Ototoxicity: Preparation Is Key

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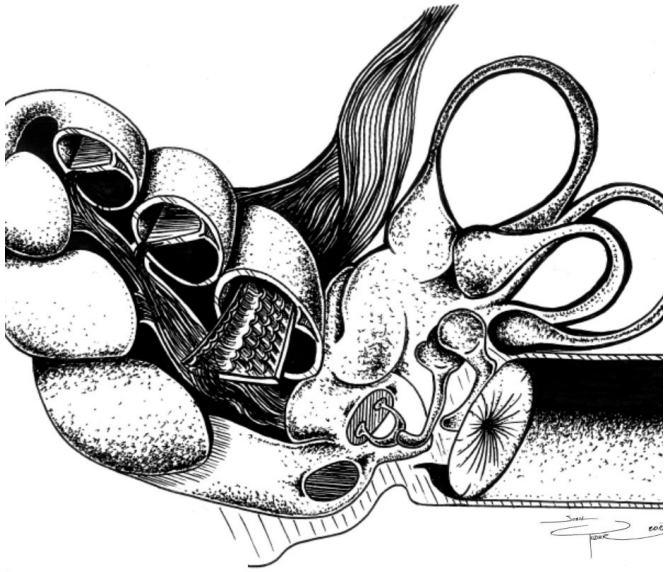


Image courtesy of Joshua Yoder

Ototoxicity refers to the potential for certain chemicals or drugs to cause damage to the inner ear and result in hearing loss or balance problems.

AVAILABLE GUIDANCE

Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route

Guidance for Industry and Review Staff

- The dermal irritation and potential for delayed contact hypersensitivity of the new formulation should be evaluated.

- The ability of the drug product to penetrate an intact tympanic membrane should be determined and the exposure to the middle and inner ears in an animal model should be estimated when this barrier is or is not intact.

- If the drug product is expected to reach the middle or inner ear during clinical use or is introduced directly to those regions, evaluation of the auditory brainstem response, as well as microscopy of relevant otic tissues, including a cytocochleogram, should be included in acute and/or repeat-dose studies conducted by intratympanic administration.

OTOTOXICITY TESTING

The Why

Ensuring a compound that accesses the middle and inner ear is safe for the auditory system.

Is your compound…

- ✓ Directly administered?
- ✓ In a class of risk?
- ✓ Are otic tissues a target?

RECOMMENDATIONS

1. Understand the limitations for dosing a small compartment
2. Solution vs. suspension
3. Have a clear understanding of your dosing regimen and compound pharmacokinetics/pharmacodynamics
4. Be sure you speak to the agency regarding your plans forward

TYPES OF INDICATIONS

Including, but not limited to:

Types of Hearing Loss

Age-Related Hearing Loss

Aminoglycoside ototoxicity

Cisplatin ototoxicity

Noise-Induced hearing loss

IDPN-Induced vestibular dysfunction

Niemann-Pick's Disease

Meniere's Disease

Otosclerosis

Tinnitus

Physical Injury to ear or brain

LIST OF KNOWN OTOTOXICANTS
PAGE 1

Re: Ototoxic drugs that can cause hearing loss or tinnitus | Mayo Clinic Connect

Certain prescription drugs can, over time, have an ototoxic effect on your hearing, causing hearing loss and ear ringing from tinnitus. Ototoxicity may be reversible or may be permanent, depending on the type of medication used, dosage and duration of treatment. There are many medications that have been listed as potentially ototoxic drugs, including antidepressants, antibiotics, and many painkillers.

Here is a list of medications that can potentially cause tinnitus.

Salicylates – Aspirin and aspirin containing products
Non-Steroidal Anti-Inflammatory Drugs (NSAIDS) – Advil, Aleve, Anaprox, Clinoril, Feldene, Indocin, Lodine, Motrin, Nalfon, Naprosyn, Nuprin, Poradol, & Voltarin.
Antibiotics – Aminoglycosides, Erythromycin, Vancomycin
Aminoglycosides – Streptomycin, Kanamycin, Neomycin, Gantamycin, Tobramysin, Amikacin, and Netilmicin
Erythromycin – EES, Eryc, E-mycin, Ilosone, Pediazole and new derivatives of Erythromycin, Biaxin, & Zithromax
Vancomycin – Vincocin
Loop Diuretics – Lasix, Endecrin, and Bumex
Chemotherapy Agents – Cisplatin, Nitrogen Mustard, and Vincristine
Quinine – Aralen, Atabrine (for treatment of malaria), Legatrin, and Q-Vel Muscle Relaxant

Ototoxic drugs that worsen tinnitus
Many prescription and nonprescription medicines can worsen ringing in the ears (tinnitus).

Starting a new medication or increasing your dose of ototoxic prescription drugs can make symptoms of tinnitus more pronounced.

LIST OF KNOWN OTOTOXICANTS
PAGE 2

Medicines that commonly cause tinnitus or make tinnitus worse include some of the following:

Antibiotics
Antidepressants
Anti-inflammatory medicines
Blood pressure medicine
Heart medicines
Local anesthetic agents
Medicines used to treat cancer
Medicine used to treat Parkinson's disease
Radiation therapy to the head or neck
Some vitamins or mineral supplements, such as niacin or vitamin A
Water pills (diuretics)

The signs of ototoxicity, in order of frequency
Symptoms of ototoxic drug reaction include:

a) Development of tinnitus in one or both ears.

b) Intensification of existing tinnitus or the appearance of a new sound.

c) Fullness or pressure in the ears other than being caused by infection.

d) Awareness of hearing loss in an unaffected ear or the progression of an existing loss.

e) Development of vertigo or a spinning sensation usually aggravated by motion which may or may not be accompanied by nausea."

HOW DO WE ASSESS OTOTOXICITY?

The How

Based on three primary endpoints:

1. Hearing assessment – Auditory Brainstem Response (ABR)

2. Evaluating the organ of Corti – Cytocochleogram

3. Histopathology of Relevant Otic Structures – Otic Histopathology

CAPABILITIES NECESSARY FOR ASSESSING OTOTOXICITY

Specialized dosing routes

- Middle Ear Catheter (unilateral/bilateral)
- Posterior Semicircular Canal (PSCC)
- Transtympanic
- Post-Auricular/Transbullar
- Intracochlear
- External Auditory Canal

Hearing Evaluations

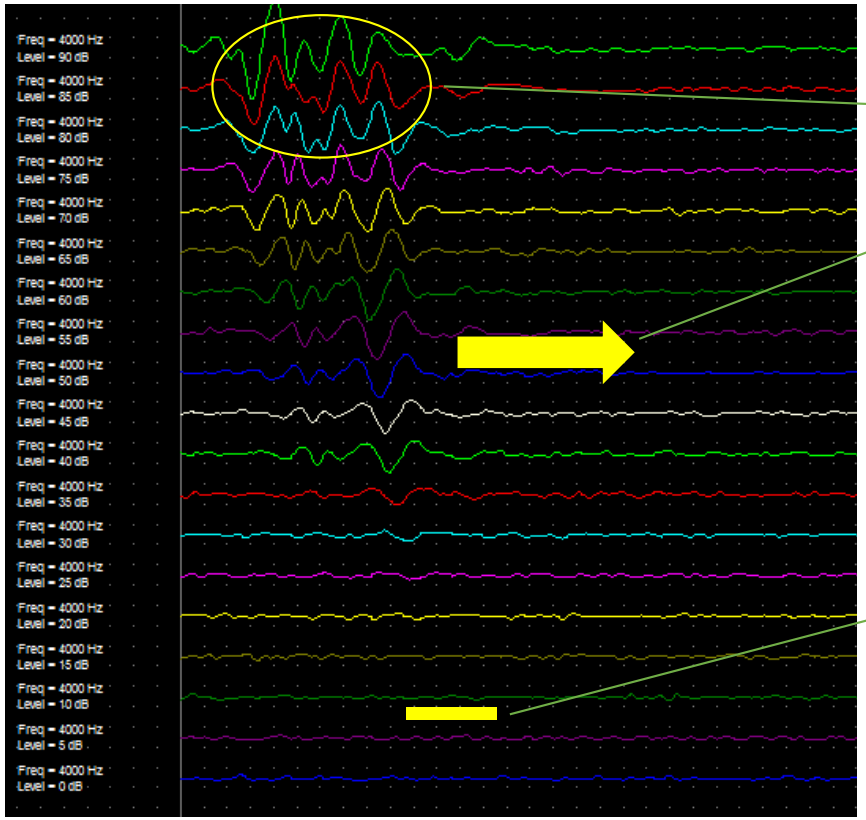
- Auditory Brainstem Response (ABR)
- Distortion Product Otoacoustic Emissions (DPOAE)

Clinical and Vestibular Assessments

Pathology Assessments:

- Cytocochleogram whole mounts and cell counts
- Otic Histopathology Evaluations

AUDITORY BRAINSTEM RESPONSE (ABR)



Tone Response (an average of thousands of fast 'beeps,' measured over time).

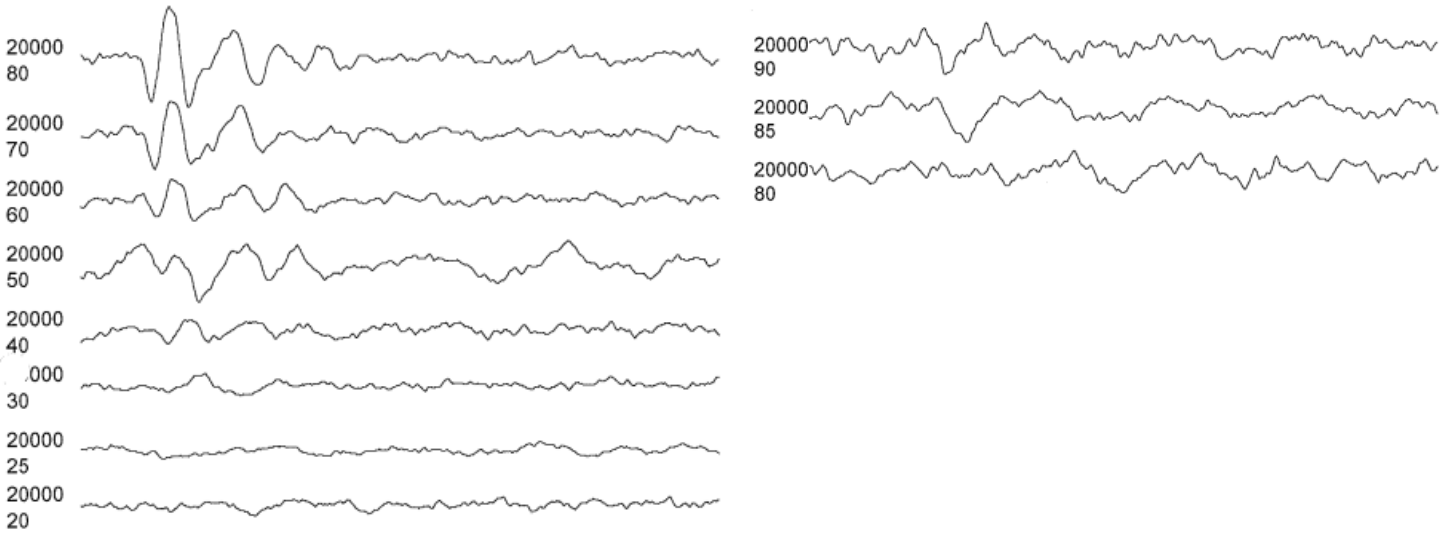
Latency Shift (response waveform moves to the right) indicating as the intensity lowers the brain takes longer to recognize the tone (milliseconds)

Region of threshold (the subject no longer registers a tone signal between these lines). In this case, threshold is between 5 and 10dB.

Image courtesy of Charles River Labs

ABR

ABR recording pre- and post-aminoglycoside treatment



Images courtesy of Charles River Labs

DISTORTION PRODUCT OTOACOUSTIC EMISSIONS (DPOAE)

Two simultaneous tones presented in descending intensities, resultant emission recorded for amplitude and threshold:

- Ratio of F1/F2=1.22 of the center frequency
- L1≥L2
- DP=2F1-F2

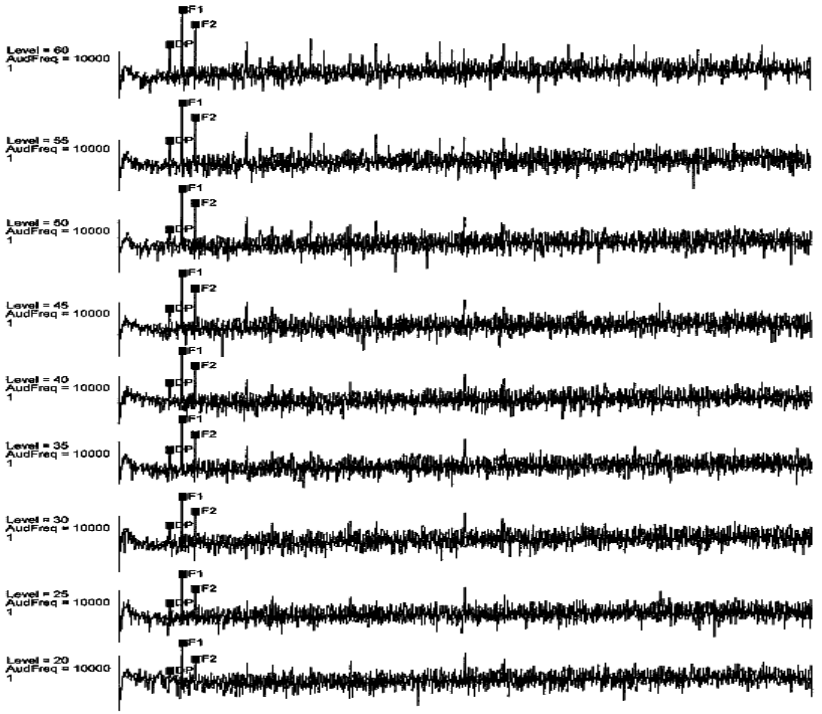


Image courtesy of Charles River Labs

CYTOCOCHLEOGRAM

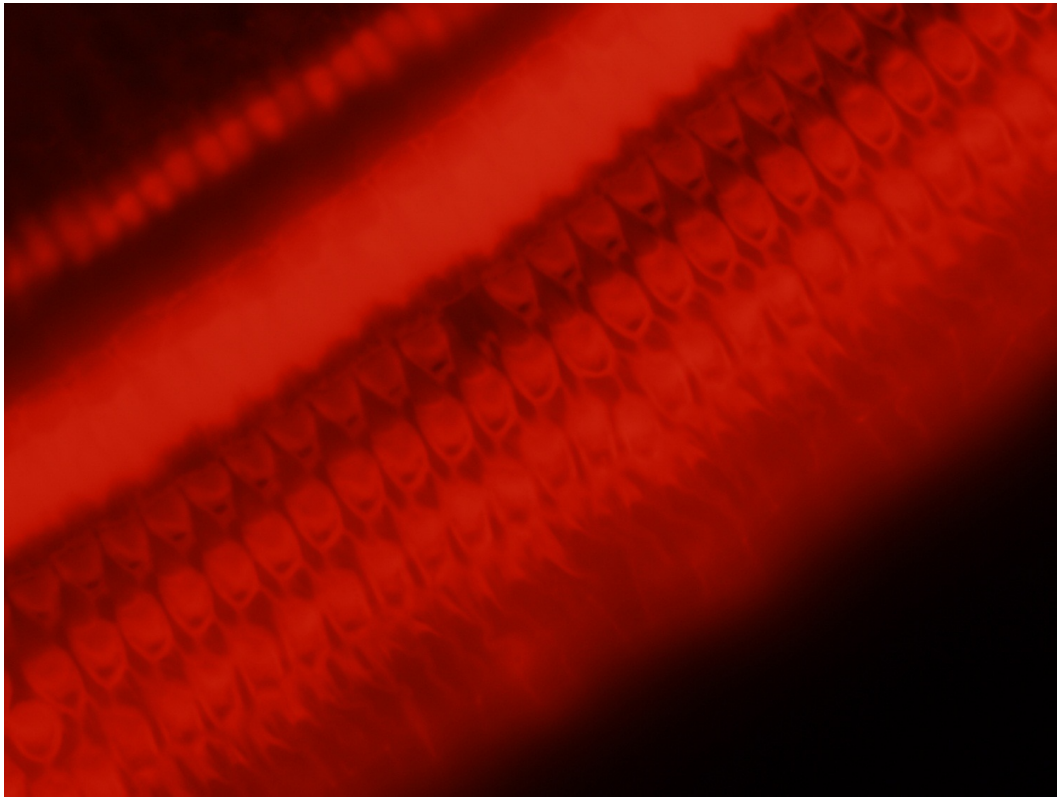


Image courtesy of Charles River Labs
Phalloidin Stained Organ Of Corti

CYTOCOCHLEOGRAMS

Guinea pig cytocochleogram

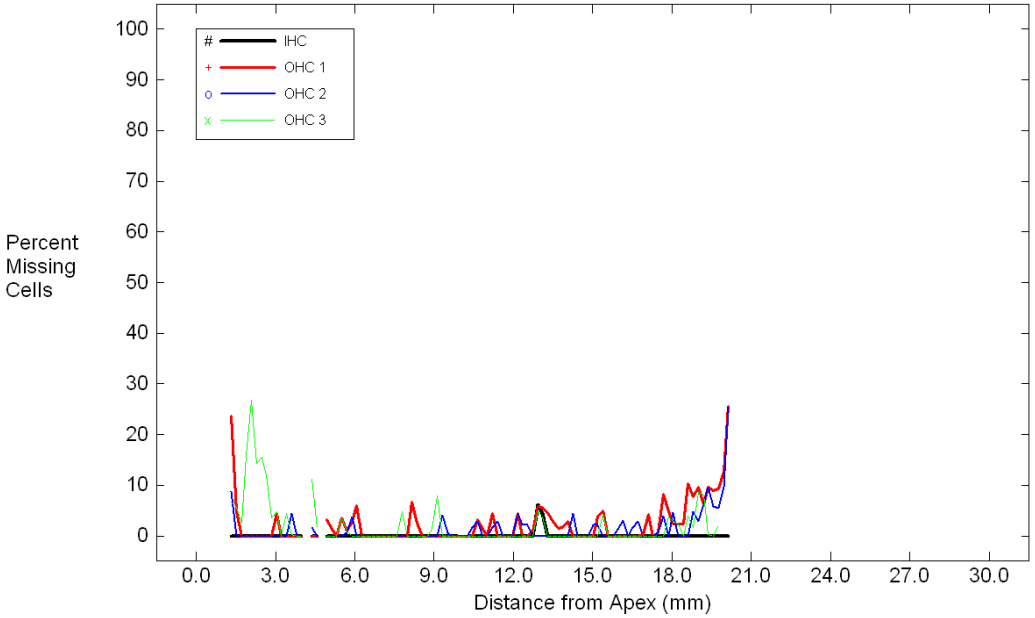


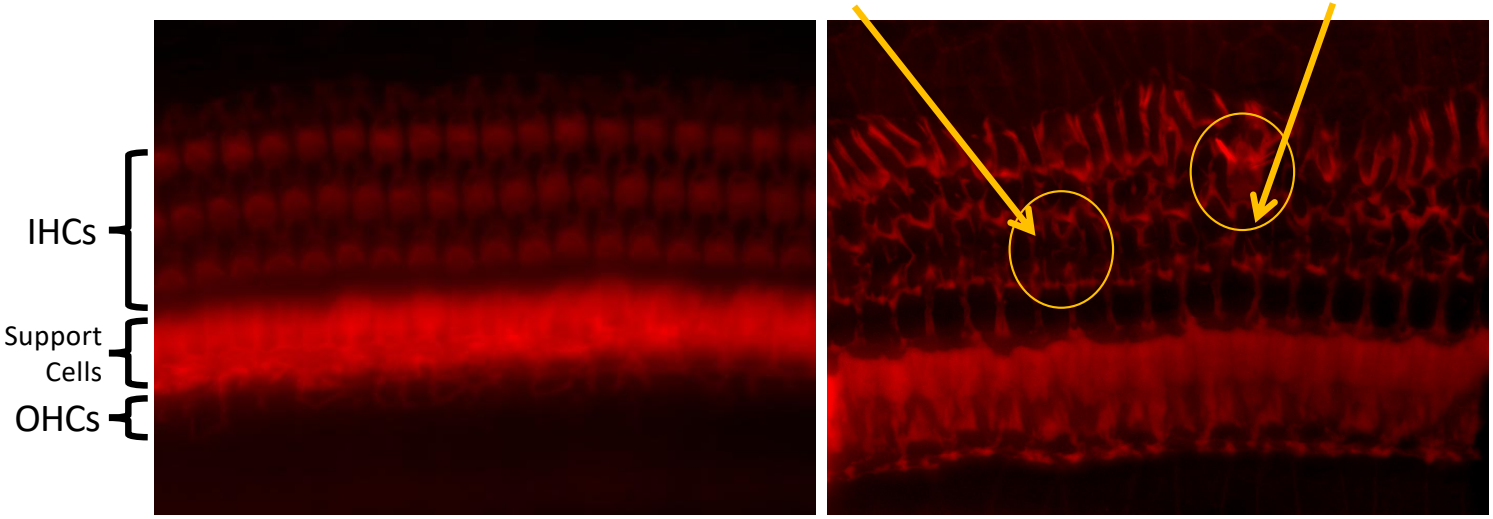
Image courtesy of Charles River Labs

Hair cell totals from each region of the cochlea are entered in the cytogram software for reporting

CYTOCOCHLEOGRAM

Single, present hair cell

'X' shaped scar visible where cell was



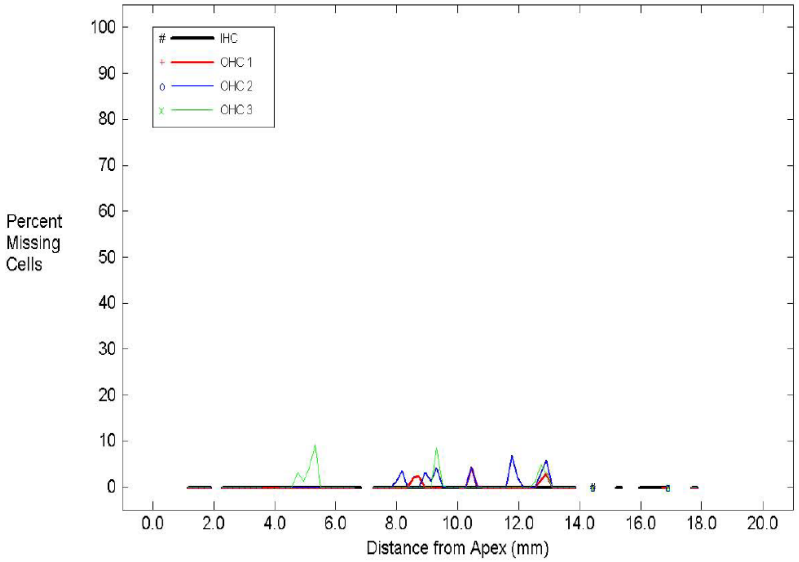
Healthy Hair Cells

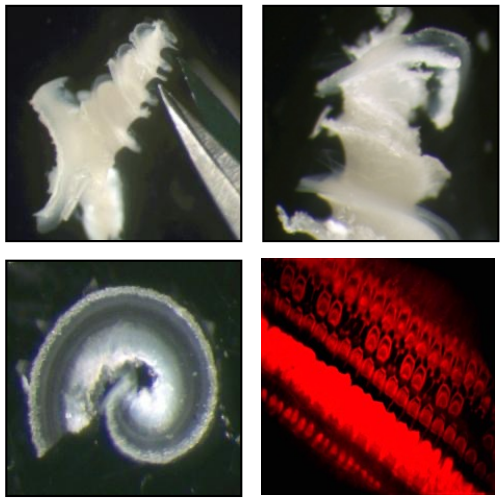
Damaged/Missing Hair Cells

The hair cells are placed on multiple slides and read using a confocal microscope, under low-light conditions to prevent photo-bleaching. The tissues are stained with AlexaFluor568 Phalloidin. Images Courtesy of Charles River Labs – Phalloidin stained Organ of Corti

CYTOCOCHLEOGRAM

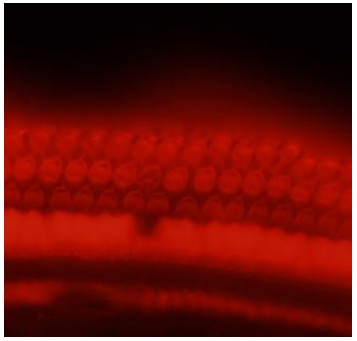
Representation of missing hair cells throughout the cochlea:



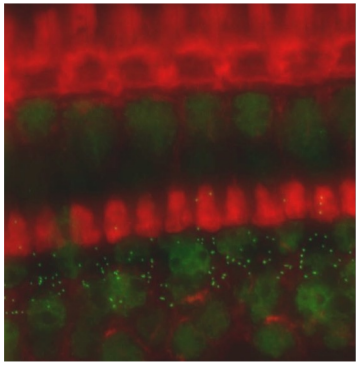


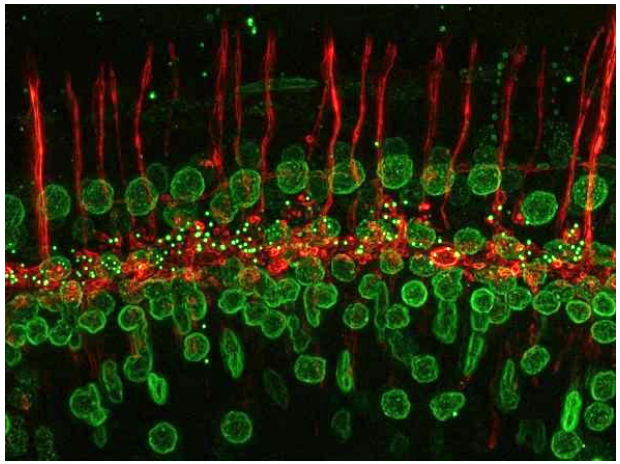
Images Courtesy of Charles River Labs

INNER EAR FLUORESCENT MARKERS

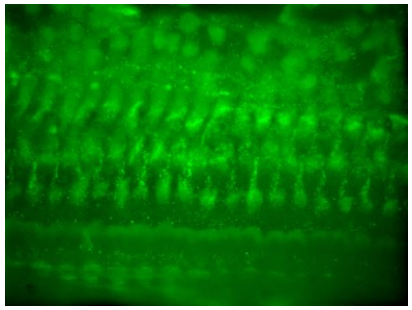


Above: Phalloidin
Below: Phalloidin and Myosin

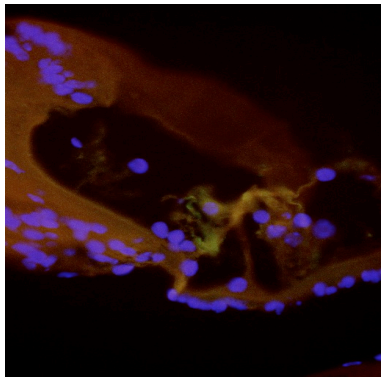




Above: Phalloidin, Myosin 7a, and CTBP2



Above: Myosin
Below: CTBP2



Images Courtesy of Charles River Labs

UTRICLE

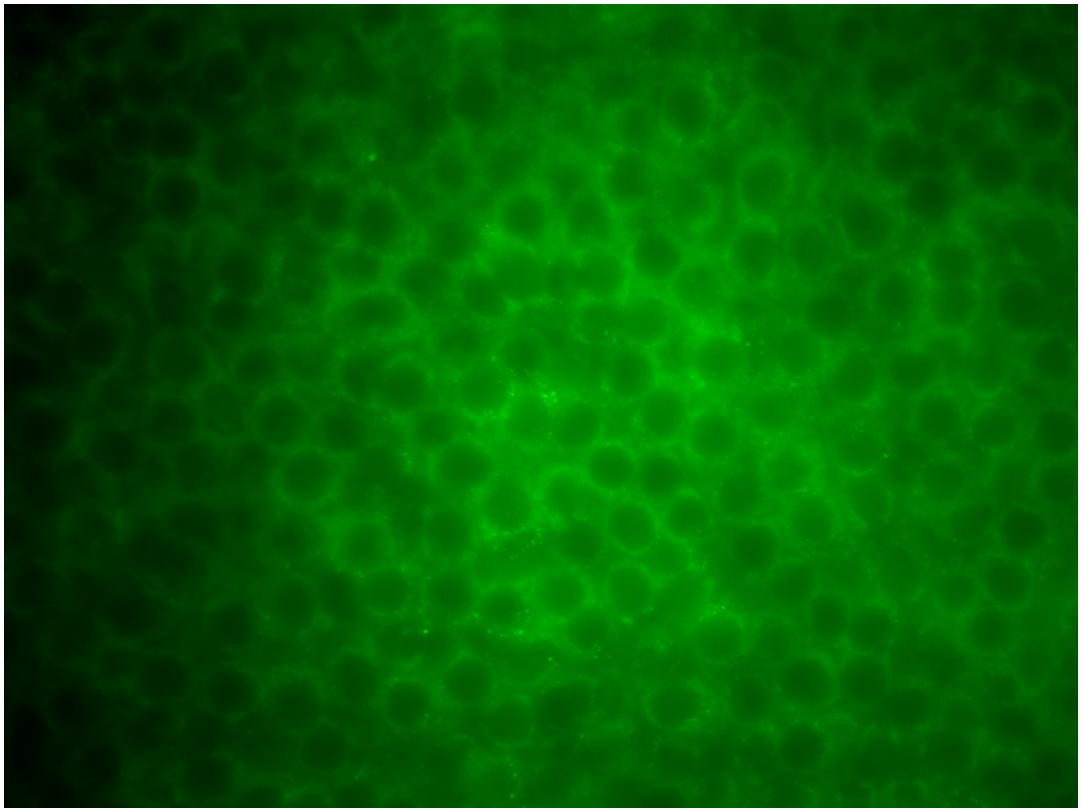


Image Courtesy of Charles River Labs
Myosin 7a stained Utricle

ADDITIONAL CAPABILITIES FOR OTOTOXICITY

Perilymph Collections
Cerebrospinal Fluid (CSF)
Otic tissues for PK analysis
Eustachian Tube Sectioning
Vestibular Assessments
Utricle/Saccule whole mounts and Histomorphometry
Specialized staining for relevant otic organs

- Phalloidin
- CTBP2
- Synaptophysin
- Myosin 7a
- Glur2

OTOTOX

PREPARATION, PITFALLS, AND PROGRESS

PREPARATION FOR OTIC STUDIES

Understanding the nuances of what are required for each study.

- Each Study is custom built
  - Includes, but not limited to:
    - Otic Histopathology
    - Cytocochleograms
    - Auditory Brainstem Responses
  - May also include
    - Blood collections
    - CSF
    - Perilymph
    - Cochlear Tissues
- Time constraints within each endpoint
- Most studies require staggered starts to accommodate one or more endpoints
- Species Selection

SPECIES SELECTION

Rodent and Non-Rodent

- Select species that are compatible with your drug
- Consideration of the ear anatomy
- Species Options
  - Rat
  - Guinea Pig
  - Cat
  - Non-Human Primate
  - Chinchilla
  - Mouse (efficacy studies primarily)

STUDY DESIGNS VARY

Example

Design will include males and females

Pretest ABR and prior to term ABR

Dosing Transtympanic

Animals held for approximately 28 days

Termination includes perfusion with saline and fixative

Includes pathology of relevant otic tissues

Includes cytocochleograms

Good Laboratory Practices (GLP)

PITFALLS OF "ADD-ON" OTIC ENDPOINTS TO TOXICITY STUDIES

- A study may need to be designed in a different way to accommodate the otic endpoints
  - Can lead to non-ideal designs or non-interpretable data
- Preparations for tissue are extensive and require specialty supplies on hand in adequate quantities.
  - Requires scrambling to obtain materials, space, and personnel.
- Specialty trained staff are required to perform ABR, Naprosyn (Nx), Decal, Trim, Embed, Section, and to create and read whole mounts of cochlea.
  - Scheduling these requires careful consideration of all endpoints included and their time constraints. Last-minute add-ons have recently become a bigger issue/concern as they are happening.

ADDRESSING AUDITORY SYSTEM AND FORWARD THINKING

Specifically for compounds outside scope

- Historical approach to sensory systems
- Regulatory guidance for otic safety
- What can we do to be proactive?
  - Elect testing prior to clinic
  - Elect to assess particular assays on tox studies
  - Test the ear tissues during distribution studies
  - Relevant use of toxicokinetic animals on early tox studies
  - Screen all compounds via *in vitro* assay (HEI-OC1 Cells)

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