# APPENDIX VI

# Microbiology Report

**Microbiology Surveillance/Diagnostic Summary Report**

**for**

# Experiment # E2186.01

## Effect of Oxybenzone on Fertility and Early Embryonic Development in Sprague-Dawley Rats (Segment I)

Principal Investigator: Amy L. Inselman, Ph.D.

Prepared by: [redacted]

Sung G. Kim, Ph.D.
Surveillance/Diagnostic Program
Office of Scientific Coordination
National Center for Toxicological Research

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## Microbiological Surveillance Summary Report for E2186.01

Microbiological surveillance samples from rooms 121,106, and 116 in building 53A were examined for the period between January 14, 2013 and June 4, 2013. These samples consisted of sentinel animals, quarantine animals, cage waste, cage water, and feed samples.

**SUMMARY OF RESULTS:**

**A. Sentinel Animals**: A total of eight sentinel animals were examined for the following:

1. **Serology Screening**: Blood from each animal was collected, allowed to clot and the serum was separated. The serum was analyzed by Multiplex Fluorescent Immunoassay (MFI) for the presence of specific antibodies at the IDEXX Research Animal Laboratory Animal Diagnostic Laboratory (IDEXX-RADIL), Columbia, Missouri. The laboratory serology method and viral/mycoplasma agent for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

**Serology Test**

**Rat Panel**

Kilhain Rat Virus (KRV)
*Mycoplasma pulmonis*
NS1 (Generic Parvo)
Pneumonia Virus of Mice (PVM)
Rat Coronavirus (RCV)
Rat Minute Virus (RMV)
Rat Parvovirus (RPV)
Rat Theilovirus (RTV)
Sendai Virus
Toolan's H- I

## Results of serology tests for Murine Virus and Mycoplasma Antibodies

| Date | Incidence of Antibody In Sentinel Animals | Positive Serologic Reaction for |
|---|---|---|
| 2/25/2013 | 0/2 | none positive |
| 4/8/2013 | 0/2 | none positive |
| 6/4/2013 | 0/4 | none positive |

2. **Additional Sentinel Animal Screening**: In addition to the serology testing, all sentinel animals are examined for the following:

(a) Ectoparasites
Results: No ectoparasites were detected on sentinel animals.

(b) Endoparasites: (wet mount at 40X and 200X magnifications)
Results: No pathogenic endoparasites were detected in sentinel animals.

(c) Bacterial pathogens
Results: No pathogenic microorganisms were detected in sentinel animals.

*The following specific rodent pathogens would be detected under routine culturing procedures:

*Bordetella bronchiseptica*
*Citrobacter freundii*
*Corynebacterium kutscheri*
*Erysipelothrix rhusiopathiae*
*Helicobacter hepaticus* (detected via PCR)
*Klebsiella oxytoca*
*Klebsiella pneumoniae*
*Listeria monocytogenes*
*Pasteurella pneumotropica*
*Pasteurella multocida*
*Pseudomonas aeruginosa*
*Salmonella* sp.
*Streptococcus pneumoniae*

**B. Animal Husbandry Supplies Monitoring**:

1. **Cage Water**: One cage water sample was tested for the presence of *Psuedomonas aeruginosa.*

   Results: The sample contained no *Pseudomonas aeruginosa.*

2. **Processed Water:** Samples of processed drinking water for the animals were tested on a regular basis for total bacterial count and for the presence of *Pseudomonas aeruginosa.*

   Results: No samples exceeded the NCTR standards (<100 bacterial cfu/ml) and contained no *Pseudomonas aeruginosa.*

3. **Room Environment Monitoring**: Swab samples are taken quarterly from each animal room to monitor sanitation procedures. Conventional animal rooms with >200 cfu of bacteria or >20 cfu of mold would be reported as exceeding the standard. Any suspect bacterial pathogens and toxin-producing molds would be identified and reported.

   Results: A total of six room surface swabs were taken during the study period. All swabs met the NCTR standards. No bacterial pathogens or toxin-producing molds were detected.

4. **Cage Waste:** A total of twenty-three waste samples were screened for bacterial pathogens during the study period.

   Results: No bacterial pathogens were detected.

5. **Animal Feed**: Samples of animal feed received from an outside vendor are tested for total bacteria and mold, and for specified bacterial pathogens and toxin-producing molds. Any suspected bacterial pathogens or toxin-producing molds would be identified and reported.

   Results: Diet Preparation Department identified four processed feed samples designated for use on E 2186.01. Microbiological analysis detected no bacteria or molds present in these samples.

**C. Quarantine Animals**: A total of four quarantine animals were screened from January 14, 2013 to February 25, 2013. The quarantine animals were examined for the following:

1. **Serology Screening**: Blood from each animal was collected, allowed to clot and the serum was separated. The serum was analyzed by Multiplex Fluorescent Immunoassay (MFI) for the presence of specific antibodies at the

Research Animal Diagnostic Laboratory, University of Missouri, Columbia, Missouri. The laboratory serology method and viral/mycoplasma agent for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

**Serology Test**

**Rat Panel**

Kilhain Rat Virus (KRV)
*Mycoplasma pulmonis*
NS1 (Generic Parvo)
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Rat Coronavirus (RCV)
Rat Minute Virus (RMV)
Rat Parvovirus (RPV)
Rat Theilovirus (RTV)
Sendai Virus
Toolan's H- I

## Results of serology tests for Murine Virus and Mycoplasma Antibodies

| Date | Incidence of Antibody In Quarantine Animals | Positive Serologic Reaction for |
|---|---|---|
| 1/14/2013 | 0/2 | none positive |
| 2/25/2013 | 0/2 | none positive |

2. **Additional Quarantine Animal Screening**: In addition to the serology testing, all quarantine animals are examined for the following:

(a) Ectoparasites:
Results: No ectoparasites were detected on sentinel animals.

(b) Endoparasites: (wet mount at 40X and 200X magnifications)
Results: No pathogenic endoparasites were detected in quarantine animals.

(c) Bacterial pathogens

Results: No pathogenic microorganisms were detected in quarantine animals.

*The following specific rodent pathogens would be detected under routine culturing procedures:

*Bordetella bronchiseptica*
*Citrobacter freundii*
*Corynebacterium kutscheri*
*Erysipelothrix rhusiopathiae*
*Helicobacter hepaticus* (detected via PCR)
*Klebsiella oxytoca*
*Klebsiella pneumoniae*
*Listeria monocytogenes*
*Pasteurella pneumotropica*
*Pasteurella multocida*
*Pseudomonas aeruginosa*
*Salmonella* sp.
*Streptococcus pneumoniae*