day and 17.7 g/day, respectively. The mean initial (Day -1) and final (Day 180) food consumption of control females were 12.3 g/day and 12.4 g/day, respectively. There were no treatment-related changes in food consumption.
5. **Hematology:** No drug-related effects were observed.
6. **Clinical Chemistry:** No treatment-related changes were observed.
7. **Urinalysis:** No treatment-related changes were observed.
8. **Organ Weights:** The organ weights were changed for the following organs in males: prostate (absolute: 110% of control, control = 802.8 mg; relative to body weight: 110% of control at 100 mg/kg and 115% of control at 200 mg/kg, control = 196.3 mg/100 g), kidney (relative to body weight: 103 to 104 % of control at 100 to 200 mg/kg, control = 552.9 mg/100 g), and brain (relative to body weight: 103 to 104% of control at 50 to 200 mg/kg, control = 474.1 mg/100 g). No other significant treatment-related changes were observed.
9. **Gross Pathology:** No treatment-related changes were observed.
10. **Histopathology:** No treatment-related changes were observed.
11. **Toxicokinetics:** Blood samples were drawn from rats (3/sex/group/time point) at 1 and 24 hours postdose after 3 and 5 months of daily dosing. The plasma was analyzed for ADL8-2698 using a validated HPLC/MS method. ADL8-2698 was not detected in the plasma of either sex after 3 months of treatment. Following 5 months of daily treatment, ADL8-2698 in male rats was not detectable at one hour after all dose levels. At 24 hours postdose at 200 mg/kg, a mean plasma concentration of 21 ng/ml was observed in males. However, in females, detectable level of drug was observed at both the time points at 100 (52.4 ng/ml at 1 h and 21.2 ng/ml at 24 h) and 200 (62.3 ng/ml at 1 h and 27.9 ng/ml at 24 h) mg/kg dose levels. There was no dose proportionality at 100 and 200 mg/kg doses.

In a 6-month oral toxicity study of ADL8-2698 in rats, animals were treated with ADL8-2698 at 0, 50, 100, and 200 mg/kg/day dose levels. The no observed effect level (NOEL) may be considered as 200 mg/kg. The above-mentioned doses did not allow the identification of any target organ of toxicity. There was no mention whether the highest tested dose was the maximum feasible dose. However, previous data indicated saturation of absorption at oral doses higher than 200-mg/kg. In addition, intravenous studies in rats and dogs showed very low order of toxicity at plasma concentrations that were 100 times greater than that obtained after oral dose in rats. It is to be mentioned here that previous acute oral toxicity studies in mice and rats, LY246736 did not produce any lethality or clinical signs of toxicity at 500 mg/kg dose.

Dog

A Subchronic Toxicity Study With Alvimopan Administered Orally to Beagle Dogs for 1 Month (Study No. TOX RPT 9/D02793)

3. One month subacute oral toxicity of LY246736 dihydrate in Beagle dogs (Study No. D02793).

Testing Laboratory:

Sponsor's laboratory.

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: January 26, 1993

Study Completed: August 13, 1993

Animals: Male (9.1 ± 0.8 kg, mean \pm SD) and female (8.9 ± 0.4

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kg) Beagle dogs; initial ages were 8 to 11 months

Methods: LY246736 dihydrate was orally administered at doses of 0, 10, 30 and 100 mg/kg, once daily for 1 month, to four groups of six dogs each. Each group consisted of 3 M and 3 F dogs. LY246736 dihydrate was prepared in a PEG [redacted] and administered in gelatin capsules.

All dogs were observed frequently on the first day of drug administration. Thereafter, animals were observed several times daily during the week and at least once on weekends and company holidays.

Body weights were recorded before study initiation, at weekly intervals during drug administration and at study termination

Food consumption was estimated visually each day.

Hematological parameters were determined twice before study initiation, at week 1 and near study termination.

Clinical chemistry parameters were determined twice before study initiation, at week 1 and near study termination. Enzyme induction by LY246736 dihydrate was assessed by determining the activities of the hepatic enzymes 7-ethoxyresorufin O-deethylase and benzphetamine N-demethylase, and by measuring total hepatic cytochrome P-450 content.

Urinalysis was done at pretest and near study termination.

Physical examinations and ophthalmic examinations were done pretest and near the end of the study. ECGs were obtained at pretest and at 0 and 0.5 hours after drug administration on day 2, Week 2 and near study termination.

Organ weights were determined at termination of the study.

Gross pathological examinations and histopathological evaluations were done at termination of the study. Plasma concentrations of LY246736 were determined 5 minutes after drug administration on Day 1, Day 15 and near study termination.

Results:

1. **Observed Effects:** In the 10 mg/kg group, no treatment-related clinical observations were seen.

In the 30 mg/kg group, one male had 1 incident of emesis and 1 female had 6 incidents of emesis. One male had 1 incident of mucoid stool. One male had 2 incidents of soft stool. One female had 5 incidents of swollen vulva. One female had 3 incidents of swollen vulva with discharge.

In the 100 mg/kg group, one male had 5 incidents of emesis and two females had a total of 3 incidents of emesis. One male had 1 incident of mucoid stool. Two males had a total of 4 incidents of soft stool and two females had a total of 3 incidents of soft stool. One female had 1 incident of runny stool. 1 female had 8 incidents of swollen vulva. One female had 6 incidents of swollen vulva with discharge.

2. Mortality: None.

3. Body Weight: No treatment-related changes in body weight occurred during the study.

4. Food Consumption: No treatment-related changes in food consumption occurred during the study.

5. Hematology: No treatment-related changes in hematologic parameters occurred during the study.

6. Blood Chemistry: No treatment-related changes in blood chemistry occurred during this study. No significant changes were seen in 7-ethoxysorufin O-deethylase or benzphetamine N-demethylase activities, or in total hepatic cytochrome P-450 content.

7. Urinalysis: No treatment-related changes in urinalysis parameters occurred during this study.

8. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment-related clinical findings were revealed by the physical examination conducted at the end of the study. No ophthalmic abnormalities were found. LY246736 dihydrate had no effect on cardiac rate, rhythm, conduction, or repolarization.

9. Organ Weights: No treatment-related changes in organ weights were seen.

10. Gross Pathology: No treatment-related changes in gross pathology were seen.

11. Histopathology: No treatment-related histologic changes were seen.

12. Plasma Levels of the Drug: Plasma concentrations of LY246736 dihydrate were determined at 0.5, 1, 2, 4, 8, 12 and 24 hours after drug administration on Days 1 and 28. On Day 1, the mean peak plasma levels (C_{max}) were 30.9 ± 14.9 ng/ml (mean \pm SE) for the 10 mg/kg group, 74.6 ± 15.6 ng/ml for the 30 mg/kg group and 92.9 ± 10.6 ng/ml for the 100 mg/kg group. In general, peak plasma levels occurred 4-12 hours after LY246736 dihydrate administration. On Day 28, the mean peak plasma levels were 27.0 ± 5.5 ng/ml for the 10 mg/kg group, 92.1 ± 9.0 ng/ml for the 30

Page 21

mg/kg group and 133.5 ± 29.3 ng/ml for the 100 mg/kg group.

In summary, the maximum no effect oral dose of LY246736 dihydrate in dogs was 100 mg/kg. The only clinically relevant observations were incidents of emesis and diarrhea. The doses of LY246736 dihydrate that were employed did not allow the identification of any target organs for toxicity. In order to elucidate clinical signs of toxicity and target organs for toxicity, the sponsor would need to repeat the study while using sufficiently higher doses of LY246736 dihydrate. Plasma levels of LY246736 dihydrate reflected poor absorption from the gastrointestinal tract.

A Subchronic Toxicity Study in Beagle Dogs Given Daily Intravenous Doses of Alvimopan for 1 Month (Study No. TOX RPT 8/D04992)

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2. One month subacute i.v. toxicity of LY246736 dihydrate in Beagle dogs (Study No. D04992).

Testing Laboratory:

Sponsor's laboratory.

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: November 13, 1992

Study Completed: August 13, 1993

Animals: Male (8.8 ± 0.9 kg, mean \pm SD) and female (8.6 ± 1.9 kg) Beagle dogs; initial ages were 7 to 12 months.

Methods: LY246736 dihydrate was intravenously administered at doses of 0, 0.05, 0.2 and 2.0 mg/kg, once daily for 1 month, to four groups of six dogs each. Each group consisted of 3 M and 3 F dogs. Vehicle was Sterile Water for Injection, USP with pH adjusted to approximately 10.5 with NaOH.

All dogs were observed frequently on the first day of drug administration. Thereafter, animals were observed several times daily during the week and at least once on weekends and company holidays.

Body weights were recorded before study initiation, at weekly intervals during drug administration and at study termination

Food consumption was estimated visually each day.

Hematological parameters were determined twice before study initiation, at week 1 and near study termination.

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Page 17

Clinical chemistry parameters were determined twice before study initiation, at week 1 and near study termination. Enzyme induction by LY246736 dihydrate was assessed by determining the activities of the hepatic enzymes 7-ethoxyresorufin O-deethylase and benzphetamine N-demethylase, and by measuring total hepatic cytochrome P-450 content.

Urinalysis was done at pretest and near study termination.

Physical examinations and ophthalmic examinations were done pretest and near the end of the study. ECGs were obtained at pretest and at 0 and 0.5 hours after drug administration on day 2, Week 2 and near study termination.

Organ weights were determined at termination of the study.

Gross pathological examinations and histopathological evaluations were done at termination of the study. Plasma concentrations of LY246736 were determined 5 minutes after drug administration on Day 1, Day 15 and near study termination.

Results:

1. Observed Effects: No treatment-related clinical signs were observed during the study. One male dog, given 2.0 mg/kg of LY246736 dihydrate, had one instance of soft, mucoid stool on Day 23.
2. Mortality: None
3. Body Weight: No treatment-related changes in body weight occurred during the study.
4. Food Consumption: No treatment-related changes in food consumption occurred during the study.
5. Hematology: In the 2.0 mg/kg group, erythrocytes ($10^6/\text{mm}^2$) and hemoglobin (g/dl) were significantly decreased (14% and 15%, respectively) in females and prothrombin time (secs) was significantly decreased (5%) in males.
6. Blood Chemistry: No treatment-related changes in blood chemistry were seen. No significant changes were seen in 7-ethoxyresorufin O-deethylase or benzphetamine N-demethylase activities, or in total hepatic cytochrome P-450 content.
7. Urinalysis: No treatment-related changes in urinalysis parameters were seen.
8. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment-related clinical findings were revealed by the physical examination conducted at the end of the study. No

Page 18

ophthalmic abnormalities were found. LY246736 dihydrate had no effect on cardiac rate, rhythm, conduction, or repolarization.

9. Organ Weights: No treatment-related changes in organ weights were seen.

10. Gross Pathology: No treatment-related changes in gross pathology were seen.

12. Histopathology: No treatment-related histologic changes were seen

12. Plasma Levels of the Drug: Plasma concentrations of LY246736 dihydrate, observed 5 minutes after i.v. administration, were approximately 120 ng/ml at 0.05 mg/kg, 430 to 520 ng/ml at 0.2 mg/kg and 3500 to 4000 ng/ml at 2.0 mg/kg. The half-life of LY246736 dihydrate at all doses was approximately 10 minutes. Plasma levels were proportional to dose, similar in males and females, and did not significantly change over the one month of treatment.

In summary, the maximum no effect i.v. dose of LY246736 dihydrate in dogs was 0.2 mg/kg. A few hematological parameters in the 2.0 mg/kg group were significantly different from control values, but associated quantitative differences were relatively moderate and may not reflect any biological significance. The doses of LY246736 dihydrate that were employed did not allow the identification of any target organs for toxicity. In order to elucidate clinical signs of toxicity and target organs for toxicity, the sponsor would need to repeat the study while using sufficiently higher doses of LY246736 dihydrate. Plasma levels of LY246736 dihydrate reflected relatively short half-lives.

Study Title: 4-Week Oral Toxicity Study of Alvimopan in Beagle Dogs

Key Study Findings: In a 4-week oral (capsule) toxicity study of alvimopan in beagle dogs, animals were treated at 0, 100, 250, 500 and 1000 mg/kg/day for 4 weeks. The target organ of toxicity could not be identified in the absence of any organ toxicity. The NOAEL appeared to be 1000 mg/kg/day.

Study No.: 808-011

Volume # and Page #: EDR NDA 21-775: pharmtox\tox\808-011.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: March 22, 2002

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: Alvimopan, R009340, 98.9%

Methods: This study was conducted to examine the potential toxicity of alvimopan following twice daily oral capsule administration in beagle dogs for four consecutive weeks. Four groups of five male and five female dogs (n = 5/sex/group) were administered alvimopan twice daily (approximately four hours apart) via gelatin capsules for four consecutive weeks at 100, 250, 500, and 1000 mg/kg/day (50, 125, 250, and 500 mg/kg bid). Control animals (three male and three female dogs) received empty gelatin capsules. All dogs received a single dose on Day 1 followed by four days of blood collection for toxicokinetic (TK) analysis and then began 28 consecutive days of twice daily dosing on Day 5. Following the second dose on Day 32 (28 days of dosing), the control group and the first three dogs/sex/treatment group were euthanized. The remaining two dogs/sex/treatment group remained on study to conduct the 48, 72, and 96 hour toxicokinetic collections and then the dogs were euthanized without further evaluation.

Doses: 100, 250, 500, and 1000 mg/kg/day (50, 125, 250, and 500 mg/kg bid) by oral route (capsule)

Basis of Dose Selection: Doses were selected based on the results of previous studies. However, details of these previous studies were not mentioned.

Species/Strain: Beagle dogs

Number/Sex/Group (Main Study): 5/sex/group for alvimopan and 3/sex/group for control

Route and Formulation: Oral, Capsule

Satellite Groups for Toxicokinetics or Recovery: None

Age: Five to six months

Study Design: The following table shows the study design (from page 70 of the report of sponsor's submission).

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	3	3
2*	100	5	5
3*	250	5	5
4*	500	5	5
5*	1000	5	5

*The last two dogs/sex/treatment group (highest animal numbers in Groups 2-5) were bled at 48, 72, and 96 hours postdose (of the second daily dose) following the last day of dosing. These animals were not necropsied, but were euthanized and discarded.

Observation and Times:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a daily basis.

Ophthalmoscopic Examinations: All animals were examined at pretest and again prior to necropsy.

Electrocardiographic Examinations: All dogs received an electrocardiographic (ECG) examination at pretest and again prior to necropsy.

Hematology: Hematology examinations were conducted on all animals at pretest and prior to termination.

Clinical Chemistry: Clinical chemistry was conducted on all animals at pretest and prior to termination.

Urinalysis: Urine analyses were conducted on all animals at pretest and prior to termination.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following table (from page 393 of sponsor's submission) shows the organs that were weighed (as indicated by #) from all animals at necropsy:

336

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NDA 21-775

The following list constitutes the full complement of organs and tissues:

-
- | | |
|---|--|
| - Adrenal (2)# | - Heart# |
| - Aorta | - Kidney (2)# |
| - Bone with marrow [femur] | - Liver [3 sections collected, 2 examined] |
| - Bone with marrow [rib] | - Lung with mainstem bronchi (2) |
| - Bone with marrow [sternum] | - Lymph nodes: mandibular (2), and mesenteric |
| - Bone marrow smear* | - Mammary gland [process females only] |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons]# | - Nictitans gland (2) |
| - Eye including optic nerve (2) | - Pancreas |
| - Gallbladder | - Pituitary# |
| - Gastrointestinal tract: | - Prostate# |
| esophagus | - Salivary gland, mandibular (2) |
| stomach [cardia, fundus, pylorus] | - Sciatic nerve |
| duodenum | - Skeletal muscle, biceps femoris |
| jejunum | - Skin |
| ileum | - Spinal cord [cervical, thoracic, and lumbar] |
| cecum | - Spleen# |
| colon | - Thymus# |
| rectum | - Thyroid/parathyroid (2)# |
| - Gonads: | - Tongue |
| ovary (2)# | - Trachea |
| testis (2)# with epididymis (2) | - Urinary bladder |
| - Gross lesions | - Uterus [both horns]/Cervix# |
| | - Vagina |
-

*Bone marrow smears were collected at the scheduled sacrifice and M/E ratios were calculated for all animals in Groups 1 and 5.

(2) Paired organ

#Weighed

Histopathology: Adequate Battery: yes (X), no ()

Peer review: yes (X), no ()

Histopathological examinations were conducted on the tissues shown in the above table of sponsor's submission from all dose groups at the scheduled necropsies.

Toxicokinetics: Blood samples were collected from all animals for the determination of the plasma concentrations of alvimopan. Blood samples were collected from the jugular vein at predose and at 0.25, 1, 4, 8, 24, 48, 72, and 96 hours postdose on Day 1 following a single dose administration. Blood samples were collected on Day 32 from all surviving animals at predose and at 0.25, 1, 4, 8, and 24 hours postdose following the second daily

dose and from two dogs/sex from each treatment group (Groups 2-5) at 48, 72, and 96 hours postdose of the second daily dose.

Results:

Mortality: There was no mortality.

Clinical Signs: Treatment-related clinical signs included soft, watery, or mucoid feces.

Body Weights: The mean initial and final body weight of control males were 9.17 and 9.68 kg, respectively. The mean initial and final body weight of control females were 9.28 and 10.40 kg, respectively. There were no treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males were 133.3 and 136.7 g/animal/day, respectively. The mean initial and final food consumption in control females were 200.0 and 140.7 g/animal/day, respectively. Treated males appeared to consume more foods than control animals on a daily basis, however, no such difference was observed in females.

Ophthalmoscopic Examinations: No treatment-related changes were observed.

Electrocardiographic (ECG) Examinations: There were no treatment-related changes.

Hematology: There were no treatment-related changes.

Clinical Chemistry: No treatment-related effects on clinical chemistry parameters were observed.

Urinalysis: No treatment-related effects on urinalysis parameters were observed.

Gross Pathology: There were no significant treatment-related macroscopic changes seen in either male or female dogs at the terminal necropsy.

Organ Weights: There were no treatment-related organ weight changes.

Histopathology: There were no treatment-related histopathologic changes.

Toxicokinetics: Plasma concentrations of alvimopan were low and did not exceed 388 ng/ml (at 250 mg/kg) after a single dose and 266 ng/mL (at 250 mg/kg bid) after repeated dose administration. Plasma concentrations of alvimopan generally declined to <10 ng/ml by 24 hours post dose. Overall, exposure to alvimopan appeared to be dose proportional up to 500 mg/kg/day but not up to 1000 mg/kg/day. Plasma concentrations of ADL 08-0011 were minimal (< 30 ng/mL) following oral administration of alvimopan. Generally, the exposure to alvimopan was found to be greater than ADL 08-0011. The

mean toxicokinetic parameters for alvimopan and ADL 8-0011 in dogs are shown in the following tables (from 362 and 363 of the report of sponsor's submission).

Mean ± SD (N=5/sex) Alvimopan Toxicokinetic Parameters Following Twice Daily Oral Administration of Alvimopan to Male and Female Dogs for 28 Days					
Dosage level (mg/kg)	Sex	C _{max} (ng/mL)	T _{max} ^a (hr)	AUC(0-8 hr) (ng•hr/mL)	AUC(0-24 hr) (ng•hr/mL)
50 bid	M	76.3 ± 35.8	4-8	404 ± 193	837 ± 418
	F	64.2 ± 21.2	0-8	270 ± 146	471 ± 289
	M&F	70.2 ± 28.5	0-8	337 ± 176	654 ± 390
125 bid	M	125 ± 53	0-8	632 ± 226	1360 ± 560
	F	84.3 ± 24.3	0-4	498 ± 234	794 ± 425
	M&F	105 ± 44	0-8	565 ± 228	1080 ± 550
250 bid	M	167 ± 69	1-8	1000 ± 300	2250 ± 1070
	F	128 ± 83	1-8	711 ± 439	1800 ± 1230
	M&F	147 ± 75	1-8	856 ± 387	2020 ± 1110
500 bid	M	108 ± 57	1-8	710 ± 403	1500 ± 940
	F	155 ± 31	4-8	971 ± 219	2100 ± 420
	M&F	131 ± 50	1-8	841 ± 335	1800 ± 760

Note: Dogs were initially administered a single dose, then after 96 hours the dogs were dosed twice daily (approximately 4 hours apart) for 28 days; parameters determined after the second daily dose
a: Range; 0 corresponds to predose

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306

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ENTEREG™ (alvimopan)

NDA 21-775

Mean ± SD (N=3-5/sex) ADL 08-0011 Toxicokinetic Parameters Following Twice Daily Oral Administration of Alvimopan to Male and Female Dogs for 28 Days				
Dosage level (mg/kg)	Sex	C _{max} (ng/mL)	T _{max} (hr)	AUC(0-24 hr) (ng·hr/mL)
50 bid	M	2.10 ± 0.85	4-8	38.4 ± 17.7
	F	5.26 ± 5.32	0-1	68.4 ± 66.1
	M&F	3.68 ± 3.82	0-8	53.4 ± 46.3
125 bid	M	8.22 ± 6.21	4-24	132 ± 104
	F	1.76 ± 0.61	4-24	33.1 ± 12.1
	M&F	4.63 ± 5.13	4-24	77.2 ± 82.8
250 bid	M	2.94 ± 1.28	4-24	53.6 ± 23.7
	F	6.57 ± 10.11	8-24	88.7 ± 149.6
	M&F	5.02 ± 7.44	4-24	73.7 ± 108.3
500 bid	M	3.56 ± 1.89	24	58.3 ± 26.7
	F	5.34 ± 7.58	4-24	100 ± 150
	M&F	4.67 ± 5.89	4-24	84.2 ± 116.6

Note: Dogs were initially administered a single dose, then after 96 hours the dogs were dosed twice daily (approximately 4 hours apart) for 28 days; parameters determined after the second daily dose
a: Range; 0 corresponds to predose

In a 4-week oral (capsule) toxicity study of alvimopan in beagle dogs, animals were treated at 0, 100, 250, 500 and 1000 mg/kg/day for 4 weeks. The target organ of toxicity could not be identified in the absence of any organ toxicity. The NOAEL appeared to be 1000 mg/kg/day. The C_{max} and AUC_{0-24h} at 1000 mg/kg/day were 131 ng/ml (about 13 times human C_{max}) and 1800 ng·hr/ml (approximately 45 times human AUC), respectively (mean AUC_{0-12h} and C_{max} for alvimopan in humans at oral dose of 12 mg b.i.d. dose were 40.2 ng·hr/ml and 10.98 ng/ml, respectively).

Study Title: 2-Week Intravenous Toxicity Study of ADL 08-0011-0 in Beagle Dogs

Key Study Findings: In a 2-week intravenous toxicity study of ADL 08-0011-0 (a metabolite of alvimopan) in beagle dogs, animals were treated at 0.5, 1, and 2 mg/kg/day for 2 weeks. The target organ of toxicity could not be identified in the absence of any organ toxicity. The NOAEL appeared to be 2 mg/kg/day.

Study No.: 808-019

Volume # and Page #: EDR NDA 21-775: pharmtox\tox\808-019.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: May 29, 2002

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: ADL 08-0011-0, 202002, 99.9%

Methods: This study was conducted to evaluate the potential toxicity of ADL 08-0011-0 (amide hydrolysis metabolite of alvimopan) following 2-weeks of once daily intravenous administration in beagle dogs. Three groups of four male and four female dogs (n = 4/sex/group) were administered the test article once daily via intravenous bolus injection for two consecutive weeks at 0.5, 1.0, or 2.0 mg/kg/day at a dose volume of 1 ml/kg. Control animals received the vehicle, 0.9% Sodium Chloride for Injection, USP, at the same dose volume as the treated groups.

Doses: 0.5, 1.0, 2.0 mg/kg/day, i.v. bolus injection

Basis of Dose Selection: Doses were selected based on the results of previous studies. However, details of these previous studies were not mentioned.

Species/Strain: Beagle dogs

Number/Sex/Group (Main Study): 4/sex/group

Route and Formulation, dose volume: Intravenous, solution, 1 ml/kg

Satellite Groups for Toxicokinetics or Recovery: None

Age: 4 to 4.5 months

Study Design: The following table shows the study design (from page 14 of the report of sponsor's submission).

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	4	4
2	0.5	4	4
3	1.0	4	4
4	2.0	4	4

Observation and Times:

Mortality: All animals were examined twice daily for mortality.

Clinical Signs: All animals were examined once daily for clinical signs.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded on a weekly basis.

Ophthalmoscopic Examinations: All animals were examined at pretest and again prior to necropsy.

Electrocardiographic (ECG) Examinations: All dogs received an ECG examination at pretest and again prior to necropsy.

Hematology: Hematology was conducted on all animals at pretest and prior to termination.

Clinical Chemistry: Clinical chemistry was conducted on all animals at pretest and prior to termination.

Urinalysis: Urine analyses were conducted on all animals at pretest and prior to termination.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following table (from page 393 of sponsor's report) shows the organs that were weighed (as indicated by #) from all animals at necropsy:

**Appears This Way
On Original**

244

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NDA 21-775

The following list constitutes the full complement of organs and tissues:

-
-
- | | |
|---|--|
| <ul style="list-style-type: none"> - Adrenal (2) # - Aorta - Bone with marrow [femur] - Bone with marrow [sternum] - Bone with marrow [rib] - Bone marrow smear* [2 collected] - Brain [cerebrum, midbrain, cerebellum, medulla/pons] # - Eye including optic nerve (2) - Gallbladder - Gastrointestinal tract: <ul style="list-style-type: none"> esophagus stomach [cardia, fundus, and pylorus] duodenum jejunum ileum cecum colon rectum - Gonads: <ul style="list-style-type: none"> ovary (2) # testis (2) # epididymis (2) - Gross lesions - Heart # | <ul style="list-style-type: none"> - Injection site [2 collected] - Kidney (2) # - Liver [3 sections collected; 2 examined] # - Lung [2 sections examined] - Lymph node, mandibular [2 collected; 1 examined] - Lymph node, mesenteric - Mammary gland [process females only] - Nictitans gland - Pancreas - Pituitary # - Prostate # - Salivary gland, mandibular [2 collected; 1 examined] - Sciatic nerve - Skeletal muscle, biceps femoris - Skin - Spinal cord [cervical, thoracic, and lumbar] - Spleen # - Thymus # - Thyroid/parathyroid (2) # - Tongue - Trachea - Urinary bladder - Uterus [both horns] with cervix # - Vagina |
|---|--|
-

*Bone marrow smears were collected at the scheduled sacrifice and held.

Organ weighed

(2) Paired organ.

Appears This Way
On Original

808-019

244

Histopathology: Adequate Battery: yes (X), no ()
Peer review: yes (X), no ()

Histopathological examinations were conducted on the tissues shown in the above table of sponsor's submission from all dose groups at the scheduled necropsies.

Toxicokinetics: Blood samples were collected from the jugular vein at predose and at 0.25, 0.5, 1, 4, and 24 hours postdose on Days 1 and 14.

Results:

Mortality: There was no mortality.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial and final body weight of control males were 9.365 and 9.675 kg, respectively. The mean initial and final body weight of control females were 7.158 and 7.233 kg, respectively. There were no treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males were 278.39 and 282.82 g/animal/day, respectively. The mean initial and final food consumption in control females were 256.04 and 233.18 g/animal/day, respectively. Food consumption was increased in the treated animals during week 1 of the treatment. In males during week 1, food consumption was found to be 130%, 120% and 126% of control at 0.5, 1.0 and 2.0 mg/kg/day, respectively. In females during week 1, food consumption was found to be 118%, 125% and 1115% of control at 0.5, 1.0 and 2.0 mg/kg/day, respectively.

Ophthalmoscopic Examinations: No treatment-related changes were observed.

Electrocardiographic Examinations: There were no treatment-related changes.

Hematology: No treatment-related changes were observed.

Clinical Chemistry: There were no treatment-related effects on clinical chemistry parameters.

Urinalysis: No treatment-related effects on urinalysis parameters were observed.

Gross Pathology: There were no significant treatment-related macroscopic changes seen in either male or female dogs at the terminal necropsy.

Organ Weights: There were no treatment-related organ weight changes seen in either sex at the terminal necropsy.

Histopathology: There were no treatment-related microscopic lesions in either sex.

Toxicokinetics: The exposure to ADL 08-0011 appeared to be dose-related from Days 1 to 14 over the dose range of 0.5 to 2 mg/kg/day. There did not appear to be any gender-related differences in the exposure to ADL 08-0011 across dose groups. The toxicokinetic parameters are shown in the following table (from page 203 of the report of sponsor's submission).

Mean ± SD (N=4) ADL 08-0011 Toxicokinetic Parameters Following Daily Intravenous Bolus Injection of ADL 08-0011-0 to Male and Female Dogs for 14 Days					
Dosage level (mg/kg/day)	Sex	Day	C _{max} ^a (ng/mL)	AUC(0-4 hr) (ng•hr/mL)	AUC(0-24 hr) ^b (ng•hr/mL)
0.5	M	1	316 ± 119	450 ± 111	482 ± 101
	F	1	278 ± 36	403 ± 64	403 ± 64
	M	14	436 ± 132	540 ± 103	559 ± 113
	F	14	479 ± 41	478 ± 84	537 ± 95
1	M	1	612 ± 116	1270 ± 480	1320 ± 430
	F	1	698 ± 87	1040 ± 290	1090 ± 210
	M	14	1200 ± 540	1270 ± 600	1710 ± 690
	F	14	1240 ± 160	1340 ± 210	1620 ± 310
2	M	1	1250 ± 350	1980 ± 510	2450 ± 300
	F	1	1500 ± 310	2080 ± 520	2330 ± 700
	M	14	1990 ± 360	2370 ± 600	3340 ± 650
	F	14	1890 ± 240	2220 ± 640	2930 ± 960

a: On Day 1, C_{max} was observed at 15 minutes, the first blood collection time point after dosing (except for male dog# 111 and female dog# 113 in the 0.5 mg/kg/day dose group when C_{max} was observed at 30 minutes post dose); on Day 14, C_{max} was observed at 5 minutes, the first blood collection time point after dosing

b: For individual animals where the last quantifiable plasma ADL 08-0011 concentration was at 4 hours, AUC(0-4 hr) was used to estimate AUC(0-24 hr)

In a 2-week intravenous toxicity study of ADL 08-0011 (a metabolite of alvimopan) in beagle dogs, animals were treated at 0.5, 1, and 2 mg/kg/day for 2 weeks. There were no significant toxicology findings to identify the target organ of toxicity. The NOAEL appeared to be the highest tested dose (2 mg/kg/day). The mean (combined male and female) C_{max} and AUC_{0-4h} value at 2.0 mg/kg/day was found to be approximately 1940 ng/ml (54 times the human C_{max} at the proposed therapeutic dose) and 2295 ng.hr/ml (1.4 times the human AUC at the proposed therapeutic dose of 12 mg b.i.d.), respectively (mean AUC_{0-α} and C_{max} for ADL 08-0011 in humans following oral dose of 12 mg b.i.d. of alvimopan were 1642.5 ng.hr/ml and 35.73 ng/ml, respectively. Data obtained from study 14CL119).

A Chronic Toxicity Study with Alvimopan Administered Orally to Beagle Dogs for 6 Months (Study No. TOX RPT 13/D02393)

6-Month Oral Toxicity Study in Dogs (Study No. D02393)

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IND 56, 553

5

Testing Laboratory: ██████████**Date Started:** August 4, 1993**Date Completed:** February 2, 1994**GLP Compliance:** A statement of compliance is included.

Animals: Beagle dogs (both sexes) were used in this study. Animals were approximately 6 to 15 months old and the initial mean body weights for males and females were 10.0 kg and 9.1 kg, respectively.

Drug Batch: ADL8-2698, Lot 284MH2.

Methods: Beagle dogs (4/sex/group) were administered orally (semi-solid matrix capsule containing polyethylene glycol (PEG) ██████ at single daily doses of 10, 30 and 100 mg/kg for 6-month period. The control animals received capsules containing PEG ██████ only. The doses were selected based on the results of a previous 1-month oral (gavage) toxicity study in dogs at 0, 10, 30, or 100 mg/kg dose levels. No treatment-related effects were observed in this study except slight higher occurrences of emesis compared to control at 30 and 100 mg/kg doses and soft stools at 100 mg/kg dose. The doses selected for the current study were the same as those used in the 1-month oral toxicity study. The high dose was selected as 100 mg/kg because previous data indicated saturation of absorption at doses \geq 100 mg/kg. In addition, previous intravenous 1-month study demonstrated very low toxicity at plasma levels $>$ 4000 ng/ml. Animals were monitored daily for clinical signs of toxicity and morbidity/mortality. Body weights were recorded weekly. Food consumption was estimated visually. Ophthalmic examination was performed on Test Day -14 and Day 170. Physical examinations were conducted on test Day -12 and Day 170. Blood samples were collected prior to initiation of dosing and on Days 57, 120, and 176 for hematology and clinical chemistry. Urinalysis was done prior to initiation of dosing and on Days 92 and 175. Dogs were sacrificed at the end of the treatment period and subjected to a gross necropsy (all dogs). Stool samples were collected for fecal occult blood prior to initiation of dosing and on Days 92 and 175. The following organs were weighed: kidneys, liver, heart, ovaries, testes, prostate, adrenals, thyroid with parathyroid, brain, and pituitary. Histopathological examinations were done on the following tissues collected from all animals: adrenals, aorta, bone (femur and sternum) and bone marrow, brain stem, cerebrum, cerebellum, epididymides, esophagus, eyes, gall bladder, Harderian gland, heart, lacrimal gland, large intestine (cecum, colon, rectum), small intestine (duodenum, ileum, jejunum), kidneys, liver, lungs, lymph nodes, mammary glands, ovaries, pancreas, parathyroid, peripheral nerve (sciatic), pituitary gland, prostate, salivary gland, seminal vesicle, skeletal muscles, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus plus cervix, and vagina.

Results:

IND 56, 553

6

1. **Observed Effects:** Abnormal stool (mucoid, loose, runny, or soft stool) was observed at a low incidence in all treatment groups, however, it was prevalent in dogs at 100 mg/kg dose level. Alopecia was observed in 3 of 8 dogs at 100 mg/kg between Days 49 and 75. Besides these, emesis and salivation was seen in very low incidences and was considered incidental.
2. **Mortality:** None.
3. **Body Weight:** The mean initial (Day -1) and final (Day 181) body weights of control males were 9.62 kg and 10.10 kg, respectively. The mean initial (Day -1) and final (Day 181) body weights of control females were 9.27 kg and 10.82 kg, respectively. The mean final body weight of high dose (100 mg/kg) males and females were 114% and 83% of control, respectively. The growth curves are shown below:

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IND 56, 553

7

Figure D-1.2. Mean Body Weight.

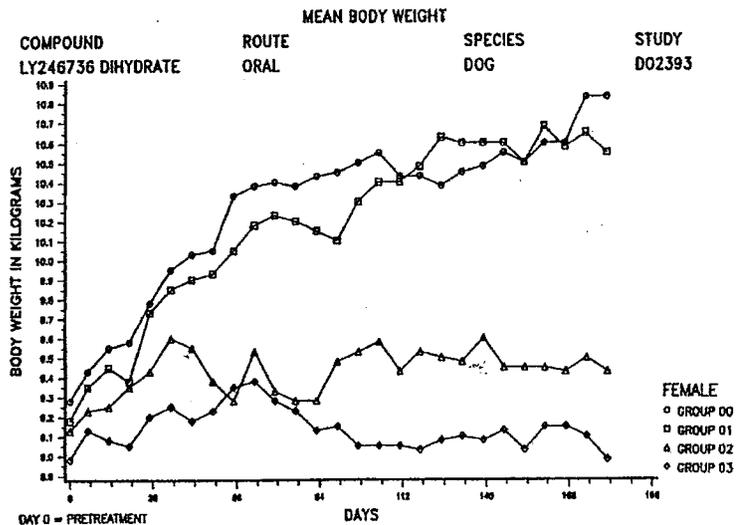
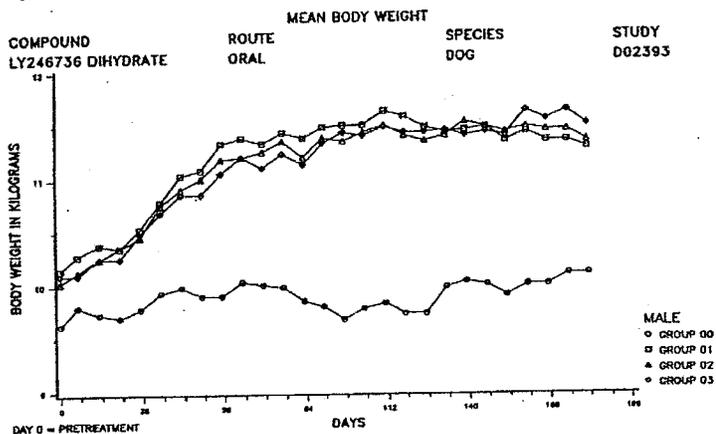


Figure D-1.1. Mean Body Weight.



IND 56, 553

8

4. **Food Consumption:** There were no treatment-related changes in qualitative food consumption.
5. **Ophthalmic Examination:** No treatment-related changes were observed.
6. **Physical Examination:** There were no treatment-related changes.
7. **Hematology:** There were no significant drug-related effects.
8. **Clinical Chemistry:** The blood urea nitrogen values were decreased on day 057 (71% of control, control = 16.77 mg/dL) and day 176 (66% of control, control = 18.40 mg/dL) in high dose males. Creatinine values were decreased significantly on day 057 (80% of control, control = 0.90 mg/dL) in high dose males. Gammaglutamyl transferase values were decreased (60% of control, control = 6.75 IU/L) on day 057 in high dose females. Globulin values were increased (116% of control, control = 2.825 g/dL) in high dose females on day 057. There were no other significant treatment-related changes.
9. **Urinalysis:** No treatment-related changes were observed.
10. **Fecal Occult Blood:** There was no compound-related observation.
11. **Organ Weights:** Relative (to body weight) kidney weight was increased (126% of control, control = 0.42 g/100 g) in high dose females. No other significant treatment-related changes were observed.
12. **Gross Pathology:** No treatment-related changes were observed.
13. **Histopathology:** No drug-related effects were observed.
14. **Toxicokinetics:** Blood samples were drawn from dogs at 4 and 12 hours after dosing on Day 176. The plasma was analyzed for ADL8-2698 using a validated HPLC/MS method. ADL8-2698 was detected in the plasma of either sex at 30 mg/kg dose groups at 4 (male: 11.3 ng/ml; female: 8.73 ng/ml) and 12 (male: 9.93 ng/ml; female: no detectable plasma level was obtained) hour time point. At 100 mg/kg, mean plasma concentrations of drug in male (18.4 ng/ml) and female (18.3 ng/ml) dogs were found to be similar at 4 h after treatment. At 12 h after treatment with 100 mg/kg, the plasma level declined to a mean of 10.6 ng/ml in males (2 of 4 dogs) and 8.5 ng/ml in one female dog (undetectable in other dogs). There appeared to be no demonstrable sex difference in the plasma concentrations of ADL8-2698. Plasma concentrations tended to be higher at 4 hours than 12 hour postdose. Due to interference of one peak, meaningful data could not be obtained on Days 0 and 91. Therefore, no conclusions could be drawn regarding steady state concentration or accumulation of the drug in the plasma.

In a 6-month oral toxicity study of ADL8-2698 in dogs, animals were treated with ADL8-2698 at 0, 10, 30, or 100 mg/kg/day dose levels. The no observed effect level (NOEL) may be considered as 100 mg/kg. The above-mentioned doses did not allow the identification of any target organ of toxicity. There was no mention whether the highest dose was the maximum feasible dose.

IND 56, 553

9

However, previous data indicated saturation of absorption at doses ≥ 100 mg/kg. In addition, previous intravenous 1-month study demonstrated very low toxicity at plasma levels > 4000 ng/ml.

6.6.6.4 Genetic Toxicology

The Effect of Alvimopan on the Induction of Reverse Mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames Test (Study No. 93031AMS3684)

4. Induction of reverse mutations in *Salmonella typhimurium* and *Escherichia coli* using the Ames test.

Testing Laboratory:

Sponsor's laboratory

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: March 4, 1993

Study Completed: September 1, 1993

Strains: *S. typhimurium*: TA1535, TA1537, TA98 and TA100. *E. coli*: WP2uvrA

Methods: The Ames test was used to study the potential of LY246736 dihydrate to produce bacterial mutation. Studies were conducted in the presence of metabolic activation (S9 mixture) or in the absence of metabolic activation. Doses of LY246736 dihydrate to be used were determined in preliminary experiments and were delineated as 312.5, 625, 1250, 2500 and 5000 ng/plate.

The positive control agent in the Ames test without metabolic activation was N-ethyl-N'-nitro-N-nitrosoguanidine for TA1535 and TA100 strains of *S. typhimurium* and the WP2uvrA strain of *E.*

Page 25

coli. The positive control agent in the Ames test without metabolic activation was 9-aminoacridine for the TA1537 strain of S. typhimurium. The positive control agent in the Ames test without metabolic activation was 2-nitrofluorene for the TA98 strain of S. typhimurium. The positive control agent in the Ames test with metabolic activation was 2-aminoanthracene for all strains studied.

The nonactivated assay was conducted by combining 0.05 ml of the appropriate dilution of LY246736 dihydrate, 0.1 ml of the appropriate bacterial tester strain, and 2.5 ml of diluted top agar. For the activated assay, 2 ml of the diluted top agar and 0.5 ml of the S9 mixture were used. Plates were inverted and incubated for approximately 48 hours at approximately 37°C. Revertant colony counts were then assessed. Criteria used for results were that bacteria treated with the vehicle control and positive control agent must yield data consistent with historical data and that a positive increase in number of revertant colonies was defined as a 2-fold increase over control values at 2 successive LY246736 dihydrate doses.

Results: In the Ames test without metabolic activation, LY246736 dihydrate (312.5 to 5000 µg/plate) did not increase the revertant colony counts. The positive control agents did increase the revertant colony counts.

In the Ames test with metabolic activation, LY246736 dihydrate (312.5 to 5000 µl/plate) did not increase the revertant colony counts. The positive control agent 2-aminoanthracene did increase the revertant colony counts.

Study Title: Ames Assay with ADL 08-0011

Key Findings: Negative.

Study No.: AA57TL.503.BTL

Volume # and Page #: EDR NDA 21-775: pharmtox\tox\aa57tl503btl.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: May 17, 2002

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ADL 08-0011, Batch 203004, 99.4%

Methods: ADL 08-0011-0 (a metabolite of alvimopan), was tested using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli*

tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. In this study, the assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of ADL 08-0011. In the initial toxicity-mutation assay, the following dose levels were tested: 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg per plate. No positive mutagenic response was observed in the initial assay. Based on the findings of the initial assay, the following doses were selected for the confirmatory assay: 75, 200, 600, 1800 and 5000 µg per plate.

Cell Line: *Salmonella typhimurium* TA1535, TA1537, TA 98, TA 100 and *Escherichia coli* tester strain WP2 uvrA.

Dose Selection Criteria:

Basis of Dose Selection: Cytotoxicity and solubility.

Range Finding Studies: In the initial range finding assay, the following dose levels were tested: 2.5, 7.5, 25, 75, 200, 600, 1800 and 5000 µg per plate. No positive mutagenic response was observed in the initial assay. Based on the findings of the initial assay, the following doses were selected for the confirmatory mutagenicity assay: 75, 200, 600, 1800 and 5000 µg per plate.

Test Agent Stability: The results of the analysis of dosing solution confirmed that the test article was within the acceptable range (10% of the theoretical target concentration).

Metabolic Activation System: The S9 liver microsomal fraction was prepared from livers obtained from Aroclor 1254 pretreated (single dose of 500 mg/kg, i.p.) male SD rats.

Controls:

Negative Controls: Dimethylsulfoxide (DMSO, final Concentration = 3.7%)

Positive Controls: Without metabolic activation: sodium azide (1.0 µg/plate) for TA 1535, TA100; 9-aminoacridine (75 µg/plate) for TA1537 and 2-nitrofluorene (1.0 µg/plate) for TA 98; Methylmethanesulfonate (1000 µg/plate) for WP2 uvrA. With metabolic activation: 2-aminoanthracene (1.0 µg/plate for all strains except 10 µg/plate for WP2 uvrA).

Comments: None

Exposure Conditions:

Incubation and Sampling Times: 48 to 72 hours

Doses Used in the Definitive Study: 75, 200, 600, 1800 and 5000 µg per plate.

Study Design: The assay was performed in two phases, using the plate incorporation method. The first phase or preliminary dose ranging experiment was conducted to establish the dose range for the confirmatory assay. The second phase is a full mutation assay.

Analysis:

No. of Replicates: All dose levels of test article, vehicle control and positive control were plated in triplicate.

Counting Method: The colonies were counted by using an automated colony counter.

Criteria for Positive Results: The following are the criteria for a positive response: 1) a dose-related increase in the mean revertants of at least one tester strain over a minimum of two increasing concentrations of test article, and/or 2) a reproducible biologically relevant positive response (For TA 98, TA 100 and WP2 uvrA: the number of revertants is at least twice as high as compared to the spontaneous reversion rate or control. For Strain TA 1535 and TA1537: the number of revertants is at least three times higher than the control) for at least one of the test points in at least one strain with or without metabolic activation.

Results:

Study Validity: The study was considered valid as all criteria for a valid study were met.

Study Outcome: Negative. The summary of results is shown in the following table (from page 38 of the sponsor's report).

**Appears This Way
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NDA 21-775

Bacterial Mutation Assay
Summary of Results

Table 22

Test Article Id : ADL 08-0011-0
Study Number : AA57TL.503.BTL Experiment No : B3

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (ug/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	11 ± 2	213 ± 8	15 ± 2	6 ± 1	11 ± 2
75	10 ± 2	228 ± 17	16 ± 4	6 ± 2	11 ± 2
200	10 ± 1	243 ± 37	16 ± 2	4 ± 2	11 ± 3
600	12 ± 2	222 ± 14	22 ± 4	7 ± 2	11 ± 2
1800	12 ± 4	219 ± 21	20 ± 4	5 ± 1	10 ± 0
5000	12 ± 2	198 ± 20	19 ± 1	5 ± 2	12 ± 4
Positive	130 ± 2	653 ± 23	360 ± 41	316 ± 91	83 ± 39

Liver Microsomes: Rat liver S9

Dose (ug/plate)	TA98	TA100	TA1535	TA1537	WP2 uvrA
Vehicle	17 ± 2	183 ± 35	18 ± 4	7 ± 1	17 ± 3
75	18 ± 5	194 ± 17	14 ± 2	7 ± 2	17 ± 2
200	19 ± 3	170 ± 20	15 ± 2	7 ± 2	13 ± 1
600	21 ± 4	160 ± 5	12 ± 5	6 ± 3	19 ± 2
1800	17 ± 3	178 ± 19	15 ± 2	8 ± 1	14 ± 1
5000	18 ± 1	171 ± 3	14 ± 3	7 ± 2	13 ± 3
Positive	230 ± 49	554 ± 40	147 ± 13	80 ± 4	111 ± 22

Vehicle = Vehicle Control
Positive = Positive Control
Plating aliquot: 50 µL

Study No. AA57TL.503.BTL

38

Mutagenicity Test on Alvimopan in an *In Vitro* Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells (Study No. 15488-0-437)

1. Chromosomal aberration assay in Chinese hamster ovary (CAW) cells.

Testing Laboratory:

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study started: January 27, 1993

Study completed: May 11, 1993

Methods: Chinese hamster ovary cells (CAW-WBL) were initiated by seeding approximately 1.5×10^6 cells per 75 cm² flask into 10 ml of complete McCoy's 5a medium. One day after culture initiation, the cultures were treated with test drugs at predetermined doses for 24 hours. Aberration assays were done without metabolic activation and with metabolic activation premix (S9 fraction derived from the liver of male Sprague-Dawley rats which had been previously treated with Aroclor 1254). Negative controls were cultures which contained only cells and culture medium. Solvent controls were cultures containing cells, culture medium and the solvent for the test drug. Positive control agents were mitomycin C for studies without metabolic activation and cyclophosphamide for studies with metabolic activation. One hundred cells from each replicate culture at four dose levels of the test drug and from each of the negative and solvent control cultures were analyzed for the different types of chromosomal aberrations. At least 25 cells were analyzed for chromosomal aberrations from one of the positive control cultures. A significant increase in chromosomal aberrations was statistically

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defined as a significant increase, compared to pooled negative and solvent controls, at the 0.01 level using the Fischer's Exact Test.

Results: No significant increase in cells with chromosomal aberrations was observed in cultures treated with either 838, 1680, 2510 or 3350 µg/ml of LY246736 dihydrate, with or without metabolic activation.

The positive control agents mitomycin C and cyclophosphamide produced significant increases in cells with chromosomal aberrations.

Study Title: Chromosome Aberration Assay Using Chinese Hamster Ovary (CHO) Cells with ADL 08-0011

Key findings: Negative.

Study No: AA57TL.331.BTL

Study Type: *In vitro* cytogenetic assay with mammalian cells.

Volume #, and Page #: EDR 21-775: pharmtox\tox\aa57tl331btl.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: May 6, 2002

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: ADL 08-0011, 203004, 99.4%

Formulation/Vehicle: Solution/Dimethyl sulfoxide (DMSO)

Methods: ADL 08-0011-0 (metabolite of alvimopan) was tested in the chromosome aberration assay using Chinese hamster ovary (CHO) cells in both the absence and presence of an Aroclor-induced S9 activation system. A preliminary assay was performed to establish the dose range for the chromosome aberration assay. In the chromosome aberration assay, the cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9 activated test system, and all cells were harvested at 20 hours after treatment initiation. Two hours prior to the scheduled cell harvest, Colcemid (0.1 µg/ml) was added to duplicate flasks for each treatment condition and the flasks returned to the incubator until cell collection. A concurrent toxicity test was also conducted in

both the non-activated and the S9 activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. Cell viability was determined by trypan blue dye exclusion.

Two hours after the addition of Colcemid, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization, mounted and dried on glass slides and stained with 5% Giemsa. The percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. A minimum of 200 metaphase spreads were examined and scored for chromatid-type (chromatid and isochromatid breaks and exchange figures such as quadriradials, triradials, and complex rearrangements) and chromosome-type (chromosome breaks and exchange figures such as dicentrics and rings) aberrations. Chromatid gaps and isochromatid gaps were recorded but not included in the analysis. Polyploid and endoreduplicated cells were also evaluated from each treatment flask per 100 metaphase cells scored.

Cell Line: Chinese Hamster Ovary (CHO) cells

Dose Selection Criteria:

Basis of Dose Selection: Solubility and cytotoxicity.

Range finding studies: In the preliminary assay, the maximum tested dose was 3846 µg/ml. Visible precipitate was observed in treatment medium at this dose level of 3846 µg/ml. Dose levels ≤ 1154 µg/ml were soluble in treatment medium. Doses were selected based on cell growth inhibition relative to the solvent control. Substantial toxicity (at least 50% cell growth inhibition, relative to the solvent control) was not observed at any dose level in the non-activated and in the S9-activated. In the absence of 50% cytotoxicity, the following dose range was chosen based on the solubility of the test article in the medium: 240 to 3846 µg/ml for all three treatment groups.

Test Agent Stability: The chromosome aberration assay was repeated due to a possible discrepancy in the results of the dosing solution analysis of the dosing solutions prepared on 25 May 2002. The samples initially analyzed by [REDACTED] were not all within 10% of the label claim. The backup samples retained by [REDACTED] were analyzed by [REDACTED] and the results were within 10% of the label claim.

For the retest of the chromosome aberration assay, the dosing preparations were prepared prior to dosing and the analysis samples were sent to [REDACTED] for concentration verification. The results of the analysis indicated that all the dosing preparation concentrations were within ±10% of target concentrations.

Metabolic Activation System: S9 liver microsomal fraction of rats treated with Aroclor 1254 (500 mg/kg, single, i.p. injection) were used in this study.

Controls:

Negative Controls: Distilled water

Positive Controls:

Without S9 mix: Mitomycin C (MMC, 0.1 and 0.2 µg/ml final concentration)

With S9 mix: Cyclophosphamide (CP, 10 and 20 µg/ml final concentrations)

Comments: None

Exposure Conditions:

Incubation and Sampling Times:

Non-activated system: 4 or 20 hours

Activated system: 4 hours

Samples were collected approximately 20 hours after initiation of the treatment for both the systems.

Doses Used in Definitive Study:

+S9: 480.75, 961.5 and 1923 µg/ml

-S9 (4 hr): 480.75, 961.5 and 1923 µg/ml

-S9 (20 hr): 961.5, 1923 and 3846 µg/ml

Study Design: The study design is shown in the following table (from page 14 of the sponsor's study report).

Adolor Corporation

ENTEREG™ (alvimopan)

NDA 21-775

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
-S9	4 hr	16 hr	240, 480.75, 961.5, 1923, 3846
	20 hr	0 hr	240, 480.75, 961.5, 1923, 3846
+S9	4 hr	16 hr	240, 480.75, 961.5, 1923, 3846

Analysis:

No. of Replicates: Two/dose

Counting Method: Manual

Criteria for Positive Results: The test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p < 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls was considered as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations was considered as negative.

Results:

Study Validity: The test was considered valid as the positive control and solvent controls fulfilled the requirements for a valid test.

Study Outcome: Negative. The following table shows the summary of results of this assay (from page 26 of the sponsor's report).

Adolor Corporation

ENTEREG™ (alvimopan)

NDA 21-775

TABLE 10
SUMMARY

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Structural (%)
DMSO	-	4	7.5	200	0.005 ±0.071	3.5	0.5
ADL-08-0011-0							
480.75	-	4	8.4	200	0.005 ±0.071	3.5	0.5
961.5	-	4	8.5	200	0.005 ±0.071	5.0	0.5
1923	-	4	7.4	200	0.010 ±0.100	6.0	1.0
MMC, 0.2	-	4	9.7	200	0.200 ±0.501	4.5	16.0**
DMSO	+	4	9.8	200	0.005 ±0.071	3.0	0.5
ADL-08-0011-0							
480.75	+	4	8.8	200	0.005 ±0.071	3.0	0.5
961.5	+	4	6.2	200	0.015 ±0.158	3.5	1.0
1923	+	4	7.1	200	0.020 ±0.172	5.0	1.5
CP, 10	+	4	6.8	200	0.165 ±0.411	5.0	15.0**
DMSO	-	20	8.3	200	0.025 ±0.186	2.0	2.0
ADL-08-0011-0							
961.5	-	20	7.4	200	0.005 ±0.071	2.5	0.5
1923	-	20	7.6	200	0.050 ±0.240	2.5	4.5
3846	-	20	5.9	200	0.025 ±0.254	2.0	1.0
MMC, 0.1	-	20	8.0	200	0.125 ±0.361	2.5	11.5**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p ≤ 0.05; **, p ≤ 0.01; using Fisher's exact test.

The Effect of Alvimopan on the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells (Study No. 930421MLA3684)

3. Induction of forward mutation at the thymidine kinase locus of L5178Y mouse lymphoma cells.

Testing Laboratory:

Sponsor's laboratory.

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: April 19, 1993

Study Completed: August 20, 1993

Methods: The TK^{-/-} mouse lymphoma cell assay was used to study the potential of LY246736 to produce mammalian cell mutation. Studies were conducted in the presence of metabolic activation (S9 mixture) or in the absence of metabolic activation. The positive test agent in the presence of metabolic activation was ethylmethanesulfonate; in the absence of metabolic activation, 3-methylcholanthrene. The number of viable colonies and the number of TK^{-/-} mutant colonies were assessed.

Concentrations of LY246736 dihydrate (1000, 1200, 1400, 1600, 1800, 2000, 2200, and 2400 µg/ml) to be used in the absence of metabolic activation were determined in preliminary toxicity tests. Concentrations of LY246736 dihydrate (1000, 1500, 2000, 2400, 2600, 2800, and 3000 µg/ml) to be used in the presence of metabolic activation were determined in preliminary toxicity and precipitation tests. Incubation of TK^{-/-} cells with LY246736 dihydrate was performed in a roller drum (20 to 30 rpm) at approximately 37°C for approximately 4 hours. To allow expression of TK^{-/-} mutants, the cells were incubated in a roller drum at approximately 37°C for approximately 48 hours. Prepared plates of cells and cloning medium were then incubated for 12 ± 2 days at approximately 37°C in a humidified 95%/5%:air/CO₂

Page 24

environment. Criteria used for results were that cell colonies treated with the vehicle control and positive control agent must yield data consistent with historical data and that a positive increase in number of mutant colonies was defined as a 2-fold increase over control values at 2 successive LY246736 dihydrate doses.

Results: In the mutation assay without metabolic activation, LY246736 dihydrate (1000 to 2400 $\mu\text{g/ml}$) had no effect on mutation frequency (TK⁺ mutants per 1×10^6 colony forming cells) or mutation index (mutation frequency of treated culture divided by mutation frequency of solvent control). The positive control agent ethylmethanesulfonate increased mutation frequency and mutation index.

In the mutation assay with metabolic activation, LY246736 dihydrate (1000 to 3000 $\mu\text{g/ml}$) had no effect on mutation frequency or mutation index. The positive control agent 3-methylcholanthrene increased mutation frequency and mutation index.

The Effect of Alvimopan Given Orally for Two Consecutive Days on the Induction of Micronuclei in Bone Marrow of ICR Mice (Study No. 930331MNT3684)

**Appears This Way
On Original**

2. Micronucleus test in mice.

Testing Laboratory:

Sponsor's laboratory.

Compliance with Good Laboratory Practices and Quality Assurance Requirements:

Sponsor provided statements of compliance with good laboratory practice regulations and quality assurance requirements for this nonclinical laboratory study.

Study Started: March 25, 1993

Study Completed: June 25, 1993

Animals: 15 M and 15 F ICR mice. Males weighed between 30.3 to 35.2 g and females weighed between 25.2 and 30.1 g; ages were approximately 8 to 9 weeks.

Methods: LY246736 dihydrate in 10% aqueous acacia solution was administered on 2 consecutive days by gavage at doses of 500, 1000 and 2000 mg/kg to three groups of 10 mice each; each group consisted of 5 males and 5 females. Selection of doses was based upon a preliminary dose range-finding study. The test article was tested to toxic levels; i.e., the maximum tolerated dose or one half of the median lethal dose. Since the test article did not elicit a toxic response, it was tested at a maximum dose of 2000 mg/kg. The positive control agent was cyclophosphamide.

Approximately 24 hours after the second drug treatment, mice were sacrificed, femurs were removed, and bone marrow slides were prepared. (It should be pointed out that it is recommended in the guidelines that if two treatments are employed, a minimum of 2 samples should be obtained between 20 and 48 hr after the last dose.) A combined total of 1000 polychromatic (PCE) and normochromatic erythrocytes (NCE) were counted for each animal using a differential cell counter; the ratio of the number of PCE

Page 23

to the number of NCE provides an index of toxicity. A total of 1000 PCEs were examined for each animal and evaluated for the presence of micronuclei. Criteria used for results were that animals treated with the vehicle control and positive control agent must yield MN frequencies consistent with historical data.

Results: LY246736 dihydrate did not produce any significant increases in micronucleated polychromatic erythrocytes or changes in the PCE/NCE ratio. The positive control agent cyclophosphamide produced significant increases in micronucleated polychromatic erythrocytes.

2.6.6.5 Carcinogenicity

The sponsor did not include any carcinogenicity study report in this submission.

2.6.6.6 Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

A Combined Segment I and Segment II Study of Alvimopan Administered Orally to CD Rats (Study No. R12693 and R12793)

REPRODUCTIVE TOXICOLOGY

Rats

1. Modified Segment I Reproductive Toxicity Study in Male and Females (Report No. R12693 and No. R12793)

Testing Laboratory: _____

Compliance with Good Laboratory Practices and Quality Assurance Requirements: Certification of compliance was provided by the sponsor.

Appears This Way
On Original

Although the sponsor submitted male data as Report No. R12693 and female data as Report No. R12793, the males and females were treated with LY246736 dihydrate and mated to one another. Thus, there was a single reproductive toxicity study.

Study Started: November 6, 1993

Study Completed: November 28, 1994

Animals: Male (Mean body weight of 383.1 g; 12 weeks of age) and female (Mean body weight of 220.1 g; 9 weeks of age) CD rats.

Methods: Sponsor selected LY246736 dihydrate doses of 0, 50, 100 and 200 mg/kg/day for the present study that were based upon results from a 6-month oral toxicity study in rats (Report No. R05693). The sponsor stated that data in Report No. R05693 indicated that oral doses higher than 200 mg/kg/day would not be expected to produce greater systemic exposure in rats. Thus, four groups of male rats (20/group) were orally administered 0, 50, 100 and 200 mg/kg/day of LY246736 dihydrate by gavage, respectively, for 4 weeks prior to mating (prematuring phase), during cohabitation (1:1) for up to 15 days and through 7 total weeks of LY246736 dihydrate treatment. Four groups of female rats (20/group) were orally administered 0, 50, 100 and 200 mg/kg/day of LY246736 dihydrate by gavage, respectively, for 2 weeks prior to mating (prematuring phase), during cohabitation (1:1) for up to 15 days, and through Day 19 of gestation (gestation phase). Vehicle was 10% aqueous acacia solution; LY246736 dihydrate concentrations of 11.1, 22.1 and 44.1 mg/ml were used for the 50, 100 and 200 mg/kg/day doses, respectively.

All rats were observed daily for survival and general condition. In the case of males, body weights were measured weekly. Detailed physical examinations were done on body weighing days. Food consumption was measured weekly only during the prematuring period. In the case of females, postmating body weights were obtained on Days 0, 3, 6, 10, 14, 17 and 20 of gestation. Detailed physical examinations were done on body weighing days and termination day. Food consumption was measured weekly during the prematuring phase and on Days 0, 3, 6, 10, 14, 17 and 20 during the gestation phase.

Reproductive performance was assessed by evidence of copulation (expelled and/or retained copulatory plugs) and presence of vaginal sperm in the female. Rat pairs were separated on the day that mating was confirmed.

In the case of males, sperm were collected from the right cauda epididymis and a small portion of the vas deferens by the diffusion method on the day of termination. Relative sperm concentrations were estimated with an automated sperm motility analyzer. Parameters of percent motile sperm, percent

progressively motile sperm, linear index, amplitude of lateral head displacement, beat/cross frequency, curvilinear velocity, average path velocity and straight-line velocity were determined. Sperm aliquots were microscopically examined for sperm breakage.

Rats were sacrificed by carbon dioxide asphyxiation. Weights of kidneys, heart, liver, spleen, testes, epididymis, prostate, adrenals, pituitary and brain were determined in all animals. Gross pathological assessments of kidneys, heart, liver, spleen, thymus, testes, epididymis, prostate, adrenals, pituitary, cerebrum, cerebellum and brain stem were made in all animals. Histopathological evaluations were limited to the testes and epididymis; only 6 animals in the 0 and 200 mg/kg/day groups, respectively, were histopathologically examined.

In the case of females, on Day 20 of gestation, live fetuses were delivered by Caesarean section. Adult rats were then sacrificed by carbon dioxide asphyxiation on Day 20 of gestation. Weights of maternal kidneys, heart, liver, spleen, ovaries, uterus, brain, adrenals, and pituitary were determined in all animals. Gross pathological assessments of kidneys, heart, liver, spleen, thymus, ovaries, uterus, cerebellum, cerebrum, brain stem, adrenals, and pituitary were made in all animals.

Ovaries and uteri were removed, uteri weighed and number of corpora lutea determined. Uteri were opened; number of implantations, live and dead fetuses and resorptions were determined. Live fetuses were weighed; live and dead fetuses were examined externally for anatomical anomalies and gender determination. One-half of each litter was sacrificed and fixed in Bouin's stain for visceral examination. The remaining pups of each litter were sacrificed, eviscerated and processed for skeletal examination.

Results:

Males

1. Observed Effects: There were no treatment-related clinical signs of toxicity.
2. Mortality: There was no treatment-related mortality.
3. Body Weight: Initial mean body weight of control males was 384.0 g. Final mean body weight of control males was 590.2 g. There were no treatment-related effects on body weight.
4. Food Consumption: Initial mean food consumption of control males was 28.7 g/day. Mean food consumption of control males on Day 28 of treatment was 29.5 g/day. There were no treatment-related effects on food consumption during the pre-mating phase.

5. Reproductive Performance: As illustrated in the following table, there were no treatment-related effects on fertility and general reproductive performance in males.

Fertility and General Reproductive Performance of Males

Treatment Dose (mg/kg/day, p.o.)	Vehicle	LY246736 Dihydrate		
	0	50	100	200
Parameter measured:				
Male data				
# Paired	20	20	20	20
# Died (After mating)	0	0	0	0
# Successfully Mating	18/20	19/20	20/20	19/20
Precoital period (days)	2.7	3.9	2.0	2.8

6. Sperm Evaluation: Control males had 15.56 million sperms/ml of fluid from the right cauda epididymis and a small portion of the vas deferens. There were no treatment-related effects on sperm concentration.

As shown in the following table, there were no treatment-related effects on sperm motion characteristics.

Summary of Epididymal Sperm Motion Characteristics

Motile Characteristic	LY246736 Dihydrate (mg/kg/day, p.o.)			
	0	50	100	200
Motile (%)	89.0	79.6	78.6	91.5
Progressively Motile (%)	20.3	18.3	17.8	21.3
Linear Index (%)	74.6	72.6	75.9	73.4
Lateral Head Displacement (μ)	17.7	16.5	16.1	17.4
Beat/Cross Frequency (Hz)	13.5	12.7	14.7	13.4
Curvilinear Velocity (μ/sec)	338.7	310.6	305.9	330.6
Average Path Velocity (μ/sec)	213.5	199.7	184.6	208.9
Straight-line Velocity (μ/sec)	155.6	144.5	137.3	150.1

There was 6.6, 14.0, 20.5 and 5.8% sperm breakage in the 0, 50, 100 and 200 mg/kg/day groups, respectively.

Thus, there were no treatment-related effects on sperm concentrations, sperm motion characteristics and sperm breakage

7. Organ Weights: There were no treatment-related effects on organ weights.

8. Gross Pathology: There were no treatment-related gross pathological lesions.

9. Histopathology: There were no treatment-related histopathological lesions. However, histopathological evaluations were limited to the testes and epididymis; only 6 animals in the 0 and 200 mg/kg/day groups, respectively, were histopathologically examined. Furthermore, the 2 males in the 200 mg/kg/day group that mated but did not impregnate were not histopathologically examined. Thus, it would seem advisable to conduct histopathological examinations for at least the remaining male rats in the 0 and 200 mg/kg/day groups.

Females

Dams

1. Observed Effects: There were no treatment-related clinical signs of toxicity.

2. Mortality: There was no treatment-related mortality

3. Body Weight: Initial mean body weight of control females was 221.6 g. Mean body weight of control rats on Day 14 (last day of pre-mating phase) was 248.4 g. There were no treatment-related effects on body weight during the pre-mating phase of the study. Mean body weight of control rats on Day 0 of the gestation phase was 252 g. Mean body weight of control rats on Day 20 of the gestation phase was 420 g. There were no treatment-related effects on body weight during the gestation phase of the study.

4. Food Consumption: Mean food consumption of control females on Day 7 of the pre-mating phase was 19.9 g/day. Mean food consumption of control females on Day 14 of the pre-mating phase was 19.6 g. There were no treatment-related effects on food consumption during the pre-mating phase. Mean food consumption of control females on Days 0-3 of gestation was 23.2 g/day. Mean food consumption of control females on Days 17-30 of gestation was 29.3 g/day. There were no treatment-related effects on body weight during the gestation phase.

5. Reproductive Performance: As illustrated in the following table, there were no treatment-related effects on fertility and general reproductive performance in females.

Fertility and General Reproductive Performance of Females

Treatment Dose (mg/kg/day, p.o.)	Vehicle 0	<u>LY246736 Dihydrate</u>		
		50	100	200
<u>Parameter measured:</u>				
<u>Female data</u>				
# Paired	20	20	20	20
# Died during pregnancy	0	1*	1	0
# Non-pregnant	2	2	3	2
# With total resorption	0	0	0	0
# With live fetuses on Day 20	16	17	17	17

*Sacrificed to avoid natural delivery

6. Organ Weights: There were no treatment-related effects on organ weights.

7. Gross Pathology: There were no treatment-related gross pathological lesions.

8. Maternal and Litter Data: As shown in the following table there were no treatment-related effects on the indicated parameters for dams and litters.

Summary of Maternal and Litter Data in a Modified Segment I Reproductive Toxicity in Rats

Treatment Dose (mg/kg/day, p.o.)	Vehicle 0	<u>LY246736 Dihydrate</u>		
		50	100	200
<u>Parameter measured:</u>				
<u>Dam data</u>				
# Dosed rats	20	20	20	20
# Gravid rats	16	17	17	17
# Aborted	0	0	0	0
# which died	0	1*	1	0
Mean Weight Change (g)	85	85	94	93
<u>Litter data</u>				
# Corpora lutea/dam	17.0	19.0	18.9	18.5
# Implantations/dam	15.7	17.2	14.4	16.4
# Preimplantation losses/dam	1.4	1.8	4.5	2.2
# Resorptions/dam	0.7	1.2	1.3	0.7
# Living fetuses/dam	15.0	16.0	13.1	15.6
Mean fetal weight (g)	3.66	3.67	3.78	3.67

*Sacrificed to avoid natural delivery

As shown in the following table for the F₁ litters, there were no treatment-related effects on external anomalies and variations and visceral anomalies and variations. However, there were treatment-related increases in skeletal variations. The 200 mg/kg/day dose produced incomplete ossification of cervical vertebra, ischium and occipital bone.

Summary of Anomalies and Variations in the F₁ Generation in a Modified Segment I Reproductive Toxicity Study in Rats

Treatment Dose (mg/kg/day, p.o.)	Vehicle 0	<u>LY246736 Dihydrate</u>		
		50	100	200
# Litters examined	16	16	16	17
# Fetuses examined	240	256	210	266
<u>External anomalies</u> (# of fetuses/# of litters affected)				
Head/neck-jaw absent	1/1	---	---	---
Head/neck-mouth-absent	1/1	---	---	---
Head/neck-nares-absent	1/1	---	---	---
# Litters examined	16	16	16	17
# Fetuses examined	240	256	210	266
<u>External variations</u> (# of fetuses/# of litters affected)				
Forelimb Hematoma	---	---	---	1/1
Head/Neck Hematoma	3/2	2/2	---	3/3
Filamentous Perineum on Tail	---	---	1/1	---
Displaced testicles	---	---	1/1	---
# Litters examined	16	16	16	17
# Fetuses examined	123	133	109	137
<u>Visceral anomalies</u> (# of fetuses/# of litters affected)				
Cavitation of Kidney	8/4	1/1	1/1	2/2

# Litters examined	16	16	16	17
# Fetuses examined	123	133	109	137
<u>Visceral variations</u> (# of fetuses/# of litters affected)				
Dark Adrenals	3/2	1/1	2/2	---
Hematoma in Kidney	---	---	1/1	---
Misshapen kidney	---	---	1/1	---
# Litters examined	15	16	15	17
# Fetuses examined	117	123	101	129
<u>Skeletal variations</u> (# of fetuses/# of litters affected)				
Incomplete ossification of vertebral column	3/2	1/1	3/2	10/6
Incomplete ossification of ischium	---	---	---	3/3
Incomplete ossification of occipital bone	2/1	---	2/2	6/3

In summary, there were no treatment-related effects of LY246736 dihydrate during a modified Segment I reproductive toxicity study of fertility in males. There were no treatment-related effects of LY246736 dihydrate in pregnant females. However, LY246736 dihydrate produced skeletal variations in fetuses; 200 mg/kg/day of LY246736 dihydrate produced incomplete ossification of cervical vertebra, ischium and occipital bone.

Study Title: A Segment I Fertility and Reproductive Performance Study of Alvimopan Administered Intravenously to Sprague Dawley Rats

Key Study Findings: In a Segment I study in rats, animals were administered alvimopan at 0, 0.2, 2 and 5 mg/kg/day by i.v. route. There were no apparent treatment-related adverse effects on the reproductive parameters of male or female rats or on the development of fetuses. There was an apparent treatment-related increase in the number of pre-implantation loss. However, these increases were neither dose-related and nor statistically significant. The NOAEL for reproductive toxicity for alvimopan, under the conditions of this study was considered as 5 mg/kg/day for both male and female animals.

Study No.: R10094, R09994

Volume #, and page #: EDR: NDA 21-775: pharmtox\tox\pd-fr-016-0701.pdf

Conducting Laboratory and Location: _____**Date of Study Initiation:** April 13, 1994**GLP Compliance:** A statement of compliance was included.**QA Reports:** yes (X) no ().**Drug, Lot #, and % Purity:** Alvimopan, Lot No. 284MH2, 90.4%

Methods: This Segment I study was conducted to assess the reproductive and early embryonic developmental toxicity of alvimopan dihydrate in rats. Alvimopan was administered intravenously to rats (20/sex/group) at 0, 0.2, 2 or 5 mg/kg/day (dose volume = 1.0 ml/kg). Males were treated for 4 weeks prior to cohabitation, throughout 14 days of cohabitation and until termination. Females were treated for 2 weeks prior to cohabitation, throughout cohabitation and continuing through Gestation Day 6 (GD6). The females were euthanized on GD20 for assessment of reproductive parameters and fetal external examinations. The males were euthanized after approximately 7 weeks of treatment. Sperms were collected for evaluation of relative concentration, motility characteristics and breakage. The testes and left epididymides were weighed and preserved for histopathologic evaluations.

Doses: 0, 0.2, 2 and 5 mg/kg/day, i.v.**Species/Strain:** Sprague Dawley rats**Number/Sex/Group:** 20/sex/group**Route, Formulation, Volume:** Intravenous, solution in distilled water, 1 ml/kg**Satellite Groups used for Toxicokinetics:** None.

Study Design: Four groups of 40 animals (20/sex/group, a total of 160 animals) were used in this study. The study design is shown below (from page 10 of the report of the sponsor's submission).

Treatment Group	Animals per Group		Dose Level (mg/kg b.w./day)	Dose Route
	Male	Female		
00	20	20	0.0 ⁽¹⁾	I.V.
01	20	20	0.2	I.V.
02	20	20	2.0	I.V.
03	20	20	5.0	I.V.

(1) vehicle only – sterile water, pH approximately 10.5.

Parameters and Endpoints Evaluated: Animals were observed for clinical signs and mortality on a daily basis. Food consumption and body weight was recorded weekly. Females were euthanized on Gestation Day 20 and the ovaries and uterus were removed, the uterus was weighed and the numbers of corpora lutea were recorded for each ovary. The uterus was opened and the number and distribution of implantations, live and dead fetuses and resorptions were recorded. Live and dead fetuses were examined externally for anatomical anomalies and gender determination. Fetuses were examined for variations, deviations and malformations. Male animals were euthanized following the termination of the mating period, after about 7 weeks of treatment. Each animal was subjected to a gross necropsy and the epididymides, testes, seminal vesicles and prostate were collected and preserved for histopathologic evaluations. In addition, sperm count and motility were also determined.

Results:

Mortality: There were no treatment-related mortalities. One male rat at 5 mg/kg/day was found moribund, following dosing, and was sacrificed for humane reason. Clinical signs for this animal included blood around the left ear, pale color and lethargy. One female rat at 0.2 mg/kg/day died following a dose administration accident. These deaths were considered due to dosing accidents and were not considered treatment-related.

Clinical Signs: There were no significant treatment-related clinical signs. It is to be mentioned here that alopecia was observed in both the control and treated animals.

Body Weight: The mean initial (Day 0) and final (Day 49) weights of the control males were 381.3 and 503.6 g, respectively. The mean initial (Day 0) and final (Day 14) body weight of control females were 228.8 and 258.4 g, respectively. There were no significant treatment-related changes.

Food Consumption: The mean initial and final food consumption in control males were 27.3 and 26.0 g/animal/day, respectively. The mean initial and final food consumption in control females were 18.8 and 19.2 g/animal/day. There were no significant treatment-related changes.

Toxicokinetics: None.

Necropsy: No significant treatment-related changes were observed in F0 males or F0 females when compared to control.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Pre- and Post-implantation loss etc.): There were no treatment-related changes in mating/fertility index, corpora lutea, pre- or post-implantation losses, and early and late resorptions. However, although not dose-related and not statistically

significant, there is an apparent treatment-related increase in the number of pre-implantation loss (as shown in the following table). No treatment-related differences were observed in relative sperm count, sperm motility characteristics or sperm breakage. Fertility and reproductive parameters for hysterectomized rats on GD 20 are shown in the following table:

Parameter	0 mg/kg/day	0.2 mg/kg/day	2.0 mg/kg/day	5.0 mg/kg/day
N=	17	16	20	18
Mating Index, %	95	94.7	100	95
Fertility Index, %	NA	NA	NA	NA
Corpora lutea/dam	17.66	17.44	17.40	17.61
Implant sites/dam	16.88	16.38	14.65	17.39
Pre-implantation loss, %	4.55	6.28	14.96	1.89
Post-implantation loss, %	NA	NA	NA	NA
Resorptions/litter				
- Total	1.24	0.88	1.05	1.22
- early	1.24	0.88	1.00	1.22
- late	0	0	0.05	0
Live fetuses/dam	15.65	15.50	13.55	16.17
Dead fetuses/dam	0	0	0	0

NA: Data not available.

In an intravenous Segment I fertility and reproductive performance study in rats, animals were administered alvimopan at 0, 0.2, 2 and 5 mg/kg/day. There were no apparent treatment-related adverse effects on the reproductive parameters of male or female rats or on the development of fetuses. There was an apparent treatment-related increase in the number of pre-implantation loss. However, these increases were neither dose-related and nor statistically significant. The NOAEL for reproductive toxicity for alvimopan, under the conditions of this study was considered as 5 mg/kg/day for both male and female animals. In the absence of toxicokinetic data, exposure level could not be compared with that of human.

Study Title: Segment II Intravenous Teratology Study in the Rabbit

Key Study Findings: In the Segment II teratology study in the rabbit, four groups of dams (20/group) were administered intravenous bolus injection of alvimopan at 5, 10, and 15 mg/kg/day from days 6 to 18 of gestation. Overall, no external, visceral and skeletal changes were observed in the pups that could be attributed to alvimopan treatment. The NOAEL for maternal toxicity and developmental toxicity was determined as 15 mg/kg/day. Alvimopan was not considered teratogenic in the rabbit at the tested doses.

Study No.: 4401-002/14TX004

Volume #, and Page #: EDR: NDA 21-775: pharmtox\tox\4401-002.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: February 17, 2002

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ().

Drug, Lot #, and % Purity: Alvimopan, Lot 01-0088, 99.09%

Methods: In this study, timed mated New Zealand White rabbits were randomly assigned to four dosage groups (Groups I through IV, n = 20/group). Alvimopan, and the vehicle (0.9% aqueous sodium chloride solution), were administered intravenously (bolus injection) once daily to these female rabbits on gestation days 6 (GD6) through GD18 at dosages of 0, 5, 10 and 15 mg/kg/day (6 ml/kg).

Doses: 0, 5, 10 and 15 mg/kg/day by slow i.v. bolus injection

Species/Strain: New Zealand White rabbits

Number/Group: 20/group

Route, Formulation, Volume: Intravenous, solution, 6 ml/kg.

Satellite Groups Used for Toxicokinetics: None.

Study Design: The following table (from page 29 of the sponsor's report) shows the study design.

2.7.1. Dosage Administration

Dosage Group	Dosage* (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers
I	0 (Vehicle)	0	6	20	8601-8620
II	5	.83	6	20	8621 - 8624, 4495 ^b , 8626 - 8640
III	10	1.7	6	20	8641-8660
IV	15	2.5	6	20	8661-8680

a. The test article was considered 100% pure for the purpose of dosage calculations.
 b. Rabbit 8625 was removed from study due to reduced body weight and was replaced with rabbit 4495.

Parameters and Endpoints Evaluated: All rabbits were observed daily for mortality and for general appearance and clinical signs. Body weights were recorded on a daily basis. Food consumption values were recorded daily throughout the study. Blood samples were collected from five rabbits per Groups II through IV on GD6 and GD18 at predose and at approximately 15 minutes, 30 minutes, 60 minutes and 8 hours postdose. The surviving rabbits were Caesarean-sectioned on GD29 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number of corpora lutea in each ovary was recorded. The uterus was excised and examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. All fetuses were weighed and examined for gross external and skeletal alterations.

Results:

Mortality (Dams): There were no mortalities in this study.

Clinical Signs (Dams): Treatment-related clinical signs included localized alopecia (limbs, underside or back), soft or liquid feces, ungroomed coat, lacrimation and scanty feces. However, the incidences were not dose-related and the signs were observed in only a few rabbits at any dosage group.

Body Weight (Dams): The initial and final body weights of control animals were 3.39 and 3.69 kg, respectively. There were no significant treatment-related changes.

Food Consumption (Dams): The mean initial and final food consumption in control animals were 174.6 and 161.0 g/animal/day, respectively. There were no treatment-related changes.

Toxicokinetics: The exposure to alvimopan and ADL 08-0011 was dose-related from GD6 through GD18. Exposure to alvimopan was found to be greater than that of the metabolite, ADL 08-0011. The exposure to alvimopan appeared similar on GD6 and GD18; however, the exposure to the metabolite ADL 08-0011 appeared to increase between GD6 and GD18. The mean AUC_{0-1h} value at 15 mg/kg/day at GD6 and GD18 were 205- and 277-times the human exposure at the proposed human dose of 12 mg b.i.d. (mean AUC_{0-12h} and C_{max} for alvimopan in humans at oral dose of 12 mg b.i.d. dose 40.2 ng.hr/ml and 10.98 ng/ml). The mean toxicokinetic parameters are shown in the following table (from page 190 of the sponsor's report).

The data are summarized in the following tables:

Mean ± SD (N=5) Alvimopan Toxicokinetic Parameters Following Intravenous Bolus Injection of Alvimopan to Pregnant Rabbits from Gestation Day 6 to 18				
Dosage level (mg/kg/day)	DG 6		DG 18	
	C _{max} ^a (ng/mL)	AUC(0-1 hr) (ng·hr/mL)	C _{max} ^a (ng/mL)	AUC(0-1 hr) ^b (ng·hr/mL)
5	6130 ± 1710	2770 ± 780	4480 ± 1920	2170 ± 860
10	10000 ± 3800	4930 ± 1540	9350 ± 2270	4320 ± 980
15	18300 ± 6600	8200 ± 2550	23800 ± 5000	11100 ± 2200

a: C_{max} was observed at 15 minutes, the first blood collection time point after dosing
 b: Although on DG 18 alvimopan was measurable in plasma 8 hours post dose for many samples, the profile was not characterized sufficiently between 1 and 8 hours to include the 8 hour post dose concentrations in the AUC calculations

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Mean ± SD (N=5) ADL 08-0011 Toxicokinetic Parameters Following Intravenous Bolus Injection of Alvimopan to Pregnant Rabbit on Gestation Day 6			
Dosage level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (hr) ^c	AUC(0-8 hr) (ng·hr/mL)
5	23.5 ± 2.8	0.25	22.8 ± 21.9
10	46.3 ± 5.3	0.25	113 ± 71
15	71.3 ± 10.2	0.25	143 ± 102

c: Range

Mean ± SD (N=5) ADL 08-0011 Toxicokinetic Parameters Following Intravenous Bolus Injection of Alvimopan to Pregnant Rabbits on Gestation Day 18				
Dosage level (mg/kg/day)	C _{max} (ng/mL)	T _{max} (hr) ^c	AUC(0-8 hr) (ng·hr/mL)	AUC(0-24 hr) (ng·hr/mL)
5	28.3 ± 16.9	0.25 - 1.0	158 ± 133	333 ± 359
10	47.9 ± 12.8	0.25 - 1.0	265 ± 147	675 ± 419
15	94.0 ± 19.3	0.25 - 0.5	378 ± 165	839 ± 457

c: Range

Terminal and Necroscopic Evaluations: C-Section Data (Implantation Sites, Pre- and Post-Implantation Loss etc.): Caesarean-sectioning and litter parameters were unaffected by alvimopan treatment up to 15 mg/kg/day. The number of corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the four dosage groups and there were no significant treatment-related changes. There were no dead fetuses. The following table (from page 53 of the sponsor's report) shows the maternal intrauterine observations.

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PROTOCOL 4401-007: INTRAVENOUS DEVELOPMENTAL TOXICITY STUDY OF ALVINOPAN (ADD. N. 3698) IN RABBITS
(SPONSOR'S STUDY NUMBER: 14TK004)

TABLE 7 (PAGE 1) CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	5	10	15
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	20(100.0)	20(100.0)	19(95.0)	19(95.0)
ABORTED AND SACRIFICED	N(%)	0(0.0)	0(0.0)	0(0.0)	1(5.3)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	20	20	19	18
CORPORA LUTEA	MEAN±S.D.	11.2 ± 1.0	10.9 ± 1.0	10.8 ± 2.3	11.6 ± 2.2
IMPLANTATIONS	MEAN±S.D.	9.1 ± 1.9	9.6 ± 1.5	9.0 ± 2.5	9.8 ± 2.1
LITTER SIZES	MEAN±S.D.	8.9 ± 1.9	9.4 ± 1.6	9.4 ± 2.5	9.4 ± 2.2
LIVE FETUSES	N	178	168	163	170
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.5	0.4 ± 0.7	0.3 ± 0.5
EARLY RESORPTIONS	N	3	1	3	4
LATE RESORPTIONS	N	2	2	5	2
DOES WITH ANY RESORPTIONS	N(%)	4(20.0)	2(10.0)	8(42.1)	6(33.3)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	20(100.0)	20(100.0)	19(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	20(100.0)	20(100.0)	19(100.0)	18(100.0)

^a Dosage occurred on days 6 through day 18 of gestation.

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Offspring (Malformations, Variations etc.): One Fetus (8673-8) in the 15 mg/kg/day group had gastroschisis (the liver, intestines and the urinary bladder protruded through the abdominal opening). Soft tissue examination confirmed the gastroschisis and revealed moderate dilation of the left renal pelvis. There were no other gross external alterations. One fetus in each of the 5 and 15 mg/kg/day dosage groups had a circumcorneal hemorrhage in the right eye and was attributed to trauma during processing. Absence of the intermediate lobe of the lung was observed in 2 and 3 fetuses from 1 and 2 litters in the 5 and 10 mg/kg/day groups, respectively. The fetal alterations observed in this study were not considered treatment-related for the following reasons: 1) the incidences were not dose-related, 2) alterations were seen in only one fetus, and 3) the litter incidence was within the testing facility historical control ranges. Overall, no external, visceral and skeletal changes were observed that could be attributed to alvimopan treatment. Alvimopan was not considered teratogenic in the rabbit. Fetal external, visceral and skeletal alterations following i.v. alvimopan administration in the rabbit are summarized in the following table.

Parameter	0 mg/kg/day	5.0 mg/kg/day	10 mg/kg/day	15 mg/kg/day
Litters Evaluated (N)	20	20	19	18
Fetuses Evaluated (N)	178	188	163	170
Live (N)	178	188	163	170
External Alterations (Fetal Incidence)				
Gastroschisis	0	0	0	1**
Fetal Visceral Aberrations (Fetal Incidence)				
Eyes: Circumcorneal hemorrhage	0	1	0	1
Lungs: Intermediate lobe absent	0	2	3	0
Liver: Protruded through abdominal opening	0	0	0	1**
Kidneys: Dilated, moderate	0	0	0	1**
Intestine: Protruded through abdominal opening	0	0	0	1**
Bladder: Protruded through abdominal opening	0	0	0	1**
Skeletal Aberrations (Fetal Incidence)				
Skull				
Irregular ossification	5	0	1	4
Hyoid				
ALA, angulated	1	0	1	3
Ribs				
Not ossified (10 th rib, left)	0	0	0	1**
Sternum				
Sternal centra: fused	0	3	6	4
Sternal centra: asymmetric	0	1	0	1
Vertebrae				

Thoracic: unilateral ossification	0	0	0	1**
<u>Pelvis</u>				
Pubis, not ossified	1	0	0	0

** : Fetus 8673-8 had other visceral alterations.

In the Segment II teratology study in the rabbit, four groups of dams (20/group) were administered intravenous bolus injection of alvimopan at 5, 10, and 15 mg/kg/day from days 6 to 18 of gestation. Overall, no external, visceral and skeletal changes were observed in the pups that could be attributed to alvimopan treatment. The NOAEL for maternal toxicity and developmental toxicity was determined as 15 mg/kg/day. Alvimopan was not considered teratogenic in the rabbit at the tested doses. The exposure to alvimopan appeared similar on GD6 and GD18; however, the exposure to the metabolite ADL 08-0011 appeared to increase between GD6 and GD18. The mean AUC_{0-1h} value at 15 mg/kg/day at GD6 and GD18 were approximately 205 and 277 times the human AUC at the proposed human dose of 12 mg b.i.d. (mean AUC_{0-12h} and C_{max} for alvimopan in humans at oral dose of 12 mg b.i.d. dose were 40.2 ng.hr/ml and 10.98 ng/ml, respectively).

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Embryofetal Development

Study Title: Intravenous Segment III Perinatal and Postnatal Developmental Toxicity Study in the Rat

Key Study Findings: In the Segment III perinatal and postnatal developmental study in the rat, four groups of dams (25/group) were given daily intravenous injections of 2, 5 and 10 mg/kg/day of alvimopan from gestation day 7 through lactation day 20. The NOAEL for maternal and reproductive toxicity was determined as 10 mg/kg/day. In this study, both alvimopan and its metabolite were found in the milk. It appears that the drug and its active metabolite are capable of being excreted through the breast milk. Alvimopan was not found to be teratogenic in the rat at the tested doses.

Study No.: 4401-001 (14TX003)

Volume #, and Page #: EDR NDA 21-775: pharmtox\tox\4401-001.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: October 30, 2001

GLP Compliance: A statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: Alvimopan, Lot 01-0088, 100%

Methods: Pregnant female SD rats (25 rats per group) were administered alvimopan intravenously at 0, 2, 5 or 10 mg/kg/day (Groups I through IV respectively). The dose volume was 4 ml/kg. An additional three rats per group were assigned to the satellite groups (Groups II through IV) for toxicokinetic (TK) evaluations and milk collection. Alvimopan or vehicle (0.9% Sodium Chloride Injection, USP) was administered once daily on day 7 of gestation (GD7) through day 14 of lactation (DL 14) to rats assigned to the satellite groups, DL 20 (rats assigned to the main study) or GD 24 (rats that did not deliver a litter). At approximately 90 days of age, the F1 generation rats within each dosage group were assigned to cohabitation for 16 days. Surviving female rats were sacrificed on GD21, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed.

Doses: 0, 2, 5 and 10 mg/kg/day, i.v.

Species/Strain: Sprague/Dawley rats

Number/Group: 25/group

Route, Formulation, Volume: Intravenous, 4 ml/kg.

Satellite Groups Used for Toxicokinetics: 3/group

Study Design: The following table shows the study design. The following table shows the study design (from page 26 of the sponsor's report).

2.7.1. Fo Generation Rats

Dosage Group	Dosage ^a (mg/kg/day)	Concentration (mg/mL)	Volume (mL/kg)	Number of Female Rats	Assigned Rat Numbers	
					Main Study Rats	Satellite Study Rats
I	0 (Vehicle)	0	4	25	12201 - 12225	N/A
II	2	0.5	4	25 + 3 ^b	12226 - 12250 ^c	2552 - 2554
III	5	1.25	4	25 + 3 ^b	12251 - 12275	2555 - 2557
IV	10	2.5	4	25 + 3 ^b	12276 - 12299 ^c , 12300	2558 - 2560

- a. The test article was considered 100% pure for the purpose of dosage calculations.
 b. Additional rats per group constituted a satellite group for toxicokinetic evaluation and milk sample collection.
 c. Rat 12250 and 12299 were placed in the satellite study for blood and milk sample collection on DL 14 due to the mortality that occurred during blood collection on DG 18.
 N/A - Not Applicable.

2.7.2. F1 Generation Rats

Dosage Group	Number of Rats/Sex	Maternal Dosage (mg/kg/day) ^a	Assigned Numbers	
			Male Rats	Female Rats
I	25	0 (Vehicle)	14001-14025	14101-14125
II	25	2	14026-14050	14126-14150
III	25	5	14051-14075	14151-14175
IV	25	10	14076 - 14082, 19225 ^a , 14084 - 14100	14176-14200

- a. Male rat 14083 was removed from study due to escape from its cage and replaced with male rat 19225.

Parameters and Endpoints Evaluated:

F0 Generation Female Rats

All F0 generation rats were observed for mortality twice daily. The rats were observed for clinical signs, abortions, premature deliveries and deaths daily before dosage, approximately 60 minutes after dosage administration and once on the day of sacrifice. Body weights were recorded weekly during acclimation, on GDO, daily during the dosage period and at sacrifice. Food consumption values were recorded on GDs 0, 7, 10, 12, 15, 18, 20 and 25 and DLs 1, 4, 7, 10 and 14. Maternal behavior was evaluated on DLs 1, 4, 7, 14 and 21. Blood samples were collected on GDs 7 and 18 and DL 6 at approximately 15, 30 and 60 minutes postdosage from each rat assigned to the satellite groups. Milk samples were collected on DL 14 at approximately 1 hour postdosage from all rats assigned to the satellite study and three randomly selected rats from the vehicle control group.

After completion of the 21-day postpartum period, female rats assigned to the main study were sacrificed and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The number and distribution of implantation sites was recorded.

F1 Generation Male and Female Rats

All F1 generation male and female rats were observed for mortality twice daily. These rats were also examined for clinical observations and general appearance once weekly during the postweaning period. Body weights for male rats were recorded weekly during the postweaning period and at sacrifice. Body weights for female rats were recorded weekly during the postweaning period and on GDs 0, 7, 10, 14, 17, 20 and 21. Food consumption values for male rats were recorded weekly except during cohabitation. Food consumption values for female rats were recorded weekly during the postweaning period except during cohabitation and on GDs 0, 7, 10, 14, 17 and 21. Beginning at day 24 postpartum (pp), one male rat and one female rat from each litter were evaluated in a passive avoidance test for learning, short-term retention and long-term retention. Beginning at approximately 70 days pp, one male rat and one female rat from each litter were evaluated in a water-M-maze test for overt coordination, swimming ability, learning and memory.

At approximately 90 days of age, the F1 generation rats within each dosage group were assigned to cohabitation for 16 days. Surviving female rats were sacrificed on GD21, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The rats were examined for number and distribution of corpora lutea, implantation sites and live and dead fetuses. Placentae were also examined. Uteri of apparently nonpregnant rats were examined to confirm the absence of implantation sites. Each fetus was weighed and examined for sex and gross external alterations. Surviving male rats were sacrificed approximately one week following the start of Caesarean-sectioning of the female rats. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Testes and epididymides of male rats were excised and grossly evaluated.

Results:

Mortality (Dams): There was no mortality.

Clinical Signs (Dams): No significant treatment-related clinical signs were observed.

Body Weight (Dams): The initial (GD0) and final (GD20) body weights of control animals were 238.5 and 397.5 g, respectively. There were no significant treatment-related changes.

Food Consumption (Dams): The initial and final food consumption values for control females were 24.4 and 25.0 g/animal/day, respectively. There were no significant treatment-related changes except on GD10 (91.6% of control) and GD12 (91% of control).

Toxicokinetics (Dams): Rats were exposed to alvimopan and ADL 08-0011 in a dose-related manner from GD7 through DL14. On DL14, approximately 1 hour after treatment, alvimopan was detectable in the milk at higher concentrations than that were found in the plasma. The metabolite, ADL 08-0011, was also detectable in the milk on DL14. The mean AUC_{0-1h} value for alvimopan at 10 mg/kg/day at GD7, GD18 and DL6 were 11-, 15-, and 6-fold human exposure at the proposed human dose of 12 mg b.i.d. (mean AUC_{0-12h} and C_{max} for alvimopan in humans at oral dose of 12 mg b.i.d. dose 40.2 ng.hr/ml and 10.98 ng/ml). The toxicokinetic data are summarized in the following table (from page 403 of the sponsor's report).

Mean ± SD (N=3) Alvimopan Toxicokinetic Parameters Following Intravenous Bolus Injection of Alvimopan to Pregnant Rats from Gestation Day 7 to Lactation Day 14						
Dosage level (mg/kg/day)	DG 7		DG 18		DL 6	
	C _{max} ^a (ng/mL)	AUC(0-1 hr) (ng•hr/mL)	C _{max} ^a (ng/mL)	AUC(0-1 hr) (ng•hr/mL)	C _{max} ^a (ng/mL)	AUC(0-1 hr) (ng•hr/mL)
2	129 ± 21	66.2 ± 8.1	214 ± 120	113 ± 53	107 ^b	72.6 ^c
5	328 ± 31	169 ± 11	632 ± 369	329 ± 188	240 ± 167	125 ± 75
10	920 ± 69	458 ± 48	1150 ± 270	608 ± 177	509 ^b	249 ^b

a. C_{max} was observed at 15 minutes; the first blood collection time point after dosing
 b: N=2
 c: N=1

The mean plasma concentrations of alvimopan and ADL 08-0011 in the plasma and milk are shown in the following table (from page 412 of sponsor's report).

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Table 7. Individual and Mean (±SD) Alvimopan Concentrations (ng/mL) in Plasma and Milk Following Intravenous Bolus Injection of 2, 5 or 10 mg/kg Alvimopan to Lactating Rats on DL 14

Dosage level
2 mg/kg/day

Rat#	Analyte Concentration (ng/mL)		
	Plasma	Milk	
	Alvimopan	Alvimopan	ADL 08-0011
2552			
2553			
12250 ^a			
Mean	NC	98.2	0.314
SD		9.6	0.067

5 mg/kg/day

Rat#	Analyte Concentration (ng/mL)		
	Plasma	Milk	
	Alvimopan	Alvimopan	ADL 08-0011
2555			
2556			
2557			
Mean	22.8	170	1.38
SD	11.0	20	0.18

10 mg/kg/day

Rat#	Analyte Concentration (ng/mL)		
	Plasma	Milk	
	Alvimopan	Alvimopan	ADL 08-0011
12299 ^a			
2559			
2560			
Mean	32.1	483	3.52
SD	16.5	61	1.00

Notes:

Plasma and milk samples were collected approximately 1 hour following dose administration (milk but not plasma samples were also collected from 3 rats in the main study control group). ADL 08-0011 was not detectable in any of the plasma samples. Alvimopan was detectable in the milk sample (13.6 ng/mL) collected from 1/3 rats in the main study control group; the reason for this is unknown. Neither alvimopan (except for one sample; 17.9 ng/mL) nor ADL 08-0011 was detectable in any of the 4 pooled plasma samples collected from pups of each litter.

NC = Not calculated (treated as 0) since <50% of the plasma samples were above the quantitation limit.

a: Animal from main study group to replace animal in satellite group that died following blood collections on DG 18.

Terminal and Necroscopic Evaluations: C-Section Data (Implantation Sites, Pre- and Post-Implantation Loss etc.): Pregnancy rate, number of implantation sites, gestation index (number of dams with one or more liveborn pups/number of pregnant rats), and the numbers of dams with stillborn pups were comparable

among the four dosage groups. The following table shows the C-section data (from page 65 of the sponsor's report).

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PROTOCOL 4401-001: INTRAVENOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTIVE TOXICITY STUDY OF ALVIMOPAN (ADL 8-2698) IN SPRAGUE-DAWLEY RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: 14TX003)

TABLE B11 (PAGE 1): NATURAL DELIVERY OBSERVATIONS - SUMMARY - F0 GENERATION FEMALE RATS

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DOSAGE GROUP		I	II	III	IV	
DOSAGE (MG/KG/DAY) ^a		0 (VEHICLE)	2	5	10	
RATS ASSIGNED TO NATURAL DELIVERY		N	25	25	25	25
PREGNANT	N(%)	23 (92.0)	21 (84.0)	25 (100.0)	25 (100.0)	
DELIVERED LITTERS	N(%)	23 (100.0)	21 (100.0)	25 (100.0)	25 (100.0)	
DURATION OF GESTATION ^b	MEAN ± S.D.	22.6 ± 0.5	22.7 ± 0.5	22.8 ± 0.4	22.9 ± 0.3	
IMPLANTATION SITES PER DELIVERED LITTER	N MEAN ± S.D.	376 16.3 ± 1.7	317 15.8 ± 2.1	406 16.2 ± 1.7	384 16.0 ± 1.9	
DAMS WITH STILLBORN PUPS	N(%)	3 (13.0)	3 (14.3)	3 (12.0)	3 (8.0)	
DAMS WITH NO LIVEBORN PUPS	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
GESTATION INDEX ^d	† N/N	100.0 33/ 33	100.0 21/ 21	100.0 25/ 25	100.0 25/ 25	
DAMS WITH ALL PUPS DYING DAYS 1-4 POSTPARTUM	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
DAMS WITH ALL PUPS DYING DAYS 5-21 POSTPARTUM	N(%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	

[] = NUMBER OF VALUES AVERAGED

- a. Dosage occurred on day 7 of gestation through day 20 of lactation.
- b. Calculated as the time (in days) elapsed between confirmed mating (arbitrarily defined as day 0) and the time (in days) the first pup was delivered.
- c. Excludes values for dams that were sacrificed on day 14 of lactation for blood and milk sample collection; implantation sites were not recorded.
- d. Number of rats with live offspring/number of pregnant rats.

PROTOCOL 4401-001: INTRAVENOUS DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTIVE TOXICITY STUDY OF ALVIMOPAN (ADL 8-2698) IN SPRAGUE-DAWLEY RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: 14TX003)

TABLE B12 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a 1		0 (VEHICLE)	2	5	10
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS					
N		23	21	25	25
PUPS DELIVERED (TOTAL)					
N		361	315	395	376
MEAN±S.D.		15.7 ± 1.9	15.0 ± 2.2	15.8 ± 1.9	15.0 ± 1.8
LIVEBORN					
MEAN±S.D.		15.5 ± 1.7	14.8 ± 2.1	15.7 ± 2.0	14.9 ± 1.9
N(%)		356(98.6)	312(99.0)	392(99.2)	373(99.2)
STILLBORN					
MEAN±S.D.		0.2 ± 0.7	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.4
N(%)		5(1.4)	3(1.0)	3(0.8)	3(0.8)
PUPS FOUND DEAD, MORIBUND SACRIFICED, OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	3/356(0.8)	1/312(0.3)	6/392(1.5)	3/373(0.8)
DAYS 2-4	N/N(%)	5/353(1.4)	1/311(0.3)	3/386(0.8)	2/370(0.5)
DAYS 5-7	N/N(%)	0/348(0.0)	3/310(1.0)	5/383(1.3)	2/368(0.5)
DAYS 8-14	N/N(%)	3/348(0.9)	3/307(1.0)	2/378(0.5)	2/366(0.5)
DAYS 15-21	N/N(%)	1/345(0.3)	0/287(0.0)	0/376(0.0)	1/350(0.3)
VIABILITY INDEX b					
†		97.8	99.4	97.7	98.6
N/N		348/356	310/312	383/392	368/373
LACTATION INDEX c					
†		98.8	98.0	98.2	98.6
N/N		344/348	287/293d	376/383	349/354d

DAY(S) = DAY(S) POSTPARTUM

- a. Dosage occurred on day 7 of gestation through day 20 of lactation.
- b. Number of live pups on day 4 postpartum/Number of liveborn pups on day 1 postpartum.
- c. Number of live pups on day 21 postpartum/Number of live pups on day 4 postpartum.
- d. Excludes values for litters in which the dam was sacrificed on day 14 of lactation for blood and milk sample collection.

Offspring (Malformations, Variations etc.): There were no treatment-related effects on learning, short-term retention, long-term retention or response inhibition and water maze performance in the F1 generation male and female rats. Mating and fertility of the F1 generation rats were unaffected by the treatment. All Caesarean-section and litter observations had comparable incidences in the

Methods: In this study, one group of five male and five female rabbits received a single dermal administration of the test article at a dose of 1000 mg/kg. Alvimopan was administered dermally to the shaved area. The delineated area was moistened with distilled water and the test article was then spread evenly over the delineated area and covered with gauze dressing. Animals were observed for clinical signs on a daily basis. Animals were examined for dermal toxicity (erythema and edema) on Day 1 and daily thereafter (Day 1-14).

Results: Clinical signs included dermal irritation at the site of test article application, dark material around the facial area, decreased defecation, fecal staining and clear ocular discharge. Alvimopan was classified as a slight irritant to the dermal tissue. The primary irritation index was calculated as 0.60 (0.00: Nonirritant; 0.01-1.99: slight irritant; 2.00-5.00 moderate irritant; 5.01-8.00 severe irritant as per dermal evaluation criteria by Environmental Protection Agency).

Study Title: Acute Dermal Irritation Study of Alvimopan in Rabbits

Key Study Findings: Alvimopan did not produce any erythema or edema at any of the observation period. The primary irritation index for alvimopan was determined to be zero (0). Alvimopan was classified as a non-irritant to rabbit skin under the conditions of the experiment.

Study No.: 808-014

Volume #, and Page #: EDR: NDA 21-775: pharmtox\tox\808-014.pdf

Conducting Laboratory and Location: _____

Date of Study Initiation: March 4, 2002

GLP Compliance: The statement of compliance was included.

QA Reports: yes (X) no ()

Drug, Lot #, and % Purity: Alvimopan, Lot No. R009340, 98.9%

Formulation: Powder

Methods: Alvimopan (0.5 g) was administered once on Day 1 via dermal application to a single site on the back of each animal for a four hour exposure period. The test site was evaluated for erythema and edema within 30-60 minutes and at 24, 48, and 72 hours following patch removal and was graded utilizing the "Draize" scale. Observations for mortality and body weights were also conducted during the course of the study.

Group Assignments – Induction Phase			
Group Number	Dose Level	Number of Animals	
		Male	Female
1	0% Untreated Control	0	0
2	100% (HCA) ^a	5	5
3	100% Treated	10	10

^aHexylcinnamic Aldehyde (HCA) was used undiluted (100%) as a positive control.

Group Assignments – Challenge Phase			
Group Number	Dose Level	Number of Animals ^b	
		Male	Female
1	50% ADL 8-2698	5	5
2	50% (HCA) ^a	5	5
3	50% ADL 8-2698	10	10

^aHexylcinnamic Aldehyde (HCA) was used diluted with mineral oil (50%) as a positive control.

^bThe same animals were used for the Induction and Challenge Phases.

In the range finding assay, the test article was administered to four sites (2/site) on each animal (one concentration per site) at concentrations of 25, 50, 75, and 100% (w/w), via topical patch application. Applications were observed and graded at 24 and 48 hours postdose.

In the induction phase, patches were applied to all test animals as described before. The patch application was repeated at the same site once a week for the next two weeks for a total of three, six-hour exposures (Days 1, 8, and 15). Dermal evaluations were recorded at 24 and 48 hours after each induction exposure was completed, according to an established grading method. After the last induction exposure, the animals were left untreated for two weeks before the challenge phase.

In the challenge phase, the test group and the positive control animals previously exposed during the induction period and the untreated control animals were challenged two weeks after the last induction exposure (Day 29) using patches as described before. The responses were graded at 24 and 48 hours after patch removal.

Dermal irritation evaluations were conducted according to the following scale (from page 12 of the sponsor's report).

Sensitization Grading Method	
Score	Observation
0	No visible change
0.5	Very faint erythema, usually patchy
1	Faint erythema, usually confluent
2	Moderate erythema
3	Severe erythema with or without edema

The sensitization was classified according to the following grade (from page 13 of the sponsor's report).

Sensitization Classification		
Sensitization Rate (%)	Grade	Classification
0	-	Nonsensitizer
>0-8	I	Weak sensitizer
9-28	II	Mild sensitizer
29-64	III	Moderate sensitizer
65-80	IV	Strong sensitizer
81-100	V	Extreme sensitizer

Results: For the range-finding exposures, no erythema or edema was observed at any application site on any animal at any observation interval. Based on these results, the test article was administered at 100% for the induction exposures and at 50% in the challenge phase. The positive control (hexylcinnamic aldehyde) responded in an expected manner indicating the validity of the experiment. Very few animals showed a dermal reaction after the challenge exposure. At 24-hours after patch removal one of 10 control animals had slight erythema and two of 20 test group animals had slight erythema. At 48-hours after patch removal no erythema was observed in any control or test animal. The sensitization index for the control and treated group was determined to be 10%. Based on these results, alvimopan was considered a non-sensitizer. The results are shown in the following table (from page 20 of the sponsor's report).

Study Number 808-015
 Skin Sensitization Study (Buehler Method) of Alvimopan (ADL 8-2698) in the Guinea Pig

Table 2 **Summary of Dermal Scores – Challenge (Male and Female)**

Group	Number of Animals	Total 24 Hour Score	Number of Animals with Scores of ≥1	Total 48 Hour Score	Number of Animals with Scores of ≥1	Severity Index (SI)		Incidence Index (II)	Sensitization Incidence Index (SII)
						24 Hour Score	48 Hour Score		
0% Untreated Control/ 50% Treated	10	1	1	0	0	0.1	0.0	1/10	10%
Positive Control 100% Induction/ 50% Challenge	10	10.5	6	7	6	1.05	0.7	9/10	90%
Test Group 100% Treated/ 50% Treated	20	2.5	2	0	0	0.125	0	2/20	10%

2.6.6.9 Discussion and Conclusions

Nonclinical toxicity of alvimopan and its metabolite, ADL 8-0011, has been studied in several single and repeated dose (up to 6 months) toxicity studies in mice, rats and dogs using oral and intravenous routes of administration. Overall, alvimopan showed a very low order of toxicity in mice, rats and dogs. Generally, the highest tested dose did not produce any significant organ toxicity, which could be partly attributed to its poor systemic absorption following oral administration. As a result, the target organ of toxicity could not be identified from these studies. In repeated-dose toxicity studies, the NOAEL was approximately 500 to 5000 fold greater than the anticipated daily oral clinical dose (0.24- 0.48 mg/kg/day based on a 50-kg body weight). Alvimopan did not exhibit any potential genotoxicity in any of the battery of genotoxicity tests.

In addition, toxicity studies were also conducted directly with ADL 08-0011, the amide hydrolysis metabolite of alvimopan to evaluate its potential toxicity, as exposure to ADL 08-0011 was relatively low in the repeated dose oral toxicity studies in rats and dogs following administration of alvimopan. The results of these studies show that ADL 08-0011 also has a low order of toxicity, even when administered by bolus i. v. injection for up to 2 weeks. ADL 08-0011 was also not genotoxic and was not associated with any toxic effects at dose levels up to 8 mg/kg/day.

In reproductive toxicity studies in rats and rabbits by oral and intravenous route, alvimopan did not cause any adverse effect on the reproductive parameters of male or female rats and rabbits. Alvimopan was not found to be teratogenic in rats and rabbits at

the tested doses under the conditions of the experiments. Alvimopan appears to have the potential to cause eye and skin irritation in the rabbit.

In conclusion, nonclinical studies conducted with alvimopan supported an assurance of safety for its proposed oral use in adult male and female patients at the proposed human therapeutic dose of 12-24 mg/day or 0.24- 0.48 mg/kg/day based on a 50-kg body weight.

2.6.6.10 Tables and Figures

Tables and figures are inserted in the appropriate sections of the text.

LABELING

1 Page(s) Withheld

 Trade Secret / Confidential

 ✓ Draft Labeling

 Deliberative Process

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Alvimopan is a relatively selective, peripherally active μ -opioid receptor antagonist intended for the management of postoperative ileus (POI), a serious condition following abdominal or pelvic surgery. Multiple factors contribute to both the development and duration of POI, including exogenous and endogenous opioids. Opioid-based regimens are the most common treatments to effectively manage post-surgical pain. However, morphine and other μ -opioid receptor agonists can prolong the duration of POI through delayed gastric emptying, reduced gastrointestinal (GI) motility, and disrupted colonic myoelectric activity. Alvimopan antagonizes the peripheral effects of opioids on gastrointestinal motility and secretion by binding to gastrointestinal tract μ -opioid receptors. The sponsor is seeking marketing approval for alvimopan to accelerate time to recovery of gastrointestinal function following abdominal or pelvic surgery.

In this NDA, the sponsor has submitted the following preclinical pharmacology and toxicology studies in support of alvimopan: pharmacology; absorption, distribution, metabolism and excretion studies in rats and dogs; toxicology studies: acute toxicity studies in mice and rats, 2-week i.v. toxicity study in rats, 2-week i.v. toxicity study with ADL 08-0011 in rats, 1-month oral toxicity study in rats, 4-week oral toxicity study in rats, 6-month oral toxicity study in rats, 1-month oral and i.v. toxicity study in dogs, 4-week oral toxicity study in dogs, 2-week i.v. toxicity study in dogs, 6-month oral toxicity study in dogs; reproductive: combined oral Segment I and II studies in rats, intravenous Segment I and II studies in rats, intravenous segment II study in rabbits; genotoxicity: Ames assay with alvimopan and ADL 08-0011 (active metabolite), chromosome aberration assay in Chinese Hamster Ovary (CHO) cells with alvimopan and ADL 08-0011, the mouse lymphoma cell (L5178Y/TK^{+/+}) forward mutation test and *in vivo* oral mouse bone marrow micronucleus assay; special toxicity study: primary ocular irritation in the rabbit, acute dermal toxicity study in the rabbit, and guinea pig skin sensitization test.

Alvimopan and its metabolite, ADL 08-0011, have been shown to be highly potent and relatively selective μ -opioid receptor antagonists as demonstrated by a series of binding and functional assays using cloned human (μ , δ , κ), rat whole brain (μ , δ), and guinea pig cortex (κ) opioid receptors. The K_i values for cloned human μ , δ and κ receptors are 0.44 nM, 10 nM and 100 nM, respectively. In mice, alvimopan antagonized the peripheral effects of opioids on gastrointestinal motility and secretion by binding to gastrointestinal tract μ -opioid receptors. Alvimopan competitively antagonized the effects of morphine on contractility in isolated guinea pig ileum preparations. Alvimopan also dose-dependently produced diarrhea in morphine-dependent mice with limited CNS effect, suggesting more selective peripheral action. This limited access of alvimopan to the CNS allows it to act peripherally without sacrificing the central analgesic effects of opioids. Overall, alvimopan possesses various pharmacological characteristics (relatively high selectivity for the peripheral μ -opioid receptors, absence of agonist activity and limited access to the CNS at doses that reverse peripheral effects) that support its use for the management of post operative ileus.

Systemic absorption of alvimopan after oral doses was low in animals, with less than 10% of the administered dose reached the systemic circulation. In humans given a 12 mg solution, or two 6 mg capsules orally, the mean absolute bioavailability was 14 and 6%, respectively. In humans, mean plasma AUC_{0-12h} and C_{max} for alvimopan after 12 mg oral b.i.d. dose for 4.5 days were approximately 40.2 ng.hr/ml and 10.98 ng/ml, respectively (data obtained from the study 14CL119). The mean $AUC_{0-\infty}$ and C_{max} for ADL 08-0011 in humans following oral dose of 12 mg b.i.d. alvimopan for 4.5 days were 1642.5 ng.hr/ml and 35.73 ng/ml, respectively (data obtained from study 14CL119). Systemic plasma clearance in humans was slower than animals. The volume of distribution is comparable across species. The estimated half-life in rats and dogs was approximately 2.0 and 0.2 hrs, respectively. The plasma half-life in humans given an intravenous dose of 12 mg was 5.3 hr compared to 2.0 and 0.2 hours in rats and dogs, respectively. The pharmacologically active metabolite, ADL 08-0011, was formed in all species following oral or intravenous administration of alvimopan, including humans. Generally, plasma concentrations of ADL 08-0011 after oral doses of alvimopan were greater than those of the parent drug in mice, rats, and human, but were less than those of the parent drug in dogs. Plasma protein binding of alvimopan was modest or low across species. Free fractions ranged from 20-30% for humans, 56% for rats, 67% for mice, and 72% for dogs. Plasma protein binding of ADL 08-0011 was species dependent with free fractions ranging from 4.5% for humans to 62% for mice. Distribution of alvimopan in rats was generally limited to the gastrointestinal tract with little distribution to peripheral tissues. Alvimopan and ADL 08-0011 were excreted through breast milk in rats after i.v. administration. Alvimopan was primarily metabolized to ADL 08-0011 in humans, and to glucuronide and sulfate conjugates in rats and dogs. Neither alvimopan nor ADL 08-0011 inhibited any of the 5 major human CYP isozymes in human liver microsomes or hepatocytes. Alvimopan and ADL 08-0011 were substrates for the p-glycoprotein (Pgp) transporter. Alvimopan was primarily excreted via the bile and feces. In rats and dogs, urinary excretion ranged from 25-30%.

Acute intravenous and oral toxicity studies were conducted in rats and mice. Alvimopan was non-lethal to rats at an i.v. dose of 20 mg/kg and an oral dose of 500 mg/kg. In mice, alvimopan was nonlethal at 500 mg/kg when administered orally. It is to be mentioned here that these are the only doses tested in acute toxicity studies in mice and rats. Subacute, subchronic and chronic toxicity studies were conducted in mice, rats and dogs after oral and intravenous administration.

In a 13-week oral (gavage) toxicity study in CD-1 mice, alvimopan was tested at 100, 300, 600 and 1000 mg/kg/day. The NOAEL was considered as 1000 mg/kg/day. In this study, alvimopan was well tolerated with maximum plasma concentrations of 79.2 ng/ml (about 8-fold human C_{max} at the proposed therapeutic dose) observed at 1 hour after a single 1000 mg/kg dose.

In a 2-week i.v. toxicity study in rats, animals received an i.v. bolus injection of alvimopan at 0, 1, 5, and 10 mg/kg/day. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL was considered as 10 mg/kg/day.

In a 2-week i.v. toxicity study in rats, animals received an i.v. bolus injection of ADL 08-0011 at 2, 4, and 8 mg/kg/day. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL may be considered as 8 mg/kg/day. The mean C_{max} and AUC_{0-4h} value at 8 mg/kg/day at Day 14 was found to be approximately 2365 ng/ml (65 times of human C_{max} at the proposed therapeutic dose) and 3590 ng.hr/ml (2 times of human AUC), respectively.

In a 1-month oral gavage toxicity study in rats, animals were treated with alvimopan at 0, 50, 100 and 200 mg/kg/day. There were some effects on hematology (neutrophil, thrombocyte and erythrocyte counts were lower in male rats compared to control) and blood chemistry (triglyceride levels were significantly lower in females at 200 mg/kg/day) parameters at 200 mg/kg/day. The NOAEL was determined as 100 mg/kg/day. The tested doses did not allow the identification of any target organ of toxicity. The mean plasma levels on Day 0 and Day 29 ranged from 2.8 to 20.5 ng/ml with an overall average of 13 ng/ml, which is approximately 1.2 times the human C_{max} exposure at the proposed therapeutic oral dose of 12 mg b.i.d.

In a 4-week oral gavage toxicity study in Fischer 344 rats, animals received oral doses of alvimopan at 100, 250 and 500 mg/kg bid or 200, 500 and 1000 mg/kg/day for 4 weeks. The target organ could not be identified in the absence of any significant organ toxicity. The NOAEL was considered as 1000 mg/kg/day. The mean C_{max} and AUC_{0-4h} value at 1000 mg/kg/day was 7.7 ng/ml and 67.75 ng.hr/ml, respectively, which is about 1.7 times the human AUC at the proposed therapeutic oral dose of 12 mg b.i.d.

In a 6-month oral gavage toxicity study in rats, animals were administered alvimopan at 0, 50, 100 and 200 mg/kg/day. The NOAEL was considered as 200 mg/kg/day. The tested doses did not allow the identification of any target organ of toxicity. There was no mention whether the highest tested dose was the maximum feasible dose (MFD). The

C_{max} at 200 mg/kg/day was 27.9 ng/ml at 24 hr, which was approximately 2.5 times the human C_{max} at the proposed therapeutic oral dose of 12 mg b.i.d.

In a 1-month oral gavage study in Beagle dogs, animals were administered with alvimopan at 0, 10, 30 and 100 mg/kg/day. The NOAEL was considered as 100 mg/kg/day. The target organ of toxicity could not be identified in the absence of any significant organ toxicity. In general, peak plasma level was 133 ng/ml at 100 mg/kg/day on Day 28, which is about 12 times the human C_{max} at the proposed therapeutic dose.

In a 1-month intravenous study in Beagle dogs, animals were administered alvimopan at 0, 0.05, 0.2 and 2.0 mg/kg/day. The NOAEL was considered as 0.2 mg/kg/day. There were some hematological effects (erythrocytes and hemoglobin were significantly decreased) at 2.0 mg/kg/day, however, these were not considered of any biological significance. The target organ of toxicity could not be identified in the absence of any significant organ toxicity. It appears that higher doses could have been tested. The mean AUC_{0-α} value on Day 28 at 2.0 mg/kg/day was 1211 ng·hr/ml, which is about 30 times the human AUC at the proposed therapeutic oral dose of 12 mg b.i.d.

In a 4-week oral (capsule) toxicity study of alvimopan in beagle dogs, animals were treated at 0, 100, 250, 500 and 1000 mg/kg/day for 4 weeks. The target organ of toxicity could not be identified in the absence of any organ toxicity. The NOAEL appeared to be 1000 mg/kg/day. The C_{max} and AUC_{0-24h} at 1000 mg/kg/day were 131 ng/ml (about 12 time human C_{max}) and 1800 ng·hr/ml (45 times of human AUC), respectively.

In a 2-week intravenous toxicity study of ADL 08-0011 (a metabolite of alvimopan) in beagle dogs, animals were treated at 0.5, 1, and 2 mg/kg/day for 2 weeks. There were no significant toxicology findings to identify the target organ of toxicity. The NOAEL appeared to be the highest tested dose (2 mg/kg/day). The mean C_{max} and AUC_{0-4h} value at 2.0 mg/kg/day was found to be approximately 1940 ng/ml (54 times the human C_{max} at the proposed therapeutic dose) and 2295 ng·hr/ml (1.4 time the human AUC at the proposed therapeutic dose of 12 mg b.i.d.), respectively.

In a 6-month oral (capsule) toxicity study in Beagle dogs, animals were administered alvimopan at 0, 10, 30 or 100 mg/kg/day. The NOAEL was considered as 100 mg/kg/day. The tested doses did not allow the identification of any target organ of toxicity. The maximum plasma concentration of 18.0 ng/ml was achieved at 100 mg/kg/day at 4 hr after treatment, which is about 1.7-fold the human C_{max} at the proposed therapeutic oral dose of 12 mg b.i.d.

Alvimopan was not genotoxic in the Ames test, the mouse lymphoma cell (L5178Y/TK⁺) forward mutation test, the chromosome aberration test in Chinese Hamster Ovary (CHO) cells, and the oral mouse micronucleus test. The pharmacologically active metabolite ADL 08-0011 was also negative in both the Ames test and chromosome aberration test in CHO cells. Overall, alvimopan and its active metabolite, ADL 08-0011, do not appear to have genotoxic potential.

The sponsor did not include reports of any carcinogenicity study with alvimopan.

In a combined fertility and reproductive performance and teratology study (Segment I and II) in rats, animals were tested at 50, 100 and 200 mg/kg/day. There were no treatment-related effects on male and female fertility. However, alvimopan produced some skeletal variations (incomplete ossification of cervical vertebra, ischium and occipital bone) at 200 mg/kg/day. Alvimopan was not considered teratogenic in rats at the tested doses.

In an intravenous fertility and reproductive performance (Segment I) study in rats, animals were administered alvimopan at 0, 0.2, 2 and 5 mg/kg/day. There were no apparent treatment-related adverse effects on the reproductive parameters of male or female rats or on the development of fetuses. There was an apparent treatment-related increase in the number of pre-implantation loss. However, these increases were neither dose-related and nor statistically significant. The NOAEL for reproductive toxicity was considered as 5 mg/kg/day for both male and female animals.

In the Segment II teratology study in the rabbit, animals were administered intravenous bolus injection of alvimopan at 5, 10, and 15 mg/kg/day from days 6 to 18 of gestation. Overall, no external, visceral and skeletal changes were observed in the pups that could be attributed to alvimopan treatment. The NOAEL for maternal toxicity and developmental toxicity was determined as 15 mg/kg/day. Alvimopan was not considered teratogenic in the rabbit at the tested doses.

In the intravenous Segment III perinatal and postnatal developmental study in the rat, animals were given daily intravenous injections of 2, 5 and 10 mg/kg/day of alvimopan from gestation day 7 through lactation day 20. The NOAEL for maternal and reproductive toxicity was determined as 10 mg/kg/day. In this study, both alvimopan and its metabolite were found in the milk. It appears that the drug and its active metabolite are capable of being excreted through the breast milk.

Alvimopan caused eye irritation when applied at a dose of 0.0420 g in the rabbit eye. Alvimopan was classified as a mild irritant to the ocular tissue of the rabbit. Administration of 0.1 g of alvimopan powder or 0.1 ml of alvimopan suspension resulted in slight to diffuse conjunctival redness. Alvimopan was classified as a slight irritant to the dermal tissue. However, alvimopan did not cause any sensitization reaction in the guinea pig.

Alvimopan is a relatively selective, peripherally active μ -opioid receptor antagonist intended for the management of postoperative ileus (POI), a condition observed following abdominal or pelvic surgery. The systemic toxicity of alvimopan and its metabolite ADL 08-0011 was adequately evaluated following sufficiently high oral and intravenous doses in mice, rats and dogs. Alvimopan and ADL 08-0011 did not cause any significant toxicity when administered in sufficiently high oral or i.v. doses in any species tested. In addition, adequate safety pharmacology studies were also conducted with alvimopan, which did not show any potential safety concern. Alvimopan and its

active metabolite ADL 08-0011 did not show any potential for genotoxicity. In fertility and reproductive performance study in rats, alvimopan did not cause any adverse effect. It was not teratogenic in rats or rabbits. The proposed human oral dose for alvimopan is 12 mg b.i.d. or 24 mg/day or 0.48 mg/kg/day (based on 50 kg body weight), which is equivalent to 17.8 mg/m². The highest tested oral doses in rats (200 mg/kg/day) and dogs (100 mg/kg/day) in 6-month studies were approximately 67.4 and 112.3 times the proposed human dose (17.8 mg/m²), respectively, based on body surface area.

In conclusion, the nonclinical studies conducted on alvimopan provide adequate assurance of safety for its proposed oral use as indicated in the draft labeling. Therefore, from a preclinical standpoint, this NDA may be approved.

The labeling of alvimopan conforms to the format specified under 21CFR, Subpart B. However, the suggested changes described in the text, should be incorporated.

Conclusions: This submission contains adequate studies for the marketing approval of alvimopan. This submission meets the guidelines and satisfies the criteria for marketing authorization of alvimopan and appears to be safe for the proposed use.

Unresolved Toxicology Issues (if any): None.

Recommendations: From a preclinical standpoint, this NDA may be approved.

Suggested Labeling: The sponsor should be asked to change the proposed label of EnteregTM as suggested in the text of the review.

Signatures:

Reviewer Signature

Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

Supervisor Signature _____ Concurrence Yes ___ No ___

Jasti B. Choudary, B.V. Sc., Ph.D.
Supervisory Pharmacologist, HFD-180

**This is a representation of an electronic record that was signed electronically and
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/s/

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