CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-362

ENVIRONMENTAL ASSESSMENT

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmaceutical Science/Immediate Office

Memorandum

Date: April 27, 2009

From: Raanan A. Bloom, Ph.D.
OPS/IO/PARS

To: Kati Johnson,
ODEII/ DMEP

Through: Jon Clark, M.S.
OPS/IO/PARS

Subject: **NDA 22-362: Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension, packaged in 2.65 and 5.3 grams packets.**

Review of Environmental Assessment

Sponsor: Daiichi Sankyo Pharma Development, a division of Daiichi Sankyo, Inc.

A. Background

Daiichi Sankyo Pharma Development requests approval of Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension for treatment of primary hypercholesterolemia (NDA 22-362). An Environmental Assessment (EA) has been submitted pursuant to 21 CFR part 25.

B. Discussion

The following review was conducted by Ruth Ganunis, Ph. D., under contract to CDER/OPS on April 20, 2009, and approved by Raanan A. Bloom, Ph.D., OPS/IO/PARS, Senior Environmental Officer.

Executive Summary

NDA 22-362 requests approval of Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension for treatment of primary hypercholesterolemia. An EA for related application NDA 21-141 for Welchol™ Tablets was previously reviewed and a FONSI was issued (2000). Upon approval of supplement S-017 to NDA 21-141 (2008), the FDA requested that an update to the Welchol™ EA be provided with future applications or supplements (a copy of the communications between the FDA and Daiichi Sankyo are provided in appendix I).

The EA submitted with this application (NDA 22-362) is intended to address the request for updated information, and is to be considered as an add-on to the EA for NDA 21-141. The EA includes updated 5 year usage estimates, an updated aquatic assessment, and a new terrestrial assessment.

Colesevelam hydrochloride is a cross-linked polymer of (poly)allylamine alkylated with 1-bromodecane and 6-bromohexyltrimethylammonium bromide that is highly stable. Colesevelam hydrochloride is not soluble in water or hydrocarbon solvents. Colesevelam hydrochloride is not metabolized and is expected to enter the environment unchanged. No environmentally relevant rapid depletion mechanism is identified. Colesevelam hydrochloride may enter the aquatic and terrestrial environment from patient use and disposal.

The fate and effect reports with regard to the aquatic environment were previously submitted and reviewed under related Welchol™ Tablet NDA 21-141. Only the 5 year production forecast and $EIC_{aquatic}$ calculation are updated here. The largest total requirement for colesevelam hydrochloride over the next five years is <600 metric tons in the year 2014. The corresponding $EIC_{aquatic}$, assuming no metabolism and the worst case scenario of all material entering the aquatic environment, is 12.94 g/L. The most sensitive aquatic species is algae, with an EC_{50} of 67.2 mg/L. The ratio of EC_{50}/MEEC is 5,193, which is significantly greater than the tier 2 assessment factor of 100. Colesevelam hydrochloride is expected to be nontoxic in the aquatic environment.

Colesevelam hydrochloride is insoluble. Upon entry into the waste water at the treatment plant, it is expected to remain as suspended particles and settle out into biosolids. Colesevelam hydrochloride will enter the terrestrial environment through the application of biosolids to land. The FDA requested that Daiichi Sankyo update their EA with regard to safety to terrestrial organisms. Based on the five year total requirement of <600 metric tons, and assuming no metabolism and the worst case scenario that all material enters the terrestrial environment, an $EEC_{terrestrial}$ was calculated to be 1.61 mg/kg. The firm provided toxicity data for plants and earthworms. For both plants and earthworms, the EC_{50} was found to be >1,000 mg/kg. The ratio of EC_{50}/MEEC is >621, which is greater than the tier 2 assessment factor of 100. Colesevelam hydrochloride is expected to be nontoxic in the terrestrial environment.

A FONSI is recommended.

Review of July 31, 2008, Environmental Assessment

BACKGROUND:

I. DATE: 31-JUL-2008

Reference is also made to NDA 21-141, Welchol™ (colesevelam hydrochloride) Tablets, approved 5/26/2000

II. APPLICANT: Daiichi Sankyo Pharma Development,
A Division of Daiichi Sankyo, Inc.

III. ADDRESS: 399 Thornall St.
Edison, NJ 08837

IV. PROPOSED ACTION:

a. Requested Approval: Daiichi Sankyo is requesting approval for Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension, packaged in 2.65 and 5.3 grams packets. This EA has been submitted pursuant to 21 CFR part 25.
b. Need for Action: Welchol™ (colesevelam hydrochloride) Tablets are currently approved for use. This application provides for Welchol™ (Colesevelam Hydrochloride) Powder for Oral Suspension for the treatment of primary hypercholesterolemia and to improve glycemic control in adults with Type II diabetes mellitus as an adjunct to diet and exercise.
c. Locations of Use: Hospital, clinics and patients homes throughout the United States.
d. Disposal Sites: Empty or partially empty containers from U.S. hospitals, pharmacies or clinics will be disposed of according to hospital, pharmacy or clinic procedures. Empty or partially empty containers from home use typically will be disposed by a community's solid waste management system which may include landfills, incineration and recycling. Minimal quantities of the unused drug may be disposed in the sewer system.

ADEQUATE

V. IDENTIFICATION OF CHEMICALS

Established Name (USAN): Colesevelam hydrochloride

Brand/Proprietary Name/Trade name: Welchol™

Chemical Name: Trans-4-(aminomethyl)cyclohexanecarboxylic acid

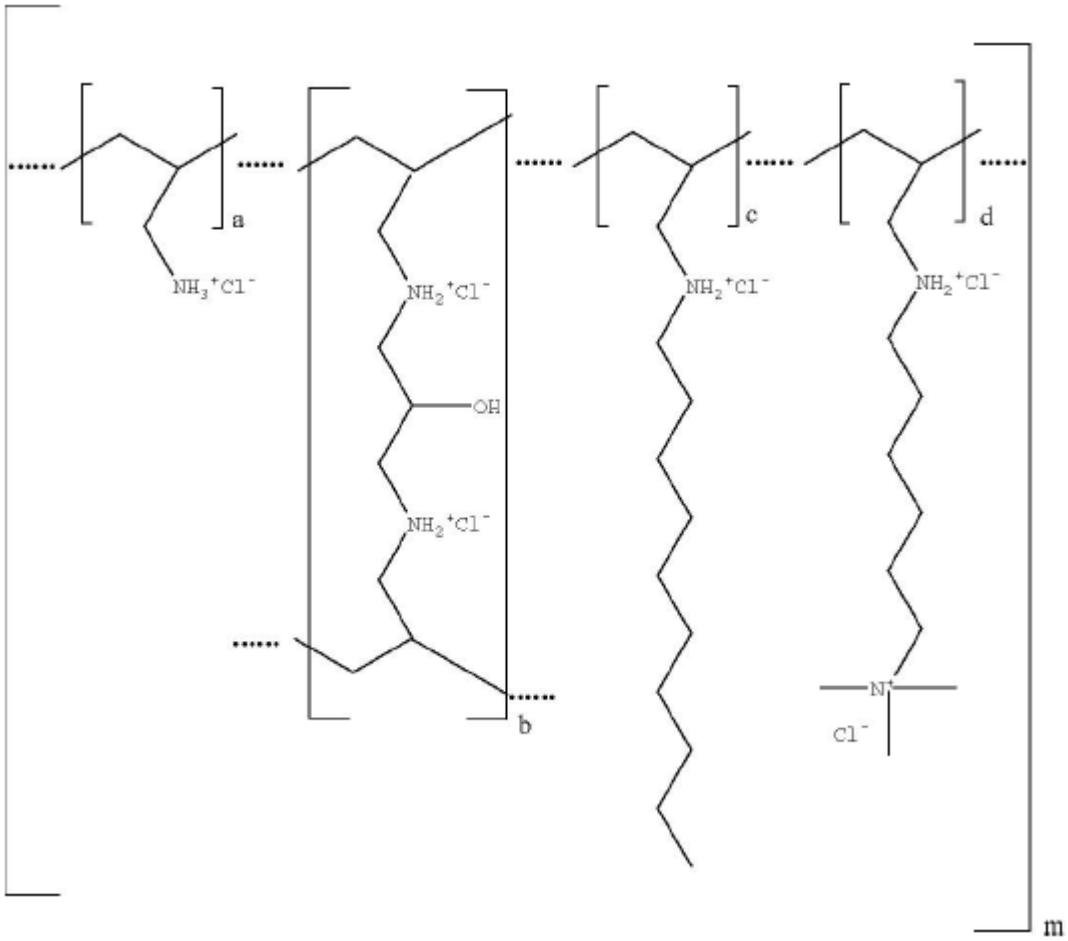
CAS Number: 182815-44-7

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:

Since colesevelam hydrochloride is a cross-linked polymer, each particle is one molecule due to multiple covalent cross-links between polymer chains. The molecular weight of a particle can be calculated from the diameter of the particle, the pycnometric density of colesevelam hydrochloride, and the conversion factor from grams to atomic mass units (amu). Using the pycnometric density of colesevelam hydrochloride of 1.10 g/cm^3, the molecular weight of a 25 m diameter particle of colesevelam hydrochloride is equal to 5.4×10^{15} amu.

Structural Formula:



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 network7

ADEQUATE

VI. ENVIRONMENTAL ISSUES

Physical and Chemical Characterization

Substance of Interest:
Colesevelam hydrochloride

Physical Description:
White to off-white powder

Water Solubility:
Colesevelam hydrochloride is a highly cross-linked polymer that is insoluble in all tested aqueous and organic solvents, including water, 0.1 N HCl/50 ºC, 1 N ammonium hydroxide, methylene chloride, acetonitrile, and methanol. Only a minimal amount of colesevelam hydrochloride could be solubilized and extracted from the tested solvents, with the exception of 1N ammonium hydroxide where an ammonium chloride salt was formed (Note that 1 N ammonium hydroxide is not representative of environmental conditions) (refer to Welchol™ Tablet NDA 21-176).

Dissociation Constant(s):
Colesevelam hydrochloride is a polyectrolyte and there is no discrete dissociation constant. Acid-base titration was used to quantify both the primary and secondary titrable amines in colesevelam hydrochloride. The total titrable amines of colesevelam hydrochloride are from the poly-allylamine hydrochloride starting material, and range from 4.4 to 4.8 mmoles of amine per gram of colesevelam hydrochloride, on an anhydrous basis (refer to Welchol™ Tablet NDA 21-176).

Log Octanol/Water Partition Coefficient:
Colesevelam hydrochloride is not soluble in water or hydrocarbon solvents. Therefore, the octanol/water partition coefficient can not be determined.

Vapor Pressure:
Colesevelam hydrochloride has no vapor pressure because it has such a high molecular weight. Water is the only volatile compound found in colesevelam hydrochloride during heating from ambient to 160 ºC. Above 160 ºC the compound begins to decompose (refer to Welchol™ Tablet NDA 21-176).

Impurities:
There are no impurities in the drug substance at a level greater than 1%.

Depletion mechanisms
The ready biodegradability of colesevelam hydrochloride was evaluated using OECD methods, with carbon dioxide evolution as the indicator for biodegradation (refer to Welchol™ Tablet NDA 21-176). Since biodegradation is measured at 2-3%, it is concluded that colesevelam hydrochloride is not readily biodegradable.

The hydrolytic stability of colesevelam hydrochloride was evaluated (refer to Welchol™ Tablet NDA 21-176). Colesevelam hydrochloride is very stable in acid and base solutions, and is not prone to hydrolysis.

Photostability studies were conducted on colesevelam hydrochloride following ICH guidelines (refer to Welchol™ Tablet NDA 21-176). Colesevelam hydrochloride was found to be stable under the conditions tested of UV-B, daylight and fluorescent bulbs.

No environmentally relevant rapid depletion mechanism is identified.

Expected Introduction Concentration

Daiichi Sankyo projected the maximum annual production volume of colesevelam hydrochloride for direct use in all formulations and for all indications based on five-year production estimates to be <600 metric tons per year.

Aquatic concentration:
Colesevelam hydrochloride is not soluble in water. Therefore, use of the $EIC_{aquatic}$ algorithm in the FDA guidance document significantly overestimates the expected aquatic concentrations, and is a conservative approach. Using this approach, the maximum $EIC_{aquatic}$ for the next five years, assuming no losses due to metabolism or degradation, is 12.94 g/L.

Terrestrial concentration:
Colesevelam hydrochloride is an insoluble compound and has a density of 1.1 g/cm^3. Upon entry into the waste water at the treatment plant, it is expected to remain as suspended particles and settle out into biosolids. The estimated environmental concentration in biosolids-amended soils, assuming no losses due to metabolism or degradation, is 1.61 mg/kg (Appendix V). This value was calculated using the FDA recommended approach (Appendix I).

Atmospheric concentration:
Since colesevelam hydrochloride has no vapor pressure, it is not expected to enter the atmospheric environment.

Maximum Expected Environmental Concentration (MEEC) from use:
The expected environmental introduction concentrations are considered to be the maximum expected environmental concentrations.

Environmental Effects:

Aquatic organisms:
Studies involving acute toxicity to activated sludge microorganisms in a wastewater treatment plants, acute toxicity to unicellular alga *Selenastrum capricornutum*, acute toxicity to the water flea *Daphnia magna*, and acute toxicity to the zebrafish *Branchydanio rerio* were previously conducted and submitted to Welchol™ Tablet NDA 21-176. In each case the tests were conducted according to OECD guidelines. The tests and the results were previously reviewed and found adequate. The test results are summarized in the table below.

Terrestrial organism:
The firm conducted a 14 day study addressing the acute toxicity of colesevelam hydrochloride to the earthworm, *Eisenia fetida,* according to OECD Guideline 207. The range of concentrations tested was 0 (control) though 1,000 mg colesevelam hydrochloride/kg dry soil. Worm survival, general health and behavior were monitored. The estimated 14 day LC_{50} value for earthworm survival was estimated to be >1,000 mg colesevelam hydrochloride/kg dry soil, the highest concentration tested. The NOEC was 1,000 mg colesevelam hydrochloride/kg dry soil.

A 19 day study addressing the potential effects of colesevelam hydrochloride on seedling emergence and early seedling growth of three non-targeted terrestrial plant species was conducted in accordance with OECD Guideline 208. The range of concentrations tested was 0 (control) though 1,000 mg colesevelam hydrochloride/kg dry soil. One species of monocotyledonous plants (Oat, *Avena sativa*) and two species of dicotyledonous plants (Radish, *Raphanus sativus* and lettuce, *Lactuca sativa*) were tested. The EC_{50} was estimated to be >1,000 mg colesevelam hydrochloride/kg dry soil, the highest concentration tested. The NOEC was 1,000 mg colesevelam hydrochloride/kg dry soil.

Summary of Environmental Effects:

Test	Result
Activated Sludge Respiration Inhibition test	EC_{50} > 300 mg/L (loading rate) NOEC = 300 mg/L (loading rate)
Acute Algal Growth Inhibition (72 hours)	EC_{50} (growth curve) = 67.2 mg/L (loading rate) EC_{50} (growth rate) >201.6 mg/L (loading rate) NOEC (growth curve) = 25.3 mg/L (loading rate) NOEC (growth rate) = 50.4 mg/L (loading rate)
Acute Daphnid Immobility (48 hours)	EC_{50} > 100 mg/L (loading rate) NOEC = 100 mg/L (loading rate)
Acute Fish Mortality (96 hours)	LC_{50} > 100 mg/L (loading rate) NOEC = 100 mg/L (loading rate)
Plant Seedling Emergence and Growth (19 days)	EC_{50} > 1,000 mg/kg dry weight (nominal) NOEC = 1,000 mg/kg dry weight (nominal)
Acute Earthworm Mortality (14 days)	EC_{50} > 1,000 mg/kg dry weight (nominal) NOEC = 1,000 mg/kg dry weight (nominal)

Assessment Factor
Colesevelam hydrochloride is expected to be the only substance entering the environment. Although colesevelam hydrochloride is insoluble and exposure in the terrestrial environment is of primary concern, the firm evaluates the potential effects on both the aquatic and terrestrial environments. The toxicity value derived from the most sensitive aquatic species (algal growth EC_{50} = 67.2 mg/L) and the toxicity value derived from the most sensitive terrestrial species (for both plants and earthworm EC_{50} > 1,000 mg/kg dry weight) were compared to the respective aquatic and terrestrial Maximum Expected Environmental Concentrations (MEEC). The calculation of the quotient EC_{50}/MEEC is summarized in the following table.

	Aquatic Environment	Terrestrial Environment
Lowest observed EC_{50} or LC_{50}	67,200 g/L	>1,000 mg/kg
Maximum Expected Environmental Concentration (MEEC)	12.94 g/L	1.61 mg/kg
Hazard Quotient (EC_{50}or LC_{50}/MEEC)	5,193	>621

In both cases, the quotient EC_{50} or LC_{50}/MEEC is greater than the tier 2 assessment factor of 100. The results indicate that colesevelam hydrochloride is not expected to be toxic to aquatic or terrestrial organisms at the highest projected use volume for the next 5 years.

ADEQUATE

VII. MITIGATION MEASURES

No mitigation measures are needed because no potential adverse environmental effects were identified.

ADEQUATE

VIII. ALTERNATIVES

No alternatives to the proposed action are proposed because no potential adverse environmental effects were identified.

ADEQUATE

IX. PREPARER

The job title and qualifications of the preparers were provided (p. 20)

ADEQUATE

X. REFERENCES

Provided.

ADEQUATE

XI. APPENDIX

Appendix I (Confidential): FDA Correspondences

Appendix II (Confidential): Environmental Review of the Manufacturing and Packaging of Welchol Powder for Oral Suspension.

Appendix III (Confidential): Five-year US Forecast

Appendix IV (Confidential): EIC Aquatic Calculation

Appendix V (Confidential): EEC Terrestrial Calculation

Appendix VI (Non-Confidential): Data Summary Table

Appendix VII (Confidential): Colesevelam Hydrochloride: Acute Toxicity to the Earthworm *Eisenia fetida*

Appendix VIII (Confidential): Colesevelam Hydrochloride: Effects on the Seedling Emergence of Non-target Terrestrial Plants

Appendix IX (Confidential): GLP Compliance Statement

ADEQUATE

C. Comments and Conclusions

Based on an evaluation of the information provided in this EA and previous EAs, in FDA guidance, and on the scientific validity of the "no effects" conclusions of the EA, no significant adverse environmental impacts are expected from the introduction of colesevelam hydrochloride residues into the environment due approval of Welchol™.

A Finding of No Significant Impact (FONSI) is recommended.

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/s/

Raanan Bloom
4/28/2009 02:31:35 PM
ENV ASSESSMENT

Jon E. Clark
4/29/2009 12:58:32 PM
CHEMIST

Environmental Assessment - Finding of No Significant Impact

NDA 22-362

Welchol™ (Colesevelam Hydrochloride) Powder for Oral Suspension

Food and Drug Administration
Center for Drug Evaluation and Research
4/27/2009

FINDING OF NO SIGNIFICANT IMPACT

NDA 22-362

Welchol™ (Colesevelam Hydrochloride) Powder for Oral Suspension

The National Environmental Policy Act of 1969 (NEPA) requires Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

NDA 22-362 requests approval of Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension for the treatment of primary hypercholesterolemia and to improve glycemic control in adults with Type II diabetes mellitus as an adjunct to diet and exercise. In support of its application, Daiichi Sankyo Pharma Development prepared an environmental assessment (attached) in accordance with 21 CFR Part 25 which evaluates the potential environmental impact from the use and disposal of this product.

The Food and Drug Administration, Center for Drug Evaluation and Research, has carefully considered the potential environmental impact due to approval of this application and has concluded that this action will not have a significant effect on the quality of the human environment. Therefore, an environmental impact statement will not be prepared.

PREPARED BY:

Raanan A. Bloom, Ph.D.
Senior Environmental Officer
Office of Pharmaceutical Science

CONCURRED BY:

Jon Clark, M.S.
Associate Director for Policy
Office of Pharmaceutical Science

CONCURRED BY:

Moheb Nasr, Ph.D.
Director, Office of New Drug Quality Assessment
Office of Pharmaceutical Science

Attachment: July 31, 2008, Environmental Assessment (confidential appendices removed)

Environmental Assessment for Welchol™ (Colesevelam Hydrochloride)

Prepared for
Daiichi Sankyo Pharma Development
399 Thornall Street
Edison, New Jersey 08837

Prepared by
Gradient Corporation
20 University Road
Cambridge, MA 02138

July 31, 2008

Table of Contents
Environmental Assessment

1 DATE

31 July 2008

2 NAME OF APPLICANT

Daiichi Sankyo Pharma Development, a Division of Daiichi Sankyo, Inc.

3 ADDRESS

399 Thornall St.
Edison, NJ 08837

4 DESCRIPTION OF PROPOSED ACTION

4.1 REQUESTED APPROVAL

Daiichi Sankyo Pharma Development is filing a New Drug Application (NDA 22-362) pursuant to section 505(b) of the Federal Food, Drug, and Cosmetic Act for Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension, packaged in 2.65 and 5.3 grams packets. As requested and agreed to by FDA (see Appendix I - Confidential), this updated and revised environmental assessment has been submitted pursuant to 21 CFR Part 25 and its format is in accordance with FDA guidance (FDA, 1998).

4.2 NEED FOR ACTION

The proposed action is to seek approval for Welchol™ (colesevelam hydrochloride) Powder for Oral Suspension for the treatment of primary hypercholesterolemia and to improve glycemic control in adults with Type II diabetes mellitus as an adjunct to diet and exercise. The maximum dose will be 3.75 g colesevelam hydrochloride anhydrous per day and the duration of use could be lifetime.

4.3 LOCATIONS OF USE

Welchol Powder for Oral Suspension is manufactured in the U.S. in compliance with applicable state and federal environmental regulations (see Appendix II - Confidential), and will be used in hospitals, clinics and/or by patients in their homes throughout the U.S.

4.4 DISPOSAL SITES

At U.S. hospitals, pharmacies, or clinics, empty or partially empty packets will be disposed of according to hospital, pharmacy, or clinic procedures. From patients with in-

home use, empty or partially empty containers in residences will typically be disposed of by a community's solid waste management system, which may include landfills, incineration, and/or recycling. Minimal quantities of the unused drug may be disposed to sewer or septic systems.

5 IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

5.1 Nomenclature

5.1.1 Established Name (USAN)

Colesevelam hydrochloride

5.1.2 Brand/Proprietary Name/Trade name

Welchol™

5.1.3 Chemical Name

Trans-4-(aminomethyl)cyclohexanecarboxylic acid

5.1.4 CAS Number

182815-44-7

5.1.5 Molecular Formula

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

5.1.6 Molecular Weight

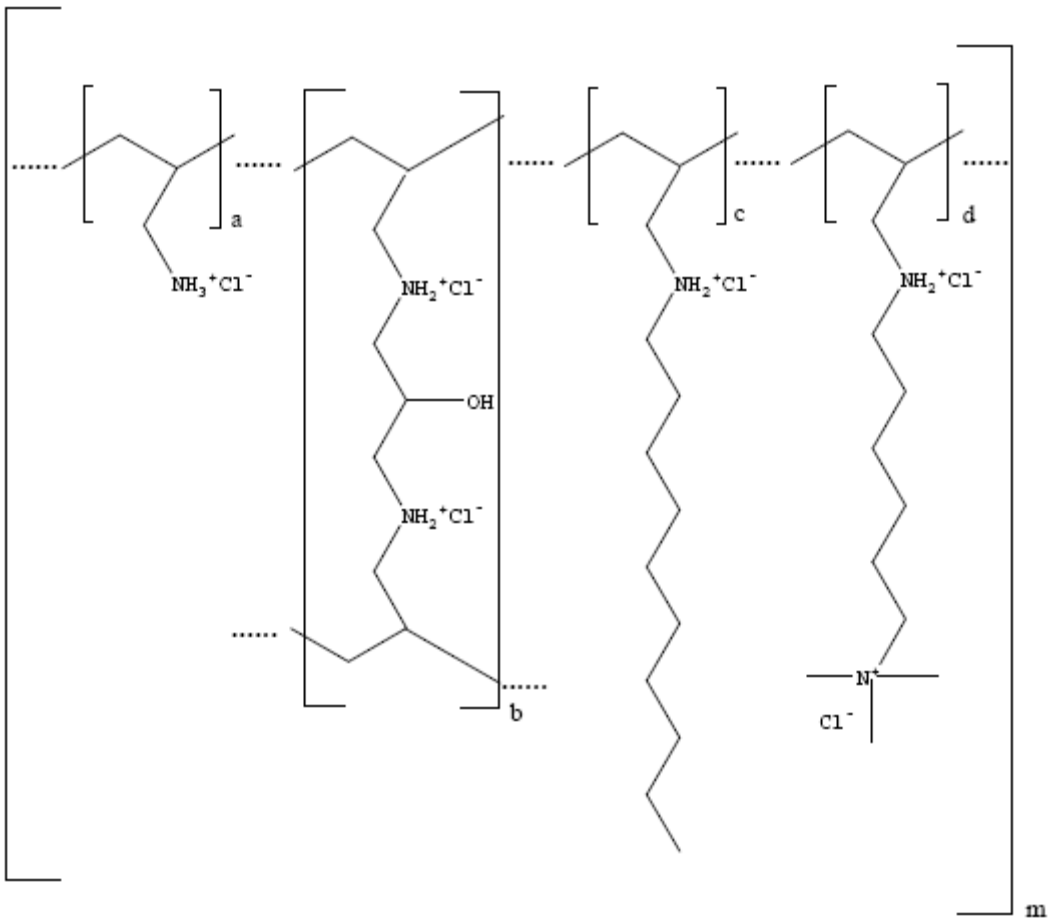
Because colesevelam hydrochloride is a cross-linked polymer, molecular weight does not have the same meaning as for a molecule with a defined discrete structure. Each particle is one molecule due to multiple covalent cross-links between polymer chains. Therefore, the molecular weight of an individual particle is equal to the weight of the particle itself. Because the density of the particle is independent of the particle size, the weight of the particle is proportional to the particle size. Analogous to the molecular weight distribution of soluble polymers, the molecular weight distribution of a cross-linked polymer is a function of the distribution of particle sizes. The molecular weight of a particle can be calculated from the diameter of the particle, the pycnometric density of colesevelam hydrochloride, and the conversion factor from grams to atomic mass units (amu). Using the pycnometric density of colesevelam hydrochloride of 1.10 g/cm^3, the

molecular weight of a 25 µm diameter particle of colesevelam hydrochloride is equal to 5.4×10^{15} amu.

5.1.7 Structural Formula

The colesevelam hydrochloride chemical structure is illustrated in Figure 1.

Figure 1: Colesevelam Hydrochloride Chemical Structure



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

The structure of colesevelam hydrochloride can be represented as a tetrapolymer of allylamine, N-decylallylamine, N-(6-trimethylammoniumhexyl)allylamine and N,N'-diallyl-1,3-diamino-2-hydroxypropane. The 2-hydroxypropyl-1,3-diyl group is formed during the cross linking and the N-decyl and N-(6-trimethylammonium) hexyl groups are derived from the alkylation step. No regular order of the groups is implied by the structure, crosslinking and alkylation are expected to occur randomly along the polymer chains. The mole fraction of protonated amines is ≥ 97%. The polymer is depicted in the hydrochloride form; less than 5% of the halides are bromide.

6 ENVIRONMENTAL ISSUES

6.1 Environmental Fate of Released Substances

6.1.1 Identification of Substances of Interest

Colesevelam hydrochloride

6.1.2 Physical and Chemical Characterization

Physical Description
White to off-white powder

Water Solubility:
Colesevelam hydrochloride is a highly cross-linked polymer that is insoluble in all tested aqueous and organic solvents. Various solvents were used in order to solubilize and extract any and potential leachables in colesevelam hydrochloride. The tested solvents include water, 0.1 N HCl/50 ºC, 1 N ammonium hydroxide, methylene chloride, acetonitrile, and methanol. A 2.5 g sample of colesevelam hydrochloride was suspended in 40 ml of the solvent to be tested. The suspension was agitated at room temperature (except where the temperature is specified) for 16 hours. The suspended solids were separated, the extraction solution was evaporated, and the dried solids were weighed. Table 1 below summarizes the amount of leachables extracted from representative lots of colesevelam hydrochloride. Only a minimal amount of colesevelam hydrochloride could be solubilized and extracted from the tested solvents, except for 1N ammonium hydroxide where an ammonium chloride salt was formed (Note that 1 N Ammonium hydroxide is not representative of environmental conditions) (refer to Welchol™ Tablet NDA 21-176).

Table 1. Solubility of colesevelam hydrochloride in various solvents (refer to Welchol™ Tablet NDA 21-176)

Solvents	Leachables extracted (WT%)			
	TKFC404-1500	TLMC006-1842	TMAC015-1868	TNCC404-221-20
Water	ND	0.94	ND	1.34
0.1 N HCl	ND	1.20	ND	0.62
0.1 N HCl/50ºC	0.52	ND	0.69	0.70
1 N Ammonium hydroxide	ND	16.22	ND	15.84
Methanol	ND	-0.12	ND	-0.22
Acetonitrile	ND	0.01	ND	-0.10
Methylene chloride	ND	0.03	ND	0.03

Dissociation Constant(s):
Colesevelam hydrochloride is a polyectrolyte and there is no discrete dissociation constant. Acid-base titration was used to quantify both the primary and secondary titrable amines in colesevelam hydrochloride. The total titrable amines of colesevelam hydrochloride are from the poly-allylamine hydrochloride starting material, and range from 4.4 to 4.8 mmoles of amine per gram of colesevelam hydrochloride, on an anhydrous basis. The experimental value differs from the theoretical amine content of 5.0 mmoles of amine per gram of colesevelam hydrochloride. This difference is likely due to the broad titration curve associated with the titration of polyelectrolytes that results in a small percentage of the amines not being titrated (refer to Welchol™ Tablet NDA 21-176).

Log Octanol/Water Partition Coefficient:
Since colesevelam hydrochloride is not soluble in water or hydrocarbon solvents, the octanol/water partition coefficient can not be determined.

Vapor Pressure:
Colesevelam hydrochloride has no vapor pressure because it has such a high molecular weight. TGA-FT-IR data confirm that the only volatile compound in colesevelam hydrochloride during heating from ambient to 160 ºC is water. At higher temperatures the compound begins to decompose (refer to Welchol™ Tablet NDA 21-176).

Impurities:
There are no impurities in the drug substance at a level greater than 1%.

6.1.3 Environmental Depletion Mechanisms

Biodegradability:
The ready biodegradability of colesevelam hydrochloride was evaluated in a GLP compliant study using OECD guideline 301B (refer to Welchol™ Tablet NDA 21-176). Carbon dioxide (CO_2) evolution as indicator for biodegradation was measured over a 28-day test period. The following treatments were tested: a negative control, a positive

control (sodium benzoate), a toxicity control (sodium benzoate + colesevelam hydrochloride), and the test substance (colesevelam hydrochloride). The toxicity control showed no evidence for inhibition of biodegradation and the test was considered valid because:

- The biodegradation of the positive control exceeded 60% within ten days of first reaching 10%;
- The final cumulative CO_2 yield from the blanks was 51.4 mg per 3 liters of medium (*i.e.*, less than 120 mg per 3 liters required by the guideline); and
- The difference in replicate values for the test substance were less than 20%.

Overall, the results from this study indicate that colesevelam hydrochloride is not readily biodegradable as the biodegradation is only 2-3%.

Hydrolytic Stability:
The hydrolytic stability of colesevelam hydrochloride was evaluated in an acid and in a base solution (refer to Welchol™ Tablet NDA 21-176). Approximately 1 g of colesevelam hydrochloride was suspended in 10 mL of 1 N NaOH and 10 mL of 1 N HCl. The mixtures were stored at 60 ºC for 12 hours. After centrifugation, the supernatant was diluted 1:5 and analyzed by gas chromatography and ion chromatography. Both the acid- and base-treated samples showed no detectable peaks by gas chromatography. Using ion chromatography, the total level of stability-indicating impurities was less than 0.1%. These results indicate that colesevelam hydrochloride is very stable in acid and base solution and is not prone to hydrolysis.

Photolytic Stability:
Photostability studies were conducted on colesevelam hydrochloride following ICH guidelines using UV-B, daylight, and fluorescent bulbs (1.2 million-lux hours and 200 Watt hours/m^2) with the sample placed in an open Petri dish. Colesevelam hydrochloride was found to be stable under these conditions (refer to Welchol™ Tablet NDA 21-176).

6.1.4 Environmental Concentrations

6.1.4.1 Expected Environmental Introduction Concentrations (EIC) From Use

The projected maximum annual production volume of colesevelam hydrochloride for direct use in all formulations and for all indications based on five-year production estimates was utilized for all calculations (see Appendix III – Confidential).

Aquatic Environment

Since colesevelam hydrochloride is not soluble in water, estimating its expected concentration in the aquatic environment is problematic. A conservative approach was taken by using the $EIC_{aquatic}$ algorithm in the FDA guidance document (FDA, 1998) and

following specific FDA recommendations (see Appendix I - Confidential). The $EIC_{aquatic}$ based on the FDA guidance document and using the maximum expected annual volume use for the next five years is **12.94 µg/L** (ppb) (see Appendix IV - Confidential). Due to the insolubility of colesevelam hydrochloride, this $EIC_{aquatic}$ significantly overestimates expected concentrations and is therefore a very conservative approach. Furthermore, this calculation assumes that all the drug substance produced is used, evenly distributed throughout the USA, and no metabolism or depletion mechanisms exist.

Terrestrial Environment

Colesevelam hydrochloride is an insoluble compound, and upon entry into a waste water treatment plant, will remain as suspended particles and settle out into biosolids due to its density (1.1 g/cm^3). Hence, potential risks to terrestrial receptors (*e.g.*, soil microorganisms, land plants, and soil invertebrates) from exposure to land-applied biosolids are possible. The estimated environmental introduction concentration in biosolids-amended soils is **1.61 mg/kg** (ppm) (see Appendix V - Confidential) and was calculated using an FDA recommended approach (see Appendix I - Confidential).

Atmospheric Environment

Colesevelam hydrochloride has no vapor pressure and is not expected to volatilize and enter the atmospheric environment.

6.1.4.2 Maximum Expected Environmental Concentration (MEEC) From Use

Since no environmental depletion mechanisms were identified for colesevelam hydrochloride, the expected environmental introduction concentrations in the aquatic environment (12.94 µg/L) and in the soil (1.61 mg/kg) can be considered the maximum expected environmental concentration in the aquatic environment ($MEEC_{aquatic}$) and in the terrestrial environment ($MEEC_{soil}$). The $MEEC_{aquatic}$ and the $MEEC_{soil}$ will be compared to the predicted environmental effect concentrations in these respective environmental compartments to calculate potential environmental risks.

6.1.5 Summary

Colesevelam hydrochloride is a cross-linked polymer of (poly)allylamine alkylated with 1-bromodecane and 6-bromohexyltrimethylammonium bromide that is highly stable and does not appear to rapidly degrade biologically. Because it is possible that this compound could enter both the aquatic and terrestrial environments, the following section describes studies performed to assess the potential effects of colesevelam hydrochloride on these environmental compartments.

6.2 Environmental Effects of Released Substances

6.2.1 Acute Toxicity to Activated Sludge Microorganisms in a Wastewater Treatment Plant

6.2.1.1 Objective

The study was performed to estimate possible effects of colesevelam hydrochloride on sludge microorganisms in sewage treatment plants. This GLP compliant study was performed according to OECD Guideline 209 and EU-Guideline 87/302, using activated sludge from a sewage treatment plant treating predominantly domestic sewage (refer to Welchol™ Tablet NDA 21-176).

6.2.1.2 Test Design

Since colesevelam hydrochloride is not soluble in water, it was ground to a fine powder and the appropriate amounts were weighed and added to the different treatments. Colesevelam hydrochloride concentrations presented in this report are calculated from the nominal loading rate and given as "loading of colesevelam hydrochloride/L". Five concentrations of the test substance colesevelam hydrochloride (19, 40, 75, 151, and 300 mg/L), three concentrations of the positive control substance 3,5-dichlorophenol (5, 15, and 45 mg/L), and two negative controls (tap water) were tested. The microbial inoculum was a preparation of activated sludge collected on the day of the test. The concentration of the microbial inoculum was adjusted to 4 g of dry weight/L, which gives a final amount of 1.6 g dry weight/L in the test medium. The inoculum was kept aerated before beginning the test. After mixing the inoculum with synthetic sewage solution and appropriate amounts of control or test substance solutions, the samples were aerated for a contact time of 3 hours at 20 °C. Negative controls were run as first and as last sample. After incubation, the respiration rates were determined in closed bottles using an oxygen-sensitive electrode. The inhibition of respiration was calculated from the respiration rates using the mean value of the negative controls as 100%.

6.2.1.3 Results

The test results fulfilled the criteria for validity: the respiration rates of the two control samples were within 15% of each other (actual value: ±1.8%) and the EC_{50} of 3,5-dichlorophenol was in the acceptable range of 5 to 30 mg/L (actual EC_{50}: 12.1 mg/L). The test substance did not inhibit the respiration rates of the bacteria up to 300 mg/L, the highest concentration utilized in the test.

6.2.1.4 Conclusion

The EC_{50} value for colesevelam hydrochloride could not be calculated since 50% inhibition was not obtained in any of the test substance treatments. Consequently, the EC_{50} value for colesevelam hydrochloride is higher than 300 mg/L (loading rate) and the No Observed Effect Concentration (NOEC) is considered to be 300 mg/L (loading rate).

6.2.2 Acute toxicity to the Unicellular Alga *Selenastrum capricornutum*

6.2.2.1 Objective

A *Selenastrum capricornutum* growth inhibition and GLP compliant study according to OECD Guideline 201 and EC guideline 92/69/EEC Part C.3, was performed to determine the possible effects of colesevelam hydrochloride on the growth of a unicellular green algae species (refer to Welchol™ Tablet NDA 21-176).

6.2.2.2 Test Design

An aqueous extract of colesevelam hydrochloride was prepared by adding 168 mg to 750 ml of double distilled water/concentrated nutrient medium (8:1). This aqueous extract was the stock solution from which the five test substance concentrations in the study were obtained by dilution. The concentrations in terms of dilutions of the loading rate were: 12.5, 25.3, 50.4, 100.8, and 201.6 mg/L. These concentrations were spaced by a factor of less than 2.2 according to the guidelines. The highest test concentration should inhibit algal growth by at least 50%, the lowest one should not markedly effect algal growth. The selected concentrations cover these specifications, since a preliminary test with one test substance concentration (100.8 mg/L) inhibited algal growth by 38.9% (based on area under growth curve) or 11.2% (based on average growth rate). The five test concentrations were tested against one negative control [double distilled water/ concentrated nutrient medium (8:1)]. There were three replicates for each test and control culture. The cell count of the algae was about 10^4 cells/mL at the start of the exposure in each vessel. In each vessel, the cell density of the algae was determined at 24, 48, and 72 hours after the onset of incubation and the pH was measured at the beginning and at the end of the incubation period. Possible test substance effects were determined by comparison of the areas under the growth curves and by comparison of the growth rates. Because the soluble components of the test substance are not known, there is no appropriate method of analysis available. Therefore, no determination of the actual concentration was performed and concentrations are given as “loading of colesevelam hydrochloride/L”.

6.2.2.3 Results

All criteria for validity of the test were fulfilled: increase in cell density in the control cultures was about 83-fold (in comparison to a the ≥ 16-fold recommended increase) and showing optimal growth, algal growth inhibition was more than 50% in the two highest

test concentrations and there was no inhibition in the two lowest test concentrations, and maximum change in pH of test cultures and controls was 0.3.

After 72 hours of incubation, algal growth was slightly enhanced at loadings of colesevelam hydrochloride of 12.5 and 25.3 mg/L. At 50.4, 100.8, and 201.6 mg/L, algal growth was inhibited by about 41 to 92% based upon the areas under the growth curve, and by about 9 to 44% based upon the growth rates. This effect was not anticipated as the test substance is insoluble in water. Analysis of cation and anion concentrations in the medium loaded with the test substance demonstrated that effects on algal growth were likely due to an anion-exchange effect (*i.e.*, phosphate: 0.2 mg/L in medium loaded with test substance *versus* 1.25 mg/L in medium, sulfate: 0.3 *versus* 6.8 mg/L). Therefore, this finding is not considered to be related to the test compound. No changes were found for cations.

6.2.2.4 Conclusion

A 72h-EC_{50} for colesevelam hydrochloride was derived based on the area under the growth curve: 67.2 mg/L (loading rate). A 72h-EC_{50} based on average growth rate could not be calculated and is estimated as higher than 201.6 mg/L (loading rate). The 72h-NOECs based on area under the growth curve and average growth rate are 25.3 and 50.4 mg/L (loading rate), respectively.

6.2.3 Acute Toxicity to the Water Flea *Daphnia magna*

6.2.3.1 Objective

The purpose of this study was to determine the acute toxicity of colesevelam hydrochloride to the daphnid, *Daphnia magna*. The criterion for effect was immobilization. The study was conducted under GLP and in accordance with OECD Guideline 202 (refer to Welchol™ Tablet NDA 21-176).

6.2.3.2 Test Design

Because the test substance is insoluble in water, a limit test with a loading rate of 100 mg/L was performed. The test substance was ground in a mortar and added to dilution water (reconstituted water according to ISO 6341) at a loading rate of 100 mg/L. This preparation was stirred for 24 hours in the dark using a magnetic stirrer. The filtrate (filtration by a pleated filter followed by a 0.2 µm sterile filter) was then used for the test. Neonates of *Daphnia magna*, hatched from ephippia and not more than 24-hours old, were exposed to the filtrate. One negative control group was exposed to dilution water only. Twenty (20) daphnids each, divided into 4 replicates (5 daphnids each), were used for the test substance group and for the control group. There is no method of analysis of the soluble components of the test substance available. Therefore, no determination of

the actual concentration of colesevelam hydrochloride was performed and concentrations are given as “loading of colesevelam hydrochloride/L”.

6.2.3.3 Results

The quality criteria for the OECD guideline were fulfilled: immobilization of control group daphnids was 0% at the end of the test (guideline: maximum of 10%), there were no control daphnids trapped at the water surface, dissolved oxygen concentrations in the test vessels were higher than 8.3 mg/L at each time determined (guideline: higher than 3 mg/L), and pH of the test substance was stable during the test. There was no immobilization in the test substance group at 24 or 48 hours.

6.2.3.4 Conclusion

Colesevelam hydrochloride did not affect mobility of *Daphnia magna* at a loading rate of 100 mg colesevelam hydrochloride/L (*i.e.*, the NOEC). The 48h-EC_{50} is therefore estimated as being higher than 100 mg/L (loading rate).

6.2.4 Acute Toxicity to the Zebrafish *Branchydanio rerio*

6.2.4.1 Objective

The purpose of this study was to determine the acute toxicity of colesevelam hydrochloride to the zebrafish, *Brachydanio rerio.* The criterion for effect was mortality, but animals were also observed for alterations and changes in behavior. The study was conducted under GLP and in accordance with OECD Guideline 203 (refer to Welchol™ Tablet NDA 21-176).

6.2.4.2 Test Design

Because the test substance is insoluble, a limit test with a loading rate of 100 mg colesevelam hydrochloride/L was performed. The test substance was ground in a mortar and added to dilution water at a loading rate of 100 mg/L. This preparation was stirred for 24 hours in the dark using a magnetic stirrer. A filtrate (Miracloth® filter, Calbiochem) was then used for the test. Local tap water was mixed with deionized water to achieve the appropriate water hardness as required by the guidelines (< 250 mg $CaCO_3$/L). This water was used for holding of the fish and for the control and test medium. Seven (7) fish (each approximately 3 cm long) were used for the test substance group and for one control group (dilution water only). No analytical method is available to determine the soluble components of the test substance. Therefore, the actual concentration of colesevelam hydrochloride was not determined and concentrations are given as “loading of colesevelam hydrochloride/L”.

6.2.4.3 Results

The quality criteria for the OECD guideline were fulfilled: constant pH and temperature conditions were maintained, no mortality occurred in the negative control group, and the dissolved oxygen concentration was higher than 60% of the air saturation value throughout the test. No mortality was noted in the test substance group. The swimming activity of the fish of the test substance group was slightly lower for about 48 hours when compared to the control animals. At the beginning of the test, the test medium was slightly cloudy and after a while small particles were seen at the bottom of the test vessel. These small particles in the filtrate were presumably the cause of the slightly different behavior of the fish in the test substance group.

6.2.4.4 Conclusion

Colesevelam hydrochloride does not affect survival of zebrafish at a loading rate of 100 mg/L (*i.e.*, the NOEC). The 96h-LC_{50} is estimated as being higher than 100 mg/L (loading rate).

6.2.5 Acute toxicity to the Earthworm *Eisenia fetida*

6.2.5.1 Objective

The purpose of this study was to determine the 14-day acute toxicity of colesevelam hydrochloride to the earthworm, *Eisenia fetida.* The study was conducted according to GLP standards (see Appendix IX - Confidential) and in accordance with OECD Guideline 207. The final study report is included in Appendix VII - Confidential.

6.2.5.2 Test Design

The earthworms (*Eisenia fetida*) used in this study were from an in-house culture, which originated from Vickers Farms in Orlando, Florida. Initial weights obtained from a representative sample of 10 worms ranged from 338 to 599 mg per worm. The artificial soil used for the test was prepared to approximate a sandy loam soil (*i.e.*, 70% industrial silica sand, 20% kaolinite clay, and 10% sphagnum peat based on dry weight equivalents). Calcium carbonate, $CaCO_3$, was added to the soil to adjust the pH to 6.0 ± 0.5. The test chambers were exposed to a continuous light cycle at a target intensity range between 400 and 800 Lux. The treatments and controls were each replicated four times, and each replicate contained 10 worms for a total of 40 worms per treatment. Due to insolubility of colesevelam hydrochloride in both water and organic solvents, the test substance was added as a dry powder to each exposure treatment. Soils were dosed with nominal calculated concentrations of 0 (control), 15.7, 31.3, 62.5, 125, 250, 500, and 1,000 mg colesevelam hydrochloride/kg dry soil and a hydration level of approximately 35% of the soil dry weight. A positive control (2-chloroacetamide) was tested at 0, 13, 25, 50, and 100 mg/kg. On days 7 and 14, worm survival and observations of general

health and behavior were noted (*e.g.*, normal, lethargic, elongation, absence of burrowing, discoloration, etc.). Any organisms that were not accounted for on day 14 (*i.e.*, not found) were considered dead. On termination, the weights of surviving earthworms were measured on a replicate basis. In addition, soil moisture, pH and temperature were determined.

6.2.5.3 Results

After 14 days of exposure to colesevelam hydrochloride, mortality was 0% in the control and all test substance treatments. Hence, the OECD criterion for acceptability of less than 10% mortality in the control was fulfilled. Burrowing behavior was not affected after 7 days of exposure. Weight loss in the study did not appear to demonstrate a concentration-dependent response. The calculated 7-and 14-day LC_{50} value for earthworm survival in the reference toxicant test was 36.6 mg 2-chloroacetamide/kg dry soil. This value is within the historical range of LC_{50} values (*i.e.*, 30.1 to 82.6 mg/kg) for this toxic standard.

6.2.5.4 Conclusion

The estimated 14-day LC_{50} value for earthworm survival was estimated to be >1,000 mg colesevelam hydrochloride/kg dry soil, the highest concentration tested. Based on absence of mortality and sublethal observations, the NOEC was 1,000 mg colesevelam hydrochloride/kg dry soil.

6.2.6 Acute Toxicity to Terrestrial Plants

6.2.6.1 Objective

The purpose of this study was to determine the potential effects of colesevelam hydrochloride on seedling emergence and early seedling growth (*i.e.*, phytotoxicity rating, shoot height, and dry weight) of three non-target terrestrial plant species (*i.e.*, oat, radish, and lettuce). The study was conducted according to GLP standards (see Appendix IX - Confidential) and in accordance with OECD Guideline 208. The final study report is included in Appendix VIII - Confidential.

6.2.6.2 Test Design

One species of monocotyledonous plants (Oat, *Avena sativa*) and two species of dicotyledonous plants (Radish, *Raphanus sativus* and lettuce, *Lactuca sativa*) were tested. The test soil was characterized as sandy loam soil, based upon the USDA textural classification. It was a mixture of two topsoils collected at a depth of approximately 6-10" at a site that was not expected to have received direct application of pesticides or herbicides prior to collection. The definitive emergence test was performed at nominal soil concentrations of 0 (control), 10.2, 25.6, 64.0, 160, 400 and 1,000 mg/kg dry soil.

There were four replicate pots each containing 10 seeds for lettuce and five seeds for radish and oat. The seeds were planted on the same day as the test substance incorporation. The in-life phase was terminated 19 days after introduction of the test substance, which corresponded to 14 days of exposure after 50% of the control seedlings had emerged. The definitive test was performed in an environmentally regulated greenhouse. Temperature and relative humidity were recorded continuously. Subsamples of the control and treated soils were collected for pH readings. Observations of seedling emergence and phytotoxic ratings (*i.e.*, visual injury assessments) were first performed on test day 5 and then on a weekly basis for all species. Visual injury assessments were made on a scale of 0 to 100, with 0 indicating no injury and 100 indicating maximum effect (100% non-emergence or mortality). The in-life phase was terminated a minimum of 19 days after introduction of the test substance, which corresponded to 14 days of exposure after 50% of the control seedlings had emerged. At test termination, the numbers of live and dead plants, if observed, were recorded along with the visual assessments. Shoot heights and individual dry weight were recorded at the end of the test.

6.2.6.3 Results

In the emergence test for each species, there were no statistically significant reductions in emergence due to colesevelam hydrochloride. The percent emergence for oat in the control was 100% and ranged from 85 to 100% in all treated soils. The percent emergence for radish in the control was 100% and ranged from 95 to 100% in all treated soils. The percent emergence for lettuce in the control was 83% and ranged from 63 to 95% in all treated soils. There was one replicate pot in the 64.0 mg/kg dry soil treatment that only displayed a percent emergence value of 30%. However, this was likely due to natural variation of the germination rate and was not considered to be a concentration dependent response of this species to the colesevelam hydrochloride exposure. Only one lettuce plant died in the 25.6 mg/kg treatment, but this did not result in a statistically significant reduction in survival for this species. None of the species tested displayed any slight to severe visual injury ratings (i.e., ratings >10) during the test.

The percent difference in the shoot heights for the treated oat, radish, and lettuce seedlings in comparison to the control seedlings ranged from -10% to 8%, from -7% to 7%, and from -7% to 3% for all treatment levels, respectively. There were no statistically significant reductions in shoot height for any of the treatment levels tested.

The percent difference in the individual dry weights for the treated oat, radish, and lettuce seedlings in comparison to the control seedlings ranged from -11% to 16%, from 1% to 34%, and from -15% to 4% for all treatment levels, respectively. Although there appears to be a concentration dependent stimulation in the individual dry weights for the radish, this was not considered to be a deleterious effect of the test substance. There were no statistically significant reductions in shoot weight for any of the treatment levels tested.

6.2.6.4 Conclusion

Colesevelam hydrochloride does not affect plant emergence, shoot height, and shoot weight at concentrations up to 1,000 mg/kg dry soil (*i.e.*, the NOEC). The EC_{50} is estimated as being higher than 1,000 mg/kg dry soil.

6.3 Environmental Assessment

Colesevelam hydrochloride is expected to be the only substance entering the environment. Since colesevelam hydrochloride is insoluble in water, the exposure compartment of primary concern is the terrestrial environment. However, a conservative approach was taken by also including an evaluation of the potential effects of colesevelam hydrochloride on the aquatic environment. Table 2 shows the toxicity profile of colesevelam hydrochloride derived from the most sensitive aquatic and terrestrial species, as well as the expected maximum environmental concentrations and the hazard ratio in the aquatic and terrestrial compartments.

Table 2. Environmental Assessment Summary

	Aquatic Environment	**Terrestrial Environment**
Lowest observed EC_{50}/LC_{50}	67,200 µg/L	>1,000 mg/kg
Maximum Expected Environmental Concentration (MEEC)	12.94 µg/L	1.61 mg/kg
Hazard Quotient (EC_{50}-LC_{50}/MEEC)	5,193	>621

The toxicity value derived for the most sensitive aquatic species (*i.e.*, algal growth 72h-EC_{50}: 67.2 mg/L), was compared to the Maximum Expected Environmental Concentration (MEEC) of colesevelam hydrochloride in the aquatic environment, which was calculated on the basis of the 5th year projected use volume (see Appendix IV - Confidential). Similarly, the lowest toxicity value derived from the terrestrial studies (*i.e.*, >1,000 mg/kg dw), was compared to the MEEC of colesevelam hydrochloride in soil, which was calculated on the basis of the 5th year projected use volume (see Appendix V - Confidential). These analyses indicate that:

- The lowest observed EC_{50} or LC_{50} for colesevelam hydrochloride derived from Tier 2 acute aquatic toxicity testing (fish, aquatic invertebrate, alga) is more than 5,000 times greater than the $MEEC_{aquatic}$ (*i.e.* in the POTW influent, not taking into account dilution).
- The lowest observed EC_{50} or LC_{50} for colesevelam hydrochloride derived from Tier 2 acute terrestrial toxicity testing (earthworm, plants) is more than 500 times greater than the $MEEC_{soil}$.

According to the FDA guidance document (FDA, 1998), no further testing is needed if the lowest observed EC_{50} or LC_{50} derived from Tier 2 acute toxicity testing is 100 times

higher than the MEEC. No effects to activated sludge organisms are anticipated due to the low toxicity of colesevelam hydrochloride to activated sludge organisms (*i.e.*, LC_{50} > 300 mg/L). Similarly, no effects on soil microorganisms are expected based on the low toxicity of colesevelam hydrochloride to sludge microorganisms (cfr. recommended approach by FDA shown in Appendix I - Confidential).

In conclusion, no effects are expected on the aquatic and terrestrial environment from societal use of colesevelam hydrochloride at the highest projected use volume for the next 5 years and using conservative assumptions for calculating its predicted environmental concentration.

7 MITIGATION MEASURES

No adverse environmental effects associated with the proposed action were identified, and therefore, no mitigation measures are needed.

8 ALTERNATIVES TO THE PROPOSED ACTION

No adverse environmental effects associated with the proposed action were identified, and therefore, no alternatives to the proposed action are proposed.

9 LIST OF PREPARERS

Company	Name	Job Title	Qualifications
Gradient Corporation	Tim Verslycke	Senior Environmental Toxicologist	Ph.D. (Applied Biological Sciences)
Gradient Corporation	Manu Sharma	Principal	M.S. (Civil Engineering), Licensed Professional Engineer (Environmental)
ABC Laboratories	John Aufderheide	Senior Toxicologist	B.S. (Biology)
ABC Laboratories	Brian Lee	Associate Field Specialist	B.S. (Agronomy)
Daiichi Sankyo Pharma Development	Martins Adeyemo[1]	Senior Director	Ph.D., DABT

[1] Reviewer

10 REFERENCES

FDA. (1998). Guidance for Industry. Environmental Assessment of Human Drug and Biologics Applications, U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER). July. Revision 1.

11 APPENDICES

Appendices I, II, VII, VIII and IX are considered confidential and not for public disclosure.

Additionally, information of the marketing estimates and the calculations of the Expected Introduction Concentrations (EIC), if disclosed, would provide competitors with confidential information on the market share of the product. For this reason, the information included in Appendices III, IV and V are also claimed as confidential and not for public disclosure.

Appendix VI is considered non-confidential and provides a data summary.

APPENDIX VI: DATA SUMMARY TABLE (NON-CONFIDENTIAL)

PHYSICAL/CHEMICAL CHARACTERIZATION	
Water Solubility	Not water soluble
Dissociation Constant(s)	Polyelectrolyte with no discrete dissociation constant
Log Octanol/Water Partition Coefficient (Log K_{ow})	N/A (Not soluble in hydrocarbon solvents)
Vapor Pressure	No vapor pressure (*i.e.*, $<10^{-5}$ Pa) because of its high molecular weight
DEPLETION MECHANISMS	
Hydrolysis	Not susceptible to hydrolysis
Photolysis	Not susceptible to photolysis
ENVIRONMENTAL EFFECTS	
Activated Sludge Respiration Inhibition test	>300 mg/L (loading rate; EC_{50})
Acute Algal Growth Inhibition (72 hours)	67.2 mg/L (loading rate; EC_{50})
Acute Daphnid Immobility (48 hours)	>100 mg/L (loading rate)
Acute Fish Mortality (96 hours)	>100 mg/L (loading rate)
Plant Seedling Emergence and Growth (19 days)	>1,000 mg/kg dry weight (nominal)
Acute Earthworm Mortality (14 days)	>1,000 mg/kg dry weight (nominal)

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Moheb Nasr
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