

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

218881Orig1s000

PRODUCT QUALITY REVIEW(S)



| | | |
|-----------------|--|--------------|
| Title: | NDA IQA Template Title Page- EXEC SUMMARY | |
| Document ID: | OPQ-ALL-TEM-0040 | |
| Effective Date: | 18 Sep 2023 | Revision: 00 |
| Total Pages: | 4 | |



Template Revision: 03

Office of Pharmaceutical Quality

New Drug Application (NDA) Integrated Quality Assessment Template

NDA Executive Summary

1. Application/Product Information

| | | | |
|--|---|--------------------|------------------|
| NDA Number | 218881 | | |
| Applicant Name | ELI LILLY AND COMPANY | | |
| Drug Product Name | Inluriyo (imlunestrant) tablets | | |
| Dosage Form | Tablet | | |
| Proposed Strength(s) | 200 mg | | |
| NDA Classification | Type 1 - NME | | |
| Route of Administration | Oral | | |
| Maximum Daily Dose | 400 mg | | |
| Rx/OTC Dispensed | Rx | | |
| Proposed Indication | <p>INLURIYO is an estrogen receptor antagonist indicated for:</p> <ul style="list-style-type: none"> treatment of adults with ER-positive, HER2-negative, ESR1-mutated advanced or metastatic breast cancer with disease progression following at least one line of endocrine therapy. | | |
| Drug Product Description | <p>INLURIYO tablets contain 200 mg imlunestrant and are white, film coated- capsule-shaped tablets, with "LILLY" on one side and "1717" and elongated 4-point starburst on the other side</p> | | |
| Co-packaged product information | N/A | | |
| Device information | N/A | | |
| Storage Temperature/ Conditions | <p>Store at 20°C to 25°C (68°F to 77°F). Excursions between 15°C to 30°C (59°F to 86°F) are permitted [see USP Controlled Room Temperature].</p> | | |
| Review Team | Discipline | Primary | Secondary |
| | <i>Drug Substance</i> | Raymond Frankewich | Haripada Sarker |

| | | | |
|-----------------|-------------------------------|---------------------|--|
| | <i>Drug Product/ Labeling</i> | Olen Stephens | Xing Wang David Claffey (labeling) |
| | <i>Manufacturing</i> | Ann-Marie Afrifa | Christine Falabella |
| | <i>Biopharmaceutics</i> | Min Kang | Anitha Govada |
| | <i>Microbiology</i> | Ann-Marie Afrifa | Christine Falabella |
| | <i>EA</i> | Xiaoqin Wu | James Laurenson |
| | <i>RBPM</i> | Utkarsh Desai | |
| | <i>ATL</i> | Xing Wang | |
| Consults | None | | |

2. Final Overall Recommendation - Approval

3. Action Letter Information

a. Expiration Dating: An expiration dating period of 36 months may be granted when stored at the proposed storage conditions.

**b. Additional Comments for Action
None**

4. Basis for Recommendation:

a. Summary of Rationale for Recommendation:

The applicant has provided sufficient information to assure the identity, strength, purity, and quality of the proposed drug product. All associated manufacturing, testing, packaging facilities were deemed acceptable based on prior inspection history. Although FDA does not agree with some of the results in the EA, the agency has concluded that the submitted information is sufficient to determine whether approval of this drug application may significantly affect the quality of the human environment, and thus the EA is adequate for approval, per 21 CFR 25.15(a). Based on the submitted information, FDA has determined that approval of this application is not expected to have significant effects on the quality of the human environment. Therefore, FDA is issuing a finding of no significant impact (FONSI), and an environmental impact statement will not be prepared, per 21 CFR 25.15(b), 25.40, and 25.41. Based on the OPQ review team's evaluation of the information provided in the submission, OPQ recommends APPROVAL of NDA 218881 for INLURIYO (imlunestrant) tablets, for oral use.

b. Is the overall recommendation in agreement with the individual discipline recommendations? Yes

Recommendation by Subdiscipline:

| | | |
|-------------------------|---|-----------------|
| Drug Substance | - | Adequate |
| Drug Product | - | Adequate |
| Quality Labeling | - | Adequate |
| Manufacturing | - | Adequate |
| Biopharmaceutics | - | Adequate |
| Microbiology | - | N/A |

Environmental Assessment: Review & FONSI - Adequate
QPA for EA(s): No

5. Life-Cycle Considerations
Established Conditions per ICH Q12: No
Comments: None

Comparability Protocols (PACMP): No
Comments:

Additional Lifecycle Comments: None

Application Technical Lead Name and Date:

Xing Wang

08/22/2025



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Wang

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CHAPTER IV: LABELING

1.0 PRESCRIBING INFORMATION

Assessment of Product Quality Related Aspects of the Prescribing Information: The associated labeling is generally acceptable for approval with the addition of edits/additions proposed below. At the time this review is due, the labeling negotiations with the applicant are on-going.

1.1 HIGHLIGHTS OF PRESCRIBING INFORMATION

| Item | Items in Proposed Labeling | Assessor's Comments |
|--|----------------------------|---|
| Product Title in Highlights | | |
| Established name(s) ¹ | Adequate | INLURIYO (imlunestrant) tablet |
| Route(s) of administration | Adequate | Oral |
| Dosage Forms and Strengths Heading in Highlights | | |
| Summary of the dosage form(s) and strength(s) in metric system | Adequate | Tablets: 200 mg |
| Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored". | N/A | |
| For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient-use). Other package terms include pharmacy bulk package and imaging bulk package. | N/A | |
| If the drug product contains an active ingredient that is a salt, clearly state whether the strength is based on the active moiety (e.g., Tablets: 10 mg of drug-x) or active ingredient (e.g., Tablets: 10 mg of drug-x hydrochloride). | Adequate | Tosylate salt; strength represented on the basis of the active moiety |

¹ Established name = [Drug] [Route of Administration] [Dosage Form]

1.2 FULL PRESCRIBING INFORMATION

1.2.1 Section 2 (DOSAGE AND ADMINISTRATION)

| Item | Items in Proposed Labeling | Assessor's Comments |
|---|----------------------------|--|
| DOSAGE AND ADMINISTRATION section | | |
| Special instructions for product preparation (e.g., reconstitution and resulting concentration, dilution, compatible diluents, storage conditions needed to maintain the stability of the reconstituted or diluted product) | N/A | "Swallow the tablets whole. Do not split, crush, or chew the tablets. (b) (4) _____ _____" |
| Important administration instructions supported by product quality information (e.g., do not crush or chew extended-release tablets, instructions for mixing with food) | Adequate | "Swallow the tablets whole. Do not split, crush, or chew the tablets. (b) (4) _____ _____" |
| For parenteral products: include statement: <i>"Parenteral drug products must be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit"</i> | N/A | |
| If there is a USP monograph for the drug product and it contains a labeling requirement, ensure the labeling requirement is fulfilled. Note the labeling requirement may be applicable to another section of the PI (e.g., Section 11). | N/A | |
| For radioactive products, include radiation dosimetry for the patient and | N/A | |

| | | |
|--|--|--|
| healthcare practitioner(s) who administer the drug | | |
|--|--|--|

1.2.2 Section 3 (DOSAGE FORMS AND STRENGTHS)

| Item | Items in Proposed Labeling | Assessor's Comments |
|--|----------------------------|--|
| DOSAGE FORMS AND STRENGTHS section | | |
| Available dosage form(s) | Adequate | Tablets |
| Strength(s) in metric system | Adequate | 200 mg |
| If the active ingredient is a salt, apply the USP Salt Policy per FDA Guidance. Clearly state whether the strength is based on the active moiety (e.g., Tablets: 10 mg of drug-x) or active ingredient (Tablets: 10 mg of drug-x hydrochloride). | Inadequate | "Tablets: 200 mg of imlunestrant" This edit was added to the working document in the clinical divisions SharePoint to be sent to the applicant. |
| A description of the identifying characteristics of the dosage forms, including shape, color, coating, scoring, imprinting, and color and clarity of the solution, when applicable | Adequate | "white, film coated-capsule shaped tablet, with "LILLY" on one side and "1717" and elongated 4-point starburst on the other side" |
| Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored" | N/A | |
| For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient-use). Other package type terms include pharmacy bulk package and imaging bulk package. | N/A | |

Section 11 (DESCRIPTION)

| Item | Items in Proposed Labeling | Assessor's Comments |
|--|----------------------------|--|
| DESCRIPTION section | | |
| Proprietary and established name(s) | Adequate | INLURIYO |
| Dosage form(s) and route(s) of administration | Inadequate | Tablet "for oral administration" This edit was added to the working document in the clinical divisions SharePoint to be sent to the applicant. |
| If the active ingredient is a salt, apply the USP Salt Policy and include the equivalency statement per Salt Guidance and MAPP . For example: "TRADENAME contains 100 mg of drug-x (equivalent to 123.7 mg of drug-x hydrochloride)" | Inadequate | INLURIYO contains 200 mg of imlunestrant (equivalent to 265.66 mg of imlunestrant tosylate) This edit was added to the working document in the clinical divisions SharePoint to be sent to the applicant. |
| List names of all inactive ingredients. Use USP/NF names in alphabetical order. Avoid brand names. | Adequate | |
| For parenteral injectable dosage forms, include the name and quantities of all inactive ingredients. For ingredients added to adjust the pH or make isotonic, include the name and statement of effect. | N/A | |
| If alcohol is present, must provide the amount of alcohol in terms of percent volume of absolute alcohol | N/A | |
| Sterility statement (if applicable) | N/A | |
| Pharmacological/Therapeutic class | Adequate | Estrogen receptor (b) (4) |
| Chemical name, structural formula, molecular weight | Adequate | |
| If radioactive, statement of important nuclear characteristics. | N/A | |
| Other important chemical or physical properties (such as pKa or pH) | Adequate | |

Section 11 (DESCRIPTION) Continued

| Item | Items in Proposed Labeling | Assessor's Comments |
|--|----------------------------|---------------------|
| For oral prescription drug products, include gluten statement (if applicable) | N/A | |
| Remove statements that may be misleading or promotional (e.g., "synthesized and developed by Drug Company X," "structurally unique molecular entity") | Adequate | |
| If there is a USP monograph for the drug product and it contains a labeling requirement, ensure the labeling requirement is fulfilled. Note the labeling requirement may be applicable to another section of the PI (e.g., Section 2). | N/A | |

1.2.4 Section 16 (HOW SUPPLIED/STORAGE AND HANDLING)

| Item | Items in Proposed Labeling | Assessor's Comments |
|--|----------------------------|---|
| HOW SUPPLIED/STORAGE AND HANDLING section | | |
| Available dosage form(s) | Adequate | Tablets |
| Strength(s) in metric system | Adequate | 200 mg |
| Available units (e.g., bottles of 100 tablets) | Adequate | 28-count or 56-count bottle configuration |
| Identification of dosage forms (e.g., shape, color, coating, scoring, imprinting, and color and clarity of the solution, when applicable); Include NDC(s) | Adequate | white capsule-shaped tablet with "LILLY" on one side and "1717" and an elongated 4-point starburst on the other side. |
| Assess if the tablet is scored. If product meets guidelines and criteria for a scored tablet, state "functionally scored" | N/A | |
| For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient-use). Other package terms include pharmacy bulk package and imaging bulk package. | N/A | |
| Special handling about the supplied product (e.g., protect from light, refrigerate). If there is a statement to "Dispense in original container," provide reason why (e.g., to protect from light or moisture, to maintain stability, etc.). For hazardous drugs, state "DRUG X is a hazardous drug. Follow applicable special handling and disposal procedures. ^x " with x numerical citation to "OSHA Hazardous Drugs." | N/A | |

1.2.5 Other Sections of Labeling

1.2.6 Manufacturing Information After Section 17 (for drug products)

| Item | Items in Proposed Labeling | Assessor's Comments |
|---|----------------------------|---|
| Manufacturing Information After Section 17 | | |
| Name and location of business (street address, city, state, and zip code) of the manufacturer, distributor, and/or packer | Adequate | Marketed by: Lilly USA, LLC, Indianapolis, IN 46285, USA |

2.0 PATIENT LABELING

Assessment of Product Quality Related Aspects of Patient Labeling (e.g., Medication Guides, Instructions for Use, Patient Information):

| Item | Items in Proposed Labeling | Assessor's Comments about Carton Labeling |
|---|----------------------------|---|
| Established name ² | Adequate | INLURIYOä (en-loo-ree-yoh) |
| Special preparation instructions (if applicable) | N/A | Swallow tablets whole. <ul style="list-style-type: none"> Do not chew, crush, or split the tablets. <div style="background-color: gray; width: 100px; height: 15px; margin-left: 100px;">(b) (4)</div> |
| Storage and handling information (if applicable) | N/A | |
| If the product contains a desiccant, ensure the desiccant has a warning (e.g., "Do not eat.") and the size and shape of the desiccant differs from the dosage form. | N/A | |
| Active ingredient(s) (if applicable) | Inadequate | Imlunestrant [added] ' <i>tosylate</i> ' This edit was added to the working document in the clinical divisions SharePoint to be sent to the applicant. |
| Alphabetical listing of inactive ingredients (if applicable) | Adequate | croscarmellose sodium, hydroxypropyl cellulose, magnesium stearate, microcrystalline cellulose. |
| Name and location of business (street address, city, state, and zip code) of manufacturer, distributor, and/or packer | Adequate | Marketed by: Lilly USA, LLC, Indianapolis, IN 46285, USA |

3.0 CONTAINER AND CARTON LABELING

² Established name = [Drug] [Route of Administration] [Dosage Form]

| Item | Items in Proposed Labeling | Assessor's Comments about Carton Labeling |
|---|----------------------------|---|
| Established name ³ , (font size and prominence) | Inadequate | <p>Include 'tablets' to the established name on the primary front display of the bottle label.</p> <p>This edit was added to the information request document in the clinical divisions SharePoint to be sent to the applicant.</p> |
| Strength(s) in metric system | Adequate | |
| Route(s) of administration | N/A | Oral is implicit for tablets |
| If the active ingredient is a salt, include the equivalency statement per Salt Guidance and MAPP . | Inadequate | <p>Include a salt equivalence statement on the side panel of the bottle label.</p> <p>This edit was added to the information request document in the clinical divisions SharePoint to be sent to the applicant.</p> |
| Net contents (e.g., tablet count, volume of liquid) | Adequate | |
| "Rx only" displayed on the principal display | Adequate | |
| NDC | Adequate | |
| Lot number and expiration date | Adequate | |
| Storage conditions. If applicable, include a space on the carton labeling for the user to write the new beyond-use-date (BUD). | Adequate | |
| For injectable drug products for parental administration, use appropriate package type term (e.g., single-dose, multiple-dose, single-patient-use). Other package terms include pharmacy bulk package and imaging bulk package, and these products require a "Not for direct infusion" statement. | N/A | |
| For parenteral injectable dosage forms, include the name and quantities of all active and inactive ingredients in alphabetical order. For ingredients added to adjust the pH or make isotonic, include the name and statement of effect. | N/A | |

| | | |
|--|----------|--|
| If alcohol is present, must provide the amount of alcohol in terms of percent volume of absolute alcohol | N/A | |
| Linear Bar code | Adequate | |

| Item | Items in Proposed Labeling | Assessor's Comments about Carton Labeling |
|---|----------------------------|--|
| Name of manufacturer/distributor /packer | Adequate | |
| If there is a Medication Guide, must include a statement about dispensing a Medication Guide to each patient. | N/A | |
| No text on Ferrule and Cap overseal, unless a cautionary statement is required. | N/A | |
| If there is a USP monograph for the drug product and it contains a labeling requirement, ensure the labeling requirement is fulfilled. | N/A | |
| When a drug product differs from the relevant USP standard of strength, quality, or purity, as determined by the application of the tests, procedures, and acceptance criteria set forth in the relevant compendium, its difference shall be plainly stated on its label. | N/A | |
| And others, if space is available. | N/A | <p>If there is room on the bottle label, include a statement to, 'Dispose unused medication via a take-back option if available. Do NOT flush down the toilet.'</p> <p>This edit was added to the information request document in the clinical divisions SharePoint to be sent to the applicant.</p> |

Assessment of Carton and Container Labeling: Adequate; labeling negotiations are on-going, but none of the proposed changes are controversial and the applicant is likely to accept without much resistance.

³ Established name = [Drug] [Route of Administration] [Dosage Form]

ITEMS FOR ADDITIONAL ASSESSMENT

Edits noted above have been included in working documents on the clinical division's SharePoint site to be sent in a collated list of changes.

Overall Assessment and Recommendation:

Adequate with the changes noted above. Labeling is currently on-going.

Primary Labeling Assessor Name and Date: Olen Stephens 5/23/2025

Secondary Assessor Name and Date: David Claffey 5/23/2025



Olen
Stephens

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Comments: Edits are noted in the document and are to be communicated to the applicant.



David
Claffey

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CHAPTER VI: BIOPHARMACEUTICS

| | |
|---|---|
| NDA Number | NDA 218881-ORIG-1 |
| Assessment Cycle Number | 1 |
| Drug Product Name/ Strength | Inluriyo® ¹ (Imlunestrant) Tablet, 200 mg |
| Route of Administration | Oral |
| Applicant Name | Eli Lilly and Company |
| Therapeutic Classification/ OND Division | DOI |
| Proposed Indication | Indicated as monotherapy for the treatment of patients with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative, estrogen receptor 1 (ESR1)-mutated advanced or metastatic breast cancer previously treated with an endocrine-based regimen |
| Primary Reviewer | Min Kang, PharmD, MS |
| Secondary Reviewer | Anitha Govada, PhD |
| Tertiary Reviewer | Angelica Dorantes, PhD |
| Assessment Recommendation | Adequate |

EXECUTIVE SUMMARY:

The Applicant submitted this 505(b)(1) NDA 218881 for Inluriyo® (Imlunestrant [LY3484356]) Tablet, 200 mg. Imlunestrant (also referred to as LY3484356 tosylate) is an immediate release film coated tablet, indicated for the treatment of adults with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), estrogen receptor 1 (ESR1)-mutated advanced or metastatic breast cancer previously treated with an endocrine based regimen.

Assessment Summary:

This Biopharmaceutics Review focuses on evaluating (1) the in vitro dissolution method and acceptance criterion as a quality control (QC) test, and (2) the bridging between the clinical and the proposed to-be-marketed drug product.

¹ FDA conditionally accepted the proprietary name of INLURIYO on 6/1/2023:
[\\CDSESUB1\EVSPROD\nda218881\0001\m1\us\proprietary-names.pdf](https://cdsesub1\EVSPROD\nda218881\0001\m1\us\proprietary-names.pdf)

- In Vitro Dissolution Method and Acceptance Criterion:**

The Applicant proposed dissolution method and acceptance criterion are found acceptable for the Quality Control (QC) testing of Inluriyo® (Imlunestrant) Tablets, 200 mg, at batch release and on stability as the following:

| Approved Dissolution Method and Acceptance Criterion for Inluriyo® (Imlunestrant) Tablet, 200 mg | | | | |
|--|--------|---------------|-----------|------------------------|
| Apparatus | Speed | Volume/Temp | Medium | Acceptance Criterion |
| USP II (Paddle) | 75 rpm | 900 mL/ 37 °C | 0.1 N HCl | Q = (b) (4)% at 20 min |

| CQAs | Initial Risk Ranking | Comments | Updated Risk Ranking after Assessment | Comments |
|-------------|----------------------|---|---------------------------------------|--|
| Dissolution | Medium | Low soluble drug substance with pH-dependent solubility | Low | The proposed dissolution method exhibits discriminating ability towards unacceptable (b) (4) quality, which is determined by a combination of several (b) (4) properties, attributes, and (b) (4) parameters. Ensuring the quality of (b) (4) for consistent and adequate drug release/dissolution is critical from biopharmaceutics perspective because the proposed drug product composes of (b) (4) |

- Product Bridging:**

The three primary registration batches (CMXVP, CMXVS, CMXVT) were used in the pivotal/clinical study (EMBER-3). However, for the proposed to-be-marketed drug product, the Applicant's proposed manufacturing site is different ((b) (4)) from the manufacturing site used to manufacture the clinical batches as well as the

primary registration/stability batches ((b) (4)). Therefore, the Applicant was requested to submit the comparative dissolution profile data of the two sites using the proposed QC dissolution method via IR #2 dated 4/10/25. Based on the data from the Applicant's response submitted on 1/31/2025, very rapid dissolution was observed ($\geq 85\%$ in 15 min) for the products manufactured at the two sites, and therefore, the bridging is adequately supported and established. The Applicant confirmed that there were no changes to the formulation, nor manufacturing process between the batches of drug product manufactured at the two sites.

List of Submissions Being Assessed:

| eCTD sequence # | Date of response | Document |
|-----------------|------------------|---|
| 0001 | 10/31/2024 | Original NDA submission |
| 0009 | 1/31/2025 | Quality/Response ² to Quality/Biopharmaceutics IR #1 dated 1/22/2025 |
| 0027 | 05/05/2025 | Quality/Response ³ to Quality/Biopharmaceutics IR #2 dated 4/10/25 |

Concise Description of Outstanding Issues (list bullet points with key information and update as needed):

None.

Overall Biopharmaceutics Recommendation:

From the Biopharmaceutics perspective, NDA 218881-ORIG-1 for Inluriyo® (Imlunestrant) Tablet, 200 mg, is **Adequate** and recommended for Approval.

² <\\CDSESUB1\EVSPROD\nda218881\0009\m1\us\quality-information-amendment.pdf>

³ <\\CDSESUB1\EVSPROD\nda218881\0027\m1\us\m1-11-1-quality-information-amendment.pdf>

B.1 BCS DESIGNATION

Assessment: A BCS designation was not requested, nor required.

Solubility: Based on the Applicant's submitted solubility data (Table 1), Imlunestrant Tosylate drug substance is low soluble.

Table 1: Solubility (mg/mL) of Imlunestrant Tosylate in Aqueous Media at 37°C

| Solvent | Solubility of Imlunestrant Tosylate (mg/mL) | Solubility Description ^a |
|-------------------------|---|-------------------------------------|
| Water | 0.019 ± 0.000 | Insoluble |
| 0.1N HCl | 1.7 ± 0.0 | Slightly soluble |
| 0.01N HCl | 0.34 ± 0.01 | Very slightly soluble |
| pH 4.5, Acetate (USP) | 0.098 ± 0.001 | Insoluble |
| pH 6.0, Phosphate (USP) | 0.015 ± 0.001 | Insoluble |
| pH 7.5, Phosphate (USP) | <0.0005 ^b | Insoluble |
| 0.1N NaOH | >10.5 ^c | Sparingly soluble |
| SGF ^d | 0.54 ± 0.00 | Very slightly soluble |
| FaSSIF ^d | 0.007 ± 0.000 | Insoluble |
| FeSSIF ^d | 3.1 ± 0.0 | Slightly soluble |

^a Solubility descriptions are consistent with the current USP, Ph.Eur., and Japanese Pharmacopoeias.

^b Concentration was measured to be <LOQ of the HPLC method, defined as 0.0005 mg/mL.

^c At the measured concentration, all of the loaded solids have been dissolved.

^d SGF: Simulated Gastric Fluid; FaSSIF/FeSSIF: Fasted/Fed State Simulated Intestinal Fluid.

Permeability: Based on the Mass Balance/Absolute Bioavailability Study ⁴ to determine the disposition of radioactivity in healthy adults following oral administration of a single dose of 400 mg, Imlunestrant Tosylate drug substance is likely highly permeable.

Reviewer's comments:

A BCS designation was not requested by the Applicant, nor required by the FDA. Based on the submitted solubility data above, this Reviewer considers imlunestrant tosylate as a low soluble drug substance (reduced solubility with increase in pH). Based on the provided information, Imlunestrant (LY3484356) is likely a high permeability drug substance. According to the BCS criteria, high permeability can be concluded when (i) absolute bioavailability is ≥85%, or (ii) ≥85% of the administered dose is recovered in urine as unchanged (parent drug), or (iii) ≥85% as the sum of parent drug, Phase 1 oxidative and Phase 2 conjugative metabolites, and (iv) for metabolites in feces, only oxidative and conjugative metabolites can be considered.

⁴ M.5.3.3.1. Mass Balance Study (J2J-MC-JZLE): <\\CDSESUB1\EVSPROD\nda218881\0001\m5\53-clin-stud-rep\533-rep-human-pk-stud\5331-healthy-subj-pk-init-tol-stud-rep\j2j-mc-jzle\jzle-04-csr-synopsis-and-body.pdf>

Based on the Applicant's mass balance data⁵, Imlunestrant recovered in urine as unchanged accounted for only about 0.278% of the administered dose, although the remainder of the Imlunestrant, which accounted for about 61.8%, was recovered in feces. In the form of metabolites, M2 (sulfate conjugate) was the most abundant in the feces accounting for a mean of 20.9% and additional metabolites resulting from oxidation accounted for about 5.1% according to the Applicant. Combining the amounts of unchanged parent drug (Imlunestrant), oxidative and conjugative metabolites, about 87.8% of Imlunestrant drug substance are recovered in feces; therefore, based on the mass-balance study result, Imlunestrant is likely a highly permeable drug substance. However, since the Applicant did not pursue an official claim for Imlunestrant as BCS-2, the final BCS designation (Class 2 or Class 4) decision remains inconclusive regarding its permeability.

B.2 DISSOLUTION METHOD AND ACCEPTANCE CRITERION

Assessment: Adequate

Table 2: Approved Dissolution Method and Acceptance Criterion for Imlunestrant Tablet, 200 mg (T1)

| Apparatus | Speed | Volume/ Temp | Medium | Acceptance Criterion |
|-----------------|--------|---------------|-----------|------------------------------------|
| USP II (Paddle) | 75 rpm | 900 mL/ 37 °C | 0.1 N HCl | Q = ^{(b) (4)} % at 20 min |

(b) (4)

⁵ M.5.3.3.1. Mass Balance Study (J2J-MC-JZLE): [\\CDSESUB1\EVSPROD\nda218881\0001\m5\53-clin-stud-rep\533-rep-human-pk-stud\5331-healthy-subj-pk-init-toi-stud-rep\j2j-mc-jzle\jzle-04-csr-synopsis-and-body.pdf](#)

B.2.4. Dissolution Data and Acceptance Criterion:**Table 5: Registration Batches**

| Batch Number | CMXVP | CMXVS | CMXVT |
|----------------------------------|--|--|--|
| Drug Substance Batch Number | C18071754-J22004A C18071754-J22004B | C18071754-J22005A C18071754-J22005B | C18071754-J22007A C18071754-J22007B |
| Theoretical Batch Size (tablets) | (b) (4) | | |
| Manufacturing Site | | | |
| Date of Manufacture | Sep 2022 | Sep 2022 | Oct 2022 |
| Batch Use | Clinical, Primary Stability | Clinical, Primary Stability | Clinical, Primary Stability |

Table 6: Dissolution Profile

| BATCH | Replicate | 5 min | 10 min | 15 min | 20 min | 30 min | 45 min | 60 min |
|-------|-----------|---------|--------|--------|--------|--------|--------|--------|
| CMXVT | 1 | (b) (4) | | | | | | |
| | 2 | | | | | | | |
| | 3 | | | | | | | |
| | 4 | | | | | | | |
| | 5 | | | | | | | |
| | 6 | | | | | | | |
| | 7 | | | | | | | |
| | 8 | | | | | | | |
| | 9 | | | | | | | |
| | 10 | | | | | | | |
| | 11 | | | | | | | |
| | 12 | | | | | | | |
| | 13 | | | | | | | |
| | 14 | | | | | | | |
| | 15 | | | | | | | |
| | 16 | | | | | | | |
| | 17 | | | | | | | |
| | 18 | | | | | | | |
| | Average | 90 | 98 | 98 | 98 | 99 | 99 | 99 |
| | Max | (b) (4) | | | | | | |
| | Min | (b) (4) | | | | | | |
| | %RSD | 5.5 | 1.9 | 1.4 | 1.3 | 1.1 | 0.9 | 0.8 |

| BATCH | Replicate | 5 min | 10 min | 15 min | 20 min | 30 min | 45 min | 60 min |
|---------|-----------|---------|--------|--------|--------|--------|--------|--------|
| CMXVP | 1 | (b) (4) | | | | | | |
| | 2 | (b) (4) | | | | | | |
| | 3 | (b) (4) | | | | | | |
| | 4 | (b) (4) | | | | | | |
| | 5 | (b) (4) | | | | | | |
| | 6 | (b) (4) | | | | | | |
| | 7 | (b) (4) | | | | | | |
| | 8 | (b) (4) | | | | | | |
| | 9 | (b) (4) | | | | | | |
| | 10 | (b) (4) | | | | | | |
| | 11 | (b) (4) | | | | | | |
| | 12 | (b) (4) | | | | | | |
| | 13 | (b) (4) | | | | | | |
| | 14 | (b) (4) | | | | | | |
| Average | 89 | 98 | 98 | 98 | 99 | 99 | 99 | |
| Max | (b) (4) | | | | | | | |
| Min | (b) (4) | | | | | | | |
| %RSD | 7.6 | 2.0 | 1.3 | 1.3 | 1.0 | 1.0 | 1.1 | |
| CMXVS | 1 | (b) (4) | | | | | | |
| | 2 | (b) (4) | | | | | | |
| | 3 | (b) (4) | | | | | | |
| | 4 | (b) (4) | | | | | | |
| | 5 | (b) (4) | | | | | | |
| | 6 | (b) (4) | | | | | | |
| | 7 | (b) (4) | | | | | | |
| | 8 | (b) (4) | | | | | | |
| | 9 | (b) (4) | | | | | | |
| | 10 | (b) (4) | | | | | | |
| | 11 | (b) (4) | | | | | | |
| | 12 | (b) (4) | | | | | | |
| | 13 | (b) (4) | | | | | | |
| | 14 | (b) (4) | | | | | | |
| Average | 85 | 98 | 99 | 99 | 99 | 99 | 99 | |
| Max | (b) (4) | | | | | | | |
| Min | (b) (4) | | | | | | | |
| %RSD | 6.9 | 1.0 | 1.1 | 1.2 | 1.1 | 1.2 | 1.1 | |

Reviewer's Comments:

Based on the dissolution data of the primary registration batches which are also the pivotal/clinical batches, the proposed drug product shows a very rapid dissolution with $\geq 90\%$ within 10 minutes using the proposed QC dissolution method. This Reviewer finds the Applicant's proposed "Q= (b) (4)% in 20 minutes" to be acceptable based on the submitted data. Further tightening of the dissolution acceptance criterion (e.g., Q= (b) (4)% in 15 minutes) is not deemed necessary because the Applicant's proposed dissolution method's discriminating ability won't be any more sensitive at 15 minutes than 20 minutes in identifying the failing batches. For example, based on Figures 3 and 5 above, the out-of-range batches (yellow) that gets rejected at 20 minutes will also get rejected at 15 minutes; and since being overly discriminating may potentially fail a perfectly fine bioequivalent batch, this Reviewer finds the Applicant's proposed dissolution acceptance criterion at "Q= (b) (4)% in 20 minutes" to be reasonable.

B.3. PRODUCT BRIDGING

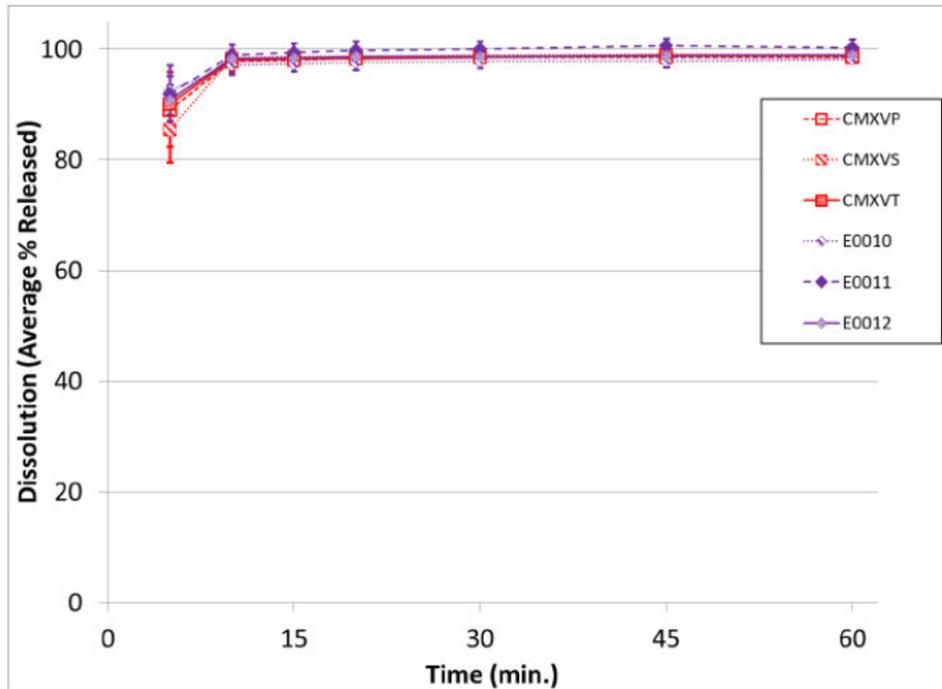
Assessment: Adequate

The three primary registration batches (CMXVP, CMXVS, CMXVT) were used in the pivotal EMBER-3 study (J2J-MC-JZLC) as described in Table 7. However, for the proposed to-be-marketed drug products, the Applicant's proposed manufacturing site is different ((b) (4)) from the manufacturing site used to manufacture the clinical batches as well as the primary registration/ stability batches ((b) (4)). Therefore, the Applicant was requested to submit the comparative dissolution data for the two sites using the proposed QC dissolution method via IR #2 dated 4/10/25. Based on the data provided in the Applicant's response submitted on 1/31/2025, very rapid dissolution (e.g., ≥85% in 15 min) was observed for the products manufactured at the two sites as described in Figure 7 below, and therefore, the bridging is supported and adequately established without a need to compare the f₂-values. The Applicant also confirmed that there were no changes to the formulation (i.e., T1 Tablet, 200 mg), nor manufacturing process (i.e., (b) (4)) between the batches of drug product manufactured at the two sites.

Table 7: Summary of Imlunestrant Capsule and Tablet Formulations tested in the clinical studies throughout the product's development.

| Formulation | C1 Capsule, 50 mg | T1 Tablet, 200 mg | | |
|------------------|--------------------------------------|--|--|---|
| | C2 Capsule, 100 mg | | | |
| Manufacturer | (b) (4) | | | |
| Use | Clinical | Clinical | Clinical Registration Stability | Clinical Proposed To-Be Marketed Product |
| Clinical Studies | Phase 1: J2J-MC-JZLA (lead-in) | Phase 1: J2J-MC-JZLA J2J-MC-JZLB Biopharmaceutic and clinical pharmacology: J2J-MC-JZLD J2J-MC-JZLE J2J-MC-JZLG J2J-MC-JZLI J2J-MC-JZLK Phase 3: J2J-MC-JZLC | Phase 1: J2J-MC-JZLF Biopharmaceutic and clinical pharmacology: J2J-MC-JZLG J2J-MC-JZLI J2J-MC-JZLK Phase 3: J2J-MC-JZLC | Future clinical studies, to be determined |

Figure 7: Dissolution Data of Primary Stability Batches ((b) (4): CMXVP, CMXVS, and CMXVT) and Process Validation Batches ((b) (4) : E0010, E0011, and E0012) using the proposed Dissolution Method for QC Testing



B.4. BIOWAIVER REQUEST

Assessment: Not Applicable (single strength drug product)

BIOPHARMACEUTICS LIST OF DEFICIENCIES

None

Appendix I. Biopharmaceutics Information Requests

1. Biopharmaceutics Information Request (IR) #1 conveyed to the Applicant on 1/22/2025.

We note that the registration stability batches are not the same batches used in the supportive clinical trials (including EMBER-3). Please submit a schematic representation (e.g., table, diagram, etc.) of the development of your proposed drug product from the (i) initial formulation to the (ii) formulation used in the clinical study (including pivotal clinical study) and the (iii) proposed to-be-marketed product, along with the batch numbers. Specify the changes in your formulation compositions, manufacturing sites and process, etc. that occurred throughout the development, along with the studies (in vitro dissolution and/or in vivo/ clinical study) bridging those products.

Applicant's Response received on 1/31/2025 (SDN-9)

2. Biopharmaceutics Information Request (IR) #2 conveyed to the Applicant on 4/10/2025.

We note that the pivotal/clinical batches and your proposed to-be-marketed (TBM)/commercial batches were manufactured at different manufacturing sites ((b) (4) vs. (b) (4)). A change in drug product manufacturing site should be supported by a scientific bridge to the pivotal/clinical batch manufacturing site. In order to bridge the batches manufactured (b) (4) and (b) (4) , please provide the comparative in vitro dissolution profile/data (individual [n=12], mean, range, %RSD at 5, 10, 15, 20, 30, 45, and 60 minutes, table and graph) between the pivotal/clinical batches and TBM/commercial batches using the proposed QC dissolution method [USP Apparatus II (Paddle) with 900 mL in 0.1 N HCl at 75 rpm]. Provide the batch numbers, age of batch and storage conditions at the time of dissolution testing in your response.

Applicant's Response received on 5/5/2025 (SDN-27)



Min (Sammie)
Kang

Digitally signed by Min (Sammie) Kang
Date: 8/15/2025 09:33:00AM
GUID: 5c6f0111000a97b812e3de3aa8a3ef20



Angelica
Dorantes

Digitally signed by Angelica Dorantes
Date: 8/15/2025 09:39:08AM
GUID: 502d0913000029d59f1c87e0a380c7f7

CHAPTER III: ENVIRONMENTAL

R REGIONAL INFORMATION

1. Summary

Eli Lilly and Company, the Applicant, submitted an environmental assessment (EA) for NDA-218881-ORIG-1 regarding the drug product Imlunestrant (200 mg Tablet), which is for the treatment of adult patients with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), estrogen receptor 1 (ESR1)-mutated advanced or metastatic breast cancer previously treated with endocrine therapy. The screening level expected introduction concentration (EIC) of imlunestrant in the aquatic environment is 0.21 ppb and the refined EIC is 0.042 ppb, both below the EA categorical exclusion level of 1 ppb (21 CFR 25.31(b)). Due to the potential of imlunestrant for interaction with estrogen and androgen hormone pathways in organisms, which indicates potential extraordinary circumstances, the Applicant determined—and FDA agreed—that imlunestrant does not qualify for categorical exclusion from an EA, per 21 CFR 25.21 and FDA 2016 guidance [1]. Therefore, the Applicant submitted an EA including a series of studies to evaluate the environmental fate and effects of imlunestrant.

FDA has conducted a thorough review and assessment of the EA and concurs with the majority of the submitted data and analyses, except for the estimated terrestrial (soil) EIC and expected environmental concentration (EEC) of imlunestrant. Nevertheless, the agency has determined that these disagreements, which have been documented in the EA review, do not affect the overall conclusion of the EA.

In conclusion, FDA has determined that the submitted information is sufficient to enable the agency to determine whether approval of this application may significantly affect the quality of the human environment, and therefore the EA is deemed adequate for approval, per 21 CFR 25.15(a). Furthermore, based on the submitted information, FDA has concluded that approval of this application is not expected to have significant effects, and thus an environmental impact statement will not be prepared. A Finding of No Significant Impact (FONSI) for this application is recommended, per 21 CFR 25.15(b), 25.40, and 25.41.

2. Substance of Interest

The Applicant identified the active pharmaceutical ingredient (API) of the drug product, imlunestrant ($C_{29}H_{24}F_4N_2O_3$, molecular weight = 524.51 g/mol as free base), as the sole substance of interest for this EA. Imlunestrant is a noncovalent-binding selective estrogen receptor degrader (SERD) and pure antagonist of wild-type and mutant

estrogen receptor α (ER α). It binds to wild-type and mutant ER, and reduces ER activity via direct degradation and antagonism, resulting in sustained inhibition of ER-dependent gene transcription and cell growth. All concentrations of imlunestrant in this EA are based on the active moiety (i.e., free base).

No information of excipients was provided in this EA, which is consistent with FDA 1998 guidance [2].

3. Expected Introduction Concentration (EIC) and Expected Environmental Concentration (EEC) of Imlunestrant

The Applicant estimated peak annual sales of imlunestrant in the U.S. 10 years post-launch and used the high-end production of 10,000 kg/year for a conservative estimation of EICs and EECs. The calculated EICs and EECs of imlunestrant in the aquatic and terrestrial (soil) environments are summarized in the table below.

| Compartment | Sub-environment | EIC | EEC |
|-------------|-----------------|--|-----------------|
| Aquatic | Surface water | EIC = 0.21 ppb; Refined EIC = 0.042 ppb | 0.0042 ppb* |
| | Sediment | 0.227 mg/kg wet weight (ww) or 1.04 mg/kg dry weight (dw) | Same as the EIC |
| Terrestrial | Soil | 0.00187 mg/kg ww* | 0.037 mg/kg ww* |

*FDA considers underestimated. See FDA’s comments in the review section.

3.1 EIC and EEC of imlunestrant in surface water

Based on the high-end production of 10,000 kg/year, the Applicant calculated the EIC-water of imlunestrant to be 0.21 ppb, according to the FDA 1998 guidance [2]. To further refine the EIC, the Applicant considered potential reduction factors such as wastewater treatment plant (WWTP) removal and human metabolism. The Applicant applied the SimpleTreat (v4.0.9) model using assay-obtained partition coefficient data to predict the fate of imlunestrant during wastewater treatment process. Results show that approximately 80% of imlunestrant will be removed from WWTP effluent due to binding to sludge. Accordingly, the Applicant calculated the refined EIC-water to be $0.21 \text{ ppb} \times (1 - 80\%) = 0.042 \text{ ppb}$ and the consequent EEC-water to be 0.0042 ppb by applying a dilution factor of 10 to the refined EIC-water.

The Applicant also discussed the metabolism and excretion of imlunestrant by humans but didn’t use this mechanism for EIC refinement. According to the submitted information, a total of 97.6% of administered imlunestrant can be excreted from human (97.3% in feces and 0.3% in urine), predominantly as the parent compound imlunestrant (61.8% in feces). The most abundant metabolite is the sulfate conjugate (20.9% in

feces). Other oxidized metabolites account for 12.7% in feces with individual amount less than or equal to 5.1%.

3.2 EIC of imlunestrant in sediment

Based on the refined EIC-water of 0.042 ppb, the Applicant calculated the concentration of imlunestrant in freshly deposited sediment to be 0.227 mg/kg ww (or 1.04 mg/kg dw), following the European Chemicals Agency (ECHA) 2016 guidance [3].

3.3 EIC and EEC of imlunestrant in soil

The Applicant calculated the EIC of imlunestrant in biosolids to be 0.635 mg/kg dw, based on the production of 10,000 kg/year, the annual generation amount of biosolids in the U.S. (1.256E+10 kg dw/year), and the SimpleTreat-predicted proportion of imlunestrant binding to WWTP sludge (80%). The Applicant then estimated the EIC of imlunestrant in digested biosolids to be 1.27 mg/kg dw, based on the assumption that the digestion process may reduce the solids level by 50%.

Based on the EIC-digested biosolids (1.27 mg/kg dw), the Applicant calculated the EIC-soil to be 0.00187 mg/kg ww following the ECHA 2016 guidance [3]. They used the default parameters in the guidance including the biosolids application rate of 0.5 kg/m²/year, which FDA considers underestimated (see FDA's comment in the review section). The Applicant also estimated a worse-case EEC in soil to be 0.00187 mg/kg ww × 20 years × 1 time/year = 0.037 mg/kg ww assuming no depletion of imlunestrant in soil after 20 years of biosolids application.

4. Physicochemical Properties and Environmental Fate of Imlunestrant

The Applicant submitted physicochemical data of imlunestrant as summarized in the table below.

| Physicochemical parameter | Method | Results |
|---|------------------|---|
| Water solubility | OECD 105 (20 °C) | 39 ppm (pH 5); 1.58 ppm (pH 7); 0.587 ppm (pH 9) |
| Melting point | In-house test | No melting point prior to onset of decomposition at ~245 °C |
| Dissociation constant (pK _a) | In-house test | 3.67 (base), 7.72 (base), 8.18 (acid) |
| Octanol-water partition coefficient (logK _{ow}) | OECD 123 (25 °C) | 3.29 (pH 5); 5.13 (pH 7); 5.55 (pH 9) |
| Organic carbon-water partition coefficient (K _{oc}) | OECD 106 | Sludge: mean 3.06E+04 L/kg (2.81E+04–3.18E+04 L/kg) |

| Physicochemical parameter | Method | Results |
|---------------------------|--------|--|
| | | Soil: mean 7.79E+05 L/kg (2.98E+05–1.43E+06 L/kg) |

Imlunestrant is ionizable across the environmental range of pH values. As pH increases, imlunestrant becomes less ionized and more hydrophobic, as reflected in its decreasing water solubility and increasing logK_{ow} values. The K_{oc} values of imlunestrant in WWTP sludge and soil indicate that imlunestrant may bind significantly to the sludge and is unlikely to permeate into groundwater in soil.

The Applicant also provided experimental and model-predicted data on environmental fate of imlunestrant in WWTP and the aquatic and terrestrial (soil) environments. Data are summarized in the table below.

| Environ. Fate Study | Method | Results |
|--|--|---|
| WWTP removal | SimpleTreat (v4.0.9) using the K _{FOC} value obtained from OECD 106 | Imlunestrant binding to sludge: 80% |
| Hydrolysis | OECD 111 (50 °C, anoxic, 5 days) | Degradation: < 3% at pH 4, 7, 9 DT ₅₀ (25 °C) > 1 year |
| Aerobic biodegradation in sludge | OECD 314B (20 °C, 28 days) | Mass balance at day 28: <ul style="list-style-type: none"> • CO₂: 0.2% applied radioactivity (AR) • Extractable sludge: 41.3% AR <ul style="list-style-type: none"> ○ Imlunestrant: 27.6% AR ○ Degradation products: 13.7% AR • Non-extractable sludge: 46.6% AR DT ₅₀ of imlunestrant in extractable sludge: 2.26 days |
| Aerobic biotransformation in water-sediment system | OECD 308 (20 °C, 102 days, two test systems including one with high organic carbon (OC) content and the other with low OC content) | Mass balance at day 102: <ul style="list-style-type: none"> • CO₂: 0.26–0.61% AR • Water: 0.2–0.8% AR • Extractable sediment: 59.6–62.6% AR; ≥ 99% as imlunestrant • Non-extractable sediment: 14.7–31.0% AR DT ₅₀ of imlunestrant: <ul style="list-style-type: none"> • Water: 1.64–2.25 days • Extractable sediment: 382–704 days • Total system (water + extractable sediment): 179–1310 days |
| Aerobic biotransformation in soil | OECD 307 (20 °C, 120 days) | Mass balance at day 120: <ul style="list-style-type: none"> • CO₂: 0.27–0.57% AR • Extractable soil: 59.8–70.6% AR; all as imlunestrant, little transformation • Non-extractable soil: 32.7–46.6% AR DT ₅₀ of imlunestrant in extractable soil: 200–310 days |

| Environ. Fate Study | Method | Results |
|---|---|--|
| Bioaccumulation in fish (bluegill <i>L. macrochirus</i>) | OECD 305 (exposure for 21 days, depuration for 60 days) | Steady-state $BCF_{ss} = 188\text{--}241$ L/kg (total radioactivity), 53–95 L/kg (imlunestrant) Growth-corrected, lipid-normalized kinetic $BCF_{KGL} = 274\text{--}500$ L/kg (total radioactivity) |

Following patient use, imlunestrant and related metabolites may enter the surface water upon the discharge of WWTP effluent to receiving waters, and the terrestrial environment from agricultural land application of WWTP biosolids. Imlunestrant is expected to significantly partition to WWTP sludge solids, resulting in a reduction of the aqueous concentration.

Based on the environmental fate data, the Applicant concludes that imlunestrant is stable with respect to hydrolysis and not readily biodegradable. Imlunestrant is not expected to volatilize and will not enter the atmospheric environment. Imlunestrant may be persistent in the aquatic environment, mainly in sediment. In the OECD 308 test, imlunestrant was observed to rapidly partition to the underlying sediment layer after application to the water phase, due to its high adsorption property. A significant portion of imlunestrant can be irreversibly incorporated into sediment and soil and form non-extractable residues, which are assumed to have low remobilization potential and not bioaccessible. The transformation of imlunestrant was negligible in both water-sediment systems (OECD 308 test) and soil systems (OECD 307 test). Although the $\log K_{ow}$ values of imlunestrant are greater than 3 across the environmental pH values, indicating the potential for bioaccumulation in fish, the bioconcentration study (OECD 305 test) shows that imlunestrant is not expected to be bioaccumulative in aquatic organisms such as fish.

5. Environmental Effects

The Applicant conducted acute and chronic ecotoxicity studies to evaluate the adverse effects of imlunestrant on microorganisms in sewage sludge, freshwater species representing three trophic levels (i.e., algae, daphnia, and fish), sediment invertebrates representing three different taxonomic groups and three different sediment-based habitats and feeding strategies, and terrestrial species representing primary producers, consumers, and decomposers (i.e., plants, invertebrates, and microbes). The six plant species represent types of plants grown on agricultural fields that would receive biosolids application and that belong to different families of 4 dicotyledonous and 2 monocotyledonous species.

The ecotoxicity results including no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), median effective concentration (EC_{50}), and median lethal concentration (LC_{50}) values are summarized in the table below.

| Environment | Organism | Method | Duration | Endpoint | Results |
|-------------------------------|--|-------------------------------|--------------------------------|--|--|
| Sewage sludge | Microbes | OECD 209 | 3 hr | Respiration inhibition | NOEC ≥ 1,000 ppm |
| Aquatic | Green algae (<i>R. subcapitata</i>) | OECD 201 | 72 hr | Yield and growth rate | NOEC = 2.5 ppb; EC ₅₀ = 33 ppb |
| | Water flea (<i>D. magna</i>) | OECD 202 | 48 hr | Immobilization | NOEC = 140 ppb; EC ₅₀ = 200 ppb |
| | | OECD 211 | 21 d | Survival, growth, and reproduction | NOEC = 3.32 ppb; EC ₅₀ = 187 ppb |
| | Fish (fathead minnow <i>P. promelas</i>) | Non-GLP acute toxicity screen | 4 days post-hatch | Embryo hatching success, survival, and symptoms of toxicity | NOEC = 61.1 ppb |
| | | OECD 203 | 96 hr | Juvenile mortality and symptoms of toxicity | NOEC = 61.1 ppb; LC ₅₀ = 113 ppb |
| | | OECD 240 | 227 d | Fecundity, fertility, hatch, survival, sex ratio, growth (length and weight), tubercle scores, vitellogenin, gonadosomatic index | NOEC = 0.43 ppb LOEC = 1.8 ppb |
| | Midge (<i>C. riparius</i>) | OECD 218 | 28 d | Emergence and development | NOEC = 68 mg/kg dw; EC ₅₀ = 224 mg/kg dw |
| | Oligochaete (<i>L. variegatus</i>) | OECD 225 | 28 d | Growth and reproduction | NOEC = 139 mg/kg dw; EC ₅₀ = 334 mg/kg dw |
| Amphipod (<i>H. azteca</i>) | EPA OPPTS 850.1770 | 42 d | Survival, growth, reproduction | NOEC = 283 mg/kg dw; EC ₅₀ = 705 mg/kg dw | |
| Terrestrial (soil) | Microbes | OECD 216 | 28 d | Nitrogen transformation | NOEC ≥ 761 mg/kg dw |
| | Earthworm (<i>E. fetida</i>) | OECD 207 | 14 d | Mortality and weight change | NOEC ≥ 750 mg/kg dw |
| | Collembola (<i>F. candida</i>) | OECD 232 | 28 d | Mortality and reproduction | NOEC = 309 mg/kg dw EC ₅₀ > 1,000 mg/kg dw |
| | Onion (<i>A. cepa</i>), soybean (<i>G. max</i>), ryegrass (<i>L. perenne</i>), radish (<i>R. sativus</i>), sunflower (<i>H. annuus</i>), tomato (<i>S. lycopersicum</i>) | OECD 208 | 21 d | Germination, shoot height and weight, survival, and visible phytotoxic effects | NOEC = 111 mg/kg dw; EC ₅₀ = 476 mg/kg dw |

Prior to conducting the multigenerational reproduction and development study (OECD 240), the Applicant conducted exposure trials with three fish species including fathead minnow, zebrafish *Danio rerio*, and medaka *Oryzias latipes*, to determine the fish species that is comparatively sensitive to imlunestrant and to establish appropriate

concentrations for the definitive test. During the 21-day exposure period, reductions in reproduction and growth (length and weight) of larval were observed in fathead minnow compared to the solvent control, while no or less apparent reductions were observed in zebrafish and medaka.

In the OECD 240 test with fathead minnow, significant increases in the growth of male and female fish were observed in the F0 and F1 generations, with the NOEC of 0.43 ppb and LOEC of 1.8 ppb for F1 male fish. A significant decrease in hatching success was also observed in the F1 generation at the highest test concentration (7.1 ppb). No significant treatment-related effects were observed in the F2 generation. The overall NOEC and LOEC of imlunestrant in this study are 0.43 ppb and 1.8 ppb, respectively.

For terrestrial organisms, the most sensitive species are sunflower *H. annuus* and tomato *S. lycopersicum*. Statistically significant reductions in emergence and/or shoot growth were observed in these two species, and the overall NOEC and LOEC are 111 mg/kg dw and 333 mg/kg dw, respectively. Compared to sunflower and tomato, ryegrass and radish were less sensitive to imlunestrant with a LOEC of 1,000 mg/kg dw. No significant effects were observed in onion and soybean as well as other tested soil organisms.

6. Environmental Risk Assessment

To assess the environmental risk of imlunestrant, the Applicant calculated the quotient as the ratio of the effect concentration (NOEC, EC₅₀, or LC₅₀) to the maximum expected environmental concentration (MEEC; i.e., EIC or EEC whichever is greater). The calculated quotients range from 65 to 23,809,524 in sewage sludge, surface water, sediment, and soil, all of which are greater than the minimum required factor of 10. No sub-lethal effects were observed at concentrations equal to the EIC or EEC. Based on these results, the Applicant concludes that no adverse environmental effects are identified from the use of imlunestrant for the treatment of patients with breast cancer. The release of imlunestrant to sewage treatment plants and subsequently to the environment does not pose an environmental risk.

Assessment: Adequate

Environmental Review:

The main goals of this environmental review, per 21 CFR 25.15(a) and (b), and 25.40, are to determine (1) whether the EA contains sufficient information to enable the agency to determine whether the proposed action may significantly affect the quality of the

human environment; and (2) if so, whether the proposed action will significantly affect the environment. Regarding the main findings of the submitted EA for NDA-218881-ORIG-1:

- The Applicant identified the API imlunestrant as the sole substance of interest for this EA. This is appropriate according to the FDA 1998 guidance [2], which notes that the EA should focus on the active moiety and the predominant structurally related substances expected to enter or exist in the environment.
- The EIC and refined EIC values of imlunestrant in the aquatic environment, including surface water and sediment, are acceptable. FDA independently developed an estimate of the production for imlunestrant, and the result is lower than the Applicant's high-end estimate (10,000 kg/year). Thus, the Applicant's usage of 10,000 kg/year for the EIC calculation is considered conservative. The Applicant applied a predicted WWTP removal rate to further reduce the EIC of imlunestrant in water. The SimpleTreat-predicted distribution of imlunestrant to WWTP sludge (80%), based on the assay-obtained partition coefficient data rather than SimpleTreat default parameters, is reasonable. The high $\log K_{ow}$ and K_{oc} (sludge) values of imlunestrant indicate its high hydrophobicity and substantial potential for binding to sludge. The Applicant did not consider human metabolism for the EIC refinement, since more than 97% of administered imlunestrant can be excreted from human body, mostly as the parent compound, and no data was provided regarding the pharmacological activities of metabolites compared to the parent compound. No degradation was assumed in water or sludge during wastewater treatment. Overall, FDA considers that the refinement approach is reasonable and agrees with the refined EIC-water of 0.042 ppb. Regarding the EEC-water, FDA considers that the standard dilution factor of 10 in the FDA 1998 guidance [2] has become less applicable as water scarcity and water reuse increases, and thus the refined EIC-water (0.042 ppb) should be used as the EEC for a conservative risk assessment. For the EIC in sediment, FDA has examined the equations and parameters in the ECHA 2016 guidance [3] as well as other guidance including the European Commission Technical Guidance Document on Risk Assessment [4] and the European Medicines Agency (EMA) 2024 guidance [5], and determined that the calculation is appropriate and the result is acceptable.
- The EIC and EEC of imlunestrant in the terrestrial (soil) environment may be underestimated. The Applicant's estimated EIC-digested biosolids (1.27 mg/kg dw) is acceptable as it is close to our estimated EIC-biosolids (1.18 mg/kg dw), but FDA does not agree with the use of default sludge application rate of 0.5 kg/m²/year in the ECHA 2016 guidance [3] for EIC-soil calculation, as this application rate is much lower than the typical application rates of biosolids in the U.S. which can be up to 20 dry tons/acre/year (i.e., 4.5 kg/m²/year) for agricultural land and 100 dry tons/acre/year (i.e., 22.4 kg/m²/year) for reclamation sites [6]. By using the high-end application rate of biosolids (22.4 kg/m²/year), FDA estimated a high-end EIC-soil of

imlunestrant to be 0.095 mg/kg dw and a worse-case EEC-soil to be 1.9 mg/kg dw after 20 years of biosolids application.

- The submitted physicochemical properties and environmental fate data for imlunestrant are adequate. Although no data was provided on the volatility of imlunestrant, FDA considers imlunestrant not volatile based on its chemistry and agrees with the Applicant that the water and terrestrial compartments rather than the air compartment need assessment. Based on its physicochemical properties, imlunestrant will likely undergo substantial partitioning to sludge, reducing its concentrations in the WWTP effluent. Once released to the environment, imlunestrant is expected to partition predominantly to sediment in the aquatic environment and remain relatively immobile in soil. Imlunestrant has low potential for bioaccumulation in fish and thus its risk to animals higher in the aquatic food chain is expected to be negligible. The OECD 308 test results show that after a 102-day incubation, less than 1% of the introduced imlunestrant remained in water, and over 98% was found in sediment, including ~60% existing as the extractable fraction and the rest existing as non-extractable bound residues. The extractable portion of imlunestrant in sediment is considered bioaccessible/bioavailable and could be released back to the water phase under certain environmental conditions. As to the terrestrial environment, the OECD 307 test results show that after a 120-day incubation in soil, about 60–70% of introduced imlunestrant existed as the extractable fraction and 33–47% as non-extractable bound residues. Imlunestrant is not readily biodegradable and minimal biotransformation was observed in both water-sediment systems and soil. Based on the submitted information, imlunestrant is considered persistent in the aquatic and terrestrial environments, and a significant portion (about 60–70%) of imlunestrant in sediment and soil could be bioaccessible/bioavailable.
- The submitted acute and chronic ecotoxicity data of imlunestrant are adequate. Imlunestrant exhibited low to moderate toxic effects on test aquatic organisms including algae, water flea, fish, and sediment-dwelling invertebrates, and terrestrial organisms including collembola and plants. As expected, fish are the most sensitive organisms to imlunestrant due to its endocrine-related activity. Imlunestrant was observed to increase fish growth and reduce fish hatching success with a NOEC of 0.43 ppb.
- The approaches that the Applicant used for environmental risk assessments of imlunestrant in the aquatic and terrestrial environments generally adhere to the FDA 1998 guidance [2]. Based on the submitted information, FDA estimated the predicted no-effect concentrations (PNECs) of imlunestrant to be 0.043 ppb for surface water, 6.8 mg/kg dw for sediment, and 11.1 mg/kg dw for the terrestrial environment. FDA then calculated the risk quotient (RQ), i.e., the ratio of EIC (or refined EIC) to PNEC, to be 0.98 for surface water, 0.15 for sediment, and 0.009 (worse-case 0.17) for soil,

all below the threshold value of 1. While the RQ value in surface water is close to 1, considering the conservative estimate of the production amount, the metabolic and treatment degradation, and environmental depletion mechanisms (such as the rapid partitioning of imlunestrant from the water phase to sediment as discussed above), the adverse effects of imlunestrant on freshwater species, if there are any, are unlikely to be significant at the expected level of exposure.

- Despite the conclusion that significant environmental effects from the use of imlunestrant are not expected, FDA recommends that precautionary mitigation measures be considered due to the persistence of imlunestrant in the environment and its hormonal effects (per FDA 2016 guidance [1]). Therefore, FDA recommends the following regarding labeling:
 1. In the FULL PRESCRIBING INFORMATION, Section 16 “HOW SUPPLIED/STORAGE AND HANDLING”, include the statement “Dispose unused medication via a take-back option if available. Otherwise, follow FDA instructions for disposing medication in the household trash, www.fda.gov/drugdisposal. Do NOT flush down the toilet.”
 2. In the PATIENT INFORMATION, include the statement "Dispose unused medication via a take-back option if available. Otherwise, follow FDA instructions for disposing medication in the household trash, www.fda.gov/drugdisposal. Do NOT flush down the toilet.”

Overall Assessment and Recommendation:

Overall, the submitted information in the EA of imlunestrant is sufficient to enable FDA to determine whether the proposed action may significantly affect the quality of the human environment. Therefore, the EA is adequate for approval.

Based on the information available to date, FDA has concluded that no significant environmental effects are expected from the approval of NDA-218881-ORIG-1. A Finding of No Significant Impact (FONSI) is recommended for this application.

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Wu

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James
Laurenson

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Finding of No Significant Impact

NDA-218881-ORIG-1 Imlunestrant

200 mg Tablet, 400 mg Once Daily

Food and Drug Administration

Center for Drug Evaluation and Research

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

Eli Lilly and Company, the Applicant, is requesting approval of NDA-218881-ORIG-1 for the drug product Imlunestrant (200 mg Tablet) which is indicated for the treatment of adult patients with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), estrogen receptor 1 (ESR1)-mutated advanced or metastatic breast cancer previously treated with an endocrine-based regimen. The active pharmaceutical ingredient, imlunestrant, is a noncovalent-binding selective estrogen receptor degrader (SERD) and pure antagonist of wild-type and mutant estrogen receptor α (ER α).

In support of their application, the Applicant submitted an environmental assessment (EA) for Imlunestrant (attached). The EA evaluates the potential environmental effects from the use of this drug product. The FDA Center for Drug Evaluation and Research (CDER) EA Team has reviewed and assessed the EA and considered potential adverse effects due to approval of this drug product application. Although FDA does not agree with some of the results in the EA, the agency has concluded that the submitted information is sufficient to determine whether approval of this drug application may significantly affect the quality of the human environment, and thus the EA is adequate for approval, per 21 CFR 25.15(a).

Based on the submitted information, FDA has determined that approval of this application is not expected to have significant effects on the quality of the human environment. Therefore, FDA is issuing a finding of no significant impact (FONSI), and an environmental impact statement will not be prepared, per 21 CFR 25.15(b), 25.40, and 25.41.

Attachment: Environmental Assessment for the Use of Imlunestrant in Patients with Breast Cancer

Environmental Assessment for the Use of Imlunestrant in Patients with Breast Cancer

**Eli Lilly and Company
Lilly Corporate Center
Indianapolis, IN 46285**

October 2024

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Environmental Assessment for the Use of Imlunestrant in Patients with Breast Cancer

Description of the Proposed Action

Requested Approval

Eli Lilly and Company has filed a supplement to the NDA for INLURIYO (imlunestrant) pursuant to the section 505(b) of the Federal Food, Drug, and Cosmetic Act. Imlunestrant will be marketed as 200 mg tablets packaged in white high density polyethylene bottles with plastic closures containing an aluminum foil induction heat seal. An Environmental Assessment (EA) is being submitted for imlunestrant pursuant to 21 C.F.R. § 25 (1997).

Imlunestrant is not categorically excluded from an assessment of environmental impact as stated in 21 C.F.R. § 25.31 (1997), because extraordinary circumstances (21 C.F.R. § 25.21, 1997) for the proposed action exist (i.e., approval of the NDA). Extraordinary circumstances exist because imlunestrant has the potential to interact with estrogen and androgen hormone pathways in environmental organisms (FDA Q&A for drugs with E, A, T activity, 2016). Therefore, an EA has been conducted to evaluate the potential to produce developmental or reproductive effects in environmental organisms following exposure to imlunestrant. The use of imlunestrant will result in one major pathway to the environment: sewage treatment facilities receiving influent from the general public. Wastes generated from production facilities are regulated by federal, state, and local environmental protection agencies and are not considered in this environmental assessment.

Need for Action

Imlunestrant is a selective pure antagonist of wild type and mutant oestrogen receptor α (ER α or ESR1), and potently inhibits transcription of ER α target genes, selectively inhibiting the proliferation of ER+ cancer cells. The current application requests approval for use of imlunestrant as a single agent for the treatment of adults with estrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), estrogen receptor 1 (ESR1)-mutated advanced or metastatic breast cancer previously treated with endocrine therapy.

Locations of Use

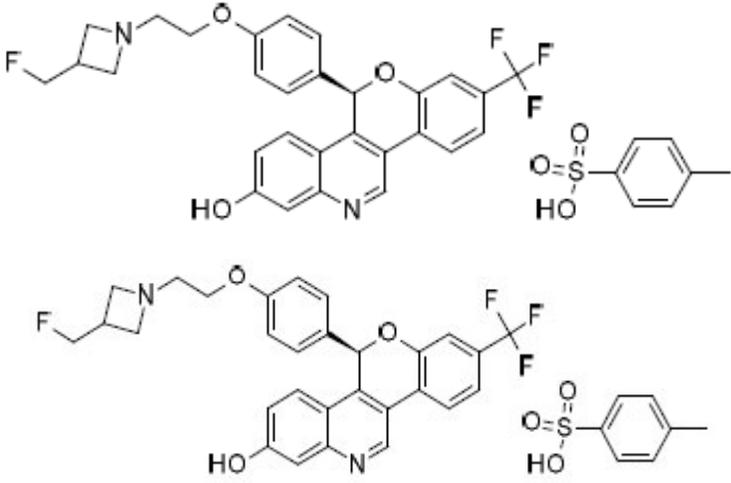
The location of the use of imlunestrant will be primarily in the patient's home and workplace. There is no reason to expect use to be concentrated in a particular geographic region.

Disposal Sites

Empty or partially empty packages containing imlunestrant will typically be disposed of by a community solid waste management system, which may include landfills, incineration, and recycling, although minimal quantities of unused drug could be disposed of in the sewer system.

Identification of the Chemical Substance

Table 1. Identification of Imlunestrant

| | |
|---|---|
| Established Name (USAN) | Imlunestrant |
| Synonym | LY3484356 |
| Brand/Proprietary/ Tradename | INLURIYO |
| Chemical Abstracts Service (CAS) Number | 2408840-41-3 (tosylate salt) 2408840-26-4 (free base) |
| Chemical Name (CAS) | (<i>R</i>)-5-[4-[2-[3-(fluoromethyl)azetidin-1-yl]ethoxy]phenyl]-8-(trifluoromethyl)-5H-chromeno[4,3-c]quinolin-2-ol 4-methylbenzenesulfonate |
| Structural Formula |  |
| Molecular Formula | $C_{29}H_{24}F_4N_2O_3 \cdot C_7H_8O_3S$ (tosylate salt) $C_{29}H_{24}F_4N_2O_3$ (free base) |
| Molecular Weight | 696.72 $g \cdot mol^{-1}$ (tosylate salt) 524.51 $g \cdot mol^{-1}$ (free base) |

Environmental Issues

Experimental studies were conducted to determine the physical-chemical properties, environmental fate, and ecotoxicity of imlunestrant. Studies were performed in accordance with U.S. FDA Good Laboratory Practice regulations and followed OECD or U.S. EPA test guidelines. Physical-chemical properties are used to inform the conduct of environmental fate and toxicity studies, and to identify potential risks related to the environmental fate of imlunestrant. Environmental fate studies are used to predict the distribution of imlunestrant in the

environmental compartments (i.e., sewage treatment plant, surface water, sediment, soil) and potential exposure routes. Ecotoxicity studies were conducted with: microorganisms from a sewage treatment plant; aquatic species from three trophic levels, which include a multigeneration reproduction and development study with fish; sediment-dwelling species representing different sediment-based habitats and feeding strategies; and soil organisms representing three different trophic levels. All concentrations of imlunestrant in this risk assessment are based on the active moiety (i.e., free base).

Environmental Fate of Imlunestrant

Physical-chemical properties and environmental fate characteristics of imlunestrant are described below, a data summary table provided in [Appendix A](#) and study summaries included in [Appendix C](#). The physical-chemical properties determined experimentally were melting temperature, decomposition temperature, dissociation constant (pKa), water solubility, octanol-water partition coefficient, and the adsorption of imlunestrant to soils and sludge biosolids. The environmental fate studies evaluated the behavior and degradation potential of imlunestrant in sludge biosolids, water-sediment systems, and soil; and the bioaccumulation potential in fish.

Physical and Chemical Characterization

Thermogravimetric analysis (TGA) demonstrates that imlunestrant does not melt prior to onset of decomposition at approximately 245°C, which indicates that imlunestrant will not volatilize into the atmosphere ([Table 3.2.S.1.3-1](#)).

Imlunestrant is ionizable across the environmental range of pH values with pKa values of 7.72 (base), 3.67 (base), and 8.18 (acid).

The melting temperature, decomposition temperature, and pKa were determined in-house at Eli Lilly and Company, and were not determined following OECD or U.S. EPA test guidelines or in accordance with Good Laboratory Practice regulations.

Water Solubility

The column elution method ([OECD 1995](#), Guideline No. 105) was used to determine water solubility of imlunestrant in aqueous buffers at pH 5, 7 and 9 to be 39, 1.58, and 0.587 mg·L⁻¹, respectively. In water, imlunestrant is ionized at lower pH values which increases its water solubility ([Study 151K-108](#)).

Octanol-Water Partition Coefficient

The log₁₀-transformed octanol-water partition coefficient (log K_{OW}) was determined using the slow-stirring method ([OECD 2022](#), Guideline No. 123) at pH 5, 7 and 9 to be 3.29, 5.13 and 5.55, respectively. As pH values increase, imlunestrant becomes less ionized and more hydrophobic as reflected in increasing log K_{OW} values ([Study 151K-109](#)).

Since the log K_{OW} values for imlunestrant were > 3 at pH values across the environmental range (i.e., pH 5, 7, 9), the potential for bioaccumulation in fish was evaluated experimentally (Study 151A-202).

Partition Coefficient in Sewage Sludge and Soil

Freundlich adsorption partition coefficients (K_F) were experimentally determined by equilibrating radiolabeled imlunestrant in sludge biosolids from 3 different sewage treatment plants and in 4 soils of varying characteristics (i.e., organic carbon content, pH and texture) (Study 151E-160; OECD 2000a, Guideline No. 106).

For sludge biosolids, adsorption K_F values normalized to organic carbon content (K_{FOC}) ranged from 2.66×10^4 to 3.15×10^4 L·kg⁻¹. The K_{FOC} , BIOSOLIDS geometric mean of 2.96×10^4 L·kg⁻¹ indicated imlunestrant would bind significantly to biosolids in a sewage treatment plant. Since sludge biosolids can be applied to agricultural soil as fertilizer, risk to the soil compartment was assessed.

The adsorption K_F values for soil were observed to be greater than those for sludge and ranged from 1.61×10^4 to 5.65×10^4 L·kg⁻¹ with geometric mean of 2.49×10^4 L·kg⁻¹. The high adsorption properties of imlunestrant were also reflected in the aquatic sediment degradation study (Study 151E-161) where imlunestrant quickly partitioned to the underlying sediment layer after application to the water phase with half-lives of 1.64 and 2.25 days. $K_{F,SOIL}$ values are used to estimate predicted environmental concentrations, where relevant, because there was no correlation ($R^2 = 0.0007$) between $K_{F,SOIL}$ and organic carbon content (Appendix D).

The sludge and soil partition coefficients indicate that imlunestrant is unlikely to permeate into groundwater.

Environmental Depletion Mechanisms

Patient Metabolism

The disposition of imlunestrant was evaluated following a single oral dose of 400 mg containing 100 μ Ci of [¹⁴C]-imlunestrant in healthy non-childbearing women (Study JZLE, 2.7.2.2.2.1.1). Results showed a mean recovery of total radioactivity of 97.3% (range: 90% to 101%) was excreted in feces and 0.3% (range: 0.1% to 0.5%) was excreted in urine. Imlunestrant was the predominant component in feces, accounting for a mean of 61.8% of the radioactive dose. The most abundant metabolite in feces was the sulfate conjugate that accounted for a mean of 20.9% of the radioactive dose. Additional metabolites resulting from oxidation were identified in feces and totaled 12.7% of the radioactive dose but individually accounted for a mean of less than or equal to 5.1%.

Hydrolysis

Less than 3% of radiolabeled imlunestrant degraded in aqueous buffers at pH 4, 7, and 9 when incubated for 5 days at 50°C in the dark under abiotic and anoxic conditions (Study 151E-163A;

OECD 2004b, Guideline No. 111). Therefore, imlunestrant is stable with respect to hydrolysis at these pH values and half-lives at 25°C were estimated to be greater than 1 year.

Transformation in Sewage Sludge Systems

Incubation of radiolabeled imlunestrant activated sewage sludge for 28 days at 20°C under aerobic conditions resulted in some primary degradation and minimal evidence of ultimate degradation (Study 151E-162; OECD 2008, Guideline No. 314B). After 28 days, 47% of the applied radioactivity was unextractable from sludge solids. In the extractable fraction, 28% of the applied radioactivity was identified as parent imlunestrant and 14% as degradation products. The DT₅₀, SLUDGE (observed time to disappearance of 50% of imlunestrant to degradation products or to unextractable radioactivity) was 2.26 days. Although a ready biodegradability test was not conducted, imlunestrant is not considered readily biodegradable given that there was less than 1% evolution of radiolabeled carbon in this study.

Transformation in Aquatic Sediment Systems

Radiolabeled imlunestrant was incubated in 2 aquatic sediment systems and their associated water for 102 days at 20°C (Study 151E-161; OECD 2002a, Guideline No. 308). One system had a high organic carbon content and fine texture (HOC) and the other a low organic carbon content and a coarse texture (LOC). At the end of the study, mean mass balance was 94.3% (HOC) and 76.0% (LOC) of the applied radioactivity with 0.2% (HOC) and 0.8% (LOC) in the overlying water, and ≤ 0.6% recovered as ¹⁴CO₂. While mass balance was low in the LOC sediment on Day 102, it ranged from 86.4 to 101.4% of applied radioactivity on all other sampling days; mass balance for HOC sediment ranged from 92.5 to 100.4% at all intervals. The low mass balance is believed to be due to incomplete or inefficient combustion of LOC sediment. In sediment, 62.6% (HOC) and 59.6% (LOC) of applied radioactivity was extractable and ≥99% of the extractable radioactivity was identified as imlunestrant; negligible transformation was observed in the chromatography. The amount of non-extractable radioactivity in sediment increased over the course of the study and was 31.0% (HOC) and 14.7% (LOC) of the applied radioactivity on Day 102. Several extraction strategies (i.e., solvents of varying polarity and dielectric constants, ethylenediaminetetraacetic acid) were attempted on the post-extracted sediment solids to remove the unextractable residues, but only small amounts could be recovered. For the purpose of kinetic analysis, the non-extractable radioactivity was assumed to have low remobilization potential and not bioaccessible. In total aquatic sediment systems, the DT₅₀ of imlunestrant to transformation products and non-bioavailable residue was 1310 (HOC) and 179 days (LOC).

Transformation in Soil Systems

Transformation of radiolabeled imlunestrant was evaluated in 4 soils of varying characteristics (i.e., organic carbon content, pH, texture and microbial biomass) incubated for 120 days at 20°C (Study 151E-169; OECD 2002b, Guideline No. 307). At the end of the study, mass balance for all soil types ranged from 100.6% to 108.0% of applied radioactivity, ≤ 0.53% evolved as ¹⁴CO₂ (or volatile gases), and unextractable residues ranged from 32.7 to 46.6% of applied radioactivity. For all soil types, all of the extractable radioactivity (59.8 to 70.6% of applied

radioactivity) was identified as imlunestrant; little transformation was observed in the chromatography. Supplemental extractions of the post-extracted soil solids (i.e., solvents of varying polarity and dielectric constants) were not successful in removing the non-extractable residues. For the kinetic analyses, the non-extractable residues were assumed to have low remobilization potential and not bioaccessible. The DT_{50} of imlunestrant (disappearance to transformation products and non-bioavailable residue) ranged from 200 to 310 days.

Bioconcentration in Fish

The bioaccumulation potential of imlunestrant was evaluated by measuring the bioconcentration factor (BCF) in fish (*Lepomis macrochirus*) following aqueous exposure ([Study 151A-202](#); [OECD 2012a](#), Guideline No, 305).

During the uptake phase, fish were exposed to radiolabeled imlunestrant at time-weighted mean measured concentrations of 1.7 or 19 $\mu\text{g}\cdot\text{L}^{-1}$. Fish were periodically sacrificed, and total radioactive residues were measured in water and in whole fish tissue until Day 21 of uptake when tissue concentrations reached steady-state (i.e., 3 consecutive sampling intervals on days 14, 19, 21 were within $\pm 20\%$ of each other with no statistically significantly increasing trend over time). Concentrations of parent imlunestrant were also determined in select water and tissue samples on Day 22 of uptake.

After 22 days of uptake, fish were transferred to clean water to begin the depuration phase. Fish were periodically sacrificed, and total radioactive residues were measured in water and in whole fish tissue until tissue concentrations were $\leq 5\%$ of steady-state. At 19 $\mu\text{g}\cdot\text{L}^{-1}$, tissue concentrations steadily declined over time until Day 28 of depuration when concentrations were 3.2% of steady-state. At 1.7 $\mu\text{g}\cdot\text{L}^{-1}$, concentrations in tissue steadily declined to 5.0% of steady-state after 60 days of depuration.

The steady-state BCF (BCF_{SS}) values based on total radioactivity at exposure concentrations of 1.7 and 19 $\mu\text{g}\cdot\text{L}^{-1}$ were 188 and 241 $\text{L}\cdot\text{kg}^{-1}$, respectively. Based on only parent imlunestrant, BCF_{SS} values were 53 and 95 $\text{L}\cdot\text{kg}^{-1}$. The BCF values based on chemical specific analysis of imlunestrant were lower than those based on total radioactivity due to both low extraction efficiency (approximately 25% to 58%) and apparent metabolism of imlunestrant in fish (since some radioactivity extracted from the tissues was not imlunestrant).

To calculate the kinetic BCF ($BCF_K = k_1 \div k_2$), the uptake and depuration rate constants (k_1 and k_2 , respectively) were derived by fitting a first-order kinetic model using the sequential method. BCF_K at 1.7 and 19 $\mu\text{g}\cdot\text{L}^{-1}$ were 302 and 203 $\text{L}\cdot\text{kg}^{-1}$, respectively. BCF_K values normalized to 5% lipid content and corrected for growth dilution (BCF_{KgL}) were 500 and 274 $\text{L}\cdot\text{kg}^{-1}$.

Environmental Concentrations of Imlunestrant

Expected Introductory Concentration

The expected introductory concentration (EIC) is derived for each environmental compartment where imlunestrant is likely to amass. Depletion or accumulation mechanisms that occur prior to

the introduction of imlunestrant into the environmental compartment are applied where relevant (e.g., patient metabolism prior to entry into publicly-owned treatment works system, adsorption to sludge biosolids prior to entry into receiving waters).

Surface Water

Eli Lilly and Company estimates the peak annual sales of imlunestrant in the United States 10 years post-launch to be less than 10,000 kg (active moiety). To conservatively estimate concentrations in the environment, 10,000 kg of imlunestrant produced for direct use will be used. The expected introduction concentration (EIC) of imlunestrant at the point of entry into the aquatic environment is calculated to be $0.21 \mu\text{g}\cdot\text{L}^{-1}$ using the following default values and assumptions:

- all imlunestrant produced for direct use in a year is consumed and enters the POTW;
- all drug product usage is proportional to the population and the amount of wastewater generated;
- the total volume of water entering POTW is $1.305 \times 10^{11} \text{ L}\cdot\text{day}^{-1}$ (Seiple et al. 2017);
- there is no metabolism of imlunestrant.

$$\text{EIC}_{\text{AQUATIC}} = \frac{10,000 \text{ kg imlunestrant} \times 1,000,000,000 \frac{\mu\text{g}}{\text{kg}}}{1.305 \times 10^{11} \frac{\text{L water}}{\text{day}} \times 365 \text{ days}} = 0.21 \frac{\mu\text{g}}{\text{L}}$$

During wastewater treatment, some imlunestrant will be removed from the aqueous phase through binding to sewage sludge solids. SimpleTreat v4.0.9 (Struijs 2014, 2015) is used to estimate binding to sewage sludge solids in a POTW using the default parameters for municipal facilities with a primary settling tank and the following model inputs:

- the amount of imlunestrant entering the POTW (i.e., emission rate input) is $27.3 \text{ kg}\cdot\text{day}^{-1}$ ($10,000 \text{ kg}\cdot\text{year}^{-1} \div 365 \text{ day}\cdot\text{year}^{-1}$);
- no degradation occurs during wastewater treatment (i.e., degradation rate input is 0 day^{-1}), due to the high DT_{50} values in the sewage sludge transformation study (Study 151E-162);
- no volatilization or stripping occurs during treatment, due to the high melting point of imlunestrant;
- a default Henry's Law Constant of $1 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$;
- $K_{\text{FOC, BIOSOLIDS}}$ of $2.96 \times 10^4 \text{ L}\cdot\text{kg}^{-1}$ (geometric mean for biosolids from 3 different POTW Study 151E-160), which was transformed within the model to partition coefficients for raw sewage and for activated sludge.

With these inputs, the SimpleTreat model estimates that 80.0% of imlunestrant will be removed from POTW effluent due to binding to sludge solids. The EIC_{AQUATIC} after adsorption to sewage sludge solids is calculated to be 0.042 µg·L⁻¹ [0.21 µg·L⁻¹ × (1 – 0.80)].

Sediment

Imlunestrant is expected to rapidly partition to sediment upon entry into receiving waters from a POTW. In the water-sediment transformation study (Study 151E-161), imlunestrant partitioned from the water to sediment with DT₅₀ values of ≤ 2.25 days. Once in the sediment, approximately half of the imlunestrant will remain bound to sediment and is not expected to be remobilized.

The expected introductory concentration in freshly deposited sediment (EIC_{SEDIMENT}) is calculated using equations R.16-6, R.16-7, R.16-16, R.16-34, and R.16-74 (modified) as well as default values in table R.16-8 of REACH (ECHA 2016). Since the concentration is derived in freshly deposited sediment, the properties for suspended matter are applied.

Since K_{F, SOIL} does not correlate with organic carbon content (Appendix D), the geometric mean for K_{F, SOIL} (2.49×10⁴ L·kg⁻¹; Study 151E-160) is used as the partitioning coefficient to suspended solids (K_{pSUSP}). Using equation R.16-7, the partitioning coefficient to the suspended matter compartment (K_{SUSP-WATER}) is calculated to be 6226 m³·m⁻³:

$$K_{SUSP-WATER} = F_{WATER,SUSP} + F_{SOILD,SUSP} \times \frac{K_{pSUSP}}{1000} \times RHO_{SOLID}$$

$$K_{SUSP-WATER} = 0.9 \frac{m^3_{WATER}}{m^3_{SOLID}} + 0.1 \frac{m^3_{SOLID}}{m^3_{SUSP}} \times \frac{2.49 \times 10^4 \frac{L_{WATER}}{kg_{SOLID}}}{1000 \frac{L_{WATER}}{m^3_{WATER}}} \times 2500 \frac{kg_{SOLID}}{m^3_{SOLID}} = 6226 \frac{m^3_{SUSP}}{m^3_{WATER}}$$

Using equation R.16-16, the bulk density of suspended matter (RHO_{SUSP}) is calculated to be 1150 kg·m⁻³:

$$RHO_{SUSP} = F_{solid,SUSP} \times RHO_{SUSP} + F_{SUSP} \times RHO_{WATER} + F_{AIR,SUSP} \times RHO_{AIR}$$

$$RHO_{SUSP} = 0.1 \frac{m^3_{SOLID}}{m^3_{SUSP}} \times 2500 \frac{kg_{SOLID}}{m^3_{SOLID}} + 0.9 \frac{m^3_{WATER}}{m^3_{SUSP}} \times 1000 \frac{kg_{WATER}}{m^3_{WATER}} + 0 \frac{m^3_{AIR}}{m^3_{SUSP}} \times 1.3 \frac{kg_{AIR}}{m^3_{AIR}}$$

$$= 1150 \frac{kg_{SUSP}}{m^3_{SUSP}}$$

The expected introductory concentration in sediment on a wet weight basis ($EIC_{\text{SEDIMENT, WET}}$) is calculated to be $227 \mu\text{g}\cdot\text{kg}^{-1}$. The concentration is derived using the partitioning coefficient for suspended matter ($6226 \text{ m}^3\cdot\text{m}^{-3}$), the bulk density of suspended matter ($1150 \text{ kg}\cdot\text{m}^{-3}$), the EIC_{AQUATIC} ($0.042 \mu\text{g}\cdot\text{L}^{-1}$), and equation R.16-34. EIC_{AQUATIC} is used to conservatively estimate the amount of imlunestrant that could partition to sediment.

$$EIC_{\text{SEDIMENT, WET}} = \frac{K_{\text{SUSP-WATER}}}{\text{RHO}_{\text{SUSP}}} \times EIC_{\text{AQUATIC}} \times 1000 \frac{\text{L}_{\text{WATER}}}{\text{m}^3_{\text{WATER}}}$$

$$EIC_{\text{SEDIMENT, WET}} = \frac{6226 \frac{\text{m}^3_{\text{SUSP}}}{\text{m}^3_{\text{WATER}}}}{1150 \frac{\text{kg}_{\text{SUSP}}}{\text{m}^3_{\text{SUSP}}}} \times 0.042 \frac{\mu\text{g}}{\text{L}} \times 1000 \frac{\text{L}_{\text{WATER}}}{\text{m}^3_{\text{WATER}}} = 227 \frac{\mu\text{g}}{\text{kg}}$$

The conversion factor ($\text{CONV}_{\text{SUSP}}$) necessary to convert the concentration in sediment on a wet weight basis to a concentration on a dry weight basis is derived using a modified version of equation R.16-74 and the bulk density of suspended matter ($1150 \text{ kg}\cdot\text{m}^{-3}$).

$$\text{CONV}_{\text{SUSP}} = \frac{\text{RHO}_{\text{SUSP}}}{F_{\text{SOLID, SUSP}} \times \text{RHO}_{\text{SOLID}}}$$

$$\text{CONV}_{\text{SUSP}} = \frac{1150 \frac{\text{kg}_{\text{SUSP}}}{\text{m}^3_{\text{SUSP}}}}{0.1 \frac{\text{m}^3_{\text{SOLID}}}{\text{m}^3_{\text{SUSP}}} \times 2500 \frac{\text{kg}_{\text{SOLID}}}{\text{m}^3_{\text{SOLID}}}} = 4.6 \frac{\text{kg}_{\text{SUSP}}}{\text{kg}_{\text{SOLID}}}$$

The expected introductory concentration of imlunestrant in sediment on a dry weight basis (EIC_{SEDIMENT}) is calculated to be $1.04 \text{ mg}\cdot\text{kg}^{-1}$. The concentration is derived using $\text{CONV}_{\text{susp}}$ ($4.6 \text{ kg}\cdot\text{kg}^{-1}$) and the $EIC_{\text{SEDIMENT, WET}}$ ($227 \mu\text{g}\cdot\text{kg}^{-1}$):

$$EIC_{\text{SEDIMENT}} = EIC_{\text{SEDIMENT, WET}} \times \text{CONV}_{\text{SUSP}}$$

$$EIC_{\text{SEDIMENT}} = 227 \frac{\mu\text{g}}{\text{kg, wet}} \times 4.6 \frac{\text{kg}_{\text{SUSP}}}{\text{kg}_{\text{SOLID}}} \times \frac{\text{mg}}{1000 \mu\text{g}} = 1.04 \frac{\text{mg}}{\text{kg}}$$

Soil

The EIC of imlunestrant in soil (EIC_{SOIL}) is calculated because imlunestrant has a $K_{\text{OC, SLUDGE}}$ greater than $1000 \text{ L}\cdot\text{kg}^{-1}$ and is not readily biodegradable. The transfer of imlunestrant to the terrestrial environment via binding to sewage sludge biosolids and subsequent application to agricultural soil is assumed.

Seiple et al. (2017) estimate that POTW in the United States generate a total of $1.256 \times 10^{13} \text{ g}$ biosolids (dry) $\cdot\text{year}^{-1}$ (or $3.44 \times 10^{10} \text{ g}$ dry biosolids $\cdot\text{day}^{-1}$), of which approximately 50%

(6.23×10^{12} g dry biosolids \cdot year $^{-1}$) is treated and beneficially used. Of the beneficially used biosolids, 35% (2.18×10^{12} g dry biosolids \cdot year $^{-1}$ or 5.97×10^9 g dry biosolids \cdot day $^{-1}$) is applied to land as digested sludge (i.e., treated with an anaerobic digester) and approximately 22% (1.40×10^{12} g dry biosolids \cdot year $^{-1}$ or 3.84×10^9 g dry biosolids \cdot day $^{-1}$) is land applied as non-digested sludge (Seiple et al. 2017). The remaining biosolids are sent to municipal landfills, incinerated or stored in biosolids landfills (NEBRA 2007). Assuming that 80% of imlunestrant binds to biosolids in a POTW, the concentration of imlunestrant in (dry) biosolids ($EIC_{\text{BIOSOLIDS}}$) is $0.635 \text{ mg}\cdot\text{kg}^{-1}$.

$$EIC_{\text{BIOSOLIDS}} = \frac{27.3 \frac{\text{kg}}{\text{day}} \times 1,000,000 \frac{\text{mg}}{\text{kg}} \times 0.80}{3.44 \times 10^{10} \frac{\text{g biosolids}}{\text{day}} \times \frac{\text{kg}}{1000 \text{ g}}} = 0.635 \frac{\text{mg}}{\text{kg}}$$

Since the digestion process reduces the solids level by 50% (Struijs 2014), the concentration in digested sludge ($EIC_{\text{DIG BIOSOLIDS}}$) was determined. Biodegradation during digestion was not considered, because the potential for biodegradation under anaerobic conditions in sludge is not known. The $EIC_{\text{DIG BIOSOLIDS}}$ is calculated to be $1.27 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sludge ($0.635 \text{ mg}\cdot\text{kg}^{-1} \div 0.5$).

The expected introductory concentration of imlunestrant in soil (EIC_{SOIL}) is derived using equations R.16-48 and default values in tables R-16-8, R.16-15 of REACH (ECHA 2016).

The concentration of imlunestrant in soil after an initial sludge application (EIC_{SOIL}) is calculated to be $0.00187 \text{ mg}\cdot\text{kg}^{-1}$. The value is derived using $EIC_{\text{DIG BIOSOLIDS}}$ ($1.27 \text{ mg}\cdot\text{kg}^{-1}$), a default dry sludge application rate of $0.5 \text{ kg}\cdot\text{m}^{-2}\cdot\text{year}^{-1}$ ($APPL_{\text{BIOSOLIDS}}$), a default mixing depth in soil of 0.20 m ($DEPTH_{\text{SOIL}}$), and a bulk soil density of $1700 \text{ kg}\cdot\text{m}^{-3}$ (RHO_{SOIL}) and equation R.16-48:

$$EIC_{\text{SOIL}} = \frac{EIC_{\text{DIG BIOSOLIDS}} \times APPL_{\text{BIOSOLIDS}}}{DEPTH_{\text{SOIL}} \times RHO_{\text{SOIL}}}$$

$$EIC_{\text{SOIL}} = \frac{1.27 \frac{\text{mg}}{\text{kg}} \times 0.5 \frac{\text{kg}_{\text{dry wt}}}{\text{m}^2 \cdot \text{yr}} \times 1 \text{ yr}}{0.20 \text{ m} \times 1700 \frac{\text{kg}}{\text{m}^3}} = 0.00187 \frac{\text{mg}}{\text{kg}}$$

Expected Environmental Concentration

The expected environmental concentration (EEC) is the concentration of imlunestrant that organisms would be exposed to in the respective environmental compartment. The EEC is derived by applying appropriate adjustments to the EIC that consider the behavior of imlunestrant in the corresponding compartment (e.g., dilution, degradation, accumulation).

Biosolids

In the sludge biosolids transformation study ([Study 151E-162](#)), minimal transformation was observed during the 28-day study and the loss of imlunestrant was primarily due to irreversible adsorption to biosolids. Therefore, no correction is applied and $EIC_{BIOSOLIDS}$ ($0.635 \text{ mg}\cdot\text{kg}^{-1}$) is taken as $EEC_{BIOSOLIDS}$.

Surface Water

The expected environmental concentration for the aquatic environment ($EEC_{AQUATIC}$) is calculated after dilution of POTW effluent into receiving waters. As described by FDA ([1996](#)), a dilution factor of 10 is applied to $EIC_{AQUATIC}$ ($0.042 \text{ }\mu\text{g}\cdot\text{L}^{-1}$) and $EEC_{AQUATIC}$ is calculated as $0.0042 \text{ }\mu\text{g}\cdot\text{L}^{-1}$.

Soil

Since imlunestrant does not appear to be easily degraded, a worst-case scenario is assumed where biosolids application to agricultural soil takes place for 20 consecutive years.

Chemicals may be removed from soil via volatilization, leaching during rain events and biodegradation, as described by REACH ([ECHA 2016](#)). Given the melting point (175°C), the K_F in soil (geometric mean: $2.49 \times 10^4 \text{ L}\cdot\text{kg}^{-1}$), and the DT_{50} in soil (200 to 310 days at 20°C), these sources of removal are not considered.

The maximum concentration after 20 years of biosolids application is $0.037 \text{ mg}\cdot\text{kg}^{-1}$ ($0.00187 \text{ }\mu\text{g}\cdot\text{kg}^{-1} \times 20 \text{ years}$) and taken as the expected environmental concentration in soil (EEC_{SOIL}).

Sediment

Imlunestrant is not expected to undergo transformation in sediment. In the water-sediment study ([Study 151E-161](#)), total system DT_{50} values were 1310 and 179 days (HOC and LOC, respectively). Since little transformation of imlunestrant was observed, no adjustments were applied and $EIC_{SEDIMENT}$ ($1.04 \text{ mg}\cdot\text{kg}^{-1}$) is taken as $EEC_{SEDIMENT}$.

Summary of Environmental Fate

Imlunestrant will enter the environment primarily through patient use. Although patient metabolism of imlunestrant is extensive, a total residue approach was followed for this risk assessment. Assuming 10,000 kg of imlunestrant is administered to patients annually, the expected introductory concentration in a POTW ($EIC_{AQUATIC}$) could be as high as $0.21 \text{ }\mu\text{g}\cdot\text{L}^{-1}$. Within a POTW, imlunestrant will partition to biosolids at a concentration of $0.635 \text{ mg}\cdot\text{kg}^{-1}$ ($EIC_{BIOSOLIDS}$) and reduce $EIC_{AQUATIC}$ to $0.042 \text{ }\mu\text{g}\cdot\text{L}^{-1}$. If biosolids are applied to land, imlunestrant could enter the terrestrial compartment at an expected introductory concentration of $0.00187 \text{ mg}\cdot\text{kg}^{-1}$ soil (EIC_{SOIL}). Imlunestrant is not expected to undergo substantial degradation in soil and 20 consecutive years of biosolids application to soil could result in an expected environmental concentration of $0.037 \text{ mg}\cdot\text{kg}^{-1}$ soil (EEC_{SOIL}). Following treatment in a POTW,

imlunestrant will enter receiving surface waters at an expected environmental concentration of $0.0042 \mu\text{g}\cdot\text{L}^{-1}$ ($\text{EEC}_{\text{AQUATIC}}$), where it will quickly partition to sediment solids at a concentration of $1.04 \text{ mg}\cdot\text{kg}^{-1}$ ($\text{EEC}_{\text{SEDIMENT}}$). Imlunestrant will become irreversibly incorporated into the sediment with a low potential for remobilization. In surface waters, imlunestrant is not expected to accumulate in aquatic organisms such as fish. Imlunestrant is not expected to volatilize and will not enter the atmospheric environment.

Table 2. Summary of Expected Environmental Concentrations of Imlunestrant

| Compartment | EIC | EEC | MEEC |
|------------------------|---|--|--|
| AQUATIC (POTW) | $0.042 \mu\text{g}\cdot\text{L}^{-1}$ | $0.042 \mu\text{g}\cdot\text{L}^{-1}$ | $0.042 \mu\text{g}\cdot\text{L}^{-1}$ |
| AQUATIC (SURFACEWATER) | -- | $0.0042 \mu\text{g}\cdot\text{L}^{-1}$ | $0.0042 \mu\text{g}\cdot\text{L}^{-1}$ |
| BIOSOLIDS | $0.635 \text{ mg}\cdot\text{kg}^{-1}$ | $0.635 \text{ mg}\cdot\text{kg}^{-1}$ | $0.635 \text{ mg}\cdot\text{kg}^{-1}$ |
| SOIL | $0.00187 \text{ mg}\cdot\text{kg}^{-1}$ | $0.037 \text{ mg}\cdot\text{kg}^{-1}$ | $0.037 \text{ mg}\cdot\text{kg}^{-1}$ |
| SEDIMENT | $1.04 \text{ mg}\cdot\text{kg}^{-1}$ | $1.04 \text{ mg}\cdot\text{kg}^{-1}$ | $1.04 \text{ mg}\cdot\text{kg}^{-1}$ |

EEC = expected environmental concentration, EIC = expected introductory concentration,

MEEC = maximum expected environmental concentration (i.e., EIC or EEC, whichever is greater),

POTW = publicly-owned treatment works

Environmental Effects of Imlunestrant

The environmental effects studies conducted with imlunestrant are described below, a data summary table provided in [Appendix B](#), and study summaries included in [Appendix C](#). The environmental effect studies evaluated acute toxicity within a POTW (i.e., microorganisms in sewage biosolids), acute toxicity to three trophic levels of aquatic species (i.e., algae, daphnia, fish) and one terrestrial species (i.e., earthworm), and chronic toxicity to species representing three trophic levels within the aquatic (i.e., algae, daphnia, fish), sediment (i.e., species representing three different taxonomic groups and three different sediment-based habitats and feeding strategies), and terrestrial (i.e., primary producers, consumers and decomposers) environments.

Tier 1 Testing

Sewage Treatment Plant Microorganisms

Microorganisms from activated sewage sludge (collected from the aeration basin of a sewage treatment plant that primarily receives domestic sewage) were exposed to imlunestrant at nominal concentrations of 10,000, 100,000 and 1,000,000 $\mu\text{g}\cdot\text{L}^{-1}$ in a range-finding test ([Study 151E-159](#); [OECD 2010](#), Guideline No. 209). After three hours of exposure, there was no significant inhibition of total respiration at any concentration tested and definitive testing at higher or lower concentrations were not pursued. The median effective concentration (EC_{50}) was $> 1,000,000 \mu\text{g}\cdot\text{L}^{-1}$ and the no-observed-effect concentration (NOEC) was $1,000,000 \mu\text{g}\cdot\text{L}^{-1}$.

Tier 2 Testing

Algae

A green alga (*Raphidocelis subcapitata*), undergoing exponential growth, was exposed for 72 hours in growth media at 7 geometric mean measured concentrations of imlunestrant ranging from 1.3 to 367 $\mu\text{g}\cdot\text{L}^{-1}$, under static conditions (Study 151P-108; OECD 2011, Guideline No. 201). Measured concentrations of imlunestrant ranged from 109 to 84.0% of nominal at 0-hour and declined to 12.6 to 26.7% of nominal at 72-hour. The decline in concentration was likely due to adsorption to the test vessels, among other factors, as evidenced by the abiotic control. For growth rate, the EC_{50} and NOEC was 33 $\mu\text{g}\cdot\text{L}^{-1}$ and 2.5 $\mu\text{g}\cdot\text{L}^{-1}$, respectively.

Aquatic Invertebrate

In a 48-hour acute toxicity test, *Daphnia magna* neonates were exposed to 5 mean measured concentrations of imlunestrant ranging from 15.7 to 286 $\mu\text{g}\cdot\text{L}^{-1}$, where exposure solutions were renewed daily (Study 151A-179; OECD 2004a, Guideline No. 202). At 0-hour, measured concentrations ranged from 52.6 to 65.2% of nominal and declined to 35.0 to 47.7% of nominal after 24 hours; the exposure period between 24-hour and 48-hour displayed a similar pattern of decline. Mean measured concentrations ranged from 50.5% to 57.1% of nominal with a coefficient of variation ranging from 11.1 to 32.1%. After 48-hours of exposure, there was 100% immobility at 286 $\mu\text{g}\cdot\text{L}^{-1}$; immobility or other physical signs of toxicity were not observed at imlunestrant concentrations < 286 $\mu\text{g}\cdot\text{L}^{-1}$. The EC_{50} was 200 $\mu\text{g}\cdot\text{L}^{-1}$ and the NOEC (and no-immobility concentration) were 140 $\mu\text{g}\cdot\text{L}^{-1}$.

Fish

Prior to conducting the multigeneration reproduction and development study in fish (Study 151A-185), a non-GLP acute toxicity screen was conducted with both fathead minnow (*Pimephales promelas*) embryos and juvenile fathead minnow (data summarized here and in report for Study 151A-185). For both exposures, the nominal concentrations of imlunestrant were 4.1, 13.5, 45, 150, 500 $\mu\text{g}\cdot\text{L}^{-1}$ with a solvent control (100 μL dimethylformamide $\cdot\text{L}^{-1}$). Both exposures were conducted in a flow-through diluter similar to that used in the fish full-life cycle study, where test solutions were split between two replicate aquaria (one for the embryo exposure and one for the juvenile exposure). Concentrations of LY3484356 were measured in each replicate prior to initiation of their respective exposures.

For the embryo exposure, 50 embryos were distributed between 2 incubation cups for each treatment and control group, and the exposure continued to 4 days post-hatch. Endpoints evaluated during the embryo exposure were hatching success, post-hatch survival and symptoms of toxicity.

For the juvenile exposure (similar to OECD 2019, Guideline No. 203), 10 fish were added to each treatment and control group, and the exposure continued for 96 hours. Endpoints evaluated during the juvenile exposure were mortality and symptoms of toxicity. The median lethal concentration (LC_{50}) is 114 $\mu\text{g}\cdot\text{L}^{-1}$, calculated as the geometric mean between 61.1 $\mu\text{g}\cdot\text{L}^{-1}$ (i.e.,

highest concentration with no mortality) and 213 $\mu\text{g}\cdot\text{L}^{-1}$ (lowest concentration with 100% mortality).

The results of both exposures are presented below:

Table 3. Summary of Acute Toxicity Screen of Imlunestrant with Fathead Minnow

| Nominal Test Concentration ($\mu\text{g}\cdot\text{L}^{-1}$) | Mean Measured Test Concentration ($\mu\text{g}\cdot\text{L}^{-1}$) | Embryo Hatching Success (%) | Embryo Post-Hatch Survival (%) | Juvenile Mortality (%) |
|--|--|-----------------------------|--------------------------------|------------------------|
| Solvent control | Not detected | 98.0 | 100 | 0.0 |
| 4.1 | 1.8 | 98.0 | 95.9 | 0.0 |
| 13.5 | 5.4 | 94.0 | 97.9 | 0.0 |
| 45 | 17.2 | 96.0 | 95.8 | 0.0 |
| 120 | 61.1 | 98.0 | 95.9 | 0.0 |
| 500 | 213 | 96.0 | 4.2 | 100 |

Soil Microorganisms

The long-term effect of imlunestrant on a natural population of soil microorganisms was evaluated by measuring their nitrogen transformation activity at 5 nominal concentrations of imlunestrant ranging from 7.6 to 761 $\text{mg}\cdot\text{kg}^{-1}$ (dry) soil over 28 days ([Study 151E-170](#); [OECD 2000b](#), Guideline No. 216). Solvent stocks of imlunestrant were used to spike aliquots of sand that were amended into sediment after solvent evaporation. Since measured concentrations of spiked sand (after evaporation) approximated nominal concentrations (85 to 135% of nominal), the results of this study are based on nominal concentrations. Nitrate content was measured on Day 0 and 28, and nitrate formation rates were calculated. Nitrate formation rate was higher in all imlunestrant treatments compared to the control with percent inhibition values ranging from -13.4 to -4.9%. Since percent inhibition was < 25% for all concentrations of imlunestrant, the NOEC and LOEC were 761 and >761 $\text{mg}\cdot\text{kg}^{-1}$ (dry) soil, respectively.

Soil Invertebrate

In an acute toxicity test, earthworms (*Eisenia fetida*) were exposed to 5 nominal concentrations of imlunestrant ranging from 3.1 to 750 $\text{mg}\cdot\text{kg}^{-1}$ (dry) soil ([Study 151P-113](#); [OECD 1984](#), Guideline No. 207). The study began with adults (< 1 year old with clitellum) and evaluated mortality and body weight changes after 14 days. Solvent stocks of imlunestrant were used to spike aliquots of sand that were amended into sediment after solvent evaporation. Analysis of dosing solution, measured prior to application to soil, approximated nominal (95.9% of nominal); therefore, results are based on nominal concentrations. At 9.0 $\text{mg}\cdot\text{kg}^{-1}$, 1 worm was missing and assumed dead on Day 7 and 1 worm was lethargic and exhibited loss of muscle tone. No other signs of physical toxicity or mortality were observed at any concentration of

imlunestrant. The LC₅₀ was empirically estimated to be >750 mg·kg⁻¹ (dry) soil. The NOEC and LOEC were 750 and >750 mg·kg⁻¹ (dry) soil, respectively.

Terrestrial Plants

In a seedling emergence test, six species of terrestrial plants were exposed to 6 nominal concentrations of imlunestrant ranging from 4.1 to 1000 mg·kg⁻¹ (dry) soil (Study 151P-109; OECD 2006, Guideline No. 208). The species tested were: onion (*Allium cepa*), ryegrass (*Lolium perenne*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), radish (*Raphanus sativus*) and tomato (*Solanum lycopersicum*). These species represent types of plants grown on agricultural fields that would receive sludge application and that belong to 6 different families of 4 dicotyledonous and 2 monocotyledonous species. Seeds were sown into soil treated with imlunestrant and evaluated for emergence, survival, shoot height, shoot dry weight, and visible phytotoxic effects after 21 days (i.e., ≥14 days after 50% control emergence). Solvent stocks of imlunestrant were used to spike aliquots of sand that were amended into sediment after solvent evaporation. Analysis of dosing solution, measured prior to application to soil, approximated nominal (85.5 to 127% of nominal); therefore, results are based on nominal concentrations.

For all species tested, there were no observations of phytotoxicity that were not reflected in the shoot height or shoot weight measurements or that occurred in a concentration-dependent manner.

For onion and soybean, exposure to imlunestrant had no statistically significant effects in any endpoint. The NOEC and LOEC for these species were 1000 and >1000 mg·kg⁻¹ (dry) soil, respectively.

There were statistically significant effects in shoot height and dry shoot weight at 1000 mg·kg⁻¹ for ryegrass (27 and 35% reduced from solvent control, respectively) and radish (29 and 58% reduced from solvent control, respectively). The NOEC and LOEC for these species was 333 and 1000 mg·kg⁻¹ (dry) soil, respectively.

The most sensitive species were sunflower and tomato. For sunflower, there were statistically significant effects in emergence at 333 and 1000 mg·kg⁻¹ (35 and 10% reduced from solvent control, respectively). For tomato, there were statistically significant effects in emergence, shoot height and dry shoot weight at 333 (16, 17 and 44% reduced from solvent control, respectively) and 1000 mg·kg⁻¹ (21, 47 and 76% reduced from solvent control, respectively). For these species, the NOEC and LOEC were 111 and 333 mg·kg⁻¹ (dry) soil, respectively.

Tier 3 Testing

Aquatic Invertebrate

In a 21-day chronic toxicity test, *D. magna* were exposed to 6 mean measured concentrations of imlunestrant ranging from 1.06 to 413 µg·L⁻¹, where exposure solutions were renewed daily (Study 151A-181B; OECD 2012b, Guideline No. 211). The exposure started with juvenile daphnia (< 24-hour post-release) and evaluated their survival, growth (dry body weight, total body length) and reproduction. Measured concentrations of new solutions ranged from 90.9 to

118% of the nominal, while old solutions ranged from 25.6 to 85.2% of nominal. Measured concentrations declined between renewals and the decline in measured concentration increased with decreasing test concentration. Although measured concentrations were not within $\pm 20\%$ of initial between renewals, the decline in concentration was consistent between each sampling interval, and demonstrate that daphnia within each treatment group were exposed to consistent conditions throughout the 21-day exposure. Mean measured concentrations ranged from 69.2% to 82.6% of nominal, with a CV ranging from 21.4 to 56.8%. A significant decrease in dry weight (20% reduced from control) and length was observed at 125 and 413 $\mu\text{g}\cdot\text{L}^{-1}$. The most sensitive endpoint was reproduction which was reduced at concentrations $\geq 10.5 \mu\text{g}\cdot\text{L}^{-1}$. Based on reproduction, the NOEC and lowest-observed-effect concentration (LOEC) were 3.32 and 10.5 $\mu\text{g}\cdot\text{L}^{-1}$, respectively.

Aquatic Vertebrate

To address the mode of action of imlunestrant (i.e., selective pure antagonist of wild type and mutant human oestrogen receptor α) and potential developmental and reproductive toxicity to aquatic vertebrates, a multigenerational reproduction and development study ([Study 151A-185](#)) was conducted following OECD Guideline No. 240 ([OECD 2015](#)) with modifications to suit fathead minnow (*Pimephales promelas*).

Fish Sensitivity Screen

There are differences in the physiological roles and pharmacological specificity of estrogen receptor subtypes in fish. Prior to conducting the multigenerational reproduction and development study, exposure trials were conducted with fathead minnow, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) to (1) determine if one fish species is substantially more sensitive to imlunestrant exposure than another and (2) establish appropriate concentrations for the definitive test. The 21-day exposures began with reproductively active groups of adult fish (identified using a 7-day pre-exposure evaluation period). Fish were exposed to imlunestrant at nominal concentrations of 1.5, 15 and 150 $\mu\text{g}\cdot\text{L}^{-1}$ with a solvent control (100 μL dimethylformamide $\cdot\text{L}^{-1}$). Each exposure was conducted under flow-through conditions using an exposure system similar to that used in the multigenerational reproduction and development study, with two replicate aquaria for each treatment and control group. During exposure, reproductive groups (4 females, 2 males for fathead minnows; 5 females, 5 males for zebrafish and medaka) were evaluated daily for survival, unusual behavior, fecundity and fertility. In the last week of the reproductive group exposure, embryos were collected and incubated within each treatment and control group (1 incubation cup containing 20 embryos per replicate; 2 incubation cups per control or imlunestrant treatment group) and the embryo/larval exposure continued to 21-days post-hatch. Endpoints evaluated for the embryo/larval exposure were hatching success, post-hatch survival, total length and wet weight. For each fish species, samples of test solution were collected prior to exposure initiation and at test termination to measure concentrations of imlunestrant. Reproduction in fathead minnow appeared to be reduced at nominal concentrations of 15 (31% reduced) and 150 $\mu\text{g}\cdot\text{L}^{-1}$ (79% reduced) compared to the solvent control, while no reduction was apparent for zebrafish and medaka. In the embryo/larval exposure, a

concentration-dependent response in all treatment groups for total length (2 to 9% reduced) and wet weight (13 to 32% reduced) was apparent for fathead minnow, and less apparent for zebrafish and medaka. For each exposure, concentrations of imlunestrant were measured prior to exposure initiation and at termination, and measured concentrations were similar to those in the multigenerational reproduction and development study.

Multigeneration Reproduction and Development Test with Fathead Minnow

The multigeneration reproduction and development study exposed fathead minnow to five concentrations of imlunestrant under flow-through conditions with a clean water control. An organic solvent carrier was avoided through the use of a concentrated water stock buffered to pH 4. The exposure was continuous over three generations: the reproductive phase of the parental (F0) generation, the full life cycle of the first filial (F1) generation and the embryo phase of the second filial (F2) generation. Four replicate test chambers were maintained for each treatment and control group. Concentrations of imlunestrant were measured weekly throughout the exposure. Due to the highly adsorptive properties of imlunestrant, measured concentrations across all generations were slightly variable with coefficient of variation ranging from 17.2 to 58.2%. Variability was greatest in the lowest treatment group due to the highly adsorptive nature of the test substance, the low nominal test concentration, the duration of the study, and the quantitation limits of the analytical method. Considering the overall length of exposure (i.e., 227 total days) and that the exposures were conducted in 3 different exposure systems (i.e., one for each generation), the measured concentrations for each treatment group demonstrated good consistency both within and between generations (and exposure systems):

Table 4. Summary of Mean Measured Concentrations in Multigeneration Reproduction and Development Test with Fathead Minnow

| Nominal Test Concentration ($\mu\text{g}\cdot\text{L}^{-1}$) | Mean Measured Test Concentration ($\mu\text{g}\cdot\text{L}^{-1}$) | | |
|--|--|-------------------|---------------------|
| | F0 generation | F1 generation | F2 generation |
| Control (clean water) | Not detected | Not detected | Not detected |
| 0.039 | 0.015 ± 0.0065 | 0.019 ± 0.011 | 0.022 ± 0.00778 |
| 0.16 | 0.092 ± 0.028 | 0.11 ± 0.020 | 0.10 ± 0.0157 |
| 0.63 | 0.44 ± 0.13 | 0.43 ± 0.17 | 0.52 ± 0.118 |
| 2.5 | 1.7 ± 0.36 | 1.8 ± 0.31 | 1.6 ± 0.495 |
| 10 | 8.1 ± 3.0 | 7.1 ± 1.54 | 6.9 ± 0.431 |

F0 reproductive groups (i.e., 2 males and 4 females per replicate) were exposed to mean measured concentrations ranging from 0.015 to $8.1 \mu\text{g}\cdot\text{L}^{-1}$ with a (clean water) control for 32 days during which survival, fecundity, fertility, tubercle scores (i.e., male secondary sex characteristic), body length, body weight, and general observations of toxicity were evaluated. There were no statistically significant effects in any treatment group on fecundity (cumulative number of eggs, eggs per female per reproductive day) or fertility. At the highest concentration

(8.1 $\mu\text{g}\cdot\text{L}^{-1}$) there was a significant increase in female body weight compared to the negative control. Embryos were collected from F0 reproduction groups to begin the F1 exposure.

F1 fish were exposed to mean measured concentrations of 0.019, 0.11, 0.43, 1.8 and 7.1 $\mu\text{g}\cdot\text{L}^{-1}$ with a negative control beginning as embryos (approximately 4 days to completion of hatch) and reared to reproductively active, sexually mature adults (156 days post-hatch) to evaluate reproduction. Apical and diagnostic (i.e., indicators of endocrine-related modality) endpoints were evaluated following completion of hatch (i.e., hatching success, time to hatch), upon formation of reproductive groups (i.e., survival, body length, body weight, plasma vitellogenin, macroscopic gonadal sex, sex ratio, tubercle scores), and after a 35-day reproduction assessment (i.e., survival body length, body weight, fecundity, fertility, macroscopic and histological gonadal sex, gonadosomatic index, gonad histopathology, tubercle scores).

Upon completion of hatch (3 to 4 days), there was a significant decrease in hatching success at the highest concentration (7.1 $\mu\text{g}\cdot\text{L}^{-1}$), but not in time to hatch.

At formation of reproductive groups (i.e., 2 males and 4 females per replicate) on day 156 post-hatch, there was an unusual low number of males in all replicates of the control (i.e., 2 to 6 males per replicate, compared to 8 to 18 males per replicate across all treatment groups). Statistical comparisons for the surplus fish (i.e., those not chosen for reproductive groups) were hindered for male-related endpoints, due to the poor sample size in the control (i.e., 0 to 4 males per replicate). Since there did not seem to be a concentration-dependent response for surplus male wet weight (significant increase at 1.8 $\mu\text{g}\cdot\text{L}^{-1}$), and tubercle scores (significant decrease at 0.43 $\mu\text{g}\cdot\text{L}^{-1}$), any differences to the control were not considered biologically meaningful. Plasma vitellogenin for surplus male fish did display a concentration-dependent response and the significant increase at 7.1 $\mu\text{g}\cdot\text{L}^{-1}$ was potentially treatment-related. Surplus female fish displayed significant increases in total length and wet weight at 7.1 $\mu\text{g}\cdot\text{L}^{-1}$; significant effects were not observed for any other female-related endpoint.

For the 35-day F1 reproduction assessment, there were no significant effects in any treatment group for survival, time to first spawn, fecundity, or fertility. Males from the reproductive groups displayed significant increases in total length, GSI and increased interstitial (i.e., Leydig) cells in testes at 7.1 $\mu\text{g}\cdot\text{L}^{-1}$, and increases in wet weight at 1.8 and 7.1 $\mu\text{g}\cdot\text{L}^{-1}$. Significant effects were not observed in other male-related endpoints. There was a significant increase in both total length and wet weight in females from the reproductive groups at 7.1 $\mu\text{g}\cdot\text{L}^{-1}$. Significant effects were not observed in other female-related endpoints. Embryos were collected from F1 reproduction groups to begin the F2 exposure.

The F2 generation embryos were exposed to mean measured concentrations ranging from 0.022 to 6.9 μg LY3484356/L with a negative control through completion of hatch. There were no significant treatment-related effects in any treatment group compared to the negative control.

Across all three generations, the most sensitive endpoints were length and weight of F1 male fish from the reproductive groups, which was significantly increased at 1.8 and 7.1 $\mu\text{g}\cdot\text{L}^{-1}$ compared to the control. The diagnostic endpoints indicate that imlunestrant may interact with the

endocrine system of fathead minnow at concentrations $\geq 7.1 \mu\text{g}\cdot\text{L}^{-1}$. The overall NOEC and LOEC for the F1 generation is 0.43 and $1.8 \mu\text{g}\cdot\text{L}^{-1}$, respectively.

Sediment Invertebrates

Endobenthic, Surface and Subsurface Feeder

An endobenthic sediment-dwelling invertebrate, midge (*Chironomus riparius*), was exposed to 7 geometric mean measured concentrations of imlunestrant ranging from 1.8 to $459 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sediment (Study 151A-184; OECD 2004c, Guideline No. 218). The study began with first instar larvae and evaluated adult emergence and development rate of males and females over 28 days. To aid in quantification, isotopically dilute stock solutions of radiolabeled imlunestrant were used to spike aliquots of sand that were amended into sediment after evaporation. Measured concentrations of total radioactivity in the sediment ranged from 84.8 to 100% of nominal at the start of the study and were consistent over the course of the study, with a coefficient of variation ranging from 1.3 to 18% and no signs of degradation based on fractionation. There were statistically significant reductions in emergence at 174 and $459 \text{ mg}\cdot\text{kg}^{-1}$ (39% and 100% reduced from solvent control, respectively). Development rate was significantly reduced at $174 \text{ mg}\cdot\text{kg}^{-1}$ for males (9% reduced from solvent control), while no significant effects were determined for females. Emergence and male development rate were the most sensitive endpoints and established the NOEC and LOEC as 68 and $174 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sediment, respectively.

Endobenthic, Subsurface Feeder

An endobenthic, sediment-ingesting invertebrate, oligochaete (*Lumbriculus variegatus*), was exposed to 7 geometric mean measured concentrations of imlunestrant ranging from 0.85 to $465 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sediment (Study 151A-204; OECD 2007, Guideline No. 225). The study began with synchronized adults and evaluated reproduction and biomass (dry weight and ash-free dry weight) after 28 days. To aid in quantification, isotopically dilute solvent stock solutions of radiolabeled imlunestrant were used to spike aliquots of sand that were amended into sediment after solvent evaporation. Measured concentrations of total radioactivity in the sediment ranged from 77.9 to 97.1% of nominal on Day 0, 81.9 to 108% of nominal on Day 7, and 78.3 to 89.2% of nominal on Day 28. There were no signs of degradation at any sampling interval based on fractionation. At $465 \text{ mg}\cdot\text{kg}^{-1}$, there were statistically significant reductions in reproduction, dry weight and ash-free dry weight (30%, 59%, 52.4% reduced from solvent control, respectively). Reproduction, dry weight and ash-free dry weight were the most sensitive endpoints and established the NOEC and LOEC as 139 and $465 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sediment, respectively.

Epibenthic, Surface Feeder

An epibenthic, surface feeding invertebrate, amphipod (*Hyalella azteca*), was exposed to 6 geometric mean measured concentrations of imlunestrant ranging from 3.2 to $935 \text{ mg}\cdot\text{kg}^{-1}$ (dry) sediment for 42 days (Study 151A-203; EPA 1996, draft OPPTS Guideline No. 850.1770). The study began with 7- to 8-day old juveniles that were exposed to imlunestrant spiked sediment for 28 days, then transferred to clean water for 14 days to assess reproductive output, growth (body

length and dry weight), and survival. To aid in quantification, isotopically dilute solvent stock solutions of radiolabeled imlunestrant were used to spike aliquots of sand that were amended into sediment after solvent evaporation. Measured concentrations of total radioactivity in the sediment ranged from 87.3% to 99.1% of nominal on Day 0 and 68.2% to 97.6% of nominal on Day 28. There were no signs of degradation at any sampling interval based on fractionation. At 935 mg·kg⁻¹, there were statistically significant reductions in all endpoints with the following percent reduction from solvent control: 16% for survival, 14% for male body length, 25% for male dry weight, 17% for female body length, 28% for female dry weight, and 70% for reproduction. The most sensitive endpoints were survival, reproduction and growth and established the NOEC and LOEC as 283 and 935 mg·kg⁻¹ (dry) sediment, respectively.

Soil Invertebrate

In a chronic toxicity test, springtails (*Folsomia candida*) were exposed to 8 nominal concentrations of imlunestrant ranging from 16.3 to 1000 mg·kg⁻¹ (dry) soil for 28 days ([Study S22-07858](#); [OECD 2012c](#), Guideline No. 232). The study started with synchronized juvenile springtails (9 to 11 days old) and evaluated mortality and reproduction after 28 days. Imlunestrant was weighed directly into sand and the sand carrier amended into soil; therefore, the results of this study are based on nominal concentrations. At the two highest concentrations (556 and 1000 mg·kg⁻¹) there were statistically significant reductions in reproduction (20 and 19% reduced from control, respectively). Therefore, the NOEC and LOEC were 309 and 556 mg·kg⁻¹ (dry) soil, respectively.

Risk Assessment

To assess the environmental risk of imlunestrant to the environment, the quotient of the appropriate effect concentration (i.e., LC₅₀, EC₅₀, NOEC) from each environmental effect study and the maximum expected environmental concentration (MEEC; i.e., EIC or EEC, whichever is greater) for the associated compartment was compared to the minimum assessment factor for each testing tier (i.e., assessment factor must be greater than 1000 for tier 1 testing, greater than 100 for tier 2 testing, and greater than 10 for tier 3 testing).

Table 5. Aquatic Environment – Effect Concentrations Compared to Expected Environmental Concentrations

| Aquatic Environment | | | | |
|--|--|----------------------------|-----------------------------|----------------------------|
| Environmental Effects Study | Effect Concentration (µg·L ⁻¹) | MEEC (µg·L ⁻¹) | Effect Concentration ÷ MEEC | Required Assessment Factor |
| TIER 1 – Acute Toxicity | | | | |
| Sewage microorganisms (3-hour, respiration) | EC ₅₀ >1,000,000 | 0.042 | > 23,809,524 | ≥1000 |
| TIER 2 – Acute Toxicity, Base Set | | | | |
| <i>Daphnia magna</i> (48- hour, immobilization) | EC ₅₀ 200 | 0.042 | 4762 | ≥100 |
| <i>Pimephales promelas</i> (96-hour, mortality) | LC ₅₀ 113 | 0.042 | 2691 | ≥100 |
| <i>Raphidocelis subcapitata</i> (72-hour, growth rate) | EC ₅₀ 33 | 0.042 | 786 | ≥100 |
| TIER 3 – Chronic Toxicity | | | | |
| <i>Daphnia magna</i> (21-day, reproduction) | NOEC 3.32 | 0.0042 | 790 | ≥10 |
| <i>Pimephales promelas</i> (227-day, multi-generation, reproduction and development) | NOEC 0.43 | 0.0042 | 102 | ≥10 |

EC₅₀ = median effect concentration; MEEC = maximum environmental concentration,
 NOEC = no-observed effect concentration

Table 6. Aquatic Sediment Environment – Effect Concentrations Compared to Expected Environmental Concentrations

| Aquatic Sediment Environment | | | | |
|--|---|-----------------------------|-----------------------------|----------------------------|
| Species | Effect Concentration (mg·kg ⁻¹) | MEEC (mg·kg ⁻¹) | Effect Concentration ÷ MEEC | Required Assessment Factor |
| TIER 3 – Chronic Toxicity | | | | |
| <i>Chironomus riparius</i> (28-day, emergence) | NOEC 68 | 1.04 | 65 | ≥10 |
| <i>Lumbriculus variegatus</i> (28-day, reproduction) | NOEC 139 | 1.04 | 134 | ≥10 |
| <i>Hyalella azteca</i> (42-day, reproduction) | NOEC 283 | 1.04 | 272 | ≥ 10 |

MEEC = maximum environmental concentration, NOEC = no-observed effect concentration

Table 7. Terrestrial Environment – Effect Concentrations Compared to Expected Environmental Concentrations

| Terrestrial Environment | | | | |
|---|---|-----------------------------|-----------------------------|----------------------------|
| Species | Effect Concentration (mg·kg ⁻¹) | MEEC (mg·kg ⁻¹) | Effect Concentration ÷ MEEC | Required Assessment Factor |
| TIER 2 – Acute Toxicity | | | | |
| Soil Microflora (28-day, nitrogen transformation) | EC ₅₀ >761 | 0.037 | >20568 | ≥ 100 |
| Terrestrial Plants (21-day, emergence) | NOEC 111 | 0.037 | 3000 | ≥ 100 |
| <i>Eisenia fetida</i> (14-day, mortality) | EC ₅₀ >750 | 0.037 | >20270 | ≥ 100 |
| TIER 3 – Chronic Toxicity | | | | |
| <i>Folsomia candida</i> (28-day, reproduction) | NOEC 309 | 0.037 | 8351 | ≥ 10 |

EC₅₀ = median effect concentration; MEEC = maximum environmental concentration, NOEC = no-observed effect concentration

The calculated assessment factors in all cases are greater than the required factors and in no case were sub-lethal effects observed at concentrations equal to the MEEC. These results indicate that the release of imlunestrant to sewage treatment plants and subsequently to the environment does not pose an environmental risk.

Other Issues

No other issues were identified.

Summary

Following patient use, imlunestrant and related metabolites enter the environment through POTW. In POTW, imlunestrant is expected to partition to the solids resulting in a reduction of the aqueous concentration. Upon discharge to receiving waters from a POTW, imlunestrant will enter the surface waters. The expected environmental concentration of imlunestrant in water is not expected to affect aquatic organisms based on the toxicity of imlunestrant to sewage biosolids microorganisms and surface water species representing three trophic levels (i.e., algae, invertebrate and fish). In surface water, imlunestrant will partition to the sediment and become irreversibly bound with low potential for remobilization. The expected environmental concentration of imlunestrant in sediment is not expected to affect sediment organisms based on the toxicity of imlunestrant to invertebrate species representing three different taxonomic groups and three different sediment-based habitats and feeding strategies. Imlunestrant may persist in the aquatic environment, mainly in sediment. Imlunestrant may enter the terrestrial environment from agricultural land application of POTW biosolids. The maximum concentration of imlunestrant expected in soil is not expected to affect terrestrial organisms based on the lack of toxicity of imlunestrant to several species representing three different trophic levels (i.e., primary

producers, consumers and decomposers). The amount of imlunestrant that humans could be exposed to by drinking surface water with the maximum expected environmental concentration of imlunestrant would be substantially less than the therapeutic dose. In summary, no adverse environmental effects have been identified from the use of imlunestrant in the treatment of patients with breast cancer.

Mitigation Measures

No mitigation measures are needed, as no adverse environmental effects have been identified in this environmental assessment from any use of imlunestrant. This action has no known effects on endangered or threatened species or historic properties.

Alternatives to the Proposed Action

As no adverse environmental effects have been identified from any use of imlunestrant, there is no need for alternatives to the proposed action.

List of Preparers

Author

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See [Appendix E](#) for curriculum vitae

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Nonconfidential Appendices

Appendix A: Summary of Physical-Chemical and Environmental Fate Studies with Imlunestrant

| Study Type | Result | | | Study No., Year (Guideline) |
|--|--|--------------------------------------|--|-------------------------------|
| Melting Point | No melting point prior to onset of decomposition at ~245°C | | | In-house (3.2.S.1.3) |
| pKa | 7.72 (base), 3.67 (base), and 8.18 (acid) | | | In-house (3.2.S.1.3) |
| Water Solubility (at 20°C) | pH 5 39 mg·L ⁻¹ | pH 7 1.58 mg·L ⁻¹ | pH 9 0.587 mg·L ⁻¹ | 151K-108, 2023 (OECD 105) |
| Log K_{ow} (at 25°C) | pH 5 3.29 | pH 7 5.13 | pH 9 5.55 | 151K-109, 2023 (OECD 123) |
| Ready Hydrolysis (Half-life at 25°C) | pH 4 > 1 year | pH 7 > 1 year | pH 9 > 1 year | 151E-163A, 2024 (OECD 111) |
| Adsorption Characteristics^a | | K _F (L·kg ⁻¹) | K _{FOC} (L·kg ⁻¹) | 151E-160, 2024 (OECD 106) |
| | Soil 1 | 1.61×10 ⁴ | 2.98×10 ⁵ | |
| | Soil 2 | 2.20×10 ⁴ | 1.38×10 ⁶ | |
| | Soil 3 | 1.90×10 ⁴ | 2.38×10 ⁶ | |
| | Soil 4 | 5.65×10 ⁴ | 1.82×10 ⁶ | |
| | Soil Geomean: | 2.49×10 ⁴ | 1.16×10 ⁶ | |
| | Sludge 1 | 1.06×10 ⁴ | 3.15×10 ⁴ | |
| | Sludge 2 | 1.03×10 ⁴ | 3.08×10 ⁴ | |
| | Sludge 3 | 9.47×10 ³ | 2.66×10 ⁴ | |
| | Sludge Geomean: | 1.01×10 ⁴ | 2.96×10 ⁴ | |
| Biodegradation in Sludge, 28 days at 20°C (% of Applied Radioactivity) | Distribution Profile at 28 days | | | 151E-162, 2024 (OECD 314B) |
| | Mass Balance | | 90.6% | |
| | Extractable | | 41.3% | |
| | Non-extractable | | 46.6% | |
| | Imlunestrant | | 27.6% | |
| | CO ₂ | | 0.2% | |
| | Degradation Products | | 13.7% | |
| | DT ₅₀ at 20°C | | 2.26 days | |

(continued)

Appendix A: Summary of Physical-Chemical and Environmental Fate Studies (concluded)

| | | | | |
|---|--|------------------------|------------------------|------------------------------|
| Degradation in Aquatic Sediment, 102 days, Aerobic Conditions at 20°C^b (% Applied Radioactivity) | Sediment System | HOC | LOC | 151E-161, 2024 (OECD 308) |
| | Distribution of Radioactivity at 102 days | | | |
| | Mass Balance | | | |
| | Water | 0.2% | 0.8% | |
| | Sediment – Extractable | 62.6% | 59.6% | |
| | Sediment – Non-extractable | 31.0% | 14.7% | |
| | CO ₂ | 0.26% | 0.61% | |
| | Total System, Radiochemical Profile at 102 days | | | |
| | Imlunestrant | 62.6% | 59.0% | |
| | Transformation Products | 0.0% | 0.6% | |
| | Disappearance Rates (days) | | | |
| | DT ₅₀ at 20°C, Water | 2.25 | 1.64 | |
| | DT ₅₀ at 20°C, Sediment | 382 | 704 | |
| DT ₅₀ at 20°C, Total System | 1310 | 179 | | |
| Degradation in Soil, 120 days, Aerobic Conditions at 20°C^c (% of Applied Radioactivity) | Distribution of Radioactivity at 120 days | | | 151E-169, 2024 (OECD 307) |
| | Mass Balance | 100.6 – 108.0% | | |
| | Extractable | 59.8 – 70.6% | | |
| | Non-extractable | 32.7 – 46.6% | | |
| | CO ₂ | 0.27 – 0.57% | | |
| | Soil Extracts, Radiochemical Profile at 120 days | | | |
| | Imlunestrant | 59.8 – 70.6% | | |
| | Transformation Products | 0.0 – 0.0% | | |
| | Disappearance Rate | | | |
| DT ₅₀ at 20°C | 200 – 310 days | | | |
| Bioaccumulation Bioconcentration Factor in Fish^f (based on total residue) | Mean Water Concentration | 1.7 µg·L ⁻¹ | 19 µg·L ⁻¹ | 151A-202, 2024 (OECD 305) |
| | Steady-state BCF | 188 L·kg ⁻¹ | 241 L·kg ⁻¹ | |
| | Growth-Corrected, Lipid-Normalized Kinetic BCF | 500 L·kg ⁻¹ | 274 L·kg ⁻¹ | |

Abbreviations: BCF = bioconcentration factor; DT₅₀ = time to disappearance of 50% of imlunestrant; HOC = high organic carbon content sediment; K_F = Freundlich adsorption coefficient; K_FOC = K_F normalized to organic carbon content; K_{OW} = octanol-water partition coefficient; LOC = low organic carbon content sediment; pK_a = dissociation constant; ND = not detected.

- a Determined at 5 concentrations of radiolabeled imlunestrant in each matrix, spanning over 2 orders of magnitude
- b Range based on measurements in 2 water-sediment systems of varying organic carbon content and texture.
- c Range based on measurements in 4 soils of varying organic carbon content, pH, clay content, microbial biomass.

Appendix B: Summary of Environmental Toxicity Studies with Imlunestrant

| Study Type | NOEC (LOEC) ^a | L(E)C ₅₀ ^a | Study, Year (Guideline) |
|--|---|--|----------------------------------|
| Sewage Treatment Plant | | | |
| Sewage microorganisms (respiration inhibition, 3 hr) | 1,000,000 ^b µg·L ⁻¹ | >1,000,000 ^b µg·L ⁻¹ | 151E-159, 2024 (OECD 209) |
| Surface Water | | | |
| Water Flea – <i>Daphnia magna</i> (acute immobilization, 48 hr) | 140 µg·L ⁻¹ | 200 µg·L ⁻¹ | 151A-179A, 2024 (OECD 202) |
| Green Algae – <i>Raphidocelis subcapitata</i> (growth rate, 72 hr) | 2.5 µg·L ⁻¹ (7.4 µg·L ⁻¹) | 33 µg·L ⁻¹ | 151P-108, 2024 (OECD 201) |
| Water Flea – <i>Daphnia magna</i> (21-day chronic growth, reproduction, and survival) | 3.32 µg·L ⁻¹ (10.5 µg·L ⁻¹) | 187 µg·L ⁻¹ | 151A-181B, 2024 (OECD 211) |
| Fathead Minnow – <i>Pimephales promelas</i> (227-day, multi-generation, reproduction and development) | 0.43 µg·L ⁻¹ (1.8 µg·L ⁻¹) | NC | 151A-185, 2024 (OECD 240) |
| Sediment | | | |
| Midge – <i>Chironomus riparius</i> (28-day chronic emergence rate and development rate) | 68 mg·kg ⁻¹ (174 mg·kg ⁻¹) | 224 mg·kg ⁻¹ | 151A-184, 2024 (OECD 218) |
| Oligochaete – <i>Lumbriculus variegatus</i> (28-day chronic growth and reproduction) | 139 mg·kg ⁻¹ (465 mg·kg ⁻¹) | 334 mg·kg ⁻¹ | 151A-204, 2024 (OECD 225) |
| Amphipod – <i>Hyalella azteca</i> (42-day chronic survival, growth, reproduction) | 283 mg·kg ⁻¹ (935 mg·kg ⁻¹) | 705 mg·kg ⁻¹ | 151A-203, 2024 (EPA 850.1770) |

(continued)

Appendix B. Environmental Toxicity Studies with Imlunestrant (concluded)

| Study Type | NOEC (LOEC) ^a | L(E)C ₅₀ ^a | Study, Year (Guideline) |
|--|--|--|-------------------------------|
| Soil | | | |
| Soil Microorganisms (28-day, nitrogen transformation) | 761 ^b mg·kg ⁻¹ (>761 ^b mg·kg ⁻¹) | >761 ^b mg·kg ⁻¹ | 151E-170, 2024 (OECD 216) |
| Terrestrial Plants (germination, fresh shoot height and weight, 14-days after 50% control emergence) | | | |
| Onion (<i>Allium cepa</i>) | 1000 mg·kg ⁻¹ (>1000 ^b mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | 151P-109, 2024 (OECD 208) |
| Ryegrass (<i>Lolium perenne</i>) | 333 mg·kg ⁻¹ (1000 mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | |
| Soybean (<i>Glycine max</i>) | 1000 mg·kg ⁻¹ (>1000 ^b mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | |
| Sunflower (<i>Helianthus annuus</i>) | 111 mg·kg ⁻¹ (333 mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | |
| Radish (<i>Raphanus sativus</i>) | 333 mg·kg ⁻¹ (1000 mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | |
| Tomato (<i>Solanum lycopersicum</i>) | 111 mg·kg ⁻¹ (333 mg·kg ⁻¹) | 476 mg·kg ⁻¹ | |
| Earthworm – <i>Eisenia fetida</i> (14-day acute mortality and weight change) | 750 mg·kg ⁻¹ (>750 ^b mg·kg ⁻¹) | >750 ^b mg·kg ⁻¹ | 151P-113, 2024 (OECD 207) |
| Collembola – <i>Folsomia candida</i> (28-day chronic mortality and reproduction) | 309 mg·kg ⁻¹ (556 mg·kg ⁻¹) | >1000 ^b mg·kg ⁻¹ | S22-07858, 2024 (OECD 232) |

Abbreviations: hr = hour; L(E)C₅₀ = median lethal concentration (LC₅₀) or median effective concentration (EC₅₀);

LOEC = lowest-observed-effect concentration; NC = not calculated due to insufficient biological response;

NOEC = no-observed-effect concentration.

^a Concentration in sediment and soil based on dry weight.

^b Highest concentration tested.

Appendix C: Report Summaries

Study 151K-108

| | | | |
|--------------------------------|---|--|---|
| Report Title: | LY3484356 – Determination of Water Solubility by the Column Elution Method following OECD Guideline 105 | | |
| Guidance Document: | OECD 105 | | |
| Compliance: | Good Laboratory Practices | | |
| Study Number: | 151K-108 | | |
| Report Date: | September 2023 | | |
| Materials & Methods | | | |
| Test Substance | Name: | LY3484356 tosylate (LSN3513322) | |
| | Potency: | 74.8% as LY3484356 | |
| Analytical Method | Instrument: | Agilent Technologies 1200 Series with Applied Biosystems/MDS Sciex API 5000 LC-MS-MS System and QJet Ion Guide | |
| | Analytical column: | THERMO Betasil C18 (50.0 mm x 2.1 mm, 5 µm) | |
| Buffers | pH 5 | 0.08 M acetate | |
| | pH 7 | 0.05 M phosphate | |
| | pH 9 | 0.05 M borate | |
| Experimental Methods | Method: | Column Elution | |
| | Column support: | Chromosorb WHP silica (100 to 120 mesh) | |
| | Temperature control: | Temperature regulated water bath with water jacketed glass column | |
| | Flow rates: | 1 st run: 1.0 mL·min ⁻¹ | |
| | | 2 nd run: 0.5 mL·min ⁻¹ | |
| | Replication: | Seven samples at each flow rate | |
| | Column temperature: | 20 ± 0.1°C | |
| Sample preparation: | Eluate was filtered (0.5 µm) and diluted 1:1 in methanol; subsequent dilution in 1:1 methanol:water | | |
| Results | | | |
| Buffer | Variation | | Water Solubility (mg LY3484356·L ⁻¹) |
| | Within a Flow Rate (Coefficient of Variation) | Between Flow Rates (% Difference) | |
| pH 5 | ≤ 4.6% | 2.4% | 39.0 |
| pH 7 | ≤ 3.2% | 0.019% | 1.58 |
| pH 9 | ≤ 4.3% | 8.4% | 0.587 |

Study 151K-109

| | | | |
|--------------------------------|---|--|--------------|
| Report Title: | LY3484356-Determination of the 1-Octanol/Water Partition Coefficient by the Slow-Stirring Method following OECD Guideline 123 | | |
| Guidance: | OECD 123 | | |
| Compliance: | Good Laboratory Practices | | |
| Study Number: | 151K-109 | | |
| Report Date: | September 2023 | | |
| Materials & Methods | | | |
| Test Substance | Name: | LY3484356 tosylate (LSN3513322) | |
| | Potency: | 74.8% as LY3484356 | |
| Analytical Method | Instrument: | Agilent Technologies 1200 Infinity Series with Applied Biosystems/ MDS Sciex API 5000 LC-MS-MS System and QJet Ion Guide | |
| | Analytical Column: | THERMO Betasil C18 (50.0 mm x 2.1 mm, 5µm) | |
| Buffers | pH 5 Buffer | 0.08 M acetate | |
| | pH 7 Buffer | 0.05 M phosphate | |
| | pH 9 Buffer | 0.05 M borate | |
| Experimental Methods | Method: | Slow-stirring | |
| | Replication: | 3 test vessels per pH | |
| | Temperature: | 25 ± 1°C | |
| | Equilibration Time: | Approximately 24 hours for all pH conditions | |
| | Sampling Intervals: | pH 5: 5 samplings over 3 days pH 7: 5 samplings over 6 days pH 9: 5 samplings over 4 days | |
| Results | | | |
| pH of Aqueous Phase | Mean Mass Balance, Range | Overall Mean K_{OW} ± Standard Deviation | Log K_{OW} |
| 5 | 116 to 119% | 1949 ± 86 | 3.29 |
| 7 | 96.0 to 110% | 134184 ± 13116 | 5.13 |
| 9 | 98.1 to 102% | 352806 ± 21610 | 5.55 |

Compound: Imlunestrant (LY3484356)

Study 151E-163A

| | | | |
|--------------------------------|--|---|-------------------------|
| Report Title: | [¹⁴ C] LY3484356: An Evaluation of Hydrolysis as a Function of pH following OECD Guideline 111 | | |
| Guidance: | OECD 111 | | |
| Compliance: | Good Laboratory Practices | | |
| Study Number: | 151E-163A | | |
| Report Date: | February 2024 | | |
| Materials & Methods | | | |
| Test Substance | <i>Name:</i> | [¹⁴ C] LY3484356 tosylate | |
| | <i>Radiopurity:</i> | 98.8% | |
| | <i>Specific activity:</i> | 55.5 µCi·mg ⁻¹ as LY3484356 tosylate | |
| Buffers (sterile, anoxic) | <i>pH 4.0 Buffer</i> | 10 mM acetate | |
| | <i>pH 7.0 Buffer</i> | 10 mM phosphate | |
| | <i>pH 9.0 Buffer</i> | 10 mM borate | |
| Incubation (in dark) | <i>Test concentration:</i> | 0.13 mg [¹⁴ C]LY3484356·L ⁻¹ | |
| | <i>Test vessels:</i> | 10-mL sterile amber glass vials (capacity: 12.0 mL) | |
| | <i>Temperature:</i> | 50 °C | |
| | <i>Duration:</i> | Five days | |
| Analysis | <i>Sampling:</i> | For each pH, three vessels were sacrificed on days 0 and 5. One of them was used for pH verification and two for LSC and HPLC analyses. | |
| | <i>Analytical methods:</i> | Liquid scintillation counting (LSC) to assess concentration of radioactivity High Performance Liquid Chromatography with UV and beta-radioactivity detectors (HPLC/UV/β-RAM) to assess distribution of radioactivity | |
| Results | | | |
| Buffer | Material Balance | % Loss After 5 Days | Half-Life at 25 °C |
| pH 4 | 98.6 to 102.8% | 1.4% | Estimated to be >1 year |
| pH 7 | 99.7 to 105.6% | 0.5% | Estimated to be >1 year |
| pH 9 | 100.9 to 103.5% | 0.5% | Estimated to be >1 year |

Study 151E-160

| | | | | | | | | |
|--------------------------------------|---|---|-------------|------------|-------------|------------------|-----------|-----------|
| Report Title: | [¹⁴ C] LY3484356 - Adsorption/Desorption Characteristics in Representative Soils and Activated Sludge Solids Following OECD Guideline 106 | | | | | | | |
| Guidance: | OECD 106 | | | | | | | |
| Compliance: | Good Laboratory Practices | | | | | | | |
| Study Number: | 151E-160 | | | | | | | |
| Report Date: | July 2024 | | | | | | | |
| Materials & Methods | | | | | | | | |
| Test Substance | Name: | [¹⁴ C] LY3484356 tosylate | | | | | | |
| | Specific radioactivity: | 73.7 μCi·mg ⁻¹ as LY3484356 | | | | | | |
| | Radiochemical purity: | 98.8% | | | | | | |
| Solids | | Soils | | | | Activated sludge | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 |
| | OC, % | 5.4 | 1.6 | 0.8 | 3.1 | 33.38 | 33.72 | 39.80 |
| | Sand, % | 15.3 | 63.5 | 83.1 | 33.5 | -- | -- | -- |
| | Silt, % | 56.2 | 16.7 | 8.1 | 36.1 | -- | -- | -- |
| | Clay, % | 28.5 | 8.1 | 8.8 | 30.4 | -- | -- | -- |
| | CEC, mmol·100g ⁻¹ pH (0.01M CaCl ₂) | 24.3 7.2 | 16.4 6.2 | 9.8 4.6 | 19.9 5.1 | -- 6.3 | -- 5.7 | -- 5.8 |
| Analytical Methods | Total radioactivity: | Liquid Scintillation Counting (LSC) | | | | | | |
| | Distribution of radioactivity: | HPLC/β-RAM | | | | | | |
| Conditions Tiers 2 and 3 | Test vessels: | Pyrex® glass tubes | | | | | | |
| | Background solution: | Aqueous 0.01 M CaCl ₂ | | | | | | |
| | Temperature: | 18.3 to 20.3 °C | | | | | | |
| | Soil: solution ratio: | 1:100 | | | | | | |
| | Sludge: solution ratio: | 1:100 | | | | | | |
| | Pre-equilibration: | Solids were pre-equilibrated with aqueous 0.01 M CaCl ₂ for 12 to 16 hours prior to test substance application | | | | | | |
| | Separation of solids: | Centrifugation | | | | | | |
| Tier 2 <i>Adsorption Kinetics</i> | Test concentrations: | Soils 1 and 2: ~200 μg·L ⁻¹ Soils 3 and 4: ~2000 μg·L ⁻¹ | | | | | | |
| | | Sludge solids 1 and 3: ~400 μg·L ⁻¹ Sludge solids 2: ~200 μg·L ⁻¹ | | | | | | |
| | Periods of equilibration: | 2, 4, 6, 24, and 48 hours for soils 2, 4, 6, and 24 hours for activated sludge solids | | | | | | |
| <i>Desorption Kinetics</i> | Method set up | Parallel method | | | | | | |
| | Periods of equilibration: | 2, 4, 6, and 24 hours | | | | | | |
| Tier 3 <i>Adsorption Isotherm</i> | Test concentrations: | Soils 1 and 2: 0, 10, 50, 100, 150, 200 μg·L ⁻¹ Soils 3 and 4: 0, 40, 500, 1000, 1500, 2000 μg·L ⁻¹ | | | | | | |
| | | Activated sludge 1: 0, 10, 50, 100, 150, 200 μg·L ⁻¹ Activated sludge 2 and 3: 0, 10, 100, 200, 300, 400 μg·L ⁻¹ | | | | | | |
| | Equilibration period: | 24 hours | | | | | | |
| <i>Desorption Isotherm</i> | Method set up: | Serial method | | | | | | |
| | Equilibration period: | 24 hours | | | | | | |

(continued)

Study 151E-160 (concluded)

| Results | | | | | | |
|--|---|--------------------------------|---------------------------------|--------------------------------|-------------------------------|---------------------------------|
| Tier 2: Kinetics | Distribution coefficients, L·kg ⁻¹ | | | | | |
| | Adsorption | | | Desorption | | |
| Solid Type | K _d ^{ads} | K _{OC} ^{ads} | K _d ^{des} | K _{OC} ^{ads} | | |
| Soil 1 | 1.61×10 ⁴ | 2.98×10 ⁵ | 4.20×10 ⁴ | 7.78×10 ⁵ | | |
| Soil 2 | 2.29×10 ⁴ | 1.43×10 ⁶ | 4.06×10 ⁴ | 2.54×10 ⁶ | | |
| Soil 3 | 5.54×10 ³ | 6.92×10 ⁵ | 2.32×10 ⁴ | 2.90×10 ⁶ | | |
| Soil 4 | 2.15×10 ⁴ | 6.95×10 ⁵ | 9.09×10 ⁴ | 2.93×10 ⁶ | | |
| Sludge 1 | 1.07×10 ⁴ | 3.18×10 ⁴ | 1.62×10 ⁴ | 4.80×10 ⁴ | | |
| Sludge 2 | 1.06×10 ⁴ | 3.18×10 ⁴ | 1.63×10 ⁴ | 4.87×10 ⁴ | | |
| Sludge 3 | 9.99×10 ³ | 2.81×10 ⁴ | 1.35×10 ⁴ | 3.80×10 ⁴ | | |
| Tier 3: Isotherms | Parameters of Freundlich equation | | | | | |
| | Adsorption | | | Desorption | | |
| Solid Type | 1/n | K _F ^{ads} | K _{FOC} ^{ads} | 1/n | K _F ^{des} | K _{FOC} ^{des} |
| Soil 1 | 1.45 | 1.61×10 ⁴ | 2.98×10 ⁵ | 1.30 | 4.10×10 ⁴ | 7.59×10 ⁵ |
| Soil 2 | 1.10 | 2.20×10 ⁴ | 1.38×10 ⁶ | 1.05 | 3.91×10 ⁴ | 2.45×10 ⁶ |
| Soil 3 | 1.38 | 1.90×10 ⁴ | 2.38×10 ⁶ | 1.07 | 2.88×10 ⁴ | 3.60×10 ⁶ |
| Soil 4 | 1.14 | 5.65×10 ⁴ | 1.82×10 ⁶ | 1.01 | 9.33×10 ⁴ | 3.01×10 ⁶ |
| Sludge 1 | 0.824 | 1.06×10 ⁴ | 3.15×10 ⁴ | 1.10 | 1.63×10 ⁴ | 4.84×10 ⁴ |
| Sludge 2 | 0.942 | 1.03×10 ⁴ | 3.08×10 ⁴ | 1.16 | 1.69×10 ⁴ | 5.07×10 ⁴ |
| Sludge 3 | 0.967 | 9.47×10 ³ | 2.66×10 ³ | 0.959 | 1.29×10 ⁴ | 3.63×10 ⁴ |
| --=not determined; CEC=cation exchange capacity; OC=organic carbon content | | | | | | |

Study 151A-202

| | | | |
|--------------------------------|--|--|--|
| Study Title: | LY3484356 – An Aqueous Exposure Bioaccumulation Test with the Bluegill (<i>Lepomis macrochirus</i>) following OECD Guideline 305 | | |
| Guidance: | OECD 305 | | |
| Compliance: | GLP | | |
| Study Number: | 151A-202 | | |
| Report Date: | September 2024 | | |
| Materials & Methods | | | |
| Test Substance | Name: | LY3484356 tosylate | |
| | Potency: | 74.8% as LY3484356 | |
| Radiotracer | Name: | [¹⁴ C]-LY3484356 tosylate | |
| | Radiochemical purity: | 98.45% | |
| | Specific activity: | 30.4 µCi/mg as LY3484356 | |
| Test Organism | Species: | <i>Lepomis macrochirus</i> (bluegill) | |
| | Source: | Osage Catfisheries, Inc (Osage Beach, Missouri) | |
| | Age at initiation: | Juveniles, 18 months (Hatched 02 July 2021) | |
| | Size at initiation: | Total length: 5.99 ± 0.40 cm; 5.3 – 6.5 cm Wet weight: 2.66 ± 0.44 g; 2.04 – 3.32 g | |
| Test Design | Dilution Water: | Test facility well water, UV sterilized and filtered | |
| | Carrier Solvent: | Dimethylformamide (DMF) | |
| | Exposure System | Continuous-flow diluter | |
| | | Temperature-controlled water baths | |
| | | 6 volume additions of solution per test chamber per day | |
| | Biomass loading: | Flow-through: 0.47 g·L ⁻¹ ·day ⁻¹ | |
| | | Instantaneous: 2.99 g·L ⁻¹ | |
| | Nominal Treatment Levels | Low level: 2.7 µg LY3484356·L ⁻¹ | |
| | | High level: 27 µg LY3484356·L ⁻¹ | |
| | | Solvent control (100 µL DMF·L ⁻¹) | |
| | Duration: | Low level: 22-day uptake, 60-day depuration | |
| | | High level: 22-day uptake, 28-day depuration | |
| | Replication: | 1 replicate per treatment and control | |
| | | 90 fish per replicate at initiation | |
| Feeding: | Sera Vipran; 2% of body weight once daily | | |
| Test chambers – uptake: | 127-L Teflon®-lined stainless steel aquaria filled with 80 L of test solution | | |
| Test chambers – depuration: | 54-L Teflon®-lined stainless steel aquaria filled with 45 L of test solution | | |
| Lipid Analyses: | Whole Fish Tissue: extraction and weighing | | |
| Analytical Measurements | Matrices and methodology: | Stock solutions: LSC, LC/MS/MS | |
| | | Water: LSC, LC/MS/MS | |
| | | Whole Fish Tissue: Combustion and LSC; extraction and LC/MS/MS | |

(continued)

Study 151A-202 (concluded)

| Results | | |
|--|---|---|
| Test Conditions | Temperature: | 21.3 – 22.1°C |
| | pH: | 8.0 – 8.6 |
| | Dissolved oxygen: | ≥5.7 mg·L ⁻¹ (≥66% of air saturation) |
| | Conductivity: | 340 – 390 µS/cm |
| | Total Hardness as CaCO ₃ : | 144 – 160 mg·L ⁻¹ |
| | Total Alkalinity as CaCO ₃ : | 176 – 198 mg·L ⁻¹ |
| | Total Organic Carbon: | 41.25 – 51.90 mg C·L ⁻¹ (uptake) |
| | Photoperiod: | 16 hours light (562 to 605 lux) and 8 hours dark with a 30-minute transition period |
| Bioconcentration Parameters (Whole Fish) Based on Total Radioactivity ¹ | | |
| C _w (concentration in water; µg LY3484356 equivalents ⁻¹) | 1.7 | 19 |
| k _g (growth rate constant; day ⁻¹): | 0.01045 | 0.01039 |
| k ₁ (overall uptake rate constant; L·kg ⁻¹ ·day ⁻¹): | 12.5 | 25.7 |
| k ₂ (overall depuration rate constant; day ⁻¹): | 0.0414 | 0.1266 |
| k _{2g} (growth-corrected depuration rate constant; day ⁻¹): | 0.0310 | 0.1162 |
| C _f (concentration in fish at steady-state; µg LY3484356·kg ⁻¹): | 319 | 4,584 |
| L _n (lipid normalization factor to 5% lipid by weight): | 1.236 | 1.236 |
| BCF _{SS} (steady-state BCF; L·kg ⁻¹): | 188 | 241 |
| BCF _{SSL} (lipid-normalized steady-state BCF; L·kg ⁻¹): | 232 | 298 |
| BCF _K (kinetic BCF; L·kg ⁻¹): | 302 | 203 |
| BCF _{Kg} (growth-corrected kinetic BCF; L·kg ⁻¹): | 404 | 222 |
| t _{1/2g} (growth-corrected half-life; day): | 22.4 | 6.0 |
| BCF _{KL} (lipid-normalized kinetic BCF; L·kg ⁻¹): | 373 | 251 |
| BCF _{KgL} (growth-corrected lipid-normalized kinetic BCF; L·kg ⁻¹): | 500 | 274 |
| Bioconcentration Parameters (Whole Fish Tissue) Based on Parent LY3484356 | | |
| Treatment Level (µg·L ⁻¹): | 1.4 | 14 |
| C _f (chemical concentration in fish at steady-state; µg·kg ⁻¹): | 74.6 | 1328 |
| C _w (chemical concentration in water; µg·L ⁻¹): | 1.4 | 14 |
| BCF _{SS} (steady-state BCF; L·kg ⁻¹): | 53 | 95 |
| BCF _{SSL} (lipid-normalized steady-state BCF; L·kg ⁻¹): | 65 | 117 |

Study 151E-162

| | | |
|---|--|---|
| Report Title: | Biodegradation of ¹⁴ C-LY3484356 in Activated Sludge Following OECD Guidelines 314B | |
| Guidance: | OECD 314B | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151E-162 | |
| Report Date: | September 2024 | |
| STUDY SUMMARY | | |
| Materials & Methods | | |
| Test Substance | Name: | [¹⁴ C]-LY3484356 tosylate |
| | Radiopurity: | 98.8% |
| | Specific activity: | 73.72 μCi·mg ⁻¹ as LY3484356 |
| Test System | Test system: | Activated sludge re-suspended in supplemented natural sewage (~ 2,500 mg solids·L ⁻¹) |
| | Source of activated sludge: | Wastewater Treatment Facility (primarily residential) |
| | Controls: | Abiotic, toxicity and viability control |
| | Test vessels: | 1 gallon amber bottles |
| | Vessel volume(s): | 2 L for biotic vessels 1 L for abiotic, toxicity, and viability controls |
| Incubation Conditions | Incubation system: | Vessels containing activated sludge were actively aerated and connected to a series of volatile traps |
| | Test duration: | 28 days |
| | Dosed concentration: | 90.4 μg ¹⁴ C-LY3484356·L ⁻¹ |
| | Temperature: | 19.5 to 21.3 °C |
| | Sampling time points: | Days 0 (5 min & 3 or 4 hr), 1, 2, 5, 7, 9, 12, 15, 21, 28 |
| | Replication: | Two biotic vessels One vessel for abiotic, toxicity and viability control |
| Analysis | Total radioactivity: | Liquid scintillation counting Matrices: Sludge suspensions, concentrated sludge extracts, post-extraction sludge solids (after combustion), volatile traps |
| | Distribution of radioactivity: | HPLC-UV/β-RAM Matrix: Concentrated sludge extracts |
| | | |
| Results | | |
| Mean Distribution of Radioactivity after 28 days | Total Recovery: | 90.6% AR |
| | Extractable Residues: | 41.3% AR |
| | Unextractable Residues: | 46.6% AR |
| | Cumulative ¹⁴ CO ₂ : | 0.2% AR |
| | [¹⁴ C] LY3484356: | 27.6% AR |
| | Total Degradation Products: | 13.7% AR |
| | No. of Major Degradation Products (≥10% AR): | 1 |
| KINETIC Endpoints | Rate constant: | 0.5929 day ⁻¹ |
| | DT ₅₀ : | 2.26 days |
| %AR = % of applied radioactivity; DT ₅₀ : time to dissipation of 50% of AR | | |

Compound: Imlunestrant (LY3484356)

Study 151E-161

| | | | | |
|--------------------------------|--|--|-------------------|------------------|
| Report Title: | Aerobic Transformation of ¹⁴ C LY3484356 in Aquatic Sediment Systems following OECD Guideline 308 | | | |
| Guidance: | OECD 308 | | | |
| Compliance: | GLP | | | |
| Study Number: | 151E-161 | | | |
| Report Date: | September 2024 | | | |
| Materials & Methods | | | | |
| Test Substance | Name: | ¹⁴ C -LY3484356 tosylate | | |
| | Radiopurity: | 98.8% | | |
| | Specific Activity: | 73.7 μCi·mg ⁻¹ as LY3484356 | | |
| Test Systems | | HOC | LOC | |
| | Sediment | Sand/Clay/Silt (%): | 15.79/31.73/52.48 | 74.08/6.80/19.12 |
| | | Organic Carbon (%): | 4.38 | 0.50 |
| | | Moisture Content (%): | 118.29 | 30.41 |
| | | pH: | 6.63 | 7.02 |
| | Water | Microbial Biomass (ug/g): | 398.7 | 125.9 |
| | | pH: | 7.12 | 7.05 |
| Dissolved Oxygen (mg/L): | | 9.34 | 8.54 | |
| Incubation Conditions | Test Duration: | 102 days | | |
| | Water:Sediment Ratio: | 3.5 to 1 (v:v) | | |
| | Temperature/Light: | 20 ± 2 °C in dark | | |
| | Aeration: | Aerobic, flow-through air | | |
| | Concentration in Water: | 613 μg ¹⁴ C -LY3484356·L ⁻¹ | | |
| | Replication: | 2 vessels per sampling interval | | |
| Analysis | Sampling Intervals: | 0, 7, 14, 28, 56, 102 days (water+sediment) 8 hours and Day 1 (water) | | |
| | Processing: | <u>Water</u> : Decant overlying water layer Preserve with addition of acetonitrile <u>Sediment</u> : Extract sediment layer three times using tetrahydrofuran + 0.5% NH ₄ OH | | |
| | Methods: | <u>LSC</u> - Total ¹⁴ C in water layer, sediment extract, combusted post-extracted sediment solids, and volatile trapping solutions <u>HPLC/β-RAM</u> - ¹⁴ C profile of test substance and transformation products in water and sediment extracts | | |
| Controls | Viability Control: | Degradation of ¹⁴ C -glucose in absence of test substance | | |
| | Toxicity Control: | Degradation of ¹⁴ C -glucose in presence of test substance | | |

(continued)

Study 151E-161 (concluded)

| Results | | | | |
|--|------------------|------|------------------|------|
| Distribution of ¹⁴ C (% of applied ¹⁴ C) | HOC ^a | | LOC ^a | |
| Total Test Systems | | | | |
| Mean material balance at day 102 | 94.3% | | 76.0% | |
| ¹⁴ C-LY3484356 at day 0 ^b | 99.9% | | 100.5% | |
| ¹⁴ C -LY3484356 at day 102 ^b | 62.6% | | 59.0% | |
| ¹⁴ CO ₂ (cumulative) at day 102 | 0.26% | | 0.61% | |
| Volatile ¹⁴ C compounds (cumulative) at day 102 | 0.06% | | 0.00% | |
| Total transformation products at day 102 | 0.0% | | 0.6% | |
| Major transformation products (>10% AR) | none | | none | |
| Water Layers | | | | |
| Total ¹⁴ C residues at day 102 | 0.2% | | 0.8% | |
| Sediment Layers | | | | |
| Extractable ¹⁴ C residues at day 102 | 62.6% | | 59.6% | |
| ¹⁴ C -LY3484356 in extracts at day 102 ^b | 62.6% | | 59.0% | |
| Non-extractable ¹⁴ C residues at day 102 ^c | 31.0% | | 14.7% | |
| Kinetic Analyses ^d | | | | |
| Disappearance at 20°C | HOC | | LOC | |
| | Model | Days | Model | Days |
| DT ₅₀ of LY3484356 in water layer | SFO | 2.25 | FOMC | 1.64 |
| DT ₅₀ of LY3484356 in sediment extract | SFO | 382 | FOMC | 704 |
| DT ₅₀ of LY3484356 in total test system | FOMC | 1310 | FOMC | 179 |
| %AR = % of applied ¹⁴ C; SFO = simple first-order kinetics; FOMC = first-order multi-compartment; HOC = high organic carbon sediment system; LOC = low organic carbon sediment system | | | | |
| ^a Viability and toxicity controls produced >30% of applied ¹⁴ C as ¹⁴ CO ₂ within 14 days; | | | | |
| ^b Determined by HPLC/β-RAM | | | | |
| ^c Irreversible binding was considered a disappearance pathway | | | | |
| ^d Derived using FOCUS (FORum for the Co-ordination of pesticide fate models and their USE) guidance | | | | |

Compound: Imlunestrant (LY3484356)

Study 151E-169

| | | | | | |
|--------------------------------|---|--|-----------------|----------------|----------------|
| Report Title: | Aerobic Transformation of [¹⁴ C]-LY3484356 in Soil following OECD Guideline 307 | | | | |
| Guidance: | OECD 307 | | | | |
| Compliance: | Good Laboratory Practices | | | | |
| Study Number: | 151E-169 | | | | |
| Report Date: | September 2024 | | | | |
| Materials & Methods | | | | | |
| Test Substance | Name: | [¹⁴ C]-LY3484356 tosylate | | | |
| | Radiopurity: | 98.45% | | | |
| | Specific Activity: | 30.4 µCi/mg as LY3484356 | | | |
| Test Soils | Soil Identification: | Soil 1 | Soil 2 | Soil 3 | Soil 4 |
| | Sand %/Silt %/Clay %: | 81.2/6.4/12.4 | 62.3/15.9/21.8 | 32.4/34.5/33.1 | 30.5/49.5/20.0 |
| | USDA Textural Class: | Sandy loam | Sandy clay loam | Clay loam | Loam |
| | Organic Carbon (OC): | 1.0% | 2.4% | 3.6% | 4.3% |
| | Microbial Biomass (µg·g ⁻¹): | 131.5 | 655.7 | 125.6 | 995.0 |
| | pH (soil:water 1:1): | 4.7 | 6.6 | 4.8 | 7.8 |
| | WHC: | 46.0% | 40.7% | 48.1% | 55.5% |
| | Moisture Content: | 10.59% | 21.98% | 19.94% | 25.54% |
| Incubation Conditions | Duration: | 120 days | | | |
| | Temperature/Light: | 20 ± 2 °C in dark | | | |
| | Aeration: | Aerobic, flow-through humidified air with volatile trapping train post-test vessel | | | |
| | Concentration: | 4.08 µg [¹⁴ C]-LY3484356·g ⁻¹ dry soil | | | |
| | Replication: | 2 vessels per soil per sampling interval | | | |
| Analyses | Sampling Intervals: | 0, 7, 14, 28, 62, 120 days | | | |
| | Processing: | Extract soil layer three times using THF + 0.1% NH ₄ OH | | | |
| | Methods: | <u>Total radioactivity</u> of soil extracts, combusted post-extracted soil solids, and volatile trapping solutions by LSC <u>Radioactivity profile</u> of parent and transformation products in soil extracts by HPLC/β-RAM | | | |
| Controls | Viability Control: | Mineralization of ¹⁴ C-glucose in absence of test substance | | | |
| | Toxicity Control: | Mineralization of ¹⁴ C-glucose in presence of test substance | | | |

(continued)

Study 151E-169 (concluded)

| Results | | | | |
|--|---|---------|----------|---------|
| Soil Identification | Soil 1 | Soil 2 | Soil 3 | Soil 4 |
| | Range, all intervals ($\mu\text{g}\cdot\text{g}^{-1}$) | | | |
| Microbial Biomass | 44.4-153 | 149-645 | 92.2-184 | 475-733 |
| Day 0 Mean Results | Distribution of Radioactivity (% of applied ^{14}C) | | | |
| ^{14}C in Soil Extracts | 99.6% | 93.4% | 96.5% | 94.4% |
| ^{14}C Unextractable Residues | 3.1% | 6.1% | 4.9% | 5.2% |
| ^{14}C Collected in Gas Traps | NA | NA | NA | NA |
| ^{14}C Material Balance | 102.6% | 99.5% | 101.4% | 99.6% |
| ^{14}C -LY3484356 in Extracts | 99.6% | 93.4% | 96.6% | 94.4% |
| Transformation Products in Extracts | 0.0% | 0.0% | 0.0% | 0.0% |
| Day 120 Mean Results | Distribution of Radioactivity (% of applied ^{14}C) | | | |
| ^{14}C in Soil Extracts | 61.8% | 59.8% | 70.6% | 67.6% |
| ^{14}C Unextractable Residues | 45.6% | 46.6% | 34.7% | 32.7% |
| ^{14}C Collected in Gas Traps | 0.53% | 0.22% | 0.14% | 0.38% |
| ^{14}C Material Balance | 108.0% | 106.6% | 105.4% | 100.6% |
| ^{14}C -LY3484356 in Extracts | 61.8% | 59.8% | 70.6% | 67.6% |
| Transformation Products in Soil Extracts | 0.0% | 0.0% | 0.0% | 0.0% |
| Kinetic Analyses | Disappearance Rates (Days at 20 °C) | | | |
| DT ₅₀ of LY3484356 | 202 | 200 | 310 | 272 |
| DT ₉₀ of LY3484356 | 670 | 665 | 1030 | 902 |
| <p>Note: All results based on simple first-order (SFO) kinetics</p> <p>NA: Not applicable because not measured on Day 0</p> <p>DT₅₀ and DT₉₀ are the time to disappearance of 50% and 90% of LY3484356 from soil, irreversible binding was considered a disappearance pathway; WHC = maximum water holding capacity</p> <p>Kinetic analyses followed FOCUS (FORum for the Co-ordination of pesticide fate models and their USE) guidance</p> | | | | |

Compound: Imlunestrant (LY3484356)

Study 151E-159

| | | |
|--|--|--|
| Report Title: | LY3484356: Activated Sludge Respiration Inhibition Test Following OECD Guideline 209 | |
| Guidance Document: | OECD 209 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151E-159 | |
| Reporting Date: | February 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Reference Substance | Name: | 3,5-dichlorophenol |
| | Purity: | 99.6% |
| Test Conditions | Nominal Test Concentrations: | 10, 100, and 1000 mg LY3484356·L ⁻¹ |
| | Addition of Test Substance: | Weighed directly into test vessel |
| | Activated Sludge Source: | Wastewater Treatment Facility (primarily residential) |
| | Sludge Solids Concentration in Test Vessels: | 1574 mg total suspended solids·L ⁻¹ |
| | Test Vessels: | 1000-mL glass Erlenmeyer flasks |
| | Agitation: | Aerated with ambient laboratory air and mixed with stir bar/stir plate |
| | Temperature: | 19.7 to 21.7°C |
| | Incubation: | 3 hours |
| Measurements | Dissolved oxygen: | YSI Model 5000 dissolved oxygen meter |
| Results | | |
| Nominal Concentration (mg LY3484356·L ⁻¹) | Total Respiration Rate (mg O ₂ ·L ⁻¹ ·h ⁻¹) | Percent Inhibition (%) |
| Negative Control | 33.2 ^a | Not applicable |
| 10 | 35.7 | -7.40 |
| 100 | 35.6 | -7.10 |
| 1000 | 34.2 ^b | -2.93 |
| 1000 (Abiotic Control) | -3.5 | Not applicable |
| ^a average of 2 replicates; respiration rates in controls: 20.1 and 22.1 mg O ₂ ·g sludge (dry weight) ⁻¹ ·h ⁻¹ ; coefficient of variation for respiration rate of negative control: 6.6% ^b average of 3 replicates EC ₅₀ for total respiration inhibition by 3,5-dichlorophenol: 12.5 mg·L ⁻¹ | | |
| Conclusions | | |
| NOEC | 1000 mg LY3484356·L ⁻¹ | |
| EC ₅₀ | >1000 mg LY3484356·L ⁻¹ | |

Compound: Imlunestrant (LY3484356)

Study 151A-179A

| | | | |
|--|---|--|---------|
| Study Title: | LY3484356 – A 48-Hour Static-Renewal Acute Toxicity with the Cladoceran (<i>Daphnia magna</i>) following OECD Guideline 202 | | |
| Guidance Document: | OECD 202 | | |
| Compliance: | Good Laboratory Practices | | |
| Study Number: | 151A-179A | | |
| Report Date: | April 2024 | | |
| Materials & Methods | | | |
| Test Substance | Name: | LY3484356 tosylate | |
| | Potency | 74.8% as LY3484356 | |
| Test Organism | Species: | <i>Daphnia magna</i> | |
| | Source: | Test Facility cultures | |
| | Age at initiation: | < 24 hours | |
| Exposure Design | Dilution water: | UV Sterilized filtered well water (freshwater) | |
| | Duration: | 48 hours | |
| | Test Groups: | 5 treatment levels with a negative control (dilution water), solvent control (0.1 mL DMF·L ⁻¹) | |
| | Nominal test concentrations: | 0.031, 0.063, 0.13, 0.25, 0.50 mg LY3484356·L ⁻¹ | |
| | Replication: | 5 daphnids per replicate | |
| | | 4 replicates per test group | |
| | Test chambers: | 250-mL glass beakers loosely covered with petri dish | |
| | Test solution volume: | ~220 mL | |
| Test solution renewal: | At 24-Hour | | |
| Environmental Conditions | Photoperiod: | 16 hours light (569 lux), 8 hours dark | |
| | Dissolved oxygen: | 7.9 to 8.8 mg/L (87 to 98% of air saturation at 20°C) | |
| | pH: | 8.0 to 8.5 | |
| | Temperature: | 19.8 to 20.4°C | |
| Analytical Measurements | Method: | LC/MS/MS | |
| | Sampling frequency: | Measured at 0-, 24- and 48-Hour | |
| Biological Data | Observation frequency: | ~4.5-Hour after test initiation, 24- and 48-Hour | |
| | Observations: | Immobility, clinical signs of toxicity | |
| Results | | | |
| Mean Measured Concentration (mg LY3484356·L ⁻¹) | Cumulative Percent Immobile | | |
| | ~4.5-Hour | 24-Hour | 48-Hour |
| Negative Control | 0 | 0 | 0 |
| Solvent Control | 0 | 0 | 0 |
| 0.016 | 0 | 0 | 0 |
| 0.035 | 0 | 0 | 0 |
| 0.066 | 0 | 0 | 0 |
| 0.14 | 0 | 0 | 0 |
| 0.29 | 0 | 0 | 100 |
| At 48 hours, all surviving daphnids appeared normal and healthy in the control and all treatment groups. | | | |
| Conclusions | | | |
| 48-Hour EC ₅₀ (95% confidence interval): | 0.20 (0.14 to 0.29) mg LY3484356·L ⁻¹ | | |
| No immobility concentration: | 0.14 mg LY3484356·L ⁻¹ | | |
| NOEC: | 0.14 mg LY3484356·L ⁻¹ | | |

Compound: Imlunestrant (LY3484356)

Study 151P-108

| | | |
|--------------------------------|---|--|
| Report Title: | LY3484356 – A 72-Hour Toxicity Test with the Freshwater Alga (<i>Raphidocelis subcapitata</i>) Following OECD Guideline 201 | |
| Guidance Document: | OECD 201 | |
| Compliance: | GLP | |
| Study Number: | 151P-108 | |
| Report Date: | September 2024 | |
| <i>Materials & Methods</i> | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 free base |
| Test Organism | Name: | Freshwater Green Alga (<i>Raphidocelis subcapitata</i>) |
| | Source: | Test facility cultures |
| Exposure Design | Dilution water: | Algal Assay Procedure (AAP) medium |
| | Duration: | 72 hours |
| | Carrier Solvent: | Dimethylformamide (DMF) |
| | Nominal test concentrations: | 4.1, 10, 26, 64, 160, 400, and 1000 µg LY3484356·L ⁻¹ |
| | Controls: | Solvent control (100 µL DMF·L ⁻¹) |
| | | Abiotic control (no algae, 25 µg LY3484356·L ⁻¹) |
| | | Negative control (dilution water) |
| | Replication: | 6 replicates for negative and solvent control |
| | | 3 replicates for each LY3484356 treatment |
| | | 2 replicates for abiotic control |
| Initial cell density: | 10,000 cells per mL | |
| Test vessels: | 250-mL glass Erlenmeyer flasks with sterile, foam stoppers | |
| Test solution volume: | 100 mL (sterile) | |
| Environmental Conditions | Agitation device: | Mechanical shakers set a ~ 100 rpm |
| | Light intensity: | 5,407 to 6,025 lux |
| | pH: | 7.3 to 8.6 |
| | Temperature: | 25.0 to 25.5°C |
| Analytical Measurements | Analytical method: | HPLC/MS/MS |
| | Sampling intervals: | 0, 24, 48, and 72 hours |
| Biological Data | Observations: | Cell counts (hemacytometer), cell appearance |
| | Observation frequency: | 0 (inoculum culture only), 24, 48, and 72 hours |

(continued)

Study 151P-108 (concluded)

| Results | | | |
|--|--|--|--|
| Geometric Mean Measured Concentration ($\mu\text{g LY3484356}\cdot\text{L}^{-1}$) | Mean Yield ($\text{cells}\cdot\text{mL}^{-1}$) | Mean Growth Rate (hours^{-1}) | |
| Negative control | 2,573,333 | 0.0770 | |
| Solvent control | 3,133,333 | 0.0798 | |
| 1.3 | 2,660,000 | 0.0775*# | |
| 2.5 | 2,800,000 | 0.0781*# | |
| 7.4 | 2,346,667* | 0.0755* | |
| 24 | 90,667* | 0.0320* | |
| 59 | 191,333* | 0.0378* | |
| 144 | 580,333* | 0.0562* | |
| 367 | 459,000* | 0.0533* | |
| * = Significant statistical difference compared to solvent control ($p < 0.05$) | | | |
| # = not considered biologically meaningful considering the allowable ($\text{CV} < 7\%$) and actual ($\text{CV} = 1.5$ to 2.3%) variability of control replicates | | | |
| Conclusions | | | |
| Endpoint | NOEC ($\mu\text{g LY3484356}\cdot\text{L}^{-1}$) | LOEC ($\mu\text{g LY3484356}\cdot\text{L}^{-1}$) | EC ₅₀ ($\mu\text{g LY3484356}\cdot\text{L}^{-1}$) |
| Growth Rate | 2.5 | 7.4 | 33 |
| Yield | 2.5 | 7.4 | 12 |

Study 151A-181

| | | |
|--------------------------------|--|--|
| Study Title: | LY3484356 – A Semi-Static Life-Cycle Toxicity Test with the Cladoceran (<i>Daphnia magna</i>) following OECD Guideline 211 | |
| Guidance: | OECD 211 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151A-181 | |
| Report Date: | October 2024 (Amended) | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Test Organism | Species: | <i>Daphnia magna</i> |
| | Source: | Testing facility cultures |
| | Age at initiation: | < 24 hours |
| Exposure Design | Dilution water: | UV Sterilized filtered well water (freshwater) |
| | Solvent: | Dimethylformamide (0.1 mL DMF·L ⁻¹) |
| | Route of administration: | Water |
| | Test chambers: | 250 mL glass beakers loosely covered with a plastic petri dish |
| | Test solution volume: | Approximately 200 mL |
| | Test solution renewal: | Daily |
| | Photoperiod: | 16 hours light (736 lux): 8 hours darkness (30-min transition) |
| | Duration: | 21 days |
| | Controls: | Negative (dilution water) control Solvent (DMF) control |
| | Target treatment levels: | 1.5, 4.8, 15, 49, 156 and 500 µg LY3484356·L ⁻¹ |
| | Replication: | 10 replicates for the negative control |
| | | 20 replicates for the solvent control group |
| | | 10 replicates for each LY3484356 treatment group |
| 1 daphnid per replicate | | |
| Feeding: | YCT, vitamins, algae once daily | |
| Analytical Measurements | Method: | LC/MS/MS |
| | Lower limit of quantitation: | 0.100 µg LY3484356·L ⁻¹ |
| | Frequency: | New solutions on Day 0, 7, 14 and 20 Old solutions on Day 1, 8, 15 and 21 |
| Environmental Conditions | pH: | 8.0 – 8.7 |
| | Dissolved oxygen: | ≥ 7.9 mg·L ⁻¹ (≥ 87% air saturation at 20 °C) |
| | Temperature: | 19.0 – 20.3°C |
| | Hardness: | 124 – 152 mg·L ⁻¹ as CaCO ₃ |
| | Alkalinity: | 160 – 182 mg·L ⁻¹ as CaCO ₃ |
| Specific Conductivity: | 309 – 368 µS·cm ⁻¹ | |

(continued)

Study 151A-181 (concluded)

| Results | | | | | |
|---|-----------------------------------|--|--|--------------------|---------------|
| Mean Measured Concentration (µg LY3484356·L ⁻¹) | Mean Percent Survival | No. Live Neonates Per Surviving Parent (Mean ± SD) | No. Live Neonates Per Parent At Start (Mean ± SD) ¹ | Growth (Mean ± SD) | |
| | | | | Dry Weight (mg) | Length (mm) |
| Negative Control | 80 | 231 ± 25.2 | 231 ± 25.2 | 1.17 ± 0.118 | 4.64 ± 0.325 |
| Solvent Control | 95 | 219 ± 13.2 | 219 ± 13.2 | 1.22 ± 0.0900 | 4.73 ± 0.177 |
| 1.06 | 80 | 217 ± 5.98 | 217 ± 5.98 | 1.12 ± 0.119 | 4.70 ± 0.142 |
| 3.32 | 80 | 213 ± 28.2 | 213 ± 28.2 | 1.22 ± 0.0771 | 4.88 ± 0.172 |
| 10.5 | 100 | 207 ± 13.9* | 207 ± 13.9* | 1.20 ± 0.121 | 4.71 ± 0.133 |
| 39.9 | 90 | 202 ± 11.4* | 202 ± 11.4* | 1.14 ± 0.156 | 4.71 ± 0.123 |
| 125 | 70 | 116 ± 17.1* | 116 ± 17.1* | 0.953 ± 0.106* | 4.35 ± 0.113* |
| 413 | 80 | 85.9 ± 22.2* | 85.9 ± 22.2* | 0.595 ± 0.114* | 3.81 ± 0.187* |
| <p>* Statistically significant reduction compared to solvent control (Jonckheere Terpstra Step-Down trend test, p ≤ 0.05)</p> <p>¹ Since there were no effects on survival in any treatment group compared to the solvent control and mortality did not follow a concentration-response pattern, replicates with parental mortality were excluded from the calculations of offspring per parental daphnid.</p> | | | | | |
| Conclusion | | | | | |
| Most Sensitive Endpoint | NOEC | | LOEC | | |
| Reproduction | 3.32 µg LY3484356·L ⁻¹ | | 10.5 µg LY3484356·L ⁻¹ | | |

Study 151A-185

| | | |
|---|--|--|
| Study Title: | LY3484356 –A Multigeneration, Reproduction and Development Toxicity Test with the Fathead Minnow (<i>Pimephales promelas</i>) Following OECD Guideline 240, with Modifications | |
| Guidance: | OECD 240 | |
| Compliance: | FDA Good Laboratory Practices | |
| Study Number: | 151A-185 | |
| Report Date: | October 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Test Organism | Species: | <i>Pimephales promelas</i> (fathead minnow) |
| | Age at pre-exposure initiation: | approximately 5 months (i.e., reproductively active adults) |
| Pre-Exposure Design | Spawning groups: | 40 groups, 2 males, 4 females per group |
| | Spawning groups selected: | 24 groups Fecundity: 25.5 to 48.0 eggs per female per day |
| Exposure Design | Exposure phases and duration: | F0 Reproduction: 32 days |
| | | F1 Embryo to Sexual Maturity: 160 days |
| | | F1 Reproduction: 35 days |
| | | F2 Embryo to Hatch: ~5 days |
| | Nominal test concentrations: | 0.039, 0.16, 0.63, 2.5 and 10 µg LY3484356·L ⁻¹ with a negative control (dilution water only) |
| | Dilution water: | Laboratory well water (UV sterilized and 0.45-µm filtered) |
| | Stock solution: | 2500 µg LY3484356·L ⁻¹ in pH 4 RO water |
| | Exposure system: | Separate exposure systems for each phase |
| | | Continuous-flow diluter using pH-adjusted stock |
| | | Temperature-controlled room |
| | Test chambers: | Photoperiod: 16 hours light: 8 hours darkness with a 30-minute transition period |
| | | F0, F1 reproduction: 19-L glass aquaria, 10-L water with 3 spawning substrates |
| | | F1, F2 embryo: 9-L glass aquaria, 7-L water with 2 egg incubation cups |
| | | F1 days 28 to 81: 19-L glass aquaria, 17-L water F1 days 82 to 160: 33-L glass aquaria, 30-L water |
| | Replication: | 4 replicates for each test concentration |
| F0, F1 reproduction: 2 males, 4 females per replicate | | |
| F1, F2 embryo: 2 egg incubation cup per replicate, 25 embryos per cup F1 post-hatch: 25 fish per replicate | | |
| Turnover rate: | At least 6 volume additions per day | |
| Biomass loading: | Instantaneous: 1.6 g fish·L ⁻¹ | |
| | Flowing solution: 0.11 g fish·L ⁻¹ ·day ⁻¹ | |
| Feeding: | Frequency: typically 2 to 3 times daily | |
| | F0, F1 reproduction: Sera Vipan, brine shrimp nauplii | |
| | F1 0 to 28 dph: Sera Micron, brine shrimp nauplii | |
| | F1 29 to 143 dph: Sera Flora, brine shrimp nauplii F1 ≥ 143 dph: Sera Vipan, brine shrimp nauplii | |

(continued)

Study 151A-185 (continued)

| | | | | | | |
|--|----------------------|--|----------------------|----------------------|----------------------|------------------------|
| Analytical Measurements | Sampling frequency: | Test solutions from 1 replicate sampled weekly, replicates alternated each week. | | | | |
| | Method: | LC/MS/MS | | | | |
| | LLOQ: | 0.0150 µg LY3484356·L ⁻¹ | | | | |
| Results | | | | | | |
| Exposure Conditions | | | | | | |
| Exposure period: | F0 Generation | F1 Generation | F2 Generation | | | |
| pH: | 7.9 – 8.3 | 7.7 – 8.3 | 8.0 – 8.2 | | | |
| Dissolved oxygen (mg·L ⁻¹): | 6.2– 8.2 | 5.6 – 8.2 | 8.1 – 8.2 | | | |
| Temperature (°C): | 24.1 – 25.5 | 23.2 – 25.8 | 24.0 – 25.6 | | | |
| Conductivity (µS·cm ⁻¹): | 349 – 406 | 338 – 400 | 364 – 385 | | | |
| Hardness (mg·L ⁻¹ as CaCO ₃): | 148 – 160 | 140 – 168 | 148 – 168 | | | |
| Alkalinity (mg·L ⁻¹ as CaCO ₃): | 178 – 188 | 176 – 196 | 182 – 188 | | | |
| Illumination (lux): | 540 – 909 | 590 – 967 | 667 – 994 | | | |
| Biological Measurements | | | | | | |
| Nominal Test Concentration (µg LY3484356·L ⁻¹) | Control | 0.039 | 0.16 | 0.63 | 2.5 | 10 |
| F0 Generation – Reproduction | | | | | | |
| Mean Measured Conc. (µg LY3484356·L ⁻¹) | < LLOQ | 0.015 | 0.092 | 0.44 | 1.7 | 8.1 |
| Survival (%) | 95.8 | 100 | 95.8 | 95.8 | 100 | 100 |
| Cumulative Eggs Produced | 2654 | 2882 | 2825 | 3273 | 2944 | 2128 |
| Eggs per Female per Day | 21.0 | 22.5 | 22.3 | 25.6 | 23.0 | 16.6 |
| Fertility (%) | 97.8 | 97.1 | 97.5 | 97.2 | 97.1 | 98.0 |
| Time to First Spawn | 1.3 | 1.3 | 3.3 | 1.5 | 1.5 | 4.5 |
| Total Length, Females (mm) | 48.8 | 49.6 | 48.7 | 49.4 | 48.9 | 51.0 |
| Total Length, Males (mm) | 61.5 | 59.6 | 60.4 | 60.3 | 59.5 | 61.4 |
| Wet Weight, Females (g) | 1.33 | 1.40 | 1.42 | 1.38 | 1.38 | 1.54* |
| Wet Weight, Males (g) | 3.40 | 3.19 | 3.05 | 3.10 | 3.12 | 3.65 |
| Tubercle Scores, Females | 0 | 0 | 0 | 0 | 0 | 0 |
| Tubercle Scores, Males | 32 | 32 | 29 | 32 | 34 | 34 |
| F1 Generation – Embryo to Sexual Maturity ^b | | | | | | |
| Mean Measured Conc. ^a (µg LY3484356·L ⁻¹) | < LLOQ | 0.019 | 0.11 | 0.43 | 1.8 | 7.1 |
| Hatching Success (%) | 100 | 92.5† | 99.0 | 99.0 | 99.0 | 86.5* |
| Time to Hatch (days) | 4.0 | 4.5 | 4.8 | 4.6 | 4.4 | 4.5 |
| Post-Hatch Survival (%) | 93.0 | 96.0 | 98.0 | 96.0 | 92.0 | 90.9 |
| Sex Ratio (% Males) | 17.6 | 51.0‡ | 50.9‡ | 53.2‡ | 65.0‡ | 54.5‡ |
| Total Length, Females ^c (mm) | 50.0 | 50.5 | 50.4 | 51.8 | 48.5 | 56.7* |
| Total Length, Males ^c (mm) | 60.4 | 60.7 | 64.3 | 65.3 | 64.3 | 62.5 |
| Wet Weight, Females ^c (g) | 1.32 | 1.36 | 1.37 | 1.36 | 1.24 | 2.01* |
| Wet Weight, Males ^c (g) | 2.53 | 2.67 | 2.97 | 3.01 | 3.11§ | 2.85 |
| Tubercle Scores, Females ^c | 0 | 0 | 0 | 0 | 0 | 0 |
| Tubercle Scores, Males ^c | 30 | 24 | 24 | 23§ | 24 | 26 |
| Vitellogenin, Females ^c (ng/mL) | 3.61x10 ⁷ | 3.11x10 ⁷ | 1.94x10 ⁷ | 2.79x10 ⁷ | 1.31x10 ⁷ | 8.55x10 ⁶ |
| Vitellogenin, Males ^{c#} (ng/mL) | 2.05x10 ⁴ | 4.19x10 ² | 7.22x10 ² | 1.27x10 ⁵ | 4.55x10 ⁴ | 1.64x10 ⁶ * |

(continued)

Compound: Imlunestrant (LY3484356)

Study 151A-185 (concluded)

| F1 Generation – Reproduction | | | | | | |
|--|--------------------------------------|-------|--------------------------------------|-------|-------|-------|
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 |
| Cumulative Eggs Produced | 4377 | 5215 | 6168 | 5871 | 3777 | 4255 |
| Eggs per Female per Day | 31.3 | 37.3 | 44.1 | 42.0 | 27.0 | 30.4 |
| Fertility (%) | 97.7 | 98.6 | 98.9 | 98.3 | 99.0 | 98.9 |
| Time to First Spawn | 1.3 | 1.3 | 1.3 | 1.3 | 1.8 | 2.0 |
| Total Length, Females (mm) | 53.0 | 52.3 | 54.4 | 54.9 | 52.0 | 57.0* |
| Total Length, Males (mm) | 63.0 | 61.1 | 66.4 | 67.3 | 68.3 | 68.5* |
| Wet Weight, Females (g) | 1.61 | 1.66 | 1.76 | 1.80 | 1.54 | 2.10* |
| Wet Weight, Males¶ (g) | 3.56 | 3.35 | 4.41† | 4.07 | 4.78* | 4.54* |
| Tubercle Scores, Females | 0 | 0 | 0 | 0 | 0 | 0 |
| Tubercle Scores, Males | 29 | 27 | 32 | 32 | 34 | 36 |
| GSI, Females | 16.6 | 14.9 | 14.4 | 14.2 | 15.6 | 18.6 |
| GSI, Males | 1.11 | 0.825 | 1.13 | 0.983 | 1.21 | 1.74* |
| F2 Generation – Embryo to Completion of Hatch | | | | | | |
| Mean Measured Conc. (µg LY3484356·L ⁻¹) | < LLOQ | 0.022 | 0.10 | 0.52 | 1.6 | 6.9 |
| Hatching Success (%) | 97.0 | 91.8† | 99.0 | 90.4† | 99.0 | 97.5 |
| Time to Hatch (days) | 3.7 | 3.5 | 3.8 | 3.8 | 3.6 | 3.9 |
| <p>a Mean measured concentrations for entire F1 exposure (i.e., from embryo through reproduction phase)</p> <p>b 1 fish of undetermined macroscopic gonadal sex in 0.11 µg·L⁻¹ excluded from statistics</p> <p>c Measurements of surplus fish (i.e., those not continued into F1 reproduction phase)</p> <p>* Statistically significant adverse effect compared to control (p < 0.05)</p> <p>† Statistically significant difference compared to control (p < 0.05), not considered biologically meaningful due to the lack of a concentration-dependent response</p> <p>‡ Statistically significant difference compared to control (p < 0.05), not considered biologically meaningful due to the lack of a concentration-dependent response and unusually low percentage of males in all negative control replicates (n=2 to 6) relative to all treatment group replicates (n=8 to 18) prior to formation of reproductive groups</p> <p>§ Statistically significant difference compared to control (p < 0.05), not considered biologically meaningful due to the lack of a concentration-dependent response, reduced number of control replicates (n=3), unusually low number of males in all control replicates (rep. A: 4 males, rep. B: 0 males, rep. C: 1 male, rep. D: 3 males) relative to all treatment group replicates (4 replicates per treatment group, 6 to 16 males per replicate)</p> <p>¶ Although wet weight of males from F1 reproductive groups displays no concentration-dependent response, the statistically significant increases at the two highest treatment groups is conservatively assumed to be treatment-related due to the increase in length and weight of females in the highest treatment group</p> <p># While the control replication is poor, the response in the highest treatment group cannot be discounted because all treatment groups display a concentration-dependent response</p> <p>Abbreviations: Conc. = concentration, dph = days post-hatch, GSI = gonadosomatic index, NOEC = no-observed effect concentration, LLOQ = lower limit of quantitation; LOEC = lowest observed effect concentration, RO = reverse osmosis</p> | | | | | | |
| Conclusions | | | | | | |
| Most Sensitive Endpoint | NOEC (µg LY3484356·L ⁻¹) | | LOEC (µg LY3484356·L ⁻¹) | | | |
| F1 Surplus Male Body Weight | 0.43 | | 1.8 | | | |

Compound: Imlunestrant (LY3484356)

Study 151A-184

| | | | |
|--|---|--|--|
| Report Title: | LY3484356 – A Prolonged Sediment Toxicity Test with <i>Chironomus riparius</i> Using Spiked Sediment Following OECD Guideline 218 | | |
| Guidance: | OECD 218 | | |
| Compliance: | Good Laboratory Practices | | |
| Study Identification | 151A-184 | | |
| Report Date: | October 2024 | | |
| Materials & Methods | | | |
| Test Substance | Name: | LY3484356 tosylate | |
| | Potency: | 74.8% as LY3484356 | |
| Radiotracer | Name: | [¹⁴ C]-LY3484356 tosylate | |
| | Radiochemical purity: | 98.45% | |
| | Specific activity: | 30.4 µCi·mg ⁻¹ as LY3484356 | |
| Test Organism | Species: | <i>Chironomus riparius</i> | |
| | Source: | Test facility cultures | |
| | Age at initiation: | First instar larvae (~3 days post hatch) | |
| Test Sediment | Type: | Artificial Sediment | |
| | Organic matter source: | Sphagnum peat | |
| | % Sand/Silt/Clay | 77/8/15 | |
| | % Organic carbon: | 1.4 | |
| | pH: | 7.5 | |
| Test Design | Test medium: | Formulated sediment with peat moss and (overlying) dilution water | |
| | Dilution Water: | Well water, UV sterilized and 0.45 µm filtered | |
| | Carrier solvent: | 1:1 of tetrahydrofuran:reverse osmosis water | |
| | Test substance application: | Isotopically dilute working stock solutions added to sand, THF/RO evaporated prior to mixing spiked sand into sediment | |
| | Duration: | 28 days | |
| | Exposure design: | Static | |
| | Nominal test concentrations: | 0 (control), 0 (solvent control), 2.0, 5.1, 13, 32, 80, 200 and 500 mg LY3484356·kg ⁻¹ dry weight | |
| | Replication: | 4 replicates per test concentration | |
| | | 20 midges per replicate | |
| | Feeding: | Ground TetraMin® flake food | |
| | Aeration: | >1 bubble per second | |
| | Test vessels: | 1-quart glass jars | |
| Amount of sediment and overlying water: | ~2 cm of sediment and ~8 cm of overlying water | | |
| Environmental Conditions (measurements in overlying water) | Photoperiod: | 16 hours light, 8 hours darkness with 30 minute transition period at 554 lux | |
| | Temperature: | 19.7 to 20.9°C | |
| | Dissolved oxygen: | ≥7.2 mg·L ⁻¹ (≥79% of air saturation value) | |
| | pH: | 8.1 to 8.8 | |
| | Ammonia, as NH ₃ : | < 0.17 (limit of quantitation) to 16.0 mg·L ⁻¹ | |

(continued)

Study 151A-184 (concluded)

| | | | |
|--|--|---|------------------------------|
| Analytical Measurements | Sampling Intervals: | Stock solutions: prior to Day 0 | |
| | | Sediment, overlying water, pore water: Days 0, 28 | |
| | Matrices measured and methodology: | Stock solutions: LSC and LC/MS/MS | |
| | | Overlying and Pore Water: LSC | |
| | | Sediment: combustion and LSC | |
| | Sediment extracts: fractionation followed by LSC | | |
| Biological Data | Observations: | Emergence, development time, abnormal behavior | |
| Results | | | |
| Geometric Mean Measured Concentration a (mg LY3484356·kg ⁻¹): | Mean Emergence Ratio | Mean Male Development Rate | Mean Female Development Rate |
| Negative Control | 0.95 | 0.0733 | 0.0643 |
| Solvent Control | 0.99 | 0.0660 | 0.0608 |
| 1.8 | 0.98 | 0.0705 | 0.0670 § |
| 4.6 | 1.00 | 0.0735 | 0.0678 § |
| 11 | 0.94 | 0.0714 | 0.0655 |
| 31 | 0.93 | 0.0708 | 0.0641 |
| 68 | 0.95 | 0.0693 | 0.0621 |
| 174 | 0.60* | 0.0602 † | 0.0579 |
| 459 | 0.0 | -- | -- |
| <p>1. Concentrations in sediment (as dry weight) based on total radioactivity, as LY3484356 equivalents</p> <p>2. Treatment group was excluded from all statistical evaluation of the endpoints.</p> <p>* Statistically significant difference compared to the solvent control ($p \leq 0.05$) using Fisher Exact/Bonferonni-Holm test.</p> <p>§ Statistically significant difference when compared to the solvent control ($p \leq 0.05$) using two-tailed Dunnett's Multiple Comparison test. However, these increases were not considered biologically relevant as the data did not follow a dose response pattern.</p> <p>† Statistically significant difference when compared to the solvent control ($p \leq 0.05$) using two-tailed William's Multiple Comparison test.</p> <p>-- Data not calculable due to 0% emergence.</p> | | | |
| Conclusions | | | |
| Most Sensitive Endpoints | NOEC (mg LY3484356·kg ⁻¹) | LOEC (mg LY3484356·kg ⁻¹) | |
| Emergence and Male Development Rate | 68 | 174 | |

Compound: Imlunestrant (LY3484356)

Study 151A-204

| | | |
|--|--|---|
| Report Title: | LY3484356 – A Prolonged Sediment Toxicity Test with <i>Lumbriculus variegatus</i> Using Spiked Sediment Following OECD Guideline 225 | |
| Guidance: | OECD 225 | |
| Compliance: | Good Laboratory Practices | |
| Study Identification | 151A-204 | |
| Report Date: | September 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.9% as LY3484356 |
| Radiotracer | Name: | [¹⁴ C] LY3484356 tosylate |
| | Radiochemical purity: | 98.45% |
| | Specific activity: | 30.4 μCi·mg ⁻¹ LY3484356 |
| Test Organism | Species: | Synchronized, Adult <i>Lumbriculus variegatus</i> |
| | Source: | Test Facility cultures |
| | Age at initiation: | 14 days post-synchronization |
| Test Sediment | Type: | Formulated sediment composed of quartz sand, kaolin clay and sphagnum peat moss |
| | %sand/silt/clay | 79/4/17 |
| | pH: | 7.6 |
| | % organic carbon: | 1.6 |
| Test Design | Test vessels: | 1-quart glass mason jars containing: |
| | Amount of sediment and overlying water: | ~2 cm of sediment (~137 g, dry weight) |
| | | ~9.5 cm of overlying water (600 mL) |
| | Overlying water: | Test Facility well water (UV sterilized and filtered) |
| | Carrier: | 1:1 tetrahydrofuran:reverse osmosis water with methanol (THF:RO:MeOH) |
| | Test substance application: | Isotopically dilute working stock solutions added to sand, THF:RO:MeOH evaporated prior to mixing spiked sand into sediment |
| | Duration: | 28 days |
| | Exposure design: | Static |
| | Control(s): | Negative control (neat sand) |
| | | Solvent control (THF:RO:MeOH spiked sand) |
| | Nominal test concentrations: | 1.0, 2.0, 6.0, 19, 56, 167 and 500 mg LY3484356·kg ⁻¹ sediment (dry weight) |
| | Replication: | 4 replicates in each treatment group |
| | | 6 replicates in each control group |
| 10 oligochaetes per replicate | | |
| Feeding: | Organic wheat grass powder | |
| Aeration: | >1 bubble per second | |
| Environmental Conditions (measurements in overlying water) | Photoperiod: | 16 hours light (180 lux), 8 hours darkness |
| | Temperature: | 20.0 to 20.6°C |
| | Dissolved oxygen: | ≥6.2 mg·L ⁻¹ (≥68% of air saturation) |
| | pH: | 8.3 to 8.6 |
| | Ammonia: | <LOQ to 4.57 mg·L ⁻¹ , as NH ₃ |

(continued)

Study 151A-204 (concluded)

| Analytical Measurements | Sampling Intervals: | Days 0, 7 and 28 | |
|---|--|---|-------------------------------|
| | Matrices measured and methodology: | Sediment: Combustion + LSC | |
| | | Sediment extracts: Fractionation + LSC | |
| | | Overlying and Pore Water: LSC | |
| Biological Data | Endpoints: | Survival, dry weight, ash-free dry weight | |
| Results | | | |
| Geometric Mean Measured Concentration ^a (mg LY3484356·kg ⁻¹) | Mean Total Number of Living Oligochaetes at Test End | Mean Dry Weight (mg) | Mean Ash Free Dry Weight (mg) |
| Negative Control ^b | 41 | 39.2 ± 3.80 | 26.2 ± 3.16 |
| Solvent Control ^b | 37 | 33.5 ± 3.55 | 23.3 ± 1.47 |
| 0.85 | 43 | 37.0 ± 3.34 | 26.3 ± 2.05 |
| 1.8 | 36 | 41.0 ± 5.70 | 27.2 ± 3.75 |
| 5.5 | 39 | 40.2 ± 7.45 | 27.1 ± 2.94 |
| 17 | 42 | 45.0 ± 3.47 | 28.0 ± 2.09 |
| 49 | 34 | 42.9 ± 5.03 | 25.3 ± 0.82 |
| 139 | 33 | 29.2 ± 7.45 | 20.9 ± 3.05 |
| 465 | 26* | 13.8 ± 3.54* | 11.1 ± 2.65* |
| ^a Concentrations in sediment (as dry weight) based on total radioactivity, as LY3484356 equivalents ^b Control was less than Limit of Quantitation, 50 dpm * Statistically significant difference from solvent control | | | |
| Conclusions | | | |
| Most Sensitive Endpoint | NOEC (mg LY3484356·kg ⁻¹) | LOEC (mg LY3484356·kg ⁻¹) | |
| Total number of oligochaetes, dry weight | 139 | 465 | |

Compound: Imlunestrant (LY3484356)

Study 151A-203

| | | |
|--|--|--|
| Study Title: | LY3484356 – A Life Cycle Toxicity Test with the Freshwater Amphipod (<i>Hyalella azteca</i>) Using Spiked Sediment following U.S EPA (draft) OPPTS Number 850.1770 | |
| Guidance: | EPA 600/R-99/064; EPA 850.1770 (draft) | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151A-203 | |
| Report Date: | September 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Radiotracer | Name: | [¹⁴ C]-LY3484356 tosylate |
| | Radiochemical purity: | 98.45% |
| | Specific activity: | 30.4 μCi·mg ⁻¹ as LY3484356 |
| Test Organism | Species: | <i>Hyalella azteca</i> |
| | Source: | Test facility cultures |
| | Age at initiation: | 7-8 days |
| Test Sediment | Type: | Natural sediment |
| | %sand/silt/clay: | 64/31/5 |
| | % organic carbon: | 3.2 |
| | pH: | 6.0 |
| Test Design | Test system: | 100 mL (3.2 cm) natural sediment and 175 mL (4.0 cm) overlying water, dosed in sediment |
| | Overlying Water: | Well water, UV sterilized and filtered |
| | Carrier: | 50/50 tetrahydrofuran/reverse osmosis water working stock solutions |
| | Test substance application: | Isotopically dilute working stock solutions added to sand, THF/RO evaporated prior to mixing spiked sand into sediment |
| | Duration: | 42 days |
| | Nominal test concentrations: | 0 (control), 0 (solvent control), 4.0, 12, 37, 111, 333 and 1000 mg LY3484356·kg ⁻¹ sediment (dry weight) |
| | Replication: | 12 biological replicates per level: 4 replicates sacrificed on day 28, 8 replicates sacrificed on day 42 10 amphipods per replicate |
| | Feeding: | Ground suspension of TetraMin® flake food and YCT |
| Test vessels: | 300-mL glass beakers with stainless steel 250 μm mesh screen covered holes. | |
| Environmental Conditions (measurements in overlying water) | Photoperiod: | 16 hours light (623 lux), 8 hours darkness |
| | Dissolved Oxygen: | 6.5 to 8.6 mg·L ⁻¹ (76 to 100% saturation at 23°C) |
| | Temperature: | 22.1 to 23.7°C (measured in the test vessels) |
| | pH: | 8.0 to 8.5 |
| | Ammonia: | < 0.17 to 0.928 mg·L ⁻¹ as NH ₃ ⁺ |
| Analytical Measurements | Sampling Intervals: | Day 0, 28 |
| | Methods: | Stock solutions: LC/MS/MS and LSC |
| | | Overlying and pore water: LSC |
| | | Sediment: Combustion and LSC |
| | | Sediment extracts: HPLC/β-RAM |
| Biological Data | Endpoints | Survival, growth, reproduction, abnormal behavior |

(continued)

Study 151A-203 (concluded)

| Results | | | | | | |
|---|--|------------------|---------------------------------------|-----------------|--------------|--------------------------------------|
| Geometric Mean Measured Sediment Concentration ^a (mg LY3484356·kg ⁻¹) | Day 42 Endpoints (Mean ± Standard Deviation) | | | | | |
| | Mean Percent Survival | Body Length (mm) | | Dry Weight (mg) | | Number of Young Per Surviving Female |
| | | Male | Female | Male | Female | |
| Negative Control ^b | 93.8 ± 7.4 | 6.64 ± 0.41 | 5.91 ± 0.29 | 1.64 ± 0.24 | 1.27 ± 0.16 | 21.7 ± 3.8 |
| Solvent Control ^b | 96.3 ± 7.4 | 6.32 ± 0.36 | 5.92 ± 0.13 | 1.32 ± 0.10 | 1.21 ± 0.11 | 23.6 ± 4.4 |
| 3.2 | 95.0 ± 7.6 | 6.45 ± 0.40 | 6.00 ± 0.25 | 1.38 ± 0.16 | 1.26 ± 0.20 | 25.3 ± 4.4 |
| 10 | 95.0 ± 10.7 | 6.45 ± 0.24 | 5.91 ± 0.21 | 1.50 ± 0.18 | 1.21 ± 0.12 | 23.8 ± 7.8 |
| 36 | 91.3 ± 6.4 | 6.60 ± 0.23 | 6.04 ± 0.17 | 1.47 ± 0.14 | 1.34 ± 0.14 | 20.7 ± 8.4 |
| 93 | 96.3 ± 5.2 | 6.34 ± 0.55 | 5.75 ± 0.20 | 1.39 ± 0.17 | 1.19 ± 0.12 | 21.9 ± 7.0 |
| 283 | 91.3 ± 6.4 | 6.56 ± 0.42 | 6.12 ± 0.44 | 1.51 ± 0.21 | 1.43 ± 0.25 | 22.9 ± 3.9 |
| 935 | 80.0 ± 17.7* | 5.41 ± 0.33* | 4.90 ± 0.68* | 0.99 ± 0.19* | 0.87 ± 0.37* | 7.0 ± 6.9* |
| ^a Concentrations in sediment (as dry weight) based on total radioactivity, as LY3484356 equivalents ^b Control less than Limit of Quantitation, ~0.0700 mg LY3484356 equivalent/kg. * Statistically significant difference from solvent control (p < 0.05) | | | | | | |
| Conclusions | | | | | | |
| Most Sensitive Endpoint | NOEC (mg LY3484356·kg ⁻¹) | | LOEC (mg LY3484356·kg ⁻¹) | | | |
| Survival, Growth, Reproduction | 283 | | 935 | | | |

Study 151P-113

| | | |
|--------------------------------|---|--|
| Report Title: | LY3484356 – An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate Following OECD Guideline 207 | |
| Guidance Document: | OECD 207 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151P-113 | |
| Report Date: | September 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Test Organism | Species: | Earthworm (<i>Eisenia fetida</i>) |
| | Source: | Laboratory cultures |
| | Age at Initiation: | Adult (< 1 year old, with clitellum) |
| Test Soil | Type: | Artificial Soil |
| | Components: | 10% sphagnum peat |
| | | 20% kaolin clay |
| | | 70% sand (1:1 play sand:silica) |
| Organic carbon content: | 1% calcium carbonate | |
| Exposure Design | Test Duration: | 5.3% |
| | Nominal Test Concentrations: | 14 days |
| | Controls: | 3.1, 9.0, 28, 83, 250 and 750 mg LY3484356·kg ⁻¹ soil (dry weight) |
| | Carrier Solvent: | Negative control and solvent control |
| | Test Substance Application: | Methanol (MeOH) |
| | Replication: | LY3484356 stock solutions in MeOH spiked onto sand. MeOH allowed to evaporate. Spiked sand mixed into soil. |
| | Test Chambers: | 4 replicates per control or treatment group 10 earthworms per replicate |
| | Soil Mass per Chamber: | 1 L glass jar, covered with perforated plastic wrap and secured with a rubber band ~750 g artificial soil |
| Test Conditions | Photoperiod: | Continuous light at 517 to 693 lux |
| | Temperature: | 18.8 to 21.6°C |
| | pH: | 6.8 to 7.9 |
| | Moisture Content: | 31 to 34% |
| Dose Confirmation | Sampling: | Primary dosing stock solution |
| | Method: | LC/MS/MS |
| Biological Data | Observations: | Mortality, physiological and behavioral abnormalities, and body weight change |

(continued)

Study 151P-113 (concluded)

| Results | | | |
|--|--|---|--|
| Nominal Concentration ^a (mg LY3484356·kg ⁻¹) | Mean Mortality ^b (%) | Mean Body Weight Change ^b (g) | |
| Negative Control | 0 | -0.03 | |
| Solvent Control | 0 | -0.02 | |
| 3.1 | 0 | -0.03 | |
| 9.0 | 2.5 | -0.03 | |
| 28 | 0 | -0.01 | |
| 83 | 0 | -0.01 | |
| 250 | 0 | -0.03 | |
| 750 | 0 | -0.03 | |
| ^a Concentrations based on soil dry weight | | | |
| ^b No statistically significant differences from solvent control | | | |
| Conclusions | | | |
| Endpoint | NOEC (mg LY3484356·kg ⁻¹) | LOEC (mg LY3484356·kg ⁻¹) | LC ₅₀ (mg LY3484356·kg ⁻¹) |
| Mortality | 750 | >750 | >750 |
| Body Weight Gain | 750 | >750 | -- |

Study 151E-170

| | | |
|--|--|---|
| Report Title: | LY3484356: Soil Microorganisms - Nitrogen Transformation Test Following OECD Guideline 216 | |
| Guidance Document: | OECD 216 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151E-170 | |
| Reporting Date: | September 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Soil | Source: | Greensboro Soil Plot (Easton, MD) |
| | pH | 6.4 |
| | Sand Content: | 68.1% |
| | Organic Carbon Content (TOC): | 0.9% |
| | Microbial Biomass (% of TOC): | 107.0 $\mu\text{g}\cdot\text{g}^{-1}$ (1.2%) |
| | Water Holding Capacity (WHC): | 26.3% |
| Exposure Design | Nominal Test Concentrations: | 7.6, 38.2, 76.4, 382.5, and 761.2 mg LY3484356·kg ⁻¹ soil dry weight |
| | Control: | Soil without test substance or methanol |
| | Carrier Solvent: | Methanol (MeOH) |
| | Test Substance Application: | LY3484356 stock solutions in MeOH spiked onto sand. MeOH allowed to evaporate. Spiked sand mixed into soil. |
| | Test Chambers: | 8 oz. Glass French Squares |
| Test Conditions | Temperature: | 19.1 to 21.0 °C |
| | pH | 5.65 to 6.36 |
| | Moisture Content | 46.6 to 60.2% of WHC |
| | Incubation: | 28 Days |
| Measurements | Nitrate Concentration: | HPLC-UV |
| | Dose Mixture (spiked sand) | HPLC-UV |
| Results | | |
| Nominal Concentration ^a (mg LY3484356·kg ⁻¹) | Mean Nitrate Formation Rate ^{a,b} ± Standard Deviation (mg NO ₃ ·kg ⁻¹ ·day ⁻¹) | Percent Inhibition (%) |
| Control | 6.69 ± 0.53 | Not Applicable |
| 7.6 | 7.59 ± 0.46 | -13.4 |
| 38.2 | 7.47 ± 0.21 | -11.6 |
| 76.4 | 7.52 ± 0.19 | -12.3 |
| 382.5 | 7.39 ± 0.48 | -10.4 |
| 761.2 | 7.02 ± 0.66 | -4.9 |
| ^a concentrations based on soil dry weight | | |
| ^b average of 3 replicates | | |
| Conclusions | | |
| Endpoint | NOEC | EC ₅₀ |
| Nitrate Formation | 761.2 mg LY3484356·kg ⁻¹ | >761.2 mg LY3484356·kg ⁻¹ |

Compound: Imlunestrant (LY3484356)

Study 151P-109

| | | |
|--|--|---|
| Report Title: | LY3484356 – A Toxicity Test to Determine the Effects on Seedling Emergence and Growth of Terrestrial Plants following OECD Guideline 208 | |
| Guidance Document: | OECD 208 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | 151P-109 | |
| Report Date: | September 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Test Soil | Type: | Artificial soil |
| | Components: | Kaolinite clay, industrial quartz sand and peatmoss, with limestone to buffer pH |
| | % Sand/Silt/Clay: | 86% sand, 5% silt, 9% clay |
| | pH (1:1 soil:water): | 6.6 |
| | Organic carbon: | 1.2% |
| Test Species | Monocots: | Onion (<i>Allium cepa</i>) |
| | | Ryegrass (<i>Lolium perenne</i>) |
| | Dicots: | Soybean (<i>Glycine max</i>) |
| | | Sunflower (<i>Helianthus annuus</i>) |
| | | Radish (<i>Raphanus sativus</i>) |
| Tomato (<i>Solanum lycopersicum</i>) | | |
| Exposure Design | Test concentrations: | 0 (negative control), 0 (solvent control), 4.1, 12, 37, 111, 333 and 1000 mg LY3484356·kg ⁻¹ soil (dry weight) |
| | Carrier solvent: | Methanol |
| | Test vessels: | 16 cm diameter x 12 cm deep plastic plant pots |
| | Test substance application: | Dosing stock in methanol added to sand, methanol evaporated prior mixing sand premix into test soil |
| | Replication: | Four replicates with five seeds each for monocots Ten replicates with two seeds each for dicots |
| | Vessel arrangement: | Randomized complete block design |
| | Watering: | An initial top-watering on Day 0, followed with sub-irrigation through saucers as needed |
| Environmental conditions | Test Environment: | Environmentally-controlled greenhouse |
| | Temperature: | 17.27 to 30.52°C |
| | Relative humidity: | 16.2% to 78.1% (photosynthetically active radiation) |
| | Photoperiod: | 16-hour light: 8-hour dark |
| | Light intensity: | 74.1 to 237.0 μE·m ⁻² ·s ⁻¹ |
| | Exposure duration: | 21 days (≥14 days after 50% control emergence) |
| Analytical Measurements | Sampling: | Primary dosing stock solution |
| | Analytical method: | LC/MSMS |
| Exposure Assessment | Endpoints: | Emergence, Survival, Shoot Height, Dry Shoot Weight and Plant Condition (qualitative only) |

(continued)

Study 151P-109 (concluded)

| Results | | | | | | | | |
|--|--|--------|---------------------------------------|-------|-------|---------------------------------------|--------|--------------------|
| Endpoint | Nominal Concentration (mg LY3484356·kg ⁻¹ dry weight) | | | | | | | |
| | NC | SC | 4.1 | 12 | 37 | 111 | 333 | 1000 |
| <i>Onion (Allium cepa)</i> | | | | | | | | |
| Emergence | 4.5 | 5.0 | 4.5 | 4.8 | 4.5 | 4.8 | 5.0 | 4.8 |
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Height (cm) | 9.3 | 9.2 | 8.6 | 7.8 | 8.9 | 8.7 | 8.6 | 8.2 |
| Dry Weight (g) | 0.0106 | 0.0069 | 0.007 | 0.008 | 0.008 | 0.008 | 0.009 | 0.008 |
| <i>Ryegrass (Lolium perenne)</i> | | | | | | | | |
| Emergence (%) | 5.0 | 4.5 | 5.0 | 5.0 | 4.8 | 4.5 | 4.5 | 4.3 |
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Height (cm) | 23.6 | 25.9 | 25.8 | 24.8 | 23.1 | 24.3 | 23.7 | 19.0* |
| Dry Weight (g) | 0.025 | 0.035 | 0.036 | 0.035 | 0.030 | 0.039 | 0.033 | 0.023 ^a |
| <i>Soybean (Glycine max)</i> | | | | | | | | |
| Emergence (%) | 1.8 | 1.9 | 1.9 | 2.0 | 1.8 | 1.9 | 2.0 | 1.8 |
| Survival (%) | 100 | 100 | 100 | 95 | 100 | 100 | 100 | 100 |
| Height (cm) | 10.7 | 10.4 | 11.1 | 10.8 | 11.9 | 11.6 | 12.1 | 11.6 |
| Dry Weight (g) | 0.33 | 0.31 | 0.34 | 0.35 | 0.37 | 0.41 | 0.38 | 0.35 |
| <i>Sunflower (Helianthus annuus)</i> | | | | | | | | |
| Emergence (%) | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 1.3* | 1.8* |
| Survival (%) | 100 | 100 | 100 | 90 | 100 | 100 | 100 | 100 |
| Height (cm) | 17.8 | 17.0 | 18.9 | 18.0 | 19.8 | 19.6 | 14.3 | 18.1 |
| Dry Weight (g) | 0.40 | 0.34 | 0.41 | 0.41 | 0.49 | 0.54 | 0.30 | 0.37 |
| <i>Radish (Raphanus sativus)</i> | | | | | | | | |
| Emergence (%) | 2.0 | 1.8 | 2.0 | 2.0 | 1.8 | 1.9 | 1.6 | 2.0 |
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Height (cm) | 7.7 | 7.6 | 7.8 | 8.2 | 9.0 | 9.5 | 7.5 | 5.4* |
| Dry Weight (g) | 0.125 | 0.135 | 0.132 | 0.132 | 0.160 | 0.178 | 0.105 | 0.057* |
| <i>Tomato (Solanum lycopersicum)</i> | | | | | | | | |
| Emergence (%) | 1.6 | 1.9 | 1.9 | 1.9 | 1.9 | 1.8 | 1.6* | 1.5* |
| Survival (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Height (cm) | 4.3 | 4.1 | 3.8 | 4.1 | 4.0 | 4.3 | 3.4* | 2.2* |
| Dry Weight (g) | 0.041 | 0.039 | 0.033 | 0.047 | 0.043 | 0.046 | 0.022* | 0.009* |
| * Significant statistical difference compared to the solvent control | | | | | | | | |
| ^a Although not statistically different, reduction from solvent control is biologically meaningful | | | | | | | | |
| Abbreviations: NC =- negative control, SC = solvent control | | | | | | | | |
| Conclusions | | | | | | | | |
| Species | Sensitive Endpoint(s) | | NOEC (mg LY3484356·kg ⁻¹) | | | LOEC (mg LY3484356·kg ⁻¹) | | |
| Onion (<i>Allium cepa</i>) | -- | | 1000 | | | >1000 | | |
| Ryegrass (<i>Lolium perenne</i>) | Height, Weight | | 333 | | | 1000 | | |
| Soybean (<i>Glycine max</i>) | -- | | 1000 | | | >1000 | | |
| Sunflower (<i>Helianthus annuus</i>) | Emergence | | 111 | | | 333 | | |
| Radish (<i>Raphanus sativus</i>) | Height, Weight | | 333 | | | 1000 | | |
| Tomato (<i>Solanum lycopersicum</i>) | Emergence, Height, Weight | | 111 | | | 333 | | |

Compound: Imlunestrant (LY3484356)

Study S22-07858

| | | |
|--------------------------------|---|---|
| Report Title: | LY3484356: Effects on the Reproduction of the Springtail <i>Folsomia candida</i> Willem (Collembola, Isotomidae) in Artificial Soil | |
| Guidance Document: | OECD 232 | |
| Compliance: | Good Laboratory Practices | |
| Study Number: | S22-07858 | |
| Report Date: | August 2024 | |
| Materials & Methods | | |
| Test Substance | Name: | LY3484356 tosylate |
| | Potency: | 74.8% as LY3484356 |
| Test Organism | Species: | <i>Collembola (Folsomia candida)</i> |
| | Source: | Laboratory culture |
| | Age at Initiation: | Female juvenile (9 to 11 days old) |
| Test Soil | Type: | Artificial Soil |
| | Components: | 5.0% sphagnum-peat |
| | | 20.0% kaolin clay |
| | | 74.8% fine industrial sand |
| | | 0.22% calcium carbonate |
| Water Holding Capacity: | 43.74% | |
| pH: | 6.1 | |
| Exposure Design | Test Duration: | 28 days |
| | Nominal Test Concentrations: | 0, 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg LY3484356·kg ⁻¹ soil dry weight |
| | Control: | Untreated control (no test substance) |
| | Test Substance Application: | Test item added to pre-moistened test substrate using quartz sand |
| | Replication: | 8 replicates for each control |
| | | 4 replicates for each test concentration |
| | | 10 organisms per replicate |
| | Test Vessels: | 250 mL tinted glass vessels, closed with screw lids containing a hole for ventilation |
| Soil Mass per Vessel: | 30 g soil (dry weight) | |
| Feeding: | Granulated dry baker's yeast on Day 0 and 14 | |
| Test Conditions | Photoperiod: | 16 hours light (448 to 730 lux), 8 hours dark |
| | Temperature: | 19.8 to 20.5°C |
| | pH: | 5.7 to 5.8 |
| | Water Content: | 51.2 to 57.2% of WHCt |
| Biological Data | Observations: | Mortality and reproduction |

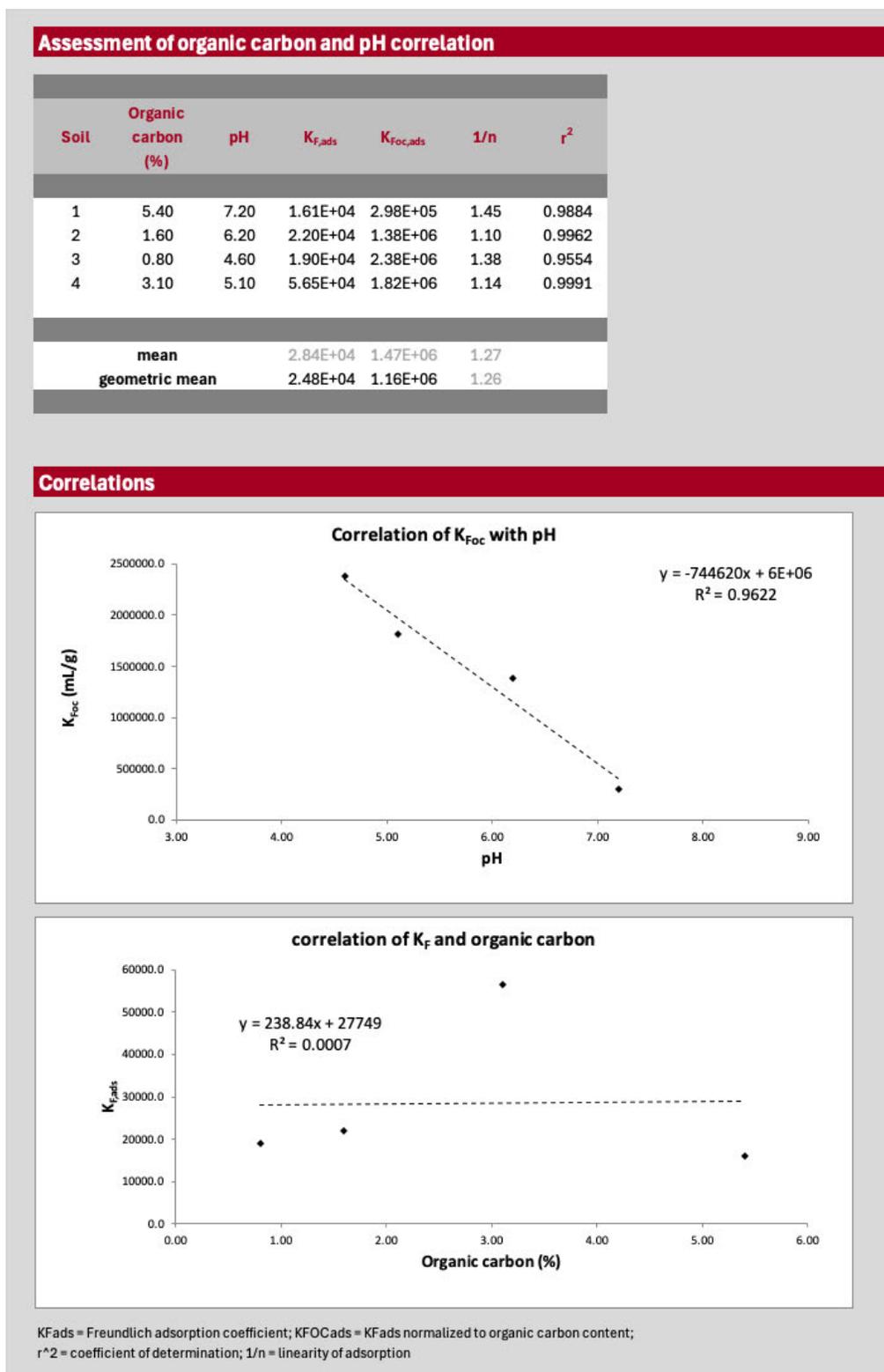
(continued)

Study S22-07858 (concluded)

| Results | | |
|--|---|--|
| Nominal Concentration ^a (mg LY3484356·kg ⁻¹) | Mean Mortality ^b (%) | Mean Reproduction ± SD ^b (Number of Juveniles) |
| Untreated control ^c | 2.9 | 809.6 ± 129.3 |
| 16.3 | 7.5 | 765.0 ± 133.7 |
| 29.4 | 0.0 | 876.8 ± 129.8 |
| 52.9 | 0.0 | 884.0 ± 100.5 |
| 95.3 | 2.5 | 925.3 ± 140.0 |
| 171 | 10.0 | 650.0 ± 210.6 |
| 309 | 0.0 | 793.3 ± 75.7 |
| 556 | 7.5 | 648.0 ± 159.8 |
| 1000 | 7.5 | 658.5 ± 111.3 |
| <p>^a Concentrations based on soil dry weight</p> <p>^b No statistically significant reductions compared to pooled control</p> <p>^c one replicate excluded from evaluation because on day 28 the lid of the test vessel was observed to be not tightly closed and the test soil appeared to be dried out</p> <p>SD = Standard deviation</p> | | |
| Conclusions | | |
| Endpoint | NOEC ^e (mg LY3484356·kg ⁻¹) | LOEC ^e (mg LY3484356·kg ⁻¹) |
| Mortality | 1000 | >1000 |
| Reproduction | 309 | 556 |

Compound: Imlunestrant (LY3484356)

Appendix D: Assessment of Organic Carbon and pH Correlation with Adsorption of Imlunestrant to Soils



Compound: Imlunestrant (LY3484356)

Appendix E: Curriculum Vitae of Preparers

Michael R. Lee

Lilly Research Laboratories, Indianapolis, IN

MS. Biology, University of Massachusetts, Dartmouth 2015

B.S. Biology, Rhode Island College 2005

Previous Experience: Biologist, Smithers Viscient (2006 to 2013). Directed chronic early-life stage to multigenerational aquatic toxicology studies with several freshwater and marine vertebrate and invertebrate species. Trained study directors and technical staff on aquatic vertebrate and invertebrate husbandry and chronic testing techniques both internal and external to Smithers Viscient. Associate Scientist, Lilly Research Laboratories (2013 to 2024). Designed and monitored environmental studies to support risk assessments for global product registrations and manufacturing of animal health products and human pharmaceuticals. Prepared environmental risk assessments for animal health and human pharmaceutical products to US FDA CVM and EMA. Authored publications and presentations in the field of environmental toxicology and fate.

Current Responsibility: Advisor, Health, Safety and Environmental. Prepare environmental risk assessments for animal and pharmaceutical products for submission to the FDA and Europe. Prepares guidelines for production facilities for containment of active products. Designs and monitors GLP environmental chemistry, fate, and toxicity studies.

Professional Activities:

Member: Society of Environmental Toxicology and Chemistry



Xiaoqin
Wu

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James
Laurenson

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Xing
Wang

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XING WANG
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