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Comparative Auditory Brainstem Response (ABR) Thresholds: Rat, Cat, Guinea Pig, and Nonhuman Primate

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ABSTRACT

There is a definite need to better monitor drug-mediated effects on neurodegeneration, neuroinflammation, and information processing (feature detection), which includes markers for progression of synaptic plasticity and neurogenesis in ototoxicity screening protocols. The field of otology acknowledges that given the heterogeneity and complexity of auditory function, a single biomarker is unlikely to identify or explain predictive ototoxicity or restorative efficacy of a novel therapeutic. We present historical control baseline Auditory Brainstem Response (ABR) data derived from over 1,500 purpose-bred laboratory animals and summarized as part of approved research studies submitted for the New Drug Application (NDA) process to the U.S. FDA. These baseline assessments were conducted as part of standard nonclinical ototoxicity screening assessment studies using ABR screening to quantify the progression of auditory damage over the course of dose administrations, that is confirmed by both histopathology and cytocochleograms. This single report differentiates the critical ABR baseline characteristics in purpose bred laboratory animals. Five hundred nine rats (254M, 255F), 503 cats (208M, 295F), 406 guinea pigs (203M, 203F), and 92 nonhuman primates (53M, 39F) have been examined using a standard structured GLP-compliant study protocol. Some general common features of using ABR evaluations are identified that may support animal selection for future ototoxicity screening safety assessment studies. The impetus of publishing historical control data is to support the potential use of novel statistical comparisons with normative and current control data to reduce the total number of animals used in future research.

ABBREVIATION

GLP: Good Laboratory Practices; FDA: ABR: Auditory Brainstem Response

INTRODUCTION

The measurement of ABRs in laboratory animals are designed to detect and characterize changes in the sensory aspects of nervous system function that result from exposure to chemical and drug substances prior to license approval for use in humans. The techniques involve neurophysiological measurements from adult animals that are sensitive to changes in the function of auditory sensory systems. These procedures can be used in two ways: 1) to detect the progression of sensory dysfunction produced by compounds in the absence of relevant information – standard Tier II drug safety assessment protocols; or 2) when there are reasons to expect that the small

compartment [1] of auditory transduction processes are specifically sensitive to the class of test compounds being investigated (e.g. aminoglycoside antibiotics) [2].

The source materials used in developing a well-structured ototoxicity protocol come through a process of harmonization that has blended testing strategies of multiple federal regulatory agencies. A harmonized ototoxicity study design is developed through blending the testing guidance and requirements of the U.S. Environmental Protection Agency's Toxic Substances Control Act [3], the subsequent EPA administrative guideline, Code of Federal Regulations (CFR), 40 CFR 798.6855 [4] the U.S. Food and Drug Administration's (FDA) Good Laboratory Practices [5] and "Guidance for Industry: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route" [6]. The FDA Guidance states that the ability of the new drug 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. 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 ABR, 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.

Over 40 years ago, Stebbins & Rudy [7] first expressed their hope that behavioral toxicology establish common procedures for animals which may yield rapid and early evidence of toxicity, but in a precise, controlled, and unequivocal manner. The ABR has revealed its true worth in ototoxicity screening procedures due to its ability to accurately and reliably reveal the earliest possible signs of cytotoxicity, i.e., at a stage when the effects noted may not be completely irreversible. The specific procedures employed during a study are selected on a case-by-case basis. The procedures are selected based on information available at the time of the study design, notable signs of toxicity observed during the conduct of other regulatory based safety assessment studies, and/or the therapeutic target for the drug development program. ABR thresholds were championed by Galambos and colleagues [8,9] and are now used by most ototoxicity screening laboratories to provide objective measures of auditory nerve and brainstem disorders [10,11].

ABR as a measure of progression of cytotoxicity

One of the most compelling and critical design questions in developing a protocol to assess ototoxic liability for NDA submissions is the selection of an in vivo biomarker of cytotoxicity or auditory dysfunction that can be repeated across the prescribed dosing regimen. However, the screening test chosen (ABR) in support of licensure approval is not the definitive endpoint for safety required under current regulatory and administrative control policies. The ABR should be used simply as a functional measure of the VIII cranial nerve and brainstem and any decision regarding safety assessment of the test article must await further analysis [12]. The critical data in these tests remains postmortem histopathology.

What we are interested in demonstrating in the safety assessment profile of a new drug is not limited to just the question of cell damage (cytotoxicity), but rather how rapid the progression of cell death occurs following a single dose administration or during the period of repeating therapeutic dose administrations. The ABR is not the definitive dependent measure of ototoxicity. In nonclinical ototoxicity studies, it is imperative to describe the onset of organ damage, its accrual, predictors of damage progression, as well as the effects of the drug on health-related quality of life in future patients who may later receive the test article [13]. Recent work suggests that changes in auditory function can be used to track the natural history of disease progression. In most contemporary standard ototoxicity studies, the ABR is the selected screening tool. In structured drug development programs, it is critical to distinguish between treatment-related effects and background 'normal variability' when interpreting results.

For research facilities generating data for regulatory submissions for agency review and possible licensure, it is imperative that the data are valid, reliable, and generated within a well-controlled environment. In order to meet the strict standards of all drug approval agencies, researchers are required to use Good Laboratory Practices (GLP), defined in medical product development regulations, for nonclinical laboratory studies. The GLP regulations are found in United States Code (USC) 21 CFR Part 58.1: Good Laboratory Practice for Nonclinical Laboratory Studies. Fourteen years ago

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a program was established to develop an organized and harmonized pharmaceutical approval program that is a stepwise process involving an evaluation of both animal and human efficacy and safety data. In 2006 the International Commission on Harmonization (ICH) established a universal lexicon related to the testing of new molecular entities in both animals and humans prior to licensure. The term for all animal research was established to be "nonclinical" research and human based data is referred to as "clinical" data (https://database.ich.org/sites/default/files/M3_R2__Guideline.pdf)

These regulations set the minimum basic requirements for: study conduct, personnel, facilities, equipment, written protocols, operating procedures, study reports, and a system of quality assurance oversight for each study to help assure the safety of FDA-regulated product. Historical control data (HCD) can be a valuable tool in contextualizing results from single studies against previous studies performed under similar conditions [14-17].

The ABR is used simply as the functional measure of the cochlea, auditory nerve and brainstem over the time course of the study [18,19]. Small compartment neurotoxicity can be permanent or recoverable [1]. It is the ABR that has been incorporated into most nonclinical safety assessment studies to accurately establish the etiology of progressive hearing loss. Mechanical or oxidative (metabolic) damage to auditory pathways are not necessarily permanent. While several cellular, structural, and physiologic functions can cause hearing decline, there are numerous "natural" restorative processes that can completely reverse damage which influence the risk-to-benefit analysis for a compound.

Institutional normative data is advantageous [20] if the data were obtained using: 1) the same laboratory, 2) study design, 3) experimental methods, 4) assessment criteria, and 5) if the studies used for comparison were conducted contemporaneously [20,21]. There is also value and advantages of comparative data review from large, statistically powerful external databases conducted in other laboratories if collecting data on similar strains of laboratory animals given regulatory and political pressures exist to decrease animal use in safety assessment studies [21,22]. Ototoxicity screening is intended to measure biological responses to test article exposure. Responses can be highly variable, with limited opportunity for control of extrinsic sources. It is critical to distinguish between treatment-related effects and background 'normal variability' when interpreting these results [23]. HCD can be a valuable tool in contextualizing results against previous studies performed under similar conditions.

By providing a relatively large and robust series of normative control ABR data from four commonly used purpose-bred laboratory animals in standard nonclinical toxicity studies, we provide a firm foundation for maintaining the ABR as the preferred method in ototoxicity screening. With these data in mind we acknowledge the critical endpoints remain histopathology and cytocochleograms, which provide the regulatory and legally defensible weight of evidence needed to establish the risk-to-benefit profile for the compound.

ABR thresholds are not hearing thresholds

ABR thresholds are not behaviorally based "hearing thresholds". Szymanski et al. [24] concluded that the relationship between physiological and behavioral auditory thresholds is dependent upon organismal variables and stimuli characteristics, in addition to electrophysiological recording parameters and may show differences of up to 20 dB between the two measures. These ABR potentials represent sensory or neural responses from lower levels of the auditory system as they are transmitted up to the cortex.

The waveforms recorded in the ABR arise from the auditory nerve and brainstem structures [25]. The simplest view of the genesis of the ABR is that each wave arises from a single anatomical site. Waves beyond II are now commonly believed to represent brainstem level activity. The early waves (I and II) are consistent in time and amplitudes to suggest their sources are from structures on the same side as the auditory stimulus presentation. Later waves (III, IV, and V) may come from structures that receive ipsilateral, contralateral, or bilateral inputs from the auditory periphery [26-32].

Interestingly, several studies have reported noticeable differences between the information processing and waveform latencies recorded in rodent and human ABRs. Differences in the localization of the exact brainstem lamina that represents the source of scalp recorded ABR waves are believed to occur due to differences in the processing of auditory stimuli across

species. In contrast to humans, wave II in the mouse ABR has been suggested to be generated by the posterior ventral cochlear nucleus and wave V by the lateral lemniscus and inferior colliculus. For example, in rats the amplitude of wave II is the largest, wave III is the smallest and wave V is not commonly used for the evaluation of ABR hearing thresholds [33]. Borg [34] was early in advocating for the ABR as being suitable for the determination of auditory thresholds and the assessment of hearing losses, and 40 years later the ABR remains critically important in nonclinical hearing research.

As described by Nolan [35] hearing loss is a multifactorial disease governed by both genetic and environmental factors (noise, ototoxic drugs) [36]. Worldwide projections indicate 432 million adults are affected by disabling hearing loss (defined as thresholds >40 dB; hearing loss in the better hearing ear averaged across 0.5, 1, 2, and 4 kHz; WHO, 2018). When these data are identified by sex the prevalence of disabling hearing loss is greater in men compared to women, equating to 242 million men and 190 million women worldwide. Women have shorter ABR latencies then men [37] and Picton [38] has suggested that these sex-differences are most likely due to the longer length of the basilar membrane in men.

Studies in aged animals have given great insight into the histological deficit with degenerative changes in the sensory hair cells, the spiral ganglion neurons, and the stria vascularis [39,40]. Progress has been made in identifying genetic markers for early onset congenital hearing loss and describing their role in normal cochlear function [41,42]. The genetic risk factors and the molecular pathways they modulate contribute to the hearing loss developed over the lifespan and remain to be clearly elucidated.

METHODS

Animals and groups

All subjects were purpose bred laboratory animals purchased from US Department of Agriculture (USDA) breeding facilities that had prior site review and formal approval by the Institutional Animal Care and Use Committee (IACUC) at Charles River Laboratories (Mattawan, MI).

Sprague-Dawley rats, domesticated shorthair cats, albino guinea pigs, and cynomolgus monkeys were used for comparative historical and cross-species data collections. Table 1 summarizes the animals ordered and used on these studies. Extra animals are generally added to the study census to ensure adequate numbers of animals are available following ABR testing that ensures a standard randomization process for study selections.

All animal colony and testing rooms are maintained on a 12 hr light/dark cycle with monitored access to food and free access to normal facility tap water. All housing, maintenance, environmental atmospheric controls and procedures were approved by the IACUC and continuously monitored to ensure strict compliance with veterinary standards of care guidelines established by AAALAC and the applicable US drug and chemical regulatory agencies that oversee contract research facilities in the U.S.

ABRs

ABRs were conducted using the same equipment, standard operating procedures, and test frequencies for all animals on this study. Only, the specific anesthesia induction and maintenance procedures were modified for the species of animal being tested. Differential anesthesia procedures between species are required in order to provide a similar level of hypnosis (sleep), analgesia, motor suppression, and body temperature control [43].

Auditory function tests were conducted in double-walled sound-attenuating chambers (RE-246, Acoustic Systems) under anesthesia (see Table 1). Standard procedure sets right ear evaluations to be conducted first, followed by the left ear using an ascending frequency method of stimulus presentation, with the lowest frequency in each ear increasing in intensity through the highest frequency of each ear.

Table 1: Summary table for the number of animals ordered for studies and those reported on study following randomization based on ABR results.

	Rat		Cat		Guinea Pig		Monkey	
	Male	Female	Male	Female	Male	Female	Males	Female
Ordered	338	338	318	448	290	313	64	43
Reported	254	255	211	298	203	201	53	39
Numerical Difference	84	83	107	150	87	112	11	4
Percent Difference	24.8%	24.5%	33.6%	33.5%	30.0%	35.6%	17.2%	9.3%

Scalp recordings of brainwave activity is unduly affected by muscle movement artifacts, therefore anesthesia /sedation must be used for the completion of animal ABRs. Smith & Mills [44] directly compared ABR interpeak intervals and amplitude

ratios from awake versus anesthetized animals using CNS depressant anesthetics (barbiturate) versus non-CNS depressant (non-barbiturate) agents. The stability of response thresholds and the small magnitude of latency and amplitude changes with a ketamine and xylazine regimen demonstrated that accurate electrophysiological measures of hearing sensitivity and auditory brainstem activity could be obtained in anesthetized animals, provided that temperature and other parameters are maintained within normal physiological limits. Ketamine anesthesia has been widely used for ABR assessments in humans [45], and laboratory animals [46-48]. For nonhuman primates, a volatile anesthetic (isoflurane) was used to allow for longer testing periods without muscle movement artifacts and for better body temperature control. Hypothermia from prolonged anesthesia monitoring will affect the accurate measurement of ABRs [49]. In our own experience general anesthesia using isoflurane is uneventful. Doyle & Fria [49] and Ros et al. [50] have confirmed the recording of normal brainstem recordings of all five ABR peaks. There were no statistically significant differences in mean latencies or median amplitudes using inhaled anesthetics like isoflurane.

Open field ABRs were recorded using a 5 millisecond tone burst stimuli, with a 0.5 millisecond gate time, presented every 20 milliseconds by an RZ6 Auditory Processor with high bandwidth range of up to 115 dB at a sampling rate of up to 200 kHz. The speaker was placed approximately 4 centimeters from the auditory canal meatus. The auditory processor is integrated with BioSigRZ software (TDT Inc., Alachua, FL, USA), and delivered binaurally via MF1 Multi-Field Magnetic Speakers designed for use with all four species of animals related to this study report. The MF1 speaker has an ultrasonic range of up to 65 kHz. The PC-based computer software provides standard configuration files to conduct ABR evaluations in all four of species in this report, as well as complete calibration files for the speakers and microphones used to conduct the ABRs in the laboratory.

At each test frequency, each tone-burst level was varied between 0 to 100 dB SPL in 5- or 10-dB steps (if no signal waveform was generated at 80dB, the technician proceeds to 100dB SPL for confirmation, but no greater). Responses were measured via subdermal needle electrodes, vertex-to-mastoid, with the ground at the contra-lateral ear. A MedusaRA4PA preamplifier/digitizer coupled with a RA4LI amplifier (TDT) that electronically increased the weak signal by 20X, and then the waveform was digitally filtered using a 300 Hz high-pass filter and 3 kHz low-pass filter with a 60Hz notch filter. A maximum of 1024 artifact-free waveforms were averaged to produce a final ABR trace, coupled with worksheet post-processing filtration of 300 Hz corner filtration, a 2-pole filter order, and a scaling of 1.5. Analysis was based on inspection of stacked waveforms. Suprathreshold stimuli were presented at a minimum of 100 times, and as the test stimulus approached threshold values the presentation rate was increased to a minimum of 512 presentations. The ABR threshold was subjectively defined as the lowest SPL to produce a repeatable waveform greater than isoelectric background wave amplitudes. Each technician is trained to identify and document these threshold values in real time.

Threshold differences between the left and right ears for 4, 10, and 20 kHz stimuli and between male and female subjects for each species were analyzed using VasserStats©:Website for Statistical Computation, Physical Sciences Resource Center, Vassar College (1998-2020, Dr. Richard Lowry; www.vassarstats.net) Since all samples sizes were greater than 30 subjects an F statistic was used; with $p < 0.05$.

Inter-rater reliability:

Inter-rater reliability assessment was assured by comparing each technician's threshold values of historical control data of over 100 ABRs standards. Guidelines for Reporting Reliability and Agreement Studies (GRRAS) have been proposed by Kottner et al. [51] and Gerke et al. [52].

Reliability scores were determined by a Kappa-statistic like analysis determination between the institutional scientific subject matter expert (SMEs) for ABRs and an outside independent rater which is considered as an internationally-renowned expert in auditory neuroscience and electrophysiology, with over 40 years of hearing research and publication history (Dr. David Dolan, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI). A bank of ABRs independently scored by Dr. Dolan are used as standards and the technicians independently review and score those ABRs standard files. Zegers et al. [53] (2010) have proposed that "a K-value between 0.00 and 0.20 was classified as 'slight'; between 0.21 and 0.40 as 'fair'; between 0.41 and 0.60 as

'moderate'; between 0.61 and 0.80 as 'substantial'; and between 0.81 and 1.00 as 'almost perfect' ". The current laboratory interobserver agreement related to threshold values was > 0.81.

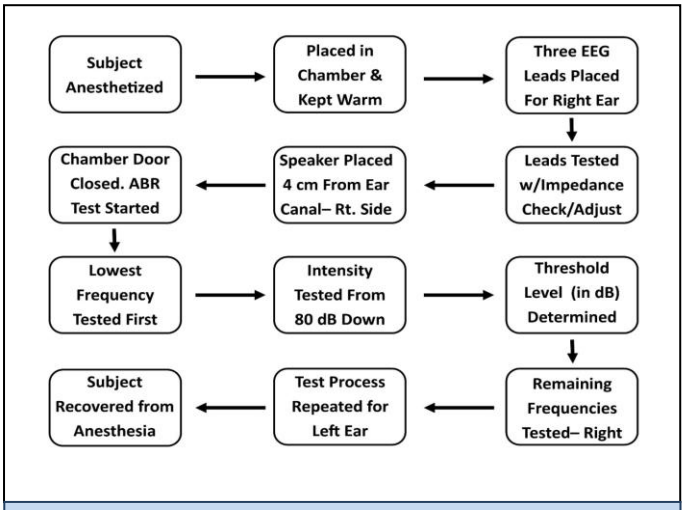

Figure 1: Process flow chart of ABR procedures conducted in rats, cats, guinea pigs, and nonhuman primates. All animals were tested with the same frequencies (4, 10, and 20 kHz) using the same equipment and procedures.

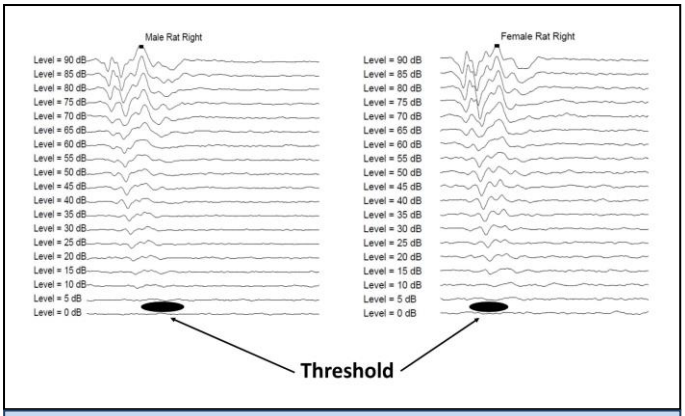

Figure 2: Effects of stimulus intensity (loudness) on representative auditory brainstem responses (ABRs) from one male (left panel) and one female (right panel) control-treated Sprague-Dawley rat. The average waveforms are shown for sound pressure levels (SPLs) from 13 to 80 dB. Both figures show ABR data from the right ear. The ABR threshold was subjectively defined as the lowest SPL to produce a repeatable waveform greater than isoelectric background wave amplitudes.

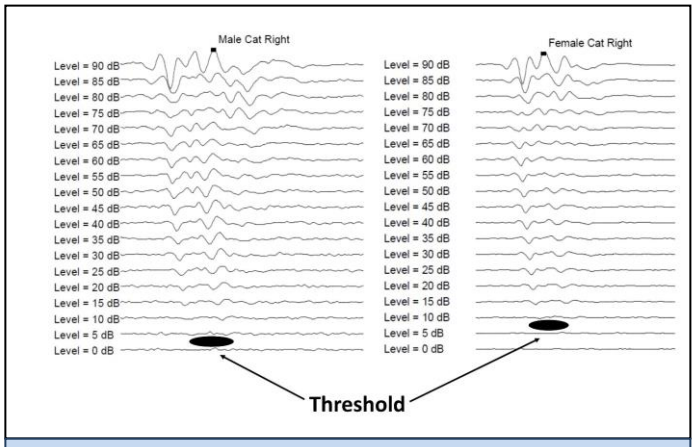

Figure 3: Effects of stimulus intensity (loudness) on representative auditory brainstem responses (ABRs) from one male (left panel) and one female (right panel) control-treated purpose bred, laboratory domestic cats. The average waveforms are shown for sound pressure levels (SPLs) from 13 to 80 dB. Both figures show ABR data from the right ear. The ABR threshold was subjectively defined as the lowest SPL to produce a repeatable waveform greater than isoelectric background wave amplitudes.

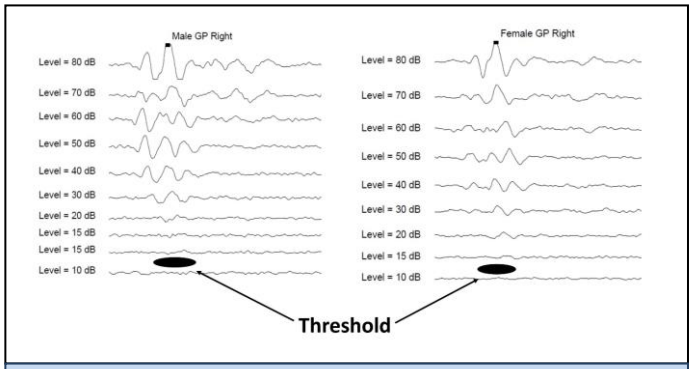

Figure 4: Effects of stimulus intensity (loudness) on representative auditory brainstem responses (ABRs) from one male (left panel) and one female (right panel) control-treated pigmented guinea pigs. The average waveforms are shown for sound pressure levels (SPLs) from 13 to 80 dB. Both figures show ABR data from the right ear. The ABR threshold was subjectively defined as the lowest SPL to produce a repeatable waveform greater than isoelectric background wave amplitudes.

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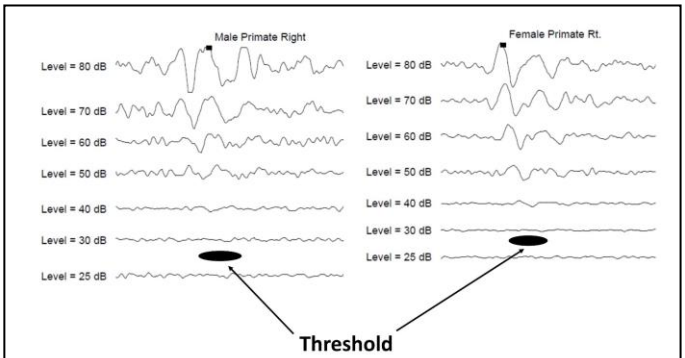

Figure 5: Effects of stimulus intensity (loudness) on representative auditory brainstem responses (ABRs) from one male (left panel) and one female (right panel) control-treated cynomolgus monkeys. The average waveforms are shown for sound pressure levels (SPLs) from 13 to 80 dB. Both figures show ABR data from the right ear. The ABR threshold was subjectively defined as the lowest SPL to produce a repeatable waveform greater than isoelectric background wave amplitudes.

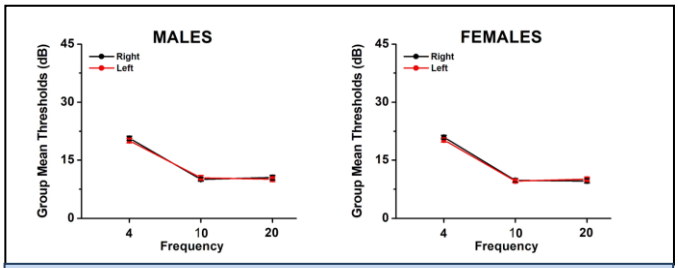

Figure 6: Group mean ABR thresholds (+/- 1 SEM), expressed in dBs, for 4, 10, and 20 kHz test frequencies for both right (black symbols) and left (red symbols) ears of male (left panel) and female (right panel) Sprague-Dawley rats. There were no statistically significant differences between males and females at any tested frequency.

Figures 2, 3, 4, and 5 are representative selected graphic presentations of the ABR waveforms determined for the mid-frequency (10 kHz) auditory stimulus from a standard (guidepost) three-frequency spectrum of 4, 10, and 20 kHz for rats, cats, guinea pigs, and monkeys, respectively. The wavelengths are ordered vertically from high (90-100 dB) amplitude sound pressure levels (top) to lowest (0 db) in 10 dB steps presented to each anesthetized animal using the psychophysical "method of limits" on a perceived loudness dimension from male (left panel) and female (right panel) subject of each species. Each wave complex represents the pooled, filtered average of a maximum of 1,024 artifact free stimulus presentations in each animal's right ear. The exception are the primate ABRs, which are averaged much longer, up to 5,000 presentations near threshold. Time from stimulus presentation is expressed in milliseconds (ms) from left to right on each waveform complex. The threshold values determined by the subjective visual inspection by trained technicians from the cumulative ABR waveform data available on the computer monitor at the time of testing are reproduced here for presentation purposes.

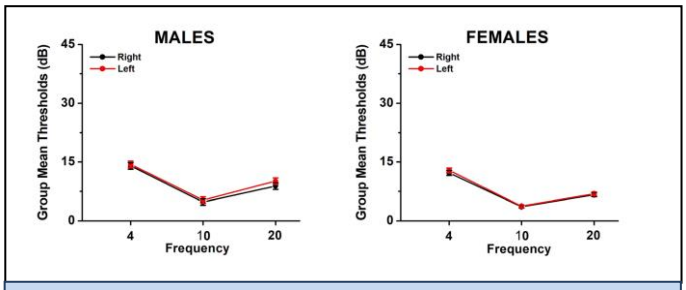

Figure 7: Group mean ABR thresholds (+/- 1 SEM), expressed in dBs, for 4, 10, and 20 kHz wavelength bands for both right (black symbols) and left (red symbols) ears of male (left panel) and female (right panel) laboratory short-haired cats.

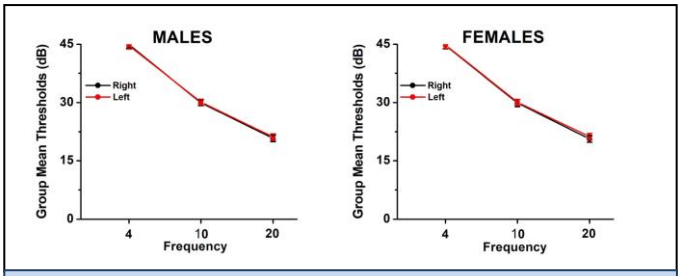

Figure 8: Group mean ABR thresholds (+/- 1 SEM), expressed in dBs, for 4, 10, and 20 kHz wavelength bands for both right (black symbols) and left (red symbols) ears of male (left panel) and female (right panel) purpose-bred laboratory guinea pigs.

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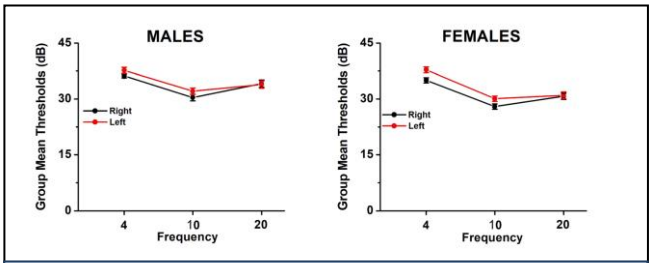

Figure 9: Group mean ABR thresholds (+/- 1 SEM), expressed in dBs, for 4, 10, and 20 kHz wavelength bands for both right (black symbols) and left (red symbols) ears of male (left panel) and female (right panel) purpose-bred laboratory cynomolgus monkeys.

Table 2: Anesthesia used to sedate animals to conduct ABRs. Each animal received identical pretreatments and anesthesia induction procedures for the four species used on the study. (refer to Flecknell, [43]).

Species	Time-to-Effect	Premedication	Anesthesia Induction	Allowable Supplement	Recovery
NHP	10 mins	Ketamine 10 mg/kg IM Atropine 0.04 mg/kg IM	Isoflurane up to 3% INH EVAP	Lactated Ringers Solution 2mL/kg/hr	NA
Rat	15 mins	NA	Ketamine 40 mg/kg IP Dexmedetomidine 0.25 mg/kg IP	Up to 1 full dose of anesthesia	Antisedan 2.5 mg/kg SC*
Guinea Pig	15 mins	NA	Ketamine 40 mg/kg IP Dexmedetomidine 0.25 mg/kg IP	Up to 1 full dose of anesthesia	Antisedan 2.5 mg/kg SC*
Cat	15 mins	NA	Ketamine 10 mg/kg IM Dexmedetomidine 0.04 mg/kg IM	Up to 1 full dose of anesthesia	Antisedan 0.4 mg/kg IM*

*Antisedan is administered at this dose or 1:1 volume Dexmedetomidine, whichever is greater

Table 3: Rats: Summary data for ABR assessments of drug- and experimentally-naïve purpose bred laboratory Sprague-Dawley rats. The grand means of stimulus intensity levels (expressed in decibels [dB] are shown for 3 reference stimuli used for threshold testing.

	ABR Frequency					
	4 kHz Right	4 kHz Left	10 kHz Right	10 kHz Left	20 kHz Right	20 kHz Left
254 Males						
MEAN (dB)	20.65	20.08	10.04	10.44	10.51	10.10
STD DEV (dB)	8.54	9.12	7.68	9.18	9.42	9.83
S.E.M. (dB)	0.54	0.58	0.48	0.58	0.59	0.62
Left v Right	p = 0.47		p = 0.60		p = 0.63	
MODE (dB)	21	21	6	21	21	6
MEDIAN (dB)	21	19	8	21	19	8
MIATA (dB)	4.62 ± 0.24		4.13 ± 0.38		3.97 ± 0.24	
Range (dB)	0 - 23		0 - 17		0 - 18	

# > 8 dB	36		30		24	
255 Females						
MEAN (dB)	20.95	20.23	9.78	9.61	9.64	9.53
STD DEV (dB)	7.88	8.40	7.39	7.62	8.79	8.96
S.E.M. (dB)	0.50	0.53	0.47	0.48	0.56	0.57
Left v Right	**p = 0.32**		**p = 0.79**		**p = 0.88**	
MODE (dB)	21	21	0	0	0	6
MEDIAN (dB)	21	20	8	8	8	7
MIATA (dB)	4.23 ± 0.23		3.84 ± 0.22		3.51 ± 0.22	
Range (dB)	0 - 17		0 - 17		0 - 19	
# > 8 dB	28		28		18	
Males vs Females	**p = 0.69**	**p = 0.86**	**p = 0.70**	**p = 0.27**	**p = 0.28**	**p = 0.50**

MIATA: Mean Inter-aural Threshold Asymmetry

Table 4: Cats: Summary data for ABR assessments of drug- and experimentally-naïve purpose bred laboratory cats. The grand means of stimulus intensity levels (expressed in decibels [dB] are shown for 3 reference stimuli sed for threshold testing.

	ABR Frequency					
	4 kHz Right	**4 kHz Left**	**10 kHz Right**	**10 kHz Left**	**20 kHz Right**	**20 kHz Left**
209 Males						
MEAN (dB)	13.97	14.37	4.80	5.31	8.85	9.23
STD DEV (dB)	12.10	11.76	6.61	7.03	8.47	9.26
S.E.M. (dB)	0.84	0.84	0.84	0.84	0.84	0.84
Left v Right	**p = 0.73**		**p = 0.45**		**p = 0.66**	
MODE (dB)	8	11	0	0	6	6
MEDIAN (dB)	12	12	2	2	6	6
MIATA (dB)	4.18 ± 0.28		3.32 ± 0.25		4.21 ± 0.28	
Range (dB)	0 – 21		0 – 19		0 – 22	
# > 8 dB	25		26		30	
295 Females						
MEAN (dB)	12.19	12.85	3.62	3.73	6.64	6.9
STD DEV (dB)	10.68	10.34	5.65	6.06	8.60	8.18
S.E.M. (dB)	0.62	0.61	0.33	0.35	0.50	0.48
Left v Right	**p = 0.45**		**p = 0.66**		**p = 0.82**	
MODE (dB)	14	9	0	0	0	0
MEDIAN (dB)	11	11	1	1	4	6
MIATA (dB)	4.13 ± 0.21		2.62 ± 0.18		4.04 ± 0.32	
Range (dB)	0 – 21		0 – 16		0 – 24	
# > 8 dB D.L.	35		19		39	
Males vs Females	**p = 0.08**	**p = 0.12**	**p = 0.03 ***	**p = 0.007 ****	**p = 0.004 ****	**p =0.003 ****

MIATA: Mean Inter-aural Threshold Asymmetry; * $p < 0.05$; ** $p < 0.01$

Table 5: Guinea Pigs: Summary data for ABR assessments of drug- and experimentally-naïve purpose bred laboratory pigmented guinea pigs. The grand means of stimulus intensity levels (expressed in decibels [dB] are shown for 3 reference stimuli used for threshold testing.

	ABR Frequency					
	4 kHz Right	4 kHz Left	10 kHz Right	10 kHz Left	20 kHz Right	20 kHz Left
203 Males						
MEAN (dB)	44.87	44.63	29.97	30.14	20.81	21.14
S.D. (dB)	12.42	12.12	10.93	10.41	12.49	11.66
S.E.M. (dB)	0.87	0.85	0.77	0.73	0.88	0.82
Laft v Right	**p = 0.84**		**p = 0.89**		**p = 0.78**	
MODE (dB)	36	36	27	28	17	16
MEDIAN (dB)	43	42	28	28	17	18
MIATA (dB)	5.07 ± 0.33		3.89 ± 0.24		4.05 ± 0.22	
Range (dB)	0 – 18		0 – 18		0 - 14	
# > 8 dB D.L.	36		22		26	
203 Females						
MEAN (dB)	44.70	44.78	29.89	30.10	20.65	21.24
S.D. (dB)	12.85	12.31	11.37	10.60	11.99	11.38
S.E.M. (dB)	0.91	0.87	0.80	0.75	0.85	0.81
Left v Right	**p = 1.0**		**p = 0.84**		**p = 0.61**	
MODE (dB)	43	36	23	26	16	18
MEDIAN (dB)	41	42	27	28	17	19
MIATA (dB)	4.58 ± 0.25		3.93 ± 0.24		4.25 ± 0.23	
Range (dB)	0 – 19		0 - 17		0 - 19	
# > 8 dB	33		24		19	
Males v Females	**p = 0.88**	**p = 0.89**	**p = 0.92**	**p = 1.0**	**p = 0.89**	**p = 0.92**

MIATA: Mean Inter-aural Threshold Asymmetry

Table 6: Monkeys: Summary data for ABR assessments of drug- and experimentally-naïve purpose bred laboratory cynomolgus monkeys. The grand means of stimulus intensity levels (expressed in

	ABR Frequency					
	4 kHz Right	4 kHz Left	10 kHz Right	10 kHz Left	20 kHz Right	20 kHz Left
53 Males						
MEAN (dB)	36.17	37.68	30.41	32.11	34.13	33.90
STD DEV (dB)	3.9	5.85	6.38	6.26	7.25	7.25
S.E.M. (dB)	0.54	0.80	0.88	0.86	0.99	0.99
Left v Right	**p = 0.56**		**p = 0.54**		**p = 0.92**	
MODE (dB)	34	37	28	34	33	27
MEDIAN (dB)	34	37	28	34	33	27
MIATA (dB)	4.45 ± 0.65		5.21 ± 0.76		6.98 ± 0.81	
Range (dB)	0 – 26		0 – 22		0 - 31	
# > 8 dB	10		11		16	
39 Females						
MEAN	35.02	37.82	27.97	30.10	30.74	31.00

(dB)						
STD DEV (dB)	4.30	4.78	4.59	4.43	5.18	5.80
S.E.M. (dB)	0.69	0.77	0.73	0.71	0.83	0.93
Left v Right	p = 0.50		p = 0.39		p = 0.81	
MODE (dB)	33	36	28	29	29	28
MEDIAN (dB)	35	37	28	29	31	32
MIATA (dB)	3.72 ± 0.56		3.56 ± 0.52		5.13 ± 0.50	
Range (dB)	0 - 14		0 - 13		0 - 11	
# > 8 dB	4		4		8	
Males vs Females	p = 1.0	p = 0.84	p = 0.47	p = 0.65	p =0.40	p = 0.49

MIATA: Mean Inter-aural Threshold Asymmetry

Table 7: ABR thresholds asymmetries in rats, cats, guinea pigs and monkeys. The percentage of right ear to left ear threshold differences are expressed as a percentage of total subject population of each species.

Animal Species	ABR Frequency Band Tested					
	4 kHz		10 kHz		20 kHz	
	Male	Female	Male	Female	Male	Female
Rat	42.62%	40.63%	41.03%	41.83%	37.45%	39.44%
Cat	41.69%	41.82%	38.30%	37.98%	47.45%	44.23%
Guinea Pig	47.05%	48.51%	45.09%	41.58%	46.07%	50.49%
NHP	49,05%	71.79%	56.60%	64.10%	49.05%	51.28%

Percentage of Subjects with Right Threshold < Left Threshold

Tables 3, 4, 5, and 6 are the corresponding group summary data derived from the reported sample sizes from cats, rats, guinea-pigs and monkeys represented by the Figures, above. As shown in these ABR Summary Tables (3 through 6), there are no physiologically meaningful sex differences in ABR thresholds for our standard 3 wavelength guideposts. There were no statistically significant male-female differences in measured thresholds for 4, 10, or 20 kHz stimuli in rats, guinea pigs, and monkeys. There were differences between male and female cats on the measured ABR thresholds for both left and right ears at the 10 and 20 kHz stimuli, however, while statistically significant the group mean differences were limited to less than 3 dB in SPL and are not considered physiologically meaningful.

Our historical data do not support the mandatory inclusion of both sexes in ototoxicity studies based solely on baseline ABR threshold differences. However, the cellular mechanisms involved in oxidative stress, initiation of apoptotic pathways, pharmacokinetics, or active metabolite production associated with the test article itself [54], may be critical factors used justify selection and use of the most sensitive gender for this specific test article of interest based on either the area-under-the-curve kinetics or Cmax achieved in the cochlear endolymph. The summary data demonstrate the highest auditory threshold values are consistently from the low frequency (4 kHz) stimuli but as shown in Figure 5, below the 3-point audiograms from these four species of commonly used experimental animals do not show strict frequency dependency.

Figures 6 through 9 show the group mean threshold values for 3 standard frequecy bands, 4, 10, and 20 kHz, in drug- and experimentally naïve rats, cats, guinea pigs, and monkeys, respectively.

The audiograms for rats, cats, and monkeys show a similar shaped “bent linear” function from low to high frequencies. The 10 kHz frequency standard demonstrating the lowest ABR threshold for rats, cats, and monkeys. Guinea pigs, on the other hand show a negative linear function with frequency, the higher the frequency the more sensitive guinea pigs are in ABR assessments. For the lowest test frequency band of 4 kHz, the group mean ABR thresholds resulted in a rank order of cats < rats < monkey < guinea pigs; the mid-frequency band (10 kHz) resulted in a rank order of cats < rats < monkeys ≡

guinea pigs; and the highest standard test frequency (20 kHz) resulted in a ranking of cats < rats < monkeys < guinea pigs.

DISCUSSION

There are no clear sex/gender differences in electrophysiological auditory thresholds in rats, cats, guinea pigs or monkeys in this laboratory. There are several published studies appearing in peer-reviewed scientific journals that do report sex/gender effects in the ABR [38]. For example, Charlton et al. [55] reported statistically different ABR thresholds in male and female Long Evans and Brattleboro rats. However, in these data, ABR thresholds differed across all groups tested by as little as 6 dB at 8 kHz and by as much as 24 dB at 42 kHz test frequencies. The ABR thresholds for the click stimuli were the same for all groups in the recent Charlton et al. study [55] (25 dB), but the peak sensitivity for the rats was between 8 and 16 kHz, with higher thresholds at both higher and lower frequencies. Significant sex differences in the ABRs were not found at all frequencies but were obtained at some of the lowest and some of the highest frequencies tested and are similar those reported by Popelar et al. [56]. As noted by the authors, it is not yet known whether these statistically significant ABR sex differences occur at ecologically "meaningful" frequencies.

Human data show that women have shorter latencies and larger amplitude waveform when compared to males [57]. However, any differences in head circumference or scalp/skull thickness that may serve to augment the total electrical impedance of the EEG circuitry in conducting ABRs is negligible [38,58-61].

There have been a considerable number of studies investigating the binaural differences in auditory cues available to a variety of different species, including the rat [62,63], cat [64-66], guinea pigs [67-71], and the monkey [72,73]. Sininger & Cone-Wesson [74,75] reported ABR asymmetries in human infants with right ear dominance. Subsequently, Keefe et al. [76] reported the results of over 2000 ABRs in human infants and found right-ear dominance for some of the response and noise amplitudes, but never showed left-ear dominance. The table below, shows the percentage of right-ear dominance for ABR thresholds in each of the four species.

In our data rats and cats showed minor left ear dominance (>50% of animals had lower left ear thresholds when compared to the right). Guinea pigs also showed a minor left ear dominance in the 4 and 10 kHz audible range of ABR test frequencies. However, male guinea pigs showed a very slight left ear dominance, with females showing no binaural threshold differences at the highest tested frequency of 20 kHz. Monkeys showed the greatest binaural difference thresholds of all four species tested. Females showed a unilateral threshold sensitivity for the right ear at the 4, 10 kHz test frequencies with only a minor binaural disparity at the high frequency of 20 kHz. Male monkeys showed only a minor unilateral difference threshold at 10 kHz. Whether these binaural difference thresholds have any ethological basis is not known but the difference may be the result of "order effects" since it is our standard practice to test right ears prior to left ears.

Our historical control data demonstrate a minimal impact on binaural threshold differences in any of the four species including rats, cats, guinea pigs, or monkeys with less than 8 dB binaural differences at 4, 10, and 20 kHz standard test frequencies. These historical records may suggest that future ototoxicity studies may be limited to the most drug-sensitive gender of experimental animals, only. males or females, but not both.

Guinea pigs are the most commonly reported animal species used in nonclinical auditory research and the ABR thresholds and resulting ABR audiograms demonstrate the reason why – guinea pigs show a direct inverse correlation between ABR thresholds and test frequencies used in standard ABR tests. Since there is a general progression of hearing loss in drug-induced cytotoxicity in the cochlea with high frequency loss initiating the cascade followed by mid-frequency loss and low frequency loss occurring only after long term drug treatments. The dynamic range of hearing in the guinea pig is linear and can provide the most sensitive measure of the onset and development of ototoxicity since this species has the lowest thresholds at the highest frequency (near the basal end of the cochlea) initially of all three other species described in this report.

Atcherson & Stoody [58] remind us that regardless of sample size, ABR thresholds are subjective measurements. Each ABR waveform in this and all laboratory settings have undergone

one critically important but basic statistical procedure – averaging. Each waveform is the amalgamated composition of a finite number of microvoltage samples collected on the scalp, which is amplified and digitized in an attempt to cancel out random background noise (EEG, or electrocorticogram [ECoG]) without allowing the desired stimulus evoked potential to continue to develop in the average. The separate waveforms collected for each rat, cat, guinea pig, or monkey is then compared with normative data collected from the larger group of 509 rats, 503 cats, 406 guinea pigs and 92 nonhuman primates. Normative data typically yield the central tendency (Tables 1 through 4) of the species, as well as the variability in the group (standard deviation or standard error of the mean). According to Atcherson & Stoody, [58] if an animal has a measurement that exceeds 2 standard deviations above or below the mean there is a greater likelihood there is an abnormality. While using this 2 SD method is common, there are reports of other SD values such as 2.5 or 3 SD; 2 SD threshold is considered a "conservative" setpoint [77]. Physiological (ABR) and behavioral auditory thresholds are not static, and the history of psychophysics tells us that there are no absolute thresholds; transience prevails. Transient threshold shifts occur in all sensory systems. No single ABR threshold shift should be used to define ototoxicity.

Hearing losses have the effect of reducing the perceived intensity level of the stimulus presentations in the ABR (wave amplitudes), which some assume should cause a prolongation of all or most ABR waveforms, as if the technician was decreasing the sound intensity (SPL: sound pressure levels); in reality this is rare [78]. Low-frequency hearing loss can be associated with a normal ABR because of the bias of tone or click stimulus intensity to the basal end of the cochlea [74]. A subject with moderate to severe sensorineural hearing loss could also have a normal ABR with all relative and absolute latencies within the normative range [75]. In clinical experience and published reports of animal ototoxicity studies appearing in peer-reviewed scientific journals, when audiometric pure-tone averages are 70 dB or greater (hearing level in humans or ABR thresholds in animals) it is not uncommon to have absent ABRs. When the thresholds at 2 kHz is no greater than 40 dB SPL, and the three audiometric average is not greater than 50 dB SPL, it has been reported that 80% of all ABRs can be normal [75,77]. The "perfect" ABR will generally only be true with optimal recording conditions, high stimulus intensity conditions, a very quiet and cooperative subject, and a very healthy animal subject with no comorbidities, such as inflammation, infection, or morphological damage [79].

The lack of significant threshold shifts in ABR waveforms, positive or negative, is not convincing evidence of drug safety or the lack of test article induced ototoxicity. Other critical parameters must be included in the standard ototoxicity study report – such as cytocochleograms and histopathology.

Compared to behavioral thresholds, physiological methods tend to overestimate thresholds, particularly at higher frequencies [80-82], in part because ABRs are limited by the electrical noise that obscures the amplified scalp responses near thresholds. Auditory function audiograms have consistently shown transient and Temporary Threshold Shifts (TTS) over repeated testing [83]. If the transient threshold represents a true shift in baseline then the difference between the final and original baselines is considered the drug-induced permanent threshold shift (DIPTS, or simply a PTS). Since drug-induced threshold shifts may show recovery during subsequent ABR re-tests, not all threshold shifts represent "hearing loss", "ototoxicity", or cochlear cell death. The time course and extent of the TTS is most likely related to anti-apoptotic mechanisms, natural cellular repair, or tolerance. The primary objectives of ototoxicity safety assessment studies are, of course, to identify permanent threshold shifts that are subsequently confirmed to be the result of anatomical/structural damage induced by the test compound. Previous animal (noise-induced) ototoxicity studies have shown that auditory threshold shifts of up to 40 dB failed to be clinically diagnosed as Permanent Threshold Shifts (PTS) by histopathological confirmation [84-86] chinchilla. Similar to ABRs, the studies using DPOAEs to track risk progression do not reliably identify the PTS, either [87- 89]. While ABRs and DPOAEs are supportive biomarkers for the identification of risk onset, and the relative rate of progression of that risk over the course of drug exposures, the definitive assay in all toxicology programs remains postmortem histopathology.

Methods for choosing an appropriate sample size in animal experiments is critical in conformity to international standards for use of animals in research (the 3R's). As Sponsors diminish

the number of animals on studies to control costs, the animal census may threaten the risk of obtaining inconclusive results and requiring a secondary run of the study to bolster the statistical power of planned comparisons. By using a more efficient experimental design we can, for a given number of animals, reduce this risk of failure.

In standard toxicology safety assessment protocols planned statistical comparisons are made to compare test article treatments back to a control group or add positive and negative control groups and schedule statistical plan that includes making all pairwise comparisons possible. These studies are inherently sensitive due to the reduced multiple testing burden, but the sensitivity can be further maximized by comparing back to historical control data, as well. Study designs employing non-concurrent controls, such as historical control data that use scientifically valid surrogate endpoints and statistical methods, such as Bayesian analyses, should be considered to determine if they may be appropriately used in these preclinical ototoxicity study protocols. Conditions where such animal data could meet the threshold for approval are conditions that typically involve scientifically valid information that is available in the public domain (i.e., study reports appearing in peer-reviewed scientific journals).

We acknowledge that institutional historical control data is imperative to maintain valid and reliable data for regulatory review for safety assessments. Using the exact same equipment, parameters and techniques to evaluate the onset, progression, and absolute magnitudes of small compartment toxicity of the auditory system reduces the likelihood of false alarms and misses [90]. Scheuren [91] has described two loudness standards that currently exist for the industry. One standard maintained at the Deutsches Institut für Normung (DIN), is referred to as DIN 45631 [92]. Under the International Standards Organization (ISO) standard #532-1 also refers to this as the "Zwicker method" (ISO 532:1975; DIN 45631) [93]. At present the stimulus features that are used in ABR evaluations have no industrial standards between manufacturers of stimulus generators used in these evaluations [94]. The second loudness standard is known as ISO 532-2 and referred to as the "Moore/Glasberg method". In the United States, the American National Standards Institute (ANSI), sets this standard under ANSI S3.4-2007 [95] and is the model accepted by the Acoustical Society of America. Equipment manufacturers operating under the ISO and the ANSI do not necessarily use the same stimulus features that are the basis of ABR threshold evaluations worldwide. Despite these issues, there remains solid grounds to publish historical control data set [96].

Historical control data contains no sensitive or proprietary information and should be uncontroversial to publish more widely. Currie & Dodds [97] supported the open publication of normative historical control data for several additional reasons:

1. Aggregated data can provide additional insights into the biology of the test system. Predictive models used in computational toxicology may also benefit from access to additional datasets that may have benefits of harmonizing other datasets to improve the practice of pathology. These successes could then extend into other areas of diagnostic or research practices.
2. Streamlining the regulatory process has the potential benefit for both the regulators and the registrants. Public access to historical control data will simplify requests for additional evidence
3. A data-driven approach towards regulatory and administrative decisions based on historical control data from other laboratories may also increase public confidence in the process. And,
4. there is also the ethical argument that researchers should be trying to maximize the value gained from animal testing data.

In conclusion, large samples of normative ABR data from four common purpose-bred laboratory animals suggests that:

1. there are no meaningful differences between male and female ABR thresholds
2. auditory sensitivity ranking: Cats > Rats > NHPs > Guinea Pigs
3. there are species differences in sensitivity range: lower thresholds imply greater sensitivity
4. most neural damage induced by drugs starts near the base of the cochlea [98-100]- high frequency (20 kHz), therefore the guinea pig may be the better test system for these study designs

5. monkeys have the greatest differential binaural sensitivity (MIATA): 6dB in high frequencies; GPs have binaural differences in low frequency range

6. female cats show the most sensitivity in all species and genders tested here: most sensitive in 10 and 20 kHz ranges, and the low frequency range (4kHz) the female cat remains more sensitive than all other subjects

7. female rats show the greatest disparity between "most often reported thresholds" (mode) in the 10 and 20 kHz range and the central tendency of the group averaged frequencies (Median), followed by cats, and

8. guinea pigs and monkeys show the greatest equality in threshold distributions (mode vs median) showing stock/strain stability.

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REFERENCES

1. Gauvin DV, Yoder JD, Tapp RL, Baird TJ. (2017). Small Compartment Toxicity: CN VIII and Quality of Life: Hearing Loss, Tinnitus, and Balance Disorders. Int J Toxicol. 36: 8-20.
2. Abernathy MM. (2015). The Safety Pharmacology of Auditory Function. Handb Exp Pharmacol. 229: 267-290.
3. USC, United States Code. (2011). Title 15, Chapter 53. Toxic Substances Control Act, sections 2601 to 2697.
4. US EPA, United States Environmental Protection Agency. (1998). Health Effects Test Guidelines: OPPTS 870.6855 Neurophysiology: Sensory Evoked Potentials.
5. US FDA, United States Food and Drug Administration. (2018). Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route.
6. US FDA, United States Food and Drug Administration. (2019). Title 21--Food And Drugs, Chapter I--Food And Drug Administration, Department Of Health And Human Services, Subchapter A—General Part 58.Good Laboratory Practice For Nonclinical Laboratory Studies (as amended).
7. Stebbins WC, Rudy MC. (1978). Behavioral ototoxicology. Environ Health Perspect. 26: 43-51.
8. Galambos R, Despland PA. (1980). The auditory brainstem response (ABR) evaluates risk factors for hearing loss in the newborn. Pediatr Res. 14: 159-163.
9. Hecox K, Galambos R. (1974). Brain stem auditory evoked responses in human infants and adults. Arch Otolaryngol. 99: 30-33.
10. Norton SJ, Gorga MP, Widen JE, Folsom RC, Sininger Y, et al. (2000). Identification of neonatal hearing impairment: summary and recommendations. Ear Hear. 21: 529-535.
11. Starr A, Rance G. (2015). Auditory Neuropathy. In: "Handbook of Clinical Neurology", Vol. 129: 495-508.
12. Sauter T, Runge CL. (2012). Early latency auditory evoked potentials in retrocochlear disease and auditory neuropathy spectrum disorder. In: "Auditory Electrophysiology: A clinical guide". New York, Stuttgart: Thieme pp 237-258.
13. Rance G, Starr A. (2015). Pathophysiological mechanisms and functional hearing consequences of auditory neuropathy. Brain. 138: 3141-3158.
14. Ghadessi M, Tang R, Zhou J, Rong Liu, Chenkun Wang, et al. (2020). A roadmap to using historical controls in clinical trials - by Drug Information Association Adaptive Design Scientific Working Group (DIA-ADSWG). Orphanet J Rare Dis 15: 69. Published 2020 Mar 12.
15. Brooks AC, Foudoulakis M, Schuster HS, Wheeler JR. (2019). Historical control data for the interpretation of ecotoxicity data: are we missing a trick? Ecotoxicology. 28: 1198-1209.
16. Viele K, Berry S, Neuenschwander B, Billy Amzal, Fang Chen, et al. (2014). Use of historical control data for assessing treatment effects in clinical trials. Pharm Stat. 13: 41-54.
17. Kim MO, Harun N, Liu C, Khoury JC, Broderick JP. (2018). Bayesian selective response-adaptive design using the historical control. Stat Med. 37: 3709-3722.
18. Starr A, Picton TW, Sininger Y, Hood LJ, Berlin CI. (1996). Auditory Neuropathy. 119: 741-753.
19. Starr A, Hamilton AE. (1976). Correlation between confirmed sites of neurological lesions and abnormalities of far-field auditory brainstem responses. Electroencephalogr Clin Neurophysiol. 41: 595-608.

20. Sininger YS. (1992). Establishing clinical norms for auditory brainstem response. Am J Audiology. 1: 16-18.
21. Greim H, Gelbke HP, Reuter U, Thielmann HW, Edler L. (2003). Evaluation of historical control data in carcinogenicity studies. Hum Exp Toxicol. 22: 541-549.
22. Ema M, Endoh K, Fukushima R, Sakiko Fujii, Hiroaki, et al. (2014). Historical control data on developmental toxicity studies in rodents. Congenit Anom (Kyoto). 54: 150-161.
23. Brooks AC, Foudoulakis M, Schuster HS, Wheeler JR. (2019). Historical control data for the interpretation of ecotoxicity data: are we missing a trick?. Ecotoxicology. 28: 1198-1209.
24. Szymanski MD, Bain DE, Kiehl K, Pennington S, Wong S, et al. (1999). Killer whale (Orcinus orca) hearing: auditory brainstem response and behavioral audiograms. J Acoust Soc Am. 106: 1134-1141.
25. Jewett DL. (1970). Volume-conducted potentials in response to auditory stimuli as detected by averaging in the cat. Electroencephal Clin Neurophysiol. 28: 609-618.
26. Achor LJ, Starr A. (1980a) Auditory brainstem responses in the cat I: Intracranial and extracranial recordings. Electroencephal Clin Neurophysiol. 48: 154-173.
27. Achor LJ, Starr A. (1980b). Auditory brainstem responses in the cat II: Effects of lesions. Electroencephal Clin Neurophysiol. 48: 174-190.
28. Buchwald JS, Huang CM. (1975). Far-field acoustic response: Origins in the cat. Science. 189: 382-384.
29. Møller AR, Jannetta PJ, Bennett M, Moller MB. (1981). Intracranially recorded responses from the human auditory nerve: New insights into the origin of brainstem evoked potentials (BSEP). Electroencephal Clin Neurophysiol. 52: 18-27.
30. Wada SI, Starr A. (1983a) Generation of auditory brainstem responses (ABRs). I. Effects of injection of a local anesthetic (procaine HCL) into the trapezoid body of guinea pigs and cat. Electroencephal Clin Neurophysiol. 56: 326-339.
31. Wada SI, Starr A. (1983b). Generation of auditory brainstem responses (ABRs). II. Effects of surgical section of the trapezoid body on the ABR in guinea pigs and cat. Electroencephal Clin Neurophysiol. 56: 340-351.
32. Wada SI, Starr A. (1983c). Generation of the auditory brainstem responses (ABRs). III. Effects of lesions of the superior olive, lateral lemniscus and inferior colliculus on the ABR in guinea pig. Electroencephal Clin Neurophysiol. 56: 352-366.
33. Overbeck GW, Church MW. (1992). Effects of tone burst frequency and intensity on the auditory brainstem response (ABR) from albino and pigmented rats. Hear Res. 59: 129-137.
34. Borg E. (1982). Auditory thresholds in rats of different age and strain. A behavioral and electrophysiological study. Hear Res. 8: 101-115.
35. Nolan LS. (2020). Age-related hearing loss: Why we need to think about sex as a biological variable. J Neurosci Res 10.1002/jnr.24647.
36. Bowl MR, Dawson SJ. (2019). Age-related hearing loss. Cold Spring Harbor Perspectives in Medicine 9: a033217.
37. Hall JW. (2007). New Handbook of Auditory Evoked Responses. Boston, MA: Pearson.
38. Picton TW. (2010). Human Auditory Evoked Potentials. 1st ed. San Diego, CA: Plural Publishing.
39. Keithley EM. (2019). Pathology and mechanisms of cochlear aging. J Neurosci Res.
40. Ohlemiller KK. (2004). Age-related hearing loss: The status of Schuknecht's typology. Curr Opin Otolaryngol & Head and Neck Surg. 12: 439-443.
41. Bowl MR, Brown SDM. (2018). Genetic landscape of auditory dysfunction. Hum Molecul Genet. 27: R130-R135.
42. Ingham NJ, Pearson SA, Vancollie VE, Rook V, Lewis MA, et al. (2019). Mouse screen reveals multiple new genes underlying mouse and human hearing loss. PLoS Biology. 17: e3000194.
43. Flecknell P. (2003). Laboratory Animal Anesthesia (2nd Edition). San Diego, CA: Elsevier Academic Press.
44. Smith DI, Mills JH. (1989). Anesthesia effects: auditory brain-stem response. Electroencephalogr Clin Neurophysiol. 72: 422-428.
45. Sasaki H. (1991). Nihon Jibiinkoka Gakkai Kaiho. 94: 1834-1843.
46. Ruebhausen MR, Brozoski TJ, Bauer CA. (2012). A comparison of the effects of isoflurane and ketamine

anesthesia on auditory brainstem response (ABR) thresholds in rats. Hear Res. 287: 25-29.

47. Cederholm JM, Froud KE, Wong AC, Ko M, Ryan AF, et al. (2012). Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse. Hear Res. 292: 71-79.
48. Lima JP, Ariga S, Velasco I, Schochat E. (2012). Effect of the ketamine/xylazine anesthetic on the auditory brainstem response of adult gerbils. Braz J Med Biol Res. 45: 1244-1247.
49. Doyle WJ, Fria TJ. (1985). The effects of hypothermia on the latencies of the auditory brain-stem response (ABR) in the rhesus monkey. Electroencephalogr Clin Neurophysiol. 60: 258-266.
50. Ros C, Soler C, García de Carellán Mateo A. (2017). Comparison of the brainstem auditory evoked responses during sevoflurane or alfaxalone anaesthesia in adult cats. Vet Anaesth Analg. 44: 1085-1090.
51. Kottner J, Audigé L, Brorson S, Allan Donner, Byron J Gajewski, et al. (2011). Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. J Clin Epidemiol. 64: 96-106.
52. Gerke O, Möller S, Debrabant B, Halekoh U, Odense Agreement Working Group. (2018). Experience Applying the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) Indicated Five Questions Should Be Addressed in the Planning Phase from a Statistical Point of View. Diagnostics (Basel). 8: 69.
53. Zegers M, de Bruijne MC, Wagner C, Groenewegen PP, van der Wal G, et al. (2010). The inter-rater agreement of retrospective assessments of adverse events does not improve with two reviewers per patient record. J Clin Epidemiol. 63: 94-102.
54. Jilanchi S, Talebi A, Nematbakhsh M. (2018). Cisplatin Alters Sodium Excretion and Renal Clearance in Rats: Gender and Drug Dose Related. Adv Biomed Res. 7: 54.
55. Charlton PE, Schatz KC, Burke K, Paul MJ, Dent ML. (2019). Sex differences in auditory brainstem response audiograms from vasopressin-deficient Brattleboro and wild-type Long-Evans rats. PLoS One. 14: e0222096.
56. Popelar J, Groh D, Mazelova J, Syka J. (2003). Cochlear function in young and adult Fischer rats. Hear Res 186: 75-84.
57. Hall JW 3rd, Buss E, Grose JH. (2007). The binaural temporal window in adults and children. J Acoust Soc Am. 121: 401-410.
58. Atcherson SR, Stoody TM. (2012). "Auditory Electrophysiology: A Clinical Guide". New York, NY: Thieme.
59. Jalaei B, Zakaria MN, Mohd Azmi MH, Nik Othman NA, Sidek D. (2017). Gender Disparities in Speech-evoked Auditory Brainstem Response in Healthy Adults. Ann Otol Rhinol Laryngol. 126: 290-295.
60. Angrisani RM, Bautzer AP, Matas CG, Azevedo MF. (2013) Auditory brainstem response in neonates: influence of gender and weight/gestational age ratio. Rev Paul Pediatr. 31: 494-500.
61. Angrisani RM, Azevedo MF, Carvallo RM, Albuquerque DinizIV EM, Ferraro AA, et al. (2013). Electrophysiological characterization of hearing in small for gestational age premature infants. Codas. 25: 22-28.
62. Harrison JM, Downey P. (1970). Intensity changes at the ear as a function of the azimuth of a tone source: a comparative study. J Acoust Soc Am. 47: 1509-1518.
63. Koka K, Jones HG, Thornton JL, Lupo JE, Tollin DJ. (2011). Sound pressure transformations by the head and pinnae of the adult Chinchilla (Chinchilla lanigera). Hear Res. 272: 135-147.
64. Phillips DP, Calford MB, Pettigrew JD, Aitkin LM, Semple MN. (1982). Directionality of sound pressure transformation at the cat's pinna. Hear Res. 8: 13-28.
65. Rice JJ, May BJ, Spirou GA, Young ED. (1992). Pinna-based spectral cues for sound localization in cat. Hear Res. 58: 132-152.
66. Roth GL, Kochhar RK, Hind JE. (1980). Interaural time differences: implications regarding the neurophysiology of sound localization. J Acoust Soc Am. 68: 1643-1651.
67. McAlpine D, Grothe B. (2003). Sound localization and delay lines--do mammals fit the model? Trends Neurosci. 26: 347-350.
68. McAlpine D, Jiang D, Palmer AR. (1996). Interaural delay sensitivity and the classification of low best frequency

binaural responses in the inferior colliculus of the guinea pig. Hear Res. 97: 136-152.

69. Palmer AR, King AJ. (1982). The representation of auditory space in the mammalian superior colliculus. Nature. 299: 248-249.
70. Palmer AR, Rees A, Caird D. (1990). Interaural delay sensitivity to tones and broad band signals in the guinea pig inferior colliculus. Hear Res. 50: 71-86.
71. Shackleton TM, Palmer AR. (2010). The time course of binaural masking in the inferior colliculus of guinea pig does not account for binaural sluggishness. J Neurophysiol. 104: 189-199.
72. Slee SJ, Young ED. (2010). Sound localization cues in the marmoset monkey. Hear Res. 260: 96-108.
73. Spezio ML, Keller CH, Marrocco RT, Takahashi TT. (2000). Head-related transfer functions of the Rhesus monkey. Hear Res. 144:73-88.
74. Sininger YS, Cone-Wesson B. (2004). Asymmetric cochlear processing mimics hemispheric specialization. Science. 305: 1581.
75. Sininger YS, Cone-Wesson B. (2006). Lateral asymmetry in the ABR of neonates: evidence and mechanisms. Hear Res; 212: 203-211.
76. Keefe DH, Gorga MP, Jesteadt W, Smith LM. (2008). Ear asymmetries in middle-ear, cochlear, and brainstem responses in human infants. J Acoust Soc Am. 123: 1504-1512.

Ünsal S, Gündüz M. (2019). Assessment of Latency and Amplitude Based on Polarity Change in Auditory-Evoked Brainstem Responses of Normal Hearing Individuals. Otolaryngol (Sunnyvale). 9: 375.

77. Don M, Eggermont JJ, Brackmann DE. (1979). Reconstruction of the audiogram using brain stem responses and high-pass noise masking. Ann Otol Rhinol Laryngol Suppl 3 Pt 2 Suppl. 88: 1-20.
78. Atcherson SR. (2012). The auditory brainstem response. In: "Auditory Electrophysiology: A clinical guide". pp. 67- 83. New York NY: Thieme.
79. Bauch CD, Olsen WO. (1988). Auditory brainstem responses as a function of average hearing sensitivity for 2,000-4,000 Hz. Audiology. 27: 156-163.
80. Dallos P, Cheatham MA. (1976a). Compound action potential (AP) tuning curves. J Acoust Soc Am 59: 591-597.
81. Dallos P, Cheatham MA. (1976b). Production of cochlear potentials by inner and outer hair cells. J Acoust Soc Am. 60: 510-512.
82. Harrison RV, Aran JM, Erre JP. (1981). AP tuning curves from normal and pathological human and guinea pig cochleas. J Acoust Soc Am. 69: 1374-1385.
83. Miller JD. (1963). Deafening effects of noise on the cat. Acta Oto-Laryngologica. Suppl. 176: 729-764.
84. Liberman MC. (1978). Auditory nerve responses from cats raised in a low-noise chamber. J Acoust Soc Am. 6: 442-455.
85. Mulroy MJ, Curley FJ. (1982). Stereociliary pathology and noise-induced threshold shift: a scanning electron microscopic study. Scan Electron Microsc. (Pt 4): 1753-1762.
86. Hamernik RP, Ahroon WA, Patterson JH Jr, Qiu W. (2002). Relations among early postexposure noise-induced threshold shifts and permanent threshold shifts in the chinchilla. J Acoust Soc Am. 111: 320-326.
87. Müller J, Janssen T. (2008). Impact of occupational noise on pure-tone threshold and distortion product otoacoustic emissions after one workday. Hear Res. 246: 9-22.
88. Shupak A, Tal D, Sharoni Z, Oren M, Ravid A, et al. (2007). Otoacoustic emission in early noise-induced hearing loss. Otol & Neurotol. 28: 745-752.
89. Ohlmiller KK. (2012). Current issues in noise-induced hearing loss. In: "Translational perspectives in auditory neuroscience: Special topics". pp 1-34. Plural Publishing: San Diego, CA.
90. Sininger YS. (1992). Establishing Clinical Norms for Auditory Brainstem Response. Am J Audiol. 1: 16-18.
91. Scheuren J. (2014). ISO 532 – Living and working with alternative loudness standards, in Proceedings of InterNoise, Melbourne, Australia.
92. DIN 45631: Berechnung des Lautstärkepegels und der Lautheit aus dem Geräuschspektrum – Verfahren nack Zwicker (Calculation of loudness level and loudness from the sound spectrum – Zwicker method), Berlin, Germany, (1991).

93. ISO. International Organization for Standardization, ISO 532:1975. (2012). Acoustics – Method for Calculating Loudness Level Stage 90.92, Geneva, Switzerland.
94. Sottek R. (2014). Improvements in calculating the loudness of time varying sounds. Inter Noise 2014.
95. ANSI. American National Standard. (2007). Procedure for the Computation of Loudness of Steady Sounds, ANSI Standard S3.4-2007, Acoustical Society of America, Melville, NY.
96. Moore BC. (2015). Differences between ANSI S3.4-2007 and the proposed ISO532-2. J Acoust Soc Am 138, 1889.
97. Currie RA, Dodds L. (2017). The benefits and challenges of opening toxicology control data. Toxicol Res (Camb). 6: 578-579.
98. Rybak LP, Talaska AE, Schacht J. (2008). Drug-Induced Hearing Loss. In: J Schacht, AN Popper & RR Fay (eds). Auditory trauma, protection, and repair. pp. 219-256. New York, NY: Springer.
99. Stebbins WC, McGiinn CS, Angela M, Feitosa G, Moody DB, et al. (1981). Animal models in the study of ototoxic hearing loss. In: S.A. Lerner, G.J. Matz, & J.E. Hawkins, Jr. (eds). Aminoglycoside Ototoxicity. pp. 5 -26. Boston, MA: Little, Brown and Company.
100. Iurato S, Henderson D, Quaranta A, Salvi RJ. (1999). Introduction. In: "Ototoxicity: Basic Science and Clinical Applications". Ann NY Acad Sci, 884: ix-xi.