

**APPENDIX VI**  
**Microbiology Report**

**Microbiology Surveillance/Diagnostic Summary Report**

**for**

**Experiment # E2187.01**

**Effect of Oxybenzone on Embryo Fetal Development  
in Sprague-Dawley Rats (Segment II)**

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

Prepared by:



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 E-2187.01

Microbiological surveillance samples from building 53A/116 were examined for the period between July 9, 2012 and September 27, 2012. These samples consisted of sentinel animals, quarantine animals, cage waste, cage water, and feed samples.

### SUMMARY OF RESULTS:

**A. Sentinel Animals:** A total of four 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 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)  
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
8/13/2012	0/2	none positive
9/17/2012	0/2	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:** A total of two cage water samples were tested for the presence of *Pseudomonas aeruginosa*.

Results: No samples contained *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 between July 9, 2012 and September 27, 2012. All swabs met the NCTR standards. No bacterial pathogens or toxin-producing molds were detected.

- 4. Cage Waste:** A total of five waste samples were screened for bacterial pathogens between July 9, 2012 and September 27, 2012.

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 two processed feed samples designated for use on E 2187.01. Microbiological analysis detected no bacteria or molds present in these samples.

- C. Quarantine Animals:** A total of ten quarantine animals were screened from July 9, 2012 to September 27, 2012. 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)  
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 Quarantine Animals	Positive Serologic Reaction for
7/10/2012	0/2	none positive
7/16/2012	0/2	none positive
8/14/2012	0/2	none positive
8/21/2012	0/2	none positive
8/28/2012	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*