# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

**208090Orig1s000**

# PHARMACOLOGY REVIEW(S)

**Tertiary Pharmacology/Toxicology Review**

**From**: Timothy J. McGovern, Ph.D.,
ODE Associate Director for Pharmacology and Toxicology, OND IO
**NDA:** 208090
**Agency receipt date:** December 12, 2014
**Drug:** Xtampza ER (oxycodone) capsules
**Sponsor:** Collegium Pharmaceutical, Inc.

**Indication:** Management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate

**Reviewing Division:** Division of Anesthesia, Analgesia, and Addiction Products

**Introductory Comments:** This is a 505(b)(2) application for an extended-release formulation of oxycodone. The maximum theoretical daily dose (MTDD) for oxycodone is 1.5 g/day for an opioid-tolerant individual. Excipients in the drug product formulation include myristic acid, yellow beeswax and carnauba wax at maximum daily exposures of (b) (4) mg, (b) (4) mg, and (b) (4) mg, respectively, based on a maximum daily intake of thirty-seven 40 mg capsules. (b) (4)

The Division pharmacology/toxicology reviewer, Dr. Grace Lee, and supervisor, Dr. Dan Mellon, concluded that the nonclinical data do not support approval of Xtampza ER for the indication listed above based on inadequate safety information related to the three primary excipients listed above when the product is used up to the MTDD for oxycodone. Specific concerns for this product relate to the lack of supportive chronic toxicology, reproductive and developmental toxicology, and carcinogenicity data. However, Drs. Lee and Mellon do allow for the possibility that the product could be approved with dose limitations in the label based on the available safety information for these excipients and in consideration of the overall risk-benefit for the indicated patient population. Under this scenario, post-marketing requirements (PMRs) would be established to further assess potential safety concerns for doses up to the MTDD of oxycodone and include chronic toxicology studies in rodents and non-rodents, a reproductive and developmental toxicology battery in rats and rabbits, and carcinogenicity studies in rats and mice. The studies could be conducted with a mixture of beeswax, carnauba wax, and myristic acid using ratios comparable to the drug product formulation. The Division indicated that some their recommended PMRs could be reconsidered should the sponsor provide appropriate post-marketing data to demonstrate that some or all of the excipients were not systemically absorbed, there were no unexpected contaminants from environmental or apicultural practices, and if chronic toxicity data did not identify concerning findings.

**Evaluation:** The FDA's 2005 Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients recommends a battery of nonclinical studies to support long-term use of novel excipients that includes safety pharmacology, absorption, distribution, metabolism, and excretion (ADME) studies, a standard battery of genetic

toxicology studies, general toxicity studies of chronic duration in two species, a battery of reproductive and developmental toxicity studies, and an evaluation of carcinogenicity studies in rats and mice. The guidance incorporates flexibility and states that existing human data for some excipients can substitute for certain nonclinical safety data, and an excipient with documented prior human exposure, such as use in previously approved products or GRAS status as a direct food additive, under circumstances relevant to the proposed use may not require evaluation in the full battery of toxicology studies. The guidance also allows for the request of additional safety studies for previously used excipients when the proposed use differs significantly from the previous use; in some cases it may be necessary for the safety database for a previously used substance to be brought up to current standards. Therefore, in certain cases a more limited dataset, such as evaluation of chronic general toxicity alone, could be considered supportive of the safety of excipients already used in approved products for which the proposed daily exposure exceeds that of their maximum exposure in previously approved products or for which there is previous human exposure by other means such as through the diet. In other cases, the guidance does indicate that additional studies may be requested for previously used excipients when the proposed use differs significantly from the previous use.

The Division's recommendations are consistent with the flexibility incorporated in the FDA guidance although I am not in complete agreement with them as discussed below. In the case of this NDA, the Division is not recommending the full battery of studies. Safety pharmacology or genetic toxicology studies are not required based on the previous experience with these substances as pharmaceutical excipients or direct food additives and the toxicology studies being requested address the lack of ADME data endpoints. The Division's recommendations for chronic toxicity, reproductive toxicity, and carcinogenicity studies are based on the novel proposed use of these excipients and inadequacy of the current safety database to support clinical exposure associated with oxycodone doses of greater than 320 mg/day. The sponsor's submission of appropriate ADME data could support a waiver for some of the recommended PMRs, assuming environmental and apicultural contamination issues are adequately addressed.

Based on the identified data, waxes appear to be of relatively low toxicity. They generally have been assumed to be indigestible, though no actual data are available to support this assumption. A 90-day study on a combination of beeswax and carnauba wax was conducted by the sponsor and did not identify any treatment-related effects. Longer term studies of carnauba wax and various hydrocarbon components of beeswax are available in the literature with many providing safety margins ranging from 2-fold to 1500-fold. Identified toxicities related to ingestion of mineral oils include histiocytosis in the liver and lymph nodes and cystic degeneration or angiectasis in the liver; the findings suggest that some components of the waxes may be absorbed. Although exposure margins are indicated from the studies conducted, the studies did not include all standard endpoints and there are limited data for the main class of fatty acid esters. Additionally, there are limited data to evaluate the potential for reproductive and developmental toxicities as well as carcinogenic effects.

Human experience related to use of beeswax includes a WHO identified daily average dietary exposure of 10 to 100 mg/person; 90th and 95th percentiles are estimated to be 350 mg/day and < 650 mg/day, respectively. The European Food Safety Authority estimates consumption in the Netherlands to be up to 1290 mg/day. Yellow or white wax has also been used as an excipient in FDA-approved oral drug products up to ~ (b) (4) mg/day for chronic us (b) (4)

Human experience related to use of carnauba wax includes a Joint FAO/WHO Expert Committee on Food Additives conclusion that an acceptable daily intake was 0-7 mg/kg (420 mg/day for a 60 kg person); the estimated consumption of carnauba wax in Europe is estimated to be 180 mg at the 95th percentiles for adults. Carnauba wax has also been used in an FDA-approved oral drug product up to (b) (4) mg/day for chronic use, a level that is less than half the anticipated exposure for the current product at the MTDD for oxycodone.

Myristic acid is a saturated fatty acid with no identified direct toxicity data available. It is a multipurpose food additive permitted for direct addition to food for human consumption as per 21 CFR 172.860. Studies of 150 days to 18 weeks duration were conducted with palmitic acid and lauric acid with NOAELs exceeding 5 g/kg/day; the data were not available for direct review. Excessive intake of saturated fats is considered a risk factor for coronary heart disease. The estimated mean daily consumption of myristic acid in food in the US has declined over the years dropping to 2.22 g/day in a 2011/2012 survey from 5.8 g/day in the 1960s; data from eastern Finland averaged 14 g/day.

In addition to the described gaps in the nonclinical data to support the safety of the three excipients associated with exposures of greater than 320 mg oxycodone/day, questions persist as to the purity levels of the beeswax and the final drug formulation. The nonclinical review team has communicated with the Product Quality reviewers regarding a detailed analysis of the beeswax. According to Dr. Mellon, the sponsor has agreed to set appropriate specifications for (b) (4) and will analyze the beeswax for possible environmental and apicultural contaminants.

**Conclusion:**

I agree with the Division pharmacology/toxicology conclusion that there are inadequate nonclinical data to support the approval of Xtampza ER at daily doses greater than 320 mg/day up to the MTDD of 1.5 g oxycodone per day due to a lack of adequate nonclinical safety data related to three excipients: beeswax, carnauba wax, and myristic acid. In addition, (b) (4) I agree with the reviewers' recommendation to require chronic general toxicology studies. In general, when an excipient has been used in previously approved products by a similar route of administration and for similar durations of use, general toxicity studies are sufficient in the absence of an identified safety signal to address concerns with increased daily exposure.

The Division also recommends that a battery of developmental and reproductive toxicology and carcinogenicity studies be conducted to further assess the potential risk of the excipients. These recommendations are based on the Division reviewers' concerns with the limited nonclinical data available, the significant increase in daily exposures through use of Xtampza compared to previous use, questions regarding the contamination profile of the beeswax, and the potential for this formulation to be used across multiple drug platforms. As noted previously, the FDA guidance does allow for such a request to address identified data gaps. Additionally, endpoints related to reproductive and carcinogenic effects can be difficult to assess in human populations. Therefore, the Division's request for these studies is not without some merit. While I understand the concerns raised by the reviewers, I believe that the conduct of chronic toxicology studies adequately address the primary concerns associated with increased daily exposures, especially since these excipients are either present in FDA-approved products intended for chronic use (beeswax and carnauba wax), are listed in the CFR as a direct food additive with no limit on use and with associated human consumption of 2 g/day or more (myristic acid), and the data that are available with the excipients or related substances do not identify concerns in these areas.

Given the potential benefit of this product to the intended patient population, it is reasonable to consider approved dose limits that could be supported by the available nonclinical data and human experience. The overall nonclinical and human experience data for the excipients appear to provide strong support for a maximum clinical dose of 320 mg oxycodone or 8 capsules, based primarily on a comparison to products listed in FDA's Inactive Ingredient Database (carnauba wax) and estimates of daily human intake in the US (beeswax and myristic acid). This degree of dose limitation appears to be reasonably safe; additional nonclinical studies such as chronic, reproduction and carcinogenicity studies, testing a mixture of the excipients would further characterize potential safety concerns at this dose level (320 mg) and could also support eventual approval of clinical doses up to the MTDD. Under this scenario, it would be appropriate to approve the application from a nonclinical perspective and require the submission of the additional safety information related to the excipients as PMRs.

There is reduced nonclinical support for dose limits up to 14-16 capsules that is based on more limited human consumption data from the 1960s (myristic acid) or the Netherlands (beeswax). However, should the overall risk: benefit evaluation support approval at this dose level, the above comments regarding PMRs apply.

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**

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/s/

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TIMOTHY J MCGOVERN
10/16/2015

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

# PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION
**Secondary Review**

| | |
|---|---|
| Application number: | 208090 |
| Supporting document/s: | 1, 11, 16, 19, 23, 28, 30 |
| Applicant's letter date: | December 12, 2014, March 11, 2015, April 6, 2015, May 1, 2015, June 16, 2015, August 7, 2015, August 10, 2015 |
| CDER stamp date: | December 12, 2014, March 11, 2015, April 6, 2015, May 1, 2015, June 16, 2015, August 7, 2015, August 10, 2015 |
| Product: | Xtampza ER (oxycodone) capsules |
| Indication: | Management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate |
| Applicant: | Collegium Pharmaceutical, Inc. |
| | 780 Dedham Street Suite 800 |
| | Canton, MA 02021 |
| Review Division: | Anesthesia, Analgesia, and Addiction Products |
| Reviewer: | Grace S. Lee, PhD, DABT |
| Supervisor/Team Leader: | R. Daniel Mellon, PhD |
| Division Director: | Sharon Hertz, MD |
| Project Manager: | Ayanna Augustus, PhD, RAC |

## Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 208090 are owned by Collegium Pharmaceutical, Inc. or are data for which Collegium Pharmaceutical, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 208090 that Collegium Pharmaceutical, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly

available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208090.

**Background and Recommendation**

Collegium Pharmaceuticals submitted NDA 208090 in support of Xtampza, an extended-release oxycodone formulation with purported abuse deterrent properties. This is a 505(b)(2) application that relies in part on the Agency's previous finding of safety for Oxycontin (NDA 22272).

The Agency and Collegium first discussed the development program at a preIND meeting in March of 2007. At that time, the final formulation was not set; however, the Division did note that no additional studies for oxycodone would be required for a 505(b)(2) application that was relying upon the Agency's previous finding of safety for a chronic oxycodone drug product. At the end-of-phase 2 (EOP2) meeting in March of 2010, the Agency specifically noted that the safety of the excipients would have to be justified up to the maximum theoretical daily dose (MTDD) of the drug product. The safety of the product was also discussed the preNDA meeting in April of 2014. At that time, a literature-based justification for the safety of novel excipients was prepared and submitted as part of the meeting package; however, detailed review was not possible at that time due to competing priorities. The Division did note that the levels of myristic acid, yellow beeswax, carnauba wax, and hypromellose were technically novel (b) (4) Although preliminary review suggested adequate safety for myristic acid and hypromellose, the Division specifically expressed concern about the justification for the beeswax and carnauba wax based on the limited toxicity data identified. The Agency specifically noted that an alternative literature-based safety justification "approach does result in some risk to your development program, as we may not ultimately agree that adequate data exist to support the safety of the excipients when the product is dosed up to the MTDD of oxycodone." We provided the following comments based on the preliminary review:

**Based upon the preliminary review of the summary information provided, we are providing the following comments. These comments should not be deemed to be a complete list of concerns with the excipient data provided to date.**

1. **Although you have stated that the components of the waxes are poorly absorbed and the metabolites, if absorbed, are metabolized to compounds occurring endogenously, it is not clear how the exposure to these compounds compares to the levels found endogenously. If the levels that would occur after consumption of the MTDD exceed**

**the endogenous levels, further studies may be required to determine that there are no safety concerns for these excipients.**

2. **If you elect to justify the safety of the waxes based on the components of the waxes, specifically delineate what data exist for each component at the worse-case maximum daily dose via the drug product, list what data are missing, and provide adequate justification for why the lack of those missing data should not be deemed a deficiency for the NDA.**

3. **As [redacted] the beeswax and carnauba wax [redacted] we may evaluate the existing safety data for the mixture or components both independently for each excipient as well as combined for a total wax MTDD of [redacted] mg per day.**

4. **Based on the dietary levels predicted for both waxes, [redacted] previous clinical experience is lacking to support safety at the MTDD.**

5. **Since both of the waxes are derived from natural products, your NDA must include information regarding the potential impurities in the wax derived from the environment. Of particular concern is the potential for residual [redacted] Purity information should be provided either in the NDA or via reference to an acceptable master file for the excipient material.**

6. **Given the potential for significant wax accumulation in the gastrointestinal tract, we are concerned that there may be GI obstruction with the material with large doses of this drug product.**

In the discussion that followed, "The Agency emphasized that a concern with reference to literature articles on natural products is that it is unlikely that adequate data will be available to assure that the batches tested are representative of the drug product excipients proposed for use. Lack of adequate data to show comparability may preclude extrapolating safety from published literature."

Dr. Grace Lee completed the primary review of this NDA application and has concluded that there are adequate data to support the safety of the drug substance impurity specifications, the drug product degradant specifications, and for many of the drug product formulation excipients. However, Dr. Lee has recommended a complete response due to the lack of adequate nonclinical information to justify the safety of three excipients when the drug product is used up to the MTDD of 1.5 g/day oxycodone for an opioid-tolerant individual. She specifically notes that there are inadequate nonclinical data for the MTDD of beeswax, carnauba wax, and myristic acid. She recommends that chronic repeat-dose toxicology studies, standard reproductive and developmental

toxicology studies, and carcinogenicity studies be completed for a mixture of these three compounds using ratios comparable to the drug product formulation.

I concur that the safety justification for these three excipients is lacking, particularly given the facts that the complete chemical composition of the beeswax and carnauba wax are unknown, we lack adequate data to understand the potential absorption, distribution, and metabolism of these complex chemical mixtures, and the products may contain trace levels of environmental contaminants (b) (4) In this memorandum, I will discuss each of these excipients briefly and I will also discuss the proposal outlined by Dr. Lee regarding the levels of these excipients that would appear to be supported by previous human experience, in case the Agency considers setting dosing limitations in the labeling. In addition, I will discuss the challenge of quality control for naturally-derived commercial products such as beeswax, as I believe this has implications regarding the overall safety evaluation of this drug product formulation.

Regarding the options for dose limitations in labeling, should they be considered given the risk-benefit of the drug product formulation, the table below summarizes the total doses of each of these excipients and the levels that would be comparable to that at the upper limits of dietary consumption or adequately justified via use in comparable FDA-approved drug products:

**Table 1: Summary of Novel Excipients and Dietary Justification for Safety**

| Component | Dose per 40 mg (oxycodone HCl equivalent) capsule (mg) | MTDD (1500 mg Oxycodone HCl equivalents = 37 pills) (mg) | Maximum Daily Dose via IID (mg) | Estimated Maximum Amount Consumed in U.S. (mg) | Maximum Amount Estimated in any Country (mg) |
|---|---|---|---|---|---|
| Oxycodone base | (b) (4) | 1500 | -- | -- | -- |
| Yellow Beeswax | (b) (4) | (b) (4) | 47<br>(<1 pill) | < 650<br>(8 pills) | Up to 1290 (The Netherlands)<br>(16 pills) |
| Carnauba wax | (b) (4) | (b) (4) | 1160<br>(14 pills) | 0-420 (ADI)<br>(5 pills) | |
| Myristic acid | (b) (4) | (b) (4) | | 2220 (2011-2012 data)<br>(10 pills) | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 5800 (1960s)<br>(27 pills) | |
| Overall Maximum Daily Oxycodone Dose Supported (HCl eq) | | | 560 mg<br>(14 pills) | 320 mg<br>(8 pills) | 640 mg<br>(16 pills) |

If one considers the limited human experience taking into consideration the upper limit of beeswax estimated to be consumed in The Netherlands, the largest daily dose of carnauba wax in any FDA approved oral drug product, and the safety of a Western Diet in the 1960s, one could justify the safety of 14-16 pills per day of this formulation. However, as noted in the following sections on food grade beeswax, carnauba wax, and myristic acid, the levels (b) (4) as per the Food Chemicals Codex for these excipients could be as high as (b) (4) mcg/pill from these three excipients alone. A total of 8 pills could result in up to (b) (4) mcg/day (b) (4) A total of 14 pills could result in up to (b) (4) mcg/day (b) (4) The Applicant has tested several batches of beeswax, carnauba wax, and myristic acid and demonstrated that these excipients do not have appreciable levels (b) (4); however, they do not test the final drug product and do not have a specification (b) (4) in the drug product. Ideally, the Applicant should set a total drug product specification (b) (4) in the final drug product (b) (4) and become familiar with the newly finalized (b) (4) document.

Based on the data summarized by Dr. Lee and briefly reiterated in this memorandum, I concur with Dr. Lee's conclusion that there are inadequate nonclinical data to support the approval of NDA 208090 given the MTDD of oxycodone and with her recommendations for nonclinical studies. Of specific concern are the findings of histiocytosis and granulomatous foci of macrophages in mesenteric lymph nodes, and combined cystic degeneration or angiectasis in the liver in studies testing various hydrocarbons, the potential for hypercholesterolemia and elevated triglycerides with high doses of myristic acid, the lack of an adequate characterization of the chemical composition of these complex mixtures, the lack of adequate characterization of the potential for absorption of components of these chemical mixtures, and the lack of adequate control over the potential for environmental or apicultural contaminants in the beeswax. As noted by Dr. Lee, if the Division were willing to consider limiting the

maximum recommended daily dose to not more than 8 of the 40 mg oxycodone equivalent capsules (MDD 320 mg), the safety of the drug product formulation could be supported by previous human experience, at the discretion of the medical officer. If such a limit were instituted, then the NDA may be approved and the recommended studies be completed as post-marketing requirements. Once these PMRs were completed, and if the data support the safety of the higher levels of these excipients, the limitation could be removed.

If the Applicant sets a specification (b) (4) in the drug product formulation (b) (4) could be considered reasonable, if the risk-benefit were deemed acceptable.

The following studies should be completed prior to approval without dosing limitations in labeling or as PMRs if dosing limitations are a clinically-viable option pending the risk-benefit assessment:

1. Conduct a chronic (6-month) repeat-dose general toxicology study in the rat model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

2. Conduct a chronic (9-month) repeat-dose general toxicology study in the dog model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

3. Conduct a fertility and early embryonic development study in the rat model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

4. Conduct an embryo-fetal development study in the rat model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

5. Conduct an embryo-fetal development study in the rabbit model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

6. Conduct a pre- and post-natal development study in the rat model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

7. Conduct a carcinogenicity assessment in the rat model testing a mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

8. Conduct a carcinogenicity assessment in the mouse model testing the mixture of beeswax, carnauba wax, and myristic acid that is representative of the drug product composition.

9. Complete a detailed analysis of the beeswax employed in your drug product for potential residual levels of environmental and apicultural sources of contaminants and provide justification or the limits of detection, the safety of the levels of contaminants present, and the need for route testing of the beeswax used in the manufacturing of your drug product.

As a possible alternative to some of the above testing, it may be possible to provide a more detailed analysis of the components of the absorption, if any, of components of the beeswax and carnauba wax. This maybe completed, in part, by analysis of potential leachables from the mixture of beeswax and carnauba wax when exposed to simulated gastric fluids, including bile acids. If adequate data can be provided to clearly demonstrate that there is no absorption of components of the wax and adequate data regarding the purity control concerns for environmental and apicultural contaminants is provided, then the reproductive and developmental studies may be waived. Likewise, if the repeat-dose toxicology studies clearly demonstrate no preneoplastic lesions and purity issues are addressed, the Sponsor may be able to submit a waiver request for the carcinogenicity studies as per the FDA guidance document *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf.

**Comment on Labeling**

I concur with Dr. Lee's labeling recommendations. In addition, should the Agency consider approving this drug product with dosing limitations based on the lack of adequate nonclinical toxicology data for these novel excipients, I recommend that a statement to such effect be included in Section 13.2 Animal Toxicology. This is consistent with the 21 CFR §201.57 which states the following:

> (ii) *13.2 Animal toxicology and/or pharmacology.* Significant animal data necessary for safe and effective use of the drug in humans that is not incorporated in other sections of labeling must be included in this section (e.g., specifics about studies used to support approval under §314.600 or §601.90 of this chapter, the absence of chronic animal toxicity data for a drug that is administered over prolonged periods or is implanted in the body).

Recommended text:

### 13.2 Animal toxicology

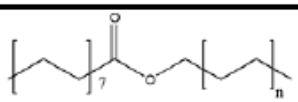
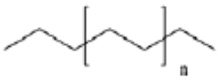
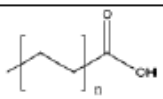
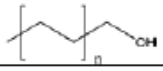
The levels of beeswax, carnauba wax, and myristic acid in this drug product have not been adequately tested for safety in chronic toxicity or reproductive and developmental toxicity studies in animals when Xtampza is used to administer oxycodone doses of greater than XX [320, 560, or 640] mg/day.

**Beeswax**

At the MTDD of Xtampza, a person would consume up to (b) (4) mg of beeswax.

Beeswax is produced by honey bees (*Apis mellifera*, L). Worker bees use the wax they produce to make their combs. Bees synthesize wax from the carbohydrates they consume (i.e., honey sugars fructose, glucose, and sucrose). The bees secrete the wax from four pairs of wax glands located on their ventral abdomen. The wax is gathered by the hind limbs and processed by mouth tools prior to being employed to build the honeycomb structure (Bogdanov, 2015).

Beeswax is an extremely complex chemical mixture containing over 300 different substances (Tulluch, 1980). Not all of those substances have been specifically identified to date, as summarized in the table below reproduced from the EFSA report (EFSA, 2007) which was derived from the data of Aichholz and Lorbeer (Aichholz and Lorbeer, 1999):

| Components | *A. mellifera* L. (%) | General structural formulas |
|---|---|---|
| Esters total [m] | 57.4 |  |
| monoesters | 40.8 | |
| hydroxymonoesters | 9.2 | |
| diesters | 7.4 | |
| Hydrocarbons total | 15.7 |  |
| alkanes | 12.8 | |
| alkenes | 2.9 | |
| Free fatty acids total | 18.0 |  |
| Free fatty alcohols total | 0.6 |  |
| Total | 91.7 | |

m: only the structural formula of alkylesters of palmitic acid is shown as example

The 2015 USP Food Chemicals Codex (FCC) states that yellow beeswax is the "purified wax from the honeycomb of *Apis mellifera* L. (Fam. Apidae), and consists primarily of myricyl palmitate (myricin), cerotic acid and ester, and some high-carbon paraffins." Further, they state that "Yellow beeswax is insoluble in water and sparingly soluble in cold alcohol. Boiling alcohol dissolves cerotic acid and part of the myricin." The structure of myricyl palmitate is depicted below:

**Figure 1: Myricyl palmitate (myricin)**

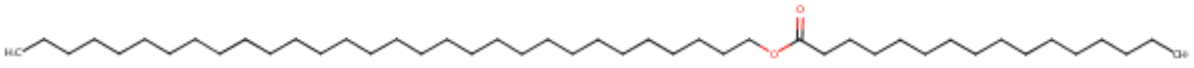
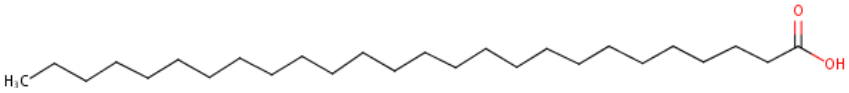


**Figure 2: Cerotic acid**




The FCC notes that yellow wax should have a melting range between 62 and 65°C, an acid value between 18 and 24, and an ester value between 72 and 77.

(b) (4)

Regulatory Status

In the U.S., beeswax (yellow and white) is generally recognized as safe (GRAS) as a direct food substance as per 21 CFR §184.1973. As per this regulation, the wax is refined by melting "in hot water to which sulfuric acid or alkali may be added to extract impurities. The resulting wax is referred to as yellow beeswax. White beeswax is produced by bleaching the constituent pigments of yellow beeswax with peroxides, or preferably it is bleached by sun light." As per the CFR, the ingredient must meet the specifications of the Food Chemicals Codex. Beeswax may be used in food at levels not to exceed good manufacturing practice. The CFR goes on to state the following:

> Current good manufacturing practice results in a maximum level, as served, of: 0.065 percent for chewing gum as defined in §170.3(n)(6) of this chapter; 0.005 percent for confections and frostings as defined in §170.3(n)(9) of this chapter; 0.04 percent for hard candy as defined in §170.3(n)(25) of this chapter; 0.1 percent for soft candy as defined in §170.3(n)(38) of this chapter; and 0.002 percent or less for all other food categories.

Beeswax is used to make the soft gelatin capsules of dietary supplements, as a glazing and coating agent, in chewing gums, and water-based flavored drinks. Dietary intake of beeswax has been estimated by several organizations. According to the World Health Organization (WHO), the average dietary exposure to beeswax per consumer was estimated to be 10 to 100 mg/person/day. The 90$^{th}$ percentile was estimated to be 350 mg/person/day. They conclude that the 95$^{th}$ percentile would be < 650 mg/person per day (WHO, 2006). The European Food Safety Authority estimates that up to 1290 mg/day could be obtained taking into consideration consumption in The Netherlands (EFSA, 2007). Taking into consideration the dietary intake and the amount of beeswax in Xtampza, dietary consumption does not support the safety of Xtampza up to the MTDD of the drug product.

| | **Daily Dose Beeswax via Diet (mg)** | **Dietary Coverage for Xtampza (number of 40 mg oxycodone HCl eq. capsules)** |
|---|---|---|
| Average | 10-100 | 1 or less |

| | | |
|---|---|---|
| 90th percentile | 350 | 4 |
| 95th percentile (WHO) | < 650 | 8 |
| 95th (EFSA, The Netherlands) | 1290 | 16 |

Waxes are composed of similar chemical constituents, such as long-chain alkanes, alcohols, and acids. Hargrove estimates that human diets rich in whole grains and honeycomb as a food staple could contain 1-2 grams of wax constituents per day (Hargrove, et al., 2004); however this is still below the (b) (4) grams per day of beeswax and carnauba wax when Xtampza is used up to the MTDD.

As per the FDA Inactive Ingredients Database (IID), yellow wax has been used in FDA-approved oral drug products with a maximum dosage form of 16.8 mg; however that drug product is only approved for a subchronic indication. As per the IID, there was a drug product approved by FDA in 1962 for a chronic-use indication containing 15.79 mg of yellow wax which, to the best of our knowledge, could result in a maximum of 47.37 mg yellow wax. Likewise, as per the IID, white wax has been used with a maximum potency of 18.36 mg per dosage form for a subchronic indication. There was an FDA approved chronic use oral drug product that contained white wax with a maximum potency of 15.3 mg that was approved in 1960. As this product has been discontinued since 1998, there are no labels available online to determine a total daily dose. (b) (4)

ADME

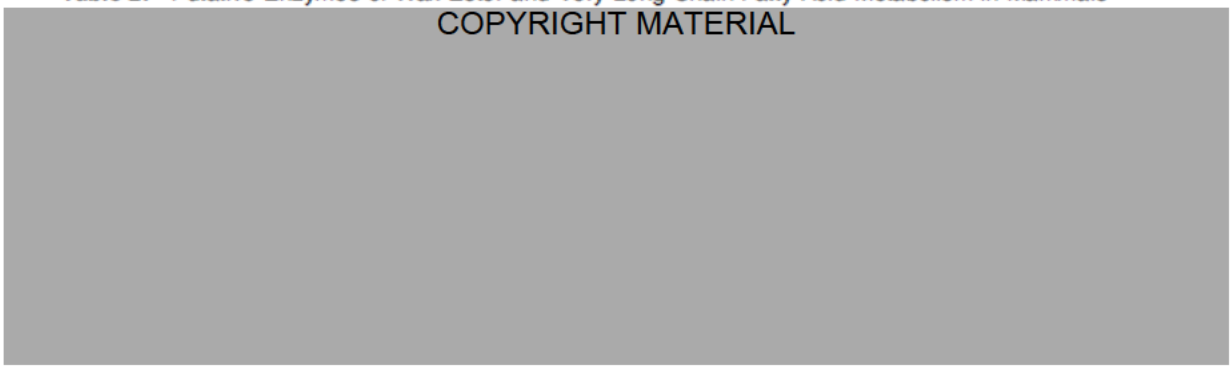
Beeswax has long been assumed to be indigestible for several reasons:

1. Beeswax has a high melting point (62-65°C) and therefore should not melt at body temperature (37°C).
2. Beeswax is insoluble in water and has a hydrophobic surface making it difficult of digestive enzymes to hydrolyze the product.

However, as noted by the WHO, there are no actual data to support this assumption and traditional ADME studies would be "difficult to perform because of its highly complex composition" assuming it is digested and absorbed at all (WHO, 2006). The WHO report does state that "There is evidence that some solubilization of beeswax is mediated by bile acids, at least in some species." This is interesting given the food effect noted in Xtampza (see clinical pharmacology review). Some species are able to digest beeswax. For example, the Greater Wax Moth, *Galleria mellonella* larvae are able to consume some components of beeswax as a nutrient (Opdyke, 1976) and some seabirds have evolved to be able to metabolize beeswax via a bile-dependent, pancreatic esterase (Place, 1992) presumable due to the use of wax esters in their diet of marine organisms.

There are some ADME data for the major constituents of beeswax, and these data suggest that the ester components in beeswax could be hydrolyzed in the intestinal lumen to their corresponding alcohols and acids. This hydrolysis is thought to occur via carboxyl ester hydrolase in the presence of bile salts (Place, 1992;Hargrove, et al., 2004). The resulting alcohols and acids could be absorbed passively via the mucosal epithelia and ultimately either used to make phospholipids or oxidized to the long chain fatty acid and metabolized via β-oxidation (Hargrove, et al., 2004). Studies have also suggested that long chain hydrocarbons can be absorbed by mammals and further metabolized to some extent. The putative enzymes that could be involved are summarized in the table below, reproduced from the Hargrove publication (Hargrove, et al., 2004).

**Table 2.** Putative Enzymes of Wax Ester and Very Long Chain Fatty Acid Metabolism in Mammals[a]




These data suggest that components of the wax could possibly be absorbed to some extent and metabolized, particularly in the presence of bile acids. This also supports the conclusion that chronic toxicity studies could help to characterize the potential impact of the compounds on tissues, particularly the pancreas, liver, intestines, and gall bladder.

Toxicology Data

The EFSA reports states that "Experimental biochemical and toxicological studies on beeswax as such are lacking. However, the Panel considered that the safety of beeswax could be evaluated on the basis of published toxicological studies on its main constituents (EFSA, 2007)."

The only toxicology study with actual beeswax was conducted by the Applicant and submitted during the review cycle. As noted in Dr. Lee's review, this 90-day toxicology study that tested a combination of both beeswax and carnauba wax, did not suggest any clear evidence of toxicity at the highest dose tested. She did note that there were some sporadic findings in the pancreas and salivary glands in the original report that only included analysis of high dose animal and we requested additional information.

Upon full evaluation of these tissues, no clear treatment-related effect was noted. However, this study alone is not long enough to support a chronic indication.

Long-term studies have been conducted with some of the major components in beeswax and have been reported in the published literature. Dr. Lee reviewed these studies in detail. Therefore, I am only summarizing the results and how they inform the safety of this drug product in the table below.

**Table 2: Chronic Toxicology Data for Classes of Compounds in Beeswax**

| **Major Component Classes** | **% in Beeswax (MTDD mg)** | **Representative Compounds** | **Chronic Toxicology Data** | **Reported NOAEL** | **Estimated Safety Margin (on a mg/m² basis)** | **Reference** |
|---|---|---|---|---|---|---|
| Fatty acid esters<br>~(C38-C64) | 57.4%<br>~1713 mg | Carnauba wax | 7-month dog | 1% Diet<br>Dose is unknown as food consumption data not reported<br>Estimated as 18 g food/kg and 10 kg body weight[1] = 1800 mg carnauba wax/10 kg dog = 180 mg/kg | 3.5x | (Parent, et al., 1983a) |
| Long-chain Free Fatty acids<br>~(C20-C36) | 18%<br>~537 mg | D-003 (isolated from sugar cane wax | 6-month rat | 1000 mg/kg | 18x | (Gamez, et al., 2002) |
| | | | 2-year rat | 1500 mg/kg | 27x | (Gamez, et al., 2007) |
| | | | 18-month mouse | 1500 mg/kg | 14x | (Noa, et al., 2009) |
| Hydrocarbons | 15.7% | P70(H) Mineral Oil (Class 1 | 12-month | 1200 mg/kg (lymph node | 25x | (WHO, |

[1] http://www.iacuc.ucsf.edu/Proc/awDogNorm.asp

| | | | | | | |
|---|---|---|---|---|---|---|
| ~(C23-C41) | ~469 mg | Mineral Oil C27) | rat | weight changes at 1200 mg/kg) | | 2002)<br><br>ADI 0-10 mg/kg |
| | | | 24-month rat | < 60 mg/kg (lymph node & hepatic changes at all doses; tested 60-1200 mg/kg) | | (WHO, 2002)<br><br>ADI 0-10 mg/kg |
| | | P100(H) Mineral Oil (Class 1 Mineral Oil C29) | 12-month rat | 1200 mg/kg | 25x | (WHO, 2002) |
| | | | 24-month rat | < 60 mg/kg (lymph node & hepatic changes at all doses; tested 60-1200 mg/kg) | | (WHO, 2002) |
| | | N70(H) Mineral Oil (Class 2 Mineral Oil C23) | 90-day Rat | Focal histiocytosis liver and lymph nodes, accumulation in tissues | | (WHO, 2012) |
| | | P15(H) Mineral Oil (Class 3 Mineral Oil C17) | 90-day Rat | Focal histiocytosis, liver and lymph nodes, accumulation in tissues | | (WHO, 2012) |
| Long-chain Free Fatty Acid alcohols<br><br>~(C28-C35) | 0.6%<br><br>~18 mg | D-002 (isolated from beeswax) | 12-month rat | 1000 mg/kg | 538x | (Rodeiro, et al., 1998) |
| | | | 12-month dog | 250 mg/kg | 1500x | (Aleman, et al., 2001) |
| | | Policosanol (isolated from | 12-month rat | 50 mg/kg or | 27x or | (Aleman, et al., |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | sugar cane wax) | | 500 mg/kg? | 269x | 1994b) |
| | | | 12-month dog | 180 mg/kg? | 1080x | (Mesa, et al., 1994) |
| | | | 2-year rat | 500 mg/kg | 269x | (Aleman, et al., 1994a) |
| | | | 18-month mouse | 500 mg/kg | 136x | (Aleman, et al., 1995) |
| Total | 91.7% | | | | | |

As noted in the table above, although there are apparent safety margins for representative compounds in the class of compounds in beeswax in the published studies, not all standard studies have been completed, there are limited chronic data for the main class of fatty acid esters, and the data for hydrocarbons suggests the possibility for adverse effects on mesenteric lymph nodes and liver, assuming the lower molecular weight hydrocarbons are actually absorbed. As such, chronic toxicology studies with the material tested should be completed. As there is an overlap in the components of beeswax and carnauba wax, the study should include both waxes.

As noted by Dr. Lee, there is also a paucity of data on reproductive and developmental effects of major components of beeswax, as summarized by the table below.

**Table 3: Reproductive and Developmental Data for Classes of Compounds in Beeswax**

| **Major Component Classes** | **% in Beeswax (MTDD mg)** | **Representative Compounds** | **Reproductive and Developmental Toxicology Data** | **Reported NOAEL** | **Estimated Safety Margin (on a mg/m$^2$ basis)** | **Reference** |
|---|---|---|---|---|---|---|
| Fatty acid esters | 57.4% ~1713 mg | | None | | | |
| Long-chain Free Fatty acids | 18% ~537 mg | D-003 (isolated from sugar cane wax) | Rat Fertility and Early Embryonic Development & Embryo-fetal Development & | 1000 mg/kg | 18x | (Rodriguez, et al., 2004) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Pre- and Postnatal Development | | | |
| | | | Rat Embryo-fetal Development | 1000 mg/kg | 18x | (Rodriguez, et al., 2003) |
| | | | Rat Pre- and Postnatal Development | 1000 mg/kg | 18x | (Rodriguez, et al., 2006) |
| | | | Rabbit Embryo-fetal Development | 1000 mg/kg | 36x | (Rodriguez, et al., 2004) |
| Hydrocarbons | 15.7%<br>~469 mg | | None | | | |
| Long-chain Free Fatty Acid alcohols | 0.6%<br>~18 mg | D-002 (isolated from beeswax) | Rat Embryo-fetal Development | 1000 mg/kg | 538x | (Rodriguez, et al., 1998) |
| | | | Rabbit Embryo-fetal Development | 1000 mg/kg | 1075x | (Rodriguez, et al., 1998) |
| | | Policosanol (isolated from sugar cane wax) | Rat Fertility and Early Embryonic Development & Embryo-fetal Development & Pre- and Postnatal Development | 500 mg/kg | 269x | (Rodriguez and Garcia, 1994) |
| | | | Rat Embryo-fetal Development | 500 mg/kg | 269x | (Rodriguez and Garcia, 1994) |
| | | | Rat Pre- and Postnatal Development | 500 mg/kg | 269x | (Rodriguez and Garcia, 1998) |
| | | | Rat Multigenerational Study | 500 mg/kg | 269x | (Rodriguez, et al., 1997) |
| | | | Rabbit Embryo-fetal | 1000 | 1075x | (Rodriguez and Garcia, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Development | mg/kg | | 1994) |

Theoretical Purity Concerns

As stated previously, beeswax is a very complex chemical mixture. The beeswax used in Xtampza is obtained from (b) (4) [redacted] No data are provided to confirm this.

Bogdanov summarizes the possible source of contaminants in bee products (e.g., honey, propolis, and wax), and classifies them as either environmental or apicultural in origin. That is, contaminants may arise from the environment the bees live in or may be introduced by the beekeepers as they maintain their hives. He summarizes these in the figure below, reproduced from his publication (Bogdanov, 2005).

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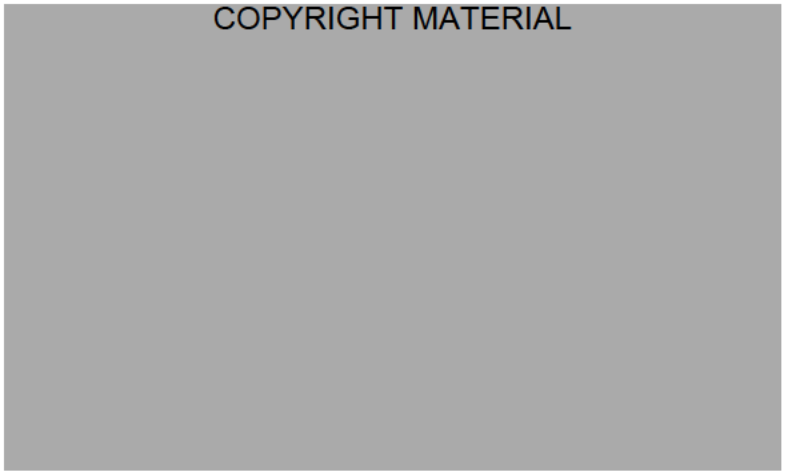

**Figure 1.** The contamination sources for the bee colony. GMO: genetically modified organisms; AFB: american foul brood; EFB: european foulbrood, SHB: small hive beetle.

(b) (4)

As such is not clear that this method of regulation and testing will be fully adequate. As noted by Bogdanov, fungicides, acaricides, antibiotics, and even leachables from wood protectants and paints used to maintain the hive could theoretically be present in bee products.

There are apparently no specific wax specifications to test for contaminants and Bogdanov recommends that since beeswax is regarded as a food additive, the same maximum residue limits (MRLs) used for honey could be applied to wax used for pharmaceuticals (Bogdanov, 2015).

The Applicant states that they are only using (b) (4) material supplied by (b) (4) and that the supplier tests the wax (b) (4), however, these methods do not specifically examine the product for residual (b) (4) contaminants. The Applicant also states (b) (4) that Collegium tested three lots of the material (b) (4) The report (b) (4) as noted in the table below.

**Figure 3: (b) (4) Impurity Test Results for Yellow Beeswax (b) (4)**

(b) (4)

However, there is no specification listed in the drug product to account for all possible sources (b) (4) in the drug product and there is indication that there will be routine testing in the drug product or the sourced materials.

**Carnauba Wax (CAS 8015-86-9)**

At the MTDD of Xtampza, a person would consume up to (b) (4) mg of carnauba wax per day.

Carnauba wax is derived from the fronds of the Brazilian wax palm, *Copernicia cerifera,* Martius. Carnauba wax is also a complex chemical mixture of aliphatic esters (wax esters), $\alpha$-hydroxyl esters, and cinnamic aliphatic diesters. It also contains free acids, free alcohols, hydrocarbons, and resins (JECFA, 1998). Carnauba wax is considered insoluble in water and has a melting range of between 80-86°C. (b) (4)

As noted in Dr. Lee's review, the actual chemical composition can vary significantly in content. In Europe, the general composition is summarized in the table below:

**Table 4: General Content of Carnauba Wax (EFSA, 2012)**

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In the U.S., the 2015 USP Food Chemical Codex only requires specific tests for acid value (fats and related substances) of between 2 and 7, an ester value between 71 and 88, a saponification value between 78 and 95, and an unsaponifiable matter value of between 50.0% and 55.0%.

Regulatory Status

In the U.S., carnauba wax is classified as GRAS as a general purpose food additive and a direct food substance in food products with no limitation other than good manufacturing practice (21 CFR §582.1978 and §184.1978). As per §184.1978 carnauba wax is marketed in five grades designated 1-5 with Grades 4 and 5 representing "the bulk of commercial trade volume. These commercial grades consist chiefly of C24 to C32 normal saturated monofunctional fatty acids and normal saturated monofunctional primary alcohols." The product must comply with the specifications outlined in the Food Chemicals Codex.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the use in foods in 1992 and concluded that an acceptable daily intake was 0-7 mg/kg (420 mg/day for a 60 kg person). (b) (4) This is also illustrated by the estimated consumption of carnauba wax in Europe, as noted in the table below:

| | **Toddlers** (12-35 months) | **Children** (3-9 years) | **Adolescents** (10-17 years) | **Adults** (>18 years) | **Elderly (> 65 years)** |
|---|---|---|---|---|---|
| | mg/kg bw/day | mg/kg bw/day | mg/kg bw/day | mg/kg bw/day | mg/kg bw/day |
| **Estimated exposure using MPL** | | | | | |
| • Mean exposure | 2.6-4.6 | 1.6-4.5 | 0.9-2.1 | 0.7-1.7 | 0.8-1.5 |
| • Exposure 95th | 3.1-8.1 | 3.2-7.6 | 1.9-3.8 | 1.5-3.0 | 1.9-2.7 |

At the upper limit of the 95% in adults, a dose of 3 mg/kg equals only 180 mg per day for a 60 kg person.

As noted in Dr. Lee's review, the FDA has approved an oral drug product with a clinical use consistent with chronic human use that would result in a maximum daily intake of up to (b) (4) mg carnauba wax. As such, there is an FDA previous finding of safety for this level of carnauba wax.

ADME Data

We have not found any publically available data regarding the absorption, distribution, metabolism, and elimination of carnauba wax. This is likely do to the complexity of the chemical mixture, the lack of water solubility of the wax, and the high melting point. As such, it is commonly believed that carnauba wax is not absorbed to any appreciable level (EFSA, 2012); however, actual absorption and metabolism data do not appear to exist to support this belief. The EFSA notes that "The major constituents of carnauba wax are esters of acids and alcohols with average chain lengths of C26 to C32" and "Generally intact long-chain fatty acid esters are poorly absorbed. However, the alcohols and acids generated from the hydrolysis of carnauba wax constituents in the intestinal tract can be absorbed and incorporated into normal cellular metabolic

pathways." Therefore, although absorption is not predicted to be high, they cannot exclude the possibility that components or metabolic degradants of the wax could be absorbed.

Toxicology Data

The Applicant cited several older published toxicology studies with carnauba wax that were not conducted in accordance with GLPs. These included a 90-day rat study (Rowland, et al., 1982), a 28-week dog study (Parent, et al., 1983a), and a subchronic and reproductive and developmental toxicology study in the rat (Parent, et al., 1983b) that included fertility and early embryonic development endpoints, some limited embryo-fetal development assessments, and some pre- and post-natal development assessments (no behavioral endpoints in F1 generation). Given the endpoints in the study, one could consider this study an attempt at fertility and pre- and post-natal development in the rat model. As only gross necropsy and histopathology were completed for the F1 generation after 13 weeks, it is possible that overt signs of teratogenicity would have been detected, but without skeletal evaluations Dr. Lee did not feel that this could be considered an adequate embryo-fetal study.

| **Study** | **NOAEL** | **HED (mg/60 kg, bsa)** | **Safety Margin at MTDD*** | **Reference** |
|---|---|---|---|---|
| 90-Day Rat Dietary General Toxicology | 10%<br>Males 8.8 g/kg<br>Females 10.2 g/kg | <br>85161 mg<br>98710 mg | <br>28.5x<br>33x | (Rowland, et al., 1982) |
| 28-Week Dog Dietary General Toxicology | 1%<br>Dose is unknown as food consumption data not reported<br>Estimated as 18 g food/kg and 10 kg body weight[2] = 1800 mg carnauba wax/10 kg dog = 180 mg/kg | 6000 mg | 2x | (Parent, et al., 1983a) |
| 4-week, Gestational, and 13-week Rat Dietary | 1%<br>Males 0.81 g/kg | 7839 | 2.6x | (Parent, et al., 1983b) |

[2] http://www.iacuc.ucsf.edu/Proc/awDogNorm.asp

| | | | | |
|---|---|---|---|---|
| Reproduction and General Toxicology | Females 0.67 g/kg | 6484 | 2.2x | |

*Based on MTDD of (b) (4) mg/60 kg person

Collectively, these data do suggest adequate safety margins for the MTDD of carnauba wax for the endpoints examined.

However, as Dr. Lee's review notes, chronic rodent toxicology, true embryo-fetal development in two species, and carcinogenicity studies in two species have not been completed for carnauba wax.

Inorganic Impurities

According to the Applicant, as per the compendial specifications, carnauba wax includes a (b) (4) test with a limit of NMT (b) (4) ppm. (b) (4) The Applicant has tested three lots of carnauba wax (b) (4) as summarized in the table below:

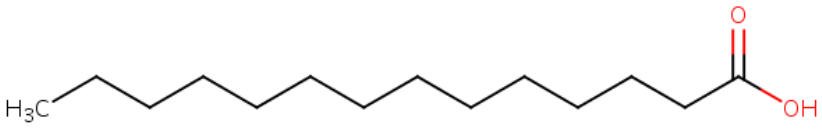
**Figure 4: (b) (4) Impurity Test Results for Carnauba Wax (b) (4)**

(b) (4)

However, the final drug product is not tested and there are no specifications (b) (4) in the drug product formulation.

**Myristic Acid (CAS 544-63-8)**

Myristic acid is a saturated 14-carbon fatty acid that is found in animal and vegetable fats with the following chemical structure.




At the MTDD of Xtampza, a person would consume up to (b) (4) mg of myristic acid ( (b) (4) g/day) via this drug product.

Regulatory Status

Myristic acid is a multipurpose food additive permitted for direct addition to food for human consumption as per 21 CFR §172.860. There is no limit designated in the CFR other than its use in accordance with good manufacturing practice.

As noted in Dr. Lee's review, the estimated daily consumption of myristic acid in food has changed over the years as diets have attempted to decrease saturated fat consumption. For example, in a food consumption study in the 1960s, a U.S. cohort of men averaged 5.8 g/day and a cohort of men in Eastern Finland averaged 14 g/day. In contrast, a 2011 to 2012 survey concluded that the mean intake in the U.S. was estimated at 2.22 g/day. Obviously the total daily intake of myristic acid via Xtampza (up to (b) (4) g/day) would also be added to the normal dietary intake.

Myristic acid was found in one FDA-approved generic oral drug product (currently discontinued). The Inactive Ingredient Database (IID) does not list a maximum potency and the labeling is not available to determine the duration of the indication.

ADME

Myristic acid is absorbed across the gastrointestinal tract and are either stored as triglycerides or catabolized via ω-, α-, or β-oxidation and the tricarboxylic acid pathways.

Toxicology

As noted in Dr. Lee's review, we are not aware of any toxicology studies for free myristic acid in the public domain. According to a JECFA review, an 18-week dietary study in male rats testing lauric acid (a 12-carbon saturated fatty acid) suggest a NOAELs of >6 g/day and a 150-day dietary study in rats testing palmitic acid (a 16-carbon saturated fatty acid) suggests a NOAEL of >5 g/kg/day, suggesting that a subchronic toxicology study with myristic would likely also have a NOAEL of > 5 g/kg/day (Burdock and

Carabin, 2007). This would correlate to a human equivalent dose of >48.3 g for a 60 kg person, based on body weight comparisons. These studies are not available for review.

According to the 2015 USP Food Chemical Codex, the specification (b) (4) in myristic acid is NMT (b) (4) mg/kg. At the MTDD of myristic acid via use of this product a person could consume up to (b) (4) mcg/day (b) (4) via the myristic acid alone.

Human Observations

As noted by Burdock and Carabin, "Excessive intake of saturated fats (of which myristic acid is a key component) in humans is generally considered to be a driver of elevated blood triglyceride and cholesterol levels and, therefore, is thought by many – but not all – clinicians to be a risk factor for coronary heart disease (CHD), at least in the United States." They go on to state that "Of particular relevant to this monograph, however, is the exceptional recognition provided myristic acid. In the reported analysis, it showed a clear positive correlation with CHD mortality. Moreover, in discussion of this fact, it is noted to be the single fatty acid most responsible for induction of hypercholesterolemia, not only repressing hepatic low density lipoprotein (LDL) receptor synthesis, but also directly stimulating hepatic LDL synthesis (Bellizzi and coworkers, 1994)" (Burdock and Carabin, 2007).

Given the possibility that components in waxes, such as D-002, D-003, and policosanol have been purported to reduce cholesterol levels, a toxicology study testing the mixture of beeswax, carnauba wax, and myristic acid would seem to be the most clinically relevant study to be completed for this drug product. This could also minimize the number of animals used to support this drug product formulation up to the MTDD of oxycodone. However, caution should be noted that the mixture may have differential cholesterol and triglyceride effects than testing of the individual products alone. Further, the mixture with the waxes could prevent the systemic absorption of myristic acid, which has implications with respect to the excipient being listed in the IID and reference to the IID as sole justification for the safety of such high levels of the free fatty acid. Nonetheless, given the lack of adequate data on such high levels of free fatty acids, I agree that further studies of the combination would address these outstanding questions and could be used to support the safety of this drug product when used up to the MTDD.

Inorganic Impurities

According to the Applicant, myristic acid has a (b) (4) limit of NMT (b) (4) ppm as per compendial specifications. Collegium has tested six lots of myristic acid used in the manufacturing of the drug product (b) (4) The results are presented below:

**Figure 5: (b) (4) Impurity Test Results for Myristic Acid (b) (4)**


However, the final drug product is not tested and there are no specifications (b) (4) in the drug product formulation.

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RICHARD D MELLON
09/29/2015

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

# PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | 208090 |
| Supporting document/s: | 1, 11, 16, 18,19, 23, 28, 30 |
| Applicant's letter date: | December 12, 2014, March 11, 2015, April 6, 2015, April 24, 2015, May 1, 2015, June 16, 2015, August 7, 2015, August 10, 2015 |
| CDER stamp date: | December 12, 2014, March 11, 2015, April 6, 2015, May 1, 2015, June 16, 2015, August 7, 2015, August 10, 2015 |
| Product: | Xtampza ER |
| Indication: | Management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate |
| Applicant: | Collegium Pharmaceutical, Inc.<br>780 Dedham Street Suite 800<br>Canton, MA 02021 |
| Review Division: | Anesthesia, Analgesia, and Addiction Products |
| Reviewer: | Grace S. Lee, PhD, DABT |
| Supervisor/Team Leader: | R. Daniel Mellon, PhD |
| Division Director: | Sharon Hertz, MD |
| Project Manager: | Ayanna Augustus, PhD, RAC |

*Template Version: September 1, 2010*

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Any information or data necessary for approval of NDA 208090 that Collegium Pharmaceutical, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 208090.

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# 1 Executive Summary

## 1.1 Introduction

Collegium Pharmaceutical, Inc. (Collegium) has submitted NDA 208090 for Xtampza ER, an extended-release (ER) microsphere-in-capsule oxycodone formulation containing excipients that are intended to confer abuse-deterrent properties. Xtampza ER is formulated in equivalent strengths of 10, 15, 20, 30, and 40 mg oxycodone HCl per capsule and is intended for twice-daily (BID) dosing. The indication for this product is management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatment options are inadequate. This NDA is a 505(b)(2) application and is relying on the Agency's findings of safety and the description of the pharmacology and toxicology of oxycodone in the label of OxyContin® (NDA 022272).

## 1.2 Brief Discussion of Nonclinical Findings

All impurities in the drug substance and drug product are controlled at acceptable levels. Xtampza ER contains excipients that are intended to confer abuse-deterrent properties. The microspheres contain oxycodone (b) (4) When calculated for the maximum theoretical daily dose (MTDD) of oxycodone HCl (1.5 g/day; or (b) (4) g/day for oxycodone base), the levels of myristic acid, beeswax, carnauba wax, and hypromellose (b) (4) To support the safety of the levels of these excipients in this product, the Applicant has submitted the literature-based safety assessments of four excipients along with a 90-day toxicity study of a beeswax/carnauba wax (b) (4) administered by oral capsules in dogs.

The literature indicates that orally administered hypromellose passes through the gastrointestinal tract largely unabsorbed and unchanged, behaving as non-nutritive fiber, and thus the level of hypromellose in Xtampza ER at MTDD does not pose a safety concern. However, the Applicant has submitted inadequate information to justify the safety of myristic acid, beeswax, and carnauba wax when the drug product is used up the maximum theoretical daily dose. (b) (4) Myristic acid is a saturated fatty acid (SFA) with cholesterol-raising effects. When myristic acid and some other SFAs are consumed from food, the harmful effects of SFAs are counterbalanced with beneficial effects of other nutrients (such unsaturated fatty acids) from food. However, when the large amount of myristic acid is consumed from Xtampza ER products at the MTDD, it is unclear that myristic acid would not pose any safety concern. Beeswax and carnauba wax are natural products and complex chemical mixtures. The Applicant's safety justification is largely based on published data on some of the major components of beeswax, but these data do not characterize

a significant fraction of the chemicals in beeswax, at least 8% of which are not even identified. Moreover, the published toxicology studies do not provide complete safety assessment of carnauba wax and all major components of beeswax because there are major gaps in characterizing toxicity profiles of these substances in the literature. As such, the Applicant's safety assessment of myristic acid, beeswax, and carnauba wax is not adequate.

For these novel excipients, estimated daily intake from food consumption or an acceptable daily intake (established by USDA or JECFA) can be used to establish the level that would be safe to use in the Xtampza product. The daily intake of myristic acid from food consumption in the U.S. is estimated to be up to 2.2 g/day, JECFA's estimated dietary exposure to beeswax is <650 mg/day, and JECFA's established ADI is 0-7 mg/kg/day or 420 mg/day for a 60 kg person. Based on review of FDA-approved drug products, the Division can confirm that there is an FDA-approved oral drug with an indication consistent with chronic human use with a maximum daily intake/consumption of (b) (4) mg/day carnauba wax. Thus, 8 capsules of 40 mg Xtampza ER ( (b) (4) mg of myristic acid, (b) (4) mg of beeswax, and (b) (4) mg of carnauba wax) do not pose a safety concern based upon previous human experience in the U.S.

However, if the benefits of the Xtampza ER product outweigh the risks, the following points are worth taking into consideration regarding the safety assessment of these novel excipients:

1) It is unclear whether exposure to isolated myristic acid of (b) (4) g/day from the Xtampza ER product at the MTDD (without counterbalancing with beneficial fatty acids) poses a safety concern due to its potential cholesterol-raising effects. If the dose is limited to 8 pills a day, the total daily dose of myristic acid via the Xtampza ER product is (b) (4) g/day, which is less than the estimated mean intake of 2.2 g/day in the U.S. diet. If the dose is limited to 16 pills a day, the total daily dose of myristic acid via the Xtampza ER product is (b) (4) g/day, which is (b) (4) the estimated mean intake of 2.2 g/day in the U.S. diet. Consumption of the product at high doses would be additive to the impact of a person's diet.

2) Although the JECFA's estimated dietary exposure to beeswax is <650 mg/day, the EU Scientific Committee on Food (SCF)'s estimated daily intake of beeswax is up to 1290 mg/day in the Netherlands. Note that the main issue of beeswax is incomplete characterization of toxicity profile of beeswax due to lack of required toxicity data, but the 90-day dog study using a beeswax/carnauba wax (b) (4) does not identify any specific toxicity and available published toxicity data of components of beeswax (specifically hydrocarbons) suggest the potential for infiltrating cell histiocytosis in the mesenteric lymph nodes and cystic degeneration or angiectasis in the liver. These data suggest that some component(s) of the beeswax are likely absorbed systemically.

3) Although ADME data of beeswax or carnauba wax do not exist, it is widely assumed, but unproven, that absorption of components of these waxes from the gastrointestinal tract would be limited.

Thus, if the benefits outweigh the risk, (b) (4) capsules of 40 mg Xtampza ER ( (b) (4) mg of myristic acid, (b) (4) mg of beeswax, and (b) (4) mg of carnauba wax[1]) can be considered for initial approval with postmarketing requirements of all nonclinical studies that are listed below to resolve deficiencies.

The active ingredient oxycodone has been used in the FDA-approved referenced drug product Oxycontin®, and its mechanism of action, pharmacodynamics, and toxicity profiles have been well-characterized.

## 1.3 Recommendations

### 1.3.1 Approvability

From a nonclinical pharmacology toxicology perspective, there are inadequate information to support the safety of three novel excipients (myristic acid, beeswax, and carnauba wax) when the drug product is administered up to the maximum theoretical daily dose of oxycodone. A complete response is recommended.

Deficiency

You have not provided adequate safety justification for three of the excipients in your drug product formulation, specifically myristic acid, beeswax, and carnauba wax when your drug product is administered up to the maximum theoretical daily dose of the drug product. Specifically, your literature-based safety justification does not address significant fractions of the wax components and you have not provided data to show that no components of the waxes are absorbed from the gastrointestinal tract. Finally, the impact of the maximum theoretical daily dose of myristic acid in your drug product has not been adequately characterized.

Information Needed to Resolve Deficiencies:

Provide adequate safety assessment of myristic acid, beeswax, and carnauba wax by conducting the following toxicity studies using (b) (4) myristic acid, beeswax, and carnauba wax:

(1) Chronic general toxicity studies, including a 9-month study in dogs and a 6-study in rats

(2) Reproductive and developmental toxicity studies, including fertility and early embryonic developmental toxicity study in rats, embryo-fetal developmental

[1] Given the previous finding of safety of (b) (4) mg/day of carnauba wax, a dose of (b) (4) mg of carnauba wax is also reasonably justified to support 16 pills per day of this formulation.

toxicity studies in rats and rabbits, and pre- and postnatal developmental toxicity study in rats

(3) Carcinogenicity studies in rats and mice, or submit documentation providing scientific justification that carcinogenicity data are not necessary as discussed in the FDA guidance document titled: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf.

Batches of beeswax and carnauba wax used in nonclinical toxicity studies should be the same or representative batches used in the drug product to be marketed.

### 1.3.2 Additional Non Clinical Recommendations

None

### 1.3.3 Labeling

The table below contains the draft labeling submitted by the Applicant, the changes proposed by the Reviewer, and the rationale and comments for the proposed changes. The recommended changes from the proposed labeling are in red (additions) or strikeout font.

**Table 1 Proposed Label for Xtampza ER**

| Applicant's proposed labeling | Reviewer's proposed changes | Rationale for changes |
|---|---|---|
| 8 USE IN SPECIFIC POPULATIONS | 8 USE IN SPECIFIC POPULATIONS | |
| 8.1 Pregnancy<br><br>*Risk Summary* | 8.1 Pregnancy<br><br>*Risk Summary* | |
| (b) (4) | | Note that for animal doses expressed in terms of |

| | | |
|---|---|---|
| (b) (4) | | human dose, an adult human dose of 160 mg/day is used in the Applicant's proposed labeling to be consistent with labeling of the reference drug OxyContin®.<br><br>Neurobehavioral findings in two published papers (Davis et al., 2010 and Sithisarn et al., 2013) are included. |
| | In the U.S. general population, the estimated background risk of major | |

| | | |
|---|---|---|
| | birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.<br><br><u>Data</u><br><br><u>*Animal Data*</u><br><br>Studies with oral doses of oxycodone hydrochloride in rats up to 8 mg/kg/day and rabbits up to 125 mg/kg/day, equivalent to 0.5 and 15 times an adult human dose of 160 mg/day, respectively on a $mg/m^2$ basis, did not reveal evidence of harm to the fetus due to oxycodone. In a pre- and postnatal toxicity study, female rats received oxycodone during gestation and lactation. There were no drug-related effects on reproductive performance in these females or any long-term developmental or reproductive effects in pups born to these rats. Decreased body weight was found during lactation and the early post-weaning phase in pups nursed by mothers given the highest dose used (6 mg/kg/day, equivalent to approximately 0.4-times an adult human dose of 160 mg/day, on a $mg/m^2$ basis). However, body weight of these pups recovered. In (b) (4) published studies, offspring of pregnant rats administered oxycodone | |

| | | |
|---|---|---|
| | during gestation have been reported to exhibit (b) (4) [redacted] | |
| **13 NONCLINICAL TOXICOLOGY** | **13 NONCLINICAL TOXICOLOGY** | |
| 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility<br><br>*Carcinogenesis*<br>(b) (4) [redacted]<br><br>*Mutagenesis*<br><br>Oxycodone was genotoxic in the mouse lymphoma (b) (4) at concentrations of 50 mcg/mL or greater with metabolic activation and at 400 mcg/mL or greater without metabolic activation. Clastogenicity was observed with oxycodone in the presence of metabolic activation in one chromosomal aberration assay in human lymphocytes at concentrations greater than or equal to 1250 mcg/mL at 24 but not 48 hours of exposure. In a second chromosomal aberration assay with human lymphocytes, no structural clastogenicity was observed either with or without metabolic activation; however, in the | 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility<br><br>*Carcinogenesis*<br>(b) (4) [redacted]<br><br>*Mutagenesis*<br><br>Oxycodone was genotoxic in the mouse lymphoma (b) (4) at concentrations of 50 mcg/mL or greater with metabolic activation and at 400 mcg/mL or greater without metabolic activation. Clastogenicity was observed with oxycodone in the presence of metabolic activation in one chromosomal aberration assay in human lymphocytes at concentrations greater than or equal to 1250 mcg/mL at 24 but not 48 hours of exposure. In a second chromosomal aberration assay with human lymphocytes, no structural clastogenicity was observed either with or without metabolic activation; however, in the | A few minor edits are made. |

| | | |
|---|---|---|
| absence of metabolic activation, oxycodone increased numerical chromosomal aberrations (polyploidy). Oxycodone was not genotoxic in the following assays: Ames S. typhimurium and E. coli test with and without metabolic activation at concentrations up to 5000 (b) (4)/plate; chromosomal aberration test in human lymphocytes (in the absence of metabolic activation) at concentrations up to 1500 (b) (4)/mL, and with activation after 48 hours of exposure at concentrations up to 5000 (b) (4)/mL; and in the in vivo bone marrow micronucleus assay in mice (at plasma levels up to 48 (b) (4)/mL).<br><br>*Impairment of Fertility*<br><br>In a study of reproductive performance, rats were administered a once daily gavage dose of the vehicle or oxycodone hydrochloride (0.5, 2, and 8 mg/kg). Male rats were dosed for 28 days before cohabitation with females, during the cohabitation and until necropsy (2-3 weeks post-cohabitation). Females were dosed for 14 days before cohabitation with males, during cohabitation and up to (b) (4) 6. Oxycodone hydrochloride did not affect reproductive function in male or female | absence of metabolic activation, oxycodone increased numerical chromosomal aberrations (polyploidy). Oxycodone was not genotoxic in the following assays: Ames S. typhimurium and E. coli test with and without metabolic activation at concentrations up to 5000 (b) (4) mcg/plate; chromosomal aberration test in human lymphocytes (in the absence of metabolic activation) at concentrations up to 1500 (b) (4) mcg /mL, and with activation after 48 hours of exposure at concentrations up to 5000 (b) (4) mcg /mL; and in the in vivo bone marrow micronucleus assay in mice (at plasma levels up to 48 (b) (4) mcg /mL).<br><br>*Impairment of Fertility*<br><br>In a study of reproductive performance, rats were administered a once daily gavage dose of the vehicle or oxycodone hydrochloride (0.5, 2, and 8 mg/kg). Male rats were dosed for 28 days before cohabitation with females, during the cohabitation and until necropsy (2-3 weeks post-cohabitation). Females were dosed for 14 days before cohabitation with males, during cohabitation and up to (b) (4) Gestation Day 6. Oxycodone hydrochloride did not affect reproductive | |

| | | |
|---|---|---|
| rats at any dose tested (≤ 8 mg/kg/day). | function in male or female rats at any dose tested (≤ 8 mg/kg/day).<br><br>13.2 Animal Toxicology | If Xtampza ER is ultimately approved with dosing limitations based the maximum daily use of 8-(b) (4) capsules, consideration should be given to including a statement regarding the lack of adequate chronic toxicology studies to support higher doses. This is appropriate for Section 13.2 as per the CFR. |

# 2 Drug Information

## 2.1 Drug

**CAS Registry Number:** 76-42-6

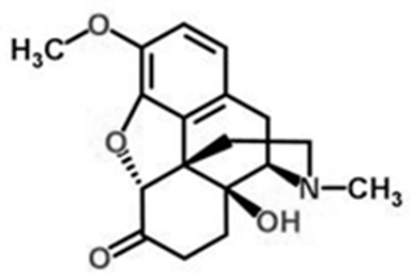
**Generic Name:** Oxycodone

**Code Name:** (b) (4)

**Chemical Name:** 4,5α-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one

**Molecular Formula/Molecular Weight:** $C_{18}H_{21}NO_4$/ 315.57 g/mol

**Structure or Biochemical Description:**




**Pharmacologic Class:** Opioid agonist (FDA Established Pharmacologic Class)

## 2.2 Relevant INDs, NDAs, BLAs, and DMFs

| IND/NDA/MF | Drug/Compound | Sponsor/Applicant | Division/Office | Status |
|---|---|---|---|---|
| IND 75786 | Oxycodone DETERx sustained | Collegium | DAAAP | Active |
| NDA 022272 | OxyContin® (reference drug) | Purdue Pharma L.P. | DAAAP | Approved |
| MF (b) (4) | (b) (4) | | ONDQA | Adequate |
| MF (b) (4) | | | ONDQA | Adequate |

## 2.3 Drug Formulation

Xtampza ER (Oxycodone DETERx®) is an abuse-deterrent, extended-release (ER) capsule oral formulation, which contains microspheres with a median particle size of approximately (b) (4) microns. The microspheres contain oxycodone bas (b) (4) Xtampza ER capsules are manufactured in 5 capsule strengths containing the equivalent of 10, 15, 20, 30, and 40 mg oxycodone HCl (expressed in terms of oxycodone base, 9, 13.5, 18, 27, and 36 mg, respectively). In this review, the dosage strengths are referred to as HCl equivalent strengths as the Applicant referred it in the NDA submission.

**Table 2 Composition of the drug product [taken directly from 3.2.P.1 Description and Composition of the Drug Product, p.5]**

| Component | Reference to Quality Standard | Function | Dose Strength (Oxycodone Hydrochloride Equivalent) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 40 mg | 30 mg | 20 mg | 15 mg | 10 mg |
| | | | Quantity per Capsule (mg) | | | | |
| | | | | | | | (b) (4) |
| Oxycodone Base | In-house standard | Drug substance | | | | | |
| | | | | | | | (b) (4) |
| Myristic Acid | FG/NF | | | | | | |
| Yellow Beeswax | NF | | | | | | |
| Carnauba Wax | NF | | | | | | |
| Stearoyl Polyoxyl-32 Glycerides | NF | | | | | | |
| | | | | | | | (b) (4) |
| Magnesium Stearate | NF | | | | | | |
| Colloidal Silicon Dioxide | NF | | | | | | |
| | | | | | | | (b) (4) |
| | | | | | | | (b) (4) |
| Hypromellose Capsule Shell[b] | | | | | | | |
| | | | | | | | (b) (4) |

API = active pharmaceutical ingredient; DMF = Drug Master File; FG = food grade; NF = National Formulary

[a] Equivalent to 40, 30, 20, 15, and 10 mg oxycodone HCl, respectively. During development, capsule strengths are expressed in terms of oxycodone HCl equivalent. Commercial strengths will be expressed in terms of oxycodone base (see proposed label in Module 1.14.1.3).

[b] The qualitative and quantitative composition of the hypromellose capsule shells are provided in Table 2.

[c] The qualitative and quantitative composition for the printing ink is listed in Table 3.

**Table 3 Composition of hard capsule shells [taken directly from 3.2.P.1 Description and Composition of the Drug Product, p.6]**

| Component | Reference to Quality Standard | Function | Dose Strength (Oxycodone Hydrochloride Equivalent)/ Capsule Size | | | | |
|---|---|---|---|---|---|---|---|
| | | | 40 mg/ 00 | 30 mg/ 0 | 20 mg/ 1 | 15 mg/ 2 | 10 mg/ 3 |
| | | | Quantity per Capsule[a] (mg) | | | | |
| **Capsule Cap (color)** | | | Flesh Opaque | Light Grey Opaque | Rich Yellow Opaque | Swedish Orange Opaque | Ivory (b) (4) |
| Titanium Dioxide | USP/NF | (b) (4) | | | | | |
| Yellow Iron Oxide | USP/NF | | | | | | |
| Red Iron Oxide | USP/NF | | | | | | |
| Black Iron Oxide | USP/NF | | | | | | |
| Hypromellose | USP/NF | | | | | | |
| Water | Potable per USP | | | | | | |
| **Capsule Body (color)** | | | White (b) (4) | | | | |
| (b) (4) | | | | | | | |

NF = National Formulary; USP = United States Pharmacopoeia

(b) (4)

Determination of the maximum daily dose of oxycodone

The maximal dosing information for oxycodone is relevant to this review in that the ICH specifications for impurity levels for the drug substance and the drug product as well as the acceptable levels of total amounts of inactive ingredients are based on the total daily dose of the drug substance. Based on clinical use data, DAAAP has determined that the maximum theoretical daily dose (MTDD) of oxycodone HCl for this product in an opioid-tolerant population could be as high as 1.5 g ( (b) (4) g/day for oxycodone base).

## 2.4 Comments on Novel Excipients

The excipients in Xtampza ER capsules, their levels, and the acceptability of the levels are presented in Table 4. (b) (4)

Titanium dioxide and iron oxides (b) (4)

use levels are

justified by the relevant Code of Federal Regulations (CFR) references. Following administration of Xtampza ER (40 mg capsules) at the MTDD, an opioid-tolerant individual would be exposed to (b) (4) mg of titanium dioxide and (b) (4) mg of red iron oxide. Titanium dioxide can be used up to 1% by weight of the food as a color additive as per 21 CFR§73.575, and iron oxides is listed as GRAS, which can be used as an indirect human food ingredient with no limitation other than current GMP as per 21 CFR §186.1374. The Applicant states that the excipients complied with USP/NF.

**Table 4 Excipients in Xtampza ER formulation with their amounts and acceptability of the amounts**

| Excipient | Weight per 40 mg capsule, mg | Total daily intake for 37 capsules, mg | Acceptability, Rationale |
|---|---|---|---|
| Myristic acid | (b) (4) | (b) (4) | No; GRAS with no limits except GMP |
| Yellow beeswax | | | No, GRAS but with GMP limitations; For JECFA, ADI of <650 mg/day |
| Carnauba wax | | | No, Max: 300 mg (IID), GRAS but with GMP limitations; For JECFA, ADI of 420 mg/day |
| Stearoyl polyoxyl-32-glycerides | | | Yes, Max: 480 mg (IID) |
| Magnesium stearate | | | Yes, Max: 256.4 mg (IID) |
| Colloidal silicon dioxide | | | Yes, Max: 100 mg (IID) |
| Hypromellose (b) (4) | | | Yes, Max: 480 mg (IIDG), data to show lack of absorption |

IID: FDA Inactive Ingredients Database

Justification for the safety of the levels of myristic acid, yellow beeswax, carnauba wax, and hypromellose was provided in the NDA submission. Background information and safety justifications on four novel excipients is described in this section, and more detailed information on beeswax and carnauba wax is included in Appendices 1-5. The information presented in this review is mainly from the Applicant's submitted safety assessment on these excipients, along with the Reviewer's literature search information.

**Myristic acid**: Myristic acid [chemically known as n-tetradecanoic acid (14:0)] is a saturated fatty acid (SFA) of 14 carbons without any double bonds between carbon atoms. Myristic acid is found ubiquitously in fats throughout the plant and animal

kingdom. Myristic acid occurs naturally in butter acids (such as nutmeg butter to the extent of 80%), lovage oil, coconut oil, mace oil, cire d'abeille (natural wax) in palm seed fats (20%), and in most animal and vegetable fats, especially in sperm whale oil. Myristic acid is used as a multipurpose food additive, including a flavor adjuvant, defoaming agent, and coating on fresh citrus fruits. Myristic acid is a key endogenous component of human cellular biochemical machinery and adipose tissue, and it is also a key component of a typical, high saturated fat Western diet. Dietary myristic acid intake ranks third among the SFA consumed in the U.S., accounting for approximately 9% of SFA intake and stands next to palmitic acid ([16:0] ~55% of SFA intake) and stearic acid ([18:0] ~25% of SFA intake). As per the evaluation prepared by the Joint FAO/WHO Committee on Food Additives in 1973 (JECFA, 1974), "myristic, palmitic, and stearic acid and their salts are normal products of the metabolism of fats and their metabolic fate is well-established. Provided that the contribution of the cations does not add excessively to the normal body load there is no need to consider the use of these substances in any different light to that of dietary fatty acids."

In a cohort study, groups of 8-49 men from 16 cohorts, aged 40-59 years old, living in the U.S., Finland, the Netherlands, Italy, Greece, the former Yugoslavia, or Japan recorded their food intake in the 1960s. During that time, protein, total fat, and overall fatty acid composition were analyzed, and in 1987, researchers collected all foods from the same regions in which men were living during the baseline survey in the 1960s and in the same season (±2 months) during which the food intakes had been recorded for analysis of food composition. Based on the average food intake, consumption of myristic acid was found to range from 0.8 g/day (Japan) to 14 g/day (East Finland), including 5.8 g/day for the U.S. cohort, 8.0 g/day for the Netherlands cohort, and 11 g/day for the West Finland cohort (de Vries et al. 1971).

A prospective cohort study of 80,082 women in the U.S. (Hu, et al., 1999) showed that individual SFA intakes, except for 12:0, declined over time (1980-1990). The primary contributors to palmitic acid and stearic acid intakes in this study included beef as a main dish, cheese, beef as a sandwich or mixed dish, and hamburgers, whereas other SFAs (4:0–14:0, including myristic acid) were primarily from dairy products. According to the What We Eat in America, The National Health and Nutrition Examination Surveys (NHANES), 2011-2012 Survey, the mean individual myristic acid intake is 2.22 g/day. Estimates from this survey were based on data from 4,801 male and female individuals aged 20 years and over (USDA, 2014).

Pharmaceutically, myristic acid is used in oral and topical formulations. Myristic acid is present in the Inactive Ingredient Database (IID) without the maximum potency specified and is declared GRAS with no limits, except GMP requirements. Administration of Xtampza ER at the MTDD of 1.5 g (i.e., the MTDD for oxycodone for an opioid-tolerant individual) results in an exposure to MA of (b) (4) mg/day, and this

amount of myristic acid has not been previously used in any FDA-approved oral drug product. (b) (4)

Toxicity data specific to myristic acid are scarce. Myristic acid *per se* has not been tested in publicly available subchronic or longer-term animal studies, but related fatty acid analogs are reported by JECFA (1997) to have been tested (information obtained from (Burdock and Carabin, 2007). A NOAEL of >6 g/kg/day was reported in male rats following dietary exposure to lauric acid (12:0) for 18 weeks, and a NOAEL of >5 g/kg/day was reported in rats following dietary exposure to palmitic acid (16:0) for 150 days. A number of reports have documented that dietary fats using different food sources have an influence on the development of mammary tumors (by promotion step) in rats and mice, however, it appears that isolated myristic acid has not been directly tested for carcinogenic potential in animals.

In humans, accumulating evidence has indicated that dietary fat has a potential role in the etiology of cardiovascular disease, certain types of cancers, and perhaps obesity through its contribution to excessive energy intake, and thus, dietary guidelines over the past several decades have proposed a limit in the total intake of dietary fat (Lichtenstein, et al., 1998). However, more recent epidemiologic studies and controlled clinical trials have indicated that the effect of fats depends not only on the quantity, but also on their composition in specific fatty acids. Fatty acids are categorized as being saturated, monounsaturated, or polyunsaturated, and fats contain a mixture of these different kinds of fatty acids (Wang, et al., 2013). Polyunsaturated fatty acids (PUFAs) are fatty acids containing two or more double bonds and can be classified in n-3 fatty acids and n-6 fatty acids. Both types of fatty acids are precursors of signaling molecules with opposing effects, that modulate membrane microdomain composition, receptor signaling and gene expression (Schmitz and Ecker, 2008). In Westernized diet, the predominant dietary PUFAs are n-6 fatty acids. The predominant n-6 fatty acid is arachidonic acid (AA), whereas typical n-3 fatty acids are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are competitive substrates for the enzymes and products of arachidonic acid metabolism. DHA- and EPA-derived eicosanoids antagonize the pro-inflammatory effects of n-6 fatty acids. The number of carbon atoms in biological systems is typically between C14 and C24, with the C16 and C18 fatty acids being the most common in humans.

SFAs with at least 14 carbons are absorbed in the small intestine, packaged in chylomicrons, secreted into the lymph system, and enter the bloodstream via the thoracic duct. However, lauric acid (12:0) or SFA with less carbons are readily absorbed and directly transported to the liver via the portal vein. The formation of a mixed micelle with bile acids is necessary for absorption of lipids (Flock and Kris-Etherton, 2013). Under normal conditions, FA absorption is known to occur mainly in

the jejunum, in well-differentiated enterocytes located in the upper 2/3 of the villi (Buttet, et al., 2014). A high-fat diet/meal may trigger an overflow of dietary fat absorption capacity in the proximal part of intestine and thus trigger the recruitment of the distal part of the intestine for their absorption.

Metabolic studies in humans have established that the type of fat, but not total amount of fat, predicts serum cholesterol levels and different types of fats have different health effects (Hu, et al., 2001). Specifically, SFAs with 12–16 carbon atoms tend to increase plasma total, LDL, and HDL cholesterol levels, whereas intake of SFAs with 4:0–10:0 carbon atoms do not. Moreover, stearic acid (18:0) does not have a cholesterol-raising effect in comparison with oleic acid (18:1) (Hu, et al., 1999). Although it is not always consistent, the literature reports that among the cholesterol-raising SFAs, myristic acid appears to be more potent than lauric acid or palmitic acid. Additionally, results from epidemiologic studies and controlled clinical trials have indicated that replacing saturated fat with unsaturated fat is more effective in lowering risk of coronary heart disease (CHD) than simply reducing total fat consumption. Moreover, prospective cohort studies and secondary prevention trials have provided strong evidence that a higher intake of n-3 PUFAs lowers risk of CHD. These trials indicate that the right types of fatty acids and other components of diet are more important than total amount of fat in reducing coronary risk. Thus, recent national dietary guidelines have shifted the emphasis from total fat reduction to distinguishing different types of fat.

Literature also suggests that dietary fat is possibly involved in the development of several cancers, including breast cancer and colorectal cancer although the literature data are not entirely consistent. Similar to the effects of dietary fat on CHD, the effect of fats in the development of tumors depends not only on the quantity, but also on their composition in specific fatty acids. In the literature, it documents that increased concentrations of SFAs with 12:0–14:0 carbon atoms and arachidonic acid (AA) might be associated with increased risk of colorectal cancer, whereas increased concentrations of SFAs with 2:0–4:0 carbon atoms and eicosanopentaenoic acid (EPA) seem to protect against colorectal cancer (Nkondjock, et al., 2003). SFAs, including lauric and palmitic acids, have been shown to activate pattern recognition receptors, leading to enhanced expression of pro-inflammatory target gene products (Lee, et al., 2010). However, thus far, the data on effect of myristic acid in the development of cancers are very limited.

At this time, research on how specific SFAs contribute to CHD and on the role each specific SFA plays in other health outcomes is not sufficient to make a specific conclusion or global recommendations for all persons to remove saturated fats from their diet (German and Dillard, 2004). However, as mentioned above, the evidence suggests that SFAs with 4:0–10:0 carbon atoms are neutral with respect to cholesterol-increasing properties and their ability to modulate LDL metabolism; lauric, myristic, and

palmitic acids have cholesterol-raising potential, and stearic acid appears to be neutral in its cholesterol-raising potential. Moreover, a limited number of controlled studies suggest that myristic acid is the most potent cholesterolemic dietary SFA.

If administered oxycodone via Xtampza ER up to the MTDD of 1.5 g, opioid-tolerant individuals would be exposed to myristic acid of (b) (4) g/day, and (b) (4) the myristic acid intake of 2.22 g/day from daily food consumption. Although literature indicates the effect of myristic acid is more pronounced, hypercholetesterolemic and some other effects of myristic acid appear to be similar to those of lauric acid and palmitic acid. Based on this information, the Reviewer considers that it is reasonable to compare exposure to myristic acid from Xtampza ER products at the MTDD with combined mean intakes of C12, C14, and C16 SFAs from daily food and beverage consumption (17.31 g/day; see Table 5).

However, it also needs to be considered that some harmful effects of SFAs in dietary fats are counterbalanced by potential beneficial effects of other nutrients such as monounsaturated and polyunsaturated fatty acids. Administration of myristic acid without other beneficial fatty acids would cause more potent adverse effects than when it is taken from food consumption. Therefore, it is still unclear whether exposure to myristic acid of (b) (4) g/day from Xtampza ER products at the MTDD does not pose a safety concern.

**Table 5 Nutrient intakes from food and beverages: mean amounts consumed per individual ages 20 and over, in the U.S., 2011-2012 [Information from USDA, 2014]**

| | Food energy (kcal) | Total fat (g) | Saturated fat (g) | Myristic acid (14:0)(g) | SFA (12:0+ 14:0+16:0) (g) | Mono-unsaturated fat (g) | Poly-unsaturated fat (g) |
|---|---|---|---|---|---|---|---|
| Males | 2567 | 96.2 | 30.9 | 2.54 | 20.24 | 34.8 | 22.7 |
| Females | 1834 | 69.0 | 22.3 | 1.92 | 14.55 | 24.3 | 16.8 |
| Males and females | 2191 | 82.2 | 26.5 | 2.22 | 17.31 | 29.4 | 19.7 |

**Beeswax:** Beeswax [CAS: 8012-89-3] is a refined wax from honey combs. The beeswax is produced in wax glands located in the abdomen of honeybees of the genus Apis. It is a complex mixture of saturated and unsaturated linear and complex monoesters, hydrocarbons, free fatty acids, free fatty alcohols, and other unknown exogenous substances. The general content of beeswax from *A. mellifera* is shown in Table 6.

**Table 6 Composition of *A. mellifera* beeswax [taken from (EFSA, 2007)]**



Beeswax has a long history of usage in cosmetics, medicine, and other related purposes. Modern day food applications of beeswax include its use as a component in dietary food supplements (soft gelatin capsules and tablets), glazing and coating agent, texturizer for chewing-gum base and carrier for food additives (including flavors and colors). A slab of honeycomb is often included in bottles of honey sold in food stores. The "Codex General Standard for Food Additives" (GSFA, 2015), which sets the conditions under which permitted food additives may be used in all foods, has classified beeswax into carrier, emulsifier, glazing agent, stabilizer, and thickener.

The Joint FAO/WHO Expert Committee on Food Additives (in 2005) calculated that the dietary exposure to beeswax would be < 650 mg per person per day (40 mg from coatings + 6.5 mg from gum + 340 mg from tablets + 60 mg from capsules + 200 mg from water-based drinks). The estimate was based on the very conservative assumption that a person would consume all foods (and tablets or capsules) containing beeswax at the highest percentile in each food category and that all those foods contained beeswax (information from (Munro, et al., 2015). Similarly, the EU Scientific Committee on Food (SCF) updated on the safety in use of beeswax and estimated its daily intake based on the assumptions that a person would consume all the proposed foods and tablets or capsules at the 95th percentile and that beeswax would be used in the proposed applications at the maximum usage level (EFSA, 2007). The EU SCF estimated daily intake of beeswax as up to 1290 mg/day (1050 mg from capsules + 40 mg from glazings + 197 mg from soft drinks).

Beeswax is listed as GRAS with GMP limitation and present in the FDA's Inactive Ingredient Database (IID) for oral capsules and tablets, and topical formulations. However, administration of Xtampza ER at the MTDD of 1.5 g results in an exposure to beeswax of (b) (4) mg/day or (b) (4) mg/kg/day for a 60 kg person (b) (4) Although beeswax has a long history of usage in food and other related purposes and widely used, the total daily intakes from these sources are still relatively smal (b) (4)

(b) (4) Thus, beeswax is considered a novel excipient due to level of exposure in Xtampza ER and it has to be adequately qualified for safety.

Studies assessing the absorption, distribution, metabolism, and excretion (ADME) of beeswax itself are not available. However, beeswax is generally considered indigestible due to its high melting point (62-65$^{o}$C) that limits its dissolution at body temperatures, water insolubility, and hydrophobicity that makes it difficult for digestive enzymes and intestinal microflora to affect its breakdown. In the literature, there are ADME studies conducted on extracts of beeswax or plant waxes showing chemical structural similarities to beeswax. For instance, an ADME study in dogs using radiolabeled triglyceride ([$^{14}$C]triolein) and wax ester ([1-$^{14}$C]cetyl oleate or cetyl[1-$^{14}$C]oleate), 87.9% of [$^{14}$C]triolein was absorbed from the margarine, whereas only 3.6% of ([1-$^{14}$C]cetyl oleate or a slightly less than 20% of cetyl[1-$^{14}$C]oleate was absorbed (Place, 1992).
Based on the available information, long-chain fatty acid esters and long-chain n-alkanes present in beeswax are predicted not to be significantly absorbed from the diet. However, long-chain fatty acid esters can be hydrolyzed into their corresponding long-chain fatty acids and alcohols, a process involving lipases in the presence of bile salts, and then these free fatty acid and alcohols can be absorbed by passive or carrier mediated absorption by the mucosal epithelial cell (Hargrove, et al., 2004). N-alkanes can be absorbed and converted to their corresponding long-chain alcohols and long-chain fatty acids. These metabolites can be further metabolized into normal cellular constituents. Metabolism of long-chain alcohols in the cell occurs primarily by either synthesis of phospholipids or oxidation to the corresponding long-chain fatty acid. Fatty acids are metabolized by β-oxidation in normal endogenous pathways. The same metabolic pathway is expected for both long-chain fatty acids and long-chain alcohols present in beeswax.

To qualify the safety of beeswax, the Applicant has not conducted all safety toxicity studies in accordance with the CDER guidance on the safety evaluation of excipients. Instead, the Applicant conducted literature-based safety assessment. Because toxicity studies on beeswax, except for the Ames test (with negative mutagenic results), are not available in the literature, safety of beeswax was evaluated on the basis of published toxicity studies conducted with the main constituents of beeswax and plant waxes with chemical and structural similarities to beeswax. The following are substances that were evaluated in the published papers.

(1) Fatty acid esters: Jojoba oil, Oleyl palmitate, and Carnauba wax.
(2) Long chain fatty acids: D-003 (isolated from sugar cane wax)
(3) Hydrocarbons (n-alkanes and n-alkenes): Low-melting point paraffin wax and Class 1 mineral oil
(4) Long chain fatty alcohols: D-002 (extracted from beeswax), and Policosanol (isolated from sugar cane wax).

The Applicant's submitted papers are listed in Table 7. The detailed composition of substances listed above and the review of the toxicity studies are presented in

Appendix 3, but toxicity studies conducted with carnauba wax are presented in Appendix 2.

**Table 7 Published toxicology studies submitted by Collegium for safety assessment of beeswax**

| Study Type \ Constituent | | (1) Fatty acid esters (57%) | (2) Long chain fatty acids (18%) | (3) Hydrocarbons (15%) | (4) Long chain fatty alcohols (<1%) |
|---|---|---|---|---|---|
| General Toxicology | Rat (Rodent) | (1) Refined jojoba oil, 7-day<br>(2) Oley palmitate, 14- & 28-day<br>(3) Carnauba wax, 90-day [CW-1] | (1) D-003, acute & 90-day<br>(2) D-003, 6-month [BW-1] | (1) Low-melting point paraffin wax or mineral oil, 28- & 90-day, (summary)<br>(2) Mineral oil class I, 12-month (summary) [BW-8] | (1) D-002, 14-day, 90-day, 12-month [BW-2]<br>(2) Policosanol, 12-month [BW-4] |
| | Dog (Non-rodent) | (1) Carnauba wax, 7-month [CW-3] | ND | ND | (1) D-002, 12-month **[BW-3]**<br>(2) Policosanol, 12-month **[BW-5]** |
| Carcino-genicity | Rat | ND | (1) D-003, 24-month **[BW-6]** | (1) Mineral oil class I, 24-month (summary) [BW-8]<br>(2) Composite mineral oil, 24-month (summary) [BW-9] | (1) Policosanol, 24-month [BW-10] |
| | Mouse | ND | (2) D-003, 18-month **[BW-7]** | ND | (1) Policosanol, 18-month [BW-11] |
| Repro and Develop Toxicology | FEED (Rat) | (1) Carnauba wax with 13-week treatment of F1 offspring | (1) D-003 [BW-12] | ND | (1) Policosanol [BW-15] |

| Study Type \ Constituent | | | (1) Fatty acid esters (57%) | (2) Long chain fatty acids (18%) | (3) Hydrocarbons (15%) | (4) Long chain fatty alcohols (<1%) |
|---|---|---|---|---|---|---|
| | | | CW-2 | | | |
| | EFD | Rat | ND | (1) D-003 BW-12, BW-13 | ND | (1) D-002 BW-15<br>(2) Policosanol BW-16 |
| | | Rabbit (second species) | ND | (1) D-003 BW-12 | ND | (1) D-002 BW-15<br>(2) Policosanol BW-16 |
| | PPND (Rat) | | (1) Carnauba wax CW-2 | (1) D-003 BW-14 | ND | (1) Policosanol BW-16, BW-17, BW-18 |

ND = No Data
FEED = Fertility and early embryonic development; EFD = Embryo-fetal development; PPND = Pre- and postnatal development
Study titles for corresponding superscripted annotations can be found in Appendices 2 and 3, and reviews of these studies are also in these appendices.

Here are major highlights from the published toxicology papers.

(1) Apparently, toxicity studies of D-003 and policosanol were conducted (b) (4) Studies using D-003 were conducted in compliance with or consistent with the (b) (4) GLP, and a few studies were conducted based on OECD GLPs. Decreases in cholesterol, triglycerides, and platelet aggregation, and an increase in bleeding time were observed in D-003-dosed groups of males and females in the 6-month rat study. Decreased total cholesterol levels were observed in policosanol-treated dogs in the one-year study.

(2) For studies using hydrocarbons, summarized information was obtained from WHO Food Additives Series 50. In the GLP carcinogenicity rat study with a 12-month interim sacrifice, dietary administration of P70H mineral oil (medium and low viscosity, class I) and P100H mineral oil (high viscosity) was associated with increased weight of mesenteric lymph nodes and increased severity of infiltrating cell histiocytosis in the mesenteric lymph nodes. An increased severity of small granulomatous foci of macrophages was also observed in the mesenteric lymph nodes of males and females in another carcinogenicity study in rats fed diet containing the composite medium-viscosity white mineral oil. Increased incidence and severity of vacuolation of periportal hepatocytes were observed in the first study, but the investigators did not consider this indicative of an adverse

effect but rather a marker of prolonged exposure to mineral oil. There was also increased incidence of combined cystic degeneration or angiectasis in the liver of male rats in the first study. These findings were also noted in the shorter-term studies using mineral oils or low-melting point paraffin wax.

(3) There were no test article-related tumor findings or reproductive and developmental findings in the submitted papers.

(4) A large number of published toxicity studies were conducted with long-chain fatty alcohols such as D-002 or policosanol, however, beeswax only contains less than 1% long-chain fatty alcohols. As shown in Table 7, a number of different types of toxicity studies are not available (ND, no data) for the rest of major constituents of beeswax in the literature. Thus, safety assessment of the main constituents of beeswax is not complete.

In addition to the literature-based safety assessment, the Applicant conducted a 2-week dose-ranging study and 90-day toxicity study of a beeswax/carnauba wax (b) (4) administered by oral capsules in dogs. The review of these dog studies of a beeswax/carnauba wax (b) (4) is presented in Appendix 1. There were no test article-related findings in the dog studies of a beeswax/carnauba wax (b) (4)

As beeswax is a natural product and complex chemical mixtures, a significant fraction of beeswax (about 8% in Table 7) has not been even characterized. The exposure level of beeswax from the Xtampza ER product at the MTDD is (b) (4) mg/day. That means patients taking the Xtampza ER product at the MTDD are exposed to (b) (4) mg of unknown substances. (b) (4) Moreover, the manufacturing process of beeswax batches is essentially (b) (4) (see Appendix 4). All these are safety concerns and cannot be addressed when the safety of beeswax is evaluated only based on toxicity profiles of major components.

Overall, safety assessment of beeswax based on the available published papers on major components of beeswax along with the 90-day toxicity dog study using a beeswax/carnauba wax (b) (4) is not sufficient to qualify of use of beeswax in the Xtampza ER product at the MTDD. Complete toxicity profile of beeswax itself, not just major components, must be adequately characterized for safety, and beeswax batches used in nonclinical toxicity studies should be the same or representative batches used in the drug product to be marketed.

**Carnauba wax:** Carnauba wax [CAS: 8015-86-9; also known as palm wax or Brazilian wax] is obtained from leaf buds and leaves of the Brazilian carnauba palm, *Copernicia cerifera*. (b) (4)

(b) (4) Carnauba wax is one of the hardest and highest-melting point natural waxes and is a complex mixture of compounds containing predominantly aliphatic, cinnamic, and hydroxycarboxylic acid esters, constituting around 80% (w/w) of the mixture. The general content of carnauba wax is shown in Table 8, and more detailed information on these constituents is presented in Appendix 2. From a chemical point of view, the fatty acids ester fraction in carnauba wax would correspond to beeswax fatty acid esters. Thus, the Applicant used literature data on carnauba wax as part of safety assessment of beeswax as mentioned above.

**Table 8 General Content of Carnauba Wax [taken from (EFSA, 2012)]**

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Carnauba wax is widely used in cosmetics, certain foods, and pharmaceutical formulations. According to 21 CFR §184.1978, carnauba wax is used in food with no limitation other than current GMP practice. The "Codex General Standard for Food Additives" (GSFA, 2015) has classified the food uses of carnauba wax into acidity regulator, anticaking agent, bulking agent, carrier, and glazing agent. The Joint FAO/WHO Expert Committee on Food Additives established an acceptable daily intake (ADI) of 0-7 mg/kg (420 mg for a 60 kg person) to carnauba wax in 1992 (JECFA, 1998).

As per the IID, Carnauba wax is used as a pharmaceutical excipient in an FDA-approved chronic oral drug product with a maximum potency of 300 mg per dosage unit. Based on review of FDA-approved drug products, the Division can confirm that there is an FDA-approved oral drug with an indication consistent with chronic human use with a maximum daily intake/consumption of (b) (4) mg/day carnauba wax. Administration of Xtampza ER at the MTDD of 1.5 g would result in an exposure to carnauba wax of (b) (4) mg/day or (b) (4) mg/kg/day for a 60 kg person (b) (4) . Thus, carnauba wax is considered a novel excipient due to level of exposure in Xtampza ER and it must be adequately qualified for safety.

ADME data of carnauba wax itself are not available. In the literature, there are data for structurally similar extracts from plant waxes. Based on the available information, long-chain fatty acid esters and long chain n-alkanes present in carnauba wax are predicted

not to be significantly absorbed from the diet. However, long-chain fatty acid esters can be hydrolyzed into their corresponding fatty acids and alcohols and subsequently absorbed (Hargrove, et al., 2004). Likewise, n-alkanes can be absorbed. These absorbed compounds are incorporated into normal cellular metabolic pathways.

To qualify the safety of carnauba wax, the Applicant conducted literature-based safety assessment of carnauba wax, along with a 2-week dose-ranging study and 90-day toxicity study of a beeswax/carnauba wax (b) (4) administered by oral capsules in dogs. The review of these dog studies of a beeswax/carnauba wax (b) (4) can be found in Appendix 1, and the review of the published papers on carnauba wax can be found in Appendix 2. There were no test article-related findings in the dog studies of a beeswax/carnauba wax (b) (4) The submitted published papers consisted of a 90-day toxicity study in rats, a 7-month toxicity study in dogs, and a reproduction and subchronic feeding study in rats [essentially a fertility and pre- and postnatal study]. The Applicant also cited negative results from the Ames test (using *S. typhimurium* TA1535, TA1537, TA1538, and TA98 and *S. cerevisiae* D4) of carnauba wax, which was summarized in the WHO Food Additive Series 30 (Ekelman K.B. and Chang, 1993). In the Applicant's submitted toxicity papers, there were no test article-related findings, except the changes in several clinical pathology parameters. In the reproduction and subchronic rat study, where F1 offspring were fed diets containing 0.1, 0.3, and 1% carnauba wax for 13 weeks, increased cholesterol levels were observed in all carnauba wax-fed groups of F1 males and females in a dose-dependent manner. In addition, free fatty acid levels were decreased in the F1 males and females fed with 0.3 and 1% carnauba wax. In contrast, increased free fatty acid levels were observed in dogs that were fed diets containing 0.1, 0.3, and 1% carnauba wax for 7 months.

The published toxicology studies were not conducted in compliance of the GLP, and the quality of the study designs (e.g., no inclusion of physical and neurobehavioral development assessment in the reproduction study) and data presentations (e.g., no histology tables) are not optimal. Additionally, for the complete safety assessment of carnauba wax, the following studies are missing: a chronic rodent study, embryo-fetal developmental toxicity studies in two species, and carcinogenicity studies in mice and rats. Thus, safety assessment of carnauba wax based on the available published papers along with the 90-day toxicity dog study using a beeswax/carnauba wax (b) (4) is not sufficient to qualify safety of the amount of carnauba wax in the Xtampza ER product at the MTDD.

**Hypromellose (b) (4):** Hypromellose [also known as hydroxypropyl methylcellulose or HPMC] is a partly *O*-methylated and *O*-(2-hydroxypropylated) cellulose, and it is available in several grades that vary in viscosity and extent of substitution. Grades are distinguished by a four-digit reference number or by specifying the apparent viscosity, in mPa s, of a 2% w/w aqueous solution at 20°C. When distinguished by reference number, the first two digits refer to the approximate percentage content of the methoxy group; the second two digits refer to the approximate percentage content of the hydroxyl propoxy group, calculated on a dry basis.

Hypromellose is included in the FDA Inactive Ingredients Database (IID) in the following dosage forms: ophthalmic preparations; oral capsules, suspensions, syrups, and tablets; and topical and vaginal preparations. Administration of Xtampza ER at the MTDD of 1.5 g results in an exposure to hypromellose of (b) (4) mg/day (b) (4)

An acceptable daily intake (ADI) has been not established for hypromellose, but the FAO/WHO Joint Expert Committee on Food Additives has recommended an intake of 30 g/day as the upper safe level of dietary fiber, which would include the consumption of modified celluloses including hypromellose (JECFA, 1990). An exposure to hypromellose of approximately (b) (4) g/day falls within the JECFA's recommended intake of dietary fiber. In addition, ADME studies in rats and humans demonstrated that ingested hypromellose is minimally absorbed and metabolized, and is excreted almost exclusively in the feces. In male and female Sprague-Dawley rats that were administered a single dose of 500 mg/kg of $^{14}$C-labeled hypromellose (Place, 1992), >99% of the administered $^{14}$C was excreted via the feces, ~1% was excreted via the urine, ~0.07% was excreted in the expired air, and ~0.05% was found in the bile. Analysis of the carcass and tissues revealed that only approximately 0.2% of the administered $^{14}$C remained and that it was present primarily in the gastrointestinal (GI) tract. In the same study, after 5 consecutive doses of 500 mg/kg, recovery of radioactivity was found primarily in the feces (97% in males, 102% in females) with trace amounts in the urine and with no evidence of tissue accumulation. In 25 adult humans (23 males and 2 females) who ingested hypromellose (Methocel HG USP 1828) in doses ranging from 0.6-8.9 g on three separate occasions, approximately 97% of the dose (determined as methoxy groups) was recovered from feces within 96 hours (Knight, et al., 1952).

The toxicological monograph for the JECFA evaluation concluded that modified celluloses as a group have low toxicity. Available ADME data show orally administered hypromellose to pass through the mammalian GI tract largely unabsorbed and unchanged, behaving as non-nutritive fiber, and an intake of 30 g/day has been recommended as the upper safe level of dietary fiber in general by JEFCA. It is thus concluded that (b) (4) g/day of hypromellose does not pose a safety concern.

## 2.5 Comments on Impurities/Degradants of Concern

***Impurities in the drug substance***

For impurities in the drug substance for a MDD of drug substance ≤ 2 g/day according to the ICH Q3A(R2) guidance, the qualification threshold is 0.15% or 1.0 mg per day intake (whichever is lower), whereas the identification threshold is 0.1% or 1.0 mg per day intake (whichever is lower). As mentioned, DAAAP has determined that the MTDD of oxycodone hydrochloride salt is 1.5 g (MTDD of (b) (4) g/day for oxycodone base) based on clinical use data. All drug substance impurity specifications meet the ICH Q3A(R2) guidance specification (Table 9). Note that (b) (4) (b) (4) has adequately qualified (b) (4) for genotoxic

potential. Based on the weight-of-evidence, it was concluded that (b) (4) does not appear to present a significant genetic toxicology concern and can therefore be regulated as a typical DS impurity according to ICH Q3A(R2) thresholds for qualification. The Applicant has proposed that (b) (4) be controlled as an individual unspecified impurity at (b) (4)% and has not listed it as an identified impurity in the proposed specifications.

**Table 9 Acceptance criteria specifications for the oxycodone base drug substance**

| Impurity | Specification | Maximum Daily Exposure | Acceptable? |
|---|---|---|---|
| (b) (4) | | | Yes |
| | | | Yes |
| | | | Yes |

***Impurities in the drug product***

For impurities in the drug product for a MDD of drug substance falling between 10 mg and 2 g according to the ICH Q3B(R2) guidance, the qualification threshold is 0.2% or 3 mg per day intake (whichever is lower), whereas the identification threshold is 0.2% or 2 mg per day intake (whichever is lower). Release specifications for two identified drug product impurities/degradants in the drug product are considered acceptable, but the proposed specification for unspecified impurity in the drug product (NMT (b) (4)%) (b) (4) was communicated to the Applicant in the 74-day letter, and the Division requested (b) (4)

**Table 10 Acceptance criteria specifications for the oxycodone base drug substance**

| Impurity | Specification | Maximum Daily Exposure | Acceptable? |
|---|---|---|---|
| (b) (4) | | | Yes |
| | | | Yes |
| | | | Yes |

*Upon the FDA's request, the specification for unspecified impurity in the drug product (b) (4)% to NMT (b) (4)%.

### 2.6 Proposed Clinical Population and Dosing Regimen

Xtampza ER capsules are planned to be marketed in 10, 15, 20, 30, and 40 mg capsules intended for twice-daily (BID) dosing in adults. The indication for Xtampza ER is management of pain severe enough to require daily, around-the-clock, long-term opioid treatment and for which alternative treatments are inadequate.

### 2.7 Regulatory Background

The Applicant is submitting NDA 208090 via the 505(b)(2) regulatory pathway and is relying on the Agency's previous findings of safety and efficacy for OxyContin® (NDA 022272). IND 75786 was opened on June 6, 2007 by Collegium. The EOP2 and PreNDA meetings were held with Collegium on March 30, 2010 and May 16, 2014, respectively. At these meetings, DAAAP repeatedly communicated to Collegium that any novel excipients (b) (4) must be adequately qualified for safety, as recommended by the FDA guidance for industry: *Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*, available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079250.pdf. The Division also stated that alternative safety justification in lieu of actual toxicology studies, the literature-based approach does result in some risk to the development program, as adequate data might not exist to support the safety of the excipients when the product is dosed up to the MTDD of oxycodone.

## 3 Studies Submitted

### 3.1 Studies Reviewed

Study title: A 90-Day Oral (Capsule) Toxicity Study of a Beeswax/Carnauba Wax (b) (4) in Beagle Dogs [Study No. (b) (4) 724003]

Study title: A 14-Day BID Oral (Capsule) Dose Range-Finding Toxicity Study of a Beeswax/Carnauba Wax (b) (4) in Beagle Dogs [Study No. (b) (4) 724002; non-GLP]: A brief review summary is presented under the Dose Selection section in the 90-day toxicity study review.

Note these studies were conducted to qualify novel excipients, beeswax and carnauba wax, and thus, the review of these studies can be found in Appendix 1.

Publication Title: Prenatal oxycodone exposure impairs spatial learning and/or memory in rats (Davis, et al., 2010)

The review of this published paper can be found under 9.3 Prenatal and Postnatal Development.

### 3.2 Studies Not Reviewed

None

### 3.3 Previous Reviews Referenced

None

# 4 Pharmacology

### 4.1 Primary Pharmacology

No primary pharmacology studies were conducted for NDA 208090.

### 4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted for NDA 208090.

### 4.3 Safety Pharmacology

No safety pharmacology studies were conducted for NDA 208090.

# 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

No pharmacokinetics/ADME studies were conducted for NDA 208090.

### 5.2 Toxicokinetics

No toxicokinetics studies were conducted for NDA 208090.

# 6 General Toxicology

### 6.1 Single-Dose Toxicity

No single-dose toxicity studies were conducted for NDA 208090.

### 6.2 Repeat-Dose Toxicity

No repeat-dose toxicity studies of API (oxycodone) were conducted for NDA 208090.

# 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

No Ames studies were conducted for NDA 208090.

### 7.2 *In Vitro* Assays in Mammalian Cells

No *in vitro* genotoxicity studies in mammalian cells were conducted for NDA 208090.

### 7.3 *In Vivo* Clastogenicity Assay in Rodent

No *in vivo* genotoxicity studies were conducted for NDA 208090.

### 7.4 Other Genetic Toxicity Studies

No other genotoxicity studies were conducted for NDA 208090.

## 8 Carcinogenicity

No carcinogenicity studies were conducted for NDA 208090.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

No fertility and early embryonic developmental toxicity study was conducted for NDA 208090.

### 9.2 Embryonic Fetal Development

No embryonic fetal developmental toxicity studies were conducted for NDA 208090.

### 9.3 Prenatal and Postnatal Development

No prenatal and postnatal developmental toxicity was conducted for NDA 208090.
However, the Applicant submitted one published paper on the pre- and postnatal developmental study and review of the study is presented below.

**Publication Title: Prenatal oxycodone exposure impairs spatial learning and/or memory in rats (Davis, et al., 2010)**

It does not state that whether the study was conducted in a GLP-compliant manner.

**Methods:** Groups of Sprague Dawley female rats (64-70 days of age; number of rat not specified) were orally (gavage; 1 mL/kg) administered oxycodone HCl or the water vehicle. In the oxycodone group, rats were administered 10 mg/kg/day for 5 days, and then the dose was escalated by 0.5 mg/kg/day for 10 days to a final dose of 15 mg/kg/day, which was maintained for 15 days. After 28 days of treatment, the females were harem bred to proven breeder males (3 females: 1 male), with the males rotated daily. Dosing continued through cohabitation and gestation until parturition.

Signs of opioid withdrawal such as weight loss, diarrhea, and irritability were monitored daily from pregnant females. Pregnant dams were individually housed from Gestation Day (GD) 17 until parturition, when litters were culled to 10 pups. Pups were weaned at Postnatal Day (PND) 21. Three separate matings were conducted to generate sufficient subjects for the behavioral testing. The exposed offspring remain undisturbed until testing commenced.

Behavioral testing, consisting of radial arm maze, T-maze, and Morris water maze, was performed in male F1 offspring so that the behavioral parameters were not confounded by fluctuations in hormones from estrous cycling. To avoid litter effects, only one male per litter was assigned to each of the testing groups, and the rats were 4–6 months of age at the time testing was initiated.

The rats for the radial arm maze and T-maze testings were food-restricted to 85% of their free-feeding body weight. There was no difference in the initial adult body weights between the control and oxycodone groups, nor a difference in the amount of time that was needed to reach 85% of the free-feeding weight. During all phases of the radial arm maze and T-maze training, rats were maintained at 85–90% of their pre-testing body weight. Shortly before testing, rats were supplemented by approximately 0.5 g of a Maypo (a sweetened maple-flavored oatmeal cereal) that served as the food reinforce for the radial arm maze and T-maze tasks.

Radial arm maze testing was performed in three phases: shaping (at least 2 days of social shaping), acquisition (10 massed trials for each subject), and retention (3 trials for 7 days).

T-maze testing consisted of four phases: shaping (same as radial arm maze), preference testing (7 trials a day for 5 days), acquisition (6 trials per day with a maximum latency of 60 s per trial for 10 days), and retention (5 days after the last day of acquisition in the same manner as the acquisition trial).

Water maze testing consisted of three phases: shaping (by placing the rats on the escape platform for 10 s before Trial 1 on the first day of testing), acquisition (4 trials per day for 7 days), and retention (4 trials for each rat for one day 7 days after the last day of acquisition). Two separate water maze experiments were conducted that differed by only the intertrial interval. For the first experiment, the interval between all four trials in each training day was 40–60 min (long intertrial interval), whereas for the second experiment, the intertrial interval was 15–30 min (short intertrial interval).

**Results:**

Reproductive effects of F0 animals: No table summarizing the reproductive outcome of F0 animals is included in the paper. It states that there were no oxycodone-related effects on number of females that became pregnant and sustained pregnancy, in gestation length, weight gain during pregnancy, and litter size.

F1 offspring:

It states that in all of the breedings, pups in the oxycodone group had lower birth weights (approximately 10%), and the body weights remained lower than pups in the control group as they underwent opioid withdrawal across the first few days. However, body weight differences resolved by the end of the first postnatal week. It also states

that their studies revealed that neonatal withdrawal activated the hypothalamic–pituitary–adrenal (HPA) axis as shown by a 3-fold elevation of corticosterone levels on PND 1, which was completely recovered by PND 2. Behavioral indices of spontaneous and precipitated withdrawal were noted, with pups having increased abdominal stretching, rolling, and twisting, especially when withdrawal was precipitated by injection of the opioid antagonist naloxone.

Radial arm maze: Results from the radial arm maze testing are shown in Figure 1. Prenatal oxycodone had no effect on overall percentage of correct choices in the radial arm maze or working memory errors, but higher percentages of reference memory errors were noted in F1 offspring in the oxycodone group.



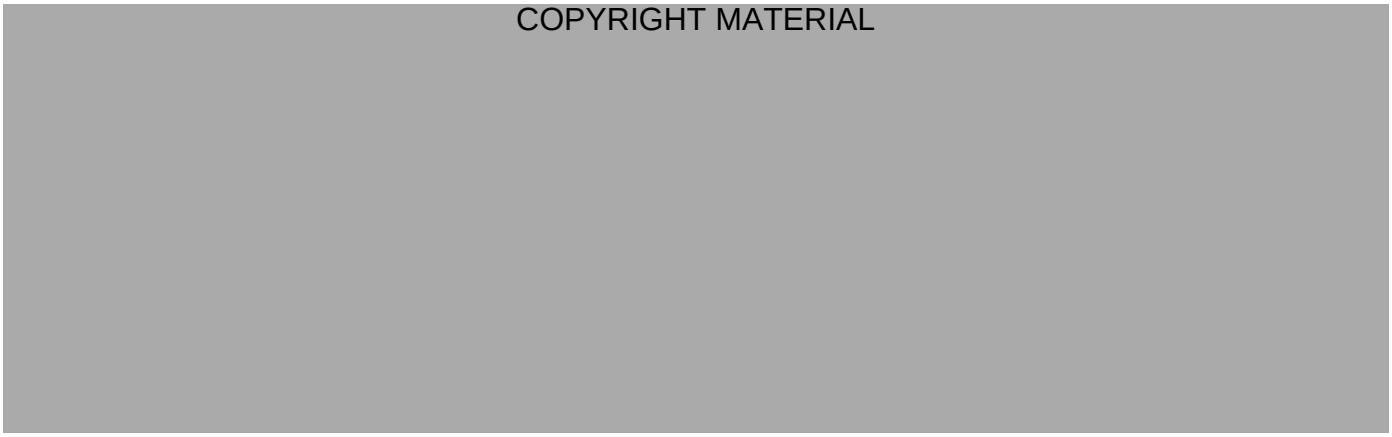


**Figure 1 Results from the radial arm maze, N=6 for prenatal water and N=7 for prenatal oxycodone [taken directly from the published paper, p.30]**

T-maze: Results from the T-maze testing are shown in Figure 2. Prenatal oxycodone had no effect on acquisition of a spatial T-maze task, however, it affected retention 5 days after acquisition. The percent correct choices were decreased across days in F1 offspring in the oxycodone group (approximately 20% fewer correct choices).



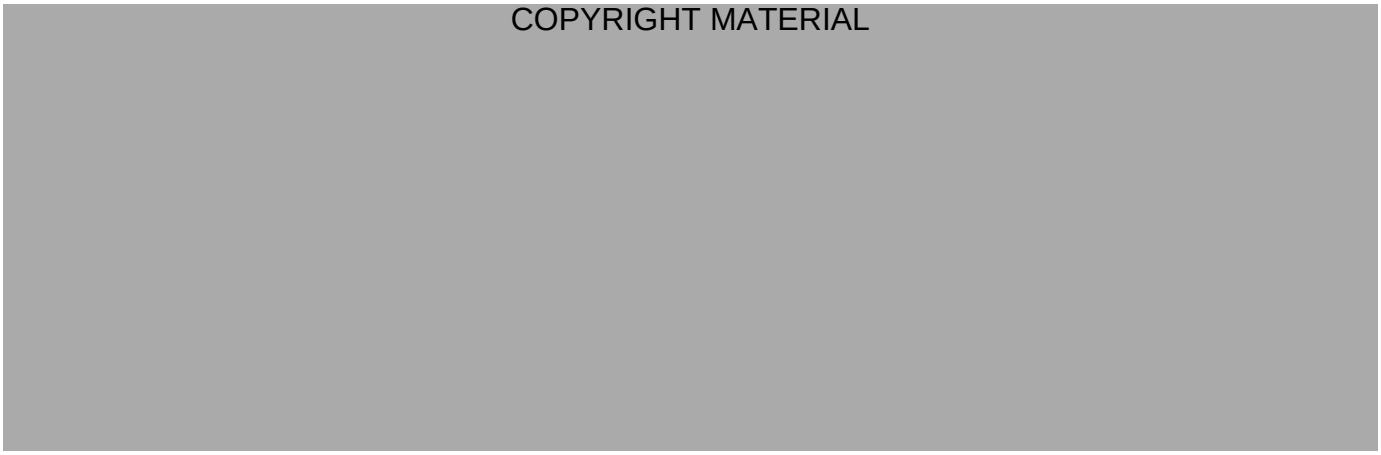


**Figure 2 Results from the spatial T-maze task, N=4 for prenatal water and N=5 for prenatal oxycodone [taken directly from the published paper, p.30]**

Morris water maze: Results from the Morris water maze testing with the long intertrial interval are shown in Figures 3 and 4. F1 offspring in the oxycodone group had a deficit in Morris water maze performance on Trial Day 2 when the latency to locate and the distance traveled to the escape platform were over 60% greater than F1 offspring in the control group (Figure 3). On Day 2, rats in the oxycodone used spatial strategies to a less degree, compared to the rats in the control group (Figure 4). Rats in the oxycodone group had fewer trials using a direct search or focused search strategy and more trials, where the wall-hugging strategy was used. However, the difference in latency and distance was transient, as performance of rats in the oxycodone group was somewhat similar to rats in the control group by Day 3 of training (Figure 3). By Day 3, the profile of the search strategies used across trials was not different between two groups (Figure 4).

When the Morris water maze was performed in a separate group of rats using a short intertrial interval (15-30 min), there were no oxycodone-related effects. A figure describing results from the maze using a short intertrial interval is included in the paper.

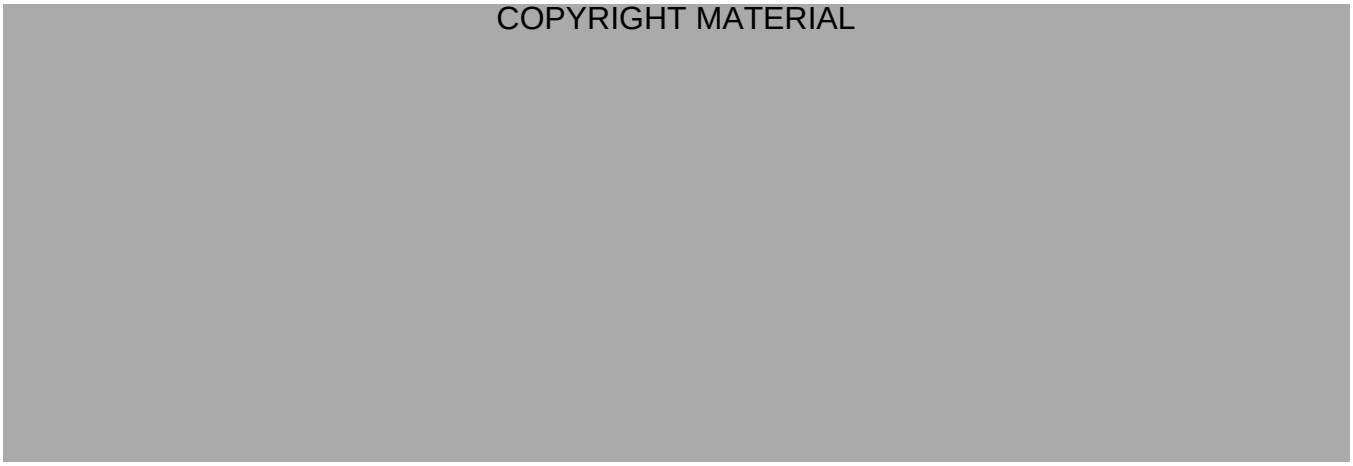



**Figure 3 Results from the Morris water maze with the long intertrial interval (40-60 min), N=8 for both groups [taken directly from the published paper, p.31]**

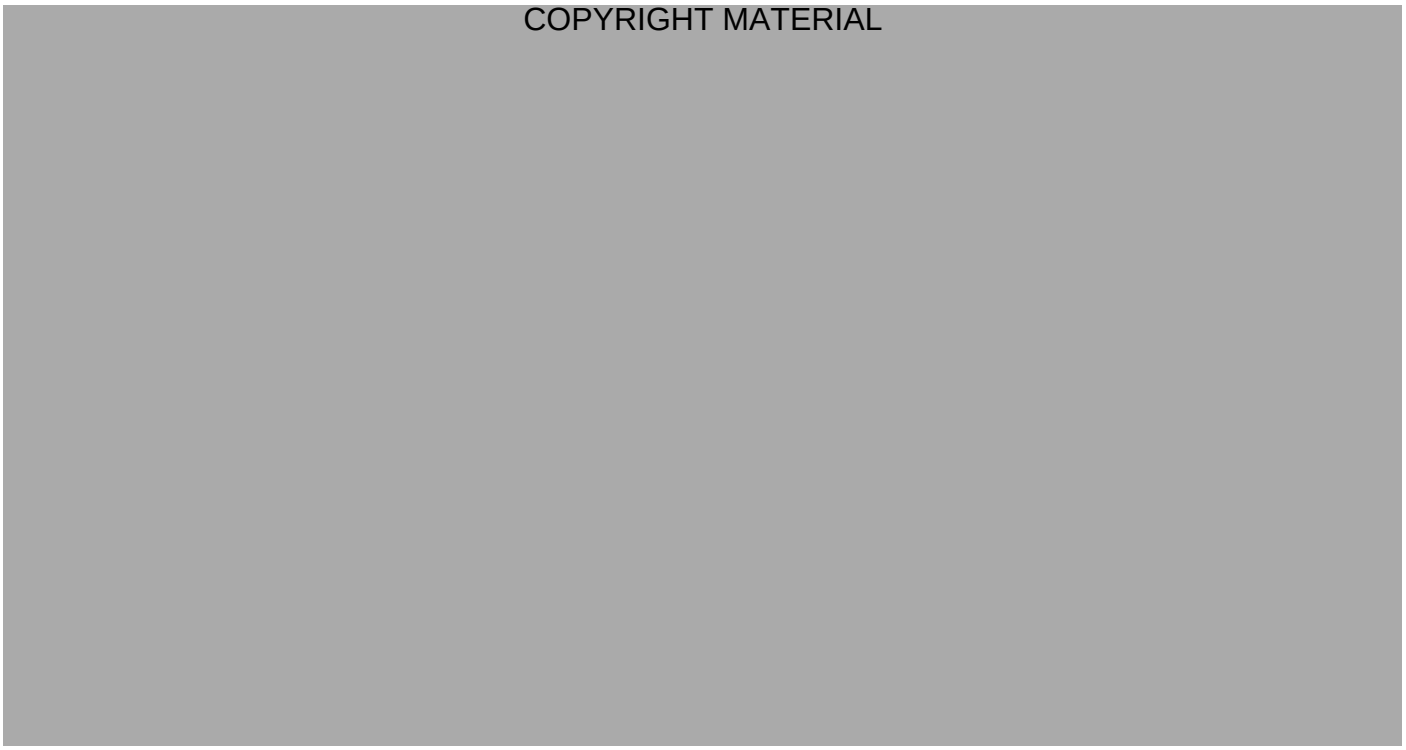



**Figure 4 Results of a spatial or a non-spatial search strategy from the Morris water maze with the long intertrial interval (40-60 min), N=8 for both groups [taken directly from the published paper, p.31]**

# 10 Special Toxicology Studies

No special toxicology studies were conducted for NDA 208090.

# 11 Integrated Summary and Safety Evaluation

The Applicant is submitting NDA 208090 via the 505(b)(2) regulatory pathway and is relying on the Agency's previous findings of safety and efficacy for OxyContin® (NDA 022272). The Applicant also intends to leverage nonclinical information in the currently approved OxyContin® labeling to inform their product labeling. Accordingly, no nonclinical study reports using oxycodone were submitted under this NDA, but the Applicant has submitted published literature on pharmacology and pharmacokinetics of opioids, including oxycodone. Review of these submitted papers, except the pre- and postnatal study in rats (Davis et al., 2010) presented above, are not included in this review as these papers did not impact on the overall scientific understanding of oxycodone and thereby do not impact drug labeling. The information on oxycodone described below is mainly from the OxyContin® label.

Oxycodone is a full opioid agonist and is relatively selective for the mu receptor, although it can bind to other opioid receptors at higher doses. The principal therapeutic action of oxycodone is analgesia, and specific CNS opioid receptors for endogenous

compounds with opioid-like activity have been identified throughout the brain and spinal cord and are thought to play a role in the analgesic effects of this drug. However, the precise mechanism of the analgesic action is still unknown.

Like all full opioid agonists, there is no ceiling effect to analgesia for oxycodone. Clinically, dosage is titrated to provide adequate analgesia and may be limited by adverse reactions, including respiratory and central nervous system (CNS) depression.

Oxycodone produces respiratory depression by direct action on brain stem respiratory centers. The respiratory depression involves both a reduction in the responsiveness of the brain stem respiratory centers to increases in CO tension and to electrical stimulation. Oxycodone depresses the cough reflex by direct effect on the cough center in the medulla, and antitussive effects may occur with doses lower than those usually required for analgesia. Oxycodone causes miosis, even in total darkness. Pinpoint pupils are a sign of opioid overdose, however, marked mydriasis rather than miosis may be seen with hypoxia in the setting of oxycodone overdose.

Oxycodone at therapeutic doses produces peripheral vasodilation (arterial and venous) and decreased peripheral resistance, and it also inhibits baroreceptor reflexes. Histamine release and/or peripheral vasodilation may results in pruritus, flushing, red eyes, sweating, and/or orthostatic hypotension.

In addition to the effects on the central nervous and cardiovascular systems, oxycodone affects the gastrointestinal tract and other smooth muscles. Oxycodone causes a reduction in motility associated with an increase in smooth muscle tone in the antrum of the stomach and duodenum. Food digestion in the small intestine is delayed, and propulsive contractions are decreased. Propulsive peristaltic waves in the colon are decreased while tone may be increased to the point of spasm resulting in constipation. Other opioid-induced effects may include a reduction in gastric, biliary, and pancreatic secretions, spasm of sphincter of Oddi, and transient elevations in serum amylase.

Opioids inhibit the secretion of endogenous hormones such as ACTH, cortisol, testosterone, and luteinizing hormone in humans. They also stimulate prolactin, growth hormone secretion, and pancreatic secretion of insulin and glucagon. Opioids have been shown to have a variety of effects on the immune system in in vitro and animal models. Overall, the effects of opioids appear to be modestly immunosuppressive.

The pharmacokinetics of oxycodone in the humans and animals are well-understood. Systemic exposure to oxycodone in humans is known to increase dose-proportionally. Oxycodone is extensively metabolized to noroxycodone, oxymorphone, and noroxymorphone, and then these are subsequently glucuronidated. The primary metabolic pathway of oxycodone is CYP3A-mediated N-demethylation to noroxycodone, which is further oxidized to noroxymorphone. Noroxycodone and noroxymorphone are the major circulating metabolites. The secondary metabolic pathways of oxycodone include CYP2D6-mediated O-demethylation to oxymorphone.

Oxymorphone is present only at relatively low concentrations in the plasma and undergoes further metabolism to form its glucuronide and noroxymorphone.

Noroxycodone exhibits very weak anti-nociceptive potency, compared to oxycodone. Noroxymorphone is active at opioid receptors, but it does not appear to cross the blood-brain barrier to a significant extent. Oxymorphone has been shown to be active and possessing analgesic activity, but its contribution to the analgesic effect following oxycodone administration is thought to be clinically insignificant. Other metabolites (α- and β-oxycodol, noroxycodol, and oxymorphol) may be present at very low concentrations and demonstrate limited penetration into the brain, as compared to oxycodone.

Oxycodone and its metabolites are excreted primarily via the kidney. The amounts of free and conjugated oxycodone in the urine were reported to be 8.9%, whereas the amounts of free noroxycodone and free and conjugated noroxymorphone were 23% and 14%, respectively. Free and conjugated oxymorphone contributed about 11%. The total plasma clearance was approximately 1.4 L/min in adults.

Mutagenic and fertility effects of oxycodone were examined in vitro and/or in vivo animal studies, but animal carcinogenicity studies have not been conducted for oxycodone. Oxycodone was genotoxic in the mouse lymphoma assay at concentrations of 50 mcg/mL or greater with metabolic activation and at 400 mcg/mL or greater without metabolic activation. Clastogenicity was observed with oxycodone in the presence of metabolic activation in one chromosomal aberration assay in human lymphocytes at ≥1250 mcg/mL at 24 hours, but not 48 hours of exposure. In a second chromosomal aberration assay with human lymphocytes, no structural clastogenicity was observed either with or without metabolic activation; however, in the absence of metabolic activation, oxycodone increased numerical chromosomal aberrations (polyploidy).

Oxycodone was not genotoxic in the following assays: Ames *S. typhimurium* and *E. coli* test with and without metabolic activation at concentrations up to 5000 mcg/plate, chromosomal aberration test in human lymphocytes (in the absence of metabolic activation) at concentrations up to 1500 mcg/mL, and with activation after 48 hours of exposure at concentrations up to 5000 mcg/mL, and in the in vivo bone marrow micronucleus assay in mice (at plasma levels up to 48 mcg/mL).

Oxycodone hydrochloride did not affect reproductive function in male or female rats at any dose tested (0.5, 2, and 8 mg/kg/day) in a fertility and early embryonic developmental toxicity study. Male rats were dosed for 28 days prior to cohabitation with females, during the cohabitation, and until necropsy (2-3 weeks post-cohabitation), whereas female rats were dosed for 14 days prior to cohabitation with males, during cohabitation, and up to Gestation Day 6. Embryo-fetal developmental toxicity studies with oral doses of oxycodone hydrochloride in rats up to 8 mg/kg/day and rabbits up to 125 mg/kg/day (equivalent to 0.5 and 15 times an adult human dose of 160 mg/day, respectively on a mg/m$^2$ basis) did not reveal evidence of fetal adverse effects. In a pre- and postnatal toxicity study in female rats received oxycodone during gestation and

lactation, there were no drug-related effects on reproductive performance in these females or any long-term developmental or reproductive effects in pups born to these rats. Decreased body weight was found during lactation and the early post-weaning phase in pups nursed by mothers given the highest dose used (6 mg/kg/day, equivalent to approximately 0.4-times an adult human dose of 160 mg/day, on a mg/m$^2$ basis). However, body weight of these pups recovered.

In the published paper by Davis et al. (2010), transient impairments in spatial learning and memory were observed in adult F1 male offspring from dams that were administered oxycodone. In this study, adult female Sprague-Dawley rats were treated for 28 days via oral gavage with ascending doses of oxycodone up to a final dose of 15 mg/kg/day. Dosing at 15 mg/kg/day continued through breeding and gestation until parturition. Animals in the control group were dosed with water. Male F1 offspring at 4-6 months of age were tested on the radial arm maze, T-maze, and Morris water maze (with short and long intertrial intervals). Prenatal oxycodone exposure resulted in a deficit in the radial arm maze characterized by a greater number of reference memory errors, especially in the beginning of testing. In the T-maze, F1 offspring in the oxycodone group exhibited a modest deficit in retention of the task when assessed 5 days after acquisition training. In the Morris water maze, F1 offspring in the oxycodone group had poorer acquisition when there was a long intertrial interval. The spatial learning deficit was characterized by an increased latency to find and a greater distance traveled to the platform. These data were corroborated by analysis of the behavioral search strategy, which showed a decreased use of spatial strategies and an increase in non-spatial strategies, especially wall-hugging, in F1 offspring in the oxycodone group, as compared to F1 offspring in the control group on Day 2 of acquisition. There was no oxycodone-related deficit when the Morris water maze was conducted using a short intertrial interval.

Similarly, prenatal exposure to oxycodone was shown to be associated with subtle, transient changes in cardiovascular response to acute stress in male rats (Sithisarn, et al., 2013). In this study, pregnant Sprague-Dawley rats were intravenously administered control (normal saline vehicle) or oxycodone (2 mg/kg) by slow injection over 10 min via the atrial cannula from GD 8 to 21 and PNDs 1, 3 and 5. F1 pups in each litter were fostered to untreated foster dams on PND 5 and were weaned on PND 25. Arterial BP telemetry probes were implanted in F1 pups on PND 27-30. F1 offspring were classically conditioned by following a pulsed tone (CS+) with tail shock on PNDs 40 and 75. A steady tone (CS−) was not followed by shock. Blood pressure (BP) and heart rate (HR) were recorded during resting periods and conditioning. On PD 40, F1 offspring in the oxycodone group had a larger amplitude of the conditional BP increase during the stress response (+3.9 in the oxycodone group vs. +1.7 mm Hg in the control group; baseline mean BP was114.8 and 118.3 mm Hg in the oxycodone and control groups, respectively). Rats in the both groups were able to discriminate between CS+ and CS−. There were no oxycodone-related effects on the increased amplitude of the conditional BP on PD 75.

All impurities in the drug substance and drug product are controlled at acceptable levels. Xtampza ER contains excipients that are intended to confer abuse-deterrent properties. The microspheres contain oxycodone [redacted] When calculated for the MTDD of oxycodone HCl (1.5 g/day; or [redacted] g/day for oxycodone base), the levels of myristic acid, beeswax, carnauba wax, and hypromellose [redacted] To qualify the safety of the levels of these excipients in the Xtampza ER product, the Applicant has submitted the literature-based safety assessments of four excipients along with a 90-day toxicity study of a beeswax/carnauba wax [redacted] administered by oral capsules in dogs. There were no test article-related findings in the 90-day dog study of a beeswax/carnauba wax [redacted] and thus, the NOAEL was the high dose the safety margin from this study is presented in Table 11.

**Table 11 Safety Margin Calculation (based on a human exposure to beeswax/ carnauba wax of [redacted] mg [redacted] mg/kg for a 60 kg person] from Xtampza ER products at the MTDD)**

| Total Daily Dose of Beeswax/Carnauba Wax [redacted] (mg/kg/day) in the 90-Day Dog Study | Safety Margin on mg/m$^2$ Basis |
|---|---|
| 500 | 2.7 |
| 1000 | 5.4 |
| 2000 | 11 |

The following are the Reviewer's conclusion on the Applicant's safety assessments of the novel excipients.

(1) Myristic Acid: Myristic acid is a saturated fatty acid (SFA), which a key endogenous component of human cellular biochemical machinery and adipose tissue and a key component of a typical, high saturated fat Western diet. [redacted] In general, the literature indicates that myristic acid and some other SFAs such as lauric acid and palmitic acid have similar harmful effects, including hypercholesterolemia. As these SFAs have similar effects, it is reasonable to compare exposure to myristic acid from the Xtampza ER product at the MTDD with combined mean intakes of these SFAs from daily food and beverage consumption (17.31 g/day). However, it also needs to be considered that some harmful effects of SFAs in dietary fats are counterbalanced by potential beneficial effects of other nutrients in diet such as monounsaturated and polyunsaturated fatty acids. Administration of myristic acid without other beneficial fatty acids would cause more potent adverse effects than when it is

taken from dietary consumption. Therefore, it is still unclear whether exposure to myristic acid of (b) (4) g/day from the Xtampza ER product at the MTDD does not pose a safety concern.

(2) Beeswax: Beeswax is a natural product and complex chemical mixture, and a significant fraction of beeswax (~8%) has not been even characterized. (b) (4)

The toxicology data of beeswax are not available in the literature, and thus, safety assessment of beeswax was evaluated on the basis of published toxicity studies conducted with the main constituents of beeswax and plant waxes with chemical and structural similarities to beeswax in the literature. However, there are still major gaps in characterizing toxicity profiles of these substances because all toxicology data of major components are not available in the literature. Overall, safety assessment of beeswax based on the available published papers on major components of beeswax along with the 90-day toxicity dog study using a beeswax/carnauba wax (b) (4) is not sufficient to qualify the amounts of beeswax in the Xtampza ER product at the MTDD. Toxicity profile of beeswax itself, not just major components, should be characterized for the adequate safety qualification, and beeswax batches used in nonclinical toxicity studies should be the same or representative batches used in the drug product to be marketed.

(3) Carnauba Wax: There are some toxicology studies of carnauba wax in the literature, but these published toxicology studies have poor quality of the study designs and data presentations. Moreover, for the complete safety assessment of carnauba wax, the following studies are missing: a chronic rodent study, embryo-fetal developmental toxicity studies in two species, and carcinogenicity studies in mice and rats. Thus, safety assessment of carnauba wax based on the available published papers along with the 90-day toxicity dog study using a beeswax/carnauba wax (b) (4) is not sufficient to qualify the amount of carnauba wax in the Xtampza ER product at the MTDD.

(4) Hypromellose: Available ADME data in animals and humans show orally administered hypromellose to pass through the gastrointestinal tract largely unabsorbed and unchanged, behaving as non-nutritive fiber, and an intake of 30 g/day has been recommended as the upper safe level of dietary fiber in general by JEFCA. Thus, it is concluded that (b) (4) g/day of hypromellose in the Xtampza ER product at the MTDD does not pose a safety concern.

As mentioned above, myristic acid, beeswax, and carnauba wax have not been fully characterized in terms of safety. (b) (4)

(b) (4)

Beeswax and carnauba wax are natural products and complex chemical mixtures. The Applicant's safety justification is largely based on published data on some of the major components of beeswax, but these data do not characterize a significant fraction of the chemicals in beeswax, many of which are not even identified. Moreover, the available published toxicology studies do not provide complete safety assessment of carnauba wax and all major components of beeswax as there are major gaps in characterizing complete toxicity profiles of these substances in the literature. As such, the Applicant's safety assessment of myristic acid, beeswax, and carnauba wax is not adequate.

In order to qualify the safety of these excipients, the Applicant must provide adequate safety assessment of myristic acid, beeswax, and carnauba wax by conducting the following toxicity studies using (b) (4) myristic acid, beeswax, and carnauba wax:

(1) Chronic general toxicity studies, including a 9-month study in dogs and a 6-study in rats

(2) Reproductive and developmental toxicity studies, including fertility and early embryonic developmental toxicity study in rats, embryo-fetal developmental toxicity studies in rats and rabbits, and pre- and postnatal developmental toxicity study in rats

(3) Carcinogenicity studies in rats and mice, or submit documentation providing scientific justification that carcinogenicity data are not necessary.

Batches of beeswax and carnauba wax used in nonclinical toxicity studies should be the same or representative batches used in the drug product to be marketed.

For these novel excipients, estimated daily intake from food consumption or an acceptable daily intake (established by USDA or JECFA) can be used to establish the level that would be safe to use in the Xtampza product. The daily intake of myristic acid from food consumption in the U.S. is 2.2 g/day, JECFA's estimated dietary exposure to beeswax is <650 mg/day, and JECFA's established ADI is 0-7 mg/kg/day or 420 mg/day for a 60 kg person. Based on review of FDA-approved drug products, the Division can confirm that there is an FDA-approved oral drug with an indication consistent with chronic human use with a maximum daily intake/consumption of (b) (4) mg/day carnauba wax. Thus, 8 capsules of 40 mg Xtampza ER ( (b) (4) mg of myristic acid, (b) (4) mg of beeswax, and (b) (4) mg of carnauba wax) do not pose a safety concern based on estimates of the upper limit of U.S. dietary consumption of beeswax.

However, if the benefits of the Xtampza ER product outweigh the risks, the following points are worth taking into consideration regarding the safety assessment of these novel excipients:

1) It is unclear whether exposure to isolated myristic acid of (b) (4) g/day from the Xtampza ER product at the MTDD (without counterbalancing with beneficial fatty

acids) poses a safety concern due to its potential cholesterol-raising effects. If the dose is limited to 8 pills a day, the total daily dose of myristic acid via the Xtampza ER product is (b) (4) g/day, which is (b) (4) the estimated mean intake of 2.2 g/day in the U.S. diet. If the dose is limited to 16 pills a day, the total daily dose of myristic acid via the Xtampza ER product is (b) (4) g/day, which is (b) (4) the estimated mean intake of 2.2 g/day in the U.S. diet. Consumption of the product at high doses would be additive to the impact of a person's diet.

2) Although the JECFA's estimated dietary exposure to beeswax is <650 mg/day, the EU Scientific Committee on Food (SCF)'s estimated daily intake of beeswax is up to 1290 mg/day in the Netherlands. Note that the main issue of beeswax is incomplete characterization of toxicity profile of beeswax due to lack of required toxicity data, but the 90-day dog study using a beeswax/carnauba wax (b) (4) does not identify any specific toxicity and available published toxicity data of components of beeswax (specifically hydrocarbons) suggest the potential for infiltrating cell histiocytosis in the mesenteric lymph nodes and cystic degeneration or angiectasis in the liver. These data suggest that some component(s) of the beeswax are likely absorbed systemically.

3) Although ADME data of beeswax or carnauba wax do not exist, it is widely assumed, but unproven, that absorption of components of these waxes from the gastrointestinal tract would be limited.

Thus, if the benefits outweigh the risk, (b) (4) capsules of 40 mg Xtampza ER ( (b) (4) mg of myristic acid, (b) (4) mg of beeswax, and (b) (4) mg of carnauba wax[2]) can be considered for initial approval with postmarketing requirements of all nonclinical studies that are listed below to resolve deficiencies.

# 12 Appendix/Attachments

Appendix 1: Review of a 90-Day Oral Toxicity Study of a Beeswax/Carnauba Wax (b) (4) in Beagle Dogs
Appendix 2: Review of Published Toxicity Studies with Carnauba Wax along with Information on Structural Constituents of Carnauba Wax
Appendix 3: Review of Published Toxicity Studies with Major Components of Beeswax
Appendix 4: Manufacturing Process and Process Control of Beeswax
Appendix 5: Manufacturing Process and Process Control of Carnauba Wax
Appendix 6: Reference List

[2] Given the previous finding of safety of (b) (4) mg/day of carnauba wax, a dose of (b) (4) mg of carnauba wax is also reasonably justified to support 16 pills per day of this formulation.

## Appendix 1: Review of a 90-Day Oral Toxicity Study of a Beeswax/ carnauba Wax (b) (4) in Beagle Dogs

**Study title: A 90-Day Oral (Capsule) Toxicity Study of a Beeswax/Carnauba Wax (b) (4) in Beagle Dogs [Final Report and Amendment to the Final Report No. 1]**

| | |
|---|---|
| Study no.: | (b) (4) 724003 |
| Study report location: | EDR |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | September 8, 2014 |
| GLP compliance: | Yes, US FDA GLP Regulations (21 CFR Part 58) |
| QA statement: | Yes |
| Drug, lot #, and % purity: | (b) (4) beeswax/ carnauba wax (b) (4) Batch No. 10479/14 of beeswax, (b) (4) Batch No. 18076 of carnauba wax |

**Key Study Findings**

- In the 90-day toxicology study, beagle dogs (4/sex/group) were orally administered beeswax/carnauba wax (b) (4) in capsules twice daily approximately 8 hours apart at doses of 0 (control; G1), 500 (LD; G2), 1000 (MD; G3), or 2000 (HD; G4) mg/kg/day. The dogs in the control group were received the same number of empty capsules as HD group.

- Analyses of dosing formulation and toxicokinetics were not conducted as the analyses would be challenging due to the nature of test article as mixtures of multiple components. Histology evaluation was mainly performed on tissues from the control and HD groups.

- Emesis frequently occurred in all dose groups, with higher incidences being observed in the control and HD groups. Due to the high incidences of emesis in the control and HD groups and lack of data on systemic exposure to the test article, the Reviewer was initially concerned whether all dogs were adequately exposed to the test article as it was intended, and it could not conclude that animals in the HD group were exposed to a higher level of the test article than animals in the MD or LD groups. In the response to the Reviewer's concern, the Applicant analyzed the incidence of emesis in greater detail and showed that the actual influence of emesis on exposure was lower than it may appear based on

the summary tables in the study report and concluded that all groups were adequately exposed during the study despite high incidences of emesis.

- There were no test article-related effects on any of parameters evaluated in the study.

- Given all shortcomings of the study mentioned above, this 90-day study is not optimal. However, the Reviewer considered this study is sufficient for the subchronic toxicity assessment in dogs for beeswax and carnauba wax, and high dose (2000 mg/kg/day) was considered the NOAEL for the study.

| Methods | |
|---|---|
| Doses: | 0, 250 (LD), 500 (MD), and 1000 (HD)mg/kg/dose BID (0, 500, 1000, and 2000 mg/kg/day, respectively) |
| Frequency of dosing: | Twice daily (approximately 8 hours apart) |
| Route of administration: | Oral (capsule) |
| Dose volume: | The test article was administered using tared, (b) (4) capsules. The study report states that 1 to 4 capsules containing the test article was dispensed daily for each dog (Groups 2, 3, and 4). However, the Reviewer concludes that at least 2 capsules were daily given to dogs since dogs were dosed twice daily. The study report also states that dogs in the control group (Group 1) received the same number of empty capsules for each sex as the dogs in the high dose group (3 or 4 capsules/dog/day). |
| Formulation/Vehicle: | Empty capsules (the same number of the capsules as for the HD group) |
| Species/Strain: | Beagle dogs |
| Number/Sex/Group: | 4 |
| Age: | Approximately 9 to 10 months old at the initiation of dosing |
| Weight: | 8.0-11.1 kg for males; 5.8-8.1 kg for females at randomization (4 days prior to the initiation of dosing) |
| Satellite groups: | None |
| Unique study design: | The characterization of the test article and toxicokinetic analysis was not performed because beeswax and carnauba wax are mixtures of multiple constituents of similar structures of chemicals. |
| Deviation from study protocol: | There were no deviations that affected the integrity of the study or impacted the study design. |

**Dose Selection**

Dose selection for this 90-day toxicity study was based on results from a 14-day dose range-finding study [Study No. (b) (4) 724002; non-GLP]. In the 14-day study, beagle dogs (2/sex/group) were orally administered beeswax/carnauba wax (b) (4) in capsules twice daily (approximately 8 hours apart) at doses of 0 (control; G1), 500 (LD; G2), 1000 (MD; G3), or 2000 (HD; G4) mg/kg/day. The dogs in the control group received the same number of empty capsules as HD group. The study included the standard parameters for a general toxicity study, except dosing formulation analyses, TK evaluation, ECG measurement, and histology evaluation. A few occurrences of emesis containing white material were noted after dosing in the HD

males and females, whereas emesis containing capsule material was noted in the control males and females and HD males and females with a similar number of occurrences. The mean thymus weights were decreased in the HD male and female groups, relative to the control group (-27 and -49%, respectively), but the changes in mean thymus weight in the LD and MD groups (-4 and +23 % in the LD and MD males, respectively; -2 and -4% in the LD and MD female groups, respectively) appeared random. Thus, the decreased mean thymus weights in the HD group were not considered test article-related. In addition, a decrease in mean ovaries weights was noted in the test article-dosed groups (-8, -25, and -23% in the LD, MD, and HD groups, respectively), whereas an increase in mean prostate weight was observed in the test article-dosed groups (+100, +56, and +28% in the LD, MD, and HD groups). In the 90-day toxicity study, there were decreased prostate weights in the test article-dosed groups. Thus, the increased prostate weights in the 14-day study were not considered test article-related. On the other hand, decreased ovaries weights were also observed in the 90-day study (see below for more details).

**Observations and Results**

**Mortality**

Viability was checked twice daily (once in the morning and once in the afternoon).

There was no test article-related mortality. All animals survived to the scheduled necropsy.

**Clinical Signs**

Clinical observations were performed daily, and detailed physical examinations were performed weekly. During the dosing period, animals were observed at the time of each dose administration and 1-2 hours postdose.

Yellow material in feces (likely due to the presence of beeswax/carnauba wax in the feces) was found in 2 MD males, 2 MD females, 3 HD males, and 4 HD females on several occasions. Emesis frequently occurred in all dose groups, with higher incidences being observed in the control and HD groups. Occurrences of emesis containing white, yellow, and/or capsule material are presented in Table 12.

Due to the high incidences of emesis in the control and HD groups along with lack of data on systemic exposure to the test article, it was unknown whether all dogs were adequately exposed to the test article as intended, and it was uncertain that animals in the HD group were exposed to a higher level of the test article than animals in the MD or LD groups. This concern was communicated to the Applicant, and the Applicant analyzed the incidence of emesis in greater detail, showed that the actual influence of emesis on exposure was lower than it may appear based on the summary tables present in the study report and concluded that all groups were adequately exposed during the study despite high incidences of emesis. See below the Special Evaluation section for more information.

All other observations in the test article-dosed groups were considered incidental because similar incidences were also observed in animals in the control group or a single or a few incidences were observed in a single animal in the test article-dosed group(s) without a dose-response relationship.

**Table 12 Emesis findings during the treatment period [Total occurrence/Number of animals], emesis containing food not presented**

| Dose Group (mg/kg/day) / Emesis Occurrence | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| Dose 1: Time of dose | | | | | | | | |
| Emesis containing white material | 4/3 | 1/1 | 0/0 | 0/0 | 2/2 | 0/0 | 1/1 | 4/3 |
| Emesis containing yellow material | 1/1 | 2/2 | 0/0 | 1/1 | 0/0 | 0/0 | 0/0 | 1/1 |
| Emesis containing capsule material | 1/1 | 0/0 | 0/0 | 1/1 | 2/1 | 1/1 | 1/1 | 1/1 |
| Dose 1: 1-2 hours postdose | | | | | | | | |
| Emesis containing white material | 12/3 | 0/0 | 4/3 | 16/4 | 13/4 | 0/0 | 4/2 | 20/4 |
| Emesis containing yellow material | 2/2 | 4/2 | 1/1 | 4/3 | 0/0 | 0/0 | 0/0 | 5/3 |
| Emesis containing capsule material | 9/3 | 2/2 | 2/2 | 10/4 | 9/3 | 4/3 | 9/3 | 15/4 |
| Dose 2: Time of dose | | | | | | | | |
| Emesis containing white material | 0/0 | 0/0 | 0/0 | 1/1 | 2/2 | 1/1 | 0/0 | 1/1 |
| Emesis containing yellow material | 0/0 | 0/0 | 0/0 | 2/1 | 1/1 | 0/0 | 0/0 | 1/1 |
| Emesis containing capsule material | 0/0 | 0/0 | 0/0 | 1/1 | 0/0 | 0/0 | 0/0 | 1/1 |
| Dose 2: 1-2 hours postdose | | | | | | | | |
| Emesis containing white material | 4/4 | 1/1 | 4/2 | 13/4 | 14/4 | 6/2 | 3/2 | 27/4 |
| Emesis containing yellow material | 0/0 | 1/1 | 4/3 | 1/1 | 0/0 | 1/1 | 1/1 | 4/2 |
| Emesis containing capsule material | 1/1 | 0/0 | 3/3 | 8/4 | 7/2 | 5/2 | 4/3 | 10/4 |

**Body Weights**

Body weights were measured weekly during the dosing period and on the day prior to the first day of the scheduled necropsy (nonfasted). Final body weights (fasted) were recorded prior to the scheduled necropsy.

There were no test article-related effects on mean body weight or mean body weight gains.

In the test article-dosed female groups, there was a dose-dependent decrease in mean body weight gains between Weeks 0 and 13 (-50%, -62.5%, and -75% in the LD, MD, and HD groups, relative to the control group). However, the actual mean body weights in these groups were slightly lower than in the control group (7.0 g, 7.2 g, and 7.1 g in the LD, MD, and HD groups, versus 7.5 g in the control group on Week 13). Moreover, decreased mean body weights or body weight gains were not noted in the test article-dosed male groups. Thus, decreased mean body weight gains in test article-dosed females were considered incidental.

**Food Consumption**

Food weights were recorded daily throughout the study period until the day prior to the scheduled necropsy, and the weekly averages were reported for the corresponding body weight intervals.

There were no test article-related changes in mean food consumption. Any changes were considered normal variations as the changes appeared random and their magnitudes were not substantial.

**Ophthalmoscopy**

Ophthalmic examinations were performed during Weeks -1 and 12, using an indirect ophthalmoscope and slit lamp biomicroscope preceded by pupillary dilation with a mydriatic agent.

No ocular lesions were observed in any animal during Weeks -1 and 12.

**ECG**

Heart rate and waveform intervals (PR, QRS, RR, QT, and QTcV [QTc Van de Water's correction]) were recorded during Weeks -1 and 12. During Week 12, ECGs were recorded at approximately 2 hours postdose.

There were no test article-related effects on heart rate or ECG measurements.

The mean heart rate (HR) in the male HD group (95 beats per minute [bpm]) was lower (-23%), relative to the male control group (123 bpm) on Week 12. However, this effect was not due to the test article because the mean HR in the male HD group on Week -1 (99 bpm) was similar to what was noted in this group on Week 12. Moreover, one control male had a relatively high HR on Week 12 (163 bpm), which caused the mean HR in the control group high.

Likewise, an increase in mean PR interval in the male HD group (707 ms) on Week 12 (+35% relative to the control group [524 ms]) was not considered test article-related because one HD male had a high value (924 ms), which caused the higher mean value in the HD group, whereas one control male had a low value (369 ms), which caused the lower mean value on Week 12.

**Hematology**

Blood samples for hematology and coagulation evaluations were collected from all animals prior to the initiation of dosing (Week -1), during Week 6, and at the scheduled necropsy (Week 12/13). The animals were fasted overnight prior to blood collection.

There were no test article-related effects on any hematology or coagulation parameters examined in this study. Any changes were considered background variations.

Hematology and coagulation parameters examined [taken directly from the study report, p.25]

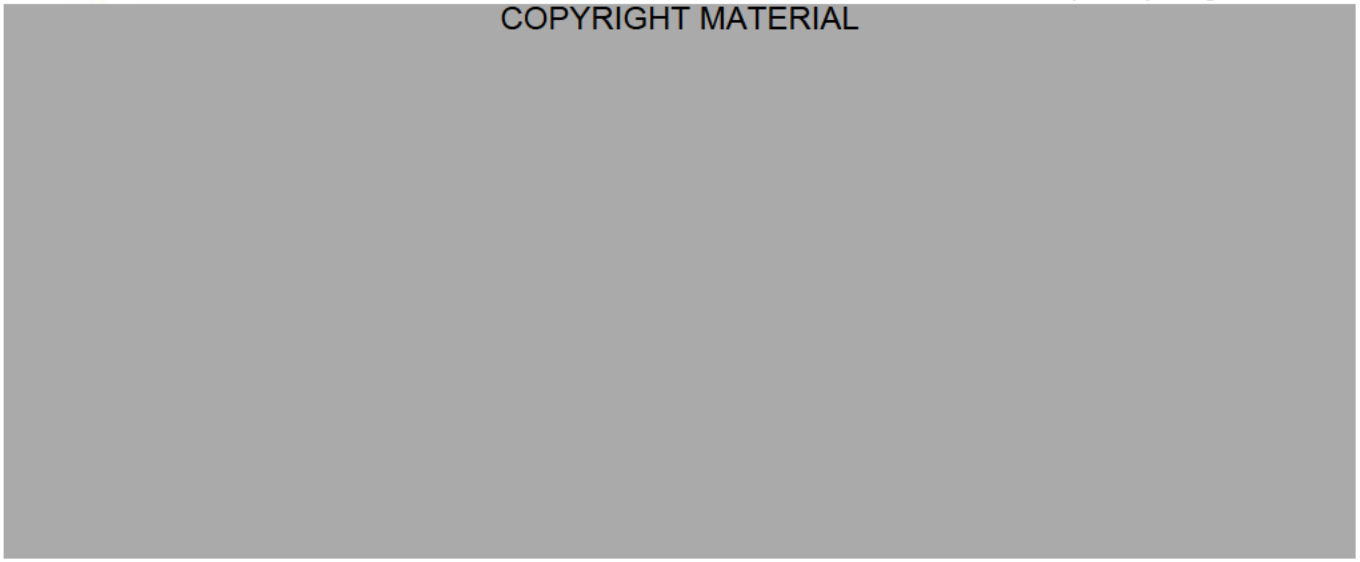


**Clinical Chemistry**

Blood samples for serum chemistry evaluations were collected from all animals prior to the initiation of dosing (Week -1), during Week 6, and at the scheduled necropsy (Week 12/13). The animals were fasted overnight prior to blood collection.

There were no test article-related effects on serum chemistry parameters. Any changes were considered background variations.

Serum chemistry parameters examined [taken directly from the study report, p.25]

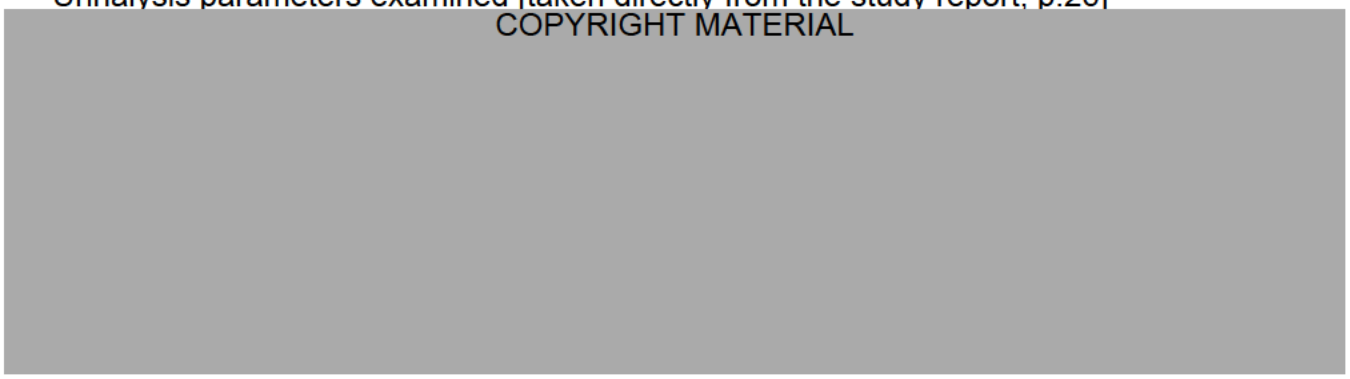



**Urinalysis**

Urine samples for urinalysis were collected from all animals prior to the initiation of dosing (Week -1), during Week 6, and at the scheduled necropsy (Week 12/13). The animals were fasted while urine was collected overnight using cage pans.

There were no test article-related effects on urinalysis parameters examined in the study. Any changes were considered background variations.

Urinalysis parameters examined [taken directly from the study report, p.26]

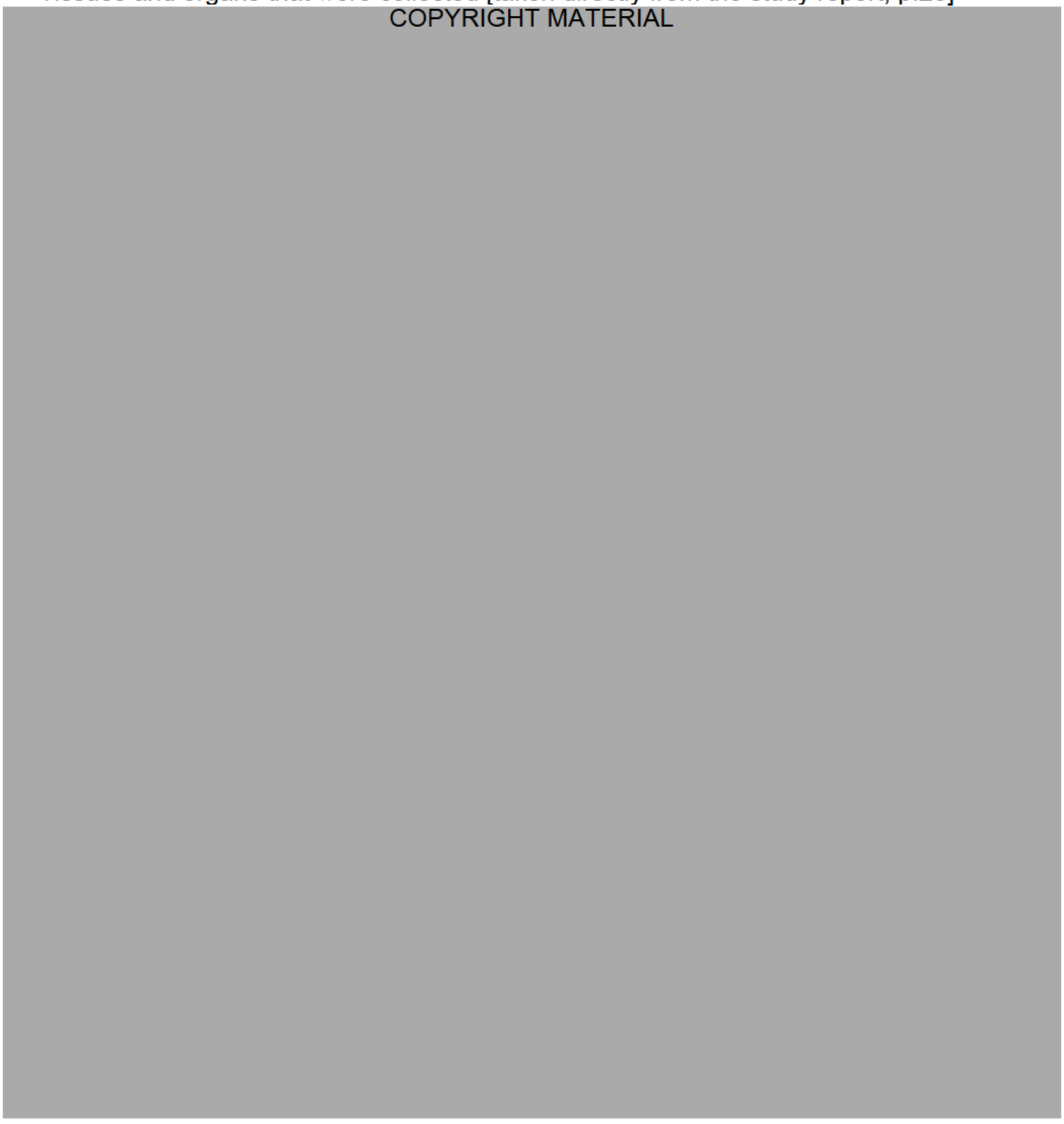



**Gross Pathology**

Complete necropsies were performed on all animals. Animals were initially sedated with an intramuscular injection of acepromazine and then were euthanized by an intravenous injection of sodium pentobarbital and exsanguinated. Tissues and organs listed below were collected and placed in 10% neutral-buffered formalin, except as noted below.

There were no test article-related macroscopic findings.

Tissues and organs that were collected [taken directly from the study report, p.28]




**Organ Weights**

Selected organs were weighed at the scheduled necropsy. Paired organs were weighed together. Organ to final body weight and organ to brain weight ratios were calculated.

Organs that were weighted [taken directly from the study report, p.29]

Changes in certain organ weights that were observed in the test article-dosed groups are presented in Table 13. However, none of these changes are considered test article-related adverse findings because there were no correlated histology findings, and most of changes were observed in one gender or the opposite direction of changes was observed between males and females. Note that decreased ovaries weights were also seen in the 14-day dog study. Although this finding is not considered adverse in the current study due to lack of any correlated histology finding, this finding should be monitored for a longer-term study.

**Table 13 Changes in organ weights from the 3-month dog study**

| Dose Group (mg/kg/day) / Organ | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| Pituitary gland (g) | 0.0679 | 0.0760 (+12%) | 0.0661 (-3%) | 0.0681 (+0.3%) | 0.0438 | 0.0484 (+11%) | 0.0584 (+33%) | 0.0564 (+29%) |
| Pituitary gland/BW | 0.00071 | 0.00075 (+6%) | 0.00065 (-8%) | 0.00069 (-3%) | 0.00062 | 0.00071 (+16%) | 0.00084* (+36%) | 0.00082* (+33%) |
| Thymus (g) | 5.20 | 6.10 (+17%) | 6.75 (+30%) | 7.38 (+42%) | 5.81 | 2.37* (-59%) | 3.62 (-38%) | 3.69 (-37%) |
| Thymus/ BW | 0.053 | 0.058 (+9%) | 0.065 (+23%) | 0.076 (+43%) | 0.082 | 0.035 (-57%) | 0.051 (-38%) | 0.055 (-33%) |
| Thyroids-parathy (g) | 0.6883 | 0.7917 (+15%) | 0.6611 (-4%) | 0.6624 (-4%) | 0.6622 | 0.4447 (-33%) | 0.4383 (-34%) | 0.4927 (-26%) |
| Thyroids-parathy/ BW | 0.007 | 0.008 (+14%) | 0.007 (0%) | 0.007 (0%) | 0.009 | 0.007 (-22%) | 0.006 (-33%) | 0.007 (-22%) |
| Prostate (g) | 8.83 | 6.88 (-22%) | 6.35 (-28%) | 5.53 (-37%) | - | - | - | - |
| Prostate/ BW | 0.089 | 0.066 (-26%) | 0.061* (-32%) | 0.056** (-37%) | - | - | - | - |
| Ovaries (g) | - | - | - | - | 0.9323 | 0.7260 (-22%) | 0.7313 (-22%) | 0.7557 (-19%) |
| Ovaries/ BW | - | - | - | - | 0.013 | 0.011 (-15%) | 0.010 (-23%) | 0.011 (-15%) |
| Uterus-CX (g) | - | - | - | - | 6.68 | 4.23 (-37%) | 3.37 (-50%) | 4.91 (-27%) |
| Uterus-CX / BW | - | - | - | - | 0.096 | 0.063 (-34%) | 0.049 (-49%) | 0.074 (-23%) |

**Histopathology**

Collected tissues listed above were stained with hematoxylin and eosin and then initially examined microscopically from all animals in the control and HD groups. In addition, gross lesions were examined from all animals, including animals in the LD and MD groups.

Upon the information request from the Division, potential test article-related target tissues, pancreas and mandibular salivary gland, were histologically evaluated in the LD and MD groups of males and females, and histology data were submitted as Study (b) (4) 724003 Amendment to the Final Report No. 1 as well as in the Amendment to the Final Pathology Report No. 1.

**Adequate Battery:** Yes

**Peer Review:** Peer review was not included in the original final report, but Peer review was added to the amendment to the final report.

**Histological Findings:**

Potential test article-related histology findings upon the review of the original final report are presented in Table 14.

Several pancreatic findings were noted in the HD group, and these findings were observed in one male and one female. The HD male (Animal 6141) had minimal fibroplasia, mild single cell necrosis, minimal decreased zymogen granules, and minimal acinar cell hyperplasia. The HD female (Animal 6170) had minimal single cell necrosis, mild decreased zymogen granules, and minimal acinar atrophy.

In the mandibular salivary gland, atrophy, fibrosis, and chronic inflammation were observed in the HD male that had pancreatic findings. In addition, 2 HD males and 1 HD female had mononuclear cell infiltrate in the mandibular salivary gland.

Slightly increased incidences of mixed cell or mononuclear cell infiltrate in the stomach and trachea of HD group of males and females were not considered test article-related as the incidences were low.

**Table 14 Histopathology findings in the 3-month dog study (data from the original final report)**

| Dose Group (mg/kg/day) / Tissue | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| | | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| **Pancreas** | | | | | | | | | |
| Atrophy, acinar | Total | 0 | - | - | 0 | 0 | - | - | 1 |
| | min | 0 | | | 0 | 0 | | | 1 |
| Decreased zymogen | Total | 0 | - | - | 1 | 0 | - | - | 1 |
| | min | 0 | | | 1 | 0 | | | 0 |

| Dose Group (mg/kg/day) / Tissue | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| | | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| granules | mild | 0 | | | 0 | 0 | | | 1 |
| Fibroplasia | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | | | 1 | 0 | | | 0 |
| Hyperplasia, acinar cell | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | | | 1 | 0 | | | 0 |
| Necrosis, single cell | Total | 1 | - | - | 1 | 0 | - | - | 1 |
| | min | 1 | | | 0 | 0 | | | 1 |
| | mild | 0 | | | 1 | 0 | | | 0 |
| **Salivary gland, mandibular** | | | | | | | | | |
| Atrophy | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | | | 1 | 0 | | | 0 |
| Fibrosis | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | | | 1 | 0 | | | 0 |
| Infiltrate, mononuclear cell | Total | 0 | - | - | 2 | 0 | - | - | 1 |
| | min | 0 | | | 2 | 0 | | | 1 |
| Inflammation, chronic | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | | | 1 | 0 | | | 0 |
| **Stomach** | | | | | | | | | |
| Infiltrate, mixed cell, submucosal | Total | 0 | - | - | 1 | 0 | - | - | 0 |
| | min | 0 | - | - | 1 | 0 | | | 0 |
| Infiltrate, mononuclear, submucosal | Total | 1 | - | - | 0 | 0 | - | - | 2 |
| | min | 1 | | | 0 | 0 | - | - | 2 |
| **Trachea** | | | | | | | | | |
| Infiltrate, mononuclear cell | Total | 0 | - | - | 1 | 0 | - | - | 1 |
| | min | 0 | - | - | 1 | 0 | - | - | 1 |

Because histology findings in the pancreas and mandibular salivary gland could be test article-related, an information request was sent to the Applicant to perform histological evaluation on these tissues for the LD and MD groups of males and females and to provide historical control incidences for findings (mean and range) in these tissues from the testing laboratory. In addition, an assessment of the toxicologic significance of the pancreatic findings was also requested.

In response to the information request from the Division, the Applicant provided additional histology data of pancreas and mandibular salivary gland in the LD and MD groups and also re-evaluation of these tissues in the HD group, along with a formal pathology peer review of these tissues. Historical control incidences for findings in these tissues from the testing laboratory were also submitted.

Histology data in the pancreas and mandibular salivary gland, which were included in Amendment to the Final Report No. 1, are presented in Table 15. In the pancreas, single cell necrosis was observed in all test article-dosed groups in a dose-independent manner, and one control male also had minimal single cell necrosis. The historical control data provided (Table 16) also had occurrence of single cell necrosis or increased apoptosis although the incidence was low. Overall, the Applicant concluded that the finding of single cell necrosis in the pancreas was not considered test article-related, and the Reviewer agreed with their conclusion.

After finishing the histology evaluation on pancreas and mandibular salivary gland, the study, and peer review pathologists discussed the results and the appropriate terminology and diagnoses were mutually agreed upon, and differences of opinion between the study and reviewing pathologists were resolved with agreement on the final diagnoses. A consensus was reached with both pathologists that findings of acinar cell hyperplasia, decreased zymogen granules, fibroplasia, and acinar atrophy in the pancreas were not present in the sections examined, and these findings were removed from the histology data in the study report amendment. In the Applicant's response, it stated that regarding zymogen granule depletion, a consensus was reached that individual acini and groups of acini were still well-endowed with zymogen granules and the pancreas did not reach a threshold for a finding of zymogen granule depletion. Regarding atrophy and fibroplasia, what was initially called atrophy and fibroplasia in the pancreas was subsequently attributed to cellular variability of the pancreas in dogs in which smaller acini near small pancreatic islets result in a prominent interstitium. In regards to hyperplasia, what was initially called hyperplasia in the pancreas was a staining artifact in a focus of acinar cells.

**Table 15 Histology findings in the pancreas and mandibular salivary gland from Amendment to the Final Report No. 1**

| Dose Group (mg/kg/day) / Tissue | | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| | | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| **Pancreas** | | | | | | | | | |
| Necrosis, single cell | Total | 1 | 2 | 2 | 1 | 0 | 2 | 1 | 1 |
| | min | 1 | 2 | 2 | 0 | 0 | 2 | 1 | 1 |
| | mild | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| **Salivary gland, mandibular** | | | | | | | | | |
| Atrophy | Total | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | min | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Fibrosis | Total | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | min | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Infiltrate, mononuclear cell | Total | **2** | 2 | 2 | 2 | 0 | 2 | 2 | 1 |
| | min | **2** | 2 | 2 | 2 | 0 | 2 | 2 | 1 |
| Inflammation, chronic | Total | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| | min | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

For the findings of minimal chronic inflammation, minimal fibrosis, and minimal atrophy that were reported in the mandibular salivary gland from one HD male, the Applicant concluded that these findings were not considered test article-related. The Applicant stated that this constellation of findings has been observed as a background finding in dogs and provided two references about these findings. In the provided reference by (Sato, et al., 2012), it states that focal fibrosis with atrophy and loss of the acini is frequently seen in salivary gland (especially in the parotid gland) of the beagle dogs, and focal inflammatory cell infiltration, occasionally accompanied by lymph follicle formation is also common around the ducts or acini of salivary gland of the beagles. Mild focal chronic inflammation is quite a common incidental finding in canine salivary glands, which has been reported in about 5% of normal beagle dogs (Greaves, 2007).

**Table 16 Historical Control Data of Beagle Dogs (b) (4)**
**(Range of Study Dates: 2/10-11/13, Age: 16-67 weeks)**

| | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|
| | | Total occurrence | Total tissue examined | % | Total occurrence | Total tissue examined | % |
| **Pancreas** | | | | | | | |
| Apoptosis, increased | total | 1 | 95 | 1.05 | 1 | 96 | 1.04 |
| | min | 1 | | | 0 | | |
| | mild | 0 | | | 1 | | |
| Atrophy, acinar | total | 0 | 95 | 0 | 2 | 96 | 2.08 |
| | min | 0 | | | 2 | | |
| Infiltrate, mononuclear | total | 3 | 95 | 3.16 | 0 | 96 | 0 |
| | min | 3 | | | 0 | | |
| Necrosis, single cell | total | 1 | 95 | 1.05 | 1 | 96 | 1.04 |
| | min | 1 | | | 1 | | |
| **Mandibular salivary gland** | | | | | | | |
| Exudate, inflammatory | total | 1 | 98 | 1.02 | | | |
| | min | 1 | | | | | |
| Infiltrate, mononuclear | total | 8 | 98 | 8.16 | 13 | 98 | 13.44 |
| | min | 8 | | | 12 | | |
| | mild | | | | 1 | | |
| Inflammation, acute | total | 0 | 98 | 0 | 1 | 98 | 1.02 |
| | mild | 0 | | | | | |
| Inflammation, subacute | total | 2 | 98 | 2.04 | 0 | 98 | 0 |
| | min | 2 | | | 0 | | |
| Necrosis, coagulative | total | 0 | 98 | 0 | 1 | 98 | 1.02 |
| | mild | 0 | | | 1 | | |

Overall, the Applicant concluded that there were no test article-related histology findings in the study, and the Reviewer agreed with the Applicant's conclusion.

In addition to histology evaluation on the pancreas and mandibular salivary gland of the LD and MD groups, the Division also requested the Applicant to conduct histological evaluation on all other tissues for the LD and MD groups because it was unknown whether all dogs were adequately exposed to the test article as it was intended, and it could not conclude that animals in the HD group were exposed to a higher level of the test article than animals in the MD or LD groups for the following reasons. First, analyses of the test article formulations and toxicokinetic parameters were not included in the study. Second, emesis findings were noted in all dose groups, with higher incidences in the control and HD groups.

Instead of performing histology evaluation on all tissues for the LD and MD groups, the Applicant analyzed the incidence of emesis in a greater detail and has concluded that the actual influence of emesis on exposure was much lower than it may appear. See below the Special Evaluation section for details.

**Special Evaluation (Applicant's Response to the Information Request Regarding the Emesis Occurrences in the Study)**

As mentioned in the last part of the Histopathology section above, the Division was concerned whether all dogs were adequately exposed to the test article as it was intended, and it could not conclude that animals in the HD group were exposed to a higher level of the test article than animals in the MD or LD groups due to high incidences of emesis in the control and HD groups and lack of TK data, which could verify the systemic exposure to the test article. Moreover, dosing formulation analyses were not carried out in the study.

In the response to the Division's concern, the Applicant analyzed the incidence of emesis in greater detail and has concluded that the actual influence of emesis on exposure was lower than it may appear based on the summary tables in the study report for the following two reasons. First, due to the way emesis findings were recorded, multiple emetic findings (i.e., emesis containing white material, emesis containing food, emesis containing yellow material, etc.) for one episode of emesis could have been interpreted as multiple emetic events. Table 17 presents number of doses associated with emesis observation in the study. Second, based on the detailed observations made with emesis events (such as the presence of capsule or yellow material), only a subset of the emetic events were likely to contain test article. On analyzing the number of doses associated with emesis observations containing capsule material and emesis containing yellow material, the percent of doses associated with these events was reduced relative to the overall incidence of emesis (Table 17). The mean numbers of doses associated with emesis containing yellow or capsule material were 3.5, 1.5, 2.5, and 7 for the control, LD, MD, and HD males, respectively, and 5, 2.75, 3.75, and 10.75 for the control, LD, MD, and HD females, respectively. Based on this analysis, despite the high incidence of emesis, the Applicant concluded that all groups were adequately exposed during the study. Furthermore, since the HD group was administered significantly higher test article than the MD and LD groups (2000 mg/kg/day versus 1000 and 500 mg/kg/day, respectively), the observed frequency of emesis in the HD group would not have resulted in a lower exposure than

the MD or LD group. For instance, the maximum number of doses associated with emesis in individual HD animals was 33, which was equivalent to 18.3% of the total number of doses (this is below 50%) and thus, this animal was still exposed to a higher amount than in the MD animals. Overall, the Applicant did not believe that the data warrant additional histological evaluation on all tissues from the MD and LD groups and additional histological evaluation was not conducted. This 90-day toxicity study (without dosing formulation and TK analyses, and with histology evaluation, mainly performed on the control and HD tissues, along with frequent occurrences of emesis) is not optimal, but the Reviewer accepts the Applicant's justification and considers that it is not necessary to conduct another 90-day toxicity study or additional histology evaluation on tissues from the LD and MD groups. However, as mentioned in the Novel Excipient section above, due to lack of adequate chronic toxicity assessment of beeswax and carnauba wax, the Reviewer recommends a 9-month toxicity dog study using a beeswax/carnauba wax (b) (4)

**Table 17 Number of doses associated with emesis observation in the 90-day dog study**

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| | 0 | 500 | 1000 | 2000 | 0 | 500 | 1000 | 2000 |
| No of doses with Emesis[a] | 10.5 (5.8%) | 3.5 (1.9%) | 7.5 (4.2%) | 15.25 (8.5%) | 14.75 (8.2%) | 5 (2.8%) | 10.75 (6.0%) | 23 (12.8%) |
| No of doses with Emesis[b] | 3.5 (1.9%) | 1.5 (0.8%) | 2.5 (1.4%) | 7 (3.9%) | 5 (2.8%) | 2.75 (1.5%) | 3.75 (2.1%) | 10.75 (6.0%) |

Total number of doses: 180 doses (BID)
[a]Emesis containing food, white material, yellow material, and/or capsule material
[b]Emesis containing only yellow material or capsule material

**Toxicokinetics**

Toxicokinetic analysis was not performed in the study. As the test article [beeswax/ carnauba wax (b) (4) consisted of various constitutes, it would be challenging to measure plasma concentrations of the test article.

**Dosing Solution Analysis**

Analyses of the test article formulations were not conducted for this study. The test article supplied by Collegium was administered in neat capsules, without any further modification.

The report of test article preparation and homogeneity prepared by Collegium states that the test article for the toxicity study in dogs was (b) (4) beeswax and

carnauba wax with a median size of approximately (b) (4) micron (micrometer) (b) (4) i.e. beeswax and carnauba wax present (b) (4) with a median size of approximately (b) (4) micron. (b) (4)

## Appendix 2: Review of Published Toxicity Studies with Carnauba Wax along with Information on Structural Constituents of Carnauba Wax

**Table 18 Structural Constituents of Carnauba Wax [Information from (Vandenburg and Wilder, 1970) and (JECFA, 1998)]**

| Composition | Amount | Description |
|---|---|---|
| Aliphatic esters | 38-40% | Straight-chain acids with even-numbered carbon chains from C24 to C28 and straight-chain alcohols with even-numbered carbon chains from C30 to C34 |
| ω-Hydroxy aliphatic esters | 12-14% | Straight-chain hydroxy acids with even-numbered carbon chains from C22 to C28, straight-chain acids with even-numbered carbon chains from C24 to C28, straight-chain monohydric alcohols with even-numbered carbon chains from C24 to C34 and dihydric alcohols with even-numbered carbon chains from C24 to C34 |
| Cinnamic aliphatic esters | 25-30% | (1) p-hydroxycinnamic aliphatic diesters (p-hydroxycinnamic acid and dihydric alcohols with even-numbered carbon chains from C24 to C34; 20-23%)<br>(2) p-methoxycinnamic aliphatic diesters (p-methoxycinnamic acid and dihydric alcohols with even-numbered carbon chains from C24 to C34; 5-7%) |
| Free alcohols | 10-12% | Straight-chain alcohols with even-numbered carbon chains from C30 to C34 |
| Free acids | 5-7% | Straight-chain acids with even-numbered carbon chains from C24 to C28 |
| Hydrocarbons | 0.3-1% | Straight-chain odd-numbered carbon chains from C27 to C31 |
| Triterpene diols | 0.4% | |

**Table 19 Studies Reviewed for Carnauba Wax**

| No. | Title | Reference |
|---|---|---|
| General Toxicology | | |
| CW-1 | Short-term toxicity study of carnauba wax in rats | Rowland et al., 1982 |
| CW-2 | Subchronic feeding study of carnauba wax in beagle dogs | Parent et al., 1983 |
| Reproductive and Developmental Toxicology | | |
| CW-3 | Reproduction and subchronic feeding study of carnauba wax in rats | Parent et al., 1983 |

**Publication Title: Short-term toxicity study of carnauba wax in rats (Rowland, et al., 1982)**

**Methods:** Groups of Wistar rats (15/sex/group; 5 weeks at the study initiation, body weight not specified) were fed diets containing 0, 1, 5, or 10% carnauba wax (CW) or 10% cellulose powder (CP) for 13 weeks. Additionally, groups of Wistar rats (5/sex/group) were fed diets containing 0, 5, or 10% carnauba wax or 10% cellulose powder (CP) for 2 or 6 weeks. The calculated mean intakes of carnauba wax were 0.8, 4.2, and 8.8 g/kg/day for males and 0.9, 4.6, and 10.2 g/kg/day for females for the 1, 5, and 10% CW diets, respectively, over the 13-week period.

The carnauba wax was supplied by Rowntree Mackintosh Ltd, York, and complied with the Food Chemicals Codex (1972) specification.

Body weight and food and water consumption were recorded at 2-5 days intervals up to Day 14 and weekly thereafter.

At the end of each dosing period, blood samples were collected for hematology evaluation, which consisted of the following parameters: hemoglobin concentration, packed cell volume, erythrocyte count, total leukocyte counts, and differential leukocyte counts (at the end of 2- and 6-week feeding periods only).

At the end of each feeding period, the blood samples were collected for serum chemistry evaluation, which consisted of the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactic dehydrogenase.

At the end of each feeding period, the urine samples were collected for urinalysis, which consisted of the following parameters: specific gravity and volume for 0-6 hour and 16-20 hour intervals. Microscopic constituents and the semi-quantitative estimation of protein, glucose, bile salts, ketones, and blood were also included. In addition, at the end of 6- and 13-week feeding periods, the same measurements were made on urine collected in the first 2 hours after a water load of 25 mL/kg, and the number of cells in urine was also counted at Week 13.

At the end of each feeding period, animals were sacrificed by an intravenous injection of pentobarbital. A complete macroscopic examination was performed for all animals and the following organs were weighted: adrenal glands, brain, gonads, heart, kidneys, liver, pituitary gland, spleen, thyroid gland, stomach, small intestine, and cecum. Histopathology was evaluated the following organs: adrenal glands, cecum, colon, esophagus, brain, eyes, gonads, Harderian gland, heart, kidneys, liver, lungs, lymph nodes, mammary gland, nerves, pancreas, pituitary gland, prostate, rectum, salivary gland, seminal vesicle, skeletal muscle, small intestine, spinal cord, spleen, stomach, trachea, thymus, thyroid gland, urinary bladder, and uterus. Paraffin-wax sections of the tissues from half the control rats and from those given 10% CW or 10% CP were stained with hematoxylin and eosin for histopathology examination.

**Results:**

Clinical signs: A table on clinical observations is not included in the paper. It states that there were no deaths, and rats appeared normal throughout the study.

Body weights and food and water consumption: A table with body weight and food and water consumption is presented in the paper. There were no treatment-related effects on body weight and water consumption.

In male and female groups fed 10% CW or 10% CP had slightly higher mean food consumption, compared to the control group (18.1, 20.5, and 21.2 g/rat/day for males in the control, 10% CW, and 10% CP groups, respectively; 14.6, 16.1, and 15.9 g/rat/day for females in the control, 10% CW, and 10% CP groups, respectively).

Hematology: A summary table on hematology data from the control and 10% CW groups on Weeks 2, 6, and 13 is included in the paper, and there were no carnauba wax-related effects.

On Week 2, there was a slight, but statistically significant increase in mean red blood count in the male 10% CW group, compared to the control group, but the change was not observed in females and also in other time points. Thus, the increase was considered incidental.

Serum chemistry: No table on serum chemistry data is included in the paper. It states that there were similar activities of the serum enzymes between control and test article-treated groups.

Urinalysis: A urinalysis table with specific gravity, volume, and urinary cells from the control and 10% CW groups on Weeks 2 and 13 is included in the paper. There were no carnauba wax-related effects on urinalysis parameters presented in the table. It also states that bile, blood, glucose, and ketones were not present in urine on Weeks 2, 6, and 13, and the concentrations of albumin were similar in all groups.

Gross examination: No table on macroscopic findings is included in the paper. It states that there were no carnauba wax-related macroscopic findings.

Organ weights: A table on relative organ weights from the control, 10% CW, and 10% CP groups is presented in the paper.

There were no carnauba wax-related changes in organ weights. A decrease in mean pituitary gland weight (relative to body weight) was seen in the male 10% CW group on Week 6 (-31% relative the control group), however, the change was considered incidental because no decrease was noted in 10% CW-fed females on Week 6 and 10% CW-fed males and females on Week 13.

Histopathology examination: No histology table is included in the paper. It states that there were no carnauba wax-related histology findings.

It states that inflammatory cell infiltration and focal necrosis in the liver and interstitial pneumonitis in the lung were noted, but the incidence and severity of these lesions were comparable between groups.

**Publication Title: Reproduction and subchronic feeding study of carnauba wax in rats (Parent, et al., 1983b)**

**Methods:** Groups of 25 male and 25 female Wistar rats (age not specified, weighing 220-225 g for males and 150-175 g for females at the time of arrival) were fed diets containing 0, 0.1 (LD), 0.3 (MD), or 1% (HD; w/w) carnauba wax for 4 weeks, and then a single male and a single female from the same group were cohabited for 7 days or until confirmed mating. Female rats that did not have confirmed mating by the 7$^{th}$ day were cohabited with second males of the same group for additional 7 days. The procedure was repeated for a third time, and females that did not then become pregnant were considered infertile.

The carnauba wax used in this study was obtained from Frank B. Ross Co. Inc., Jersey City, NJ and was incorporated into the diet using a Hobart blender.

F0 animals:

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly for the F0 animals, except during mating. F0 females were weighed on Gestation Days (GDs) 0, 6, 11, 15, and 20.

F1 offspring:

Total litter weights were recorded at birth and on Postnatal Days (PNDs) 4, 14, and 21. The number of pregnant females, number of alive or dead pups born, and survival of the offspring were recorded. On PND 4, each litter culled to 10 pups with equal numbers of

males and females if possible. On PND 21, at least one but not more than 2 male and 2 female F1 animals per litter were randomly selected, and these animals were fed diets containing 0, 0.1 (LD), 0.3 (MD), or 1% (HD) carnauba wax for 13 weeks. Mean intake of carnauba wax calculated on the basis of food consumption over the 13 weeks was 0.08, 0.25, and 0.81 g/kg/day for males and 0.09, 0.27, and 0.67 g/kg/day for females at levels of 0.1, 0.3, and 1.0%, respectively.

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly for the F1 animals. Ophthalmic examinations were performed on F1 animals at the time of weaning and at the end of the 13-week feeding period.

Blood samples for clinical pathology evaluation were collected from F1 animals at weaning using 10 animals/sex/group that were not selected to continue the 13-week feeding group, and on then randomly selected F1 animals/sex/group after 6 and 13 weeks of feeding.

The hematology evaluation consisted of the following parameters: hematocrit, erythrocyte count, total and differential leukocyte counts, prothrombin time, and levels of hemoglobin.

The serum chemistry evaluation consisted of the following parameters: glucose, urea nitrogen, protein, bilirubin, sodium, potassium, chloride, total free fatty acids, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase.

A complete macroscopic examination was performed for all animals (F0 animals and F1 animals). However, organ weight and histopathological examinations were performed in only F1 animals that were fed diets containing carnauba wax for 13 weeks. The following organs were weighted: adrenal glands, epididymides, heart, liver, kidneys, ovaries, spleen, testes, thyroid/parathyroid gland, and uterus. Histopathology was evaluated in the control and HD groups of F1 animals, and the following organs histologically evaluated: adrenal glands, brain, epididymides, eyes, heart, kidneys, large intestine, liver, lungs, lymph nodes (mesenteric), ovaries, pancreas, pituitary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, small intestine, spinal cord, spleen, sternum and marrow, stomach, testes, thyroid/parathyroid gland, urinary bladder, uterus, and grossly abnormal tissues. For the LD and MD groups, only grossly abnormal tissues were histologically examined.

**Results:**

F0 and F1 animals:

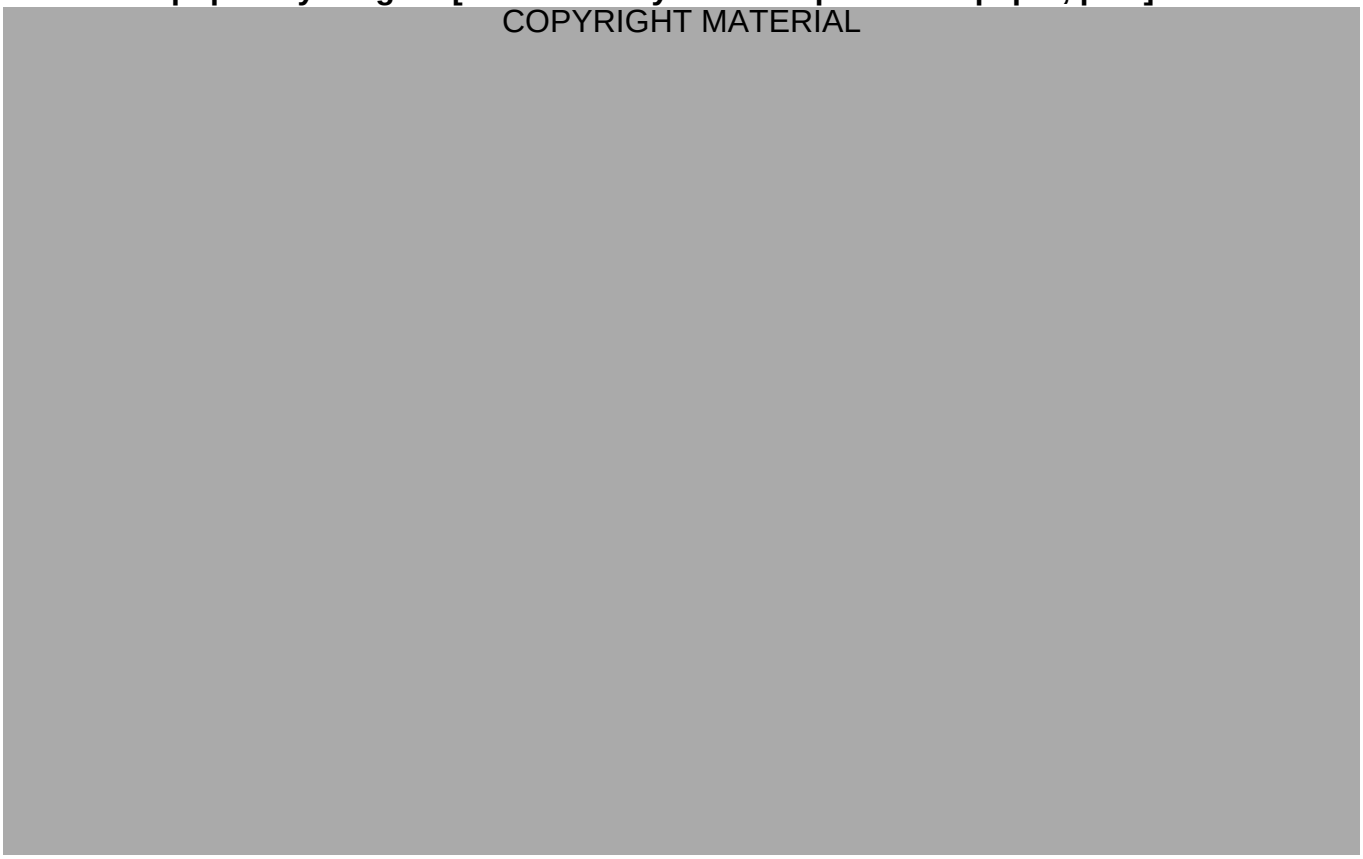
Clinical signs: A table of clinical observations is not included in the paper. It states that there were no carnauba wax-related adverse clinical signs.

Body weights and food consumption: There are no tables on body weight and food consumption in the paper, except F0 pup body weight until PND 21 (see Table 20). It states that body weights in the carnauba wax-fed groups were comparable to those in the control group. It also states that intermittent significant differences in food consumption were noted among groups of males and females, but no consistent or significant pattern was noted.

Reproduction: Reproductive data are shown in Table 20, and there were no effects of carnauba wax on reproductive parameters of F1 females.

**Table 20 Reproductive assessment of F0 rats fed diets containing carnauba wax and F1 pup body weights [taken directly from the published paper, p.90]**





F1 offspring:

Ophthalmic examination: No table on ophthalmic findings is included in the paper. It states that there was no carnauba wax-related finding.

Hematology: A summary table on hematology data after 90 days of feeding is included in the paper. There were no test article-related effects on hematology parameters in F1 animals fed diets containing carnauba wax for 90 days. It also states that there were no carnauba wax-related effects on hematology parameters after 45 days of feeding.

Serum chemistry: A summary table on serum chemistry data after 90 days of feeding is included in the paper. Mean free fatty acid levels were decreased in the MD and HD groups of F1 males and females, whereas cholesterol levels were increased in all carnauba wax-fed groups of F1 males and females in a dose-dependent manner (Table 21).

A slight increase in blood urea nitrogen in the HD group of males and females and a dose-independent decrease in ALT in the carnauba wax-fed groups of males were not considered test article-related since the changes were noted in only one gender or were small in magnitude.

**Table 21 Changes in serum chemistry parameters of F1 rats fed diets containing carnauba wax for 90 days**

| Dose Group (% in diet) / Parameter | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.1% | 0.3% | 1.0% | 0 | 0.1% | 0.3% | 1.0% |
| Cholesterol (mg/dL) | 75 | 92 (+23%) | 93 (+24%) | 96 (+28%) | 102 | 116 (+14%) | 117 (+15%) | 121 (+19%) |
| Free fatty acid (mcM/L) | 906 | 814 (-10%) | 577 (-36%) | 554 (-39%) | 770 | 812 (+5%) | 493 (-36%) | 575 (-25%) |
| BUN (mg/dL) | 17.2 | 17.2 | 17.9 | 20.1 (+17%) | 19.0 | 20.0 | 19.0 | 21.0 (+11%) |
| ALT (RF units) | 26.2 | 23.4 (-11%) | 20.0 (-24%) | 21.9 (-16%) | 24.0 | 21.0 | 21.0 | 23.0 |

Gross examination: No table on macroscopic findings is included in the paper. It states that there was no carnauba wax-related macroscopic finding in F0 and F1 animals.

Organ weights: A table on relative organ weights is presented in the paper.

There were no test article-related changes in mean organ weights of F1 animals.
A high mean uterus weight was observed in the HD F1 female group, compared to the control group (+157% [0.59% of body weight in the HD group vs. 0.23% of body weight in the control group]), however, without any other changes, the toxicological significance of the increase is unknown.

Histopathology examination: No table on histopathological findings is included in the paper. It states that there were no carnauba wax-related histology findings.

**Publication Title: Subchronic feeding study of carnauba wax in beagle dogs (Parent, et al., 1983a)**

**Methods:** Groups of 6 male and 6 female beagle dogs (4-7 months old; weights not specified) were fed diets containing 0, 0.1 (LD), 0.3 (MD), or 1% (HD; w/w) carnauba wax for 28 consecutive weeks.

Carnauba wax (Lot No. 5470) was obtained from the Frank B. Ross Co., Jersey City, NJ. Carnauba wax was mixed dry with Purina Dog Meal using a Hobart blender. Diets were prepared weekly and stored at -4°C until used. All diets were analyzed for carnauba wax concentration by extraction with chloroform, fractionation using methanol chloroform mixtures, and subsequent gravimetric analysis of the extract.

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly.

Gross eye examinations and blood and urine sample collection for clinical pathology evaluation were carried out prior to dosing, after 11 and 26 weeks of feeding.

The hematology evaluation consisted of the following parameters: hematocrit, erythrocyte count, total and differential leukocyte counts, prothrombin time, and levels of hemoglobin.

The serum chemistry evaluation consisted of the following parameters: glucose, urea nitrogen, total fatty acids, cholesterol, total protein, albumin, globulin, and activities of alkaline phosphatase and alanine aminotransferase (ALT).

The urinalysis consisted of the following parameters: specific gravity, pH, glucose, albumin, ketones, and microscopic elements.

After 28 weeks of feeding, all animals were sacrificed by an intravenous injection of pentobarbital. A complete macroscopic examination was performed for all animals and the following organs were weighted: adrenal glands, brain, heart, kidneys, liver, pituitary gland, and thyroid gland. Histopathology was evaluated the following organs: adrenal glands, aorta, bone marrow, brain, eyes, gall bladder, gonads, heart, kidneys, large intestine, liver, lungs, lymph nodes, mammary gland, pancreas, pituitary gland, prostate, small intestine, spinal cord, spleen, stomach, thyroid gland, urinary bladder, uterus, and grossly abnormal tissues.

**Results:**

Clinical signs: A table on clinical observations is not included in the paper. It states that there were no carnauba wax-related adverse clinical signs.

Body weights and food consumption: There are no tables on body weight and food consumption in the paper. It states that body weight and food consumption in the carnauba wax-fed groups were comparable to those in the control group.

Ophthalmic examination: No table on ophthalmic findings is included in the paper. It states that there was no carnauba wax-related finding.

Hematology: A summary table on hematology data after 26 weeks of feeding is included in the paper, and there were no test article-related effects. It states that there were no carnauba wax-related effects after 11 weeks of feeding.

Serum chemistry: A summary table on serum chemistry data after 26 weeks of feeding is included in the paper. After 26 weeks, mean free fatty acid levels were increased in all carnauba wax-fed groups of males, but the increase was not dose-related (Table 22). Females in the LD group exhibited increased mean free fatty acid levels, but females in the MD and HD groups had decreased levels after 26 weeks. It states that observations made after 11 weeks of feeding were comparable between groups.

**Table 22 Changes in serum chemistry parameter from the 28-week dog study**

| Dose Group (% in diet) / Parameter | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.1% | 0.3% | 1.0% | 0 | 0.1% | 0.3% | 1.0% |
| Free fatty acid (mcM/L) | 138 | 412 (+199%) | 325 (+136%) | 317 (+130%) | 250 | 327 (+31%) | 198 (-21%) | 223 (-11%) |

Urinalysis: No table on urinalysis is included in the paper. It is states that there were no carnauba wax-related effects on urinalysis parameters.

Gross examination: No table on macroscopic findings is included in the paper. It states that there were no carnauba wax-related macroscopic findings.

Organ weights: A table on relative organ weights is presented in the paper.

There were no carnauba wax-related changes in organ weights. A slight decrease in adrenal gland weights was seen in all carnauba wax-fed groups of males and females, however, the changes were not considered test article-related due to the small changes with lack of a dose-response relationship.

**Table 23 Changes in relative organ weights (% of body weight) from the 28-week dog study**

| Dose Group (% in diet) / Parameter | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 0.1% | 0.3% | 1.0% | 0 | 0.1% | 0.3% | 1.0% |
| Adrenals | 0.0128 | 0.0114 (-11%) | 0.0115 (-10%) | 0.0101 (-21%) | 0.0150 | 0.0132 (-12%) | 0.0134 (-11%) | 0.0137 (-8.7%) |

Histopathology examination: No histology table is included in the paper. It states that there were no carnauba wax-related histology findings. The most common histology finding was slight to mild chronic enteritis as evidenced by slight congestion, prominence of lymphocytes and plasma cells in the lamina propria, and occasional hyperplasia of the lymphoid tissue. It also states that the incidence of this lesion was comparable between groups.

## Appendix 3: Review of Published Toxicity Studies with Main Components of Beeswax

The Applicant conducted the literature-based safety assessment of beeswax using available toxicity data of major components of beeswax (by using the compounds extracted from beeswax or structurally similar compounds), and the following compounds were used. The information was obtained from the Applicant's submission.

(1) Fatty acid esters: Saturated alkyl palmitates (C38 – C52) and unsaturated alkyl esters of oleic acid (C46 – C54) constituting the predominant structures
- Jojoba oil: 97% (w/w) plant wax esters of monounsaturated, straight-chain acids and alcohols with high molecular weights (C16-C24-26)
- Oleyl palmitate: C34 ester of palmitic acid and oleyl alcohol
- Carnauba wax: Complex mixture of compounds with a fatty acid ester fraction that corresponds to beeswax fatty acid esters

(2) Long-chain fatty acids: Unbranched saturated molecules with even carbon numbers from C20 to C36 with tetracosanoic acid (C24) as the most abundant free fatty acid in *A. mellifera* beeswax
- D-003: A mixture of very long-chain fatty acids, isolated from sugar cane wax, in which octacosanoic acid (C28) represents the major component followed by triacontanoic acid (C30), dotriacontanoic (C32), and etratriacontanoic acid (C34)

(3) Hydrocarbons (n-alkanes and n-alkenes): Second major constituents of beeswax, with odd chain n-alkanes (C23 – C31) predominating
- Class 1 mineral oil and high-melting point microcrystalline waxes: characterized by a high content of ≥ C25 hydrocarbons, corresponding with the beeswax hydrocarbon fraction in which chain lengths higher than C25 account for more than 98% of total hydrocarbon content.

(4) Long-chain fatty alcohols: Free fatty alcohols with C33 (0.3% - 1.8%) and C35 (0.3%) alcohols
- D-002: A mixture of high molecular-weight aliphatic alcohols, extracted from beeswax, which composed roughly of 27% triacontanol (C30), 17% octacosanol (C28), 17% dotriacontanol (C32), 15% hexacosanol (C23), 13% tetracosanol (C24), and 2% tetratriacontanol (C34)
- Policosanol: A mixture of long-chain primary aliphatic alcohols, isolated from sugar cane wax, containing C28 octacosanol (66%), C30 tricontanol (12%), C26 hexacosanol (7%), and other minor alcohols
- *Note that the carbon lengths of long-chain fatty alcohols in D-002 and policosanol are not corresponding to those in beeswax even though D-002 is extracted from beeswax*.

Published papers submitted by the Applicant were reviewed (Table 24) and the summarized below. For general toxicology studies, longer-term studies were reviewed, but shorter-term studies were not reviewed unless the data were relevant to make appropriate assessment of beeswax or its components.

**Table 24 Published toxicology studies submitted by Collegium for safety assessment of beeswax**

| Study Type \ Constituent | | (1) Fatty acid esters (57%) | (2) Long chain fatty acids (18%) | (3) Hydrocarbons (15%) | (4) Long chain fatty alcohols (<1%) |
|---|---|---|---|---|---|
| General Toxicology | Rat (Rodent) | (1) Refined jojoba oil, 7-day<br>(2) Oley palmitate, 14- & 28-day<br>(3) Carnauba wax, 90-day CW-1 | (1) D-003, acute & 90-day<br>(2) D-003, 6-month BW-1 | (1) Low-melting point paraffin wax or mineral oil, 28- & 90-day, (summary)<br>(2) Mineral oil class I, 12-month (summary) BW-8 | (1) D-002, 14-day, 90-day, 12-month BW-2<br>(2) Policosanol, 12-month BW-4 |
| | Dog (Non-rodent) | (1) Carnauba wax, 7-month CW-3 | ND | ND | (1) D-002, 12-month **BW-3**<br>(2) Policosanol, 12-month **BW-5** |
| Carcino-genicity | Rat | ND | (1) D-003, 24-month **BW-6** | (1) Mineral oil class I, 24-month (summary) BW-8<br>(2) Composite mineral oil, 24-month (summary) BW-9 | (1) Policosanol, 24-month BW-10 |
| | Mouse | ND | (2) D-003, 18-month **BW-7** | ND | (1) Policosanol, 18-month BW-11 |

| Study Type \ Constituent | | | (1) Fatty acid esters (57%) | (2) Long chain fatty acids (18%) | (3) Hydrocarbons (15%) | (4) Long chain fatty alcohols (<1%) |
|---|---|---|---|---|---|---|
| Repro and Develop Toxicology | FEED (Rat) | | (1) Carnauba wax with 13-week treatment of F1 offspring CW-2 | (1) D-003 BW-12 | ND | (1) Policosanol BW-15 |
| | EFD | Rat | ND | (1) D-003 BW-12, BW-13 | ND | (1) D-002 BW-15<br>(2) Policosanol BW-16 |
| | | Rabbit (second species) | ND | (1) D-003 BW-12 | ND | (1) D-002 BW-15<br>(2) Policosanol BW-16 |
| | PPND (Rat) | | (1) Carnauba wax CW-2 | (1) D-003 BW-14 | ND | (1) Policosanol BW-16, BW-17, BW-18 |

ND = No Data
FEED = Fertility and early embryonic development; EFD = Embryo-fetal development; PPND = Pre- and postnatal development
Study titles for corresponding superscripted annotations can be found in Tables 19 and 25.

**Table 25 Published Studies Reviewed for Beeswax**

| | No. | Title | Reference |
|---|---|---|---|
| General Toxicology | | | |
| Long chain fatty acid | BW-1 | Six-month toxicity study of oral administration of D-003 in Sprague Dawley rats | Gámez et al., 2002 |
| Hydrocarbon | BW-8 | 12-month toxicity and 2-year carcinogenicity feed study in rats using P70(H) and P100(H) mineral oils- Summarized info | Vavasour and Chen, WHO Food Additive 50 |
| Long chain fatty alcohol | BW-2 | Preclinical oral toxicology in rats of D-002, a natural drug with antiulcer effects | Rodeiro et al., 1998 |
| | BW-3 | One-year dog toxicity study of D-002, a mixture of aliphatic alcohols | Alemán et al., 2001 |
| | BW-4 | A 12-month study of policosanol oral toxicity in Sprague Dawley rats | Alemán et al., 1994 |
| | BW-5 | Toxicity of policosanol in beagle dogs: one-year study | Mesa et al., 1994 |

| | No. | Title | Reference |
|---|---|---|---|
| Carcinogenicity | | | |
| Long chain fatty acid | BW-6 | Long-term carcinogenicity of D-003, a mixture of high molecular weight acids from sugarcane wax, in Sprague Dawley rats: A 24 months study | Gámez et al., 2007 |
| | BW-7 | Study of the long-term carcinogenicity potential of D-003, a mixture of high molecular weight sugarcane wax acids, in mice | Noa et al., 2009 |
| Hydrocarbon | BW-8 | 12-month toxicity and 2-year carcinogenicity feed study in rats using P70(H) and P100(H) mineral oils- Summarized info | Vavasour and Chen, WHO Food Additive 50 |
| | BW-9 | 2-year carcinogenicity feed study in rats using composite medium-viscosity white mineral oil Summarized info | |
| Long chain fatty alcohol | BW-10 | Carcinogenicity of policosanol in mice: an 18-month study | Alemán et al., 1995 |
| | BW-11 | Carcinogenicity of policosanol in Sprague Dawley rats: a 24-month study | Alemán et al., 1994 |
| Reproductive and Developmental Toxicology | | | |
| Long chain fatty acid | BW-12 | Evaluation of the reproductive and developmental toxicity of the D-003, a mixture of long-chain fatty acids, in rats and rabbits | Rodríguez et al., 2004 |
| | BW-13 | Lack of developmental toxicity of D-003: a mixture of long-chain fatty acids in rats | Rodríguez et al., 2003 |
| | BW-14 | Perinatal/postnatal study of D-003, a mixture of long-chain fatty acids, in rats | Rodríguez et al., 2006 |
| Long chain fatty alcohol | BW-15 | Developmental toxicity of D-002 (a mixture of aliphatic primary alcohols) in rats and rabbits | Rodríguez et al., 1998 |
| | BW-16 | Teratogenic and reproductive studies of policosanol in the rat and rabbit | Rodríguez and García, 1994 |
| | BW-17 | Evaluation peri- and post-natal toxicity of policosanol in rats | Rodríguez and García, 1998 |
| | BW-18 | Multigeneration reproduction study of policosanol in rats | Rodríguez et al, 1997 |

# General Toxicology

## (1) Fatty Acid Esters

See the review of studies of carnauba wax in Appendix 2.

## (2) Long-Chain Fatty Acid

**Publication Title: Six-month toxicity study of oral administration of D-003 in Sprague Dawley rats (Gamez, et al., 2002)**

**Methods:** Groups of Sprague Dawley rats (20/sex/group; 6-8 weeks old, weighing 160-220 g at arrival; a 2-week acclimation period) were administered D-003 at oral (gavage) doses of 0 (acacia gum/water), 250 (LD), 500 (HD), or 1000 (HD) mg/kg/day (volume of 5-10 mL/kg) for 6 months. In addition, 14 rats/sex/group for the control and HD groups were included to assess the reversibility of the D-003 effects after the 30-day recovery period. These recovery rats were also used to assess the effects of D-003 on bleeding time. It states that the study was conducted by Cuban GLP.

D-003 was obtained from the Chemistry Department of the Center of Natural Products, Havana City, Cuba, and its purity checked by gas chromatography. D-003 was suspended in acacia gum prepared in distilled water (10 mg of acacia gum/ml water), and the dosing formulations were prepared weekly.

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly.

Blood samples were collected for hematology evaluation after 6 months of dosing, and the evaluation included following: hemoglobin, hematocrit, lymphocytes, neutrophils, monocytes, eosinophils, and total leukocyte counts.

Blood samples were collected for serum chemistry evaluation after 6 months of dosing, and the evaluation included following: glucose, cholesterol, triglycerides, creatinine, uric acid, urea and the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), alkaline and acid phosphatases, as well as acetylcholinesterase.

Platelet aggregation and bleeding time were determined in the recovery groups of males and females (14/sex/group). It states that coagulation factors (prothrombin time [PT] and kaolin-activated thromboplastin-time [KPT]) were determined in eight male main study animals as these factors tend to be highly variable in females. Bleeding time was only determined in rats in the control and HD groups after the tip of the immersed tails was cut from anesthetized rats. Platelet aggregation and coagulation factors were evaluated after 6 months of dosing, whereas bleeding time was evaluated prior to dosing, after 3 and 6 months of dosing, and after the 30-day recovery period.

Rats in the main study portion were sacrificed after 6 months of dosing, and rats in the recovery portion were sacrificed after the 30-day recovery period. Animals were terminally anesthetized with diethyl ether, and blood samples were collected from the abdominal aorta. Macroscopic examination was performed for all animals and the following organs were weighted: adrenal glands, heart, kidneys, liver, lungs, prostate

gland, spleen, testis, thymus, and uterus. Histopathology was evaluated the following organs: lymph nodes (mandibular and mesenteric), red marrow, pituitary gland, thyroid and parathyroid, larynx/trachea, bronchi, salivary glands, tongue, esophagus, stomach (glandular and non-glandular), small intestine (duodenum, jejunum, and ileum), large intestine (caecum, colon, and rectum), pancreas, seminal vesicles, penis, urinary bladder, vagina, ovary, skeletal muscle, skin and subcutis, eyes, Harderian glands, Zymbal gland, sciatic nerve, cerebrum, cerebellum, and spinal cord. Histology was performed the collected tissues from the animals from the control and HD groups and animals with macroscopic findings.

**Results:**

Viability and clinical signs: No table summarizing clinical observation is included in the paper. It states that all animals were sacrificed at the scheduled necropsy, and there were no D-003-related adverse clinical signs.

Body weight and food consumption: A mean body weight table (baseline and after 6 months) is included in the paper. There was no D-003-related effect on mean body weight. It also states that food consumption was not affected by D-003.

Hematology: A summary table describing hematology evaluation after 6 months of dosing is included in the paper. There were no D-003-related effects on hematology parameters.

Serum chemistry: A summary table describing serum chemistry evaluation after 6 months of dosing is included in the paper. A dose-dependent decrease in mean cholesterol and a slight decrease (but not dose-dependent) in mean triglycerides were observed in the test article-dosed groups of males and females (Table 26).

**Table 26 Changes in serum chemistry parameters from the 6-month rat study of D-003**

| Dose Group (mg/kg) / Parameter | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 250 | 500 | 1000 | 0 | 250 | 500 | 1000 |
| Cholesterol (mmol/L) | 1.91 | 1.61 (-16%) | 1.32 (-31%) | 1.30 (-32%) | 2.58 | 2.14 (-17%) | 2.10 (-19%) | 1.85 (-28%) |
| Triglycerides (mmol/L) | 1.28 | 1.11 (-13%) | 1.15 (-10%) | 1.15 (-10%) | 1.02 | 0.89 (-13%) | 0.82 (-20%) | 0.94 (-8%) |

Coagulation: Data on coagulation parameters are presented in Tables 27-28. A dose-dependent decrease in platelet aggregation was observed in all D-003 treated groups (a decrease of 51, 54, and 55% in the LD, MD, and HD groups, respectively, relative to the control group). In addition, rats in the HD group exhibited an increase in bleeding time after 3 and 6 months of dosing (at 3 months after dosing, an increase of 53% in HD males and 58% in HD females, relative to the respective control groups; at 6 months, an

increase of 56% in HD males and 58% in HD females, relative to the respective control groups). However, KPT and PT were not affected by D-003.

**Table 27 Platelet aggregation and coagulation [taken directly from the published paper, p.382]**



**Table 28 Bleeding time (sec) of D-003 [taken directly from the published paper, p.382]**

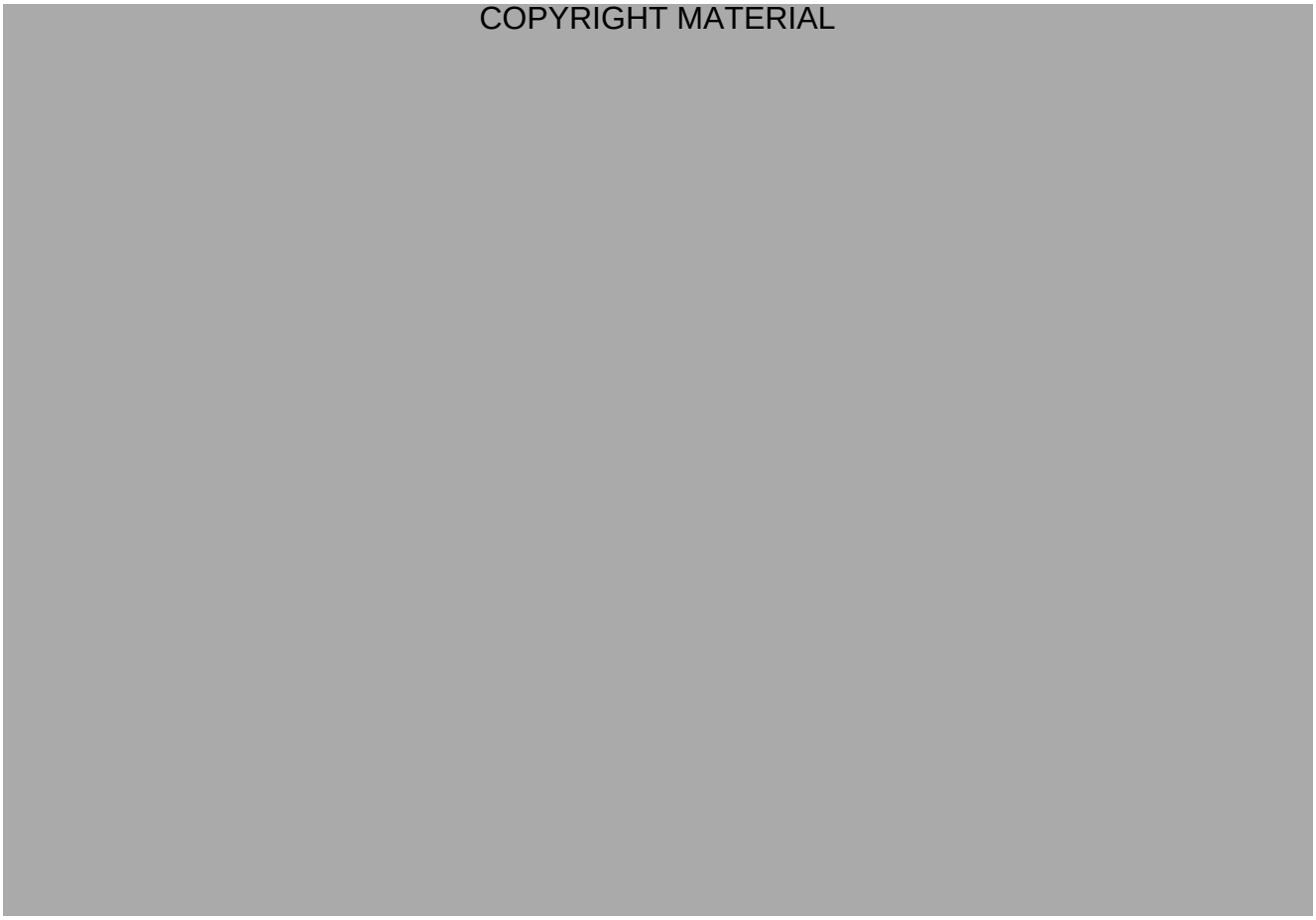


Gross examination: No table summarizing macroscopic findings is included in the paper. It states that there was no D-003-related macroscopic finding.

Organ weights: No table summarizing organ weights is included in the paper. It states that there was no D-003-related effect on organ weights.

Histopathology examination: A histology table is included in the paper. Hyperplasia on Langerhans pancreatic cells was noted in 2 HD males (Table 29). The paper describes that this lesion is reported as spontaneous in rats given *ad libitum* access to food.

**Table 29 Histology findings from the 6-month rat study of D-003 [taken directly from the published paper, p.383]**




## (3) Hydrocarbons

See the Carcinogenicity Section.

## (4) Long-chain fatty alcohols

**Publication Title: Preclinical oral toxicology in rats of D-002, a natural drug with antiulcer effects (Rodeiro, et al., 1998)**

The study consisted of subacute, subchronic, and chronic studies in rats.

**Methods:** Sprague Dawley rats (6-8 weeks age, weighing 150 to 200 g at arrival; a 7-day acclimation period) were used in the study. Rats had free access to water and food *ad libitum*.

D-002 was supplied by Centro de Productos Naturales, Centro Nacional de Investigaciones Cientificas (Havana City, Cuba).

The composition of the batch (021093) used in this study (92% purity) was as follows: triacontanol, 26.63%; octacosanol, 17.49%; dotriacontanol, 16.95%; hexacosanol, 15.34%; tetracosanol, 13.24%, and tetratriacontanol, 2.23%.

For the subacute study, groups of rats (5/sex/group) were daily administered D-002 at oral (gavage) doses of 0 (10 mg/mL acacia gum/water), 2000, 3000, or 5000 mg/kg/day (10 mL/kg) for 14 days. Viability and clinical signs were observed twice daily, and body weight was measured at the beginning, after 7 days of dosing and the end of the study.

For the subchronic study, groups of rats (12/sex/group) were daily administered D-002 at oral (gavage) doses of 0 (10 mg/mL acacia gum/water), 5, 25, 125, or 625 mg/kg/day (10 mL/kg) for 90 days. Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly.

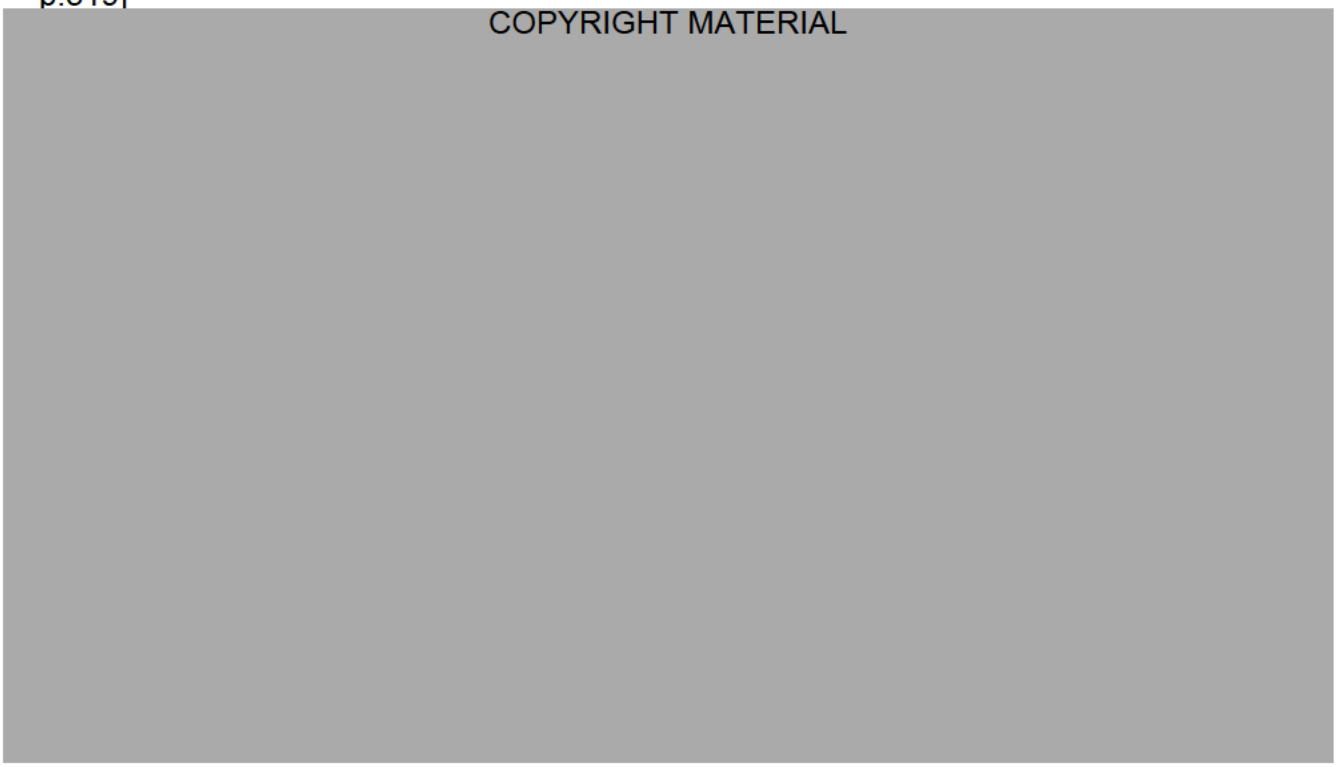
For the chronic study, groups of rats (20/sex/group) were daily administered D-002 at oral (gavage) doses of 0 (10 mg/mL acacia gum/water), 250, 500, or 1000 mg/kg/day (10 mL/kg) for 1 year. Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly during the first 13 weeks of dosing and then monthly until the scheduled sacrifice.

Blood samples were collected for hematology evaluation at the end of the dosing period for the subchronic and chronic studies, and the evaluation included following: hemoglobin, hematocrit, and total and differential counts of leukocytes. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Blood samples were collected for serum chemistry evaluation at the end of the dosing period for subacute, subchronic, and chronic studies, and the evaluation included following: glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, acetylcholinesterase (AChE), acid and alkaline phosphatases, creatinine kinase, and urea. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Macroscopic examination was performed for all animals for subacute, subchronic, and chronic studies, and the following organs were weighted: liver, heart, kidneys, spleen, lungs, and thymus. It states that the collection of tissues for histopathological evaluation was performed according to the criteria of the U.S. National Toxicology Program (Chhabra et al., 1990 (Chhabra, et al., 1990), and the tissues for histopathological evaluation presented in Chhabra et al. (1990)'s paper are shown below. Tissue samples were fixed in 10% buffered formaldehyde, embedded in paraffin, cut, and stained with hematoxylin and eosin for examination by light microscopy.

Tissues for histopathological evaluation [taken directly from Chhabra et al., (1990), p.319]




**Results:**

*Subacute study:* No table summarizing mortality, clinical observation, or body weight is included in the study. It states that no mortality or toxicity signs were observed during the subacute study, and there were no D-002-related changes in body weight gains. Data summarizing serum chemistry parameters and organ weights are presented in the paper, and there were no D-002-related effects on these parameters. No histology data are presented in the table, but it states that there were no D-002-related histology findings.

*Subchronic study:* A table summarizing mortality from the subchronic and chronic studies is included in the paper (Table 30), and it states that the cause of all deaths was due to dosing errors. A table or figure describing body weight is not included in the paper, but it states that there were no D-002-related changes in body weight. Data summarizing hematology, serum chemistry, organ weights, and histology are presented in the paper, and there were no D-002 related effects on these endpoints.

**Table 30 Mortality from the subchronic and chronic studies, and the papers states that all deaths were due to dosing errors [taken directly from the published paper, p.157]**



*Chronic study:* As mentioned above, mortality data from the chronic study are included in the paper (Table 30), and it states that the cause of all deaths were due to dosing errors. Figures describing body weights of males and females are included in the paper, and apparently, there were sight decreases in mean body weights in the 500 and 1000 mg/kg groups of females from 4 months of dosing. Data summarizing hematology, serum chemistry, organ weights, and histology are presented in the paper, and there were no D-002 related effects on these endpoints. In the 1000 mg/kg group, two males had inflammatory infiltrate in the liver, and one female had focal necrosis in the liver, and none from other groups had any liver findings. Because of low incidences of the liver findings in the high dose group, the findings were considered incidental.

**Publication Title: One-year dog toxicity study of D-002, a mixture of aliphatic alcohols (Aleman, et al., 2001)**

**Methods:** Groups of beagle dogs (4/sex/group; 10-12 weeks age, weighing 8-12 kg at arrival; a 15-day acclimation period) were daily administered D-002 at oral (gavage) doses of 0 (10 mg of acacia gum/mL water), 50, or 250 mg/kg/day (8 mL/kg) for 1 year.

The composition of the batch used in this study (92% purity) was as follows: triacontanol, 26.63%; octacosanol, 17.49%; dotriacontanol, 16.95%; hexacosanol, 15.34%; tetracosanol, 13.24%, and tetratriacontanol, 2.23%.

Viability and clinical signs were observed daily, and body weight was measured monthly.

Ophthalmological examination was performed prior to dosing and at Week 52.

Blood samples were collected for hematology evaluation (hemoglobin and hematocrit) prior to dosing and every 3 months up to the end of the study.

Blood samples were collected for serum chemistry evaluation prior to dosing and every 3 months up to the end of the study, and the evaluation included following: glucose, aspartate aminotransferase (AST), alanine aminotransferases (ALT), acid and alkaline phosphatases, creatinine, and acetyl cholinesterase.

Macroscopic examination was performed for all animals, and the following organs were weighted: liver, kidneys, heart, lungs, spleen, thymus, adrenals, testes, and prostate. For histopathological evaluation, the tissue samples were collected for the following organs: brain (cerebellum, medulla, hypophysis [pituitary gland]), eyes, larynx, trachea, bronchi, lungs, tongue, esophagus, salivary glands, stomach, duodenum, jejunum, ileum, cecum, colon, rectus, thymus, pancreas, liver, skeletal muscle, aorta, heart, bone marrow, skin, kidneys, adrenals, bladder, testes, penis, prostate, uterus, vagina, ovaries, lymph node, thyroid, parathyroid, and bone. These tissue samples were fixed in 10% formaldehyde, embedded in paraffin, cut, and stained with hematoxylin and eosin.

**Results:**

Viability and clinical signs: No table summarizing survival or clinical observations is included in the paper. It states that all dogs were survived until the scheduled sacrifice, and there were no abnormal clinical signs.

Body weight: Figures describing body weight (apparently over the first 12 months) are included in the paper. The X-axis describing time interval says “weeks”, however, it appears to be a typo since body weight was measured monthly. There were no D-002-related effects on mean body weights.

Hematology: The values for hemoglobin and hematocrit are included in the study, and these parameters were not affected by D-002.

Serum chemistry: Tables summarizing serum chemistry parameters are presented in the paper. There were no D-002-related effects on serum chemistry parameters.

Gross examination: The paper does not mention about macroscopic findings. It appears that there were no D-002-related macroscopic findings.

Organ weights: Tables summarizing organ weights are presented in the paper, and apparently, there were no D-002-related effects on organ weights.

There was a slight increase in mean liver weight in D-002-dosed groups of males and females (Table 31). Because the small magnitude of the changes and no other correlated histological and liver enzyme changes, this decrease in liver weight was not considered D-002-related. Additional changes in D-002-dosed groups of females (20%

increase in the mean lung weight in the 250 mg/kg group and up to 100% increase in the adrenals in the 50 and 250 mg/kg groups, compared to the control group) were also considered incidental because the changes were only seen in females and no other correlated findings were observed.

**Table 31 Changes in organ weight (absolute) in dogs administered D-002 for 1 year**

| Dose Group (mg/kg) / Organ | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | 0 | 50 | 250 | 0 | 50 | 250 |
| Liver | 2.35 | 2.85 (+21%) | 2.80 (+19%) | 2.41 | 2.58 (+7%) | 2.64 (+10%) |

Histopathology examination: A histology table as shown below (Table 32) is included in the paper. The paper states that there were no D-002-related histological findings.

Adenitis was observed in one male each in 50 and 250 mg/kg groups. As the finding was occurred in only males with the low incidence, its toxicological significance is unknown.

Thyroiditis was observed in one male each in 50 and 250 mg/kg groups, but this finding was also seen in one control female. Thus, this finding was considered incidental.

**Table 32 Histopathological findings in dogs administered D-002 for 1 year [taken directly from the paper, p.184]**



**Publication Title: A 12-month study of policosanol oral toxicity in Sprague Dawley rats (Aleman, et al., 1994b)**

**Methods:** Groups of Sprague Dawley rats (20/sex/group; 6-8 weeks age, weighing 150 to 200 g at arrival; a 7-day acclimation period) were daily administered policosanol at oral (gavage) doses of 0 (10 mg/mL acacia gum/water), 0.5, 5, 50, or 500 mg/kg/day (10 mL/kg) for 12 months.

It states that rats had free access and moderate dietary restriction.

Viability and clinical signs were observed daily, and body weight was measured weekly during the first 13 weeks of the dosing period and then monthly until the scheduled sacrifice. Food consumption was recorded weekly.

Blood samples were collected for hematology evaluation at the end of the dosing period, and the evaluation included following: hemoglobin, hematocrit, and total and differential counts of leukocytes. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

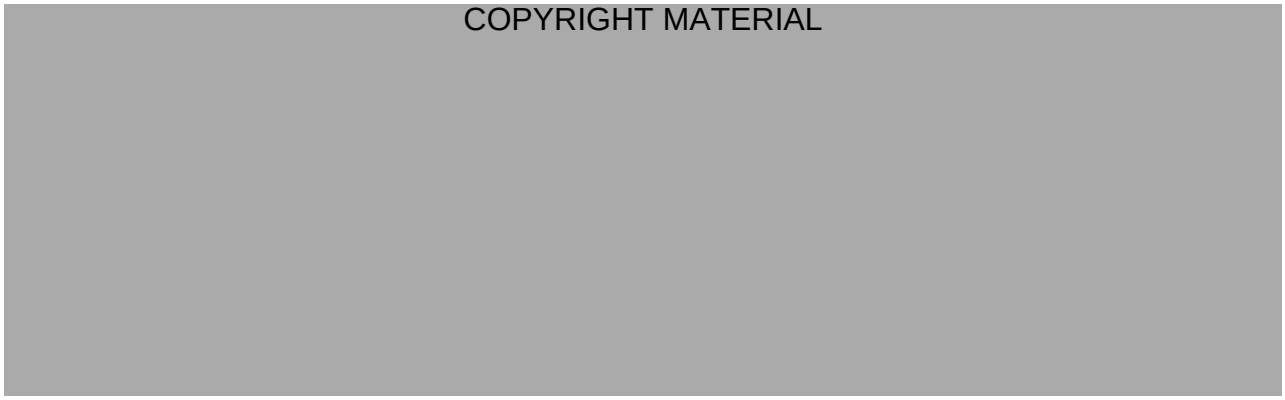
Blood samples were collected for serum chemistry evaluation at the end of the dosing period, and the evaluation included following: glucose, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acetylcholinesterase (AChE), acid and alkaline phosphatases, and creatinine kinase. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, heart, kidneys, spleen, lungs, and thymus. It states that the collection of tissues for histopathological evaluation was performed from all organs according to Stevens (Stevens and Gallo, 1986) and Gallo (1986). However, the specific tissues for histopathologic evaluation are not presented in the present paper and Stevens and Gallo (1986)'s article. Tissue samples were fixed in 10% buffered formaldehyde, embedded in paraffin, cut, and stained with hematoxylin and eosin for histopathological examination by light microscopy. An additional ultrastructural study were conducted using liver tissues from the control, 5, and 500 mg/kg/day groups (five animals/group), which were stained with lead acetate and uranyl citrate.

**Results:**

Viability and clinical signs: Mortality data are presented in Table 33. There were no differences in mortality between the control and policosanol-dosed groups. It states that the deaths occurred in the study was attributed to dosing errors, which were verified by the macroscopic examination. It also states that there were no clinical signs of toxicity.

**Table 33 Mortality percent during the 12-month dosing period of policosanol [taken directly from the paper, p.78]**



Body weight: Figures describing body weight during the 12-month dosing period are included in the paper. There were no policosanol-related effects on mean body weight.

Hematology: Tables summarizing hematology parameters are presented in the paper. Apparently, there were no policosanol-related effects on hematology parameters.

There were slight decreases in neutrophil levels at 500 mg/kg/day (decreases of 14% in males and 21% in females, compared to the respective control group). Due to the slight decrease without any other changes, its toxicological significance is unknown.

Serum chemistry: Tables summarizing serum chemistry parameters are presented in the paper. There were no policosanol-related effects on serum chemistry parameters.

Gross examination: The paper does not mention about macroscopic findings. It appears that there were no policosanol-related macroscopic findings.

Organ weights: Tables summarizing organ weights are presented in the paper. There were no policosanol-related effects on organ weights.

Histopathology examination: The paper includes summary tables for non-neoplastic lesions and ultrastructural lesions in the liver, and the paper states that there were no policosanol-related histopathology findings. However, the summary tables for non-neoplastic lesions from all collected tissues do not present findings from the highest dose group (500 mg/kg/day). It could not be ruled out that the 500 mg/kg/day group had no lesions. However, without any explanation, the Reviewer should not make any assumption, and based on the data presented in the paper, it is concluded there were no policosanol-related effects up to 50 mg/kg/day and any conclusion cannot be made for 500 mg/kg/day group.

**Publication Title: Toxicity of policosanol in beagle dogs: one-year study (Mesa, et al., 1994)**

**Methods:** Groups of beagle dogs (4/sex/group; 10-12 weeks age, weighing 9-14 kg at arrival; a 15-day acclimation period) were daily administered policosanol at oral (gavage) doses of 0 (10 mg of acacia gum/mL water), 30, or 180 mg/kg/day (5 mL/kg) for 52 weeks.

Relative concentrations of each alcohol in the batch were: octacosanol (67%), Triacontanol (14%), hexacosanol (8%), tetracosanol (5%), heptacosanol (3%), nonacosanol (< 1%), dotriacontanol (< 1%), and tetratriacontanol (< 1%). Policosanol suspensions were freshly prepared 2 hours before use.

Viability and clinical signs were observed daily, and body weight was measured monthly.

Ophthalmological examination was performed prior to dosing and at Week 52.

Hematology parameters were not evaluated in the study.

Blood samples were collected for serum chemistry evaluation prior to dosing and at Weeks 8, 12, 24, and 52, and the evaluation included following: glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, creatinine, and bilirubin. Prior to blood collection, animals were fasted overnight 12 hours. In addition, blood samples were collected for evaluation of lipid profile (total cholesterol, triglycerides, and HDL-C) prior to dosing and at Weeks 8, 24, 36, and 52.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, heart, kidneys, spleen, lungs, thymus, pancreas, suprarenals [adrenal gland], and testes. For histopathological evaluation, the tissue samples were collected for the following organs: brain (cerebellum, medulla, hypophysis [pituitary gland]), eyes, larynx, trachea, bronchi, lungs, tongue, esophagus, salivary glands, stomach, duodenum, jejunum, ileum, cecum, colon, rectus, thymus, pancreas, liver, skeletal muscle, aorta, heart, bone marrow, skin, kidneys, adrenals, bladder, testes, penis, prostate, uterus, vagina, ovaries, lymphatic node, thyroid, parathyroid, and bone. These tissue samples were fixed in 10% formaldehyde, embedded in paraffin, cut, and stained with hematoxylin and eosin.

**Results:**

Viability and clinical signs: No table summarizing mortality and clinical observations is included in the paper. It states that all dogs were survived until the scheduled sacrifice, and there were no abnormal clinical signs or ophthalmologic findings.

Body weight: A table with initial weights and final weights is included in the paper. There were no policosanol-related effects on mean body weight.

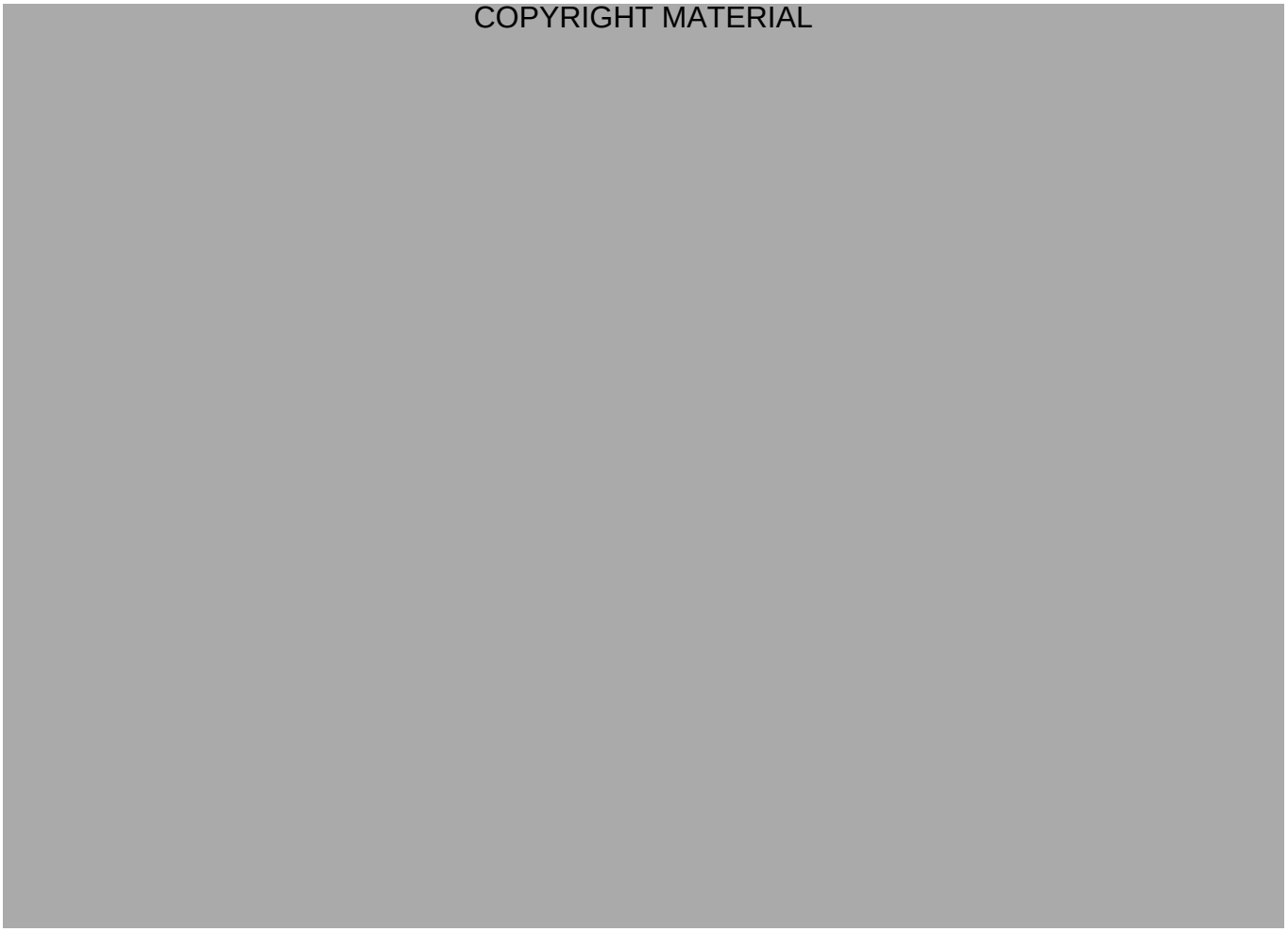
Hematology: Hematology parameters were not evaluated in the study.

Serum chemistry: A table summarizing serum chemistry parameters in males is included in the paper. There were no policosanol-related effects on serum chemistry parameters in males. The paper also states that there were no policosanol-related effects in females as well.

Lipid profile: A table summarizing serum lipid profile values from combined males and females and a figure describing mean percent reduction in serum cholesterol are presented in the paper. As shown in Table 34, there a decrease in total cholesterol levels in the 30 and 180 mg/kg groups. At Week 52, over 20% decreases in mean total cholesterol levels were noted (23% and 27% in the 30 and 180 mg/kg groups, respectively, relative to their respective baseline values). Triglyceride and HDL-C values were not affected by policosanol.

**Table 34 Serum lipid profile values (mmol/L) of beagle dogs (males and females) treated orally with policosanol for 52 weeks [taken directly from the paper, p.85]**





Gross examination: The paper does not mention about macroscopic findings. It appears that there were no policosanol-related macroscopic findings.

Organ weights: A table summarizing organ weights from males is presented in the paper, and the paper states that were no significant differences between control and policosanol-dosed groups of males and females.

According to the table, relative to the control group, organ weight changes in the male 180 mg/kg group were observed in the following: -22% in the liver, -40% in the spleen, +80% in the thymus, and +150% in the suprarenal (right only). Without corresponding histological findings, the toxicological significance of these changes is unknown.

Histopathology examination: No histology table is included in the paper. It states that there were no policosanol-related histological findings.

## Carcinogenicity

### (1) Fatty Acid Esters
None

### (2) Long-Chain Fatty Acid

**Publication Title: Long-term carcinogenicity of D-003, a mixture of high molecular weight acids from sugarcane wax, in Sprague Dawley rats: A 24 months study (Gamez, et al., 2007)**

**Methods:** Groups of Sprague Dawley rats (60/sex/group; 5-7 weeks age at arrival; a 15-day acclimation period) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum-water), 50 (LD), 500 (MD), or 1500 (HD) mg/kg/day (10 mL/kg) for 5 days a week for 24 months.

It states that rats had free access and moderate dietary restriction (approximately a 20% reduction in average *ad libitum* intake after growing [from Week 13]).

D-003, supplied by the Plants of Natural Products (National Centre for Scientific Research, Havana City, Cuba), was obtained through sugarcane wax saponification and further extraction and purification in organic solvents. The composition of D-003, assessed through a validated gas chromatography method was as follows: 1-tetracosanoic (0.9%), 1-pentacosanoic (0.5%), 1-hexacosanoic (1.0%), 1-heptacosanoic (3.0%), 1-octacosanoic (40.0%), 1-nonacosanoic (3.0%), 1-triacontanoic (18.0%), 1-hentriacontanoic (1.0%), 1-dotriacontanoic (8.0%), 1-tritriacontanoic (2.0%), 1-tetratriacontanoic (12.0%), 1-pentatriacontanoic (0.5%), and 1-hexatriacontanoic (4.0%). Long-term (5 years) studies have confirmed the stability of this substance. D-003 was suspended in acacia gum-water (10 mg/mL) after confirming that it was stable under these conditions and suspensions were prepared weekly.

This study was conducted by Cuban GLP and audited by the Quality Assurance Unit.

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly during the first 13 weeks of the dosing period and then monthly until the scheduled sacrifice.

Blood samples were collected for hematology evaluation at the end of the dosing period, and the evaluation included the following: hemoglobin and red blood cell and white blood cell counts. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Blood samples were collected for serum chemistry evaluation at the end of the dosing period, and the evaluation included following: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), alkaline and acid phosphatases, albumin, cholesterol,

triglycerides, and glucose. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, kidneys, heart, spleen, lungs, thymus, adrenals, gonads, and brain. It states that the collection of tissues for histopathological evaluation was performed according to the criteria of the U.S. National Toxicology Program (Chhabra et al., 1990 (Chhabra, et al., 1990), and the tissues for histopathologic evaluation presented in Chhabra et al. (1990)'s paper is shown below.

Tissues for histopathologic evaluation [taken directly from Chhabra et al., (1990), p. 319]



**Results:**

Viability and clinical signs: A table summarizing viability or clinical observation is not included in the paper. It states that there were no differences in survival days between the control and D-003-dosed groups (for males, 670.5 and 684.7 days, respectively; for females, 682.7 and 676.2 days, respectively). Similarly, no differences in mortality rates were observed between the control and D-003-dosed groups (for males, 31.7% [19/60] and 26.7% [48/180], respectively; for females, 35.0% [21/60] and 34.4% [62/180], respectively). Furthermore, there were no D-003-related adverse clinical signs.

Body weight and food consumption: A table summarizing body weight and food consumption is not included in the paper. It states that there were no D-003-related effects on mean body weight and food consumption.

Hematology: A table summarizing hematology evaluation is not included in the paper. It states that there were no D-003-related effects on hematology parameters.

Serum chemistry: A table summarizing serum chemistry evaluation is not included in the paper. It states that there were no D-003-related effects on serum chemistry parameters, except a serum total cholesterol reduction in the MD and HD groups.

Gross examination: A table summarizing macroscopic evaluation is not included in the paper. It states that there was no D-003-related macroscopic finding. No table was included.

Organ weights: A table summarizing organ weights is not included in the paper. It states that there was no D-003-related effect on organ weights.

Histopathology examination: A summary table summarizing total tumor occurrences and a table with each type of neoplastic lesions (malignant and benign) are included in the paper.

There were no D-003-related tumor findings.

For non-neoplastic lesion, glomerulonephrosis was most frequently observed in males and females, with slightly higher incidences in the control group of males and females.

**Publication Title: Study of the long-term carcinogenicity potential of D-003, a mixture of high molecular weight sugarcane wax acids, in mice (Noa, et al., 2009)**

**Methods:** Groups of OF1 mice (50/sex/group; 4-7 weeks age, weighing 22-24 g at arrival; a 15-day acclimation period) were administered D-003 at oral (gavage) doses of 0 (10 mg/mL acacia gum-water), 50 (LD), 500 (MD), or 1500 (HD) mg/kg/day (10 mL/kg) for 6 days a week for 18 months.

It states that mice had free access and moderate dietary restriction (approximately a 20% reduction in average *ad libitum* intake after growing [from Week 13]).

The batch of D-003 used in the study, supplied by the Plants of Natural Products (National Centre for Scientific Research, Havana City, Cuba), had the following composition: tetracosanoic (0.2%), pentacosanoic (0.4%), hexacosanoic (2.1%), heptacosanoic (2.4%), octacosanoic (40.1%), 1-nonacosanoic (3.0%), triacontanoic (17.4%), hentriacontanoic (1.0%), dotriacontanoic (8.6%), tritriacontanoic (2.1%), tetratriacontanoic (12.6%), pentatriacontanoic (0.5%), hexatriacontanoic (4.0%), which was assessed through a validated gas chromatography method. For dosing, D-003 was suspended in 10 mg/mL acacia gum-water, after corroborated its stability in such a vehicle. Suspensions were prepared weekly.

It states that this study design was consistent with OECD 451 Guideline and GLP guidelines of the OECD.

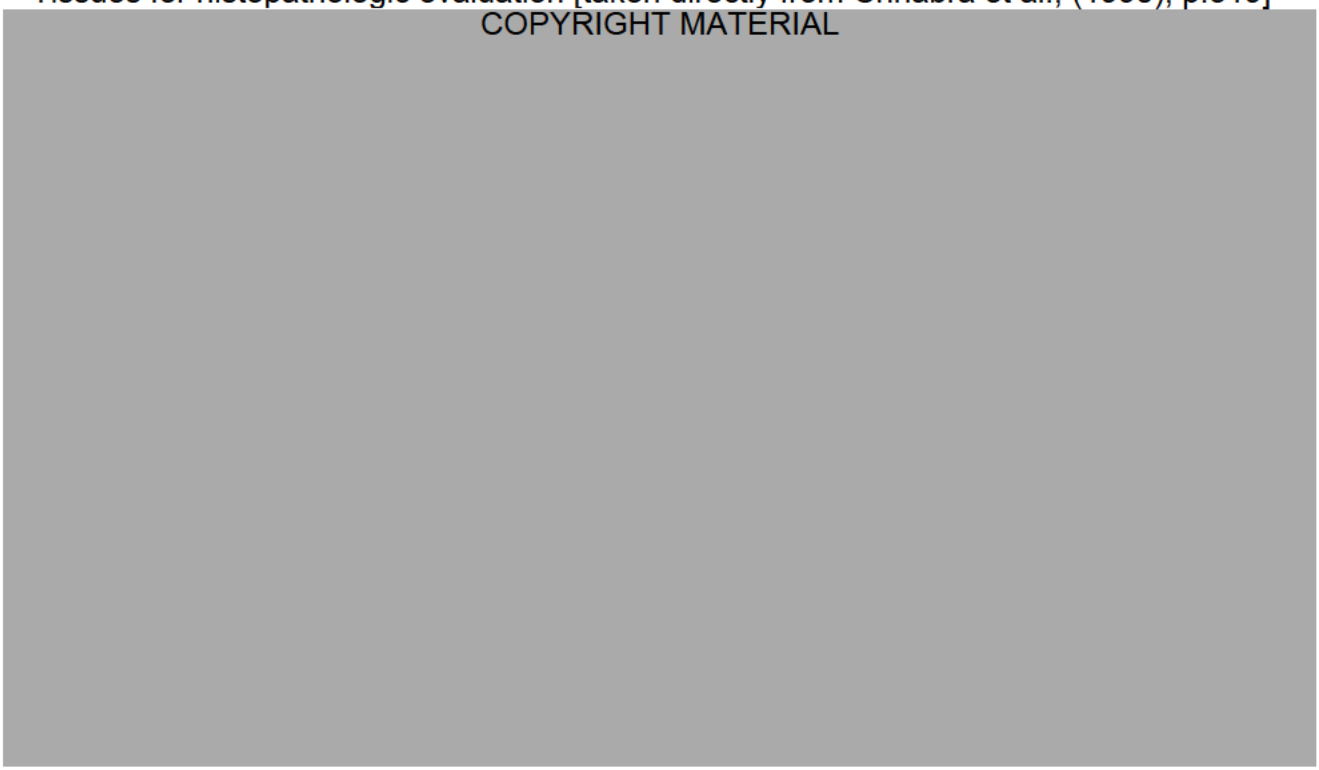
Viability and clinical signs were observed twice daily, and body weight and food consumption were measured weekly during the first 13 weeks of the dosing period and then monthly until the scheduled sacrifice.

Blood samples were collected for hematology evaluation at the end of the dosing period, and the evaluation included following: hemoglobin, hematocrit, and platelet counts. Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Blood samples were collected for serum chemistry evaluation at the end of the dosing period, and the evaluation included following: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Prior to blood collection, animals were fasted overnight 12 hours with water *ad libitum* until the scheduled sacrifice.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, kidneys, heart, spleen, lungs, thymus, gonads, and brain. It states that the collection of tissues for histopathological evaluation was performed according to the criteria of the U.S. National Toxicology Program (Chhabra et al., 1990 (Chhabra, et al., 1990), and the tissues for histopathologic evaluation presented in Chhabra et al. (1990)'s paper is shown below. Histopathological examination was performed in all animals of all groups. Approximately 43 tissues and all gross lesions were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 mcm with a rotary microtome, and stained with hematoxylin and eosin for histopathological examination by light microscopy.

Tissues for histopathologic evaluation [taken directly from Chhabra et al., (1990), p.319]



**Results:**

Viability and clinical signs: A table summarizing survival or clinical observations is not included in the paper. It states that there were no differences in survival days between the control and D-003-dosed groups (for males, 531.6 and 535.7 days, respectively; for females, 535.8 and 544.0 days, respectively). Similarly, no differences in mortality rates were observed between the control and D-003-dosed groups (for males, 20.0% [10/50] and 12.0% [18/150], respectively; for females, 10.0% [5/50] and 5.3% [8/150], respectively). Furthermore, it states that there were no D-003-related adverse clinical signs.

Body weight and food consumption: A table summarizing body weight or food consumption is not included in the paper. It states that there were no D-003-related effects on mean body weight and food consumption.

Hematology: A table summarizing hematology evaluation is not included in the paper. It states that there were no D-003-related effects on hematology parameters (hemoglobin, hematocrit, and platelet counts).

Serum chemistry: A table summarizing serum chemistry evaluation is not included in the paper. It states that there were no D-003-related effects on serum chemistry parameters (ALT and AST).

Gross examination: A table summarizing macroscopic findings is not included in the paper. It states that there was no D-003-related macroscopic finding.

Organ weights: A table summarizing organ weights is not included in the paper. It states that there was no D-003-related effect on organ weights.

Histopathology examination: A summary table summarizing total tumor occurrences and a table with each type of neoplastic lesions (malignant and benign) are included in the paper.

There were no D-003-related tumor findings.

For non-neoplastic lesions, uterine hyperplasia and ovarian cysts were frequently observed in both control and D-003-dosed groups with slightly higher incidences in the control group.

## (3) Hydrocarbons

The following information was obtained from Mineral oils (medium- and low-viscosity) and paraffin waxes (Vavasour and Chen, 2015), WHO Food Additives Series: 50)

1. A combined 12-month toxicity and 2-year carcinogenicity study was conducted in rats that were fed diets containing P70(H) mineral oil (medium and low viscosity, Class I) or P100(H) mineral oil (high viscosity). The study also included a recovery group after 12-month of treatment. The studies were conducted in compliance with U.S. FDA GLP Regulations and the OECD Principles of GLP. Each portion of the study consisted of the five groups (0 [control], 60, 120, 240, and 1200 mg/kg/day), and the concentrations in the diet were adjusted to achieve a constant target dose throughout the study. Groups of 50 male and 50 female animals were used in the main (24-month) study, with additional groups of 30 male and 30 female animals for the reversibility phase (for 12-month treatment followed by a 12-month recovery period). Of the 30 animals/sex/group in the reversibility phase, 10 animals/sex/group were killed after the 12-month feeding period. Additional groups of at least five females were included to evaluate tissue hydrocarbon concentrations at 3, 6, 12, 18, and 24 months in both phases of the study.

The parameters evaluated in the study were clinical observations, body weight, food consumption, ophthalmology, hematology, serum chemistry, urine parameters, organ weights (including mesenteric lymph nodes), and macroscopic and microscopic examinations. Analyses for mineral hydrocarbon were performed on the liver, kidneys, mesenteric lymph nodes, and spleen from female animals. Histopathological examination of 48 tissues (including the liver, spleen, mesenteric and mandibular lymph nodes, Peyer patches, kidney, and bone marrow) was conducted for all animals in the control and 1200 mg/kg/day in the main (24-month) study and at the 12-month sacrifice. From animals in the 60, 120, or 240 mg/kg groups in the main study, only the lungs, liver, mesenteric lymph nodes, spleen, and kidneys were examined; the mesenteric lymph nodes and livers of animals in all groups in the recovery study were also examined.

There were no treatment-related effects on survival, clinical signs, body weight, food consumption, ophthalmology, clinical pathology parameters. Furthermore, there were no treatment-related macroscopic findings. Neither oil was carcinogenic in the study.

Dietary administration of both oils was associated with increased weight of mesenteric lymph nodes and increased severity of infiltrating cell histiocytosis; increased incidence and severity of vacuolation of periportal hepatocytes; increased incidence of combined cystic degeneration or angiectasis in the liver of male rats, with no dose–response relationship; and a quantifiable, reversible accumulation of mineral hydrocarbons in the liver to a similar level regardless of dose but dependent on the type of mineral oil. The effects were more marked in rats treated with P70H oil than with P100H oil, and there were sex-related differences in response. Below, these findings for each oil are described in detail.

After 24 months of treatment of medium-viscosity white mineral oil (P70H), a dose-related, statistically significantly increase in mean mesenteric lymph node weight (absolute and relative to body and brain weight) was observed in all P70H-treated groups of female rats and in the 1200 mg/kg group of males. Mesenteric lymph node weights were also statistically significantly increased in males at 1200 mg/kg group after

12 months of treatment, but this effect was no longer significant after the 12-month recovery period, owing to an increased value for this parameter in the control group. No significant differences in mesenteric lymph node weights were observed in females after 12 months of treatment, although after the 12-month recovery period, a slight but significant increase was seen in females at the two higher doses (240 and 1200 mg/kg). The severity, but not the incidence, of infiltrating cell histiocytosis of the mesenteric lymph nodes was increased in all P70H-treated groups after 24 months of treatment. In addition, there was a slight increase in the severity of this lesion in animals in the 1200 mg/kg group (the only group assessed) at the 12-month sacrifice. After the 12-month recovery period, these changes were still apparent in all groups, at severity greater than or comparable to that in the animals treated for 24 months.

A dose-related increase in the incidence and severity of vacuolation of periportal hepatocytes was observed in the livers of males in all P70H-treated groups. A smaller increase in severity only was seen in treated female rats, because the incidence and severity of portal vacuolation were already high in female, compared with male, controls. The increased incidence and severity of vacuolation of periportal hepatocytes was still evident in males after the 12-month recovery period. The investigators did not consider this indicative of an adverse effect but rather a marker of prolonged exposure to mineral oil. An increased incidence of combined angiectasis and cystic degeneration (focal sinusoidal dilatation) was also observed in all P70H-treated male groups, compared with the control group at the 24-month sacrifice. This lesion was minimal, and the incidence was similar in all P70H-treated groups. While the incidence of this lesion was not increased over that in controls in males after the recovery period, data from the 12-month sacrifice were not available to assess the significance of this observation.

Histological analysis revealed quantifiable amounts of hydrocarbon in the livers of P70H-treated animals; the hepatic concentrations of mineral hydrocarbon in animals in the 1200 mg/kg group reached nearly a maximum within 3 months and increased slowly up to 24 months of treatment. The values for animals in the 60, 120, and 240 mg/kg groups at 12 and 24 months were 1200–1500 mcg/g tissue, which were similar to each other and were approximately 60% of those of animals in the 1200 mg/kg group (1800 and 2300 mcg/g tissue, respectively). During the recovery period, the hepatic concentrations had dropped substantially by 6 months and had returned to background levels by 12 months. The amount of mineral hydrocarbon in the spleen and mesenteric lymph nodes of most treated animals at higher doses was below the practical limit of reliable quantification.

After 24 months of treatment with high viscosity white mineral oil (P100H), statistically significant increases in mean mesenteric lymph node weights (absolute and relative to body weight and brain weight) were observed in females in the 1200 mg/kg group.
This effect was not evident at lower doses or in male rats or after the recovery period. A marginal increase in severity, but not the incidence, of infiltrating cell histiocytosis in the mesenteric lymph nodes was observed in all P100H-treated groups at 24 months. No

clear dose–response relationship was apparent in the males, and no effects were observed at 12 months of treatment.

A slight increase in the incidence and severity of vacuolation of periportal hepatocytes was observed in the livers in all P100H-treated groups of males and females. An increase in the incidence of combined angiectasis and cystic degeneration (focal sinusoidal dilatation) was observed in all treated male groups, without a dose–response relationship. This lesion was not apparent at the 12-month sacrifice. In view of the nature and severity of the response, the investigators did not consider the increased grade of vacuolation to be indicative of an adverse toxicological effect but rather a marker of prolonged administration of white oil.

Histological analysis revealed quantifiable amounts of hydrocarbon in the livers of P100H-treated animals; the hepatic concentrations of mineral hydrocarbon in animals in the 1200 mg/kg group reached nearly a maximum within 3 months (900 mcg/g tissue) and increased slowly up to 24 months of treatment. The concentrations in the liver at 12 and 24 months were similar in all P100H-treated groups, suggesting that a steady-state level had been reached. After cessation of treatment, the hepatic levels had dropped substantially by 6 months and had returned to background levels by 12 months. Mineral hydrocarbon was found in mesenteric lymph nodes and spleen at some times. The concentrations in the spleen and mesenteric lymph nodes of most treated animals at the higher doses were below the practical limit of reliable quantification. Detectable residues of mineral hydrocarbon were not found consistently in the kidneys.

2. In another carcinogenicity study, groups of Fischer 344 rats (50/sex/group) were fed diets containing 2.5% or 5% of the composite medium-viscosity white mineral oil (equivalent to 1250 and 2500 mg/kg/day) continually for 104 weeks. The oil mixture used in the study was a blend of equal quantities of eight commercially available paraffinic white mineral oils (mineral oil, medium- and low-viscosity, Class I), and the physical properties of the blended mineral oil were intermediate between those of P70H (medium and low viscosity, Class I) and N70H (medium and low viscosity, Class II).

Parameters examined in the study were body weight, food consumption, hematology, serum chemistry, organ weights, and macroscopic and microscopic evaluations. A full necropsy was performed on all animals, the major organs were weighed, and histological was performed on the following tissues: liver, mesenteric lymph node, heart, and spleen.

There were no treatment-related effects on survival, abnormal clinical signs, and clinical pathology parameters. Body weights and food consumption of males and females fed 5% mineral oil were slightly increased. In the group given 5%, the absolute weights of the liver and kidney were increased in males, and the absolute and relative weights of the submaxillary gland were reduced in females. The increased absolute organ weights were attributed to the slightly increased body weights of males at this concentration.

There were no treatment-related tumors in the study.

An increased severity of small granulomatous foci of macrophages was observed in the mesenteric lymph nodes of males and females at 2.5 and 5% relative to the respective control groups.

## (4) Long-Chain Fatty Alcohols

**Publication Title: Carcinogenicity of policosanol in mice: an 18-month study (Aleman, et al., 1995)**

**Methods:** Groups of Swiss mice (20/sex/group; young adults, actual age not specified, weighing 22-24 g at the start of the study; a 7-day acclimation period) were daily administered policosanol at oral (gavage) doses of 0 (vehicle: 10 mg/mL acacia gum/water), 50, or 500 mg/kg/day (5 mL/kg) for 18 months.

Only one batch of policosanol (L-C-20) was used throughout the study.

It states that mice had free access and moderate dietary restriction (20 g/cage/day or 4 g/mouse/day; approximately a 20% reduction in average *ad libitum* intake).

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly during the first 13 weeks of the dosing period and then monthly until the scheduled sacrifice.

Blood samples were not collected for clinical pathology evaluation.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, kidneys, heart, spleen, lungs, and thymus. Tissues for histopathological evaluation were collected as following: heart, aorta, lymph nodes (mandibular and mesenteric), spleen, bone with marrow, thymus, pituitary, thyroid with parathyroid, adrenal glands, trachea, lungs, salivary glands, esophagus, stomach (glandular and non-glandular), small intestine (duodenum, jejunum, and ileum), large intestine (caecum, colon, and rectum), pancreas, liver, kidneys, urinary glands, testes, prostate, seminal vesicles, skeletal muscle, skin and subcutis, eyes, Harderian glands, sciatic nerve, and brain (cerebrum and cerebellum). Tissue samples were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

**Results:**

Viability and clinical signs: A table summarizing survival analysis using different statistical tests is included in the paper. There were no differences in mortality between the control and policosanol-dosed groups. At the end of the study, the survival rates

were 73, 79, and 74% for males, and 80, 79, and 80% for females in the 0, 50, and 500 mg/kg groups, respectively.

Body weight: Figures summarizing body weights of males and females during the 18-month dosing period are included in the paper. There were no policosanol-related effects on mean body weight.

Clinical Pathology: Not performed.

Gross examination: The paper does not mention about macroscopic findings. It appears that there were no policosanol-related macroscopic findings.

Organ weights: A table summarizing organ weights is presented in the paper. There were no policosanol-related effects on organ weights.

Histopathology examination: The paper includes a summary table for neoplastic lesions. There were no policosanol-related neoplastic lesions.

Hepatocellular carcinoma was observed in 3 males and 1 female in the 50 mg/kg group, but none in the 500 mg/kg group. Because of the lack of a dose-response occurrence, this finding was not considered policosanol-related.

**Publication Title: Carcinogenicity of policosanol in Sprague Dawley rats: a 24-month study (Aleman, et al., 1994a)**

**Methods:** Groups of Sprague Dawley rats (55/sex/group; 6-8 weeks old, weighing 150-200 g at the start of the study; a 15-day acclimation period) were daily administered policosanol at oral (gavage) doses of 0 (vehicle: acacia gum/water), 50, or 500 mg/kg/day (5 mL/kg) for 24 months.

Only one batch of policosanol (L-C-20) was used throughout the study.

It states that rats had free access and moderate dietary restriction (approximately a 20% reduction in average *ad libitum* intake after growing).

Viability and clinical signs were observed daily, and body weight and food consumption were measured weekly during the first 13 weeks of the dosing period and then monthly until the scheduled sacrifice.

Blood samples were not collected for clinical pathology evaluation.

Macroscopic examination was performed for all animals and the following organs were weighted: liver, kidneys, heart, spleen, lungs, and thymus. It states that the collection of tissues for histopathological evaluation was performed according to the criteria of the

U.S. National Toxicology Program (Chhabra, et al., 1990), and the tissues for histopathologic evaluation presented in Chhabra et al. (1990)'s paper is shown below.

Tissues for histopathologic evaluation [taken directly from Chhabra et al., (1990), p.319]



**Results:**

Viability and clinical signs: A table summarizing survival analysis using different statistical tests and figures describing survival percentage according to the ten time intervals selected for the statistical analysis are included in the paper. There were no statistically significant differences in mortality between the control and policosanol-dosed groups.

Body weight: Figures summarizing body weights of males and females during the 24-month dosing period and a table summarizing body weight values at baseline and after 24 months are included in the paper. A slight decrease in mean body weight was observed in policosanol-dosed groups after 24 months (-8.5% and -4.1% in the 50 and 500 mg/kg/day groups for males; -3.2% and -4.9% in the 50 and 500 mg/kg/day groups for females, relative to the respective control groups).

Clinical Pathology: Not performed.

Gross examination: The paper does not mention about macroscopic findings. It appears that there were no policosanol-related macroscopic findings.

Organ weights: The paper does not mention or include a table on organ weights.

Histopathology examination: The paper includes summary tables summarizing neoplastic lesions for males and females. There were no policosanol-related neoplastic lesions.

# Reproductive and Developmental Toxicology

## (1) Fatty Acid Esters

None

## (2) Long-Chain Fatty Acid

**Publication Title: Evaluation of the reproductive and developmental toxicity of the D-003, a mixture of long-chain fatty acids, in rats and rabbits (Rodriguez, et al., 2004)**

This study consisted of (1) fertility and developmental rat study and (2) embryo-fetal developmental rabbit study.

D-003 was obtained from the Chemistry Department of the Centre of Natural Products, La Habana, Cuba, and its purity checked by gas chromatography. D-003 suspension was prepared in 1% acacia gum/water.

It states that the study was conducted under the Cuban GLP guidelines and study protocols were consistent with the OECD and ICH guidelines.

*(1) Fertility and Developmental Rat Study:*

**Methods:** Groups of Sprague Dawley rats (15 males and 30 females/group; 10-12 weeks old, weighing 123 g for males and 98 g for females at the start of the study) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 500, or 1000 mg/kg/day (10 mL/kg). Male rats were dosed 4 weeks prior and during cohabitation, whereas female rats were dosed for 15 days prior to mating, throughout cohabitation and gestation to Lactation Day (LD) 21.

Viability and clinical signs were observed daily.

For mating, two female rats were housed with one male rat and examined daily for the evidence of mating (presence of copulatory plug or sperm in a vaginal lavage) and the day with evidence of mating was designated Gestation Day (GD) 0.

All female rats were permitted natural delivery. On the Postnatal Day (PND) 1, F1 pups were counted, weighed, examined externally, and sexed. On PND 4, litters were reduced by random culling to four per sex. The survival rate and body weight of pups were recorded on PNDs 4, 7, 14, and 21. Their physical development was observed as

following: pinna unfolding on PND 4, hair growth on PND 10, incisor eruption on PND 12, eye opening on PND 15, ear opening on PND 13, testis descent on PND 25, and vaginal opening on PND 37. The following behavior developments were evaluated: the surface righting reflex on PND 4, righting in air, visual placing, and auditory startle on PND 14; pupillary, corneal, and palpebral reflexes on PND 17; the locomotor development (pivoting, crawling, and walking) and huddling behavior on PNDs 4, 7, 10, and 14. Activity in the open field measurements (squares entered, urination/defecation, and standing on hindlimbs) were evaluated on PND 21.

At weaning, one male and one female were selected for post-weaning evaluation. Body weights were recorded once a week from weaning until breeding. F1 females and males of the same dose group, but of different litters, were cohabited in a 1:1 ratio between Postnatal Weeks 10 and 12. F1 females that delivered were euthanized within 1 week of delivery and the number of metrial glands was recorded. The F2 pups were weighed, sexed, and examined for external malformations on PND 1. F2 pups were euthanized with an overdose of ether and discarded without further examination within 1 week of delivery.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

F0 females:

Viability and clinical signs: It states that the dams did not exhibit unusual behavior or clinical signs of toxicity.

Body weight and food consumption: A mean body weight gain table for F0 female rats during the gestation and lactation periods is included study. There was a slight, dose-independent decrease in mean body weight gain in the D-003-treated groups between LDs 1 and 21 (-16% and -12% in the 500 and 1000 mg/kg groups, respectively, compared to the control group). It states that food consumption was not affected by D-003.

Reproductive effects of F0 animals: The reproductive parameters of F0 females are shown in Table 35, and there were no D-003-related effects on these parameters.

**Table 35 Reproductive parameters of F0 females [taken directly from the published paper, p.1980]**



F1 animals:

Pup survival and body weight: The above table also contains the pup survival and body weights. There were no D-003-treated effects on the pup survival and their body weights.

Physical and behavioral development: Four tables summarizing physical and behavioral development evaluation are presented in the paper, and these parameters were not affected by the D-003 treatment.

Post-weaning body weight: Figures summarizing the post-weaning body weights of F1 rats of males and females are included in the paper, and there were no D-003-related effects. Moreover, a table on body weight gain of F1 female rats during the gestation period is presented in the table, and there were no D-003-related effects.

Reproductive parameters of F1 rats: The reproductive parameters of F1 females are shown in Table 36, and there were no D-003-related effects on these parameters.

**Table 36 Reproductive parameters of F1 females [taken directly from the published paper, p. 1982]**



*(2) Embryo-fetal Developmental Rabbit Study*:

**Methods:** Groups of New Zealand White rabbits (27 mated females/group; 2.5-3.5 kg at the time of mating) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 500, or 1000 mg/kg/day (5 mL/kg) on GDs 6-18.

All rabbits were C-sectioned on GD 29. For each litter, the number of corpora lutea and the position and number of implantation sites were recorded, and all fetuses were weighed, sexed, and externally examined. Approximately half of the fetuses from each litter were subject to the visceral examination (including the internal examination of the heart and freehand coronal section of Bouin-fixed heads), and remaining fetuses were subject to the skeletal examination.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

Viability and clinical signs: It states that the dams did not exhibit unusual behavior or clinical signs of toxicity.

Body weight and food consumption: A mean body weight gain table for rabbits during the gestation period is included in the paper, and there was no D-003-related effect. It also states that food consumption was not affected by D-003.

Reproductive effects: The reproductive parameters of rabbits are shown in Table 37, and there were no D-003-related effects on these parameters.

**Table 37 Reproductive parameters of rabbits [taken directly from the published paper, p.1983]**



Fetal developmental parameters: The above table also contains the number of live fetuses and fetal body weight, and there were no D-003-treated effects on these parameters. Furthermore, the paper includes a summary table on fetal external, visceral, and skeletal observations, and there were no D-003-related fetal findings.

**Publication Title: Lack of developmental toxicity of D-003: a mixture of long-chain fatty acids in rats (Rodriguez, et al., 2003)**

D-003 was obtained from the Chemistry Department of the Centre of Natural Products, La Habana, Cuba, and its purity checked by gas chromatography. D-003 suspension was prepared in 1% acacia gum/water.

It states that the study was conducted under the Cuban GLP guidelines and study protocols were consistent with the OECD and ICH guidelines.

**Methods:** Groups of Sprague Dawley rats (25 mated females/group; 6-7 weeks old; a 2-week acclimation period before mating) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 5, 100, or 1000 mg/kg/day (10 mL/kg) on Gestation Days (GD) 6 through 15. In addition, a positive control group was included in the study. The rats in the positive control group (11 mated females) were administered cyclophosphamide at an intraperitoneal dose of 50 mg/kg on GD 15. The day with evidence of mating was designated GD 0.

The paper does not state the observation of viability and clinical signs and measurement of body weight and food consumption. However, there is a table on body weight gain with the following intervals: GDs 0-6, 6-15, 15-20, and 0-20.

Rats were C-sectioned on GD 20. For each litter, the numbers of corpora lutea, implantation sites, resorption, and live and dead fetuses were recorded, and all fetuses were weighed, sexed, and externally examined. Approximately half of the fetuses from each litter were subject to the visceral examination (Bouin-fixed and sectioned, including the head), and remaining fetuses were subject to the skeletal examination after cleared and stained with Alizarin Red S.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

Viability and clinical signs: It states that the dams did not exhibit clinical signs of toxicity.

Body weight and food consumption: A table summarizing body weight gains during the gestation and lactation periods is included in the paper. There was no D-003-treated effect on mean body weight gain. The paper does not state or include any food consumption data.

Reproductive parameters: The reproductive parameters of females are shown in Table 38, and there were no D-003-related effects on these parameters. The positive control (an intraperitoneal dose of 50 mg/kg cyclophosphamide on GD 15) yielded expected results.

**Table 38 Reproductive parameters of females given D-003 [taken directly from the published paper, p.91]**

Fetal developmental parameters: The above table also contains the number of live fetuses and fetal body weight, and there were no D-003-treated effects on these parameters. Furthermore, the paper includes a summary table describing fetal external, visceral, and skeletal observations, and there were no D-003-related fetal findings.

In the 100 mg/kg group, there were two fetal incidences of malformations (one fetus with absent tail, syndactyly, and anus agenesis; the other fetus with kinky tail and syndactyly). Because the low incidences occurred only in the mid-dose group, these findings were considered incidental.

**Publication Title: Perinatal/postnatal study of D-003, a mixture of long-chain fatty acids, in rats (Rodriguez, et al., 2006)**

**Methods:** Groups of Sprague Dawley rats (25 mated females/group; ~12 weeks old at arrival; a 2-week acclimation period prior to mating) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 500, or 1000 mg/kg/day (10 mL/kg) beginning at Gestation Day (GD) 15 and through gestation until Lactation Day (LD) 21. The day with evidence of mating was designated GD 0.

D-003 was obtained from the Chemistry Department of the Centre of Natural Products, (Havana, Cuba), and its purity checked by gas chromatography. D-003 suspension was prepared in 1% acacia gum/water.

Viability and clinical signs were observed daily, and body weights and food consumption were measured on GDs 0, 6, 15, and 21 and LDs 1, 7, 14, and 21.

All female rats naturally delivered. The day of birth was designated as Postnatal Day (PND) 0 (equivalent to LD 0). Pups were sexed, weighed, and examined on PNDs 1, 7, 14, and 21. On PND 4, litters were culled to eight pups (four males and four females). Physical and behavior development was evaluated in the following parameters: pinna unfolding, hair growth, incisor eruption, eye opening, ear opening, surface righting, geotaxis negative, air righting, auditory startle, visual placing, pupilar reflex, corneal reflex, parpebral reflex, testes descent, and vaginal opening.

One male and one female F1 pups from each litter were randomly selected to maintain and evaluate the reproductive potential, and the rest of F1 pups were sacrificed on PND 21 and were subject to the visceral examination. F0 females were also sacrificed on LD 21. When selected F1 animals reached sexual maturity, they were paired within the same dose group (but avoiding brother–sister mating). Pregnant F1 females were allowed to deliver, and F2 offspring were evaluated on PND 1 for visceral malformation.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

F0 females:

Viability and clinical signs: It states that one F0 female in the 500 mg/kg dose group was found dead, which was attributed to a gavage error.

Body weight and food consumption: A mean body weight gain table for F0 female rats during the gestation and lactation periods is included in the paper. There was a slight, dose-independent decrease in mean body weight gain in the D-003 treated groups between LDs 1 and 21 (-48% and -13% in the 500 and 1000 mg/kg/dose groups, respectively, relative to the control group). A table with food consumption is included in the paper, and there was no apparent D-003-related effect on food consumption.

Reproductive effects of F0 animals: The reproductive parameters of F0 females are shown in Table 39, and there were no D-003-related effects on these parameters.

**Table 39 Reproductive parameters of F0 females [taken directly from the published paper, p.225]**



F1 animals:

Pup survival and body weight: A table summarizing pup survival and body weights is included in the paper. There were no D-003-treated effects on the pup survival and their body weights.

Physical and behavioral development: A table summarizing physical and behavioral development evaluation is presented in the paper, and these parameters were not affected by the D-003 treatment.

Gestational body weight and food consumption: Tables summarizing body weight gains and food consumption of F1 females during the gestation period are included in the paper, and there were no D-003-related effects.

Reproductive parameters of F1 rats: The reproductive parameters of F1 females are shown in Table 40, and there were no D-003-related effects on these parameters. In addition, there is a table on survival and body weight of F2 pups on PND 1, and there was no D-003-related effect. The paper also states that no malformation was observed in these pups.

The mean fertility index was slightly decreased in the 500 and 1000 mg/kg groups (a decrease of 10% in each group, relative to the control group). However, because the magnitude of the change was minor, this change was not considered D-003-related.

**Table 40 Reproductive parameters of F1 females [taken directly from the published paper, p.227]**



### (3) Hydrocarbons

None

### (4) Long-Chain Fatty Alcohol

**Publication Title: Developmental toxicity of D-002 (a mixture of aliphatic primary alcohols) in rats and rabbits (Rodriguez, et al., 1998)**

D-002 was supplied by the Centre of Natural Products, Chemistry Branch. The batch composition was as follows: triacontanol (26.63%), octacosanol (17.49%), dotriacontanol (16.95%), hexacosanol (15.34%), tetracosanol (13.24%), tetratriacontanol (2.23%), and other well-known and non-active compounds (7.12%). All suspensions were prepared daily in 10 mg/mL of gum acacia solution.

It does not state whether the study was conducted under the Cuban GLP guidelines or study protocols were consistent with the OECD and ICH guidelines.

**Methods:** Groups of Sprague Dawley rats (25 mated females/group; age not specified, weighing 175-240 g at mating) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 100, 320, or 1000 mg/kg/day (10 mL/kg) on Gestation Days

(GD) 6 through 15. Groups of New Zealand White rabbits (16-20 mated females/group; age not specified, weighing 2.5-3.5 kg at mating) were administered D-003 at oral (gavage) doses of 0 (1% acacia gum/water), 100, 320, or 1000 mg/kg/day (10 mL/kg) on Gestation Days (GD) 6 through 18. The day with evidence of mating was designated GD 0.

Viability and clinical signs were observed daily, and body weight was measured daily during the dosing period and on the day of sacrifice. The paper does not state about measurement of food consumption.

Rats and rabbits were C-sectioned on GDs 20 and 29, respectively. For each litter, the numbers of corpora lutea, implantation sites, resorptions, and live and dead fetuses were recorded. The uteri of apparently non-pregnant animals were stained with a 10% solution of sodium sulfide and examined for evidence of implantation. All fetuses were weighed, sexed, and externally examined. Approximately one-half of the rat fetuses from each litter were subject to the fresh visceral examination, and remaining rat fetuses were subject to the skeletal examination after cleared and stained with Alizarin Red S. All rabbit fetuses were subject to both visceral and skeletal examinations.

It does not states that the litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

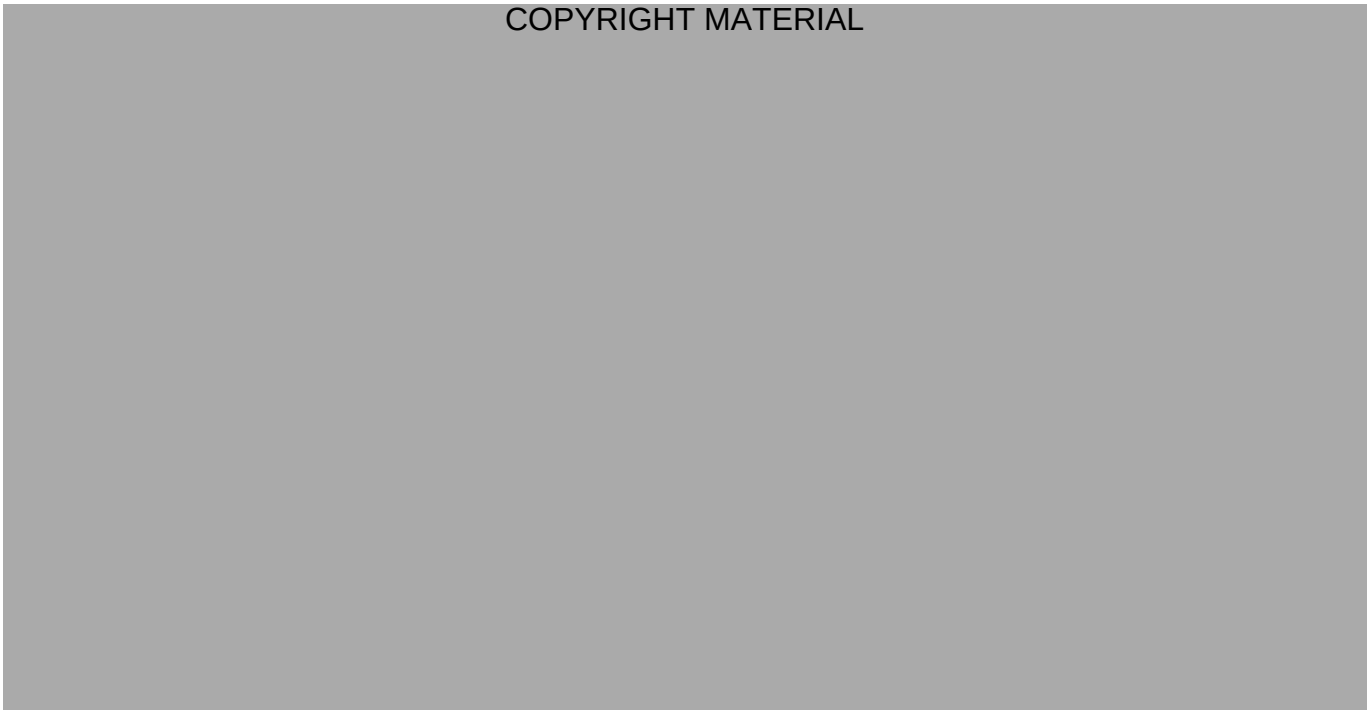
Viability and clinical signs: It states that the dams did not exhibit clinical signs of toxicity.

Body weight and food consumption: The body weight data are presented in Tables 41 and 42 for rats and rabbits, respectively. There was no D-002-treated effect on mean body weight gain. The paper does not state or include any food consumption data.

Reproductive parameters: The reproductive parameters of female rats are shown in Table 41, and there were no D-002-related effects on these parameters.

**Table 41 Gestational body weights and reproductive parameters of female rats given D-002 [taken directly from the published paper, p.314]**





The reproductive parameters of rabbits are shown in Table 42, and there were no D-002-related effects on these parameters.

The mean preimplantation loss in the control, 100, 320, and 1000 mg/kg groups of rabbits was 11.8, 13.2, 13.5, and 21.1%, respectively, whereas the mean postimplantation loss was 6.0, 1.7, 4.7, and 8.9%, respectively. Thus, a slightly higher preimplantation loss was noted in the 1000 mg/kg groups. Because dosing initiated after implantation was complete, this finding was not considered D-002-related.

**Table 42 Gestational body weights and reproductive parameters of female rabbits given D-002 [taken directly from the published paper, p.315]**



Fetal developmental parameters: The above tables also contain the number of live fetuses and fetal body weights, and there were no D-002-treated effects on these parameters in both rats and rabbits. Furthermore, the paper includes tables summarizing fetal external, visceral, and skeletal observations in rats and rabbits, and there were no D-002-related fetal findings.

In the 320 and 1000 mg/kg groups of rabbits, slightly higher fetal male to female ratios (1.42 and 1.58, respectively) along with slight higher mean fetal weights (32.8 and 33.7 g, respectively, vs. 29.3 g in the control group). Because the change was minor in magnitude, the findings were also not considered D-002-related.

In the 320 mg/kg group of rats, a slightly higher number of fetuses had <6 ossified sternal centra, (13 out of 235 fetuses), compared to the control group (6 out of 207 fetuses). Because the incidence was slightly increased only in the mid-dose group (not high-dose group), the finding was considered incidental. A slightly higher number of rabbit fetuses with <6 ossified sternal centra in the 1000 mg/kg group (8 out of 78 fetuses), compared to the control group (5 out of 114 fetuses). Because this finding is a relatively common variation finding in rabbits and only slightly increased incidence was observed, the finding was considered incidental. Similarly, in the 320 mg/kg group of rabbits, there were two fetal incidences of hemivertebra with fused ribs. Because the low incidence occurred only in the mid-dose group, these findings were considered incidental.

**Publication Title: Teratogenic and reproductive studies of policosanol in the rat and rabbit (Rodriguez and Garcia, 1994)**

The study consisted of (1) fertility and reproductive rat study, (2) embryo-fetal developmental rat study, and (3) embryo-fetal developmental rabbit study.

Technical grade policosanol was supplied by Centro Nacional de Investigaciones Cientificas (CNIC; La Habana, Cuba). The test sample was analyzed for chemical purity by gas chromatography and was determined to be 92% pure. All suspensions were prepared in 1% (10 mg/mL) aqueous gum acacia solution.

It does not state whether the study was conducted under the Cuban GLP guidelines and study protocols were consistent with the OECD and ICH guidelines.

*(1) Fertility and Reproductive Rat Study*:

**Methods:** Groups of Sprague Dawley rats (30 females/group; 8-10 weeks at the start of the study; a 2-week acclimation period) were administered policosanol at oral (gavage) doses of 0 (1% acacia gum/water), 5, 50, or 500 mg/kg/day. The number of males in this portion of the study was not specified. Male rats were dosed 60 days prior and during cohabitation, whereas female rats were dosed for 2 weeks prior to mating, throughout cohabitation and gestation to Lactation Day (LD) 21. The day with evidence of mating was designated Gestation Day (GD) 0.

Viability and clinical signs were observed daily, and it appears that body weights were measured on GDs 0, 6, 13, and 21. The paper does not state about the measurement of food consumption.

Fifteen female rats per group were C-sectioned on GD 21, and remaining females were allowed to deliver. For the females that were sacrificed on GD 21, the number and position of corpora lutea, implantation sites, resorptions, and live and dead fetuses were recorded. All fetuses were weighed, sexed, and externally examined. Approximately half of the rat fetuses from each litter were subject to the fresh visceral examination, and remaining rat fetuses were subject to the skeletal examination after cleared and stained with Alizarin Red S. For the females allowed to deliver, litters were sexed and weighted on Postnatal Day (PND) 1 and were culled to 8 pups (4 males and 4 females as extent as possible). Pup survival, body weight, and any obvious neurological deficits were examined, and pups were sacrificed on PND 21.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

Viability and clinical signs of F0 females: It states that the dams did not exhibit clinical signs of toxicity, and no preterminal deaths were noted. It also states that no unusual maternal behavior was observed.

Body weight of F0 females: It states that body weight was not affected by policosanol.

Reproductive and F1 development parameters: The reproductive and F1 observations are shown in Tables 43 and 44, and there were no policosanol-related effects on these parameters.

It states that no alterations in the external or visceral morphology of fetuses were observed. During the skeletal examination, one fetus from the control exhibited wavy ribs and one fetus in the 5 mg/kg group had fused ribs.

**Table 43 Reproductive and fetal parameters from rats that were C-sectioned on GD 21 in the fertility and reproductive portion [taken directly from the published paper, p.112]**

**Table 44 Reproductive and F1 parameters from rats that were allowed to deliver in the fertility and reproductive portion [taken directly from the published paper, p.112]**



*(2) Embryo-fetal developmental toxicity study in rats:*

**Methods:** Groups of Sprague Dawley rats (22-24 mated females/group; 8-10 weeks old at the start of the study; a 2-week acclimation period) were administered policosanol at oral (gavage) doses of 0 (1% acacia gum/water), 5, 50, or 500 mg/kg/day from Gestation Day (GD) 6 through 15. The day with evidence of mating was designated GD 0.

Viability and clinical signs were observed daily, and body weights were measured on GDs 0, 6, 13, and 19. The paper does not state about the measurement of food consumption.

Rats were C-sectioned on GD 19, and the number and position of corpora lutea, implantation sites, resorptions, and live and dead fetuses were recorded. All fetuses were weighed, sexed, and externally examined. Approximately half of the rat fetuses from each litter were subject to the visceral examination after preserved in Bouin, and remaining rat fetuses were subject to the skeletal examination after cleared and stained with Alizarin Red S.

**Results:**

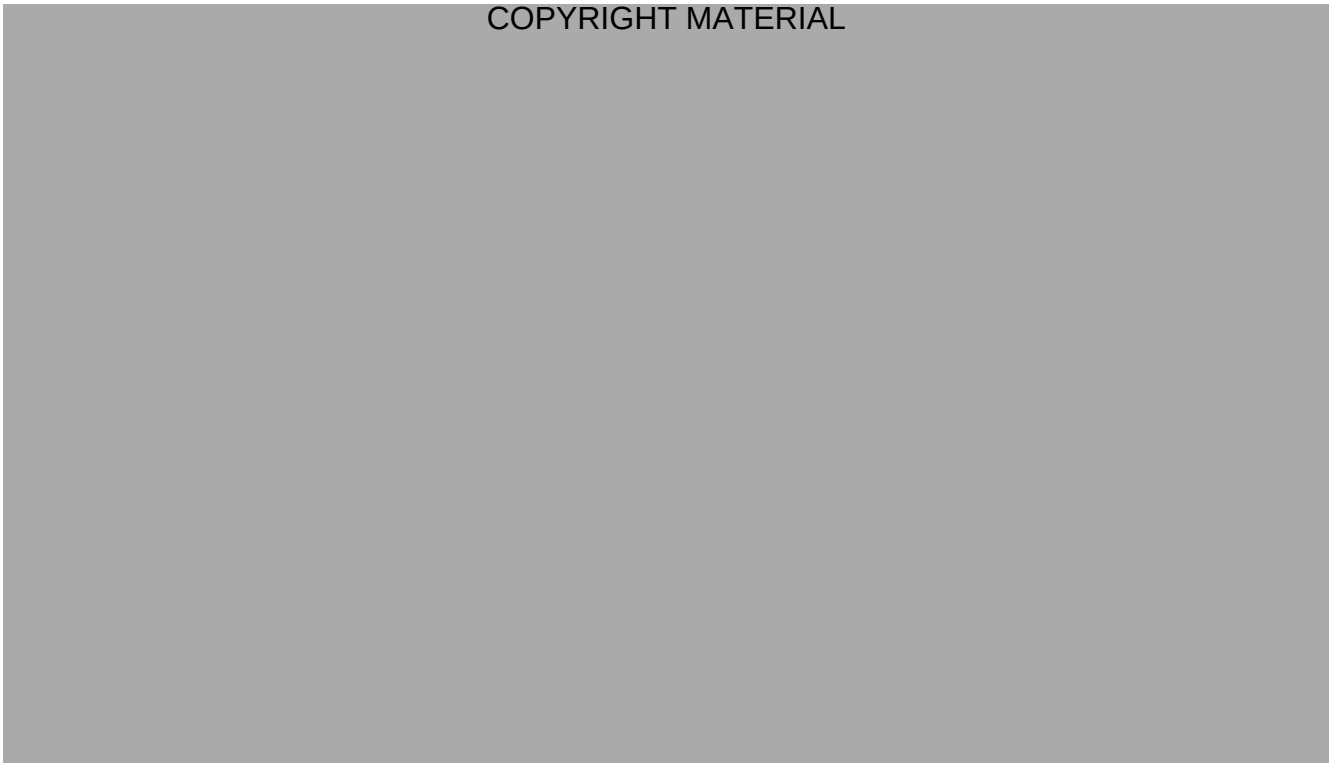
Viability and clinical signs: It states that there were no preterminal, deaths and dams did not exhibit adverse clinical signs.

Body weight: It states that body weight was not affected by policosanol.

Reproductive and fetal development parameters: The reproductive fetal observations are shown in Table 45, and there were no policosanol-related effects on these parameters, except malformations observed in fetuses in the 50 and 500 mg/kg groups. Two fetuses in the 50 mg/kg group (from two different litters) and two fetuses in the 500 mg/kg group (from a single litter) had bilateral exophthalmos. One fetus with bilateral exophthalmos in the 50 mg/kg group also exhibited agnathia, low-set ears, and a digit deformity on the right forelimb. Incidence of this finding was 0.7 (2/280 fetuses) and 0.6 (2/315 fetuses). In the paper, it states that these values are in the incidence range of exophthalmos (0.4-1.5) in historical control data reported for Sprague-Dawley rats (Manson and Kang, 1989), and one fetus with this finding was also observed in the authors' control groups of Sprague-Dawley rats (1/917 fetuses, with the incidence of 1/270 in an individual study). Thus, the exophthalmos finding was considered incidental.

**Table 45 Reproductive and fetal parameters from rats that were C-sectioned on GD 19 in the embryonic and fetal development toxicity portion [taken directly from the published paper, p.110]**





*(3) Embryo-fetal developmental toxicity study in rabbits:*

**Methods:** Groups of New Zealand White rabbits (15 mated females/group; 4-5 months of age at the arrival; a 2-week acclimation period) were administered policosanol at oral (gavage) doses of 0 (1% acacia gum/water), 500, or 1000 mg/kg/day (total volume of 10 mL) from GDs 6 to 18.

Rabbits were C-sectioned on GD 29. For each litter, the number and position of implantation sites, resorptions, and live and dead fetuses were recorded, and all fetuses were weighed, externally examined, internally sexed, and examined both for visceral and skeletal abnormalities.

Viability and clinical signs were observed daily, and body weights were measured on GDs 6, 13, 18, and 29. The paper does not state about the measurement of food consumption.

The litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

Viability and clinical signs: It states that there were no preterminal deaths, and the dams did not exhibit adverse clinical signs.

Body weight: It states that body weight was not affected by policosanol.

Reproductive and fetal development parameters: The reproductive fetal observations are shown in Table 46, and there were no policosanol-related effects on these parameters.

**Table 46 Reproductive and fetal parameters from rabbits that were C-sectioned on GD 29 in the embryonic and fetal development toxicity portion [taken directly from the published paper, p.111]**

**Publication Title: Evaluation peri- and post-natal toxicity of policosanol in rats (Rodriguez and Garcia, 1998)**

Technical grade Policosanol was supplied by Centro Nacional de Investigaciones Cientificas (CNIC; La Habana, Cuba).

It does not state that whether the study was conducted under the Cuban GLP guidelines and study protocols were consistent with the OECD and ICH guidelines.

**Methods:** Groups of Sprague Dawley rats (16 mated females/group; 8-10 weeks old at the start of the study; a 2-week acclimation period) were administered policosanol at oral (gavage) doses of 0 (1% acacia gum/water; 10 mg/mL), 5, 50, or 500 mg/kg/day (5 mL/kg) from Gestation Days (GD) 6 to Lactation Day (LD) 21. The day with evidence of mating was designated GD 0.

It does not state about viability check and clinical sign observation and measurement on food consumption. Body weights were measured on GDs 0, 6, 13, and 21 and LDs 1, 7, and 21.

All female rats naturally delivered. The day of birth was designated as Postnatal Day (PND) 0 (equivalent to LD 0). On PND 1, litters were examined for litter size, number of live and dead pups, external defects, sex, and weight of pups, and then litters were culled to 5 pups (one male and four females to the extent as possible). Pup survival and body weights were recorded on PND 7, 14, and 21. Physical and behavior development was evaluated in the following parameters: pinna unfolding on PND 4, hair growth on PND 7, incisor eruption on PND 10, ear opening on PND 14, eye opening on PND 15, descent testes on PND 21, vaginal opening on PND 37, surface righting on PND 2, air righting on PND 14, auditory startle on PND 17, visual placing on PND 17, and corneal reflex on PND 17.

One male and one female F1 pups from each litter were maintained and evaluated the reproductive potential, and the rest of female F1 pups were sacrificed on PND 21 and were subject to the visceral examination. F0 females were also sacrificed on LD 21. Sexually mature F1 animals were paired within the same dose group (but avoiding brother–sister mating). The same procedure was followed for observation and data collection for F2 offspring. It appears that F2 animals were maintained until at least PND 21 for males and PND 37 for females as the data on these PNDs are presented in the paper.

It does not state that the litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

F0 females:

Viability and clinical signs: It states that there were no preterminal deaths, and there were no unusual behavior or clinical signs of toxicity.

Body weight: It states that body weight was not affected by policosanol treatment.

Reproductive effects of F0 animals: The reproductive parameters of F0 females are shown in Table 47, and there were no policosanol-related effects on these parameters.

**Table 47 Reproductive parameters of F0 females and F1 pup data [taken directly from the published paper, p.2]**

F1 animals:

Pup survival and body weight: Data on pup survival and body weights during the pre-weaning period are included in the table above. There were no policosanol-related effects on the pup survival and their body weights.

Physical and behavioral development: Two tables summarizing physical and behavioral development evaluation are included in the paper, and these parameters were not affected by the policosanol treatment.

Reproductive parameters of F1 rats: The reproductive parameters of F1 females are shown in Tables 48-49, and there were no policosanol-related effects on these parameters.

**Table 48 Fertility parameters of F1 females [taken directly from the published paper, p.4]**

**Table 49 Reproductive parameters of F1 females and F2 pup data [taken directly from the published paper, p.4]**



F2 animals:

Pup survival and body weight: Data on pup survival and body weights of F2 animals during the pre-weaning period are included in the table above. There were no policosanol-related effects on the pup survival and their body weights.

Physical and behavioral development: Two tables summarizing physical and behavioral development evaluation of F2 pups are included in the paper, and these parameters were not affected by the policosanol treatment.

**Publication Title: Multigeneration reproduction study of policosanol in rats (Rodriguez, et al., 1997)**

The relative concentration of each alcohol in the batch were: octacosanol (67%), triacontanol (14%), hexacosanol (8%), tetracosanol (5%), heptacosanol (3%), nonacosanol (< 1%), dotriacontanol (< 1%), and tetratriacontanol (< 1%). All suspensions were prepared in 10 mg/mL aqueous gum acacia solution. Gas chromatography was used to measure policosanol in the suspension, and it states that these analyses indicated all expected concentrations for these suspensions.

It states that the study was based on guidelines and GLPs of the OECD.

**Methods:** Groups of Sprague Dawley rats (15 males and 30 females/group; 6-8 weeks old at the initiation of dosing) were administered policosanol at oral (gavage) doses of 0 (1% acacia gum/water), 5, 50, or 500 mg/kg/day. It states that policosanol was administered to rats of both sexes throughout three successive generations, but the paper does not specify how early dosing was initiated in the offspring.

Viability and clinical signs were observed daily, and body weights were measured weekly. It does not state about measurement of food consumption. When the F0 animals were approximately 16 weeks of age, two females were cohabitated with a single F0 male for a period of 3 weeks. Females with evidence of mating were cage individually. The length of gestation was recorded for each dam. The females were allowed to deliver, and the number of pups per litter, sex distribution, and gross abnormalities were evaluated. On Postnatal Day (PND) 4, litters were culled to 8 pups randomly. Survival and body weight of offspring were recorded on PNDs 1, 4, 7, 14, and 21, and the development of the pups was observed.

For F0 animals, 15 males and 30 females were used for mating, and for the subsequent generations, 10 males and 20 females were used for mating. For each generation, animals were mated twice. F0 animals were mated to produce F1a offspring, and F1a pups were sacrificed after weaning. Two weeks later, the second mating of F0 animals was performed to generate F1b offspring. Among F1b animals, one male and one female (per litter-probably) were randomly selected at weaning and maintained to generate F2a and F2b animals. Similarly, F3a and F3b offspring were generated from selected F2b animals.

It states that at necropsy, macroscopic examination was performed in all generations, and histopathology examinations were performed on the organs with gross findings.

In addition, the following behavioral tests were carried out on the F3b offspring after PND 21: righting on a surface, air righting, corneal, pirmal and pain reflexes, auditory startle, and visual placing.

It does not state that the litter was used as an experimental unit and data were expressed as mean per litter and per group.

**Results:**

It states that there were no policosanol-related clinical signs in the pregnant or lactating females. Also, it states that body weights and length of gestation were not affected by policosanol treatment.

There are 6 tables summarizing reproductive performance data in the paper. Each table contains reproductive data for each generation for each mating, and the endpoints presented in the table are following: the number of mated females, the number of pregnant females, total number of live pups (at birth, PND 4 [post-culling], and weaning), mean pup weight (at PND 4 and weaning), and indices of fertility, gestation, viability, and lactation. There were no policosanol-related effects on endpoints evaluated in the study.

The fertility index and mean number of live pups at birth were occasionally lower in the 50 and 100 mg/kg groups as shown in Table 50. As these changes were not consistent and the magnitudes of the changes were relatively small, the findings were not considered policosanol-related.

**Table 50 Fertility index and mean number of live pups per litter at birth of different generations [FI values taken directly from 6 tables in the published paper]**

| | F1a (first mating) | | F1b (second mating) | | F2a (first mating) | | F2b (second mating) | | F3a (first mating) | | F3b (second mating) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose Group | FI[a] of F0 | Live pups[b] | FI[a] of F0 | Live pups[b] | FI[a] of F1b | Live pups[b] | FI[a] of F1b | Live pups[b] | FI[a] of F2b | Live pups[b] | FI[a] of F2b | Live pups[b] |
| Control | 86 | 11.6 | 87 | 10.6 | 95 | 11.2 | 75 | 10.5 | 85 | 10.6 | 85 | 11.3 |
| 5 mg/kg | 87 | 9.6 | 83 | 10.9 | 85 | 11.6 | 85 | 11.1 | 85 | 9.6 | 95 | 10.2 |
| 50 mg/kg | 91 | 8.9 | 70 | 9.4 | 90 | 10.7 | 90 | 10.5 | 75 | 11.9 | 85 | 11.4 |
| 500 mg/kg | 79 | 11 | 79 | 8.4 | 90 | 11.7 | 70 | 9.6 | 90 | 10.7 | 85 | 10.6 |

[a]Fertility Index (FI) = (No. of pregnant females/No. of mated females) x 100
[b]Mean No. of live pups per litter at birth are presented. The Reviewer calculated this value by using the data presented in the paper (total No. of live pups at birth and No. of pregnant rats for each group)

The paper also states that there were no policosanol-related effects on any development parameters evaluated in the F3b offspring.

## Appendix 4: Manufacturing Process and Process Control of Beeswax

The following information is obtained from 3.2.A.3 Excipients-Yellow Beeswax.

Yellow beeswax for Xtampza ER is manufactured [redacted] In response to the information request from the FDA regarding the control of residual [redacted] in beeswax, the Applicant states that the [redacted] beeswax used in Xtampza ER is sourced from specific suppliers that are known [redacted] to provide beeswax that tests negative [redacted]

[redacted] A flow diagram depicting the yellow beeswax manufacturing process is shown in Figure 6 [redacted]


**Figure 5 Flow diagram for yellow beeswax manufacturing process [taken directly from 3.2.A.3 Excipients-Yellow Beeswax, p.8]**

(b) (4) tests the (b) (4) yellow beeswax according to Table 51 below. As compared to (b) (4) the content of beeswax is not thoroughly analyzed.

**Table 51 (b) (4) yellow beeswax specifications [taken directly from 3.2.A.3 Excipients-Yellow Beeswax, p.9]**

| Test (Method) | Acceptance Criteria |
|---|---|
| (b) (4) | |

(b) (4)

As the FDA requested to provide justification that the Applicant's assessment (b) (4) is adequate to fully characterize the potential for all possible residual (b) (4) Collegium provided some additional information on assessment (b) (4) in beeswax. The supplier (b) (4) chose to evaluate (b) (4)

Based on this evaluation and testing, the supplier has found and chosen sources of (b) (4) beeswax that test negative for (b) (4)

[redacted] each lot of [redacted] beeswax. This testing uses validated methods [redacted] The list [redacted] that are tested was submitted to the FDA. The list [redacted] and MRL limits are the same [redacted]

Upon receipt of a lot of [redacted] yellow beeswax, NF [redacted] Collegium has verified the lot by analyzing [redacted] by a second testing laboratory, [redacted] This laboratory also uses validated methods [redacted] The list [redacted] analyzed and MRL limits are the same [redacted]

To date, three lots of [redacted] yellow beeswax were tested [redacted] which were also tested for the supplier [redacted] All lots have passed USP/EP specifications. Although lots of [redacted] yellow beeswax are tested [redacted] However, it states that the supplier has found and chosen sources of [redacted] beeswax that test negative [redacted]

Overall, a quality control process of beeswax is not as robust [redacted]

The manufacturer's specifications for yellow beeswax do not include a test [redacted] nor is such a test included in the USP/NF as a requirement for compliance to the monograph. Collegium has investigated this material for the presence of [redacted] impurities [redacted] in yellow beeswax by sending three lots to an outside contract laboratory [redacted] Analysis was conducted [redacted]

**Table 52 (b) (4) impurity test results for yellow beeswax (b) (4) [taken directly from 3.2.A.3 Excipients-Yellow Beeswax, p.12]**

(b) (4)

The FDA requested Collegium to ensure that the beeswax used in the preparation of the drug product, does not contain protein allergens, and in the response, Collegium reiterated wide use of beeswax in food applications and searched for any allergic reactions of beeswax reported in the literature. Some of the key points are summarized here.

Modern day food applications of beeswax include its use as a component in dietary food supplements (soft gelatin capsules and tablets), glazing and coating agent, texturizer for chewing-gum base and carrier for food additives (including flavors and colors). Based on the available data, oral exposure to beeswax from a variety of sources is extremely common. (b) (4) Additionally, the Applicant conducted literature search using PubMed with the search terms (beeswax AND allerg* AND food) in all fields and found 3 articles concerning beeswax allergies. All allergic responses are found in topical products, where contact allergies with the skin and lips were reported, and there were no articles that report allergic reactions with patients who have ingested beeswax. The Applicant also pointed out that the manufacturing process of yellow beeswax involves (b) (4) The manufacturing process for Xtampza ER (b) (4)

## Appendix 5: Manufacturing Process and Process Control of Carnauba Wax

Carnauba wax is manufactured (b) (4)

A flow diagram depicting the carnauba wax NF manufacturing process at shown in Figure 7 (b) (4)

(b) (4)

(b) (4)


**Figure 6 Flow diagram for the carnauba wax manufacturing process [taken directly from 3.2.A.3 Excipients-Carnauba Wax, p.13]**

(b) (4)

(b) (4)

See Table 53 below.

**Table 53 Excipient testing specification summary for carnauba wax, NF [taken directly from 3.2.A.3 Excipients-Carnauba Wax, p.18]**

| Test | Specification |
|---|---|
| **Compendial Tests** | |
| (b) (4) | |

(b) (4)

(b) (4)

The results showed that levels of (b) (4) impurities observed in carnauba wax do not pose a safety risk.

**Table 54 (b) (4) impurity test results for carnauba wax (b) (4) [taken directly from 3.2.A.3 Excipients-Carnauba Wax, p.17]**

(b) (4)

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GRACE S LEE
09/22/2015

RICHARD D MELLON
09/22/2015

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 208090

**NDA Number: 208090** **Applicant: Collegium Pharmaceutical, Inc.** **Stamp Date: 12/12/2014**

**Drug Name: Xtampza (Oxycodone DETERx® Capsules)** **NDA/BLA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | X | | Note that this is a 505(b)(2) application, and the Applicant relies on information from the literature as well as the Agency's previous findings of safety for OxyContin for toxicology studies. |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | | | Not applicable<br>Note that this is a 505(b)(2) application, and the Applicant relies on information from the literature as well as the Agency's previous findings of safety for OxyContin for toxicology studies. |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route? | | | Not applicable<br>Note that this is a 505(b)(2) application, and the Applicant relies on information from the literature as well as the Agency's previous findings of safety for OxyContin for toxicology studies. |
| 7 | Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | | | The Applicant conducted one GLP-compliant GLP study to qualify the novel excipients (yellow beeswax and carnauba wax), however, the study report of this study has not been submitted. We contacted the Applicant to determine the intent of this study, and the Applicant |

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 208090

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| | | | | replied that they felt the weight-of-evidence based on literature was adequate in the absence of the study report. |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | | X | The Applicant provided a literature-based justification for the levels of novel excipients along with the final study report of a 14-week dog study using beeswax and carnauba wax (non-GLP) and the study protocol of a 90-day dog study using beeswax and carnauba wax (GLP). Upon preliminary review, in the absence of the final study report of the 90-day dog study and adequate characterization of chronic toxicity study of beeswax, the submitted information is not considered adequate for review to evaluate whether these excipient levels are adequately qualified. However, the Applicant claims that the literature review alone is adequate to support the safety, therefore, the Agency will consider this deficiency to be a review issue and not a filing issue. |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | X | | Revision of labeling is needed according to the PLLR, with permission from the Applicant. |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | | X | The Applicant addressed the issues of oxycodone impurities (b) (4) which were raised at the preNDA meeting. However, the proposed specification for unspecified impurity in the drug product ((b) (4)%) yields above the ICH Q3B limit of 2 mg/day. This issue will be conveyed to the Applicant. See below. |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | | | Defer to CSS |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | Not applicable |

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 208090

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __Yes_____**

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Following the filing review of you NDA submission, we have identified the following potential review issues:

1. Upon preliminary review, your NDA does not appear to contain adequate information to justify the safety of the drug product formulation. Specifically, a complete toxicity profile of beeswax was not included in your NDA submission. Submit the final study report of the 90-day dog study using beeswax and carnauba wax [Study Number (b) (4) 724003] as soon as possible; however, we cannot guarantee that this study will be reviewed during this review cycle, and submission of the final study report could impact the action date for this application. In addition, you do not appear to have provided a chronic toxicity profile of beeswax or chronic toxicity studies using saturated, unbranched long-chain free fatty acids. We also note that you only provided tertiary review articles containing only summary data for certain constituents of beeswax (e.g. toxicity studies using oleyl palmitate or Class I mineral oil). We recommend that you provide copies of the original source materials (primary references) for these studies if at all possible. As we have previously communicated, a final determination of the adequacy of the safety assessment of the novel excipients based on a detailed review of all submitted toxicological data (including reproductive and developmental toxicity and carcinogenicity data) will be made during the NDA review cycle, and inadequate toxicology data could be potentially approval issues.

2. The proposed specification for unspecified impurity in the drug product (NMT (b) (4) %) (b) (4)

| | |
|---|---|
| Grace S. Lee, PhD, DABT | February 6, 2015 |
| Reviewing Pharmacologist | Date |
| R. Daniel Mellon, PhD | February 6, 2015 |
| Team Leader/Supervisor | Date |

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GRACE S LEE
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