

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

**APPLICATION NUMBER: 21-141 and 21-176**

**PHARMACOLOGY REVIEW(S)**

New file

NDA 21-141(capsules)  
NDA 21-176 (tablets)

Review completion date: April 25, 2000

**DRUG:** Colesevelam HCl (Welchol™)

**INDICATION:** Use alone or in combination with an HMG-CoA reductase inhibitor as adjunctive therapy to diet and exercise for the reduction of elevated LDL cholesterol in patients with primary hypercholesterolemia

**TEAM LEADER MEMO TO FILE REGARDING  
PRECLINICAL PHARMACOLOGY/TOXICOLOGY ISSUES  
FOR NDA 21-141 and NDA 21-176 Colesevelam HCl (Welchol™)  
(CAPSULES AND TABLETS)**

**SUMMARY OF PRINCIPLE PHARMACOLOGY/TOXICOLOGY ISSUES  
WITH NDA 21-141 AND NDA 21-176**

The following discussion is based upon the primary NDA pharmacology review provided by Gemma Kuijpers, Ph.D., reviewing pharmacologist. The reader is referred to the primary pharmacology review for further information on the studies discussed below.

**INTRODUCTION:**

The product covered by this NDA represents an essentially non-absorbed bile acid sequestrant for the proposed indication of reduction of elevated LDL cholesterol in patients with primary hypercholesterolemia. This is to be used as adjunctive therapy to diet and exercise and may be used alone or in combination with an HMG-CoA reductase inhibitor. Chemically, it is poly(allylamine hydrochloride) cross-linked with epichlorohydrin and then alkylated with decylbromide and 6-trimethylammonium-hexylbromide. Excipients include magnesium stearate, microcrystalline cellulose, and silicone dioxide

Welchol™ acts as a bile acid sequestrant. By binding bile acids in the intestine and impeding their reabsorption, the hepatic enzyme, cholesterol 7- $\alpha$  hydroxylase, is upregulated to increase the conversion of cholesterol to bile acids. In response to this, transcription and activity of HMG-CoA reductase is also increased which, in turn, increases the number of surface-active LDL receptors. The net effect is to decrease serum LDL cholesterol.

The key preclinical/nonclinical studies considered in the evaluation of the safety and labeling of this product are briefly outlined below:

**PHARMACOKINETICS:**

There were two pharmacokinetic studies (one rat, one dog) performed to examine Absorption, Distribution, Metabolism and Elimination. Both studies were performed with [<sup>14</sup>C]-labeled compound. In the dog study, dosing with unlabeled compound for 28 days was followed by a single dose of labeled compound. Elimination was primarily by the fecal route.

The results indicate that in both species, small amounts of polymer or soluble low molecular weight degradants are absorbed systemically. In dogs, small quantities of radiolabeled material were detected in the liver, bile, spleen, mesenteric lymph nodes, cecum, rectum and small intestine. Results with colesevelam pretreatment for 28 days in dogs suggest that repeated

dosing does not change the absorption or distribution of this product. The effect of chronic treatment on absorption is unknown.

This product can be considered to be essentially not absorbed and the primary reviewer has recommended that determinations of safety margins of animal findings relative to human exposure be based on mg/kg comparisons rather than the body surface area comparisons commonly used by the agency when plasma level data are unavailable. The team leader agrees with this assessment. However, this does not eliminate concern for systemic effects, which could still be a concern with chronic dosing. As discussed in the toxicology section, secondary effects related to the pharmacodynamic effects of the drug (e.g., changes in absorption of fat-soluble vitamins) do occur. In addition, there is no data to determine if chronic dosing (>6 months) leads to changes in absorption.

#### **TOXICOLOGY:**

The following toxicology studies were reviewed (see primary review for details):  
90 day and 6 month dietary toxicology studies in rats  
13 week and 1 year oral toxicity studies in dogs (capsule administration)

#### **Brief summary of results:**

Perhaps the most consistent finding across the above referenced studies relate to a hemorrhagic syndrome noted in several organs. Death in high dose male rats was attributed to this syndrome. A decreased absorption of fat-soluble vitamins was detected by measuring plasma levels of Vitamins A, E and D, which support this conclusion. It is likely that vitamin K (not measured directly) was also decreased which could be related to the hemorrhagic syndrome. A decrease in APTT was noted in the 90-day rat study, which also supports this conclusion. Changes in APTT are not mentioned in the other toxicity studies. The decrease in absorption of these vitamins was considered in the design of the chronic carcinogenicity studies, which were performed with vitamin supplementation.

Elevations of transaminases (1-2X) were noted in the rat studies, which apparently had no histological correlates. This was not noted in the dog studies.

No Adverse Effect Levels (NOAEL) for both species were approximately three-fold the proposed human dose based on mg/kg comparisons. (Note that while in the absence of plasma level data the agency generally bases comparisons on body surface area, in the case of essentially non-absorbed compounds, a mg/kg comparison is considered appropriate). The pharmacology team leader recommends that a statement \_\_\_\_\_ should be considered in the label.

#### **GENETIC TOXICOLOGY:**

Testing the potential for genetic toxicity of insoluble compounds presents technical problems for achieving appropriate exposure, which is critical to the execution of these studies. To address this problem, the *in vitro* standard battery of genetic toxicology testing was performed with an acid extract of colesevelam in 0.1 N HCl at 50°C for three days.

Bacterial Reverse Mutation assay (Ames test)  
*In Vitro* Chromosomal Aberration Assay (CHO cells)

Parent compound (not an extract) was used for the *in vitro* Chromosomal Aberration Assay (Mouse micronucleus)

In addition, Ames and CHO tests were performed with 4 degradants that were detected during long term stability studies (note: these were soluble and tested directly).

#### RESULTS:

Based on endpoints commonly used to determine appropriate dose selection (cellular toxicity, limit dose and/or presence of precipitate) the studies were all performed at adequate doses and can be considered to be valid studies.

1. The results in the Ames tests were uniformly and clearly negative for the extracts and degradants. This indicates a lack of mutagenic effect of degradants or the extract.
2. The results of the *in vitro* CHO cell assays were equivocal (see discussion below)
3. The results of the *in vivo* mouse micronucleus test with parent compound were apparently negative, but this assay is considered to be inappropriate for non-absorbed compounds (see discussion below).

#### COMMENTS ON CHO ASSAY OF IN VITRO CLASTOGENIC POTENTIAL:

There were mixed findings with the CHO assay, an indicator of clastogenic potential. In the test with the extract of parent compound, there was a statistically significant positive finding in the presence of metabolic activation at a dose of 100 µg/ml with 4 hour incubation. It is noted that precipitate was detected at all doses tested which included the low dose of 25 µg/ml tested with 4 hour incubation in the presence of metabolic activation. In addition, precipitate was noted as low as 7 µg/ml in the 20-hour continuous treatment in the absence of metabolic activation. In general, when the presence of precipitation is used as a dose selection endpoint, the goal is to achieve the lowest dose at which precipitate occurs. Thus, 25 µg/ml (or perhaps even lower doses) could be considered an appropriate high dose for this experiment. This experiment exceeded that level. Therefore, the apparent positive finding at 100 µg/ml may not be relevant to clinical exposure. There was no confirmatory study in the presence of metabolic activation, therefore it is not known if this is a reproducible finding. However, as noted below, there were equivocal findings with two degradants, which could correlate with a positive finding with the extract of the parent compound tested in the presence of metabolic activation. The team leader considers this finding equivocal until further studies are done to clarify this.

In the tests with the degradants, the CHO assay with decylamine HCl suggested a positive finding in the absence of metabolic activation at 15 µg/ml with 4 hour incubation. In the 20 hour incubation without metabolic activation, the high dose of 13 µg/ml resulted in no finding of clastogenicity. Although the dose is lower, it would be expected that the longer exposure could have resulted in a similar positive finding as noted in the 4 hour exposure. Therefore, the team leader considers that the apparent positive finding was not reproducible but, in the absence of additional confirmatory data, concludes that the results with decylamine HCl in the CHO assay are equivocal.

The results in the CHO assay with the second degradant, didecylamine HCl, were negative.

The results in the CHO assay with the third degradant, aminohexyltrimethyl ammonium chloride HCl were positive with a 4 hour exposure at 5000 µg/ml in the absence of metabolic activation. In the 20 hour exposure without metabolic activation, there was no statistically significant

finding, but there was a slight increase in structural aberrations at this dose. As concluded with the decylamine HCl, this reviewer considers these findings to be equivocal.

The results in the CHO assay with the fourth degradant, 6-Decylaminoethyltrimethyl ammonium chloride HCl, were negative.

In conclusion, although the apparent positive findings were not clearly reproducible, the fact that they occurred with the extract of parent in the presence of metabolic activation and with multiple degradants in the absence of metabolic activation provides cause for concern. The team leader recommends that these findings be considered equivocal until further data proves otherwise.

#### COMMENTS ON THE MOUSE MICRONUCLEUS ASSAY OF *IN VIVO* CLASTOGENIC POTENTIAL:

The mouse micronucleus assay is considered to be a measure of the potential for clastogenic effects in an *in vivo* model. In general, it is considered less sensitive than the *in vitro* assays, but has the advantage of being an indicator of what could happen in an *in vivo* situation and accounts for *in vivo* metabolism, pharmacokinetics and DNA-repair processes. The principle of the assay is to expose the animal to sufficiently high doses to expose bone marrow to adequate levels of drug, then measure the occurrence of micronuclei (an indicator of clastogenicity) in the polychromatic erythrocytes. To have an appropriately performed assay, it is necessary that the bone marrow be exposed to the test agent or its metabolites. The sponsor used 5000 µg/kg as an acceptable "limit dose" for this assay and there was an indication of bone marrow toxicity noted as changes in polychromatic to normochromatic erythrocyte ratios which normally suffices as an adequate dose selection endpoint. However, since this compound is essentially not absorbed, it is likely that the effect on bone marrow toxicity was a secondary effect. Since the bone marrow was not exposed to significant amounts of the drug product or degradants, this assay is not appropriate for this compound. While it is likely that a non-absorbed compound is not likely to be a concern for genetic toxicity findings systemically, consideration has to be given to the fact that the gastrointestinal tract will be exposed to high levels of the compound and thus, in terms of g.i. exposure, the micronucleus test is not an adequate assessment of *in vivo* clastogenic potential.

#### CONCLUSIONS REGARDING GENETIC TOXICOLOGY FINDINGS WITH WELCHOL™:

The pharmacology team leader concludes that the findings in the Ames were adequately performed and clearly negative for both parent compound and degradants.

The findings in the *in vitro* CHO cell clastogenicity assay yielded equivocal results with the extract of parent compound in the presence of metabolic activation and with the degradants decylamine HCl and aminohexyltrimethyl ammonium chloride HCl with 4 hour incubation in the absence of metabolic activation. There is insufficient evidence to make the determination that the results indicate a clear positive for this assay. However, the findings in multiple assays are cause for concern and the fact that the findings weren't reproduced in the 20 hour incubations with lower doses do not specifically address this issue without further data to support a clear "positive" or "negative" determination. Therefore, the team leader recommends that the results of the CHO assays be described as "equivocal" in the label.

The *in vivo* mouse micronucleus test, while apparently performed to criteria considered adequate to ensure sufficient dosing (limit dose and changes in PCE:NCE ratio), do not provide an adequate assessment of *in vivo* clastogenicity since the drug is essentially not absorbed.

---

#### **CARCINOGENICITY:**

The results of carcinogenicity assessments in rats and mice are extensively covered in Dr. Kuijper's reviews and have been presented to the executive Carcinogenicity Assessment Committee (e-CAC) for comment. The minutes of the e-CAC meeting are provided in Dr. Kuijper's review. Dose administration was performed by dietary route. Since there was evidence of decreased absorption of fat-soluble vitamins, the studies were designed to include vitamin supplementation for control and test groups. Additionally, a non-supplemented control group was included. Due to the study design, the most appropriate comparison for statistical calculations of tumor incidence is to use the vitamin-treated control group. Initially, exposure was calculated to deliver specific doses to the animals. Due to the lack of obvious toxicity endpoint to justify the dose selection, the sponsor attempted to use exposure to 5% of diet as a dose selection endpoint justification. The 5% levels were not reached immediately during the rat study. The e-CAC considered that since 85% of the maximum dose was achieved by week 20, the dose levels were considered adequate for an evaluation. For mice, the exposure level was less than 5% of diet (~2%), but changes in body weight and decreased in serum vitamin E levels were considered to be sufficient to justify dose selection.

In rats, there was a statistically significant increase in the incidence of pancreatic acinar cell adenoma in male rats. There was a statistically significant increase in thyroid C-cell adenomas in female rats without an increase in thyroid C-cell hyperplasia or carcinoma and an increase (though not statistically significant) of adenomas in male rats. There was also an increase of thyroid C-cell hyperplasia in male rats, which suggests that there was a potentially biologically relevant effect in male rats as well. The primary reviewer recommends that both the pancreatic findings and thyroid findings be discussed in the label. The team leader agrees with this assessment.

There appeared to be no evidence of effects on tumor incidence in mice.

#### **REPRODUCTIVE TOXICOLOGY:**

The sponsor adequately performed the standard series of reproductive tests. These included:  
Segment I (dietary, rat): treatment in males 4 weeks pre-mating then 3 weeks of cohabitation, treatment in females 2 weeks pre-mating up to day 7 of presumed gestation. Doses 0, 0.2, 1 and 2 g/kg/day.

Segment II (dietary, rat): treatment days 7-17 of presumed gestation in females. Doses 0, 0.3, 1 and 3 g/kg/day.

Segment III (gavage, rat): females dosed from day 6 of presumed gestation until day 20-23 postpartum. Examination of F0, F1 and F2 generations. Doses 0.1, 0.3 and 1 g/kg/day.

Segment II (gavage, rabbit): treatment in females from day 6-18 of presumed gestation. Doses of 0.1, 0.5 and 1 g/kg/day.

Doses represent at least a 13-fold multiple of human exposure based on mg/kg comparisons. No adverse effects on reproduction, fertility, or fetal development were noted in rats and no adverse effects on fetal development were noted for rabbits.

## CONCLUSIONS

The preclinical issues for the proposed use of this product have been sufficiently addressed so the pharmacology team leader can concur with the primary reviewer to recommend that the applications for NDA s 21-141 and 21-176 should be approved (AP) from a pharmacology standpoint pending appropriate modifications to the label. The following key issues should be discussed in the label (see primary review for text to be communicated to sponsor):

### CARCINOGENICITY

- a. Statistically significant increase in incidence of pancreatic acinar cell adenoma in male rats.
- b. Statistically significant increase in thyroid C-cell adenomas in female rats. Also, since there was a non-statistically significant finding in males which could be relevant, this should also be included in the label.

### GENETIC TOXICOLOGY

- a. Negative findings in Ames bacterial mutagenesis assay
- b. Equivocal findings in the CHO *in vitro* clastogenicity assay with extract of the parent compound in the presence of metabolic activation and also with two degradants in the absence of metabolic activation (4-h incubation) tested separately.
- c. References to \_\_\_\_\_ should be deleted \_\_\_\_\_

### REPRODUCTIVE TOXICOLOGY

Category B: No adverse effects on reproduction, fertility or fetal development were noted.



**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA****KEY WORDS:** polymer, bile acid sequestrant, cholesterol, hypercholesterolemia

**NDA NUMBER:** 21,141 (Capsules)  
21,176 (Tablets)

**DRUG:** COLESEVELAM HYDROCHLORIDE (WELCHOL)

**Reviewer Name:** Gemma A. Kuijpers, Ph.D.

**Division Name:** Division of Metabolic and Endocrine Drug Products

**HFD #** 510 (DMEDP)

**Review Completion Date:** April 11, 2000

**Date of submission:** July 30, 1999

**Information to sponsor:** Yes (X) (Labeling Comments)

**Sponsor:** Geltex Pharmaceuticals, Inc., MA

**Manufacturer for drug substance:** \_\_\_\_\_

**Drug substance:** colesevelam hydrochloride

**Geltex Compound Nr.:** GT31-104HB

**USAN:** colesevelam hydrochloride

**Trade Name:** Welchol®

**Chemical Name:** allylamine polymer with 1-chloro-2,3-epoxypropane, [6-(allylamino)-hexyl]trimethylammonium chloride and N-allyldecylamine, hydrochloride (IUPAC)

**CAS Registry Number:** 182815-44-7

**Molecular Formula:**  $(C_3H_8NCl)_2(C_9H_{20}N_2OCl_2)_1(C_{13}H_{28}NCl)_7(C_{12}H_{28}N_2Cl_2)_6$

**Molecular Weight:** 212 g/mole (one polymer subunit)

**Drug Class:** Bile acid sequestrant

**Drug Product:** Capsules (375 mg), or Tablets (625 mg)

**Structure:** See page 3

**CLINICAL INFORMATION**

**Indication:** Adjunctive therapy to diet and exercise for the reduction of elevated LDL cholesterol in patients with primary hypercholesterolemia

**Clinical formulation:** Tablet or Capsules

**Strength:** 625 mg (tablet), 375 mg (capsule)

**Route of administration:** Oral

**Disclaimer - use of sponsor's material:** Summary Tables and Figures from the electronic submission have been used in the Review

**Relevant INDs/NDAs/DMFs:** IND \_\_\_\_\_ (colesevelam hydrochloride)  
NDA 20,926 (Renagel)

**RECOMMENDATION CODE:** AP**APPEARS THIS WAY  
ON ORIGINAL**

**CONTENTS**

	Page
INTRODUCTION.....	4
NONCLINICAL PHARMACOLOGY.....	5
NONCLINICAL PHARMACOKINETICS.....	6
GENERAL TOXICOLOGY.....	10
CARCINOGENICITY.....	17
Rat Carcinogenicity Study.....	17
Mouse Carcinogenicity Study.....	41
Addendum.....	62
Exec CAC Documents.....	65
GENETIC TOXICOLOGY.....	70
REPRODUCTIVE TOXICOLOGY.....	135
SPECIAL TOXICITY.....	155
SUMMARY AND EVALUATION.....	158
RECOMMENDATION.....	163
LABELING REVIEW.....	164
ATTACHMENT 1.....	165
(Statistical Reviews of Carcinogenicity Studies)	

*Handwritten initials: A, D*

*Handwritten: /S/ U*

*Handwritten: 4/19/00*

---

Gemrna Kuijpers, Ph.D.

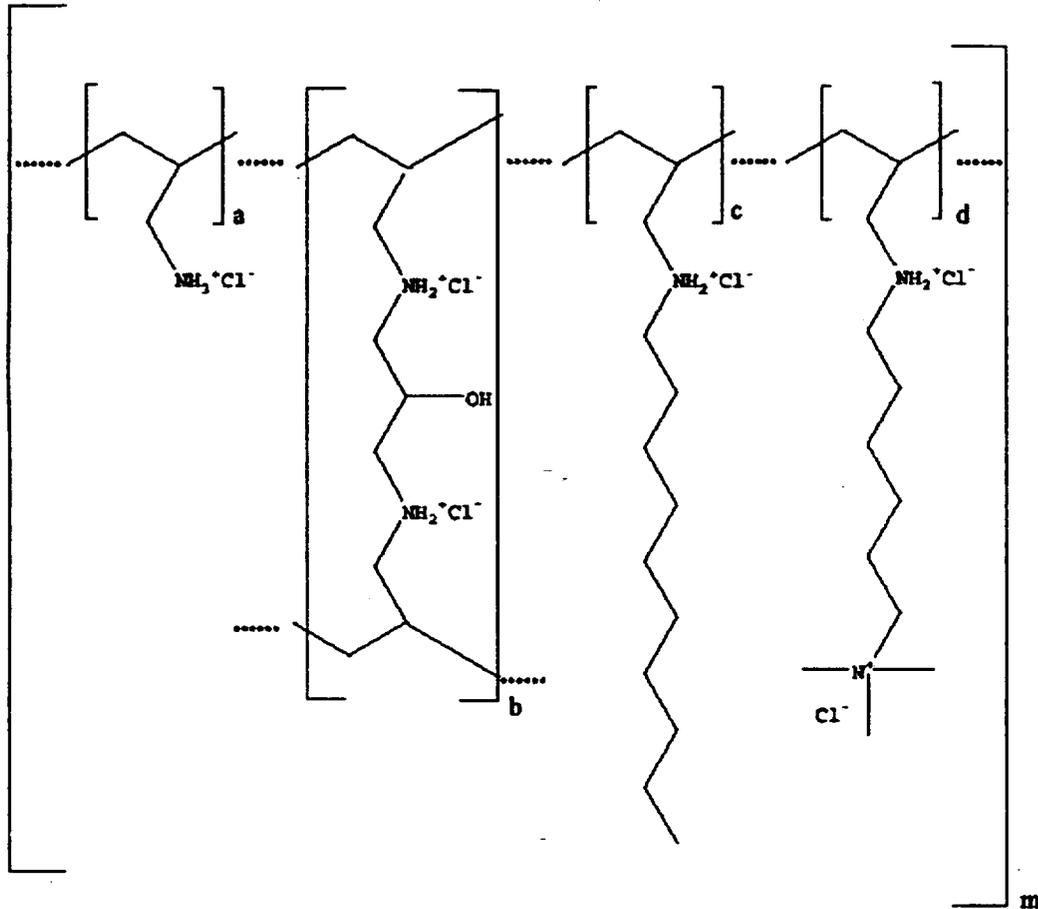
*Handwritten: /S/*

*Handwritten: 4/19/00*

*Handwritten: see Team leader Memo to file*

Cc:  
NDA Arch  
HFD-510  
HFD-510/Kuijpers/Steigerwalt/Shen/Koch

**Figure 5.2-1: Chemical Structure of Colesevelam Hydrochloride**



Where:

- |   |  |          |
|---|--|----------|
| a | = number of primary amine groups           | a = 0.14 |
| b | = number of cross-linked amine groups      | b = 0.12 |
| c | = monoquat alkylated amine groups          | c = 0.34 |
| d | = decylbromide alkylated amine groups      | d = 0.40 |
| m | > 100 to indicate extended polymer network |          |

**APPEARS THIS WAY  
ON ORIGINAL**

## INTRODUCTION

Bile acid sequestrants are drugs that bind bile acids and can lower serum cholesterol levels. Cholesterol is excreted from the body by hepatic secretion into bile. The cholesterol molecule can be secreted directly into bile either as cholesterol, or as bile acids after modification of the alkyl side chain to a carboxylic acid and the addition of hydroxyl groups to the steroid nucleus. Although a major part of intestinal bile acids are reabsorbed, the bulk of sterol excretion occurs by fecal excretion of bile acids. The mechanism by which bile acid sequestrants lower serum cholesterol is to increase bile acid excretion. The enhanced excretion of the bile acids from the liver into the GI tract increases their synthesis from cholesterol. This causes a compensatory increase in LDL cholesterol uptake by the hepatocyte via the LDL receptor. The net effect is a decrease in serum LDL cholesterol levels.

Colesevelam hydrochloride is poly(allylamine hydrochloride) cross-linked with epichlorohydrin and then alkylated with approximately equal amounts of decylbromide and 6-trimethylammonium-hexylbromide. Approximately \_\_\_\_\_ of the amines are alkylated. About \_\_\_\_\_ of the amines are protonated. The polymer is highly charged at physiological pH. The bile acid binding effect is maximal at about pH 7.

Colesevelam hydrochloride is a polymeric substance that is insoluble in aqueous solution. The polymer is sized as particles with a mean size between \_\_\_\_\_  $\mu\text{m}$  in diameter. The size distribution is specified such that : \_\_\_\_\_ in diameter. Particles of this size are essentially not absorbed from the gastrointestinal tract. Also, the particles are cationic and face a significant barrier to absorption through the highly anionic mucus layer that lines the entire intestine.

**APPEARS THIS WAY  
ON ORIGINAL**

## NONCLINICAL PHARMACOLOGY

Colesevelam hydrochloride has been evaluated for efficacy as a bile acid sequestrant in a number of nonclinical *in vitro* and *in vivo* studies (Table 5.2-1).

**Table 5.2-1: Nonclinical Pharmacodynamic Studies**

SECTION NUMBER	STUDY TITLE	SPECIES	TREATMENT DURATION	DOSE	GLP
5.2.3.1	Effect of Cholestagel (GT31-104HB) on Bile Acid Binding Kinetics <i>in vivo</i> (Study No. GT-02-EF-7)	--	--	--	No
5.2.3.2	Effect of Cholestagel (GT31-104HB) on Bile Acid Excretion in Rats and Hamsters (Study No. GT-02-EF-9)	Rat Hamster	2 to 3 days	0.2 to 0.8% 0.15 to 0.6%	No
5.2.3.3	Effect of Cholestagel (GT31-104HB) Alone and in Combination with Lovastatin on Plasma Cholesterol Levels in Dogs (Study No. GT-02-EF-8)	Dog	14 days	300, 1000 mg/kg	No

### Summary of Nonclinical Pharmacology

Colesevelam hydrochloride was shown to bind bile acids *in vitro* in a mixed bile acid solution. The binding data indicate that colesevelam hydrochloride binds approximately 3 mmoles per gram of polymer. No evidence for cooperative kinetics was uncovered.

When compared to cholestyramine, colesevelam hydrochloride was shown to have greater binding affinity for glycocholic acid (GC). However, the affinities for TC, GCDC, GDC, TDC and TDC were very similar for the two compounds. The suppression of bile acid binding to colesevelam hydrochloride by oleic acid was less than that observed with cholestyramine.

The bile acid binding characteristics of colesevelam hydrochloride were evaluated in rats and hamsters by measuring fecal excretion of bile acids. In rats, colesevelam hydrochloride, at doses of 0.2 to 0.8% in the diet, caused a dose-related increase in fecal bile acid excretion. In the hamster, colesevelam hydrochloride, at doses of 0.15 to 0.6% in the diet, caused a dose-dependent increase in fecal bile acid excretion. On a weight basis, colesevelam hydrochloride was approximately twice as potent as cholestyramine in increasing fecal bile acid excretion in these animal models.

The profile of bile acids and of lipid metabolism in hamsters resembles that of humans. The rat differs from humans and hamsters in that it generates predominately taurine-conjugated bile acids.

The cholesterol-lowering properties of colesevelam hydrochloride were evaluated in chow-fed beagle dogs. Colesevelam hydrochloride alone reduced serum cholesterol by 8 and 20 percent when administered at oral doses of 300 and 1000 mg/kg/day, respectively, for 15 days. Lovastatin (5 mg/kg/day) alone reduced serum cholesterol by 19%. When combined with lovastatin (5 mg/kg/day), colesevelam hydrochloride reduced serum cholesterol by 33 and 55% at 300 and 1000 mg/kg/day, respectively. Thus, the reductions obtained with the combination were greater than with either agent alone.

In conclusion, the data on *in vitro* bile acid binding, and on *in vivo* bile acid excretion in rats and hamsters and plasma cholesterol in dogs, support the mechanism of action of colesevelam hydrochloride as a bile acid sequestering polymer.

## NONCLINICAL PHARMACOKINETICS

Two pharmacokinetic studies were carried out to examine absorption, distribution, and excretion (Table 5.2-2). Metabolism studies were not done. Polymer absorption was studied using radiolabeled compound.

**Table 5.2-2: Nonclinical Pharmacokinetics Studies**

SECTION NUMBER	STUDY TITLE	SPECIES	TREATMENT DURATION	DOSE (g/kg)	GLP
5.2.5.1	Absorption, Distribution and Excretion of [ <sup>14</sup> C]-Cholestagel (GT31-104HB) Following a Single Oral Dose in the Rat. (Study No. GT-02-PK-3)	Rat	1 day	3	Yes
5.2.5.2	Pharmacokinetics, Tissue Distribution and Excretion of Radioactivity in Male Beagle Dogs Following a Single Oral Administration of [ <sup>14</sup> C]-Cholestagel with and without a One-Month Pretreatment with Unlabeled Cholestagel. (Study No. GT-02-PK-5)	Dog	1 day	1	Yes

### Absorption, Distribution and Excretion of [<sup>14</sup>C]-Cholestagel (GT31-104HB) Following a Single Oral Dose in the Rat. (Study No. GT-02-PK-3)

#### METHODS

Rats (n=6) were dosed once orally via gavage with radiolabeled drug followed by unlabeled compound at a target dose of 3 g/kg/day via the diet. 96h after dose rats were euthanized, and tissues taken for analysis. Urine and feces was collected 0-24h, 24-48h, 48-72h, 72-96h.

#### RESULTS

One rat had a small amount of radioactivity (0.01% of radioactivity) in the 0-24h urine sample. Approximately 99% of the mean total radioactive dose administered was recovered in the feces by 48h. Blood and plasma had no detectable <sup>14</sup>C levels. Of all tissues examined at 96h postdose (liver, spleen, kidneys, mesenteric lymph nodes, muscle, stomach, small and large intestine, stomach and intestinal contents), only the stomach contained a small amount of radioactivity in 5 out of 6 animals (mean value 0.1 ug eq/g tissue), representing ca. 0.01% of total administered radioactive dose.

#### CONCLUSION

Following oral administration of labeled [<sup>14</sup>C]-colesevelam hydrochloride, the radiolabel is almost completely eliminated within 48 hours via the feces. One animal out of six excreted a small amount of radiolabel in the urine. A minor amount of radioactivity is retained in the stomach.

### Pharmacokinetics, Tissue Distribution, and Excretion of Radioactivity in Male Beagle Dogs Following a Single Oral Administration of [<sup>14</sup>C]-Cholestagel with and without a One Month Pretreatment with Unlabeled Cholestagel (Study No. GT-02-PK-5)

#### METHODS

Beagle dogs (6 per group) were administered empty capsules (Group 2) or capsules containing unlabeled colesevelam hydrochloride (Group 3) at a dose level of 1 g/kg for 28 consecutive days, respectively. Following the 28-day pretreatment, animals in Groups 2 and 3 were administered <sup>14</sup>C-colesevelam hydrochloride at a target dose level of 100 mg/kg and 167 µCi/dog. Group 1, which had only one animal, was untreated for the duration of the study. Animals were sacrificed at 72 hours after dosing of <sup>14</sup>C-colesevelam hydrochloride and blood, tissue, urine, and feces were

measured for radioactivity at 0 to 6, 6 to 12, 12 to 24, 24 to 48, and 48 to 72 hour periods in all dogs. Tissue Collection: Following barbiturate injection, terminal blood, bile and selected tissues (heart, kidneys, liver, lungs, liver, mesenteric lymph nodes, skeletal muscle, spleen, caecum, colon, rectum, small intestine, stomach, stomach and intestinal contents) were collected from Group 2 and 3 animals 72 hours following the administration of  $^{14}\text{C}$ -colesevelam hydrochloride.

## RESULTS

The levels of radioactivity in blood and plasma were below the lower limit of quantification in both test groups. Small quantities of radioactivity were found in the liver, spleen, mesenteric lymph nodes, caecum, rectum, and small intestine. No activity was found in kidney, stomach, muscle, or stomach and GI content.

The tissue radioactivity concentrations are presented in Table 4. The liver, mesenteric lymph nodes, spleen, rectum and its content and small intestine from Group 2 animals contained minimal quantities of radioactivity. The liver, bile, caecum, small intestine and rectum contents contained minimal quantities of radioactivity in Group 3 animals. The activity ranged from 0.009 (small intestine, Group 3) to 0.034 (spleen, Group 2)  $\mu\text{g eq/g}$  tissue.

Mean Concentration of Radioactivity in Tissues of Male Beagle Dogs Following a Single Oral Administration of  $^{14}\text{C}$ -CholestaOcl Without a One Month Pretreatment With Unlabelled CholestaGel

Group 2: At a Mean Dose of 100 mg/kg

Samples	72 h <sup>a</sup>		
	Concentration of Radioactivity, $\mu\text{g eq/g}$	Blood and Tissue to Plasma Ratio	Percent of Dose
Plasma <sup>b</sup>	0.000 $\pm$ 0.000	n/a	n/a
Blood	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Bile	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Heart	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Kidneys	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Liver	0.022 $\pm$ 0.055	n/a	0.003 $\pm$ 0.006
Lungs	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Lymph Nodes (Mesenteric)	0.012 $\pm$ 0.028	n/a	0.000 $\pm$ 0.000
Muscle (Leg adductor)	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Spleen	0.034 $\pm$ 0.004	n/a	0.001 $\pm$ 0.003
Caecum	0.018 $\pm$ 0.029	n/a	0.000 $\pm$ 0.000
Caecum - Contents	n/a	n/a	0.000 $\pm$ 0.000
Colon	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Colon - Contents	n/a	n/a	0.000 $\pm$ 0.000
Rectum	0.012 $\pm$ 0.030	n/a	0.000 $\pm$ 0.000
Rectum - Contents	n/a	n/a	0.005 $\pm$ 0.008
Small Intestine	0.012 $\pm$ 0.029	n/a	0.002 $\pm$ 0.004
Small Intestine - Contents	n/a	n/a	0.000 $\pm$ 0.000
Stomach	0.000 $\pm$ 0.000	n/a	0.000 $\pm$ 0.000
Stomach - Contents	n/a	n/a	0.000 $\pm$ 0.000

<sup>a</sup> Mean  $\pm$  S.D., N=6. The values for individual animals are presented in Appendixes 2, 3, 4 and 5.

<sup>b</sup> Plasma concentration units are  $\mu\text{g eq/ml}$ .

n/a Not applicable.

Mean Concentration of Radioactivity in Tissues of Male Beagle Dogs Following a Single Oral Administration of <sup>14</sup>C-CholestaGel With a One Month Pretreatment With Unlabelled CholestaGel

Group 3: At a Mean Dose of 100 mg/kg

Samples	72 h <sup>a</sup>		
	Concentration of Radioactivity, µg eq/g	Blood and Tissue to Plasma Ratio	Percent of Dose
Plasma <sup>b</sup>	0.000 ± 0.000	n/a	n/a
Blood	0.000 ± 0.000	n/a	0.000 ± 0.000
Bile	0.000 ± 0.001	n/a	0.000 ± 0.000
Heart	0.000 ± 0.000	n/a	0.000 ± 0.000
Kidneys	0.000 ± 0.000	n/a	0.000 ± 0.000
Liver	0.013 ± 0.023	n/a	0.002 ± 0.004
Lungs	0.000 ± 0.000	n/a	0.000 ± 0.000
Lymph Nodes (Mesenteric)	0.000 ± 0.000	n/a	0.000 ± 0.000
Muscle (Leg adductor)	0.000 ± 0.000	n/a	0.000 ± 0.000
Spleen	0.000 ± 0.000	n/a	0.000 ± 0.000
Caecum	0.025 ± 0.030	n/a	0.000 ± 0.000
Caecum - Contents	n/a	n/a	0.000 ± 0.000
Colon	0.000 ± 0.000	n/a	0.000 ± 0.000
Colon - Contents	n/a	n/a	0.000 ± 0.000
Rectum	0.000 ± 0.000	n/a	0.000 ± 0.000
Rectum - Contents	n/a	n/a	0.001 ± 0.003
Small Intestine	0.005 ± 0.021	n/a	0.001 ± 0.002
Small Intestine - Contents	n/a	n/a	0.000 ± 0.000
Stomach	0.000 ± 0.000	n/a	0.000 ± 0.000
Stomach - Contents	n/a	n/a	0.000 ± 0.000

<sup>a</sup> Mean ± S.D., N=6. The values for individual animals are presented in Appendices 2, 3, 4 and 5.

<sup>b</sup> Plasma concentration units are µg eq/ml.

n/a Not applicable.

The major part of the radioactivity was recovered in the feces with negligible amounts detected in the urine and cage rinses. The results for Group 2 and 3 on tissue distribution and recovery were similar. Approximately 75% and 71% of doses were recovered in the feces of Group 2 and 3 animals over 72h. The total radioactivity recovered over 72 h was approximately 75 and 73% for Group 2 and 3 animals. After 72h recovery was very small. It is unclear why the recovery was not complete.

### CONCLUSION

Small quantities of radioactivity were found in the liver, spleen, mesenteric lymph nodes, caecum, rectum, and small intestine. Urinary excretion of <sup>14</sup>C-colesevelam hydrochloride was minimal and a major part of the radioactivity was recovered in the feces.

The data indicate a small amount of absorption, either of polymer degraded in the GI tract or soluble low molecular weight degradants present in the administered dose. The results suggest that a one-month colesevelam hydrochloride pretreatment did not affect absorption and distribution of the compound.

APPEARS THIS WAY  
ON ORIGINAL

BEST POSSIBLE COPY

One study of potential drug interactions was carried out in a canine model.

**Table 5.2-9: Drug Interaction Study**

SECTION NUMBER	STUDY TITLE	SPECIES	TREATMENT DURATION	DOSE	GLP
5.2.6.1	Effect of Cholestagel (GT31-104HB) on the Oral Bioavailability of <sup>3</sup> H-Tetracycline, <sup>3</sup> H-Verapamil, <sup>3</sup> H-Quinidine, <sup>3</sup> H-Valproic Acid, <sup>14</sup> C-Warfarin, Lisinopril, and Metoprolol in Male Beagle Dogs (Study No. GT-02-PK-4)	Dog	1 day	100 mg/kg	Yes

**Effect of Cholestagel (GT-31-104HB) on the Oral Bioavailability of <sup>3</sup>H-Tetracycline, <sup>3</sup>H-Verapamil, <sup>3</sup>H-Quinidine, <sup>3</sup>H-Valproic Acid, <sup>14</sup>C-Warfarin, Lisinopril, and Metoprolol in Male Beagle Dogs (GT-02-PK-4)**

The purpose of this study was to determine the relative bioavailability of a number of drugs administered orally alone or in combination with colesevelam hydrochloride in male beagle dogs.

**METHODS**

In a repeat-measure design, 8 male beagle dogs each received a single oral dose of the following compounds with and without a simultaneous administration of colesevelam hydrochloride at a target dose of 100 mg/kg: 3H-tetracycline (target dose of 25 mg/kg, 100 uCi/dog); 3H-verapamil (target dose of 10 mg/kg, 50 uCi/dog); 3H-quinidine (target dose of 15 mg/kg, 50 uCi/dog); 3H-valproic acid (target dose of 15 mg/kg, 50 uCi/dog); <sup>14</sup>C-warfarin (target dose of 0.5 mg/kg, 20 uCi/dog); lisinopril (target dose of 0.2 mg/kg using —); and metoprolol (target dose of 1.5 mg/kg using —). Serial blood samples were collected from each animal at the following sampling times: 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose. Pharmacokinetic parameters ( $C_{max}$ ,  $T_{max}$ , and  $AUC_{0-t}$ ) were determined using WinNon and the statistical analysis employed (ANOVA).

**RESULTS**

Overall, these results indicate that there was no significant effect on the rate ( $T_{max}$ ) and extent ( $C_{max}$  and  $AUC_{0-t}$ ) of radioactivity, or parent compound, following administration of 3H-tetracycline, 3H-verapamil, 3H-quinidine, 3H-valproic acid, <sup>14</sup>C-warfarin, lisinopril, and metoprolol when administered in combination with colesevelam hydrochloride at a mean dose of 100 mg/kg.

**CONCLUSION**

The results of this study indicate that colesevelam hydrochloride does not alter the pharmacokinetic properties of the seven drugs used in this study.

**APPEARS THIS WAY  
ON ORIGINAL**

## GENERAL TOXICOLOGY

### REPEAT-DOSE TOXICITY STUDIES

The repeat-dose toxicity of colesevelam hydrochloride was evaluated by oral administration in rats and dogs. These studies are listed in Table 5.3-2.

**Table 5.3-2: Repeat Dose Toxicity Studies**

SECTION NUMBER	STUDY TITLE	SPECIES	TREATMENT DURATION	DOSE G/KG	GLP
5.3.2.1	A 90 Day Oral (Diet) Toxicity Study with Cholestagel™ (GT31-104HB) in Rats (Study No. GT-02-TX-9)	Rat	90 days	0, 0.3, 1.5, 3.0	Yes
5.3.2.2	A 13-Week Capsule Toxicity Study with Cholestagel™ (GT31-104HB) in Dogs (Study No. GT-02-TX-10)	Dog	13 weeks	0, 0.2, 0.67, 2.0	Yes
5.3.2.3	A Six-Month Oral (Diet) Toxicity Study of Cholestagel (GT31-104HB) in Rats (Study No. GT-02-TX-19)	Rat	6 months	0, 0.2, 1.2, 2.4	Yes
5.3.2.4	One-Year Oral Toxicity Study in the Beagle Dogs with a 4-Week Recovery Period (Study No. GT-02-TX-28)	Dog	1 year	0, 0.2, 0.6, 2.0	Yes

### A 90- Day Oral (Diet) Toxicity Study of Cholestagel (GT31-104HB) in Rats (Study No. GT-02-TX-9)

#### METHODS

Sprague-Dawley ——— rats (15/sex/group) were exposed to colesevelam hydrochloride in the diet at targeted dose levels of 0.3, 1.5, or 3.0 g/kg/day (Groups 3, 4, 5). 15 male and 15 female rats received cellulose ——— at a targeted dose level of 3.0 g/kg/day in the diet and served as treated controls (Group 2). 15 male and 15 female rats received basal diet and served as untreated controls (Group 1).

#### RESULTS

Mortality in HD males, probably due to hemorrhagic syndrome.

Body weight decrease in HD males, and food consumption elevated in HD in comparison to basal diet control.

Hematology: Significant effects only in males

**Table 5.3-3: Group Mean Hematology (Mean ± S.D.) Values for Week 13 Males**

GROUP NO. (DOSE)	WBC (tham/ $\mu$ L)	RBC (m/ $\mu$ L)	HGB (g/dL)	HCT (%)	APTT (SEC)
1 (0.0 g/kg)	9.8 ±2.14	7.58 ±0.236	15.6 ±0.38	44.5 ±1.09	22.9 ±7.64
2 (3.0 g/kg) Cellulose	9.3 ±0.81	7.69 ±0.411	15.6 ±0.78	45.1 ±2.66	22.4 ±3.54
3 (0.3 g/kg)	9.0 ±2.25	7.38 ±1.452	14.7 ±2.79	43.7 ±8.72	25.7 ±4.99
4 (1.5 g/kg)	9.9 ±2.75	7.72 ±0.276	15.8 ±0.51	46.2 ±1.40	22.2 ±4.89
5 (3.0 g/kg)	10.1 ±5.50	5.84 ±1.946**	12.5 ±3.96 <sup>†</sup>	36.0 ±12.09 <sup>†</sup>	34.9 ±6.36 <sup>†</sup>

<sup>†</sup>P < 0.05; P values vs. control by Dunnett's t-test

\*\*P < 0.01; P values vs. control by Dunnett's t-test

Clinical Chemistry:  
Elevated transaminases and decreases in serum vitamins in MD and HD animals.

**Table 5.3-5: Group Mean Clinical Chemistry (Mean ± S.D): Week 13**

GROUP NUMBER (DOSE)	MALE			FEMALE		
	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (µg/mL)	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (µg/mL)
1 (0.0 g/kg)	0.26 ± 0.051	4.50 ± 0.866	24.45 ± 3.622	0.12 ± 0.045	5.08 ± 0.838	30.24 ± 5.961
2 (3.0 g/kg) cellulose	0.29 ± 0.112	5.10 ± 0.780	25.88 ± 6.104	0.12 ± 0.039	6.69 ± 1.682**	38.10 ± 5.674
3 (0.3 g/kg)	0.27 ± 0.054	5.06 ± 0.905	32.52 ± 5.847*	0.15 ± 0.051	7.30 ± 1.541**	39.81 ± 12.130
4 (1.5 g/kg)	0.30 ± 0.113	3.66 ± 0.534	31.92 ± 10.131	0.13 ± 0.044	4.81 ± 0.610	50.52 ± 16.796**
5 (3.0 g/kg)	0.13 ± 0.061*	1.39 ± 0.629	10.56 ± 4.559**	0.09 ± 0.033	2.72 ± 0.793**	24.58 ± 10.600

\* P ≤ 0.05; P values vs. control by Dunnett's t-test  
\*\* P ≤ 0.01; P values vs. control by Dunnett's t-test

Pathology: Macroscopic hemorrhagic syndrome in various organs in HD males. Microscopic tissue hemorrhage and inflammation, and tissue changes due to hemorrhage.

### CONCLUSION

Mortality in HD males due to hemorrhagic syndrome. Decreases in red cell indices and increased APTT in HD males. Elevated transaminases and decreased serum vitamins (A, E, D) in MD and HD, most pronounced in males. Macroscopic and microscopic hemorrhagic tissue changes in HD males. The no observable adverse effect level (NOAEL) for 3-month dietary exposure to colesevelam hydrochloride was 0.3 g/kg/day.

### Thirteen (13)-Week Capsule Toxicity Study with Cholestagel (GT31-104HB) in Dogs (Study No. GT-02-TX-10)

#### METHODS

Male and female beagles were assigned to four groups (6/sex/group). Capsule preparations were adjusted weekly based on body weights and were designed to administer 0.2, 0.67, and 2.0 g/day for 13 weeks. The control group received empty gelatin capsules.

#### RESULTS

One HD male was sacrificed on Day 76 with dehydration, anorexia, renal dysfunction. Relation to treatment was unclear.

Increased incidence of discolored or liquid feces, and red discharge in MD and HD. Few feces in HD males. Body weight decreased intermittently in HD, and food consumption lowered in HD.

Clinical Chemistry:

Table 5.3-6: Summary of Fat-Soluble Vitamins (Mean ± S.D.) for Week 14

GROUP NUMBER (DOSE)	MALE			FEMALE		
	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (µg/mL)	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (ng/mL)
1 (0.0 g/kg)	1.31 ± 0.212	12.8 ± 4.03	64.4 ± 15.61	1.02 ± 0.056	11.3 ± 0.98	63.5 ± 10.0
2 (0.2 g/kg)	1.16 ± 0.096	7.44 ± 0.700*	68.3 ± 7.60	1.11 ± 0.314	10.5 ± 2.48	77.1 ± 5.48
3 (0.67 g/kg)	1.23 ± 0.231	7.08 ± 0.433*	54.9 ± 17.14	0.937 ± 0.1528	7.61 ± 2.932	60.8 ± 8.78
4 (2.0 g/kg)	1.04 ± 0.302	4.04 ± 1.187*	46.8 ± 9.06	0.860 ± 0.2589	3.60 ± 0.556*	42.4 ± 15.27*

\*P ≤ 0.05; P values vs. control by Dunnett's t-test; N=4

Table 5.3-7: Summary of Clinical Chemistry Data (Mean ± S.D.) for Week 14 Males

GROUP NO. (DOSE)	Chol. (mg/dL)	Calcium (mg/dL)	Chloride (mmol/L)	Alk. Phos IU/L	I Phos. mg/dL
1 (0.0 g/kg)	176 ± 28.2	10.7 ± 0.38	121 ± 2.2	62 ± 3.3	6.2 ± 0.26
2 (0.2 g/kg)	113 ± 10.3*	10.5 ± 0.33	122 ± 1.9	87 ± 18.0*	6.1 ± 0.50
3 (0.67 g/kg)	124 ± 6.6*	10.2 ± 0.21	125 ± 1.0*	77 ± 10.8*	5.6 ± 0.20
4 (2.0 g/kg)	102 ± 6.0*	10.2 ± 0.32	128 ± 2.1*	117 ± 72.3*	6.9 ± 0.38*

\*P ≤ 0.05; P values vs. control by Dunnett's t-test; N=4

Table 5.3-8: Summary of Clinical Chemistry Data (Mean ± S.D.) for Week 14 Females

GROUP NO. (DOSE)	Chol. (mg/dL)	Calcium (mg/dL)	Chloride (mmol/L)	Alk. Phos IU/L	I Phos. mg/dL
1 (0.0 g/kg)	176 ± 14.4	10.8 ± 0.31	119 ± 3.5	94 ± 18.5	5.7 ± 0.33
2 (0.2 g/kg)	144 ± 25.5	10.4 ± 0.14	122 ± 1.6	96 ± 24.1	5.3 ± 0.5
3 (0.67 g/kg)	136 ± 40.0	10.6 ± 0.15	122 ± 2.1	74 ± 13.0	5.8 ± 0.41
4 (2.0 g/kg)	84 ± 12.1*	10.3 ± 0.29	127 ± 4.3*	97 ± 19.3	6.8 ± 0.61*

\*P ≤ 0.05; P values vs. control by Dunnett's t-test; N=4

No significant effects on organ weights.  
No pathology observations.

#### CONCLUSION

Mortality (1 out of 6 animals) with unclear cause in HD males. Abnormal or few feces in MD and HD. BW and FC decreased in HD. Decreases in Vitamins A, E, D in MD and HD males and females. Decreased serum cholesterol in LD, MD and HD males and females. Increased serum alkaline phosphatase in LD, MD, HD males, and increased serum phosphorus in HD males and females. Increased serum chloride in MD and/or HD of both sexes. No drug-related macroscopic or microscopic effects. NOAEL < 0.2 g/kg/day.

#### A 6 Month Oral (Diet) Toxicity Study of Cholestagel (GT31-104HB) in Rats (Study No. GT-02-TX-19)

#### METHODS

Ten (10) male and 10 female rats per group were exposed to colesevelam hydrochloride in the diet at targeted dose levels of 0.2, 1.2, or 2.4 g/kg/day for 26 weeks. 10 male and 10 female rats received cellulose \_\_\_\_\_ at a targeted dose level of 2.4 g/kg/day in the diet and served as treated controls. The highest dose was chosen based upon observations of significant toxicity at 3 g/kg/day in GT-02-TX-9. 10 male and 10 female rats received basal diet and served as untreated controls.

#### RESULTS

No test article-related adverse clinical signs, body weight change, or ophthalmologic findings. Two (2) animals were found dead during the study: one HD male on Day 63 and a female from the basal control group on Day 170. The cause of death of the control animal was unknown. Gross postmortem findings for the male were unremarkable, and clinical signs did not precede death.

Food consumption: Increased in MD and HD compared to the basal control diet group.

Hematology: No effects at Weeks 13 or 26.

Clinical Chemistry:

Elevated transaminases (ALT, AST: 1.5-2x) in HD males and females, and decreases in serum vitamins in MD and HD animals. Vitamin E decreased as compared to control values at Week 26 in HD males and females. Vitamin D levels decreased in MD and HD males and females as compared to basal control values at Week 26.

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 5.3-9: Group Mean Clinical Chemistry Values (Mean ± S.D.): Week 26**

GROUP NUMBER (DOSE)	MALE			FEMALE		
	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (µg/mL)	VITAMIN A (µg/mL)	VITAMIN E (µg/mL)	VITAMIN D (ng/mL)
1 (0.0 g/kg)	0.32 ± 0.043	6.57 ± 1.445	13.02 ± 2.912	0.17 ± 0.040	7.45 ± 1.044	24.80 ± 5.587
2 (2.4 g/kg) cellulose	0.39 ± 0.057	6.11 ± 1.641	10.45 ± 1.947	0.15 ± 0.036	5.97 ± 1.297	30.48 ± 4.449
3 (0.2 g/kg)	0.45 ± 0.081*	7.04 ± 2.372	10.19 ± 3.932	0.16 ± 0.027	9.46 ± 1.010	28.04 ± 13.896
4 (1.2 g/kg)	0.40 ± 0.046	5.99 ± 1.184	6.66 ± 4.456*	0.20 ± 0.043	7.91 ± 2.408	17.09 ± 2.522
5 (2.4 g/kg)	0.28 ± 0.124	1.79 ± 0.456*	5.07 ± 3.975*	0.18 ± 0.035	3.91 ± 0.826**	21.38 ± 1.537

\*P ≤ 0.05; P values vs. control by Dunnett's t-test; N=10

\*\*P ≤ 0.01; P values vs. control by Dunnett's t-test

Organ weights: Ovary weights increased in HD females as compared to basal control, not as compared to cellulose control.

Gross pathology: No macroscopic lesions.

Histopathology:

Fibrosis of the pancreas in 2 of 10 HD males, lymphoid depletion of the mandibular lymph node in 2 of 10 HD males, and hemorrhage in the thymus in 2 of 10 HD males. Foreign material, possibly test article, was present in the lumen of the gastrointestinal tract of HD animals. Hemorrhage of the lacrimal gland in 2 of 10 HD males. Retrobulbar hemorrhage in the eyes in 2 of 10 HD females. Dilatation of the lumen of the uterus in 4 of 10 HD females.

#### CONCLUSION

Elevated transaminases in HD, and decreases in serum vitamins in MD and HD animals. Ovary weights increased in HD females. Histopathology changes including hemorrhage in HD males. Dilatation of uterine lumen in HD females. NOAEL 0.2 g/kg/day.

#### One Year Oral Toxicity Study in the Beagle Dogs with a 4 Week Recovery Period (Study No. GT-02-TX-28)

##### METHODS

Four (4) groups of 4 dogs/sex/group were given single doses of colesvelam hydrochloride for one (1) year. The dose levels were 0, 200, 600, and 2000 mg/kg/day in loose filled capsules. Two (2) additional dogs/sex/group were included in the control and high-dose groups (for a total of 6 dogs/sex/group) as recovery groups followed for 4 weeks at the end of dosing.

## RESULTS

Clinical signs: occasional vomiting and loose/liquid feces in animals from all groups, including the control animals.

Body weights: Reduced in HD females throughout the study.

Food consumption: No effect.

Water consumption: Increased in MD and HD animals.

Hematology changes: Slight to moderate decreases in RBC and Hb, and increase in MCV in MD males and HD males and females.

Clinical chemistry changes: Decreases in phospholipid and cholesterol levels, decreases in fat-soluble vitamins, and increases in chloride levels mainly in HD groups. No significant effects on blood folic acid levels.

Table 5.3-13: Group Mean Clinical Chemistry Week 52

DOSE GROUP	MALES			FEMALE		
	VITAMIN A µg/mL	VITAMIN D µg/mL	VITAMIN E µg/mL	VITAMIN A µg/mL	VITAMIN D µg/mL	VITAMIN E µg/mL
1 (0 mg/kg)						
Mean	0.62	73.68	7.08	0.58	72.13	8.27
S.D.	0.13	13.49	0.65	0.09	8.94	2.04
N	6	6	6	6	6	6
2 (200 mg/kg)						
Mean	0.60	65.55	5.14	0.54	70.71	6.63
S.D.	0.08	12.39	1.09	0.13	15.14	2.97
N	4	4	4	4	4	4
3 (600 mg/kg)						
Mean	0.83	45.52**	2.54***	0.50	48.57**	3.64*
S.D.	0.61	13.84	2.59	0.03	10.58	1.41
N	4	4	4	4	4	4
4 (2000 mg/kg)						
Mean	0.39*	16.68***	0.54***	0.33***	15.91***	1.41***
S.D.	0.09	5.48	0.56	0.02	4.57	2.32
N	6	6	6	6	6	6

\*P ≤ 0.05; \*\*P ≤ 0.01; \*\*\*P ≤ 0.001, P values vs. control

Urinalysis changes: Increases in urine volume mainly in HD groups, possibly following increased water consumption.

Hematology and clinical chemistry returned to normal levels following the 4-week recovery period.

Gross pathology:

One HD female had a diverticulum and prominent Peyer's patches surrounded by thickened and reddened mucosa in the ileum. Pale thyroids seen in one HD male and one HD female.

Organ weights:

Lungs – significant decrease in absolute and relative organ weights in HD males and relative organ weights in the MD males.

Heart – significant increase in relative organ weights in HD females.

Kidneys - significant increase in relative organ weights in HD females

Histopathology:

Ulceration, gastric metaplasia and diverticulitis in the HD females with gross ileal findings.

A low incidence of apparently non-dose related intestinal lesions occurred in treated animals. No histological thyroid lesions found. Collecting tubule vacuolation was reduced in HD animals.

Reduced collecting tubule vacuolation was still observed in the kidneys of HD females.

**CONCLUSION**

Decreased body weight in HD females. Decreases in red cell indices in MD and HD males and females. Decreases in phospholipid and cholesterol levels, decreases in fat-soluble vitamins, and increases in chloride levels mainly in HD groups. Clinical pathology changes were reversible. Decrease in lung weight in males, and increases in heart and kidney weights in females. Possibly test article-related intestinal lesions in both sexes. Collecting tubule vacuolation reduced in HD animals. NOAEL 200 mg/kg/day.

**APPEARS THIS WAY  
ON ORIGINAL**

# RAT CARCINOGENICITY STUDY

## GENERAL INFORMATION

**Study Title:** A Dietary Carcinogenicity Study of Cholestagel® in the Albino Rat  
**Study Number:** GT-02-TX-25  
**Volume Numbers:** Electronic NDA Submission (no paper copy)  
**Test Facility:** [ ]  
**Study Period:** November 1996 - November 1998  
**Date of Submission:** July 30, 1999  
**QA Report:** Yes  
**Dose-range-finding study:** None

## STUDY PROTOCOL AND METHODS

**Study Type:** Diet  
**Species/strain:** Rat, HSD:SD strain  
**Number of animals:** Main Study: 60/sex/dose group; Satellites: 10/sex/group  
**Age at start of study:** Approximately 6 weeks  
**Weight at start of study:** 142-205g (males), 117-160g (females)  
**Animal housing:** Individually  
**Drug Name:** Colesevelam hydrochloride (Cholestagel, GT31-104HB)  
**Drug Batch number(s):** Batch Nrs CHOG 9604 1459, TKFC406-1502, CHOG 9605 1587, CHOG 9606-1604, 056329, TLMC 002 1830, TMAC 010 1852, TMAC 011 1853  
**Drug Analysis:** Samples taken from each batch before and after treatment period with that particular batch  
**Drug Homogeneity:** Not assessed  
**Drug Stability:** 2 weeks, room temperature  
**Vehicle employed:** Powdered certified rat chow  
**Preparation of diets:** Test article was mixed with rodent chow for ca. 15 min in a V-blender, prepared weekly, stored at room temperature. Appropriate dose level (mg/kg) was attained by adding required amount of test article to chow, based on animal's body weight and food consumption.

### Doses:

Group		Dose (g/kg/day)	Main Study N/sex/group	Satellites N/sex/group
1	Control	0 (basal diet)	60	10
2	Control*	0 (basal diet*)	60	10
3	LD*	0.4	60	10
4	MD*	1.2	60	10
5	HD*	2.4	60	10
6	Health screen	-	10	-

#### \*Vitamin-supplemented diet:

Vitamin A - supplemented with an additional 1250 IU/kg  
 Vitamin D - supplemented with an additional 750 IU/kg  
 Vitamin E - supplemented with an additional 640 IU/kg  
 Vitamin K - supplemented with an additional 20 ppm

**Relation to Clinical Use:** Recommended starting dose: 6x625mg (3750mg) per day, equivalent to 62.5mg/kg/day.

**CAC Concurrence:** Not available

**Route of Administration:** Oral (diet)  
**Frequency of Drug Administration:** Daily  
**Control groups:** Two groups, one with vitamin-supplemented diet (n=60)  
**Interim Sacrifices:** None  
**Special Study Group(s):** Health screen: 10/sex/dose group (for blood sampling and gross pathology examination)  
**Unscheduled Sacrifices or Deaths:** See Results (Mortality)  
**Statistics:** *Sponsor's evaluation:* Mortality and tumour data: Proc Chronic program  
*CDER evaluation:* See CDER Biometrics Review

## **STUDY RESULTS**

### **Clinical Observations**

No treatment-related clinical signs.

#### **Incidence of masses at necropsy (Main and Satellite Study)**

Group		Dose (g/kg/day)	MALES	FEMALES
			Number per 70 (%)	Number per 70 (%)
1	Control 1	0	21 (30%)	41 (59%)
2	Control 2*	0	28 (40%)	45 (64%)
3	LD*	0.4	23 (33%)	44 (63%)
4	MD*	1.2	19 (27%)	42 (60%)
5	HD*	2.4	15 (21%)	43 (63%)

\*Vitamin-supplemented diet

### **Mortality**

*Note: Survival graphs are attached to this review (p. 28)*

Mortality was fairly high in male Group 1 (82%) and Groups 2, 3, and 4 (65%-75%). Mortality was decreased in male Group 5 (42%). According to the CDER Reviewers' statistical analysis the linear trend in mortality was statistically significant.

Mortality was moderate in all females groups (32%-52%) and was not drug treatment-related.

#### **Mortality (Main Study)**

Group		Dose (g/kg/day)	MALES	FEMALES
			Number per 60 (%)	Number per 60 (%)
1	Control 1	0	49 (82%)	19 (32%)
2	Control 2*	0	41 (68%)	31 (52%)
3	LD*	0.4	45 (75%)	24 (40%)
4	MD*	1.2	39 (65%)	19 (32%)
5	HD*	2.4	25 (42%)	29 (48%)

\*Vitamin-supplemented diet

### **Body Weight**

*Note: Body weight graphs are attached to this review (p. 29)*

Small reduction in BW and BW gain in first 52 weeks, particularly in first 26 weeks of study in HD males, as compared to Group 1 controls, and occasionally to Group 2 controls.

At approximately Week 90 all male animals started to lose weight.

#### **Body weight in males**

Body weight	Ctr 1	Ctr 2	LD	MD	HD
wk 52 (g)	558	538	547	550	517
wk 52 (%)	104%	100%	102%	102%	96%
wk 104 (g)	487	517	514	500	483
wk 104 (%)	94%	100%	99%	97%	93%

**Body weight gain in males**

BW gain (g)	Ctr 1	Ctr 2	LD	MD	HD
wk 0-26	330	322	331	327	303
wk 26-52	46	38	31	40	35
wk 0-104	316	336	313	303	293

**Food Consumption:**

*Males:* Slight increase in LDm in first 90 weeks of study, and slight to moderate increase in MDm and HDm through week 104 of the study.

*Females:* Slight increase in MDf and HDf through week 104 of the study.

**Food consumption values at Week 52 and Week 104**

	Ctr 1	Ctr 2	LD	MD	HD
Food consumption (g/animal) at wk 52					
MALES	156	161	163	176	178
FEMALES	116	113	116	121	125
Food consumption (g/animal) at wk 104					
MALES	134	144	144	144	164
FEMALES	122	120	120	128	134

**Calculated intake of test article:**

The calculated values shown in the table below are over the whole 104-week dosing period.

**Mean overall calculated intake (g/kg/day)**

Group	LD	MD	HD
Target dose (g/kg/day)	0.4	1.2	2.4
Calculated dose (g/kg/day)			
MALES	0.38	1.18	2.39
FEMALES	0.39	1.18	2.39
Calculated dose (% of target dose)			
MALES	96%	98%	99%
FEMALES	99%	98%	99%

**Test article concentrations in the diet**

Group	LD	MD	HD
Target dose (g/kg/day)	0.4	1.2	2.4
DIET LEVELS MALES (g/kg)			
Week 1	2.7	8.01	16.0
Week 13	7.4	22.1	41.5
Week 26-27	8.9	26.0	48.4
Week 52-53	9.8	27.4	50.4
Week 104	9.7	27.9	51.4

Group	LD	MD	HD
Target dose (g/kg/day)	0.4	1.2	2.4
DIET LEVELS FEMALES (g/kg)			
Week 1	2.9	8.7	17.4
Week 13	6.2	18.6	35.0
Week 26-27	6.7	20.7	38.1
Week 52-53	7.1	21.2	39.0
Week 104	7.0	20.8	39.0

**Toxicokinetics:**

No data

**Ophthalmology:**

No treatment-related changes

**Hematology**

Weeks 26, 52, 104: At some of the time points, there were differences in lymphocyte count (decrease) and/or segmented neutrophil count (increase) in MD and HD, m and f, and in eosinophil count (decrease) in HDm. Changes were slight (lymphocytes) to moderate (eosinophils), and their biological significance is unclear.

**Serum vitamin levels**

(Data from satellite animals)

Vitamin D:

Large decrease in serum vitamin D levels in HD males in all weeks assayed (26, 52, 79, 104). Control levels in females (groups 1 and 2) were much higher than in males.

**Vitamin D levels (Week 52)**

Group	Dose (g/kg/day)	MALES		FEMALES	
		25,OH-Vit D (ng/ml)		25,OH-Vit D (ng/ml)	
1	Control 1	0	10.2	29.0	
2	Control 2*	0	13.2	37.8	
3	LD*	0.4	10.6	28.9	
4	MD*	1.2	13.0	28.1	
5	HD*	2.4	4.5**	31.2	

\*Vitamin-supplemented diet; \*\* significantly different from groups 1 and 2

**Vitamin D levels (Week 104)**

Group	Dose (g/kg/day)	MALES		FEMALES	
		25,OH-Vit D (ng/ml)		25,OH-Vit D (ng/ml)	
1	Control 1	0	6.1	18.2	
2	Control 2*	0	7.6	19.9	
3	LD*	0.4	6.6	21.1	
4	MD*	1.2	7.0	19.6	
5	HD*	2.4	2.1**	16.4	

\*Vitamin-supplemented diet; \*\* significantly different from groups 1 and 2

Vitamin E:

Decrease in serum Vitamin E levels in HD males in Week 104, but not in Weeks 26, 52, 79. No effects in females at any time.

**Vitamin E levels (Week 52)**

Group	Dose (g/kg/day)	MALES		FEMALES	
		Vit E (ug/ml)		Vit E (ug/ml)	
1	Control 1	0	19.5	13.5	
2	Control 2*	0	25.3	20.7	
3	LD*	0.4	33.0	25.5	
4	MD*	1.2	29.9	23.4	
5	HD*	2.4	21.1	21.3	

\*Vitamin-supplemented diet;

**Vitamin E levels (Week 104)**

Group		Dose (g/kg/day)	MALES	FEMALES
			Vit E (ug/ml)	Vit E (ug/ml)
1	Control 1	0	52.5	47.7
2	Control 2*	0	69.1	63.2
3	LD*	0.4	72.2	65.2
4	MD*	1.2	74.9	60.5
5	HD*	2.4	50.0**	59.6

\*Vitamin-supplemented diet; \*\* significantly different from group 2

**Organ Weights:**

No data

**Gross pathology:**

**Gross pathology findings (all animals, ie, preterminal and terminal sacrifice)\***

Group #		Males					Females				
		Ctrl1	Ctrl2	LD	MD	HD	Ctrl1	Ctrl2	LD	MD	HD
N examined		60	60	60	60	60	60	60	60	60	60
N preterminal		49	41	45	39	25	19	31	24	19	29
N terminal		11	19	15	21	35	41	29	36	41	31
Kidney	Foci pale	4	5	11	13	21	1	2	2	0	2
	Discoloration	14	12	7	3	2	1	3	1	1	2
Lung	Foci pale	3	5	1	8	7	0	0	0	0	0
	Cyst	0	1	2	4	3	0	0	0	0	0
	Area raised	2	4	5	10	10	10	18	20	23	24
	Area pale	0	5	4	6	12	8	17	9	5	13
	Nodule	1	0	0	1	4	0	1	2	1	1
Pancreas	Nodule	0	0	1	1	4	0	0	0	2	0
Lacrimal gland	Foci pale	4	4	3	5	2	2	2	3	7	8
Liver	Cyst	0	1	2	4	3	6	6	2	7	12
Uterus	Material dark	-	-	-	-	-	2	3	2	2	8

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

**Histopathology**

**NOTE: Histopathology was performed in Groups 1, 2, and 5 on retained tissues from all animals, but in Groups 3 and 4 only on tissues from animals that were euthanized preterminally or found dead! (Satellite animals were not analyzed). Nevertheless, data show that in LD and MD groups histopathology findings were often reported for more animals than preterminal ones only.**

**Neoplastic histopathology findings (all animals, ie, preterminal and terminal sacrifice)\***

Group #		Males					Females				
		Ctrl1	Ctrl2	LD	MD	HD	Ctrl1	Ctrl2	LD	MD	HD
N preterminal		49	41	45	39	25	19	31	24	19	29
N terminal		11	19	15	21	35	41	29	36	41	31
Pancreas	Acinar cell adenoma (B)	0/60	0/60	0/45	2/40	3/60	0/60	0/59	0/24	0/21	0/60
	%	0	0	0	5	5	0	0	0	0	0
Thyroid	C-cell adenoma (B)	8/60	7/60	7/49	5/41	13/60	20/60	11/60	4/28	5/23	19/60
	%	13	12	14	12	22	33	18	14	22	32

Harderian gland	Adenocarcinoma (M)	0	0	0	0	0	0/60	0/60	1/25	0/20	2/60
	%	0	0	0	0	0	0	0	4	0	3

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

**Non-neoplastic histopathology findings (all animals, i.e., preterminal and terminal sacrifice)\***

Group #		Males					Females				
Group		Ctrl	Ctrl2	LD	MD	HD	Ctrl1	Ctrl2	LD	MD	HD
<i>N preterminal</i>		49	41	45	39	25	19	31	24	19	29
<i>N terminal</i>		11	19	15	21	35	41	29	36	41	31
Bone marrow	Increased erythropoiesis	2/60	2/60	1/45	2/40	8/60	0/60	2/60	0/24	0/19	1/60
	%	3	3	2	5	13	0	3	0	0	2
Kidney	Chronic progressive nephropathy	59/60	60/60	55/57	55/57	60/60	40/60	29/60	22/33	20/28	36/60
	%	98	100	96	96	100	67	14	67	71	60
Liver	Biliary cyst	1/60	1/60	0/51	3/51	2/60	4/60	6/60	4/36	6/37	12/60
		2	2	0	6	3	7	10	11	16	20
Lung	Granuloma	0/60	0/60	1/51	0/51	6/60	1/60	2/60	2/47	1/41	3/60
	%	0	0	2	0	10	2	3	4	2	5
Pancreas	Acinar cell hyperplasia	1/60	1/60	0/45	2/40	2/60	0	0	0	0	0
	%	2	2	0	5	3	0	0	0	0	0
Thymus	Hemorrhage	0/58	2/60	3/49	1/46	4/60	1/59	2/60	0/29	1/24	4/60
	%	0	3	6	2	7	2	3	0	4	7
Thyroid	C-cell hyperplasia	7/60	8/60	3/49	8/41	13/60	10/60	11/60	5/28	4/23	8/60
	%	12	13	6	20	22	17	18	18	17	13

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

**Note:**

Sponsor's neoplastic incidence summary tables in APPENDIX 1

**STATISTICAL ANALYSIS OF TUMOR FINDINGS**

**Sponsor's statistical analysis**

Mortality and tumor data were statistically analyzed using PROC CHRONIC. Tumor data analysis was only conducted on Groups 1, 2 and 5, as animals from Groups 2 and 3 were not all examined.

**Mortality**

Statistical analysis of mortality data showed that in males there was a significant decrease in mortality rates at increasing dose levels (heterogeneity test; two-tailed Tarone's test). Pairwise comparison of Group 2 with Groups 3, 4, and 5 showed a significant difference in mortality rate between Group 2 and Group 5.

**Tumors**

There were no statistically significant increases of tumor incidences in Group 5 animals. The increased incidence of thyroid C-cell adenoma in male animals was not statistically significant.

There were significant variations in the control groups for uterine benign endometrial stromal polyp and lymph node lymphosarcoma (Sponsor's Table A below). The incidence of both tumors

was decreased in Group 2 as compared to Group 1 and the decrease was significant for incidental (and pooled) uterine polyp and for fatal lymph node lymphosarcoma.

**Table A: Summary results of Proc Chronic analysis on female and male data sets with significant heterogeneity test(s).**

Sex	Organ / Tissue	Lesion type	Tumor Classification	Number of Tumor-Bearing Animals / Sample Size			P-VALUE <sup>1</sup>			
				Group 1	Group 2	Group 5	Heterogeneity Test <sup>2</sup>	Group 1 vs Group 2 <sup>3</sup>	Group 1 vs Group 5 <sup>3</sup>	Group 2 vs Group 5 <sup>3</sup>
♀	Uterus	Benign endometrial stromal polyp	F=fatal	0	0	0	---	---	---	---
			I=incidental	13	3	4	<b>0.03203</b>	<b>0.04288</b>	0.97817	0.39248
			Pooled=F+I	13/60	3/60	4/60	<b>0.03203</b>	<b>0.04288</b>	0.97817	0.39248
♂	Lymph Node	Lympho-sarcoma	F=fatal	3	0	0	<b>0.01066</b>	<b>0.04387</b>	0.98887	---
			I=incidental	0	2	0	0.08548	0.27347	---	0.97368
			Pooled=F+I	3/60	2/60	0/60	<b>0.04948</b>	0.36537	0.98887	0.97368

Significant p-values are in bold.

<sup>1</sup> Two-tailed test.

<sup>3</sup> One-tailed test.

Sponsor's conclusion: Dietary administration of colesevelam hydrochloride to Sprague Dawley rats for up to 2 years did not produce any tumorigenic effect.

Reviewers comment: Since thyroid C-cell adenoma was increased in parallel with C-cell hyperplasia in Group 5 HD males, the thyroid C-cell preneoplastic and/or neoplastic findings are suggestive of a drug-related effect.

### CDEP reviewers statistical analysis

(APPENDIX 2)

The CDER reviewer analyzed the dose-tumor positive linear trend of ALL groups. Although the Sponsor claimed that the tumor data analysis was only conducted on Groups 1,2, and 5 due to the fact that microscopic examinations were not performed on all tissues/organs of animals in Groups 3 and 4, the CDER Reviewer included Groups 3 and 4 in her tumor data analysis because the number of histopathology findings in these groups was not less than 45% of the total (in other words, a sufficient number of tissues from (preterminal) animals was still examined). The result was as follows:

#### Incidence rates and P-values of tumor types showing significant trends

Organ	Tumor	Tumor-Bearing animal	P-value
Pancreas	Acinar cell adenoma (5106)	0,0,0,2,3	0.002*

\*statistical significance at level 0.025 (rare tumor)

CDER Reviewer also performed a pairwise comparison of the two control groups and Group 5. The pairwise test also showed a statistical significant increase in the incidence of pancreas acinar cell adenoma in Group 5 (P<0.05, Exact)

## **SUMMARY AND EVALUATION**

Colesevelam hydrochloride is a non-absorbed polymeric bile acid sequestrant indicated for lowering of serum cholesterol levels. A 104-week dietary carcinogenicity study was carried out in rats to investigate the carcinogenic potential of the compound. Animals in Control Group 2 and the three drug dose Groups 3, 4 and 5 received a diet supplemented with Vitamins A, E, D and K to prevent secondary effects of vitamin depletion due to reduced absorption of these fat-soluble vitamins.

### **Dose selection**

The dietary concentration of the test compound in HD male rats achieved a 5% level at week 46-47 and remained at that level throughout the study. The dietary concentration of the test compound in HD female rats achieved a 4% level by week 62 and remained at that level throughout the study.

It can be argued that the high dose in the female rat study was too low, i.e., 4% rather than the recommended 5% in a study in which toxicity is not a dose-limiting factor.

The high doses used in the rat carcinogenicity study, for both male and female animals (2.4 g/kg/day), were approximately 30 times the maximum recommended human dose (4.5 g/day), when compared on the basis of kg body weight. Since this drug is virtually not absorbed the most appropriate dose comparison is on the basis of body weight.

### **Rat and human dose comparison**

Rat dose group	Dose (g/kg/day)	Multiple of maximum recommended human dose*
LD	0.4 g/kg/day	5.3x
MD	1.2 g/kg/day	16x
HD	2.4 g/kg/day	32x

Maximum recommended human dose is 4.5 g/day, or 75 mg/kg for a 60 kg person

### **Mortality, Body weight, Food consumption, Hematology, Serum vitamin levels**

In the rat carcinogenicity study with colesevelam hydrochloride mortality was decreased in Group 5 HD males as compared to Group 1 and Group 2 controls. Body weight was slightly reduced in HD males as compared to Group 1 controls and occasionally to Group 2 controls, mainly due to an effect occurring in the first 52 weeks of the study. Food consumption was slightly and dose-dependently increased in all treated male and female groups throughout the study. Occasional changes with unclear significance were seen in white blood cell differential count in MD and/or HD males and females. Vitamin D levels were significantly decreased in HD males throughout the study. Vitamin E levels were decreased at Week 104 but not Week 52 in HD males.

### **Gross pathology**

In Group 5 males there was an increase in the incidence of pale foci in the kidneys. This may be related to the decreased mortality in this HD group. Group 5 males also had slightly decreased body weights. This may have been the cause of a decreased severity of chronic progressive nephropathy (with no effect on incidence) in this group leading to decreased incidence of kidney discoloration. No other drug-related macroscopic findings were seen in males and no findings were seen in females.

### **Histopathology findings**

#### **Neoplastic findings**

NOTE: LD and MD animals were only evaluated histopathologically when they were found dead or were sacrificed preterminally. For that reason, tumor findings are only available from preterminal LD and MD animals. Thus, only statistical analysis of tumor incidences in control and HD animals is possible.

**Pancreas:** In male rats, there was an incidence of pancreatic acinar cell adenoma in MD and HD animals (2/40 and 3/60). The tumor was not seen in control or LD males. These data suggest that

the pancreatic acinar cell tumor incidence in the males is drug-related. The tumor was not seen in female animals.

**Thyroid:** In male rats, an increased incidence of thyroid C-cell (calcitonin-producing cell) adenoma was observed in HD animals as compared to control groups 1 and 2. In females, thyroid C-cell adenoma incidence was also increased in HD animals when compared to control group 2 (vitamin-supplemented group) but not when compared to control group 1.

In males, the thyroid C-cell tumor incidence was fairly high in the two control groups (8/60 and 7/60). The incidence in the HD group (13/60) was approximately 2x the control incidence. It is unclear from these data whether the increased tumor incidence in males was drug-related.

In females the incidence in HD animals (19/60) was approximately 2x the incidence in Group 2 controls (11/60). It is unclear from the data whether the occurrence of this tumor in females is drug-related.

**Harderian gland:** In female animals there was an increased incidence of Harderian gland adenocarcinoma in LD and HD animals (1/25 and 2/60). In MD females this tumor was not seen. The tumor was not seen in male animals. The drug-relatedness of this finding in the females is not clear.

#### Non-neoplastic findings

**Pancreas:** Acinar cell hyperplasia was increased in mid and high dose males.

**Thyroid:** C-cell hyperplasia was increased in HD males. Sponsor believes this is due to decreased mortality in the HD male group. This is a possible explanation. However, if this was the case, other tissue hyperplasias or lesions would also be expected to be increased in HD males but not in females. It is not clear from the data whether this was the case. The increase in C-cell hyperplasia in the HD males coincided with an increase in thyroid C-cell adenoma, suggesting a drug-related effect. However, while in HD females thyroid C-cell adenoma incidence was also increased, thyroid hyperplasia was not, arguing for the increased survival of HD males underlying the increased hyperplasia incidence.

The severity but not the incidence of chronic progressive nephropathy was decreased in HD males. Sponsor views this as being related to the increased survival in this group. Findings related to nephropathy were indeed also decreased in the HD males (gastric mineralization, cardiomyopathy, parathyroid hyperplasia). However, the cause of the decreased severity of the nephropathy is unclear. Possibly, the reduced body weight in the HD males was responsible for this effect.

#### CDER Biometrics evaluation (APPENDIX 2):

According to a dose-tumor trend test among all groups there was a significant positive trend for pancreas acinar cell adenoma. A pairwise test between the two control groups and HD Group 5, also showed statistical significance for pancreas acinar cell adenoma.

#### CONCLUSIONS

Colesevelam chloride was tested in a rat bioassay for 104 weeks at doses up to 32 times the maximum recommended human dose. Mortality and body weight were decreased in the HD male group. Food consumption was increased in all treated males and females. There were decreases in Vitamin D and Vitamin E levels in HD males.

Tumor findings included an increased incidence of pancreas acinar cell adenoma in MD and HD males. This increase was statistically significant. Thyroid C-cell adenoma appeared increased in HD males, but the finding was not statistically significant. The apparent increase in Harderian gland tumors in females was also not statistically significant.

#### QUESTION TO EXEC CAC

What tumors are relevant for the clinical situation and which ones do you suggest should be mentioned in the label?

**NDA 21-223**  
**Cholestagel**  
**Rat Carcinogenicity Study**

**Histopathology Inventory**  
**(Tissues retained)**

Sponsor Study #GT-02-TX-25		
Species: Rat	Pathology	
	Groups 1,2,5	Groups 3,4**
Abnormalities	X	X
Adrenals	X	X
Aorta (thoracic)	X	X
Blood film		
Bone (sternum)	X	X
Bone marrow (sternum)	X	X
Brain	X	X
Cecum	X	X
Colon	X	X
Duodenum	X	X
Epididymides	X	X
Esophagus	X	X
Eyes	X	X
Gall bladder	X	X
Harderian glands	X	X
Heart	X	X
Ileum	X	X
Jejunum	X	X
Kidneys	X	X
Lacrimal glands	X	X
Larynx		
Liver	X	X
Lungs (all lobes)	X	X
Lymph nodes, mandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary gland (inguinal)	X	X
Optic nerves	X	X
Ovaries	X	X
Pancreas	X	X
Pituitary	X	X
Parathyroids	X	X
Pharynx		
Prostate	X	X
Rectum*	X	X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle (quadriceps)	X	X
Skin (inguinal)	X	X
Spinal cord (cervical)	X	X
Spleen	X	X
Stomach	X	X
Teeth		
Testes	X	X

**APPEARS THIS WAY  
ON ORIGINAL**

Thymus	X	X
Thyroid	X	X
Tongue	X	X
Tonsils		
Trachea	X	X
Ureter		
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal glands		

\*tissue retained but not processed

\*\*Group 3 and 4: tissue from preterminal animals only examined

**APPEARS THIS WAY  
ON ORIGINAL**

# BEST POSSIBLE COPY

FIGURE I

PROJECT NO. 3798

## SURVIVAL OF RATS MALES

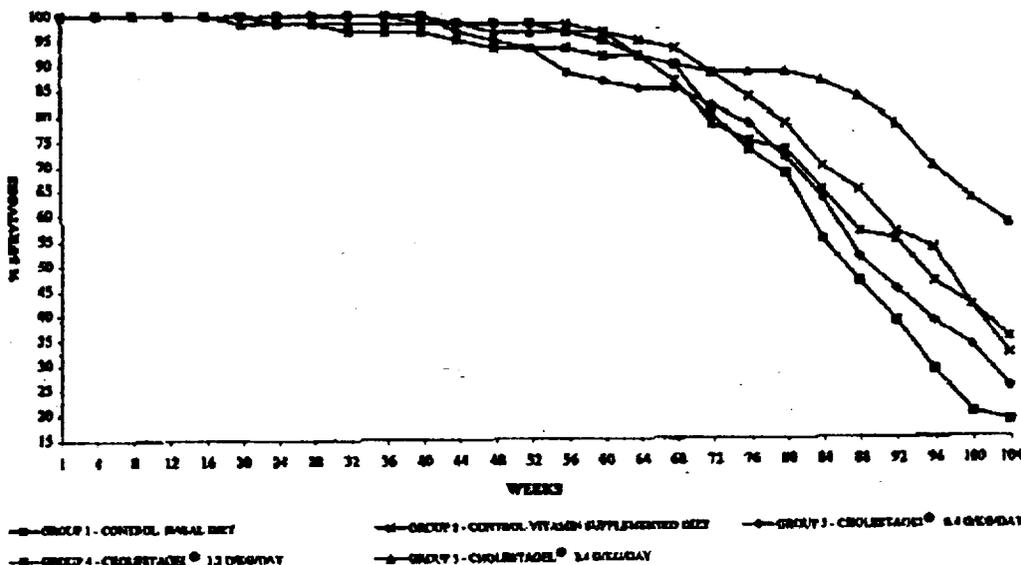


FIGURE II

PROJECT NO. 3799

## SURVIVAL OF RATS FEMALES

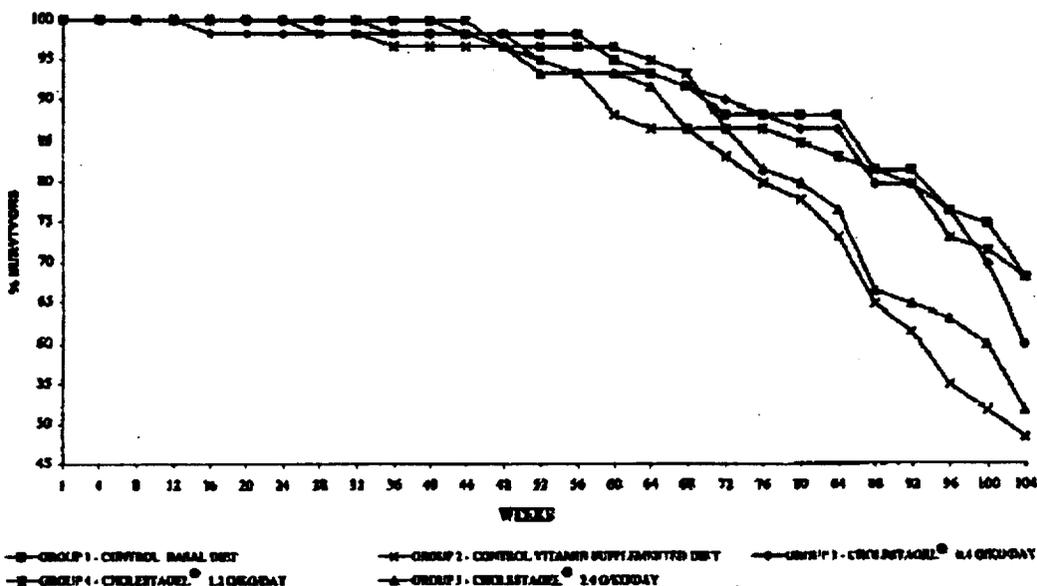
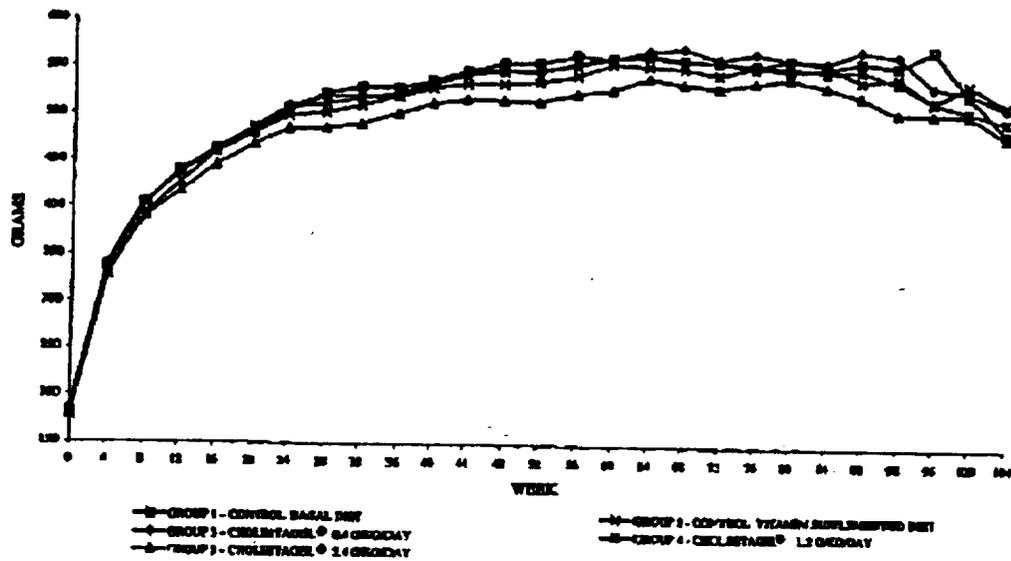


FIGURE III

PROJECT NO. 5789

### GROUP MEAN BODY WEIGHT MAIN STUDY - MALES

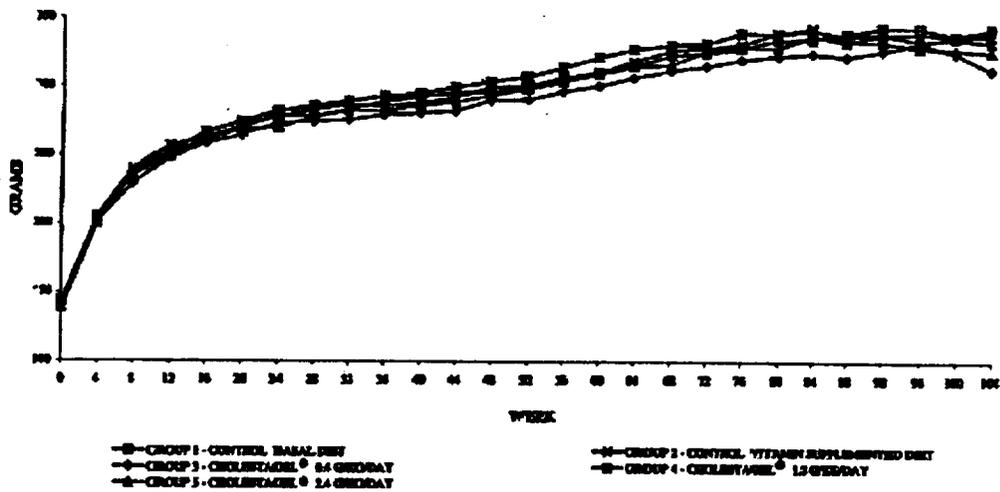


- 124 -

FIGURE IV

PROJECT NO. 5789

### GROUP MEAN BODY WEIGHT MAIN STUDY - FEMALES



- 125 -

# APPENDIX 1

Sponsor's histopathology summary tables

TABLE NO.: 1B  
PROJECT NO.: 87889

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

		SEX :				
		— M A L E —				
		DOSE GROUP :				
		1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		60	60	60	60	60
GROUP 1	CONTROL BASAL DIET	GROUP 4 CHOLESTAGEL® 1.2 G/KG/DAY				
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET	GROUP 5 CHOLESTAGEL® 2.4 G/KG/DAY				
GROUP 3	CHOLESTAGEL® 0.4 G/KG/DAY					
ADRENAL	-TOTAL EXAMINED	60	60	47	43	60
	-#-CORTICAL ADENOMA	2	2	0	0	1
	-#-BENIGN PHEOCHROMOCYTOMA	20	9	8	3	7
	-#-MALIGNANT PHEOCHROMOCYTOMA	3	2	3	1	3
BONE MISCELLANEOUS	-TOTAL EXAMINED	1	0	0	1	0
	-#-OSTEOMA	1	0	0	0	0
BONE STERNUM	-TOTAL EXAMINED	60	60	45	40	60
	-#-OSTEOSARCOMA	1	0	0	0	0
BRAIN	-TOTAL EXAMINED	60	60	45	40	60
	-#-GLIOMA	1	1	0	0	0
CAVITY ABDOMINAL	-TOTAL EXAMINED	0	1	0	2	2
	-#-MALIGNANT SCIRRHOMA	0	0	0	1	0
DUODENUM	-TOTAL EXAMINED	60	60	46	40	60
	-#-ADENOCARCINOMA	0	0	0	0	1

12300

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 67989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1	CONTROL BASAL DIET	GROUP 4	CHOLESTAGEL®	1.2 G/KG/DAY		
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET	GROUP 5	CHOLESTAGEL®	2.4 G/KG/DAY		
GROUP 3	CHOLESTAGEL® 0.4 G/KG/DAY					
		SEX :				
		DOSE GROUP :				
		NO. OF ANIMALS IN DOSE GROUP :				
		MALE				
		1	2	3	4	5
		60	60	60	60	60
HEART	-TOTAL EXAMINED	60	60	46	39	60
	-MALIGNANT ENDOCARDIAL SARCOMA	0	0	2	0	0
JEWELRY	-TOTAL EXAMINED	60	60	46	41	60
	-M-ADENOCARCINOMA	0	0	1	1	0
KIDNEY	-TOTAL EXAMINED	60	60	57	57	60
	-M-NEPHROBLASTOMA	0	0	0	1	1
	-M-LIPOMA	0	0	1	0	0
	-M-TUBULAR CELL CARCINOMA	1	0	1	0	0
	-M-HEMANGIOSARCOMA	0	0	0	1	0
LIVER	-TOTAL EXAMINED	60	60	51	51	60
	-M-HEPATOCELLULAR ADENOMA	0	0	0	0	1
LUNG	-TOTAL EXAMINED	60	60	51	51	60
	-M-ALVEOLAR/BRONCHIOAL ADENOMA	0	0	0	1	0
LYMPH NODE	-TOTAL EXAMINED	30	29	24	30	18
	-M-LYMPHOSARCOMA	2	2	2	4	0
	-M-HISTIOCYTIC SARCOMA	1	0	0	0	0

ASDA

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CICLESTAGEL® 1.2 G/KG/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CICLESTAGEL® 2.4 G/KG/DAY				
GROUP 3 CICLESTAGEL® 0.4 G/KG/DAY						
NO. OF ANIMALS IN DOSE GROUP :	SEX :	MALE				
	DOSE GROUP :	1	2	3	4	5
		60	60	60	60	60
<b>PANCREAS GLAND</b>						
-TOTAL EXAMINED		60	60	47	40	50
-FIBROADENOMA		0	1	1	1	0
-ADENOCARCINOMA		0	1	0	0	0
<b>PANCREAS</b>						
-TOTAL EXAMINED		60	60	46	40	60
-ACINAR CELL ADENOMA		0	0	0	2	3
-ISLET CELL CARCINOMA		0	2	2	0	2
-MIXED ACINAR-ISLET CELL CARCINOMA		1	0	0	0	0
-ISLET CELL ADENOMA		0	0	1	0	1
<b>PITUITARY</b>						
-TOTAL EXAMINED		60	60	52	44	60
-ADENOMA, PARS DISTALIS		5	11	5	4	12
-ADENOMA, PARS INTERMEDIA		0	1	0	0	0
<b>SKIN MISCELLANEOUS</b>						
-TOTAL EXAMINED		27	37	22	24	24
-SQUAMOUS CELL CARCINOMA		1	0	1	0	1
-BENIGN KERATOCARCINOMA		1	1	2	0	0
-BASAL CELL CARCINOMA		1	1	0	0	0
-SEBACEOUS ADENOMA		0	1	0	0	0
-SQUAMOUS CELL PAPILLOMA		0	0	0	1	1

- 1885 -

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTAGE <sup>®</sup> 1.2 G/KG/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTAGE <sup>®</sup> 2.4 G/KG/DAY				
GROUP 3 CHOLESTAGE <sup>®</sup> 0.4 G/KG/DAY						
SEX :		MALE				
DOSE GROUP :		1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		60	60	60	60	60
<b>SUBCUTANEOUS TISSUE</b>						
	-TOTAL EXAMINED	50	6	8	11	5
	-M-FIBROSARCOMA	3	1	0	0	1
	-M-HEMANGIOSARCOMA	1	0	0	1	0
	-M-MALIGNANT SARCOMA	1	0	0	1	0
	-F-LIPOMA	0	0	1	0	0
	-F-FIBROMA	2	1	3	1	3
	-M-INDIFFERENTIATED SARCOMA	0	0	1	0	0
<b>TESTIS</b>						
	-TOTAL EXAMINED	60	60	48	40	60
	-M-INTERSTITIAL CELL ADENOMA	2	0	0	0	0
	-M-SEKOLI CELL TUMOR	0	0	0	1	0
	-M-NEOTHELIOMA	0	0	1	0	1
<b>THYMUS</b>						
	-TOTAL EXAMINED	58	60	49	46	60
	-F-BENIGN THYMOMA	0	0	0	1	0
<b>THYROID</b>						
	-TOTAL EXAMINED	60	60	49	41	60
	-M-C-CELL CARCINOMA	0	1	2	0	0
	-F-C-CELL ADENOMA	0	7	7	5	13
	-F-FOLLICULAR CELL ADENOMA	0	1	0	0	0

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
 PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
 CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTAGEL® 1.2 G/KG/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTAGEL® 2.4 G/KG/DAY				
GROUP 3 CHOLESTAGEL® 0.4 G/KG/DAY						
NO. OF ANIMALS IN DOSE GROUP :	SEX :	R A T E				
	DOSE GROUP :	1	2	3	4	5
		60	60	60	60	60
TONGUE	-TOTAL EXAMINED	60	60	45	40	60
	-SQUAMOUS CELL CARCINOMA	0	0	0	0	1
URINARY BLADDER	-TOTAL EXAMINED	60	60	45	40	60
	-TRANSITIONAL CELL PAPILLOMA	0	1	0	0	0
MESENTERY	-TOTAL EXAMINED	2	2	1	3	0
	-FIBROMA	0	0	0	1	0
ZYMBAL'S GLAND	-TOTAL EXAMINED	0	1	0	2	0
	-CARCINOMA	0	1	0	2	0

§ -NEOPLASIA    ¶ -NECROSIS    @ -HALLIBRANDT

APPEARS THIS WAY  
 ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

		SEX :				
		FEMALE				
		1	2	3	4	5
DOSE GROUP :						
NO. OF ANIMALS IN DOSE GROUP :		60	60	60	60	60
ADRENAL	-TOTAL EXAMINED	60	60	30	25	60
	-B-CORTICAL ADENOMA	4	1	2	0	1
	-B-BENIGN PHEOCHROMOCYTOMA	1	0	0	0	0
	-B-MALIGNANT PHEOCHROMOCYTOMA	0	0	0	1	1
BONE MARROW	-TOTAL EXAMINED	60	60	24	19	60
	-B-MYELOID LEUKEMIA	0	1	0	0	0
BRAIN	-TOTAL EXAMINED	60	60	24	19	60
	-B-GLIOMA	2	2	1	0	0
PANCREATIC GLAND	-TOTAL EXAMINED	60	60	26	20	60
	-B-ADENOCARCINOMA	0	0	1	0	2
TESTIS	-TOTAL EXAMINED	60	60	24	19	60
	-B-LEIOMYOSARCOMA	0	0	0	0	1
KIDNEY	-TOTAL EXAMINED	60	60	33	28	60
	-B-NEPHROBLASTOMA	0	0	0	0	1
	-B-TUBULAR CELL ADENOMA	1	1	0	0	0
	-B-LIPOMA	0	1	0	0	1

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
 PROJECT NO.: 8798

DISTRIBUTION OF NEOPLASTIC LESIONS  
 CARCINOGENICITY GROUP - ALL ANIMALS

GROUP	DIET	SEX :	FEMALE					
			DOSE GROUP :	1	2	3	4	5
GROUP 1	CONTROL BASAL DIET							
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET							
GROUP 3	CHOLESTAGEL® 0.4 G/KG/DAY							
GROUP 4	CHOLESTAGEL® 1.2 G/KG/DAY							
GROUP 5	CHOLESTAGEL® 2.4 G/KG/DAY							
		NO. OF ANIMALS IN DOSE GROUP :	60	60	60	60	60	
<b>LIVER</b>								
		-TOTAL EXAMINED	60	60	36	37	60	
		-#-HEPATOCELLULAR ADENOMA	3	1	1	1	2	
		-#-CHOLANGIOMA	2	0	0	0	0	
<b>LYMPH NODE</b>								
		-TOTAL EXAMINED	30	40	37	35	41	
		-#-LYMPHOSARCOMA	4	0	1	1	2	
		-#-HISTIOCYTIC SARCOMA	1	2	0	0	0	
<b>SPLEEN</b>								
		-TOTAL EXAMINED	60	60	43	40	60	
		-#-FIBROSARCOMA	24	23	26	22	22	
		-#-ADENOCARCINOMA	0	7	0	9	5	
		-#-ADENOMA	0	2	1	0	0	
<b>OVARY</b>								
		-TOTAL EXAMINED	60	60	34	29	60	
		-#-BENIGN LYFOMA	1	0	0	0	0	
<b>PANCREAS</b>								
		-TOTAL EXAMINED	60	60	24	21	60	
		-#-ISLET CELL ADENOMA	1	0	0	0	0	
		-#-ISLET CELL CARCINOMA	0	0	0	3	1	

APPEARS THIS WAY  
 ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1 CONTROL BASAL DIET		GROUP 4 CHOLESTAGEL® 1.2 G/KG/DAY				
GROUP 2 CONTROL VITAMIN SUPPLEMENTED DIET		GROUP 5 CHOLESTAGEL® 2.4 G/KG/DAY				
GROUP 3 CHOLESTAGEL® 0.4 G/KG/DAY						
		SEX :				
		DOSE GROUP :				
NO. OF ANIMALS IN DOSE GROUP :		FEMALE				
		1	2	3	4	5
		60	60	60	60	60
PITUITARY	-TOTAL EXAMINED	60	60	50	47	60
	-#-ADENOMA, PARS DISTALIS	34	27	29	28	24
	-#-CARCINOMA, PARS DISTALIS	1	1	0	1	0
CLITORAL GLAND	-TOTAL EXAMINED	4	3	3	7	9
	-#-SQUAMOUS CELL PAPILLOMA	0	0	0	0	1
SKIN MISCELLANEOUS	-TOTAL EXAMINED	13	6	6	9	10
	-#-BENIGN KERATOCARCINOMA	1	0	0	0	0
	-#-SQUAMOUS CELL CARCINOMA	1	0	0	0	0
	-#-SQUAMOUS CELL PAPILLOMA	0	0	0	1	0
SPINAL CORD CERVICAL	-TOTAL EXAMINED	60	60	24	19	60
	-#-GLIOMA	0	0	1	0	0
SUBCUTANEOUS TISSUE	-TOTAL EXAMINED	8	2	5	4	4
	-#-HEMANGIOSARCOMA	1	1	0	0	0
	-#-FIBROMA	0	0	2	1	1
	-#-FIBROSARCOMA	1	0	0	2	0
	-#-CARCINOSARCOMA	1	0	0	0	0

- 1210 -

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
PROJECT NO.: 87989

INCIDENCE OF NEOPLASTIC LESIONS  
CARCINOGENICITY GROUP - ALL ANIMALS

GROUP 1	CONTROL BASAL DIET	GROUP 4	CHOLESTAGEL® 1.2 G/KG/DAY			
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET	GROUP 5	CHOLESTAGEL® 2.4 G/KG/DAY			
GROUP 3	CHOLESTAGEL® 0.4 G/KG/DAY					
		SEX :				
		— FEMALE —				
		1	2	3	4	5
		NO. OF ANIMALS IN DOSE GROUP :				
		60	60	60	60	60
THYRUS		-TOTAL EXAMINED				
		88	68	29	24	60
-B-BENIGN THYROID		1	0	0	0	0
-M-MALIGNANT SCHWANNOMA		0	0	1	0	0
-M-MALIGNANT THYROID		0	0	1	0	0
THYROID		-TOTAL EXAMINED				
		60	60	28	23	60
-B-C-CELL ADENOMA		20	11	4	5	19
-M-C-CELL CARCINOMA		2	0	2	0	0
-B-FOLLICULAR CELL ADENOMA		0	0	0	0	1
-M-FOLLICULAR CELL CARCINOMA		0	0	0	1	0
URINARY BLADDER		-TOTAL EXAMINED				
		59	50	24	20	59
-B-TRANSITIONAL CELL PAPILLOMA		0	0	0	0	1
UTERUS		-TOTAL EXAMINED				
		60	60	55	56	60
-M-CARCINOSARCOMA		0	1	0	0	0
-B-SQUAMOUS CELL PAPILLOMA		0	1	0	0	1
-B-BENIGN ENDOMETRIAL STROMAL POLYP		13	3	2	9	4
-M-LEIOMYOSARCOMA		0	1	0	0	0
-M-MALIGNANT SCHWANNOMA		1	2	3	0	4
-M-SQUAMOUS CELL CARCINOMA		1	0	2	1	0
-M-ADENOCARCINOMA		1	1	1	0	0
-M-ENDOMETRIAL STROMAL SARCOMA		1	0	0	0	0

- 4211 -

APPEARS THIS WAY  
ON ORIGINAL

TABLE NO.: 18  
 PROJECT NO.: 87909

INCIDENCE OF NEOPLASTIC LESIONS  
 CARCINOGENICITY GROUP - ALL ANIMALS

		SEX :				
		----- FEMALE -----				
		1	2	3	4	5
NO. OF ANIMALS IN DOSE GROUP :		60	60	60	60	60
GROUP 1	CONTROL BASAL DIET					
GROUP 2	CONTROL VITAMIN SUPPLEMENTED DIET					
GROUP 3	CHOLESTAGEL® 0.4 G/KG/DAY					
GROUP 4	CHOLESTAGEL® 1.2 G/KG/DAY					
GROUP 5	CHOLESTAGEL® 2.4 G/KG/DAY					
VAGINA						
	-TOTAL EXAMINED	59	60	56	19	58
	-IN-MALIGNANT SARCOMA	1	1	0	0	0
ZYMBAL'S GLAND						
	-TOTAL EXAMINED	0	0	0	1	0
	-IN-CARCINOMA	0	0	0	1	0

♠ - NEOPLASIA    ♠ - BENIGN    ♠ - MALIGNANT

APPEARS THIS WAY  
 ON ORIGINAL

# MOUSE CARCINOGENICITY STUDY

## GENERAL INFORMATION

**Study Title:** A Dietary Carcinogenicity Study of Cholestagel® in the Albino Mouse  
**Study Number:** GT-02-TX-24  
**Volume Numbers:** Electronic NDA Submission (no paper copy)  
**Test Facility:** [ ]  
**Study Period:** November 1996 - November 1998  
**Date of Submission:** July 30, 1999  
**QA Report:** Yes  
**Dose-range-finding study:** None

## STUDY PROTOCOL AND METHODS

**Study Type:** Diet  
**Species/strain:** Mice, Swiss Cr:CD<sup>R</sup>-1(ICR)BR strain  
**Number of animals:** Main Study: 50/sex/dose group; Satellites: 15/sex/group  
**Age at start of study:** Approximately 6 weeks  
**Weight at start of study:** 23-35g (males), 15-27g (females)  
**Animal housing:** Individually  
**Drug Name:** Colesevelam hydrochloride (Cholestagel, GT31-104HB)  
**Drug Batch number(s):** Batch Nrs CHOG 9604 1459, CHOG 9605 1587, TLMC 002 1830, TMAC 010 1852, TMAC 011 1853  
**Drug Analysis:** Samples taken from each batch before and after treatment period with that particular batch  
**Drug Homogeneity:** Not assessed  
**Drug Stability:** 2 weeks, room temperature  
**Vehicle employed:** Powdered certified rodent chow  
**Preparation of diets:** Test article was mixed with rodent chow for ca. 15 min in a V-blender, prepared weekly, stored at room temperature

### Doses:

Group		Dose (g/kg/day)	Main Study N/sex/group	Satellites N/sex/group
1	Control	0 (basal diet)	50	15
2	Control*	0 (basal diet*)	50	-
3	LD*	0.3	50	15
4	MD*	1.0	50	15
5	HD*	3.0	50	15
6	Health screen	-	10	-

#### \*Vitamin-supplemented diet:

Vitamin A - supplemented with an additional 1250 IU/kg  
 Vitamin D - supplemented with an additional 750 IU/kg  
 Vitamin E - supplemented with an additional 640 IU/kg  
 Vitamin K - supplemented with an additional 20 ppm

**Relation to Clinical Use:** Recommended starting dose: 6x625mg (3750mg) per day, equivalent to 62.5mg/kg/day.  
**CAC Concurrence:** Not available  
**Route of Administration:** Oral (diet)  
**Frequency of Drug Administration:** Daily  
**Control Employed:** Two groups, one with vitamin-supplemented diet (n=60)  
**Interim Sacrifices:** None

**Special Study Group(s):** Health screen: 10/sex/dose group (for blood sampling and gross pathology examination)

**Unscheduled Sacrifices or Deaths:** See Results (Mortality)

**Statistics:** *Sponsor's evaluation:* Mortality and tumour data: Proc Chronic program  
*CDER evaluation:* See CDER Biometrics Review (APPENDIX 2)

**STUDY RESULTS**

**Clinical Observations**

No treatment-related clinical signs.

**Incidence of masses at necropsy**

Group		Dose (g/kg/day)	MALES	FEMALES
			Number per 65 (%)	Number per 65 (%)
1	Control 1	0 (basal diet)	5 (8%)	7 (11%)
2	Control 2*	0 (basal diet*)	4 (6%)	4 (6%)
3	LD*	0.3	9 (14%)	2 (3%)
4	MD*	1.0	5 (8%)	3 (5%)
5	HD*	3.0	4 (6%)	1 (1.5%)

\*Vitamin-supplemented diet

**Mortality**

*Note: Survival graphs are attached to this review (p. 51)*

Mortality appeared slightly but non-dose-dependently decreased in drug treated animals as compared to control Groups 2. According to CDER Reviewer there was no significant difference in survival between treatment groups.

**Mortality**

Group		Dose (g/kg/day)	MALES	FEMALES
			Number per 50 (%)	Number per 50 (%)
1	Control 1	0 (basal diet)	28 (56%)	34 (68%)
2	Control 2*	0 (basal diet*)	35 (70%)	33 (66%)
3	LD*	0.3	32 (64%)	26 (52%)
4	MD*	1.0	26 (52%)	26 (52%)
5	HD*	3.0	29 (58%)	30 (60%)

\*Vitamin-supplemented diet

**Body Weight**

*Note: Body weight graphs are attached to this review (p.52)*

No significant effects.

Male body weights appeared to decrease slightly in all groups after approximately 72 weeks.

**Food Consumption:**

Very slight increases in HD groups (males and females) throughout the study

**Calculated intake of test article:**

The calculated values shown in the table below are over the whole 104-week dosing period.

**Mean overall calculated intake (g/kg/day)**

Group	LD	MD	HD
Target dose (g/kg/day)	0.3	1.0	3.0
Calculated dose (g/kg/day)			
MALES	0.29	0.97	3.03
FEMALES	0.31	1.03	3.08

Calculated dose (% of target dose)			
MALES	97	97	101
FEMALES	103	103	103

**Test article concentrations in the diet**

Group	LD	MD	HD
Target dose (g/kg/day)	0.3	1.0	3.0
DIET LEVELS MALES (g/kg)			
Week 1	1.23	4.11	12.3
Week 13	1.90	6.56	18.5
Week 26-27	2.15	7.00	21.0
Week 52-53	2.18	7.09	21.3
Week 104	1.83	5.83	21.0

Group	LD	MD	HD
Target dose (g/kg/day)	0.3	1.0	3.0
DIET LEVELS FEMALES (g/kg)			
Week 1	1.16	3.85	11.5
Week 13	1.49	4.83	13.9
Week 26-27	1.68	5.60	16.8
Week 52-53	1.76	5.86	16.8
Week 104	1.89	5.10	13.7

**Toxicokinetics:**

No data

**Ophthalmology:**

No treatment-related changes

**Hematology**

No significant effects

**Serum vitamin levels**

(Data from satellite animals)

Vitamin D:

Non-dose-dependent increase in LD, MD, and HD females as compared to controls (Group 1) receiving unsupplemented diet. Data for group 2 controls were not given (reason unclear). The effect seems to be an effect of vitamin supplementation, and not a drug effect.

**Vitamin D levels (Week 52) (average data for 5 satellite animals)**

Group		Dose (g/kg/day)	MALES	FEMALES
			25,OH-Vit D (ng/ml)	25,OH-Vit D (ng/ml)
1	Control 1	0	34.7	32.7
2	Control 2*	0	(no data) (?)	(no data) (?)
3	LD*	0.4	39.2	48.8
4	MD*	1.2	39.9	52.8
5	HD*	2.4	40.9	51.1

\*Vitamin-supplemented diet

**Vitamin D levels (Week 103) (average data for 5 satellite animals)**

		MALES	FEMALES

Group		Dose (g/kg/day)	25,OH-Vit D (ng/ml)	25,OH-Vit D (ng/ml)
1	Control 1	0	32.0	34.7
2	Control 2*	0	-	-
3	LD*	0.4	30.7	41.7
4	MD*	1.2	39.0	41.7
5	HD*	2.4	20.9	48.7

\*Vitamin-supplemented diet

#### Vitamin E:

Small decreases in MD and HD, males and females, as compared to LD (Group 3). Although data for Group 2 were not available this looks like a drug-related effect

#### Vitamin E levels (Week 52) (average data for 5 satellite animals)

Group		Dose (g/kg/day)	MALES	FEMALES
			Vit E (ug/ml)	Vit E (ug/ml)
1	Control 1	0	2.67	2.74
2	Control 2*	0	-	-
3	LD*	0.4	6.2	5.15
4	MD*	1.2	4.66	6.12
5	HD*	2.4	5.78	4.34

\*Vitamin-supplemented diet

#### Vitamin E levels (Week 103) (average data for 5 satellite animals)

Group		Dose (g/kg/day)	MALES	FEMALES
			Vit E (ug/ml)	Vit E (ug/ml)
1	Control 1	0	10.6	12.5
2	Control 2*	0	-	-
3	LD*	0.4	18.7	18.0
4	MD*	1.2	15.0	12.6
5	HD*	2.4	13.1	14.6

\*Vitamin-supplemented diet

#### Organ Weights:

No data

#### Gross pathology:

#### Gross pathology findings (all animals, ie, preterminal and terminal sacrifice)\*

Group #		Males					Females				
Group		Ctrl1	Ctrl2	LD	MD	HD	Ctrl1	Ctrl2	LD	MD	HD
N examined		50	50	50	50	50	50	50	50	50	50
N preterminal											
N terminal											
Kidney	Discoloration	1	9	7	5	4	7	9	7	5	5
Liver	Cyst	1	0	2	2	3	2	1	1	1	4
	Discoloration	1	0	2	6	5	6	3	1	3	4
Lymph node	Discoloration	0	0	4	3	3	3	2	4	0	2
Lymph node mesenteric	Discoloration	1	1	1	3	2	3	1	4	2	3
Uterus	Dilatation	-	-	-	-	-	0	0	0	1	2

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

## Histopathology

Retained tissues from all animals in all groups were examined

### Neoplastic histopathology findings (all animals, ie, preterminal and terminal sacrifice)\*

Group #		Males					Females				
Group		Ctr1	Ctr2	LD	MD	HD	Ctr1	Ctr2	LD	MD	HD
N		50	50	50	50	50	50	50	50	50	50
Liver	Hemangiosarcoma (M)	0	1	4	3	2	1	1	1	0	0
Harderian gland	Adenoma (B)	2	5	2	2	4	1	0	2	0	3

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

### Non-neoplastic histopathology findings (all animals, ie, preterminal and terminal sacrifice)\*

Group #		Males					Females				
Group		Ctr1	Ctr2	LD	MD	HD	Ctr1	Ctr2	LD	MD	HD
N		50	50	50	50	50	50	50	50	50	50
Duodenum	Mucosal hyperplasia	0	0	0	2	1	0	0	0	0	1
Eye	Corneal mineralization	0	1	1	4	3	4	8	9	3	5
Harderian gland	Hyperplasia	0	0	1	1	2	0	0	0	0	0
Heart	Fibrosis	1	1	2	3	4	0	0	0	2	0
Ilium	Ileitis	0	0	0	1	1	0	0	0	0	0
Jejunum	Jejunitis	0	0	1	2	2	0	0	0	0	0
Kidney	Dilatation Bowman's capsules	0	0	0	0	0	0	1	2	4	9
Liver	Biliary cyst	1	1	0	0	3	0	0	0	1	1
Lung	Pleuritis	0	0	2	3	3	0	1	0	0	0
Stomach	Erosion	2	4	1	4	6	1	1	4	2	4
	Ulcer	0	1	1	2	0	0	0	0	1	1

\*Pathology findings entered in this Table are findings that suggest a drug-related effect in either males or females

#### Note:

Sponsor's neoplastic and non-neoplastic incidence summary tables in APPENDIX 1

#### Factors contributory to death

The cause of death of the preterminal decedent animals was either neoplastic (e.g. pulmonary and lymphoid tumors) or non-neoplastic (e.g. amyloidosis or glomerulonephritis). There were no treatment related effects in the distribution of neoplastic or non-neoplastic lesions on the causes of death in preterminal animals.

BEST POSSIBLE COPY

**STATISTICAL ANALYSIS OF TUMOR FINDINGS**

**Sponsor's statistical analysis**

Mortality and tumor data were statistically analyzed using PROC CHRONIC. Tumor data analysis was done on all groups.

**Mortality**

Statistical analysis of mortality data showed that there was no significant difference in mortality rate among the five groups.

**Tumors**

The statistical analysis of female neoplastic data revealed no statistically significant results. In males, significant results in the heterogeneity test were found for three data sets, i.e., liver malignant hepatocellular carcinoma (incidence 1-3-8-2-5), spleen malignant hemangiosarcoma (0-2-0-1-0), and thyroid benign follicular cell adenoma (0-0-0-2-0). However, there was no dose-related increase in tumor incidence for any of these three tumors, and pairwise comparison for two of these tumors revealed the significant effects to occur in dose groups other than the high dose group (LD in liver, MD in thyroid).

**Table A: Summary results<sup>S</sup> of Proc Chronic analysis on male data sets with significant heterogeneity test(s).**

Organ / Tissue	Lesion Type	Tumor Classification	Number of Tumor-Bearing Animals / Sample Size					P-VALUE	
			Group 1	Group 2	Group 3	Group 4	Group 5	Heterogeneity HP-99 groups 1, 2, 3, 4, 5	Trend test
Liver	Malignant hepatocellular carcinoma	P-fatal	1	3	3	2	3	0.76312	0.49437
		I-incident	0	0	5 <sup>B</sup>	0	2	0.88235	0.68283
		Pooled-F+I	1/50	3/50	8 <sup>B</sup> /50	2/50	5/50	0.04976	0.68532
Spleen	Malignant hemangiosarcoma	P-fatal	0	0	0	1	0	0.46092	0.55169
		I-incident	0	2	0	0	0	0.82196	0.92679
		Pooled-F+I	0/50	2/50	0/50	1/50	0/50	0.13418	0.89515
Thyroid	benign follicular cell adenoma	P-fatal							
		I-incident	0/50	0/50	0/50	2 <sup>A</sup> /50	0/50	0.83648	0.57134
		Pooled-F+I							

**Sponsor's conclusion:** Dietary administration of colesevelam hydrochloride to CD mice for up to 2 years did not produce any tumorigenic effect.

**CDER reviewers statistical analysis (APPENDIX 2)**

The CDER Reviewer tested the dose-tumor positive linear trend in tumor incidence of all groups and for all tissues/organs. There was no statistically significant dose-tumor positive linear trend in male or female mice.

## **SUMMARY AND EVALUATION**

Colesevelam hydrochloride is a non-absorbed polymeric bile acid sequestrant indicated for lowering of serum cholesterol levels. A 104-week dietary carcinogenicity study was carried out in mice to investigate the carcinogenic potential of the compound. Animals in Control Group 2 and the three drug dose Groups 3, 4, and 5 received a diet supplemented with Vitamins A, E, D and K to prevent secondary effects of vitamin depletion due to reduced absorption of these fat-soluble vitamins.

### **Dose selection**

The dietary concentration of the test compound in HD male mice achieved a 2% level at week 20 and remained at that level throughout the study. The dietary concentration of the test compound in HD female mice achieved a 1.5% level by week 20 and increased transiently to 1.9% by week 79. It can be argued that the high doses in the mouse study were too low, i.e., less than the recommended 5% in a study in which toxicity is not a dose-limiting factor.

The high doses used in the mouse carcinogenicity study, for both male and female animals (3.0 g/kg/day), were approximately 40 times the maximum recommended human dose (4.5 g/day), when compared on the basis of kg body weight. Since this drug is virtually not absorbed the most appropriate dose comparison is on the basis of body weight.

### **Mouse and human dose comparison**

Mouse dose group	Dose (g/kg/day)	Multiple of maximum recommended human dose*
LD	0.3 g/kg/day	4x
MD	1.0 g/kg/day	13x
HD	3.0 g/kg/day	40x

Maximum recommended human dose is 4.5 g/day, or 75 mg/kg for a 60 kg person

### **Mortality, Body weight, Food consumption, Hematology, Serum vitamin levels**

In the mouse carcinogenicity study with colesevelam hydrochloride there was no drug-related effect on mortality. Body weight was not significantly affected by drug treatment. Food consumption was slightly increased in HD males and females throughout the study. There were no significant hematological effects. Although data from control group 2 were lacking, Vitamin D levels appeared to be increased as compared to control group 1 in all female drug dose groups throughout the study. There was no drug-related effect on Vitamin D levels in either sex. Vitamin E levels also appeared increased as compared to control group 1 in both male and female dose groups. However, as in rats, Vitamin E levels were decreased by drug treatment in a dose-related manner.

### **Gross pathology**

There was a slight dose-related decrease in the incidence of kidney discoloration in drug treated male mice. In the liver, an increased incidence of cysts appeared to occur in male and female HD animals, as well as a dose-related increase in discoloration in drug-treated male groups. Lymph node discoloration appeared to be increased in drug-treated male groups. The incidence of uterus dilatation was slightly increased in MD and HD females. The reasons for these findings are unclear.

### **Histopathology findings**

#### **Neoplastic findings**

**Liver:** In male mice, an increased incidence of hemangiosarcoma occurred in LD, MD and HD animals (4/50, 3/50, 2/50, respectively, vs. 1/50 in control group 2). However, there was no clear positive dose-relationship.

**Harderian gland:** In female animals there was an increased incidence of Harderian gland adenocarcinoma in LD and HD animals (2/50 and 3/50). However, in MD females this tumor was

not seen. There was no drug dependence of the incidence of this tumor in male animals. The relationship to drug treatment of this tumor in females is unclear.

Non-neoplastic findings

There were a number of findings in a variety of organs that appeared to be related to drug treatment. The most prominent finding was in the kidney in females (capsule dilatation):

*Kidney:* Drug dose-dependent dilatation of Bowman's capsules in females

*GI tract:* Duodenal mucosal hyperplasia in males, ileitis in males, jejunitis in males, stomach erosion and/or ulcer in males and females.

*Liver:* Biliary cyst in MD and/or HD males and females

*Lung:* Pleuritis in males

*Heart:* Fibrosis in males

*Eye:* Corneal mineralization in males

*Harderian gland:* Hyperplasia in males

The cause and significance of these findings was unclear.

CDER Biometrics evaluation (APPENDIX 2):

The CDER Reviewer tested the dose-tumor positive linear trend in tumor incidence of all groups and for all tissues/organs for male and female mice. There was no statistically significant dose-tumor positive linear trend in male or female mice. Thus, the Reviewers' conclusion was consistent with the Sponsor's conclusion on tumorigenicity.

CONCLUSIONS

Colesevelam chloride was tested in a mouse bioassay for 104 weeks at doses up to 40 times the maximum recommended human dose. Food consumption was slightly increased in all treated males and females. There appeared to be a dose-related decrease in Vitamin E levels in males and females.

There were no statistically significant increases in tumor incidences in male or female mice.

APPEARS THIS WAY  
ON ORIGINAL

**NDA 21-223  
Cholestagel  
Mouse Carcinogenicity Study**

**Histopathology Inventory  
(Tissues Retained)**

Sponsor Study #GT-02-TX-24	
Species: Mouse	Pathology
	Groups 1,2,3,4,5
Abnormalities	X
Adrenals	X
Aorta (thoracic)	X
Blood film	
Bone (sternum)	X
Bone marrow (sternum)	X
Brain	X
Cecum	X
Colon	X
Duodenum	X
Epididymides	X
Esophagus	X
Eyes	X
Gall bladder	X
Harderian glands	X
Heart	X
Ileum	X
Jejunum	X
Kidneys	X
Lacrimal glands	X
Larynx	
Liver	X
Lungs (all lobes)	X
Lymph nodes, mandibular	X
Lymph nodes, mesenteric	X
Mammary gland (inguinal)	X
Optic nerves	X
Ovaries	X
Pancreas	X
Pituitary	X
Parathyroids	X
Pharynx	
Prostate	X
Rectum*	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle (quadriceps)	X
Skin (inguinal)	X
Spinal cord (cervical)	X
Spleen	X
Stomach	X
Teeth	
Testes	X
Thymus	X
Thyroid	X

**APPEARS THIS WAY  
ON ORIGINAL**

Tongue	X	
Tonsils		
Trachea	X	
Ureter		
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal glands		

\*tissue retained but not processed

**APPEARS THIS WAY  
ON ORIGINAL**