EPA Registration Jacket
43813-27 Vol. 4

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Antimicrobials Division (7510C)
1200 Pennsylvania Avenue NW
Washington, D.C. 20460

NOTICE OF PESTICIDE:
_x_ Registration
__ Reregistration

(under FIFRA, as amended)

EPA Reg. Number: 43813-27

Date of Issuance:

Term of Issuance:

Name of Pesticide Product: ECONEA TECHNICAL

Name and Address of Registrant (include ZIP Code):

Janessen Pharmaceutica, Inc.
1125 Trenton-Harbourton Road
Titusville, NJ 08560-0200

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

1. Make the labeling changes listed below before you release the product for shipment:

a. Add the phrase "EPA Registration Number 43813-27".

Signature of Approving Official:

Dennis H Edwards Jr for

Marshall Swindell
Product Manager Team-33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Date: 042307

EPA Form 8570-6

The Confidential Statement of Formula dated January 4th, 2007, is acceptable.

2. Submit three (3) copies of the final printed label prior to releasing this product for sale.

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA sec. 6(e). Your release for shipment of the product constitutes acceptance of these conditions.

A stamped copy of the label is enclosed for your records.

Sincerely,

Dennis H Edwards Jr for

Marshall Swindell
Product Manager 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Enclosure: (Stamped Labeling)

ECONEA® Technical

Anti-fouling Preservative

For Formulating Use Only

ACTIVE INGREDIENT:	
Tralopyril*	93.2%
INERT INGREDIENTS:	6.8%
TOTAL:	100.0%

* CAS# 122454-29-9

KEEP OUT OF REACH OF CHILDREN

DANGER **POISON**

See side panel for first aid and additional precautionary statements

EPA Reg. No.: 43813-xx

EPA Est. No.: 241-MO-001

Manufactured for: **JANSSEN PHARMACEUTICA**
1125 Trenton-Harbourton Road
Titusville, NJ 08560

ACCEPTED
with COMMENTS
in EPA Letter Dated:
042307
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
43813-27

® Registered Trademark of Janssen Pharmaceutica

Rev. 03/07

ACCEPTED
with COMMENTS
in EPA Letter Dated:

042307

Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No. 43813-27

PRECAUTIONARY STATEMENT
HAZARDS TO HUMANS AND DOMESTIC ANIMALS
DANGER

Fatal if swallowed. Harmful if inhaled or absorbed through the skin. Causes moderate eye irritation. Avoid breathing dust. Avoid contact with skin, eyes, or clothing. Wash hands thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove contaminated clothing and wash clothing before reuse.

Handler Personal Protective Equipment (PPE):

- Wear long-sleeved shirt, long pants, socks, shoes, and chemical resistant natural rubber gloves.
- Wear protective eyewear such as goggles, face shield or safety glasses.
- Wear dust filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, R, P, or HE filter.

Pesticide User Safety Requirements:
Discard clothing and other absorbent materials that have been heavily contaminated with this product. Do not reuse them. Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

FIRST AID	
If swallowed	-Call a poison control center or doctor immediately for treatment advice. -Have person sip a glass of water if able to swallow. -Do not induce vomiting unless told to do so by a poison control center or doctor. -Do not give anything by mouth to an unconscious person.
If inhaled	-Move person to fresh air. -If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. -Call a poison control center or doctor for further treatment advice
If in eyes	-Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. -Call a poison control center or doctor for treatment advice.
If on skin or clothing	-Take off contaminated clothing. -Rinse skin immediately with plenty of water for 15-20 minutes. -Call a poison control center or doctor for treatment advice.

HOT LINE NUMBER:

For information on this pesticide product (including health concerns, medical emergencies, or pesticide incidents) call the National Pesticide Telecommunications Information Center at 1-800-858-7378.

For chemical emergency assistance (spill, leak, fire, or accident), call Chem Trec at 1-800-424-9300.

Have the product container with you when calling a poison control center or doctor, or going for treatment.

NOTE TO PHYSICIAN

Probable mucosal damage may contraindicate the use of gastric lavage.

ACCEPTED
with COMMENTS
in EPA Letter Dated:
042307
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
43813-27

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
This product is intended for use only during industrial formulation processes producing antifouling products for control of hard fouling organisms.
Formulators using ECONEA are responsible for providing additional data to support registration of their end-use product(s).

STORAGE AND DISPOSAL

PROHIBITIONS: Do not contaminate water, food or feed by storage and disposal.
STORAGE: DO NOT mix or store this product or solutions of this product in a manner inconsistent with its labeling.
DISPOSAL: Pesticide wastes may be acutely hazardous. Improper disposal is a violation of Federal Law.
PESTICIDE DISPOSAL: Pesticide, mixtures, or equipment rinse waters that cannot be chemically reprocessed must be disposed of according to applicable federal, state or local procedures. Contact your State Pesticide or Environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.
CONTAINER DISPOSAL: Completely empty liner by shaking and tapping sides and bottom to loosen clinging particles. Empty residue into formulation equipment. Then dispose of liner in a sanitary landfill or by incineration if allowed by State and local authorities. If burned, stay out of smoke. If drum is contaminated and cannot be reused, dispose of in the same manner.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

NOTICE OF WARRANTY

Janssen Pharmaceutica warrants that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of use or application, all of which are beyond the control of Janssen Pharmaceutica. To the extent permitted by law, Janssen Pharmaceutica disclaims any liability for consequential, special or indirect damages resulting from the use, handling, application, storage or disposal of this product or for damages in the nature of penalties, and the buyer and user waive any right that they may have to such damages. **To the extent consistent with applicable law, Janssen Pharmaceutica makes no warranties of merchantability or of fitness for a particular purpose or any other express or implied warranty except as stated above.**

Material to be added to a Mini-Jacket
(in the case where an e-Jacket exists)

Reg. No. 43813-27

Send to SIG: check box ☑

This material is:

- ☐ New stamped-accepted label
- ☐ New CSF
- ☐ Notification
- ☐ Final Printed Label
- ☑ Other: Copy of Science Review (OEPC)

Instructions: Attach this notice on top of the material. It must be clipped all together and there should be NO STAPLES in the material. Then give the material with this coversheet to staff in the Information Services Center (Room 230).

Reviewer's Name: Karen Lee

Phone: 308-6237 **Division:** AD

Date: 5/31/07

SJG
6/1/07

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES,
AND TOXIC SUBSTANCES

February 15, 2007

MEMORANDUM

SUBJECT: ***Econea*** Occupational Exposure Re-Assessment Using a Revised Dermal Toxicity Endpoint: Update of November 15, 2006 Review for a New Active Ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)*,(a.k.a., *Econea*), Proposed as an Antifoulant in the Manufacturing-Use Product (MUP) **ECONEA™ Technical** (EPA File Symbol 43813-ET, 93.2% a.i.), and in the Formulated End-Use Product (EP) **Sigma Nexxium 20 Antifouling** (EPA File Symbol 11350-GL, 3.4% a.i..).

TO: Dennis Edwards, Chief
Marshall Swindell, Product Manager, Team 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

FROM: Doreen Aviado, Biologist, Team Two Doreen Aviado 2/15/07
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

THRU: Nader Elkassabany, Team Leader, Team Two NCook for 2/21/07
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Norm Cook, Chief 2/21/07
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

DP Barcodes: D295928, D327535, D327536 & D330452 & D335509

Pesticide Chemical/No.: MUP: 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl) (AC 303268, R107894, CL 303268, AF028) *Econea* / 119093.
EP: *Econea* /119093 and *Sea-Nine 211* (C-9211, RH-287, or Kathon 287T) / 128101

Registrants: Janssen Pharmaceutica Inc. (MUP); and Sigma Coatings USA (EP)

EPA File Symbols: 43813-ET: *ECONEA™ Technical* (MUP);
11350-GL: *Sigma Nexxium 20 Antifouling* (EP)

MRID No.: 468466-02

EXECUTIVE SUMMARY

Presented herein is a revised assessment as an update to the November 15, 2006 occupational exposure review memorandum, based on a newly selected dermal toxicity endpoint (i.e., a systemic NOAEL ≥ 1000 mg/kg/day) which replaces the one used initially (i.e., oral NOAEL <6.3 mg/kg/day) to determine short-term and intermediate-term dermal risks for shipyard painters in contact with this new antifoulant active ingredient, *Econea.* In addition, the level of concern associated with the new endpoint has also been revised to reflect a target margin of exposure (Target MOE) of 100 for the dermal route, replacing the initial Target MOE of 300. In light of the updated revisions to the toxicological hazard characterization for *Econea*, the dermal risk outcomes for most occupational scenarios have improved.

The Antimicrobials Division (AD), Product Management Team 33, requested that the Risk Assessment and Science Support Branch (RASSB) conduct an occupational exposure assessment for a proposed ready-to-use antifoulant paint product, ***Sigma Nexxium 20 Antifouling***, containing a new antifoulant active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a., *Econea*). The active ingredient *Econea* is a degradate of an agricultural use insecticide-miticide (Chlorfenapyr, PC Code 129093) which is registered primarily for foliar applications to greenhouse grown ornamentals and fruiting vegetables. Chlorfenapyr as the precursor for *Econea* is metabolized to form this new active.

Econea is slated for registration as a technical grade active (93.2% a.i.) in Janssen Pharmaceutica's formulator source product ***ECONEA™ Technical*** (EPA File Symbol 43813-ET); intended solely as a manufacturing-use product (MUP) in formulating antifoulant paints.[1] Each formulator using this MUP is responsible for obtaining EPA registration for their pesticide end-use product (EP). At present, the one proposed formulated end-use product (EP) under AD/RASSB exposure review is Sigma Coatings' ***Sigma Nexxium 20 Antifouling*** (EPA File Symbol 11350-GL), a self-polishing antifouling coating for commercial application to underwater hulls of boats (i.e., pleasure, military and commercial vessels) in fresh water and marine environments.[2] This EP is for use in commercial or government shipyards only and

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[1] Formulator source products, such as *ECONEA™ Technical*, which are used as the technical active to manufacture pesticide end products are typically not assessed for human exposure concerns as part of the registration process. The Agency assumes that occupational workplace safety standards set by the Occupational Safety and Health Administration (OSHA) for industrial manufacturing facilities and any specified personal protective equipment (PPE) on the MUP label (or chemical MSDS) are adequate to protect workers in contact with such chemicals/source products during formulation. Also, the Agency does not impose human exposure data requirements (*Series 875 Data Guidelines*) for MUPs, only for typical end-use products (EPs) which address handler/postapplication exposure.

[2] In 2004 *Sigma Coatings USA* applied for an experimental use permit (EUP) for a *Sigma Nexxium 710 Antifouling* paint formulation containing 3.25% *Econea* and 3.26% Sea-Nine 211. A preliminary screening-level human exposure assessment was conducted at that time with no risk concern outcomes (AD/RASSB Review Memorandum for D310254 by C. Walls, Chemist, dated November 23, 2004). AD recently received a registration application for an additional proposed *Econea*-based antifoulant EP as of September, 2006. The EP is TRILUX 44-White (EPA File Symbol 2693-EEN) from International Paint LLC containing a co-biocide mixture of 3.9% Econea and 4.12% Zinc pyrithione

contains a co-biocide mixture of 3.4% *Econea* a.i., and 3.4% *Sea-Nine 211* a.i. (C-9211, RH-287, or Kathon 287T). *Econea* will control the growth of barnacles and other hard fouling organisms and *Sea-Nine 211* is added to control the growth of algae. Since *Sea-Nine 211* is already registered with the Agency for similar antifoulant use patterns (PC Code 128101) only the new active *Econea* was evaluated in a screening-level assessment for occupational exposure concerns.

Chemical-specific worker exposure monitoring data were not submitted, or cited, for use in assessing exposures for industrial shipyard workers as painters and handlers of ***Sigma Nexxium 20 Antifouling*** paint. However, certain data were provided by Janssen Pharmaceutica, Inc. in the form of an occupational exposure report (the most recent version as MRID 468466-02) dated May 15, 2006, entitled "*Revision 1 to MRID No. 46751303: Screening Level Occupational Exposure Assessments For R107894 (AC or CL 303268) As An Antifoulant In Paint Applied To Underwater Hulls of Pleasure, Military, and Commercial Craft.*" This report supports the use pattern for the MUP, but is specifically intended to satisfy human exposure data needs for the formulated ***Sigma Nexxium 20 Antifouling*** EP and addresses potential occupational exposure concerns for representative use patterns. The submitted assessments are intended to qualitatively evaluate the potential worker exposures during shipyard painting operations and address, in a broad sense, the Human Exposure Data requirements under Series 875 Guidelines.[3] As a conservative screening tool the report also includes quantitative dermal/inhalation exposure estimates and calculated MOEs for different painter scenarios (i.e., paint mixer/loader/applicator scenarios) based on surrogate data from the Pesticide Handlers Exposure Database (PHED).

An evaluation of non-dietary occupational exposures was conducted by AD/RASSB on representative scenarios for exposure routes of concern based on the product use patterns and the toxicity of the active ingredient. The assessment relied on certain Agency standard values and surrogate data sources to develop scenarios; including surrogate unit exposure data taken from PHED, Version 1.1 (U.S. EPA, 1998). Registrant-submitted information on shipyard worker observations (MRID 468466-02) was used as "Product Use" and "Description of Human Activity" data to aid in calculating handler doses.

Endpoints for this risk assessment were obtained from the AD/RASSB toxicology memoranda written in support of the registration action for the technical source MUP product ***ECONEA™ Technical*** (U.S. EPA, 2006; U.S. EPA, 2007). Based on submitted acute toxicity

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(PC Code 088002). This product has been submitted for RASSB review of leach rate study data (October, 2006), but not for a worker exposure assessment.

[3] The data covered in the report covers the minimum requirements for an EP (i.e., data to address GLN 875.1700 and 875.2700 *Product Use Information*, and GLN 875.2800 *Description of Human Activity* to better characterize the nature of the potential application/post-application exposures). The quantitative exposure estimates in the report follow the Agency's initial recommendation from a March 7, 2001 pre-registration meeting, to submit a 'human health exposure risk assessment' as an alternative to generating any Application and/or Post-Application Guideline Studies under GLN 875.1100/GLN 875.1200 *Dermal Exposure Outdoor/Indoor* and GLN 875.1300/GLN 875.1400 *Inhalation Exposure Outdoor/Indoor*.

studies, *Econea* (93.2% a.i., MUP) is most acutely toxic via the oral (Toxicity Category I - DANGER) and inhalation (Toxicity Category II) routes and the MUP concentration does not elicit dermal sensitization. In the case of the formulated ***Sigma Nexxium 20 Antifouling*** paint, the EP product appears to be of moderate toxicity for eye irritation (Toxicity Category II) and acute oral and primary skin irritation (Toxicity Category III). Study data indicate that the formulation is a dermal sensitizer. The ***Sigma Nexxium 20 Antifouling*** draft product labeling includes the signal word "CAUTION" and precautionary statements for handlers to wear at a minimum, a powered air purifying respirator (PAPR) and indirect references to other personal protective equipment (PPE) in the form of gloves and protective clothing.

The short-term (ST)/intermediate-term (IT) dermal endpoint for *Econea* is the systemic NOAEL $\geq$ 1000 mg/kg/day from an upgraded 90-day dermal study in the rat (U.S. EPA, 2007). This dermal endpoint replaces the oral NOAEL <6.3 mg/kg/day (90-day oral toxicity study in the rat) previously selected for conducting the dermal assessment presented in the November 15, 2006 review memorandum.

A 90-day inhalation toxicity study in the rat was used for the ST/IT inhalation endpoint. The NOAEL could not be calculated so the LOAEL air concentration of <20mg/m^3 was selected based on local irritation effects of the dorsal region of the nose. Since the adverse effects were due to point-of-entry localized irritation, it was assumed that an inhalation reference dose (RfD) could be estimated as 5.7 mg/kg/day if needed as an alternate to the air concentration LOAEL in developing exposure scenario risk determinations, as per Agency methodologies (U.S. EPA, 1989 and 1994).

The level of concern (LOC) for *Econea* ST/IT dermal route exposures is 100, and for inhalation route exposures it is 300 [i.e., a margin of exposure (MOE) less than 100 (dermal) and 300 (inhalation) indicates potential risk concerns] for occupational scenarios. The LOCs are based on uncertainty factors of 10x interspecies extrapolation, 10x intraspecies variation, and in the case of inhalation route exposure, a 3x for data gaps where a LOAEL was selected for lack of a NOAEL. Dermal penetration factors were not assigned, nor an inhalation absorption factor applied since the endpoints were derived from route-specific studies. A body weight of an average male adult (i.e., 70 kg) was used to estimate exposure doses.

Occupational Exposure Summary

Occupational handler risks were assessed for shipyard workers in contact with antifoulant paint containing *Econea*. All occupational exposure scenarios were assumed to be short-term (ST), 1 to 30 days, and intermediate-term (IT), occurring for periods of 30 days to 6 months. No endpoints were identified for long-term (LT) exposure scenarios (i.e., exceeding 6 months duration). Handler dermal and inhalation exposure scenarios were identified for shipyard workers involved with painting operations using ***Sigma Nexxium 20 Antifouling***; including paint tenders who perform ancillary tasks of mixing/loading paints, and paint applicators engaged in predominantly airless sprayer applications, and to a minor degree, brush/roller touch-up painting.

The handler exposures were estimated at baseline (i.e., single layer clothing, no gloves or respirator) and for various personal protective equipment (PPE) scenarios where different clothing attire (i.e., gloves and Tyvek coveralls) and respirator options (i.e., dust/mist, organic/vapour, powered air and supplied air respirators) are employed, as applicable. The Agency generally is not concerned with a calculated MOE greater than or equal to the target MOE (i.e., level of concern). Results from the screening-level occupational exposure assessment indicate that the following handler scenarios exceed the Agency's level of concern [i.e., MOEs below the target of 100 (dermal) and 300 (inhalation)] and therefore pose potential risks:

Industrial Shipyard Scenarios:

Paint Applicators (Airless Sprayer- primary):

ST/IT dermal exposure: Baseline MOE = 87 (using Agency estimates for amt. handled);

ST/IT inhalation exposure: Baseline MOE = 7;
PPE-PHED Organic Vapour Respirator MOE = 60.

All other scenarios evaluated yielded exposure estimates which do not pose a risk concern (i.e., MOEs well above the Agency targets). The handler assessment confirms the need for clear labeling language requiring dermal and respiratory PPE during product use and adherence of industrial safety standards for workers engaged in commercial antifoulant applications/handling.

Occupational postapplication exposures were not assessed. No exposure data have been submitted to the Agency to determine the extent of postapplication exposures for worker scenarios. It is assumed that exposures in industrial settings following painting operations will be less than handler exposure during painting. Inhalation concerns post-treatment should be minimal due to the non-volatile nature of the active ingredient (1.9×10^{-8} kPa at 20°C) and the lack of aerosol/spray mist generation once boat hull painting is done. *Econea* is unlikely to volatilize appreciably at room temperature during clean-up tasks. Dermal contact with painted surfaces is also not a concern since workers do not remain in work areas or re-enter as fresh paint is drying.

Data Limitations and Uncertainties

The following items are some of the data limitations and uncertainties associated with the occupational exposure assessment:

- Chemical-specific exposure monitoring data were not available; therefore as is policy, the Agency used surrogate data sources and standard approaches which may not realistically estimate exposure during actual use conditions. Surrogate unit exposure values were taken from the Pesticide Handlers Exposure Database (PHED) (USEPA, 1998). (See Appendix A for a summary of PHED data).

- AD/RASSB relied upon professional judgment of industrial practices, product labeling use rates/methods and certain registrant-provided inputs (taken from submitted data

MRID 468466-02) for estimating quantity handled/day and surface area painted/day. It is not known if the observational data for shipyard painting operations (MRID 468466-02) may actually underestimate the quantity handled/area painted (e.g., application rate of 500 sq ft/hr and 3000 sq ft/day painted surface area). Therefore, Agency default values for estimating amount handled were also used for a comparison assessment.

Review Outcome and Recommendations

At this time the screening-level assessment supports a "conditional" registration of the new active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a., *Econea*) and its intended commercial antifoulant use patterns, with the following considerations:

ECONEA™ Technical MUP 93.2% a.i. (EPA File Symbol 43813-ET):

- As a note to PM Team 33 regulatory management, the ***ECONEA™ Technical*** MUP draft product labeling of May 2, 2002 is incomplete. Specific formulator use directions covering the industrial application methods/use rates were not cited on the label nor provided in the form of a technical bulletin.

- Also, precautionary labeling statements for this powder formulation will need revising according to FIFRA guidance for Toxicity Category I-II products which carry the "DANGER" Signal Word. Specifically, the addition (beyond the cited protective eyewear, e.g., goggles, face shield or safety glasses) of clear PPE statements for handlers to wear long-sleeved shirt, long pants, socks and shoes, chemical-resistant gloves, and a dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C).

Sigma Nexxium 20 Antifouling EP 3.4% a.i. (EPA File Symbol 11350-GL):

- The PPE precautionary labeling statements are not acceptable on the draft labeling/technical bulletin submitted May 7, 2002 for the ***Sigma Nexxium 20 Antifouling*** EP. Based on acute toxicity (Categories II-III) and primary use in airless spray applications, appropriate PPE must include clothing and respiratory protection reflective of industry standards for commercial handlers of antifoulant paints. Specifically, PPE shall include use of protective eyewear (e.g., goggles, face shield or safety glasses), statements for handlers to wear long-sleeved shirt, long pants, socks and shoes, and chemical-resistant gloves. The labeling already states that *"While spraying and/or sanding boat surfaces, wear a minimum of a powered air purifying respirator (PAPR) jointly approved by the Mining Enforcement and Safety Administration and the National Institute for Occupational Safety and Health."* Due to concerns for spray paint operators in confined/enclosed spaces, the respiratory protection statement can be revised to include *"...wear a supplied-air respirator (SAR) or powered air purifying respirator (PAPR)..."*.

- Since the EP is a dermal sensitizer, the labeling must state: *Prolonged or Frequently Repeated Skin Contact May Cause Allergic Reactions in Some Individuals*. Additional

dermal PPE statements should be considered as: *Wear a Tyvek hooded coverall or the exact equivalent, impervious gloves and impervious footwear that protects the lower legs.* The precautionary labeling should also include: *Unprotected persons should be kept out of the treatment areas within walking distance of XXX feet* (i.e., registrant-specified distance, such as 100 feet).

- The EP labeling includes certain restrictions for commercial shipyard use only. However, the PM Team 33 may wish to require additional, prominent, use restriction statements such as: *For Professional Application Only, Not for Use by Private Applicators or Marinas.*

- The occupational assessment covers use patterns specified for the formulated EP, ***Sigma Nexxium 20 Antifouling*** as a shipyard antifoulant coating. If this EP use pattern is amended to include uses outside of commercial shipyards, for smaller craft in marinas or D-I-Y applications, AD/RASSB will need to conduct a revised assessment of potential risks.

1.0 INTRODUCTION

1.1 Purpose

In support of registration for ***ECONEA ™ Technical*** MUP, as the a.i. technical source product, and the formulated ***Sigma Nexxium 20 Antifouling*** paint EP, *Janssen Pharmaceutica Inc.* (MUP registrant) and *Sigma Coatings USA* (EP registrant) jointly submitted registration applications to the Agency in May, 2002. The Antimicrobials Division (AD), Product Management Team 33, requested that the Risk Assessment and Science Support Branch (RASSB) review the registration application for the new antifoulant active ingredient, *1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)* (a.k.a. *Econea)* in its proposed use in the formulated end-use product (EP) ***Sigma Nexxium 20 Antifouling***, a self-polishing antifouling coating containing a co-biocide mixture of 3.4% *Econea* a.i., and 3.4% *Sea-Nine 211* a.i. (C-9211, RH-287, or Kathon 287T).

As part of the review AD/RASSB conducted an initial occupational exposure assessment of the EP, as presented in the November 15, 2006 review memorandum. This assessment is now revised and updated to reflect the selection of a new dermal toxicity endpoint (i.e., a systemic NOAEL $\geq$ 1000 mg/kg/day) which replaces the one used initially (i.e., oral NOAEL <6.3 mg/kg/day) to determine short-term and intermediate-term dermal risks for shipyard painters in contact with this new antifoulant active ingredient, *Econea.* In addition, the level of concern associated with the new endpoint has also been revised to reflect a target margin of exposure (Target MOE) of 100 for the dermal route, replacing the initial Target MOE of 300.

1.2 Criteria for Conducting Exposure Assessments

An occupational exposure assessment is required for an active ingredient if (1) certain toxicological criteria are triggered <u>and</u> (2) there is potential exposure to handlers (mixers, loaders, applicators, etc.) during use or to persons entering treated sites after application is

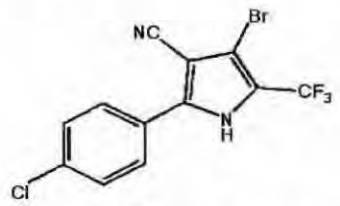
complete. For *Econea*, both criteria are met in the proposed use as a commercial antifoulant coating.

As part of this review of ***Sigma Nexxium 20 Antifouling***, an exposure assessment was conducted to determine if there are any non-dietary occupational exposure concerns for adult handlers during mixing/loading/application of antifoulant coatings in shipyards and any workplace postapplication concerns. The Agency reviewed the draft product labeling and technical data sheet, submitted screening-level assessment (MRID 468466-02), and the toxicity profile/hazard characterization for this compound, in order to assess occupational exposures.

1.3 Chemical Identification and Physical/Chemical Properties

Econea is a metabolite (AC 303268 or CL 303268) of the registered insecticide *Chlorfenapyr* (PC Code 129093). It functions as an uncoupler of oxidative phosphorylation in the mitochondria of cells as the mode of action. The physical/chemical characteristics of *Econea* are identified in Tables 1 and 2 as follows:

Table 1. Chemical Nomenclature/Product Identification for *Econea* Technical Grade Active

Compound	The chemical structure of AC 303268 is as follows:  *Econea*
Common name	*Econea* known as AC 303268 (trade name), R107894, Econea 028 or AF028.
PC Code	119093
IUPAC name*	1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)
CAS #	122454-29-9
Empirical Formula	$C_{12}H_5BrClF_3N_2$
MUP/EP formulations	Formulated as *ECONEA ™ Technical* (MUP containing 93.2% AC 303268) and *Sigma Nexxium 20 Antifouling* (EP containing 3.4% AC 303268 and 3.4% Sea-Nine 211 as co-biocides)

Source: Table based on AD/RASSB Product Chemistry Review for AC 303268 TGAI, by R. Quick, Chemist, dated August 27, 2003, (D289033); also, an AD/RASSB Environmental Fate Assessment of Econea Technical by S. Gowda, Chemist, dated August 17, 2006 (D330789). * International Union of Pure and Applied Chemistry designation (IUPAC).

Table 2. Physicochemical Properties of *Econea* Technical Grade Active	
Parameter	**Value**
Physical state	TGAI is a powder
Color	Off-white to pale yellow-brown
Odor	Slightly sweet, marzipan-like
Melting point/range	252.3 - 253.4 °C
pH	5.16 at 22°C (0.1% w/v dispersion in water)
Relative density	1.714 g/ml (s.d. 0.007)
Water solubility (mg/L at 20°C)	solvent / solubility freshwater (pH 4.9) 0.17 mg/L (Low Water Solubility) seawater (pH 8.1) 0.16 mg/L
Vapor pressure at 25°C	1.9×10^{-8} kPa at 20°C and 4.6×10^{-8} kPa at 25°C (non-volatile)
Dissociation constant (pK_a)	$pK_a = 7.08$ at 26°C
Octanol/water partition coefficient Log(K_{ow})	log $P_{ow} = 3.5$ at pH 5
UV/visible absorption spectrum	Wavelengths of maximum absorbance are 281.4, 281.9, and 223.9 for acidic, neutral and alkaline conditions, respectively.

Source: Table based on AD/RASSB Product Chemistry Review for AC 303268 TGAI, by R. Quick, Chemist, dated August 27, 2003, (D289033).

1.4 Use Profile

The ***Sigma Nexxium 20 Antifouling*** EP is for commercial application (in commercial or government shipyards only) to metal and fiberglass boat hulls to control barnacle growth in fresh and salt water. The antifoulant coating is to be applied primarily by airless sprayer, yet a brush or roller may be used for touch-up and repair only. By restricting the use applications to commercial handlers in shipyards it is inferred that this EP will not be sold for use by either residential do-it-yourself (DIY) applicators on recreational boats, or private operators in marinas and small boatyards.

In a pre-registration meeting of March 7, 2001, the registrants (*Janssen Pharmaceutica Inc.* and *Sigma Coatings USA)* noted that the new active ingredient has been in use as an antifoulant in parts of Europe since 2000 (i.e., in Italy, Greece and Spain). They emphasized use of the EP for coating commercial vessels and government and Navy ships; and they noted that use on pleasure craft is not intended at present but may be considered by Sigma at a future date. An overview of the product use applications is shown in Table 3.

Table 3. *Sigma Nexxium 20 Antifouling* Use Overview: Proposed Use Applications Based on Draft Product Labeling/Technical Data Sheet	
Formulation	A Ready-to-Use (RTU) Viscous Self-Polishing Antifouling Paint containing 3.4% *Econea* and 3.4% *Sea-Nine 211* as co-biocides. (Net Contents: 5 U.S. Gallons). Color: Redbrown (5299)- flat
Active Ingredient % (PC Code)	*Econea* (AC 303268) 3.4% (119093)
Product Density	12.5 lbs/gal mass density (0.425 lbs a.i./gallon). Solids content 55% by volume.
Labeling Restrictions/Precautions	Applicators are Commercial/Professional Handlers – (NOT private operators or D-I-Y applicators. NOT for application by homeowners.) *Commercial Users:* "For Commercial Use Only." "For Use in Commercial or Government Shipyards Only".
Product Use/Application Rates/Methods	*Product Use Directions:* "For use on metal and fiberglass boat hulls to control barnacle growth in fresh and salt water. Do not apply below 36°F unless care is taken to insure absence of frost/ice. Surfaces to be painted shall be free of dirt, oil, grease and other surface contaminants. Apply in accordance with Sigma's directions or specifications given in the Technical Data Sheet for 5299." *Application Rates/Methods:* "Airless Spray" painting equipment is recommended for *Sigma Nexxium 20 Antifouling* application (2100-2500 p.s.i. specified nozzle pressure; 0.021 -0.027 inch nozzle orifice). "Brush/Roller" may be used for touch-up and repair only. • Paint may be thinned with a paint thinner (max 2% in volume). • Paint should be stirred well before use, preferably by means of a mechanical mixer, to ensure homogeneity. • Theoretical Spreading Rate: 220 ft ^{2}/gal at 4 mils. dry film thickness as optimal (range: 3 mils/294 ft ^{2}/gal to 6 mils/147 ft ^{2}/gal). • Minimum Drying Times: *Touch dry* - after 1 hour; *Overcoating interval* – minimum 8 hours; *Refloating time* – minimum 12 hours.
Personal Protective Equipment (PPE) and Precautionary Statements	*Signal Word:* CAUTION. *Warning:* Keep out of Reach of Children. *Precautionary Statements:* "Harmful if inhaled. Causes moderate eye irritation. Avoid contact with eyes or clothing. Avoid breathing spray mist. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove contaminated clothing and wash clothing before reuse." *PPE for Applicators and other Handlers:* • (As per Draft Label): "While spraying and/or sanding boat surfaces, wear a minimum of a powered air purifying respirator (PAPR) jointly approved by the Mining Enforcement and Safety Administration and the National Institute for Occupational Safety and Health." • (As per Technical Data Sheet 5299): "Gloves and fresh air mask recommended, see safety sheets 1430, 1431 and relevant material safety data sheet."

Source: *Sigma Nexxium 20 Antifouling* draft product labeling and technical data sheet (no. 5299) dated May 7, 2002.

2.0 TOXICOLOGICAL HAZARD AND ENDPOINT SELECTION

Endpoints for the risk assessment were obtained from the AD/RASSB toxicology memoranda written in support of the registration action for the technical source MUP product ***ECONEA ™ Technical*** (U.S. EPA, 2006; U.S. EPA, 2007); especially, the revised February 5, 2007 document by Toxicologists S.L. Malish and J. Chen, entitled "*Toxicity Study Review of Econea-Technical (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]) and Toxicology Endpoints for Risk Assessment*". The review summarized the acute toxicity and subchronic studies for the technical active and the revised selection of toxicological endpoints for assessing occupational/residential exposure risks.[4] An overview is presented in Tables 4 and 6.

2.1 Acute Toxicity

Based upon acceptable guideline studies reviewed for the technical source MUP, ***ECONEA ™ Technical*** (93.2% a.i. powder), *Econea* shows severe acute toxicity via the oral route (Toxicity Category I - DANGER) and is moderately toxic via inhalation (Toxicity Category II). It is mildly toxic through the dermal route, and from primary eye/skin irritation studies (Toxicity Categories III and IV). The MUP concentration does not elicit dermal sensitization.

In the case of the formulated ***Sigma Nexxium 20 Antifouling*** paint, the EP product appears to be of moderate toxicity (Toxicity Category II – eye irritation). It is moderately toxic for acute oral and primary skin irritation (Toxicity Category III) and mildly toxic via the dermal route (Toxicity Category IV). However, study data indicate that the formulation is a dermal sensitizer. The registrant requested a waiver for the acute inhalation data requirement based on the rationale that the active is not respirable due to the particle size distribution (5-10 microns), but the Agency denied the data waiver request since particles of that size range are still inhalable. It is not known if the registrant has further addressed this data gap. The acute toxicity data for *Econea* MUP and EP are summarized below in Tables 4 and 5.

[4] The inclusion in the toxicity study review of residential endpoints is based on any non-dietary incidental oral ingestion scenarios that may be developed for this active ingredient. Since *Econea* is an antifoulant for paints used in fresh water, the possibility of incidental oral ingestion of residues in potable drinking water might occur.

Table 4. Acute Toxicity Data on *ECONEA™ Technical* (93.2% a.i. powder) MUP				
Guideline No.	**Study Type**	**MRID #**	**Results**	**Toxicity Category**
870.1100	Acute Oral – Rat (Limit Test) **	45673915	LD_{50} Not established *[Acceptable/Guideline]*	I
870.1200	Acute Dermal - Rabbit**	45673916	LD_{50} > 2000 mg/kg for both males and females *[Acceptable/Guideline]*	III
870.1300	Acute Inhalation – Rat	45673917**	LC_{50} = 790 mg/m^3 (males) LC_{50} = 790 mg/m^3 (females) LC_{50} = 770 mg/m^3 (combined) *[Unacceptable/Guideline]* based on the fact that the percentage of particles below 4 um at the low and mid-dose level did not meet the EPA recommendations.	III
		46846601*	LC_{50} = <510 mg/ m^3 (males) LC_{50} = <510 mg/ m^3 (females) LC_{50} = <510 mg/ m^3 (combined) *[Acceptable/Guideline]*	II
870.2400	Primary Eye Irritation – Rabbit (Limit Test)**	46539401	Mildly irritating *[Acceptable/Guideline]*	III
870.2500	Primary Skin Irritation – Rabbit (Limit Test)**	45673918	Mildly irritating based on very slight erythema, but no edema at 72 hours *[Acceptable/Guideline]*	IV
870.2600	Dermal Sensitization - Guinea Pig**	45673919	Not a dermal sensitizer *[Acceptable/Guideline]*	N/A

* revised memorandum of February 5, 2007 from S.L. Malish and J. Chen to N. Cook {Toxicity Review of *Econea*-Technical (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]) and Toxicology Endpoints for Risk Assessment } for D323129, D327538, D328778, D330458 and D335209.
** memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole…[ECONEA technical]: Review of Toxicology data submitted in Support of Registration).

Table 5. Acute Toxicity Data on *Sigma Nexxium 20 Antifouling* (3.4% a.i.) paint EP				
Guideline No.	**Study Type**	**MRID #**	**Results**	**Toxicity Category**
870.1100	Acute Oral – Rat	45673202	LD_{50} = 5068 mg/kg (males) (95% CI: 4850 – 5303) LD_{50} = 3869 mg/kg (females) (95% CI: 3754 -3987) *[Acceptable/Guideline]*	III
870.1200	Acute Dermal - Rabbit	45673203	LD_{50} = > 5050 mg/kg (males) LD_{50} = > 5050 mg/kg (females) LD_{50} = > 5050 mg/kg (combined) *[Acceptable/Guideline]*	IV
870.1300	Acute Inhalation - Rat		Data Gap - No Data *[Waiver Denied]* *	Unknown
870.2400	Primary Eye Irritation - Rabbit	45673204	Moderate irritation based on corneal opacity, erythema, and chemosis. *[Acceptable/Guideline]*	II
870.2500	Primary Skin Irritation - Rabbit	45673205	Based on observations of erythema (resolved within 7 days) and edema (resolved within 48 hours). *[Acceptable/Guideline]*	III
870.2600	Dermal Sensitization - Guinea Pig	45673206	Modified Buehler method *[Acceptable/Guideline]*	Dermal Sensitizer

Source: Table based on AD/PSB Acute toxicity Review for EPA Reg. No. 11350-GL (*Sigma Nexxium 20 Antifouling)* by C. Jiang, Chemist, dated May 18, 2005 (D316716). * Waiver for acute inhalation is denied due to particle size (5-10 microns) considered inhalable.

2.2 Dose Response Assessment

Based on the toxicity review, study data for ***ECONEA ™ Technical*** (93.2% a.i.) indicate that *Econea* is not mutagenic, nor a developmental toxicant. However, study data show that neurotoxic effects in rats were seen (decreased motor activity in males, and axonal degeneration in the peroneal nerve in females). It is unknown if *Econea* is associated with reproductive or carcinogenic effects since toxicity studies to address these concerns have not been submitted to support the technical active or product registrations.

The short-term (ST)/intermediate-term (IT) dermal endpoint for *Econea* is the systemic NOAEL ≥ 1000 mg/kg/day from an upgraded 90-day dermal study in the rat (U.S. EPA, 2007). This dermal endpoint replaces the oral NOAEL <6.3 mg/kg/day (90-day oral toxicity study in the

rat) previously selected for conducting the dermal assessment presented in the November 15, 2006 review memorandum. The 90-day dermal rat study (MRID 46802201) was initially found to be deficient for lack of performed lung histopathology, but upgradeable. The Agency requested that lung pathology be evaluated to note the frequency of lung lesions at the low and intermediate dose levels to better define the NOAEL/LOAEL. The registrant responded by submitting additional data (MRID 47006601) to upgrade the original study report. Upon review, these data were found to be acceptable, satisfying guideline requirements and upgrading the study to "ACCEPTABLE-GUIDELINE".

A 90-day inhalation toxicity study in the rat was used for the ST/IT inhalation endpoint. The NOAEL could not be calculated so the LOAEL air concentration of <20mg/m^3 was selected based on local irritation effects of the dorsal region of the nose. An absorption factor was not applied to inhalation exposures because the endpoint was derived from an inhalation route-specific study. Since the adverse effects were due to point-of-entry localized irritation, it was assumed that an inhalation reference dose (RfD) could be estimated as 5.7 mg/kg/day if needed as an alternate to the air concentration LOAEL in developing exposure scenario risk determinations, as per Agency methodologies (U.S. EPA, 1989 and 1994).

The level of concern (LOC) for *Econea* ST/IT dermal route exposures is 100, and for inhalation route exposures it is 300 [i.e., a margin of exposure (MOE) less than 100 (dermal) and 300 (inhalation) indicates potential risk concerns] for occupational scenarios. The LOCs are based on uncertainty factors of 10x interspecies extrapolation, 10x intraspecies variation, and in the case of inhalation route exposure, a 3x for data gaps where a LOAEL was selected for lack of a NOAEL. Dermal penetration factors were not assigned, nor an inhalation absorption factor applied since the endpoints were derived from route-specific studies.

A summary of dose levels and endpoints selected for use in human risk assessments of *Econea* are presented below in Table 6. Endpoints for adult dermal and inhalation routes of exposure will be used in this assessment.

Table 6. Summary of Toxicological Doses and Endpoints for *ECONEA™ Technical* for Use in Human Risk Assessment

Exposure Scenario	Dose Used in Risk Assessment	Target MOE for Risk Assessment	Study and Toxicological Effects
Acute and Chronic Dietary	Risk assessment not required based on use pattern.		
Non-Dietary Occupational/Residential Exposures			
Incidental Oral Short-Term (1-30 days) and Intermediate-Term (1–6 months) (Occupational /Residential)	**NOAEL** = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intraspecies variation, 3x for lack of a NOAEL in females)	**Subchronic (90 Day) Oral - Rat** **LOAEL** = 6.3 mg/kg/day (LDT) for females based on microscopic findings of the brain and spinal cord. *Classification: Acceptable/Guideline*

Table 6. Summary of Toxicological Doses and Endpoints for *ECONEA™ Technical* for Use in Human Risk Assessment			
Exposure Scenario	**Dose Used in Risk Assessment**	**Target MOE for Risk Assessment**	**Study and Toxicological Effects**
Dermal* Short-Term (1-30 days) and Intermediate-Term (1–6 months) (Occupational)	**Dermal Signs:** **NOAEL** = 100 mg/kg/day **Systemic+:** **NOAEL** = ≥ 1000 mg/kg/day (HDT, limit dose)	Target MOE = 100 (Dermal signs and systemic) (10x interspecies extrapolation, 10x intraspecies variation +)	**Subchronic (90 Day) Dermal - Rat** **Dermal Signs:** **LOAEL** = 300 mg/kg/day based on the appearance of sores. **Systemic+:** **LOAEL** > 1000 mg/kg/day *Classification: Acceptable/Guideline*
Dermal Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		
Inhalation ** Short-Term (1-30 days) and Intermediate (1 – 6 months) Term (Occupational)	**NOAEL** could not be calculated (<20mg/m3) (LDT). [Since toxicity was due to point-of-entry effects (i.e., local irritation of the dorsal region of the nose), an **Inhalation RfD** was estimated as 5.7 mg/kg/day.]	Target MOE = 300 10x interspecies extrapolation, 10x intraspecies variation, 3x conversion of LOAEL to NOAEL	**Subchronic (90 Day) Inhalation Toxicity – Rat (Phase I)** **LOAEL** is 20-mg/m^3 based on the findings observed in the nasal section N1 to N3 and included chronic and subacute inflammation , ulcerations, exudates, epithelial hyperplasia, hyperkeratosis and degeneration of the olfactory epithelium. Decreased body weight gain was also seen at this dose level. *Classification: Acceptable/Guideline*
Inhalation Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern.		
Cancer	No cancer data available.		

Source: Table based on AD/RASSB revised memorandum of February 5, 2007 from S.L. Malish and J. Chen to N. Cook ,*"Toxicity Study Review of Econea-Technical (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]) and Toxicology Endpoints for Risk Assessment"* (D323129, D327538, D328778, D330458 and D335209).

NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, LDT = lowest dose tested, HDT = highest dose tested (limit dose) and MOE = margin of exposure.

* Dermal – A dermal penetration factor is not established for use of a route-specific dermal endpoint.

+ The primary consideration for the short- and intermediate-term dermal exposure scenario is the systemic NOAEL/LOAEL; the dermal signs NOAEL/LOAEL is of secondary (minimal) importance.

** Inhalation – Where an Inhalation Reference Dose (RfD) is needed the following conversion equation was used: Estimated Inhalation RfD (mg/kg/day) = (Toxic concentration 20-mg/m^3 x Adult daily inhalation rate 20 m^3/day) / Adult body weight 70 kg. This approach assumes that the same air concentration would cause equivalent effects in both rat and human receptors. (Guidance from J. Chen based on U.S. EPA, 1989 & 1994.)

4.0 OCCUPATIONAL EXPOSURE ASSESSMENT

4.1 Occupational Exposure/Risk Pathway

Sigma Nexxium 20 Antifouling is a ready-to-use paint containing 3.4% *Econea*. The label limits the applications to commercial applicators in commercial or government shipyards. The labeling states that at a minimum a PAPR respirator jointly approved by the Mine Safety and Health Administration and the National Institute for Occupational Safety and Health (NIOSH) must be worn while spraying paint, sanding, or sandblasting boat surfaces. The labeling indirectly recommends gloves (technical data sheet) but does not instruct painters to wear protective clothing such as protective eyewear (safety glasses, goggles or face shield), chemical resistant gloves, long sleeved cotton shirt, long pants, and hat. ***Sigma Nexxium 20 Antifouling*** is applied at 220 sq. ft/gallon as the optimal spreading rate (dry film thickness of 4 mils). The labeling cites an overcoating interval of 8 hours minimum, so only one full coat is assumed to be applied per day.

Based on the use patterns specified for ***Sigma Nexxium 20 Antifouling***, AD/RASSB has determined that there is a potential for exposures to mixers, loaders, applicators, or other handlers associated with the new active ingredient, *Econea*. Table 7 presents the exposure scenarios assessed in this document.

Table 7. Shipyard Exposure Scenarios Associated with Occupational Handlers of *Econea* in *Sigma Nexxium 20 Antifouling*

Representative Use	Method of Application	Exposure Scenario	Application Rate (% a.i.)
Use Site Category: IX - Antifoulant Paints			
Antifoulant paint applications to boat hulls	Paint Tenders: • Open pour liquid mixing/loading	ST/IT : Dermal and Inhalation (Baseline and PPE)	3.4% (RTU)
	Paint Applicators: • airless sprayer (primary) • bush/roller (secondary)	ST/IT : Dermal and Inhalation (Baseline and PPE)	3.4% (RTU)

ST= short-term (1 to 30 days), IT=intermediate-term (1 to 6 months), RTU = ready-to-use formulation.

Industrial shipyard operations are closely regulated as an industry sector by The U.S. Department of Labor's Occupational Safety & Health Administration (OSHA). These include oversight of worker safety practices during painting operations, inclusive of antifoulants. OSHA has set compliance standards [1915.35] for clothing PPE and respiratory protection during shipyard spray applications and hand applications (brush or roller). In particular, powered air purifying respirators (PAPRs) are cited when ambient air ventilation is adequate, and air-line respirators (i.e., supplied-air respirators – SAR)

are specified while painting/working in confined or enclosed spaces. Since limited PPE is specified on the ***Sigma Nexxium 20 Antifouling*** labeling (powered air purifying respirator- PAPR) and technical data sheet (gloves/fresh air mask) several PPE scenarios will be assessed. Typical industrial safety practices include the following PPE: Hood/head cover, safety glasses/goggles, appropriate respirator, coveralls, Tyvek paper suit, gloves, safety shoes and barrier cream for exposed facial skin as appropriate. Handlers review labels and Material Safety Data Sheets (MSDS) and comply with OSHA regulations (ACC, 2005).

4.2 Occupational Handlers Exposures and Risks

Chemical-specific worker exposure monitoring data were not submitted or cited in support of this new antifoulant (*Econea*) to assess exposures for industrial shipyard workers as painters and handlers of ***Sigma Nexxium 20 Antifouling*** paint. However, certain data were provided by Janssen Pharmaceutica, Inc. in the form of an occupational exposure report (the most recent version as MRID 468466-02) dated May 15, 2006, entitled "*Revision 1 to MRID No. 46751303: Screening Level Occupational Exposure Assessments For R107894 (AC or CL 303268) As An Antifoulant In Paint Applied To Underwater Hulls of Pleasure, Military, and Commercial Craft.*" The assessments are intended to evaluate the potential worker exposures during commercial painting operations. As a conservative screening tool the submitted report included qualitative observations of shipyard workers and quantitative dermal/inhalation exposure estimates and calculated MOEs for different painter scenarios (i.e., paint mixer/loader/applicator scenarios) based on surrogate data from the Pesticide Handlers Exposure Database (PHED).

An evaluation of non-dietary occupational exposures was conducted by AD/RASSB on representative scenarios for exposure routes of concern based on the ***Sigma Nexxium 20 Antifouling*** product labeling use patterns and the toxicity of the active ingredient. (See Table 8.) No chemical-specific exposure data are available. Therefore, the assessment relied on certain Agency standard values and surrogate data sources to develop scenarios; including surrogate unit exposure data taken from PHED, Version 1.1 (U.S. EPA, 1998). Certain registrant-submitted information on shipyard worker observations (MRID 468466-02) was used to aid in calculating handler doses at baseline and when PPE is worn:

Shipyard Workers Engaged in Boat Hull Painting Operations

The assessment presented is based on label specifications for ***Sigma Nexxium 20 Antifouling*** :

- the mass density of the product (12.51 lb/gal) and
- spreading rate (220 sq.ft/gal) as optimal at 4 mils dry film thickness
- 3.4% a.i., Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl) (*R107894*, PC Code:119093)

The submitted data (MRID 468466-02) cites the following painting operation observations. These observations were presented based on shipyard activities. When boats are drydocked, the hulls are high-pressure washed with water, sanded or grit-blasted as necessary to remove old coatings, coated with primers, then painted with antifoulants (and other coatings as needed). Actual antifoulant painting activities represent a small fraction of the overall time spent in refurbishing drydocked vessels:

- *Paint Tenders:* 1-3 paint stations/worker will be tended for paint applicators so it is estimated that they handle 3X the amount of paint (a.i.) as a painter. 5-gal pails of paint are handled from pallets and taken to pressure units where thinners may be added and paint mixing conducted using an electric-powered agitator. Coatings are poured (open pour) from pail-to-pail. Typical PPE is Tyvek coveralls over street clothes; leather safety boots; hard hats; eye protection; and heavy rubber gloves. (*Note: no respiratory protection.*)

- *Paint Applicators (Airless Sprayer):* The paint station consists of the pressure unit for the airless spraygun. A take-up hose in a can of paint supplied the pressure unit, which sent paint through a 30 meter hose to the handheld spraygun. The painter (applicator) worked from the floor or from the basket of a man-lift. PPE in addition to the standard safety boots and hard hats, typically included a range of clothing ensembles based on the coating being applied. For an antifoulant, clothing may include long pants and long-sleeved shirts to full PPE: Tyvek coveralls; woven fabric hood under a hard hat; eye protection; respirator; and gloves. "During a normal work shift, a worker will spend approximately 6 hours painting. He will handle the most paint when the entire hull is being coated to 4 mils, on average 500 sq. ft. per hour."

- Unit Exposures are from PHED where the following PPE and corresponding protection factors were incorporated:

- long pants, long sleeved shirts (baseline)
- additional layer of clothes as in Tyvek coveralls (50% protection factor),
- chemical resistant gloves (90% protection factor when no gloved data are available),
- dust/mist respirator (80% protection factor)
- organic vapour respirator (90% protection factor).

- Other protection factors applied are the assigned protection factors (APF) from OSHA's National Institute for Occupational Safety and Health (NIOSH) for powered air purifying (PAPR) and supplied air (SAR) respirators (NIOSH, 2004):

- PAPR-full-facepiece /SAR-full-facepiece respirator (50X protection factor as

1/50th of airborne contaminants inhaled)

- SAR-full-facepiece pressure demand (1000X protection factor as 1/1000th of airborne contaminants inhaled).

Assumptions and factors used in the shipyard scenario assessments detailed in Table 8 are described below:

- **Unit Exposures (UE):** ***Shipyard Antifoulant Painters (Applicators)***
  Dermal and inhalation unit exposure values were taken from the Pesticide Handler Exposure Database. Antifoulant paints are applied primarily by airless sprayers in shipyards and dry docks (ACC, 2005). Therefore, for professionals applying *Econea*-based antifoulant paint to ships, the PHED dermal and inhalation unit exposure values for ***airless sprayer*** applications (PHED scenario 23) were utilized in this assessment (USEPA, 1998). The dermal unit exposure is 38 mg/lb a.i. for ungloved replicates (single layer of clothing) and 14.34 mg/lb a.i. for gloved replicates. A scenario was added for the addition of an additional layer of clothes as Tyvek coveralls (50% protection factor to torso) to cover body as 13.6 mg/lb ai.

- The PHED inhalation unit exposure value for the ***airless sprayer*** application was available in terms of an air concentration (mg/m^3/% a.i.) as well as, in terms of amount handled (mg/lb a.i.). Since the inhalation toxicity endpoint was determined from an inhalation study (as opposed to an oral study), the endpoint units are given in terms of an air concentration (mg/m^3). Therefore, in order to more accurately estimate inhalation risks (MOEs), it was more appropriate to use the unit exposure value in terms of an air concentration (mg/m^3/% a.i.) rather than amount handled (mg/lb a.i.) (See Appendix A). Furthermore, although the label does require the use of a PAPR class respirator, it does not specifically identify the type of PAPR respirator (e.g., full-facepiece or hood/helmet) to be worn. Nor is there mention of special respiratory protection for work in enclosed or confined spaces (i.e., OSHA specified SAR respirators). Therefore, this assessment evaluated other PPE scenarios in addition to the baseline (i.e., no respirator) inhalation exposures; including PHED 90% PF for organic vapour respirator (10X); a PAPR(or SAR) full-face respirator that provide a 50X protection factor, and a SAR full face respirator with positive pressure that provides a 1000X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.681 mg/m^3/% a.i for baseline, 0.0681 mg/m^3/% a.i for PHED respirator, 0.0136 mg/m^3/% a.i for a PAPR(SAR) full face respirator, and 0.00068 mg/m^3/% a.i for a SAR full face respirator with positive pressure.

- Shipyard painters use ***brush and roller*** equipment for touch-up and repair as minor painting techniques. There are no chemical-specific exposure data to assess these techniques, however surrogate PHED data are available for painting with a brush. The surrogate data are based on painters wearing long pants, long sleeve shirts, no gloves, and no respirator. The PHED test subjects were monitored while painting a bathroom with a paint brush. Although the exposures while

painting a boat hull will differ, these data are judged to be adequately representative. For the ***brush and roller*** applications, dermal and inhalation unit exposure were taken from PHED Scenario 22. The dermal unit exposure is 180 mg/lb a.i. for ungloved replicates (single layer clothing; long-sleeved shirt, long pants; no gloves) and 24 mg/lb a.i. with gloves. A scenario was added for the addition of an additional layer of clothes as Tyvek coveralls (50% protection factor to torso) yielding 22 mg/lb a.i.. The inhalation unit exposure is 0.28 mg/lb a.i. (no respirator). Baseline inhalation exposures (i.e., no respirator) as well as exposures wearing a PHED dust/mist respirator which provides 80% protection (20X), a PHED organic vapour respirator that provides a 90% protection factor (10X), and a PAPR(or SAR) full-face respirator that provide a 50X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.28 mg/lb a.i. for baseline, 0.056 mg/lb a.i. for a PHED dust/mist, 0.028 mg/lb a.i. for PHED vapour respirator, and 0.0056 mg/lb a.i. for a PAPR(or SAR) full-face respirator.

- **Unit Exposures (UE):** ***Shipyard Antifoulant Paint Tenders (Mixer/Loader)***
  For paint tenders, the most representative surrogate unit exposure data available is PHED Scenario 3, ***open mixing/loading*** of all liquids. The label does not specify mandatory use of gloves when pouring and blending the paints, so this assessment evaluated workers with and without (i.e., baseline) gloves. The dermal unit exposures are 2.9 mg/lb a.i. for ungloved replicates and 0.023 mg/lb a.i. for gloved replicates. A scenario was added for the addition of an additional layer of clothes as Tyvek coveralls (50% protection factor to torso) yielding 0.01748 mg/lb a.i.. Furthermore, although the label does require the use of a PAPR while spray painting, it does not specify respiratory protection for ancillary workers engaged in paint mixer/loader tasks (i.e., paint tenders). Therefore, this assessment evaluated the baseline inhalation exposures (i.e., no respirator) as well as exposures wearing a PHED dust/mist respirator which provides 80% protection (20X), a PHED organic vapour respirator that provides a 90% protection factor (10X), and a PAPR(or SAR) full-face respirator that provide a 50X protection factor (NIOSH, 2004). The inhalation unit exposure values are 0.0012 mg/lb a.i. for baseline, 0.00024 mg/lb a.i. for a PHED dust/mist, 0.00012 mg/lb a.i. for a PHED vapour respirator, and 0.000024 mg/lb a.i. for a full face respirator.

- **Amount handled**: Based on AD's standard assumptions and knowledge of shipyard painting practices, a shipyard painter over the course of a day may handle upwards of 50 gallons of paint via airless sprayer depending on the coating and desired film thickness (industry estimate ACC, 2005). In contrast, based on submitted observational data (MRID 468466-02) a shipyard painter will handle closer to 14 gallons of paint using an ***airless sprayer*** for 3000 sq ft of coverage (observations of 500 sq ft/hr x 6 hr/day x gal/220 sq ft = 14 gallons applied at a dry film thickness of 4 mils). Therefore, this ST/IT assessment used both the 14 gallon (registrant observational data) and 50 gallon (Agency default) estimates for comparison. For ***brush and roller***, as a minor painting scenario, Agency standard estimates of 5 gals/day were used (or 63 lbs/day, assuming paint has a density of

12.51 lbs/gal) equating to 1100 sq ft of coverage at a maximum. For ***open mixing/loading***, paint tenders handle 3X the amount of paint as do airless spray painters. Therefore 42 gallons and 150 gallons were estimated using registrant data and Agency assumptions (3 x 14 gals for airless = 42 gallons/day; 3 x 50 gals for airless = 150 gallons/day).

- **Exposure time**: (For a*irless sprayer* scenario only.) As previously mentioned, the inhalation unit exposure for the airless sprayer was provided in terms of an air concentration (mg/m^3/% a.i.) due to the use of an inhalation (not oral) toxicity study. Since the inhalation toxicity study was based on the animals being exposed to the chemical for 6 hours per day, the worker exposure and MOE equation had to be modified to account for the amount of time that the worker is actually exposed to the active ingredient. In this case, it was assumed that it would take a professional shipyard painter 6 hours to accomplish this task using an airless sprayer (MRID 468466-02, and ACC, 2005).

Table 8. ST/IT Exposures and MOEs for Shipyard Painters of *Sigma Nexxium 20 Antifouling* Paint *

Scenario	Application Rate (% ai)	Dermal UE (mg/lb ai)	Dermal PPE	Inhalation Air Conc UE (mg/m3/%ai)	Respiratory PPE	Amount Handled (lb/day) [a]	Dermal Dose (mg/kg/day) [b]	Dermal MOE [c] Target = 100	Exposure Time (hr/day)	Inhalation Air Conc MOE [d] Target = 300
Airless sprayer	3.4% *Econea*	38	Baseline	0.681	Baseline	175 (625)	3.23 (11.54)	310 **(87)**	6.0	**7**
		14.34	Gloves	0.0681	PHED Respirator	175 (625)	1.22 (4.35)	820 (230)		**60**
		13.6	Tyvek coveralls	0.0136	PAPR-face resp	175 (625)	1.2 (4.13)	833 (242)		400
		--	--	0.00068	SAR-Full-face resp. w/ pos. pressure	175 (625)	--	--		6000
Scenario	**Application Rate (% ai)**	**Dermal UE (mg/lb ai)**	**Dermal PPE**	**Inhalation UE (mg/lb ai)**	**Respiratory PPE**	**Amount Handled (lb/day) [a]**	**Dermal Dose (mg/kg/day) [b]**	**Dermal MOE [c]**	**Inhalation Dose [b] (mg/kg/day)**	**Inhalation MOE [c]**
Brush & Roller	3.4% *Econea*	180	Baseline	0.28	Baseline	63	6	167	0.0086	663
		24	Gloves	0.056	PHED Dust/Mist	63	0.734	1362	0.002	2850
		22	Tyvek coveralls	0.028	PHED Respirator	63	0.673	1486	0.0009	6333
		--	--	0.0056	PAPR-face resp	63	--	--	0.0002	28,500
Paint Tenders: (open pour mix/load)		2.9	Baseline	0.0012	Baseline	525 (1877)	0.74 (3)	1351 (333)	0.0003 (0.0011)	19,000 (5,182)
		0.023	Gloves	0.00024	PHED Dust/Mist	525 (1877)	0.006 (0.021)	166,667 (47,619)	0.00006 (0.00022)	95,000 (25,909)
		0.01748	Tyvek coveralls	0.00012	PHED Respirator	525 (1877)	0.0045 (0.016)	222,222 (62,500)	0.00003 (0.00011)	190,000 (51,818)
		--	--	0.000024	PAPR-face resp	525 (1877)	--	--	0.000006 (0.000022)	950,000 (259,091)

Note: * Values in parentheses ( ) denote calculated estimates based on Agency default assumptions for amount handled. UE = Unit Exposure, as derived from PHED Version 1.1, August, 1998 "PHED Surrogate Exposure Guide". PHED values as described within this review.

PHED values for Dermal/Inhalation UE and PPE: Baseline Dermal = "Single Layer, No Gloves" to represent a single layer of work clothes (long-sleeve shirt, long pants) and no protective gloves; Dermal PPE = the use chemical-resistant gloves and coveralls added to the baseline clothing scenario. Baseline Inhalation = No respirator used; Respiratory PPE = Use of a specified respirator: PHED Dust/Mist, PHED Respirator (organic vapour), PAPR full-face, and SAR full-face with positive pressure.

a: lb/day = gal/day x 12.51 lb/gal as paint density: 14 gallons or (50 gallons) Airless; 5 gallons Paint Brush; 42 gallons or (150 gallons) Paint tenders.

b: Dose (mg/kg/day) = app rate (%ai) x UE (mg/lb ai) x quantity handled (lb/day) / BW (70 kg).

c: MOE = ST/IT NOAEL (mg/kg/day) / Dose (mg/kg/day) where dermal systemic ST/IT NOAEL ≥1000 mg/kg/day and inhalation ST/IT NOAEL = 5.7 mg/kg/day.

d: air conc inhalation MOE = (ST/IT NOAEL of 20 mg/m^3 x animal exposure time 6 hrs/day) / [(UE mg/m^3/% ai x % ai (not fraction a.i.) x worker exposure time hr/day) x (1.25 m^3 per hour inhalation rate)].

4.3 Occupational Postapplication Exposure

Occupational postapplication exposures were not assessed. No exposure data have been submitted to the Agency to determine the extent of postapplication exposures for worker scenarios. It is assumed that exposures in industrial settings following painting operations will be less than handler exposure during painting. Inhalation concerns post-treatment should be minimal due to the non-volatile nature of the active ingredient (1.9×10^{-8} kPa at 20°C) and the lack of aerosol/spray mist generation once boat hull painting is done. *Econea* is unlikely to volatilize appreciably at room temperature during clean-up tasks. Dermal contact with painted surfaces is also not a concern since workers do not remain in work areas or re-enter as fresh paint is drying.

5.0 ASSESSMENT OF DATA GAPS AND UNCERTAINTIES

The non-dietary occupational exposure assessment was conducted for the new antifoulant active ingredient *Econea*, in the end-use paint product, ***Sigma Nexxium 20 Antifouling*** to identify any potential exposure concerns associated with this new pesticide. The following items are some of the data limitations and uncertainties for this assessment:

- Chemical-specific exposure monitoring data were not available; therefore as is policy, the Agency used surrogate data sources and standard approaches which may not realistically estimate exposure during actual use conditions. Surrogate unit exposure values were taken from the Pesticide Handlers Exposure Database (PHED) (USEPA, 1998). (See Appendix A for a summary of PHED data).

- AD/RASSB relied upon professional judgment of industrial practices, product labeling use rates/methods and certain registrant-provided inputs (taken from submitted data MRID 468466-02) for estimating quantity handled/day and surface area painted/day. It is not known if the observational data for shipyard painting operations (MRID 468466-02) may actually underestimate the quantity handled/area painted (e.g., application rate of 500 sq ft/hr and 3000 sq ft/day painted surface area). Therefore, Agency default values for estimating amount handled were also used for a comparison assessment.

6.0 REFERENCES

ACC. 2005. American Chemistry Council (ACC). Antifouling Biocides and Paints. Presented to USEPA Antimicrobials Division by ACC Biocides Panel and NPCA Marine Antifoulants Committee. June 8, 2005.

NIOSH. 2004. NIOSH Respirator Selection Logic 2004. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health. October 2004.

U.S. EPA. 1989 and 1994. Interim Methods for Development of Inhalation Reference Doses. (1989, EPA/600/8-88/066F). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. (1994, EPA/600/8-90/066F). U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA. 1998. PHED Surrogate Exposure Guide. Estimates of Worker Exposure from the Pesticide Handler Exposure Database Version 1.1. Washington, DC: U.S. Environmental Protection Agency. August, 1998.

U.S. EPA. 2006. Toxicity Study Review of *Econea*-Technical [*1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)*] and Toxicology Endpoints for Risk Assessment. Memorandum from S.L. Malish, Ph.D., and J. Chen, Toxicologists, U.S.EPA, RASSB/AD to Norm Cook, Chief, RASSB/AD. Dated June 29, 2006. DP Barcodes D323129, D327538, D328778, and D330458.

U.S. EPA. 2007. *(Revised Memorandum)* Toxicity Study Review of *Econea*-Technical [*1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)*] and Toxicology Endpoints for Risk Assessment. Memorandum from S.L. Malish, Ph.D., and J. Chen, Toxicologists, U.S.EPA, RASSB/AD to Norm Cook, Chief, RASSB/AD. Dated February 5, 2007. DP Barcodes D323129, D327538, D328778, D330458 and D335209.

cc: Doreen Aviado/RASSB/AD
Chemical File
Circulation File

APPENDIX A: Summary of PHED data

The Pesticide Handlers Exposure Database (PHED):

The Pesticide Handlers Exposure Database (PHED) has been developed by a Task Force consisting of representatives from Health Canada, the U.S. Environmental Protection Agency (EPA), and the American Crop Protection Association (ACPA). PHED provides generic pesticide worker (i.e., mixer/loader and applicator) exposure estimates. The dermal and inhalation exposure estimates generated by PHED are based on actual field monitoring data, which are reported generically (i.e., chemical specific names not reported) in PHED. It has been the Agency's policy to use "surrogate" or "generic" exposure data for pesticide applicators in certain circumstances because it is believed that the physical parameters (e.g., packaging type) or application technique (e.g., aerosol can), not the chemical properties of the pesticide, attribute to exposure levels. [Note: Vapor pressures for the chemicals in PHED are in the range of E-5 to E-7 mm Hg.] Chemical-specific properties are accounted for by correcting the exposure data for study specific field and laboratory recovery values as specified by the PHED grading criteria.

PHED handler exposure data are generally provided on a normalized basis for use in exposure assessments. The most common method for normalizing exposure is by pounds (lbs) of active ingredient (ai) handled per replicate (i.e., exposure in mg per replicate is divided by the amount of ai handled in that particular replicate). These unit exposures are expressed as mg/lb ai handled. This normalization method presumes that dermal and inhalation exposures are linear based on the amount of active ingredient handled.

For the assessment of Shipyard workers using airless spraying techniques to apply *Econea*-based antifoulant paints, the PHED data for the Airless Sprayer scenario (Scenario 23) was used. Since a route-specific inhalation toxicity endpoint for *Econea* was selected for determining risk, the normalized inhalation unit air concentration (0.681 mg/m3/%ai) from PHED was used for exposure dose calculations. Attached are the raw data for the air concentrations in PHED study 0467 during airless sprayer application (exterior house stain containing 0.5% ai fungicide applied to a residence). The air concentrations are reported in this spreadsheet as monitored in the study (mg/m3) as well as "unit exposures (UE)" normalized to lb ai handled (mg/m3/lb ai) and also normalized by % ai in the stain (mg/m3/%ai). They are actually "unit air concentrations", not "exposures". The % ai in the final treatment solution/antifoulant coating is used as the normalization variable rather then lb ai handled (i.e., mg/m3/%ai).

PHED Airless Sprayer Study 0467 Data – (exterior house stain application to residential home 0.5% a.i. fungicide)

Record I.D.	Total AI applied (lb)	Volume handled (gallons)	Final Conc (lb ai/gal)	Treatment Solution (% ai)	Air Sample Time (min)	Spray Rate (gal/hour)	Air Amount (ug)	Air LOQ (ug)	Air Volume (liter)	Average Flow Rate (l/min)	Air Conc. Study (ug/L)	Air Conc. Study (mg/m3)	Air Conc. Normalized (mg/m3/lb ai)	Air Conc. Normalized (mg/m3/%ai)
0467*C*01	0.1667	5	0.03334	0.5	17	17.6	4.3	2	34	2	0.126	0.126	0.759	0.253
0467*E*01	0.1667	5	0.03334	0.5	25	12.0	13.6	2	50	2	0.272	0.272	1.632	0.544
0467*G*01	0.1667	5	0.03334	0.5	20	15.0	10	2	40	2	0.250	0.250	1.500	0.500
0467*I*01	0.1667	5	0.03334	0.5	16	18.8	14.4	2	32	2	0.450	0.450	2.699	0.900
0467*K*01	0.1667	5	0.03334	0.5	18	16.7	13.7	2	36	2	0.381	0.381	2.283	0.761
0467*O*01	0.1667	5	0.03334	0.5	13	23.1	13.7	2	26	2	0.527	0.527	3.161	1.054
0467*A*01	0.1667	5	0.03334	0.5	27	11.1	8.8	2	54	2	0.163	0.163	0.978	0.326
0467*M*01	0.1667	5	0.03334	0.5	16	18.8	4.4	2	32	2	0.138	0.138	0.825	0.275
0467*B*01	0.1667	5	0.03334	0.5	13	23.1	20.1	2	26	2	0.773	0.773	4.638	1.546
0467*F*01	0.1667	5	0.03334	0.5	17	17.6	5.4	2	34	2	0.159	0.159	0.953	0.318
0467*H*01	0.1667	5	0.03334	0.5	11	27.3	5.5	2	22	2	0.250	0.250	1.500	0.500
0467*J*01	0.1667	5	0.03334	0.5	11	27.3	12.8	2	22	2	0.582	0.582	3.490	1.164
0467*D*01	0.1667	5	0.03334	0.5	21	14.3	16.8	2	42	2	0.400	0.400	2.400	0.800
0467*L*01	0.1667	5	0.03334	0.5	12	25.0	7.2	2	24	2	0.300	0.300	1.800	0.600
0467*N*01	0.1667	5	0.03334	0.5	13	23.1	8.7	2	26	2	0.335	0.335	2.007	0.669
Geometric mean	0.1667	5	0.033	0.5	16.0	18.7	9.5	2	32.1	2	0.297	0.297	1.783	0.594
Arithmetic mean	**0.1667**	**5**	**0.033**	**0.5**	**16.7**	**19.4**	**10.6**	**2**	**33.3**	**2**	**0.340**	**0.340**	**2.041**	**0.681**
std	5.7E-17	0	0.000	0.0	4.9	5.2	4.8	0	9.8	0	0.184	0.184	1.101	0.367
median	0.1667	5	0.033	0.5	16.0	18.8	10.0	2	32.0	2	0.300	0.300	1.800	0.600
75%tile	0.1667	5	0.033	0.5	19.0	23.1	13.7	2	38.0	2	0.425	0.425	2.549	0.850
90%tile	0.1667	5	0.033	0.5	23.4	26.4	15.8	2	46.8	2	0.560	0.560	3.358	1.120
maximum	0.1667	5	0.033	0.5	27.0	27.3	20.1	2	54.0	2	0.773	0.773	4.638	1.546

Record I.D.= Record identification from PHED study code 0467. Airless sprayer (3000 psi).
Volume handled represents 5 gallons of house stain sprayed. 5 gal covered 750 to 1250 sq. ft.
Final Concentration (lb ai/gal) = actual concentration used during the study (total lb ai / gallons sprayed).
Treatment solution (% ai) = 0.5% ai as reported in PHED study code 0467 (fungicide in house stain).
Air sample time (minutes) = the exposure time for each replicate in PHED study 0467 that the personal air pump ran.
Spray rate (gal/hour) = volume sprayed (5 gal) / (exposure duration in minutes/60 min per hour).
Air amount (ug) = amount of chemical analyzed on each air sampler.
Air LOQ (ug) = limit of quantification for air samplers, note: all values were detectable.
Air volume (liters) = volume of air sampled per replicate.
Average flow rate (l/min) = personal air samplers run at 2 l/min.
Air concentration in study = air amount (ug) / Air Volume (L); equivalent to mg/m3; Air Concentration normalized (mg/m3/lbai) = Air concentration in study / lb ai handled per replicate.
Air Concentration normalized (mg/m3/%ai) = Air concentration in study / % ai (0.5% ai used, not weight fraction of 0.005).

DATA PACKAGE BEAN SHEET

Date: 21-Feb-2007

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-6341 Room# PY1 S-8828

Risk Manager Reviewer: Norman Cook NCOOK

Sent Date: 23-Aug-2006 Calculated Due Date: 30-Mar-2007 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NON-FOOD USE;OUTDOOR;OTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 12-Jan-2007 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title:

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #: 335209

Assigned To	Date In	Date Out	
Organization: AD / RASSB	12-Jan-2007	21-Feb-2007	Last Possible Science Due Date: 22-Sep-2006
Team Name: RASSB2	12-Jan-2007	21-Feb-2007	Science Due Date:
Reviewer Name: Aviado, Doreen	12-Jan-2007	21-Feb-2007	Sub Data Package Due Date:

Contractor Name:

* * * Studies Sent for Review * * *

No Studies

* * * Additional Data Package for this Decision * * *

Printed on Page 2

* * * Data Package Instructions * * *

Sub-bean for potential revision of exposure/MOE assmt based on add'l toxicity data. NCook

Material to be added to a Mini-Jacket
(in the case where an e-Jacket exists)

Reg. No. 43813-ET 27

Send to SIG: check box ☒

This material is:

- ☒ New stamped-accepted label
- ☐ New CSF
- ☐ Notification
- ☐ Final Printed Label
- ☒ Other: Registration Notice and DCRs

Instructions: Attach this notice on top of the material. It must be clipped all together and there should be NO STAPLES in the material. Then give the material with this coversheet to staff in the Information Services Center (Room 230).

Reviewer's Name: Karen Leary

Phone: 308-6237 Division: AD

Date: 5/17/07

Explore Registrations

Reg Number: 43813-ET Reg. Type: Product Registration - Section 3 Status: Under Review (02-May-2002)

Name: ECONEA TECHNICAL <View Registration Details>

(No New Receipts)

S:	Submission Type	OPP Rec'vd Date	Resubmission	Description

- D: Pending; 220066; 43813-ET; A41; NEW AI;
  - DP 292015;AD / RASSB; Complete Date
  - S: 802542 12/13/2006; Pending Product
  - S: 798301 8/23/2006; Pending Product A
  - S: 794761 5/31/2006; New Registration;
  - S: 792764 4/4/2006; Pending Product A
  - S: 791292 3/9/2006; New Registration;
  - S: 790457 2/15/2006; New Registration;
  - S: 789834 2/3/2006; Pending Product A
  - S: 787676 12/6/2005; New Registration;
  - S: 785105 10/5/2005; Pending Product A
  - S: 782775 8/10/2005; Miscellaneous Re
  - S: 781775 7/18/2005; Pending Product A
  - S: 778775 5/11/2005; Pending Product A
  - S: 778408 5/4/2005; Pending Product A
  - S: 774033 1/24/2005; New Registration;
  - S: 772850 1/11/2005; New Registration;
  - S: 771841 12/16/2004; Pending Product
  - S: 765679 6/4/2003; New Registration;
  - S: 759391 1/21/2004; New Registration;
  - S: 741978 6/4/2003; New Registration;

Decision Sequence: 220066

Action: A41 NEW AI;NON-FOOD USE;OUTDOOR;OTHER USES;

Number: 43813-ET Original Decision:

Name: ECONEA TECHNICAL

Decision Status: PENDING (28-Mar-2003)

Organization Owner: AD / RMB1

Team Owner: RM 33

FFS Start Date 22-Jun-2004 Received by Risk Manager: 19-Mar-2003

Due Date 08-Jan-2007 FFS Amt Expected: $82,500

Negotiated Due Date: 23-Apr-2007 FFS Amt Refunded:

FFS Amt Received: $82,500

Comments:

Dennis H Edwards
4/23/07

Recommendation of Division Directors Negotiated Due Dates		
Decision#:	Registration# 43813-ET	Petition #:
Fee Category: A - 49	PRIA Decision Time Frame: 180 days	
Submitted by: Marshall Swindell	Branch: RMBI	Date: 03/20/2007
Company: Janessen Pharmaceutica, Inc		
Original Due Date: March 30, 2007	Proposed New Due Date: April 9, 2007	
Previous Negotiated Due Dates: No		
Is the "Fix" in-house? No	If not, date "Fix" expected: April 8, 2007	
Issue (describe in detail): Need to reconcile issues associated the persistence of the chlorfenapyr degradate, Econea, which is the active ingredient subject to this regulatory action for a technical and two EPs.		
NA		
Janessen Pharmaceutica, Inc is requesting an extension until April 9, 2007 to allow inter-office reconciliation of persistence issues.		
Other Comments/Deficiency Type: Product Chemistry: _ Acute Tox: ___ Efficacy: ____ Labeling: ____ Other: _ None at this time.___		
Approve: ✓	Disapprove:	
If disapproved, action to be taken:		
OD or DOD Signature: Marshall Swindell		Date: 3-30-07

Recommendation of Division Directors Negotiated Due Dates		
Decision#: 220066	Registration#: 43813-ET	Petition #: N/A
Fee Category: A-41	PRIA Decision Time Frame: 21 months	
Submitted by: Karen Leavy	Branch: RMBI	Date: 04/06/07
Company: Janssen Pharmaceutica, Inc.		
Original Due Date: 03/30/07	Proposed New Due Date: 4-23-07	
Previous Negotiated Due Dates: yes, 4-9-07		
Is the "Fix" in-house? Yes	If not, date "Fix" expected:	

Issue (describe in detail):

Additional time are needed to reconcile issues associated with Chlorfenapry degradate, Econea, which is the active ingredient subject to this regulatory action subject to this regulatory action. Meeting held with EFED, AD and Anne Lindsay on 4/4/07 to discuss these issues.

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates):

This is the second negotiation for this registration application. The company has requested an additional time extension of two weeks to allow time for these issues to be addressed.

Rationale for Proposed Due Date: This time extension will allow time to reconcile issues and revise the briefing memo for this new active ingredient.

Other Comments/Deficiency Type:

Product Chemistry: __ Acute Tox: ___ Efficacy: ____ Labeling: ____ Other: ___

Approve:	Disapprove:

If disapproved, action to be taken:

OD or DOD Signature: Anne E Lindsay	Date: 4/6/07

eruckert@mwe.com
04/06/2007 11:36 AM

To Betty Shackleford/DC/USEPA/US@EPA, Dennis Edwards/DC/USEPA/US@EPA
cc bgoodwin@janus.jnj.com
bcc
Subject Econea//Janssen EPA File Symbol 43813-// Extension request

Betty,
This will confirm that Janssen is willing to extend the date for the Econea registration decision from Monday, April 9, 2007 to Monday April 23, 2007. As you are aware, there is significant interest in the registration progress of this product in both the private and public sectors because of the significant copper environmental issue it helps address. To that end, there will be a series of meetings during the April 17 -20 time frame to consider the next steps for the chemical. It would be appreciated that if at all possible, the registration decision not necessarily take the full two weeks to be made. However, we can understand, given people's schedules that the full time might be required. We do appreciate the Agency's efforts to complete the registration of the compound. Ed Ruckert Counsel for Janssen

Edward M. Ruckert
McDermott Will & Emery LLP
600 - 13th Street, NW
Washington, DC 20005
202.756.8214
202.756.8087 FAX

****
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Recommendation of Division Directors Negotiated Due Dates		
Decision#: 220066	Registration#: 43813-ET	Petition #: N/A
Fee Category: A-41	PRIA Decision Time Frame: 21 months	
Submitted by: Karen Leavy	Branch: RMBI	Date: 04/06/07
Company: Janssen Pharmaceutica, Inc.		
Original Due Date: 03/30/07	Proposed New Due Date: 4-23-07	
Previous Negotiated Due Dates: yes, 4-9-07		
Is the "Fix" in-house? Yes	If not, date "Fix" expected:	

Issue (describe in detail):

Additional time are needed to reconcile issues associated with Chlorfenapry degradate, Econea, which is the active ingredient subject to this regulatory action subject to this regulatory action. Meeting held with EFED, AD and Anne Lindsay on 4/4/07 to discuss these issues.

Describe Interactions with Company (describe when contacted and company's response including response to previous negotiated due dates):

This is the second negotiation for this registration application. The company has requested an additional time extension of two weeks to allow time for these issues to be addressed.

Rationale for Proposed Due Date: This time extension will allow time to reconcile issues and revise the briefing memo for this new active ingredient.

Other Comments/Deficiency Type:

Product Chemistry: __ Acute Tox: ___ Efficacy: ____ Labeling: ____ Other: ____

Approve:	Disapprove:

If disapproved, action to be taken:

OD or DOD Signature: Anne E Lindsay	Date: 4/6/07

DECISION PKG. NO. 220066 SUBM. DUE DATE 4/23/07

SUBMISSION BAR CODE # 802542 REVIEWER (MU)

CODING FORM FOR APPLICATIONS FOR REGISTRATION/AMENDMENTS

FILE SYMBOL/REG NO. 4-3813-ET PM 33 ACTION CODE A47

DESCRIPTOR PRIA **FQPA**____ **NFQPA**____

[ ] CHILD RESISTANT PACKAGING: [ ] REQUIRED [ ] NOT REQUIRED

REGISTRATION TYPE: [ ] CONDITIONAL [ ] UNCONDITIONAL [ ] RESTRICTED USE

DATE ON APPLICATION	EPA RECEIVE DATE	PM RECEIVE DATE
12 / 11 / 06	12 / 13 / 06	12 / 14 / 06

METHOD OF SUPPORT		FORMULATORS EXEMPTION	
[ ] CITE-ALL	[ ] SELECTIVE	[ ] SUBMITTED	[ ] NOT SUBMITTED
[ ] NOT SUBMITTED	[ ] N/A	[ ] N/A	

REVIEW(S) REQUESTED	DATA PACK #	DATE SENT	DUE DATE	DATE RETURNED
CHEMISTRY				
EFFICACY				
ACUTE TOX.				
RASSB TOX.				
ENVIRON. FATE				
FISH/WILDLIFE				
OTHER: :				

STATUS_____

RESPONSE CODE 1180 RESPONSE DATE 4/23/07

SCIENCE GROUP	DIVISION	BRANCH	SECTION	CSF Y/N	LABEL Y/N
CHEMISTRY	AD	EASSB	CTT		
EFFICACY	AD	EASSB	EET		

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Antimicrobials Division (7510C)
1200 Pennsylvania Avenue NW
Washington, D.C. 20460

NOTICE OF PESTICIDE:
_x_ Registration
__ Reregistration

(under FIFRA, as amended)

EPA Reg. Number: 43813-27

Date of Issuance:

Term of Issuance:

Name of Pesticide Product: ECONEA TECHNICAL

Name and Address of Registrant (include ZIP Code):

Janessen Pharmaceutica, Inc.
1125 Trenton-Harbourton Road
Titusville, NJ 08560-0200

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered/reregistered under the Federal Insecticide, Fungicide and Rodenticide Act.

Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

1. Make the labeling changes listed below before you release the product for shipment:

a. Add the phrase "EPA Registration Number 43813-27".

Signature of Approving Official:

Dennis H Edwards Jr. for

Marshall Swindell
Product Manager Team-33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Date: 042307

EPA Form 8570-6

The Confidential Statement of Formula dated January 4th, 2007, is acceptable.

2. Submit three (3) copies of the final printed label prior to releasing this product for sale.

If these conditions are not complied with, the registration will be subject to cancellation in accordance with FIFRA sec. 6(e). Your release for shipment of the product constitutes acceptance of these conditions.

A stamped copy of the label is enclosed for your records.

Sincerely,

Dennis H Edwards for

Marshall Swindell
Product Manager 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Enclosure: (Stamped Labeling)

ECONEA® Technical

Anti-fouling Preservative

For Formulating Use Only

ACTIVE INGREDIENT:
Tralopyril*.....93.2%

INERT INGREDIENTS:	6.8%
TOTAL:	100.0%

* CAS# 122454-29-9

KEEP OUT OF REACH OF CHILDREN

DANGER POISON

See side panel for first aid and additional precautionary statements

EPA Reg. No.: 43813-xx

EPA Est. No.: 241-MO-001

Manufactured for: **JANSSEN PHARMACEUTICA**
1125 Trenton-Harbourton Road
Titusville, NJ 08560

ACCEPTED
with COMMENTS
in EPA Letter Dated:
042307
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
43813-27

® Registered Trademark of Janssen Pharmaceutica

Rev. 03/07

ACCEPTED
with COMMENTS
in EPA Letter Dated:
042307
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
43813-27

PRECAUTIONARY STATEMENT
HAZARDS TO HUMANS AND DOMESTIC ANIMALS
DANGER

Fatal if swallowed. Harmful if inhaled or absorbed through the skin. Causes moderate eye irritation. Avoid breathing dust. Avoid contact with skin, eyes, or clothing. Wash hands thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Remove contaminated clothing and wash clothing before reuse.

Handler Personal Protective Equipment (PPE):

- Wear long-sleeved shirt, long pants, socks, shoes, and chemical resistant natural rubber gloves.
- Wear protective eyewear such as goggles, face shield or safety glasses.
- Wear dust filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, R, P, or HE filter.

Pesticide User Safety Requirements:
Discard clothing and other absorbent materials that have been heavily contaminated with this product. Do not reuse them. Follow the manufacturer's instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

FIRST AID	
If swallowed	-Call a poison control center or doctor immediately for treatment advice. -Have person sip a glass of water if able to swallow. -Do not induce vomiting unless told to do so by a poison control center or doctor. -Do not give anything by mouth to an unconscious person.
If inhaled	-Move person to fresh air. -If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. -Call a poison control center or doctor for further treatment advice
If in eyes	-Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. -Call a poison control center or doctor for treatment advice.
If on skin or clothing	-Take off contaminated clothing. -Rinse skin immediately with plenty of water for 15-20 minutes. -Call a poison control center or doctor for treatment advice.

HOT LINE NUMBER:

For information on this pesticide product (including health concerns, medical emergencies, or pesticide incidents) call the National Pesticide Telecommunications Information Center at 1-800-858-7378.

For chemical emergency assistance (spill, leak, fire, or accident), call Chem Trec at 1-800-424-9300.

Have the product container with you when calling a poison control center or doctor, or going for treatment.

NOTE TO PHYSICIAN

Probable mucosal damage may contraindicate the use of gastric lavage.

ACCEPTED
with COMMENTS
in EPA Letter Dated:
042307
Under the Federal Insecticide, Fungicide, and Rodenticide Act as amended, for the pesticide, registered under EPA Reg. No.
43813-27

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.
This product is intended for use only during industrial formulation processes producing antifouling products for control of hard fouling organisms.
Formulators using ECONEA are responsible for providing additional data to support registration of their end-use product(s).

STORAGE AND DISPOSAL

PROHIBITIONS: Do not contaminate water, food or feed by storage and disposal.
STORAGE: DO NOT mix or store this product or solutions of this product in a manner inconsistent with its labeling.
DISPOSAL: Pesticide wastes may be acutely hazardous. Improper disposal is a violation of Federal Law.
PESTICIDE DISPOSAL: Pesticide, mixtures, or equipment rinse waters that cannot be chemically reprocessed must be disposed of according to applicable federal, state or local procedures. Contact your State Pesticide or Environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.
CONTAINER DISPOSAL: Completely empty liner by shaking and tapping sides and bottom to loosen clinging particles. Empty residue into formulation equipment. Then dispose of liner in a sanitary landfill or by incineration if allowed by State and local authorities. If burned, stay out of smoke. If drum is contaminated and cannot be reused, dispose of in the same manner.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA.

NOTICE OF WARRANTY

Janssen Pharmaceutica warrants that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of use or application, all of which are beyond the control of Janssen Pharmaceutica. To the extent permitted by law, Janssen Pharmaceutica disclaims any liability for consequential, special or indirect damages resulting from the use, handling, application, storage or disposal of this product or for damages in the nature of penalties, and the buyer and user waive any right that they may have to such damages. **To the extent consistent with applicable law, Janssen Pharmaceutica makes no warranties of merchantability or of fitness for a particular purpose or any other express or implied warranty except as stated above.**

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES
Antimicrobial Division

Thursday, January 04, 2007

REG. NO. OR FILE SYMBOL:		43813-ET
DP Barcode		D318296
MRID:		None
SUBJECT: (name of product)		Econea Technical
DOCUMENT TYPE		Product Chemistry Review
Manufacturing-Use [ ]	**OR**	**End-Use [x]**
INGREDIENTS (PC Codes):		119093
CAS Number		122454-29-9
TEST LAB:		None
SUBMITTER:		Janssen Pharmaceutica, Inc.
GUIDELINES:		None
COMMODITIES:		
REVIEWER:		Ian Blackwell
APPROVER:		Karen Hicks
COMMENT:		

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES
Antimicrobial Division

TO:	**Marshall Swindell, PM 33/ Karen Leavy** PM Team 33
FROM:	Ian Blackwell, Biologist Product Science Branch, CT Team Antimicrobial Division (7510P)
THRU:	Karen P. Hicks, CT Team Leader Product Science Branch Antimicrobial Division (7510C)
THRU:	Michele E. Wingfield, Chief Product Science Branch Antimicrobial Division (7510C)
APPLICANT:	Janssen Pharmaceutica
Action code:	A41
Due date:	Jan. 8, 2007

Product Formulation

Active Ingredient(s)	Percent by Weight
Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)	93.2

I **BACKGROUND:** Janssen Pharmaceutica has submitted CSFs and chemistry studies to support their product, Econea Technical. This submission includes the following:

1 A basic CSF of 43813-ET dated 4/12/02. The registrant later submitted CSFs dated 11/30/06 and 1/4/07.

2 The following reports: MRID Numbers

- 456739-01 through 456739-07
- 456958-01 through 456958-02
- 465451-01
- 467784-01

II **FINDINGS:**

1 The Upper and Lower Certified Limits (UCL and LCL) of the 1/4/2007 CSF are acceptable. (LCLs are not required for impurities.)

A The LCL of the 11/30/2006 CSF is unacceptable.
B The percents by weight of the 4/12/2002 CSF do not add up to 100%.

2 The label declaration of percent of active ingredient matches that of the submitted ("Rev. 01/04") product label.

3 The reports of the chemical identification, description of materials, and manufacturing process. As this is an integrated product, the formulation process is not required.

4 The discussion of impurities and chemical analysis are acceptable.

5 The Certified Limits presented in the technical report have a problem with the certified limits. The Certified Limits of the 1/4/7 CSF are acceptable.

6 The Analytical Methods for the active ingredient are acceptable.

7 The report of the physical state, color (Munsell reference!) and odor are acceptable. The stability to elevated temperatures and metals is acceptable.

8 The pH test is acceptable.

9 The following assay reports are acceptable:

A 830.7050. UV/visible absorption
B 830.7200. Melting Point/ Melting Range
C 830.7300. Density/ Relative Density
D 830.7370. Dissociation Constant in Water
E 830.7950. Vapor Pressure

10 Determination of boiling point is not required for this solid product.

11 The registrant does not appear to have had a Generator Column Method water solubility assay (830.7860) conducted for this product. This will be considered a data gap for this product.

12 The active ingredient is cleared for use in pesticide products.

III CONCLUSIONS:

1 The 1/4/2007 CSF (basic formulation) is acceptable. All prior CSFs (including the 4/12/2002 and 11/30/2006 CSFs) are unacceptable

2 The label declaration of the percent of active ingredient is acceptable.

3 The registrant must address the absence of the Generator Column Method water solubility assay (830.7860) for this product.

4 The other product chemistry requirements for 43813-ET are satisfied.

PRODUCT CHEMISTRY REVIEW

4. CONFIDENTIAL STATEMENT OF FORMULA

4a. Type of formulation and source registration
☐ Non-integrated formulation system [ ]
☐ Are all TGAI used registered? Yes [X] No [ ]

☐ Integrated formulation system [X]

☐ if "ME-TOO", specify EPA Reg. # of existing product:

4b. Clearance of inerts for non-food or food use:
Cleared for food use under 40 CFR §180.1001: Yes [ ] No [ ] NA [X]

4c. Physical state of product: white powder

4d. The chemical IDs and analytical information (including that for the TGAI), density, pH, and flammability are consistent with that given in 830.1000, Series A and 830.7300, .7000 and .6315 respectively: Yes [X] No [ ]

4h. NCs and CLs are acceptable: [X] Not acceptable [ ]

4i. Active ingredient (s)

		NC	UCL	LCL
A.	Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)	93.2	96	90

4j. For products produced by an integrated formulation system:
☐ All impurities of toxicological significance have a UCL?
Yes [X] No [ ] Not applicable []

☐ All impurities of $\geq$ 0.1% in the product have been identified?

Yes [X] No [ ] Not applicable [ ]

5. PRODUCT LABEL

5a. The active ingredients statement (chemical IDs and NC] is consistent with the CONFIDENTIAL STATEMENT OF FORMULA? Yes [x] No [ ]

5b. The formulation contains one of the following:

☐	10% or more of a petroleum distillate:	Yes [ ]	No [X]
☐	1.0% or more of methyl alcohol:	Yes [ ]	No [X]
☐	Sodium nitrite at any level:	Yes [ ]	No [X]
☐	a toxic List 1 inert at any level:	Yes [ ]	No [X]
☐	arsenic in any form:	Yes [ ]	No [X]

5c. If Yes to any of the above, does the inert ingredients statement contain a footnote indicating this? Yes [ ] No [ ] Not applicable [X]

5d. The appropriate warning statement regarding flammability or explosive characteristics of the product are listed on the label?
Yes [ ] No [ ] Not applicable [X]

5e. The storage and disposal instructions for the pesticide and container are in compliance with PR Notice 84-1 for household use products or PR Notice 83-3 for all other uses? Yes [x] No [ ]

5f. Does the product require an expiration date at which time the NC falls below the LCL (based on the one year storage stability data or other information)?
Yes [ ] No [X]

6.a **PRODUCT CHEMISTRY (Series 830 Part A)**

	Acceptance of Information	MRID No.
830.1550 Chemical ID (See Appendix)[0]	Acceptable	456739-02
830.1600 Description of Materials	Acceptable	456958-02
830.1620 Manufacturing Process[0]	Acceptable	456958-02
830.1650 Formulation Method[0]	N/A	
830.1670 Discussion of Impurities[0]	Acceptable	456739-01
830.1700 Analysis[5]	Acceptable	456739-03, 456739-04, 456739-05
830.1750 Certified Limits[6]	Acceptable	(CSF)
830.1800 Analytical Method for AIs[7]	Acceptable	456739-05

Explanation: A=acceptable; N=not acceptable; NA=technically not applicable; NR= not required,
G=data gap; U=requires upgrading; W=waived; E=EPA estimate.

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[1]See Confidential Appendix A for additional information
[2]For MP/EP products produced by an integrated formulation system.
[3]For products from a TGAI or MP.
[4]May be waived unless actual/possible impurities are of toxicological concern.
[5]Five batch analysis required for products produced by an integrated formulation system.

[6]If different from standard CLS recommended in 40 CFR 158.175, this should be discussed in Confidential Appendix A.

[7]Abbreviate method used as follows: gas chromatography (GC), infrared (IR),

6b. Physical/Chemical Properties*	Acceptance of data	Value or qualitative description	MRID No.
830.6302 Color	Acceptable	Pale yellow-brown Munsell ref. = 5Y8.5/4	456739-07
830.6303 Physical State	Acceptable	powder	456739-07
830.6304 Odor	Acceptable	"Slightly sweet, marzipan-like"	456739-07
830.6313 Stability to Normal and Elevated Temperatures, Metals and Metal Ions	Acceptable	Stable	456739-07
830.6314 Oxidation/Reduction; Chemical Incompatibility		Not required	
830.6315 Flammability/Flame Extension		Not required	
830.6316 Explodability		Not required	
830.6317 Storage Stability		Not required	
830.6319 Miscibility[2]		Not required	
830.6320 Corrosion Characteristics		Not required	
830.6321 Dielectric Breakdown Voltage		Not required	
830.7000 pH[1]	Acceptable	5.16 at 1% w/v	456739-06
830.7050 UV/Visible Absorption	Acceptable	Λ_{Max} = 281.4 nm (acidic) Λ_{Max} = 281.9 nm (neutral methanol)	456739-07
830.7100 Viscosity		n/a	
830.7200 Melting Point/Melting Range	Acceptable	252.3 °C/ 252.3 to 253.4 °C	456739-07
830.7220 Boiling Point/Boiling Range		Not required	
830.7300 Density/Relative Density/Bulk Density Pour density	Acceptable	1.714 (σ_{n-1} 0.007) at 20°C 0.521 g/mL	456739-07
830.7370 Dissociation Constant in Water	Acceptable	PKa= 7.08 at 26°C	456739-06
830.7520 Particle Size, Fiber Length, and Diameter Distribution		Not required for TGAI	
830.7550 Partition Coefficient (n-octanol/H_2O). Shake flask method	Acceptable	7.08 at 25°C	456739-06
830.7560 Partition Coefficient (n-octanol/H_2O). Generator column method.	Acceptable	Log_{POW} = 3.5	456739-06
830.7840 Water solubility: Column elution method. Shake flask method.	Acceptable	0.17 mg/L	456739-06
830.7860 Water Solubility: Generator column method.		Data Gap	
830.7950 Vapor Pressure	Acceptable	1.9×10^{-8} Pa at 20°C	456739-06

Physical and Chemical Characteristics (Series 830, Group B)

Explanation: A=acceptable; N=not acceptable; NA=technically not applicable; NR= Not required G=data gap; U=requires upgrading; W=waived; E=EPA estimate.

* Provide brief description, e.g., color--yellow or property value, e.g., density 1.25 g/cc; Unless otherwise indicated, the property should be at 25^0 C.

Solubility of 43813-ET in Various Samples	
Acetone	300.5 g/L
Ethyl Acetate	236.0 g/L
n-Heptane	7.2 g/L
Methanol	109.1 g/L
n-Octanol	85.2 g/L
Xylene	5.6 g/L

				11/15/2006				
	43813-ET							
93.2	93.2	2.796	95.996	90.404				
0.2								
0.1								
0								
0.2								
0.8								
0.02								
0.4								
0.02								
0.3								
0.3								
0.2								
0								
0.6								
0.001								
0.1								
0.1								
0.1								
0.1								
0.2								
0.3								
97.241								

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Washington, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES
Antimicrobial Division

Thursday, September 14, 2006

REG. NO. OR FILE SYMBOL:		43813-ET
DP Barcode		D328525
MRID:		467784-01
SUBJECT: (name of product)		Econea Technical
DOCUMENT TYPE		Product Chemistry Review
Manufacturing-Use [ ]	**OR**	**End-Use [x]**
INGREDIENTS (PC Codes):		119093
CAS Number		
TEST LAB:		Covance Laboratories Ltd.
SUBMITTER:		Janssen Pharmaceutica N.V.
GUIDELINES:		830.6302, 8306303, 830.6317 and 830.6320
COMMODITIES:		
REVIEWER: Ian Blackwell		
APPROVER: Karen Hicks		9/14/06
COMMENT:		

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES
Antimicrobial Division

TO: Marshall Swindell, PM 33/ Karen Leavy
PM Team 33

FROM: Ian Blackwell, Biologist
Product Science Branch, CT Team
Antimicrobial Division (7510P)

THRU: Karen P. Hicks, CT Team Leader
Product Science Branch
Antimicrobial Division (7510C)

THRU: Michele E. Wingfield, Chief
Product Science Branch
Antimicrobial Division (7510C)

APPLICANT:
Action code: A41
Due date: 1/8/2007

Product Formulation

Active Ingredient(s)	Percent by Weight
1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)	93.2%

I **BACKGROUND:** Janssen Pharmaceutica, Inc., has submitted a three-year Storage Stability/Corrosion Characteristics study in support of their application for the registration of Econea Technical.

II FINDINGS:

1 **Evaluation of the Ambient Temperature Storage** (MRID 467784-01)

2 The following Group B product chemistry data requirements are complete: 830.6302 (Color), and 830.6303 (Physical State).

3 The following Group B product chemistry data requirements are incomplete: 830.6317 (Storage Stability) and 830.6320 (Corrosion Characteristics). The applicant has not determined the concentrations of the active ingredients in the product every 3 months following the study initiation for a minimum of 1 year, but rather every six months for 3 years. The relative humidity during testing period was reported as 60% (deviation from the 50% recommended per OPPTS 830.6317). The applicant has not recorded the weight of the container at each of the test intervals to monitor weight.

4 A Good Laboratory Practices (GLP) statement was provided stating that the study was conducted in compliance with the United Kingdom (GLP Monitoring Authority, Medicines and Healthcare products Regulating Agency (MHRA)) Good Laboratory Practice Regulations 1999, Statutory Instrument 1999 No. 3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004, and the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January, 1998). A GLP statement regarding compliance with 40 CFR Part 160 was not provided. A Quality Assurance Unit statement was provided.

III RECOMMENDATIONS:

1 The storage stability study is acceptable and the product and container pass the test. The provided storage stability studies are not exactly complete in that the test facility failed to make observations three months after storage. However, in that the product was acceptable far beyond the 3-month point, the absence of 3-month data can be disregarded. In the future, for the corrosion characteristics studies, the weight of the container and observations made (e.g., "perforations," "darkening," "leaking or rust at the seam, etc.), should be recorded at each test interval.

April 28, 2006

SUBJECT: PRODUCT CHEMISTRY REVIEW OF Econea Technical

DP Barcode: D328525 **Reg. No. or File Symbol:** 43813-ET
Manufacturing-use [ ] **End-use Product** [ ]

TO: Wallace Powell, EPA Work Assignment Manager
FROM: John S. Chandler, CSS Work Assignment Manager

Product Formulation
Active Ingredients: **% by wt.:**

1H-Pyrrole-3-carbonitrile,
4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl).....93.2%

BACKGROUND:

Janssen Pharmaceutica Inc. has submitted a three-year Storage Stability/Corrosion Characteristics study in support of their application for registration of Econea Technical.

A copy of the Confidential Statement of Formula (CSF) was not provided to CSS for review. Without information regarding nominal and certified limits, CSS was not able to assess whether the concentration of the active ingredient fell outside the limits during the study. CSS has noted concentrations of actives reported in Table B of this report.

FINDINGS:

Evaluation of the Ambient Temperature Storage (MRID 467784-01)

- The following Group B product chemistry data requirements are complete: 830.6302 (Color), and 830.6303 (Physical State).

- The following Group B product chemistry data requirements are incomplete: 830.6317 (Storage Stability) and 830.6320 (Corrosion Characteristics). The applicant has not determined the concentrations of the active ingredients in the product every 3 months following the study initiation for a minimum of 1 year, but rather every six months for 3 years. The relative humidity during testing period was reported as 60% (deviation from the 50% recommended per OPPTS 830.6317). The applicant has not recorded the weight of the container at each of the test intervals to monitor weight.

- A Good Laboratory Practices (GLP) statement was provided stating that the study was conducted in compliance with the United Kingdom (GLP Monitoring Authority, Medicines and Healthcare products Regulating Agency (MHRA)) Good Laboratory

Practice Regulations 1999, Statutory Instrument 1999 No. 3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations, 2004, and the OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring ENV/MC/CHEM (98) 17 (Revised in 1997, Issued January, 1998). A GLP statement regarding compliance with 40 CFR Part 160 was not provided. A Quality Assurance Unit statement was provided.

RECOMMENDATIONS:

- The provided storage stability studies are incomplete. Long-term storage stability and corrosion characteristics are currently data gaps. Storage stability study needs to be conducted in accordance with GLP requirements outlined at 40 CFR Part 160. Per OPPTS 830.6317 (Storage Stability) and 830.6320 (Corrosion Characteristics) guidelines, the concentrations of the active ingredients in the product should be recorded at the beginning of the test period and every 3 months thereafter for a minimum of 1 year. For the corrosion characteristics studies, the weight of the container and observations made (e.g., "perforations," "darkening," "leaking or rust at the seam, etc.), should be recorded at each test interval.

PRODUCT CHEMISTRY REVIEW

I. CONFIDENTIAL STATEMENT OF FORMULA

a. Type of formulation and source registration

- Non-integrated formulation system [ ]
  - Are all TGAIs used registered? Yes [ ] No [ ]
- Integrated formulation system [ ]
- If "ME-TOO", specify EPA Reg. # of existing product:

b. Clearance of inerts for non-food or food use:
Cleared for food use under 40 CFR §180.1001: Yes [ ] No [ ] NA [ ]

c. Physical state of product:

d. The chemical IDs and analytical information (including that for the TGAIs), density, pH, and flammability are consistent with that given in 830, Group B:
Yes [ ] No [ ]

e. NCs and CLs are acceptable: Yes [ ] No [ ]

f. Active ingredient(s)

	NC (%)	LCL (%)	UCL (%)
A.			

g. For products produced by an integrated formulation system:

- All impurities of toxicological significance have a UCL?
  Yes [ ] No [ ] Not applicable [ ]

- All impurities of ≥ 0.1% in the product have been identified?
  Yes [ ] No [ ] Not applicable [ ]

II. PRODUCT LABEL

a. The active ingredients statement (chemical IDs and NC) is consistent with the CONFIDENTIAL STATEMENT OF FORMULA? Yes [ ] No [ ]

b. The formula contains one of the following:

- 10% or more of a petroleum distillate: Yes [ ] No [ ]
- 1.0% or more of methyl alcohol: Yes [ ] No [ ]
- sodium nitrite at any level: Yes [ ] No [ ]
- a toxic List 1 inert at any level: Yes [ ] No [ ]
- arsenic in any form: Yes [ ] No [ ]

c. If Yes to any of the above, does the inert ingredients statement contain a footnote indicating this? Yes [ ] No [ ] Not applicable [ ]

d. The appropriate warning statement regarding flammability or explosive characteristics of the product are listed on the label?
Yes [ ] No [ ] Not applicable [ ]

e. The storage and disposal instructions for the pesticide container are in compliance with PR Notice 84-1 for household use products or PR Notice 83-3 for all other uses?
Yes [ ] No [ ]

f. Does the product require an expiration date at which time the NC falls below the LCL (based on the one year storage stability data or other information)?
Yes [ ] No [ ]

Storage stability was not conducted in accordance with GLP requirements, and is incomplete.

Table A:
Product Chemistry (830 Series, Group A)

Data Requirements	Acceptance of Information	MRID No.
830.1550 Product Identity[1]		
830.1600 Description of Materials		
830.1620 Production Method[2]		
830.1650 Formulation Process[3]		
830.1670 Formation of Impurities[4]		
830.1700 Preliminary Analysis[5]		
830.1750 Certified Limits[6]		
830.1800 Analytical Method[7]		

Explanation: A=acceptable; N=not acceptable; NA=technically not applicable; G=data gap; U=requires upgrading; W=waived; E=EPA estimate.

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[1]See Confidential Appendix A for additional information
[2]For MP/EP products produced by an integrated formulation system.
[3]For products from a TGAI or MP
[4]May be waived unless actual/possible impurities are of toxicological concern.
[5]Five batch analysis required for products produced by an integrated formulation system.
[6]If different from standard CLs recommended in 40 CFR 158.175, this should be discussed in Confidential Appendix A.
[7]Abbreviate method used as follows: gas chromatography (GC), infrared (IR), etc.

Table B:
Physical and Chemical Characteristics (Series 830, Group B)

Physical/Chemical Properties*	Acceptance of data	Value or qualitative description	MRID No.
830.6302 Color	A	light yellow (visual observation at 25 C)	467784-01
830.6303 Physical State	A	granular powder (visual observation at 25 C)	467784-01
830.6304 Odor			
830.6313 Stability to Normal and Elevated Temperatures, Metals and Metal Ions			
830.6314 Oxidation/Reduction; Chemical Incompatibility			
830.6315 Flammability/Flame Extension			
830.6316 Explodability			
830.6317 Storage Stability	N	Sample stored for 3 years at room temperature (25 C) and 60% relative humidity. Samples were analyzed every six months (HPLC-UV) over a three year period. Tests to assess the physical state, color, interaction with the container, and active ingredient content were performed at the indicated intervals. The following concentrations of the active ingredient (average of 6 measurements) were reported during each of the test intervals: Beginning: 87.15±0.41% After 6 mos: 95.19±1.53% After 12 mos: 96.48±1.54% After 24 mos: 95.51±1.37% After 36 mos: 94.86±0.44%	467784-01
830.6319 Miscibility[2]			
830.6320 Corrosion Characteristics	N	Sample stored for 3 years at room temperature (25 C) and 60% relative humidity.	467784-01

Physical/Chemical Properties*	Acceptance of data	Value or qualitative description	MRID No.
		Samples were analyzed every six months (HPLC-UV) over a three year period. Tests to assess the physical state, color, interaction with the container, and active ingredient content were performed at the indicated intervals. No change in color, physical state or degradation of the container was observed.	
830.6321 Dielectric Breakdown Voltage			
830.7000 pH[1]			
830.7100 Viscosity			
830.7200 Melting Point/Melting Range			
830.7220 Boiling Point/Boiling Range			
830.7300 Density/Relative Density/Bulk Density			
830.7370 Dissociation Constants in Water			
830.7550/830.7560/830.7570 Partition Coefficient			
830.7840/830.7860 Water Solubility			
830.7950 Vapor Pressure			

Explanation: A=acceptable; N=not acceptable; NA=technically not applicable; G=data gap; U=requires upgrading; W=waived; E=EPA estimate.

* Provide brief description, e.g., color – yellow or property value, e.g., density 1.25 g/cc; unless otherwise indicated, the property should be at 25 C.

[1]If product is dispersible with water
[2]If product is an emulsifiable liquid

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of
Prevention, Pesticides and
Toxic Substances

June 29, 2006

MEMORANDUM

Toxicity Study Review of **Econea-Technical** (H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]) and Toxicology Endpoints for Risk Assessment

DP Barcode: D323129 ,327538, 328778, 330458
EPA Reg. No.: 43813-ET
PC Code: 119093

From: S.L. Malish, Ph.D. Toxicologist, Team 1 Steven L. Malish 7/03/06
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)[7510P]

and

Jonathan Chen, Senior Toxicologist Jonathan Chen 07/03/06
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)[7510P]

Thru: Nader Elkassabany, Ph.D., Team Leader,
Team One, RASSB/AD [7510P]

To: Norman Cook, Chief, RASSB/AD [7510P]

Synonym: Econea®028, CL 303'268, R 107894

FORMULATION:

Active Ingredient: %
weight

1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl] 94.6% a.i.

Applicant: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

Uses: Antifouling paint additive use; An incidental oral scenario is used because the product will be used in antifoulant paints used in fresh water. The possibility of incident oral ingestion, therefore, might incur. Non- Food Use. Nexxium 20 is the name of the end product.

ACTION REQUESTED: Review the following toxicity studies and propose toxicology endpoints for risk assessment.

I. Acute toxicology Profile

The acute toxicology profile is presented in Table 1.

Table 1. Acute Toxicity Data for Econea-Technical

Guideline No.	Data Requirement/Species	Conclusions/Acceptable/ Comments	Tux Cat	MRID
870.1100	Acute Oral/Rat (Limit Test)**	LD_{50} = Not established **[Acceptable/-Guideline]**	I	45673915
870.1200	Acute Dermal/Rabbit**	LD_{50} >2000 mg/kg for both males and females **[Acceptable/Guideline]**	III	45673916
870.1300	Acute Inhalation/Rat**	LC50 = 790 mg/m3 (males) LC50 = 790 mg/m3 (females) LC50 = 770 mg/m3 (combined) **[Unacceptable/Guideline]** based on the fact that the percentage of particles below 4 um at the low and mid-dose level did not meet the EPA recommendations	III	45673917

870.1300	Acute Inhalation/Rat*	LC50 = <510 mg/m3 (males) LC50 = <510 mg/m3 (females) LC50 = <510 mg/m3 (males and females) **[Acceptable/Guideline]**	**II**	46846601
870.2500	Dermal Irritation/ Rabbit (Limit Test)**	Mildly irritating based on very slight erythematic, but no edema at 72 hours **[Acceptable/Guideline]**	**IV**	45673918
870.2400	Primary Eye Irritation./Rabbit (Limit Test)**	Mildly irritating **[Acceptable/Guideline]**	**III**	46539401
870.2600	Dermal Sensitization/ Guinea Pig**	Not a dermal sensitizer **[Acceptable/Guideline]**	**N/A**	45673919

* memorandum of June 29, 2006 memorandum from S.L. Malish and J. Chen to N. Cook (Toxicity Review of Econea Technical and Toxicology Endpoints for Risk Assessment).
** memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole…[ECONEA technical]: Review of Toxicology data submitted in Support of Registration).

II. Toxicology Profile, other studies

Table 2. Toxicology Profile of Econea-Technical

Study/Purity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
870. xxxx **Mutagenicity** **Salmonella typhimurium Reverse Mutation**	NA	**This is a required study.**
870.5300 **Mutagenicity-** **Mammalian cell gene mutation assay*** HPRT in CHO in cultured cells *in vitro*	MRID 46426903 Tested up to the limits of cytotoxicity and solubility	No evidence of a concentration related positive response that induce d mutant colonies over background **Classification: acceptable/guideline**
870.5395 **Mutagenicity –** ***in vivo* micronucleus**	MRID 46426904 Tested up to the toxic dose of 15 mg/kg. Male animals were used	There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes

Study/Purity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
assay*		**Classification: acceptable/guideline**
870.870.3700 **Developmental (Rat)*** 94.6% a.i.	MRID 46426902 Doses of 0, 5, 10 or 20 mg/kg/day from days 6–19 of gestation	**Maternal LOAEL = 10 mg/kg/day** based on frequent salivation. **Maternal NOAEL = 5 mg/kg/day** **Developmental LOAEL = 10 mg/kg/day** based on decreased fetal weight **Developmental NOAEL = 5 mg/kg/day** **Classification: acceptable/guideline**
870.3200 **Subchronic (28 Day Dermal) Range-finding Study (Rat)***** 94.6% a.i.	MRID 46659702 Doses of 0, 30, 100, 300, and 1000 mg/kg/day	**NOAEL** ≤ 1000 mg/kg/day **LOAEL** >1000 mg/kg/day (HDT) **Classification: acceptable/guideline**
870.3100 **Subchronic 90 Day (Oral) Study (Rat)** 94.6% a.i. with a Pathology Peer Review***	MRID 46659701 Doses of 0, 5.2, 16.2 and 51.9 mg/kg/day in males and 6.3, 20.9, and 62.0 mg/kg/ day in females;	**Males** **NOAEL** = 5.2 mg/kg/day in males **LOAEL** = 16.2 mg/kg/day in males based on based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord. **Females** **NOAEL** = could not be determined **LOAEL** = 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. **Classification: acceptable/guideline**
870. 3250 **Subchronic (90 Day Dermal) Study (Rat)**** 94.6% a.i.	MRID 46802201 Doses of 0, 100, 300, and 1000 mg/kg/day	**Dermal:** **NOAEL** 100 mg/kg/day **LOAEL** 300 mg/kg/day based on the appearance of sores **Systemic:** **NOAEL** = 300 mg/kg/day **LOAEL** = 1000 mg/kg/day based on histopathology changes in the lungs **Classification: Not-acceptable, but upgradeable/Guideline. The study can be upgraded by noting the frequency of the lung lesions at the two lower dose levels to better define the NOAEL and LOAEL.**
No Guideline available **Inhalation**	MRID 46751302	**NOAEL**: 24 mg/m^3. **LOAEL**: 80 mg/m^3 based on reduced body

Study/Purity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
Rangefinding**	Doses of 0, 24, 80, (6 hours a day for 12 days) or 239 mg/m^3, for app. 4.5 hours. An additional group was administered 0 and 126 mg/m3 where 3/5 males died.	weight gain. **Classification: Non-guideline**
870.3465/870.6200 **Subchronic (90 Day) Inhalation Toxicity and Neurotoxicity**** **Phase I - Subchronic Toxicity)**	MRID: 46802202 0, 20, 40, and 80 mg/m^3	**Systemic (Phase I)** **Systemic** **LOAEL** was 20 mg/m^3 (male) based on decreased body weight gain. **NOAEL** in the male could not be determined (<20 mg/m3), LDT **LOAEL** was 40 mg/m^3 (females) based on decreased body weight gain. **NOAEL** in females was 20 mg/m3. **Local irritation of the dorsal region of the nose** **LOAEL for both sexes is 20-mg/m^3 based on the findings observed in the N1 for both sexes and N3 section in the females of the 20-mg/m^3 animals. The 20-mg/m^3 males exhibited chronic and subacute inflammation (Nasal section 1), ulcerations (N1), and exudate (N1), and the females had subacute inflammation (N1), epithelial hyperplasia (N1), hyperkeratosis (N1), and degeneration of olfactory epithelium (N3).** **NOAEL was not determined (<20 mg/m3)(LDT)** **Classification: acceptable/guideline**
Subchronic (90 Day) Inhalation Toxicity and Neurotoxicity** **Phase II - Neurotoxicity**	MRID: 46802202 0, 20, 40, and 80 mg/m^3	**Neurotoxicity (Phase II)** **LOAEL** in males was 40 mg/m3 based on decreased motor activity **NOAEL** in males was 20 mg/m3 **LOAEL** in females was 80 mg/m3 based on axonal degeneration observed in the peroneal nerve. **NOAEL** in females was 40 mg/m3 **Classification: acceptable/guideline**

* memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole…[ECONEA technical]: Review of Toxicology data submitted in Support of Registration) contain the Acute toxicology

LC50 data, the Executive summary for the Developmental study in rats, and the Executive summaries for the Mutagenicity studies, are also contained in the June 29, 2006 memo (below).

** memorandum of June 29, 2006 from S.L. Malish and J. Chen to N. Cook (Toxicity Review of Econea Technical and Toxicology Endpoints for Risk Assessment,) contains Executive summaries and the DERS.

*** memorandum of Jan 31, 2006 from Malish to Swindell (Subchronic (90-Day) Oral Toxicity - Rat (466597-01) with a Pathology Peer Review, and a 28-Day Repeat Dermal Toxicity – Rat (466597-02) with Econea technical (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]). These Executive summaries and the DERS are also contained in the June 29, 2006 memo above.

A. The executive summaries of the mutagenicity and subchronic studies are presented below.

1. **Salmonella typhimurium Reverse Mutation Assay**

Study not available, but is required for a complete toxicology profile.

2. *In vitro* **Mammalian Cell Gene Mutation**, MRID 46426903

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay HPRT (MRID 46426903), Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to R 107894, (∃94% pure, batch AC 12649-8 / test substance no.: 03/0238-1) in dimethylsulfoxide for 4 hours at concentrations of 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µg/mL (Trial 1, -S9) and 0.625, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 µg/mL (Trial 1, +S9), 1.25, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 µg/mL (Trial 2, -S9) and 1.25, 2.5, 5.0, 10.0, 12.5, 15.0, 17.5, and 20.0 µg/mL (Trial 2, +S9) as well as 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, and 40.0 µg/mL (Trial 3, +S9), in the presence and absence of mammalian metabolic activation derived from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

R 107894 was tested up to cytotoxic and insolubility concentrations and was not mutagenic in this *in vitro* mammalian cell gene mutation assay in the presence or absence of S9 activation. The positive controls did induce the appropriate response. **There was no evidence of a concentration-related, positive response that induced mutant colonies over background.**

This study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

3. ***In vivo*** **Mammalian Cell Cytogenetics** (Bone Marrow Micronucleus), MRID 46426904

EXECUTIVE SUMMARY: In a NMRI mouse bone marrow micronucleus assay (MRID 46426904), 5 male mice per dose were exposed once by oral gavage to R 107894 (>94% pure; test substance number: 03/0238-1, batch number: AC 12649-8) at doses of 0, 3, 6, or 12 mg/kg bw. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle used was olive oil.

R 107894 was tested at adequate doses based on a pretest using NMRI mice (details not reported) that were gavage-dosed up to a dose of 15 mg/kg bw. Results from this range-finding assay indicated acute toxicity deaths up to 15 mg/kg bw. Clinical signs of toxicity (squatting posture) in the 12-mg/kg treated animals with no distinct differences between males and females. Hence, only males were used for the main assay.

There was no mortality among the animals tested in the main assay, however, clinical signs of toxicity (squatting posture) were observed in the 12 mg/kg bw animals at both 24- and 48-hour harvest times. The mean body weights were not reported in the study report. No statistically-significant increases in the number of small or large micronucleated PCEs or NCEs were reported at any dose or harvest time compared to the concurrent solvent control. No inhibition of erythropoiesis was determined from the ratio of PCEs to NCEs. Additionally, positive controls also showed a significant increase in the percentage of micronucleated PCEs in males; therefore, the positive control elicited a clearly positive response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

4. **Developmental Toxicity - Rat**, MRID 46426902

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46426902), R107894 (94.6% a.i., batch AC12649-8) was administered to 25 time-mated Sprague-Dawley female Crl:CD rats /dose by gavage at dose levels of 0, 5, 10 or 20 mg/kg bw/day from day 6 through 19 of gestation (i.e. gd 6-19).

Treatment related maternal effects at 20 mg/kg/day included increased mortality, frequent salivation, reduced body weight gain, decreased uterine weight, deceased corrected body weight gain, increased resorptions (total, early and late) and decreased placental weight. At 10 mg/kg/day, dams exhibited frequented salivation. The maternal LOAEL is 10 mg/kg bw/day, based on frequent salivation. The NOAEL is 5 mg/kg/ bw/day.

Developmental findings also occurred at 10 and 20 mg/kg/day. Treatment-related effects at 20 mg/kg/day included decreased mean fetal weight and an increased incidence of fetuses exhibiting disturbances and delays of ossification (non/incomplete/dumbbell/biparte) of various skeletal structures (skull, sternebrae), as well as supernumerary (14th) and wavy ribs. The ossification delays were considered to

be secondary to maternal toxicity and correlated with decreased fetal weight. At 10 mg/kg/day, there was a decrease in mean fetal weight. The developmental LOAEL is 10 mg/kg/bw/day, abased on decreased fetal weight, the developmental NOAEL is 5 mg/kg bw/day.

The study is classified as **Acceptable- Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

5. **28-Day Repeat Dermal Toxicity – Rat**; MRID 46659702

EXECUTIVE SUMMARY: In a 28-day range-finding dermal toxicity study (MRID 466597-02), R107894 (94.6% a.i.; lot #12649-8) was administered to 5 rats/dose /sex at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day for 29 days (6 hours/day, 5 days/week). Results from this study will be used to determine the dose range of a proposed 90-day dermal subchronic study.

Treatment-related effects at 1000 mg/kg/day included ano-genital staining but this was not considered to be toxicologically significant. There were no mortalities during this study. Additionally, there were no significant changes to body weight, food consumption, food efficiency, organ weight, or pathology (gross or microscopic). **The LOAEL is >1000 mg/kg/day (HDT). The NOAEL is ≤1000 mg/kg/day.** Based on these results, rats would be expected to tolerate an exposure of >1000 mg/kg/day in the subsequent 90-day dermal study.

This 28-day range-finding dermal toxicity study is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a range-finding dermal toxicity study OPPTS 870.3200.

6. **Subchronic (90 Day) day dermal toxicity study - Rat**, MRID 468022-01

EXECUTIVE SUMMARY: In a 90-day dermal toxicity study (MRID 46802201), R107894 (94.6% a.i., Lot #AC 12649-8) was applied to the shaved skin of 10 rats, Crl:CD® (SD)/sex/dose at dose levels of 0, 100, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 90-day period.

One 100-mg/kg/day female died prior to study termination. A treatment-related dose-dependent increase in small dermal sores was noted in both the mid- and high-dose group. **Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.**

Histopathology findings in the lungs (increased incidence of bonchoalveolar associated lymphoid tissue, chronic inflammation, and alveolar macrophages) were observed at the high-dose for both males and females and appear to be the result of treatment. With regard to the pathology, only the control and high-dose males and females were evaluated as well as one low-dose female. Because these effects occurred in both males and females at increased frequency at the high-dose, they are considered treatment-related

and adverse. **Consequently, the systemic NOAEL is 300 mg/kg/day; the systemic LOAEL is 1000 mg/kg/day based on histopathology changes in the lungs (i.e., an increased incidence of BALT, chronic inflammation, and alveolar macrophages).**

This 90-day dermal toxicity study in the rat is **NOT ACCEPTABLE, but upgradeable. The study, however, satisfies** the guideline requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats. The study can be upgraded by noting the frequency of the lung lesions at the two lower dose levels so as to better define the NOAEL and LOAEL.

7 . **Subchronic (90-Day) Oral Toxicity** – Rat, MRID 466597-01

EXECUTIVE SUMMARY: A pathology peer review (MRID 46659701) was performed on a 90-day oral toxicity study (MRID 464422-01). In the study, R 107894 (94.6% a.i., Batch # AC 12649-8) was administered to 10 Sprague-Dawley rats/sex/dose via food at dose levels of 0, 80, 250, or 750 ppm active ingredient. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females using the analytical concentration. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The intake of test article in these rats was similar to that observed in the main study animals (i.e., 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females).
The original pathology was peer reviewed.

No unscheduled deaths occurred during the course of the study. There were no treatment-related clinical signs of toxicity, opthalmological findings, urinalysis, or macroscopic findings. Food consumption decreased in the first week of treatment in both males and females administered 750 ppm. Thereafter, food consumption in the 750-ppm group was lower than that observed in the controls; and values reached statistical significance in Weeks 1, 6, and 9 in males and Weeks 1, 4, 6-9, and 11 in females. In conjunction with the low food consumption, high-dose males had statistically-significantly lower body weights within the first week of treatment and throughout treatment; high-dose females displayed statistically significant decreases in mean body weight starting on Week 7 through Week 13. The body weight gains were low throughout the study, likely due to low food consumption. The food efficiency was reduced in high-dose animals with only the males having statistically significant decreases (Weeks 1-5, 7, 8, and 13). The mid-dose males had statistically significant decreases in mean body weight during Weeks 7-13, with corresponding decreased body weight gain and food consumption. The mid-dose females displayed only slight decrease in body weight, body weight gain, and food consumption. The recovery group displayed signs of weight gain and increased food consumption; however, the high-dose animals still had statistically-significantly decreased mean body weight and body weight gain as compared to controls. Food efficiency was increased in the treated animals during the recovery period.

Hematology data indicated only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were statistically-significantly increased in the mid- and high-dose males, which correlate to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically significant change. Low-dose males also displayed a statistically significant increase in ALP. Signs of dehydration and undernourishment were evident with statistically significant increases in phosphorus, urea, magnesium, and cholesterol; and a statistically significant decrease in glucose in high-dose males and females.

Clinical chemistry findings in the recovery group indicate reversibility of the effects. Differences in creatinine and triglycerides were generally <10% different from the main control group and were thus considered due to biological variation.

Terminal body weight was reduced a statistically significant amount in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs. Treatment-related changes include increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. The relative brain weights in the high-dose animals remained elevated after the recovery period, though the males were the only statistically significant value. However, frank effects were noted in the brain at necropsy in these recovery group animals.

Microscopic findings of the brain and spinal cord observed in the main group were as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females; moderate to marked multifocal vacuolation of the thoracic cord in the high-dose females; minimal to marked multifocal vacuolation of the lumbar cord in the mid- and high-dose males and females as well as the low-dose females. Microscopic finding of the brain and spinal cord of the animals that were in the perfusion fixation satellite group displayed treatment-related finding as follows: slight or minimal multifocal vacuolization in the frontal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the parietal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the midbrain (high-dose males; mid- and high-dose females); minimal to slight multifocal vacuolization of the pons (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the cerebellum (high-dose males; mid- and high-dose females); slight multifocal vacuolization of the medulla oblongata (high-dose female); slight multifocal vacuolization of the cervical cord (high-dose male); minimal to moderate multifocal vacuolization of the lumbar cord (mid- and high-dose males and females); minimal to marked myelin vacuolation of the cervical

dorsal root (mid- and high-dose males and females); minimal to slight myelin vacuolation of the cervical ventricle root (high-dose males); minimal to marked myelin vacuolation of the lumbar dorsal root (mid- and high-dose males; all dosed females); minimal to marked myelin vacuolation of lumbar ventricle root (mid- and high-dose males; all dosed females); and slight to moderate myelin vacuolation of the proximal sciatic nerve (high-dose males and females). The lumbar region (cauda equina) was most profoundly affected and the females appeared to be more sensitive.

Other treatment related microscopic findings in the main group were minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). Peer review of the pathology confirmed the general findings, although there were some minor differences on severity and in some instances the involvement of vacuolation in several tissues.

There were no changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal).

After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males and could not be determined in females.

This 90-day oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a subchronic (90-day) oral toxicity study OPPTS 870.3100 in the rat.

8. **Inhalation (range-finding) Toxicity Study** - Rat , MRID 46751302

EXECUTIVE SUMMARY: In a range-finding inhalation toxicity study (MRID 46751302), R107894 (93% a.i., lot no. 89000015) was administered to 5 Sprague-Dawley rats/sex/dose via nose only exposure at dose levels of 0, 24, 80 (6 hours a day for 12 days), or 240 mg/m^3 for 4 hours and 25 minutes, due to high mortality. The surviving animals were held in the animal room and observed without further exposure. Additional groups of 5 Sprague-Dawley male rats/dose were administered the test material via nose only exposure at dose levels of 0 or 120 mg/m^3 for 6 hours for one day only due to high

mortality. All surviving animals were euthanized the following day. The 24, 80, 120 or 240 mg/m^3 dose levels were determined to be equivalent to 24, 80, 126, or 236 mg/m^3, respectively, of the nominal concentration of the test substance. MMAD and GSD values were within acceptable limits.

Clinical signs, body weight, food consumption, gross and microscopic pathology, organ weight, and a functional observational battery were performed. Clinical chemistry, hematology and urinalysis were not performed.

Several unscheduled deaths occurred during the course of the study: in the 240 mg/m^3 group, 4/5 males and 2/5 females died during the first day of exposure, thus, prompting the termination of exposure at 4 hours and 25 minutes; in the 120 mg/m^3 group, 3/5 males died after a single 6 hour exposure. There were no test article-related effects in food consumption, gross or microscopic pathology, or organ weights. A decrease in body weight gain was observed in the 80-mg/m^3 rats. Observations in 240-mg/m^3 surviving rats included rales and labored respiration. At the 120-mg/m^3 exposure level, hypoactivity, increased respiration, gasping and/or vocalization upon handling were observed. No signs of toxicity were observed in the 24-mg/m^3 animals.

The LOAEL is 80 mg/m^3 based on reduced body weight gain. The NOAEL is 24 mg/m^3.

This range-finding oral toxicity study in the rat is **ACCEPTABLE-NONGUIDELINE. No guidelines are available for this type of study.**

9. **Subchronic Inhalation Toxicity and Neurotoxicity Screening Battery – Rats,** MRID 468022-02

This executive summary is for the Subchronic Inhalation Toxicity, Phase I

EXECUTIVE SUMMARY: In a combined subchronic inhalation toxicity (Phase I) and Neurotoxicity study (Phase II), R107894 (Lot no. ZR107894EXA028; 92.4% a.i.) was administered via nose-only exposure (MRID 46802202), to 10 Crl:CD (Sprague Dawley) rats/sex/dose in Phase I and 12 rats/sex/dose in Phase II at nominal concentrations of 0, 20, 40, and 80 mg/m^3. (analytical concentration equivalent to 0, 20, 40 and 80 mg/m3 of the test substance). The exposure period was 6 hours per day, 7 days per week for a minimum of 90 consecutive days. Particle size was determined and was within the accepted range.

No observable adverse effect level was 20-mg/m^3 for R107894 following measured parameters: survival, hematology, clinical chemistry, or gross pathology. Nasal lesions were observed in the 20-mg/m^3 animals and were considered a local effect.

One female rat in the Phase I inhalation toxicity study control group was found dead on study Day 9. This animal was in the control group, therefore the death was not considered test article-related. Three males in the 80-mg/m^3 Phase II neurotoxicity study

group were found dead on study Days 0, 20, and 85, respectively. Additionally, an 80-mg/m^3 female in the Phase II neurotoxicity study group was found dead on Day 0 after the first exposure. Since there were numerous deaths in the 80-mg/m^3 (high-exposure) group these deaths were considered test article-related.

Clinical signs of toxicity related to treatment included brown staining on multiple body surfaces in males and females of all three test article-treated groups. Staining, which began on Day 11 and persisted through the end of the study, was observed on the neck, forelimb, abdominal area, thoracic area, inguinal area, hindlimb, rump, anogenital area, urogenital area, and tail. Reduced mean body weights for the male and female animals of the 20- (males), 40-, and 80-mg/m^3 test groups were statistically significantly lower than the control group. There was a ≤10% reduction in body weight in the 20-mg/m^3 males and females; cumulative body weight gains were 5% lower than controls in the 20-mg/m^3 females and 17% lower in the 20-mg/m^3 males. The reduced mean body weights for 40- and 80-mg/m^3 male and females and the 20-mg/m^3 males were consistent with lower food consumption (g/animal/day), body weight gains (weekly and cumulatively) and food efficiency.

Microscopic treatment related effects were limited to the nose of all of the treated animals. The effects were observed to be localized in the dorsal region of the nose, and included inflammation, ulceration or erosion, squamous or respiratory metaplasia, and olfactory or respiratory epithelial degeneration. These changes displayed a dose-related increase in incidence and severity.

The LOAEL of R107894 for male and female is 20-mg/m^3 based on the findings observed in the N1 (Nasal section 1) for both sexes and N3 section in the females of the 20-mg/m^3 animals. The 20-mg/m^3 males exhibited chronic and subacute inflammation, ulcerations (N1), and exudate (N1), and the females had subacute inflammation (N1), epithelial hyperplasia (N1), hyperkeratosis (N1), and degeneration of olfactory epithelium (N3). The NOAEL could not be determined (<20 mg/m3)(LDT).

The systemic toxicity LOAEL was 20 mg/m3 (LDT) in the male based on a decrease in body weight gain. The NOAEL in the male could not be determined (<20 mg/m3).

The LOAEL in the female was 40 mg/m3 based on a decreased in body weight gain. The NOAEL was 20 mg/m3.

This subchronic inhalation toxicity study in the rat is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rats.

10. **Subchronic Inhalation Toxicity and Neurotoxicity Screening Battery – Rats,** MRID 468022-02

This executive summary is for the Neurotoxicity Screening Battery, Phase II

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 468022-02), groups of Crl:CD(SD) 12 Sprague-Dawley derived rats/sex/dose (**Phase II**) were exposed to R107894 (92.4% a.i. of Lot no. ZR107894EXA028) via nose only exposure at targeted dose levels of 0, 20, 40, or 80 mg/m^3 for 6 hours a day for a minimum of 90 consecutive days (~13 weeks). The particle size was within the required range.

Another phase of the study evaluating the subchronic inhalation toxicity of R107894 was performed on an additional 10 animals/sex/dose (**Phase I**) and discussed in the accompanying subchronic inhalation toxicity DER for R107894.

Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all Phase II animals (12 rats/sex /group) prior to dose administration (pretest), and at Weeks 3, 7, and 12. Cholinesterase activity was not determined. At study termination, 6 animals/sex/dose in the controls and 80-mg/m^3 groups were perfused in situ for neuropathological examination of the central and peripheral nervous system tissues

For the Phase II neurotoxicity study, after the first day of exposure, 1 male and 1 female were found dead in the 80-mg/m^3 group and were replaced. Two 80-mg/m^3 males died prior to study termination, one on Day 20 and the other on Day 85.

During FOB handling measurement, the 40- and 80-mg/m^3 animals were observed to have soiled fur, unclean fur, and increased grooming (80-mg/m^3 males) during Weeks 7 and 12. These changes correlate with the clinical toxicity findings of soiled fur and were not considered adverse neurotoxicological findings. During Week 3 FOB measurements, an overall decrease in total locomotor activity was noted in the 40- and 80-mg/m^3 males and in ambulatory activities for 80-mg/m^3 males as a result of reduced activity in sub-session measurements. During Week 12, the 80-mg/m^3 males were observed with total activity decrease compared to controls.

At neuropathology, an increase in minimal axonal degeneration was observed in the peroneal nerve in the 80-mg/m^3 animals (3/6 for both males and females) over controls (0/0 for both males and females).

The neurotoxicity LOAEL was 40 mg/m^3 in males based on decreased motor activity and 80 mg/m^3 in females based on axonal degeneration observed in the peroneal nerve. The neurotoxicity NOAEL was 20 mg/m^3 for males and 40 mg/m^3 for females.

This Neurotoxicity Screening Battery is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

III. Toxicology Endpoints for Risk Assessment

Note: These endpoints were discussed at the meeting of June 27, 2006

Rationale and Comments on the Studies used for the Toxicology Endpoints for Risk Assessment

Incidental Oral Exposure Scenario: Econea is used as an antifoulant for paints used in fresh water and the possibility of incidental oral ingestion might occur.

Dermal Exposure Scenario: The Subchronic Dermal study at the low and mid dose levels did not have lung histopathology performed. The study, therefore, was classified as Not-Acceptable. The study can be upgraded to Acceptable if the lung pathology were evaluated for the low and intermediate dose levels.

Table 3. Toxicology Endpoints for Risk Assessment for Econea-technical

Exposure Scenario	Dose Used in Risk Assessment	Target MOE for Risk Assessment	Study and Toxicological Effects
Acute and Chronic Dietary	Risk assessment not required based on use pattern		
Non-Dietary Occupational/Residential Exposures			
Incidental Oral Short-Term (1-30 days) and Intermediate-Term(1–6 months) (Occupational /Residential)	**NOAEL** = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intrapecies variation, 3x for lack of a NOAEL in females)	**Subchronic (90 Day) Oral** **LOAEL** = 6.3 mg/kg/day (LDT) for females based on based microscopic findings of the brain and spinal cord. **Classification: Acceptable/Guideline**
Dermal* Short-Term (1-30 days) and Intermediate-Term(1–6 months) (Occupational)	**NOAEL** = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intrapecies variation, 3x for the data gap	**Subchronic (90 Day) Oral** **LOAEL** = 6.3 mg/kg/day (LDT) for females based on based microscopic findings of the brain and spinal cord. **Classification: Acceptable/Guideline**
Dermal* Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		
Inhalation Short-Term (1-30 days) and Intermediate (1 – 6	**NOAEL** could not be calculated (<20mg/m3) (LDT).	Target MOE = 300 10x interspecies extrapolation, 10x	**Subchronic (90 Day) Inhalation Toxicity (Phase I)**

months) Term (Occupational)		intrapecies variation, 3x conversion of LOAEL to NOAEL	LOAEL is 20-mg/m^3 based on the findings observed in the nasal section N1 to N3 and included chonic and subacute inflamma-tion , ulcerations, exudates, epithelial hyperplasia, hyperkeratosis and degeneration of the olfactory epithelium. Decreased body weight gain was also seen at this dose level. **Classification: Acceptable/Guideline**
Inhalation Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		
Cancer	No cancer data available		

* 100% absorption factor will be used.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of
Prevention, Pesticides and
Toxic Substances

February 5, 2007

MEMORANDUM

Toxicity Study Review of **Econea-Technical** (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]) and Toxicology Endpoints for Risk Assessment

DP Barcode: D323129, 327538, 328778, 330458, 335209
EPA Reg. No.: 43813-ET
PC Code: 119093

From: S.L. Malish, Ph.D. Toxicologist, Team 1 S. L. Malish 2/9/07
Risk Assessment and Science Support Branch (RASSB)
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and

Jonathan Chen, Senior Toxicologist Jonathan Chen 02/07/2007
Risk Assessment and Science Support Branch (RASSB)
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Thru: Nader Elkassabany, Ph.D., Team Leader, Nader Elk 2/7/07
Team One, RASSB/AD [7510P]

To: Norman Cook, Chief, RASSB/AD [7510P] 2/8/07

Synonym: Econea®028, CL 303'268, R107894, Nexxium 20 (end use product)

FORMULATION:

Active Ingredient: % weight

1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl] 94.6% a.i.

Applicant: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

Uses: Antifouling paint additive use; An incidental oral scenario is used because the product will be used in antifoulant paints used in fresh water. The possibility of incident oral ingestion, therefore, might incur. Non-Food Use is expected.

ACTION REQUESTED: Review the following toxicity studies and proposed toxicology endpoints for risk assessment .

BACKGROUND: A Data Evaluation Report for the study - Merkel, D. (2006). R107894: Dermal toxicity study (90-day repeat dermal application study in rats), MRID 46802201 was originally submitted. Because the study was considered not to be acceptable, but upgradeable (with the addition of lung histopathological data at the low and intermediate dose levels), the sponsor submitted additional data (Amendment to R107894, MRID 47006601) to upgrade the original report. This final memorandum incorporates the additional data for Econea.

In this memorandum "Table 2. Toxicology Profile for Econea – Technical" and the executive summary for the 90 day dermal in rats toxicity study has also been changed to reflect these changes. Moreover, the Toxicology Endpoints for Risk Assessment for Econea - Technical (Table 3) has been revised to include the Subchronic (90 day) Dermal Toxicity for the Short and Intermediate Term Dermal Exposure Scenarios.

I . Acute Toxicology Profile

The acute toxicology profile is presented in Table 1.

Table 1. Acute Toxicity Data for Econea-Technical

Guideline No.	Data Requirement/Species	Conclusions/Acceptable/ Comments	Tox Cat	MRID
870.1100	Acute Oral/Rat (Limit Test)**	LD_{50} = Not established **[Acceptable/-Guideline]**	I	45673915
870.1200	Acute Dermal/Rabbit**	LD_{50} >2000 mg/kg for both males and females **[Acceptable/Guideline]**	III	45673916

870.1300	Acute Inhalation/Rat**	LC50 = 790 mg/m3 (males) LC50 = 790 mg/m3 (females) LC50 = 770 mg/m3 (combined) **[Unacceptable/Guideline]** based on the fact that the percentage of particles below 4 um at the low and mid-dose level did not meet the EPA recommendations	**III**	45673917
870.1300	Acute Inhalation/Rat*	LC50 = <510 mg/m3 (males) LC50 = <510 mg/m3 (females) LC50 = <510 mg/m3 (males and females) **[Acceptable/Guideline]**	**II**	46846601
870.2500	Dermal Irritation/ Rabbit (Limit Test)**	Mildly irritating based on very slight erythematic, but no edema at 72 hours **[Acceptable/Guideline]**	**IV**	45673918
870.2400	Primary Eye Irritation./Rabbit (Limit Test)**	Mildly irritating **[Acceptable/Guideline]**	**III**	46539401
870.2600	Dermal Sensitization/ Guinea Pig**	Not a dermal sensitizer **[Acceptable/Guideline]**	**N/A**	45673919

* memorandum of June 29, 2006 memorandum from S.L. Malish and J. Chen to N. Cook (Toxicity Review of Econea Technical and Toxicology Endpoints for Risk Assessment).
** memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole…[ECONEA technical]: Review of Toxicology data submitted in Support of Registration).

II. Toxicology Profile, other studies

Table 2. Toxicology Profile of Econea-Technical

Study/Purity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
870. xxxx **Mutagenicity** **Salmonella typhimurium Reverse Mutation**	NA	**This is a required study.**
870.5300 **Mutagenicity-** **Mammalian cell gene mutation assay*** HPRT in CHO in cultured cells *in vitro*	MRID 46426903 Tested up to the limits of cytotoxicity and solubility	No evidence of a concentration related positive response that induce d mutant colonies over background **Classification: acceptable/guideline**
870.5395 **Mutagenicity –** ***in vivo* micronucleus assay***	MRID 46426904 Tested up to the toxic dose of 15 mg/kg. Male animals were used	There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes **Classification: acceptable/guideline**
870.870.3700 **Developmental (Rat)*** 94.6% a.i.	MRID 46426902 Doses of 0, 5, 10 or 20 mg/kg/day from days 6–19 of gestation	**Maternal LOAEL = 10 mg/kg/day** based on frequent salivation. **Maternal NOAEL = 5 mg/kg/day** **Developmental LOAEL = 10 mg/kg/day** based on decreased fetal weight **Developmental NOAEL = 5 mg/kg/day** **Classification: acceptable/guideline**
870.3200 **Subchronic (28 Day Dermal) Range-finding Study (Rat)***** 94.6% a.i.	MRID 46659702 Doses of 0, 30, 100, 300, and 1000 mg/kg/day	**NOAEL** $\leq$ 1000 mg/kg/day **LOAEL** >1000 mg/kg/day (HDT) **Classification: acceptable/guideline**
870.3100 **Subchronic 90 Day (Oral) Study (Rat)** 94.6% a.i. with a Pathology Peer Review***	MRID 46659701 Doses of 0, 5.2, 16.2 and 51.9 mg/kg/day in males and 6.3, 20.9, and 62.0 mg/kg/ day in females;	**Males** **NOAEL** = 5.2 mg/kg/day in males **LOAEL** = 16.2 mg/kg/day in males based on based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord. **Females** **NOAEL** = could not be determined **LOAEL** = 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord.

Study/Parity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
		Classification: acceptable/guideline
870. 3250 **Subchronic (90 Day Dermal) Study (Rat)**** 94.6% a.i.	MRIDs: 46802201, 47006601 Doses of 0, 100, 300, and 1000 mg/kg/day	<u>Dermal</u>: **NOAEL** 100 mg/kg/day **LOAEL** 300 mg/kg/day based on the appearance of sores <u>Systemic</u>: **NOAEL** = ≥1000 mg/kg/day (HDT, limit dose) **LOAEL** = >1000 mg/kg/day **Classification: Acceptable - Guideline**
No Guideline available **Inhalation Rangefinding**** 93% a.i.	MRID 46751302 Doses of 0, 24, 80, (6 hours a day for <u>12 days</u>) or 239 mg/m^3, for app. 4.5 hours. An additional group was administered 0 and 126 mg/m3 where 3/5 males died.	**NOAEL**: 24 mg/m^3. **LOAEL**: 80 mg/m^3 based on reduced body weight gain. **Classification: Non-guideline**
870. 3465/870.6200 **Subchronic (90 Day) Inhalation Toxicity and Neurotoxicity**** **Phase I - Subchronic Toxicity)**	MRID: 46802202 0, 20, 40, and 80 mg/m^3	<u>Systemic</u> **(Phase I)** <u>Systemic</u> **LOAEL** was 20 mg/m^3 (male) based on decreased body weight gain. **NOAEL** in the male could not be determined (<20 mg/m3), LDT **LOAEL** was 40 mg/m^3 (females) based on decreased body weight gain. **NOAEL** in females was 20 mg/m3. <u>**Local irritation of the dorsal region of the nose**</u> **LOAEL for both sexes is 20-mg/m^3 based on the findings observed in the N1 for both sexes and N3 section in the females of the 20-mg/m^3 animals. The 20-mg/m^3 males exhibited chronic and subacute inflammation (Nasal section 1), ulcerations (N1), and exudate (N1), and the females had subacute inflammation (N1), epithelial hyperplasia (N1), hyperkeratosis (N1), and degeneration of olfactory epithelium (N3).** **NOAEL was not determined (<20 mg/m3)(LDT)** **Classification: acceptable/guideline**

Study/Purity	MRID/Dose Levels	NOAEL/LOAEL Endpoints/Classification
Subchronic (90 Day) Inhalation Toxicity and Neurotoxicity** **Phase II - Neurotoxicity**	MRID: 46802202 0, 20, 40, and 80 mg/m^3	**Neurotoxicity (Phase II)** **LOAEL** in males was 40 mg/m3 based on decreased motor activity **NOAEL** in males was 20 mg/m3 **LOAEL** in females was 80 mg/m3 based on axonal degeneration observed in the peroneal nerve. **NOAEL** in females was 40 mg/m3 **Classification: acceptable/guideline**

* memorandum of Jan 31, 2006 from McMahon to Swindell (1H-Pyrrole...[ECONEA technical]: Review of Toxicology data submitted in Support of Registration) contain the Acute toxicology LC50 data, the Executive summary for the Developmental study in rats, and the Executive summaries for the Mutagenicity studies, are also contained in the June 29, 2006 memo (below).
** memorandum of June 29, 2006 from S.L. Malish and J. Chen to N. Cook (Toxicity Review of Econea Technical and Toxicology Endpoints for Risk Assessment,) contains Executive summaries and the DERS.
*** memorandum of Jan 31, 2006 from Malish to Swindell (Subchronic (90-Day) Oral Toxicity - Rat (466597-01) with a Pathology Peer Review, and a 28-Day Repeat Dermal Toxicity – Rat (466597-02) with Econea technical (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]). These Executive summaries and the DERS are also contained in the June 29, 2006 memo above.

A. The executive summaries of the mutagenicity and subchronic studies are presented below.

1. **Salmonella typhimurium Reverse Mutation Assay**

Study not available, but is required for a complete toxicology profile.

2. *In vitro* **Mammalian Cell Gene Mutation,** MRID 46426903

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay HPRT (MRID 46426903), Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to R 107894, (∃94% pure, batch AC 12649-8 / test substance no.: 03/0238-1) in dimethylsulfoxide for 4 hours at concentrations of 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µg/mL (Trial 1, -S9) and 0.625, 1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 µg/mL (Trial 1, +S9), 1.25, 2.5, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 µg/mL (Trial 2, -S9) and 1.25, 2.5, 5.0, 10.0, 12.5, 15.0, 17.5, and 20.0 µg/mL (Trial 2, +S9) as well as 10.0, 12.5, 15.0, 17.5, 20.0, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, and 40.0 µg/mL (Trial 3, +S9), in the presence and absence of mammalian metabolic activation derived from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

R 107894 was tested up to cytotoxic and insolubility concentrations and was not mutagenic in this *in vitro* mammalian cell gene mutation assay in the presence or absence of S9 activation. The positive controls did induce the appropriate response. There was

no evidence of a concentration-related, positive response that induced mutant colonies over background.

This study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement (OPPTS 870.5300, OECD 476) for *in vitro* mutagenicity (mammalian forward gene mutation) data.

3. ***In vivo* Mammalian Cell Cytogenetics** (Bone Marrow Micronucleus), MRID 46426904

EXECUTIVE SUMMARY: In a NMRI mouse bone marrow micronucleus assay (MRID 46426904), 5 male mice per dose were exposed once by oral gavage to R 107894 (>94% pure; test substance number: 03/0238-1, batch number: AC 12649-8) at doses of 0, 3, 6, or 12 mg/kg bw. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle used was olive oil.

R 107894 was tested at adequate doses based on a pretest using NMRI mice (details not reported) that were gavage-dosed up to a dose of 15 mg/kg bw. Results from this range-finding assay indicated acute toxicity deaths up to 15 mg/kg bw. Clinical signs of toxicity (squatting posture) in the 12-mg/kg treated animals with no distinct differences between males and females. Hence, only males were used for the main assay.

There was no mortality among the animals tested in the main assay, however, clinical signs of toxicity (squatting posture) were observed in the 12 mg/kg bw animals at both 24- and 48-hour harvest times. The mean body weights were not reported in the study report. No statistically-significant increases in the number of small or large micronucleated PCEs or NCEs were reported at any dose or harvest time compared to the concurrent solvent control. No inhibition of erythropoiesis was determined from the ratio of PCEs to NCEs. Additionally, positive controls also showed a significant increase in the percentage of micronucleated PCEs in males; therefore, the positive control elicited a clearly positive response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment** time.

This study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

4. **Developmental Toxicity - Rat**, MRID 46426902

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46426902), R107894 (94.6% a.i., batch AC12649-8) was administered to 25 time-mated Sprague-Dawley female Crl:CD rats /dose by gavage at dose levels of 0, 5, 10 or 20 mg/kg bw/day from day 6 through 19 of gestation (i.e. gd 6-19).

Treatment related maternal effects at 20 mg/kg/day included increased mortality, frequent salivation, reduced body weight gain, decreased uterine weight, deceased corrected body weight gain, increased resorptions (total, early and late) and decreased placental weight. At 10 mg/kg/day, dams exhibited frequented salivation. The maternal LOAEL is 10 mg/kg bw/day, based on frequent salivation. The NOAEL is 5 mg/kg/ bw/day.

Developmental findings also occurred at 10 and 20 mg/kg/day. Treatment-related effects at 20 mg/kg/day included decreased mean fetal weight and an increased incidence of fetuses exhibiting disturbances and delays of ossification (non/incomplete/dumbbell/biparte) of various skeletal structures (skull, sternebrae), as well as supernumerary (14th) and wavy ribs. The ossification delays were considered to be secondary to maternal toxicity and correlated with decreased fetal weight. At 10 mg/kg/day, there was a decrease in mean fetal weight. The developmental LOAEL is 10 mg/kg/bw/day, abased on decreased fetal weight, the developmental NOAEL is 5 mg/kg bw/day.

The study is classified as **Acceptable- Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

5. **28-Day Repeat Dermal Toxicity – Rat**; MRID 46659702

EXECUTIVE SUMMARY: In a 28-day range-finding dermal toxicity study (MRID 466597-02), R107894 (94.6% a.i.; lot #12649-8) was administered to 5 rats/dose /sex at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day for 29 days (6 hours/day, 5 days/week). Results from this study will be used to determine the dose range of a proposed 90-day dermal subchronic study.

Treatment-related effects at 1000 mg/kg/day included ano-genital staining but this was not considered to be toxicologically significant. There were no mortalities during this study. Additionally, there were no significant changes to body weight, food consumption, food efficiency, organ weight, or pathology (gross or microscopic). **The LOAEL is >1000 mg/kg/day (HDT). The NOAEL is ≤1000 mg/kg/day.** Based on these results, rats would be expected to tolerate an exposure of >1000 mg/kg/day in the subsequent 90-day dermal study.

This 28-day range-finding dermal toxicity study is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a range-finding dermal toxicity study OPPTS 870.3200.

6. **Subchronic (90 Day) Day Dermal Toxicity Study - Rat**, MRID 468022-01 and 47006601

EXECUTIVE SUMMARY: In a 90-day dermal toxicity study (MRID 46802201, MRID 47006601), R107894 (94.6% a.i., Lot #AC 12649-8) was applied to the shaved skin of 10 rats, Crl:CD® (SD)/sex/dose at dose levels of 0, 100, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 90-day period.

One 100-mg/kg/day female died prior to study termination. A treatment-related dose-dependent increase in small dermal sores was noted in both the mid- and high-dose group. Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.

There were no other adverse findings with this substance.

The systemic NOAEL is ≥1000 mg/kg/day (HDT). The systemic LOAEL is >1000 mg/kg/day. One thousand (1000) mg/kg/day is considered the limit dose; at or above this dose, no additional testing need be done.

This 90-day dermal toxicity study in the rat is **ACCEPTABLE – GUIDELINE.** The study, satisfies the guideline requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats.

7 . **Subchronic (90-Day) Oral Toxicity – Rat**, MRID 466597-01

EXECUTIVE SUMMARY: A pathology peer review (MRID 46659701) was performed on a 90-day oral toxicity study (MRID 464422-01). In the study, R 107894 (94.6% a.i., Batch # AC 12649-8) was administered to 10 Sprague-Dawley rats/sex/dose via food at dose levels of 0, 80, 250, or 750 ppm active ingredient. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females using the analytical concentration. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The intake of test article in these rats was similar to that observed in the main study animals (i.e., 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females).
The original pathology was peer reviewed.

No unscheduled deaths occurred during the course of the study. There were no treatment-related clinical signs of toxicity, ophthalmological findings, urinalysis, or macroscopic findings. Food consumption decreased in the first week of treatment in both males and females administered 750 ppm. Thereafter, food consumption in the 750-ppm group was lower than that observed in the controls; and values reached statistical significance in Weeks 1, 6, and 9 in males and Weeks 1, 4, 6-9, and 11 in females. In conjunction with the low food consumption, high-dose males had statistically-significantly lower body weights within the first week of treatment and throughout treatment; high-dose females displayed statistically significant decreases in mean body weight starting on Week 7 through Week 13. The body weight gains were low throughout the study, likely due to low food consumption. The food efficiency was reduced in high-dose animals with only the males having statistically significant decreases (Weeks 1-5, 7, 8, and 13). The mid-dose males had statistically significant decreases in mean body weight during Weeks 7-13, with corresponding decreased body

weight gain and food consumption. The mid-dose females displayed only slight decrease in body weight, body weight gain, and food consumption. The recovery group displayed signs of weight gain and increased food consumption; however, the high-dose animals still had statistically-significantly decreased mean body weight and body weight gain as compared to controls. Food efficiency was increased in the treated animals during the recovery period.

Hematology data indicated only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were statistically-significantly increased in the mid- and high-dose males, which correlate to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically significant change. Low-dose males also displayed a statistically significant increase in ALP. Signs of dehydration and undernourishment were evident with statistically significant increases in phosphorus, urea, magnesium, and cholesterol; and a statistically significant decrease in glucose in high-dose males and females.

Clinical chemistry findings in the recovery group indicate reversibility of the effects. Differences in creatinine and triglycerides were generally <10% different from the main control group and were thus considered due to biological variation.

Terminal body weight was reduced a statistically significant amount in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs. Treatment-related changes include increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. The relative brain weights in the high-dose animals remained elevated after the recovery period, though the males were the only statistically significant value. However, frank effects were noted in the brain at necropsy in these recovery group animals.

Microscopic findings of the brain and spinal cord observed in the main group were as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females; moderate to marked multifocal vacuolation of the thoracic cord in the high-dose females; minimal to marked multifocal vacuolation of the lumbar cord in the mid- and high-dose males and females as well as the low-dose females. Microscopic finding of the brain and spinal cord of the animals that were in the perfusion fixation satellite group displayed treatment-related finding as follows: slight or minimal multifocal vacuolization in the frontal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the parietal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the midbrain (high-dose males; mid- and high-dose females);

minimal to slight multifocal vacuolization of the pons (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the cerebellum (high-dose males; mid- and high-dose females); slight multifocal vacuolization of the medulla oblongata (high-dose female); slight multifocal vacuolization of the cervical cord (high-dose male); minimal to moderate multifocal vacuolization of the lumbar cord (mid- and high-dose males and females); minimal to marked myelin vacuolation of the cervical dorsal root (mid- and high-dose males and females); minimal to slight myelin vacuolation of the cervical ventricle root (high-dose males); minimal to marked myelin vacuolation of the lumbar dorsal root (mid- and high-dose males; all dosed females); minimal to marked myelin vacuolation of lumbar ventricle root (mid- and high-dose males; all dosed females); and slight to moderate myelin vacuolation of the proximal sciatic nerve (high-dose males and females). The lumbar region (cauda equina) was most profoundly affected and the females appeared to be more sensitive.

Other treatment related microscopic findings in the main group were minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). Peer review of the pathology confirmed the general findings, although there were some minor differences on severity and in some instances the involvement of vacuolation in several tissues.

There were no changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal).

After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males and could not be determined in females.

This 90-day oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a subchronic (90-day) oral toxicity study OPPTS 870.3100 in the rat.

8. **Inhalation (range-finding) Toxicity Study** - Rat , MRID 46751302

EXECUTIVE SUMMARY: In a range-finding inhalation toxicity study (MRID 46751302), R107894 (93% a.i., lot no. 89000015) was administered to 5 Sprague-Dawley rats/sex/dose via nose only exposure at dose levels of 0, 24, 80 (6 hours a day for 12 days), or 240 mg/m^3 for 4 hours and 25 minutes, due to high mortality. The surviving animals were held in the animal room and observed without further exposure. Additional groups of 5 Sprague-Dawley male rats/dose were administered the test material via nose only exposure at dose levels of 0 or 120 mg/m^3 for 6 hours for one day only due to high mortality. All surviving animals were euthanized the following day. The 24, 80, 120 or 240 mg/m^3 dose levels were determined to be equivalent to 24, 80, 126, or 236 mg/m^3, respectively, of the nominal concentration of the test substance. MMAD and GSD values were within acceptable limits.

Clinical signs, body weight, food consumption, gross and microscopic pathology, organ weight, and a functional observational battery were performed. Clinical chemistry, hematology and urinalysis were not performed.

Several unscheduled deaths occurred during the course of the study: in the 240 mg/m^3 group, 4/5 males and 2/5 females died during the first day of exposure, thus, prompting the termination of exposure at 4 hours and 25 minutes; in the 120 mg/m^3 group, 3/5 males died after a single 6 hour exposure. There were no test article-related effects in food consumption, gross or microscopic pathology, or organ weights. A decrease in body weight gain was observed in the 80-mg/m^3 rats. Observations in 240-mg/m^3 surviving rats included rales and labored respiration. At the 120-mg/m^3 exposure level, hypoactivity, increased respiration, gasping and/or vocalization upon handling were observed. No signs of toxicity were observed in the 24-mg/m^3 animals.

The LOAEL is 80 mg/m^3 based on reduced body weight gain. The NOAEL is 24 mg/m^3.

This range-finding oral toxicity study in the rat is **ACCEPTABLE-NONGUIDELINE. No guidelines are available for this type of study.**

9. **Subchronic Inhalation Toxicity and Neurotoxicity Screening Battery – Rats,** MRID 468022-02

This executive summary is for the Subchronic Inhalation Toxicity, Phase I

EXECUTIVE SUMMARY: In a combined subchronic inhalation toxicity (Phase I) and Neurotoxicity study (Phase II), R107894 (Lot no. ZR107894EXA028; 92.4% a.i.) was administered via nose-only exposure (MRID 46802202), to 10 Crl:CD (Sprague Dawley) rats/sex/dose in Phase I and 12 rats/sex/dose in Phase II at nominal concentrations of 0, 20, 40, and 80 mg/m^3. (analytical concentration equivalent to 0, 20, 40 and 80 mg/m3 of the test substance). The exposure period was 6 hours per day, 7 days per week for a minimum of 90 consecutive days. Particle size was determined and was within the accepted range.

No observable adverse effect level was 20-mg/m^3 for R107894 following measured parameters: survival, hematology, clinical chemistry, or gross pathology. Nasal lesions were observed in the 20-mg/m^3 animals and were considered a local effect.

One female rat in the Phase 1 inhalation toxicity study control group was found dead on study Day 9. This animal was in the control group, therefore the death was not considered test article-related. Three males in the 80-mg/m^3 Phase II neurotoxicity study group were found dead on study Days 0, 20, and 85, respectively. Additionally, an 80-mg/m^3 female in the Phase II neurotoxicity study group was found dead on Day 0 after the first exposure. Since there were numerous deaths in the 80-mg/m^3 (high-exposure) group these deaths were considered test article-related.

Clinical signs of toxicity related to treatment included brown staining on multiple body surfaces in males and females of all three test article-treated groups. Staining, which began on Day 11 and persisted through the end of the study, was observed on the neck, forelimb, abdominal area, thoracic area, inguinal area, hindlimb, rump, anogenital area, urogenital area, and tail. Reduced mean body weights for the male and female animals of the 20- (males), 40-, and 80-mg/m^3 test groups were statistically significantly lower than the control group. There was a ≤10% reduction in body weight in the 20-mg/m^3 males and females; cumulative body weight gains were 5% lower than controls in the 20-mg/m^3 females and 17% lower in the 20-mg/m^3 males. The reduced mean body weights for 40- and 80-mg/m^3 male and females and the 20-mg/m^3 males were consistent with lower food consumption (g/animal/day), body weight gains (weekly and cumulatively) and food efficiency.

Microscopic treatment related effects were limited to the nose of all of the treated animals. The effects were observed to be localized in the dorsal region of the nose, and included inflammation, ulceration or erosion, squamous or respiratory metaplasia, and olfactory or respiratory epithelial degeneration. These changes displayed a dose-related increase in incidence and severity.

The LOAEL of R107894 for male and female is 20-mg/m^3 based on the findings observed in the N1 (Nasal section 1) for both sexes and N3 section in the females of the 20-mg/m^3 animals. The 20-mg/m^3 males exhibited chronic and subacute inflammation, ulcerations (N1), and exudate (N1), and the females had subacute inflammation (N1), epithelial hyperplasia (N1), hyperkeratosis (N1), and degeneration of olfactory epithelium (N3). The NOAEL could not be determined (<20 mg/m3)(LDT).

The systemic toxicity LOAEL was 20 mg/m3 (LDT) in the male based on a decrease in body weight gain. The NOAEL in the male could not be determined (<20 mg/m3).

The LOAEL in the female was 40 mg/m3 based on a decreased in body weight gain. The NOAEL was 20 mg/m3.

This subchronic inhalation toxicity study in the rat is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rats.

10. **Subchronic Inhalation Toxicity and Neurotoxicity Screening Battery – Rats**, MRID 468022-02

This executive summary is for the Neurotoxicity Screening Battery, Phase II

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 468022-02), groups of Crl:CD(SD) 12 Sprague-Dawley derived rats/sex/dose (**Phase II**) were exposed to R107894 (92.4% a.i. of Lot no. ZR107894EXA028) via nose only exposure at targeted dose levels of 0, 20, 40, or 80 mg/m^3 for 6 hours a day for a minimum of 90 consecutive days (~13 weeks). The particle size was within the required range.

Another phase of the study evaluating the subchronic inhalation toxicity of R107894 was performed on an additional 10 animals/sex/dose (**Phase I**) and discussed in the accompanying subchronic inhalation toxicity DER for R107894.

Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all Phase II animals (12 rats/sex /group) prior to dose administration (pretest), and at Weeks 3, 7, and 12. Cholinesterase activity was not determined. At study termination, 6 animals/sex/dose in the controls and 80-mg/m^3 groups were perfused in situ for neuropathological examination of the central and peripheral nervous system tissues

For the Phase II neurotoxicity study, after the first day of exposure, 1 male and 1 female were found dead in the 80-mg/m^3 group and were replaced. Two 80-mg/m^3 males died prior to study termination, one on Day 20 and the other on Day 85.

During FOB handling measurement, the 40- and 80-mg/m^3 animals were observed to have soiled fur, unclean fur, and increased grooming (80-mg/m^3 males) during Weeks 7 and 12. These changes correlate with the clinical toxicity findings of soiled fur and were not considered adverse neurotoxicological findings. During Week 3 FOB measurements, an overall decrease in total locomotor activity was noted in the 40- and 80-mg/m^3 males and in ambulatory activities for 80-mg/m^3 males as a result of reduced activity in sub-session measurements. During Week 12, the 80-mg/m^3 males were observed with total activity decrease compared to controls.

At neuropathology, an increase in minimal axonal degeneration was observed in the peroneal nerve in the 80-mg/m^3 animals (3/6 for both males and females) over controls (0/0 for both males and females).

The neurotoxicity LOAEL was 40 mg/m^3 in males based on decreased motor activity and 80 mg/m^3 in females based on axonal degeneration observed in the peroneal

nerve. The neurotoxicity NOAEL was 20 mg/m^3 for males and 40 mg/m^3 for females.

This Neurotoxicity Screening Battery is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

II. Toxicology Endpoints for Risk Assessment

Note: These endpoints were discussed at the meeting of June 27, 2006

Rationale and Comments on the Studies used for the Toxicology Endpoints for Risk Assessment

Incidental Oral Exposure Scenario: Econea is used as an antifoulant for paints used in fresh water and the possibility of incidental oral ingestion might occur.

Dermal Exposure Scenario: The Subchronic Dermal study at the low and mid dose levels did not have lung histopathology performed. The study, therefore, was classified as Not-Acceptable. The study can be upgraded to Acceptable if the lung pathology were evaluated for the low and intermediate dose levels.

Table 3. Toxicology Endpoints for Risk Assessment for Econea-technical

Exposure Scenario	Dose Used in Risk Assessment	Target MOE for Risk Assessment	Study and Toxicological Effects
Acute and Chronic Dietary	Risk assessment not required based on use pattern		
Non-Dietary Occupational/Residential Exposures			
Incidental Oral Short-Term (1-30 days) and Intermediate-Term(1–6 months) (Occupational /Residential)	**NOAEL** = <6.3 mg/kg/day (LDT) for females	Target MOE = 300 (10x interspecies extrapolation, 10x intrapecies variation, 3x for lack of a NOAEL in females)	**Subchronic (90 Day) Oral** **LOAEL** = 6.3 mg/kg/day (LDT) for females based on based microscopic findings of the brain and spinal cord. **Classification: Acceptable/Guideline**
Dermal* Short-Term (1-30 days) and Intermediate-Term(1–6 months) (Occupational)	**Dermal Signs:** **NOAEL** = 100mg/kg/day **Systemic+:** **NOAEL** = ≥1000 mg/kg/day (HDT, limit dose	Target MOE = 100 (Dermal signs and systemic) (10x interspecies extrapolation, 10x intrapecies variation+.	**Subchronic (90 Day) Dermal** **Dermal Signs:** **LOAEL** = 300 mg/kg/day based on the appearance of sores **Systemic+:** **LOAEL** >1000 mg/kg/day

			Classification: Acceptable/Guideline
Derrnal* Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		
Inhalation Short-Term (1-30 days) and Intermediate (1 – 6 months) Term (Occupational)	**NOAEL** could not be calculated (<20mg/m3) (LDT).	Target MOE = 300 10x interspecies extrapolation, 10x intrapecies variation, 3x conversion of LOAEL to NOAEL	**Subchronic (90 Day) Inhalation Toxicity (Phase I)** LOAEL is 20-mg/m^3 based on the findings observed in the nasal section N1 to N3 and included chonic and subacute inflamma-tion , ulcerations, exudates, epithelial hyperplasia, hyperkeratosis and degeneration of the olfactory epithelium. Decreased body weight gain was also seen at this dose level. **Classification: Acceptable/Guideline**
Inhalation Long-Term (6 months – lifetime) (Occupational)	Risk assessment not required based for this use pattern		
Cancer	No cancer data available		

* 100% absorption factor will be used.

+ The primary consideration for the short and intermediate dermal exposure scenario is the systemic NOAEL/LOAEL; the dermal signs NOAEL/LOAEL is of secondary (minimal) importance.

DATA PACKAGE BEAN SHEET

Date: 08-Feb-2007

* * * Registration Information * * *

Registration: 43813-ET - ECONEA TECHNICAL

Company: 43813 - JANSSEN PHARMACEUTICA INC.

Risk Manager: RM 33 - Marshall Swindell - (703) 308-6341 Room# PY1 S-8828

Risk Manager Reviewer: Demson Fuller DFULLE02

Sent Date: 23-Aug-2006 Calculated Due Date: 30-Mar-2007 Edited Due Date:

Type of Registration: Product Registration - Section 3

Action Desc: (A41) NEW AI;NON-FOOD USE;OUTDOOR;OTHER USES;

Ingredients: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-(93.2%)

* * * Data Package Information * * *

Expedite: Yes ● No Date Sent: 05-Jan-2007 Due Back:

DP Ingredient: 119093, 1H-Pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)-

DP Title: Dermal Toxicity Study

CSF Included: Yes ● No Label Included: Yes ● No Parent DP #:

Assigned To	Date In	Date Out		
Organization: AD / RASSB	12-Jan-2007	08-Feb-2007	Last Possible Science Due Date:	22-Sep-2006
Team Name: RASSB2	12-Jan-2007	08-Feb-2007	Science Due Date:	08-Jan-2007
Reviewer Name: Malish, Steven	12-Jan-2007	08-Feb-2007	Sub Data Package Due Date:	
Contractor Name:				

* * * Studies Sent for Review * * *

Printed on Page 2

* * * Additional Data Package for this Decision * * *

Printed on Page 3

* * * Data Package Instructions * * *

Please review the study to complete the risk assessment for ECONEA, EPA Reg No. 43813-ET. If you have any questions, please follow up with Marshall Swindell or Karen Leavy. Thanks.

Demson

EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Sr. Scientist
Team 2, RASSB/Antimicrobial Division

S. L. Malish 2/2/07
Jonathan Chen 02/07/07

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (90-Day Dermal) Toxicity - Rats; OPPTS 870.3250 (rodent) OECD 411.

PC CODE: 119093
DECISION No: 220066
DP BARCODE: 335209
EPA REG No: 43813-ET

TEST MATERIAL (PURITY): 1H -Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl); (94.6%)

SYNONYMS: , Econea®028, CL 303'268, R107894, Econea Technical

CITATION: Merkel, D. (2006). R107894: Dermal toxicity study (90-day repeat dermal application study in rats). Product Safety Laboratories, Dayton, New Jersey. Laboratory study number 17279, March 24, 2006. MRID 46802201 and 470066-01. Unpublished.

Merkel, D. (2006). R107894: Amendment to R107894: Dermal toxicity Study (90-day repeat dermal application study in rats). Eurofins/Product Safety Laboratories, Dayton, New Jersey. Laboratory study number 17279, Dec 8, 2006. MRID 47066-01. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Plant & Material Protection Division, Beerse, Belgium.

Background: R107894: Dermal toxicity study (90-day repeat dermal application study in rats), MRID 46802201 (2006) was previously submitted. Because the study was considered to be not acceptable, but upgradeable (with the addition of lung histopathological data at the low and intermediate dose levels), the sponsor submitted additional data (Amendment to R107894, MRID 47006601 (2006) to upgrade the original report. A statistical analysis was also done on the lung lesions and was included in the Appendix. This DER incorporates both the original data and the new data contained in the above amendment and should be considered the final report.

EXECUTIVE SUMMARY: In a 90-day dermal toxicity study (MRID 468022-01, MRID 46802201), R107894 (94.6% a.i., Lot #AC 12649-8) was applied to the shaved skin of 10 rats, Crl:CD® (SD)/sex/dose at dose levels of 0, 100, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 90-day period.

One 100-mg/kg/day female died prior to study termination. A treatment-related dose-dependent increase in small dermal sores was noted in both the mid- and high-dose group. Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.

There were no other adverse findings with this substance.

The systemic NOAEL is ≥1000 mg/kg/day (HDT). The systemic LOAEL is >1000 mg/kg/day. One thousand (1000) mg/kg/day is considered the limit dose; at or above this dose, no more testing need be done.

This 90-day dermal toxicity study in the rat is **ACCEPTABLE – GUIDELINE.** The study, satisfies the guideline requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats.

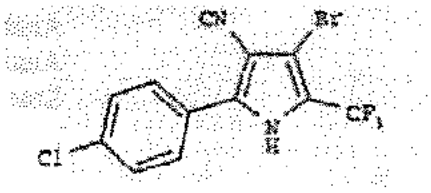
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claims statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: R107894

Description:	Light tan powdered solid stored at room temperature and not exposed to heat or direct sunlight
Lot/batch #:	AC 12649-8
Purity:	94.6 % a.i.
Compound stability:	Chemical analysis, homogeneity and stability were found in other studies in this series
CAS #:	122454-29-9
Structure	$C_{12}H_5BrClF_3N_2$



MRID 46442201

2. Vehicle and/or positive control: Distilled water (3 mL)

3. Test animals:

Species:	Rat	
Strain:	Crl:CD® (SD)	
Age/weight at study initiation:	Animals were 9 weeks old at test initiation; males weighed 302-345 grams, females weighed 211-236 grams	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually-housed in suspended stainless steel cages	
Diet:	Purina Certified Rodent Meal #5002 was available *ad libitum* during acclimation and throughout the study. There were reportedly no contaminants present in animal feed at concentrations sufficient to affect the overall study.	
Water:	Filtered water was available *ad libitum* by automatic water dispensing system; there were reportedly no contaminants present in the animal water at concentrations sufficient to affect the overall study.	
Environmental conditions:	Temperature:	19-23EC
	Humidity:	28-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	12 days	

B. STUDY DESIGN:

1. **In life dates:** Start: August 10, 2005; End: November 10, 2005

2. **Animal assignment**: Eighty (40 males and 40 females) healthy animals (i.e., those free of clinical disease or injury) with body weights within ± 20% of the mean were randomly assigned to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	10	10
Low	100	10	10
Mid	300	10	10
High	1000	10	10

3. **Dose selection rationale:** The dose levels were selected based on the results from the 28-day repeated dose study (PSL Study No. 17026) in which the neat test substance was moistened with distilled water and applied dermally at dose levels 0, 30, 100, 300, or 1,000 mg/kg/day. No evidence of dermal irritation or systemic toxicity was observed. Thus, the study authors expected to find a NOAEL by administering concentrations of 0, 100, 300, or 1,000 mg/kg/day to the rats in this study.

4. **Preparation and treatment of animal skin:** Fur was removed by clipping an area from the shoulders to the hips (i.e., greater than 10% of the body surface) from all rats during the acclimation period. Shortly before the first application and weekly thereafter, the fur of each test animal was re-clipped. A 4-ply gauze pad was used to administer the appropriate amount of substance, moistened with 0.3 milliliters of distilled water, to the dose site. The applied quantities of the test substance were adjusted weekly to individual animal body weight. The test substance/vehicle suspension was fastened to the trunk of the body with non-irritating, adhesive tape. The dressings were removed after 6 hours and animals were wiped with a 3% solution of Ivory® dishwashing liquid using clean towels, rinsed with water, and patted dry. Treatment occurred at approximately the same time each day (except during blood collection) for 5 days of any 7-day period, and the first day of application was considered Day 1. Animals that were not in their wrapping after the 6 hours of exposure were noted.

   On Days -6, -5, and -2, the "sham wrapping" spanned approximately 1 hour, 3 hours, and 6 hours, respectively. Animals were weighted, and the skin was checked for abnormalities prior to the first application of the test substance. Animals were re-clipped, as needed, throughout the study.

5. **Statistics:** Statistical analysis was conducted for body weight, daily body weight gain, daily food consumption, daily food efficiency, organ weight, and organ-to-body/brain weight ratio data. Homogeneity of variance and normality within test groups were evaluating using Bartlett's test. When variances were homogenous, treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's t-test for multiple comparisons. When variances were considered significantly different by Bartlett's test, groups were compared using Kruskal-Wallis non-parametric analysis of variance, followed by Dunn's test. A probability value of $p \leq 0.05$ was considered to be a significant difference among groups.

   For the means and standard deviations of clinical pathology quantitative data, Levene's test for variance of homogeneity (Levene, H., 1960) and the Shaprio-Wilk test for normality

(Shapiro, S.S. and Wilk, M.B., 1965) were used. If variances were considered to be not significant, groups were compared using a one-way analysis of variance (ANOVA) (Snedecor, G.W. and Cochran, W.G., 1967) followed by Dunnett's t-test for multiple comparisons (Dunnett, C.W., 1964, 1980, Tamhane, A.C., 1979). If the Shaprio-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. Where variances were considered significantly different by Shaprio-Wilk test, groups were compared using Kruskal-Wallis non-parametric analysis of variance followed by Dunn's test (Kruskal, W.H. and Wallis, W.A., 1952, Dunn, O.J., 1964).

Histopathological data concerning lung incidences of increased bronchoalveolar associated lymphoid tissue (BALT) and chronic inflammation were statistically analyzed using non-parametric approaches (Fisher's Exact Test and the Cochran-Armitage Trend Test) and using a logistic regression model. The severity data were analyzed using similar statistical approaches (see Appendix 1).

A probability value of $p \leq 0.05$ was considered to be a significant difference among groups.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were observed twice daily for signs of mortality. Cageside observations were determined daily from Day 1 to 91 (males) or 92 (females). Abnormalities were recorded.

1b. Clinical examinations: Detailed observations were recorded on Day 1 prior to test substance application, and weekly thereafter. Observations were made both when handling the animal and when placed in an open field. Clinical signs observed included, but were not limited to changes of the skin, fur, eyes, and mucous membranes and the occurrence of secretion and excretion. The animals were examined for signs of local skin irritation each Monday prior to application and again on each Friday after removing the gauze patches; animals were evaluated using the Draize method. Mean erythema and edema scores were determined for each group at each time interval; the sum of the mean erythema and mean edema for each group at each time interval was defined as the primary dermal irritation (PDI).

1c. Neurological evaluations: It was reported that animals were examined for autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern), as well as changes in gait, posture, and response to handling during clinical examinations. Additionally, the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) were recorded during clinical examinations.

2. Body weight:

Animals were weighed 3 times during acclimation, prior to initiation of the study, and weekly thereafter. Surviving animals were weighed immediately before sacrifice.

Mean body weight gains were calculated for each dose group for each interval and for the overall test interval.

3. **Food consumption:** Food consumption was determined weekly for each individual, adjusting for spillage. The method of collecting the food consumption data was not specified. Mean food consumption was reported as g food/animal/week. Food consumption ratios were calculated weekly.

4. **Ophthalmoscopic examination:** Eyes of the 100 test animals considered for the study (only total of 80 animals used) were examined by focal illumination and indirect ophthalmoscopy prior to study initiation; the eyes were re-examined on Day 87.

5. **Hematology and clinical chemistry:** Blood was collected via orbital sinus under isoflurane anesthesia on Day 86 for hematology and clinical chemistry from all surviving animals; however, clotting occurred in 2 males in dose group 100 mg/kg/day and 1 male in group 300 mg/kg/day, and 1/10 female in group 100 mg/kg/day died prior to sacrifice, thus decreasing the number of samples evaluated. Blood samples used to determine the prothrombin time and partial thromboplastin time were collected via the inferior vena cava using 3 ml syringe/21-gauge needle under isoflurane anesthesia at terminal sacrifice. All animals were fasted overnight prior to blood collections. The CHECKED (X) parameters were examined.

   a. **Hematology:** In addition to the parameters marked below, any platelet clumps that were observed were noted.

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Red cell distribution width (RDW)
X	(Activated partial thromoboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250

b. **Clinical chemistry:** Following serum chemistry analysis, serology testing was performed on a pooled sample from two animals.

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium* (K)	X	Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
X	Sorbitol dehydrogenase*		

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250

6. **Urinalysis:** Urine was not collected and analyzed for these study animals.

7. **Sacrifice and pathology**: All surviving animals were sacrificed (euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia) on schedule (Day 92 for males, Day 93 for females) and subjected to gross pathological examination. Gross lesions and tissues of interest (including those for histopathology examination) were preserved in 10% neutral buffered formalin. Histopathology examinations were conducted for all control and high-dose animals. Histopathology examinations also were conducted on gross lesions from animals in other groups. Fixed tissues were processed to paraffin-embedded sections, placed on glass microscope slides, and stained with hematoxylin and eosin. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*		Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated areas)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*	X	Mammary gland*		
X	Nose*	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250
\+ Organ weights required.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** Abnormal observations are summarized in Table 2. Treatment-related ano-genital staining was observed in all treated males and females. This observation was not considered adverse. There appears to be a treatment-related increase in small sores observed in mid- and high-dose males and females.

Table 2. Summary of Clinical Observations[a]				
Dose rate (mg/kg/day)	0	100	300	1000
MALES (Number of animals showing symptoms)				
Observations During Removal from Cage and Handling				
Fur/Skin				
Ano-genital staining	0	1 (Day 50)	8 (Days 15-91)	10 (Days 15-91)
Small sore	0	0	4 (between Days 22-36)	6 (between Days 36 and 91)
FEMALES (Number of animals showing symptoms)				
Observations During Removal from Cage and Handing				
Fur/Skin				
Ano-genital staining	0	3 (Days 50, 71, or 78)	9 (between Days 15-85)	10 (between Days 15-91)
Small Sore	0	0	1 (Day 29)	3 (between Days 50-91)

[a] Data obtained from pages 36-37 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42..
[b] One animal found dead on Day 42 prior to dosing.

2. **Mortality:** One female rat in the 100 mg/kg/day (low) dose group was found dead on Day 42 prior to dosing. Severe urogenital inflammation was noted, and the death was not attributed to the treatment.

3. **Neurological evaluations:** There were no changes to behavior noted during the study.

4. **Dermal irritation:** Due to a technical error, scores for erythema and edema were not recorded at the end of the first week of exposure following patch removal. No Primary Dermal Irritation (mean erythema + mean edema) was observed for any of the animals. Small sores were noted in 4/10 males administered 300 mg/kg/day, 6 males administered 1000 mg/kg/day, 1/10 female administered 300 mg/kg/day during, and 3/10 females administered 1000 mg/kg/day (see Table 2).

B. **BODY WEIGHT AND WEIGHT GAIN:** Mean body weight and mean body weight gain data are indicated in Table 3 below. There were no treatment-related changes in mean body weight or mean body weight gain for either males or females. A few statistically significant changes in mean body weight and mean body weight gain are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 3: Mean body weights (BW) and body weight gains (BWG) during 91 days of treatment[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (g±SD)				
Initial (Day 1)	323.9±14.0	322.8±10.7	322.4±10.0	319.3±11.7
Day 8	349.4±15.6	345.5±15.6	346.4±10.1	337.9±17.5
Day 15	367.2±18.1	371.7±17.9	366.0±14.2	355.5±21.4
Day 22	388.8±21.0	387.2±17.9	386.5±18.3	372.2±23.6
Day 29	409.1±23.7	409.2±19.4	406.6±22.4	390.5±26.4
Day 36	422.7±27.2	428.5±23.7	425.5±25.7	407.2±27.9
Day 43	439.5±29.7	443.3±20.0	437.1±27.7	413.1±34.5
Day 50	453.7±30.2	457.6±30.2	445.5±34.1	424.4±31.5
Day 57	472.5±33.2	472.7±28.9	462.8±33.7	443.1±40.3
Day 64	480.9±31.8	479.6±28.3	472.8±33.7	451.7±40.9
Day 71	487.3±36.7	481.2±24.8	478.2±31.5	458.1±40.4
Day 78	492.3±39.1	496.7±30.3	494.5±33.8	465.2±46.4
Day 85	508.1±42.4	508.2±36.7	506.1±31.5	479.5±46.9
Final (day 91)	504.8±42.7	515.7±34.0	505.7±31.4	478.6±46.6
Overall BWG Day 1-91 % Control	13.9±2.6 100%	14.8±2.4 106%	13.3±3.3 96%	12.3±3.2 88%
FEMALES (g±SD)				
Initial (Day 1)	224.6±5.1	225.5±6.6	222.8±7.0	222.9±5.6
Day 8	236.8±6.7	237.4±10.1	232.8±7.5	230.3±6.0
Day 15	241.2±6.1	243.1±8.4	235.4±9.5	234.3±9.4
Day 22	250.6±7.6	252.3±11.6	245.1±10.0	243.4±11.2
Day 29	255.9±7.4	262.0±9.7	253.4±12.7	251.4±12.5
Day 36	259.4±7.0	266.7±14.4	257.0±12.3	255.0±13.8
Day 43	266.9±12.3	273.4±14.1	259.4±15.9	256.7±13..9
Day 50	271.4±10.3	280.8±16.0	265.2±14.3	263.8±15.4
Day 57	276.9±10.9	284.3±16.8	271.0±17.2	268.3±16.0
Day 64	274.6±10.7	287.1±18.8	272.1±17.8	269.0±16.6
Day 71	274.2±13.0	290.8*±18.6	271.7±14.8	262.5±10.4

TABLE 3: Mean body weights (BW) and body weight gains (BWG) during 91 days of treatment [a]

Dose rate (mg/kg/day)	0	100	300	1000
Day 78	279.3±9.8	291.7±26.7	275.5±16.7	271.8±17.9
Day 85	283.5±14.8	303.2±21.5	284.4±18.4	280.3±17.8
Final (day 91)	279.7±10.7	294.9±16.5	278.1±17.0	278.1±18.9
Overall BWG Day 1-91 % Control	4.2±0.9 100%	5.3±1.0 126%	4.3±1.4 102%	4.2±1.3 100%

[a] Data obtained from pages 28-31 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
* Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption:** Food consumption data are indicated in Table 4 below. There were no treatment-related changes in food consumption for either males or females. A few statistically significant changes in food consumption are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 4. Average Food Consumption (FC) (g/week)[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (g±SD)				
1-8	201.2±13.0	203.4±18.5	201.1±10.8	196.0±17.8
8-15	203.3±11.6	214.8±15.7	206.3±13.9	199.8±16.7
15-22	206.5±14.4	215.3±19.9	213.3±19.3	206.5±17.5
22-29	213.3±20.5	226.2±16.9	211.9±12.0	214.5±16.6
29-36	205.4±15.5	220.0±17.9	211.1±16.7	210.8±20.2
36-43	214.3±16.8	220.9±17.0	211.9±15.2	210.1±18.3
43-50	211.1±16.1	199.2±31.6	206.4±21.4	209.9±19.4
50-57	216.8±16.7	219.4±22.8	210.2±13.4	202.8±29.4
57-64	219.2±8.3	216.4±12.7	211.5±11.9	214.3±16.9
64-71	212.3±21.4	217.5±25.5	206.7±35.6	210.6±15.2
71-78	203.7±12.8	208.1±31.1	211.8±14.2	211.3±22.8
78-85	218.6±14.3	218.6±15.8	210.2±15.3	213.9±19.2
85-91	162.8±15.3	169.0±18.8	148.6±33.4	164.1±14.3
Overall (1-91)	206.8±13.0	211.4±13.2	204.7±11.7	205.0±16.5

TABLE 4. Average Food Consumption (FC) (g/week)[a]				
Dose rate (mg/kg/day)	0	**100**	**300**	**1000**
FEMALES (g±SD)				
1-8	153.6±10.3	158.6±11.1	155.0±8.2	159.6±13.5
8-15	160.7±10.1	162.5±8.7	164.8±7.7	157.7±13.0
15-22	164.5±9.8	166.7±13.1	167.0±9.2	163.1±11.8
22-29	162.8±11.1	169.3±11.4	164.6±9.8	170.1±14.3
29-36	158.4±17.1	164.4±9.0	155.7±9.4	156.2±14.3
36-43	161.0±14.0	167.9±13.6	158.4±8.0	160.0±14.6
43-50	160.9±15.4	170.7±15.5	160.3±10.4	161.2±13.9
50-57	153.0±13.9	163.0±11.4	157.6±11.8	154.1±12.9
57-64	153.8±13.3	165.3±13.2	159.4±8.5	156.4±11.7
64-71	159.1±25.3	166.7±9.6	159.3±10.4	172.9±54.8
71-78	152.2±16.1	156.2±32.6	153.1±13.4	171.3±34.9
78-85	147.4±20.6	170.2*±21.3	158.5±11.9	159.1±15.3
85-91	114.4±11.5	115.0±20.0	112.2±8.2	119.0±11.2
Overall (1-91)	154.0±9.6	161.8±9.3	155.8±7.2	158.5±11.3

[a] Data obtained from pages 32-33 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42..
* Statistically different (p <0.05) from the control.

2. **Food efficiency:** Food efficiency data are indicated in Table 5 below. There were no treatment-related changes in food efficiency for either males or females. A few statistically significant changes in food efficiency are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 5. Average Weekly Food Efficiency (g/week)[a]				
Dose rate (mg/kg/day)	0	**100**	**300**	**1000**
MALES (g±SD)				
1-8	0.04±0.01	0.11±0.03	0.12±0.02	0.09±0.05
8-15	0.09±0.04	0.12±0.05	0.09±0.03	0.09±0.03
15-22	0.10±0.04	0.07±0.04	0.09±0.03	0.08±0.03
22-29	0.10±0.02	0.10±0.04	0.09±0.04	0.08±0.04
29-36	0.07±0.04	0.09±0.04	0.09±0.04	0.08±0.04
36-43	0.08±0.04	0.06±0.05	0.05±0.02	0.03*±0.04
43-50	0.07±0.02	0.08±0.08	0.04±0.05	0.06±0.05

TABLE 5. Average Weekly Food Efficiency (g/week)[a]				
Dose rate (mg/kg/day)	0	100	300	1000
50-57	0.09±0.03	0.07±0.03	0.08±0.03	0.09±0.06
57-64	0.04±0.03	0.03±0.03	0.05±0.03	0.04±0.01
64-71	0.03±0.05	0.01±0.05	0.02±0.10	0.03±0.02
71-78	0.02±0.07	0.07±0.07	0.08±0.06	0.03±0.03
78-85	0.07±0.04	0.05±0.06	0.05±0.08	0.07±0.02
85-91	-0.02±0.06	0.04±0.10	-0.20±0.66	-0.01±0.04
Overall (1-91)	0.06±0.01	0.07±0.01	0.06±0.01	0.06±0.01
FEMALES (g±SD)				
1-8	0.08±0.03	0.07±0.03	0.07±0.04	0.05*±0.02
8-15	0.03±0.05	0.04±0.03	0.02±0.04	0.02±0.03
15-22	0.06±0.04	0.05±0.03	0.06±0.02	0.06±0.03
22-29	0.03±0.04	0.06±0.04	0.05±0.03	0.05±0.03
29-36	0.02±0.04	0.03±0.04	0.02±0.03	0.02±0.03
36-43	0.04±0.05	0.03±0.05	0.02±0.03	0.01±0.04
43-50	0.03±0.06	0.04±0.04	0.03±0.04	0.04±0.04
50-57	0.04±0.02	0.02±0.05	0.04±0.03	0.03±0.04
57-64	-0.02±0.02	0.02*±0.03	0.01±0.02	0.00±0.02
64-71	0.00±0.04	0.02±0.04	0.00±0.03	-0.07±0.17
71-78	0.03±0.04	-0.01±0.08	0.02±0.02	0.05±0.08
78-85	0.02±0.06	0.07**±0.06	0.06±0.02	0.05±0.02
85-91	-0.04±0.06	-0.09±0.15	-0.06±0.06	-0.02±0.05
Overall (1-92)	0.03±0.01	0.03*±0.01	0.03±0.01	0.03±0.01

[a] Data obtained from pages 34-35 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
* Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test.
** Statistically different (p <0.05) from the control by Kruskal-Wallis test.

D. OPHTHALMOSCOPIC EXAMINATION: All animals were evaluated as opthalmoscopically normal, except for two 100-mg/kg/day females, which exhibited hyphema in the right eye. This symptom was deemed to be caused by trauma that might occur during certain forms of blood collection, and was not associated with exposure to an ocular toxicant. Histopathological findings related to the eye, however, were found only in one of these 2 females.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related hematology findings in male or female rats. Females administered 1000 mg/kg/day exhibited a significant increase in the average red cell distribution width; however, this finding was not considered treatment-related because there were no changes in red cell mass parameters (red blood cell count, hemoglobin, and hematocrit). Additionally, there were more treated animals (males and females) with trace anisocytosis (variation in red cell size) and macrocytosis (large cells); however, severity grades in these groups were not higher than the respective controls.

2. **Clinical chemistry:** Possible treatment-related changes to clinical chemistry parameters are presented in Table 6. In males, there was a significant increase in urea nitrogen at 1000 mg/kg/day. This increase was considered to be possibly related to treatment. However, it also was noted that values were within historical control values (Haskell Notebook Number E-98560-AN; not provided); other related parameters (creatinine, inorganic phosphorous) were comparable to control values, and there were no microscopic changes to the kidneys at this dose group. Therefore, this change was considered to be non-adverse. In females, there were significant increases observed in triglyceride levels at 1000 mg/kg/day. Although this change was considered treatment-related, it also was considered non-adverse because all but one value were within the historical control data (Haskell Notebook Number E-98560-AN; not provided). Females administered 1000-mg/kg/day also exhibited a decrease in albumin that was considered treatment-related, but not adverse because values for 5 females were within the historical data range (Haskell Notebook Number E-98560-AN; not provided) and those outside the range were only minimally below the lower limit of the reference interval. Lastly, there was an increase in alkaline phosphatase observed in 100-, 300-, and 1000-mg/kg/day females; this increase only reached statistical significance at 100 mg/kg/day. Because of the lack of a dose-response relationship, this effect was not considered treatment-related.

TABLE 6. Selected Clinical Chemistry Data[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (Mean±SD)				
Alkaline Phosphatase (U/L)	117±32	129±28	125±32	108±21
Blood Urea Nitrogen (mg/dL)	13±1	14±1	14±2	16*±2
Triglycerides (mg/dL)	62±46	85±72	80±46	72±35
Albumin (g/dL)	3.4±0.2	3.4±0.1	3.3±0.1	3.2±0.3
FEMALES (Mean±SD)				
Alkaline Phosphatase (U/L)	59±24	84*±23	80±19	75±17
Blood Urea Nitrogen (mg/dL)	16±3	15±2	16±2	17±2
Triglycerides (mg/dL)	33±7	30±7	39±12	53*±17
Albumin (g/dL)	3.7±0.3	3.6±0.2	3.6±0.2	3.4*±0.2

[a] Data obtained from pages 50-53 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
.* Statistically significant difference from control at p<0.05 by Dunnett/Tamhane-Dunnett test.

F. URINALYSIS: A urinalysis was not conducted.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Select relative organ weights are presented in Table 7 below. There were no treatment-related changes in absolute or relative (to body weight or brain weight) organ weight changes. Although significant changes in absolute and relative organ weight were observed (increased absolute male heart weight at 300 mg/kg/day; increased relative [to body weight] male adrenal and liver weight at 300 and 1000 mg/kg/day, respectively; and increased absolute and relative [to body weight] female liver weight at 1000 mg/kg/day), these differences were considered minimal and did not correspond to any gross or microscopic changes. Therefore, they were not considered to be of toxicological importance.

TABLE 7. Select Organ Weight Data[a]

mg/kg/day		0	100	300	1000
MALES					
Final body weight	Mean±SD	504.8±42.7	515.7±34.0	505.7±31.4	478.6±46.6
	N	10	10	10	10
	% control	100%	102%	100%	95%
Absolute liver weight	Mean±SD	13.90±1.95	15.34±2.23	14.00±2.21	15.47±2.42
	N	10	10	10	10
	% control	100%	110%	101%	111%
Liver-to-body weight	Mean±SD	29.46±1.94	32.06±3.17	29.73±2.96	34.97*±3.74
	N	10	10	10	10
	% control	100%	109%	101%	119%
FEMALES					
Final body weight	Mean±SD	279.7±10.7	294.9±16.5	278.1±17.0	278.1±18.9
	N	10	9[b]	10	10
	% control	100%	105%	99%	99%
Absolute liver weight	Mean±SD	8.44±0.65	8.93±0.88	9.36±1.18	9.63*±1.26
	N	10	9[b]	10	10
	% control	100%	106%	111%	114%
Liver-to-body weight	Mean±SD	33.80±2.92	33.32±2.85	37.64*±3.16	38.69**±3.87
	N	10	9[b]	10	10
	% control	100%	99%	111%	114%

[a] Data obtained and calculated from pages 41-43 in the study report.

[b] One animal found dead on Day 42 prior to dosing.
* Statistically different (p <0.01) from the control by Dunnett's Multiple Comparisons test
** Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test

2. **Gross pathology:** Gross pathology findings were limited, and there were no treatment-related findings. With regard to males, an irregularly shaped spleen was found in one 100 - mg/kg/day male, in which capsule anomaly was found during microscopic evaluations. Also, a firm mass inguinal area (0.5 x 1.0 cm) in untreated skin was found in one 100-mg/kg/day male. At 300 mg/kg/day, 1/10 animals had crooked incisors, and 1/10 animals had a discolored liver. The animal with the discolored liver also exhibited signs of subcapsular congestion. At 1000 mg/kg/day, 1/10 animals had a moderately enlarged prostate with a 0.5 x 1.5 cm mass.

   Fluid-filled uteruses were found in 4/10, 5/10, and 6/10, and 4/10 females in dose groups 0, 100 (misprinted as 25 in Appendix K), 300, and 1000 mg/kg/day, respectively. In Appendix K, there were no indications of fluid filled ovaries. These results differ slightly from the histopathology findings for the 1000 mg/kg/day dose level which state that there was one animal with fluid filled ovaries. This inconsistency does not affect the overall study results. In the control group, 1/10 females (also counted in the fluid-filled uterus animals) was found with hydronephrosis in the right kidney. At 100 mg/kg/day, 1/10 female was found with an enlarged right eye. Another 100- mg/kg/day female (animal 3974, which was found dead on Day 42) exhibited the following: a fluid-filled thorax; a discolored left kidney with hydronephrosis and gritty material, a discolored right kidney, and a distended fluid-filled urinary bladder with hemorrhagic lining.

   Microscopic pathology: Select microscopic findings are summarized in Table 8 below. Minimal, but statistically not significant (p>0.05), increases were seen in the histopathology findings in the lungs (BALT and chronic inflammation) in males and females. The statistical analysis is presented in Appendix 1.

   Increased Bronchoalveolar Associated Lymphoid Tissue (BALT) in the male showed incidence values that were statistically not significant (p>0.05) at all dose levels (4/10 to 7/10), except for the Intermediate dose (7/10, p = 0.02), vs. the Control (1/10). This result is inconsistent since the effect was not statistically significant for the High dose compared to the Controls.

   The group mean severity values were also statistically not significant (p>0.05) at all dose levels (1.3 to 2.0) vs. the Control (1.0), except for the Intermediate dose (2.0, p = 0.08). This result is inconsistent since the effect was not statistically significant for the High dose compared to the Controls.

   For chronic inflammation, incidence values were statistically not significant (p>0.05) at all dose levels (4/10 to 8/10), vs. the control (4/10) while the group mean severity values were also statistically not significant (p>0.05) at all dose levels (1.3 to 1.9) vs. the control (1.3).

   In females, the BALT incidence were statistically not significant (p>0.05) at all dose levels (3/10 to 5/10) vs. the Control (3/10), while the group mean severity values were also

statistically not significant (p>0.05) at all dose levels (1.4-1.7) vs. the control (1.0). The chronic inflammation incidence was statistically not significant (p>0.05) in all dose groups (5/10 to 7/10), vs. the control (5/10). The chronic inflammation group mean severity values was also statistically not significant (p>0.05) in all dose groups (1.1–1.2), vs. the control (1.0).

Some findings like tubular mineralization in the kidney of females and chronic inflammation in the lungs of males show considerable variation between treatment groups. Females in all dose groups, including the control group, exhibited the gross observation of fluid filled uterus. This finding was correlated in some females with dilation of the uterine horns and was attributed to the stage of estrus. One 300-mg/kg/day male (animal 3959) exhibited a cystic dilated preputial gland duct that was chronically inflamed; these findings corresponded to gross pathology findings. One high-dose male (animal 4003) exhibited chronic inflammation of the prostate; this observation corresponded to gross pathology findings.

For the one low-dose female that died during the study, severe urogenital inflammation of the kidney papilla and urinary bladder was observed. The cause was speculated to be an ascending infection. One high-dose female had two sets of some organs and tissues.

TABLE 8. Summary of Histopathology Incidences[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (Number of animals showing symptoms /Number of animals evaluated)				
Kidney				
Infiltrate, Mononuclear Cell	4/10	0/0	0/0	4/10
Mineralization, Tubular	0/10	0/0	0/0	0/10
Nephropathy	6/10	0/0	0/0	8/10
Lung				
Increased Bronchoalveolar Associated Lymphoid Tissue (BALT)	1/10 (1.0)	4/10 (1.3)	7/10 (2.0)	4/10 (1.5)*
Inflammation, Chronic	4/10 (1.3)	4/10 (1.3)	8/10 (1.9)	7/10 (1.3)
Nose				
Infiltrate, Lymphocytic, Nasopharynx	3/10	0/0	0/0	7/10
FEMALES (Number of animals showing symptoms /Number of animals evaluated)				
Kidney				
Infiltrate, Mononuclear Cell	1/10	0/1	0/0	4/11
Mineralization, Tubular	2/10	1/1	0/0	7/11
Nephropathy	1/10	0/1	0//0	1/11

TABLE 8. Summary of Histopathology Incidences[a]				
Dose rate (mg/kg/day)	0	**100**	300	**1000**
Liver				
Inflammation, Chronic	8/10	0/1	0/0	11/11
Lung				
Increased Bronchoalveolar Associated Lymphoid Tissue (BALT)	3/10 (1.0)	3/10 (1.7)	5/10 (1.4)	5/10 (1.6)
Inflammation, Chronic	5/10 (1.0)	5/10 (1.2)	6/10 (1.2)	7/10 (1.1)
Nose				
Infiltrate, Lymphocytic, Nasopharynx	10/10	0/0	0/0	9/10
Uterus				
Dilatation, Horns	4/10	5/6	5/6	6/11

[a] Data obtained from pages 220-227 in the study report (MRID 46802201) and p. 10 in the study report (MRID 47006601).
() = Group mean where the sum of the severities are divided by the number affected.
* Noted as 5 (1.4) in MRID document.

III. DISCUSSION AND CONCLUSIONS:

A. **CONCLUSIONS:** The dermal administration of 100, 300, or 1000 mg/kg/day of R107894 to rats over a 90-day period resulted in no adverse effects. Treatment-related changes were observed in the 1000-mg/kg/day animals that included increased ano-genital staining (males and females), increased urea nitrogen in males, increased triglyceride levels in females, and decreased albumin in females. None of these findings were considered adverse because of the type of effect, values fell within historical control values, or there was a lack of corresponding findings. One female in the 100-mg/kg/day dose group was found dead on Day 42; however, given that the animal was in the low-dose group and urogenital inflammation unrelated to the treatment was noted, the death was not attributed to the intake of R107894. The NOAEL was reportedly 1000 mg/kg/day.

There appears to be a treatment-related increase in small sores that is dose related. Limited detail is provided with regard to these sores as they are only footnoted in the clinical observation tables. These sores appear to be a dermal reaction to the test material and not a systemic affect. These sores are noted in both the mid- and high-dose group. **Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.**

Additionally, because historical control data were not provided for the clinical chemistry parameters, our reviewers could not validate whether increases in 1000-mg/kg/day male urea nitrogen and 1000-mg/kg/day female triglycerides values were within historical control data. The same is true for the decreases in albumin for 1000-mg/kg/day females. Our reviewers, however, were able to review data provided by Charles River for rats 21 weeks of age (age

when blood was examined), which confirms the investigator's conclusions (Charles River, 2006). Therefore, these affects are not believed to be adverse.

Histopathology findings in the kidneys and nose are not considered treatment-related because they are only present in one sex and there are no corresponding changes observed in the animals.

Although, histopathology findings in the lungs (increased incidence of BALT and chronic inflammation) were observed in both males and females, no statistical difference ($p<0.05$) was seen in either the incidence or the severity of the responses. In the intermediate dose males, the statistical analysis noted that when the incidence and severity parameters were measured, the large number of affected animals were not statistical outliers; the result, however, was inconsistent since the effect was not statistically significant for the high dose compared to the control.

There were no other adverse findings with this substance.

The **systemic** NOAEL is ≥1000 mg/kg/day (HDT); The systemic LOAEL is >1000 mg/kg/day. One thousand (1000) mg/kg/day is considered the limit dose; at or above this dose, no more testing need be done.

B. MAJOR STUDY DEFICIENCIES: None

C. Minor deficiencies not upgradeable

- Hematology and clinical chemistry evaluations were conducted for only one time period. Thus, it is difficult to discern if statistically significant data is incidental or toxicologically significant.

STUDY CLASSIFICATION: This 90-day dermal toxicity study in the rat is considered **ACCEPTABLE - GUIDELINE.** Since the NOAEL is ≥1000 mg/kg/day (limit dose), OPP guidelines note that no further toxicity testing is required. The study satisfies the GUIDELINE requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats.

D. REFERENCES:

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APPENDIX 1

MEMORANDUM

DATE: February 2, 2007

Histopathology data of a study R107894: Dermal toxicity study (90-data repeat dermal application study in rats) with MRID 46802201 concerning lung incidences of increased bronchoalveolar associated lymphoid tissue (BALT) and chronic inflammation (CI) were analyzed statistically using non-parametric approaches (Fisher's exact test and the Cochran-Armitage trend test) and using a logistic regression model. Overall, the dose-response effect was not statistically significant at the five percent level except if you only compare the Controls against the Intermediate dose (300 mg/kg) for BALT in Males. The large numbers of affected animals for the Intermediate dose in males were not statistical outliers based on their residuals from the logistic model.

The severity data were analyzed using similar statistical approaches, treating the non-affected animals as having a score of zero. The results were similar.

Data

The following tables were provided by EPA.

Table 1. Lung Histopathology Incidence - Males

	Males			
Parameter	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (300 mg/kg/day)	High (1000 mg/kg/day)
BALT	1 (1.0)	4 (1.3)	7 (2.0)	5 (1.4)
Chronic Inflammation	4 (1.3)	4 (1.3)	8 (1.9)	7 (1.3)

() = Group mean where the sum of the severities are divided by the number affected.

Table 2. Lung Histopathology Incidence - Females

	Females			
Parameter	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (300 mg/kg/day)	High (1000 mg/kg/day)
BALT	3 (1.0)	3 (1.7)	5 (1.4)	5 (1.6)
Chronic Inflammation	5 (1.0)	5 (1.2)	6 (1.2)	7 (1.1)

() = Group mean where the sum of the severities are divided by the number affected.

For the severity analyses, the raw data giving the disease severities for each affected animal were

analyzed.

Note that these data show two inconsistencies. First, it appears that the Table footnotes have a typo, since the numbers in parentheses are the sums of the severities divided by the number affected, and not *vice versa*. These "group means" were not used in the following analyses. Second, there was a slight inconsistency between the two data sets, because the number of Male animals with severity 1 or more for BALT at the High dose was 4 instead of the 5 in Table 2; Table 3 below has the corrected data as per the raw data. The statistical analyses were performed on the corrected data; however, the difference was minor and would have only slightly changed the p-value if left as presented. An excel spreadsheet is provided with the raw data and calculations of incidences and severity scores.

Table 3. Corrected Lung Histopathology Incidence - Males

	Males			
Parameter	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (300 mg/kg/day)	High (1000 mg/kg/day)
BALT	1 (1.0)	4 (1.3)	7 (2.0)	4 (1.5)
Chronic Inflammation	4 (1.3)	4 (1.3)	8 (1.9)	7 (1.3)

() = Group mean where the sum of the severities are divided by the number affected.

Non-Parametric Approach – Incidence Data

The Fisher exact test tests for any association between the dose group and the response, i.e., whether the probability of being affected varies with the dose level. This is a conditional test given the total numbers of animals with BALT or CI and the total numbers tested at each dose level (10). The Cochran-Armitage trend test tests for whether the proportion of affected animals is linearly related to the dose. The exact version of the Cochran-Armitage test was used, which tests the significance of the estimated dose slope coefficient given the total numbers of animals with BALT or CI and the total numbers tested at each dose level (10).

The main analysis used all the data for each parameter (disease) and gender. To evaluate the possibility that the higher increases for males in the Intermediate dose group might be outliers, the same analyses were repeated without the data from that dose group to see if the conclusions were affected. To compare each treated group against the control, the same analyses were performed using only the control group and one of the treated groups. The results are shown in Table 4. Any p-values below 0.05 are statistically significant at the five percent level, i.e., show a statistically significant dose-response.

Table 4. Fisher and Cochran-Armitage Trend Tests for Yes/No Response

Dose groups	Parameter	Gender	P-value for Fisher exact test (two-sided)	P-value for exact Cochran-Armitage trend test (two-sided)
All	BALT	Females	0.71	0.35
All	BALT	Males	0.07	0.23
All	CI	Females	0.89	0.34
All	CI	Males	0.21	0.08
Exclude Intermediate	BALT	Males	0.23	0.12
Exclude Intermediate	CI	Males	0.47	0.18
Control and Low	BALT	Females	1.00	1.00
Control and Low	BALT	Males	0.30	0.30
Control and Low	CI	Females	1.00	1.00
Control and Low	CI	Males	1.00	1.00
Control and Intermediate	BALT	Females	0.65	0.65
Control and Intermediate	BALT	Males	0.02	0.02
Control and Intermediate	CI	Females	1.00	1.00
Control and Intermediate	CI	Males	0.17	0.17
Control and High	BALT	Females	0.65	0.65
Control and High	BALT	Males	0.14	0.14
Control and High	CI	Females	0.65	0.65
Control and High	CI	Males	0.37	0.37

The analyses of all the data show no statistically significant results at the five percent level. At the ten percent level, the Fisher test shows an association between dose and response for BALT in Males and the Cochran-Armitage test shows a linear trend between dose and response for CI in Males. These marginally significant responses become non-significant when the data for Males at the Intermediate dose are excluded, suggesting that those data are weak outliers. The comparisons between the Control group and each treated group are all not statistically significant except for the comparison between the Control and Intermediate groups for BALT in Males; this result is inconsistent since the effect was not statistically significant for the High dose compared to the Controls.

Logistic Regression Approach – Incidence Data

Using logistic regression the probability of a response was modeled as $g(\alpha + \beta \times \text{dose})$, where g is the inverse logit function, $g(x) = 1/\{1+\exp(-x)\}$. The p-value is the statistical significance of

the dose coefficient β, i.e., testing whether the coefficient equals zero. The p-values are shown in Table 5.

Table 5. Logistic Regression for Yes/No Response

Parameter	Gender	P-value for Logistic Regression
BALT	Females	0.33
BALT	Males	0.21
CI	Females	0.31
CI	Males	0.16

None of the p-values were statistically significant at the five or ten percent levels, which demonstrate no dose-response relationship using the logistic regression model. Furthermore, none of the Pearson or deviance residuals exceeded the nominal critical value of 2, implying that the responses for Males in the Intermediate dose group are not outliers.

Non-Parametric Approach – Severity Data

The Fisher exact test tests for any association between the dose group and the response severity, i.e., whether the probability of being affected at a given severity level varies with the dose level. Unaffected animals were treated as having a severity of 0. This is a conditional test given the total numbers of animals with BALT or CI and the total numbers tested at each dose level (10). The Cochran-Armitage trend test is not applicable for the severity data since there are more than two levels.

As above, the main analysis used all the data for each parameter (disease) and gender. To evaluate the possibility that the higher increases for males in the Intermediate dose group might be outliers, the same analyses were repeated without the data from that dose group to see if the conclusions were affected. To compare each treated group against the control, the same analyses were performed using only the control group and one of the treated groups. The results are shown in Table 6. Any p-values below 0.05 are statistically significant at the five percent level, i.e., show a statistically significant dose-response.

Table 6. Fisher Exact Tests for Association Between Dose and Severity of Response

Dose groups	Parameter	Gender	P-value for Fisher exact test (two-sided)
All	BALT	Females	0.59
All	BALT	Males	0.08
All	CI	Females	0.96
All	CI	Males	0.42
Exclude Intermediate	BALT	Males	0.51
Exclude	CI	Males	0.72

Dose groups	Parameter	Gender	P-value for Fisher exact test (two-sided)
Intermediate			
Control and Low	BALT	Females	0.32
Control and Low	BALT	Males	0.30
Control and Low	CI	Females	1.00
Control and Low	CI	Males	1.00
Control and Intermediate	BALT	Females	0.54
Control and Intermediate	BALT	Males	0.08
Control and Intermediate	CI	Females	1.00
Control and Intermediate	CI	Males	0.21
Control and High	BALT	Females	0.31
Control and High	BALT	Males	0.37
Control and High	CI	Females	0.65
Control and High	CI	Males	0.48

These results for response severity are similar to those for the Yes/No response. The analyses of all the data show no statistically significant results at the five percent level. At the ten percent level, the Fisher test shows an association between dose and response severity for BALT in Males. These marginally significant responses become non-significant when the data for Males at the Intermediate dose are excluded, suggesting that those data are weak outliers. The comparisons between the Control group and each treated group are all not statistically significant except for the comparison between the Control and Intermediate groups for BALT in Males (p-value=0.08); this result is inconsistent since the effect was not statistically significant for the High dose compared to the Controls.

Logistic Regression Approach – Severity Data

Using cumulative logistic regression the probability of a response at severity less than or equal to s was modeled as $g(\alpha(s) + \beta \times \text{dose})$, where $s >= 0$ and g is the inverse logit function $g(x) = 1/\{1+\exp(-x)\}$. The intercept varies with the severity level but the slope is constant. A severity of zero corresponds to no disease being found. The p-value is the statistical significance of the dose coefficient β, i.e., testing whether the dose coefficient equals zero. The p-values are shown in Table 5.

Table 7. Logistic Regression for Severity of Response

Parameter	Gender	P-value for Logistic Regression
BALT	Females	0.21
BALT	Males	0.46
CI	Females	0.30
CI	Males	0.28

None of the p-values were statistically significant at the five or ten percent levels, which demonstrate no dose-response relationship using the logistic regression model.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Jan 31, 2006

MEMORANDUM

Review Subchronic (90-Day) Oral Toxicity - Rat (466597-01) and a 28-Day Repeat Dermal Toxicity – Rat (466597-02) with 1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]

DP Barcode: D323129
EPA Reg. No.: 43813-ET
PC Code: 119093

From: S.L. Malish, Ph.D. Toxicologist, Team 1 S.L. Malish 1/31/06
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD)[7510C]

To: Marshall Swindell, PM 33
PM Team Reviewer: Karen Leavy
Regulatory Management Branch
Antimicrobial Division [7510C]

Thru: Kathryn Montague, Team Leader, Kathryn V Montague 2/1/06
Team One, RASSB/AD [7510C].

and

Norm Cook, Chief, RASSB/AD [7510C] 2/8/06

Synonym: Econea®028, CL 303'268, R 107894

FORMULATION:

Active Ingredient:	% weight
1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]	94.6% a.i.

Laboratory: WIL Research Laboratories, LLC, Ashland fpr MRID 466597-01 and Product Safety Laboratories for MRID 466597-02.

Applicant: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

Uses: Antifouling Agent

ACTION REQUESTED: Review the following toxicology studies: Subchronic (90-Day) Oral Toxicity - Rat (466597-01) and a 28-Day Repeat Dermal Toxicity – Rat (466597-02). Complete Data Evaluation Reports (DER) are included.

1. **28-Day Repeat Dermal Toxicity** – Rat; MRID 466597-02

EXECUTIVE SUMMARY: In a 28-day range-finding dermal toxicity study (MRID 466597-02), R107894 (94.6% a.i.; lot #12649-8) was administered to 5 rats/dose /sex at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day for 29 days (6 hours/day, 5 days/week). Results from this study will be used to determine the dose range of a proposed 90-day dermal subchronic study.

Treatment-related effects at 1000 mg/kg/day included ano-genital staining but this was not considered to be toxicologically significant. There were no mortalities during this study. Additionally, there were no significant changes to body weight, food consumption, food efficiency, organ weight, or pathology (gross or microscopic). **The LOAEL is >1000 mg/kg/day (HDT). The NOAEL is ≤1000 mg/kg/day.** Based on these results, rats would be expected to tolerate an exposure of >1000 mg/kg/day in the subsequent 90-day dermal study.

This 28-day range-finding dermal toxicity study is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a range-finding dermal toxicity study OPPTS 870.3200.

2. **Subchronic (90-Day) Oral Toxicity** - Rat; MRID 466597-01

EXECUTIVE SUMMARY: A pathology peer review (MRID 46659701) was performed on a 90-day oral toxicity study (MRID 464422-01). In the study, R 107894 (94.6% a.i., Batch # AC 12649-8) was administered to 10 Sprague-Dawley rats/sex/dose via food at dose levels of 0, 80, 250, or 750 ppm active ingredient. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females using the analytical concentration. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The intake of test article in these rats was similar to that observed in the main study animals (i.e., 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females).
The original pathology was peer reviewed.

No unscheduled deaths occurred during the course of the study. There were no treatment-related clinical signs of toxicity, opthalmological findings, urinalysis, or macroscopic findings. Food

consumption decreased in the first week of treatment in both males and females administered 750 ppm. Thereafter, food consumption in the 750-ppm group was lower than that observed in the controls; and values reached statistical significance in Weeks 1, 6, and 9 in males and Weeks 1, 4, 6-9, and 11 in females. In conjunction with the low food consumption, high-dose males had statistically-significantly lower body weights within the first week of treatment and throughout treatment; high-dose females displayed statistically significant decreases in mean body weight starting on Week 7 through Week 13. The body weight gains were low throughout the study, likely due to low food consumption. The food efficiency was reduced in high-dose animals with only the males having statistically significant decreases (Weeks 1-5, 7, 8, and 13). The mid-dose males had statistically significant decreases in mean body weight during Weeks 7-13, with corresponding decreased body weight gain and food consumption. The mid-dose females displayed only slight decrease in body weight, body weight gain, and food consumption. The recovery group displayed signs of weight gain and increased food consumption; however, the high-dose animals still had statistically-significantly decreased mean body weight and body weight gain as compared to controls. Food efficiency was increased in the treated animals during the recovery period.

Hematology data indicated only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were statistically-significantly increased in the mid- and high-dose males, which correlate to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically significant change. Low-dose males also displayed a statistically significant increase in ALP. Signs of dehydration and undernourishment were evident with statistically significant increases in phosphorus, urea, magnesium, and cholesterol; and a statistically significant decrease in glucose in high-dose males and females.

Clinical chemistry findings in the recovery group indicate reversibility of the effects. Differences in creatinine and triglycerides were generally <10% different from the main control group and were thus considered due to biological variation.

Terminal body weight was reduced a statistically significant amount in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs. Treatment-related changes include increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. The relative brain weights in the high-dose animals remained elevated after the recovery period, though the males were the only statistically significant value. However, frank effects were noted in the brain at necropsy in these recovery group animals.

Microscopic findings of the brain and spinal cord observed in the main group were as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females; moderate to marked multifocal vacuolation of the

thoracic cord in the high-dose females; minimal to marked multifocal vacuolation of the lumbar cord in the mid- and high-dose males and females as well as the low-dose females. Microscopic finding of the brain and spinal cord of the animals that were in the perfusion fixation satellite group displayed treatment-related finding as follows: slight or minimal multifocal vacuolization in the frontal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the parietal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the midbrain (high-dose males; mid- and high-dose females); minimal to slight multifocal vacuolization of the pons (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the cerebellum (high-dose males; mid- and high-dose females); slight multifocal vacuolization of the medulla oblongata (high-dose female); slight multifocal vacuolization of the cervical cord (high-dose male); minimal to moderate multifocal vacuolization of the lumbar cord (mid- and high-dose males and females); minimal to marked myelin vacuolation of the cervical dorsal root (mid- and high-dose males and females); minimal to slight myelin vacuolation of the cervical ventricle root (high-dose males); minimal to marked myelin vacuolation of the lumbar dorsal root (mid- and high-dose males; all dosed females); minimal to marked myelin vacuolation of lumbar ventricle root (mid- and high-dose males; all dosed females); and slight to moderate myelin vacuolation of the proximal sciatic nerve (high-dose males and females). The lumbar region (cauda equina) was most profoundly affected and the females appeared to be more sensitive.

Other treatment related microscopic findings in the main group were minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). Peer review of the pathology confirmed the general findings, although there were some minor differences on severity and in some instances the involvement of vacuolation in several tissues.

There were no changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal).

After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males and could not be determined in females.

This 90-day oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a subchronic (90-day) oral toxicity study OPPTS 870.3100 in the rat.

EPA Reviewer: Steven L. Malish, Ph.D. , Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Toxicologist
Team 1, RASSB/Antimicrobial Division

Steven L. Malish 1/24/06
Jonathan Chen 01/31/06

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Repeat Dermal Toxicity - Rat; OPPTS 870.3200; OECD 411

PC CODE: 119093
DECISION#: 220066

DP BARCODE: 323129
EPA Reg. No.: 43813-ET

TEST MATERIAL (PURITY: 1H-pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl); (94.6% a.i.)

SYNONYMS: Econea®028, R107894

CITATION: Merkel D. (2005). R170894: Dermal Toxicity Study (28-Day Repeat Dermal Application Study in Rats). Product Safety Laboratories, Dayton, NJ. Study No. 17026, September 19, 2005. MRID 466597-02. Unpublished.

SPONSOR: Janssen Pharmaceutical N.V., Plant & Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium

EXECUTIVE SUMMARY: In a 28-day range-finding dermal toxicity study (MRID 46659702), R107894 (94.6% a.i.; lot #12649-8) was administered to 5 rats/dose /sex at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day for 29 days (6 hours/day, 5 days/week). Results from this study will be used to determine the dose range of a proposed 90-day dermal subchronic study.

Treatment-related effects at 1000 mg/kg/day included ano-genital staining but this was not considered to be toxicologically significant. There were no mortalities during this study. Additionally, there were no significant changes to body weight, food consumption, food efficiency, organ weight, or pathology (gross or microscopic). **The LOAEL is >1000 mg/kg/day (HDT). The NOAEL is ≤1000 mg/kg/day.** Based on these results, rats would be expected to tolerate an exposure of >1000 mg/kg/day in the subsequent 90-day dermal study.

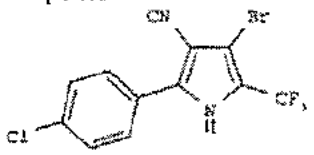
This 28-day range-finding dermal toxicity study is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a range-finding dermal toxicity study OPPTS 870.3200.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 1H-pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl); R107894

Description:	Light tan solid material stored at room temperature and not exposed to heat or direct sunlight
Lot/Batch #:	Lot # AC 12649-8
Purity:	94.6% a.i.
Compound Stability:	Not Reported
CAS #:	Not Reported
Structure:	

2. Vehicle and/or positive control: Distilled water (lot/batch # and purity not reported)

3. Test animals:

Species:	Rat	
Strain:	Crl:CD®(SD) IGS BR VAF/Plus®	
Age/weight at study initiation:	Approximately 9 weeks of age / 281-322 grams (males) and 180-224 grams (females)	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Animals were individually housed in suspended, stainless-steel cages.	
Diet:	Purina Certified Rodent Meal® #5002M (PMI Nutrition International) was provided *ad libitum*. No contaminants that could interfere with the study outcome were found during trace analyses.	
Water:	Filtered tap water was supplied by an automatic water dispensing system *ad libitum*. Results of the water analysis showed that contaminant levels were acceptable and within regulatory standards.	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	54-69%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN:

1. In life dates:
Start: 3/9/05
End: 4/7/05

2. Animal assignment: Animals were assigned randomly by computer-generated assignment to the test groups noted in Table 1.

TABLE 1: Study design

Test Group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	5	5
Low	30	5	5
Intermediate-Low	100	5	5
Intermediate-High	300	5	5
High	1000	5	5

3. Dose selection rationale: The dose levels were selected based on information provided by the Sponsor. These doses selected will be used to bracket the dose-range of a proposed 90-day dermal subchronic study.

4. Preparation and treatment of animal skin: During acclimation, the fur of each test animal (n=60) was clipped from the shoulder to the hips, resulting in an area of at least 10% of the body surface. Care was taken to avoid abrading the skin. Acclimatization to the dosing procedure was performed on days -6, -5, and -2, where the animals were "sham wrapped" for 1, 3, and 6 hours, respectively. Prior to the first application, the animals were weighed, re-clipped, and the skin was examined for any abnormalities. During application, an appropriate amount of the test material was applied to a gauze pad, which was moistened with 0.3 mL of distilled water. After application, the trunk of the animal was then wrapped with nonirritating tape to avoid ingestion of the test material or dislocation of the patch. Dosing occurred at approximately the same time each day (±2 hours) 6 hours/day, 5 days/week over a course of 29 days (minimum of 20 exposures). After the 6-hour exposure, the wrappings were removed, and the entire application site was cleansed with a ~3% solution of Ivory® Dishwashing Liquid using a clean towel to remove any residual test substance and gently patted dry with a clean pad. It was noted whether animals were out of the wrapping at patch removal. The first day of dosing was designated as Day 1 of the study. Rats in the control group were exposed to the vehicle using the same procedure as described for the treated rats.

5. Statistics: Treated animals were compared to control groups using a One-Way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison test. Data were evaluated for homogeneity of variances and normality by the Bartlett's test. Data that was statistically significant of the Bartlett's test was further evaluated with a non-parametric method (Kruskal-Wallis). Significance was judged at $p<0.05$.

C. METHODS:

1. Observations:

1a. Cageside Observations: Mortality and morbidity were examined twice daily. Additionally, the dose site of each animal was examined for signs of erythema and edema on each Friday after patch removal using the Draize et al.[1] method. The mean erythema and mean edema scores were determined for each group at each time interval. The Primary Dermal Irritation (PDI) was

[1] Draize J.H., Woodward, G. and Calvery, H.O. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82:377-390.

defined as the sum of the mean erythema and the mean edema for each group at each time interval.

1b. Clinical Examinations: Detailed clinical observations were recorded on Day 1 (prior to treatment) and weekly thereafter (prior to patch application). Examinations were conducted while animals were both handled and placed in an opened field. Examinations included, but were not limited to, the following: changes to the skin, fur, eyes, and mucous membranes; and occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern).

1c. Neurological Evaluations: Changes in gait, posture and response to handling, as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming or repetitive circling), or bizarre behavior (e.g., self-mutilation or walking backward) were recorded.

2. Body weight: Individual body weights were recorded 4 times during the acclimation period. Test animals were weighed on Day 1 before dosing and weekly thereafter. Mean daily body weight gains and overall body weight gains were calculated. All animals were weighed immediately prior to sacrifice (fasted body weight).

3. Food consumption: Individual food consumption was determined weekly. Mean daily food consumption and mean daily food efficiency (defined as body weight gain divided by food consumption) were calculated and reported.

4. Ophthalmoscopic examination: An ophthalmoscopic examination was not conducted.

5. Hematology & Clinical Chemistry: Examination of hematology and clinical chemistry endpoints was not conducted.

6. Urinalysis: A urinalysis was not conducted.

7. Sacrifice and Pathology: Surviving animals were euthanized at the scheduled sacrifice (day 30) by exsanguination from the abdominal aorta under isoflurane anesthesia and subjected to gross pathological examination, which included an initial examination of external surfaces and orifices, as well as the cranial, thoracic, and abdominal cavities and their contents. Gross lesions were noted, and tissues of interest and any gross lesions were retained in 10% neutral buffered formalin. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta, thoracic*	XX	Brain*+
	Salivary glands*		Heart*+		Peripheral nerve*
	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	XX	Spleen*+		Eyes (optic nerve)*
	Jejunum*		Thymus*+		GLANDULAR
	Ileum*				Adrenal gland*+
	Cecum*		UROGENITAL		Lacrimal gland
	Colon*		Kidneys*+		Parathyroid*
	Rectum*		Urinary bladder*		Thyroid*
XX	Liver*+		Testes*+		OTHER
	Gall bladder* (not rat)		Epididymides*+		Bone (sternum and/or femur)
	Bile duct* (rat)		Prostate*		Skeletal muscle
	Pancreas*		Seminal vesicles*		Skin* (treated & untreated areas)
	RESPIRATORY		Ovaries*+		All gross lesions and masses*
	Trachea*		Uterus*+		
	Lung*		Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250
\+ Organ weights required.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs: Treatment-related clinical signs are presented in Table 2. All animals exposed to 1000 mg/kg/day exhibited ano-genital staining during daily observations, weekly observations, and/or at gross necropsy. Additionally, two male rats and one female rat treated with 1000 mg/kg/day exhibited a red stain around the nose and mouth during daily observations. Ano-genital staining also was observed once in two 300-mg/kg/day female rats; however, this occurrence was considered incidental and not considered treatment related.

TABLE 2. Treatment-related clinical signs

	Dose rate [mg/kg/day]				
	0	30	100	300	1000
Male					
Fur/Skin	0	0	0	0	5 (2[b]); 1(-[c])
Female					
Fur/Skin	0	0	0	2(2[b])	5(2[b]); 1(-[c])

[a] Data obtained from pages 220-21 in the study report. An entry of 0 indicates that all animals in the group appeared normal when evaluated for the specified observation, or that all animals did not exhibit the specific clinical sign. An entry greater than 0 indicates the number of animals in the group that exhibited the specific clinical sign. The number in the parenthesis (if present) represents the score given for the observed clinical sign.
[b] Score of 2 indicates urine stained/wetness.
[c] Animal #1171 had two small sores on bottom lip.

Small sores on the lower lip was observed during detailed observation and/or at necropsy in one male and one female treated with 1000 mg/kg/day. These findings were considered to be minor injuries sustained at the animal feed jar and not treatment related.

2. Mortality: There were no mortalities during this study.

3. Neurological Evaluations: There were no changes to gait, posture, and response to handling. Additionally, there were no clonic or tonic movements, stereotypies (e.g., excessive grooming and repetitive circling), or bizarre behavior (e.g., self-mutilation and walking backward)

4. Dermal Irritation: Dermal irritation was not observed in this study. The following animals were found out of their wrappings: animal #1176 (1000 mg/kg/day) for 9 out of 20 exposures; animal #1145 (30 mg/kg/day) for 5 out of 20 exposures; and animal #1167 (300 mg/kg/day) for 3 out of 20 exposures. According to the study author, these events did not compromise the outcome of the study.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights and body weight gains are presented in Table 3. There were no treatment-related or statistically significant changes in body weight or body weight gain throughout the study.

TABLE 3. Average body weights and body weight gains during treatment

Dose rate [mg/kg/day]	Body Weights (g±SD)					Total Weight Gain	
	Day 1	Day 8	Day 15	Day 22	Day 29	g	% of control
Male							
0	308.8±16.7	338.0±19.2	368.6±23.0	398.2±27.6	423.8±31.6	115.0	---
30	309.8±8.7	339.6±19.9	369.6±14.9	386.0±31.4	412.6±32.4	102.8	-11%
100	314.4±13.3	345.2±14.9	369.6±14.9	395.0±22.5	417.2±26.7	102.8	-11%
300	310.4±10.5	344.6±8.8	369.6±6.4	395.4±6.5	417.0±5.3	106.6	-7%
1000	308.2±15.3	333.4±24.5	360.8±27.4	383.0±29.8	409.2±27.5	100.0	-12%
Female							
0	200.8±8.9	219.8±8.9	225.8±16.7	238.0±10.8	241.4±9.0	40.6	---
30	202.4±9.4	222.2±23.3	234.4±24.0	242.4±27.7	245.0±24.9	42.6	+5%
100	203.8±16.3	220.4±16.9	230.2±22.3	240.0±17.2	251.4±17.6	47.6	+17%
300	199.8±14.1	216.6±17.0	220.0±20.7	227.8±22.0	234.8±20.1	35.0	-14%
1000	199.8±11.9	215.4±17.2	225.4±19.2	233.6±22.8	242.2±20.7	42.4	+4%

[a] Data obtained from pages 22-23 in the study report; total weight gain and % change was calculated by the reviewer.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. Food consumption: Food consumption data are presented in Table 4. There were no treatment-related or statistically-significant changes in food consumption throughout the study.

TABLE 4. Average food consumption during treatment

Dose rate [mg/kg/day]	Food Consumption (g/day±SD)				Total Food Consumption	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	g/day	% of control
Male						
0	27.6±1.4	28.2±2.2	30.5±2.1	31.5±2.2	29.4±1.9	---
30	28.1±3.3	27.6±3.1	28.1±3.0	29.6±3.1	28.4±3.0	-3%
100	28.3±1.7	27.8±2.0	28.6±2.9	29.5±2.1	28.5±2.0	-3%

Dose rate [mg/kg/day]	Food Consumption (g/day±SD)				Total Food Consumption	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	g/day	% of control
300	29.5±2.2	29.6±1.4	30.9±1.6	32.0±1.4	30.5±1.4	+4%
1000	27.9±2.1	28.2±2.3	29.9±1.8	31.0±1.9	29.3±1.7	-1%
Female						
0	19.5±1.8	19.9±1.7	21.1±3.1	20.9±2.0	20.4±2.0	---
30	20.9±1.6	22.1±1.7	22.1±1.6	22.4±1.8	21.9±1.5	+7%
100	21.5±2.8	22.4±2.3	23.5±3.8	23.3±1.8	22.7±2.3	+4%
300	17.9±2.0	17.9±2.2	20.0±2.0	19.9±1.4	18.9±0.8	-7%
1000	20.2±1.2	20.2±1.1	21.5±1.0	21.4±1.3	20.8±1.1	+2%

[a] Data obtained from page 24 in the study report; % change was calculated by our reviewers.

2. **Food efficiency:** Food efficiency data are presented in Table 5. There were no treatment-related or statistically-significant changes in food efficiency throughout the study.

TABLE 5. Average food efficiency during treatment

Dose rate [mg/kg/day]	Food Efficiency (±SD)				Total Food Efficiency	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	Ratio	% of control
Male						
0	0.15±0.03	0.15±0.03	0.14±0.03	0.12±0.03	0.14±0.02	---
30	0.15±0.05	0.14±0.04	0.10±0.04	0.13±0.02	0.13±0.02	-7%
100	0.15±0.01	0.12±0.04	0.13±0.03	0.11±0.02	0.13±0.02	-7%
300	0.16±0.03	0.12±0.04	0.12±0.03	0.10±0.01	0.12±0.01	-14%
1000	0.13±0.04	0.14±0.01	0.10±0.03	0.12±0.02	0.12±0.01	-14%
Female						
0	0.14±0.02	0.04±0.07	0.08±0.04	0.02±0.04	0.07±0.02	---
30	0.13±0.03	0.08±0.01	0.05±0.04	0.02±0.05	0.07±0.01	0%
100	0.11±0.02	0.06±0.04	0.06±0.04	0.07±0.03	0.07±0.01	0%
300	0.13±0.05	0.02±0.08	0.05±0.03	0.05±0.04	0.07±0.02	0%
1000	0.11±0.03	0.07±0.05	0.05±0.03	0.06±0.02	0.07±0.02	0%

[a] Data obtained from page 25 in the study report; % change was calculated by our reviewers.

D. OPHTHALMOSCOPIC EXAMINATION: An ophthalmoscopic examination was not conducted.

E. BLOOD ANALYSES: Blood analyses were not conducted.

F. URINALYSIS: A urinalysis was not conducted.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Select organ weight findings are presented in Table 6. There were no treatment-related or statistically-significant changes to absolute organ weights or organ-to-body weight ratios for both sexes throughout the study. Females administered 300 mg/kg/day exhibited a statistically-significant decrease in spleen-to-brain weight; however, this finding was not dose-related and did not correspond to any gross or microscopic pathology. Consequently, this finding was considered incidental.

TABLE 6. Select relative organ weights

Relative organ weight (gx1000±SD)	Dose rate (mg/kg/day)				
	0	30	100	300	1000
Male					
Spleen-to-brain	0.33±0.03	0.34±0.08	0.28±0.01	0.32±0.03	0.31±0.06
Female					
Spleen-to-brain	0.31±0.06	0.24*±0.01	0.26±0.01	0.28±0.04	0.26±0.03

[a] Data obtained from page 25 in the study report; % change was calculated by reviewer.
* Statistically different ($p<0.05$) from the control

2. **Gross pathology:** There were no treatment-related gross pathology findings. One 300-mg/kg/day female (animal #1167) exhibited mottled kidneys, and one 1000-mg/kg/day male (animal #1171) showed small sores on its lower lip. These findings were considered to be isolated.

3. **Microscopic pathology:** There were no treatment-related microscopic pathology findings. Minimal to slight/mild multifocal chronic inflammation of the liver was observed in animals from all treatment groups, including the control group, and was not considered treatment-related. Minimal lymphoid depletion was noted in the marginal zone of the spleen in one 1000-mg/kg/day female; however, this finding was considered isolated and not related to treatment.

III. DISCUSSION AND CONCLUSIONS: The dermal exposure to R107894 did not result in toxicity at 1000 mg/kg/day. Based on these findings, the LOAEL is >1000 mg/kg/day in male and female rats, the NOAEL is ≤1000 mg/kg/day. In the subsequent 90-day dermal study, rats would be expected to tolerate an exposure of 1000 mg/kg/day.

STUDY CLASSIFICATION: This 28-day range-finding dermal toxicity study is **ACCEPTABLE - GUIDELINE** because it satisfies the guideline requirement for **a** dermal toxicity study **OPPTS** 870.3200.

EPA Reviewer: Steven L. Malish, Ph.D. , Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Toxicologist
Team 2, RASSB/Antimicrobial Division

Steven L. Malish 1/24/06
Jonathan Chen 01/31/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (90-Day) Oral Toxicity - [rat]; OPPTS 870.3100 [§82-1]; OECD 408.

PC CODE: 119093
DECISION#: 220066

DP BARCODE: D323129
EPA REG. NO.: 43813-ET

TEST MATERIAL (PURITY): 1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]; (94.6% a.i.)

SYNONYMS: Econea®028, CL 303'268, R 107894

CITATION: Radovsky, A. (2005). Pathology Peer Review Statement of R 107894 Subchronic toxicity study in Sprague-Dawley rats, administration via the diet over 3 months and a recovery period of 4 weeks. WIL Research Laboratories, LLC, Ashland, OH. Project No.:50C0238/03021. September 28, 2005. MRID 466597-01. Unpublished.

Mellert, W., K. Deckart, W. Kaufmann, et al. (2004). R 107894 Subchronic toxicity study in Sprague-Dawley rats, administration via the diet over 3 months and a recovery period of 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Project No.:50C0238/03021, October 14, 2004. MRID 46442201. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

EXECUTIVE SUMMARY: A pathology peer review (MRID 46659701) was performed on a 90-day oral toxicity study (MRID 46442201). In the study, R 107894 (94.6% a.i., Batch # AC 12649-8) was administered to 10 Sprague-Dawley rats/sex/dose via food at dose levels of 0, 80, 250, or 750 ppm active ingredient. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females using the analytical concentration. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The intake of test article in these rats was similar to that observed in the main study animals (i.e., 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females).

The original pathology was peer reviewed.

No unscheduled deaths occurred during the course of the study. There were no treatment-related clinical signs of toxicity, opthalmological findings, urinalysis, or macroscopic findings. Food consumption decreased in the first week of treatment in both males and females administered 750 ppm. Thereafter, food consumption in the 750-ppm group was lower than that observed in the controls; and values reached statistical significance in Weeks 1, 6, and 9 in males and Weeks 1, 4, 6-9, and 11 in females. In conjunction with the low food consumption, high-dose males had statistically-significantly lower body weights within the first week of treatment and throughout treatment; high-dose females displayed statistically-significant decreases in mean body weight starting on Week 7 through Week 13. The body weight gains were low throughout the study, likely due to low food consumption. The food efficiency was reduced in high-dose animals with only the males having statistically-significant decreases (Weeks 1-5, 7, 8, and 13). The mid-dose males had statistically-significant decreases in mean body weight during Weeks 7-13, with corresponding decreased body weight gain and food consumption. The mid-dose females displayed only slight decrease in body weight, body weight gain, and food consumption. The recovery group displayed signs of weight gain and increased food consumption; however, the high-dose animals still had statistically-significantly decreased mean body weight and body weight gain as compared to controls. Food efficiency was increased in the treated animals during the recovery period.

Hematology data indicated only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were statistically-significantly increased in the mid- and high-dose males, which correlate to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically-significant change. Low-dose males also displayed a statistically-significant increase in ALP. Signs of dehydration and undernourishment were evident with statistically-significant increases in phosphorus, urea, magnesium, and cholesterol; and a statistically-significant decrease in glucose in high-dose males and females.

Clinical chemistry findings in the recovery group indicate reversibility of the effects. Differences in creatinine and triglycerides were generally <10% different from the main control group and were thus considered due to biological variation.

Terminal body weight was reduced a statistically significant amount in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs. Treatment-related changes include increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. The relative brain weights in the high-dose animals remained elevated after the recovery period, though the males were the only

are specified while painting/working in confined or enclosed spaces. Since limited PPE is specified on the ***Sigma Nexxium 20 Antifouling*** labeling (powered air purifying respirator- PAPR) and technical data sheet (gloves/fresh air mask) several PPE scenarios will be assessed. Typical industrial safety practices include the following PPE: Hood/head cover, safety glasses/goggles, appropriate respirator, coveralls, Tyvek paper suit, gloves, safety shoes and barrier cream for exposed facial skin as appropriate. Handlers review labels and Material Safety Data Sheets (MSDS) and comply with OSHA regulations (ACC, 2005).

4.2 Occupational Handlers Exposures and Risks

Chemical-specific worker exposure monitoring data were not submitted or cited in support of this new antifoulant (*Econea*) to assess exposures for industrial shipyard workers as painters and handlers of ***Sigma Nexxium 20 Antifouling*** paint. However, certain data were provided by Janssen Pharmaceutica, Inc. in the form of an occupational exposure report (the most recent version as MRID 468466-02) dated May 15, 2006, entitled "*Revision 1 to MRID No. 46751303: Screening Level Occupational Exposure Assessments For R107894 (AC or CL 303268) As An Antifoulant In Paint Applied To Underwater Hulls of Pleasure, Military, and Commercial Craft.*" The assessments are intended to evaluate the potential worker exposures during commercial painting operations. As a conservative screening tool the submitted report included qualitative observations of shipyard workers and quantitative dermal/inhalation exposure estimates and calculated MOEs for different painter scenarios (i.e., paint mixer/loader/applicator scenarios) based on surrogate data from the Pesticide Handlers Exposure Database (PHED).

An evaluation of non-dietary occupational exposures was conducted by AD/RASSB on representative scenarios for exposure routes of concern based on the ***Sigma Nexxium 20 Antifouling*** product labeling use patterns and the toxicity of the active ingredient. (See Table 8.) No chemical-specific exposure data are available. Therefore, the assessment relied on certain Agency standard values and surrogate data sources to develop scenarios; including surrogate unit exposure data taken from PHED, Version 1.1 (U.S. EPA, 1998). Certain registrant-submitted information on shipyard worker observations (MRID 468466-02) was used to aid in calculating handler doses at baseline and when PPE is worn:

Shipyard Workers Engaged in Boat Hull Painting Operations

The assessment presented is based on label specifications for ***Sigma Nexxium 20 Antifouling*** :

- the mass density of the product (12.51 lb/gal) and
- spreading rate (220 sq.ft/gal) as optimal at 4 mils dry film thickness
- 3.4% a.i., Pyrrole-3-carbonitrile, 4-bromo-2-(p-chlorophenyl)-5-(trifluoromethyl) (*R107894*, PC Code:119093)

statistically-significant value. However, frank effects were noted in the brain at necropsy in these recovery group animals.

Microscopic findings of the brain and spinal cord observed in the main group were as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females; moderate to marked multifocal vacuolation of the thoracic cord in the high-dose females; minimal to marked multifocal vacuolation of the lumbar cord in the mid- and high-dose males and females as well as the low-dose females. Microscopic finding of the brain and spinal cord of the animals that were in the perfusion fixation satellite group displayed treatment-related finding as follows: slight or minimal multifocal vacuolization in the frontal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the parietal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the midbrain (high-dose males; mid- and high-dose females); minimal to slight multifocal vacuolization of the pons (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the cerebellum (high-dose males; mid- and high-dose females); slight multifocal vacuolization of the medulla oblongata (high-dose female); slight multifocal vacuolization of the cervical cord (high-dose male); minimal to moderate multifocal vacuolization of the lumbar cord (mid- and high-dose males and females); minimal to marked myelin vacuolation of the cervical dorsal root (mid- and high-dose males and females); minimal to slight myelin vacuolation of the cervical ventricle root (high-dose males); minimal to marked myelin vacuolation of the lumbar dorsal root (mid- and high-dose males; all dosed females); minimal to marked myelin vacuolation of lumbar ventricle root (mid- and high-dose males; all dosed females); and slight to moderate myelin vacuolation of the proximal sciatic nerve (high-dose males and females). The lumbar region (cauda equina) was most profoundly affected and the females appeared to be more sensitive.

Other treatment related microscopic findings in the main group were minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). Peer review of the pathology confirmed the general findings, although there were some minor differences on severity and in some instances the involvement of vacuolation in several tissues.

There were no changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal).

After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males and could not be determined in females.

This 90-day oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a subchronic (90-day) oral toxicity study OPPTS 870.3100 in the rat.

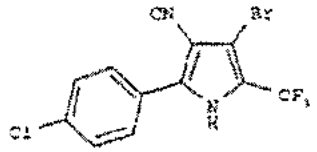
COMMENTS: This is a revised Executive Summary only and does not alter the conclusions of the original review. This summary incorporates the findings of the pathology peer review.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl], R 107894

Description:	Powder, white to light brown
Lot/Batch #:	AC 12649-8
Purity:	94.6% a.i.
Compound Stability:	Expiration date April 30, 2008 at room temperature; the date of production was May-June 2000. The study report verifies that the test substance was 94.6% pure just prior to study initiation and was stored in the dark at room temperature. The test substance is expected to be stable for at least 3 years before recertification is needed.
CAS # of TGAI:	122454-29-9
Structure:	

2. Vehicle and/or positive control: Food

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley Crl:CD® (Charles River)
Age/weight at study initiation:	Approximately 42±1 days old / 171.8-216.3 g for males and 145.3-174.3 g for females
Source:	Charles River, Germany
Housing:	Rats were individually housed in DK IfI stainless steel wire mesh cages (Becker & Co. Castro-Rauxel, Germany). An absorbent dust free paper (SSNIFF, Soest, Germany) was under the cages.
Diet:	Powdered, Kliba maintenance diet rat-mouse meal (Provimi Kliba SA, Kaiseraugst, Switzerland) was available *ad libitum*, except during overnight fast before blood withdrawal. Chemical and microbiological contaminants in the diet were analyzed, the data were not provided but available from the laboratory.
Water:	Tap water was available *ad libitum*. Analysis results from Frankenthal and Technical Services of BASF Aktiengesellschaft for contaminants in the water were not provided, but were stated to be suitable according to German Drinking Water Regulation (Trinkwasserverordnung).
Environmental conditions:	Temperature: 20-24°C Humidity: 30-70% Air changes: not stated Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	6 days

B. STUDY DESIGN:

1. In life dates: Start: September 9, 2003 End: January 15, 2004

2. Animal assignment: Animals were assigned to the test groups noted in Table 1 below using computer generated random numbers. Two satellite groups were employed with the 11 groups consisting of 5 rats/sex/dose which underwent perfusion fixation, and R1, which is a recovery group with a 4-week period with no exposure to the test article after the 3 months of dosing which underwent immersion fixation.

TABLE 1. Study Design[a]

Test Group	Target Dose (ppm)	Administered Dose Males (mg/kg/day)	# Male	Administered Dose Females (mg/kg/day)	# Female
0H (Control)	0	0	10	0	10
1H (Low)	80	5.2	10	6.3	10
2H (Mid)	250	16.2	10	20.9	10
3H (High)	750	51.9	10	62.0	10
0S (Control)	0	0	10	0	10
1S (Low)	80	5.1	10	6.8	10
2S (Mid)	250	16.2	10	20.7	10
3S (High)	750	51.6	10	61.4	10

[a] Data taken from pages 21 and 44 of the study report.
H = main groups
S = satellite groups (5 rats/sex/dose perfusion fixation after 3 months and 5 rats/sex/dose clinical pathology and target organ pathology after 4-week recovery)

3. Dose selection rationale: Doses were selected based on the request of the sponsor, the rationale for dose selection was not provided.

4. Dose formulation preparation and analysis: Dose formulations were prepared every 4 weeks by mixing appropriate amounts of test substance in a small amount of powdered Kliba maintenance diet (rat-mouse meal) for a premix, which was then mixed for about 10 minutes in a Ruberg EM 100 laboratory mixer with the appropriate amount of diet to obtain the desired concentrations. The dose formulations were stored at ambient temperatures. Concentration of the dose formulations were tested during Weeks 1 and 11. Stability was evaluated in a 50 mg/kg formulation prior to study initiation. Samples were stored in a freezer. Homogeneity and concentration were analyzed in all concentrations at the start and the end (samples randomly selected from food hoppers) of the administration period.

Results:

Homogeneity Analysis: The samples from the test diets were generally above 90% the nominal concentration and the standard deviations were low, thus, demonstrating these samples were homogeneous. The average % of nominal concentration was 94.8±1.6%, 96.5±1.2%, and 96.0±1.0% for the 80-, 250-, and 750-ppm dose formulations, respectively, at the start of the administration period (September 11, 2003). The average % of nominal concentration was 100.8±3.3%, 93.7±1.2%, and 90.4±0.1% for the 80-, 250-, and 750-ppm dose formulations, respectively at the end of the administration period (December 15, 2003; pp. 470-471).

Stability Analysis: The test article (50 mg/kg) in the food was found to be stable at ambient conditions for up to 32 days (p. 468). After 12 days, the sample was 92.3% of the nominal concentration and after 32 days it was 93.7%.

Concentration Analysis: The mean analytical test diet concentrations were found to be 78.2±3.1, 238±4.8, or 699±22.8 mg/kg of the diet, for the 80-, 250-, and 750-ppm dose formulations, respectively (pp. 470-471).

The analytical data indicated that the samples were stable for the 32 day utilization period. The variance between nominal and actual dosage to the animals was less than ±10% with a range of 90.4% to 100.8%, thus, the dose formulation was prepared properly.

5. Statistics: For body weight, body weight change, food consumption, and food efficiency, each treatment group was compared to the control group using Dunnett's test (two-sided). Clinical pathology, except reticulocytes and differential leukocytes, were evaluated by a non-parametric one-way Kruskal-Wallis test (two-sided). If the data were significant then a Wilcoxon-test (two-sided) test was employed. The urinalysis parameters, except volume, appearance, and specific gravity, were evaluated by a pair-wise Fischer's exact test. Organ weights were evaluated by the one-way Kruskal-Wallis test (two-sided). If the data were significant then a Wilcoxon-test (two-sided) test was employed. The statistical tests were appropriate tests for this study.

C. METHODS:

1. Observations:

1a. Cageside Observations: Animals were inspected twice a day during the week and once a day on the weekends or public holidays.

1b. Clinical Examinations: Detailed clinical observations were conducted in an open arena on Day 0, then weekly thereafter. Clinical parameters examined were as follows: behavior during handling, fur, skin, posture, salivation, respiration, arousal/activity level, tremors, convulsions,

abnormal movements, impaired gait, lacrimation, palpebral closure, exopthalmus, feces (consistency/appearance) urine, and pupil size.

1c. Neurological Evaluations: Neurological tests were not performed.

2. Body weight: Animals were weighed before exposure, on Day 0 and weekly thereafter.

3. Food consumption: Food consumption (g/animal/day) was measured weekly. Food efficiency was calculated. The calculation is provided below:

$$\frac{BW_x - BW_y}{FC_{y\,to\,x}} \times 100 = \text{Food efficiency for day x}$$

BW_x = Body weight on day x (g)
BW_y = Body weight on day y (g)
$FC_{y\,to\,x}$ = mean food consumption for day y to day x; calculated as mean consumption on day x, multiplied with the number of days from day y to day x (g)

Compound intake calculations were determined from food consumption and animal weights, the calculation is below.

$$\frac{FC_x - C}{BW_x} = \text{Substance intake for day x}$$

BW_x = Body weight on day x (g)
FC = mean daily food consumption for day x (g)
C = concentration in the diet on day x (mg/kg)

4. Ophthalmoscopic examination: Eyes were examined in all animals three days prior to study initiation and in the control and high-dose groups on Day 85.

5. Hematology & Clinical Chemistry: Blood was collected on Day 92/93 of the main testing groups (10 rats/sex/dose) and on Day 122 of the satellite groups (5 rats/sex/dose) via retroorbital sinus plexus in isofluorane-anaesthetized rats in the morning after an overnight fast. The CHECKED (X) parameters were examined in all animals.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		Heinz bodies
	(Thromboplastin time)		Red cell morphology
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Albumin/Globulin ratio
X	Sodium*	X	Glucose*
	ENZYMES* (2 or more)	X	Total bilirubin
X	Alkaline phosphatase (AP)	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Globulin
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
	Ornithine decarboxylase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

6. Urinalysis: Urine was collected on Day 88 on the main group without analyses of the satellite groups using steel pans placed under the cages for at least 16 hours. The CHECKED (X) parameters were examined in all animals.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

7. Sacrifice and Pathology: All animals from the main and satellite, subset group R1 (recovery animals), that died and those sacrificed (decapitated) on schedule were subjected to detailed autopsy. The CHECKED (X) tissues were collected and examined microscopically from the

control and high-dose animals, except the duodenum, brain, spinal cord, and gross lesions were examined in all of the animals in the main group. In the satellite group, the brain, spinal cord, and duodenum were examined in all animals that were not examined for neuropathology (i.e., R1, recovery animals). The tissues were preserved in neutral buffered formaldehyde (4%), embedded in paraplast, sectioned, and stained with hematoxylin. Brain and lumbar spinal cord sections for all main group and satellite group recovery animals were also sent out for peer review. In addition, the organs marked XX were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic nerve)*
X	Esophagus*	X	Bone marrow (femur)*	X	Spinal cord (cervical, thoracic, and lumbar)*
X	Stomach (fore and glandular)*	X	Lymph nodes (mandibular and mesenteric)*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland*
X	Colon*	X	Urinary bladder*	X	Parathyroid*
X	Rectum*	XX	Testes*+	X	Thyroid*
XX	Liver*+	XX	Epididymides*+		Zymbal gland
	Gall bladder (not rat)*	X	Prostate*		OTHER
	Bile duct (rat)	X	Seminal vesicles*	X	Bone (sternum with marrow)
X	Pancreas*	XX	Ovaries*+	X	Bone (femur knee joint and marrow)
	RESPIRATORY	X	Oviducts	X	Skeletal muscle
X	Trachea*	X	Vagina	X	Skin
X	Lung*	XX	Uterus*+	X	Mammary gland (female)
X	Nose*			X	All gross lesions and masses*
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.
\+ Organ weights required for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

From the satellite groups, subset II, 5 rats/sex/dose were anesthetized (Narcoren®; 4 mL/kg) and sacrificed by perfusion fixation. Sorensen phosphate buffer was the rinsing solution, and Karnovsky was the fixative solution. The perfused animals were examined grossly as best as possible. In the perfused satellite group, the brain was weighed without the olfactory bulb, and the brain, spinal cord, and gross lesions were examined microscopically. The peripheral nerve tissues were embedded in epoxy resin, sectioned, stained with Azure II-Methylene blue-basic Fuschsin (Ambf), except the gasserian ganglia and gastrocnemius muscle, which were preserved in 4% formaldehyde. The brain, brain associated organs/tissues, spinal cord, gasserian ganglia, and gastrocnemius muscle were embedded in paraplast, sectioned, and stained with hematoxylin eosin stain. The CHECKED (X) tissues were collected and examined microscopically from the control and high-dose animals. The brain (frontal and parietal lobes), cerebellum, cervical dorsal root ganglia, lumbar spinal cord, lumbar dorsal root ganglia, lumbar ventral root, midbrain, and

pons slides for all terminal sacrifice satellite animals were examined by a peer review pathologist. The (XZ) tissues were analyzed in all of the animals.

	PERIPHERAL NERVOUS SYSTEM		BRAIN CROSS SECTIONS
X	Dorsal root ganglion 3 of (C3-C6)	XZ	Frontal
XZ	Dorsal root fiber (C3-C6)	XZ	Parietal lobe with diencephalon
XZ	Ventral root fiber (C3-C6)	XZ	Midbrain with occipital and temporal lobes
X	Dorsal root ganglion, 3 of (L1-L4)	XZ	Pons
XZ	Dorsal root fiber (L1-L4)	XZ	Cerebellum
XZ	Ventral root fiber (L1-L4)	XZ	Medulla oblongata
XZ	Proximal sciatic nerve		BRAIN ASSOCIATED ORGAN/TISSUES
X	Proximal tibial nerve (at knee)	X	Eyes
X	Distal tibial nerve (at lower leg)	X	Retina
X	Gasserian ganglia with nerve	X	Optic nerve
X	Gastrocnemius muscle		SPINAL CORD (CROSS AND LONGITUDINAL SECTIONS
		XZ	Cervical cord (C3-C6)
		XZ	Lumbar cord (L1-L4)

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity: There were no clinical signs of toxicity.

2. Mortality: All animals survived until scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN: Table 2a summarizes the body weight data for the main group. Table 2b summarizes the body weight data for the satellite group (both the perfusion and recovery groups). Body weights in 750-ppm males of the main and satellite groups were statistically-significantly less than the concurrent controls from Week 1 until Week 13. The 250-ppm males in the main group displayed a statistically-significant decrease in weight as compared to controls from Weeks 5-13. Although the body weights in the 250-ppm satellite males were also reduced as compared to the controls, the reduction was not of the same magnitude observed in the main group and the weights did not achieve statistical significance. In the main study, body weight changes in the 750-ppm males were statistically-significantly lower than controls from Week 1-13 with approximately 20-30% differences throughout. The 750-ppm males displayed statistically-significant decreases in weight gain throughout treatment, and the 250-ppm main study males displayed statistically-significant decreased weight gains during Weeks 4-13. The overall body weight gain in the main study males was lower in all treatment groups as compared to the control, but the difference was only significant in the mid- and high-dose males. While the body weight gain was significantly reduced in 750-ppm satellite males, the reduction was lower in both the low- and mid-dose groups than was observed in the main study groups.

In the main study group, the 750-ppm female's body weights were lower than those of the controls in the first week and became statistically-significant after Week 4 and for the remainder of the study. Body weights in the 750-ppm female satellite group were also reduced compared to the control with statistically-significant results for all weeks except Week 2. The 250-ppm main group females had a reduction in weight that reached statistical significance only on Week 12. Body weight gain was only affected in the high-dose females with reductions noted in both the main and satellite groups. The body weight gain in 750-ppm main group females was statistically-significantly lower than controls during Weeks 3-13, but the differences were 20-30% throughout the study. The 750-ppm satellite females had statistically-significant lower body weight gains during Weeks 1-13, except Week 2.

During the recovery period there were no statistically-significant differences in the body weights of treated males and females compared to the controls. The body weights in high-dose males and females, however, remained lower than the control. There was a dose-related increase in body weight gains during the recovery period in both males and females.

Table 2a. Average Body Weights and Total Body Weight Gains during 90 Days of Treatment in the Main Group[a]

ppm n=10	Body Weights (g±SD) % change from control						Total Weight Gain	
	Week -1	Week 1	Week 4	Week 7	Week 10	Week 13	g	%
Male								
0	200.0±9.2	262.3±15.9	389.7±30.7	461.2±39.4	509.1±37.9	527.0±41.8	327.0	-
80	199.3±7.9 -0	264.8±16.3 +1	379.2±17.9 -2	438.7±25.6 -5	489.8±33.1 -4	507.8±39.3 -4	308.5	94
250	201.5±10.4 +1	254.6±16.5 -3	369.5±26.7 -5	417.8±35.5** -9	457.9±38.5** -10	472.2±38.4** -10	270.7	83**
750	197.5±12.7 -1	240.9±12.3* -8	342.6±16.5** -12	384.5±19.8** -17	404.5±25.8** -21	414.4±28.7** -21	216.9	66**
Female								
0	161.9±7.0	190.8±12.6	252.6±19.0	276.0±28.5	294.6±32.6	307.9±31.8	146.0	-
80	159.9±7.2 -1	187.3±9.5 -2	243.5±18.2 -4	271.0±21.7 -2	286.9±21.4 -3	299.0±20.4 -3	139.1	95
250	160.4±9.9 -1	187.6±13.0 -2	239.5±13.4 -5	266.0±18.6 -4	283.5±19.9 -4	292.8±20.8 -5	132.4	91
750	161.1±8.8 -1	182.6±9.9 -4	226.6±14.9** -10	241.7±17.0** -12	253.8±16.6** -14	264.3±18.1** -14	103.2	71**

a Data obtained from pp. 81-82 and 85-86 in the study report.
* Statistically different ($p<0.05$) from the control

** Statistically different ($p<0.01$) from the control.

Table 2b. Average Body Weights and Total Body Weight Gains during 90 Days of Treatment and 4 Weeks of Recovery in the Satellite Group[a]

ppm	Exposure I1 and R1 (n=10)						Recovery R1 (n		
	Body Weights (g±SD) % difference				Total Weight Gain in Exposure		Body Weights (g±SD) % difference		
	Week -I	Week 1	Week 7	Week 13	g	%	Week 14	Week 17	
Male									
0	200.5±84	261.0±11.1	450.3±32.5	518.7±32.6	318.2	-	501.8±19.5	526.5±23.2	
80	200.2±8.4 -0	255.5±19.3 -2	451.9±30.0 +0	527.8±35.4 +2	327.6	103	525.9±37.6 +5	555.1±47.0 +5	
250	199.6±11.7 -1	258.4±17.1 -1	432.3±27.8 -4	498.3±33.2 -4	298.7	94	506.6±24.3 +1	542.4±34.6 +3	
750	198.5±6.0 -1	239.0±12.2** -8	365.4±23.1** -19	413.1±24.4** -20	214.6	67**	448.3±28.0* -11	489.2±25.5 -7	
Female									
0	161.8±7.2	190.1±9.4	270.4±23.0	302.5±17.8	140.7	-	305.1±16.1	313.1±17.9	
80	158.5±7.3 -2	185.8±12.4 -2	255.3±21.0 -6	288.7±25.4 -5	130.2	93	299.9±26.9 -2	315.5±31.9 +1	
250	161.8±6.4 0	191.6±10.2 +1	269.4±13.3 -0	306.4±21.1 +1	144.6	103	318.1±12.6 +4	334.3±13.7 +7	
750	157.6±8.7 -3	178.4±9.7* -6	240.1±9.3** -11	261.2±12.1** -14	103.6	74**	282.1±11.4 -8	298.0±17.5 -5	

a Data obtained from pp. 83-84 and 87-88 in the study report.
* Statistically different (p<0.05) from the control
** Statistically different (p<0.01) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: In the main study (satellite group discussed below), food consumption in 750-ppm treated males during the first week was approximately 19% lower and in the females was 12%, as indicated in Table 3a below. Food consumption remained lower than the controls throughout the main study in the 750-ppm group in males and females, but the difference only reached statistical significance in males during Weeks 1, 6, and 9, and in females during Weeks 1, 4, 6-9, and 11. The 250-ppm males had a slight decrease (3-7%) in food consumption until Week 11, after that the food consumption was similar to the controls. Food consumption in the 250-ppm females varied from the control, but the differences were likely due to variability as weekly changes were just as likely to be greater or lesser than the controls. An incidental statistically-significant decrease was observed in the 80-ppm females on Week 7.

TABLE 3a. Average Food Consumption (g/animal/day) during 90 Days of Treatment for the Main Group[a]

ppm (n=10)	Food consumption (g/animal/day±SD) % difference				
	Week 1	Week 4	Week 7	Week 10	Week 13
Male					
0	25.4±2.2	26.0±3.6	26.8±2.7	26.0±1.9	22.5±1.3
80	24.6±1.4 -4	25.8±1.7 -1	26.7±2.2 -1	27.4±1.9 +6	23.7±1.5 +6
250	23.7±2.3 -7	24.8±1.8 -5	25.9±2.1 -3	24.9±1.9 -4	23.5±1.7 +4
750	20.7±1.4** -19	24.7±1.7 -5	26.5±1.7 -1	25.3±1.7 -3	22.5±2.5 0
Female					
0	19.3±1.9	21.5±1.5	22.2±1.9	21.6±2.1	19.0±1.4
80	19.0±1.2 -2	20.4±1.3 -5	20.4±1.1* -8	21.0±1.1 -3	18.9±1.1 -1
250	19.0±1.3 -2	21.0±1.3 -3	21.7±1.8 -2	22.2±1.9 +3	19.8±1.4 +5
750	16.9±1.7** -12	19.3±1.5** -10	20.0±0.9** -10	19.4±1.1* -10	18.0±1.1 -5

a Data obtained from pp. 73-74 and 77-78 in the study report.
* Statistically different ($p<0.05$) from the control
** Statistically different ($p<0.01$) from the control.

As was observed in the main study animals, food consumption was lower in the 750-ppm satellite males and females compared to the controls throughout the 13 weeks of treatment (Table 3b below), but the difference only reached statistical significance in males during Weeks 1, 2, 4, 6, 7, 9, and 11, and in females during Weeks 1-4, 6-8, 10, 12, and 13. The largest decrease in food consumption was observed in the first week of treatment in both the 750-ppm males and

females with approximately 20 and 18% decreases observed. The 250-ppm males had a slight decrease in food consumption in the initial week, but the differences from the control through the remainder of the study were sporadic and were likely due to variation and not treatment-related effects. During the initial week of recovery (Week 14), all male and female treatment groups consumed more food than the controls. The 750-ppm males continued to consume greater amounts than the controls until the final week where the food consumption was similar to the controls. Mid-dose females consistently ate more than the controls during the recovery period. None of the changes in the recovery period, however, achieved statistical significance, possibly due to the small number of animals remaining (i.e., 5 rats/sex/dose).

TABLE 3b. Average Food Consumption (g/animal/day) during 90 Days of Treatment and 4 Weeks of Recovery for the Satellite Group[a]

ppm	Food Consumption (g/animal/day±SD) % difference				
	Treatment I1 and R1 (n=10)			Recovery R1 (n=5)	
	Week 1	Week 7	Week 13	Week 14	Week 17
Male					
0	25.3±1.5	26.5±2.1	25.1±1.2	24.4±2.1	25.8±2.3
80	24.8±1.8 -2	27.0±1.8 +2	26.5±1.4 +5	25.3±1.5 +4	25.7±2.7 -1
250	24.1±1.9 -5	27.3±1.9 +3	25.6±2.7 +2	26.3±2.0 +8	27.0±3.1 +5
750	20.3±1.3** -20	24.1±2.2* -9	24.7±1.6 -2	27.2±1.7 +12	26.0±1.8 +1
Female					
0	19.4±1.4	21.6±1.9	21.0±2.3	20.2±0.9	21.6±1.3
80	19.0±1.8 -2	21.0±1.9 -3	21.2±2.2 +1	21.5±1.7 +7	22.3±2.8 +3
250	19.4±1.5 +0	21.5±0.8 -0	21.5±1.0 +2	22.3±0.8 +10	22.4±1.0 +4
750	15.9±1.1** -18	19.1±1.1** -12	19.1±1.0* -9	21.4±1.6 +6	21.2±1.1 -2

a Data obtained from pp. 75-76 and 79-80 in the study report.
* Statistically different (p<0.05) from the control
** Statistically different (p<0.01) from the control.

2. <u>Compound consumption</u>: In the main study, based on the measured concentration of the compound in the 80-, 250-, and 750-ppm dose formulations, the average daily intakes were 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females. In the satellite study, based on the nominal concentrations, the expected doses would have been 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females.

3. **Food efficiency:** Select weekly food efficiency values are provided in Tables 4a (main study) and 4b (satellite study). In the main study , the 750-ppm males and females had decreased feed efficiency with only the males displaying statistically-significant values as compared to the controls (Weeks 1-5, 7, 8, and 13). There was general decrease in food efficiency in the 250- and 80-ppm males and females, but the low-dose males and females had sporadic increases. A few incidences of statistically-significant decreased feed efficiency were observed at Week 5 for 80-ppm males, Weeks 5 and 7 for 250-ppm males, and Week 4 in the 250-ppm females.

TABLE 4a. Average Food Efficiency (g animal/g food) during 90 Days of Treatment for the Main Group[a]

ppm (n=10)	Food Efficiency (g body weight/ g food weight±SD)				
	Week 1	Week 4	Week 7	Week 10	Week 13
Male					
0	34.9±3.8	19.5±3.9	10.2±4.1	6.1±4.3	-1.6±4.2
80	38.1±6.2	17.7±1.4	8.8±2.9	7.6±2.7	-2.4±3.9
250	32.0±2.1	16.8±2.6	3.1±3.2**	4.7±4.0	-2.5±2.6
750	30.0±3.8*	15.1±4.4*	4.4±3.6**	2.8±2.4	-7.0±7.0*
Female					
0	21.0±5.5	15.6±5.0	1.7±4.4	3.1±4.9	-0.6±5.1
80	20.6±3.6	10.6±4.6	5.8±6.7	1.2±5.6	-1.5±5.0
250	20.3±4.7	9.5±6.3*	5.9±5.5	2.5±5.0	6.9±21.0
750	18.2±3.8	11.8±5.4	2.8±6.5	0.1±6.9	1.8±4.0

a Data obtained from pp. 97-98 and 102-103 in the study report.
* Statistically different ($p<0.05$) from the control
** Statistically different ($p<0.01$) from the control.

In the satellite group, feed efficiency (Table 4b, below) was lower than the controls in the 750-ppm group, but the difference only reached statistical significance in males during Weeks 3-8, and in females during Weeks 3 and 5. After the test article was removed from the feed the 750-ppm males and females displayed an increase in feed efficiency during all 4 weeks with Weeks 14 and 15 in the males being statistically significant as compared to controls. The 250-ppm males had slight decreases in food efficiency, but the decreases were sporadic and did not reach statistical significance.

TABLE 4b. Average Food Efficiency (g animal/g food) during 90 Days of Treatment and 4 Weeks of Recovery for the Satellite Group[a]

ppm	Food Efficieney (g body weight/ g food weight±SD)				
	Treatment I1 and R1 (n=10)			Recovery R1 (n=5)	
	Week 1	Week 7	Week 13	Week 14	Week 17
Male					

0	34.2±5.4	9.8±2.8	1.0±2.7	2.7±1.9	5.2±3.5
80	31.7±9.0	7.9±2.3	3.5±3.2	2.6±0.7	3.4±3.4
250	34.8±2.8	6.8±2.3	4.3±2.8	4.4±2.3	3.9±2.3
750	28.4±5.6	1.7±3.8**	2.5±3.8	16.0±3.7**	5.8±3.0
Female					
0	20.8±3.7	-0.9±5.5	-0.9±3.6	5.8±5.7	5.3±6.0
80	20.3±2.8	5.2±5.8	-0.5±3.7	0.7±1.1	4.6±3.9
250	21.7±4.2	1.1±4.0	3.2±2.1	5.9±4.1	5.3±3.8
750	18.5±3.9	4.2±4.6	3.5±5.8	12.2±4.2	8.4±4.1

a Data obtained from pp. 99-101 and 104-106 in the study report.
* Statistically different (p<0.05) from the control
** Statistically different (p<0.01) from the control.

D. OPHTHALMOSCOPIC EXAMINATION: No effects related to the test article were observed. Corneal stippling and remainder of the pupillary membrane were noted in a few rats but these findings were not related to treatment.

E. BLOOD ANALYSES:

1. **Hematology:** Prothrombin time measured at the end of treatment (main study group) was statistically-significantly decreased in the males (all treatment groups) and females (750-ppm group). The decrease in females appeared to be dose-related, but the males did not display the same relation to dose (Table 5a below). The platelet counts measured after treatment (main study group) were increased in mid- and high-dosed males and all female groups with possible relation to dose. In the females the increase in platelets is equivalent to the decrease in prothrombin time. In the males, however, there was no increase in platelets in the low-dose group, but a decrease in prothrombin time was observed. There were statistically-significant, but slight (<5%), increases in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in 750-ppm males. The 250- and 750-ppm females showed slight (<5%), but statistically-significant, increases in MCHC. It was concluded that they were due to normal variation and not related to treatment. This is confirmed by the values observed after the recovery period, which showed similar slight (≤5%) differences between the treatment group and the control.
2.

TABLE 5a. Selected Hematology Parameters for Main Study[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
MCV	MEAN	51.1	51.2	50.6	51.8	52.7	51.2	52.1	51.3
fL	S.D.	2.2	1.6	1.5	2.5	1.8	1.8	1.0	1.5
	% [b]	-	+0	-1	+1	-	-3	-1	-3
	N	10	10	10	10	10	10	10	10

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
MCH Fmol	MEAN	1.06	1.05	1.04	1.10*	1.12	1.10	1.13	1.11
	S.D.	0.04	0.04	0.03	0.05	0.04	0.04	0.02	0.04
	% [b]	-	-1	-2	+4	-	-2	+1	-1
	N	10	10	10	10	10	10	10	10
MCHC mmol/L	MEAN	20.66	20.52	20.62	21.25**	21.22	21.35	21.59*	21.61**
	S.D.	0.24	0.26	0.40	0.40	0.25	0.45	0.31	0.39
	%	-	-1	-0.2	+3	-	+1	+2	+2
	N	10	10	10	10	10	10	10	10
Platelets giga/L	MEAN	1144	1142	1230	1320**	1036	1075	1113	1131
	S.D.	176	109	232	125	120	150	135	277
	%	-	-0	+8	+15	-	+4	+7	+9
	N	10	10	10	10	10	10	10	10
Prothrombin time Sec	MEAN	35.0	32.2**	33.4*	31.9**	29.6	28.8	27.8	26.9**
	S.D.	1.7	1.9	1.7	2.3	2.2	1.8	1.5	1.3
	%	-	-8	-5	-9	-	-3	-6	-9
	N	10	10	10	10	10	10	10	10

a Data obtained from pages 119, 121, 131, and 133 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

After a 4-week recovery period (satellite group; R1), the only statistically-significant finding was an incidental increase in MCV in 750-ppm males (Table 5b below). The platelet counts in the males, however, still demonstrated a dose-related increase, but the prothrombin time was back to control values indicating a reversal of the effect.

TABLE 5b. Selected Hematology Parameters for Satellite Study (R1)[a]

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
MCV fL	MEAN	50.4	50.3	49.6	52.4*	53.6	52.4	51.9	51.4
	S.D.	1.2	1.1	0.9	1.6	2.6	1.8	1.9	0.9
	% [b]	-	-0	-2	+4	-	-2	-3	-4
	N	5	5	5	5	5	5	5	5
MCH Fmol	MEAN	1.03	1.03	1.02	1.08	1.13	1.10	1.09	1.10
	S.D.	0.03	0.03	0.02	0.04	0.04	0.03	0.04	0.03
	% [b]	-	0	-1	+5	-	-3	-4	-3
	N	5	5	5	10	5	5	5	5
MCHC mmol/L	MEAN	20.54	20.52	20.62	20.59	21.09	21.09	21.08	21.50
	S.D.	0.15	0.28	0.40	0.29	0.28	0.19	0.29	0.25
	% [b]	-	-0	+0	+0	-	0	-0	+2
	N	5	5	5	5	5	5	5	5
Platelets giga/L	MEAN	1110	1136	1236	1243	1114	1026	1183	1030
	S.D.	101	91	202	175	108	66	112	154
	% [b]	-	+2	+11	+12	-	-8	+6	-8
	N	5	5	5	5	5	5	5	5
Prothrombin time Sec	MEAN	32.5	32.1	33.2	32.1	29.1	28.0	28.1	28.9
	S.D.	1.4	1.8	3.0	1.8	2.3	1.8	0.9	1.7
	% [b]	-	-1	+2	-1	-	-4	-3	-1
	N	5	5	4	5	4	5	5	5

a Data obtained from pages 120, 122, 132, and 134 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

2. <u>Clinical Chemistry</u>: Selected clinical chemistry parameters are provided in Tables 6a (main study) and 6b (satellite group; R1). At the end of treatment (main study group), males demonstrated a statistically-significant increase in ALT across all treatment groups that was

apparently dose-related. The same effect was not noted in females, who had a statistically-significant decrease in ALT in the low-dose. While there were still differences in the ALT after the recovery period (Table 6b), there was no relation to dose in either the males or females; therefore, it is concluded that, in the absence of liver histopathology findings, the effects were reversed. ALP also was statistically-significantly increased in all male treatment-groups at the end of treatment. While the females also had increases across all treatment groups, only the high-dose group was increased by a statistically significant amount. After the recovery period, the ALP in the high-dose males was increased (not significantly) as compared to concurrent controls. The difference from controls, however, was lower than at the end of treatment for all groups indicating recovery. Phosphorous levels at the end of treatment were increased in a dose-dependent manner in both males and females with high-dose males and females reaching statistical significance. The values remained elevated (not significant) after the recovery period in mid- and high-dose males and females. Urea levels at the end of treatment were statistically-significantly increased in mid- and high-dose males (dose-dependent) and high-dose females. This effect was reversible with a 4-week recovery period. Creatinine was statistically-significantly increased in high-dose males after treatment. Female creatinine levels, however, displayed a statistically-significant decrease in the low- and mid-dose groups. There were no changes noted after the recovery period. Glucose was statistically-significantly decreased in high-dose males and mid- and high-dose females (dose-related). After the recovery period, the glucose was still lower (not significantly) than the control in these groups. Albumin was statistically-significantly decreased in high-dose females while globulin had a statistically significant increase in mid- and high-dose females (dose-dependent). The same was not observed in the males and was reversible after a 4-week recovery period. Triglycerides were decreased in a dose-dependent manner in males with the high-dose statistically-significantly lower than the control. While the triglycerides remained low after the recovery period in all groups, the difference was no longer related to dose. Cholesterol was increased in high-dose males (not significant) and in all treated females (statistically significant in the mid- and high-dose groups only). These changes were reversible after recovery. Magnesium was increased in high-dose males (not significant) and females (statistically significant). The values remained elevated after the recovery period; however, the results did not achieve statistical significance. There also was an incidental statistically-significant decrease in serum chloride levels in mid-dose females.

TABLE 6a. Selected Clinical Chemistry Parameters Main Group[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
ALT µkat/L	MEAN	0.65	0.76*	0.82**	1.06**	0.74	0.60*	0.65	0.80
	S.D.	0.04	0.21	0.24	0.39	0.12	0.13	0.15	0.22
	%[b]	-	+17	+26	+63	-	-19	-12	+8
	N	10	10	10	10	10	10	10	10
ALP µkat/L	MEAN	1.31	1.60*	1.47*	2.21**	0.54	0.58	0.57	0.73**
	S.D.	0.15	0.25	0.17	0.75	0.12	0.10	0.16	0.13
	%	-	+19	+12	+69	-	+7	+6	+35
	N	10	10	10	10	10	10	10	10
Phosphorus mmol/L	MEAN	2.08	2.12	2.18	2.36**	1.60	1.63	1.72	1.88**
	S.D.	0.12	0.09	0.15	0.11	0.16	0.15	0.18	0.13
	%	-	+2	+5	+14	-	+2	+8	+18
	N	10	10	10	10	10	10	10	10

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
Urea	MEAN	5.23	5.27	5.90*	7.63**	7.05	6.69	7.27	9.25**
mmol/L	S.D.	0.48	0.49	0.75	0.65	0.61	0.90	1.32	1.25
	%	-	+0.8	+12	+46	-	-5	+3	+31
	N	10	10	10	10	10	10	10	10
Creatinine	MEAN	47.1	47.5	48.1	55.8**	57.0	52.8*	51.7*	55.0
µmol/L	S.D.	3.8	2.5	2.9	5.0	4.9	4.7	4.1	3.7
	%	-	+1	+2	+19	-	-7	-9	-4
	N	10	10	10	10	10	10	10	10
Glucose	MEAN	6.72	6.85	6.23	5.59**	6.95	6.78	6.25**	5.66**
mmol/L	S.D.	0.51	0.54	0.32	0.59	0.33	0.65	0.40	0.35
	%	-	+2	-7	-17	-	-2	-10	-18
	N	10	10	10	10	10	10	10	10
Albumin	MEAN	36.18	35.72	35.75	35.31	37.99	38.04	37.65	34.82**
g/L	S.D.	0.89	1.62	1.47	1.24	2.51	1.77	1.40	0.95
	%	-	-1	-1	-2	-	0	-1	-8
	N	10	10	10	10	10	10	10	10
Globulin	MEAN	32.05	31.62	31.38	33.76	27.18	27.62	28.69**	30.98**
g/L	S.D.	1.85	2.59	1.73	2.75	1.55	1.36	1.27	1.61
	%	-	-1	-2	+5	-	+2	+6	+14
	N	10	10	10	10	10	10	10	10
Trigyeeride	MEAN	0.70	0.64	0.46	0.38*	0.45	0.45	0.48	0.45
mmol/L	S.D.	0.30	0.21	0.13	0.13	0.11	0.11	0.15	0.09
	%	-	-20	-34	-46	-	0	+7	0
	N	10	10	10	10	10	10	10	10
Cholesterol	MEAN	1.89	1.91	1.83	2.12	2.10	2.29	2.91**	3.19**
mmol/L	S.D.	0.47	0.53	0.36	0.50	0.42	0.36	0.36	0.46
	%	-	+1	-3	+12	-	+9	+39	+52
	N	10	10	10	10	10	10	10	10
Magnesium	MEAN	0.89	0.89	0.90	0.95	0.95	0.96	0.99	1.03**
mmol/L	S.D.	0.05	0.06	0.03	0.07	0.04	0.03	0.05	0.05
	%	-	0	+1	+7	-	+1	+4	+8
	N	10	10	10	10	10	10	10	10

a Data obtained from pages 135, 137, 139-140, 143-144 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

TABLE 6b. Selected Clinical Chemistry Parameters Satellite Group (R1)[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
ALT	MEAN	0.73	1.05	0.68	0.98	0.81	0.73	1.44	0.78
µkat/L	S.D.	0.03	0.62	0.07	0.54	0.24	0.10	1.22	0.21
	%[b]	-	+44	-7	+34	-	-10	+78	-4
	N	5	5	5	5	5	5	5	5
ALP	MEAN	1.26	1.27	1.09	1.47	0.50	0.45	0.46	0.51
µkat/L	S.D.	0.15	0.27	0.16	0.17	0.14	0.05	0.14	0.13
	%	-	+0.8	-14	+17	-	-10	-8	+2
	N	5	5	5	5	5	5	5	5
Phosphorus	MEAN	1.93	1.93	2.03	2.09	1.52	1.50	1.66	1.60
mmol/L	S.D.	0.08	0.07	0.18	0.11	0.16	0.21	0.12	0.24
	%	-	0	+5	+8	-	-1	+9	+5
	N	5	5	5	5	5	5	5	5
Urea	MEAN	5.60	5.67	5.54	5.75	7.03	6.78	7.42	7.24
mmol/L	S.D.	0.53	0.54	0.63	0.36	1.57	0.84	0.75	1.19
	%	-	+1	+1	+3	-	-4	+6	+3
	N	5	5	5	5	5	5	5	5

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
Creatinine µmol/L	MEAN	49.2	52.1	53.0	49.4	57.2	54.6	53.8	53.9
	S.D.	1.2	6.1	3.3	2.7	7.3	5.6	2.5	5.8
	%	-	+6	+8	+0	-	-5	-6	-6
	N	5	5	5	5	5	5	5	5
Glucose mmol/L	MEAN	6.63	7.01	7.50	6.41	7.11	6.75	6.52	6.44
	S.D.	0.48	0.29	0.55	0.42	0.41	0.23	0.43	0.44
	%	-	+6	+13	-3	-	-5	-8	-9
	N	5	5	5	5	5	5	5	5
Albumin g/L	MEAN	36.66	36.45	36.91	36.63	39.22	39.21	38.22	38.04
	S.D.	1.26	1.76	1.10	1.20	1.22	0.66	1.37	1.02
	%	-	-1	+1	0	-	0	-3	-3
	N	5	5	5	5	5	5	5	5
Globulin g/L	MEAN	32.55	33.52	32.32	30.42	28.05	29.03	29.31	28.52
	S.D.	1.84	2.62	1.83	2.07	2.29	1.20	1.48	2.25
	%	-	+3	-1	-7	-	+3	+4	+2
	N	5	5	5	5	5	5	5	5
Trigyceride mmol/L	MEAN	0.75	0.61	0.73	0.46	0.55	0.48	0.46	0.59
	S.D.	0.64	0.17	0.27	0.17	0.10	0.08	0.09	0.14
	%	-	-19	-3	-39	-	-13	-16	+7
	N	5	5	5	5	5	5	5	5
Cholesterol mmol/L	MEAN	2.05	2.10	1.90	2.07	2.32	2.17	2.67	2.43
	S.D.	0.54	0.30	0.52	0.46	0.52	0.40	0.13	0.76
	%	-	+2	-7	-1	-	-6	+15	+5
	N	5	5	5	5	5	5	5	5
Magnesium mmol/L	MEAN	0.85	0.84	0.87	0.89	0.92	0.94	0.93	0.98
	S.D.	0.05	0.03	0.01	0.04	0.07	0.05	0.03	0.04
	%	-	-1	+12	+5	-	+2	+1	+7
	N	5	5	5	5	5	5	5	5

a Data obtained from pages 136, 138, 141-142, 145-146 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

F. Urinalysis - No abnormal or treatment-related findings were observed in the urinalysis data.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: Terminal body weight was statistically-significantly reduced in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs (Table 7a below). The relative weights of heart and adrenals (not significant in females) were statistically-significantly increased in high-dose males and females. These changes are considered related to reduced body weight and not directly related to treatment as there are no macroscopic or microscopic changes observed in these organs Relative spleen weights were statistically-significantly increased in low- (absolute also increased), mid-, and high-dose females and mid-and high-dose males. While the increase in males appears to be related to a decrease in body weight, this cannot explain the increase in the low-dose females. There was a slight increase in extramedullary hematopoiesis in high-dose males and females (other groups not examined). Absolute kidney weight was decreased in mid- and high-dose males, and high-dose females with the low-dose females displaying a statistically-significant increase. This was also related to the decrease in body weight as similar decreases were observed and there were no changes in the absolute weight. Thymus absolute and relative weight ratios were statistically-significantly decreased in high-dose males. Statistically-

significant increases in epididymal and testes relative weights were increased in the high-dose males, with relative epididymides weight being statistically-significantly increased in the mid-dose males as well. These changes also are likely due to reduced body weight and are not biologically significant as there were no pathological changes in these organs. A dose-related increasing trend was observed in the absolute and relative liver weights in the females and relative weights in males. Although there are no pathology results to support hepatotoxicity, increases in clinical chemistry parameters (i.e., ALT and ALP) with reversibility noted, indicate that the liver was affected by treatment, and that the changes were due to increased metabolism of the test article. Mid- and high-dose males and high-dose females had increased relative brain weights in the main study (Table 7a below). The satellite group II which were perfuse fixed before weighed demonstrated an increase in relative brain weight in high-dose males (statistically significant) and females (not significant), a statistically-significant increase in the absolute brain weight in low-dose males, and a statistically-significant decrease in the absolute brain weight in high-dose females. Although the changes in weight may be related to changes in body weight, there were several lesions in the brain noted in histopathology including multifocal vacuolation that indicate the brain as a target organ.

TABLE 7a. Selected Absolute and Relative Organ Weights from the Main Group[a]

Dose Ppm		Males				Females			
		0	80	250	750	0	80	250	750
Number of animals		10	10	10	10	10	10	10	10
Terminal Body Wt. (g)	MEAN	489.16	469.61	436.91*	379.01**	286.34	280.84	274.38	243.15**
	S.D.	39.509	36.571	39.198	24.597	30.099	18.562	21.782	15.165
	%[b]	-	-4	-11	-23	-	-2	-4	-15
BRAIN									
Absolute (g)	MEAN	2.136	2.135	2.105	2.060	1.967	2.037	2.014	1.977
	S.D.	0.053	0.087	0.094	0.077	0.063	0.061	0.089	0.103
	%	-	-0	-1	-4	-	+4	+2	+1
Per Body Weight (‰)	MEAN	0.439	0.457	0.485*	0.545**	0.693	0.729	0.737	0.815**
	S.D.	0.032	0.033	0.040	0.035	0.071	0.059	0.055	0.055
	%	-	+4	+11	+24	-	+5	+0	+18
HEART									
Absolute (g)	MEAN	1.387	1.383	1.314	1.302	0.952	0.992	0.986	0.926
	S.D.	0.125	0.150	0.112	0.097	0.058	0.080	0.060	0.061
	%	-	-0.3	-5	-6	-	+4	+4	-3
Per Body Weight (‰)	MEAN	0.284	0.294	0.302	0.344**	0.335	0.354	0.360	0.381**
	S.D.	0.016	0.019	0.022	0.018	0.028	0.027	0.020	0.019
	%	-	+4	+6	+21	-	+6	+7	+14
LIVER									
Absolute (g)	MEAN	11.863	11.870	11.340	11.469	7.397	7.804*	7.928**	8.727**
	S.D.	1.163	1.583	1.125	1.107	0.712	0.432	0.737	0.447
	%	-	+0	-4	-3	-	+6	+7	+18
Per Body Weight (‰)	MEAN	2.424	2.519	2.597**	3.029**	2.595	2.785*	2.892*	3.594**
	S.D.	0.109	0.183	0.135	0.266	0.221	0.159	0.162	0.160
	%	-	+4	+7	+25	-	+7	+11	+39

Dose Ppm		Males				Females			
		0	80	250	750	0	80	250	750
KIDNEYS									
Absolute (g)	MEAN	3.175	3.095	2.877**	2.420**	1.979	2.111*	2.064	1.792**
	S.D.	0.212	0.283	0.318	0.213	0.143	0.157	0.169	0.158
	%	-	-3	-9	-24	-	+7	+4	-9
Per Body Weight (‰)	MEAN	0.651	0.660	0.659	0.640	0.697	0.752	0.754	0.738
	S.D.	0.044	0.043	0.042	0.055	0.075	0.043	0.058	0.059
	%	-	+1	+1	-2	-	+8	+8	+6
SPLEEN									
Absolute (g)	MEAN	0.716	0.710	0.779	0.711	0.599	0.701**	0.651	0.589
	S.D.	0.051	0.063	0.146	0.091	0.078	0.070	0.089	0.073
	%	-	-1	+9	-1	-	+17	+9	-2
Per Body Weight (‰)	MEAN	0.147	0.152	0.178**	0.189**	0.209	0.251**	0.237*	0.242**
	S.D.	0.013	0.019	0.025	0.029	0.015	0.031	0.023	0.028
	%	-	+3	+21	+29	-	+20	+13	+16
ADRENAL									
Absolute (mg)	MEAN	56.60	56.30	54.50	59.00	90.1	96.2	94.8	87.8
	S.D.	6.310	7.056	6.151	8.919	8.062	13.677	9.841	13.147
	%	-	-1	-4	+4	-	+7	+5	-3
Per Body Weight (‰)	MEAN	0.012	0.012	0.013	0.016**	0.032	0.035	0.035	0.036
	S.D.	0.001	0.001	0.001	0.002	0.005	0.007	0.003	0.06
	%	-	0	+8	+33	-	+9	+9	+13
THYMUS									
Absolute (mg)	MEAN	248.20	217.30	256.00	148.20**	303.1	294.9	300.2	281.7
	S.D.	47.735	51.968	51.223	33.819	53.949	60.080	43.741	62.029
	%	-	-12	+3	-40	-	-3	-1	-7
Per Body Weight (‰)	MEAN	0.051	0.046	0.059	0.039*	0.106	0.105	0.110	0.116
	S.D.	0.010	0.009	0.011	0.009	0.014	0.020	0.014	0.023
	%	-	-10	+16	-24	-	-1	+4	+9
EPIDIDYMIDES									
Absolute (mg)	MEAN	1.266	1.200	1.255	1.173				
	S.D.	0.107	0.076	0.102	0.131				
	%	-	-5	-1	-7				
Per Body Weight (‰)	MEAN	0.260	0.257	0.289*	0.310**				
	S.D.	0.031	0.030	0.033	0.031				
	%	-	-1	+11	+19				
TESTES									
Absolute (mg)	MEAN	3.548	3.315	3.371	3.571				
	S.D.	0.269	0.222	0.288	0.422				
	%	-	-7	-5	+1				
Per Body Weight (‰)	MEAN	0.729	0.708	0.778	0.944**				
	S.D.	0.078	0.043	0.096	0.144				
	%	-	-3	+7	+30				

a Data obtained from pages 151-154 in the study report.
b % difference from control, calculated by reviewers

* Statistically different (p <0.05) from the control
** Statistically different (p<0.01) from the control

Table 7b summarizes the brain weights (the only organ weighed) for satellite group I1 after perfuse fixation. The high-dose male and female final weights were statistically-significantly decreased, which led to increases in relative brain weight in the high-dose males (significant) and high-dose females (not significant) compared to control weights.

TABLE 7b. Selected Absolute and Relative Brain Weights in the Satellite Group (I1)[a]

		Males				Females			
Dose ppm		0	80	250	750	0	80	250	750
Number of animals		5	5	5	5	5	5	5	5
Terminal Body Wt. (g)	MEAN	512.76	503.20	467.46	383.86**	293.56	265.94	287.10	244.9**
	S.D.	29.433	36.418	42.403	25.596	22.709	20.046	22.086	10.173
	%[b]	-	-2	-9	-25	-	-9	-2	-17
BRAIN									
Absolute (g)	MEAN	2.062	2.144*	1.974	1.960	1.924	1.870	1.932	1.830**
	S.D.	0.068	0.30	0.097	0.111	0.043	0.089	0.062	0.071
	%	-	+4	-4	-5	-	-3	+0	-5
Per Body Weight (‰)	MEAN	0.403	0.428	0.425	0.512**	0.660	0.707	0.676	0.748
	S.D.	0.010	0.027	0.041	0.029	0.068	0.069	0.056	0.030
	%	-	+6	+5	+27	-	+7	+2	+13

a Data obtained from pages 155-158 in the study report.
b % difference from control, calculated by reviewers
* Statistically different (p <0.05) from the control
** Statistically different (p<0.01) from the control

In the satellite group R1, the recovery group, the high-dose males still had statistically-significant decreased body weight and the high-dose females had reduced weight that did not achieve statistical significance (Table 7c below). The treated females displayed statistically-significantly increased liver weight. A statistically-significant increase in relative testes weight of 7.91% was observed in the low-dose males; however, this was considered to be unrelated to treatment because no lesions were observed and the change was not observed in the higher doses. A statistically-significant decrease in kidney weight was also observed, but was related to the reduced body weight in this group as there were no pathological findings nor were there any other changes notes.

TABLE 7c. Selected Absolute and Relative Liver Weights in the Satellite Group (R1)[a]

		Males				Females			
Dose ppm		0	80	250	750	0	80	250	750
Number of animals		5	5	5	5	5	5	5	5
Terminal Body Wt. (g)	MEAN	495.52	521.10	500.90	452.76*	291.56	293.54	307.18	275.82
	S.D.	19.76	38.449	30.890	29.161	16.581	30.166	11.111	19.314
	%[b]	-	+5	+1	-9	-	+1	+5	-5
LIVER									
Absolute (g)	MEAN	12.35	12.998	13.218	11.226	7.106	7.792	8.122**	7.740
	S.D.	0.916	0.879	1.778	1.100	0.267	0.687	0.395	0.568
	%	-	-1	-4	-15	-	+10	+14	+9
Per Body Weight (%u)	MEAN	2.490	2.496	2.631	2.477	2.441	2.659 *	2.644 *	2.812*
	S.D.	0.098	0.063	0.218	0.149	0.101	0.070	0.094	0.205
	%	-	+0	+6	-1	-	+9	+8	+15

a Data obtained from pages 159-162 in the study report.
b % difference from control, calculated by reviewers
* Statistically different ($p < 0.05$) from the control
** Statistically different ($p < 0.01$) from the control

2. **Gross pathology:** There were no treatment-related changes in macroscopic lesions in the main and I1 satellite group

3. **Microscopic pathology:** All slides from the various treatment groups were peer reviewed and any changes are contained in the tables.

Table 8a summarizes treatment related lesions and severity of the lesions observed in the main group after treatment. Increases in the incidence of hyperplasia of the mucosal cells of the duodenum were noted in the mid- and high-dose animals with the females being more sensitive than the males.

There was a slight increase in extramedullary hematopoiesis in the spleen, which coincides with changes in the spleen weight and changes in a few hematological parameters associated with red blood cells.

The brain, thoracic and lumbar cord displayed a treatment related increase in the incidence and severity of multifocal vacuolation in the mid- and high-dose animals. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8a below. Further investigations on changes of the brain are addressed in the I1 satellite group that underwent perfusion and detailed brain necropsy.

Table 8a. Incidences of Selected Histopathology Findings After Treatment in the Main Group[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
N	10	10	10	10	10	10	10	10
Duodenum	10	10	10	10	10	10	10	10
Mucosal hyperplasia	0	0	2	2	0	0	7	8
Minimal	0	0	1	2	0	0	6	5
Slight	0	0	1	0	0	0	1	3
Spleen	10	-	-	10	10	-	-	10
Extramedullary hematopoiesis	0	-	-	1	1	-	-	3
Brain	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	1	10/9[b]	0	5/0	1/3	9/8
Minimal	0	0	1	7/6	0	5/0	1/3	3/4
Slight	0	0	0	1	0	0	0	3/1
Moderate	0	0	0	2	0	0	0	3
Focal calcification	0	0	0	1	0	0	0	0
Ventricles dilation	0	1	0	0	0	0	0	0
Moderate	0	1	0	0	0	0	0	0
Thoracic cord	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	0	0	0	0	0	2
Moderate	0	0	0	0	0	0	0	1
Marked/Severe	0	0	0	0	0	0	0	1
Lumbar cord	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	8	10	0	5	9	10
Minimal	0	0	6	0	0	3	3	0
Slight	0	0	2	2	0	2	4	0
Moderate	0	0	0	6	0	0	2	3
Marked/Severe	0	0	0	2	0	0	0	7

a Data obtained from pages 166-167 and 170 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement.

Animals that underwent *in situ* perfusion displayed changes to almost all areas of the brain and spinal cord in the mid- and high-dose animals with the females appearing to be more sensitive (Table 8b). The high-dose males displayed slight or minimal multifocal vacuolization in the

frontal lobe, parietal lobe, midbrain, pons, cerebellum, cervical cord, and lumbar cord. The high-dose females displayed slight to moderate multifocal vacuolization in the frontal lobe, parietal lobe, midbrain, pons, medulla oblongata, cerebellum, and lumbar cord. The mid-dose females displayed slight or minimal multifocal vacuolization in the frontal lobe, parietal lobe, midbrain, pons, cerebellum, and lumbar cord. However, the mid-dose males did not have any incidence of multifocal vacuolization of any area of the brain or spinal cord at any level. Almost all high-dose males displayed minimal to marked myelin vacuolation cervical dorsal root, cervical ventricle root, lumbar dorsal root, lumbar ventricle root, and proximal sciatic nerve with the lumbar region being most profoundly affected. In the high-dose females, minimal to marked myelin vacuolation cervical dorsal root, lumbar dorsal root, lumbar ventricle root, and proximal sciatic nerve with the lumbar dorsal region were most profoundly affected. The mid-dose males and females displayed minimal to slight myelin vacuolation of the cervical ventricle root, and lumbar dorsal root, lumbar ventricle root. Low-dose females had slight myelin vacuolation of the lumbar dorsal root and lumbar ventricle root. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8b below.

Table 8b. Incidences of Neurological Histopathology Findings After Treatment in the Satellite Group (I1)[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Frontal Lobe	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	2/3[b]	0	0	2	5/4
Minimal	0	0	0	1/3	0	0	2	3/4
Slight	0	0	0	1/0	0	0	0	2/0
Parietal Lobe	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	4	0	0	2	4/5
Minimal	0	0	0	2	0	0	1/2	1/2
Slight	0	0	0	2	0	0	1/0	2
Moderate	0	0	0	0	0	0	0	1
Midbrain	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	1	5/4
Minimal	0	0	0	1/3	0	0	1	2/1
Slight	0	0	0	2/0	0	0	0	2
Moderate	0	0	0	0	0	0	0	1
Pons	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	2/1	4/2

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Minimal	0	0	0	1/2	0	0	2/1	2/1
Slight	0	0	0	2/1	0	0	0	2/1
Cerebellum	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	4/3	0	0	1/2	5
Minimal	0	0	0	2/3	0	0	0/1	1/2
Slight	0	0	0	2/0	0	0	1	3/2
Moderate	0	0	0	0	0	0	0	1
Medulla Oblongata	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	0	0	0	0	2
Slight	0	0	0	0	0	0	0	2
Cervical Cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Dorsal Root Cervical	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	1/2	4	0	0	2/3	2/3
Minimal	0	0	1/2	1/3	0	0	1/2	0/1
Slight	0	0	0	1/1	0	0	1	1
Moderate	0	0	0	2/0	0	0	0	1
Ventral Root Cervical	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	0	2	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Lumbar cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	2/3	5	0	0	1/2	4/5
Minimal	0	0	2/3	2	0	0	1/2	2/3
Slight	0	0	0	2/3	0	0	0	2
Moderate	0	0	0	1/0	0	0	0	0
Lumbar Dorsal Root	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	3	5	0	2/1	4/5	5
Slight	0	0	1/2	0	0	2/1	1/2	2/3

Microscopic Observations								
Sex	**Male**				**Female**			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Minimal	0	0	2/1	1	0	0	2/3	1/0
Moderate	0	0	0	2	0	0	1/0	1
Marked/Severe	0	0	0	2	0	0	0	1
Lumbar Ventral Root	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	1/2	5	0	1	3/4	5
Slight	0	0	1/2	0	0	1	0/1	0
Minimal	0	0	0	2	0	0	3	1
Moderate	0	0	0	1	0	0	0	4
Marked/Severe	0	0	0	2	0	0	0	0
Proximal Sciatic Nerve	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	0	5	0	0	0	1
Slight	0	0	0	1	0	0	0	0
Minimal	0	0	0	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Axonal degeneration	0	0	0	0	0	0	1	1
Slight	0	0	0	0	0	0	1	1
Proximal Tibial Nerve	5	5	5	5	5	5	5	5
Axonal degeneration[c]	0	0	0	0	0	0	0	1

a Data obtained from pages 168, 171-172 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement
c Severity was not provided.

After 4 weeks of no exposure to the test article, the brain and lumbar cord still display signs of injury as summarized in Table 8c. There was a decrease in the severity of the lesions but the low-, mid- and high-dose females and mid- and high-dose males still had multifocal vacuolization of the lumbar cord. The brain was noted with multifocal vacuolization in the high-dose males and females and mid-dose females. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8c below.

Table 8c. Incidences of Brain Histopathology Findings After Treatment in the Satellite Group (R1)[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Brain	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	1/2[b]	4
Minimal	0	0	0	3	0	0	1/2	3
Slight	0	0	0	0	0	0	0	1
Lumbar cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	2/3	5	0	2	5/4	5
Minimal	0	0	2	0	0	2	2/1	1
Slight	0	0	0/1	3	0	0	2	2
Moderate	0	0	0	2	0	0	1	2

a Data obtained from pages 168, 171-172 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement

III. DISCUSSION and CONCLUSIONS

A. Results of the neurological pathology from a 90-day oral toxicity study (MRID 46442201) were confirmed in a Pathology Peer Review Statement (MRID 46659701). In the 90-day oral toxicity study, R 107894 (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]; 94.6% a.i.) was administered to 10 Sprague-Dawley rats/sex/dose at concentrations of 0, 80, 250, or 750 ppm via the feed. The doses were calculated to be equivalent 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females. The analytical concentration was on average 90.4-100.8% of the nominal concentration. Additional 5 rats/sex/group (I1) were administered the same concentrations of test article, sacrificed at the end of treatment, and *in situ* perfusion fixed for histological examination of the nervous system. Another 5 rats/sex/group (R1) were treated in the same manner and were removed from treatment after 13 weeks and allowed a 4-week recovery period, after which they were sacrificed for evaluation after immersion fixation. These additional 10 rats/sex/group were considered a satellite group. Intake of test compound was similar to that observed in the main group.

There were no treatment-related effects noted in clinical signs, mortality, ophthalmology or urinalysis in treated animals. Our reviewers agree with the study authors that the MTD was exceeded in the high-dose, because weight gain was 29% lower than the controls. Several signs of toxicity were observed in the high-dose animals such as decreased body weight; decreased food consumption; decreased prothrombin time and increased platelet counts; increased ALT, ALP, phosphorus, blood urea nitrogen, creatinine (males only), cholesterol, and magnesium, decreased glucose and triglycerides (males only); increased relative organ weights (brain, heart,

liver, spleen, adrenals, epididymides, and testes); mucosal hyperplasia of the duodenum, multifocal vacuolation of the CNS and multifocal intramyelinic vacuolation of the proximal peripheral nervous system. Although the changes in the red blood cell parameters (MCH and MCHC) were likely treatment-related due to the fact that they were consistent in the high-dose group and changes were noted in the spleen (i.e., weight and pathology), the changes were ≤5% different from the control. The clinical chemistry changes were associated with the severely reduced weight of these animals and possibly to a disrupted liver function.

The mid-dose animals displayed to a lesser extent than high-dose animals reduced weight and food consumption; decreased prothrombin time and increased platelet counts; increased ALT (males only), ALP, phosphorus, blood urea nitrogen, creatinine (males only), cholesterol (females only), and magnesium, decreased glucose and triglycerides (males only); increased relative organ weights (brain [males only], heart, liver, spleen, adrenals, epididymides, and testes); mucosal hyperplasia of the duodenum, multifocal vacuolation of the CNS and multifocal intramyelinic vacuolation of the proximal peripheral nervous system. As in the high-dose animals most signs of toxicity showed recovery except relative liver weight in females and multifocal vacuolation of the brain and lumbar cord. In the low-dose females minimal multifocal vacuolation of the brain and lumbar cord, and slight myelin vacuolation in the lumbar dorsal and ventral root was observed. After 4-week cessation of treatment the low-dose females still displayed minimal multifocal vacuolation of the lumbar cord.

Although the study authors state in many cases that these changes were reversible after a 4-week recovery period due to a lack of statistical significance after the recovery period, this may be due to the small number of animals (i.e., 5) measured after the recovery period. Many instances (i.e., platelets in mid-and high-dose males, ALT and ALP in high-dose males, phosphorous in mid- and high-dose males and females, glucose in high-dose males and all treated females, triglycerides in high-dose males, and magnesium in high-dose males and females) indicate residual effects even though the results did not achieve statistical significance.

The target organ is the white matter of the CNS (brain, spinal cord) and PNS (ventral and dorsal root) without apparent functional alteration as observed with clinical signs of toxicity. This study lacked a neurological examination (e.g., FOB), which could have greatly added to the toxicological evaluation. The Pathology Peer Review Statement generally concurred with the neurological pathology findings in the report. There are several instances where the Peer Reviewers differed on the severity of lesions and in the number of lesions; however, these differences of opinion did not change the overall findings on the neurological pathology of the report.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based micro-scopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males. The NOAEL could not be determined in females.

B. <u>STUDY DEFICIENCIES:</u> There were no major study deficiencies. Minor deficiencies included the following:

- Neurological testing was not performed; however, clinical symptoms did not indicate neurological toxicity. Considering the frank effects of the CNS and PNS, these tests would have proved very useful in determining if the lesions affected function.
- Historical control data would have been useful.
- Three slides were missing from the terminal sacrifice satellite group rats including one from an 0S male (#14) of the lumbar dorsal root and two of the central ventral root for 3S males (#73 and #75) for the peer review pathology evaluation.
- Tissues from the recovery satellite group were prepared by immersion-fixation only and not perfusion-fixed as was done for the terminal satellite group. Therefore, the recovery animals could only be compared to the main group, but the majority of the findings were in the satellite group.
- There were several instances of disagreement between the study pathologist and the review pathologist. While some of the instances were a matter of degree of involvement, there were numerous cases where the difference was between minor involvement and no involvement. The general conclusion, however, remained the same.

C. STUDY CLASSIFICATION: This subchronic (90-day) oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a 90-day exposure for a 90-day oral toxicity study OPPTS 870.3100 in the rat.

EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Sr. Scientist
Team 2, RASSB/Antimicrobial Division

S. L. Malish 6/7/06
Jonathan Chen 06/13/06

DATA EVALUATION REPORT

STUDY TYPE: Inhalation (range-finding) Toxicity Study

PC CODE: 119093
DECISION No: 220066

DP BARCODE: 327538
EPA REG No: 43813-ET

TEST MATERIAL (PURITY): 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl); (93% a.i.)

SYNONYMS: Econea®028, CL 303'268, R 107894

CITATION: Kirkpatrick, D. (2006). An inhalation toxicity range-finding study of R107894 in albino rats. WIL Research Laboratories, Ashland, Ohio. WIL-436003. January 11, 2006. MRID 46751302. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340, Beerse, Belgium

EXECUTIVE SUMMARY: In a range-finding inhalation toxicity study (MRID 46751302), R107894 (93% a.i., lot no. 89000015) was administered to 5 Sprague-Dawley rats/sex/dose via nose only exposure at dose levels of 0, 24, 80 (6 hours a day for 12 days), or 240 mg/m^3 for 4 hours and 25 minutes, due to high mortality. The surviving animals were held in the animal room and observed without further exposure. Additional groups of 5 Sprague-Dawley male rats/dose were administered the test material via nose only exposure at dose levels of 0 or 120 mg/m^3 for 6 hours for one day only due to high mortality. All surviving animals were euthanized the following day. The 24, 80, 120 or 240 mg/m^3 dose levels were determined to be equivalent to 24, 80, 126, or 236 mg/m^3, respectively, of the nominal concentration of the test substance. MMAD and GSD values were within acceptable limits.

Clinical signs, body weight, food consumption, gross and microscopic pathology, organ weight, and a functional observational battery were performed. Clinical chemistry, hematology and urinalysis were not performed.

Several unscheduled deaths occurred during the course of the study: in the 240 mg/m^3 group, 4/5 males and 2/5 females died during the first day of exposure, thus, prompting the termination of exposure at 4 hours and 25 minutes; in the 120 mg/m^3 group, 3/5 males died after a single 6 hour exposure. There were no test article-related effects in food consumption, gross or microscopic

pathology, or organ weights. A decrease in body weight gain was observed in the 80-mg/m^3 rats. Observations in 240-mg/m^3 surviving rats included rales and labored respiration. At the 120-mg/m^3 exposure level, hypoactivity, increased respiration, gasping and/or vocalization upon handling were observed. No signs of toxicity were observed in the 24-mg/m^3 animals.

The LOAEL is 80 mg/m^3 based on reduced body weight gain. The NOAEL is 24 mg/m^3.

This range-finding oral toxicity study in the rat is **ACCEPTABLE-NONGUIDELINE. No guidelines are available for this type of study.**

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: R107894, 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)

Description: Light brown powder

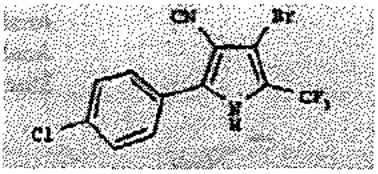
Lot/Batch #: Lot no. 89000015

Purity: 92.4% a.i. as reported by sponsor; however, HPLC analysis performed by the study laboratory indicates a purity of 93.0%

Compound Stability: Considered stable when stored under room temperature conditions; stability analysis was performed for the 90-day inhalation toxicity testing study report

CAS # of TGAI: 122454-29-9

Structure:



2. Vehicle control: Filtered air

3. Test animals:

Species: Rat

Strain: Crl:CD®(SD)

Age/weight at study Initiation: Approximately 7 weeks old for main study rats and approximately 9 weeks old for additional study rats / 217-257 g for males and 153-169 g for females in main study

Source: Charles River, Raleigh NC

Housing: Individually-housed in stainless steel, suspended wire mesh cages until exposure where animals were in nose only stainless steel exposure chambers for 6 hours.

Diet: Certified Rodent LabDiet® 5002 PMI Nutritional International, LLC *ad libitum* (except during exposure and overnight fasting period prior to necropsy)
Analysis for major nutritive constituents and significant contaminants did not indicate any contaminants that may interfere with the outcome of the study as stated in the study report.

Water: Reverse osmosis-treated on-site drinking water was provided *ad libitum* (except during exposure)
Analysis for significant contaminants did not indicate any contaminants that may interfere with the outcome of the study as stated in the study report.

Environmental conditions:
- **Temperature:** 21.5-21.6° C
- **Humidity:** 43.3-54.4%
- **Air changes:** Minimum of 10/hr
- **Photoperiod:** 12 hrs dark/12 hrs light

Acclimation period: 12 days; additional study rats were also acclimated to the exposure restraint for 2 hours on the day prior to exposure

B. STUDY DESIGN:

1. **In life dates**: Start: June 5, 2005 (main study); July 13, 2005 (additional groups)
End: June 17, 2005 (main study); July 14, 2005 (additional groups)

2. **Animal assignment:** Animals were assigned to the test groups noted in Table 1 below using computer-generated random numbers based on weight in a block design. After the main group was exposed, additional groups were exposed to 0 or 120 mg/m^3 consisting of 5 rats/males/dose.

TABLE 1: Study design

Test group	Nominal Conc. (mg/ m^3)	Analytical Conc. (mg/ m^3)	MMAD µm	GSD	Rats/sex	
					Male	Female
Control	0	-	-	-	5	5
Low (LCT)	24	24	2.0	2.30	5	5
Mid (MCT)	80	80	2.4	2.24	5	5
High (HCT)	240	239	2.5	1.94	5	5
Additional Control	0	-	-	-	5	-
Additional Exposure	120	126	2.4	1.91	5	-

3. **Dose selection rationale:** no rationale was presented on the doses selected for this range-finding study.

4. **Generation of the test atmosphere / chamber description:**

Analytical Chemistry utilized was gravimetric determination of the aerosolized particles.

The exposure chamber was an 8.6-L nose only stainless steel exposure system. The test article was micronized then aerosolized to 1-3 µm (MMAD) by a Jet-O-Mizer (Fluid Energy Aljet, Plumsteadville, Pennsylvania) into humidified ambient filtered air. Results are in Table 1 above. Temperature, relative humidity, and oxygen content were monitored throughout exposure. Time to equilibrium was not determined.

Test atmosphere concentration: Exposure concentration was determined by gravimetric methods. Samples were collected from the exposure chamber onto glass filters and weighed. Results are in Table 1 above.

Particle size determination: The aerosolized particle size was determined by a cascade impactor. Results are in Table 1 above.

5. **Statistics:** Intergroup differences were analyzed for body weight, body weight change, food consumption, continuous functional observation battery (FOB), and organ weight data via a

parametric one-way analysis of variance (ANOVA). Intergroup variance with $p<0.05$ were evaluated by Dunnett's test to compare treatment and control groups. Scalar or descriptional measurements in the FOB were analyzed via Fisher's Exact Test.

C. METHODS:

1. Observations:

1a. Cageside Observations: Animals were inspected twice daily for signs of toxicity and mortality.

1b. Clinical Examinations: Clinical examinations were conducted twice daily prior to dose administration and 1 hour after dosing. Groups that were removed from dosing were observed once daily. Detailed physical examinations were performed weekly from one week prior to dosing, and prior to scheduled necropsy.

1c. Neurological Evaluations: The following evaluations (measurements) of a modified functional observation battery were performed on Days 1 and 12 (0-1 hour after exposure): ease of removal from cage, ease of handling, lacrimation, salivation, piloerection, fur, palpebral closure, respiration, red/crusty deposits, mucus membranes/eyes/skin color, eye prominence, muscle tone, mobility, gait, convulsions/tremors, arousal, bizarre/stereotypic behavior, backing, grooming, and urination.

2. Body weight: Animals were weighed weekly beginning 2 weeks prior to exposure. Mean body weight and mean body weight changes were calculated.

3. Food consumption: Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/animal/day. Food efficiency was not calculated.

4. Ophthalmoscopic examination: Eyes were not examined.

5. Hematology & Clinical Chemistry: Blood was not collected.

6. Urinalysis: Urine was not collected.

7. Sacrifice and Pathology: All animals that died and those sacrificed on schedule were subjected to gross pathological examination (external surface; all orifices; cranial, thoracic, abdominal and pelvic cavities; and viscera) and the CHECKED (X) tissues were collected for histological examination. Tissues were fixed in 10% neutral formalin, trimmed, embedded in paraffin, sectioned, and stained with hematoxylin/eosin. All tissues listed were examined in all control and treated animals. The (XX) organs, in addition, were weighed.

	NEUROLOGIC		DIGESTIVE SYSTEM
XX	Brain	XX	Liver
X	Cerebrum level 1		CARDIOVASC./HEMAT.
X	Cerebrum level 2	XX	Spleen
X	Cerebellum with medulla/pons		
X	Spinal Cord (3 levels – cervical, thoracic and lumbar)		

II. RESULTS

A. OBSERVATIONS :

1. Clinical signs of toxicity: The single surviving 240-mg/m^3 male displayed rales and labored respiration after the 4 hour and 25 minutes single exposure during the 2 days after exposure. One of the 3 surviving females displayed yellow material on the hindlimbs, ventral trunk, and urogenital area up to 3 days after exposure; the 2 other surviving females did not display any abnormal signs. Yellow material on the forelimbs hindlimbs, trunk, neck, anogenital, and/or urogenital area also were observed in the 80-mg/m^3 rats for several days after exposure. In the additional 120-mg/m^3 treatment group, clinical signs of toxicity observed in the two survivors included hypoactivity, increased respiration, gasping and/or vocalization upon handling.

2. Mortality: Treatment-related deaths were observed in 4/5 males and 2/5 females exposed to 240 mg/m^3 either during or after the exposure. Exposure duration was reduced to 4 hours and 25 minutes due to the lethality. In the additional group exposed to 120 mg/m^3, 2/5 males died after the single 6 hour exposure.

3. Neurological Evaluations:

Day 1 Observations: Slightly soiled fur was noted in 5/5 males and 3/5 females exposed to 80 mg/m^3, and in the one surviving 240-mg/m^3 male; the increased incidence observed in 80-mg/m^3 males was statistically-significantly higher than controls. The incidence (4/5) of red deposits around the nose observed in 80-mg/m^3 females also was statistically-significantly higher than controls. The 120- mg/m^3 animals were not evaluated for FOB.

Day 12 Observations: Very soiled, crusty fur was observed in one 24-mg/m^3 female, one 80-mg/m^3 male, and three 80-mg/m^3 females. The 120-mg/m^3 animals were not evaluated for FOB.

B. BODY WEIGHT AND WEIGHT GAIN: Body weight gain for Week 0 to 1 was lower in the 80-mg/m^3 females and statistically significantly lower in the 80-mg/m^3 males (Table 2a). Substantial weight loss was observed in the surviving 120-mg/m^3 animals as noted in Table 2b.

Body weights and body weight gains (Table 2a) in the male in the 240 mg/m^3 males showed only 1 animal left and a valid comparison with the controls could not be made. Females at this dose level showed a surprising increase in body weight gain vs. the control. In the additional 1 day

treatment groups (120 mg/m^3), a body weight gain for one (1) day is considered toxicologically meaningless (Table 2b).

TABLE 2a: Mean body weights and body weight gains during 12 days of treatment[a]

Analytical Concentration (mg/m^3)		Body Weights (g)				Total Weight Gain[b]	
		Week -1	Week 1	BWG (Wk 0 to 1)	Week 1	g	% of control
Male							
0	mean±SD	229±8.6	259±16.1	30±8.1	258±18.8	29	-
	N	5	5	5	5		
24	mean±SD	230±7.8	264±6.3	34±2.1	267±5.4	37	+28
	N	5	5	5	5		
80	mean±SD	233±14.7	253±16.9	20±5.8*	250±20.1	17	-41
	N	5	5	5	5		
240	mean±SD	234±13.4	257±0.0	33±0.0	275±0.0	51	+76
	N	5	1	1	1		
Female							
0	mean±SD	158±6.1	178±10.0	20±4.6	174±13.7	16	-
	N	5	5	5	5		
24	mean±SD	160±6.5	177±8.0	17±5.3	175±8.6	15	-7
	N	5	5	5	5		
80	mean±SD	158±6.4	169±10.3	11±5.6	164±13.0	6	-63
	N	5	5	5	5		
240	mean±SD	160±5.6	181±17.8	22±10.8	188±22.2	28	+75
	N	5	3	3	3		

[a] Data obtained from pages 50-53 in the study report.
[b] Calculated by reviewer, Week -1 to 1
* Statistically different ($p < 0.05$) from the control (Dunnett's test).

TABLE 2b: Mean male body weights and body weight gains during 1 day of treatment in the additional groups [a]

Analytical Concentration (mg/m^3)		Body Weights (g)		
		Day 0	Day 1	Body Weight Gain[b] (Day 0-1)
0	mean±SD	340±21.9	338±20.1	-2
	N	5	5	5
120	mean±SD	321±40.0	297±34.7	-34
	N	5	3	5

[a] Data obtained from pages 263-264 in the study report.
[b] Calculated by our reviewer

C. FOOD CONSUMPTION:

1. Food consumption: Food consumption was unaffected by treatment.

2. Food efficiency: Food efficiency was not calculated or necessary for the short duration of this toxicity study.

D. OPHTHALMOSCOPIC EXAMINATION: Not performed.

E. BLOOD ANALYSES: Not performed.

F. URINALYSIS: Not performed.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: A statistically-significant ($p>0.01$) increase in relative liver weight was observed in the 80-mg/m^3 females, as compared to controls. This finding was not considered to be treatment-related because it was not correlated to any macro- or microscopic findings, and the absolute organ weight was not statistically-significantly different from controls. Organ weights were not evaluated in the 120-mg/m^3 animals.

2. Gross pathology: Decedents from the 240-mg/m^3 treatment group exhibited dark red areas and dark red discoloration in the lungs, which were associated with microscopic observations of congestion or hemorrhaging. The 120-mg/m^3 animals did not display any abnormalities at necropsy.

3. Microscopic pathology: Congestion of the liver and/or lungs was noted in the animals that died prior to study termination. Hemorrhagic and/or fibrinous exudates also were observed in one 240-mg/m^3 male. These observations were considered to be acute factors relating to the death of these animals, but were not evidence of test article-related toxicity. In 3/4 males that died prior to sacrifice in the 240-mg/m^3 group, centrilobular vacuolization of the liver was observed and characterized by intracytoplasmic vacuoles filed with eosinophilic, proteinacious fluid. This finding was not considered a toxic effect of R107894, but a result of anoxic conditions caused by treatment [Sykes B, E. Penny, I. Purchase (1976). Hepatocyte vacuolization and increased liver weight occurring in anoxic rats. Toxicology and Applied Pharmacology. 36:31-39]. The 120-mg/m^3 animals did not display any abnormalities at necropsy.

III. DISCUSSION AND CONCLUSIONS: In an inhalation toxicity range-finding study, R107894 (93% a.i.) was administered to 5 Sprague-Dawley [Crl:CD®(SD)] rats/sex/dose via nose only exposure at dose levels of 0, 24, 80 (6 hours a day for 12 days) , or 240 mg/m^3 for (4 hours and 25 minutes due to high mortality). Exposure duration in the 240-mg/m^3 rats was limited to the surviving animals (1/5 males and 2/5 females) were held in the animal room and observed for the study duration of 12 days and were not exposed to the test article again. Additional groups of 5 male rats were exposed to dose levels of 0 or 120 mg/m^3 for 6 hours via nose only exposure. Due to the high mortality (3/5 males) during exposure, all surviving animals were euthanized 1 day after exposure. The study author concluded that R107894 was lethal at 120 and 240 mg/m^3 if inhaled by nose only methods in rats. The 80-mg/m^3 animals displayed a yellow material on their fur, red nose deposits (females), and depression in weight gain in the first week of exposure. No other signs of toxicity were noted in the 80-mg/m^3 animals. No signs of toxicity were observed in the 24-mg/m^3 animals, therefore, this level of exposure was designated as the no-observable-effect-level for rats exposed to R107894 for 12 days by nose only methods. Based on these results, the recommended doses for a 90-day toxicity/neurotoxicity study are 0, 20, 40, and 80 mg/m^3.

Body weights and body weight gains (Table 2a) in the male in the 240 mg/m^3 males showed only 1 animal left and a valid comparison with the controls could not be made. Females at this dose level showed a surprising increase in body weight gain vs. the control. In the additional 1 day treatment groups (120 mg/m^3), a body weight gain for one (1) day is considered toxicologically meaningless (Table 2b).

In the 1.3 Result section of the original report, "5/5 females died during or after a single 4 hour and 25 minute exposure."; the results show that only 2/5 females died. This typographical error do not affect the conclusion of the study.

The LOAEL is 80 mg/m^3 based on depression in weight gain in the first week of exposure. The NOAEL is 24 mg/m^3.

B. STUDY DEFICIENCIES: Due to the fact that this study serves as a range finding study, the study deficiencies were considered minor. Study deficiencies noted included those reported as

protocol deviations by the study author (p. 36 of the original report) and the typographical error noted in the study report.

C. STUDY CLASSIFICATION: This inhalation range-finding oral toxicity study in the rat is **ACCEPTABLE-NONGUIDELINE** because there were no guidelines for this type of study.

EPA Reviewer: Steven L. Malish, Ph.D. , Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Sr. Scientist
Team 2, RASSB/Antimicrobial Division

S. L. Malish 6/15/06
Jonathan Chen 06/19/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Inhalation Toxicity [OPPTS 870.3465] and Neurotoxicity Screening Battery [OPPTS 870.6200] – Rats.

This DER is for the Neurotoxicity Screening Battery, Phase II

PC CODE: 119093 **DP BARCODE:** 328778
DECISION#: 220066 **EPA REG. NO.:** 43813-ET

TEST MATERIAL (PURITY): 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), (92.4% a.i.)

SYNONYMS: Econea®028, CL 303'268, R107894

CITATION: Beck, M. (2006). A 90-day combined nose-only inhalation toxicity and neurotoxicity study of R107894 in rats. WIL Research Laboratories, Ashland, Ohio. WIL-436004. March 31, 2006. MRID46802202. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340, Beerse, Belgium

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 468022-02), groups of Crl:CD(SD) 12 Sprague-Dawley derived rats/sex/dose (**Phase II**) were exposed to R107894 (92.4% a.i. of Lot no. ZR107894EXA028) via nose only exposure at targeted dose levels of 0, 20, 40, or 80 mg/m^3 for 6 hours a day for a minimum of 90 consecutive days (~13 weeks). The particle size was within the required range.

Another phase of the study evaluating the subchronic inhalation toxicity of R107894 was performed on an additional 10 animals/sex/dose (**Phase I**) and discussed in the accompanying subchronic inhalation toxicity DER for R107894.

Neurobehavioral assessment (functional observational battery [FOB] and motor activity testing) was performed on all Phase II animals (12 rats/sex /group) prior to dose administration (pretest), and at Weeks 3, 7, and 12. Cholinesterase activity was not determined. At study termination, 6 animals/sex/dose in the controls and 80-mg/m^3 groups were perfused in situ for neuropathological examination of the central and peripheral nervous system tissues

For the Phase II neurotoxicity study, after the first day of exposure, 1 male and 1 female were found dead in the 80-mg/m^3 group and were replaced. Two 80-mg/m^3 males died prior to study termination, one on Day 20 and the other on Day 85.

During FOB handling measurement, the 40- and 80-mg/m^3 animals were observed to have soiled fur, unclean fur, and increased grooming (80-mg/m^3 males) during Weeks 7 and 12. These

changes correlate with the clinical toxicity findings of soiled fur and were not considered adverse neurotoxicological findings. During Week 3 FOB measurements, an overall decrease in total locomotor activity was noted in the 40- and 80-mg/m^3 males and in ambulatory activities for 80-mg/m^3 males as a result of reduced activity in sub-session measurements. During Week 12, the 80-mg/m^3 males were observed with total activity decrease compared to controls.

At neuropathology, an increase in minimal axonal degeneration was observed in the peroneal nerve in the 80-mg/m^3 animals (3/6 for both males and females) over controls (0/0 for both males and females).

The neurotoxicity LOAEL was 40 mg/m^3 in males based on decreased motor activity and 80 mg/m^3 in females based on axonal degeneration observed in the peroneal nerve. The neurotoxicity NOAEL was 20 mg/m^3 for males and 40 mg/m^3 for females.

This Neurotoxicity Screening Battery is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

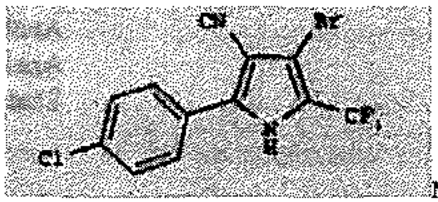
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS: (Some of these elements are also contained in Phase I, Subchronic Inhalation Toxicity Study.

A. MATERIALS:

1. Test material: Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl)

Description:	Light brown powder
Lot/batch #:	Lot no. ZR107894EXA028
Purity:	92.4% a.i.
CAS # of TGAI:	122454-29-9
Compound stability:	HPLC measurements indicated the test article was 93.4% pure compared to 93.0% measured at the initiation of the range-finding study (MRID46751302); the test compound was considered stable during the entire study.
Structure:	$C_{12}H_5BrClF_3N_2$



MRID 46442201

2. Vehicle and/or positive control: The vehicle control was filtered air.

3. Test animals:

Species:	Rat
Strain:	Crl:CD(SD)
Age/weight at study initiation:	~8 weeks old/males weighed between 222 to 286 g; females weighted between 150 to 213 g
Source:	Charles River Laboratories, Inc. (Raleigh, North Carolina)
Housing:	Individually housed in clean, stainless steel, wire-mesh cages suspended above cage-board
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® provided *ad libitum* (except during exposure and during the period of fasting prior to blood collection). The diet was analyzed for chemical composition and possible contaminants by the supplier; results were not provided in the study report.
Water:	Reverse osmosis-treated (on-site) drinking water provided *ad libitum*; delivered by an automatic watering system. Analysis for contaminants did not indicate any contaminants that may interfere with the interpretation of the results.
Environmental conditions:	**Temperature:** 22±3EC **Humidity:** 50±20% **Air changes:** ≥10/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	14 days (general study conditions) 5 days to the nose only restraint tubes with each day having an hour longer of exposure to the system.

B. STUDY DESIGN:

1. In life dates: Start: August 2, 2005; End: November 1 and 4, 2005

2. **Animal assignment:** Animals were assigned to groups in a computerized randomization procedure according to body weight. Each group consisted of 10 rats/sex/dose in Phase I and 12 rats/sex/dose in Phase II. Animals assigned to Phase II were then randomized into 4 study replicates for the functional observational battery and locomotor activity assessments. Rats were exposed in 18.1 L stainless steel conventional nose-only exposure systems to micronized aerosolized particles. The exposure period was 6 hours per day, 7 days per week for a minimum of 90 consecutive days. Food and water were withheld only during exposure periods and during a period of fasting prior to blood collection. The control groups were exposed to clean filtered air. Animals were observed daily and body weights and food intake frequency were recorded weekly for 90 to 94 days. In Phase II, functional observational battery and locomotor activity assessments were performed on all animals/sex/group prior to dosing and during study Weeks 3, 7, and 12. Table 1 presents the number of animals assigned to each study group.

TABLE 1: Study design*

Experimental parameter	Nominal dose group (mg/m^3)			
	Control	20	40	80
Total number of animals/sex/group	12/sex	12/sex	12/sex	12/sex
Behavioral testing (FOB, motor activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	6/sex	6/sex	6/sex	6/sex

3. **Dose selection rationale:** The dose levels were selected based on the results from MRID 46751302 where 5 Sprague-Dawley rats/sex/dose were exposed via nose only exposure at dose levels of 0, 24, 80, or 240 mg/m^3 for 6 hours a day for 12 days; another group of 5 Sprague-Dawley rats/sex/dose was exposed to 120 mg/m^3 (for 6 hours for one day. Mortality was noted in ≥120 mg/m^3 rats while clinical signs of toxicity were observed in ≥80-mg/m^3 rats. No signs of toxicity were observed in the 24-mg/ m^3 group.

4. **Generation of the test atmosphere / chamber description:**

Time to equilibrium was not reported.
Analytical chemistry was determined by standard gravimetric methods.

Test atmosphere concentration: Actual exposure concentrations (as total test compound formulation) of the atmosphere were determined by standard gravimetric methods. Samples were collected on glass fiber filters and held in open-faced filter holders inside nose-only chambers. Results are summarized in Table 2.

Particle size determination: Aerosol particle size determinations were performed using a cascade impactor. Mean particle sizes as mass median aerodynamic diameter (MMAD) ± geometric standard deviation (GSD) were determined during the study. Results are summarized in Table 2.

Table 2: Test article analytical data*

Test group	Nominal conc. (mg/m^3)	Analytical conc. (mg/m^3)	MMAD (Φm)	GSD
Control	0	0	0	0
Low (LCT)	20	20	2.1	0.22
Mid (MCT)	40	40	2.3	0.26
High (HCT)	80	80	2.2	0.25

*Exposure concentrations were not adjusted to the active ingredient

5. **Statistics:** All statistical analyses were performed with two-tailed tests for minimum significance levels of 1% and 5%, comparing results to the control group by sex, except as otherwise noted in the Table 3 below. Mean values were presented with the number of animals (n) used to calculate the mean and the standard deviation (SD).

TABLE 4: Statistics

Parameter	Notes
Body weight	Intergroup differences were analyzed with 1-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare test groups to the control group
	Body weight was analyzed separately for each dose group (control group males and 20 mg/m^3 group males) with repeated measures analysis of variance (RANOVA) Factors in RANOVA model included: PHASE (1= tox animals, 2 = neurotox animals); WEEK of study (weeks 1-13); and interaction of PHASE and WEEK (PHASE*WEEK)
	Random effect of animal included as the repeated measurement were analyzed with SAS® procedure PROC MIXED
	Covariance structure across time was selected by evaluating Akaike's Information Criterion (AIC) If PHASE*WEEK was not significant ($p > 0.05$), the 2 phases were compared through the PHASE main effect If PHASE*WEEK was significant at $p < 0.05$, the 2 phases were compared at each week through PHASE*WEEK and across weeks with the PHASE main effect
	Pretreatment (Week 0) data analyzed with ANOVA to compare the 2 phases prior to treatment
Body weight change	Intergroup differences were analyzed with 1-way ANOVA; if significant, Dunnett's test was used to compare test groups to the control
Food consumption	
Clinical pathology	
Organ weight	
Functional observational battery (FOB)	Intergroup differences were analyzed with 1-way ANOVA; if significant, Dunnett's test was used to compare test groups to the control Parameters that yielded scalar or descriptive data were analyzed with Fisher's Exact Test

TABLE 4: Statistics	
Parameter	**Notes**
Locomotor activity	Analysis endpoints were analyzed by sex and session with RANOVA Factors in RANOVA model included: treatment group (TRT); time interval (TIME); interaction of time interval and treatment group (TRT*TIME)
	Random effect of animal included as the repeated measurement were analyzed with the SAS® procedure PROC MIXED
	Covariance structure across time was selected by comparing AIC for first-order autoregressive homogeneous (AR[1]) and compound symmetric (CS) structures
	Monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dose levels
	Linear dose by time interaction (LinTrt*Time) was evaluated; if significant ($p < 0.05$), trend tests on treatment means were performed at 0.05 for each time interval; if not significant, trend tests were performed across the combined time intervals of the entire session
	Nonmonometric dose-responses were evaluated when no significant linear trends were detected but TRT and/or TRT*TIME interaction was significant ($p < 0.01$)
	Linear Contrasts (within RANOVA) were used for pairwise comparisons for each individual treated group with the control group ($p = 0.01$) If TRT*TIME was significant, comparisons were conducted for each time interval If only TRT was significant, the comparisons were conducted across the combined time intervals of the entire session

These statistical methods for data analysis were considered acceptable.

C. METHODS / OBSERVATIONS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily, once in the morning and once in the afternoon, for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted twice daily, prior to exposure and up to 1 hour following exposure. A detailed clinical observation was performed weekly.

2. Cholinesterase determination: Cholinesterase determination was not performed.

3. **Neurobehavioral assessment:**

a. **Functional Observational Battery (FOB):** The same technicians were used throughout testing. Whenever possible, the technicians were blind to the treatment status of the animals. FOB testing was conducted in a sound-attenuate room with a white-noise generator set to 70 ± 10 dB. Testing was done prior to initiation exposure and prior to daily exposure during Weeks 3, 7, and 12. Scoring criteria were provided for all measured parameters. The CHECKED (X) parameters were examined.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD OBSERVATIONS
X	Posture*		Reactivity*	X	Mobility
X	Biting	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
	Tremors*	X	Piloerection*	X	Convulsions*
	Abnormal Movements*	X	Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*		Abnormal movements*
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
		X	Red / crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*	X	Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*	X	Ease of removal from cage	X	Backing
X	Pain response (tail pinch) *	X	Ease of handling animal in hand	X	Time to first step (seconds)
X	Pupil response*		PHYSIOLOGICAL OBSERVATIONS		
X	Eyeblink response	X	Body weight*		NEUROMUSCULAR OBSERVATIONS
X	Forelimb extension	X	Body temperature+	X	Hindlimb extensor strength
X	Hindlimb extension	X	Catalepsy	X	Forelimb grip strength*
X	Air righting reflex+		OTHER OBSERVATIONS	X	Hindlimb grip strength*
X	Olfactory orientation			X	Landing (hindlimb) foot splay*
				X	Rotarod performance

*Required parameters; +recommended parameters

b. **Locomotor activity:** Locomotor activity was tested in all animals after FOB completion and was evaluated prior to initiation of exposure and during study Weeks 3, 7, and 12. Treatment group testing was performed by replicate sequence. All animals were tested individually. Locomotor activity was measured using the SDI Photobeam Activity System, which measures animal motor activity in a clear plastic cage using a series of infrared photobeams. To decrease distractions, the cages were surrounded by four-sided black plastic enclosures that were positioned so as to not interfere with the photobeams.

Testing was performed in a sound-attenuated room with a white noise generator set at 70±10 dB. Data were collected during 60 minute sessions at 5-minute intervals. For analysis, the data were compiled as four 15-minute subsessions measuring total motor activity and ambulatory activity. Total motor activity was defined as a combination of fine motor skills including grooming and interruption of one photobeam; ambulatory motor activity was defined as interruption of two or more consecutive photobeams.

4. **Sacrifice and pathology:** All animals that died and those sacrificed following completion of the dosing schedule were subjected to gross pathological examination. Animals were sacrificed with an injection of sodium pentobarbital and were perfused in situ with a buffered 4.0% paraformaldehyde/1.4% glutaraldehyde solution. The central and peripheral nervous systems were dissected from 6 rats/sex from the control and 80 mg/m^3 groups and preserved. The central nervous system was embedded in paraffin, sectioned, and stained with hematoxylin and eosin; the peripheral nervous system was embedded in plastic, sectioned, and stained. The whole brain (minus olfactory bulbs) was weighed and measured. The CHECKED (X) tissues were evaluated.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
	Forebrain	X	Mid-thigh
	Center of cerebrum	X	Sciatic notch
X	Midbrain (tectum, tegmentum, and cerebral peduncles)		
X	Cerebellum		**OTHER**
X	Pons	X	Sural nerves
X	Medulla oblongata	X	Tibial nerves
X	Olfactory bulbs	X	Peroneal nerves
X	Cerebral cortex	X	Lumbar dorsal root ganglia
X	Hippocampus	X	Lumbar dorsal root fibers
X	Basal ganglia	X	Lumbar ventral root fibers
X	Thalamus	X	Cervical dorsal root ganglia
X	Hypothalamus	X	Cervical dorsal root fibers
X	Central gray matter	X	Cervical ventral root fibers
	SPINAL CORD		
X	Cervical swelling		
X	Lumbar swelling		
	Thoracic swelling		
	OTHER		
	Gasserian ganglion		
X	Trigeminal ganglia / nerves		
X	Optic nerves		
X	Eyes		
X	Gastrocnemius (skeletal) muscle		

5. **Positive controls:** Positive controls were not used during this study. However, historical data were provided in Appendix F (pp. 1299-1354) that demonstrate the adequate sensitivity of the locomotor activity and FOB testing on approximately 6 week old Sprague-Dawley rats (Crl:CD®(SD)IGS BR). The WIL-99140 study, performed on April 12-13, 1999 (pp.1300-1306), tested the locomotor activity monitoring sessions on untreated rats. The WIL-99149 study, performed on May 17-21, 1999 (pp.1307-1329), tested Sprague-Dawley rats treated with physiological saline, d-amphetamine sulfate (2 or 4 mg/kg), or chlorpromazine hydrochloride (5 or 10 mg/kg). Animals were kept under standard housing conditions and were administered a single intraperitoneal injection of the appropriate test article. Locomotor activity was evaluated 4-5 days prior to dosing and on the day of administration. The animals dosed with d-amphetamine sulfate demonstrated a dose-dependent increase in locomotor activity while those dosed with chlorpromazine hydrochloride demonstrated a dose-dependent decrease in locomotor activity. The SDI-PAS locomotor activity monitoring system, therefore, was demonstrated to be sufficiently sensitive to detect a dose-related increase or decrease in locomotor activity in Sprague-Dawley rats. The WIL-99263 study, performed from December 14, 2001-October 9, 2002 (pp. 1321-1334), evaluated inter-observer reliability on FOB observations. Sprague-Dawley rats received a single oral dose of deionized water; 3, 3'-iminodipropionitrile (2000 mg/kg); or parathion (4 mg/kg). Phase I of the study consisted of untreated animals (60 rats per technician) observed for comparison of naïve stereotypic behavior; Phase II consisted of 2 rats/dose that were observed and the effects discussed; Phase III consisted of 3 rats/dose that were observed by all technicians to evaluate inter-observer reliability. After all three phases, it was concluded that the technicians fulfilled the criteria of reliable observations for FOB assessments. In WIL-99310 (pp. 1335-1354), dates of study not provided, the inter-reliability of the FOB observers were evaluated for Sprague-Dawley rats exposed to a single Mazola® corn oil; 3, 3'-iminodipropionitrile (2000 mg/kg); or parathion (4 mg/kg) via gavage or a single intraperitoneal injection of 2 mg/kg of d-amphetamine sulfate. Phase I consisted of untreated animals (60 animals per technician) observed for comparison of naïve stereotypic behavior; Phase II consisted of 2 rats/dose that were observed and the effects discussed; and Phase III consisted of 3 rats/dose that were observed by all technicians to evaluate inter-observer reliability. Observer A was found to not meet criteria of a reliable FOB observer and Observer E was found to need additional training on papillary response.

II. RESULTS:

A. OBSERVATIONS:

Mortality: After the first day of exposure, 1 male and 1 female were found dead in the 80 mg/m^3 group, but these animals were immediately replaced. The replacement animals were not fully acclimated to the nose only restraint system but they did not display signs of undue stress. Two 80 mg/m^3 males died prior to study termination, one on Day 20 and the other on Day 89. Pathology of these rats was unremarkable as to the cause of death: dark red adrenals and mediastinal lymph node was noted in one

male and the other had a prolapsed penis. These deaths were considered treatment-related due to their large number at the high dose.

B. **CHOLINESTERASE ACTIVITIES:** Cholinesterase activity was not determined and was not of concern with this test compound.

C. **NEUROBEHAVIORAL RESULTS:** Occasional variations in both the qualitative and quantitative FOB data of home cage (Table 5), sensory (Table 8), and neuromuscular (Table 9) observation were recorded, but these changes did not appear to be treatment-related due to the lack of a dose-response, and because occurrence was seen in both control and treated animals with similar frequency and/or inconsistency across test intervals.

Handling observations of soiled fur (Table 6), open field observation of grooming (Table 7), and physiological observations of body weight (Table 10) were treatment-related and correlated with other observations made during the course of the study.

1. **FOB findings:** The significant home cage observations are summarized in Table 5. However, none of the statistically significant observations are treatment-related because the findings (alert and oriented toward observer, sleeping (lying on side or curled up), sitting (standing) normally, eyelids open, or eyelids closed) do not correlate with any other findings, and occurred sporadically over the test intervals.

TABLE 5: Functional observation battery results—home cage observations[a]

Observation	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
Sitting or standing normally				
Pretest	9/12	8/12	9/12	7/11
Week 3	4/12	4/12	6/12	3/11
Week 7	0/12	2/12	1/12	2/11
Week 12	4/12	2/12	4/12	3/11
Asleep, lying on side, or curled up				
Pretest	3/12	4/12	3/12	4/11
Week 3	2/12	7/12	6/12	6/11
Week 7	2/12	4/12	2/12	7/11*
Week 12	0/12	4/12	1/12	5/11*
Alert, oriented toward observer				
Pretest	-	-	-	-
Week 3	6/12	1/12	0/12*	2/11
Week 7	10/12	6/12	9/12	2/11*
Week 12	7/12	6/12	6/12	2/11
Eyelids wide open				
Pretest	9/12	8/12	9/12	7/11
Week 3	10/12	5/12	6/12	3/11*
Week 7	9/12	7/12	9/12	4/11
Week 12	12/12	7/12*	11/12	3/11*
Eyelids completely shut				
Pretest	3/12	4/12	3/12	4/11
Week 3	1/12	4/12	3/12	3/11

TABLE 5: Functional observation battery results—home cage observations[a]

Observation	Nominal dose level (mg/m³)			
	Control	20	40	80
Week 7	1/12	3/12	2/12	7/11*
Week 12	0/12	0/12	0/12	3/11
Females				
Sitting or standing normally				
Pretest	8/12	5/12	7/12	10/11
Week 3	7/12	1/12*	3/12	5/12
Week 7	2/12	1/12	2/12	1/12
Week 12	2/12	0/12	1/12	5/12
Asleep, lying on side, or curled up				
Pretest	4/12	7/12	4/12	1/11
Week 3	1/12	0/12	1/12	3/12
Week 7	0/12	0/12	0/12	2/12
Week 12	-	-	-	-
Alert, oriented toward observer				
Pretest	0/12	0/12	1/12	0/11
Week 3	4/12	11/12*	8/12	4/12
Week 7	10/12	11/12	10/12	9/12
Week 12	10/12	12/12	10/12	6/12
Eyelids wide open				
Pretest	8/12	5/12	8/12	10/11
Week 3	11/12	12/12	10/12	7/12
Day 7	12/12	12/12	12/12	10/12
Week 12	12/12	12/12	12/12	12/12
Eyelids completely shut				
Pretest	4/12	7/12	4/12	1/11
Week 3	1/12	0/12	0/12	2/12
Week 7	0/12	0/12	0/12	1/12
Week 12	-	-	-	-

[a] Data were extracted from pp. 154-161 of the study report
Values represent incidence
* Statistically different ($p < 0.05$) from the control group, Fisher's exact test.

The high-and mid-dose females were noted with treatment-related increased incidence of slightly soiled fur at Week 12, and a decrease in clean and groomed fur compared to controls. Results are presented in Table 6.

TABLE 6: Functional observation battery results—handling observations[a]

Observation	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
Clean and groomed fur				
Pretest	12/12	12/12	12/12	11/11
Week 3	12/12	12/12	12/12	10/11
Week 7	12/12	11/12	9/12	5/11*
Week 12	12/12	12/12	8/12	8/11
Slightly soiled fur				
Pretest	-	-	-	-
Week 3	0/12	0/12	0/12	1/11
Week 7	0/12	1/12	3/12	5/11*
Week 12	0/12	0/12	4/12	2/11
Very soiled fur				
Pretest	-	-	-	-
Week 3	-	-	-	-
Week 7	0/12	0/12	0/12	1/11
Week 12	0/12	0/12	0/12	1/11
Females				
Clean and groomed fur				
Pretest	12/12	12/12	12/12	11/11
Week 3	12/12	12/12	8/12	8/12
Week 7	7/12	6/12	2/12	1/12*
Week 12	12/12	11/12	4/12*	3/12*
Slightly soiled fur				
Pretest	-	-	-	-
Week 3	0/12	0/12	4/12	4/12
Week 7	5/12	6/12	7/12	7/12
Week 12	0/12	1/12	8/12*	8/12*
Very soiled fur				
Pretest	-	-	-	-
Week 3	-	-	-	-
Week 7	0/12	0/12	3/12	4/12
Week 12	0/12	0/12	0/12	1/12

[a] Data were extracted from pp. 162-185
Values represent incidence
* Statistically different ($p < 0.05$) from the control group, Fisher's exact test.

Rearing and mean urine pool counts (urination) were incidental findings that reached statistical significance compared to controls during one or more of the test intervals in the open field observations. Mean urine pools in high-dose males displayed a decreasing trend but the values were similar to pre-treatment; thus, this finding is not considered treatment-related.

Grooming increased in the high-dose males compared to controls and pretreatment; however, only the Week 3 value reached statistical significance. Because the increase in grooming may be due to the increased amount of crusty brown material on the fur, it was considered treatment-related. Table 7 summarizes the select findings of the open field test.

TABLE 7: Functional observation battery results—open field[a]

Observation	Dose level (mg/m³)			
	Control	20	40	80
Males				
Rearing (mean±SD)				
Pretest	6.9±2.57	9.8±2.52	9.4±3.00	8.2±3.66
Week 3	6.7±4.08	7.4±3.87	8.3±3.73	8.1±2.95
Week 7	7.8±3.21	7.7±3.85	9.5±3.12	8.6±3.07
Week 12	6.7±4.12	6.6±3.32	7.3±3.45	9.7±6.53
Grooming (mean±SD)				
Pretest	1.1±1.00	1.1±1.38	1.3±1.29	0.4±0.92
Week 3	0.2±0.39	0.3±0.65	0.7±0.89	1.4±1.63*
Week 7	0.5±1.17	0.4±0.67	0.6±0.79	1.4±1.50
Week 12	0.3±1.15	0.4±0.90	0.3±0.49	0.9±0.70
Urine pools (mean±SD)				
Pretest	0.5±0.80	0.4±0.67	0.8±1.11	0.5±0.69
Week 3	1.8±1.06	0.3±0.87**	0.7±0.98*	0.5±0.69**
Week 7	1.8±1.11	0.9±1.08	1.1±1.31	0.4±0.50**
Week 12	1.8±1.14	0.9±0.67	1.5±0.80	0.6±0.81*
Females				
Rearing (mean±SD)				
Pretest	9.3±2.38	9.9±2.91	10.5±2.07	11.1±2.98
Week 3	10.3±3.74	14.4±2.15*	12.6±5.42	11.3±2.39
Week 7	13.3±2.83	14.3±1.78	13.7±2.71	12.0±3.28
Week 12	12.7±3.50	13.3±4.47	12.3±4.79	11.6±5.20
Grooming (mean±SD)				
Pretest	1.0±0.85	0.1±0.29	1.0±1.35	0.6±0.92
Week 3	0.9±1.00	0.5±0.80	1.3±1.14	1.0±1.35
Week 7	0.7±1.23	0.3±0.65	0.6±0.79	0.8±0.75
Week 12	0.4±0.90	0.6±0.51	0.3±0.49	0.9±1.24
Urine pools (mean±SD)				
Pretest	0.5±0.80	0.3±0.62	0.3±0.62	0.1±0.30
Week 3	0.3±0.62	0.1±0.29	0.2±0.58	0.1±0.29
Week 7	0.0±0.00	0.3±0.62	0.3±0.45	0.2±0.39
Week 12	0.4±0.67	0.4±0.90	0.5±0.67	0.6±1.08

[a] Data were extracted from pp. 186-201
Values represent mean number of incidence
* Statistically different ($p < 0.05$) from the control group, Dunnett's test.
** Statistically different ($p < 0.01$) from the control group, Dunnett's test.

The high-dose males displayed a statistically significant increase in the incidence of no reaction to touch at Week 12; however, the finding did not correlate to any other findings and was considered incidental (Table 8).

TABLE 8: Functional observation battery results—sensory[a]

Observation	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
No reaction to touch				
Pretest	2/12	0/12	0/12	1/11
Week 3	3/12	5/12	3/12	1/11
Week 7	0/12	1/12	1/12	0/12
Week 12	0/12	3/12	0/12	4/12*
Females				
No reaction to touch				
Pretest	0/12	1/12	0/12	0/11
Week 3	1/12	1/12	0/12	2/12
Week 7	-	-	-	-
Week 12	2/12	0/12	2/12	0/12

[a] Data were extracted from pp. 202-217
Values represent incidence
* Statistically different (p <0.05) from the control group, Fisher's exact test.

Table 9 summarizes forelimb strength of the neuromuscular measurement of the FOB. An isolated decrease in forelimb strength was observed in the high-dose females at Week 3; however, due to the isolated nature of this response and lack of a trend at other time points, this finding was not considered related to treatment.

TABLE 9: Functional observation battery results—neuromuscular[a]

Observation	Dose level (mg/m³)			
	Control	20	40	80
Males				
Forelimb strength (mean g±SD)				
Pretest	698.0±84.71	701.3±75.18	668.8±116.56	744.0±63.77
Week 3	759.8±124.95	684.8±141.38	628.6±119.74	731.7±152.38
Week 7	501.3±89.67	499.3±110.24	480.6±110.41	556.1±97.92
Week 12	1101.2±348.77	1040.3±387.28	1022.9±325.67	1033.5±453.66
Females				
Forelimb strength (mean g±SD)				
Pretest	734.7±99.87	687.6±68.24	735.4±135.80	673.5±124.65
Week 3	705.7±150.29	611.2±125.87	687.5±153.32	557.0±135.40*
Week 7	467.3±119.81	439.8±65.23	460.5±144.04	441.1±142.91
Week 12	931.3±425.72	899.3±299.98	1086.3±296.36	986.2±319.30

[a] Data were extracted from pp. 218-225; n=12 except 80 mg/m³ males where n=11 at each measurement and 80 mg/m³ females where n=11 during pretest
+ Statistically different (p <0.05) from the control group, Dunnett's test.

Table 10 summarizes the body weights of the FOB tests' physiological results. As discussed previously (see Table 5b), all treated males displayed statistically decreased body weight after treatment compared to controls, as did the high-dose females (statistically significant at Weeks 7 and 12). Mid-dose females had significantly decreased body weight as compared to controls at Week 12; this trend, although not reaching statistical significance, was also observed at Weeks 3 and 7.

TABLE 10: Functional observation battery results—physiological[a]

Observation	Dose level (mg/m³)			
	Control	20	40	80
Males				
Body weight (mean g±SD)				
Pretest	205.8±12.24	204.7±15.72	204.7±15.31	208.4±15.68
Week 3	348.2±26.51	314.5±25.82**	315.3±20.71**	295.2±31.97**
Week 7	431.6±40.45	375.8±37.81**	366.0±19.96**	350.6±37.91**
Week 12	486.9±50.38	425.4±46.74**	412.8±21.73**	397.0±46.92**
Females				
Body weight (mean g±SD)				
Pretest	167.4±7.64	167.4±12.82	165.6±13.53	167.0±9.19
Week 3	223.4±15.67	212.9±15.78	212.4±14.42	205.4±15.73
Week 7	253.5±15.45	238.3±16.27	236.8±16.25	224.5±22.62**
Week 12	270.7±19.29	256.4±18.20	247.8±18.71*	238.0±24.40**

[a] Data were extracted from pp. 226-233; n=12 except 80 mg/m³ males where n=11 at each measurement and 80 mg/m³ females where n=11 during pretest
+ Statistically different (p <0.05) from the control group, Dunnett's test.
++ Statistically different (p <0.01) from the control group, Dunnett's test.

2. **Motor activity:** Treatment-related differences were observed in total motor activity (mid- and high-dose males) and ambulatory counts (high-dose males) during Week 3, as summarized in Tables 11a and 11b. Activity levels were unaffected during Week 7 but a significant ($p \leq 0.001$) decrease in total activity during the 60-minute session was noted in the high-dose males.

   Statistically-significant linear by interval interaction ($p \leq 0.001$) was obtained from the ambulatory counts in the first 15 minutes during Week 3 for high-dose females; statistically-significant linear by interval interaction ($p \leq 0.001$) was observed for total and ambulatory counts for low-dose females during the second 15-minute interval during Week 7. These two observations were isolated and random findings that were significantly different with no apparent dose-response; thus, they are not considered treatment-related. No evidence of habituation was observed.

TABLE 11a: Motor activity (mean overall total counts for session)[a]				
Test day	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
Pretest	311.23	272.50	305.63	238.41#
Week 3	448.00	392.56	363.60*	298.11**@
Week 7	432.71	385.19	373.77	354.09
Week 12	448.13	357.67	359.54	301.80**
Females				
Pretest	235.42	270.31	282.92	227.32
Week 3	369.31	381.98	379.85	323.00
Week 7	325.71	386.69	364.71	293.38%
Week 12	362.52	399.75	426.00	309.40

[a] Data were extracted from pp. 234; n=12 except 80 mg/m³ males where n=11 at each measurement and 80 mg/m³ females where n=11 during pretest

@ Statistically different (p <0.01), F-test for treatment

% Statistically different (p <0.01) RANOVA interaction between treatment*time

Statistically different (p <0.05) RANOVA linear dose trend by time interaction

* Statistically different (p <0.05) from the control group, linear trend.

** Statistically different (p <0.01) from the control group, linear trend.

TABLE 11b: Motor activity (mean overall ambulatory counts for session)[a]				
Test day	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
Pretest	108.27	92.40	105.75	78.182#
Week 3	152.94	132.29	125.42	98.614**
Week 7	137.38	130.90	124.54	118.82
Week 12	131.96	104.29	108.17	91.14
Females				
Pretest	85.40	96.19	100.33	78.40
Week 3	139.33	140.92	149.46	116.35#
Week 7	119.33	143.98	140.88	106.79%
Week 12	130.48	143.02	158.02	110.56

[a] Data were extracted from pp. 234; n=12 except 80 mg/m³ males where n=11 at each measurement and 80 mg/m³ females where n=11 during pretest

@ Statistically different (p <0.01), F-test for treatment

% Statistically different (p <0.01) RANOVA interaction between treatment*time

Statistically different (p <0.05) RANOVA linear dose trend by time interaction

* Statistically different (p <0.05) from the control group, linear trend.

** Statistically different (p <0.01) from the control group, linear trend

F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** There were no gross pathology results that were considered to be treatment-related in the animals surviving until the end of treatment. Both high-dose males that died prior to study termination displayed dark red adrenals; one male also

had dark red mediastinal lymph nodes while the other had a prolapsed penis. Brain length and width were comparable between the treatment and control groups.

2. **Brain weight:** Table 12 summarizes brain weight data. All of the treated males displayed a dose-related increase in relative brain weights that was considered treatment-related. Although female rats displayed a (non-significant) trend of increasing relative brain weight, their relative brain weights did increase in a dose-related manner.

TABLE 12: Absolute and relative brain weights (mean±SD)[a]

Parameter	Nominal dose level (mg/m³)			
	Control	20	40	80
Males				
Body wt. (g)	498±51.5	440±45.7**	426±23.3**	401±50.9**
n	12	12	12	9
% of control	100	88	86	81
Brain wt. (g)	2.08±0.129	2.04±0.095	2.00±0.113	1.97±0.146
n	12	12	12	10
% of control	100	98	96	95
Brain/body wt. (g/100 g)	0.421±0.0341	0.466±0.0368*	0.471±0.0421*	0.492±0.0494**
n	12	12	12	9
% of control	100	111	112	117
Females				
Body wt. (g)	277±20.5	263±18.7	256±19.7*	242±23.1**
n	12	12	12	11
% of control	100	95	92	87
Brain wt. (g)	1.97±0.091	1.95±0.130	1.92±0.81	1.85±0.075*
n	12	12	12	12
% of control	100	99	97	94
Brain/body wt. (g/100 g)	0.713±0.0494	0.743±0.0421	0.754±0.0622	0.770±0.0677
n	12	12	12	11
% of control	100	104	106	108

[a] Data were extracted from pp. 347-350; Only Phase II animals were used.
* Statistically different ($p < 0.05$) from the control group, Dunnett's test.
** Statistically different ($p < 0.01$) from the control group, Dunnett's test.

3. **Neuropathology:** Brain sections were generally unremarkable; however, minimal axonal degeneration was observed in spinal nerve roots and peripheral nerves the data are summarized in Table 13. All incidences of minimal axonal degeneration in high-dose animals were comparable to controls except for the peroneal nerve with incidences of 3/6 for both the high-dose males and females.

TABLE 13. Incidence of neuropathological findings[a]

Lesion	Treatment group			
	Male		Female	
	Control	80 mg/m^3	Control	80 mg/m^3
Sciatic Nerve axonal degeneration	3/6	2/6	3/6	2/6
Sural Nerve axonal degeneration	0/6	1/6	0/6	0/5[b]
Tibial Nerve axonal degeneration	2/6	1/6	3/6	2/6
Lumbar Roots				
-dorsal fiber axonal degeneration	1/6	0/6	0/6	0/6
-ventral fiber axonal degeneration	0/6	0/6	1/6	0/6
Peroneal Nerve axonal degeneration	0/6	3/6	0/6	3/6
Trigeminal Nerve axonal degeneration	1/6	0/6	1/6	0/6

[a] Data were extracted from pp. 351-361. All treatment groups were evaluated.
All lesions were graded minimal
[b] One sural nerve was not examined

III. DISCUSSION AND CONCLUSIONS:

A. **CONCLUSIONS**: In a combined subchronic neurotoxicity and subchronic nose-only inhalation study, R107894 (Econea Technical, 92.4% a.i.) was administered to Crl:CD(SD) Sprague-Dawley derived rats (22 rats/dose) at dose levels of 0, 20, 40, or 80 mg/m^3 for approximately 13 weeks. Animals in each dose group were divided into 2 sections (10 animals/sex/Phase I-systemic toxicity; 12 animals/sex/Phase II-neurotoxicity).

In this neurotoxicity screening battery, after the first day of exposure, 1 male and 1 female were found dead in the 80 mg/m^3 group and were replaced. Two 80 mg/m^3 males died prior to study termination, one on Day 20 and the other on Day 89. All treated animals were observed with brownish staining of the fur, which contributed to observations of grooming and soiled fur in the functional observation battery. However, the dose-related increase in stained/soiled fur was not considered an adverse effect of treatment.

During Week 3 FOB measurements, an overall decrease in total locomotor activity was noted in the 40 and 80 mg/m^3 males and in ambulatory activities for 80 mg/m^3 males. During Week 12 of FOB testing, the 80 mg/m^3 males exhibited decreased total activity compared to controls.

The increase in minimal axonal degeneration of the peroneal nerve was considered treatment-related. No effect on the strength of the hindlimb (where the nerve resides) was observed.

The 20-mg/m^3 males also had significantly increased relative brain weights that were dose dependent; however, this change are not considered adverse because of a lack of macroscopic or microscopic findings and was most likely due to the decrease in weight of these animals. These increases in relative brain weights across the male

dose groups were most likely due to the decreased weight of these animals since the absolute brain weights were comparable to that of the control group; therefore, the change is not an indication of toxicological effects of R107894 (Econea Technical).

The neurotoxicity LOAEL was 40 mg/m^3 in males based on decreased motor activity and 80 mg/m^3 in females based on axonal degeneration observed in the peroneal nerve. The neurotoxicity NOAEL was 20 mg/m^3 for males and 40 mg/m^3 for females.

B. STUDY CLASSIFICATION: This neurotoxicity study is classified as **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for an Neurotoxicity Screening Battery in rats (870.6200; OECD 424).

EPA Reviewer: Steven L. Malish, Ph.D., Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Sr. Scientist
Team 2, RASSB/Antimicrobial Division

S. L. Malish 6/06
Jonathan Chen 06/13/06

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (90-Day Dermal) Toxicity - Rats; OPPTS 870.3250 (rodent) [82-3] OECD 411.

PC CODE: 119093 **DP BARCODE:** 328778
DECISION No: 220066 **EPA REG No:** 43813-ET

TEST MATERIAL (PURITY): 1H -Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl); (94.6%)

SYNONYMS: , Econea®028, CL 303'268, R107894,Econea Technical

CITATION: Merkel, D. (2006). R107894: Dermal toxicity study (90-day repeat dermal application study in rats). Product Safety Laboratories, Dayton, New Jersey. Laboratory study number 17279, March 24, 2006. MRID 46802201. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Plant & Material Protection Division, Beerse, Belgium.

EXECUTIVE SUMMARY: In a 90-day dermal toxicity study (MRID 468022-01), R107894 (94.6% a.i., Lot #AC 12649-8) was applied to the shaved skin of 10 rats, Crl:CD® (SD)/sex/dose at dose levels of 0, 100, 300, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 90-day period.

One 100-mg/kg/day female died prior to study termination. A treatment-related dose-dependent increase in small dermal sores was noted in both the mid- and high-dose group. **Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.**

Histopathology findings in the lungs (increased incidence of bonchoalveolar associated lymphoid tissue, chronic inflammation, and alveolar macrophages) were observed at the high-dose for both males and females and appear to be the result of treatment. With regard to the pathology, only the control and high-dose males and females were evaluated as well as one low-dose female. Because these effects occurred in both males and females at increased frequency at the high-dose, they are considered treatment-related and adverse. **Consequently, the systemic NOAEL is 300 mg/kg/day; the systemic LOAEL is 1000 mg/kg/day based on histopathology changes in the lungs (i.e., an increased incidence of BALT, chronic inflammation, and alveolar macrophages).**

This 90-day dermal toxicity study in the rat is **NOT ACCEPTABLE, but upgradeable. The study, however, satisfies** the guideline requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats. The study can be upgraded by noting the frequency of the lung lesions at the two lower dose levels so as to better define the NOAEL and LOAEL.

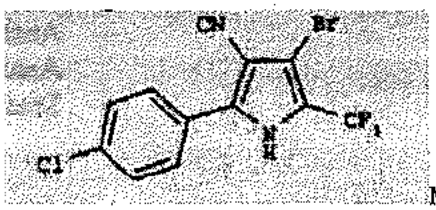
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claims statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: R107894

Description:	Light tan powdered solid stored at room temperature and not exposed to heat or direct sunlight
Lot/batch #:	AC 12649-8
Purity:	94.6 % a.i.
Compound stability:	Chemical analysis, homogeneity and stability were found in other studies in this series
CAS #:	122454-29-9
Structure	$C_{12}H_5BrClF_3N_2$



MRID 46442201

2. Vehicle and/or positive control: Distilled water (3 mL)

3. Test animals:

Species:	Rat	
Strain:	Crl:CD® (SD)	
Age/weight at study initiation:	Animals were 9 weeks old at test initiation; males weighed 302-345 grams, females weighed 211-236 grams	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually-housed in suspended stainless steel cages	
Diet:	Purina Certified Rodent Meal #5002 was available *ad libitum* during acclimation and throughout the study. There were reportedly no contaminants present in animal feed at concentrations sufficient to affect the overall study.	
Water:	Filtered water was available *ad libitum* by automatic water dispensing system; there were reportedly no contaminants present in the animal water at concentrations sufficient to affect the overall study.	
Environmental conditions:	**Temperature:**	19-23EC
	Humidity:	28-70%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	12 days	

B. STUDY DESIGN:

1. **In life dates:** Start: August 10, 2005; End: November 10, 2005

2. **Animal assignment**: Eighty (40 males and 40 females) healthy animals (i.e., those free of clinical disease or injury) with body weights within ± 20% of the mean were randomly assigned to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	10	10
Low	100	10	10
Mid	300	10	10
High	1000	10	10

3. **Dose selection rationale:** The dose levels were selected based on the results from the 28-day repeated dose study (PSL Study No. 17026) in which the neat test substance was moistened with distilled water and applied dermally at dose levels 0, 30, 100, 300, or 1,000 mg/kg/day. No evidence of dermal irritation or systemic toxicity was observed. Thus, the study authors expected to find a NOAEL by administering concentrations of 0, 100, 300, or 1,000 mg/kg/day to the rats in this study.

4. **Preparation and treatment of animal skin:** Fur was removed by clipping an area from the shoulders to the hips (i.e., greater than 10% of the body surface) from all rats during the acclimation period. Shortly before the first application and weekly thereafter, the fur of each test animal was re-clipped. A 4-ply gauze pad was used to administer the appropriate amount of substance, moistened with 0.3 milliliters of distilled water, to the dose site. The applied quantities of the test substance were adjusted weekly to individual animal body weight. The test substance/vehicle suspension was fastened to the trunk of the body with non-irritating, adhesive tape. The dressings were removed after 6 hours and animals were wiped with a 3% solution of Ivory® dishwashing liquid using clean towels, rinsed with water, and patted dry. Treatment occurred at approximately the same time each day (except during blood collection) for 5 days of any 7-day period, and the first day of application was considered Day 1. Animals that were not in their wrapping after the 6 hours of exposure were noted.

   On Days -6, -5, and -2, the "sham wrapping" spanned approximately 1 hour, 3 hours, and 6 hours, respectively. Animals were weighted, and the skin was checked for abnormalities prior to the first application of the test substance. Animals were re-clipped, as needed, throughout the study.

5. **Statistics:** Statistical analysis was conducted for body weight, daily body weight gain, daily food consumption, daily food efficiency, organ weight, and organ-to-body/brain weight ratio data. Homogeneity of variance and normality within test groups were evaluating using Bartlett's test. When variances were homogenous, treated and control groups were compared using a one-way analysis of variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's t-test for multiple comparisons. When variances were considered significantly different by Bartlett's test, groups were compared using Kruskal-Wallis non-parametric analysis of variance, followed by Dunn's test. A probability value of $p \leq 0.05$ was considered to be a significant difference among groups.

   For the means and standard deviations of clinical pathology quantitative data, Levene's test for variance of homogeneity (Levene, H., 1960) and the Shaprio-Wilk test for normality

(Shapiro, S.S. and Wilk, M.B., 1965) were used. If variances were considered to be not significant, groups were compared using a one-way analysis of variance (ANOVA) (Snedecor, G.W. and Cochran, W.G., 1967) followed by Dunnett's t-test for multiple comparisons (Dunnett, C.W., 1964, 1980, Tamhane, A.C., 1979). If the Shaprio-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test was used. Where variances were considered significantly different by Shaprio-Wilk test, groups were compared using Kruskal-Wallis non-parametric analysis of variance followed by Dunn's test (Kruskal, W.H. and Wallis, W.A., 1952, Dunn, O.J., 1964).

A probability value of $p \leq 0.05$ was considered to be a significant difference among groups.

C. METHODS:

1. Observations:

1a. **Cageside observations:** Animals were observed twice daily for signs of mortality. Cageside observations were determined daily from Day 1 to 91 (males) or 92 (females). Abnormalities were recorded.

1b. **Clinical examinations:** Detailed observations were recorded on Day 1 prior to test substance application, and weekly thereafter. Observations were made both when handling the animal and when placed in an open field. Clinical signs observed included, but were not limited to changes of the skin, fur, eyes, and mucous membranes and the occurrence of secretion and excretion. The animals were examined for signs of local skin irritation each Monday prior to application and again on each Friday after removing the gauze patches; animals were evaluated using the Draize method. Mean erythema and edema scores were determined for each group at each time interval; the sum of the mean erythema and mean edema for each group at each time interval was defined as the primary dermal irritation (PDI).

1c. **Neurological evaluations:** It was reported that animals were examined for autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern), as well as changes in gait, posture, and response to handling during clinical examinations. Additionally, the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) were recorded during clinical examinations.

2. **Body weight:** Animals were weighed 3 times during acclimation, prior to initiation of the study, and weekly thereafter. Surviving animals were weighed immediately before sacrifice. Mean body weight gains were calculated for each dose group for each interval and for the overall test interval.

3. **Food consumption:** Food consumption was determined weekly for each individual, adjusting for spillage. The method of collecting the food consumption data was not specified. Mean food consumption was reported as g food/animal/week. Food consumption ratios were calculated weekly.

4. **Ophthalmoscopic examination:** Eyes of the 100 test animals considered for the study (only total of 80 animals used) were examined by focal illumination and indirect ophthalmoscopy prior to study initiation; the eyes were re-examined on Day 87.

5. **Hematology and clinical chemistry:** Blood was collected via orbital sinus under isoflurane anesthesia on Day 86 for hematology and clinical chemistry from all surviving animals; however, clotting occurred in 2 males in dose group 100 mg/kg/day and 1 male in group 300 mg/kg/day, and 1/10 female in group 100 mg/kg/day died prior to sacrifice, thus decreasing the number of samples evaluated. Blood samples used to determine the prothrombin time and partial thromboplastin time were collected via the inferior vena cava using 3 ml syringe/21-gauge needle under isoflurane anesthesia at terminal sacrifice. All animals were fasted overnight prior to blood collections. The CHECKED (X) parameters were examined.

   a. **Hematology:** In addition to the parameters marked below, any platelet clumps that were observed were noted.

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Red cell distribution width (RDW)
X	(Activated partial thromoboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250

   b. **Clinical chemistry:** Following serum chemistry analysis, serology testing was performed on a pooled sample from two animals.

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium* (K)	X	Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
X	Alkaline phosphatase (AP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
X	Sorbitol dehydrogenase*		

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250

6. **Urinalysis:** Urine was not collected and analyzed for these study animals.

7. **Sacrifice and pathology**: All surviving animals were sacrificed (euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia) on schedule (Day 92 for males, Day 93 for females) and subjected to gross pathological examination. Gross lesions and tissues of interest (including those for histopathology examination) were preserved in 10% neutral buffered formalin. Histopathology examinations were conducted for all control and high-dose animals. Histopathology examinations also were conducted on gross lesions from animals in other groups. Fixed tissues were processed to paraffin-embedded sections, placed on glass microscope slides, and stained with hematoxylin and eosin. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*		Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin* (treated & untreated areas)
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*	X	Mammary gland*		
X	Nose*	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250
\+ Organ weights required.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** Abnormal observations are summarized in Table 2. Treatment-related ano-genital staining was observed in all treated males and females. This observation was not considered adverse. There appears to be a treatment-related increase in small sores observed in mid- and high-dose males and females.

Table 2. Summary of Clinical Observations[a]				
Dose rate (mg/kg/day)	**0**	**100**	**300**	**1000**
MALES (Number of animals showing symptoms)				
Observations During Removal from Cage and Handling				
Fur/Skin				
Ano-genital staining	0	1 (Day 50)	8 (Days 15-91)	10 (Days 15-91)
Small sore	0	0	4 (between Days 22-36)	6 (between Days 36 and 91)
FEMALES (Number of animals showing symptoms)				
Observations During Removal from Cage and Handing				
Fur/Skin				
Ano-genital staining	0	3 (Days 50, 71, or 78)	9 (between Days 15-85)	10 (between Days 15-91)
Small Sore	0	0	1 (Day 29)	3 (between Days 50-91)

[a] Data obtained from pages 36-37 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42..
[b] One animal found dead on Day 42 prior to dosing.

2. **Mortality:** One female rat in the 100 mg/kg/day (low) dose group was found dead on Day 42 prior to dosing. Severe urogenital inflammation was noted, and the death was not attributed to the treatment.

3. **Neurological evaluations:** There were no changes to behavior noted during the study.

4. **Dermal irritation:** Due to a technical error, scores for erythema and edema were not recorded at the end of the first week of exposure following patch removal. No Primary Dermal Irritation (mean erythema + mean edema) was observed for any of the animals. Small sores were noted in 4/10 males administered 300 mg/kg/day, 6 males administered 1000 mg/kg/day, 1/10 female administered 300 mg/kg/day during, and 3/10 females

administered 1000 mg/kg/day (see Table 2).

B. **BODY WEIGHT AND WEIGHT GAIN:** Mean body weight and mean body weight gain data are indicated in Table 3 below. There were no treatment-related changes in mean body weight or mean body weight gain for either males or females. A few statistically significant changes in mean body weight and mean body weight gain are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 3: Mean body weights (BW) and body weight gains (BWG) during 91 days of treatment[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (g±SD)				
Initial (Day 1)	323.9±14.0	322.8±10.7	322.4±10.0	319.3±11.7
Day 8	349.4±15.6	345.5±15.6	346.4±10.1	337.9±17.5
Day 15	367.2±18.1	371.7±17.9	366.0±14.2	355.5±21.4
Day 22	388.8±21.0	387.2±17.9	386.5±18.3	372.2±23.6
Day 29	409.1±23.7	409.2±19.4	406.6±22.4	390.5±26.4
Day 36	422.7±27.2	428.5±23.7	425.5±25.7	407.2±27.9
Day 43	439.5±29.7	443.3±20.0	437.1±27.7	413.1±34.5
Day 50	453.7±30.2	457.6±30.2	445.5±34.1	424.4±31.5
Day 57	472.5±33.2	472.7±28.9	462.8±33.7	443.1±40.3
Day 64	480.9±31.8	479.6±28.3	472.8±33.7	451.7±40.9
Day 71	487.3±36.7	481.2±24.8	478.2±31.5	458.1±40.4
Day 78	492.3±39.1	496.7±30.3	494.5±33.8	465.2±46.4
Day 85	508.1±42.4	508.2±36.7	506.1±31.5	479.5±46.9
Final (day 91)	504.8±42.7	515.7±34.0	505.7±31.4	478.6±46.6
Overall BWG Day 1-91 % Control	13.9±2.6 100%	14.8±2.4 106%	13.3±3.3 96%	12.3±3.2 88%
FEMALES (g±SD)				
Initial (Day 1)	224.6±5.1	225.5±6.6	222.8±7.0	222.9±5.6
Day 8	236.8±6.7	237.4±10.1	232.8±7.5	230.3±6.0
Day 15	241.2±6.1	243.1±8.4	235.4±9.5	234.3±9.4
Day 22	250.6±7.6	252.3±11.6	245.1±10.0	243.4±11.2
Day 29	255.9±7.4	262.0±9.7	253.4±12.7	251.4±12.5
Day 36	259.4±7.0	266.7±14.4	257.0±12.3	255.0±13.8

TABLE 3: Mean body weights (BW) and body weight gains (BWG) during 91 days of treatment[a]

Dose rate (mg/kg/day)	0	100	300	1000
Day 43	266.9±12.3	273.4±14.1	259.4±15.9	256.7±13..9
Day 50	271.4±10.3	280.8±16.0	265.2±14.3	263.8±15.4
Day 57	276.9±10.9	284.3±16.8	271.0±17.2	268.3±16.0
Day 64	274.6±10.7	287.1±18.8	272.1±17.8	269.0±16.6
Day 71	274.2±13.0	290.8*±18.6	271.7±14.8	262.5±10.4
Day 78	279.3±9.8	291.7±26.7	275.5±16.7	271.8±17.9
Day 85	283.5±14.8	303.2±21.5	284.4±18.4	280.3±17.8
Final (day 91)	279.7±10.7	294.9±16.5	278.1±17.0	278.1±18.9
Overall BWG Day 1-91 % Control	4.2±0.9 100%	5.3±1.0 126%	4.3±1.4 102%	4.2±1.3 100%

[a] Data obtained from pages 28-31 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
* Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption:** Food consumption data are indicated in Table 4 below. There were no treatment-related changes in food consumption for either males or females. A few statistically significant changes in food consumption are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 4. Average Food Consumption (FC) (g/week)[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (g±SD)				
1-8	201.2±13.0	203.4±18.5	201.1±10.8	196.0±17.8
8-15	203.3±11.6	214.8±15.7	206.3±13.9	199.8±16.7
15-22	206.5±14.4	215.3±19.9	213.3±19.3	206.5±17.5
22-29	213.3±20.5	226.2±16.9	211.9±12.0	214.5±16.6
29-36	205.4±15.5	220.0±17.9	211.1±16.7	210.8±20.2
36-43	214.3±16.8	220.9±17.0	211.9±15.2	210.1±18.3
43-50	211.1±16.1	199.2±31.6	206.4±21.4	209.9±19.4
50-57	216.8±16.7	219.4±22.8	210.2±13.4	202.8±29.4
57-64	219.2±8.3	216.4±12.7	211.5±11.9	214.3±16.9

TABLE 4. Average Food Consumption (FC) (g/week)[a]				
Dose rate (mg/kg/day)	**0**	**100**	**300**	**1000**
64-71	212.3±21.4	217.5±25.5	206.7±35.6	210.6±15.2
71-78	203.7±12.8	208.1±31.1	211.8±14.2	211.3±22.8
78-85	218.6±14.3	218.6±15.8	210.2±15.3	213.9±19.2
85-91	162.8±15.3	169.0±18.8	148.6±33.4	164.1±14.3
Overall (1-91)	206.8±13.0	211.4±13.2	204.7±11.7	205.0±16.5
FEMALES (g±SD)				
1-8	153.6±10.3	158.6±11.1	155.0±8.2	159.6±13.5
8-15	160.7±10.1	162.5±8.7	164.8±7.7	157.7±13.0
15-22	164.5±9.8	166.7±13.1	167.0±9.2	163.1±11.8
22-29	162.8±11.1	169.3±11.4	164.6±9.8	170.1±14.3
29-36	158.4±17.1	164.4±9.0	155.7±9.4	156.2±14.3
36-43	161.0±14.0	167.9±13.6	158.4±8.0	160.0±14.6
43-50	160.9±15.4	170.7±15.5	160.3±10.4	161.2±13.9
50-57	153.0±13.9	163.0±11.4	157.6±11.8	154.1±12.9
57-64	153.8±13.3	165.3±13.2	159.4±8.5	156.4±11.7
64-71	159.1±25.3	166.7±9.6	159.3±10.4	172.9±54.8
71-78	152.2±16.1	156.2±32.6	153.1±13.4	171.3±34.9
78-85	147.4±20.6	170.2*±21.3	158.5±11.9	159.1±15.3
85-91	114.4±11.5	115.0±20.0	112.2±8.2	119.0±11.2
Overall (1-91)	154.0±9.6	161.8±9.3	155.8±7.2	158.5±11.3

[a] Data obtained from pages 32-33 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42..
* Statistically different (p <0.05) from the control.

2. **Food efficiency:** Food efficiency data are indicated in Table 5 below. There were no treatment-related changes in food efficiency for either males or females. A few statistically significant changes in food efficiency are noted below; however, these changes were considered to be isolated and not dose-related.

TABLE 5. Average Weekly Food Efficiency (g/week)[a]				
Dose rate (mg/kg/day)	**0**	**100**	**300**	**1000**
MALES (g±SD)				
1-8	0.04±0.01	0.11±0.03	0.12±0.02	0.09±0.05
8-15	0.09±0.04	0.12±0.05	0.09±0.03	0.09±0.03

TABLE 5. Average Weekly Food Efficiency (g/week)[a]				
Dose rate (mg/kg/day)	0	100	300	1000
15-22	0.10±0.04	0.07±0.04	0.09±0.03	0.08±0.03
22-29	0.10±0.02	0.10±0.04	0.09±0.04	0.08±0.04
29-36	0.07±0.04	0.09±0.04	0.09±0.04	0.08±0.04
36-43	0.08±0.04	0.06±0.05	0.05±0.02	0.03*±0.04
43-50	0.07±0.02	0.08±0.08	0.04±0.05	0.06±0.05
50-57	0.09±0.03	0.07±0.03	0.08±0.03	0.09±0.06
57-64	0.04±0.03	0.03±0.03	0.05±0.03	0.04±0.01
64-71	0.03±0.05	0.01±0.05	0.02±0.10	0.03±0.02
71-78	0.02±0.07	0.07±0.07	0.08±0.06	0.03±0.03
78-85	0.07±0.04	0.05±0.06	0.05±0.08	0.07±0.02
85-91	-0.02±0.06	0.04±0.10	-0.20±0.66	-0.01±0.04
Overall (1-91)	0.06±0.01	0.07±0.01	0.06±0.01	0.06±0.01
FEMALES (g±SD)				
1-8	0.08±0.03	0.07±0.03	0.07±0.04	0.05*±0.02
8-15	0.03±0.05	0.04±0.03	0.02±0.04	0.02±0.03
15-22	0.06±0.04	0.05±0.03	0.06±0.02	0.06±0.03
22-29	0.03±0.04	0.06±0.04	0.05±0.03	0.05±0.03
29-36	0.02±0.04	0.03±0.04	0.02±0.03	0.02±0.03
36-43	0.04±0.05	0.03±0.05	0.02±0.03	0.01±0.04
43-50	0.03±0.06	0.04±0.04	0.03±0.04	0.04±0.04
50-57	0.04±0.02	0.02±0.05	0.04±0.03	0.03±0.04
57-64	-0.02±0.02	0.02*±0.03	0.01±0.02	0.00±0.02
64-71	0.00±0.04	0.02±0.04	0.00±0.03	-0.07±0.17
71-78	0.03±0.04	-0.01±0.08	0.02±0.02	0.05±0.08
78-85	0.02±0.06	0.07**±0.06	0.06±0.02	0.05±0.02
85-91	-0.04±0.06	-0.09±0.15	-0.06±0.06	-0.02±0.05
Overall (1-92)	0.03±0.01	0.03*±0.01	0.03±0.01	0.03±0.01

[a] Data obtained from pages 34-35 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
* Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test.
** Statistically different (p <0.05) from the control by Kruskal-Wallis test.

D. OPHTHALMOSCOPIC EXAMINATION: All animals were evaluated as

opthalmoscopically normal, except for two 100-mg/kg/day females, which exhibited hyphema in the right eye. This symptom was deemed to be caused by trauma that might occur during certain forms of blood collection, and was not associated with exposure to an ocular toxicant. Histopathological findings related to the eye, however, were found only in one of these 2 females.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related hematology findings in male or female rats. Females administered 1000 mg/kg/day exhibited a significant increase in the average red cell distribution width; however, this finding was not considered treatment-related because there were no changes in red cell mass parameters (red blood cell count, hemoglobin, and hematocrit). Additionally, there were more treated animals (males and females) with trace anisocytosis (variation in red cell size) and macrocytosis (large cells); however, severity grades in these groups were not higher than the respective controls.

2. **Clinical chemistry:** Possible treatment-related changes to clinical chemistry parameters are presented in Table 6. In males, there was a significant increase in urea nitrogen at 1000 mg/kg/day. This increase was considered to be possibly related to treatment. However, it also was noted that values were within historical control values (Haskell Notebook Number E-98560-AN; not provided); other related parameters (creatinine, inorganic phosphorous) were comparable to control values, and there were no microscopic changes to the kidneys at this dose group. Therefore, this change was considered to be non-adverse. In females, there were significant increases observed in triglyceride levels at 1000 mg/kg/day. Although this change was considered treatment-related, it also was considered non-adverse because all but one value were within the historical control data (Haskell Notebook Number E-98560-AN; not provided). Females administered 1000-mg/kg/day also exhibited a decrease in albumin that was considered treatment-related, but not adverse because values for 5 females were within the historical data range (Haskell Notebook Number E-98560-AN; not provided) and those outside the range were only minimally below the lower limit of the reference interval. Lastly, there was an increase in alkaline phosphatase observed in 100-, 300-, and 1000-mg/kg/day females; this increase only reached statistical significance at 100 mg/kg/day. Because of the lack of a dose-response relationship, this effect was not considered treatment-related.

TABLE 6. Selected Clinical Chemistry Data[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (Mean±SD)				
Alkaline Phosphatase (U/L)	117±32	129±28	125±32	108±21
Blood Urea Nitrogen (mg/dL)	13±1	14±1	14±2	16*±2
Triglycerides (mg/dL)	62±46	85±72	80±46	72±35
Albumin (g/dL)	3.4±0.2	3.4±0.1	3.3±0.1	3.2±0.3
FEMALES (Mean±SD)				

TABLE 6. Selected Clinical Chemistry Data[a]				
Dose rate (mg/kg/day)	0	100	300	1000
Alkaline Phosphatase (U/L)	59±24	84*±23	80±19	75±17
Blood Urea Nitrogen (mg/dL)	16±3	15±2	16±2	17±2
Triglycerides (mg/dL)	33±7	30±7	39±12	53*±17
Albumin (g/dL)	3.7±0.3	3.6±0.2	3.6±0.2	3.4*±0.2

[a] Data obtained from pages 50-53 in the study report; n=10 except n=9 in the 100 mg/kg/day females after Day 42.
.* Statistically significant difference from control at p<0.05 by Dunnett/Tamhane-Dunnett test.

F. **URINALYSIS:** A urinalysis was not conducted.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight:** Select relative organ weights are presented in Table 7 below. There were no treatment-related changes in absolute or relative (to body weight or brain weight) organ weight changes. Although significant changes in absolute and relative organ weight were observed (increased absolute male heart weight at 300 mg/kg/day; increased relative [to body weight] male adrenal and liver weight at 300 and 1000 mg/kg/day, respectively; and increased absolute and relative [to body weight] female liver weight at 1000 mg/kg/day), these differences were considered minimal and did not correspond to any gross or microscopic changes. Therefore, they were not considered to be of toxicological importance.

TABLE 7. Select Organ Weight Data[a]					
mg/kg/day		0	100	300	1000
MALES					
Final body weight	Mean±SD	504.8±42.7	515.7±34.0	505.7±31.4	478.6±46.6
	N	10	10	10	10
	% control	100%	102%	100%	95%
Absolute liver weight	Mean±SD	13.90±1.95	15.34±2.23	14.00±2.21	15.47±2.42
	N	10	10	10	10
	% control	100%	110%	101%	111%
Liver-to-body weight	Mean±SD	29.46±1.94	32.06±3.17	29.73±2.96	34.97*±3.74
	N	10	10	10	10
	% control	100%	109%	101%	119%
FEMALES					
Final body weight	Mean±SD	279.7±10.7	294.9±16.5	278.1±17.0	278.1±18.9
	N	10	9[b]	10	10
	% control	100%	105%	99%	99%

TABLE 7. Select Organ Weight Data[a]					
mg/kg/day		0	100	300	1000
Absolute liver weight	Mean±SD	8.44±0.65	8.93±0.88	9.36±1.18	9.63*±1.26
	N	10	9[b]	10	10
	% control	100%	106%	111%	114%
Liver-to-body weight	Mean±SD	33.80±2.92	33.32±2.85	37.64*±3.16	38.69**±3.87
	N	10	9[b]	10	10
	% control	100%	99%	111%	114%

[a] Data obtained and calculated from pages 41-43 in the study report.
[b] One animal found dead on Day 42 prior to dosing.
* Statistically different (p <0.01) from the control by Dunnett's Multiple Comparisons test
** Statistically different (p <0.05) from the control by Dunnett's Multiple Comparisons test

2. Gross pathology: Gross pathology findings were limited, and there were no treatment-related findings. With regard to males, an irregularly shaped spleen was found in one 100 - mg/kg/day male, in which capsule anomaly was found during microscopic evaluations. Also, a firm mass inguinal area (0.5 x 1.0 cm) in untreated skin was found in one 100-mg/kg/day male. At 300 mg/kg/day, 1/10 animals had crooked incisors, and 1/10 animals had a discolored liver. The animal with the discolored liver also exhibited signs of subcapsular congestion. At 1000 mg/kg/day, 1/10 animals had a moderately enlarged prostate with a 0.5 x 1.5 cm mass.

Fluid-filled uteruses were found in 4/10, 5/10, and 6/10, and 4/10 females in dose groups 0, 100 (misprinted as 25 in Appendix K), 300, and 1000 mg/kg/day, respectively. In Appendix K, there were no indications of fluid filled ovaries. These results differ slightly from the histopathology findings for the 1000 mg/kg/day dose level which state that there was one animal with fluid filled ovaries. This inconsistency does not affect the overall study results. In the control group, 1/10 females (also counted in the fluid-filled uterus animals) was found with hydronephrosis in the right kidney. At 100 mg/kg/day, 1/10 female was found with an enlarged right eye. Another 100- mg/kg/day female (animal 3974, which was found dead on Day 42) exhibited the following: a fluid-filled thorax; a discolored left kidney with hydronephrosis and gritty material, a discolored right kidney, and a distended fluid-filled urinary bladder with hemorrhagic lining.

Microscopic pathology: Select microscopic findings are summarized in Table 8 below. Histopathology findings in the lungs (increased incidence of BALT, chronic inflammation, and alveolar macrophages) were observed at the high-dose for both males and females and appear to be the result of treatment.

Some findings like tubular mineralization in the kidney of females and chronic inflammation in the lungs of males show considerable variation between treatment groups. Females in all dose groups, including the control group, exhibited the gross observation of fluid filled uterus. This finding was correlated in some females with dilation of the uterine horns and was attributed to the stage of estrus. One 300-mg/kg/day male (animal 3959)

exhibited a cystic dilated preputial gland duct that was chronically inflamed; these findings corresponded to gross pathology findings. One high-dose male (animal 4003) exhibited chronic inflammation of the prostate; this observation corresponded to gross pathology findings.

For the one low-dose female that died during the study, severe urogenital inflammation of the kidney papilla and urinary bladder was observed. The cause was speculated to be an ascending infection. One high-dose female had two sets of some organs and tissues.

TABLE 8. Summary of Histopathology Incidences[a]

Dose rate (mg/kg/day)	0	100	300	1000
MALES (Number of animals showing symptoms /number of animals evaluated)				
Kidney				
Infiltrate, Mononuclear Cell	4/10	0/0	0/0	4/10
Mineralization, Tubular	0/10	0/0	0/0	0/10
Nephropathy	6/10	0/0	0/0	8/10
Lung				
Increased Bronchoalveolar Associated Lymphoid Tissue (BALT)	0/10	0/0	0/0	4/10
Inflammation, Chronic	2/10	0/0	0/0	6/10
Macrophages, Alveolar	2/10	0/0	0/0	3/10
Nose				
Infiltrate, Lymphocytic, Nasopharynx	3/10	0/0	0/0	7/10
FEMALES (Number of animals showing symptoms (Number of animals evaluated)				
Kidney				
Infiltrate, Mononuclear Cell	1/10	0/1	0/0	4/11
Mineralization, Tubular	2/10	1/1	0/0	7/11
Nephropathy	1/10	0/1	0//0	1/11
Liver				
Inflammation, Chronic	8/10	0/1	0/0	11/11
Lung				
Increased Bronchoalveolar Associated Lymphoid Tissue (BALT)	1/10	0/1	0/0	3/10
Inflammation, Chronic	5/10	0/1	0/0	7/10
Macrophages, Alveolar	1/10	0/1	0/0	4/10
Nose				

TABLE 8. Summary of Histopathology Incidences[a]				
Dose rate (mg/kg/day)	0	100	300	1000
Infiltrate, Lymphocytic, Nasopharynx	10/10	0/0	0/0	9/10
Uterus				
Dilatation, Horns	4/10	5/6	5/6	6/11

[a] Data obtained from pages 220-227 in the study report

III. DISCUSSION AND CONCLUSIONS:

A. CONCLUSIONS: The dermal administration of 100, 300, or 1000 mg/kg/day of R107894 to rats over a 90-day period resulted in no adverse effects. Treatment-related changes were observed in the 1000-mg/kg/day animals that included increased ano-genital staining (males and females), increased urea nitrogen in males, increased triglyceride levels in females, and decreased albumin in females. None of these findings were considered adverse because of the type of effect, values fell within historical control values, or there was a lack of corresponding findings. One female in the 100-mg/kg/day dose group was found dead on Day 42; however, given that the animal was in the low-dose group and urogenital inflammation unrelated to the treatment was noted, the death was not attributed to the intake of R107894. The NOAEL was reportedly 1000 mg/kg/day.

There appears to be a treatment-related increase in small sores that is dose related. Limited detail is provided with regard to these sores as they are only footnoted in the clinical observation tables. These sores appear to be a dermal reaction to the test material and not a systemic affect. These sores are noted in both the mid- and high-dose group. **Consequently, a dermal NOAEL is 100 mg/kg/day; a dermal LOAEL is 300 mg/kg/day based on the appearance of sores.** Additionally, because historical control data were not provided for the clinical chemistry parameters, our reviewers could not validate whether increases in 1000-mg/kg/day male urea nitrogen and 1000-mg/kg/day female triglycerides values were within historical control data. The same is true for the decreases in albumin for 1000-mg/kg/day females. Our reviewers, however, were able to review data provided by Charles River for rats 21 weeks of age (age when blood was examined), which confirms the investigator's conclusions (Charles River, 2006). Therefore, these affects are not believed to be adverse.

Histopathology findings in the lungs (increased incidence of BALT, chronic inflammation, and alveolar macrophages) were observed at the high-dose for both males and females and appear to be the result of treatment. Our reviewers disagree that there was considerable variation between treatment groups because only the control- and high-dose males and females and one low-dose female were evaluated. Without the data from the low and high dose animals, a dose response relationship could not be established or refuted; these increases in lung histopathological findings occurred in both males and females. Other histopathology findings in the kidneys and nose are not considered treatment-related because they are only present in one sex and there are no corresponding changes observed in the animals. **Consequently, the systemic NOAEL is 300 mg/kg/day; the systemic LOAEL is 1000 mg/kg/day based on histopathology changes in the lungs (i.e., an increased incidence of BALT, chronic inflammation, and alveolar macrophages).**

C. MAJOR STUDY DEFICIENCIES:

The study can be upgraded by noting the frequency of the lung lesions at the two lower dose levels so as to better define the NOAEL and LOAEL.

E. Minor deficiencies not upgradeable

- Hematology and clinical chemistry evaluations were conducted for only one time period. Thus, it is difficult to discern if statistically significant data is incidental or toxicologically significant.

F. STUDY CLASSIFICATION: This 90-day dermal toxicity study in the rat is **NOT-ACCEPTABLE, but upgradeable . The study** satisfies the GUIDELINE requirement for a 90-day dermal toxicity study (OPPTS 870.3250; OECD 411) in rats.

E. REFERENCES:

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Jonathan Chen 06/19/06

DATA EVALUATION RECORD

STUDY TYPES: Subchronic Inhalation Toxicity [OPPTS 870.3465] and Neurotoxicity Screening Battery [OPPTS 870.6200] – Rats.

This DER is for the Subchronic Inhalation Toxicity Study, Phase I

PC CODE: 119093
DECISION No: 220066

DP BARCODE: 328778
EPA REG No: 43813-ET

TEST MATERIAL (PURITY): 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl); (92.4%)

SYNONYMS: Econea®028, CL 303'268, R107894, Econea Technical

CITATION: Beck, M. (2006). A 90-day combined nose-only inhalation toxicity and neurotoxicity study of R107894 in rats. WIL Research Laboratories, Ashland, Ohio. WIL-436004. March 31, 2006. MRID46802202. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Beerse, Belgium

EXECUTIVE SUMMARY: In a combined subchronic inhalation toxicity (Phase I) and Neurotoxicity study (Phase II), R107894 (Lot no. ZR107894EXA028; 92.4% a.i.) was administered via nose-only exposure (MRID 468022-02), to 10 Crl:CD (Sprague Dawley) rats/sex/dose in Phase I and 12 rats/sex/dose in Phase II at nominal concentrations of 0, 20, 40, and 80 mg/m^3. (analytical concentration equivalent to 0, 20, 40 and 80 mg/m3 of the test substance). The exposure period was 6 hours per day, 7 days per week for a minimum of 90 consecutive days. Particle size was determined and was within the accepted range.

No observable adverse effect level was 20-mg/m^3 for R107894 following measured parameters: survival, hematology, clinical chemistry, or gross pathology. Nasal lesions were observed in the 20-mg/m^3 animals and was considered a local effect.

One female rat in the Phase I inhalation toxicity study control group was found dead on study Day 9. This animal was in the control group, therefore the death was not considered test article-related. Three males in the 80-mg/m^3 Phase II neurotoxicity study group were found dead on study Days 0, 20, and 85, respectively. Additionally, an 80-mg/m^3 female in the Phase II neurotoxicity study group was found dead on Day 0 after the first exposure. Since there were numerous deaths in the 80-mg/m^3 (high-exposure) group these deaths were considered test

article-related.

Clinical signs of toxicity related to treatment included brown staining on multiple body surfaces in males and females of all three test article-treated groups. Staining, which began on Day 11 and persisted through the end of the study, was observed on the neck, forelimb, abdominal area, thoracic area, inguinal area, hindlimb, rump, anogenital area, urogenital area, and tail. Reduced mean body weights for the male and female animals of the 20- (males), 40-, and 80-mg/m^3 test groups were statistically significantly lower than the control group. There was a ≤10% reduction in body weight in the 20-mg/m^3 males and females; cumulative body weight gains were 5% lower than controls in the 20-mg/m^3 females and 17% lower in the 20-mg/m^3 males. The reduced mean body weights for 40- and 80-mg/m^3 male and females and the 20-mg/m^3 males were consistent with lower food consumption (g/animal/day), body weight gains (weekly and cumulatively) and food efficiency.

Microscopic treatment related effects were limited to the nose of all of the treated animals. The effects were observed to be localized in the dorsal region of the nose, and included inflammation, ulceration or erosion, squamous or respiratory metaplasia, and olfactory or respiratory epithelial degeneration. These changes displayed a dose-related increase in incidence and severity.

The local irritation LOAEL of R107894 for males and females is 20-mg/m^3 based on the findings observed in the N1 (nasal section 1) for both sexes and N3 section in the females of the 20-mg/m^3 animals. The 20-mg/m^3 males exhibited chronic and subacute inflammation (N1), ulcerations (N1), and exudate (N1), and the females had subacute inflammation (N1), epithelial hyperplasia (N1), hyperkeratosis (N1), and degeneration of olfactory epithelium (N3). The NOAEL could not be determined (<20 mg/m3)(LDT).

The systemic toxicity LOAEL was 20 mg/m3 (LDT) in the male based on a decrease in body weight gain. The NOAEL in the male could not be determined (<20 mg/m3).

The LOAEL in the female was 40 mg/m3 based on a decreased in body weight gain. The NOAEL was 20 mg/m3.

This subchronic inhalation toxicity study in the rat is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in the rats.

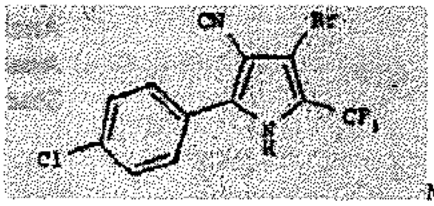
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: 1H-Pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluoromethyl), (R107894)

Description:	Light brown powder
Lot/batch #:	ZR107894EXA028
Purity:	92.4% a.i.
Compound stability:	HPLC measurements indicated the test article was 93.4% pure compared to 93.0% measured at the initiation of the range-finding study (MRID 46751302); the test compound was considered stable during the entire study.
CAS # of TGAI:	122454-29-9
Structure:	$C_{12}H_5BrClF_3N_2$



MRID 46442201

2. Vehicle and/or positive control: The vehicle control was filtered air.

3. Test animals:

Species:	Rats
Strain:	Crl:CD(Sprague Dawley)
Age/weight at study initiation:	~8 weeks old/males weighed between 222 to 286 g and females weighted between 150 to 213 g
Source:	Charles River Laboratories, Inc. (Raleigh, North Carolina)
Housing:	Individually housed in clean, stainless steel, wire-mesh cages suspended above cage-board.
Diet:	PMI Nutrition International, LLC, Certified Rodent LabDiet® provided *ad libitum* (except during exposure and during the period of fasting prior to blood collection). The diet was analyzed for chemical composition and possible contaminants by the supplier; results were not provided in the study report.
Water:	Reverse osmosis-treated (on-site) drinking water provided *ad libitum*; delivered by an automatic watering system. Analysis for contaminants did not indicate any contaminants that may interfere with the interpretation of the results.
Environmental conditions:	**Temperature:** 19.1-21.4°C **Humidity:** 38.3-53.2% **Air changes:** 10/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	Animals were housed for a 14-day acclimation period and observed twice daily for mortality, behavioral changes, and changes in appearance. During the last five days of acclimatization the rats were acclimated to the nose only restraint system for one hour on the first day and increased the hours in the restraint dosage system with additional days.

B. STUDY DESIGN:

1. In life dates: Start: August 2, 2005; End: November 1 and 4, 2005

2. **Animal assignment:** Animals were weighed and examined nine days prior to experimental dose administration, and those deemed fit for exposure were entered into a computer randomization procedure. Animals were then assigned to the groups noted in Table 1 according to body weight by the computerized randomization procedure, with individual body weights within ± 20% of the mean for each sex. Each group consisted of 10 males and females.

Test group	Test Article	Nominal conc. (mg/m^3)	Analytical conc. (mg/m^3)	No. of Rats/Sex
Group 1 (Control)	Filtered Air	0	0	10
Group 2 (LCT)	R107894	20	20	10
Group 3 (MCT)	R107894	40	40	10
Group 4 (HCT)	R107894	80	80	10

[a] Data was obtained from p. 26 of the study report;

3. **Dose selection rationale:** The dose levels were selected based on the results from a 14-day inhalation toxicity study, MRID46751302, which found exposure at 120 and 240 mg/m^3 (0.12 and 0.24 mg/L; concentration in inspired air) to be lethal and exposure at 80 mg/m^3 (0.08 mg/L) to cause minimal body weight decreases and minor clinical signs.

4. **Generation of the test atmosphere / chamber description:** The test material was micronized and aerosolized by a Jet-O-Mizer and delivered to the animals via an 18-liter stainless steel nose-only exposure system.

   Time to equilibrium was not reported.

   Standard gravimetric methods were used to determine the concentration of R107894. The exposure concentration was not adjusted for purity of the active ingredient in this powder.

   Test atmosphere concentration: Actual exposure concentrations of the atmosphere were determined by standard gravimetric methods. Samples were collected on glass fiber filters and held in open-faced filter holders inside nose-only chambers. Results are in Table 2.

   Particle size determination: Aerosol particle size determinations were performed using cascade impactors. Mean particle sizes as mass median aerodynamic diameter (MMAD) (± geometric standard deviation) were determined during the study. Results are in Table 2.

Test group	Nominal conc. (mg/m^3)	Analytical conc. (mg/m^3)*	MMAD (Φm)	GSD
Control	0	0	0	0
Low (LCT)	20	20	2.1	0.22
Mid (MCT)	40	40	2.3	0.26
High (HCT)	80	80	2.2	0.25

* Exposure concentrations were not adjusted to the active ingredient

5. **Statistics:** All statistical analyses were performed with two-tailed tests for minimum significance levels of 1% and 5% comparing the results to the control group by sex, except as noted in Table 3 below. Mean values were presented with the number of animals (N) used to calculate the mean and the standard deviation (SD).

TABLE 3. Statistical analyses	
Body weight	Intergroup differences were analyzed with 1-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare test groups to the control
	Body weight was analyzed separately for each dose group (control group males and $20mg/m^3$ group males) with repeated measures analysis of variance (RANOVA) Factors in RANOVA model: PHASE (1= tox animals, 2 = neurotox animals); WEEK of study (Weeks 1-13); interaction of PHASE and WEEK (PHASE*WEEK)
	Random effect of animal included as the repeated measurement were analyzed with SAS® procedure PROC MIXED
	Covariance structure across time was selected by evaluating Akaike's Information Criterion (AIC) If PHASE*WEEK not significant ($p > 0.05$), the 2 phases were compared through the PHASE main effect. If PHASE*WEEK was significant at ($p < 0.05$), the 2 phases were compared at each week through PHASE*WEEK and across weeks with the PHASE main effect
	Pretreatment Week 0 data analyzed with ANOVA to compare the 2 phases prior to treatment
Body weight change Food consumption Clinical pathology Organ weight	Intergroup differences were analyzed with 1-way analysis of variance (ANOVA); if significant, Dunnett's test was used to compare test groups to the control

These statistical methods for data analysis were considered acceptable. Statistical analyses of functional observation battery and locomotor activity are presented in the MRID468022-02 Neurotoxicity Study Data Evaluation Record.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily, once in the morning and once in the afternoon, for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted twice daily, prior to exposure and approximately 0 to 1 hours following exposure. A detailed clinical observation was performed weekly. A Functional Observation Battery was performed on Phase II animals and discussed in the MRID468022-02 Neurotoxicity Study Data Evaluation Record.

2. **Body weight:** Animals were weighed weekly, beginning 1 week prior to test article administration (study Week -1). In Phase I, final body weights (fasted) were measured prior to necropsy.

3. **Food consumption:** Food consumption for each animal was recorded; mean daily diet consumption was measured as g/animal/day and calculated as g food/kg body weight/day. Food efficiency was calculated as g/animal/day and g/kg/day per corresponding body weight change intervals. Body weight gained as a percent of food consumed, was also calculated. Food spillage or weighing errors were recorded as "NA", not applicable, when appropriate.

4. **Ophthalmoscopic examination:** Eyes were examined in all groups prior to the first exposure (study Week -1) and again near the end of the exposure period. Eyes were examined with a slit lamp biomicroscope after application of mydriasis.

5. **Hematology and clinical chemistry:** Blood samples were taken from the retro-orbital sinus of isoflurane-anesthetized animals prior to the scheduled necropsy at the end of study Week 13 for hematology and clinical analysis. The animals were fasted overnight preceding the blood collection. Blood was taken from the vena cava at sacrifice for activated partial thromboplastin determination. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count* and estimate[a]	X	Reticulocyte count
	Blood clotting measurements*	X	Red cell morphology (RBC Morphology)[a]
	(Thromboplastin time)		
X	Acitvated partial thromboplastime time (APTT)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

[a] Presented on individual tables if the automated differential data were verified by manual review of slides

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins (by calculation)
X	Sodium*	X	Albumin/globulin ration (A/G Ratio by calculation)
	ENZYMES (more than 2 hepatic enzymes*)	X	Glucose*
X	Alkaline phosphatase*	X	Total bilirubin
	Cholinesterase (ChE)	X	Total serum protein (TP)*
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase (LDH)		Serum protein electrophoresis
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic inhalation studies based on Guideline 870.3465

6. **Urinalysis**: Urine was collected from all animals while they were fasted overnight at the scheduled necropsy (study Week 13). The CHECKED (X) parameters were examined.

X	Appearance (color, clarity)*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)	X	Nitrate
X	Protein*	X	Urobilinogen
		X	Leukocytes

* Optional for inhalation toxicity studies

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination. Animals were sacrificed by isoflurane anesthesia followed by exsanguination. The epididymides and testes were fixed in Bouin's solution and the eyes were fixed in Davidson's solution. Bone marrow was collected from animals sacrificed in extremis and all animals at the scheduled necropsies; bone marrow was not placed in fixative and examined if scientifically warranted. All other collected tissues and organs were fixed in 10% neutral-buffered formalin. The head was preserved and, following decalcification, 6 cross-sections of the nasal cavities were prepared for microscopy. Organs and tissues were embedded in paraffin, sectioned at 4 to 8 microns, mounted on microscope slides, and stained with hematoxylin and eosin. Microscopy was performed on all collected tissues of the control and 80 mg/m^3 animals, with the exception of the brain. All gross lesions, the larynx, lungs, trachea, nasal tissues, liver, and kidneys from all animals were examined at necroscopy. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		**CARDIOVASC./HEMAT.**		**NEUROLOGIC**
	Tongue	X	Aorta, thoracic*	XX	Brain (cerebrum level 1 and 2, cerebellum with medulla/pons)*+
X	Salivary glands (mandibular)*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*		Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes (mediastinal and mesenteric)*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	Harderian glands
	Gall bladder* (not rat)	XX	Epididymides*+		**OTHER**
	Bile duct* (rat)	X	Prostate*	X	Bone (sternum and femur)
X	Pancreas*	X	Seminal vesicles*	X	Skeletal muscle (rectus femoris)
	RESPIRATORY	XX	Ovaries*+	X	Skin
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
XX	Lung (inflated with fixative after weighing)*	X	Mammary gland*		
X	Nose*	X	Cervix		
X	Pharynx*	X	Vagina		
X	Larynx*				

* Recommended for subchronic rodent studies based on Guideline 870.3465
+ Organ weights required

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Rats in all three groups exhibited brown staining on multiple body surfaces, including the neck, forelimb, abdominal area, thoracic area, inguinal area, hindlimb, rump, anogenital area, urogenital area, and tail. This staining was observed on Day 11 and persisted through the end of the study. The incidence of brown staining was greater in all three groups, although only slightly in the 20-mg/m^3 group, as compared to the control group. Other clinical findings were not considered test article-related because they were sporadic, not dose-related, and/or were common laboratory findings for rats of the age and strain utilized.

2. **Mortality:** One female rat in the Phase I inhalation toxicity study control group was found dead on study Day 9. However, since this animal was in the control group, this death was not considered test article-related.

   Three males in the 80-mg/m^3 Phase II neurotoxicity study group were found dead on study Days 0, 20, and 85, respectively. Additionally, an 80-mg/m^3 female in the Phase II neurontoxicity study group was found dead on Day 0 after the first exposure. Since there were numerous deaths in the 80-mg/m^3 (high-exposure) group these deaths were considered test article-related.

3. **Neurological evaluations:** Neurological findings are discussed in the MRID 468022-02 Neurotoxicity Study Data Evaluation Record.

B. BODY WEIGHT AND WEIGHT GAIN:

Group average body weights and cumulative weight gain data are presented in Table 4 for the combined weights of Phase I and Phase II groups. Reduced mean body weights were observed in all three test article-treated groups; however, the reduction was ≤10% in the 20-mg/m^3 males and females; cumulative body weight gains were 5% in the 20-mg/m^3 females and 17% in the 20 mg/m^3 males. The differences in cumulative body weight gains for the 20-(males), 40-, and 80-mg/m^3 animals from the control group were statistically significant. In general, throughout the study, the mean body weights for the male and female animals of the 40- and 80-mg/m^3 groups were statistically significantly lower than the control group, which is consistent with lower food efficiency observed in the these groups.

TABLE 4: Mean body weights and body weight gains during 90 days of treatment combined phases[a]

Nominal (mg/m³)	Body weights (mean g±SD; N)					Total weight gain during treatment	
	Week -1	Week 0	Week 1	Week 7	Week 13	g	% of control
Male							
0	204±10.1 22	252±14.3 22	281±21.7 22	427±39.2 22	493±45.5 22	241	100
20	205±11.0 22	252±15.4 22	272±19.1 22	389±42.3** 22	451±54.3* 22	199**	83
40	204±9.7 22	254±12.7 22	275±14.1 22	382±32.6** 22	439±39.7** 22	183**	76
80	203±10.6 21	250±16.6 22	263±21.3** 22	359±43.2** 21	415±48.5** 20	165**	68
Female							
0	167±8.4 22	190±9.9 22	202±13.4 22	259±16.8 22	278±21.1	88	100
20	168±9.6 22	189±10.9 22	203±10.2 22	251±16.4 22	273±20.3	84	95
40	168±11.0 21	189±14.7 22	198±12.4 22	243±15.2** 22	257±18.7** 22	68**	77
80	168±7.7 21	187±8.9 22	194±13.0 22	231±19.7** 22	250±20.6** 22	63**	72

[a] Data obtained from pages 118-123 in the study report.
* Statistically different ($p < 0.05$) from the control, Dunnett's tests.
** Statistically different ($p < 0.01$) from the control, Dunnett's tests.

C. FOOD CONSUMPTION:

1. **Food consumption:** Food consumption as measured as g/kg/day, were generally decreased in all of the treatment groups except 20-mg/m³ females who displayed comparable food consumption values as compared to control. Overall, mean food consumption as calculated as g/kg of animal/day of the test article-treated rats was comparable to the control group with a few exceptions. The 80-mg/m³ males showed a decrease in food consumption that was statistically different from the control during Weeks 0 to 1 and significantly increased during Weeks 3 to 4, 9 to 10, 10 to 11, and 12 to 13. A significant increase in food consumption for the 40-mg/m³ males was also noted during Weeks 9 to 10, 10 to 11, and 12 to 13. A similar result was seen for the 40-mg/m³ and 80-mg/m³ males during Week 11, but the data was not statistically different from the control. Additionally, a significant increase in food consumption was observed during Weeks 12 to 13 in the 20-mg/m³ males. The female animals in the study also had comparable food consumption values to the controls. A significant increase in food consumption was noted during Weeks 7 to 8 for the 40-mg/m³ and 80-mg/m³ females. Additionally, the 80-mg/m³ females exhibited a statistically

significant increase in food consumption during Weeks 12 to 13. These results generally correlated with the lower mean body weight and food efficiency observed in these groups. Select data are summarized in Table 5 below.

TABLE 5: Food consumption for combined groups (mean g/kg/day±SD; n)[a]

Week No.	Dose level (mg/m³)			
	Control	20	40	80
Males				
Weeks -1 to 0	95±4.3 22	96±5.3 22	94±4.8 22	95±4.6 21
Weeks 0 to 1	85±3.1 22	86±5.9 22	84±5.1 22	81±4.9* 22
Weeks 2 to 3	77±2.5 21	77±5.1 22	76±5.1 22	77±3.6 21
Weeks 4 to 5	70±4.4 22	70±3.4 22	70±3.7 22	69±4.0 21
Weeks 6 to 7	67±3.0 22	68±21.3 22	65±5.5 22	66±3.8 21
Weeks 11 to 12	59±6.2 22	60±3.0 22	61±3.3 22	62±3.5 21
Weeks 12 to 13	56±3.4 21	59±3.9* 22	59±3.6** 22	61±3.6** 20
Females				
Weeks -1 to 0	92±7.0 22	92±6.5 22	93±13.4 21	95±10.4 21
Weeks 0 to 1	89±6.9 22	91±4.3 22	88±8.0 22	87±5.3 22
Weeks 2 to 3	84±4.7 21	86±4.0 22	83±4.1 22	84±5.2 22
Weeks 4 to 5	82±17.8 21	78±11.3 22	79±9.2 22	78±6.7 22
Weeks 6 to 7	72±11.4 21	75±4.6 22	73±4.3 22	77±5.6 22
Weeks 11 to 12	71±5.5 21	73±3.8 22	72±5.2 22	74±4.9 22
Weeks 12 to 13	68±4.1 21	71±4.4 22	71±4.3 22	73±5.5** 22

[a] Data were extracted from data on pp. 142-147
* Statistically different ($p < 0.05$) from the control, Dunnett's test.
** Statistically different ($p < 0.01$) from the control, Dunnett's test.

2. <u>Food efficiency</u>: Food efficiency (measured as body weight gained as a percentage of feed consumed) was generally significantly lower in all three treatment group males from study Weeks 0 to 1 through Weeks 6 to 7. Food efficiency was lower in all three treatment group females from study Weeks 1 to 2 through Weeks 2 to 3 for the 20-mg/m³ females and through Weeks 4 to 5 for the 40- and 80-mg/m³ females. In general, there was a decrease in food efficiency in all of the treated animals as compared to the controls, with the 20-mg/m³ animals displaying comparable or slightly elevated values sporadically through the study duration, especially in the females. Table 6 summarizes select food efficiency data for the

combined Phases.

TABLE 6: Food efficiency for combined groups (mean±SD; n)[a]

Week No.	Dose level (mg/m³)			
	Control	20	40	80
Males				
Weeks -1 to 0	31.8±4.62 22	30.4±4.97 22	32.9±4.77 22	29.8±6.17 21
Weeks 0 to 1	17.8±8.60 22	12.6±4.66* 22	13.4±5.32 22	9.2±6.79** 22
Weeks 1 to 2	17.8±8.60 22	12.6±4.66* 22	13.4±5.32 22	9.2±6.79** 22
Weeks 2 to 3	15.4±2.19 21	13.5±2.49 22	11.8±2.96* 22	11.8±8.27* 21
Weeks 4 to 5	13.5±2.81 22	10.9±3.88* 22	10.1±2.65** 22	8.1±3.51** 21
Weeks 6 to 7	10.0±2.60 22	7.4±5.62 22	5.7±4.69** 22	5.9±3.37** 21
Weeks 11 to 12	5.7±2.95 22	4.5±3.74 22	3.8±3.40 22	3.3±2.06 21
Weeks 12 to 13	3.6±2.99 21	4.9±2.96 22	3.5±2.41 22	3.7±3.15 20
Females				
Weeks -1 to 0	20.0±4.83 22	18.0±6.12 22	17.3±5.88 21	15.9±5.02 21
Weeks 0 to 1	9.6±5.62 22	10.6±4.46 22	8.0±5.91 22	6.0±5.56 22
Weeks 1 to 2	12.0±4.80 22	8.7±3.60* 22	7.6±4.89** 22	7.4±3.94** 22
Weeks 2 to 3	7.8±5.39 21	6.5±3.56 22	8.0±4.94 22	6.6±4.50 22
Weeks 4 to 5	8.0±4.03 21	6.3±3.93 22	4.0±4.99* 22	2.7±5.49** 21
Weeks 6 to 7	4.7±3.41 21	3.4±3.47 22	3.4±4.70 22	4.1±3.81 22
Weeks 11 to 12	1.8±4.46 21	2.0±4.46 22	-1.8±6.40* 22	0.2±3.60 22
Weeks 12 to 13	0.0±3.26 21	0.6±5.43 22	0.5±3.58 22	2.8±4.55 22

[a] Data were extracted from data on pp. 142-147]; expressed a body weight gained as percent of feed consumed0
* Statistically different (p <0.05) from the control, Dunnett's test.
** Statistically different (p <0.01) from the control, Dunnett's test.

D. **OPHTHALMOSCOPIC EXAMINATION:** Ophthalmic examinations were performed on all animals prior to the first exposure (study Week -1) and again near the end of the exposure period. No unusual findings or ophthalmic lesions were noted for animals in any of the test article-treated groups.

E. **BLOOD ANALYSES:** Select clinical chemistry parameters are presented in Table 7, hematology data were unremarkable.

1. **Hematology:** No test article-related hematological alterations or statistically significant differences between the control and treated groups were noted.

2. **Clinical chemistry:** Blood was taken on during the scheduled necropsy at the end of study Week 13, and the summary data are presented in Table 7. Clinical chemistry results indicated that mean alkaline phosphatase levels were higher in the 20- and 80-mg/m^3 males and in the 40- and 80-mg/m^3 females. While these results were statistically different from the coinciding control group values, the individual values within the above-mentioned groups were generally within the range of the coinciding control group ranges. Since microscopic changes were not observed in the liver, the implication of these results is not known. The following additional parameters measured were statistically different from the control; however, these differences were believed to have resulted from abnormal control values:

   - Higher mean phosphorous levels were observed in the 20-, 40-, and 80-mg/m^3 males and the 40-mg/m^3 females;
   - Higher mean urea nitrogen levels were observed in the 80-mg/m^3 males and females;
   - Higher mean globulin, cholesterol, and potassium levels were exhibited by the 80 mg/m^3 females;
   - Higher mean alanine aminotransferase and aspartate aminotrasferase levels were measured for the 20-mg/m^3 females;
   - Lower mean albumin levels were exhibited by the 80-mg/m^3 females; and
   - Lower mean albumin/globulin ratio levels were observed in the 40- and 80-mg/m^3 females.

   Additionally, although these differences were considered statistically significant, they were not considered toxicologically significant. The differences did not occur in a dose-related manner and were not correlated with any histopathological findings. Other treatment-related changes were not observed.

TABLE 7: Select Clinical Chemistry Parameters in Rats at Day 90[a]

Nominal (mg/m^3)	Mean ± Standard Deviation (SD)								
	Alkaline Phosphatase (U/L)	Phosphorous (mg/dL)	Urea Nitrogen (mg/dL)	Globulin (g/dL)	Cholesterol (mg/dL)	Potassium (mEq/L)	Alanine Aminotransferase (U/L)	Aspartate Aminotransferase (U/L)	Albumin (g/dL)
Males									
0	104.0±21.4	6.7±0.50	16.2±1.89	2.7±0.19	53.0±8.6	4.80±0.281	50.0±6.8	103.0±20.8	4.3±0.18
20	140.0±17.8**	7.7±0.75**	16.4±2.78	2.7±0.26	46.0±7.6	5.03±0.523	49.0±4.6	99.0±15.4	4.3±0.10
40	119.0±15.6	7.6±0.43*	17.8±2.24	2.6±0.19	54.0±13.1	5.00±0.399	44.0±7.9	105.0±19.9	4.2±0.15
80	150.0±26.6**	7.4±0.72*	18.8±2.18*	2.7±0.16	44.0±8.3	4.86±0.476	47.0±8.8	99.0±13.0	4.1±0.19
Females									
0	67.0±22.4	6.3±1.06	18.9±2.61	2.7±0.14	65.0±12.8	4.10±0.316	39.0±4.6	94.0±10.4	4.7±0.28
20	75.0±18.1	7.3±0.92	18.9±1.54	2.9±0.19	72.0±20.8	4.32±0.405	60.0±33.7*	120.0±33.7*	4.9±0.45
40	95.0±15.8*	7.7±0.78 *	18.9±2.73	2.9±0.28	78.0±6.7	4.45±0.426	40.0±8.4	92.0±14.3	4.4±0.25
80	91.0±21.7*	7.4±1.07	21.5±2.44	3.1±0.38*	86.0±16.6*	4.65±0.453*	41.0±9.1	102.0±16.3	4.1±0.22**

[a] Data obtained from Table 41of the study report; n=10 except control females n=9
*Statistically different ($p < 0.05$) from the control using Dunnett's test.
** Statistically different ($p < 0.01$) from the control using Dunnett's test.

F. **URINALYSIS:** Urine was collected from all animals while they were fasted overnight prior to their scheduled necropsy (study Week 13). No urinalysis parameters in the test article-treated groups were statistically different from the control group.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight:** Table 8 summarizes select organ weight data. Only the animals in the inhalation subchronic toxicity study (Phase I) were used. Treatment-related observations included higher mean lung weights in all of the test article-treated female groups. Furthermore, the 80-mg/m^3 females exhibited higher mean absolute (11.3% absolute) lung weights compared to the control group, while the 20-, 40-, and 80-mg/m^3 females demonstrated higher relative (to final body weight) lung weights compared to the control group and the 80-mg/m^3 females had increased lung weight relative to brain weight. The 80-mg/m^3 males displayed increases in absolute lung weight (25%) and relative to body weight (13%) as compared to controls. These results were statistically significant, but not toxicologically significant since the higher lung weights did not correspond to any macro- or microscopic findings and were most likely due to the test article deposition in the lungs.

   Several organs weights were found to be statistically significantly different than the controls; however, these changes lacked corresponding findings to suggest a toxicological effect. Most findings were due to reduced body weight or lacked a dose-response relationship as well as lacking corresponding macro- or microscopic changes. These changes are listed below and summarized in Table 8:

   - The 80-mg/m^3 males had a 5% decrease in absolute brain weight as compared to controls;
   - The 80-mg/m^3 males had a 15% increase in epididymides weight relative to body weight as compared to controls;
   - The 40- and 80-mg/m^3 males had a 25% increase in adrenal weight relative to body weight as compared to controls;
   - The 40- and 80-mg/m^3 females had a 10 and 12% increase in brain weight relative to body weight as compared to controls, respectively;
   - The 20- and 80-mg/m^3 females had higher liver weight relative to body weight as compared to controls by 11 and 12%, respectively.

TABLE 8: Absolute and relative brain weights (mean±SD)[a]

Parameter	Nominal dose level (mg/m^3)			
	Control	20	40	80
Males				
Body wt. (g)	452±34.7	426±58.8	414±51.1	387*±48.9
% of control	100	94	92	86
Brain wt. (g)	2.08±0.10	2.08±0.08	2.03±0.11	1.97*±0.09
% of control	100	100	98	95
Brain/body wt. (g/100 g)	0.462±0.038	0.496±0.070	0.496±0.061	0.515±0.068

TABLE 8: Absolute and relative brain weights (mean±SD)[a]

Parameter	Nominal dose level (mg/m³)			
	Control	20	40	80
% of control	100	107	107	111
Liver wt. (g)	11.91±0.94	10.69±1.67	10.47±0.99	10.52±1.75
% of control	100	90	88	88
Liver/body wt. (g/100 g)	2.641±0.238	2.506±0.082	2.540±0.168	2.716±0.240
% of control	100	95	96	103
Liver/brain wt. (g/100 g)	572.845±48.881	515.972±83.181	515.526±42.216	535.996±91.391
% of control	100	90	90	94
Lungs wt. (g)	1.67±0.10	1.59±0.11	1.65±0.19	1.77±0.13
% of control	100	95	99	106
Lungs/body wt. (g/100 g)	0.369±0.023	0.378±0.033	0.390±0.028	0.461**±0.039
% of control	100	102	106	125
Lung/brain wt. (g/100 g)	80.052±4.675	76.924±5.998	81.405±7.033	90.053**±7.346
% of control	100	96	101	113
Epididymides wt. (g)	1.33±0.10	1.38±0.07	1.35±0.13	1.30±0.11
% of control	100	104	102	98
Epididymides/body wt. (g/100 g)	0.295±0.024	0.328±0.034	0.329±0.034	0.340*±0.039
% of control	100	111	112	115
Epididymides/brain wt. (g/100 g)	63.919±4.482	66.494±4.284	66.624±6.587	66.273±5.898
% of control	100	103	105	103
Adrenals wt. (g)	0.0536±0.0073	0.0580±0.0093	0.0599±0.0073	0.0579±0.0058
% of control	100	108	112	108
Adrenals/body wt. (g/100 g)	0.012±0.002	0.014±0.003	0.015*±0.002	0.015**±0.002
% of control	100	117	125	125
Adrenals/brain wt. (g/100 g)	2.579±0.374	2.791±0.427	2.951±0.334	2.957±0.378
% of control	100	108	114	115
Females				
Body wt. (g)	256±22.9	255±14.5	232*±18.3	225**±15.7
% of control	100	100	91	88
Brain wt. (g)	1.90±0.095	1.97±0.142	1.90±0.064	1.88±0.064
% of control	100	104	100	99
Brain/body wt. (g/100 g)	0.748±0.056	0.773±0.068	0.824*±0.057	0.837**±0.044
% of control	100	103	110	112
Liver wt. (g)	7.04±0.62	7.78±0.76	6.79±0.59	6.95±0.79
% of control	100	111	96	99
Liver/body wt. (g/100 g)	2.764±0.264	3.057*±0.296	2.930±0.176	3.088*±0.265
% of control	100	111	106	112
Liver/brain wt. (g/100 g)	370.248±28.682	398.552±54.484	356.901±30.223	369.542±31.913
% of control	100	108	96	100
Lungs wt. (g)	1.24±0.10	1.36±0.11	1.28±0.09	1.38*±0.12

TABLE 8: Absolute and relative brain weights (mean±SD)[a]				
Parameter	Nominal dose level (mg/m³)			
	Control	20	40	80
% of control	100	110	103	111
Lungs/body wt. (g/100 g)	0.488±0.032	0.534*±0.050	0.553**±0.028	0.616**±0.044
% of control	100	109	113	126
Lung/brain wt. (g/100 g)	65.460±4.568	69.253±5.327	67.315±4.496	73.710**±5.741
% of control	100	106	103	113
Adrenals wt. (g)	0.0723±0.0055	0.0805±0.0130	0.0717±0.0084	0.0719±0.0068
% of control	100	111	99	99
Adrenals/body wt. (g/100 g)	0.029±0.004	0.032±0.005	0.031±0.003	0.032±0.003
% of control	100	110	107	110
Adrenals/brain wt. (g/100 g)	3.810±0.328	4.112±0.743	3.766±0.374	3.832±0.381
% of control	100	108	99	101

[a] Data were extracted from pp. 275-288; n=10 except control females n=9 and n=9 for 40-mg/m³ males lungs only
* Statistically different (p <0.05) from the control group, Dunnett's test.
** Statistically different (p <0.01) from the control group, Dunnett's test.

2. **Gross pathology:** At necropsy, the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities, including viscera, were examined in all animals. Treatment-related macroscopic findings were not observed, any findings were considered incidental due to their lack of dose-response relationship and sporadic occurrence.

3. **Microscopic pathology:** Treatment-related microscopic findings were observed in numerous sections of the nose (N1 to 6) in the 80-mg/m³ males and females and less frequently in the nose of the 40-mg/m³ males and females. A few findings in N1 and N3 sections of the nose were observed in 20-mg/m³ animals with the males displaying minimal chronic and subacute inflammation, mild ulcerations, and minimal to mild exudate, all at N1; the females had minimal subacute inflammation, mild epithelial hyperplasia, and minimal hyperkeratosis at N1, as well as minimal degeneration of olfactory epithelium at N3. Treatment-related findings of the nose (N1-N6) of the 40- and 80 mg/m³ males and females included inflammation, ulceration or erosion, squamous or respiratory metaplasia, and olfactory or respiratory epithelial degeneration in the nose. Findings increased in incidence and lesions increased in severity according to dose. Other microscopic findings were not observed, or were considered incidental and not related to exposure of the test article. A summary of the incidence of selected microscopic alterations in nasal sections (N1-N6) for all groups is presented in Table 9.

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]				
Nominal concentration (mg/m³)	Control	20	40	80
Males				
N1				
Inflammation (Chronic-active)	2/10	1/10	8/10	2/10
Minimal	1/10	1/10	2/10	0/10

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]

Nominal concentration (mg/m^3)	Control	20	40	80
Mild	1/10	0/10	6/10	2/10
Inflammation (subacute) - Minimal	0/10	3/10	0/10	5/10
Ulceration	0/10	1/10	4/10	7/10
Minimal	0/10	0/10	1/10	1/10
Mild	0/10	1/10	3/10	5/10
Moderate	0/10	0/10	0/10	1/10
Exudate	1/10	4/10	8/10	7/10
Minimal	1/10	3/10	3/10	3/10
Mild	0/10	1/10	5/10	4/10
Erosion-Mild	0/10	0/10	2/10	0/10
Hypertrophy (glandular)-Minimal	0/10	0/10	1/10	0/10
N2				
Metaplasia (squamous)	0/10	0/10	0/10	4/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	3/10
Inflammation (subacute)	0/10	0/10	0/10	6/10
Minimal	0/10	0/10	0/10	2/10
Mild	0/10	0/10	0/10	4/10
Ulceration	0/10	0/10	0/10	7/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	4/10
Moderate	0/10	0/10	0/10	2/10
Exudate	0/10	0/10	0/10	7/10
Minimal	0/10	0/10	0/10	3/10
Mild	0/10	0/10	0/10	4/10
Degeneration (olfactory epithelium)-Minimal	0/10	0/10	1/10	0/10
Hyperplasia (epithelium)-Mild	0/10	0/10	0/10	1/10
N3				
Metaplasia (squamous)	0/10	0/10	0/10	3/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	2/10
Exudate	0/10	0/10	0/10	2/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	1/10
Inflammation (subacute)-Mild	0/10	0/10	0/10	2/10
Ulceration	0/10	0/10	0/10	0/10
Erosion	0/10	0/10	0/10	0/10
Degeneration (olfactory or respiratory epithelium)-Minimal	0/10	0/10	3/10	1/10
N4				
Metaplasia (squamous)-Mild	0/10	0/10	0/10	3/10
Atrophy	0/10	0/10	0/10	3/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	2/10
Exudate	0/10	0/10	0/10	4/10

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]

Nominal concentration (mg/m^3)	Control	20	40	80
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	3/10
Degeneration (olfactory or respiratory epithelium)	0/10	0/10	1/10	4/10
Minimal	0/10	0/10	1/10	2/10
Mild	0/10	0/10	0/10	2/10
Ulceration-Mild	0/10	0/10	0/10	1/10
Inflammation (subacute)	0/10	0/10	0/10	3/10
Minimal	0/10	0/10	0/10	1/10
Mild	0/10	0/10	0/10	2/10
N5				
Metaplasia (squamous)	0/10	0/10	1/10	3/10
Mild	0/10	0/10	1/10	2/10
Moderate	0/10	0/10	0/10	1/10
Atrophy-Mild	0/10	0/10	0/10	3/10
Exudate-Minimal	0/10	0/10	0/10	4/10
Inflammation (subacute)-Mild	0/10	0/10	0/10	2/10
Degeneration (olfactory epithelium)	0/10	0/10	2/10	4/10
Minimal	0/10	0/10	1/10	2/10
Mild	0/10	0/10	1/10	2/10
Ulceration-Mild	0/10	0/10	0/10	1/10
N6				
Inflammation (subacute)-Minimal	1/10	0/10	0/10	0/10
Metaplasia (respiratory epithelium)	0/10	0/10	0/10	2/10
Mild	0/10	0/10	0/10	1/10
Moderate	0/10	0/10	0/10	1/10
Exudate	0/10	0/10	0/10	4/10
Minimal	0/10	0/10	0/10	3/10
Mild	0/10	0/10	0/10	1/10
Metaplasia, squamous	0/10	0/10	0/10	3/10
Mild	0/10	0/10	0/10	2/10
Moderate	0/10	0/10	0/10	1/10
Degeneration (olfactory epithelium)-Minimal	0/10	0/10	2/10	1/10
Atrophy-Mild	0/10	0/10	0/10	1/10
Hyperplasia (epithelium)-Mild	0/10	0/10	0/10	1/10
Females				
N1				
Inflammation (chronic active)-Mild	0/9	0/10	1/10	0/10
Ulceration	0/9	0/10	6/10	6/10
Minimal	0/9	0/10	1/10	0/10
Mild	0/9	0/10	5/10	2/10

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]

Nominal concentration (mg/m³)	Control	20	40	80
Moderate	0/9	0/10	0/10	4/10
Exudate	0/9	0/10	6/10	6/10
Minimal	0/9	0/10	2/10	1/10
Mild	0/9	0/10	4/10	3/10
Moderate	0/9	0/10	0/10	2/10
Inflammation (subacute)	0/9	1/10	2/10	4/10
Minimal	0/9	1/10	0/10	0/10
Mild	0/9	0/10	1/10	4/10
Moderate	0/9	0/10	1/10	0/10
Erosion (epithelial)	0/9	0/10	4/10	1/10
Mild	0/9	0/10	2/10	1/10
Moderate	0/9	0/10	1/10	0/10
Severe	0/9	0/10	1/10	0/10
Hyperplasia (epithelial)-Mild	0/9	1/10	2/10	4/10
Hyperkeratosis-Minimal	0/9	1/10	0/10	0/10
N2				
Metaplasia (squamous)	0/9	0/10	1/10	1/10
Mild	0/9	0/10	1/10	0/10
Moderate	0/9	0/10	0/10	1/10
Ulceration	0/9	0/10	0/10	2/10
Minimal	0/9	0/10	0/10	1/10
Mild	0/9	0/10	0/10	1/10
Exudate-Minimal	0/9	0/10	0/10	3/10
Inflammation (subacute)-Minimal	0/9	0/10	1/10	1/10
Hyperplasia (epithelial)	0/9	0/10	1/10	2/10
Minimal	0/9	0/10	1/10	0/10
Mild	0/9	0/10	0/10	2/10
Degeneration (respiratory epithelium)-Minimal	0/10	0/10	1/10	1/10
N3				
Metaplasia (squamous)	0/9	0/10	0/10	4/10
Minimal	0/9	0/10	0/10	2/10
Mild	0/9	0/10	0/10	2/10
Exudate-Minimal	0/9	0/10	0/10	1/10
Degeneration (olfactory epithelium)	0/9	2/10	8/10	2/10
Minimal	0/9	2/10	8/10	1/10
Mild	0/9	0/10	0/10	1/10
Atrophy	0/9	0/10	0/10	4/10
Mild	0/9	0/10	0/10	3/10
Moderate	0/9	0/10	0/10	1/10
Degeneration (respiratory epithelium)	0/9	0/10	0/10	3/10
Minimal	0/9	0/10	0/10	2/10
Mild	0/9	0/10	0/10	1/10
Ulceration	0/9	0/10	0/10	2/10
Minimal	0/9	0/10	0/10	1/10
Mild	0/9	0/10	0/10	1/10
Erosion-Moderate	0/9	0/10	0/10	1/10

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]

Nominal concentration (mg/m^3)	Control	20	40	80
N4				
Metaplasia (squamous)	0/9	0/10	0/10	5/10
Minimal	0/9	0/10	0/10	1/10
Mild	0/9	0/10	0/10	2/10
Moderate	0/9	0/10	0/10	2/10
Atrophy	0/9	0/10	0/10	5/10
Mild	0/9	0/10	0/10	1/10
Moderate	0/9	0/10	0/10	4/10
Exudate	0/9	0/10	0/10	6/10
Minimal	0/9	0/10	0/10	4/10
Mild	0/9	0/10	0/10	2/10
Degeneration (olfactory epithelium)	0/9	0/10	4/10	2/10
Minimal	0/9	0/10	4/10	1/10
Mild	0/9	0/10	0/10	1/10
Ulceration	0/9	0/10	0/10	4/10
Mild	0/9	0/10	0/10	3/10
Moderate	0/9	0/10	0/10	1/10
Inflammation (subacute)	0/9	0/10	0/10	3/10
Minimal	0/9	0/10	0/10	2/10
Mild	0/9	0/10	0/10	1/10
Metaplasia (respiratory epithelium)	0/9	0/10	0/10	2/10
Mild	0/9	0/10	0/10	1/10
Moderate	0/9	0/10	0/10	1/10
Degeneration (respiratory epithelium)-Mild	0/9	0/10	0/10	1/10
N5				
Metaplasia (squamous)	0/9	0/10	1/10	5/10
Mild	0/9	0/10	1/10	3/10
Moderate	0/9	0/10	0/10	2/10
Atrophy	0/9	0/10	0/10	5/10
Mild	0/9	0/10	0/10	4/10
Moderate	0/9	0/10	0/10	1/10
Exudate	0/9	0/10	1/10	5/10
Minimal	0/9	0/10	1/10	4/10
Mild	0/9	0/10	0/10	1/10
Inflammation (subacute)	0/9	0/10	1/10	2/10
Minimal	0/9	0/10	1/10	1/10
Mild	0/9	0/10	0/10	1/10
Degeneration (olfactory (epithelium	0/9	0/10	7/10	6/10
Minimal	0/9	0/10	3/10	2/10
Mild	0/9	0/10	2/10	2/10
Moderate	0/9	0/10	2/10	2/10
Ulceration-Mild	0/9	0/10	1/10	2/10
Metaplasia (respiratory epithelium)-Minimal	0/9	0/10	0/10	1/10
N6				
Inflammation (subacute)-Minimal	0/9	0/10	0/10	1/10

TABLE 9: Microscopic Pathology in Nasal Sections N1-6 (incidence)[a]				
Nominal concentration (mg/m^3)	Control	20	40	80
Degeneration (olfactory epithelium)	0/9	0/10	1/10	1/10
Minimal	0/9	0/10	1/10	0/10
Mild	0/9	0/10	0/10	1/10

[a] Data were extracted from pp. 316-320; 332-338

III. DISCUSSION AND CONCLUSIONS:

A. **CONCLUSIONS:** In a subchronic (90-day) inhalation toxicity study, R107894 was administered via nose-only exposure to 10 Crl:CD (Sprague Dawley) rats/sex/dose, at 20, 40, or 80 mg/m^3 (calculated 0.018, 0.037, or 0.073 mg/L, respectively). A control group of 10 Crl:CD (Sprague Dawley) rats/sex was administered filtered air concurrently. Animals were exposed to R107894 via an 18.1L stainless steel conventional nose-only exposure system. The exposure period was 6 hours per day, 7 days a week for a minimum of 90 consecutive days.

Rats in all three groups exhibited brown staining on multiple body surfaces. The incidence of brown staining was greater in all three groups, although only slightly in the 20 mg/m^3 group, as compared to the controls. In the Phase I inhalation portion of the study, no treatment-related deaths were noted. Only one female rat in the Phase I inhalation toxicity study control group was found dead on study Day 9, this death was not considered treatment related.

Reduced mean body weights for the male and female animals of the 40- and 80-mg/m^3 males and females were statistically significantly lower than the control group, which is consistent with the lower food consumption and food efficiency observed in the these groups.

Blood samples were taken at the end of study Week 13. No test article-related hematological alterations or statistically significant differences between the control and test article-treated groups were noted. Significant clinical chemistry results indicated that mean alkaline phosphatase levels were higher for males in the 80-mg/m^3 group and for females in the 40- and 80-mg/m^3 groups. Since microscopic changes were not observed in the liver, the implication of these results is not known.

Microscopic treatment related effects were limited to the dorsal portion of the nose in the 40- and 80-mg/m^3 animals. Ulcerations and erosions were typically accompanied with inflammation and exudate but the inflammation was separately diagnosed unless there was extension into non-ulcerated/eroded areas. The margins of ulcerated/eroded areas often had hyperplasia of the epithelium as a response to the injury but was not separately diagnosed unless beside an area of injury. In the most posterior areas alterations were metaplasia or degeneration of the nasal arches. The degeneration or metaplasia of the nasal arches was sporadically accompanied by loss of the submucosal glands and was diagnosed as atrophy.

No observable adverse effect level was 20-mg/m^3 for R107894 following measured parameters: survival, hematology, clinical chemistry, or gross pathology. However, nasal lesions were observed in the 20-mg/m^3 animals and was considered a local effect.

The findings in N1 and N3 sections of the nose observed in 20-mg/m^3 animals were as follows: the males displayed minimal chronic and subacute inflammation, mild ulcerations, and minimal to mild exudate, all at N1 section; the females had minimal subacute inflammation, mild epithelial hyperplasia, and minimal hyperkeratosis at N1, as well as minimal degeneration of olfactory epithelium at N3.

Local Irritation

The **LOAEL** of R107894 for males and females is 20-mg/m^3 (LDT) based on the findings observed in the N1 and N3 sections of the 20-mg/m^3 animals. A **NOAEL** was not able to be established based on the results of this study.

Systemic Toxicity

The systemic toxicity **LOAEL** was 20 mg/m3 (LDT) in the male based on a decrease in body weight gain. The NOAEL in the male could not be determined (<20 mg/m3).

The **LOAEL** in the female was 40 mg/m3 based on a decreased in body weight gain. The **NOAEL** was 20 mg/m3.

C. STUDY CLASSIFICATION:

This subchronic inhalation toxicity study in the rat is **ACCEPTABLE-GUIDELINE** and satisfies the guideline requirement for a subchronic inhalation study OPPTS 870.3465; OECD 413 in rats.

EPA Reviewer: Steven L. Malish, Ph.D. , Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Toxicologist
Team 2, RASSB/Antimicrobial Division

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (90-Day) Oral Toxicity - [rat]; OPPTS 870.3100 [§82-1]; OECD 408.

PC CODE: 119093
DECISION#: 220066

DP BARCODE: D323129
EPA REG. NO.: 43813-ET

TEST MATERIAL (PURITY): 1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]; (94.6% a.i.)

SYNONYMS: Econea®028, CL 303'268, R 107894

CITATION: Radovsky, A. (2005). Pathology Peer Review Statement of R 107894 Subchronic toxicity study in Sprague-Dawley rats, administration via the diet over 3 months and a recovery period of 4 weeks. WIL Research Laboratories, LLC, Ashland, OH. Project No.:50C0238/03021. September 28, 2005. MRID 466597-01. Unpublished.

Mellert, W., K. Deckart, W. Kaufmann, et al. (2004). R 107894 Subchronic toxicity study in Sprague-Dawley rats, administration via the diet over 3 months and a recovery period of 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Project No.:50C0238/03021, October 14, 2004. MRID 46442201. Unpublished.

SPONSOR: Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium

EXECUTIVE SUMMARY: A pathology peer review (MRID 46659701) was performed on a 90-day oral toxicity study (MRID 46442201). In the study, R 107894 (94.6% a.i., Batch # AC 12649-8) was administered to 10 Sprague-Dawley rats/sex/dose via food at dose levels of 0, 80, 250, or 750 ppm active ingredient. These concentrations were equivalent to 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females using the analytical concentration. An additional study was performed with 10 Sprague-Dawley rats/sex/dose at the same concentration as above with 5 rats/sex/dose undergoing terminal sacrifice by *in situ* perfusion after anesthesia after 3 months of exposure and 5 rats/sex/dose undergoing a 4-week recovery period. The intake of test article in these rats was similar to that observed in the main study animals (i.e., 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females).

The original pathology was peer reviewed.

No unscheduled deaths occurred during the course of the study. There were no treatment-related clinical signs of toxicity, opthalmological findings, urinalysis, or macroscopic findings. Food consumption decreased in the first week of treatment in both males and females administered 750 ppm. Thereafter, food consumption in the 750-ppm group was lower than that observed in the controls; and values reached statistical significance in Weeks 1, 6, and 9 in males and Weeks 1, 4, 6-9, and 11 in females. In conjunction with the low food consumption, high-dose males had statistically-significantly lower body weights within the first week of treatment and throughout treatment; high-dose females displayed statistically-significant decreases in mean body weight starting on Week 7 through Week 13. The body weight gains were low throughout the study, likely due to low food consumption. The food efficiency was reduced in high-dose animals with only the males having statistically-significant decreases (Weeks 1-5, 7, 8, and 13). The mid-dose males had statistically-significant decreases in mean body weight during Weeks 7-13, with corresponding decreased body weight gain and food consumption. The mid-dose females displayed only slight decrease in body weight, body weight gain, and food consumption. The recovery group displayed signs of weight gain and increased food consumption; however, the high-dose animals still had statistically-significantly decreased mean body weight and body weight gain as compared to controls. Food efficiency was increased in the treated animals during the recovery period.

Hematology data indicated only minor (<10% difference from controls) effects in high-dose animals, except for a 15% increase in platelets in the males with a corresponding increase in females that was not significant (9%). All hematological values were comparable to controls after the 4-week recovery period. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were statistically-significantly increased in the mid- and high-dose males, which correlate to increased relative liver weights in the main group. Females displayed a lesser increase in ALP with only the high-dose group demonstrating a statistically-significant change. Low-dose males also displayed a statistically-significant increase in ALP. Signs of dehydration and undernourishment were evident with statistically-significant increases in phosphorus, urea, magnesium, and cholesterol; and a statistically-significant decrease in glucose in high-dose males and females.

Clinical chemistry findings in the recovery group indicate reversibility of the effects. Differences in creatinine and triglycerides were generally <10% different from the main control group and were thus considered due to biological variation.

Terminal body weight was reduced a statistically significant amount in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs. Treatment-related changes include increases in relative brain weights (mid- and high-dose males; high-dose females) and liver weights (mid- and high-dose males; all treated females). However, the changes in ALP and ALT enzyme were reversible after 4 weeks and the increases in the liver were likely due to increased metabolism of the test article. The relative brain weights in the high-dose animals remained elevated after the recovery period, though the males were the only

statistically-significant value. However, frank effects were noted in the brain at necropsy in these recovery group animals.

Microscopic findings of the brain and spinal cord observed in the main group were as follows: minimal to moderate multifocal vacuolation of the brain in the mid- and high-dose males and females as well as the low-dose females; moderate to marked multifocal vacuolation of the thoracic cord in the high-dose females; minimal to marked multifocal vacuolation of the lumbar cord in the mid- and high-dose males and females as well as the low-dose females. Microscopic finding of the brain and spinal cord of the animals that were in the perfusion fixation satellite group displayed treatment-related finding as follows: slight or minimal multifocal vacuolization in the frontal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the parietal lobe (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the midbrain (high-dose males; mid- and high-dose females); minimal to slight multifocal vacuolization of the pons (high-dose males; mid- and high-dose females); minimal to moderate multifocal vacuolization of the cerebellum (high-dose males; mid- and high-dose females); slight multifocal vacuolization of the medulla oblongata (high-dose female);.slight multifocal vacuolization of the cervical cord (high-dose male); minimal to moderate multifocal vacuolization of the lumbar cord (mid- and high-dose males and females); minimal to marked myelin vacuolation of the cervical dorsal root (mid- and high-dose males and females); minimal to slight myelin vacuolation of the cervical ventricle root (high-dose males); minimal to marked myelin vacuolation of the lumbar dorsal root (mid- and high-dose males; all dosed females); minimal to marked myelin vacuolation of lumbar ventricle root (mid- and high-dose males; all dosed females); and slight to moderate myelin vacuolation of the proximal sciatic nerve (high-dose males and females). The lumbar region (cauda equina) was most profoundly affected and the females appeared to be more sensitive.

Other treatment related microscopic findings in the main group were minimal to slight mucosal hyperplasia of the duodenum in the mid- and high-dose males and females that were reversible after 4 weeks. These were spongiform myelinopathy in the central and the proximal peripheral nervous system. The most severe lesions were multifocal vacuolation of the white matter and intramyelinic vacuolation of the peripheral nerves in the cauda equina (lumbar cord and root fibers). Peer review of the pathology confirmed the general findings, although there were some minor differences on severity and in some instances the involvement of vacuolation in several tissues.

There were no changes in behavior or parasympathetic movements (e.g. tremors, convulsions, impaired gait, posture, response to handling, activity, or arousal).

After 4 weeks of no treatment, microscopic lesions were still observed in the brain and lumbar region. Minimal multifocal vacuolization of the brain in high-dose males and minimal to slight multifocal vacuolization of the brain in mid- and high-dose females were observed; minimal to slight multifocal vacuolization of the lumbar in mid- and high-dose males and minimal to moderate multifocal vacuolization of the lumbar in all treated females. The lesion severity increased with dose.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and body weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males and could not be determined in females.

This 90-day oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a subchronic (90-day) oral toxicity study OPPTS 870.3100 in the rat.

COMMENTS: This is a revised Executive Summary only and does not alter the conclusions of the original review. This summary incorporates the findings of the pathology peer review.

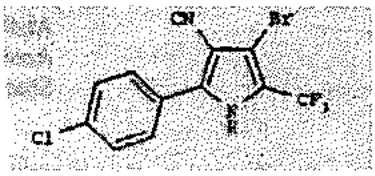
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl], R 107894

Description:	Powder, white to light brown
Lot/Batch #:	AC 12649-8
Purity:	94.6% a.i.
Compound Stability:	Expiration date April 30, 2008 at room temperature; the date of production was May-June 2000. The study report verifies that the test substance was 94.6% pure just prior to study initiation and was stored in the dark at room temperature. The test substance is expected to be stable for at least 3 years before recertificatinn is needed.
CAS # of TGAI:	122454-29-9
Structure:	

2. Vehicle and/or positive control: Food

3. Test animals:

Species:	Rats	
Strain:	Sprague-Dawley Crl:CD® (Charles River)	
Age/weight at study initiation:	Approximately 42±1 days old / 171.8-216.3 g for males and 145.3-174.3 g for females	
Source:	Charles River, Germany	
Housing:	Rats were individually housed in DK III stainless steel wire mesh cages (Becker & Co. Castro-Rauxel, Germany). An absorbent dust free paper (SSNIFF, Soest, Germany) was under the cages.	
Diet:	Powdered, Kliba maintenance diet rat-mouse meal (Provimi Kliba SA, Kaiseraugst, Switzerland) was available *ad libitum,* except during overnight fast before blood withdrawal. Chemical and microbiological contaminants in the diet were analyzed, the data were not provided but available from the laboratory.	
Water:	Tap water was available *ad libitum.* Analysis results from Frankenthal and Technical Services of BASF Aktiengesellschaft for contaminants in the water were not provided, but were stated to be suitable according to German Drinking Water Regulation (Trinkwasserverordnung).	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	not stated
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	6 days	

B. STUDY DESIGN:

1. In life dates: Start: September 9, 2003 End: January 15, 2004

2. Animal assignment: Animals were assigned to the test groups noted in Table 1 below using computer generated random numbers. Two satellite groups were employed with the I1 groups consisting of 5 rats/sex/dose which underwent perfusion fixation, and R1, which is a recovery group with a 4-week period with no exposure to the test article after the 3 months of dosing which underwent immersion fixation.

TABLE 1. Study Design[a]

Test Group	Target Dose (ppm)	Administered Dose Males (mg/kg/day)	# Male	Administered Dose Females (mg/kg/day)	# Female
0H (Control)	0	0	10	0	10
1H (Low)	80	5.2	10	6.3	10
2H (Mid)	250	16.2	10	20.9	10
3H (High)	750	51.9	10	62.0	10
0S (Control)	0	0	10	0	10
1S (Low)	80	5.1	10	6.8	10
2S (Mid)	250	16.2	10	20.7	10
3S (High)	750	51.6	10	61.4	10

[a] Data taken from pages 21 and 44 of the study report.
H = main groups
S = satellite groups (5 rats/sex/dose perfusion fixation after 3 months and 5 rats/sex/dose clinical pathology and target organ pathology after 4-week recovery)

3. Dose selection rationale: Doses were selected based on the request of the sponsor, the rationale for dose selection was not provided.

4. Dose formulation preparation and analysis: Dose formulations were prepared every 4 weeks by mixing appropriate amounts of test substance in a small amount of powdered Kliba maintenance diet (rat-mouse meal) for a premix, which was then mixed for about 10 minutes in a Ruberg EM 100 laboratory mixer with the appropriate amount of diet to obtain the desired concentrations. The dose formulations were stored at ambient temperatures. Concentration of the dose formulations were tested during Weeks 1 and 11. Stability was evaluated in a 50 mg/kg formulation prior to study initiation. Samples were stored in a freezer. Homogeneity and concentration were analyzed in all concentrations at the start and the end (samples randomly selected from food hoppers) of the administration period.

Results:

Homogeneity Analysis: The samples from the test diets were generally above 90% the nominal concentration and the standard deviations were low, thus, demonstrating these samples were homogeneous. The average % of nominal concentration was 94.8±1.6%, 96.5±1.2%, and 96.0±1.0% for the 80-, 250-, and 750-ppm dose formulations, respectively, at the start of the administration period (September 11, 2003). The average % of nominal concentration was 100.8±3.3%, 93.7±1.2%, and 90.4±0.1% for the 80-, 250-, and 750-ppm dose formulations, respectively at the end of the administration period (December 15, 2003; pp. 470-471).

Stability Analysis: The test article (50 mg/kg) in the food was found to be stable at ambient conditions for up to 32 days (p. 468). After 12 days, the sample was 92.3% of the nominal concentration and after 32 days it was 93.7%.

Concentration Analysis: The mean analytical test diet concentrations were found to be 78.2±3.1, 238±4.8, or 699±22.8 mg/kg of the diet, for the 80-, 250-, and 750-ppm dose formulations, respectively (pp. 470-471).

The analytical data indicated that the samples were stable for the 32 day utilization period. The variance between nominal and actual dosage to the animals was less than ±10% with a range of 90.4% to 100.8%, thus, the dose formulation was prepared properly.

5. Statistics: For body weight, body weight change, food consumption, and food efficiency, each treatment group was compared to the control group using Dunnett's test (two-sided). Clinical pathology, except reticulocytes and differential leukocytes, were evaluated by a non-parametric one-way Kruskal-Wallis test (two-sided). If the data were significant then a Wilcoxon-test (two-sided) test was employed. The urinalysis parameters, except volume, appearance, and specific gravity, were evaluated by a pair-wise Fischer's exact test. Organ weights were evaluated by the one-way Kruskal-Wallis test (two-sided). If the data were significant then a Wilcoxon-test (two-sided) test was employed. The statistical tests were appropriate tests for this study.

C. METHODS:

1. Observations:

1a. Cageside Observations: Animals were inspected twice a day during the week and once a day on the weekends or public holidays.

1b. Clinical Examinations: Detailed clinical observations were conducted in an open arena on Day 0, then weekly thereafter. Clinical parameters examined were as follows: behavior during handling, fur, skin, posture, salivation, respiration, arousal/activity level, tremors, convulsions,

abnormal movements, impaired gait, lacrimation, palpebral closure, exopthalmus, feces (consistency/appearance) urine, and pupil size.

1c. Neurological Evaluations: Neurological tests were not performed.

2. Body weight: Animals were weighed before exposure, on Day 0 and weekly thereafter.

3. Food consumption: Food consumption (g/animal/day) was measured weekly. Food efficiency was calculated. The calculation is provided below:

$$\frac{BW_x - BW_y}{FC_{y\,to\,x}} \times 100 = \text{Food efficiency for day x}$$

BW_x = Body weight on day x (g)
BW_y = Body weight on day y (g)
$FC_{y\,to\,x}$ = mean food consumption for day y to day x; calculated as mean consumption on day x, multiplied with the number of days from day y to day x (g)

Compound intake calculations were determined from food consumption and animal weights, the calculation is below.

$$\frac{FC_x - C}{BW_x} = \text{Substance intake for day x}$$

BW_x = Body weight on day x (g)
FC = mean daily food consumption for day x (g)
C = concentration in the diet on day x (mg/kg)

4. Ophthalmoscopic examination: Eyes were examined in all animals three days prior to study initiation and in the control and high-dose groups on Day 85.

5. Hematology & Clinical Chemistry: Blood was collected on Day 92/93 of the main testing groups (10 rats/sex/dose) and on Day 122 of the satellite groups (5 rats/sex/dose) via retroorbital sinus plexus in isofluorane-anaesthetized rats in the morning after an overnight fast. The CHECKED (X) parameters were examined in all animals.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		Heinz bodies
	(Thromboplastin time)		Red cell morphology
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

b. Clinical Chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Albumin/Globulin ratio
X	Sodium*	X	Glucose*
	ENZYMES* (2 or more)	X	Total bilirubin
X	Alkaline phosphatase (AP)	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Globulin
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)		
X	Aspartate aminotransferase (AST/also SGOT)		
	Ornithine decarboxylase		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

6. Urinalysis: Urine was collected on Day 88 on the main group without analyses of the satellite groups using steel pans placed under the cages for at least 16 hours. The CHECKED (X) parameters were examined in all animals.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

7. Sacrifice and Pathology: All animals from the main and satellite, subset group R1 (recovery animals), that died and those sacrificed (decapitated) on schedule were subjected to detailed autopsy. The CHECKED (X) tissues were collected and examined microscopically from the

control and high-dose animals, except the duodenum, brain, spinal cord, and gross lesions were examined in all of the animals in the main group. In the satellite group, the brain, spinal cord, and duodenum were examined in all animals that were not examined for neuropathology (i.e., R1, recovery animals). The tissues were preserved in neutral buffered formaldehyde (4%), embedded in paraplast, sectioned, and stained with hematoxylin. Brain and lumbar spinal cord sections for all main group and satellite group recovery animals were also sent out for peer review. In addition, the organs marked XX were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic nerve)*
X	Esophagus*	X	Bone marrow (femur)*	X	Spinal cord (cervical, thoracic, and lumbar)*
X	Stomach (fore and glandular)*	X	Lymph nodes (mandibular and mesenteric)*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*+
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland*
X	Colon*	X	Urinary bladder*	X	Parathyroid*
X	Rectum*	XX	Testes*+	X	Thyroid*
XX	Liver*+	XX	Epididymides*+		Zymbal gland
	Gall bladder (not rat)*	X	Prostate*		OTHER
	Bile duct (rat)	X	Seminal vesicles*	X	Bone (sternum with marrow)
X	Pancreas*	XX	Ovaries*+	X	Bone (femur knee joint and marrow)
	RESPIRATORY	X	Oviducts	X	Skeletal muscle
X	Trachea*	X	Vagina	X	Skin
X	Lung*	XX	Uterus*+	X	Mammary gland (female)
X	Nose*			X	All gross lesions and masses*
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.
\+ Organ weights required for 90-day oral rodent studies based on OPPTS 870.3100 guidelines.

From the satellite groups, subset I1, 5 rats/sex/dose were anesthetized (Narcoren®; 4 mL/kg) and sacrificed by perfusion fixation. Sorensen phosphate buffer was the rinsing solution, and Karnovsky was the fixative solution. The perfused animals were examined grossly as best as possible. In the perfused satellite group, the brain was weighed without the olfactory bulb, and the brain, spinal cord, and gross lesions were examined microscopically. The peripheral nerve tissues were embedded in epoxy resin, sectioned, stained with Azure II-Methylene blue-basic Fuschsin (Ambf), except the gasserian ganglia and gastrocnemius muscle, which were preserved in 4% formaldehyde. The brain, brain associated organs/tissues, spinal cord, gasserian ganglia, and gastrocnemius muscle were embedded in paraplast, sectioned, and stained with hematoxylin eosin stain. The CHECKED (X) tissues were collected and examined microscopically from the control and high-dose animals. The brain (frontal and parietal lobes), cerebellum, cervical dorsal root ganglia, lumbar spinal cord, lumbar dorsal root ganglia, lumbar ventral root, midbrain, and

pons slides for all terminal sacrifice satellite animals were examined by a peer review pathologist. The (XZ) tissues were analyzed in all of the animals.

	PERIPHERAL NERVOUS SYSTEM		BRAIN CROSS SECTIONS
X	Dorsal root ganglion 3 of (C3-C6)	XZ	Frontal
XZ	Dorsal root fiber (C3-C6)	XZ	Parietal lobe with diencephalon
XZ	Ventral root fiber (C3-C6)	XZ	Midbrain with occipital and temporal lobes
X	Dorsal root ganglion, 3 of (L1-L4)	XZ	Pons
XZ	Dorsal root fiber (L1-L4)	XZ	Cerebellum
XZ	Ventral root fiber (L1-L4)	XZ	Medulla oblongata
XZ	Proximal sciatic nerve		BRAIN ASSOCIATED ORGAN/TISSUES
X	Proximal tibial nerve (at knee)	X	Eyes
X	Distal tibial nerve (at lower leg)	X	Retina
X	Gasserian ganglia with nerve	X	Optic nerve
X	Gastrocnemius muscle		SPINAL CORD (CROSS AND LONGITUDINAL SECTIONS
		XZ	Cervical cord (C3-C6)
		XZ	Lumbar cord (L1-L4)

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity: There were no clinical signs of toxicity.

2. Mortality: All animals survived until scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN: Table 2a summarizes the body weight data for the main group. Table 2b summarizes the body weight data for the satellite group (both the perfusion and recovery groups). Body weights in 750-ppm males of the main and satellite groups were statistically-significantly less than the concurrent controls from Week 1 until Week 13. The 250-ppm males in the main group displayed a statistically-significant decrease in weight as compared to controls from Weeks 5-13. Although the body weights in the 250-ppm satellite males were also reduced as compared to the controls, the reduction was not of the same magnitude observed in the main group and the weights did not achieve statistical significance. In the main study, body weight changes in the 750-ppm males were statistically-significantly lower than controls from Week 1-13 with approximately 20-30% differences throughout. The 750-ppm males displayed statistically-significant decreases in weight gain throughout treatment, and the 250-ppm main study males displayed statistically-significant decreased weight gains during Weeks 4-13. The overall body weight gain in the main study males was lower in all treatment groups as compared to the control, but the difference was only significant in the mid- and high-dose males. While the body weight gain was significantly reduced in 750-ppm satellite males, the reduction was lower in both the low- and mid-dose groups than was observed in the main study groups.

In the main study group, the 750-ppm female's body weights were lower than those of the controls in the first week and became statistically-significant after Week 4 and for the remainder of the study. Body weights in the 750-ppm female satellite group were also reduced compared to the control with statistically-significant results for all weeks except Week 2. The 250-ppm main group females had a reduction in weight that reached statistical significance only on Week 12. Body weight gain was only affected in the high-dose females with reductions noted in both the main and satellite groups. The body weight gain in 750-ppm main group females was statistically-significantly lower than controls during Weeks 3-13, but the differences were 20-30% throughout the study. The 750-ppm satellite females had statistically-significant lower body weight gains during Weeks 1-13, except Week 2.

During the recovery period there were no statistically-significant differences in the body weights of treated males and females compared to the controls. The body weights in high-dose males and females, however, remained lower than the control. There was a dose-related increase in body weight gains during the recovery period in both males and females.

Table 2a. Average Body Weights and Total Body Weight Gains during 90 Days of Treatment in the Main Group[a]

ppm n=10	Body Weights (g±SD) % change from control						Total Weight Gain	
	Week -1	Week 1	Week 4	Week 7	Week 10	Week 13	g	%
Male								
0	200.0±9.2	262.3±15.9	389.7±30.7	461.2±39.4	509.1±37.9	527.0±41.8	327.0	-
80	199.3±7.9 -0	264.8±16.3 +1	379.2±17.9 -2	438.7±25.6 -5	489.8±33.1 -4	507.8±39.3 -4	308.5	94
250	201.5±10.4 +1	254.6±16.5 -3	369.5±26.7 -5	417.8±35.5** -9	457.9±38.5** -10	472.2±38.4** -10	270.7	83**
750	197.5±12.7 -1	240.9±12.3* -8	342.6±16.5** -12	384.5±19.8** -17	404.5±25.8** -21	414.4±28.7** -21	216.9	66**
Female								
0	161.9±7.0	190.8±12.6	252.6±19.0	276.0±28.5	294.6±32.6	307.9±31.8	146.0	-
80	159.9±7.2 -1	187.3±9.5 -2	243.5±18.2 -4	271.0±21.7 -2	286.9±21.4 -3	299.0±20.4 -3	139.1	95
250	160.4±9.9 -1	187.6±13.0 -2	239.5±13.4 -5	266.0±18.6 -4	283.5±19.9 -4	292.8±20.8 -5	132.4	91
750	161.1±8.8 -1	182.6±9.9 -4	226.6±14.9** -10	241.7±17.0** -12	253.8±16.6** -14	264.3±18.1** -14	103.2	71**

a Data obtained from pp. 81-82 and 85-86 in the study report.
* Statistically different ($p<0.05$) from the control
** Statistically different ($p<0.01$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: In the main study (satellite group discussed below), food consumption in 750-ppm treated males during the first week was approximately 19% lower and in the females was 12%, as indicated in Table 3a below. Food consumption remained lower than the controls throughout the main study in the 750-ppm group in males and females, but the difference only reached statistical significance in males during Weeks 1, 6, and 9, and in females during Weeks 1, 4, 6-9, and 11. The 250-ppm males had a slight decrease (3-7%) in food consumption until Week 11, after that the food consumption was similar to the controls. Food consumption in the 250-ppm females varied from the control, but the differences were likely due to variability as weekly changes were just as likely to be greater or lesser than the controls. An incidental statistically-significant decrease was observed in the 80-ppm females on Week 7.

TABLE 3a. Average Food Consumption (g/animal/day) during 90 Days of Treatment for the Main Group[a]

ppm (n=10)	Food consumption (g/animal/day±SD) % difference				
	Week 1	Week 4	Week 7	Week 10	Week 13
Male					
0	25.4±2.2	26.0±3.6	26.8±2.7	26.0±1.9	22.5±1.3
80	24.6±1.4 -4	25.8±1.7 -1	26.7±2.2 -1	27.4±1.9 +6	23.7±1.5 +6
250	23.7±2.3 -7	24.8±1.8 -5	25.9±2.1 -3	24.9±1.9 -4	23.5±1.7 +4
750	20.7±1.4** -19	24.7±1.7 -5	26.5±1.7 -1	25.3±1.7 -3	22.5±2.5 0
Female					
0	19.3±1.9	21.5±1.5	22.2±1.9	21.6±2.1	19.0±1.4
80	19.0±1.2 -2	20.4±1.3 -5	20.4±1.1* -8	21.0±1.1 -3	18.9±1.1 -1
250	19.0±1.3 -2	21.0±1.3 -3	21.7±1.8 -2	22.2±1.9 +3	19.8±1.4 +5
750	16.9±1.7** -12	19.3±1.5** -10	20.0±0.9** -10	19.4±1.1* -10	18.0±1.1 -5

a Data obtained from pp. 73-74 and 77-78 in the study report.
* Statistically different ($p<0.05$) from the control
** Statistically different ($p<0.01$) from the control.

As was observed in the main study animals, food consumption was lower in the 750-ppm satellite males and females compared to the controls throughout the 13 weeks of treatment (Table 3b below), but the difference only reached statistical significance in males during Weeks 1, 2, 4, 6, 7, 9, and 11, and in females during Weeks 1-4, 6-8, 10, 12, and 13. The largest decrease in food consumption was observed in the first week of treatment in both the 750-ppm males and

females with approximately 20 and 18% decreases observed. The 250-ppm males had a slight decrease in food consumption in the initial week, but the differences from the control through the remainder of the study were sporadic and were likely due to variation and not treatment-related effects. During the initial week of recovery (Week 14), all male and female treatment groups consumed more food than the controls. The 750-ppm males continued to consume greater amounts than the controls until the final week where the food consumption was similar to the controls. Mid-dose females consistently ate more than the controls during the recovery period. None of the changes in the recovery period, however, achieved statistical significance, possibly due to the small number of animals remaining (i.e., 5 rats/sex/dose).

TABLE 3b. Average Food Consumption (g/animal/day) during 90 Days of Treatment and 4 Weeks of Recovery for the Satellite Group[a]

ppm	Food Consumption (g/animal/day±SD) % difference				
	Treatment I1 and R1 (n=10)			Recovery R1 (n=5)	
	Week 1	Week 7	Week 13	Week 14	Week 17
Male					
0	25.3±1.5	26.5±2.1	25.1±1.2	24.4±2.1	25.8±2.3
80	24.8±1.8 -2	27.0±1.8 +2	26.5±1.4 +5	25.3±1.5 +4	25.7±2.7 -1
250	24.1±1.9 -5	27.3±1.9 +3	25.6±2.7 +2	26.3±2.0 +8	27.0±3.1 +5
750	20.3±1.3** -20	24.1±2.2* -9	24.7±1.6 -2	27.2±1.7 +12	26.0±1.8 +1
Female					
0	19.4±1.4	21.6±1.9	21.0±2.3	20.2±0.9	21.6±1.3
80	19.0±1.8 -2	21.0±1.9 -3	21.2±2.2 +1	21.5±1.7 +7	22.3±2.8 +3
250	19.4±1.5 +0	21.5±0.8 -0	21.5±1.0 +2	22.3±0.8 +10	22.4±1.0 +4
750	15.9±1.1** -18	19.1±1.1** -12	19.1±1.0* -9	21.4±1.6 +6	21.2±1.1 -2

a Data obtained from pp. 75-76 and 79-80 in the study report.
* Statistically different ($p<0.05$) from the control
** Statistically different ($p<0.01$) from the control.

2. Compound consumption: In the main study, based on the measured concentration of the compound in the 80-, 250-, and 750-ppm dose formulations, the average daily intakes were 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females. In the satellite study, based on the nominal concentrations, the expected doses would have been 5.1, 16.2, or 51.6 mg/kg/day, respectively, in males and 6.8, 20.7, or 61.4 mg/kg/day, respectively, in females.

3. **Food efficiency**: Select weekly food efficiency values are provided in Tables 4a (main study) and 4b (satellite study). In the main study, the 750-ppm males and females had decreased feed efficiency with only the males displaying statistically-significant values as compared to the controls (Weeks 1-5, 7, 8, and 13). There was general decrease in food efficiency in the 250- and 80-ppm males and females, but the low-dose males and females had sporadic increases. A few incidences of statistically-significant decreased feed efficiency were observed at Week 5 for 80-ppm males, Weeks 5 and 7 for 250-ppm males, and Week 4 in the 250-ppm females.

TABLE 4a. Average Food Efficiency (g animal/g food) during 90 Days of Treatment for the Main Group[a]

ppm (n=10)	Food Efficiency (g body weight/ g food weight±SD)				
	Week 1	Week 4	Week 7	Week 10	Week 13
Male					
0	34.9±3.8	19.5±3.9	10.2±4.1	6.1±4.3	-1.6±4.2
80	38.1±6.2	17.7±1.4	8.8±2.9	7.6±2.7	-2.4±3.9
250	32.0±2.1	16.8±2.6	3.1±3.2**	4.7±4.0	-2.5±2.6
750	30.0±3.8*	15.1±4.4*	4.4±3.6**	2.8±2.4	-7.0±7.0*
Female					
0	21.0±5.5	15.6±5.0	1.7±4.4	3.1±4.9	-0.6±5.1
80	20.6±3.6	10.6±4.6	5.8±6.7	1.2±5.6	-1.5±5.0
250	20.3±4.7	9.5±6.3*	5.9±5.5	2.5±5.0	6.9±21.0
750	18.2±3.8	11.8±5.4	2.8±6.5	0.1±6.9	1.8±4.0

a Data obtained from pp. 97-98 and 102-103 in the study report.
* Statistically different (p<0.05) from the control
** Statistically different (p<0.01) from the control.

In the satellite group, feed efficiency (Table 4b, below) was lower than the controls in the 750-ppm group, but the difference only reached statistical significance in males during Weeks 3-8, and in females during Weeks 3 and 5. After the test article was removed from the feed the 750-ppm males and females displayed an increase in feed efficiency during all 4 weeks with Weeks 14 and 15 in the males being statistically significant as compared to controls. The 250-ppm males had slight decreases in food efficiency, but the decreases were sporadic and did not reach statistical significance.

TABLE 4b. Average Food Efficiency (g animal/g food) during 90 Days of Treatment and 4 Weeks of Recovery for the Satellite Group[a]

ppm	Food Efficiency (g body weight/ g food weight±SD)				
	Treatment 11 and R1 (n=10)			Recovery R1 (n=5)	
	Week 1	Week 7	Week 13	Week 14	Week 17
Male					

0	34.2±5.4	9.8±2.8	1.0±2.7	2.7±1.9	5.2±3.5
80	31.7±9.0	7.9±2.3	3.5±3.2	2.6±0.7	3.4±3.4
250	34.8±2.8	6.8±2.3	4.3±2.8	4.4±2.3	3.9±2.3
750	28.4±5.6	1.7±3.8**	2.5±3.8	16.0±3.7**	5.8±3.0
Female					
0	20.8±3.7	-0.9±5.5	-0.9±3.6	5.8±5.7	5.3±6.0
80	20.3±2.8	5.2±5.8	-0.5±3.7	0.7±1.1	4.6±3.9
250	21.7±4.2	1.1±4.0	3.2±2.1	5.9±4.1	5.3±3.8
750	18.5±3.9	4.2±4.6	3.5±5.8	12.2±4.2	8.4±4.1

a Data obtained from pp. 99-101 and 104-106 in the study report.
* Statistically different (p<0.05) from the control
** Statistically different (p<0.01) from the control.

D. OPHTHALMOSCOPIC EXAMINATION: No effects related to the test article were observed. Corneal stippling and remainder of the pupillary membrane were noted in a few rats but these findings were not related to treatment.

E. BLOOD ANALYSES:

1. **Hematology:** Prothrombin time measured at the end of treatment (main study group) was statistically-significantly decreased in the males (all treatment groups) and females (750-ppm group). The decrease in females appeared to be dose-related, but the males did not display the same relation to dose (Table 5a below). The platelet counts measured after treatment (main study group) were increased in mid- and high-dosed males and all female groups with possible relation to dose. In the females the increase in platelets is equivalent to the decrease in prothrombin time. In the males, however, there was no increase in platelets in the low-dose group, but a decrease in prothrombin time was observed. There were statistically-significant, but slight (<5%), increases in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in 750-ppm males. The 250- and 750-ppm females showed slight (<5%), but statistically-significant, increases in MCHC. It was concluded that they were due to normal variation and not related to treatment. This is confirmed by the values observed after the recovery period, which showed similar slight (≤5%) differences between the treatment group and the control.
2.

TABLE 5a. Selected Hematology Parameters for Main Study[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
MCV	MEAN	51.1	51.2	50.6	51.8	52.7	51.2	52.1	51.3
fL	S.D.	2.2	1.6	1.5	2.5	1.8	1.8	1.0	1.5
	% [b]	-	+0	-1	+1	-	-3	-1	-3
	N	10	10	10	10	10	10	10	10

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
MCH Fmol	MEAN	1.06	1.05	1.04	1.10*	1.12	1.10	1.13	1.11
	S.D.	0.04	0.04	0.03	0.05	0.04	0.04	0.02	0.04
	% [b]	-	-1	-2	+4	-	-2	+1	-1
	N	10	10	10	10	10	10	10	10
MCHC mmol/L	MEAN	20.66	20.52	20.62	21.25**	21.22	21.35	21.59*	21.61**
	S.D.	0.24	0.26	0.40	0.40	0.25	0.45	0.31	0.39
	%	-	-1	-0.2	+3	-	+1	+2	+2
	N	10	10	10	10	10	10	10	10
Platelets giga/L	MEAN	1144	1142	1230	1320**	1036	1075	1113	1131
	S.D.	176	109	232	125	120	150	135	277
	%	-	-0	+8	+15	-	+4	+7	+9
	N	10	10	10	10	10	10	10	10
Prothrombin time Sec	MEAN	35.0	32.2**	33.4*	31.9**	29.6	28.8	27.8	26.9**
	S.D.	1.7	1.9	1.7	2.3	2.2	1.8	1.5	1.3
	%	-	-8	-5	-9	-	-3	-6	-9
	N	10	10	10	10	10	10	10	10

a Data obtained from pages 119, 121, 131, and 133 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

After a 4-week recovery period (satellite group; R1), the only statistically-significant finding was an incidental increase in MCV in 750-ppm males (Table 5b below). The platelet counts in the males, however, still demonstrated a dose-related increase, but the prothrombin time was back to control values indicating a reversal of the effect.

TABLE 5b. Selected Hematology Parameters for Satellite Study (R1)[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
MCV fL	MEAN	50.4	50.3	49.6	52.4*	53.6	52.4	51.9	51.4
	S.D.	1.2	1.1	0.9	1.6	2.6	1.8	1.9	0.9
	% [b]	-	-0	-2	+4	-	-2	-3	-4
	N	5	5	5	5	5	5	5	5
MCH Fmol	MEAN	1.03	1.03	1.02	1.08	1.13	1.10	1.09	1.10
	S.D.	0.03	0.03	0.02	0.04	0.04	0.03	0.04	0.03
	% [b]	-	0	-1	+5	-	-3	-4	-3
	N	5	5	5	10	5	5	5	5
MCHC mmol/L	MEAN	20.54	20.52	20.62	20.59	21.09	21.09	21.08	21.50
	S.D.	0.15	0.28	0.40	0.29	0.28	0.19	0.29	0.25
	% [b]	-	-0	+0	+0	-	0	-0	+2
	N	5	5	5	5	5	5	5	5
Platelets giga/L	MEAN	1110	1136	1236	1243	1114	1026	1183	1030
	S.D.	101	91	202	175	108	66	112	154
	% [b]	-	+2	+11	+12	-	-8	+6	-8
	N	5	5	5	5	5	5	5	5
Prothrombin time Sec	MEAN	32.5	32.1	33.2	32.1	29.1	28.0	28.1	28.9
	S.D.	1.4	1.8	3.0	1.8	2.3	1.8	0.9	1.7
	% [b]	-	-1	+2	-1	-	-4	-3	-1
	N	5	5	4	5	4	5	5	5

a Data obtained from pages 120, 122, 132, and 134 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

2. **Clinical Chemistry:** Selected clinical chemistry parameters are provided in Tables 6a (main study) and 6b (satellite group; R1). At the end of treatment (main study group), males demonstrated a statistically-significant increase in ALT across all treatment groups that was

apparently dose-related. The same effect was not noted in females, who had a statistically-significant decrease in ALT in the low-dose. While there were still differences in the ALT after the recovery period (Table 6b), there was no relation to dose in either the males or females; therefore, it is concluded that, in the absence of liver histopathology findings, the effects were reversed. ALP also was statistically-significantly increased in all male treatment-groups at the end of treatment. While the females also had increases across all treatment groups, only the high-dose group was increased by a statistically significant amount. After the recovery period, the ALP in the high-dose males was increased (not significantly) as compared to concurrent controls. The difference from controls, however, was lower than at the end of treatment for all groups indicating recovery. Phosphorous levels at the end of treatment were increased in a dose-dependent manner in both males and females with high-dose males and females reaching statistical significance. The values remained elevated (not significant) after the recovery period in mid- and high-dose males and females. Urea levels at the end of treatment were statistically-significantly increased in mid- and high-dose males (dose-dependent) and high-dose females. This effect was reversible with a 4-week recovery period. Creatinine was statistically-significantly increased in high-dose males after treatment. Female creatinine levels, however, displayed a statistically-significant decrease in the low- and mid-dose groups. There were no changes noted after the recovery period. Glucose was statistically-significantly decreased in high-dose males and mid- and high-dose females (dose-related). After the recovery period, the glucose was still lower (not significantly) than the control in these groups. Albumin was statistically-significantly decreased in high-dose females while globulin had a statistically significant increase in mid- and high-dose females (dose-dependent). The same was not observed in the males and was reversible after a 4-week recovery period. Triglycerides were decreased in a dose-dependent manner in males with the high-dose statistically-significantly lower than the control. While the triglycerides remained low after the recovery period in all groups, the difference was no longer related to dose. Cholesterol was increased in high-dose males (not significant) and in all treated females (statistically significant in the mid- and high-dose groups only). These changes were reversible after recovery. Magnesium was increased in high-dose males (not significant) and females (statistically significant). The values remained elevated after the recovery period; however, the results did not achieve statistical significance. There also was an incidental statistically-significant decrease in serum chloride levels in mid-dose females.

TABLE 6a. Selected Clinical Chemistry Parameters Main Group[a]

Observation		Male				Female			
ppm		0	80	250	750	0	80	250	750
ALT µkat/L	MEAN	0.65	0.76*	0.82**	1.06**	0.74	0.60*	0.65	0.80
	S.D.	0.04	0.21	0.24	0.39	0.12	0.13	0.15	0.22
	%[b]	-	+17	+26	+63	-	-19	-12	+8
	N	10	10	10	10	10	10	10	10
ALP µkat/L	MEAN	1.31	1.60*	1.47*	2.21**	0.54	0.58	0.57	0.73**
	S.D.	0.15	0.25	0.17	0.75	0.12	0.10	0.16	0.13
	%	-	+19	+12	+69	-	+7	+6	+35
	N	10	10	10	10	10	10	10	10
Phosphorus mmol/L	MEAN	2.08	2.12	2.18	2.36**	1.60	1.63	1.72	1.88**
	S.D.	0.12	0.09	0.15	0.11	0.16	0.15	0.18	0.13
	%	-	+2	+5	+14	-	+2	+8	+18
	N	10	10	10	10	10	10	10	10

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
Urea mmol/L	MEAN	5.23	5.27	5.90*	7.63**	7.05	6.69	7.27	9.25**
	S.D.	0.48	0.49	0.75	0.65	0.61	0.90	1.32	1.25
	%	-	+0.8	+12	+46	-	-5	+3	+31
	N	10	10	10	10	10	10	10	10
Creatinine µmol/L	MEAN	47.1	47.5	48.1	55.8**	57.0	52.8*	51.7*	55.0
	S.D.	3.8	2.5	2.9	5.0	4.9	4.7	4.1	3.7
	%	-	+1	+2	+19	-	-7	-9	-4
	N	10	10	10	10	10	10	10	10
Glucose mmol/L	MEAN	6.72	6.85	6.23	5.59**	6.95	6.78	6.25**	5.66**
	S.D.	0.51	0.54	0.32	0.59	0.33	0.65	0.40	0.35
	%	-	+2	-7	-17	-	-2	-10	-18
	N	10	10	10	10	10	10	10	10
Albumin g/L	MEAN	36.18	35.72	35.75	35.31	37.99	38.04	37.65	34.82**
	S.D.	0.89	1.62	1.47	1.24	2.51	1.77	1.40	0.95
	%	-	-1	-1	-2	-	0	-1	-8
	N	10	10	10	10	10	10	10	10
Globulin g/L	MEAN	32.05	31.62	31.38	33.76	27.18	27.62	28.69**	30.98**
	S.D.	1.85	2.59	1.73	2.75	1.55	1.36	1.27	1.61
	%	-	-1	-2	+5	-	+2	+6	+14
	N	10	10	10	10	10	10	10	10
Trigyceride mmol/L	MEAN	0.70	0.64	0.46	0.38*	0.45	0.45	0.48	0.45
	S.D.	0.30	0.21	0.13	0.13	0.11	0.11	0.15	0.09
	%	-	-20	-34	-46	-	0	+7	0
	N	10	10	10	10	10	10	10	10
Cholesterol mmol/L	MEAN	1.89	1.91	1.83	2.12	2.10	2.29	2.91**	3.19**
	S.D.	0.47	0.53	0.36	0.50	0.42	0.36	0.36	0.46
	%	-	+1	-3	+12	-	+9	+39	+52
	N	10	10	10	10	10	10	10	10
Magnesium mmol/L	MEAN	0.89	0.89	0.90	0.95	0.95	0.96	0.99	1.03**
	S.D.	0.05	0.06	0.03	0.07	0.04	0.03	0.05	0.05
	%	-	0	+1	+7	-	+1	+4	+8
	N	10	10	10	10	10	10	10	10

a Data obtained from pages 135, 137, 139-140, 143-144 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

TABLE 6b. Selected Clinical Chemistry Parameters Satellite Group (R1)[a]

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
ALT µkat/L	MEAN	0.73	1.05	0.68	0.98	0.81	0.73	1.44	0.78
	S.D.	0.03	0.62	0.07	0.54	0.24	0.10	1.22	0.21
	%[b]	-	+44	-7	+34	-	-10	+78	-4
	N	5	5	5	5	5	5	5	5
ALP µkat/L	MEAN	1.26	1.27	1.09	1.47	0.50	0.45	0.46	0.51
	S.D.	0.15	0.27	0.16	0.17	0.14	0.05	0.14	0.13
	%	-	+0.8	-14	+17	-	-10	-8	+2
	N	5	5	5	5	5	5	5	5
Phosphorus mmol/L	MEAN	1.93	1.93	2.03	2.09	1.52	1.50	1.66	1.60
	S.D.	0.08	0.07	0.18	0.11	0.16	0.21	0.12	0.24
	%	-	0	+5	+8	-	-1	+9	+5
	N	5	5	5	5	5	5	5	5
Urea mmol/L	MEAN	5.60	5.67	5.54	5.75	7.03	6.78	7.42	7.24
	S.D.	0.53	0.54	0.63	0.36	1.57	0.84	0.75	1.19
	%	-	+1	+1	+3	-	-4	+6	+3
	N	5	5	5	5	5	5	5	5

Observation ppm		Male				Female			
		0	80	250	750	0	80	250	750
Creatinine µmol/L	MEAN	49.2	52.1	53.0	49.4	57.2	54.6	53.8	53.9
	S.D.	1.2	6.1	3.3	2.7	7.3	5.6	2.5	5.8
	%	-	+6	+8	+0	-	-5	-6	-6
	N	5	5	5	5	5	5	5	5
Glucose mmol/L	MEAN	6.63	7.01	7.50	6.41	7.11	6.75	6.52	6.44
	S.D.	0.48	0.29	0.55	0.42	0.41	0.23	0.43	0.44
	%	-	+6	+13	-3	-	-5	-8	-9
	N	5	5	5	5	5	5	5	5
Albumin g/L	MEAN	36.66	36.45	36.91	36.63	39.22	39.21	38.22	38.04
	S.D.	1.26	1.76	1.10	1.20	1.22	0.66	1.37	1.02
	%	-	-1	+1	0	-	0	-3	-3
	N	5	5	5	5	5	5	5	5
Globulin g/L	MEAN	32.55	33.52	32.32	30.42	28.05	29.03	29.31	28.52
	S.D.	1.84	2.62	1.83	2.07	2.29	1.20	1.48	2.25
	%	-	+3	-1	-7	-	+3	+4	+2
	N	5	5	5	5	5	5	5	5
Trigyceride mmol/L	MEAN	0.75	0.61	0.73	0.46	0.55	0.48	0.46	0.59
	S.D.	0.64	0.17	0.27	0.17	0.10	0.08	0.09	0.14
	%	-	-19	-3	-39	-	-13	-16	+7
	N	5	5	5	5	5	5	5	5
Cholesterol mmol/L	MEAN	2.05	2.10	1.90	2.07	2.32	2.17	2.67	2.43
	S.D.	0.54	0.30	0.52	0.46	0.52	0.40	0.13	0.76
	%	-	+2	-7	-1	-	-6	+15	+5
	N	5	5	5	5	5	5	5	5
Magneslum mmol/L	MEAN	0.85	0.84	0.87	0.89	0.92	0.94	0.93	0.98
	S.D.	0.05	0.03	0.01	0.04	0.07	0.05	0.03	0.04
	%	-	-1	+12	+5	-	+2	+1	+7
	N	5	5	5	5	5	5	5	5

a Data obtained from pages 136, 138, 141-142, 145-146 of the study report.
b % difference from control, calculated by reviewers; N = number of animals
* Statistically different (p <0.05) from the control
** Statistically different (p <0.01) from the control

F. Urinalysis - No abnormal or treatment-related findings were observed in the urinalysis data.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: Terminal body weight was statistically-significantly reduced in mid- and high-dose males and high-dose females, which affected the absolute and/or relative weights of several organs (Table 7a below). The relative weights of heart and adrenals (not significant in females) were statistically-significantly increased in high-dose males and females. These changes are considered related to reduced body weight and not directly related to treatment as there are no macroscopic or microscopic changes observed in these organs Relative spleen weights were statistically-significantly increased in low- (absolute also increased), mid-, and high-dose females and mid-and high-dose males. While the increase in males appears to be related to a decrease in body weight, this cannot explain the increase in the low-dose females. There was a slight increase in extramedullary hematopoiesis in high-dose males and females (other groups not examined). Absolute kidney weight was decreased in mid- and high-dose males, and high-dose females with the low-dose females displaying a statistically-significant increase. This was also related to the decrease in body weight as similar decreases were observed and there were no changes in the absolute weight. Thymus absolute and relative weight ratios were statistically-significantly decreased in high-dose males. Statistically-

significant increases in epididymal and testes relative weights were increased in the high-dose males, with relative epididymides weight being statistically-significantly increased in the mid-dose males as well. These changes also are likely due to reduced body weight and are not biologically significant as there were no pathological changes in these organs. A dose-related increasing trend was observed in the absolute and relative liver weights in the females and relative weights in males. Although there are no pathology results to support hepatotoxicity, increases in clinical chemistry parameters (i.e., ALT and ALP) with reversibility noted, indicate that the liver was affected by treatment, and that the changes were due to increased metabolism of the test article. Mid- and high-dose males and high-dose females had increased relative brain weights in the main study (Table 7a below). The satellite group I1 which were perfuse fixed before weighed demonstrated an increase in relative brain weight in high-dose males (statistically significant) and females (not significant), a statistically-significant increase in the absolute brain weight in low-dose males, and a statistically-significant decrease in the absolute brain weight in high-dose females. Although the changes in weight may be related to changes in body weight, there were several lesions in the brain noted in histopathology including multifocal vacuolation that indicate the brain as a target organ.

TABLE 7a. Selected Absolute and Relative Organ Weights from the Main Group[a]

Dose Ppm		Males				Females			
		0	80	250	750	0	80	250	750
Number of animals		10	10	10	10	10	10	10	10
Terminal Body Wt. (g)	MEAN	489.16	469.61	436.91*	379.01**	286.34	280.84	274.38	243.15**
	S.D.	39.509	36.571	39.198	24.597	30.099	18.562	21.782	15.165
	%[b]	-	-4	-11	-23	-	-2	-4	-15
BRAIN									
Absolute (g)	MEAN	2.136	2.135	2.105	2.060	1.967	2.037	2.014	1.977
	S.D.	0.053	0.087	0.094	0.077	0.063	0.061	0.089	0.103
	%	-	-0	-1	-4	-	+4	+2	+1
Per Body Weight (‰)	MEAN	0.439	0.457	0.485*	0.545**	0.693	0.729	0.737	0.815**
	S.D.	0.032	0.033	0.040	0.035	0.071	0.059	0.055	0.055
	%	-	+4	+11	+24	-	+5	+0	+18
HEART									
Absolute (g)	MEAN	1.387	1.383	1.314	1.302	0.952	0.992	0.986	0.926
	S.D.	0.125	0.150	0.112	0.097	0.058	0.080	0.060	0.061
	%	-	-0.3	-5	-6	-	+4	+4	-3
Per Body Weight (‰)	MEAN	0.284	0.294	0.302	0.344**	0.335	0.354	0.360	0.381**
	S.D.	0.016	0.019	0.022	0.018	0.028	0.027	0.020	0.019
	%	-	+4	+6	+21	-	+6	+7	+14
LIVER									
Absolute (g)	MEAN	11.863	11.870	11.340	11.469	7.397	7.804*	7.928**	8.727**
	S.D.	1.163	1.583	1.125	1.107	0.712	0.432	0.737	0.447
	%	-	+0	-4	-3	-	+6	+7	+18
Per Body Weight (‰)	MEAN	2.424	2.519	2.597**	3.029**	2.595	2.785*	2.892*	3.594**
	S.D.	0.109	0.183	0.135	0.266	0.221	0.159	0.162	0.160
	%	-	+4	+7	+25	-	+7	+11	+39

Dose Ppm		Males				Females			
		0	80	250	750	0	80	250	750
KIDNEYS									
Absolute (g)	MEAN	3.175	3.095	2.877**	2.420**	1.979	2.111*	2.064	1.792**
	S.D.	0.212	0.283	0.318	0.213	0.143	0.157	0.169	0.158
	%	-	-3	-9	-24	-	+7	+4	-9
Per Body Weight (‰)	MEAN	0.651	0.660	0.659	0.640	0.697	0.752	0.754	0.738
	S.D.	0.044	0.043	0.042	0.055	0.075	0.043	0.058	0.059
	%	-	+1	+1	-2	-	+8	+8	+6
SPLEEN									
Absolute (g)	MEAN	0.716	0.710	0.779	0.711	0.599	0.701**	0.651	0.589
	S.D.	0.051	0.063	0.146	0.091	0.078	0.070	0.089	0.073
	%	-	-1	+9	-1	-	+17	+9	-2
Per Body Weight (‰)	MEAN	0.147	0.152	0.178**	0.189**	0.209	0.251**	0.237*	0.242**
	S.D.	0.013	0.019	0.025	0.029	0.015	0.031	0.023	0.028
	%	-	+3	+21	+29	-	+20	+13	+16
ADRENAL									
Absolute (mg)	MEAN	56.60	56.30	54.50	59.00	90.1	96.2	94.8	87.8
	S.D.	6.310	7.056	6.151	8.919	8.062	13.677	9.841	13.147
	%	-	-1	-4	+4	-	+7	+5	-3
Per Body Weight (‰)	MEAN	0.012	0.012	0.013	0.016**	0.032	0.035	0.035	0.036
	S.D.	0.001	0.001	0.001	0.002	0.005	0.007	0.003	0.06
	%	-	0	+8	+33	-	+9	+9	+13
THYMUS									
Absolute (mg)	MEAN	248.20	217.30	256.00	148.20**	303.1	294.9	300.2	281.7
	S.D.	47.735	51.968	51.223	33.819	53.949	60.080	43.741	62.029
	%	-	-12	+3	-40	-	-3	-1	-7
Per Body Weight (‰)	MEAN	0.051	0.046	0.059	0.039*	0.106	0.105	0.110	0.116
	S.D.	0.010	0.009	0.011	0.009	0.014	0.020	0.014	0.023
	%	-	-10	+16	-24	-	-1	+4	+9
EPIDIDYMIDES									
Absolute (mg)	MEAN	1.266	1.200	1.255	1.173				
	S.D.	0.107	0.076	0.102	0.131				
	%	-	-5	-1	-7				
Per Body Weight (‰)	MEAN	0.260	0.257	0.289*	0.310**				
	S.D.	0.031	0.030	0.033	0.031				
	%	-	-1	+11	+19				
TESTES									
Absolute (mg)	MEAN	3.548	3.315	3.371	3.571				
	S.D.	0.269	0.222	0.288	0.422				
	%	-	-7	-5	+1				
Per Body Weight (‰)	MEAN	0.729	0.708	0.778	0.944**				
	S.D.	0.078	0.043	0.096	0.144				
	%	-	-3	+7	+30				

a Data obtained from pages 151-154 in the study report.
b % difference from control, calculated by reviewers

* Statistically different (p <0.05) from the control
** Statistically different (p<0.01) from the control

Table 7b summarizes the brain weights (the only organ weighed) for satellite group I1 after perfuse fixation. The high-dose male and female final weights were statistically-significantly decreased, which led to increases in relative brain weight in the high-dose males (significant) and high-dose females (not significant) compared to control weights.

TABLE 7b. Selected Absolute and Relative Brain Weights in the Satellite Group (I1)[a]

		Males				Females			
Dose ppm		0	80	250	750	0	80	250	750
Number of animals		5	5	5	5	5	5	5	5
Terminal Body Wt. (g)	MEAN	512.76	503.20	467.46	383.86**	293.56	265.94	287.10	244.9**
	S.D.	29.433	36.418	42.403	25.596	22.709	20.046	22.086	10.173
	%[b]	-	-2	-9	-25	-	-9	-2	-17
BRAIN									
Absolute (g)	MEAN	2.062	2.144*	1.974	1.960	1.924	1.870	1.932	1.830**
	S.D.	0.068	0.30	0.097	0.111	0.043	0.089	0.062	0.071
	%	-	+4	-4	-5	-	-3	+0	-5
Per Body Weight (‰)	MEAN	0.403	0.428	0.425	0.512**	0.660	0.707	0.676	0.748
	S.D.	0.010	0.027	0.041	0.029	0.068	0.069	0.056	0.030
	%	-	+6	+5	+27	-	+7	+2	+13

a Data obtained from pages 155-158 in the study report.
b % difference from control, calculated by reviewers
* Statistically different (p <0.05) from the control
** Statistically different (p<0.01) from the control

In the satellite group R1, the recovery group, the high-dose males still had statistically-significant decreased body weight and the high-dose females had reduced weight that did not achieve statistical significance (Table 7c below). The treated females displayed statistically-significantly increased liver weight. A statistically-significant increase in relative testes weight of 7.91% was observed in the low-dose males; however, this was considered to be unrelated to treatment because no lesions were observed and the change was not observed in the higher doses. A statistically-significant decrease in kidney weight was also observed, but was related to the reduced body weight in this group as there were no pathological findings nor were there any other changes notes.

TABLE 7c. Selected Absolute and Relative Liver Weights in the Satellite Group (R1)[a]

		Males				Females			
Dose ppm		0	80	250	750	0	80	250	750
Number of animals		5	5	5	5	5	5	5	5
Terminal Body Wt. (g)	MEAN	495.52	521.10	500.90	452.76*	291.56	293.54	307.18	275.82
	S.D.	19.76	38.449	30.890	29.161	16.581	30.166	11.111	19.314
	%[b]	-	+5	+1	-9	-	+1	+5	-5
LIVER									
Absolute (g)	MEAN	12.35	12.998	13.218	11.226	7.106	7.792	8.122**	7.740
	S.D.	0.916	0.879	1.778	1.100	0.267	0.687	0.395	0.568
	%	-	-1	-4	-15	-	+10	+14	+9
Per Body Weight (‰)	MEAN	2.490	2.496	2.631	2.477	2.441	2.659 *	2.644 *	2.812*
	S.D.	0.098	0.063	0.218	0.149	0.101	0.070	0.094	0.205
	%	-	+0	+6	-1	-	+9	+8	+15

a Data obtained from pages 159-162 in the study report.
b % difference from control, calculated by reviewers
* Statistically different ($p < 0.05$) from the control
** Statistically different ($p < 0.01$) from the control

2. Gross pathology: There were no treatment-related changes in macroscopic lesions in the main and 11 satellite group

3. Microscopic pathology: All slides from the various treatment groups were peer reviewed and any changes are contained in the tables.

Table 8a summarizes treatment related lesions and severity of the lesions observed in the main group after treatment. Increases in the incidence of hyperplasia of the mucosal cells of the duodenum were noted in the mid- and high-dose animals with the females being more sensitive than the males.

There was a slight increase in extramedullary hematopoiesis in the spleen, which coincides with changes in the spleen weight and changes in a few hematological parameters associated with red blood cells.

The brain, thoracic and lumbar cord displayed a treatment related increase in the incidence and severity of multifocal vacuolation in the mid- and high-dose animals. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8a below. Further investigations on changes of the brain are addressed in the I1 satellite group that underwent perfusion and detailed brain necropsy.

Table 8a. Incidences of Selected Histopathology Findings After Treatment in the Main Group[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
N	10	10	10	10	10	10	10	10
Duodenum	10	10	10	10	10	10	10	10
Mucosal hyperplasia	0	0	2	2	0	0	7	8
Minimal	0	0	1	2	0	0	6	5
Slight	0	0	1	0	0	0	1	3
Spleen	10	-	-	10	10	-	-	10
Extramedullary hematopoiesis	0	-	-	1	1	-	-	3
Brain	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	1	10/9[b]	0	5/0	1/3	9/8
Minimal	0	0	1	7/6	0	5/0	1/3	3/4
Slight	0	0	0	1	0	0	0	3/1
Moderate	0	0	0	2	0	0	0	3
Focal calcification	0	0	0	1	0	0	0	0
Ventricles dilation	0	1	0	0	0	0	0	0
Moderate	0	1	0	0	0	0	0	0
Thoracic cord	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	0	0	0	0	0	2
Moderate	0	0	0	0	0	0	0	1
Marked/Severe	0	0	0	0	0	0	0	1
Lumbar cord	10	10	10	10	10	10	10	10
Multifocal vacuolation	0	0	8	10	0	5	9	10
Minimal	0	0	6	0	0	3	3	0
Slight	0	0	2	2	0	2	4	0
Moderate	0	0	0	6	0	0	2	3
Marked/Severe	0	0	0	2	0	0	0	7

a Data obtained from pages 166-167 and 170 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement.

Animals that underwent *in situ* perfusion displayed changes to almost all areas of the brain and spinal cord in the mid- and high-dose animals with the females appearing to be more sensitive (Table 8b). The high-dose males displayed slight or minimal multifocal vacuolization in the

frontal lobe, parietal lobe, midbrain, pons, cerebellum, cervical cord, and lumbar cord. The high-dose females displayed slight to moderate multifocal vacuolization in the frontal lobe, parietal lobe, midbrain, pons, medulla oblongata, cerebellum, and lumbar cord. The mid-dose females displayed slight or minimal multifocal vacuolization in the frontal lobe, parietal lobe, midbrain, pons, cerebellum, and lumbar cord. However, the mid-dose males did not have any incidence of multifocal vacuolization of any area of the brain or spinal cord at any level. Almost all high-dose males displayed minimal to marked myelin vacuolation cervical dorsal root, cervical ventricle root, lumbar dorsal root, lumbar ventricle root, and proximal sciatic nerve with the lumbar region being most profoundly affected. In the high-dose females, minimal to marked myelin vacuolation cervical dorsal root, lumbar dorsal root, lumbar ventricle root, and proximal sciatic nerve with the lumbar dorsal region were most profoundly affected. The mid-dose males and females displayed minimal to slight myelin vacuolation of the cervical ventricle root, and lumbar dorsal root, lumbar ventricle root. Low-dose females had slight myelin vacuolation of the lumbar dorsal root and lumbar ventricle root. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8b below.

Table 8b. Incidences of Neurological Histopathology Findings After Treatment in the Satellite Group (I1)[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Frontal Lobe	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	2/3[b]	0	0	2	5/4
Minimal	0	0	0	1/3	0	0	2	3/4
Slight	0	0	0	1/0	0	0	0	2/0
Parietal Lobe	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	4	0	0	2	4/5
Minimal	0	0	0	2	0	0	1/2	1/2
Slight	0	0	0	2	0	0	1/0	2
Moderate	0	0	0	0	0	0	0	1
Midbrain	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	1	5/4
Minimal	0	0	0	1/3	0	0	1	2/1
Slight	0	0	0	2/0	0	0	0	2
Moderate	0	0	0	0	0	0	0	1
Pons	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	2/1	4/2

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Minimal	0	0	0	1/2	0	0	2/1	2/1
Slight	0	0	0	2/1	0	0	0	2/1
Cerebellum	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	4/3	0	0	1/2	5
Minimal	0	0	0	2/3	0	0	0/1	1/2
Slight	0	0	0	2/0	0	0	1	3/2
Moderate	0	0	0	0	0	0	0	1
Medulla Oblongata	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	0	0	0	0	2
Slight	0	0	0	0	0	0	0	2
Cervical Cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Dorsal Root Cervical	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	1/2	4	0	0	2/3	2/3
Minimal	0	0	1/2	1/3	0	0	1/2	0/1
Slight	0	0	0	1/1	0	0	1	1
Moderate	0	0	0	2/0	0	0	0	1
Ventral Root Cervical	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	0	2	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Lumbar cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	2/3	5	0	0	1/2	4/5
Minimal	0	0	2/3	2	0	0	1/2	2/3
Slight	0	0	0	2/3	0	0	0	2
Moderate	0	0	0	1/0	0	0	0	0
Lumbar Dorsal Root	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	3	5	0	2/1	4/5	5
Slight	0	0	1/2	0	0	2/1	1/2	2/3

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Minimal	0	0	2/1	1	0	0	2/3	1/0
Moderate	0	0	0	2	0	0	1/0	1
Marked/Severe	0	0	0	2	0	0	0	1
Lumbar Ventral Root	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	1/2	5	0	1	3/4	5
Slight	0	0	1/2	0	0	1	0/1	0
Minimal	0	0	0	2	0	0	3	1
Moderate	0	0	0	1	0	0	0	4
Marked/Severe	0	0	0	2	0	0	0	0
Proximal Sciatic Nerve	5	5	5	5	5	5	5	5
Myelin vacuolation	0	0	0	5	0	0	0	1
Slight	0	0	0	1	0	0	0	0
Minimal	0	0	0	3	0	0	0	1
Moderate	0	0	0	1	0	0	0	0
Axonal degeneration	0	0	0	0	0	0	1	1
Slight	0	0	0	0	0	0	1	1
Proximal Tibial Nerve	5	5	5	5	5	5	5	5
Axonal degeneration[c]	0	0	0	0	0	0	0	1

a Data obtained from pages 168, 171-172 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement
c Severity was not provided.

After 4 weeks of no exposure to the test article, the brain and lumbar cord still display signs of injury as summarized in Table 8c. There was a decrease in the severity of the lesions but the low-, mid- and high-dose females and mid- and high-dose males still had multifocal vacuolization of the lumbar cord. The brain was noted with multifocal vacuolization in the high-dose males and females and mid-dose females. These results were confirmed by a peer review pathological examination, although a few minor discrepancies between the results of the two pathologists are noted in Table 8c below.

Table 8c. Incidences of Brain Histopathology Findings After Treatment in the Satellite Group (R1)[a]

Microscopic Observations								
Sex	Male				Female			
ppm	0	80	250	750	0	80	250	750
n	5	5	5	5	5	5	5	5
Brain	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	0	3	0	0	1/2[b]	4
Minimal	0	0	0	3	0	0	1/2	3
Slight	0	0	0	0	0	0	0	1
Lumbar cord	5	5	5	5	5	5	5	5
Multifocal vacuolation	0	0	2/3	5	0	2	5/4	5
Minimal	0	0	2	0	0	2	2/1	1
Slight	0	0	0/1	3	0	0	2	2
Moderate	0	0	0	2	0	0	1	2

a Data obtained from pages 168, 171-172 in the study report.
b Data obtained from pages 166-167 and 170 in the original study report/data obtained from pages 17-19 of the Pathology Peer Review Statement

III. DISCUSSION and CONCLUSIONS

A. Results of the neurological pathology from a 90-day oral toxicity study (MRID 46442201) were confirmed in a Pathology Peer Review Statement (MRID 46659701). In the 90-day oral toxicity study, R 107894 (1H-Pyrrole-3-carbonitrile, 4-bromo-2-[4-chlorophenyl]-5-[trifluoromethyl]; 94.6% a.i.) was administered to 10 Sprague-Dawley rats/sex/dose at concentrations of 0, 80, 250, or 750 ppm via the feed. The doses were calculated to be equivalent 5.2, 16.2, or 51.9 mg/kg/day, respectively, in males and 6.3, 20.9, or 62.0 mg/kg/day, respectively, in females. The analytical concentration was on average 90.4-100.8% of the nominal concentration. Additional 5 rats/sex/group (I1) were administered the same concentrations of test article, sacrificed at the end of treatment, and *in situ* perfusion fixed for histological examination of the nervous system. Another 5 rats/sex/group (R1) were treated in the same manner and were removed from treatment after 13 weeks and allowed a 4-week recovery period, after which they were sacrificed for evaluation after immersion fixation. These additional 10 rats/sex/group were considered a satellite group. Intake of test compound was similar to that observed in the main group.

There were no treatment-related effects noted in clinical signs, mortality, ophthalmology or urinalysis in treated animals. Our reviewers agree with the study authors that the MTD was exceeded in the high-dose, because weight gain was 29% lower than the controls. Several signs of toxicity were observed in the high-dose animals such as decreased body weight; decreased food consumption; decreased prothrombin time and increased platelet counts; increased ALT, ALP, phosphorus, blood urea nitrogen, creatinine (males only), cholesterol, and magnesium, decreased glucose and triglycerides (males only); increased relative organ weights (brain, heart,

liver, spleen, adrenals, epididymides, and testes); mucosal hyperplasia of the duodenum, multifocal vacuolation of the CNS and multifocal intramyelinic vacuolation of the proximal peripheral nervous system. Although the changes in the red blood cell parameters (MCH and MCHC) were likely treatment-related due to the fact that they were consistent in the high-dose group and changes were noted in the spleen (i.e., weight and pathology), the changes were ≤5% different from the control. The clinical chemistry changes were associated with the severely reduced weight of these animals and possibly to a disrupted liver function.

The mid-dose animals displayed to a lesser extent than high-dose animals reduced weight and food consumption; decreased prothrombin time and increased platelet counts; increased ALT (males only), ALP, phosphorus, blood urea nitrogen, creatinine (males only), cholesterol (females only), and magnesium, decreased glucose and triglycerides (males only); increased relative organ weights (brain [males only], heart, liver, spleen, adrenals, epididymides, and testes); mucosal hyperplasia of the duodenum, multifocal vacuolation of the CNS and multifocal intramyelinic vacuolation of the proximal peripheral nervous system. As in the high-dose animals most signs of toxicity showed recovery except relative liver weight in females and multifocal vacuolation of the brain and lumbar cord. In the low-dose females minimal multifocal vacuolation of the brain and lumbar cord, and slight myelin vacuolation in the lumbar dorsal and ventral root was observed. After 4-week cessation of treatment the low-dose females still displayed minimal multifocal vacuolation of the lumbar cord.

Although the study authors state in many cases that these changes were reversible after a 4-week recovery period due to a lack of statistical significance after the recovery period, this may be due to the small number of animals (i.e., 5) measured after the recovery period. Many instances (i.e., platelets in mid-and high-dose males, ALT and ALP in high-dose males, phosphorous in mid- and high-dose males and females, glucose in high-dose males and all treated females, triglycerides in high-dose males, and magnesium in high-dose males and females) indicate residual effects even though the results did not achieve statistical significance.

The target organ is the white matter of the CNS (brain, spinal cord) and PNS (ventral and dorsal root) without apparent functional alteration as observed with clinical signs of toxicity. This study lacked a neurological examination (e.g., FOB), which could have greatly added to the toxicological evaluation. The Pathology Peer Review Statement generally concurred with the neurological pathology findings in the report. There are several instances where the Peer Reviewers differed on the severity of lesions and in the number of lesions; however, these differences of opinion did not change the overall findings on the neurological pathology of the report.

The LOAEL is 16.2 mg/kg/day in males based on reduced body weight and weight gain, reduced food consumption, hematology, clinical chemistry, organ weights, and microscopic findings of the brain and spinal cord and 6.3 mg/kg/day (LDT) in females based microscopic findings of the brain and spinal cord. The NOAEL is 5.2 mg/kg/day in males. The NOAEL could not be determined in females.

B. <u>STUDY DEFICIENCIES:</u> There were no major study deficiencies. Minor deficiencies included the following:

- Neurological testing was not performed; however, clinical symptoms did not indicate neurological toxicity. Considering the frank effects of the CNS and PNS, these tests would have proved very useful in determining if the lesions affected function.
- Historical control data would have been useful.
- Three slides were missing from the terminal sacrifice satellite group rats including one from an 0S male (#14) of the lumbar dorsal root and two of the central ventral root for 3S males (#73 and #75) for the peer review pathology evaluation.
- Tissues from the recovery satellite group were prepared by immersion-fixation only and not perfusion-fixed as was done for the terminal satellite group. Therefore, the recovery animals could only be compared to the main group, but the majority of the findings were in the satellite group.
- There were several instances of disagreement between the study pathologist and the review pathologist. While some of the instances were a matter of degree of involvement, there were numerous cases where the difference was between minor involvement and no involvement. The general conclusion, however, remained the same.

C. STUDY CLASSIFICATION: This subchronic (90-day) oral toxicity study in the rat is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a 90-day exposure for a 90-day oral toxicity study OPPTS 870.3100 in the rat.

EPA Reviewer: Steven L. Malish, Ph.D. , Toxicologist
Team 1, RASSB/Antimicrobial Division
EPA Secondary Reviewer: Jonathan Chen, Ph.D., Toxicologist
Team 1, RASSB/Antimicrobial Division

S. L. Malish
Jonathan Chen

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Repeat Dermal Toxicity - Rat; OPPTS 870.3200; OECD 411

PC CODE: 119093
DECISION#: 220066

DP BARCODE: 323129
EPA Reg. No.: 43813-ET

TEST MATERIAL (PURITY: 1H-pyrrole-3-carbonitrile, 4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl); (94.6% a.i.)

SYNONYMS: Econea®028, R107894

CITATION: Merkel D. (2005). R170894: Dermal Toxicity Study (28-Day Repeat Dermal Application Study in Rats). Product Safety Laboratories, Dayton, NJ. Study No. 17026, September 19, 2005. MRID 466597-02. Unpublished.

SPONSOR: Janssen Pharmaceutical N.V., Plant & Material Protection Division, Turnhoutseweg 30, B-2340 Beerse, Belgium

EXECUTIVE SUMMARY: In a 28-day range-finding dermal toxicity study (MRID 46659702), R107894 (94.6% a.i.; lot #12649-8) was administered to 5 rats/dose /sex at dose levels of 0, 30, 100, 300, or 1000 mg/kg/day for 29 days (6 hours/day, 5 days/week). Results from this study will be used to determine the dose range of a proposed 90-day dermal subchronic study.

Treatment-related effects at 1000 mg/kg/day included ano-genital staining but this was not considered to be toxicologically significant. There were no mortalities during this study. Additionally, there were no significant changes to body weight, food consumption, food efficiency, organ weight, or pathology (gross or microscopic). **The LOAEL is >1000 mg/kg/day (HDT). The NOAEL is ≤1000 mg/kg/day.** Based on these results, rats would be expected to tolerate an exposure of >1000 mg/kg/day in the subsequent 90-day dermal study.

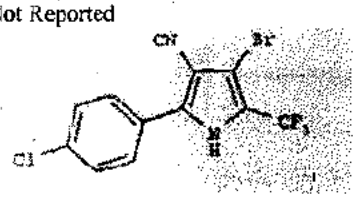
This 28-day range-finding dermal toxicity study is **ACCEPTABLE-GUIDELINE** because it satisfies the guideline requirement for a range-finding dermal toxicity study OPPTS 870.3200.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements are provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: 1H-pyrrole-3-carbonitrile,4-bromo-2-(4-chlorophenyl)-5-(trifluromethyl); R107894

Description:	Light tan solid material stored at room temperature and not exposed to heat or direct sunlight
Lot/Batch #:	Lot # AC 12649-8
Purity:	94.6% a.i.
Compound Stability:	Not Reported
CAS #:	Not Reported
Structure:	

2. Vehicle and/or positive control: Distilled water (lot/batch # and purity not reported)

3. Test animals:

Species:	Rat	
Strain:	Crl:CD®(SD) IGS BR VAF/Plus®	
Age/weight at study initiation:	Approximately 9 weeks of age / 281-322 grams (males) and 180-224 grams (females)	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Animals were individually housed in suspended, stainless-steel cages.	
Diet:	Purina Certified Rodent Meal® #5002M (PMI Nutrition International) was provided *ad libitum*. No contaminants that could interfere with the study outcome were found during trace analyses.	
Water:	Filtered tap water was supplied by an automatic water dispensing system *ad libitum*. Results of the water analysis showed that contaminant levels were acceptable and within regulatory standards.	
Environmental conditions:	**Temperature:**	19-23°C
	Humidity:	54-69%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN:

1. In life dates:
Start: 3/9/05
End: 4/7/05

2. Animal assignment: Animals were assigned randomly by computer-generated assignment to the test groups noted in Table 1.

TABLE 1: Study design

Test Group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	5	5
Low	30	5	5
Intermediate-Low	100	5	5
Intermediate-High	300	5	5
High	1000	5	5

3. Dose selection rationale: The dose levels were selected based on information provided by the Sponsor. These doses selected will be used to bracket the dose-range of a proposed 90-day dermal subchronic study.

4. Preparation and treatment of animal skin: During acclimation, the fur of each test animal (n=60) was clipped from the shoulder to the hips, resulting in an area of at least 10% of the body surface. Care was taken to avoid abrading the skin. Acclimatization to the dosing procedure was performed on days -6, -5, and -2, where the animals were "sham wrapped" for 1, 3, and 6 hours, respectively. Prior to the first application, the animals were weighed, re-clipped, and the skin was examined for any abnormalities. During application, an appropriate amount of the test material was applied to a gauze pad, which was moistened with 0.3 mL of distilled water. After application, the trunk of the animal was then wrapped with nonirritating tape to avoid ingestion of the test material or dislocation of the patch. Dosing occurred at approximately the same time each day (±2 hours) 6 hours/day, 5 days/week over a course of 29 days (minimum of 20 exposures). After the 6-hour exposure, the wrappings were removed, and the entire application site was cleansed with a ~3% solution of Ivory® Dishwashing Liquid using a clean towel to remove any residual test substance and gently patted dry with a clean pad. It was noted whether animals were out of the wrapping at patch removal. The first day of dosing was designated as Day 1 of the study. Rats in the control group were exposed to the vehicle using the same procedure as described for the treated rats.

5. Statistics: Treated animals were compared to control groups using a One-Way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison test. Data were evaluated for homogeneity of variances and normality by the Bartlett's test. Data that was statistically significant of the Bartlett's test was further evaluated with a non-parametric method (Kruskal-Wallis). Significance was judged at $p<0.05$.

C. METHODS:

1. Observations:

1a. Cageside Observations: Mortality and morbidity were examined twice daily. Additionally, the dose site of each animal was examined for signs of erythema and edema on each Friday after patch removal using the Draize et al.[1] method. The mean erythema and mean edema scores were determined for each group at each time interval. The Primary Dermal Irritation (PDI) was

[1] Draize J.H., Woodward, G. and Calvery, H.O. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. 82:377-390.

defined as the sum of the mean erythema and the mean edema for each group at each time interval.

1b. Clinical Examinations: Detailed clinical observations were recorded on Day 1 (prior to treatment) and weekly thereafter (prior to patch application). Examinations were conducted while animals were both handled and placed in an opened field. Examinations included, but were not limited to, the following: changes to the skin, fur, eyes, and mucous membranes; and occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern).

1c. Neurological Evaluations: Changes in gait, posture and response to handling, as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming or repetitive circling), or bizarre behavior (e.g., self-mutilation or walking backward) were recorded.

2. Body weight: Individual body weights were recorded 4 times during the acclimation period. Test animals were weighed on Day 1 before dosing and weekly thereafter. Mean daily body weight gains and overall body weight gains were calculated. All animals were weighed immediately prior to sacrifice (fasted body weight).

3. Food consumption: Individual food consumption was determined weekly. Mean daily food consumption and mean daily food efficiency (defined as body weight gain divided by food consumption) were calculated and reported.

4. Ophthalmoscopic examination: An ophthalmoscopic examination was not conducted.

5. Hematology & Clinical Chemistry: Examination of hematology and clinical chemistry endpoints was not conducted.

6. Urinalysis: A urinalysis was not conducted.

7. Sacrifice and Pathology: Surviving animals were euthanized at the scheduled sacrifice (day 30) by exsanguination from the abdominal aorta under isoflurane anesthesia and subjected to gross pathological examination, which included an initial examination of external surfaces and orifices, as well as the cranial, thoracic, and abdominal cavities and their contents. Gross lesions were noted, and tissues of interest and any gross lesions were retained in 10% neutral buffered formalin. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta, thoracic*	XX	Brain*+
	Salivary glands*		Heart*+		Peripheral nerve*
	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*	XX	Spleen*+		Eyes (optic nerve)*
	Jejunum*		Thymus*+		GLANDULAR
	Ileum*				Adrenal gland*+
	Cecum*		UROGENITAL		Lacrimal gland
	Colon*		Kidneys*+		Parathyroid*
	Rectum*		Urinary bladder*		Thyroid*
XX	Liver*+		Testes*+		OTHER
	Gall bladder* (not rat)		Epididymides*+		Bone (sternum and/or femur)
	Bile duct* (rat)		Prostate*		Skeletal muscle
	Pancreas*		Seminal vesicles*		Skin* (treated & untreated areas)
	RESPIRATORY		Ovaries*+		All gross lesions and masses*
	Trachea*		Uterus*+		
	Lung*		Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90-day dermal toxicity studies based on Guideline 870.3250
\+ Organ weights required.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs: Treatment-related clinical signs are presented in Table 2. All animals exposed to 1000 mg/kg/day exhibited ano-genital staining during daily observations, weekly observations, and/or at gross necropsy. Additionally, two male rats and one female rat treated with 1000 mg/kg/day exhibited a red stain around the nose and mouth during daily observations. Ano-genital staining also was observed once in two 300-mg/kg/day female rats; however, this occurrence was considered incidental and not considered treatment related.

TABLE 2. Treatment-related clinical signs

	Dose rate [mg/kg/day]				
	0	30	100	300	1000
Male					
Fur/Skin	0	0	0	0	5 (2[b]); 1(-[c])
Female					
Fur/Skin	0	0	0	2(2[b])	5(2[b]); 1(-[c])

[a] Data obtained from pages 220-21 in the study report. An entry of 0 indicates that all animals in the group appeared normal when evaluated for the specified observation, or that all animals did not exhibit the specific clinical sign. An entry greater than 0 indicates the number of animals in the group that exhibited the specific clinical sign. The number in the parenthesis (if present) represents the score given for the observed clinical sign.
[b] Score of 2 indicates urine stained/wetness.
[c] Animal #1171 had two small sores on bottom lip.

Small sores on the lower lip was observed during detailed observation and/or at necropsy in one male and one female treated with 1000 mg/kg/day. These findings were considered to be minor injuries sustained at the animal feed jar and not treatment related.

2. Mortality: There were no mortalities during this study.

3. Neurological Evaluations: There were no changes to gait, posture, and response to handling. Additionally, there were no clonic or tonic movements, stereotypies (e.g., excessive grooming and repetitive circling), or bizarre behavior (e.g., self-mutilation and walking backward)

4. Dermal Irritation: Dermal irritation was not observed in this study. The following animals were found out of their wrappings: animal #1176 (1000 mg/kg/day) for 9 out of 20 exposures; animal #1145 (30 mg/kg/day) for 5 out of 20 exposures; and animal #1167 (300 mg/kg/day) for 3 out of 20 exposures. According to the study author, these events did not compromise the outcome of the study.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights and body weight gains are presented in Table 3. There were no treatment-related or statistically significant changes in body weight or body weight gain throughout the study.

TABLE 3. Average body weights and body weight gains during treatment

Dose rate [mg/kg/day]	Body Weights (g±SD)					Total Weight Gain	
	Day 1	Day 8	Day 15	Day 22	Day 29	g	% of control
Male							
0	308.8±16.7	338.0±19.2	368.6±23.0	398.2±27.6	423.8±31.6	115.0	---
30	309.8±8.7	339.6±19.9	369.6±14.9	386.0±31.4	412.6±32.4	102.8	-11%
100	314.4±13.3	345.2±14.9	369.6±14.9	395.0±22.5	417.2±26.7	102.8	-11%
300	310.4±10.5	344.6±8.8	369.6±6.4	395.4±6.5	417.0±5.3	106.6	-7%
1000	308.2±15.3	333.4±24.5	360.8±27.4	383.0±29.8	409.2±27.5	100.0	-12%
Female							
0	200.8±8.9	219.8±8.9	225.8±16.7	238.0±10.8	241.4±9.0	40.6	---
30	202.4±9.4	222.2±23.3	234.4±24.0	242.4±27.7	245.0±24.9	42.6	+5%
100	203.8±16.3	220.4±16.9	230.2±22.3	240.0±17.2	251.4±17.6	47.6	+17%
300	199.8±14.1	216.6±17.0	220.0±20.7	227.8±22.0	234.8±20.1	35.0	-14%
1000	199.8±11.9	215.4±17.2	225.4±19.2	233.6±22.8	242.2±20.7	42.4	+4%

[a] Data obtained from pages 22-23 in the study report; total weight gain and % change was calculated by the reviewer.

C. FOOD CONSUMPTION AND EFFICIENCY:

1. Food consumption: Food consumption data are presented in Table 4. There were no treatment-related or statistically-significant changes in food consumption throughout the study.

TABLE 4. Average food consumption during treatment

Dose rate [mg/kg/day]	Food Consumption (g/day±SD)				Total Food Consumption	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	g/day	% of control
Male						
0	27.6±1.4	28.2±2.2	30.5±2.1	31.5±2.2	29.4±1.9	---
30	28.1±3.3	27.6±3.1	28.1±3.0	29.6±3.1	28.4±3.0	-3%
100	28.3±1.7	27.8±2.0	28.6±2.9	29.5±2.1	28.5±2.0	-3%

Dose rate [mg/kg/day]	Food Consumption (g/day±SD)				Total Food Consumption	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	g/day	% of control
300	29.5±2.2	29.6±1.4	30.9±1.6	32.0±1.4	30.5±1.4	+4%
1000	27.9±2.1	28.2±2.3	29.9±1.8	31.0±1.9	29.3±1.7	-1%
Female						
0	19.5±1.8	19.9±1.7	21.1±3.1	20.9±2.0	20.4±2.0	---
30	20.9±1.6	22.1±1.7	22.1±1.6	22.4±1.8	21.9±1.5	+7%
100	21.5±2.8	22.4±2.3	23.5±3.8	23.3±1.8	22.7±2.3	+4%
300	17.9±2.0	17.9±2.2	20.0±2.0	19.9±1.4	18.9±0.8	-7%
1000	20.2±1.2	20.2±1.1	21.5±1.0	21.4±1.3	20.8±1.1	+2%

[a] Data obtained from page 24 in the study report; % change was calculated by our reviewers.

2. Food efficiency: Food efficiency data are presented in Table 5. There were no treatment-related or statistically-significant changes in food efficiency throughout the study.

TABLE 5. Average food efficiency during treatment

Dose rate [mg/kg/day]	Food Efficiency (±SD)				Total Food Efficiency	
	Day 1-8	Day 8-15	Day 15-22	Day 22-29	Ratio	% of control
Male						
0	0.15±0.03	0.15±0.03	0.14±0.03	0.12±0.03	0.14±0.02	---
30	0.15±0.05	0.14±0.04	0.10±0.04	0.13±0.02	0.13±0.02	-7%
100	0.15±0.01	0.12±0.04	0.13±0.03	0.11±0.02	0.13±0.02	-7%
300	0.16±0.03	0.12±0.04	0.12±0.03	0.10±0.01	0.12±0.01	-14%
1000	0.13±0.04	0.14±0.01	0.10±0.03	0.12±0.02	0.12±0.01	-14%
Female						
0	0.14±0.02	0.04±0.07	0.08±0.04	0.02±0.04	0.07±0.02	---
30	0.13±0.03	0.08±0.01	0.05±0.04	0.02±0.05	0.07±0.01	0%
100	0.11±0.02	0.06±0.04	0.06±0.04	0.07±0.03	0.07±0.01	0%
300	0.13±0.05	0.02±0.08	0.05±0.03	0.05±0.04	0.07±0.02	0%
1000	0.11±0.03	0.07±0.05	0.05±0.03	0.06±0.02	0.07±0.02	0%

[a] Data obtained from page 25 in the study report; % change was calculated by our reviewers.

D. OPHTHALMOSCOPIC EXAMINATION: An ophthalmoscopic examination was not conducted.

E. BLOOD ANALYSES: Blood analyses were not conducted.

F. URINALYSIS: A urinalysis was not conducted.

G. SACRIFICE AND PATHOLOGY:

1. Organ weight: Select organ weight findings are presented in Table 6. There were no treatment-related or statistically-significant changes to absolute organ weights or organ-to-body weight ratios for both sexes throughout the study. Females administered 300 mg/kg/day exhibited a statistically-significant decrease in spleen-to-brain weight; however, this finding was not dose-related and did not correspond to any gross or microscopic pathology. Consequently, this finding was considered incidental.

TABLE 6. Select relative organ weights

Relative organ weight (gx1000±SD)	Dose rate (mg/kg/day)				
	0	30	100	300	1000
Male					
Spleen-to-brain	0.33±0.03	0.34±0.08	0.28±0.01	0.32±0.03	0.31±0.06
Female					
Spleen-to-brain	0.31±0.06	0.24*±0.01	0.26±0.01	0.28±0.04	0.26±0.03

[a] Data obtained from page 25 in the study report; % change was calculated by reviewer.
* Statistically different ($p<0.05$) from the control

2. **Gross pathology**: There were no treatment-related gross pathology findings. One 300-mg/kg/day female (animal #1167) exhibited mottled kidneys, and one 1000-mg/kg/day male (animal #1171) showed small sores on its lower lip. These findings were considered to be isolated.

3. **Microscopic pathology**: There were no treatment-related microscopic pathology findings. Minimal to slight/mild multifocal chronic inflammation of the liver was observed in animals from all treatment groups, including the control group, and was not considered treatment-related. Minimal lymphoid depletion was noted in the marginal zone of the spleen in one 1000-mg/kg/day female; however, this finding was considered isolated and not related to treatment.

III. DISCUSSION AND CONCLUSIONS: The dermal exposure to R107894 did not result in toxicity at 1000 mg/kg/day. Based on these findings, the LOAEL is >1000 mg/kg/day in male and female rats, the NOAEL is ≤1000 mg/kg/day. In the subsequent 90-day dermal study, rats would be expected to tolerate an exposure of 1000 mg/kg/day.

STUDY CLASSIFICATION: This 28-day range-finding dermal toxicity study is **ACCEPTABLE - GUIDELINE** because it satisfies the guideline requirement for a dermal toxicity study OPPTS 870.3200.

DATA REVIEW FOR ACUTE INHALATION TOXICITY (§81-3, 870.1300)
Limit Test with Econea Technical

Product Manager: M. Swindell
MRID No.: 468466-01
DP# 330458

Reviewer: Steven L. Malish
Study Completion Date: May 25, 2006
Lab Study No.: 19437

Testing Laboratory: Product Safety Laboratories
Author: George E. Moore

Quality Assurance (40 CFR §160.12):

Test Material: Econea Technical
Concentration: 92.4% a.i.

Species: Rat
Age: 9 – 10 weeks
Weight: Males = 300 to 348 gm; Females = 210 – 234 gm
Source: Ace animals, Boyertown, PA

Summary:

Males =
Females =
Combined =
1. LC_{50} (mg/L):

2. The estimated LC_{50} is <510 mg/m3 for males/females and males and females combined.
3. MMAD: 3.5 Fm
4. Toxicity Category: II **Classification:**

Procedure (Deviation From §81-3):

Results: All animals were found dead upon removal from the exposure tubes.

Reported Mortality

Exposure Concentration	(NUMBER DEATHS/NUMBER TESTED)		
	Males	Females	Combined
510 mg/m3	5	5	10

Chamber Atmosphere			
Dose Level	MMAD	GSD	particles < Fm

1

	Fm	Fm	
	Fm	Fm	

Chamber Environment	
Chamber Volume	
Airflow	
Temperature	^{o}C
Relative Humidity	%

Clinical Observations: Not done because all animal died during exposure.

Gross Necropsy Findings: Slightly red lungs and edema of the lungs

DATA EVALUATION REPORT

PIRATE Metabolite

Study Type: 84-2; *Salmonella typhimurium* and *Escherichia coli*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 101P (MRID 43492840)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer: Sandra Daussin, B.S.	Signature: Sandy Daussin by WJS Date: 1/11/96
Secondary Reviewer: Steven Brecher, Ph.D.	Signature: Steven Brecher by WJS Date: 1/11/96
Project Manager: William J. Spangler, Ph.D.	Signature: William J. Spangler Date: 1/11/96
Quality Assurance: Reto Engler, Ph.D.	Signature: Reto Engler by WJS Date: 1/11/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Irving Mauer, Ph.D. Date 02/16/96
Review Section IO, Toxicology Branch 1 (7509C)
EPA Secondary Reviewer: Marion Copley, DVM, DABT, Date 2/21/96
Review Section 4, Toxicology Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Salmonella typhimurium and Escherichia coli/mammalian activation gene mutation assay
OPPTS Number: 870.5265 & 870.5100 OPP Guideline Number: [§84-2]

DP BARCODE: D212558 SUBMISSION CODE: None
P.C. CODE: 129093 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): CL 303,268 (pirate metabolite and impurity, 100.3% active ingredient)

SYNONYMS: 4-Bromo-2-(p-chlorophenyl)-5-(trifluoromethyl)-pyrrole-3-carbonitrile

CITATION: Mulligan, B. (1994) Microbial Mutagenicity Plate Incorporation Assay of CL 303,268. American Cyanamid Company, Genetic Toxicology Laboratory, Princeton, NJ. Study No. 9402001. August 12, 1994. MRID 43492840. Unpublished.

SPONSOR: American Cyanamid Co., Princeton, NJ

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 43492840), strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella typhimurium or Escherichia coli WP2 uvrA- were exposed to CL 303,268 (100.3% a.i.) in dimethylsulfoxide in the presence and absence of S9 mammalian metabolic activation. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate (+/-S9). E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, and 250 µg/plate (+/-S9).

CL 303,268 (100.3% a.i.) was tested up to cytotoxic concentrations with the S. typhimurium strains and the limit of solubility, 250 µg/plate, with E. coli WP2 uvrA-. The positive controls induced the appropriate responses in the corresponding strains. CL 303,268 failed to induce a genotoxic response in any of the tester strains with the exception of a borderline positive result for the TA100 strain at the 1.0 µg/plate dose level. As the result was equivocal and a genotoxic response was not found in any of the other tester strains, CL 303,268 was determined to not be mutagenic under the conditions of the submitted study.

JANSSEN PHARMACEUTICA INC.

465451-00

May 9, 2005

Document Processing Desk (7504C)
Regulatory Management Branch I
Antimicrobial Division (7510W)
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202-4501

SUBJECT: ECONEA Technical (43813-ET)

Attn: Mr. Marshall Swindell
Product Manager 33

Enclosed are three copies of each of the following study:

Volume 1 R107894: Evaluation of the Ambient Temperature Storage Stability
Janssen Report No. AGR 458
Covance Laboratories Ltd Report Number 1073/63-D2149
May 6, 2005
OPPTS Guideline Number 830.6317 & 830.6320

MRID 46545101

This study fills the current data gap for a one-year storage stability/corrosion study. Please submit the ECONEA product chemistry for scientific review.

Sincerely,

William R. Goodwine

William R. Goodwine
Senior Director
Plant & Material Protection Division
(609) 730-2607

1125 TRENTON-HARBOURTON ROAD
TITUSVILLE, NEW JERSEY 08560-0200

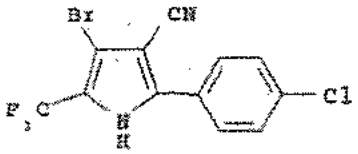
This study is classified as acceptable, and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity bacterial reverse gene mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CL 303,268
   Description: Nearly white solid
   Lot/Batch #: AC8979-44B
   Purity: 100.3% a.i.
   Stability of compound: Not specified
   CAS #: Not specified
   Structure:



   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: The test substance was stored at room temperature. Dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were from 86-106% of the nominal concentrations with coefficients of variation of 4 and 6% for the two replicate trials.

2. Control Materials:
   Negative: DMSO
   Solvent/final concentration: DMSO/0.1 mL per plate

   Positive:
   Nonactivation:

Sodium azide	not used	µg/plate	
2-Nitrofluorene	20	µg/plate	TA98, TA1538
9-Aminoacridine	50	µg/plate	TA1537
Other (list):			
N-methyl-N-nitro-N-nitrosoguanidine	10	µg/plate	*E. coli* WP2 *uvrA-*, TA100, TA1535

Activation:
2-Aminoanthracene 2.5 µg/plate all S. Typhimurium strains
2-Aminoanthracene 10 µg/plate E. coli WP2 uvrA-

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
___ phenobarbital	___ non-induced	___ mouse	___ lung
___ none		___ hamster	___ other
___ other			___ other

The S9 homogenate was purchased from Microbiological Associates, Inc., Bethesda, MD. The test S9 was purchased frozen (-80 C) and was thawed on the day of testing. The S9:cofactor mix was (1:10) and 0.5 mL of S9 mix was used per plate. The S9 mix composition included NADP, glucose-6-phosphate, potassium chloride, and magnesium chloride-6-hydrate in a sodium phosphate buffer.

4. Test organisms: S. typhimurium strains
___ TA97 x TA98 x TA100 ___ TA102 ___ TA104
x TA1535 x TA1537 x TA1538 ; list any others:
E. coli strain
x WP2 uvrA-
Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used

Preliminary cytotoxicity test: Nine dose levels (1, 5, 10, 25, 50, 100, 250, 500, or 1000 µg/plate) were evaluated with the S. typhimurium strain TA100 or E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. Vehicle and positive control groups were included.

Mutagenicity assay: S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate. E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, or 250 µg/plate. All evaluated dose levels were assayed with and without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   _x_ standard plate test
   ___ pre-incubation (___ minutes)
   ___ "Prival" modification *(i.e. azo-reduction method)*
   ___ spot test
   ___ other *(describe)*

2. Protocol: Tester strains were inoculated into nutrient broth culture 1 day prior to dosing and incubated for approximately 11 hours. Test substance and positive control substances were diluted in DMSO to specified concentrations. For the activation conditions, 0.1 mL of the appropriate tester strain culture at a density of $1x10^8$ to $2x10^9$ cells/mL, 0.5 mL of the S9 mix, and 0.1 mL of test material solution, solvent, or positive control were mixed with 2.0 mL of melted top agar supplemented with histidine, tryptophan, and biotin. The mixture was poured over a minimal agar plate. For nonactivation conditions, 0.5 mL of buffer (0.1 M sodium phosphate, 0.008 M magnesium chloride, 0.033 M potassium chloride) was substituted for the S9 mix. After incubation (37 ± 2 C for 48 ± 6 hours), the number of revertant colonies were counted by hand or using an automated colony counter. The background lawn and/or the number of colonies were examined to determine the toxicity of the test compound. Means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

   (a) Assay validity: A detailed discussion of the criteria used to determine if the assay was valid was not given. However, the study report states that the assay would be "partially repeated" if there was "excessive toxicity" and that the assay results would be compared with "recent historical control values...to gain perspective on test system performance." An assay was considered valid if the positive control materials demonstrated responses that were consistent with their "published activities".

   (b) Positive response: The test material was considered mutagenic if it caused a reproducible dose-related increase in the mean number of revertants over three dose levels with at least one positive dose level. A positive dose level was one where the increase in the mean number of revertants was at least 2-fold higher than the vehicle control for strains TA98

and TA100 and 3-fold for strains TA1535, TA1537, TA1538, and WP2 uvrA-.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: The number of colonies/plate for the preliminary assay are presented in Attachment 1 (study report page 14) of this DER. Nine dose levels (1, 5, 10, 25, 50, 100, 250, 500, and 1000 µg/plate) were evaluated with the S. typhimurium strain TA100 and E. coli WP2 uvrA- in the presence and absence of S9 activation. Single plates were used per dose and per condition. The positive controls used were 2-aminoanthracene (activation conditions) and N-methyl-N'-nitro-N-nitrosoguanidine (non-activation conditions). The test material precipitated in the test plates with concentrations ≥250 µg/plate. Toxicity was observed as "greatly reduced or no background lawns, or no bacterial growth" in the TA100 strain at doses ≥10 µg/plate (+S9) and ≥5 µg/plate (-S9). In addition, slight toxicity was observed in the TA100 strain as "reduced background lawns" at doses of 5 µg/plate (+S9) and 1 µg/plate (-S9). For the WP2 uvrA-, no toxicity was observed at any dose level with or without activation.

Based on these results, the mutation assay was performed with a dose range of 0.05-250 µg/plate +/-S9 activation.

B. Mutagenicity assay: The test results for the mutagenicity assays with CL 303,268 are presented in Attachment 1 (study report pages 15-18) of this DER. S. typhimurium strains TA98, TA100, TA1535, TA1537, or TA1538 were evaluated with CL 303,268 at concentrations of 0.05, 0.10, 0.25, 0.50, 1.0, or 5.0 µg/plate. E. coli WP2 uvrA- was tested with CL 303,268 at concentrations of 10, 25, 50, 100, or 250 µg/plate. All evaluated dose levels were assayed with and without S9 activation. Triplicate plates were used for each dose, strain, and condition. Vehicle and positive control groups were included and the assay was run in duplicate. The test material precipitated in the E. coli WP2 uvrA- 250 µg plates.

There was no significant increase in the mean number of revertant colonies in any of the test strains at any dose level/condition with the exception of the TA100 strain. For the TA100 strain, an increase in the mean number of revertant colonies was demonstrated over the dose range. In addition, at the highest dose tested that did not induce cytotoxicity (1.0 µg/plate), the mean number of revertants was 1.9x (initial trial) and 1.5x (confirmatory trial) the vehicle control. For the TA100 strain, a dose level was

considered positive if the increase in the mean number of revertants was at least 2-fold higher than the vehicle control. Toxicity was observed as "reduced background lawns" with activation and as "greatly reduced or no background lawns" without activation in all S. typhimurium strains at the 5.0 µg dose. All positive controls induced an appropriate increase in the number of revertant colonies. For the E. coli WP2 uvrA- strain, no toxicity was observed at any dose level with or without activation.

Based on the findings, the study author concluded that CL 303,268 was not mutagenic under the conditions of this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study author's conclusion that CL 303,268 was not mutagenic under the conditions of the submitted microbial gene mutation assay. CL 303,268 was assayed over an appropriate dose range as it was tested to cytotoxic concentrations with the S. typhimurium strains and to the limit of solubility (250 µg/plate) with the E. coli WP2 uvrA- strain. CL 303,268 failed to induce a genotoxic response in any of the tester strains with the exception of the borderline positive result for the TA100 strain at the 1.0 µg/plate dose level. As the result was equivocal and a genotoxic response was not found in any of the other tester strains, we agree with the conclusion that CL 303,268 was not mutagenic under the conditions of the submitted study. The sensitivity of the test system to detect mutagenesis was adequately demonstrated by the response obtained with the nonactivated and S9-activated positive controls. The study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

Pages 295-299 * Claimed confidential by submitter*

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

September 18, 2006

MEMORANDUM

Subject: Econea Ecological Effects Data Evaluation Records (D327534)

To: Karen Leavy, RMT-33
Marshall Swindell, RM-33
Dennis Edwards, Branch Chief
Regulatory Management Branch I
Antimicrobials Division (7510P)

From: Kathryn Montague, Biologist
Siroos Mostaghimi, Team Leader
Norm Cook, Branch Chief
Risk Assessment and Science Support Branch
Antimicrobials Division (7510P)

Janssen Pharmaceutica, Inc., has submitted six ecological effects studies using Econea technical or degradate in support of the registration of Econea as a marine antifoulant. The results of those studies are summarized below:

1. MRID #467513-05. Cafarella, M.A. 2006. R107894 – Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-through Conditions.

   The 96-hr shell deposition EC_{50} was 0.64 µg a.i./L, with a 95% Confidence Interval (CI) of 0.34 to 1.21 µg a.i./L.The NOEC = 0.29 µg a.i./L. This study is acceptable, and fulfills Guideline 850.1025/72-3.

2. MRID #467513-06. Hoberg, J.R. 2006. Supplemental to: R107894-Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus)* Under Flow-Through Conditions.

   The information contained in this submission is adequate to allow the original study, MRID #456740-03, to be upgraded from supplemental to acceptable. Original results were: 96-hr LD_{50} 23.71 µg a.i./L, 95% CI 19.9 - 29.3 µg a.i./L
   NOEC 10.0 µg a.i./L.

3. MRID #467513-07. Cafarella, M.A. 2006. CL322-250 – Early Life-stage Toxicity Test with Zebra Fish (*Dano rario*).

Larval Survival	Hatching Survival
NOEC: 0.069 mg a.i./L	NOEC: 0.27 mg a.i./L
LOEC: 0.14 mg a.i.;L	LOEC: 0.53 mg a.i./L
Total Length	**Wet Weight**
NOEC: ≥0.53 mg a.i./L	NOEC: ≥0.53 mg a.i./L
LOEC: >0.53 mg a.i./L	LOEC: >0.53 mg a.i./L

This study is acceptable, and fulfills Guideline 850.1300/72-4a.

4. MRID #467513-08. Sayers, L.E. 2006. CL322,248 – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions.

   The 96-hr LC_{50} was 4.3 mg a.i./L, with a 95% CI of 3.3 to 5.4 mg a.i./L. The NOEC was 2.5 mg a.i./L. The study is acceptable, and fulfills Guideline 850.1035/72-3c.

5. MRID #467513-09. Sayers, L.E. 2006. CL322,248 – Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) under Static Conditions.

   The 96-hr LC_{50} was > 89 mg a.i./L. There were no effects at the highest level tested, making the NOEC 89 mg a.i./L. The study is acceptable, fulfilling Guideline 850.1075/72-3a.

6. MRID #467513-10. Gallagher, S.P., K.H. Martin, and J.B. Beavers. 2006. CL322,248: A Dietary LC_{50} Study with the Mallard.

   The 8-day Dietary LC_{50} was >5620 ppm a.i. The no mortality concentration was 5620 ppm a.i. The NOEC was 3160 ppm a.i., due to a reduction body weight gain at higher treatment levels. The study is acceptable, and fulfills Guideline 850.2200/71-2.

Based on the results of the marine acute studies on the degradate CL322,248, three previously reserved data requirements for CL322,248 can now be dropped: Marine/estuarine bivalve acute toxicity testing (850.1025/72-3b), Marine/estuarine fish early life-stage testing (850.1300/72-4a), and Marine/estuarine invertebrate life-cycle testing (850.1400/72-4b).

The following data requirements are still outstanding for Econea:

Degradate CL322,250

850.1400/72-4b Aquatic invertebrate life-cycle testing, freshwater species
(submitted study MRID#465960-11 is supplemental, can be upgraded if additional information submitted; however, since freshwater species is less sensitive than marine/estuarine species, the marine/estuarine life-cycle test is required)

850.1400/72-4b Aquatic invertebrate life-cycle testing, marine/estuarine species
(submitted study MRID#465960-12 was invalid)

If you have any questions on the above, please contact Kathryn Montague (703-305-1243 or montague.Kathryn@epa.gov).

DATA EVALUATION RECORD
OYSTER ACUTE TOXICITY TEST (SHELL DEPOSITION)
OPPTS GUIDELINE 850.1025

1. **CHEMICAL:** Econea Technical **PC Code No.:** 119093

2. **TEST MATERIAL:** R107894(aka CL303268) **Purity:** 94.6% (p. 12)
Cas No. 122454-29-9

3. **CITATION**

Author: Mark A. Cafarella
Title: R107894 – Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions
Study Completion Date: 17 January 2006
Laboratory: Springborn Smithers Laboratories
Sponsor: Janssen Pharmaceutica N. V.
Laboratory Report ID: Springborn Smithers Study No. 13751.6157
Janssen Study No. AGR 1176
MRID No.: 467513-05

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD/US EPA
Signature: Kathryn V. Montague **Date:** 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD/US EPA
Signature: Siroos Mostaghimi **Date:** 9/19/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Crassostrea virginica*
Valve Height: 43±4.7 mm
Definitive Test Duration: 96-hr
Study Method: Flow-through
Type of Concentrations: Mean-measured and nominal concentrations reported

7. **CONCLUSIONS**

Verified Results Synopsis: 96-hr EC_{50} = 0.64 µg a.i./L, 95% CIs = 0.34 to 1.21 µg a.i./L.
NOEC = 0.29 µg a.i./L

8. **ADEQUACY OF THE STUDY**

A. **Classification: Acceptable**

B. **Rationale: Deviations from Guidelines recommendations did not affect study results.**

C. **Repairability: N/A**

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1025:

- The following information was not included in the study report: cleaning procedures upon receipt of oysters, whether oyster valves were held tightly together during grinding, if oysters with visible openings to the shell cavity were discarded, whether dead oysters were removed from the tanks, and physical/chemical properties of the test chemical.
- Filtered seawater was used. The guideline states that unfiltered seawater should be used. However, since oysters were fed an algal suspension during the test, this should not have impacted the results.
- The measured flowrate was between 50% and 72% of the nominal flow. The guideline states that the measured flowrate should between 90% and 110% of the nominal flow.
- Dilution factor of 2 used between nominal concentrations. The guideline states that the factor should not exceed 1.8. However, good dose-response results were obtained during the study.
- In the solvent control, an average of 1.8 mm of new shell growth was observed. The guideline states that a minimum of 2 mm of new shell growth should be observed in both the control and solvent control. Statistical analysis determined that there was not a significant difference between the control and solvent control, however.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
▪ Species Eastern oyster - *Crassostrea virginica*	▪ *Crassostrea virginica* used
Life Stage/Size ▪ 30-50 mm in valve height ▪ As similar in age and/or size as possible to reduce variability ▪ Standard deviation of valve height < 20% of the mean ▪ Should be in pre-spawn condition of gonadal development prior to and during test as determined by direct or histological observation of the gonadal tissue for presence of gametes	▪ 43±4.7 mm (p. 13) ▪ Study notes that oysters were of similar age (p. 13) ▪ Standard deviation less than 20% of mean ▪ Gonadal tissue examined for gametes (p. 13)
Acquisition ▪ Oysters may be cultured in laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease	▪ Oysters obtained from commercial supplier (Circle C Oysters) (p. 13)

Acclimation	
▪ Upon receipt, oysters brushed clean of fouling organisms and gradual transfer of oysters to holding water	▪ **Not discussed in study report.**
▪ Oysters held for at least 12-15 days before testing	▪ Oysters held 14 days prior to testing (p. 13)
▪ Oyster held in dilution water at test temperature for at least 2 days before used	▪ 48 hrs prior to testing, oysters held in conditions similar to testing conditions (p. 13)
▪ During holding, dissolved oxygen > 60% saturation and temperature of holding water should be same as testing water	▪ DO range between 69 to 102% of saturation (p. 13)
▪ Batch of oysters acceptable for testing if mortality over 7-day period prior to testing is <5%; if mortality between 5 and 10%, acclimation should continue for 7 additional days; if mortality >10%, entire batch should be rejected	▪ No mortality during 7-day period prior to testing. (p. 13)
▪ Oysters which appear diseased, have cracked, chipped, bored or gaping shells, or are infested with mudworms or boring sponges should not be used	▪ Oysters with boring sponges and/or mudworms discarded (p. 13)
Preparation for Definitive Test	
▪ Oysters which have been acclimated and meet condition criteria should have 3-5 mm of the shell periphery, at the rounded end, ground away with a small electric disc grinder or other appropriate device to produce a smooth, rounded, blunt profile	▪ 3-5 mm of new peripheral shell removed using fine-grit grinding wheel (p. 13)
▪ Oyster's valves should be held together tightly during grinding to avoid vibrating shell and injuring abductor muscle	▪ **Not discussed in study report.**
▪ If an opening into the shell cavity is visible, oyster should not be used	▪ **Not discussed in study report.**

B. Test System

Guideline Criteria	Reported Information
Test Chamber	
▪ Tanks should be made of chemically inert material	▪ Glass and Teflon-coated equipment used. (p. 15-17)
Temperature	
▪ Test temperature should be 20°C	▪ 19-21°C (p. 20, 25)
▪ Temporary fluctuations (less than 8 hr) within ± 5°C permissible	
▪ Should be recorded continuously	▪ Temperature monitored continuously in each replicate (p. 17)

Salinity ▪ Dilution water – salinity in excess of 12 ppt ▪ Natural seawater – weekly range of <10 ppt ▪ Artificial seawater – should not vary by more than 2 ppt ▪ Measured at beginning and end of test in each chamber.	▪ Natural seawater used (p. 14). Measured consistently at 32 ppt, measured daily in each replicate chamber. (p. 17, 25)
Dissolved Oxygen ▪ Dissolved oxygen concentrations should be at least 60% during and at the end of the test. ▪ Measurements should be made daily from the beginning to the end of the test in each chamber	▪ DO range between 4.8 and 7.9, over 60% saturation concentration (p. 25) ▪ Measured daily in each replicate chamber (p. 17)
pH ▪ Measured in each test chamber at the beginning and end of test. ▪ Dilution water should have a monthly range of <0.8 unit ▪ Artificial seawater pH should not vary by more than 0.5 unit ▪ Test should be carried out without adjustment of pH unless there is evidence of marked change, in which case the guidelines advise that test be repeated with pH adjustment to dilution water	▪ Measured daily in each replicate chamber (p. 17) ▪ pH range between 7.6 and 8.0 (p. 25) ▪ Seawater salinity consistently measured at 32 ppt (p. 25) ▪ No adjustment used.
Feeding ▪ Cultured algae may be added to dilution water sparingly as needed	▪ Concentrated volumes of algal suspension (approximately 10^7 cells/mL) added three times daily. (p. 14)
Dilution Water ▪ Constant supply of good quality unfiltered seawater should be available throughout holding, acclimation, and testing periods ▪ Natural seawater recommended, but artificial seawater with food added can be used ▪ Should be delivered at a flow rate of at least 1 and preferably 5 L/hr/oyster ▪ Flowrate should be ±10% of nominal flow ▪ Dilution water is acceptable if oysters survive and grow normally for 14 days without exhibiting signs of stress	▪ **Filtered seawater used** (p. 14) ▪ Natural seawater used (p. 14) ▪ 5.25 L/oyster/hour (p. 16) ▪ **Measured flowrate between 50% and 72% of nominal flow** (p. 27, 28) ▪ No stress reported (p. 13)

Carriers ▪ Stock solutions of test substances of low aqueous solubility may be prepared by ultrasonic dispersion or by use of organic solvents, emulsifiers, or dispersants of low toxicity to oysters ▪ When used, control oysters should be exposed to same concentration of carrier as that used in highest concentration treatment ▪ Concentration of carriers should not exceed 0.1 mL/L	▪ Acetone used as solvent (p. 15) ▪ Solution in solvent control mixing chamber contained the same amount of solvent present in the highest treatment level (0.10 mL solvent per liter dilution water). (p. 15-16)

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ Should be conducted to establish test chemical concentrations for the definitive test ▪ Test should be conducted in same way as definitive test except a widely spaced concentration series (i.e., log-interval) is used	▪ Range-finding test conducted, using the same method as the definitive test, but widely spaced concentrations (p. 19)
Doses ▪ At least 5 test concentrations should be used ▪ Dilution factor between concentrations should not exceed 1.8 ▪ Test chemical concentrations should be documented	▪ 5 doses used (0.25, 0.50, 1.0, 2.0, and 4.0 µg a.i./L) (p. 9) ▪ **Dilution factor of 2 used between concentrations** ▪ Concentrations documented (p. 9)
Number and Placement of Organisms: ▪ 20 oysters per test chamber. ▪ Impartially distributed among test chambers ▪ Spread out equidistantly from one another with left (cupped) valve down and open, unhinged ends all oriented in same direction facing the incoming flow of test solution	▪ 20 oysters per test chamber used. (p. 17) ▪ Impartially selected (p. 17) ▪ Placed equidistant from each other with valve inflow openings facing toward the flow of water (p. 17)
Duration of Test ▪ 96 hours	▪ 96 hrs
Endpoints ▪ Shell growth	▪ Shell growth
Shell Growth Measurements ▪ Oysters should be inspected at least after 24, 48, 72, and 96 hours ▪ Dead oysters should be removed ▪ Shell growth increments measured after 96 hours ▪ Record the length of the longest "finger" of new shell growth to the nearest 0.1 mm	▪ Oysters observed every 24 hrs (p. 17) ▪ **It is unclear from the study if dead oysters were removed.** ▪ New shell growth measured to nearest 0.1 mm (p. 28)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes (p. 2-3)
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	Yes (p. 1,9)
Control ▪ Mortality should not exceed 10% at end of test ▪ Minimum of 2 mm of new shell growth should be observed	▪ No mortality in control replicates ▪ 2.05 mm new shell growth observed in dilution water control. **1.8 mm new shell growth observed in solvent control.** (p. 28)
Information on test chemical (e.g., water solubility, vapor pressure, purity, stability in water and light, n-octanol/water partition coefficient, and pKa values)?	**Water solubility, vapor pressure, stability, K_{oc}, pKa not reported.** (p. 12)
Source of dilution water, the mean, standard deviation and range of salinity, pH, temperature, and dissolved oxygen during test period?	Yes (p. 14, 25)
Description of test procedures used (e.g., flow-through system, test chambers, chemical delivery system, aeration, etc.)?	Yes (p. 15-17)
Detailed information on oysters used, including the age and/or size, source, history, method of confirmation of pre-spawn condition, acclimation procedures, and food used?	Yes (p. 13)
Number of organisms tested, loading rate, and flow rate?	Yes (p. 15-17)
Methods of preparation of stock and test solutions, and test chemical concentrations used?	Yes (p. 15)
Number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period?	Yes (p. 21-22)
96-hr shell growth measurements of each oyster, the mean, standard deviation and range of measured growth at 96-hr of oysters in each concentration of test substance and control?	Yes (p. 28)
Calculated 96-hr EC_{50} and its 95% confidence limits and statistical methods used to calculate values?	Yes (p. 18-19, 22)
Graph of concentration-response curve based on 96 hr chemical concentration and shell growth measurements upon which EC50 calculated?	Yes (p. 31)
When observed, the 96 hr NOEC?	Yes (p. 22)
Raw data included?	Yes

Methods and data records reported?	Yes
Statistical methods reported?	Yes

Dose Response

Nominal Concentration (µg ai/L)	Mean Measured Concentration (µg ai/L)	Replicate Chamber		Mean Shell Deposition (mm)
		A	B	
Control	Control	1.9	2.2	2.05
Solvent Control	Solvent Control	1.6	2.0	1.80
0.25	0.18	1.9	2.0	1.95
0.50	0.29	2.1	1.5	1.8
1.0	0.60	1.0	1.2	1.1
2.0	1.0	0.7	0.4	0.55
4.0	2.3	0.0	0.0	0.0

Statistical Results

Statistical Method: The 96-hr EC_{50} value and 95% confidence intervals were determined by fitting the data to a best fit linear regression curve based on least squares. Four linear regression curves were produced, using the 4 combinations associated with log transformed concentrations vs. non-transformed concentrations and probit-transformed percent shell reductions vs. non-transformed percent shell reductions. The regression line that provided the best fit of the data was selected based on the highest associated coefficient of determination (r^2). This regression equation was then applied to calculate the EC_{50} and 95% confidence intervals, using the method of inverse prediction. The NOEC for the 96-hr exposure period was determined using the Williams' Test. The NOEC was defined as the highest concentrations at, and below which, there was no statistically significant reduction growth.

Results Synopsis: 96-hr EC_{50} = 0.66 µg a.i./L, 95% CIs = 0.43 to 0.99 µg a.i./L.
NOEC = 0.29 µg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: It is unclear from the study report if the control or solvent control was used to determine EC_{50} and 95% confidence intervals. It is also unclear if, in performing statistical analysis, the study authors differentiated between the two replicates used for each dose.

NOEC: TOXSTAT was used to determine if the solvent control and control could be pooled for analysis. They met the requirements for pooling, however, TOXSTAT could not handle the combine number of replicates . The solvent control was therefore used as the baseline for statistical comparison. The raw data did not meet assumptions for normality and homogeneity of variance, so a square-root transformation was applied. The NOEC for the 96-hr exposure period was determined using ANOVA with Dunnett's Test and William's Test.

EC50: TOXANAL was used to verify the EC50 calculation, again using the solvent control as the

baseline. Probit analysis was the appropriate analysis to determine the EC50 value.

Results Verification Synopsis: 96-hr EC_{50} = 0.68 µg a.i./L, 95% CIs = 0.6 to 0.7 µg a.i./L, slope = 4.6
NOEC = 0.29 µg a.i./L

The values for the 96-hr EC_{50} and NOEC closely match those obtained by the study authors. The values for the 95% CIs differ somewhat; without more knowledge of the program used by the study authors to perform the statistical analysis, it is difficult to determine the cause of the discrepancy.

14. REVIEWER'S COMMENTS:

No additional comments.

DATA EVALUATION RECORD
FISH ACUTE TOXICITY TEST, FRESHWATER AND MARINE
GUIDELINE OPPTS 850.1075

1. **CHEMICAL:** R107894 **PC Code No.:** 119093

2. **TEST MATERIAL:** R107894 **Purity:** NP
CAS No.: 122454-29-9
Batch No.: NP

3. **CITATION**

Author:	James R. Hoberg
Title:	Supplemental to: R107894-Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions.
Study Completion Date:	23 January 2006
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Materials Protection Division Turnhoutsweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Study No. 13751.6119 Janssen Study No. AGR 368
MRID No.:	467513-06

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD, US EPA
Signature: Kathryn V. Montague **Date:** 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD, US EPA
Signature: Siroos Mostaghimi **Date:** 9/20/06

6. **PURPOSE:** This supplemental report (MRID 467513-06) was prepared in response to the Data Evaluation Record (DER) for the exposure of juvenile sheepshead minnow to R107894 under flow-through conditions (MRID 456740-03). The study was classified as supplemental due to a number of guideline deviations. The laboratory notes that with additional or clarifying information the study may be upgraded to core. The supplemental study provides the laboratory's additional or clarifying information to each guideline deviation noted in the DER. This DER reviews the laboratory's responses to the deviations.

7. **ADEQUECY OF THE STUDY:**

A. Classification: The information contained in this submission is adequate to allow the original study, MRID #456740-03, to be upgraded from supplemental to acceptable.

B. Rationale: Adequate explanation/justification was provided to address the major Guideline deviations in the original DER.

C. Repairability: N/A

8. GUIDELINE DEVIATIONS AND RESPONSES:

a) Guideline Deviation #1: The Guideline specifies that temperature should be recorded at least hourly in one replicate throughout the test. According to the original DER, the Study Report indicates that continuous temperature monitoring was performed in replicate A of the control, but no hourly measurements were reported.

Registrant response: Temperature was recorded continuously and noted in footnote b located on page 21 of the original report.

Reviewer's comments: The response satisfies any noted deviations.

b) Guideline Deviation #2: The Guideline states that the test substance should be measured in each replicate at 0, 48, and 96 hours. According to the original DER, concentrations were not reported for each replicate (a mean value was reported for each set of replicates) and were only measured at the start (0 hours) and end of the test (96 hours).

Registrant response: The laboratory used its knowledge of the test material and allowances under guideline 850.1000 to respond to the deviation. The laboratory noted that guideline 850.1000 allows the laboratory to conduct alternating replicate analyses when using a "splitter" under flow through conditions. This condition was met by the laboratory. Guideline 850.1000 requires that the test material not degrade to less than 70%. The laboratory noted that the concentrations actually increased at termination and attributed the increases to the degradation product, CL322,250. The laboratory stated that the 48-hour test concentration analysis was not required since variability was not expected to cause a problem.

Reviewer's comments: Guidelines 850.1000 states: "although it is undesirable to allow the concentrations to decline throughout the study, the problem may be unavoidable" and also that test concentration measurements should be made at 48-hours, if variability is expected to be a problem. The response satisfies any noted deviations.

c) Guideline Deviation #3: The Guideline states that no more than 25% variation is allowed between test concentrations within the same treatment during the test. Since concentrations were only measured at the start of the test (0 hours) and at the end of the test (96 hours) and in alternate replicates, it cannot be determined whether this requirement was met.

Registrant response: See above response.

Reviewer's comments: The response satisfies any noted deviations.

d) Guideline Deviation #4: Information regarding the test substance, such as substance form, impurities, and physicochemical characteristics, was not provided.

Registrant response: The study sponsor has previously provided information regarding the test substance information, impurities, and physiochemical characteristics information in the Product Chemistry section of the original submissions under MRID 45673901, 45673906-07.

Reviewer's comments: It is recommended that the Product Chemistry section (MRID 45673901, 45673906-07) be reviewed for effects concerning aquatic systems (e.g. degradation in water and/or light). Chemical information pertaining to fish acute toxicity should be contained in the study report.

e) Guideline Deviation #5: The Guideline indicates that the hourly temperature reading should be recorded at least hourly in one replicate. The Study Report indicates that continuous monitoring was performed in one replicate, but the data is not reported.

Registrant response: The Laboratory noted that the response is the same as a) above.

Reviewer's comments: The response satisfies any noted deviations.

f) Guideline Deviation #6: The dissolved oxygen concentration fell below the recommended 75% 24 hours into the test, ranging from 68 to 73% in treatments 100, 60, and 36 µg a.i./L.

Registrant response: The oxygen level fell below 75% at the 24-hour interval and then rebounded to above the recommended level. The lower levels coincided with fish mortality and the decrease was attributed to greater oxygen consumption by fish due to chemical stress. The laboratory, through experience, noted that sheepshead minnow can survive without stress at oxygen levels near 50%. The laboratory noted that the minnows should survive at oxygen saturation levels of 68%.

Reviewer's comments: The response satisfies the noted deviation, however, there does appear to be a typo in the table provided (table 1 on page 13 of the supplemental report. In that table, the dissolved oxygen level for replicate A, at 24-hours for the nominal concentration (22 ug a.i./L), is reported to be 5.9 mg/L or 8.1%. It appears that this should be a saturation level of 81%, not 8.1%.

g) Guideline Deviation #7: It could not be determined whether the light intensity measurement was within the recommended intensity range specified in the Guideline since the units, lux, can only be converted to lm/m^2 and the recommendation is in lm.

Registrant response: The laboratory stated that the test substance is not light sensitive and that the lighting during exposure was adequate for the test organisms to survive. The laboratory does not feel that the deviation impacted the test results.

Reviewer's comments: The response satisfies any noted deviations, but the Product Chemistry section (MRID 45673901, 45673906-07) should be reviewed to verify. See d) above.

h) Guideline Deviation #8: No details were provided regarding when the fish were added to the test chambers in relation to the addition of the test substance. Also, the Study Report does not indicate if any disease treatment was administered to the fish 48 hours prior to test initiation or during the test.

Registrant response: Analysis of the test solutions during the pretest period indicated that the test substance was present at appropriate levels in the test solutions (see pg. 7 of the original report- MRID 456740-03, section 3.2.2 or page 12 of this report- MRID 467513-06). The fish were

added to the test solutions subsequent to review of the pretest analysis. No disease treatments were administered to the fish for at least 10 days prior to testing.

Reviewer's comments: The response satisfies any noted deviations.

APPENDIX A
Original Data Evaluation Report

DATA EVALUATION RECORD
Fish Acute Toxicity Test, Freshwater and Marine (OPPTS 850.1075)

1. CHEMICAL: R107894 (Econea technical) PC Code No.: 119093

2. TEST MATERIAL: R107894 Purity: 94.6% a.i.

3. CITATION

Author(s):	William Lima (Study director)
Title:	R107894 - Acute toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions
Study Completion Date:	October 16, 2001
Laboratory:	Springborn Laboratories, Inc. 790 Main Street Wareham, Massachusetts 02571-1075
Sponsor:	Janssen Pharmaceutica Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Study No. 13751.6119 Janssen Study No. AGR. 368
MRID No.:	456740-03
DP Barcode:	D289026

4. REVIEWED BY: Kathryn V. Montague, Biologist, AD/RASSB, USEPA

Signature:

Date:

5. APPROVED BY: Rick Petrie, Team Leader, AD/RASSB, USEPA

Signature:

Date:

6. STUDY PARAMETERS

Scientific Name of Test Organism:	*Cyprinodon variegatus*
Age of Test Organism at Test Initiation:	Juvenile
Definitive Study Duration:	96 hours
Type of Concentrations:	Mean measured

7. IDENTIFICATION OF TEST SUBSTANCE

Test Substance:	R107894
Trade name:	ECONEA Technical
CAS number:	122454-29-9
Description:	Not reported
Active Ingredient(s):	94.6%
Inactive Ingredient(s):	Not reported
Source:	Janssen Pharmaceutica Titusville, New Jersey
Lot or Batch Number:	AC 12395-43
Purity:	94.6%
Control Substance:	Seawater (dilution water control) and seawater with acetone (solvent control)

8. SUMMARY

An acute toxicity study was performed for R107894 technical on Sheepshead Minnow (*Cyprinodon variegatus*) in seawater. Nominal test concentration of 13, 22, 36, 60, and 100 µg a.i./L and controls (saltwater and saltwater with acetone) were tested. Groups of ten sheepshead minnows per replicate were exposed to the test concentration and the controls. The batch of chemical obtained for the study contained 94.6% of the active ingredient R107894. Mean measured concentrations of R107894 were 5.6, 10, 17, 30, and 48 µg a.i./L. Partial mortalities were observed in 3 treatment levels and total mortality occurred in the sheepshead minnows exposed to the highest treatment concentration (100 µg a.i./L).

9. CONCLUSIONS

Results Synopsis:

Moving average analysis (TOXANAL)

96-hr LD_{50}:	23.71 µg a.i./L	95% C.I.:	19.9 - 29.3 µg a.i./L
Probit slope:	probit method not used	NOEC:	10.0 µg a.i./L

10. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: Test chemical concentrations were not measured according to Guideline recommendations (see below). This results in uncertainty regarding the actual levels to which the organisms were exposed throughout the test.

C. Repairability: Not repairable.

II. GUIDELINE DEVIATIONS

- The Guideline specifies that temperature should be recorded at least hourly in one replicate throughout the test. The Study Report indicates that continuous temperature monitoring was performed in replicate A of the control but no hourly measurements were reported.

- **The Guideline states that the test substance should be measured in each replicate at 0, 48, and 96 hours. In the Study Report, concentrations were not reported for each replicate (a mean value was reported for each set of replicates) and were only measured at the start (0 hours) and end of the test (96 hours).**

- **The Guideline states that no more than 25% variation is allowed between test concentrations within the same treatment during the test. Since concentrations were only measured at start of test (0 hours) and at end of test (96 hours) and in alternate replicates, it cannot be determined whether this requirement was met.**

- Information regarding the test substance, such as substance form, impurities, and physicochemical characteristics, was not provided.

- The Guideline indicates that hourly temperature reading should be recorded at least hourly in one replicate. The Study Report indicates that continuous monitoring was performed in one replicate, but the data is not reported.

- The dissolved oxygen concentration fell below the recommended 75% 24 hours into the test, ranging from 68 to 73% in treatments 100, 60, and 36 µg a.i./L.

- It could not be determined whether the light intensity measurement was within the recommended intensity range specified in the Guideline since the units, lux, can only be converted to lm/m^2 and the recommendation is in lm.

- No details were provided on when the fish were added to the test chambers, in relation to the addition of the test substance. Also, the Study Report does not indicate if any disease treatment was administered to the fish 48 hours prior to test initiation or during the test.

21. SUBMISSION PURPOSE: Registration

22. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species is the bluegill sunfish (*Lepomis macrochirus*) and rainbow trout (*Oncorhynchus mykiss*). (Data on both cold and warm freshwater generally required.) If saltwater species required - Atlantic silverside (*Menidia menidia*).	Sheepshead minnow (*Cyprinodon variegatus*) is one of the saltwater test species identified in OPPTS 850.1075 as an acceptable test species.
Weight:	For representative sample (N=30): (p. 12)

Juvenile fish <3.0 g should be used.	Mean = 0.48 g Range = 0.19 to 1.1 g
Age: Are juvenile fish of the same age?	The Study Protocol (p. 31) indicates that the fish will be of approximately the same age and size.
Size: Longest not > 2x shortest	For representative sample (N=30): (p. 12) Mean = 25 mm Range: 19 to 36 cm
Supplier:	Aquatic BioSystems, Ft. Collins, CO (p. 11)
All fish from same source and population?	Yes
Were fish used in a previous test?	Not specified.
If wild fish used, were they quarantined for 7 days?	Not applicable.

B. Acclimation

Guideline Criteria	Reported Information
Acclimation Period: Minimum 12 days (14 days recommended)	Stock was acclimated at least 14 days (p. 11).
Holding water: Should come from same source as test dilution water (if not, acclimation to dilution water should be done gradually).	Yes.
Disease treatment: Were any disease treatments administered within 48 hr of test initiation or during testing?	No information provided (assume none administered).
Feeding: Did any feeding occur within 48 hr of test initiation?	No (p. 12)
Pretest mortality: Did fish appear stressed or did >5% die during 48 hr prior to test?	No mortalities observed (p. 12).
Water temperature: · Any changes should not exceed 3°C per day. · Fish should be held minimum of 7 days at test	· Temperature ranged from 22 to 24°C (p. 12) (recommended test temperature is 22 ± 2°C) during holding. · Fish were held for a minimum of 14 days under these conditions (p. 11).

temperature prior to testing.	

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Clean surface or ground water, seawater (estuaringe/marine species), and reconstituted water. If dechlorinated tap water used then daily chlorine analysis should be performed.	Natural filtered natural seawater used (from Cape Cod Canal, Bourne, MA) (p. 12).
Water temperature: · Recommended for sheepshead minnows: 22 ± 2.0°C. · Must be recorded in all replicates at start of test and every 24 hr. · Temperature should be recorded at least hourly in one replicate throughout the test. · Temperature should vary no more than 1.0°C in any 24-hr period. · Test system should have automatic alarm to alert temperature changes in excess of 2.0°C.	· Reported range: 22.0 -23.0°C (p. 21). · One measurement reported for start of test and for every 24 hours thereafter (p. 21). · The report indicates that continuous temperature monitoring was performed in replicate A of the control although no detailed measurements are reported (p. 17). Duplicate aquaria were kept in a water bath to maintain the temperature at 22 ± 1.0°C (p.13). · Reported temperatures did not vary by more than 1.0°C (p. 21). · Information not reported.
Dissolved Oxygen · Static tests: ≥60% saturation or for flow-through tests: ≥75% at all time. · Should be measured in each replicate at start of test and then every 24 hrs.	· Flow-through test performed with DO ≥5.4 mg O_2/L with DO range: 5.4 - 6.7 mg/L (p. 21). According to Protocol Deviations (p. 19), the DO percent saturation fell below 75% for measurement made at 24-hours when it ranged from 68 to 73% in treatments 100, 60, and 36 µg a.i./L. · Yes - DO measured daily in each replicate.
Chemical analysis of test water: · Was a chemical analysis performed on the water used in testing? · Were the required elements tested?	· The report indicates that the dilution water supply is analyzed perdiodically for potential toxicants, including pesticides, PCBs and selected toxic metals (p. 12). The water is also analyzed monthly for total organic carbon concentration. · According to the report, none of these compounds were detected at concentrations

Guideline Criteria	Reported Information
· Were any maximum concentrations exceeded?	that are considered toxic in any of the water samples analyzed. (p. 12).
Water Hardness: · Between 40 and 180 mg/L as $CaCO_3$. · Should be measured at beginning of each test.	· Not applicable (only for freshwater).
Salinity: · Should be 20±5 ppt for estuarine species. · For flow-through tests, should be recorded at the beginning of test, on day 4, and if extended, on days 7 and 14.	· Not applicable - marine species. · Salinity was recorded at start of test and at every 24-hr interval thereafter (p. 21).
Was distilled water used in making stock solutions of test water?	Not applicable.
Solvents: · Preferred solvents are dimethyl formamide, triethylene glycol, methanol, acetone, or ethanol. · Solvent concentration should not exceed 0.5 mL/L (static-renewal testing) or 0.1 mL/L (flow-through testing). · Solvent concentrations should be kept constant in the solvent control and all test solutions.	· Acetone used as solvent. · The concentration of acetone in the solvent control vessels was equivalent to the concentration of solvent present in the highest treatment level solutions (0.095 mL/L) (p. 14). · The diluter system was calibrated before and at the end of the test and was monitored daily (p. 13).
pH: · pH should not be adjusted after addition of test chemical or stock solution into the dilution water. · pH should be measured in each replicate at the beginning of every test and every 24 hrs afterwards. · pH must be monitored in low, medium, high test concentrations and must remain >6.0 and <8.0 for freshwater testing and >7.5 and <8.5 for marine testing.	· No indication given in the report that pH was adjusted. · Yes (p. 21). · Measured for each nominal concentration and control replicate on a daily basis; reported range: 8.0 to 8.1 (p. 21).
Loading: Static/static-renewal tests: ≤8 FWF/L solution, or flow-through tests: ≤0.5 FWF/L solution, per 24 hours	Fish loading was 0.11 g fish/L/day (p. 14).
Test chambers/equipment: · Material that contact stock solution, test solution, or dilution water: Glass, No. 316	· Test vessels used were constructed of glass and silicone sealant (p. 13).

Guideline Criteria	Reported Information
stainless steel, nylon screen, and perfluorocarbon plastic (e.g., Teflon). · Size: need to be large enough to maintain specified load rates of FWF. · System should be calibrated before and after each test.	· Each test aquaria measured 30 x 15 x 20 cm and water depth was maintained at 15 cm to achieve a test volume of 6.8 L (p. 14) · Yes (p. 14).
Aeration: Static test systems - aeration allowed only if oxygen levels are in danger of dropping below 60% saturation due to chemical characteristics of the test material. Test concentration must be measured during the test is aeration used. Flow-through test - no aeration of actual test vessels may be utilized.	Test solutions not aerated (p. 13).
Light: · Photoperiod - may range from 12D/12N to 16D/8N with 15 to 30 min transitions periods. · Intensity - should range from 39 to 100 lm at water surface.	· 16 hour photoperiod used with no sudden transitions (p. 13). · Light intensity measured at 70 to 80 footcandles at surface of the test solution (1 footcandle = 10.76 lm/m^2, so for aquarium with dimensions of L x W = 30 cm x 15 cm and intensity of 75 footcandles, intensity would be 36.3 lm) (p. 13).
Did the test concentrations remain at least at 80% of the nominal concentrations throughout the test? or Did mean measured concentrations accurately represent exposure levels maintained throughout the test?	No (see p. 22). The mean percent of the nominal concentration calculated with the measurements taken at 0 hour and 96 hours ranged between 43 and 51%.

D. Test Design

Guideline Criteria	Reported Information
Range-finding test: Was a range-finding test performed?	Yes (p. 17).
Test substance: · Degree of purity for technical ingredients and mixtures reported? · Percentage of each impurity reported (totaling to 100%)? · Physicochemical characteristics (i.e., solubility, vapor pressure, hydrolysis in pH 5, 7, and 9) reported?	· Active ingredient reported to be 94.6% (provided by the sponsor) (p. 11). · No information provided. · No information provided.
Was the test performed for a minimum of 96 hours?	Yes (p. 9).
Controls: Were same dilution water, conditions, procedures, and test population used for the controls?	Yes.
Introduction of fish: · Were fish added to test chambers within 30 min of or prior to addition of test substance? · Were fish introduced randomly or impartially to each replicate?	· Not specified in the report. · Yes (p. 14).
Number of test organisms: Minimum 7 fish/level required (10 is preferred).	10 fish for each test concentration and control replicate (p.14).
Did each test chamber have equal volumes of test solutions and fish?	Yes (p. 14).
Were fish fed during the treatment period?	No (p. 12).
Test concentrations: · Minimum of control and 5 concentrations in a geometric series. · Concentrations should be 50% greater than the next lowest test concentration (not to exceed 120 percent). · No more than 25% variation allowed between test concentrations within the same treatment during the test.	· 5 treatment levels (13, 22, 36, 60, and 100 µg a.i./L) and 2 controls (dilution water and solvent) (p. 9). · Concentration were >50% higher than the next lowest test concentration. · Not able to determine - concentrations were only measured at start of test (0 hours) and at end of test (96 hours)and in alternate replicates (see Table 2, p. 22).
Concentration selection: Test concentration should be selected to produce	

Guideline Criteria	Reported Information
a NOEC and, preferably, at least 2 partial mortalities (i.e., one greater than and the other less than 50%, after 96 hours).	Yes (p. 24).
Concentration analysis: · Concentration analysis must be performed at test start and then every 48 hrs. · Static tests: concentration should be measured in each replicate minimally at start, at 48 hrs, and at the end of test. · Static-renewal tests: concentration should be measured in each replicate minimally at start and end of test and just before and after each renewal. · Flow-through tests: concentration should be measured in each replicate at 0, 48, and 96 hrs, and every 96 hours thereafter as long as test continues.	Flow-through test performed. Concentrations were measured only in one replicate per test concentration at start of (0 hrs) and end of the test (96 hrs) (p. 15).

E. Reported Results

Guideline Criteria	Reported Information
Test facilities, test dates, and personnel reported?	Yes.
Were the LC50 concentration curves, LC50 values, and associated 95% CI determined for 24, 48, 72, and 96 hr (whenever sufficient data exist)?	LC50s and associated 95% CIs were calculated for 24, 48, 72, and 96 hr, however, concentration data was not measured at 24, 48, and 72 hrs (p. 25).
Was a graph of the concentration-mortality curve at test termination included in the report?	Yes (p. 27).
Control mortality: Not more than 10% (or 1 mortality if 7 to 10 control fish are used) control organisms may die during test.	No fish deaths occurred in the control groups (p. 24).
Was a NOEL for the 96-hr test reported?	Yes, a NOEC of 10 µg a.i./L was determined (p. 18).
If no NOEL determined was this due to the test chemical's aqueous solubility limit, self-dispersibility limit, or other physico-chemical limitations? Explanation should be provided.	Not applicable.
Was any abnormal behavior displayed by the test	The report indicates that there were no sublethal

Guideline Criteria	Reported Information
fish reported?	effects observed among the fish in the controls. The study does not indicate if sublethal effects were seen in the fish populations in the test concentrations (p. 18).
Were there any protocol deviations or occurrences that may have affected the final results?	The dissolved oxygen concentration dropped to 68-73% during the 24 hour interval in treatments 100, 60, and 36 µg a.i./L. The report indicates that this deviation did not impact the results (p. 19).
Were constant conditions maintained throughout the test?	Dissolved oxygen fell below 75% during the test. Also, because R107894 hydrolyzed rapidly to CL322,250, the concentrations of the test substances dropped to 43 to 51% of the nominal concentrations.
Were quality control methods and a quality assurance statement included?	Yes (pp. 3 and 4).
Were raw data presented?	Excerpted raw data was included in Appendix III (p. 58).
Were the methods of statistical analysis reported?	Yes, the statistical analysis is described in the report (p. 16).
Were the methods used in analyzing the test concentrations described? Was the accuracy of the each method (i.e., detection limit and quantification limit) provided?	Yes (p. 15 and Appendix II).

95. RESULTS SUMMARY

Summary of Percentage Mortalities During Test

Nominal Concentration (µg a.i./L)	Mean Measured Concentration (µg a.i./L)	Replicate	Cumulative Mortality (%)			
			24 hrs	48 hrs	72 hrs	96 hrs
Control	NA	A	0 (0)	0 (0)	0 (0)	0 (0)
		B	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	NA	A	0 (0)	0 (0)	0 (0)	0 (0)
		B	0 (0)	0 (0)	0 (0)	0 (0)
13	5.6	A	0 (0)	0 (0)	10 (1)	10 (1)
		B	0 (0)	0 (0)	0 (0)	0 (0)
22	10	A	0 (0)	0 (0)	0 (0)	0 (0)
		B	0 (0)	0 (0)	0 (0)	0 (0)
36	17	A	0 (0)	10 (1)	10 (1)	10 (1)
		B	0 (0)	10 (1)	20 (2)	20 (2)
60	30	A	20 (2)	50 (5)	60 (6)	60 (6)
		B	30 (3)	60 (6)	70 (7)	70 (7)
100	48	A	100 (10)	100 (10)	100 (10)	100 (10)
		B	100 (10)	100 (10)	100 (10)	100 (10)

The actual number of mortalities is shown in parentheses.

Measured Concentrations of R107894 for Flow-through Test with Sheepshead Minnow (*Cyprinodon variegatus*).

Nominal Concentration (µg a.i./L)	Measured Concentration (µg a.i./L)		
	0 hours	96 hours	Mean
Control	<1.7	<1.5	NA
Solvent Control	<1.7	<1.5	NA
13	5.1	6.1	5.6
22	9.8	11	10
36	15	19	17
60	28	33	30

100	46	49	48

Statistical Results

96-hr LD_{50}: 26 µg a.i./L 95% C.I.: 17 - 48 µg a.i./L
Probit slope: not used NOEC: 10.0 µg a.i./L

15. VERIFICATION OF STATISTICAL RESULTS:

Using moving average method (TOXANAL):
96-hr LD_{50}: 23.71 µg a.i./L 95% C.I.: 19.9 - 29.3 µg a.i./L
Probit slope: not used NOEC: 10.0 µg a.i./L

16. REVIEWER'S COMMENTS: Measurements were only performed at the initiation and end of the test, thus, without additional data, it is uncertain what the actual levels of chemical were in the treatment vessels. The Guideline deviations are listed on page 2 of this report.

DATA EVALUATION RECORD
FISH EARLY LIFE STAGE TOXICITY TEST
GUIDELINE OPPTS 850.1400

1. **CHEMICAL:** Econea degradate **PC Code No.:** **N/A (degradate of 119093)**

2. **TEST MATERIAL:** CL322250 **Purity: 88.2%**
Batch No. 1547-20

3. **CITATION**

Author:	Mark A. Cafarella
Title:	CL322,250 – Early Life-Stage Toxicity Test with Zebra Fish (*Danio rerio*)
Study Completion Date:	January 27, 2006
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N.V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Janssen Study No. AGR1177 Springborn Smithers Study No. 13751.6158
MRID No.:	467513-07

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD/US EPA
Signature: Kathryn V. Montague **Date:** 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD/US EPA
Signature: Siroos Mostaghimi **Date:** 9/20/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Danio rerio*
Age of Test Organism: Embryos <24 hours old
Definitive Test Duration: 35 days (30 days post-hatch)
Study Method: Flow-through
Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Verified Results Synopsis:

Larval Survival*	Hatching Survival
NOEC: 0.069 mg a.i./L	NOEC: 0.27 mg a.i./L
LOEC: 0.14 mg a.i.;L	LOEC: 0.53 mg a.i./L
MATC: 0.098 mg a.i./L	MATC: 0.38 mg a.i./L

Total Length	Wet Weight
NOEC: ≥0.53 mg a.i./L	NOEC: ≥0.53 mg a.i./L
LOEC: >0.53 mg a.i./L	LOEC: >0.53 mg a.i./L
MATC: 0.53 mg a.i./L	MATC: 0.53 mg a.i./L

* Most Sensitive Indicator

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: No significant deviations from Guideline requirements

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on the EPA OPPTS Guideline 850.1400:

- The Study Report did not state what the brood fish were fed.
- Data were not available in the Study Report to determine temperatures between test chambers on successive days. However, the temperature range of 24 to 26°C was within the guideline range of 25 ± 2°C.
- The Study Report does not state if the embryonic stage at the beginning of the exposure period was verified.
- At the end of the test, fish were weighed individually for wet weight only. Guideline states that dry weights are preferred.
- NOEC and LOEC values were only reported for larval survival, the most sensitive indicator.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ Recommended freshwater - rainbow trout (*Oncorhynchus myliss*), fathead minnow (*Pimephales promelas*), zebra fish (*Danio rerio*), ricefish (*Oryzias latipes*) ▪ Recommended saltwater - sheepshead minnow (*Cyprinodon variegatus*) ▪ Examples of other well documented species listed in guidelines	▪ Zebra fish (*Danio rerio*) (p. 13).
Source of organisms	▪ Broodstock maintained at Springborn Smithers Laboratories (p. 13)
Feeding Time to first feeding ▪ Rainbow trout – 19 days post-hatch ▪ Fathead minnow -- within 2 days of hatching ▪ Zebra fish -- 6-7 days after spawning ▪ Ricefish -- within 24 hrs of hatch/swim-up ▪ Sheepshead minnow -- within 24 hrs of hatch/swim-up Brood Fish Food Requirements ▪ Rainbow trout – trout food ▪ Fathead minnow – frozen brine shrimp ▪ Zebra fish – brine shrimp nauplii, flake food ▪ Ricefish – flake food ▪ Sheepshead minnow – frozen brine shrimp or flake food Newly Hatched Larvae Food Requirements ▪ Rainbow trout – does not require food ▪ Fathead minnow -- brine shrimp nauplii, newly hatched ▪ Zebra fish -- protozoa (filtered from mixed culture), protein (granules from fermentation process) ▪ Ricefish -- brine shrimp nauplii, flake food (or protozoa or rotifers) ▪ Sheepshead minnow -- brine shrimp nauplii Juveniles Food Requirements ▪ Rainbow trout – trout starter/4% body weight per day/2-4 feeds per day ▪ Fathead minnow -- brine shrimp nauplii/ad libitum ▪ Zebra fish -- brine shrimp nauplii ▪ Ricefish -- brine shrimp nauplii or flake food or rotifers/brine shrimp once daily; flake food twice daily or flake food and rotifers once daily	▪ First feeding: test day 5 (day 0 post-hatch) (p. 16). ▪ Brood food: not reported ▪ Larvae food: culture of microorganisms (i.e. rotifers) 3x daily (p. 16). ▪ Juvenile food: live brine shrimp nauplii 3x daily *ad libitum* (p. 16).

Guideline Criteria	Reported Information
▪ Sheepshead minnow -- brine shrimp nauplii/2-3 feeds per day *Other well documented fish species requirements listed in guidelines.	

B. Test System

Guideline Criteria	Reported Information
Temperature ▪ Must not differ by more than ± 1.5°C between test chambers or between successive days ▪ Measured in all test chambers weekly and preferably continuously in at least one vessel ▪ Specific test species requirements (°C): -- Rainbow trout – 10 ± 2 for embryos; 12 ± 2 for larvae and juvenile fish[1] -- Fathead minnow -- 25 ± 2 -- Zebra fish -- 25 ± 2 -- Ricefish -- 24 ± 1; 23 ± 2 for larvae and juvenile fish; this supercedes the requirement for temperature control given earlier on in the test -- Sheepshead minnow -- 25 ± 2 [1]the particular strain of rainbow trout may necessitate the use of other temperatures; brood stock must be held at the same temperature as that to be used for the eggs *Other well documented fish species requirements listed in guidelines.	▪ Temperatures differed by 2.0°C (p. 27). Data not available to determine temperatures between test chambers or successive days. ▪ Measured in each aquarium daily and continuously in one replicate of control (pp. 17 and 27). ▪ Temperature range of 24 to 26°C throughout exposure period (p. 21).
Salinity ▪ Sheepshead minnow – should be at 15-30; for any given test this shall be performed to ± 2% ▪ Should be measured in all vessels weekly	▪ Not applicable.

Guideline Criteria	Reported Information
Photoperiod ▪ Rainbow trout – darkness for larvae until one week after hatching except when they are being inspected, then subdued lighting throughout test (12-16 hr photoperiod) ▪ Fathead minnow – 16 hr ▪ Zebra fish – 12-16 hr; for any given test conditions, light regime should be constant ▪ Ricefish – 12-16 hr; for any given test conditions, light regime should be constant ▪ Sheepshead minnow – 12-16 hr; for any given test conditions, light regime should be constant *Other well documented fish species requirements listed in guidelines.	▪ Photoperiod of 16-hr light/8-hr dark with transition period (p. 15). ▪ Light intensity 50 to 60 footcandles (540 to 640 lux) measured with VWR Traceable light meter (p. 15).
Test Chambers ▪ Any glass, stainless steel, or other chemically inert vessel can be used	▪ 12 glass aquaria with silicone sealant (39 x 20 x 25 cm, 15L) (p. 15).
Handling of Embryos and Larvae ▪ Initially, may be exposed within main vessel in smaller glass or stainless steel vessels, fitted with mesh sides or ends to permit flow of test solution ▪ May be suspended from an arm arranged to move vessel up and down to induce non-turbulent flow; organisms must always be submerged ▪ Egg containers, grids or mesh should be removed after larvae hatch, except mesh should be retained to prevent escape of fish ▪ If need to transfer larvae, should not be exposed to air and nets should not be used to release fish from egg containers ▪ Post-hatch transfer time requirements: -- Rainbow trout – 14-16 days post-hatch -- Fathead minnow – once hatching is 90% -- Zebra fish – not necessary -- Ricefish – from hatch to swim-up -- Sheepshead minnow – not applicable *Other well documented species requirements listed in guidelines.	▪ Exposure of embryos and larvae initiated when embryo incubation cups distributed to each test aquaria. Cups constructed of round glass jars with bottoms cut off and replaced with screen (pp. 15 and 40). ▪ Eggs impartially transferred to each egg incubation cup using a serological pipet after cups containing control water were placed in water bath and Carolina dish containing eggs placed in same water bath (pp. 15, 16 and 40). ▪ Egg cups suspended and oscillated by rocker arm apparatus (pp. 16 and 40).

Guideline Criteria	Reported Information
Loading of Fish ▪ Randomly distributed ▪ At least 60 eggs, divided equally between at least 2 replicate test chambers per concentration ▪ Loading rate low enough to allow oxygen concentration of at least 60% saturation maintained without aeration ▪ For flow through test, loading rate not to exceed 0.5g/L/24 hr and 5g/L of solution at any time	▪ Eggs impartially distributed (p. 15). ▪ 60 eggs per cup (one cup per replicate vehicle) (p. 16). ▪ Dissolved oxygen concentrations >60% of saturation (p. 27). ▪ Loading rate 0.02 g/L per day (p. 21).
Dissolved Oxygen ▪ Should be measured in all vessels weekly ▪ Between 60 and 100% saturation	▪ DO measured in each aquarium daily (p. 17). ▪ DO between 94 and 112% of saturation (p. 27).
pH ▪ Should be measured in all vessels at beginning and end of test	▪ pH measured in each aquarium daily (p. 17). ▪ Range: 7.4 – 7.9 (p. 27).
Dilution Water ▪ Any water in which test species shows control survival at least as good as that listed below (see Survival Criteria) ▪ Dilution water samples should be taken at intervals (e.g., every 3 months) and analyzed for heavy metals, pesticides, total organic carbon, major anions and cations, and suspended solids ▪ Acceptable chemical characteristics of dilution water: particulate matter - <20 mg/L, total organic carbon - <2 mg/L, un-ionized ammonia - <1Φg/L, residual chlorine - <10Φg/L, total organophosphorus pesticides - <50 ng/L, total organochlorine pesticides plus PCBs - <50ng/L, total organic chlorine - <25mg/L	▪ Dilution water-well water supplemented with Town of Wareham well water and aerated (p. 13). ▪ Daphnid cultures successfully maintained and cultured in same source of dilution water (p. 13). ▪ Analyzed periodically for presence of pesticides, PCBs and toxic metals; none detected at concentrations considered toxic (ASTM) (p. 13). ▪ TOC concentrations measured monthly; 0.17 to 1.1 mg/L (p. 13). ▪ Total hardness and alkalinity measured weekly; 52 to 60 mg/L and 34 to 37 mg/L, respectively. ▪ Specific conductance 190 to 280 umhos/cm (p. 13).

Guideline Criteria	Reported Information
Renewal ▪ For flow-through systems, require system which continually dispenses and dilutes a stock solution of the test substance ▪ Flow rates of stock solution and dilution water should be checked at intervals during the test and should not vary by more than 10% throughout the test ▪ A flow rate equivalent to at least 5 test chamber volumes per 24 hr has found to be suitable ▪ For semi-static technique, two different renewal systems may be followed: (1) new test solutions prepared in clean vessels and surviving eggs and larvae gently transferred into new vessels or (2) test organisms are retained in test vessels while a proportion (at least 2/3) of the test water is changed	▪ Exposure system (intermittent-flow proportional diluter system) designed to provide five concentrations of test substance and a dilution water control to duplicate test aquaria (p. 14). ▪ Calibration of diluter system checked prior to test initiation and confirmed at test termination. Function of diluter monitored daily and visual check twice daily. Study Report states that diluter system functioned properly. (pp. 14 and 15). ▪ Flow rate approximately 7.0 aquarium volumes per 24-hour period, with 90% replacement time of 7 hours (p. 15).

C. Test Design

Guideline Criteria	Reported Information
Doses ▪ Five concentrations of test substance spaced by a constant factor not exceeding 3.2 ▪ Use of fewer than five concentrations, for example in limit tests, and a narrower concentration interval may be appropriate; justification required ▪ When solubilizing agent used, concentration should not be greater than 0.1 mL/L and should be same in all vessels	▪ Five nominal test concentrations of 0.031, 0.063, 0.13, 0.25 and 0.50 mg a.i./L (p. 14). ▪ Concentrations spaced by a constant factor of 2.0 (p. 14). ▪ Use of solubility agent not reported (p. 14).
Controls ▪ One dilution water control and, if relevant, one solvent control	▪ Dilution water control (p. 14).
Replicates Per Dose ▪ At least 2 replicates per concentration	▪ Two replicates per concentration and control (pp. 14 and 29).
Frequency of Analytical Determinations and Measurements ▪ During test, concentration of test substance determined at a minimum of 5x is necessary ▪ If study lasts more than 1 month, determinations should be made at least once a week	▪ Samples collected from each treatment level and control on test days 0, 5, 12, 19, 26 and 28 (i.e., day -5, 0, 7, 14, 21 and 28 post-hatch((p. 18).

Guideline Criteria	Reported Information
Duration of Test ▪ Should start as soon as possible after eggs fertilized ▪ Should continue until at least all control fish have been free-feeding ▪ Species-dependent requirements: ▪ Rainbow trout – 2 weeks after controls are free-feeding (or 60 days post-hatch) ▪ Fathead minnow – 32 days from start of test (or 28 days post-hatch) ▪ Zebra fish – 30 days post-hatch ▪ Ricefish – 30 days post-hatch ▪ Sheepshead minnow – 32 days from start of test (or 28 days post-hatch)	▪ Exposure initiated within 24 hours after fertilization (p. 11). ▪ Test duration 35 days (30 days post-hatch) (p. 11)
Survival Criteria (of controls) ▪ Hatching success (percent): -- Rainbow trout – >66 -- Fathead minnow – >66 -- Zebra fish – none provided in guidelines -- Ricefish – none provided in guidelines -- Sheepshead minnow – >75 ▪ Post-Hatch success (percent) -- Rainbow trout – 70 -- Fathead minnow – 70 -- Zebra fish – 70 -- Ricefish – 80 -- Sheepshead minnow – 80	▪ Hatching success: average 87% (p. 22). ▪ Post-hatch success: average 94% (p. 22).

D. Observations

Guideline Criteria	Reported Information
Stage of Embryonic Development ▪ Embyronic stage at beginning of exposure to the test substance should be verified as precisely as possible	▪ Embryonic stage not reported.
Hatching and Survival ▪ Observations should be made at least once daily and numbers recorded ▪ Dead embryos, larvae, and juvenile fish should be removed as soon as observed	▪ Observations made daily and numbers recorded (pp. 16 and 17). ▪ Dead embryos, larvae and juvenile fish removed when observed (p. 17).
Abnormal Appearance ▪ Number of larvae or fish showing abnormality of body form should be recorded at adequate intervals depending on duration of test ▪ Should only be removed upon death	▪ Appearance observed and recorded daily (p. 17).

Guideline Criteria	Reported Information
Abnormal Behavior ▪ Abnormalities should be recorded at adequate intervals depending on duration of test	▪ Behavior observed and recorded daily (p. 17).
Weight ▪ At end of test, all surviving fish must be weighed (individual weights preferred but if fish are especially small, may be weighed in groups by test vessel; dry weights (25 hr at 60°C) preferable to wet weights (blotted dry))	▪ At end of test, surviving fish weighed individually (wet weight) (p. 17).
Length ▪ At end of test, measurement of length recommended: standard, fork or total length (if caudal fin rot or fin erosion occurs, standard lengths should be used)	▪ At end of test, surviving fish measured individually for total length (p. 17)

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	▪ Yes (pp. 3 and 4).
Name of test and investigator, name and location of laboratory, and start/end dates of test reported?	▪ Yes (pp. 1 and 9).
Source of test material, lot number, composition, known chemical and physical properties, and identity and concentration of any solvent used reported?	▪ Yes (names, source, batch number, purity and recertification date (p. 12). Known physical/chemical properties not provided.
Nominal test concentrations, means of measured values, their standard deviation in the test vessels, and the method by which these were attained?	▪ Yes (pp. 17, 18, and 28).
Evidence that concentrations of test solutions have been satisfactorily maintained within ± 20% of mean measured values?	▪ Yes (p. 28).
Method of preparation of stock solutions and frequency of renewal?	▪ Yes (p. 14).
Information on characteristics of dilution water?	▪ Yes (p. 13).
Detailed information on feeding?	▪ Yes (p. 16).
Description of test system and test design included?	▪ Yes (pp. 14 and 15).
Water quality within test vessels?	▪ Yes (pp. 21 and 27).
NOEC and LOEC for each response included?	▪ No, only for larval survival, the most sensitive indicator (p. 23).
Concentration-response data and curves included?	▪ Yes (concentration response data).

Guideline Criteria	Reported Information
Raw data included?	▪ Only representative data (Appendix 3)
Methods and data records reported?	▪ Yes
Statistical methods reported?	▪ Yes (pp. 18 and 19)

Dose Response: At the end of the test, all surviving fish were healthy. No abnormal behavior was observed in any treatment level tested or the control. Based on the results of the study, larval survival was determined to be the most sensitive endpoint.

Mean Survival of Organisms at Hatch and Mean Survival, Total Length, and Wet Weight of Larvae at 30 Days Post-Hatch

Nominal Concentrations (mg ai/L	Mean Measured (mg ai/L)	Survival of Organisms at Hatch (%)	Larval Survival (%)	Total Length (SD)[a] (mm)	Wet Weight (SD) (mg)
Control	Control	87	94	18.9 (1.5)	53.3 (12)
0.031	0.036	78	87	18.1 (1.9)	49.5 (14)
0.063	0.069	77	93	18.9 (1.5)	53.2 (15)
0.13	0.14	87	64[b]	18.8 (1.7)	56.9 (15)
0.25	0.27	78	84	18.0 (1.9)	48.0 (16)
0.50	0.53	68	37[b]	17.5 (1.9)[c]	45.9 (17)[c]

a SD = Standard Deviation
b Statistically reduced compared to the control based on Dunnett's Test.
c Excluded from further statistical analysis due to survival effect.

Statistical Results

Statistical Method: Data obtained on larval survival and larval growth (total length and wet weight) at test termination were analyzed to identify significant differences between the treatment and control groups. Analyses were performed using the mean organism response in each treatment group rather than individual response values. Significant differences in the percent survival were evaluated after transformation (i.e., arsine square-root percentage) of the data.

The data were checked for normality and homogeneity of variance using Shapiro-Wilks Test and Bartlett's Test, respectively. The performance of the organisms exposed to each treatment level of the test substance was compared to the performance of the control using Dunnett's Test for larval survival and Williams' Test for larval length and wet weight. Any treatment levels that demonstrated a statistically significant effect on larval survival were excluded from further statistical analyses. The number of healthy fish at the end of the test, the time to start and end of hatching, the number of larvae hatching each day, the number of deformed larvae and the number of fish exhibiting abnormal behavior were not statistically analyzed due to the lack of variability observed between treatment levels and controls. TOXSTAT® was used to perform the statistical computation. The theoretical threshold

concentration expected to produce no deleterious effects at the 95% level of uncertainty was estimated as the MATC (geometric mean of the NOEC and LOEC).

Results Synopsis: .

Based on the most sensitive indicator-larval survival:

NOEC: 0.069 mg a.i/L
LOEC: 0.14 mg a.i/L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The survival of organisms at hatch, larval survival, total length, and wet weight data were analyzed to determine if significant differences existed between the control and the treatment groups. The data for each endpoint was first tested for normality and homogeneity of variances using the Shapiro Wilk's Test and Bartlett's Test, respectively. All data sets were found to be normally distributed. All data sets were also found to exhibit homogeneity of variances, with the exception of the total length data. Homogeneity of variance tests could not be performed because one group had zero variance. For the total length data set, the Kruskal-Wallis Test was used to determine any significant differences due to treatment effects. For the remaining data sets, Dunnett's Test was used to determine any significant differences due to treatment effects. A NOEC and LOEC were determined for each dataset, as well as the MATC.

Results Verification Synopsis:

Larval Survival*

NOEC: 0.069 mg a.i./L
LOEC: 0.14 mg a.i./L
MATC: 0.098 mg a.i./L

Hatching Survival

NOEC: 0.27 mg a.i./L
LOEC: 0.53 mg a.i./L
MATC: 0.38 mg a.i./L

Total Length

NOEC: ≥0.53 mg a.i./L
LOEC: >0.53 mg a.i./L
MATC: 0.53 mg a.i./L

Wet Weight

NOEC: ≥0.53 mg a.i./L
LOEC: >0.53 mg a.i./L
MATC: 0.53 mg a.i./L

* Most Sensitive Indicator

14. REVIEWER'S COMMENTS:

Deviations from the guidelines are found in Section 9 of this DER.

DATA EVALUATION RECORD
MYSID ACUTE TOXICITY TEST
GUIDELINE OPPTS 850.1035

1. **CHEMICAL:** PC Code No.: 119093

2. **TEST MATERIAL:** CL322,248 Purity: 94.5%
CAS No. 122454-29-9
Batch No. 1547-24

3. **CITATION:**

Author:	Lee E. Sayers
Title:	CL322,248 – Acute Toxicity to Mysids (*Americamysis bahia*) Under Static Conditions
Study Completion Date:	24 January 2006
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, Massachusetts 02571-1037
Sponsor:	Janssen Pharmaceutica N. V. Plant and Material Protection Division Turnhoutseweg 30 B-2340 Beerse, Belgium
Laboratory Report ID:	Springborn Smithers Study No. 13751.6160, Janssen Study No. AGR1179
MRID No.:	467513-08

4. **REVIEWED BY:** Kathryn Montague, M.S., Biologist RASSB/AD/US EPA
Signature: Kathryn V Montague Date: 09/19/06

5. **APPROVED BY:** Siroos Mostaghimi, Team Leader RASSB/AD/US EPA
Signature: Siroos - Mostaghimi Date: 9/20/06

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: *Americamysis bahia*
Age of Test Organism: <24 hrs
Definitive Test Duration: 96-hr
Study Method: Static
Type of Concentrations: Mean-measured

7. **CONCLUSIONS**

Verified Results Synopsis: 96-hr LC_{50}: 4.3 mg a.i./L 95% C.I.: 3.3 to 5.4 mg a.i./L
NOEC: 2.5 mg a.i./L

8. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale: Although there were deviations from Guideline recommendations, they did not affect the results or reliability of this study.

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.1035:

- The dissolved oxygen levels were between 37% and 108% saturation. This falls outside the guideline-stipulated range of 60% to 105%. Low dissolved oxygen levels were measured in the solvent control only. Excluding the solvent control, dissolved oxygen levels were between 5.5 and 7.8 mg/L (77% to 108% saturation). The study author notes that "gentle, oil-free aeration" was used at 72-hrs (when the low DO levels were measured in the solvent control). Because no mortalities or adverse effects were observed in the control vessels, and all analytical recoveries at 96 hrs of exposure were consistent, the study authors concluded that this deviation did not have a negative impact on the results of the study.
- The test water used (filtered natural seawater) was different from the culturing water (artificial seawater)
- The temperature changed more rapidly than the guidelines recommend (2°C in a 24-hr period vs. 1°C in a 24-hr period).
- Based on the limited information available, the salinity may have changed more rapidly than the guidelines allow (5 to 10% in a 24-hr period vs. 5% in a 24-hr period).
- The report does not state that the test chambers were covered to reduce the loss of test solution or dilution water due to evaporation. In addition, it is unknown if the chambers were placed randomly.
- Measurements were taken from composites of each dose level; it is unclear if concentrations for each individual replicate varied.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species • Mysids (*Mysidapsis bahia*; now *Americamysis*)	• *Americamysis bahia* (p. 11).
Life Stage/Size • Should be either juvenile (<24 hours old) or young adults (5 to 6 days old). • Should be of normal size and appearance for their age.	• <24 hrs old (p. 11) • No description of size and appearance.

Acquisition ▪ Mysids should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. ▪ Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. ▪ Taxonomic verification should be obtained from the commercial supplier by experienced laboratory personnel or by an outside expert.	▪ Laboratory cultures used (p. 11). ▪ Brood stock originally obtained from commercial supplier (p. 11). ▪ **Taxonomic verification not reported.**
Acclimation ▪ Within a 24-h period, changes in water temperature should not exceed 1°C, while salinity changes should not exceed 5 percent. ▪ During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.	▪ **The report states that the temperature rose from 24°C to 26°C between Hour 0 and Hour 24, then dropped back down to 24°C at Hour 48. Based on the data available in the study report, it appears that the maximum salinity changes in a 24-hr period could be between 5% and 10%. (p. 21).** ▪ **Not enough information is provided about acclimation conditions to determine if they were similar to the conditions of the testing areas.**

B. Test System

Guideline Criteria	Reported Information
Test Chamber ▪ Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.	▪ 1-L Glass beakers used as test chambers (p. 13).
▪ Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.	▪ No cover is mentioned in the report.
▪ For flow through tests, retention chambers can be constructed with netting material of appropriate mesh size.	▪ Not applicable. Static test used.

Test Substance Delivery System (flow-through tests) ▪ Proportional diluters, metering pumps, or other suitable systems should be used. ▪ System should be calibrated prior to start of test. ▪ General operation should be checked 2x daily. ▪ 24-hr flow through a test chamber should be equal to 5x the volume of the test chamber. ▪ Flow rates among chambers or across time should not vary more than 10%.	▪ Not applicable. Static test used.
Temperature ▪ The test temperature should be 25°C. ▪ Deviations from the test temperature should be not greater than ±2°C. ▪ Measured in each chamber at beginning and end of test.	▪ Temperature ranged between 24 to 27°C. (p. 21). ▪ Temperature measured daily in each chamber (p. 14).
pH ▪ Measured in each chamber at beginning and end of test.	▪ pH measured daily in each chamber (p. 14).
Salinity ▪ Salinity of 20 ± 3 ppt. ▪ Measured in each chamber at beginning and end of test.	▪ Salinity ranged between 19-21 ppt (p. 21). ▪ Salinity measured daily in each chamber (p. 14).
Dissolved Oxygen ▪ Dissolved oxygen concentration between 60 and 105 % saturation. ▪ Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment. ▪ Measured in each chamber at beginning and end of test	▪ **Overall, the DO levels ranged from 2.8 to 7.8 mg/L (37% to 108% saturation). The low DO levels occurred at the 72-hr interval when levels ranged from 37 to 44% of saturation in the solvent control (p. 21).** ▪ **Aeration performed at 72 hours (p. 17).** ▪ Dissolved Oxygen measured daily in each chamber (p. 14).
Photoperiod ▪ Photoperiod of 14 hours light and 10 hours dark, with a 15 to 30 min transition period.	▪ 14 hrs light / 10 hrs dark used. "Sudden transitions from light to dark…were avoided." (p. 12-13).
Feeding ▪ Mysids should be fed daily during testing. ▪ A recommended food is live Artemia spp. (48–hr-old nauplii).	▪ Live brine shrimp nauplii (*Artemia salina*) added daily (p. 14).

Comment [E1]: Calculated % sat using footnote values. Note that the author states on p. 19 that DO levels were as low as 37%. This may be due to rounding.

Dilution Water	
• Natural seawater or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating, and testing periods without showing signs of stress.	• Filtered natural seawater used for testing (p. 12).
• Mysids should be cultured and tested in dilution water from the same origin.	• **Testing water and culturing water are different. Artificial seawater used for culturing (p. 11).**
• Natural seawater should be filtered through a filter with a pore size of <20 □m prior to use in a test.	• 20- and 5- micron filters used (p. 12).
• Artificial seawater can be prepared by adding commercially available formulations or specific amounts of reagent-grade chemicals to deionized water (conductivity should be <1 □ohm/cm at 12°C).	• Not applicable. Natural filtered seawater used in testing.
• If artificial seawater prepared from ground or surface water source, conductivity and total organic carbon should be measured.	• Not applicable. Naural filtered seawater used in testing.
Test Substance	
• Concentration of test substance should be measured: --at beginning and end of test for static tests --at beginning and end of test and in at least one appropriate chamber whenever a malfunction is detected for flow-through tests	• Concentration measured at beginning and end of (static) test. (p. 15).
• Among replicate test chambers, measured concentrations should not vary by more than 20%.	• **Measurements were taken from the intermediate vessel prior to division into replicates at test initiation and from composites of each dose level at test termination; it is unclear if concentrations for each individual replicate varied (p. 15).**
Carriers	
• Use of carriers should be avoided , if possible, as they may serve as a carbon source for bacteria. • If solvents, solubilizing agents, or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. Preferred carriers are dimethyl formamide, triethylene glycol, acetone, or ethanol. • Concentration of carriers should not exceed 0.1 mL/L	• Acetone used as carrier at a concentration of 0.1 mL/L (p. 14).

Comment [E2]: 0's 0.4 mL to 1 L water, but then brought up to a total final volume of 4 L. So 0.1 mL/L

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test	
▪ Range finding test should be conducted to determine (1) which life stage (juvenile or young adult) is to be utilized in the definite test; and (2) the test solution concentrations for the definitive test.	▪ Two range-finding tests conducted to determine life stage and test concentrations for definitive test (p. 16).
▪ The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g. 1, 10, 100 mg/L, etc.), usually under static conditions.	▪ Concentrations of 0.010, 0.10, and 1.0 mg a.i/L used in first test and 10, 50, and 100 mg a.i./L used in second test (p. 16).
▪ A minimum of 10 mysids for each age class (juvenile or young adult) should be exposed to each concentration of test substance for up to 96 hours. The age class which is most sensitive to the test substance in the range-finding test should be utilized in the definitive test.	▪ 10 mysids used per replicate, 96-hr test duration. Mysids ≤ 24 hrs old and 5/6 days old. Mysids ≤ 24 hrs were more sensitive and therefore used in definitive test (p. 16).
Doses	
▪ At least 5 test concentrations should be used.	▪ 5 concentrations: 1.3, 2.5, 5.0, 10 and 20 mg a.i/L used (p. 17).
▪ Dilution factor between concentration should be chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g. 2, 4, 8, 16, 32, and 64 mg/L).	▪ Dilution factor of 2X (p. 17).
Controls	
▪ Every test should include controls consisting of the same dilution water, conditions, and procedures, and mysids from the same population or culture container, except that none of the test chemical is added.	▪ Control and solvent control used under same procedures and mysids. (p. 14).
Replicates Per Dose	
▪ Two or more replicates.	▪ 2 replicates per dose group and control group used. (p. 14).
Number and Placement of Organisms	
▪ A minimum of 20 mysids per concentration.	▪ 20 mysids used per concentration (p. 14).
▪ Impartially distributed among test chambers.	▪ Impartially selected (p. 14).
▪ Test chambers within the testing area should be positioned in a random manner.	▪ Unknown if chambers were positioned randomly.
▪ An equal number of mysids should be introduced into the test and control chambers.	▪ Equal number of mysids introduced into test and control chambers (p. 14).
▪ Loading of mysids in static tests should not exceed 30 mysids per liter.	▪ Loading of 20 mysids/L used (i.e., 10 mysids in 500 mL solution) (p. 13).
Duration of Test	
▪ 96 hours	▪ 96-hrs (p. 14).
Endpoints	
▪ Mortality	▪ Mortality (p. 14).

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	• Yes (pp. 3 and 4).
The nature of the test, laboratory, name of the investigator, test substance, and dates of test reported?	• Yes (pp. 1 and 18).
Control • Mortality should not exceed 10% at end of test	• No mortality reported (p. 23).
Detailed description of the test substances (e.g. the source, lot number, composition, physical and chemical properties, shelf life and storage conditions, and any carrier or additives used)?	• Yes, with the exception of physical/chemical properties (p. 11).
•Detailed information about the shrimp (e.g. common and scientific names, source of supply, age, history, weight, acclimation procedure, and feeding history)?	• Yes (pp. 11 and 12).
A description of the experimental design including the number of test solution concentrations, number of replicates, and number of shrimp per replicate?	• Yes (pp. 13 and 14).
The source of the dilution water, its chemical characteristics (e.g. salinity), and a description of any pretreatment?	• Yes (p. 12).
A description of the test chambers, the depth and volume of solution in the chamber, the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and flow rate expressed as volume additions per 24 h?	• Yes (pp. 12, 13, and 14).
The concentration of the test substance in each test chamber before the start of the test and at the end?	• Yes (p. 22).
Number of dead shrimp and measurements of water temperature, salinity, and dissolved oxygen concentration in each test chamber recorded at the protocol-designated times?	• Yes (pp. 21 and 23).
Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks?	• Yes, (pp. 14, 21, and 44).
Recorded data for the holding and acclimation period (temperature, salinity, etc.)?	• Yes (p. 11).
Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 h. A statistical test of goodness-of-fit performed?	• Yes (p. 26).

For each set of mortality data, the 48– and 96–h LC_{50} and 95 percent confidence limits calculated on the basis of the average measured concentration of the test substance. When data permits, LC_{50} values with 95 percent confidence limits should be computed for 24– and 72–h observations. The NOEC and slope of the dose-response curves calculated?	• Yes (p. 24).
The methods used in calculating the concentration-response curves and the LC_{50} values should be fully described?	• Yes (pp. 15 and 16).

Dose Response

No mortality or adverse effects were noted for mysids exposed to the controls. Although mortality of 5% (one mysid) was observed in the 1.2 and 2.5 mg a.i/L treatment levels, the mortality was within the expected range of naturally occurring variability and was not considered toxicant related.

Nominal Concentration (mg ai/L)	Mean Measured Concentration (mg ai/L)	Replicate Chamber (% after 96 hour)		Mean Mortality (% after 96 hour)
		A	B	
Control	Control	0	0	0
Solvent Control	Solvent Control	0	0	0
1.3	1.2	0	10	5
2.5	2.5	10	0	5
5.0	4.9	80	30	55
10	9.7	100	100	100
20	20	100	100	100

Statistical Results

Statistical Method: The mean measured concentrations tested and the corresponding mortality data were used to estimate LC_{50} and 95% confidence intervals (CIs). LC_{50} and 95% CI values were calculated for all durations using binomial probability (p. 15).

Results Synopsis:

Duration	LC_{50} (mg a.i./L)	95% Lower CI	95% Upper CI
24-hr	12	9.7	20
48-hr	7.1	4.9	9.7
72-hr	6.1	4.9	9.7
96-hr	4.6	2.5	9.7

NOEC through 96 hours = 2.5 mg a.i./L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: The mean measured concentrations tested and the corresponding mortality data reported in the Study Report were used to estimate the 96-hr LC_{50} and 95% confidence imerval valuess. The LC_{50} value was estimated by TOXANAL using the moving average method. The NOEC was determined using the assumption that one mortality out of twenty organisms (5%) was not significant. The LC_{50} and 95% confidence interval values were verified to be the same as the values calculated by the study author.

Results Verification Synopsis: 96-hr LC_{50}: 4.3 mg a.i./L 95% C.I.: 3.3 to 5.4 mg a.i./L
NOEC: 2.5 mg a.i./L

14. REVIEWER'S COMMENTS:

Deviations from the guidelines are found in Section 9 of this DER.