Distortion product otoacoustic emissions: towards reliable and valid early identification and monitoring of hearing in adults receiving ototoxic medication

by

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List of Abbreviations

ANOVA	analysis of variance
dB	decibel
DPOAEs	distortion product otoacoustic emissions
Expt	experiment
F	female
GM	geometric mean
HL	hearing level
ICC	intraclass correlation coefficient
Kruskal-Wallis H	Kruskal-Wallis one-way analysis of variance
L	left
M	male
MDD	minimal detectable difference
MDR-TB	multidrug-resistant tuberculosis
MMA	mixed model analysis
n	number
NA	not applicable
NH	normal hearing
OAE	otoacoustic emission
OHCs	outer hair cells
R	right
RM ANOVA	repeated measures analysis of variance
ROC	receiver operator characteristic
SD	standard deviation
SEM	standard error of measurement
SNHL	sensorineural hearing loss
SNR	signal-to-noise ratio
SPL	sound pressure level
TB	tuberculosis
UHF	ultra-high frequency
UCT	University of Cape Town

Publications as outputs from this research project

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s):

(1) **Petersen, L.**, Wilson, W., & Kathard, H. (2018). Towards the preferred stimulus parameters for DPOAEs in adults: A preliminary study. *South African Journal of Communication Disorders, 65 (1)* https://doi.org/10.4102/sajcd.v65i1.585

(2) **Petersen, L.,** Wilson, W.J., & Kathard, H. (2017). A systematic review of stimulus parameters for eliciting distortion product otoacoustic emissions from adult humans. *International Journal of Audiology,* http://dx.doi.org.ezproxy.uct.ac.za/10.1080/14992027.2017.1290282

Signed: 

Abstract

Background: Multidrug-resistant tuberculosis (MDR-TB) patients receive aminoglycosides as part of their treatment. These drugs are ototoxic, and can cause permanent damage to the cochlea, resulting in a debilitating hearing loss, which has a negative impact on an individual's quality of life. Early detection and management of an ototoxic hearing loss can minimise the impact of the hearing loss on the person's social, emotional and vocational wellbeing. While patients with MDR-TB are often very ill, it might be ideal to use an objective test that does not require active participation from the patient. In this way, the reliability and validity of the test will not be affected by the patient's state. Distortion product otoacoustic emissions (DPOAEs) at $2f_1$-f_2 are a viable option, as it evaluates cochlear function, specifically the outer hair cells, which are affected first by ototoxic medication.

Method: This thesis used a sequential study design aimed to determine the DPOAE stimulus parameters that yield (a) the highest level and the most reliable, sensitive and specific DPOAEs reported in the literature, (b) the highest level and the most reliable DPOAEs in healthy, normally hearing adults, and (c) the most sensitive and specific DPOAEs in participants with MDR-TB patients receiving ototoxic medication. High frequency pure tone audiometry (defined in this thesis as frequencies > 8 kHz) was used as the gold standard. Descriptive statistics, the intraclass correlation coefficient, Pearson's correlation coefficient and mixed model analyses were used to analyse the data.

Results: *Systematic review:* The results of the systematic review indicated an L_1/L_2 setting of 75/75 dB SPL and f_2/f_1 value from 1.20 to 1.22 yielded the highest level DPOAEs. The systematic review results for stimulus parameters that yielded the highest test-retest reliability, sensitivity and specificity were inconclusive. *Preliminary study with healthy normal-hearing participants:* The results of the preliminary study in healthy, normal-hearing participants indicated that the highest levels of DPOAEs were elicited with L_1/L_2 intensity levels of 65/65 and 65/55 dB SPL, and f_2/f_1 ratios of 1.18, 1.20 and 1.22, as determined by mixed model analyses ($p < 0.05$). These same stimulus parameters yielded the most reliable DPOAEs in both ears, as determined by intraclass correlation coefficient analysis. *Main study with healthy, normal-hearing participants:* Descriptive statistics and mixed model analysis showed stimulus intensity levels L_1/L_2 of 65/55 dB SPL, and f_2/f_1 ratios of 1.18 and 1.20, elicited the

largest DPOAEs. The ratio of 1.20 yielded the largest DPOAEs < 5000 Hz and f_2/f_1 ratio of 1.18 the largest DPOAEs ≥ 5000 Hz. The second highest DPOAE levels were elicit by L_1/L_2 = 65/65 dB SPL and f_2/f_1 = 1.18. The test-retest reliability in this sample was not influenced by changing the stimulus parameters, and DPOAEs were only unreliable at an f_2 frequency of 8 000 Hz. *Study in participants with MDR-TB:* Results in participants with MDR-TB receiving ototoxic medication indicated that the highest levels of DPOAEs were elicited with L_1/L_2 = 65/55 and an f_2/f_1 ratio of 1.18 at f_2 ≥ 5000 Hz, followed by 65/65 and 1.18. For f_2 < 5000 Hz, stimulus intensities of L_1/L_2 = 65/55 and an f_2/f_1 ratio of 1.20 yielded the largest DPOAE levels. Relating to sensitivity and specificity, the stimulus parameter combination of 65/55 dB and 1.18 detected the highest number of ears with outer hair cell damage in participants with MDR-TB receiving ototoxic medication.

Conclusion: It should be considered to use an f_2/f_1 ratio of 1.18 for f_2 ≥ 5000 Hz and 1.20 for f_2 < 5000 Hz when monitoring for ototoxicity, to assist with early identification of outer hair cell damage, in conjunction with high frequency pure tone audiometry. This finding needs to be confirmed in a larger sample of participants with MDR-TB receiving ototoxic medication.

Keywords: distortion product otoacoustic emissions, reliability, validity, sensitivity, specificity, multidrug-resistant tuberculosis

CHAPTER 1: INTRODUCTION

Multidrug-resistant tuberculosis (MDR-TB) is a communicable disease caused by bacteria that are resistant to at least two standard tuberculosis treatment drugs (World Health Organization, 2018a). The World Health Organization has declared MDR-TB a global public health crisis; it predominantly affects citizens of developing countries (World Health Organization, 2019a). South Africa, along with other high burden countries, like India, China and Russia, carry the largest share of the global burden of MDR-TB (World Health Organization, 2018a).

Treatment for MDR-TB is more complex, lengthy and expensive than for drug-susceptible tuberculosis (World Health Organization, 2019b). The intensive phase of treatment for MDR-TB often includes aminoglycoside antibiotics like kanamycin, amikacin or streptomycin, or a polypeptide antibiotic like capreomycin. These antibiotics are ototoxic and are known to cause permanent damage to the cochlea (Sagwa, Souverein, Ribeiro, Leufkens, & Mantel-Teeuwisse, 2017). These drugs mostly affect the outer hair cells in the cochlea, with damage starting at the basal end and spreading to the apical portion with prolonged exposure (Schellack & Naude, 2013). This outer hair cell (and other cellular) loss can lead to permanent hearing loss, which can affect an individual's ability to communicate and overall quality of life (Arnold et al., 2017; Harris et al., 2012). To minimise these effects, early detection and management of outer hair cell (OHC) damage due to ototoxic medication is pertinent (AAA, 2009).

The current gold standard for ototoxicity monitoring is a combination of standard pure tone audiometry at frequencies from 250 to 8000 Hz and high frequency pure tone audiometry at frequencies ≥ 8 000 Hz (AAA, 2009). Pure tone audiometry is a behavioural test that relies on the active participation of the person being tested. Patients with life-threatening illnesses, like MDR-TB, for example, might be too ill to actively participate in pure tone testing, especially at the beginning stages of treatment (Fausti et al., 1999). As a result, pure tone testing in this population might not be as reliable as desired.

Distortion product otoacoustic emissions (DPOAEs) is an objective test that does not require active participation from the individual being tested (Dhar & Hall, 2018). DPOAEs are low-level signals produced by the outer hair cells of the cochlea as by-products of travelling waves along

the basilar membrane caused by the presentation of specific acoustic stimuli to the ear (Kemp, 1998). DPOAEs provide a non-invasive manner of investigating the outer hair cells of the cochlea, the structures that are most affected by ototoxic medication like kanamycin and amikacin (Reavis et al., 2008).

DPOAEs are elicited by two pure tones with frequencies f_1 and f_2 (where f_1 is the lower frequency of the pair) and intensities of L_1 and L_2 (where L_1 is the intensity of the f_1 frequency) (Kemp, 1998). The level of the elicited DPOAE has been found to depend on the combinations of stimulus parameters used to elicit the DPOAE. These parameters include f_1 and f_2 frequencies, f_2/f_1 ratio, L_1 and L_2 intensity levels and L_1/L_2 level separation (Prieve & Fitzgerald, 2015). For example, researchers found the highest L_1/L_2 stimuli elicited the highest level DPOAEs (Beattie & Jones, 1998; Bonfils, Avan, Londero, Trotoux, et al., 1991; Dreisbach & Siegel, 2005; Mills, Feeney, & Gates, 2007). However, studies examining the sensitivity specificity of DPOAEs found moderate stimulus intensities identified auditory disorders better than higher or lower levels of L_1 and L_2 and performed better at separating individuals with normal cochlear outer hair cell function from those with outer hair cell damage (Bonfils & Avan, 1992; Chida, Fukuda, Satoh, Kashiwamukra, et al., 2001; Moulin, Bera & Collet, 1994).

The use of different DPOAE stimulus parameter combinations has also been shown to influence the test-retest reliability of DPOAEs (Beattie, Kenworthy, & Luna, 2003; Moulin, 2000a). The interaction between the stimulus parameters can also influence the DPOAE level, for example, at moderate stimulus intensities, an L_1/L_2 level separation of 10 to 15 dB yields the optimal DPOAEs. Another example of the interaction includes that, with increased stimulus frequency, the ideal f_2/f_1 ratio decreases.

DPOAEs have been used in clinical settings for auditory screening and diagnostic purposes since the mid-1990s (Dhar & Hall, 2018). While this test holds great promise as an ototoxicity monitoring tool, the preferred stimulus parameters to elicit the highest level DPOAEs in adults are yet to be determined in a systematic way. Additionally, no studies have yet been conducted on the validity of DPOAEs as an early warning of ototoxicity in MDR-TB patients in clinical settings.

When adapting or refining an existing test, like DPOAEs, it is pertinent that the reliability and validity of the test need to be considered. While there are numerous types of reliability and

validity, the current thesis focused on test-retest reliability and concurrent validity. Test-retest reliability refers to whether the test (DPOAEs in this thesis) produces similar or identical results when repeated under the same conditions (Maxwell & Satake, 2006); in other words, whether the test produces minimum variation as a result of chance (Riegelman, 2005). It is important to establish both random and systematic errors associated with the new/adapted test (Streiner & Norman, 2008). Concurrent validity, which is subsumed under criterion validity together with predictive validity, involves comparing the new/adapted test with the current gold standard (Streiner & Norman, 2008). In ototoxicity monitoring, especially high frequency pure tone audiometry is viewed as the current the gold standard (ASHA, 1994).

When building evidence for a diagnostic test, for example DPOAEs, it is important to consider existing literature and evaluate the reliability and validity of the test in a rigorous and systematic manner. Questions about the rigour of studies investigating DPOAEs and the quality of evidence for the stimulus parameters currently used in clinical practice need to be answered.

Overall aim of the thesis

The overall aim of this thesis was to systematically determine the best stimulus parameters for eliciting the highest level and most reliable DPOAEs in young adults, and to systematically investigate the concurrent validity of DPOAEs as an early indication of ototoxicity in adults receiving ototoxic medication as part of their treatment for MDR-TB.

Structure of the thesis

This thesis is structured as a thesis containing publications. It consists of the following chapters:

Chapter 1 introduces the thesis, its main topics and its structure.

Chapter 2 reviews MDR-TB, the drug regimen used to treat it and the pathophysiology of the ototoxic drugs in that regimen, as well as the need to monitor for ototoxicity in patients receiving ototoxic drugs as part of MDR-TB management.

Chapter 3 reviews DPOAEs and their potential role in ototoxicity monitoring.

Chapter 4 presents the overall thesis methodology and discusses the theoretical and philosophical positioning of the thesis.

Chapter 5 presents the problem statement and aims for all the studies included in the thesis.

Chapter 6 presents the systematic review of the stimulus parameters that yield the highest level and most reliable, sensitive and specific DPOAEs in adults. This chapter is in the form of an article published in the *International Journal of Audiology*, with an expanded methods section and the formatting and referencing adapted to match the styles used in the overall thesis.

Chapter 7 presents the preliminary study to determine the set of stimulus parameters yielding higher level and more reliable DPOAEs so that these parameters could be used in the rest of the thesis. This chapter is in the form of an article published in the *South African Journal of Communication Disorders*, with the formatting and referencing adapted to the styles used in the overall thesis.

Chapter 8 presents the main study that used the set of stimulus parameters identified in Chapter 7 to determine the stimulus parameters that elicit the highest level and most reliable DPOAEs in healthy, normally hearing adults. This chapter is in the format of an article to be submitted to the *International Journal of Audiology,* with the formatting and referencing changed to the styles used in the overall thesis.

Chapter 9 contains the study that examined the stimulus parameters that yield the highest level DPOAEs with the highest concurrent validity of DPOAEs when used to monitor for ototoxicity in adults receiving ototoxic medication to treat MDR-TB. This chapter is in the form of an article to be submitted to the *International Journal of Audiology*, with the formatting and referencing adapted to the styles used in the overall thesis.

Chapter 10 discusses the findings of the current thesis, its strengths and limitations, it's clinical and theoretical implications, and its directions for future research.

The 'thesis containing publications' format of this thesis results in some inevitable repetition of content, particularly regarding the introductory chapters of the thesis, the introductions and the methods in the included publications.

CHAPTER 2: MULTIDRUG-RESISTANT TUBERCULOSIS AND ITS CLINICAL MANAGEMENT

The chapter reviews MDR-TB, the drug regimen used to treat it, the pathophysiology of the ototoxic drugs in that regimen, and the need to monitor for ototoxicity in patients receiving ototoxic drugs as part of the MDR-TB management.

Multidrug-resistant tuberculosis

MDR-TB is defined as tuberculosis (TB) that is resistant to isoniazid and rifampicin, with or without resistance to other drugs (World Health Organization, 2018a). These two drugs are two of the most powerful components of the standard anti-tuberculosis regimen (World Health Organization, 2018a), and resistance to isoniazid and rifampicin has serious implications for prognosis as well as for cost of TB management (Department of Health, 1999). MDR-TB is a man-made problem, mainly as a result of poor TB management (Department of Health) and drug-resistant strains of the bacteria Mycobacterium tuberculosis have evolved in a variety of ways (Mukherjee et al., 2004).

In 2013, the World Health Organisation has declared MDR-TB a public health crisis (Ismail et al., 2018). South Africa is one of the countries with the highest burden of MDR-TB, alongside Russia, China and India (World Health Organization, 2019a). These countries have a disproportionately high prevalence of MDR-TB, when compared to global prevalence (Ismail et al., 2018). In South Africa, for example, the prevalence of MDR-TB is 2.1% (95% CI 1.5-2.7) among new TB cases and 4.6% (3.2-6.0) among retreatment cases (Ismail et al., 2018). TB forms part of South Africa's quadruple burden of disease and has been exacerbated by the high incidence and prevalence of the human immunodeficiency virus (HIV) (Pillay-van Wyk et al., 2016; World Health Organization, 2019a). The presence of HIV makes the individual vulnerable for TB infection and enables the progression of latent TB infection to active disease (Fätkenheuer, Taelman, Lepage, Schwenk, & Wenzel, 1999). With an increased probability of TB reinfection, the chances of developing MDR-TB is greater (Herselman, 2002).

A compounding factor is that TB mainly affects the economically active age group in South Africa, where 86.6% of the TB patients reported in 1999 were in the age group between 20 to 59 years (Kironde, 2000). It can be postulated that patients with MDR-TB will be similarly

affected. This phenomenon has obvious economic implications because people with MDR-TB cannot work while ill and is, therefore, not contributing to the economy of their household and country for that period of time.

Treating MDR-TB

Due to growing bacterial resistance to standard anti-tuberculosis drugs, medical treatment for MDR-TB must increasingly rely on drugs that are more expensive and toxic, in comparison to the standard TB treatment regimen (World Health Organization, 2019b). Currently the World Health Organization recommends the inclusion of the aminoglycosides amikacin and streptomycin in the shortened MDR-TB treatment regimen or when there is intolerability or toxicity of one of the Group A[1] or Group B[2] drugs (World Health Organization, 2019b). Until 2018, the aminoglycoside kanamycin, as well as the polypeptide antibiotic capreomycin, were also included in the MDR-TB treatment regimen (World Health Organization, 2018b). These drugs are known to have negative side effects, especially ototoxicity[3] (Arnold et al., 2017; Sturdy et al., 2011). Despite these negative side effects, aminoglycosides continue to be used to treat MDR-TB because of their effectiveness against the bacteria that cause TB and they are cheaper to produce relative to other antibiotics with less or no ototoxicity, especially in developing countries (Arnold et al., 2017; Bennett, 1996; Rybak & Ramkumar, 2007). In South Africa the national department of health adheres to the WHO treatment recommendations for MDR-TB and most treatment has been decentralised (National Department of Health, 2019). This management of MDR-TB is similar to that in other resource-constrained countries like India and Russia (Ministry of Health and Family Welfare, 2017; Yunusbaeva et al., 2019).

Ototoxicity of treatments for MDR-TB

Aminoglycosides and capreomycin cause irreversible damage to the human inner ear (Duggal & Sarkar, 2007; Reavis et al., 2011) by damaging cells in the cochlea and/or the vestibular system through mainly free radical formation (Arnold et al., 2017; Bennett, 1996; Schellack & Naude, 2013). These antibiotics enter the cochlear or vestibular fluids via the bloodstream

[1] Group A MDR-TB drugs include levofloxacin/moxifloxacin, bedaquiline, linezolid

[2] Group B MDR-TB drugs include clofazimine, cycloserine/terizidone

[3] Ototoxicity in this thesis refers to cochleotoxicity, i.e., cochlear damage due to medication

and result in intracellular biochemical and morphological changes of the cochlear outer hair cells (OHCs) or the vestibular system (Arnold et al., 2017; Barclay & Begg, 1994). Aminoglycosides and polypeptide antibiotics damage the inner ear by reactive oxygen metabolite (ROM) formation when they bind with iron (Seidman, Quirk, & Shirwany, 1999). This drug-iron complex then forms an oxidative compound that contributes to the formation of free radicals (Schacht, 1999). These free radicals cause permanent damage to the sensory cells and neurons in the inner ear due to oxidative activities with proteins and other targets (Selimoglu, 2007).

Aminglycosides' initial ototoxic effects occur in the OHCs and supporting cells at the basal portion of the cochlea, where high frequency sounds are initially processed (Henley, Weatherly, Martin, & Lonsbury-Martin, 1996). Since OHCs at the base have larger transduction currents and the larger single channel conductance than at the apical end of the cochlea, aminoglycoside entry is easier at the base (O'Sullivan et al., 2017). Prolonged use of aminoglycosides sees this damage progress toward the apical portion of the cochlea where low frequency sounds are transduced (Rybak & Ramkumar, 2007). Thus, ototoxic damage typically results in a bilateral, symmetrical high frequency sensorineural hearing loss initially and spreads to the lower frequencies with prolonged aminoglycoside treatment (Fausti, Henry, et al., 1992; Sha & Schacht, 1997). If treatment with these drugs is continued, the damage progresses from the OHCs to the inner hair cells, supporting cells and then to more central neural structures, e.g. the spiral ganglion cells (Selimoglu, 2007). This damage is typically irreversible in humans (Probst, Harris, & Hauser, 1993) and the progression of hearing loss is independent of the disease treated with aminoglycosides, as can be seen in studies conducted with participants with cystic fibrosis, MDR-TB and non-tuberculosis mycobacterium (Al-Malky, Suri, Dawson, Sirimanna, & Kemp, 2011; Fausti, Henry, et al., 1992; Harris et al., 2012; Peloquin et al., 2004). It is believed that capreomycin has a similar pathophysiology as aminoglycosides (Arnold et al., 2017).

As stated earlier, the OHCs in the cochlea which are responsible for analysing high frequencies, are damaged first when exposed to aminoglycosides or polypeptide antibiotics (Arnold et al., 2017; Rybak & Ramkumar, 2007). This damage to hair cells reduces the cochlea's ability to analyse the frequencies of incoming sounds and reduces its sensory response to low and moderate intensity sounds (Bess & Humes, 1995). These ototoxic effects in the cochlea might

remain undetected and might not immediately result in a noticeable hearing loss (Fausti et al, 1984). This problem can be exacerbated by the half-life of aminoglycosides in the inner ear being up to six months or longer after treatment has stopped (Rybak & Ramkumar, 2007)

Aminoglycosides and polypeptides show different patterns of ototoxicity, as well as variable severity and incidence rates for ototoxicity (Castillo & Roland, 2007; Selimoglu, 2007). For example, streptomycin has been found to primarily damage the vestibular system, with only a minor cochleotoxic effect (Bennett, 1996). The reported incidence of ototoxicity due to streptomycin varies and is generally below 20% (Sha & Schacht, 1997).

Kanamycin, on the other hand, is predominantly cochleotoxic (ASHA, 1994; Petersen & Rogers, 2015). Amikacin, which is a derivative of kanamycin, has the same pathophysiology as kanamycin, as do capreomycin (polypeptide antibiotic). The reported incidence of cochleotoxicity due to these antibiotics vary from 22% to 82% (Arnold et al., 2017; Fausti et al., 1984; Ghafari et al., 2019; Harris et al., 2012; Moore, Smith, & Lietman, 1984; Ramma & Ibekwe, 2012).

Research conducted on cochleotoxicity in participants with MDR-TB receiving ototoxic medication revealed similar variations in prevalence and incidence figures. A retrospective folder review (*n* = 61) by De Jager and Van Altena (2002) found 18% hearing loss in MDR-TB patients (10 to 83 years, mean age = 36 years) receiving either kanamycin (*n* = 45), streptomycin (*n* = 5), amikacin (*n* = 2) or a combination of two or all three drugs. The presence/absence of an ototoxic hearing loss was determined through serial audiograms obtained with conventional pure tone testing. Ramma and Ibekwe (2012) conducted a cross-sectional study on 49 participants (18 to 60 years, mean age = 33 years) with MDR-TB and extensively drug resistant TB (XDR-TB) receiving kanamycin, amikacin or capreomycin. They found hearing loss in 47% of their sample with conventional pure tone audiometry. Harris et al. (2012) found that 58% of their 153 participants (14 to 70 years, median age = 36 years) with MDR-TB developed hearing loss in their longitudinal study where participants were tested with conventional pure tone audiometry once a month for three months. These participants received either kanamycin (*n* = 145), streptomycin (*n* = 5) or capreomycin (*n* = 1). Ghafari et al. (2019) found 82% of their 102 participants with MDR-TB developed hearing loss due to treatment with kanamycin. In this study, pure tone testing was done up to 16 kHz which could

explain the higher incidence of hearing loss compared to De Jager and Van Altena (2002), Harris et al. (2012) and Ramma and Ibekwe (2012).

From this point onwards, the term 'ototoxicity' will be used to refer to cochleotoxicity only, for ease of reference. The varying ototoxic incidence figures for aminoglycosides and polypeptide antibiotics can be ascribed to the different levels of ototoxicity of these drugs, as well as a lack of standardised ototoxicity monitoring (Bennett, 1996). The degree of ototoxicity of individual drugs cannot be fully predicted due to inter-patient variability, even when all the risk factors for a given patient are known (Campbell & Durrant, 1993). This highlights the monitoring of hearing as being an important part of the management of patients receiving ototoxic medication (AAA, 2009; ASHA, 1994).

Importance of early detection of ototoxicity

Ototoxicity monitoring aims to achieve both early identification of and early intervention for ototoxicity (AAA, 2009). Early detection of OHC damage due to ototoxicity, i.e., before the patient notices a hearing loss, can help prevent further permanent damage to hair cells in the cochlea responsible for hearing sounds in the frequency range important for communication (Konrad-Martin et al., 2005). Hearing loss is known to negatively impact quality of life and result in impaired communication, social interaction and reduced vocational options (Cohen, Labadie, Dietrich, & Haynes, 2004; Ruben, 2000). MDR-TB affects mainly the economically active age group in SA, as mentioned earlier (Kironde, 2000). In addition to the economic effect of the illness, permanent hearing loss will compound the negative effect on quality of life in this population.

Furthermore, early detection and management of ototoxicity can guide appropriate medical management (Konrad-Martin et al., 2005). For patients with MDR-TB, the audiologist will inform the treating physician of any deterioration of hearing, resulting in either a change of treatment to less ototoxic medication, a reduction in the dosage or reducing the frequency of drug administration (Department of Health, 2019).

However, hearing conservation is not always possible in people with MDR-TB, for instance when cessation of ototoxic medication could be life threatening or when resources are unavailable to change to a more expensive, but less ototoxic drug (AAA, 2009). In these

instances, a sensitive test of OHC damage will allow more time for counselling and management to prepare the individual for an eventual hearing loss than a test that detects the damage after the person has started noticing changes in hearing (Fausti et al., 2007). Early and timely counselling prior to hearing loss being noticeable might assist the patient and their significant others in the acceptance process and prepare the patient with strategies to maximise communication (Fausti et al., 2007).

Monitoring for ototoxicity during MDR-TB treatment

Ototoxicity monitoring during treatment with aminoglycosides and polypeptides is crucial, as the likelihood for developing ototoxic hearing loss cannot be accurately predicted (Arnold et al., 2017; Selimoglu, 2007). Due to inter-patient variability, even if all risk factors for an individual are known, the occurrence and degree of ototoxic hearing loss cannot be fully predicted as is possible with aminoglycoside-induced nephrotoxicity (Campbell & Durrant, 1993). Therefore, careful monitoring of OHC function and hearing status is important during and after aminoglycoside and polypeptide treatment to either maximise hearing conservation and/or minimise the negative impact of hearing loss (Arnold et al., 2017; ASHA, 1994; Selimoglu, 2007).

For early detection of ototoxicity, a method that is both sensitive and specific to OHC damage is needed. Self-report of hearing loss is a quick and inexpensive method. However, this method lacks sensitivity and relying on self-report can cause a delay in detecting ototoxic hearing loss, as demonstrated by Ramma and Ibekwe (2012). They found a prevalence of ototoxic hearing loss in 47% of their participants with MDR- or XDR-TB when using conventional pure tone audiometry in contrast to 25% with self-report (Ramma & Ibekwe, 2012). Thus, the only way to guarantee that ototoxic damage is identified is to monitor the OHC function of all individuals receiving aminoglycoside treatment.

The current gold standard for ototoxicity monitoring is high frequency pure tone audiometry (Schellack & Naude, 2013). It is a behavioural test that relies on the patient to respond to pure tone stimuli to indicate that they have heard the sounds. To obtain thresholds, the patient must indicate the softest sounds that they heard. This test requires concentration on the part of the participant in order to yield reliable results. Often patients with MDR-TB, especially in the initial phase of treatment, are seriously ill, and cannot actively participate in this

behavioural test (Fausti et al., 1999). As a result, the test outcome can be unreliable, and not provide a valid picture of the person's hearing ability or hearing loss.

In order to reduce the amount of time needed for high frequency pure tone audiometry, Fausti et al. (1999) developed a sensitive range for ototoxicity (SRO), which provides a shorter alternative to high frequency pure tone testing. The SRO would require less concentration from a patient than the conventional way of pure tone testing. However, it still relies on the active participation of the patient, which might not be feasible for a patient with MDR-TB.

In this population, an objective test would be more ideal, in other words a test that does not require the patient's active participation in order to detect the ototoxic damage as early as possible. A reliable and valid "picture" of the patient's OHC function will enable early management that can minimise the ototoxic damage, and thus the impact on the person's quality of life. A possible test that could meet these requirements would be distortion product otoacoustic emissions.

Summary

MDR-TB has reached epidemic proportions in developing countries like South Africa, Russia and India. Part of the treatment regimen for MDR-TB includes antibiotics that are ototoxic and cause permanent damage to the inner ear. Due to inter-patient variability, it is currently not possible to accurately predict who will develop ototoxic hearing loss. As MDR-TB predominantly affects people in the economically active age group, it is important for early identification and management to monitor the hearing and OHC function of patients with MDR-TB receiving ototoxic medication, in order to maximise their productivity and quality of life. As most patients with MDR-TB are very ill, especially in the initial stages of treatment, they might be unable to provide reliable responses on a behavioural hearing test. Thus, an objective test like distortion product otoacoustic emissions might be better suited for this population.

CHAPTER 3: DISTORTION PRODUCT OTOACOUSTIC EMISSIONS

For early identification of outer hair cell (OHC) damage in patients with MDR-TB receiving ototoxic medication an objective test may be a more viable option than high frequency pure tone audiometry, which relies on active participation. This viable alternative could be distortion product otoacoustic emissions (DPOAEs). This chapter reviews distortion product otoacoustic emissions, the generating mechanism(s), stimulus parameter influence on the DPOAE performance and their potential role in ototoxicity monitoring for patients receiving ototoxic drugs as part of the MDR-TB management.

Distortion product otoacoustic emissions

Distortion product otoacoustic emissions are low-level acoustic signals emitted by the organ of Corti (the hearing organ) in the cochlea of the inner ear in response to tonal stimulation (Dhar & Hall, 2018). To elicit DPOAEs, two speakers are placed in the external ear canal to simultaneously present two pure tones, referred to as the primaries, into the ear canal. These primaries are labelled f_1 and f_2, where $f_2 > f_1$, with intensity levels L_1 and L_2. To record DPOAEs, a microphone is placed in the ear canal alongside the speakers used to present the primaries. This test can be conducted in a quiet room and does not require a soundproof or sound-treated environment. Test time is approximately one minute per ear (in co-operative subjects) and the specific information obtained by DPOAEs about auditory function cannot be obtained by any other means in such a non-invasive manner (Berlin, 1998).

It is well known that otoacoustic emissions are by-products of normal outer hair cell function (Prieve & Fitzgerald, 2015). These emissions are generated by the nonlinear motion of the basilar membrane in the cochlea and travel through the middle ear to where they are measured in the external ear canal (Allen & Fahey, 1993). The presence of DPOAEs is taken to indicate normal- or near-normal mechanical function of the cochlear OHCs (Brown, Sheppard, & Russell, 1994). It is also taken to indicate and can only be measured reliably with normal middle ear functioning as both stimuli and the response must pass through the middle ear for a DPOAE to be recorded (Keefe & Abdala, 2007).

It is also speculated that the cochlear amplifier also plays a role in otoacoustic emission (OAE) generation (Vento, Durrant, Sabo, & Boston, 2004). The healthy cochlea demonstrates non-

linear behaviour and sophisticated frequency specificity at low stimulus intensity levels (Prieve & Fitzgerald, 2015). The non-linear characteristics of the cochlea are believed to result from the active biological mechanisms of the inner ear, also known as the cochlear amplifier. In addition, non-linear activity is a consequence of the sensitivity and frequency selectivity seen in the healthy human cochlea (Allen & Fahey, 1993; Brown, Gaskill, Carlyon, & Williams, 1993). It is hypothesised that the cochlear amplifier contributes additional energy that boosts the basilar membrane vibration at the peak of the travelling wave, particularly at low stimulus intensity levels. Evidence exists that the OHCs contribute to this process (Prieve & Fitzgerald, 2015; Shera & Guinan Jr, 1999). Researchers have noted decreased auditory sensitivity, broader tuning curves and abnormal response growth when OHCs are damaged or missing (Hoth, Gudmundsdottir, & Plinkert, 2010; Prieve & Fitzgerald, 2015).

DPOAEs were discovered by David Kemp in 1978 with the DPOAE obtained at a frequency of $2f_1 - f_2$ seeing the most clinical use since the early 1990s when commercial DPOAE equipment became widely available (Dhar & Hall, 2018). DPOAEs continue to be frequently used for screening and diagnostic purposes to identify and diagnose OHC dysfunction. The most commonly used DPOAE presentation and analysis in the clinical setting is the "DPgram", which is an audiogram-like plot of the DPOAE level most commonly as a function of f_2, one of the stimulus frequencies (Lonsbury-Martin & Martin, 2002). Another type of DPOAE presentation and analysis is the DPOAE input/output function (DPOAE I/O function), also known as the DPOAE growth function (Dorn et al., 2001). With this type of DPOAE, the stimulus frequencies are kept constant while the stimulus intensities are varied and the DPOAE I/O functions are obtained for a set of discrete frequencies (Lonsbury-Martin & Martin, 2002). As a result, more information is obtained regarding cochlear function, specifically outer hair cell status; however, due to uncertainties regarding the underpinning DPOAE mechanisms and intersubject variability, the DPOAE I/O function is not routinely used in the clinic (Dorn et al., 2001; Neely, Johnson, Kopun, Dierking, & Gorga, 2009). In addition, the procedure to obtain DPOAE I/O functions is longer than for the DPgram (Dhar & Hall, 2018). According to Dhar and Hall (2018) the DPgram is currently a more efficient way of obtaining frequency-specific information about OHC function than the DPOAE I/O function.

DPOAE parameters

Determining which recording and stimulus parameters to use to elicit optimal DPOAEs from adult humans can be challenging, in no small part due to the vast number of parameters available to the user. The DPOAE level, its reliability and validity, and therefore their success/failure as a measure of auditory function, was shown to depend primarily on system calibration (Siegel, 2002) and the stimulus parameters used to elicit these emissions (Dhar & Hall, 2018). For stimulus parameters, these include (but are not limited to) the frequencies (f_1 and f_2), levels (L_1 and L_2), and durations of the two primary tones and the ratio of the frequencies (f_2/f_1). With regard to recording parameters, these include (but are not limited to) which distortion product is measured ($2f_1$-f_2, $2f_2$-f_1, etc.), the frequency against which the DPOAE is reported (e.g., f_1, f_2, the geometric mean of f_1 and f_2, the frequency of the distortion product, etc.), how the level of the DPOAE is reported (absolute level or signal-to-noise ratio [SNR]), how many averages are sampled per data point, the method of artefact rejection, and the stopping criteria. All of these have been investigated to determine the suggested parameters for eliciting DPOAEs under clinical conditions (Abdala, 1996; Lonsbury-Martin & Martin, 2002; Stover, Gorga, Neely, & Montoya, 1996; Whitehead, Stagner, McCoy, Lonsbury-Martin, & Martin, 1995).

An important consideration when choosing the stimulus and recording parameters for DPOAE testing is the feature of the emissions the user wishes to optimise (Abdala, 1996; Dhar & Hall, 2018; Lonsbury-Martin & Martin, 2002; Siegel, 2002; Stover et al., 1996; Whitehead, Stagner, et al., 1995). Possibly the simplest feature in this regard is the absolute DPOAE level. This can be misleading, however, with higher absolute DPOAE levels potentially resulting from higher levels of acoustic and/or physiological noise. A second feature of DPOAEs that could be optimised is the SNR. While this option could mitigate the problem of larger absolute DPOAE levels resulting from higher noise floors, it has the disadvantages of potentially passing a low-level DPOAE based on a strong SNR and of needing to agree on how noise levels will be determined. A third feature of DPOAEs that could be optimised is reliability. A final feature of DPOAEs that could be optimised is sensitivity and specificity to a particular disorder of interest.

The distance between the stimulus frequencies f_1 and f_2, called the f_2/f_1 ratio, needs to be close enough to cause two traveling waves on the basilar membrane to overlap, in order to

create a DPOAE (Allen & Fahey, 1993; Dhar & Hall, 2018). Early studies showed that DPOAEs with the highest level (and therefore most easily detected) were obtained in adults and neonates when the frequency separation of the stimuli, known as the f_2/f_1 ratio, was approximately 1.22 (Abdala, 1996; Gaskill & Brown, 1990; Harris et al., 1989). Following these results, an f_2/f_1 ratio of 1.2 to 1.23 is now used as the default in most clinical settings (Dhar & Hall, 2012).

However, later studies found that the ideal f_2/f_1 ratio decreases with increasing stimulus frequency. A study by Moulin, Jourdain, and Collet (1999) found the ideal frequency separation to be 1.25 for DPOAEs elicited near 500 Hz and 1.186 for those elicited near 5 000 Hz in adults. Similarly, Dreisbach and Siegel (2001) found that the DPOAE levels at stimulus frequencies ≥ 10 kHz were 5 to 10 dB higher when an f_2/f_1 ratio of approximately 1.15 was used in comparison to a ratio of 1.2. This phenomenon could indicate the presence of sharper mechanical tuning in the high frequencies (Dreisbach & Siegel, 2001). The change in the optimal frequency ratio with different stimulus frequencies provides evidence for the tonotopic organisation of the basilar membrane in the cochlea, and relates to critical band theory (Loven, 2009). Critical band theory, driven by the seminal research of Fletcher (1940), states that frequency information is processed in discrete units along the length of the basilar membrane in a different manner at the base versus the apex of the cochlea and is the basis for frequency tuning and selectivity (Loven, 2009).

With regard to f_2/f_1 ratios, the larger DPOAEs elicited by f_2/f_1 ratios of 1.18, 1.20 or 1.22 could reflect the cochlea's bandpass filter properties/function (Allen & Fahey, 1993) and give an indication of this organ's frequency selectivity (Brown et al., 1993). This assumption is generally consistent with Gaskill and Brown (1990) and Harris et al. (1989) who found DPOAE levels reached a maximum at f_2/f_1 ratios of 1.22 and 1.25 respectively, and a decline with higher or lower f_2/f_1 ratios. The DPOAE magnitude is systematically reduced if the ratio is increased or decreased from 1.22 (Abdala, 1996). Stover, Neely, and Gorga (1999) suggested the reduction of DPOAE level at higher f_2/f_1 ratios could be the effect of greater separation of the stimulus frequencies that reduces the interaction of their travelling waves on the basilar membrane, while the declines at lower f_2/f_1 ratios could result from less separation of the stimulus frequencies and greater cancellation of their travelling waves on the basilar membrane.

According to Dhar and Hall (2018) the effective stimulus intensities to elicit DPOAEs range between 40 to 70 dB SPL. As the stimulus intensity is increased, a growth in the DPOAE magnitude is seen, and at ± 70 dB SPL the DPOAE level generally reaches a plateau (Dhar & Hall, 2018). When stimulus intensities of ≥ 70 dB SPL are used, it becomes difficult to determine the source of the DPOAE, and it is believed that instrumentation and other artifacts could result in OAEs that originate from passive sources rather than active OHC function (Carter, Williams, & Seeto, 2015; Dorn et al., 2001).

Apart from absolute stimulus intensity levels, the effective L_1/L_2 separation was also found to play a role in the DPOAE magnitude. Some studies have found that a 10–15 dB level separation, with $L_1 > L_2$, produced the largest DPOAE levels for adults and term neonates (Abdala, 1996; Stover et al., 1996). To investigate the effect of stimulus intensity on the ability of DPOAEs between 500 to 8000 Hz to distinguish between children and adults with normal hearing versus those with hearing impairment, Stover et al. (1996) compared various L_1/L_2 intensities by varying L_2 from 65 dB SPL to 10 dB SPL while maintaining L_2 10 dB below L_1. Intensity levels of $L_1 = 65$ and $L_2 = 55$ dB SPL proved to be the most effective for differentiating those with hearing loss from those without (Stover et al., 1996). Similarly, Gorga, Nelson, Davis, Dorn, and Neely (2000) reported the fewest diagnostic errors (i.e. false-positive and false-negative rates) in adults with and without hearing loss when moderate L_1 intensities (such as 65 or 55 dB SPL) were used to elicit the DPOAEs, thereby establishing concurrent validity of this test against conventional pure tone audiometry.

In addition to the effects of frequency ratio and stimulus intensity, synergistic and antagonistic effects of stimulus parameters also influence the DPOAE magnitude (Harris et al., 1989; Whitehead, Stagner, et al., 1995). Harris et al. (1989) found that the f_2/f_1 ratio yielding the highest level DPOAEs decreased when the stimulus intensity was decreased. With regard to stimulus intensity levels, Whitehead, Stagner, et al. (1995) found at high stimulus intensities, the level separation of $L_1 = L_2$ yielded the highest DPOAE levels, whereas at lower intensity levels, the level separation of $L_1 > L_2$ elicited the highest DPOAE magnitudes (Whitehead, Stagner, et al., 1995). In addition, Johnson, Neely, Garner, and Gorga (2006) found that as the frequency ratio was decreased, the highest DPOAE levels were observed when the stimulus intensity levels were closer to each other (L_1 was closer to L_2).

DPOAE test-retest reliability

Apart from investigating the DPOAE stimulus parameters, researchers also examined the test-retest reliability of DPOAEs elicited using these parameters (Beattie, Caldwell, & Kenworthy, 2005; Beattie et al., 2003; Keppler et al., 2010; Ng & McPherson, 2005; Wagner, Heppelmann, Vonthein, & Zenner, 2008; Zhao & Stephens, 1999), albeit to a lesser extent than the research on the influence of stimulus parameters on DPOAE levels. Keppler et al. (2010) showed intraclass correlation coefficients (ICC) for DPOAE amplitudes of 0.89–0.96 ($p<0.001$) for tests conducted seven days apart, indicating very good test-retest reliability for stimulus intensity levels of 75/70- and 65/55 dB SPL and an f_2/f_1 ratio of 1.22. Similar studies have shown standard errors of measurement (SEM) for DPOAEs ranging from 0.67–4.8 (Beattie et al., 2005; Beattie et al., 2003; Ng & McPherson, 2005; Wagner et al., 2008), with the minimum detectable difference for changes in DPOAEs considered a real change reported by Keppler et al. (2010) to range from 0.88–2.81 dB.

DPOAE sensitivity and specificity for outer hair cell damage

Similar to reliability, very little research has been conducted into DPOAE stimulus parameters and DPOAE sensitivity and specificity to auditory disorders, especially with studies manipulating the stimulus parameters. Bonfils and Avan (1992) manipulated equi-level stimulus intensities from 42 to 72 dB SPL, in 10 dB steps, and used an f_2/f_1 ratio of 1.23. In 25 normal-hearing ears and 50 ears with hearing impairment, they found the best stimulus intensity combinations to detect sensorineural hearing loss to be L_1/L_2 = 62/62 and 52/52 dB SPL. Similarly, Chida, Fukuda, Satoh, Kashiwamura, et al. (2001) found the stimulus intensity levels of 60/60 and 60/50 dB SPL to identify OHC damage better than L_1/L_2 = 70/70 dB SPL with a fixed frequency ratio of 1.22 in 80 ears with known sensorineural hearing loss. These studies examined the stimulus intensity parameters only with fixed stimulus frequency ratios. Thus, none of these aforementioned studies manipulated the stimulus intensities and f_2/f_1 ratios simultaneously to examine the synergistic effect of these changed stimulus parameters on the DPOAE sensitivity and specificity to outer hair cell damage. The current thesis aimed to address this gap in the literature by manipulating intensity and frequency ratio simultaneously to determine the effect on DPOAE level, reliability, sensitivity and specificity.

Apart from reliability and validity, the timing of identifying OHC damage was also examined. After monitoring the hearing of TB patients undergoing aminoglycoside therapy in South Africa for eight weeks, Petersen (2005) found that DPOAEs elicited using L1/L2 = 65/55 dB SPL and an f_2/f_1 ratio of 1.22 identified hearing loss at least two weeks prior to conventional pure tone audiometry (PTA). This finding was consistent with similar results observed in DPOAEs using similar parameters in other patient groups undergoing ototoxic cisplatin therapy for cancer (Knight et al., 2007; Stavroulaki et al., 2001) or being exposed to harmful levels of noise (Edwards et al., 2010). Based on these results, DPOAEs are considered to be more sensitive than conventional PTA to the early signs of hearing loss.

DPOAEs versus high frequency pure tone audiometry for detecting outer hair cell damage

The sensitivity of DPOAEs to OHC damage has also been compared to high frequency pure tone audiometry, part of the current gold standard for ototoxicity monitoring. The current literature on the sensitivity of DPOAEs are contradictory. For example, Al-Malky et al. (2011) found a 100% sensitivity for DPOAEs to detect abnormal OHC function in their 8 participants with cystic fibrosis (CF). In contrast, Vasconcelos, Frota, Ruffino-Netto, and Kritski (2018) found 0% sensitivity for DPOAEs detecting ototoxic damage in relation to hfPTA in their 10 patients with MDR-TB receiving amikacin. The differences for sensitivity values in Al-Malky et al. (2011) and Vasconcelos et al. (2018) can be explained by the different criteria used to determine the presence of ototoxic damage. Al-Malky et al. (2011) used statistical differences between DPOAEs of participants with CF receiving aminoglycosides vs. CF participants with no aminoglycoside exposure. On the other hand, Vasconcelos et al. (2018) used DPOAE level reductions of 4 dB or more at two or more adjacent frequencies as an indicator of ototoxic damage. Reavis et al. (2008) compared DPOAEs from 0.8 to 8 kHz, using a stimulus intensity pair of 65/59 dB SPL and an f_2/f_1 ratio of 1.2, to pure tone thresholds from 0.5 to 20 kHz in 53 patients receiving either (1) cisplatin or carboplatin or (2) ototoxic antibiotics for more than three days. They found that high frequency audiometry identified OHC damage prior to DPOAEs in a third of their participants. In another third of their sample, DPOAEs identified OHC damage first, Lastly, both tests (DPOAEs and hfPTA) identified damage at the same time in a third of participants. In all instances either DPOAEs, hfPTA or both identified OHC damage

prior to conventional pure tone audiometry. Although Reavis et al. (2008) used the same DPOAE change criterion of 4 dB as Vasconcelos et al. (2018) to determine ototoxic damage, their sample size of 53 participants was much larger than that of Vasconcelos et al. (2018), which could explain the difference in sensitivity values. All these studies used a fixed L_1/L_2 and f_2/f_1 ratio and did not systematically vary the stimulus parameters to determine whether other combinations might yield different results.

According to Dreisbach and Siegel (2005), the use of optimal combinations of stimulus intensities and f_2/f_1 ratios would elicit larger DPOAEs and, therefore, more effective test protocols for identification and diagnosis of cochlear OHC function. Thus, it is imperative to systematically vary DPOAE stimulus parameters to determine whether the DPOAE level, test-retest reliability and validity can be enhanced for ototoxicity monitoring purposes.

DPOAEs versus other types of OAEs for detecting outer hair cell damage

Spontaneous OAEs (SOAEs) are low level tonal emissions that occur in the absence of an acoustic stimulus and are recorded in the ear canal (Dhar & Hall, 2018; Tubis & Talmadge, 1998). These emissions are typically present between 0.8 to 4 kHz (Bilger, Matthies, Hammel, & Demorest, 1990; van Dijk, Wit, Tubis, Talmadge, & Long, 1994). Prevalence reports of SOAEs in children and adults vary from 0-85% with the highest occurrence seen in neonates and the lowest in individuals older than 70 years (Bonfils, 1989; Burns, Arehart, & Campbell, 1992; Kuroda, 2007; Morlet et al., 1995; Strickland, Burns, & Tubis, 1985).

It is thought that SOAEs are generated within the cochlea and reflect cochlear amplifier activity (Keilson, Khanna, Ulfendahl, & Teich, 1993). There is also some suggestion that these emissions originate from minor cochlear structural irregularities that are not significant enough to adversely affect hearing thresholds (Bright, 2002). Research has shown that damage to the cochlea through e.g., ototoxins and age-related deterioration can adversely affect SOAEs (Bonfils, 1989; Kuroda, 2007; Long & Tubis, 1988). However, present SOAEs can also signal OHC damage (Penner, 1996; Ruggero, Rich, & Freyman, 1983).

Since SOAEs can be present in healthy and damaged cochleas it would not be a sensitive or specific test for ototoxicity monitoring. In addition, SOAEs seem to appear at discrete and

unpredictable frequencies and thus, would not be the preferred OAE type for monitoring OHC function.

Apart from SOAEs, OAEs can also be elicited by introducing a stimulus into the ear. Transient evoked OAEs (TEOAEs) are typically elicited with click stimuli in commercially available equipment (Lonsbury-Martin & Martin, 2001). With this broadband stimulus, the base of the cochlea is stimulated first and the apical portion later (Dhar & Hall, 2018). Thus, activation of the basal area produces a response seen in the early part of the analysis time. Stimulus energy persists in the ear for two or three milliseconds after stimulus presentation. At the same time, high frequency TEOAEs are present in the ear canal. In order to separate the eliciting stimulus from the OAE, specialised equipment is needed (Goodman, Fitzpatrick, Ellison, Jesteadt, & Keefe, 2009). In commercial equipment, recording of acoustic activity is started about two milliseconds after stimulus presentation (Goodman et al., 2009). Thus, high frequency TEOAE activity that reaches the ear canal prior to the recording window is not captured (Dhar & Hall, 2018; Goodman et al., 2009). As a result, the upper limit for TEOAE measurement with commercial equipment is approximately 4000 to 5000 Hz (Goodman et al., 2009; Kemp, 2002; Probst, Lonsbury-Martin, & Martin, 1991).

In addition to the measurement techniques used, the upper frequency limit of the TEOAE is often restricted by the frequency bandwidth of the earphones used to deliver the click stimulus. The transducers commonly used in commercial TEOAE equipment lack an energy transfer function with a sufficiently flat magnitude to extend into the high frequencies (Goodman et al., 2009). As a result, the click energy at high frequencies ≥ 5 kHz may be less than that at lower frequencies (Goodman et al., 2009). This lack of transducer transfer function contributes to the difficulty of detecting high frequency TEOAEs.

With ototoxic damage the highest frequencies are typically affected first. Results in groups of patients (Sisto et al., 2013; Stavroulaki, Apostolopoulos, Segas, Tsakanikos, & Adamopoulos, 2001) and case studies (Lonsbury-Martin & Martin, 2001) indicate that DPOAE sensitivity to OHC damage due to ototoxicity was higher compared to TEOAEs elicited by clicks. In addition, DPOAEs would also seem preferable to TEOAEs as an ototoxicity monitoring tool, due to the DPOAEs' extended dynamic range regarding hearing loss and measurement over a broader frequency range when compared to TEOAEs (Stavroulaki et al., 2001).

Another type of evoked OAE, the stimulus frequency otoacoustic emission (SFOAE), is elicited with low-level single pure tones (Abdala & Kalluri, 2017). These OAEs appear at the same frequency as the stimulus frequency (Kemp, 2002; Rasetshwane, Bosen, Kopun, & Neely, 2019). Therefore, specialised signal presentation and analysis techniques are needed to separate the SFOAE from the stimulus (Kemp, 2002), which are not readily available in commercial equipment. Thus, SFOAEs would not be the ideal tool for ototoxicity monitoring, either.

DPOAEs in patients exposed to ototoxic agents

Research in patients with MDR-TB receiving ototoxic medication yielded conflicting evidence regarding DPOAEs' ability to detect OHC dysfunction compared to standard pure tone audiometry, i.e. ≤ 8 kHz and/or high frequency audiometry. Appana, Joseph, and Paken (2016) found, in a longitudinal study including 52 adult participants with MDR-TB (15-56 years; mean age 34 years) receiving kanamycin, that DPOAEs detected ototoxic OHC damage sooner than standard or high frequency pure tone audiometry up to 12 kHz. A study by Vasconcelos et al. (2018), however, found no significant differences in DPOAEs in their longitudinal study in 10 patients (mean age 49 years; 18-69 years; 7 men, 3 women;) with MDR-TB receiving amikacin, whilst both standard and high frequency thresholds decreased significantly. Both studies used DPOAE stimulus parameters fixed at L_1/L_2 = 65/55 dB SPL and f_2/f_1 ratio = 1.22. A possible reason for the discrepant results could be the criteria used for DPOAEs to detect ototoxic damage: Appana et al. (2016) determined the presence of OHC damage with the presence or absence of DPOAEs, whereas Vasconcelos et al. (2018) used a decline of 4 dB or more at two adjacent DPOAE frequencies as an indication of OHC damage.

In contrast to DPOAE research in patients with MDR-TB, the general consensus in the literature on ototoxicity in patients with cystic fibrosis, cancer and chemical solvent exposure is that DPOAEs detect OHC dysfunction sooner than conventional pure tone audiometry but at the same time or later than hfPTA (Al-Malky et al., 2011; Govender, Govender, & Matthews, 2013; Knight, Kraemer, Winter, & Neuwelt, 2007; Reavis et al., 2008; Sisto et al., 2013). These studies all employed fixed DPOAE stimulus parameters of L_1/L_2 = 65/55 dB SPL and an f_2/f_1 ratio = 1.22, except for Reavis et al. (2008) who used L_1/L_2 = 65/59 dB SPL and an f_2/f_1 ratio = 1.2.

While DPOAEs in the general ototoxicity literature detect OHC damage after hfPTA, this test could potentially not be a viable option for very ill individuals like patients with MDR-TB as they might be unable to yield reliable and valid pure tone thresholds (Fausti et al., 1999). As DPOAEs do not rely on the active participation of the person being tested, it would be prudent to attempt to maximise the sensitivity of this test for ototoxicity to detect OHC damage as soon as possible.

In addition, while it is known that DPOAEs can detect outer hair cell damage sooner than conventional audiometry, it is worthwhile exploring whether DPOAEs can detect this damage earlier with stimulus parameters that are different to the ones currently used in clinics. Although DPOAEs can detect outer hair cell damage prior to sfPTA, it is known that outer hair cell damage can still occur up to six months after treatment with ototoxic medication was stopped. Thus, early identification of outer hair cell damage could assist in preventing permanent damage that is noticeable by the individual. In turn, preventing or minimising permanent outer hair cell damage could maximise quality of life (Konrad-Martin et al., 2005).

For ototoxicity monitoring it would be ideal to test high frequency DPOAEs to detect outer hair cell damage even earlier than emissions up to 8 kHz. Unfortunately, high frequency DPOAEs currently cannot be acquired reliably and validly with commercial equipment. In the absence of commercially available high frequency DPOAE equipment it would be prudent to determine optimal stimulus parameters for early detection of high frequency damage caused by ototoxic medication like aminoglycosides for MDR-TB treatment. By doing this, stimulus parameter sets can be customised for this population, rather than using a standard set of parameters (e.g., L_1/L_2 = 65/55 dB SPL and an f_2/f_1 ratio = 1.22) used for general diagnostic purposes.

Summary

DPOAEs provide a quick, non-invasive manner to investigate cochlear outer hair cell function, especially OHCs. Various stimulus parameters affect the DPOAE level, the test-retest reliability and validity of DPOAEs, namely the stimulus frequencies, f_2/f_1 ratios, the stimulus intensities, and the stimulus intensity separation. To optimise DPOAEs as an ototoxicity monitoring tool, the stimulus parameters need to be investigated in a systematic manner in normal-hearing, healthy participants as well as patients with MDR-TB receiving ototoxic medication.

CHAPTER 4: PROBLEM STATEMENT AND AIMS

This chapter offers the problem statement and aims for the thesis.

Problem statement

Early detection of outer hair cell (OHC) damage due to ototoxicity, i.e., before the patient notices a hearing loss, can help prevent further permanent damage to hair cells in the cochlea responsible for hearing sounds in the frequency range important for communication (Konrad-Martin et al., 2005). Currently high frequency pure tone audiometry (hfPTA) is viewed as the gold standard for ototoxicity monitoring (ASHA, 1994). As most patients with MDR-TB are very ill, especially in the initial stages of treatment, they might be unable to provide reliable responses on a behavioural hearing test. Thus, an objective test like distortion product otoacoustic emissions might be better suited for this population.

DPOAEs provide a quick, non-invasive manner to investigate cochlear outer hair cell function, especially OHCs. It is already known that DPOAEs are more sensitive than standard frequency pure tone audiometry (sfPTA) (Knight et al., 2007; Stavroulaki et al., 2002). Various stimulus parameters affect the DPOAE level, the test-retest reliability and validity of DPOAEs, namely the stimulus frequencies, f_2/f_1 ratios, the stimulus intensities, and the stimulus intensity separation. The DPOAE level, its reliability and validity, and therefore their success/failure as a measure of auditory function, was shown to depend partially on the stimulus parameters used to elicit these emissions ((Dhar & Hall, 2018; Dreisbach & Siegel, 2005). The DPOAE stimulus parameters currently used clinically have been derived from research with small sample sizes and limited/no inferential statistics. To optimise DPOAEs as an ototoxicity monitoring tool, the stimulus parameters need to be investigated in a systematic manner in normal-hearing, healthy participants as well as patients with MDR-TB receiving ototoxic medication, which this current thesis aimed to do.

With the systematic manipulation of stimulus parameters, namely the stimulus intensity and $f_1–f_2$ ratio, the performance of the $2f_1–f_2$ DPOAE in terms of level, test-retest reliability and validity DPOAE can be improved for ototoxicity monitoring for MDR-TB patients receiving ototoxic medication as part of their treatment.

Aims

The overall aim of this thesis was to determine the best stimulus parameters for eliciting the highest level and most reliable DPOAEs in young adults, and to investigate the concurrent validity of DPOAEs elicited using those parameters as an early indication of ototoxicity in adults receiving ototoxic medication as part of their treatment for MDR-TB. To achieve this overall aim, the research completed for this thesis proceeded in four phases.

Phase 1 – a systematic review of the literature

The first phase of this thesis involved a systematic review of the scientific literature on DPOAEs. It aimed to determine which combinations of stimulus parameters would be more likely to elicit DPOAEs at $2f_1–f_2$ from human adults in the standard frequency range of 0.5 to 8 kHz that were higher in:

- absolute level
- test-retest reliability
- sensitivity and specificity to cochlear lesions.

These combinations would then be the targets of the next phase of the thesis.

Phase 2 – a preliminary study of DPOAE stimulus parameters in normally hearing young adults

The second phase of the thesis involved a preliminary study of the DPOAE stimulus parameters combinations identified in phase 1. It aimed to determine which of these combinations elicited DPOAEs at $2f_1–f_2$ from a small sample of normally hearing human adults in the standard frequency range of 0.5 to 8 kHz where those DPOAEs were higher in absolute level and test-retest reliability. Short-term test-retest reliability was determined in this sample, that is participants were tested on two occasions, 24 to 48 hours apart. These stimulus parameter combinations would then be the targets of the next two phases of the thesis.

Phase 3 – a study of DPOAE stimulus parameters in normally hearing young adults

The third phase of the thesis involved a study of the DPOAE stimulus parameter combinations identified in phase 2. It aimed to determine which of these combinations elicited DPOAEs at $2f_1–f_2$ from a large sample of normally hearing human adults in the standard frequency range

of 0.5 to 8 kHz where those DPOAEs were higher in absolute level and test-retest reliability. Medium-term test-retest reliability was determined in this sample, that is participants were test on two occasions, 10 to 14 days apart.

Phase 4 – a preliminary study of the concurrent validity of DPOAEs as an early indication of ototoxic damage in adults receiving ototoxic medication as part of their treatment for MDR-TB

The fourth and final phase of the thesis involved a preliminary study of the concurrent validity of DPOAEs as an early indication of ototoxicity in adults receiving ototoxic medication as part of their treatment for MDR-TB. It aimed to determine this concurrent validity on a small sample of these adults who had undergone DPOAE and standard- and high-frequency pure tone audiometry during their treatment. Participants were tested on two occasions, 10 to 14 days apart

CHAPTER 5: METHODOLOGY

Chapter 5 follows the presentation of the problem statement and aims of the thesis in Chapter 4 by describing the methodology used for the thesis. The procedural details of the studies in the four phases will be discussed in the subsequent chapters, namely Chapters 6 to 9.

A positivist research paradigm

This thesis adopted a positivist research paradigm. This paradigm assumes that a universal truth exists (Mukherji & Albon, 2014) and research adopting positivism accepts that it should function within agreed practices and norms. An investigation employing a positivist paradigm includes observation/measurement and recording of events or phenomena in a systematic way and strives to investigate, confirm and predict law-like relationships or patterns of behaviour (Mukherji & Albon, 2014). Positivism predominantly underpins a quantitative methodological approach (Taylor & Medina, 2011).

Quantitative studies

This thesis used a series of quantitative studies to achieve its aims. In quantitative research, formalised tests and measuring instruments are used to determine the characteristics of data in numerical terms in a precise and objective manner (Maxwell & Satake, 2006). Thus, quantitative methodology aims to quantify, measure or determine the nature or extent of a problem or phenomenon. Qualitative methodology, on the other hand, is typically more concerned with describing experiences, investigating the nature of an issue and accentuating meaning (Coolican, 2017). The goal of quantitative research is predominantly to prove that the hypothesis being evaluated is either true or false (Maxwell & Satake, 2006). With a quantitative methodology adopting a positivist framework, a type of temporary reality is created “by seeking and finding empirical evidence for hypotheses, knowing that their ultimate proof will always remain questionable to some degree” (Maxwell & Satake, 2006, p.384).

Statistical testing

With the quantitative methodology, statistical tests are applied to infer the probability for finding similar between- or within-group differences in a comparable population of people studied or evaluated under similar conditions or circumstances (Maxwell & Satake, 2006). This statistical analysis can help determine whether an occurrence took place by chance. A quantitative methodology strives to maximise validity of the research process through careful sampling, suitable instrumentation and suitable statistical treatment of data. Reliability, using quantitative methods, involves choosing measures that demonstrate replicability and consistency over time, over measurement tools and over groups of participants (Mukherji & Albon, 2014).

Appropriateness of the chosen methodology

It is well known that the positivist paradigm has its disadvantages and it is pertinent to acknowledge that research conducted within this framework cannot provide all the answers. Questions about morality or lived experiences, for example, will be difficult, if not impossible, to address within this framework, and would be best answered through qualitative research.

However, the chosen paradigm and methodology were deemed appropriate for the purpose of this thesis, as the diagnostic test being evaluated is DPOAEs, which provides numeric data. In addition, the gold standard employed in this thesis, namely pure tone audiometry, also yields numerical values. Therefore, a standard quantitative methodology was used in this thesis.

The thesis is concerned with determining the stimulus parameters that yield the highest level, and most reliable and valid DPOAEs for use in ototoxicity monitoring in adults. With a quantitative methodology, the study can be replicated by others because the variables, and data collection methods are controlled (Mukherji & Albon, 2014). Additionally, by applying controlled procedures and quantifiable variables, the obtained results can assist in refining existing theory.

According to Brown (2014) the findings from a single study offer the most basic form of research evidence. A single study can contribute to the evidence but is strengthened when combined with the results of more soundly conducted studies. These studies then form the

building blocks to build a reliable vase of clinical knowledge regarding a specific phenomenon (see Figure 5.1). Findings from many methodologically sound studies are required to build a reliable and valid base of clinical knowledge regarding a phenomenon or problem. Confirming a finding from a number of studies ensures that a knowledge claim did not merely happen by chance as a result of studying certain patients, the research methods or the research setting of that one particular study. If a finding is confirmed through the results of numerous different studies clinicians can be confident in that knowledge because it was replicated through diverse research methods, data collection settings and participants.

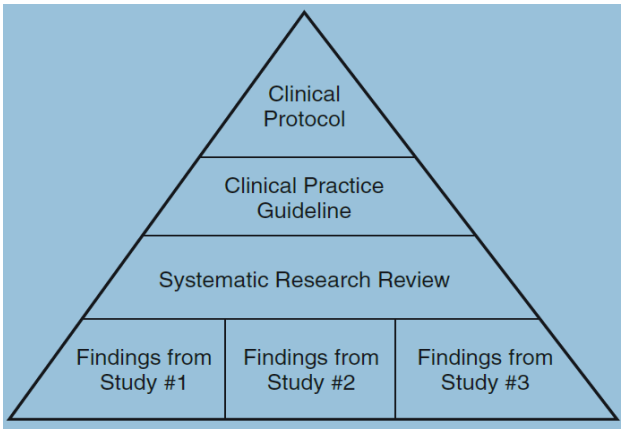

Figure 5.1 Building knowledge for practice. Source: Brown (2014)

The current thesis offers applied research with a clinical research question. A sequential research design was adopted. A sequential study design provided the elements to build the evidence step-wise (Hong, Pluye, Bujold, & Wassef, 2017), starting with a systematic review of the available literature, then a preliminary study to determine and eliminate unnecessary stimulus parameters, then confirm the optimal stimulus parameters in a larger sample of healthy, normal-hearing adults, and lastly, a preliminary study with MDR-TB participants receiving ototoxic medication. With the sequential study design, this thesis was able to answer the research question in different ways, i.e., with a systematic review, normal-hearing population and a patient population, namely a sample of participants with MDR-TB receiving ototoxic medication. By doing this, the results were triangulated by various studies with the same aims and objectives, within a short timeframe.

Validity and reliability

With validity, the concern is about whether a test measures what it says it measures (Maxwell & Satake, 2006). According to Messick (1989), construct validity is the overarching meaning of measures to which all types of validity add (see Figure 5.2). However, it is important to first obtain evidence that the test is measuring a phenomenon in a reliable fashion (Streiner & Norman, 2008). Establishing reliability is the first step in providing evidence of the value of a test, by demonstrating that measurements of a phenomenon on different occasions yield similar results (Streiner & Norman, 2008). Thereafter, face and content validity need to be considered, which are assessed subjectively (Dellinger & Leech, 2007; Streiner & Norman, 2008). More important though, is criterion validity, which is determined objectively (Streiner & Norman, 2008). Criterion validity, which subsumes concurrent and predictive validity, involves comparing the new/adapted test with the gold standard. This thesis focussed on test-retest reliability and concurrent validity of DPOAEs.

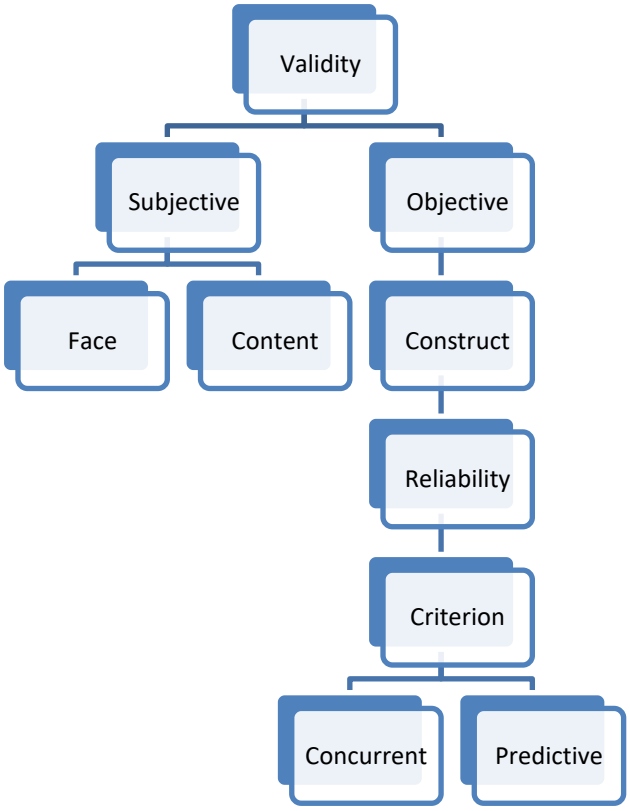

Figure 5.2 Validity framework adapted from Streiner and Norman (2008) and Dellinger and Leech (2007)

Choice of statistical tests to assess reliability

The choice of statistical tests is important, as it allows the researcher to translate data into information, that is to interpret and understand data and to relay findings in a digestible way (especially in the case of clinical questions or applied research, as the information needs to be understandable to the end user – the clinician). Inferential statistics provide the researcher with a set of tools to draw conclusions that are based on objective and repeatable mathematical procedures rather than on an interpretation of the acquired data that is subjective (Max & Onghena, 1999). However, if the statistical test is inappropriate for the intended purpose, the conclusions drawn will be invalid.

To determine test-retest reliability of a measure, many researchers would argue that the intraclass correlation coefficient (ICC) for absolute agreement is the appropriate statistical test to use. Test-retest reliability "reflects the variation in measurements taken by an instrument on the same subject under the same conditions" (Koo & Li, 2016, p. 155). The ICC examines the relationship among variables that share both their metric and variance, i.e. the variables are of a common class (McGraw & Wong, 1996). Thus, the ICC for absolute agreement would be appropriate to examine correlations between repeated measures by the same test/method (Bland & Altman, 1990).

Pearson's correlation coefficient is used to examine the correlation between measurements from two different methods/tests, that is they share neither metrics nor variance, e.g. comparing DPOAEs and pure tone air conduction thresholds (Bland & Altman, 1990; McGraw & Wong, 1996). Seeing that two different methods of measurement are used, a clear ordering of the variables (i.e. the two variables are the two methods) exist (Bland & Altman, 1990).

When examining data obtained through repeated measures, researchers are often faced by missing values and small sample sizes due to attrition. With traditional statistical approaches like analysis of variance (ANOVA), it can be difficult to deal with missing data, as the whole case gets deleted if a single measurement is missing (Field, 2013). Although there are ways to correct for missing data, these techniques can be complicated. Mixed model analysis provides a more powerful alternative to analyse data from repeated measures. This statistical test does not require complete data sets, so the whole case does not need to be deleted should there be data points missing (Field, 2013).

Summary

This thesis is situated within a positivist research paradigm and employed a quantitative methodology. While the shortcomings of such a paradigm is acknowledged, a positivist paradigm and quantitative methodology were best suited to the aims of this thesis, as the focus of this study was on DPOAEs, which yield numerical data.

CHAPTER 6: SYSTEMATIC REVIEW OF DPOAE STIMULUS PARAMETERS IN ADULTS

This chapter presents phase 1 of the thesis, in the form of a paper published in the *International Journal of Audiology*, with the methods section expanded to provide a full discussion of the methods used.

Reference: Petersen, L.[4], Wilson, W.J., & Kathard, H. (2017). A systematic review of stimulus parameters for eliciting distortion product otoacoustic emissions from adult humans. *International Journal of Audiology,* http://dx.doi.org.ezproxy.uct.ac.za/10.1080/14992027.2017.1290282.

Acronyms and abbreviations:

ANOVA – analysis of variance, dB – decibel, DPOAE – Distortion Product Otoacoustic Emission, Expt – experiment, F – female, GM – geometric mean, ICC – intraclass correlation coefficient, HL – hearing level, Kruskal-Wallis H – Kruskal–Wallis one-way analysis of variance, L – left, M – male, MDD – Minimal detectable difference, n – number, NA – not applicable, NH – normal hearing, R – right, ROC – receiver operator characteristic, RM ANOVA – repeated measures analysis of variance, SD – standard deviation, SEM – standard error of measurement, SPL – sound pressure level, UHF – ultra-high frequency.

Abstract

Objective: To review the scientific literature to determine if a set of stimulus parameters can be described to elicit distortion product otoacoustic emissions (DPOAEs) of higher absolute level and/or greater reliability in healthy adult humans and higher sensitivity and specificity in adults with cochlear lesions. *Design:* Systematic review. *Study Sample:* Searches of four electronic databases yielded 47 studies that had used different parameters to elicit DPOAEs from within or between-groups of adult humans. *Results:* The wide range of stimulus parameters used in the reviewed studies saw a wide range of reported values for DPOAE level, reliability, and sensitivity and specificity to cochlear lesions. *Conclusion:* The most commonly used stimulus parameters for eliciting DPOAEs from adult humans have included frequency

[4] The first author conceptualised the study, collected, analysed, interpreted the data and conceptualised and drafted the journal article. The first author is also the corresponding author.

ratios for the two primary tones (f_2/f_1) of between 1.04 to 1.4 and levels (L_1/L_2) of 65/55 dB SPL. The most commonly used parameters for eliciting DPOAEs of higher level in healthy adults appear to be linked to f_2/f_1 values between 1.20 to 1.22 and L_1/L_2 levels of 75/75 dB SPL. The stimulus parameters for eliciting DPOAEs of greater reliability in healthy adults and higher sensitivity and specificity in adults with cochlear lesions have yet to be clearly determined.

Keywords

Distortion product otoacoustic emissions (DPOAEs), stimulus parameters, systematic review, evidence-based practice, adults.

Introduction

Determining which stimulus and recording parameters to use to elicit distortion product otoacoustic emissions (DPOAEs) from adult humans can be challenging, in no small part due to the large number of parameters available to the user. With regard to stimulus parameters, these include but are not limited to the frequencies (f_1 and f_2) in Hertz, levels (L_1 and L_2) in dB SPL and duration in milliseconds of the two primary tones; the ratio of these frequencies (f_2/f_1); and how the primary tones are calibrated. With regard to recording parameters, these include but are not limited to which distortion product is measured (e.g., the commonly recorded $2f_1$-f_2 or others such as $2f_2$-f_1), against which frequency the distortion product is reported (e.g., against f_1, f_2, the geometric mean of f_1 and f_2, or the frequency of the distortion product), how the level of the DPOAE is reported (e.g., absolute level or signal-to-noise ratio [SNR]), how many averages are sampled per data point, the method of artifact rejection and the stopping criteria.

An important consideration when choosing which stimulus and recording parameters to use to elicit DPOAEs is the feature of the emissions the user wishes to optimise. Perhaps the simplest feature in this regard is the absolute level of the DPOAE. The task here would be to determine the parameters that elicit DPOAEs of the highest absolute level. Such a feature could be misleading, however, with higher absolute DPOAE levels potentially resulting from higher levels of acoustic and/or physiological noise.

A second feature of DPOAEs that could be optimised is the SNR. This changes the task to one of determining the stimulus and recording parameters that elicit DPOAEs of the highest level

relative to the noise floor. While this approach mitigates the problem of larger absolute DPOAE levels resulting from higher noise floors, it has the disadvantage of potentially passing a low-level DPOAE based on a strong SNR. It also raises a technical issue that the methods used to estimate noise levels in DPOAE recordings differ across manufacturers of commercially available DPOAE devices.

A third feature of DPOAEs that could be optimised is reliability. The previous task of optimising DPOAE levels now gives way to one of determining the stimulus and recording parameters that elicit the same DPOAEs when applied in the same way to the same subjects.

A final feature of DPOAEs that could be optimised is sensitivity and specificity. This task would now be to determine the stimulus and recording parameters that elicit DPOAEs with the largest differences in persons with normal hearing versus persons with hearing loss, particularly where those losses are the result of cochlear (outer hair cell) lesions.

Previous attempts to determine which stimulus and recording parameters might optimise one or more features of the DPOAE have encountered at least four significant confounds. The first confound is the high number of parameters open to manipulation and the even higher number of parameter combinations this generates. Assessing every possible combination requires excessively long test times per subject, which can be difficult to achieve even with co-operative adults.

The second confound relates to the effect size on the DPOAE of changing some parameters. This can be seen in reports that changing f_2/f_1 by up to ±0.05 or changing the L_1 minus L_2 level by 5 to 10 dB SPL can result in changes in individual DPOAE levels that fall within the reported range of DPOAE test/retest variability (e.g., Carter et al, 2015; Reavis et al, 2015). These findings challenge the precision with which the effect of changing the DPOAE stimulus and recording parameters can be measured.

The third confound relates to what physiological processes are represented by an elicited DPOAE. Ideally, the parameters used should elicit a DPOAE that most represents the processes of the cochlear amplifier. Previous research, summarised in reviews such as Gorga, Neely, and Dorn (2002), warns of the potential for high level primary tones (greater than 65 to 70 dB SPL) to saturate DPOAEs at some frequencies and/or introduce distortion generated by the DPOAE instrumentation itself. Such possibilities add to suggestions that eliciting DPOAEs of the

highest level may not represent the "optimal" DPOAEs for clinical use. Other research has developed the so-called "scissors paradigm" where the difference between L_1 and L_2 is increased if the stimulus level is decreased. Such changes are thought to elicit DPOAEs more directly related to the f_2 generation site in the cochlea compared to DPOAEs elicited when L_1 equals L_2 (Janssen, Kummer, & Arnold, 1995a, 1995b; Kummer, Janssen, Hulin, & Arnold, 2000; Whitehead, McCoy, Lonsbury-Martin, & Martin, 1995).

The fourth confound relates to the high inter-subject variability seen in DPOAEs. This leads to the high likelihood that there will be no single set of parameters that will evoke an "optimal" DPOAE at all frequencies in all subjects (Londero, Bonfils, & Avan, 2002).

Perhaps as a result of the above confounds, researchers, clinicians and manufacturers have recommended a range of DPOAE stimulus and recording parameters for clinical use often based on their own databases and adult populations. While many of these parameters have been successfully used in a wide range of clinical settings over the past few decades, this use alone is not sufficient evidence to conclude which parameters might elicit "optimal" DPOAEs from adult humans.

Methods

Aims

This study aimed to determine which stimulus parameters should be used to elicit DPOAEs from adult humans. The specific clinical question being asked was what stimulus parameters elicit DPOAEs at $2f_1 - f_2$ from human adults in the frequency range of 2 to 8 kHz where those DPOAEs are of the:

- highest absolute level.
- highest test-retest reliability
- highest sensitivity and specificity to cochlear lesions.

Research design

The current study used a systematic review design. This design allows for a meticulous summary of all available relevant primary research to answer a research question (Clarke,

2011). Along with meta-analysis, this design is regarded as high-level evidence (Cox, 2005) that can influence clinical practice.

Data collection

The PRISMA-P method (Shamseer et al., 2015) was used to identify studies for inclusion in this systematic review. Four databases were included in the literature search for articles published from 1978 to 2016: *Excerpta Medica Database* (EMBASE), *Cumulative Index to Nursing and Allied Health Literature* (CINAHL), *PubMed* and *Scopus*. The term used to search these databases was: distortion product otoacoustic emissions. This search returned 5 589 studies, of which 3 115 were removed as duplicates. The titles of the remaining 2 474 studies were reviewed and a further 1 945 were removed as their titles were deemed irrelevant to the research question. Abstracts for the remaining 529 studies were reviewed to select studies that met the inclusion criteria for the present systematic review. These criteria were that the study must have: 1) investigated DPOAEs in adult (≥ 18 years) human participants, 2) been published in the English language, peer reviewed, scientific literature, and 3) reported on a comparison of $2f_1$-f_2 DPOAE results for absolute level, SNR, reliability and/or sensitivity and specificity to cochlear lesions, where these DPOAE results were obtained using two or more f_2/f_1 and/or two or more L_1/L_2 settings within a single group of healthy adult humans (for DPOAE level and reliability) or between-groups of adult humans with or without sensorineural hearing loss (SNHL) for DPOAE sensitivity and specificity. This identified 142 studies for full review from which 43 studies were selected for final inclusion in the systematic review. Two reviewers screened the articles to be included in the study. Additional relevant studies were also sought from the reference lists of these studies, which identified a further four studies for inclusion (n=47). All studies included in the systematic review were observational and cross-sectional in design. The biases induced by only searching databases with published studies (publication bias) in the English language (language bias) are acknowledged.

Data management

All titles and abstracts were stored in EndNote versions X4 and X5. Data relating to the aims of the study were extracted, and entered onto three Excel spreadsheets, one for each aim.

Results

Table 6.1 describes 33 studies that considered the absolute level of DPOAEs ($2f_1 - f_2$) elicited using different stimulus parameters within a single group of adult humans with normal hearing. Overall, the data presented in Table 6.1 suggest that the stimulus parameters most likely to generate DPOAEs with the highest absolute level are those using an f_2/f_1 setting from 1.20 to 1.22 and an L_1/L_2 setting of 75/75 dB SPL. This conclusion is based on 13 (81.3%) of 16 studies including f_2/f_1 values from 1.20 to 1.22 among their comparisons and reporting them as eliciting the highest DPOAE absolute level, and eight (88.9%) of nine studies including the L_1/L_2 setting of 75/75 dB SPL amongst their comparisons and reporting it as eliciting the highest DPOAE absolute level.

It should be noted that some studies reported some trends for the best f_2/f_1 values and L_1/L_2 settings by f_2 frequency. For example, Dreisbach and Siegel (2001) reported lower f_2/f_1 values as eliciting higher DPOAE absolute levels as f_2 frequency was increased (and vice-versa). Beattie and Jones (1998) reported that at higher L_1/L_2 settings, equilevel stimuli elicited higher DPOAE absolute levels (e.g., L_1/L_2 = 75/75 dB SPL), but at lower L_1/L_2 settings level differences of 5 to 10 dB elicited higher DPOAE absolute levels (e.g., 65/60 or 65/55 dB SPL). Finally, some f_2/f_1 values and L_1/L_2 settings were included in only a few studies, or even a single study, which prevented a reasonable comparison of these parameters to those more commonly used across higher numbers of studies. It was noted that no studies reported DPOAE SNR results in a manner that satisfied the present systematic review's inclusion criteria.

Table 6.1: Studies that considered the level of DPOAEs ($2f_1 - f_2$) elicited using different stimulus parameters in subjects with normal hearing.

ANOVA – analysis of variance, dB – decibel, Expt – experiment, F – female, GM – geometric mean, HL – hearing level, Kruskal-Wallis H – Kruskal–Wallis one-way analysis of variance, L – left, M – male, n – number, NA – not applicable, NH – normal hearing, R – right, RM ANOVA – repeated measures analysis of variance, SPL – sound pressure level, UHF – ultra-high frequency, y – age in years.

Study	Subjects	Stimulus parameters	Findings
1 Abdala (2000). USA	10 subjects (6M, 4F), 24 to 35 y	**f_2:** 1.5, 3, 6 kHz **f_2/f_1 ratio:** 1.14; 1.20; 1.35 **L_1/L_2:** L_1 80 to 30 dB SPL in 5 dB steps. L_2 always 10 dB below L_1	**Best f_2/f_1:** 1.20 **Best L_1/L_2:** ≥60/50 dB SPL No inferential statistics used
2 Abdala (1996). USA	10 subjects (6F, 4M), 23 to 34 y	**f_2:** 1.5, 6 kHz **f_2/f_1 ratio:** 13 different ratios from 1.03 to 1.39 **L_1/L_2:** L_1 65, 60, 55, 50 dB SPL. L_2 fixed at 50 dB SPL	**Best f_2/f_1:** Overall best = 1.203 (dependent on **L_1/L_2**, 1.21 for **f_2** = 1.5 kHz, 1.19 for **f_2** = 6 kHz) **Best L_1/L_2:** 65/50 & 60/50 dB SPL Based on ANOVA
3 Beattie & Ireland (2000). USA	55 ears (55F), 20 to 26 y	**f_1/f_2 GM:** 0.531, 1, 2, 4 kHz **f_2/f_1 ratio:** 1.21 **L_1/L_2:** 55/55, 45/45, 35/35 dB SPL	**Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 55/55 dB SPL No inferential statistics used
4 Beattie & Jones (1998). USA	30 ears (30F), 21 to 30 y	**f_2:** 0.593 to 6.093 kHz (11 test frequencies) **f_2/f_1 ratio:** 1.21 **L_1/L_2:** L_1 75, 65, 55, 45 dB SPL. L_2 +5, 0, -5, -10, -15 dB SPL relative to L_1	**Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 75/75 dB SPL. If L_1=65, best 65/60 and 65/55 dB SPL. If L_1=55 dB SPL, best 55/50, 55/45 & 55/40 dB SPL Based on ANOVA

Study	Subjects	Stimulus parameters	Findings
5 Beattie et al. (2004). USA	50 ears (50F), 19 to 26 y	**f_2:** 1, 2, 4 kHz **f_2/f_1 ratio:** 1.2 **L_1/L_2:** L_1 75 to 40 dB SPL in 5 dB steps. L_2 always 10 dB below L_1	**Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** 75/65, 70/60, 65/55, 60/50 dB SPL No inferential statistics used on DPOAE absolute levels
6 Bian & Chen (2008). USA	8 ears in phase 1, 8 in phase 2, 23 to 40 y	**f_2:** 4 kHz **f_2/f_1 ratio:** 1.2 to 1.8 in 0.1 steps (phase 1); 1.15, 1.185, 1.22, 1.255, 1.29, 1.325, 1.36 (phase 2) **L_1/L_2:** L_1 and L_2 swept independently from 75 to 54 dB SPL in 3 dB steps	**Best f_2/f_1:** 1.22 to 1.25 **Best L_1/L_2:** 75/72 dB SPL No inferential statistics used
7 Bonfils et al. (1991). France	20 ears in expt 1, 18 to 28 y	**f_2: 0.**813 to 1.161 kHz; DPOAE frequency kept constant at 707.5 Hz **f_2/f_1 ratio:** 1.06 to1.38 in 0.2 steps **L_1/L_2:** 30/30 to 80/80 dB SPL in 6 dB steps	**Best f_2/f_1:** 1.22-1.30 **Best L_1/L_2:** 84/84 dB SPL No inferential statistics used
8 Chida et al. (2001). Japan	177 ears (30M, 64F), 6 to 69 y. NH – 97 ears	**f_2:** 1, 2, 4 kHz **f_2/f_1 ratio:** 1.22 **L_1/L_2:** 70/70, 60/60, 60/50 dB SPL	**Best f_2/f_1:** NA (fixed at 1.22) **Best L_1/L_2:** 70/70 dB SPL Based on ANOVA
9 Dhar et al. (1998). USA	40 ears (10M, 10F), 18 to 30 y	**f_2:** 1, 1.5, 2, 3, 4, 5, 6 kHz **f_2/f_1 ratio:** 1.22 **L_1/L_2:** 70/70, 60/60, 50/50, 40/40 dB SPL. Then for each, L_1 increased in 5 dB steps until L_1 - L_2=15 dB SPL,	**Best f_2/f_1:** NA (fixed at 1.22) **Best L_1/L_2:** 75/70 dB SPL Based on ANOVA

Study	Subjects	Stimulus parameters	Findings
		except 85/70 dB SPL, which was replaced with 80/65 dB SPL	
10 Dhar et al. (2005). USA	3 subjects (3 ears)	f_2**:** 1.55 to 1.95, 1.8 to 2.2 & 2.25 to 2.65 kHz in 4 to 8 Hz steps f_2/f_1 **ratio:** 1.053, 1.065, 1.08, 1.11, 1.14, 1.18, 1.22, 1.26, 1.30, 1.32, 1.34, 1.36 L_1/L_2**:** a) 75/75, 65/65, 45/45 dB SPL. b) L_1 65 & L_2 60, 55, 50, 45 dB SPL	**Best** f_2/f_1**:** 1.22 **Best** L_1/L_2**:** not reported Based on ANOVA
11 Dreisbach & Siegel (2001). USA	*Expt 1.* 6 ears (3F, 3M), 22 to 30 y *Expt 2.* 8 ears (3F, 5M), 19 to 30 y	*Expt 1.* f_2**:** 2, 2.5, 3, 4, 5, 6, 8 kHz f_2/f_1 **ratio:** 1.2 L_1/L_2**:** L_1 65 to 30 dB SPL in 3 dB steps. L_2 -15 dB SPL relative to L_1 *Expt 2.* f_2**:** 2, 4, 8 kHz f_2/f_1 **ratio:** 1.11 to 1.33 in steps of 0.02 L_1/L_2**:** not stated	*Expt 1.* **Best** f_2/f_1**:** NA (fixed at 1.2) **Best** L_1/L_2**:** 70/60 dB SPL *Expt 2.* **Best** f_2/f_1**:** 1.23 for 2 kHz, 1.198 for 4 kHz, 1.17 for 8 kHz **Best** L_1/L_2**:** not stated No inferential statistics used
12 Dreisbach & Siegel (2005). USA	8 subjects (5F, 3M), 22 to 32 y	f_2**:** 2, 5 kHz f_2/f_1 **ratio:** 1.2 L_1/L_2**:** L_1 70, 60, 50, 40, 30 dB SPL and L_2 varied for each L_1 from 70 to 30 dB SPL in 3 dB steps. L_1 varied for each L_2 from 70 to 30 dB SPL in 3 dB steps & L_2 70, 60, 50, 40, 30 dB SPL	**Best** f_2/f_1**:** NA (fixed at 1.2) **Best** L_1/L_2**:** 70/70 dB SPL No inferential statistics used
13 Gaskill & Brown (1990). UK	3 expts, 34 ears in total (19F, 15M),	3 expts f_1**:** variable from 0.5 to 8 kHz	**Best** f_2/f_1**:** 1.225

Study	Subjects	Stimulus parameters	Findings
	15 to 50 y. N varies within expts	**f_2/f_1 ratio:** variable from 1.075 to 1.375 **L_1/L_2:** L_1 variable from 30 to 70 dB SPL with variable **L_1/L_2** levels	**Best L_1/L_2:** Levels <60 dB SPL with $L_1 > L_2$ by 15 dB SPL No inferential statistics used on DPOAE absolute levels. Samples for individual experiments as low as n=4. Samples for individual results as low as n=1
14 Harris et al. (1989). USA	5 subjects (4M, 1F), 10 ears, 21 to 27 y	**DPOAE *f*:** 1, 2.5, 4 kHz **f_2/f_1 ratio:** 0.2 steps from 1.01 to 1.79 (1 kHz), 1.01 to 1.59 (2.5 kHz), 1.01 to 1.41 (4 kHz) **L_1/L_2:** 85/85, 75/75, 65/65 dB SPL	**Best f_2/f_1:** 1.2 **Best L_1/L_2:** 85/85 dB SPL for 1 kHz, 65/65 dB SPL for 2.5 & 4 kHz No inferential statistics used
15 Hauser & Probst (1991). Switzerland	10 subjects (5M, 5F), 20 ears, 22 to 32 y	**GM:** 1, 2, 4 kHz **f_2/f_1 ratio:** 1.25 (1 kHz), 1.23 (2 kHz), 1.21 (4 kHz) **L_1/L_2:** L_1 75 or 65 dB SPL. L_2 varied in 5 dB steps from 20 to 90 dB SPL	**Best f_2/f_1:** NA (fixed at 1.25 for 1 kHz, 1.23 for 2 kHz, 1.21 for 4 kHz) **Best L_1/L_2:** 75/65 & 65/55 dB SPL at 1 & 2 kHz, 75/75 & 65/60 dB SPL at 4 kHz No inferential statistics used
16 Johnson et al. (2006). USA	20 subjects, gender & age not stated	**f_2:** 1, 2, 4, 8 kHz **f_2/f_1 ratio:** 1.05 to 1.4 in steps of 0.05 **L_1/L_2:** L_1 from 43 to 76 dB SPL. L_2 ranged from 17 to 69 dB SPL	**Best f_2/f_1:** 1.2 when L_2<40 dB SPL, >1.2 when L_2>40 dB SPL **Best L_1/L_2:** absolute best not stated No inferential statistics used
17 Kummer et al. (2000). Germany	22 ears (12F, 10M), 19 to 35 y	**f_2:** 0.977, 1.456, 1.953, 2.979, 3.955, 5.597, 7.959 kHz **f_2/f_1 ratio:** 1.2 **L_1/L_2:** L_1 70 to 30 dB SPL in 5 dB steps. L_2 65 to 5 dB SPL in 5 dB steps (giving 61 **L_1/L_2** combinations)	**Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** $L_1 = 0.4\ L_2 + 41$ dB SPL Based on linear regression

Study	Subjects	Stimulus parameters	Findings
18 Lasky (1998). USA	*Expt 2:* 6 subjects, 21.8±1.7 y *Expt 3:* 6 subjects, 22.4±1.6 y	*Expt 2.* **f_2:** 2, 4, 8 kHz **f_2/f_1 ratio:** 1.2 **L_1/L_2:** L_1 80 to 25 dB SPL in 5 dB steps. L_2 0, -10 & -15 dB SPL relative to L_1 *Expt 3.* **f_2:** 1, 2, 4, 8 kHz **f_2/f_1 ratio:** 1.1, 1.2, 1.3 **L_1/L_2:** L_1 65 to 40 dB SPL in 5 dB steps. L_2 65 to 40 dB SPL in 5 dB steps	*Expt 2.* **Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** For higher L_1 values, L_1 should be equal to L_2 *Expt 3.* **Best f_2/f_1:** 1.2 **Best L_1/L_2:** 65/60 dB SPL No inferential statistics used
19 Londero et al. (2002). France	11 ears with NH, 20 to 39 y	**f_2:** 2, 3, 4, 5, 6, 7, 8 kHz **f_2/f_1 ratio:** 1.05 to 1.70 in steps of about 0.02 **L_1/L_2:** 70/70, 60/60 dB SPL	**Best f_2/f_1:** 1.16 to 1.24 **Best L_1/L_2:** 70/70 dB SPL No inferential statistics used
20 Lonsbury-Martin et al. (1990). USA	44 ears (10F, 12M), 21 to 30 y	*Expt 1.* **GM:** 1 to 8 kHz in 100 Hz steps **f_2/f_1 ratio:** 1.21 **L_1/L_2:** 85/85, 75/75, 65/65 dB SPL *Expt 2.* **GM:** 1, 1.2, 1.5, 2, 2.3, 2.8, 3.5, 4.3, 5.3, 6.5, 8 kHz **f_2/f_1 ratio:** 1.21 **L_1/L_2:** 85/85 to 25/25 dB SPL in 5 dB steps	*Expt 1.* **Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 85/85 dB SPL *Expt 2.* **Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 85/85 & 75/75 dB SPL for GM >1.5 kHz, 85/85 dB SPL for GM ≤1.5 kHz No inferential statistics used
21 Marcrum et al. (2016). Germany	57 ears (30 subjects), 21 to 33 y	*Expt 2.* **f_2:** 1, 2, 3, 4, 6 kHz **f_2/f_1 ratio:** 1.22 **L_1/L_2:** $L_1 = 0.4\ L_2 + 39$ dB SPL and up to 15 dB above and below this point in 3 dB steps, L_2 varied from 20 to 75 dB SPL in 5 dB steps	**Best f_2/f_1:** NA (fixed at 1.22) **Best L_1/L_2:** $L_1 = 0.49\ L_2 + 41$ dB SPL Based on linear regression and Kruskal-Wallis H

Study	Subjects	Stimulus parameters	Findings
22 Meinke et al. (2013). USA	17 ears (17M) with NH, 18 to 50 y	**f_2:** 5160 points between 0.258 to 18.023 kHz **f_2/f_1 ratio:** 1.025 to 1.5 in 0.025 steps **L_1/L_2:** 75/75, 65/55 dB SPL	**Best f_2/f_1:** 1.2 to 1.35 **Best L_1/L_2:** 75/75 dB SPL No inferential statistics used
23 Mills et al. (2007). USA	40 ears (10F, 10M), 18 to 24 y	**f_2:** 1, 2, 3, 4, 6, 8 kHz **f_2/f_1 ratio:** 1.21 & 1.28 **L_1/L_2:** L_1 85 to 20 dB SPL in 5 dB steps, L_2 always -10 dB SPL relative to L_1	**Best f_2/f_1:** 1.28 **Best L_1/L_2:** 85/75 dB SPL No inferential statistics used
24 Moulin (2000a). France	18 ears (10F, 8M), 24 to 46 y	**f_2:** 0.757, 0.879, 1, 1.257, 1.5, 2.002, 3.003, 4.004, 5.005, 6.006 kHz **f_2/f_1 ratio:** 1.02 to 1.50 in steps of 0.012 to 0.02 **L_1/L_2:** 65/60 dB SPL	**Best f_2/f_1:** 1.244 – 0.105 log(f_2), ranging from near 1.24 at f2=1 kHz to near 1.18 at f_2=5 kHz **Best L_1/L_2:** NA (L_1/L_2 fixed at 65/60 dB SPL) Polynomial function used to fit best f_2/f_1 curve
25 Neely et al. (2009). USA	322 ears (176 subjects), 11 to 80 y	**f_2:** 0.7 to 8 kHz in half-octave steps **f_2/f_1 ratio:** $1.22+\log_2(9.6/f_2)$ ($L_2/415)^2$ **L_1/L_2:** $L_1=80+0.137\log_2(18/f_2)$ (L_2-80) dB SPL. L_2 80 to -20 dB SPL in 5 dB steps	**Best f_2/f_1:** not stated **Best L_1/L_2:** L_2=80 dB SPL for f_2=0.7 to 2 kHz. L_2=70-80 dB SPL for f_2=4 or 8 kHz Polynomial function used to fit best f_2/f_1 curve
26 Nielsen et al. (1993). Denmark	10 ears (2F, 3M), 22 to 42 y	**GM:** 0.5, 1, 1.5, 2, 3, 4, 6, 8 kHz **f_2/f_1 ratio:** 1.15 to 1.4 in 0.05 steps **L_1/L_2:** 75/75 dB SPL	**Best f_2/f_1:** 1.2 to 1.25 **Best L_1/L_2:** NA (fixed at 75/75 dB SPL) No inferential statistics used

Study	Subjects	Stimulus parameters	Findings
27 Rasmussen (1993). Denmark	14 ears (3F, 4M), 25 to 55 y	**GM:** 0.5, 1, 1.5, 2, 3, 4, 6, 8 kHz **f_2/f_1 ratio:** 1.23 **L_1/L_2:** L_1 75, 70, 65 dB SPL with L_2=75 dB SPL, L_1=75 dB SPL with L_2 75, 70, 65 dB SPL	**Best f_2/f_1:** NA (fixed at 1.23) **Best L_1/L_2:** 75/75 dB SPL No inferential statistics used
28 Smurzynski et al. (1990). USA	10 ears (5 subjects), 21 to 41 y	**DPOAE *f*:** 1.53 kHz **f_2/f_1 ratio:** 1.03 to 1.4 in 0.02 steps **L_1/L_2:** 70/70 dB SPL	**Best f_2/f_1:** 1.18 **Best L_1/L_2:** NA (fixed at 70/70 dB SPL) No inferential statistics used
29 Stover et al. (1999). USA	14 subjects with NH, young adults	**f_2:** 1 to 8 kHz in half-octave steps **f_2/f_1 ratio:** 1.01 to 1.5 kHz, where f_1 was moved in 25 Hz steps for each f_2 frequency **L_1/L_2:** L_1 75 to 45 dB SPL in 5 dB steps. L_2 -10 dB SPL relative to L_1	**Best f_2/f_1:** Not stated **Best L_1/L_2:** 75/65 dB SPL No inferential statistics used
30 Vento et al. (2004). USA	36 ears (18F, 18M), 18 to 25 y	**f_2:** 2, 4, 6 kHz **f_2/f_1 ratio:** 1.01 to 1.4 **L_1/L_2:** 65/55, 50/40 dB SPL	**Best f_2/f_1:** 1.203 to 1.4 **Best L_1/L_2:** 65/55 dB SPL Based on ANOVA
31 Vinck et al. (1996). Belgium	101 ears (58F, 43M), 19 to 28 y	**f_2:** 0.696 to 6.348 kHz in 11 steps **f_2/f_1 ratio:** 1.22 **L_1/L_2:** 80/80, 75/75, 70/70 dB SPL	**Best f_2/f_1:** NA (fixed at 1.22) **Best L_1/L_2:** 75/75 dB SPL No inferential statistics used
32 Whitehead et al. (1995a). USA	16 ears (11 subjects), 18 to 44 y	**GM:** 1 to 8 kHz in 139 Hz steps **f_2/f_1 ratio:** 1.21	**Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 85/85, 85/80, 75/75, 75/70 dB SPL

Study	Subjects	Stimulus parameters	Findings
		L_1/L_2: L_1 85, 75, 65 dB SPL. L_2 0, -5, -10, -15 dB SPL relative to L_1	No inferential statistics used
33 Whitehead et al. (1995b). USA	15 ears (8 subjects), 18 to 44 y	**GM:** 2.98 kHz (15 ears); 1.39, 2.79, 5.57 kHz (7 ears) **f_2/f_1 ratio:** 1.25 for 2.98 Hz, 1.2 to 1.25 for 1.39 to 5.57 kHz **L_1/L_2:** L_1 85 dB SPL with L_2 85 to 20 dB SPL in 5 dB steps**.** L_1 85 to 20 dB SPL in 5 dB steps with L_2 85 dB SPL	**Best f_2/f_1:** NA (fixed for different GMs) **Best L_1/L_2:** 75/75, 75/70 dB SPL Note: no inferential statistics used

Table 6.2 describes 10 studies that considered the test-retest reliability of DPOAEs ($2f_1 - f_2$) elicited using different stimulus parameters within a single group of adult humans with normal hearing. Overall, the data presented in this table suggest that the stimulus parameters most likely to generate the most reliable DPOAEs are yet to be determined. This conclusion is based on variable results across the nine studies. In addition, only one study (Moulin, 2000b) examined more than one frequency ratio within a single group of subjects.

Table 6.2: Studies that considered the test-retest reliability of DPOAEs ($2f_1 - f_2$) elicited using different stimulus parameters from subjects with normal hearing.

ICC – intraclass correlation coefficient, MDD – Minimal detectable difference, SD – standard deviation, SEM – standard error of measurement. All other abbreviations as per Table 6.1.

Study	Subjects	Stimulus parameters	Test intervals	Findings
1 Beattie (2003). USA	62 ears (62F), 19 to 26 y	**f_2:** 1, 2, 4 kHz **f_2/f_1 ratio:** about 1.2 **L_1/L_2:** L_1 75 to 40 dB SPL in 5 dB steps. L_2 -10 dB relative to L_1	3 times/condition at 10 to 20 min intervals	**Best f_2/f_1:** NA (fixed at about 1.2) **Best L_1/L_2:** 65/55 dB SPL for combined f_2. Variable for individual f_2 Based on non-inferential comparison of SEM values
2 Beattie et al. (2004). USA	50 ears (50F), 19 to 26 y	**f_2:** 1, 2, 4 kHz **f_2/f_1 ratio:** about 1.2 **L_1/L_2:** L_1 75 to 40 dB SPL in 5 dB steps. L_2 -10 relative to L_1	3 times/condition at 10 to 20 min intervals	**Best f_2/f_1:** NA (fixed at about 1.2) **Best L_1/L_2:** 75/65, 70/60, 65/55 dB SPL for combined f_2. Variable for individual f_2 Based on non-inferential comparison of SEM values
3 Dreisbach et al.	25 ears (14F,	**f_2:** 2 to 4 kHz at 24 points/octave **f_2/f_1 ratio:** 1.2	4 tests each 1 to 2 weeks apart	**Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** No best

	Study	Subjects	Stimulus parameters	Test intervals	Findings
	(2006). USA	11M), 18-29 y	**L_1/L_2:** 70/60, 70/55, 60/50, 60/45 dB SPL		Based on ANOVA analysis
4	Franklin et al. (1992). USA	12 ears (5F, 7M), 19 to 44 y	**DPOAE *f*:** 1 to 8 kHz at 10 points/octave **f_2/f_1 ratio:** 1.21 **L_1/L_2:** 75/75, 65/65, 55/55 dB SPL	Short term: day 1, 2, 4, 6, 8 Long term: week 1, 2, 4, 6, 8	*Short term:* **Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 75/75 dB SPL *Long term:* **Best f_2/f_1:** NA (fixed at 1.21) **Best L_1/L_2:** 65/65 dB SPL Based on non-inferential comparisons of correlation & SEM values
5	Keppler et al. (2010). Belgium	29 ears (14F, 15M), 19 to 28 y	**f_2:** 0.841 to 8 kHz at 8 points/octave **f_2/f_1 ratio:** 1.22 **L_1/L_2:** 75/70, 65/55 dB SPL	baseline, immediate (no probe removal), immediate (after probe replacement), 60 min, 7 days	**Best f_2/f_1:** NA (fixed at 1.22) **Best L_1/L_2:** 75/70 dB SPL Based on ANOVA, ICC, SEM, MDD analyses
6	Marcrum et al. (2016) Germany	21 ears (11 subjects), 20 to 44 y	*Expt 2.* **f_2:** 1, 2, 3, 4, 6 kHz **f_2/f_1 ratio:** 1.22 **L_1/L_2:** $L_1 = 0.4\ L_2 + 39$ dB SPL and up to 15 dB SPL above and below this point in 3 dB SPL steps, L_2 was varied from 20 to 75 dB SPL in 5 dB steps	baseline, immediate (no probe removal	**Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** $L_1 = 0.49\ L_2 + 41$ dB SPL Based on linear regression and Kruskal-Wallis H

Study	Subjects	Stimulus parameters	Test intervals	Findings
7 Moulin (2000b). France	3 ears, 24 to 46 y	**f_2:** 1, 1.5, 2, 3, 4, 6 kHz **f_2/f_1 ratio:** 1.05 to 1.40 in 0.05 steps **L_1/L_2:** 65/60 dB SPL	S1: repeated at 10 weeks & 2 y. S2: repeated at 45 weeks. S3: repeated at 26 weeks	**Best f_2/f_1:** <1.28 **Best L_1/L_2:** NA (fixed at 65/60 dB SPL) Based on descriptive values only
8 Roede et al. (1993). Germany	22 ears (12R, 10L) (6F, 6M), mean age 26.3 y	*Expt 1.* **GM:** 0.8 to 8 kHz in 0.2 octave steps **f_2/f_1 ratio:** 1.21 **L_1/L_2:** 70/70, 55/55 dB SPL *Expt 2.* **GM:** 0.8 to 6 kHz in 0.2 octave steps **f_2/f_1 ratio:** 1.22 **L_1/L_2:** L_1 70 to 35 dB SPL in 5 dB steps. L_2 -6 dB SPL relative to L_1	Repeated after 3 one-week intervals & a final four-week interval (total = 6 weeks)	**Best f_2/f_1:** NA (fixed at 1.21 or 1.22) **Best L_1/L_2:** 70/70, 70/64 dB SPL Based on repeated measure SD values
9 Stuart et al. (2009). USA	16 ears (16F), 20 to 26 y	**f_2:** 1.514 to 7.568 kHz in 12 steps **f_2/f_1 ratio:** 1.2 **L_1/L_2:** 57/45, 55/40, 53/35, 51/30 dB SPL	Immediate, immediate after replacing probe, immediate with second tester, immediate with second tester after replacing probe	**Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** no best Based on ANOVA, SEM & Cronbach alpha analyses

Study	Subjects	Stimulus parameters	Test intervals	Findings
10 Wagner et al. (2008). Germany	80 ears, 19.7 to 43.3 y	**f_2:** 1, 2, 3, 4, 5, 6 kHz **f_2/f_1 ratio:** 1.2 **L_1/L_2:** $L_1 = 0.4L_2 + 39$ dB SPL. L_2 60, 50, 40, 35, 25, 20 dB SPL	*Group 1 (n=40 ears):* repeated twice on same day & once between 1 to 35 days later *Group 2 (n=40 ears):* repeated between 1 to 14 days & repeated again between 1 to 15 days	Groups 1 & 2 **Best f_2/f_1:** NA (fixed at 1.2) **Best L_1/L_2:** no best Based on SEM & Cronbach alpha analyses

Table 6.3 describes nine studies that considered the sensitivity and specificity of DPOAEs ($2f_1 – f_2$) elicited using different stimulus parameters when assessing subjects with normal hearing versus subjects with SNHL. Sensitivity was defined as the DPOAEs' ability to detect outer hair cell dysfunction when this dysfunction was actually present, with pure tone thresholds as gold standard. This value, also referred to as the true positive rate, was calculated as follows: [True positive/ (True positive + False negative)] x 100 or through ROC curve analysis. Specificity was defined as the DPOAEs' ability to indicate a negative result, i.e., that OHC dysfunction is not present, with pure tone thresholds as gold standard. This value was calculated by [True negative/ (True negative + False positive)] x 100 or ROC curve analysis. Overall, the data presented in this table suggest that the stimulus parameters most likely to produce DPOAEs of the highest sensitivity and specificity to SNHL are yet to be determined. This conclusion is based on the inconsistent use of different stimulus parameters across the different studies.

Table 6.3: Studies that considered the effects of different DPOAE ($2f_1 – f_2$) stimulus parameters when assessing subjects with normal hearing versus SNHL.

The reference standard for each study was pure tone audiometry. ROC – receiver operator characteristic. All other abbreviations as per Table 6.1.

	Study	Subjects	Stimulus parameters	Findings	Sensitivity	Specificity
1	Arnold et al. (1999). USA	All: 50 ears (21F, 29M), 17 to 37 y Unstated numbers with normal UHF & abnormal UHF hearing	f_2: 0.8 to 8 kHz in 0.1 octave steps f_2/f_1 ratio: 1.22 L_1/L_2: 75/75, 65/65, 55/55 dB SPL	Best f_2/f_1: NA (fixed at 1.22) Best L_1/L_2: no best RM ANOVA analysis used	Not provided	
2	Bonfils & Avan (1992). France	NH: 25 ears, aged 7 to 42 y	f_2: 1.129 to 8.875 kHz in 5 steps f_2/f_1 ratio: 1.23	Best f_2/f_1: NA (fixed at 1.23) Best L_1/L_2: 62/62, 52/52 dB SPL	L_1/L_2 = 52/52: 84-100%	L_1/L_2 = 52/52: 49-95% L_1/L_2 =

	Study	Subjects	Stimulus parameters	Findings	Sensitivity	Specificity
		High *f* HL: 50 ears, aged 23 to 70 y	L_1/L_2: 72/72 to 42/42 dB SPL in 10 dB steps	Based on correlation and regression analyses	L_1/L_2 = 62/62: 78-91%	62/62: 84-100%
3	Chida et al. (2001). Japan	All: 177 ears (64F, 30M), aged 6 to 69 y NH: 97 ears SNHL: 80 ears	f_2: 1, 2, 4 kHz f_2/f_1 ratio: 1.22 L_1/L_2: 70/70, 60/60, 60/50 dB SPL	Best f_2/f_1: NA (fixed at 1.22) Best L_1/L_2: 60/60, 60/50 dB SPL Based on ANOVA & ROC analyses	L_1/L_2 = 60/60: 90% L_1/L_2 = 60/50: 95%	L_1/L_2 = 60/60: 86% (at 2 kHz) L_1/L_2 = 60/50: 85% (at 2 kHz)
4	Kummer et al. (1998). Germany	NH: 20 ears (12F, 8M), 18 to 32 y SNHL: 15 ears (7 F, 8M), 16 to 76 y	f_2: 0.488 to 8.008 kHz at 11 to 15 steps/octave f_2/f_1 ratio: 1.2 L_1/L_2: $L_1 = 0.4L_2$ + 39 dB SPL, L_1 = 47 to 65 dB SPL	Best f_2/f_1: NA (fixed at 1.2) Best L_1/L_2: 57/45, 55/40, 53/35 dB SPL Based on correlation analyses	Not provided	Not provided
5	Moulin et al. (1994). France	NH: 24 ears (12F, 12M), aged 24±8.1 y SNHL: 159 ears (39F, 42M), F aged 51±19.6 y, M aged 45±14.6 y	GM: 0.706 to 5.664 kHz (f_{DPOAE} 0.5 to 5 kHz) f_2/f_1 ratio: 1.22 L_1/L_2: 80/80 to 40/40 dB SPL in 10 dB steps	Best f_2/f_1: NA (fixed at 1.22) Best L_1/L_2: 70/70 & 60/60 dB SPL Based on DPOAE prevalence & correlation analyses	Not provided	Not provided

	Study	Subjects	Stimulus parameters	Findings	Sensitivity	Specificity
6	Smurzynski et al. (1990). USA	NH: 10 ears (5 subjects), 21 to 41 y High *f* SNHL: 4 ears (2 subjects), adults	DPOAE *f*: 1.530 kHz f_2/f_1 ratio: 1.03 to 1.4 in 0.02 steps L_1/L_2: 70/70 dB SPL	Best f_2/f_1: No DPOAEs for higher f_2/f_1 when f_1 & f_2 in region of hearing impairment Best L_1/L_2: NA (fixed at 70/70 dB SPL) No inferential statistics used	Not provided	Not provided
7	Sun et al. (1996). USA	NH: 32 ears (20 subjects) SNHL: 45 ears (28 subjects) Aged 18 to 75 y	f_2: 0.5 to 6 kHz in quarter-octave steps f_2/f_1 ratio: ≈1.2 (1.18 to 1.23) L_1/L_2: 65/65, 65/50 dB SPL	Best f_2/f_1: NA (fixed at ≈1.2) Best L_1/L_2: 65/50 dB SPL for higher frequencies Based on Wilcoxon, correlation & ROC curve analyses	L_1/L_2 = 65/50 dB SPL: 83-93% L_1/L_2 = 65/65 dB SPL: 80-83%	L_1/L_2 = 65/50 dB SPL: 83-90% L_1/L_2 = 65/65 dB SPL: 83-85%
8	Sutton et al. (1994). USA	NH: 14 ears (9R, 5L) (7F, 7M), 19-48 y SNHL: same ears following exposure to 105 dB SPL at 2.8 kHz for 3 min	f_2: 4.4 kHz. f_2/f_1 ratio: 1.21 L_1/L_2: L_1 75 to 25 dB SPL in 5 dB steps, L_2= L_1 L_1 75 to 20 dB SPL in 5 dB steps. L_2 -25 dB relative to L_1	Best f_2/f_1: NA (fixed at 1.21) Best L_1/L_2: 55/30 dB SPL No inferential statistics used	Not provided	Not provided

Study	Subjects	Stimulus parameters	Findings	Sensitivity	Specificity
9 Whitehead et al. (1995a). USA	NH: 16 ears (11 subjects), 18 to 44 y SNHL: 15 ears (11 subjects), 19 to 41 y	GM: 1 to 8 kHz in 139 Hz steps f_2/f_1 ratio: 1.21 L_1/L_2: L_1 85, 75, 65 dB SPL. L_2 0, -5, -10, -15 dB relative to L_1	Best f_2/f_1: NA (fixed at 1.21) Best L_1/L_2: $L_2 < L_1$ No inferential statistics used	Not provided	Not provided

Discussion

This systematic review of DPOAE research studies suggests the following:

- The stimulus parameters most likely to generate DPOAEs ($2f_1 - f_2$) with the highest absolute level from human adults are those using an f_2/f_1 value from 1.20 to 1.22 and an L_1/L_2 setting of 75/75 dB SPL (from a review of 33 within-group studies). The 75/75 dB SPL setting for L_1/L_2 should be treated with caution, however, with higher absolute DPOAE levels potentially resulting from higher levels of noise and/or distortion generated by the DPOAE instrumentation itself (Whitehead, McCoy, et al., 1995). Such passive responses could mask the presence of a cochlear lesion (Martin, Stagner, Jassir, Telischi, & Lonsbury-Martin, 1999; Robles & Ruggero, 2001).
- The stimulus parameters most likely to generate DPOAEs with the highest test-retest reliability of DPOAEs ($2f_1 - f_2$) from human adults are yet to be determined as results from previous studies were inconclusive (from a review of 10 within-group studies – see Table 6.2).
- The stimulus parameters most likely to generate DPOAEs ($2f_1 - f_2$) with the highest sensitivity and specificity to SNHL in human adults are yet to be determined as results from previous studies were inconclusive (from a review of nine between-group studies – see Table 6.3).

No conclusions could be drawn about the stimulus parameters most likely to generate DPOAEs ($2f_1 - f_2$) with the highest SNR as none of the reviewed studies included this measure in their analyses.

Several factors limited the ability to draw conclusions from the reviewed research. First was the wide variety of parameters used across the studies to elicit DPOAEs. While some parameter settings were more commonly used, others were used in as few as a single study. There were also examples of certain DPOAE parameters being listed in the methods of some studies but the results of using those parameters were not reported in the results sections of those studies. Second was the limited participant sample size present in several of the studies. This affected the power of some studies and prevented the use of inferential statistics in other studies, with the latter limiting the interpretation of results in some studies to descriptive comparisons only. Third was an inconsistent reporting of ears versus subjects with some studies reporting DPOAE results for ears (sometimes treating left and right ear results as independent) while other studies reported DPOAE results for subjects (sometimes not reporting which ear/s had been tested in each subject).

There remains an ongoing need for research to determine a clearly defined set of preferred stimulus and recording parameters for eliciting DPOAEs of higher level and/or greater reliability in healthy adults, and higher sensitivity and specificity in adults with cochlear lesions. While this research continues, clinicians should turn to meta-analyses and large-scale studies that have considered fixed (or limited) sets of stimulus parameters for eliciting DPOAEs from adult humans. This approach would allow the clinician to identify the absolute levels, test-retest reliability and/or sensitivity and specificity to SNHL of DPOAEs that can be expected when using those fixed sets of stimulus parameters. Recent examples include Carter et al. (2015) who summarise DPOAE data suitable for reference use after recording DPOAEs from 2 672 test ears in 1 386 adult humans (DPOAE parameters used: f_2/f_1 = 1.21 and L_1/L_2 = 70/70 and 70/60 dB SPL), and Reavis, McMillan, Dille, and Konrad-Martin (2015), who report a meta-analysis of DPOAE retest variability for serial monitoring of cochlear outer hair cell function in adults (DPOAE parameters used in the 10 studies included in the meta-analysis: f_2/f_1 = 1.2 and L_1/L_2 = 75/70 and 65/65).

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CHAPTER 7: PRELIMINARY STUDY ON STIMULUS PARAMETERS

This chapter presents phase 2 of the thesis in the form of a paper published in the *South African Journal of Communication Disorders*, with the methods section expanded to provide a full discussion of the methods used.

Reference: Petersen, L.[5], Wilson, W., & Kathard, H. (2018). Towards the preferred stimulus parameters for DPOAEs in adults: A preliminary study. *South African Journal of Communication Disorders, 65 (1)* https://doi.org/10.4102/sajcd.v65i1.585.

Abstract

Background: Although distortion product otoacoustic emissions (DPOAEs) are useful in evaluating cochlear outer hair cell function, determining the optimal stimulus parameters could result in a more reliable, sensitive and specific diagnostic tool across the range of DPOAE applications. *Aim:* To identify which stimulus parameters warrant further investigation for eliciting the largest and most reliable distortion product otoacoustic emissions (DPOAEs) in adult humans. *Method:* A single group, repeated measures design involving a convenience sample of 20 normal-hearing participants between 19 and 24 years of age. *Results:* Descriptive statistics and mixed model analyses suggested L_1/L_2 intensity levels of 65/65 and 65/55 dB SPL, and f_2/f_1 ratios of 1.18, 1.20 and 1.22 elicited larger and more reliable DPOAEs in both ears. *Conclusion:* Further investigation of the 65/65 and 65/55 dB SPL intensity levels and the 1.18, 1.20 and 1.22 f_2/f_1 ratios is warranted to determine the stimulus parameters for eliciting the largest and most reliable DPOAEs in adult humans across the range of DPOAE applications.

Introduction

Distortion product otoacoustic emissions (DPOAEs) are sounds emitted from the cochlea in response to two simultaneously presented tonal stimuli. These stimuli have levels designated as

[5] The first author conceptualised the study, collected, analysed, interpreted the data and conceptualised and drafted the journal article. The first author is also the corresponding author.

L_1 and L_2 and frequencies designated as f_1 and f_2. The sensitivity of DPOAEs to outer hair cell dysfunction in the cochlea has seen them successfully used in a variety of clinical and research applications such as newborn hearing screening, diagnostic audiological assessment, ototoxicity monitoring and the study of cochlear mechanics (Dhar & Hall, 2012; Hood & Berlin, 2002).

The successful use of DPOAEs in a range of applications suggests that their optimal stimulus parameters have been determined. This is not the case (Petersen, Wilson, & Kathard, 2017). Instead, the DPOAE level has been found to depend on varying combinations of stimulus parameters including f_1 and f_2 frequencies, f_2/f_1 ratio, L_1 and L_2 intensity levels, and L_1/L_2 level separation (Prieve & Fitzgerald, 2015). Furthermore, DPOAEs have been elicited using a wide range of stimulus parameters including f_2/f_1 ratios from 1.03 to 1.79 and L_1/L_2 combinations ranging from 30/30 to 85/85 dB SPL (Petersen et al., 2017). Dreisbach and Siegel (2001) added to this complexity by reporting that the optimal f_2/f_1 ratio varies as a function of f_2 frequency, with lower f_2/f_1 values eliciting higher DPOAE levels at higher f_2 frequencies and vice-versa.

Recommended stimulus parameters have also varied depending on the application. For diagnostic purposes and/or ototoxicity monitoring, f_2/f_1 ratios have ranged from 1.20 to 1.22 and L_1/L_2 combinations from 45/35 to 65/55 dB SPL (Dhar & Hall, 2012; Hall, 2000). Hall (2000) also reported that for cochlear lesions, decreasing the stimulus levels improved DPOAE sensitivity, whereas increasing the stimulus levels improved DPOAE specificity. For screening applications, an f_2/f_1 ratio of 1.20 has often been recommended with L_1/L_2 combinations of either 65/55 or 65/65 dB SPL (Dhar & Hall, 2012; Hall, 2000). Other recommendations have included an L_1/L_2 combination of 65/55 dB SPL for its reported twofold advantage of producing a higher DPOAE level with an improved sensitivity to outer hair cell dysfunction, whereas the use of L_1/L_2 combinations above 70/70 dB SPL has been discouraged to avoid possible response artifacts that can be mistaken for DPOAEs and confusion over the source of the resulting DPOAEs (Dhar & Hall, 2012).

The variation in stimulus parameters used to elicit DPOAEs highlights the continuing need to determine the optimal DPOAE stimulus parameters across all its applications. This search needs to be driven by sound research based on a continuum of evidence that considers two factors: the

cyclical nature of knowledge creation and the quality of existing evidence informing clinical practice. The cyclical nature of knowledge creation, especially in the case of clinical practice, refers to the cycle of developing theories that are then tested by research to develop new knowledge. This new knowledge is then applied to clinical settings where it is used to refine existing theories or propose new ones (Schmidt & Brown, 2015). Patient care is improved through the repetitive nature of this cycle and its ability to generate ever-changing scientific knowledge. In the case of DPOAEs, the quality of existing evidence informing clinical practice refers to the following questions: "How rigorous is the research investigating DPOAE stimulus parameters?" and "How strong is the evidence for the stimulus parameters currently used in the clinical setting?" Asking such questions seeks to strengthen clinical practice and expand current evidence bases (Puddy & Wilkins, 2011).

In response to the above call, the authors of this study recently began to search for the optimal stimulus parameters for eliciting DPOAEs from human adults for clinical applications, especially to assist with early identification of outer hair cell damage in individuals receiving ototoxic treatment for multi-drug-resistant TB. This search began with a systematic review (Petersen et al., 2017) that first asked: "What is an 'optimal' DPOAE?" Factors such as the clinical value of DPOAE level, SNR, reliability, sensitivity and specificity to OHC dysfunction were considered, as well as confounds such as the high number of DPOAE parameters open to manipulation, the small effect sizes of changing some parameters, the physiological processes represented by DPOAEs and the high inter-subject variability seen in DPOAEs.

The review then examined 47 DPOAE studies that had met the inclusion criteria for the systematic review. Of these, 33 studies met the inclusion criteria to examine the influence of intensity and/or frequency ratio on the DPOAE level. Most of the studies were found to have small sample sizes (often fewer than 10 participants) and/or to have manipulated only one set of stimulus parameters (18 having manipulated L_1/L_2 levels at a fixed f_2/f_1 ratio, or vice versa). Of the remaining 15 studies that manipulated both intensity and frequency ratio parameters, 8 studies used 15 participants or less. Ten of the 15 studies had only used descriptive statistics when reporting their results, leaving open the possibility that any observed differences had occurred

by chance alone (interestingly, this limitation was seen in two seminal and highly cited papers on DPOAE stimulus parameters by Gaskill and Brown [1990] and Harris, Lonsbury-Martin, Stagner, Coats and Martin [1989]). Petersen et al. (2017) concluded that although some parameters are commonly used to elicit DPOAEs, only their effects on DPOAE level have been considered in limited detail (with their effect of DPOAE SNR, reliability, and sensitivity and specificity to OHC dysfunction being largely ignored), and the optimal parameters for eliciting DPOAEs in adult humans in clinical applications have yet to be determined (Petersen et al., 2017).

This study sought to expand on the findings of Petersen et al. (2017) towards a final determination of the optimal stimulus parameters for eliciting DPOAEs from human adults for clinical diagnostic applications. It considered a wide range of commonly used stimulus parameters from those reported by Petersen et al. (2017) but expanded their investigation by systematically manipulating both f_2/f_1 ratios and L_1/L_2 levels simultaneously. This study was limited to measuring the effect of stimulus parameters on DPOAE level and reliability (and not SNR or sensitivity and specificity to OHC dysfunction) in adult humans with normal hearing to manage the total number of variables under examination.

Method

For phase 2, the aim was to systematically investigate stimulus parameters to determine which intensity pair and frequency ratio combinations yield the largest amplitude and most reliable DPOAEs in normal-hearing, healthy adult participants, to define the stimulus parameters to be used in the rest of the study.

The aim for phase 2 was achieved by the following:

(1) Comparing the absolute DPOAE levels elicited for all stimulus conditions.

(2) Comparing the short-term, test-retest reliability of the DPOAEs elicited for all stimulus combinations.

Research design

A single group, repeated measures design was used for this study (Maxwell & Satake, 2006). This design, also known as a within-subjects design, was deemed appropriate to examine the influence of intensity and frequency ratio stimulus parameters on DPOAE levels. Each participant was exposed to all DPOAE stimulus parameters, and the effects of these parameters were measured and compared within each individual (Maxwell & Satake, 2006). With a repeated measures design, it was possible to establish the highest level and test-retest reliability of DPOAEs in the same participants, where the participants acted as their own controls.

A known disadvantage of using a repeated measures design is the possibility of a learning effect (Howell, 1999). However, learning effects do not influence the DPOAE test, as it is an objective measure that does not require active participation of the participant. Another disadvantage of repeated measures, namely order effects, were mitigated by starting with a different stimulus parameter combination with each participant (Maxwell & Satake, 2006).

Participants

Inclusion criteria

All participants:

(a) were between 18–30 years of age , to minimise age-related deterioration of the cochlea (Gates & Mills, 2005; Schuknecht, 1955);

(b) had normal non-diagnostic otoscopic results, to rule out possible outer or middle ear problems that could obscure measurement of cochlear outer hair cell function (Hall, 2000);

(c) had normal hearing, which is defined as thresholds of ≤15 dB hearing level (HL) (Clark, 1981, in Katz, 2002) for the frequency range 250Hz to 8000Hz;

(d) had normal middle ear functioning, which is defined as a Type A tympanogram, with compliance between 0.3 and 1.4 cm^3, middle ear pressure ranging from –150 daPa to 100 daPa and ear canal size between 1.0 and 2.0 cm^3 (Grason-Stadler, 2003) because OAEs cannot be reliably measured in the presence of a middle ear abnormality (Hall, 2000);

(e) had no self-reported history of hereditary hearing loss, significant ear disease, ear surgery or long-term noise exposure; and

(f) had no medical condition that could affect the audiological test results negatively (Hall, 2000).

Participant description

Twenty normal-hearing adult participants (15 females, 5 males, aged 19–24 years) were conveniently sampled from the staff and student population of the University of Cape Town, South Africa.

Recruitment

Recruitment commenced after obtaining ethics approval from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (HREC/REF: 512/2013) (See Appendix A) and written permission from the University of Cape Town. The participants for this aim were recruited by posting notices on UCT notice boards, and emails to undergraduate and postgraduate students in the Faculty of Health Sciences (See Appendix B). The researcher explained the study to the interested individuals and provided an information sheet with the same information in written form. The aims, procedures and ethical issues of the study were explained, as well as the commitments required to partake in the study.

Sampling

Convenience sampling (Katzenellenbogen, Joubert, & Abdool Karim, 1997) was employed by accepting each person who met the selection criteria and was willing to participate in the study.

Data collection

Instrumentation

All audiological tests were conducted in a sound treated booth and/or in a quiet room in the Audiology Clinic at the University of Cape Town.

A Welch Allyn otoscope was used for non-diagnostic otoscopic examinations. A GSI Tympstar was used for tympanometry. A GSI 61 audiometer and TDH-39 earphones with standard frequency testing capability were used for pure tone audiometry.

DPOAEs were collected with the GSI Audera, a commercially available piece of equipment, which can reliably evaluate emissions up to 8 kHz. A Brüel & Kjær 2238 class 1 handheld sound level meter was used to monitor sound levels in the quiet room.

All the equipment was calibrated according to the relevant specifications, prior to data collection. In addition, a biological check of all the equipment was done prior to testing. The following DPOAE data were recorded from each participant for each set of stimulus parameters at each f_2 frequency on each test occasion: absolute level of DPOAE, absolute level of the noise floor and the DPOAE SNR, calculated as the absolute level of the DPOAE minus the level of the noise floor.

Procedure

After ethics approval and written permission had been received, written informed consent was obtained from each participant (See Appendix C). Biographical information obtained included date of birth, age, sex and medical history.

The researcher verbally conveyed information regarding the purpose, protocol and the requirements of the study in the participant's language (English or Afrikaans), which was also shared with each participant in written form. If a participant preferred isiXhosa, a mother-tongue isiXhosa research assistant explained the study to the patient. IsiXhosa-speaking participants received written documentation in isiXhosa. All discrepancies and uncertainties were addressed.

Participants were initially screened for inclusion in the study using a live voice interview and a commercially available otoscope, audiometer, tympanometer and DPOAE device (GSI Audera 2.7, Version C). To pass the DPOAE screening, the participants had to show $2f_1$-f_2 DPOAEs at least 3 dB above the noise floor at f_2 frequencies 2, 4 and 8 kHz to tonal stimuli with an f_2/f_1 ratio of 1.2 and an L_1/L_2 setting of 65/55 dB SPL (Lonsbury-Martin, Harris, Hawkins, Stagner, & Martin,

1990). All initial testing was conducted in a sound-treated booth meeting South African National Standards (2006).

The following tests were conducted at baseline and the follow-up session in both ears: otoscopy, tympanometry, PTA and DPOAEs. Standard pure tone audiometry was conducted at baseline, and at the subsequent test session to rule out any possible hearing changes. The follow-up session took place within 24–48 hours post-baseline. If the potential participant's hearing was normal, he/she continued with the rest of the study. If a hearing loss was detected, the researcher explained the results to the participant. In addition, the researcher also provided information and emotional counselling to the participant. The participant was referred to the relevant health practitioner for management.

Before commencing the test protocol, the researcher explained the nature and purpose of all test procedures to the participants to familiarise them with the session. Participants were then given an opportunity to seek clarification and also reminded that questions could be asked at any stage during data collection. Participants were reminded that they could withdraw at any point in the study.

Participants were seated in a comfortable chair for the duration of the tests. A glass of water was provided. Testing was stopped in the case of coughing or sneezing and continued after coughing/sneezing ceased. Breaks in testing were provided for between tests.

Clear instructions were given before each procedure and repeated during the test if necessary. The same instructions were given to all participants to improve reliability.

The instructions used for pure tone testing were: "You will hear soft sounds. Each time you hear a sound, please raise your hand immediately. Even if the sound is very soft and you can barely hear it, you must still raise your hand. We will start with your right ear." If it was deemed necessary, the instructions were repeated during testing.

For standard frequency pure tone audiometry, the modified Hughson-Westlake technique was used to obtain thresholds from 250 to 8 000 Hz.

Before DPOAE measurement, participants were instructed as follows: "I am going to place a probe in your ear. It will not be painful. Your ear will just feel blocked up. Please remain quiet and still while the probe is in your ear. You do not have to do anything, even if you hear a sound. You can sit back and relax."

Distortion product otoacoustic emissions testing was conducted in a quiet room with background noise levels < 55 dB A (Lee & Kim, 1999). In-ear calibration was done prior to each DPOAE test. The $2f_1$-f_2 DPOAE measurements were obtained from each ear of each participant using the following stimulus parameters: f_2/f_1 ratios – 1.18, 1.20, 1.22, 1.24, 1.26 and 1.28; L_1/L_2 settings – 65/65, 65/55, 60/45, 60/53 and 55/40 (each level reported in dB SPL); and f_2 frequencies: 2003, 2519, 3178, 3996, 5000, 6996 and 8003 Hz. The stimulus intensity pair of 65/55 dB SPL was chosen, as it is the one used most regularly in clinics. The rest of the L_1/L_2 settings were calculated using the paradigm suggested by Kummer, Janssen, and Arnold (1998) to optimise stimulus levels, namely $L_1 = 0.42L_2 + 39$. To mitigate potential order effects, a single sequence of stimulus parameters was set, and each participant was started at a different point in this sequence. The order of ear testing was reversed for each sequential participant. The $2f_1$-f_2 DPOAEs were sampled until at least one of the two stopping rules was met: (1) the noise floor at the distortion product frequency was less than -10 dB SPL, or (2) until 32 s of artifact-free sampling had been averaged (Dille et al., 2010). DPOAEs were recorded at least twice per stimulus parameter combination, for replication purposes. Participants were seated in a comfortable chair and were instructed to remain still and quiet during the DPOAE test procedure with breaks provided as required. The DPOAE test time per participant was approximately 90 minutes per test occasion. Each participant underwent DPOAE testing on two occasions 24–48 hours apart.

Reliability and validity of data collection

Possible sources of error in measurement in this study include the instrument, tester, participant and the environment.

To enhance reliability in instrumentation, the necessary system calibration was done prior to data collection. In addition, daily calibration was done by inserting the DPOAE probe into a hard-

walled cavity and measuring the response according to manufacturer specifications. Biological checks were conducted before each data collection session, and a visual probe inspection was done prior to testing each participant to check for any obstruction in the probe tubes.

The way of measuring each participant was standardised so that the measurements would take place in the same manner across participants and test intervals. Sufficient training reduced the error variance (Streiner & Norman, 2008). By following the same measurement procedure with each participant, it is hoped that intra-tester and inter-tester reliability were enhanced. The researcher and research assistants underwent a training period prior to data collection, to become familiar with the equipment and the measurement procedure.

To minimise participant variability, the test time was kept as short as possible. Data collection took place at the same time of the day for participants, as far as possible.

The ambient noise level was controlled by conducting all testing in a soundproof booth or a sound-treated booth. A possible source of equipment noise is the computer used to collect the data, which was therefore placed as far as possible from the participant.

Face validity refers to whether the instrument appears to assess the aspect of interest (Streiner & Norman, 2008). Experts like Dreisbach and Siegel (2001) believe that DPOAEs measure outer hair cell function of the cochlea. The DPOAEs were present in all participants of the current study, and it can be surmised that their cochleae functioned normally, as they all presented with normal hearing.

Content validity implies that the instrument covers all domains of the phenomenon under investigation (Maxwell & Satake, 2006). With DPOAEs, two pure tones were sent into the ear at intensities ≤ 70 dB SPL that elicit active cochlear activity. This activity was measured in the ear canal via a sensitive microphone. The stimulus intensities were monitored throughout the test procedure while DPOAEs were recorded, to ensure that the intended L_1/L_2 were delivered into the ear canal.

Criterion validity of DPOAEs has been evaluated and determined in patient populations (patients with cancer, cystic fibrosis, etc.) receiving ototoxic medication, e.g., cisplatin and aminoglycosides (Al-Malky et al., 2011; Reavis et al., 2008; Sisto et al., 2013).

Data management

All data was imported into Excel spreadsheets. A research assistant checked 10% of the data to assure that data had been imported accurately. If data entry errors were detected, the whole dataset was rechecked. Spreadsheets were uploaded to Microsoft OneDrive, an online storage space, to a password-protected account. Only the researcher had access to the password. The data will be kept for a minimum of five years, as per the South African Medical Research Council's recommendations (SAMRC, 2018).

Data analysis

All DPOAE data were found to meet parametric assumptions following examination of these data's histograms, box-and-whisker plots and Q–Q plots (data not shown). Descriptive statistics were calculated for all DPOAE measures and correlation analyses were conducted to determine if the DPOAE results for the left and right ears were related. As these analyses showed significant correlations in DPOAE results between the ears, all further analyses of the DPOAE data were conducted for each ear separately.

Two sets of linear mixed model analyses were conducted at the 5% significance level on the DPOAE data for each f_2 value separately. Each set of analyses considered DPOAE amplitudes as dependent variables, the stimulus level combinations and frequency ratios as fixed effect independent variables, and the participants as a random effect independent variable. The first set of analyses sought to identify the presence of any main effects of level settings (L_1/L_2 in dB SPL) for all f_2/f_1 settings combined and any main effects of frequency ratio settings (f_2/f_1) for all L_1/L_2 settings combined. The second set of analyses sought to identify the presence of any main effects of the combined level (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) settings.

Finally, two-way, mixed-model, intraclass correlation coefficient (ICC) analyses for absolute agreement were conducted at the 5% significance level on the DPOAE data for each f_2 value separately to determine the level of agreement (reliability) of the absolute levels of the DPOAE recordings from the first to the second assessment occasions for each combined level (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) setting separately. The ICC examines the relationship among variables that share both their metric and variance, i.e. the variables are of a common class and would be appropriate to determine correlations between repeated measures of the same test (McGraw & Wong, 1996).

All statistical analyses were conducted using IBM SPSS Statistics versions 23 and 24 (64-bit edition).

Ethical considerations

Ethics approval was obtained from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town before initiating the study (HREC/REF: 512/2013; See Appendix A). Permission was obtained from the Department of Student Affairs and the Human Resource Department at the university prior to data collection. This study complied with the principles set out by the Declaration of Helsinki (World Medical Association, 2013).

Autonomy refers to the competent individual's right to self-determination (Katzenellenbogen et al., 1997). Providing the participants with adequate information to give informed consent to participate in the study adhered to this principle of autonomy and safeguarded their freedom of choice.

To facilitate understanding of the study, information was presented in the participant's first language (Afrikaans, English, Xhosa). Participants were given the opportunity to ask for clarification at any time during the study. It was also explained to the participant that participation in the study was voluntary and that he/she could withdraw at any stage of data collection. The participants were informed that refusal to participate in or withdrawal from the

study would not affect their life. Participants were required to sign a consent form if they agreed to take part in the study (See Appendix C).

The ethical principle of beneficence requires that the actions of the researcher are aimed at improving the well-being of the participant (Katzenellenbogen et al., 1997). If any hearing or auditory abnormalities were detected, the participant was referred to the relevant health practitioner.

Non-maleficence is the principle that refers to the researcher's obligation to not do harm (Katzenellenbogen et al., 1997). No procedures were used that could harm the participants, and all tests were non-invasive. All necessary equipment, e.g., probe tips and earphones, was cleaned with alcohol swabs after use with each participant to ensure that bacteria were not spread from one participant to the next. The researcher washed her hands with an antiseptic agent for the same reason. The researcher took all reasonable steps to ensure the participants' comfort.

The ethics principle of justice can be interpreted to mean a fair distribution of benefits of the research (Katzenellenbogen et al., 1997). Therefore, all eligible individuals were included in the study, provided that they met the selection criteria.

There were no direct benefits for the participants of the study, other than detecting possible hearing or auditory damage. There were no known risks for participants. Participants received compensation of R100 per session for travel costs and inconvenience (each test session took between 60 to 90 minutes).

Confidentiality was respected at all times and all participants' details and results were kept confidential and anonymous by assigning a number to each participant, which corresponded with the order of enrolment in the study. This number was used on all documentation pertaining to the study. Identifying information was only known to the researcher and was kept separate from collected data in a locked filing cabinet in the researcher's office. The researcher also assured the participants that no identifying information would appear in any publication or presentation forthcoming from this study.

Researcher experience implies that the researcher is competent to conduct the research (World Medical Association, 2013). The researcher is professionally qualified as an audiologist and has sufficient theoretical and clinical expertise to conduct the research. The researcher acted in an accountable and responsible manner and upheld professional standards.

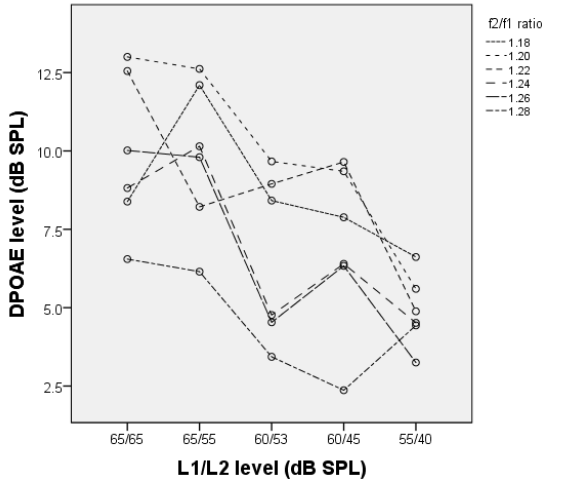
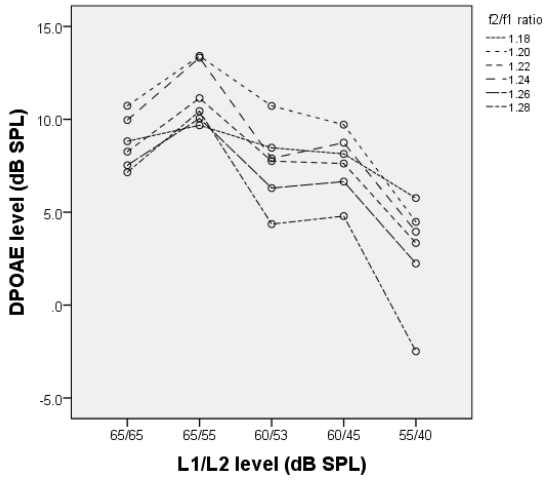
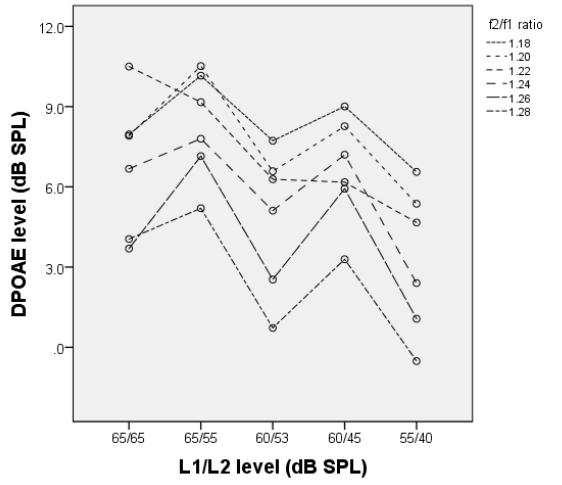
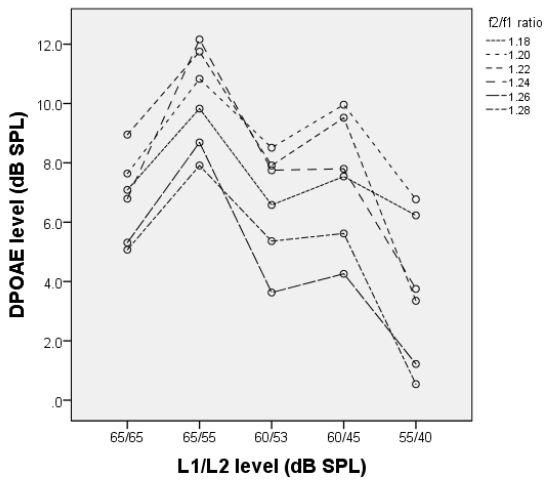
Results

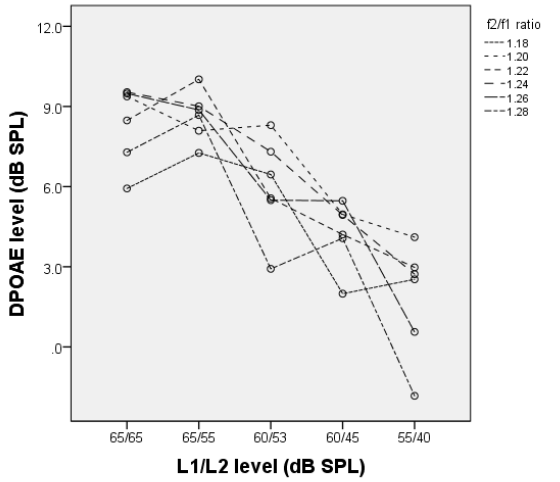
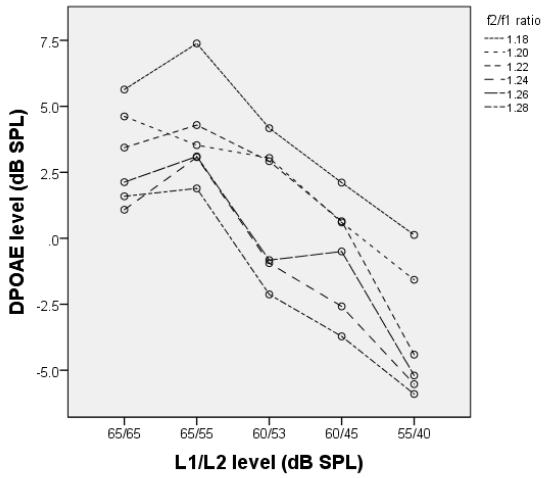
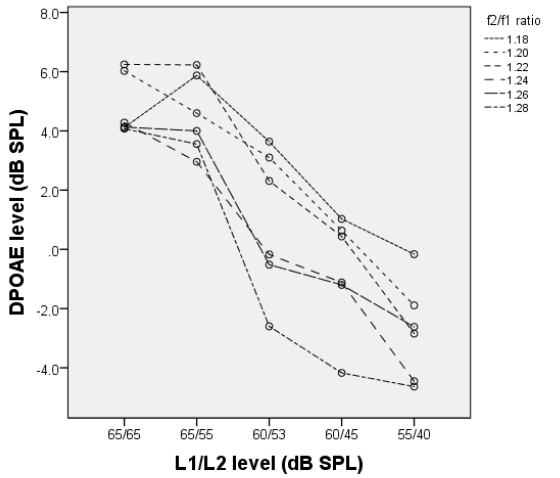
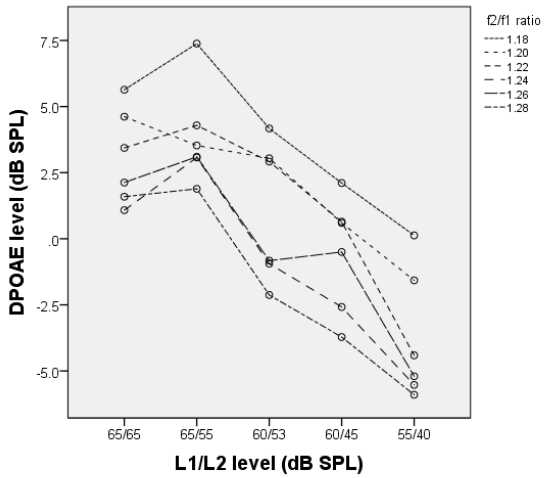
All participants included in the study had type A tympanograms and normal hearing with pure tone air conduction thresholds ≤ 15 dB HL. These results are displayed in Table 7.1.

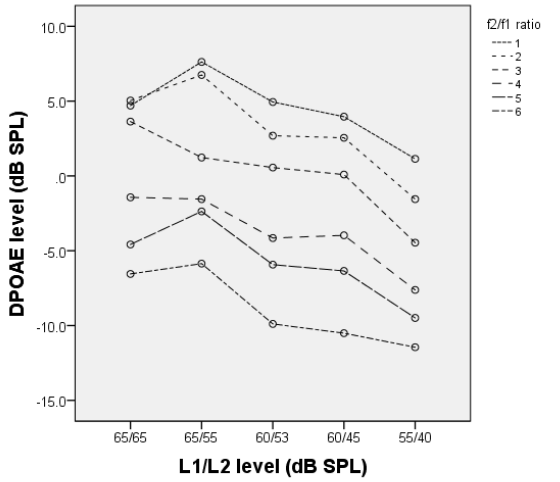
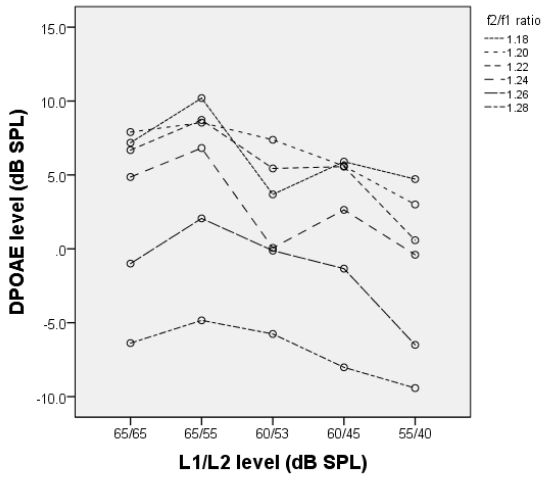
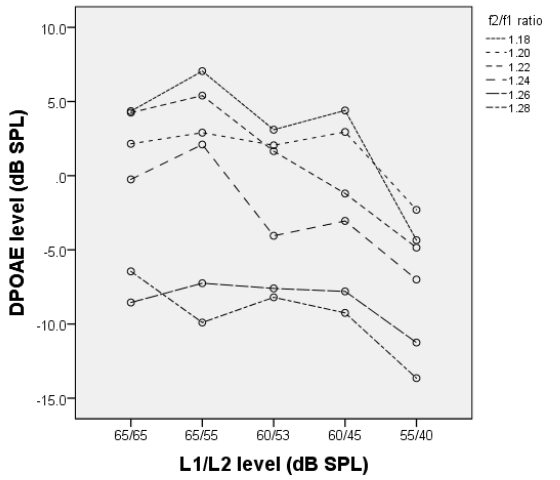
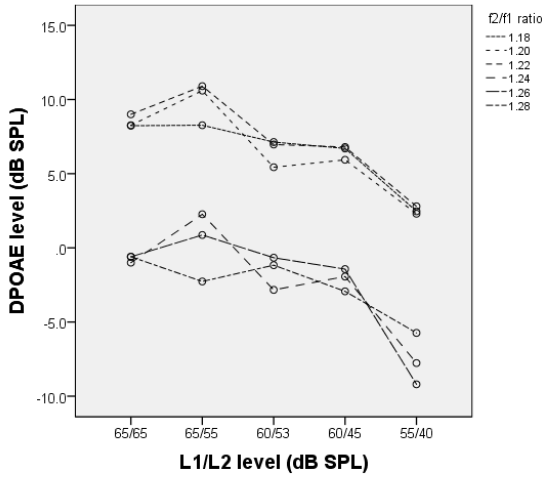
Table 7.1 Participants' tympanometry and pure tone test results

			Tympanometry								Pure Tone Audiometry (Hz)											
			Right ear				Left ear				Right ear						Left ear					
Participant ID	Age	Sex (M/F)	ECV	ml	daPa	Type	ECV	ml	daPa	Type	250	500	1000	2000	4000	8000	250	500	1000	2000	4000	8000
2	18	F	1	0,4	20	A	1	0,4	15	A	5	5	0	0	0	5	5	5	5	0	5	0
3	21	F	1,7	0,7	15	A	1,5	0,7	15	A	5	5	5	5	5	0	5	0	0	5	5	-5
5	22	F	1,6	0,5	-20	A	1,4	0,6	20	A	10	10	0	5	0	0	10	10	0	5	-5	5
6	18	F	0,9	0,7	25	A	0,9	0,9	30	A	0	0	0	5	5	-5	5	0	0	5	10	0
7	23	F	1	0,3	-10	A	0,9	0,3	0	A	5	5	10	5	10	5	0	5	5	0	5	10
9	20	F	1,5	0,6	25	A	1,2	0,6	20	A	-5	0	0	5	0	15	5	-5	10	5	10	5
10	21	F	1,7	0,9	25	A	1,7	0,9	20	A	-5	-5	0	0	10	0	0	-5	10	5	10	5
14	20	F	1,2	0,5	25	A	1,1	0,4	20	A	20	15	5	0	10	15	10	10	0	0	5	15
15	21	F	1,6	1,4	10	A	1,5	1,4	20	A	-10	-10	0	5	5	5	0	-10	5	5	5	5
16	21	F	1,1	1,3	5	A	1,2	1,3	0	A	15	5	5	5	0	5	0	0	5	0	-5	5
33	21	F	1,1	0,6	20	A	1,5	0,8	25	A	-5	0	-5	10	10	5	0	0	5	-5	10	10
36	19	F	0,8	0,4	5	A	0,8	0,3	-5	A	0	-5	0	-5	10	0	5	5	0	0	5	10
38	21	F	1,1	0,8	15	A	1	0,5	15	A	15	10	10	15	15	5	15	10	15	15	15	15
39	21	F	1,2	0,6	15	A	1,3	1,1	15	A	10	5	0	0	0	15	15	10	5	0	0	5
40	23	M	1	1,4	15	A	1,1	1,5	20	A	5	5	0	5	5	10	5	0	5	5	5	10
41	21	M	1	0,7	20	A	1	0,7	20	A	5	-5	0	5	10	0	10	0	0	10	10	0
42	23	M	1,3	1,1	35	A	1,3	1	-15	A	10	5	0	5	0	15	5	5	-5	0	5	5
43	22	F	1,2	1	-15	A	1,2	1,1	-15	A	10	0	0	5	5	15	5	10	-5	10	0	15
44	22	M	1,9	1,3	-5	A	1,7	1,1	-10	A	5	0	0	0	0	10	5	5	0	-5	-5	10
45	23	F	1	0,3	15	A	0,9	0,4	20	A	5	-5	-10	-5	0	-10	0	-10	-5	-10	0	-5

Figure 7.1 shows the DPOAE mean absolute levels for all combinations of f_2/f_1 and L_1/L_2 at each f_2 frequency for the participants at the first assessment occasion. This figure also presents the numbers of ears showing DPOAEs at each of these stimulus combinations. These results showed this study's participants were more likely to show DPOAEs of higher intensity at lower f_2 frequencies.

Right ear	Left ear
2 003 Hz	
	
2 519 Hz	
	

Right ear	Left Ear
3 175 Hz	
	
3 996 Hz	
	

Right ear	Left ear
5 000 Hz	
	
6 996 Hz	
	

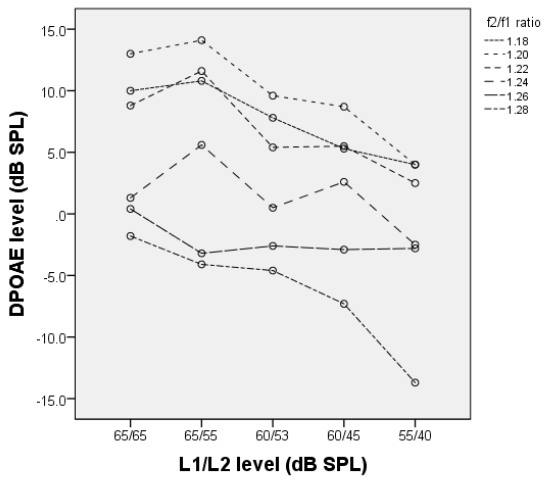
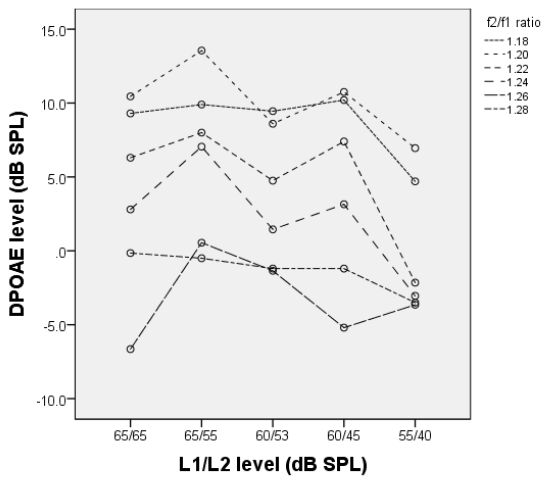
Right ear	Left ear
8 003 Hz	
	

Figure 7.1: Mean distortion product otoacoustic emission absolute levels per intensity and frequency ratio combinations for each frequency (f_2): (a), right ear at 2003 Hz; (b), left ear at 2003 Hz; (c), right ear at 2519 Hz; (d), left ear at 2519 Hz; (e), right ear at 3175 Hz; (f), left ear at 3175 Hz; (g), right ear at 3996 Hz; (h), left ear at 3996 Hz; (i), right ear 5000 Hz; (j), left ear at 5000 Hz; (k), right ear at 6996 Hz; (l), left ear at 6996 Hz; (m), right ear at 8003Hz; (n), left ear at 8003Hz.

Table 7.2 shows the results of the linear mixed model analyses for main effects of level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) settings. For all f_2 values and in both ears, these analyses showed that the 65/55 and 65/65 level settings consistently resulted in higher DPOAE levels across all f_2/f_1 settings, and the 1.18, 1.20 and 1.22 f_2/f_1 settings regularly resulted in higher DPOAE levels across all L_1/L_2 settings.

Table 7.2: Results of the mixed model analyses for statistically significant main effects ($p < 0.05$) of level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) settings.

f_2 (Hz)	Ear	Best L_1/L_2 (for all f_2/f_1 combined)*	Best f_2/f_1 (for all L_1/L_2 combined)*
2003	L	65/55	1.18, 1.20, 1.22
	R	65/55	1.20
2519	L	65/55	1.18, 1.20, 1.22
	R	65/55	1.18, 1.20, 1.22
3175	L	65/65, 65/55	1.18, 1.20, 1.22
	R	65/65, 65/55	1.20, 1.22
3996	L	65/65, 65/55	1.18, 1.20
	R	65/65, 65/55	1.18, 1.20, 1.22
5039	L	65/65, 65/55	1.18
	R	65/65, 65/55	1.18, 1.20
6351	L	65/65, 65/55	1.18, 1.20
	R	65/65, 65/55	1.18, 1.20, 1.22
8003	L	65/55	1.18
	R	65/65, /65/55	1.18, 1.20

L, left; R, right. Where more than one L_1/L_2 or f_2/f_1 is shown, the first value indicates the combination that yielded the highest DPOAE level, the next one the second highest DPOAE level, etc.

Table 7.3 shows the results of the mixed model analyses of all level and frequency settings combined. For all f_2 values and in both ears, these analyses showed that the level (dB SPL) and frequency ratio settings of 65/65 and 1.20, 65/55 and 1.22, 65/55 and 1.20, and 65/55 and 1.18 regularly resulted in higher DPOAE levels compared to other level and frequency ratio combinations.

Table 7.3: Results of the mixed model analyses of all level and frequency settings combined.

f_2 (Hz)	Ear	Best combinations of L_1/L_2 and f_2/f_1 (only combinations showing a best result on at least one occasion are shown)													
		65/65					65/55					60/53		60/45	
		1.26	1.24	1.22	1.20	1.18	1.26	1.24	1.22	1.20	1.18	1.20	1.18	1.20	1.18
2003	L				X			X	X	X	X				

f_2 (Hz)	Ear	Best combinations of L_1/L_2 and f_2/f_1 (only combinations showing a best result on at least one occasion are shown)													
		65/65					65/55					60/53		60/45	
		1.26	1.24	1.22	1.20	1.18	1.26	1.24	1.22	1.20	1.18	1.20	1.18	1.20	1.18
	R				X				X	X					
2519	L						X	X	X	X	X				
	R			X	X			X	X	X	X		X	X	X
3175	L		X	X	X			X	X	X					
	R	X		X	X			X	X						
3996	L			X	X				X	X	X	X	X		
	R			X	X				X		X				
5039	L				X	X				X	X				
	R				X	X				X			X		
6351	L			X	X	X			X		X				
	R					X				X	X		X		
8003	L					X				X	X		X		
	R				X	X			X	X	X				
Counts	-	1	1	6	11	6	1	5	10	11	10	1	5	1	1

Note: Within each f_2 and ear combination, the X's indicate L_1/L_2 and f_2/f_1 stimulus combinations that produced distortion product otoacoustic emissions levels that were significantly ($p < 0.05$) higher than other L_1/L_2 and f_2/f_1 stimulus combinations in that row.

L = left; R = right.

The results of the ICC analysis of DPOAE results obtained for each f_2 value, for right and left ears, and for every L_1/L_2 (dB SPL) and f_2/f_1 stimulus combination are not shown in this article (because of the very high number of the analyses conducted). Instead, Table 7.4 shows for each f_2 value, for right and left ears, the lowest and highest ICC absolute agreement (single) coefficients with their 95% confidence intervals from all L_1/L_2 and f_2/f_1 stimulus combinations returning significant ($p < 0.05$) ICC values. Table 7.4 also shows for each f_2 value, for right and left ears, the L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations that returned insignificant ICC values. No obvious patterns emerged regarding L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations that were more or less likely to

return better or worse ICC results for each f_2. It was noted, however, that more L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations returned insignificant ICC values for f_2 = 8003 Hz, meaning that results at this frequency were more likely to be unreliable, regardless of the L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations used.

Table 7.4: Results of the intraclass correlation coefficient absolute agreement (single) analyses of distortion product otoacoustic emissions results obtained at each f_2 value for each stimulus level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) setting.

f_2 (Hz)	Ear	For L_1/L_2 and f_2/f_1 stimulus combinations returning significant ($p < 0.05$) ICC values:			L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations returning insignificant ($p > 0.05$) ICC (single) values
		Lowest and highest ICC (single) coefficients	95% confidence intervals	L_1/L_2 (dB SPL) and f_2/f_1 stimulus combination	
2003	L	0.43	-0.02 to 0.73	65/65 and 1.22	65/65 and 1.20, 65/65 and 1.28, 65/55 and 1.22, 60/53 and 1.20, 55/40 and 1.20
		0.79	0.53 to 0.92	60/45 and 1.28	
	R	0.41	-0.05 to 0.73	65/65 and 1.28	65/55 and 1.22, 55/40 and 1.22, 55/40 and 1.20, 55/40 and 1.28
		0.91	0.78 to 0.96	65/55 and 1.20	
2519	L	0.39	-0.03 to 0.71	65/55 and 1.18	65/65 and 1.24
		0.932	0.81 to 0.97	55/40 and 1.26	
	R	0.42	-0.01 to 0.72	65/55 and 1.18	55/40 and 1.28
		0.88	0.73 to 0.95	60/53 and 1.18	
3175	L	0.26	-0.23 to 0.64	65/55 and 1.28	65/65 and 1.20, 65/65 and 1.22, 65/55 and 1.24, 60/53 and 1.18, 60/53 and 1.22, 60/45 and 1.24
		0.63	0.26 to 0.85	55/40 and 1.22	
	R	0.43	-0.01 to 0.73	60/53 and 1.22	None

f_2 (Hz)	Ear	For L_1/L_2 and f_2/f_1 stimulus combinations returning significant ($p < 0.05$) ICC values:			L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations returning insignificant ($p > 0.05$) ICC (single) values
		Lowest and highest ICC (single) coefficients	95% confidence intervals	L_1/L_2 (dB SPL) and f_2/f_1 stimulus combination	
		0.92	0.78 to 0.97	60/55 and 1.20	
3996	L	0.41	-0.05 to 0.73	65/55 and 1.18	65/55 and 1.26, 60/53 and 1.24, 55/40 and 1.28
		0.90	0.73 to 0.96	60/45 and 1.26	
	R	0.38	-0.06 to 0.70	65/65 and 1.26	65/55 and 1.28, 60/53 and 1.24, 60/45 and 1.28, 55/40 and 1.28
		0.92	0.80 to 0.97	60/53 and 1.18	
5039	L	0.54	0.08 to 0.81	65/65 and 1.20	60/53 and 1.18
		0.94	0.77 to 0.99	55/40 and 1.26	
	R	0.34	-0.17 to 0.71	60/53 and 1.22	60/45 and 1.28, 55/40 and 1.28
		0.93	0.81 to 0.98	60/45 and 1.22	
6351	L	0.49	-0.10 to 0.83	65/55 and 1.26	60/53 and 1.26, 60/45 and 1.26, 60/45 and 1.28
		0.93	0.67 to 0.99	55/40 and 1.18	
	R	0.50	-0.11 to 0.84	60/53 and 1.22	65/55 and 1.24, 60/53 and 1.26, 60/45 and 1.24, 60/45 and 1.26
		0.94	0.84 to 0.98	65/65 and 1.18	
8003	L	0.47	0.01 to 0.77	60/53 and 1.18	65/65 and 1.18, 65/65 and 1.26, 65/55 and 1.28, 60/53 and 1.20, 60/53 and 1.24, 60/53 and 1.26, 60/53 and 1.28, 60/45 and 1.20, 60/45 and 1.26, 60/45 and 1.28, 55/40 and 1.18, 55/40 and 1.24, 55/40 and 1.26, 55/40 and 1.28
		0.87	0.56 to 0.97	55/40 and 1.22	

f_2 (Hz)	Ear	For L_1/L_2 and f_2/f_1 stimulus combinations returning significant ($p < 0.05$) ICC values:			L_1/L_2 (dB SPL) and f_2/f_1 stimulus combinations returning insignificant ($p > 0.05$) ICC (single) values
		Lowest and highest ICC (single) coefficients	95% confidence intervals	L_1/L_2 (dB SPL) and f_2/f_1 stimulus combination	
	R	0.43	-0.08 to 0.77	65/55 and 1.22	65/65 and 1.24, 65/65 and 1.26, 65/55 and 1.28, 60/53 and 1.24, 60/53 and 1.28, 60/45 and 1.24, 60/45 and 1.28, 55/40 and 1.22, 55/40 and 1.26, 55/40 and 1.28
		0.99	0.79 to 1.00	60/53 and 1.26	

ICC, intraclass correlation coefficient; L, Left; R, Right.

Discussion

Overall, the L_1/L_2 combinations and f_2/f_1 ratios used in this study elicited DPOAEs of varying amplitude and reliability. An L_1/L_2 combination of 65/55 dB SPL appeared to elicit the largest DPOAEs at most f_2 values, followed by an L_1/L_2 combination of 65/65. This finding supports similar findings regarding the L_1/L_2 combinations more likely to elicit larger DPOAEs from human adults (Beattie & Jones, 1998; Vento et al., 2004). Direct comparisons between this study's findings and similar studies in the literature were difficult, however, with many studies in the literature having used higher L_1/L_2 levels than this study (Beattie et al., 2004; Hauser & Probst, 1991; Meinke et al., 2013; Whitehead, McCoy, et al., 1995). These higher L_1/L_2 levels were avoided in this study because of their higher likelihood of eliciting false negative results and artifacts (Carter et al., 2015; Dorn et al., 2001).

The f_2/f_1 ratios of 1.18, 1.20 and 1.22 appeared to elicit the largest DPOAEs at most f_2 values. This finding supports similar findings regarding the f_2/f_1 ratios that are more likely to elicit larger DPOAEs from human adults (Abdala, 1996; Dreisbach & Siegel, 2001; Gaskill & Brown, 1990) as well as supporting previous reports that the best f_2/f_1 ratio appears to decrease as f_2 increases and vice-versa (Abdala, 1996; Dreisbach & Siegel, 2001).

Stimulus parameters using an L_1/L_2 of 65/65 with an f_2/f_1 ratio of 1.20 or an L_1/L_2 of 65/55 with f_2/f_1 ratios of 1.18, 1.20 or 1.22 appeared to elicit the largest DPOAEs at most f_2 values. This result

supports similar findings regarding the L_1/L_2 and f_2/f_1 ratio parameter settings that are more likely to elicit larger DPOAEs from human adults (Beattie & Jones, 1998; Vento et al., 2004).

This study's results do not explain why the largest DPOAEs were elicited using stimulus parameters using L_1/L_2 combinations of 65/65 or 65/55 dB SPL and f_2/f_1 ratios of 1.18, 1.20 or 1.22. Regarding L_1/L_2 combinations, the larger DPOAEs elicited by stimuli with primaries of 65 (i.e., the 65/65 and 65/55 dB SPL level stimuli) could be related to the function of the cochlear amplifier (Harris et al., 1989) as stimuli with lower level primaries (L_1/L_2 levels of 60/53, 60/45 and 55/40 dB SPL) yielded lower level DPOAEs. Such a possibility would be generally consistent with Brown and Gaskill (1990) who reported DPOAE amplitude to depend more on the level of L_1 than L_2. Regarding f_2/f_1 ratios, the larger DPOAEs elicited by f_2/f_1 ratios of 1.18, 1.20 or 1.22 could reflect the cochlea's frequency selectivity and bandpass filter function or properties (Allen & Fahey, 1993). Such a possibility would be generally consistent with Gaskill and Brown (1990) and Harris et al. (1989) who found that DPOAE levels peaked at f_2/f_1 ratios of 1.22 and 1.25, respectively, with a decline with higher or lower f_2/f_1 ratios. Stover, Neely and Gorga (1999) suggested that these declines at higher f_2/f_1 ratios could result from greater separation of the primaries that lessen the interaction of their travelling waves on the basilar membrane, whereas the declines at lower f_2/f_1 ratios could result from less separation of the primaries and greater cancellation of their travelling waves on the basilar membrane.

Although some L_1/L_2 combinations and f_2/f_1 ratios clearly elicited larger DPOAEs, no L_1/L_2 combinations and f_2/f_1 ratios clearly elicited more reliable DPOAEs. This was consistent with previous reports that commonly used sets of stimulus parameters to elicit DPOAEs of similarly varying reliability (Stuart, Passmore, Culbertson, & Jones, 2009; Wagner, Heppelmann, Vonthein, & Zenner, 2008) but inconsistent with reports finding higher L_1/L_2 combinations to elicit more reliable DPOAEs (Franklin et al., 1992; Keppler et al., 2010; Roede et al., 1993). It must be noted that DPOAEs for f_2 = 8003 Hz in this study were most likely to be unreliable. This finding could indicate that any DPOAE recorded at such high f_2 frequencies is likely to be unreliable; however, such a conclusion should be interpreted with caution as varying the location of the probe

microphone has been shown to affect the calibration of the sound source at these frequencies (Siegel, 2002).

Conclusion

The study concluded that further, targeted investigation of the 65/65, 65/55 and 60/53 dB SPL intensity levels and the 1.18, 1.20, 1.22 f_2/f_1 ratios is warranted to determine the best stimulus parameters for eliciting the largest and most reliable DPOAEs in adult humans. These stimulus parameters yielded the largest and most reliable DPOAEs in this study's small sample. It is recommended that a larger sample size be used to obtain sufficient statistical power. In addition, these stimulus parameters should be investigated in individuals with hearing loss of cochlear origin to select the parameters most sensitive to outer hair cell damage.

CHAPTER 8: MAIN STUDY WITH HEALTHY, NORMAL HEARING PARTICIPANTS

This chapter presents phase 3 of the thesis in the form of an article that will be submitted to the *International Journal of Audiology*, with the methods section expanded to provide a full discussion of the methods used. For completeness, some repetition of the methodology was inevitable.

Towards the preferred stimulus parameters for distortion product otoacoustic emissions in adults

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Keywords: Distortion product otoacoustic emissions (DPOAEs), stimulus parameters, adults, reliability

List of abbreviations:

dB – decibel, DPOAE – Distortion Product Otoacoustic Emission, Expt – experiment, F – female, ICC – intraclass correlation coefficient, HL – hearing level, L – left, M – male, MMA – mixed model analysis, n – number, NH – normal hearing, R – right, SD – standard deviation, SEM – standard error of the mean, SPL – sound pressure level.

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Abstract

Objective: To determine which stimulus parameters elicit the largest and most reliable distortion product otoacoustic emissions (DPOAEs) in adult humans. *Design:* A single group, repeated measures design. *Study Sample:* A convenience sample of 65 participants, i.e., 39 females and 26 males, aged 19 to 28 years with pure tone hearing thresholds within normal limits. *Method:* With a repeated measures design, participants were tested on two occasions with tympanometry, standard pure tone audiometry and DPOAEs, 10 to 14 days apart. DPOAE stimulus parameters included f_2/f_1 ratios of 1.18, 1.20 and 1.22 and L_1/L_2 settings of 65/65, 65/55, and 60/53 dB SPL at f_2 frequencies between 2 to 8 kHz. *Results:* Descriptive statistics and mixed model analysis showed stimulus intensity levels L_1/L_2 of 65/55 dB SPL, and f_2/f_1 ratios of 1.18 and 1.20, elicited the largest DPOAEs. In terms of reliability, intraclass correlation coefficient analyses showed no difference among stimulus parameters. *Conclusion:* The study concluded that different stimulus parameters ought to be used when eliciting DPOAEs at mid frequencies vs. high frequencies (i.e., f_2 > 5000 Hz). However, further investigation in individuals with hearing loss is warranted to determine which stimulus parameters are most sensitive and specific in detecting outer hair cell damage.

Keywords

Distortion product otoacoustic emissions (DPOAEs), stimulus parameters, adults, reliability

Introduction

Distortion product otoacoustic emissions (DPOAEs) are known to be sensitive and specific to damage to the outer hair cells (OHCs) of the cochlea. Consequently, DPOAEs have been effectively used in a range of applications including new-born hearing screening, diagnostic audiological assessment and ototoxicity monitoring (Dhar & Hall, 2012). Despite these successful applications, the ideal/optimal parameters for eliciting DPOAEs in the clinic remain unconfirmed (Petersen et al., 2017). Determining the optimal parameters is needed to enhance the reliability,

sensitivity and specificity of DPOAEs across all of its applications (Hauser & Probst, 1991; Whitehead, McCoy, et al., 1995).

For research purposes, a wide range of stimulus parameters have been used to elicit DPOAEs in human subjects. These parameters have included f_2/f_1 ratios ranging from 1.03 to 1.79, and L_1/L_2 combinations ranging from 30/30 to 85/85 dB SPL (Petersen et al., 2017). For diagnostic and/or ototoxicity monitoring applications, the recommendations for f_2/f_1 ratios have ranged from 1.20 to 1.22 and L_1/L_2 combinations of 45/35 to 65/55 dB SPL (Dhar & Hall, 2012). For screening purposes 1.20 has been suggested as the desired f_2/f_1 ratio, with L_1/L_2 either 65/55 or 65/65 dB SPL (Dhar & Hall, 2012; Hall, 2000). Numerous authors such as Dhar & Hall (2012) have indicated that stimulus intensities of L_1/L_2 = 65/55 dB SPL have the twofold advantage of yielding a higher DPOAE level and enhanced sensitivity to detect OHC dysfunction. These authors also argue that L_1/L_2 intensities above 70 dB SPL yield questionable responses and may result in response artifacts that can be mistaken for DPOAEs.

Although a vast amount of research into DPOAE stimulus parameters has been conducted to date, no single set of DPOAE stimulus parameters has been identified as being optimal for eliciting DPOAES from human subjects (Petersen et al., 2017). This gap could be due to the complexities of DPOAEs and how different stimulus parameter settings interact with factors including DPOAE fine structure amplitude, reliability, sensitivity and specificity. For example, Hall (2000) reported that for OHC dysfunction, decreasing the stimulus levels enhanced DPOAE sensitivity whereas increasing stimulus levels enhanced DPOAE specificity. Similarly, Dreisbach and Siegel (2001) reported the optimal f_2/f_1 ratio varies as a function of frequency, with smaller frequency ratios eliciting higher DPOAE levels at high frequencies, and vice versa.

A vital shortcoming in many of the studies investigating DPOAE stimulus parameters to determine which combinations yield the highest level DPOAEs has been the lack of manipulating both intensity and frequency ratio parameters in the same study. In this regard, most studies have fixed the frequency ratio to one value while manipulating stimulus level, e.g., Beattie and Ireland (2000), Chida et al. (2001) and Kummer et al. (2000). When both the stimulus level and frequency ratio were manipulated, studies most often had small sample sizes (Abdala, 1996, 2000; Bonfils

& Avan, 1992; Londero et al., 2002; Petersen, Wilson, & Kathard, 2018). In addition, several studies, including seminal research, used no inferential statistics when determining the optimal stimulus parameters (Gaskill & Brown, 1990; Harris et al., 1989; Meinke et al., 2013). This prevents the full investigation of stimulus parameter interactions when seeking to determine the ideal/optimal parameters for eliciting DPOAEs (Petersen et al., 2017).

Apart from determining the stimulus parameters that elicit the highest level DPOAEs, it is also important to consider which stimulus parameters result in the most reliable DPOAEs. Such a finding could enhance the test-retest reliability of DPOAEs for diagnostic purposes (Stuart et al., 2009). A systematic review found 10 studies that manipulated stimulus parameters to determine the influence on DPOAE reliability (Petersen et al., 2017). Of the reviewed studies, nine manipulated the stimulus intensity pair, but kept the frequency ratio constant. Only one study manipulated the frequency ratio, but kept the stimulus intensity constant at L_1/L_2 = 65/60 dB SPL (Moulin, 2000a).

Thus, the aim of this study was to test a range of recommended DPOAE stimulus parameters that manipulated both stimulus level and frequency ratio to determine which parameters elicit the (1) largest and (2) most reliable DPOAEs in a sample of healthy, adult humans.

Method

Aim of phase 3

With the stimulus parameters obtained in phase 2, the aim of the third phase was to systematically investigate stimulus parameter combinations to determine which intensity and frequency ratio combinations that yielded the highest DPOAE level and most reliable DPOAEs in normal-hearing, healthy adults.

The aim in phase 3 was achieved by the following:

(1) Comparing the absolute DPOAE levels elicited for all stimulus conditions.

(2) Comparing the medium-term test-retest reliability of DPOAEs elicited for all stimulus combinations.

Research design

A single group, repeated measures design was used for this study (Maxwell & Satake, 2006). This design was deemed appropriate to examine the influence of intensity and frequency ratio stimulus parameters on DPOAE levels. With a repeated measures design, it was possible to establish the highest level and test-retest reliability of DPOAEs in the same participants, where the participants acted as their own controls.

A known disadvantage of using a repeated measures design is the possibility of a learning effect (Howell, 1999). However, learning effects do not influence the DPOAE test, as it is an objective measure that does not require active participation of the participant.

Participants

Inclusion criteria

All participants:

(a) were between 18–30 years of age, to minimise age-related deterioration of the cochlea (Gates & Mills, 2005; Schuknecht, 1955);

(b) had normal non-diagnostic otoscopic results to rule out possible outer or middle ear problems that could obscure measurement of cochlear outer hair cell function (Hall, 2000);

(c) had normal hearing, which is defined as thresholds of ≤ 15 dB hearing level (HL) (Clark, 1981, in Katz, 2002) for the frequency range 250Hz to 8000Hz;

(d) had normal middle ear functioning, which is defined as a Type A tympanogram, with compliance between 0.3 and 1.4 cm^3, middle ear pressure ranging from –150 daPa to 100 daPa and ear canal size between 1.0 and 2.0 cm^3 (Grason-Stadler, 2003) because

OAEs cannot be reliably measured in the presence of a middle ear abnormality (Hall, 2000);

(e) had no self-reported history of hereditary hearing loss, significant ear disease, ear surgery or long-term noise exposure; and

(f) had no medical condition that could affect the audiological test results negatively (Hall, 2000).

Participant description

Seventy-four participants were conveniently sampled from the staff and student population of the University of Cape Town, South Africa. Two individuals did not pass the OAE screening, and four did not have OAEs from 3000 Hz upwards when doing diagnostic OAEs and three individuals did not return for the second test. Thus, the study sample consisted of 65 healthy, otologically normal adult participants (39 females, 26 males, aged 19 to 30 years).

Recruitment and sampling

Staff and students were recruited from the University of Cape Town through advertisements distributed via official emails from the relevant departments, Vula – the university's online learning platform and notice boards on the university premises (See Appendix D). Convenience sampling (Katzenellenbogen, Joubert & Abdool Karim, 1997) was employed by accepting each person who met the selection criteria and was willing to participate in the study.

Data collection

Instrumentation

All audiological tests were conducted in a sound treated booth and/or in a quiet room in the Audiology Clinic at the University of Cape Town.

A Welch Allyn otoscope was used for non-diagnostic otoscopic examinations. A GSI Tympstar was used for tympanometry. A GSI 61 audiometer and TDH-39 earphones with standard frequency testing capability were used for pure tone audiometry.

DPOAEs were collected with the GSI Audera Version C, a commercially available piece of equipment, which can reliably evaluate emissions up to 8 kHz. A Brüel & Kjær 2238 class 1 handheld sound level meter was used to monitor sound levels in the quiet room.

All the equipment was calibrated according to the relevant specifications, prior to data collection. In addition, a biological check of all the equipment was done prior to testing.

Procedure

After ethics approval and written permission had been received, written informed consent was obtained from each participant (See Appendix E). Biographical information obtained included date of birth, age, sex and medical history (including noise exposure).

The researcher verbally conveyed information regarding the purpose, protocol and requirements of the study in the participant's language (English or Afrikaans), which was also shared with each participant in written form. If a participant preferred isiXhosa, a mother-tongue isiXhosa research assistant explained the study to the patient. IsiXhosa-speaking participants received written documentation in isiXhosa. All discrepancies and uncertainties were addressed.

Participants were initially screened for inclusion in the study using a live voice interview, non-diagnostic otoscopy, conventional frequency pure tone audiometry, tympanometry and DPOAE screening. To pass the DPOAE screening, the participants had to show $2f_1$-f_2 DPOAEs at least 3 dB above the noise floor at f_2 frequencies 2, 4 and 8 kHz to tonal stimuli with an f_2/f_1 ratio of 1.2 and an L_1/L_2 setting of 65/55 dB SPL (Lonsbury-Martin et al., 1990). All initial testing was conducted in a sound-treated booth meeting South African National Standards (2006).

The following tests were conducted at baseline and the follow-up session: otoscopy, tympanometry, PTA and DPOAEs. Standard pure tone audiometry was conducted at baseline, and at the subsequent test session to rule out any possible changes in hearing thresholds. The follow-up session took place within 10 to 14 days post-baseline. If the potential participant's hearing was normal, he/she continued with the rest of the study. If a hearing loss or ear abnormality was detected, the researcher explained the results to the participant. In addition,

the researcher also provided information and emotional counselling to the participant, who was referred to the relevant health practitioner for management.

Before commencing the test protocol, the researcher explained the nature of all test procedures to the participants to familiarise them with the session. Participants were then given an opportunity to seek clarification and also reminded that questions could be asked at any stage during data collection. Participants were reminded that they could withdraw at any point in the study.

Participants were seated in a comfortable chair for the duration of the tests. A glass of water was provided. Testing was stopped in the case of coughing or sneezing and continued after coughing/sneezing ceased. Breaks in testing were provided for between tests.

Clear instructions were given before each procedure and repeated during the test if necessary. The same instructions were given to all participants to improve reliability.

The instructions used for pure tone testing were: “You will hear soft sounds. Each time you hear a sound, please raise your hand immediately. Even if the sound is very soft and you can barely hear it, you must still raise your hand. We will start with your right ear.” If it was deemed necessary, the instructions were repeated during testing.

For pure tone audiometry, the modified Hughson-Westlake technique was used to obtain thresholds from 250 to 8 000 Hz.

Before DPOAE measurement, participants were instructed as follows: “I am going to place a probe in your ear. It will not be painful. Your ear will just feel blocked up. Please remain quiet and still while the probe is in your ear. You do not have to do anything, even if you hear a sound. You can sit back and relax.”

Distortion product otoacoustic emissions testing was conducted in a quiet room with background noise levels < 55 dBA (Lee & Kim, 1999). Ambient noise levels were measured before and during DPOAE testing. In-ear calibration was completed before each test. The $2f_1$-f_2 DPOAE measurements were obtained from each ear of each participant using the following stimulus

parameters: f_2/f_1 ratios – 1.18, 1.20, and 1.22; L_1/L_2 settings – 65/65, 65/55, and 60/53 dB SPL (these stimulus parameters were determined during the preliminary study in phase 2); and f_2 frequencies: 2003, 2519, 3178, 3996, 5000, 6996 and 8003 Hz. The stimulus intensity pair of 65/55 dB SPL was included, as it is the pair used most regularly in clinics. The rest of the L_1/L_2 settings were calculated using the paradigm suggested by Kummer et al. (1998) to optimise stimulus levels, namely $L_1 = 0.42L_2 + 39$. To mitigate potential order effects, a single sequence of stimulus parameters was set, and each participant was started at a different point in this sequence. The order of ear testing was reversed for each sequential participant. The following DPOAE data were recorded from each participant for each set of stimulus parameters at each f_2 frequency on each test occasion: absolute level of DPOAE, absolute level of the noise floor and the DPOAE SNR, calculated as the absolute level of the DPOAE minus the level of the noise floor. The $2f_1$-f_2 DPOAEs were sampled until at least one of the two stopping rules was met: (1) the noise floor at the distortion product frequency was less than -10 dB SPL, or (2) until 32 s of artifact-free sampling had been averaged (Dille et al., 2010). DPOAEs were recorded at least twice per stimulus parameter combination, for replication purposes. Participants were seated in a comfortable chair and were instructed to remain still and quiet during the DPOAE test procedure with breaks provided as required. The DPOAE test time per participant was approximately 90 minutes per test occasion. Each participant underwent DPOAE testing on two occasions, i.e., at baseline and between 10 to 14 days later.

Reliability and validity of data collection

Possible sources of error in measurement in this study include the instrument, tester, participant and the environment.

In order to enhance reliability in instrumentation, the necessary system calibration was done prior to data collection. In addition, daily calibration was done by inserting the DPOAE probe into a hard-walled cavity and measuring the response according to manufacturer specifications. Biological checks were conducted before each data collection session, and a visual probe inspection was done prior to testing each participant.

The way of testing each participant was standardised, so that the measurements took place in the same manner across participants and test intervals. Sufficient training reduced the error variance (Streiner & Norman, 2008). By following the same measurement procedure with each participant, it was hoped that intra-tester and inter-tester reliability would be enhanced. The researcher and research assistants underwent a training period prior to data collection to become familiar with the equipment and the measurement procedure.

To minimise participant variability, the test time was kept as short as possible. Data collection took place at the same time of the day for participants, as far as possible.

The ambient noise level was controlled by conducting all testing in a soundproof booth or a sound-treated booth. A possible source of equipment noise is the computer used to collect the data, which was therefore placed as far as possible from the participant.

Face validity refers to whether the instrument appears to assess the aspect of interest (Streiner & Norman, 2008). Experts like Dreisbach and Siegel (2001) believe that DPOAEs measure outer hair cell function of the cochlea. The DPOAEs were present in all participants of the current study, and it can be surmised that their cochleae functioned normally as they all presented with normal hearing.

Content validity implies that the instrument covers all domains of the phenomenon under investigation (Maxwell & Satake, 2006). With DPOAEs, two signals are sent into the ear at an intensity ≤ 70 dB SPL that elicits active cochlear activity. This activity is measured in the ear canal via a sensitive microphone. The stimulus intensities were monitored throughout the test procedure while DPOAEs were recorded, to ensure that the intended L_1/L_2 were delivered into the ear canal.

Criterion validity of DPOAEs has been evaluated and determined in patient populations (patients with cancer, cystic fibrosis, etc.) receiving ototoxic medication, e.g., cisplatin and aminoglycosides.

Data management

All data was imported into Excel spreadsheets. A research assistant checked 10% of the data to assure that data had been imported accurately. If data entry errors were detected, the whole dataset was rechecked. Spreadsheets were uploaded to Microsoft OneDrive, an online storage space, to a password protected account. Only the researcher had access to the password. The data will be kept for a minimum of five years, as per the South African Medical Research Council's recommendations (SAMRC, 2018).

Data analysis

All DPOAE data were found to meet parametric assumptions following examination of these data's histograms, box-and-whisker plots and Q–Q plots (data not shown). Descriptive statistics were calculated for all DPOAE measures, and correlation analyses were conducted to determine if the DPOAE results for the left and right ears were related. As these analyses showed significant correlations in DPOAE results between the ears, all further analyses of the DPOAE data were conducted for each ear separately.

Two sets of linear mixed model analysis (MMA) were conducted at the 5% significance level on the DPOAE data for each f_2 value separately. Each set of analyses considered DPOAE amplitudes as dependent variables, the stimulus level combinations and frequency ratios as fixed effect independent variables, and the participants as a random effect independent variable. The first set of analyses sought to identify the presence of any main effects of level settings (L_1/L_2 in dB SPL) for all f_2/f_1 settings combined and any main effects of frequency ratio settings (f_2/f_1) for all L_1/L_2 settings combined. The second set of analyses sought to identify the presence of any main effects of the combined level (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) settings.

Finally, two-way, mixed-model, intraclass correlation coefficient (ICC) analyses for absolute agreement were conducted at the 5% significance level on the DPOAE data for each f_2 value separately to determine the level of agreement (reliability) of the absolute levels of the DPOAE recordings from the first to the second assessment occasions for each combined level (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) setting separately. The ICC examines the relationship among

variables that share both their metric and variance, i.e. the variables are of a common class and would be appropriate to determine correlations between repeated measures of the same test (McGraw & Wong, 1996).

All statistical analyses were conducted using IBM SPSS Statistics versions 23 and 24 (64-bit edition).

Ethical considerations

Ethics approval was obtained from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town before initiating the study (HREC/REF: 512/2013; See Appendix A). Permission was obtained from the Department of Student Affairs and the Human Resource Department at the university prior to data collection. This study complied with the principles set out by the Declaration of Helsinki (World Medical Association, 2013).

Autonomy refers to the competent individual's right to self-determination (Katzenellenbogen et al., 1997). Providing the participants with adequate information to give informed consent to participate in the study adhered to this principle of autonomy and safeguarded their freedom of choice.

To facilitate understanding of the study, information was presented in the participant's first language (Afrikaans, English, Xhosa). Participants were given the opportunity to ask for clarification at any time during the study. It was also explained to the participant that participation in the study was voluntary and that he/she could withdraw at any stage of data collection. The participants were informed that refusal to participate in or withdrawal from the study would not affect their lives. Participants were required to sign a consent form if they agreed to take part in the study (See Appendix E).

The ethical principle of beneficence requires that the actions of the researcher are aimed at improving the well-being of the participant (Katzenellenbogen et al., 1997). If any hearing or auditory abnormalities were detected, the participant was referred to the relevant health practitioner.

Non-maleficence is the principle that refers to the researcher's obligation to not do harm (Katzenellenbogen et al., 1997). No procedures were used that could harm the participants, and all tests were non-invasive. All necessary equipment, e.g., probe tips and earphones, was cleaned with alcohol swabs after use with each participant, to ensure that bacteria were not spread from one participant to the next. The researcher washed her hands with an antiseptic agent for the same reason. The researcher took all reasonable steps to ensure the participants' comfort.

The ethics principle of justice can be interpreted to mean a fair distribution of benefits of the research (Katzenellenbogen et al., 1997). Therefore, all eligible individuals were included in the study, provided they met the selection criteria.

There were no direct benefits for the participants of the study, other than detecting possible hearing or auditory damage. There were no known risks for participants. Participants received compensation of R100 per session for travel costs and inconvenience (each test session took 60 to 90 minutes).

Confidentiality was respected at all times and all participants' details and results were kept confidential and anonymous by assigning a number to each participant, which corresponded with the order of enrolment in the study. This number was used on all documentation pertaining to the study. Identifying information was only known to the researcher and was kept separate from collected data in a locked filing cabinet in the researcher's office. The researcher also assured the participants that no identifying information would appear in any publication or presentation forthcoming from this study.

Researcher experience implies that the researcher is competent to conduct the research (World Medical Association, 2013). The researcher is professionally qualified as an audiologist and has sufficient theoretical and clinical expertise to conduct the research. The researcher acted in an accountable and responsible manner and upheld professional standards.

Results

Figure 8.1 shows the DPOAE mean absolute levels for all combinations of f_2/f_1 ratio and L_1/L_2 at each f_2 frequency for the participants at the first assessment occasion. The figure illustrates that participants were more likely to show DPOAEs of higher intensity at the lower f_2 frequencies, i.e., 2006–3175 Hz, regardless of the primaries and f_2/f_1 ratios used.

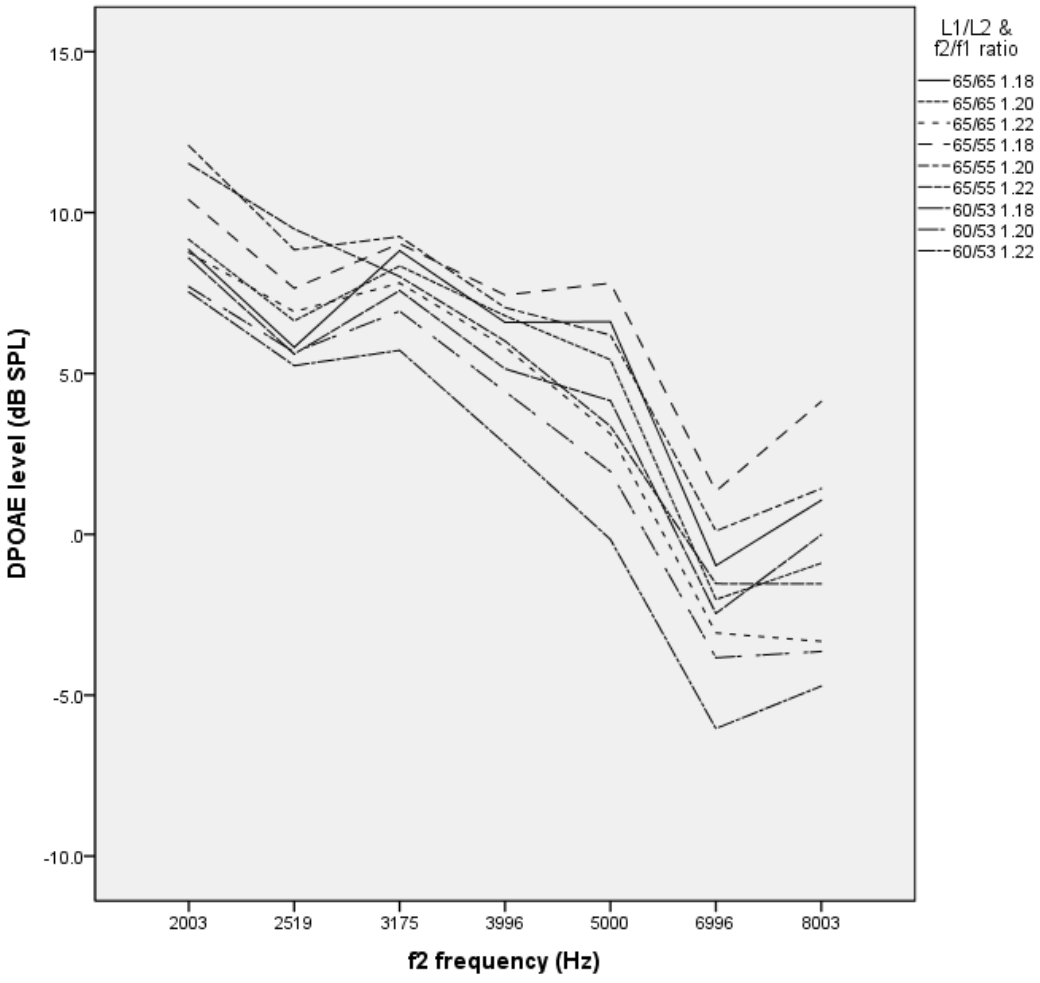

Figure 8.1: Mean DPOAE absolute levels per intensity and frequency ratio combinations for each frequency (f_2)

The MMA detected a significant main effect of stimulus intensity level (L_1/L_2) and ratio (f_2/f_1) combination, $F(8, 7618.80) = 57.37$, $p < .001$, a significant main effect of stimulus frequency (f_2) $F(6, 7618.63) = 496.27$, $p < .001$, and a significant interaction effect, $F(48, 7618.48) = 3.35$, $p < .001$.

For the main effect of level-frequency ratio combination, post-hoc analyses revealed that across all f_2 values, the L_1/L_2 level of 65/55 and f_2/f_1 ratio settings of 1.18 and 1.20 resulted in higher DPAOE intensities compared to other level and frequency ratio combinations. To explore the interaction effect, post-hoc comparisons were conducted to determine which combination of f_2/f_1 and L_1/L_2 resulted in the highest DPAOE amplitude.

Table 8.1 shows the results of the post-hoc analyses investigating, at each f_2 value, the level and frequency ratio combinations that resulted in the highest DPOAE amplitudes. For all f_2 values, these analyses showed the level (dB SPL) and frequency ratio settings of 65/55 1.18, 65/55 1.20, and 65/55 1.22 regularly resulted in larger DPOAE amplitudes compared to other level and frequency ratio combinations. As can be seen in table 8.1, the f_2/f_1 ratios of 1.20 and 1.22 yielded the highest level DPOAEs at frequencies ≤ 3 996 Hz, and 1.18 at frequencies ≥ 5 039 Hz.

Table 8.1. Best level and frequency ratio combinations at each f_2 value

f_2 (Hz)	Best L1/L2 f_2/f_1 combination/s
2003	65/55 1.20 and 65/55 1.22
2519	65/55 1.20 and 65/55 1.22
3175	All except 60/53 1.20 and 1.22
3996	All except 60/53 1.18, 1.20 and 1.22
5039	65/65 1.18 and 65/55 1.18
6351	65/55 1.18
8003	65/55 1.18

The results of the ICC analysis of DPOAE results obtained for each f_2 value, and for every L_1/L_2 (dB SPL) and f_2/f_1 stimulus combination are not shown in this paper (due to the high number of these analyses conducted). Instead, Table 8.2 shows for each f_2 value, the lowest and highest ICC absolute agreement (single) co-efficients with their 95% confidence intervals. These results show

moderate to very strong relationships between the test intervals for each of the stimulus parameters used. For each f_2 value, none of the L_1/L_2 (dB SPL) & f_2/f_1 stimulus combinations returned insignificant ICC values.

Table 8.2. ICC absolute agreement (single) analyses of DPOAE results obtained at each f_2 value stimulus level (L_1/L_2 in dB SPL) and f_2/f_1 ratio setting.

	For L_1/L_2 and f_2/f_1 stimulus combinations returning significant ($p<0.05$) ICC values:		
f_2 (Hz)	Lowest and highest ICC (single) co-efficients	95% confidence intervals	L_1/L_2 (dB SPL) & f_2/f_1 stimulus combination
2003	.514 .739	.360 - .656 .627 - .828	65/65 1.22 65/55 1.20
2519	.542 .784	.391 - .678 .687 - .859	65/65 1.22 60/53 1.18
3175	.488 .749	.331 - .635 .642 - .835	65/55 1.22 65/55 1.20
3996	.536 .753	.387 - .673 .647 - .837	65/65 1.22 65/55 1.20
5039	.519 .808	.357 - .666 .720 - .875	60/53 1.22 60/53 1.18
6351	.690 .850	.560 - .797 .777 - .905	60/53 1.20 65/55 1.18
8003	.562 .703	.406 - .701 .580 - .803	60/53 1.20 65/55 1.18

Discussion

Regarding frequency ratio, the study results show that typically f_2/f_1 of 1.20 and 1.22 yielded higher DPOAE levels at the lower frequencies (i.e., f_2 = 2003–3996 Hz) and 1.18 at the higher frequencies (i.e., 5039–8003 Hz). These results are in line with previous studies (Abdala, 1996; Dreisbach & Siegel, 2001; Harris et al., 1989), whose findings also indicate the f_2/f_1 ratio that elicits the highest DPOAE levels decrease with increased stimulus frequency.

These findings suggest that the changing frequency ratio is representative of the bandpass function in the human cochlea (Harris et al., 1989), and gives an indication of this organ's

frequency selectivity (Brown et al., 1993). Thus, the decreasing f_2/f_1 ratio with increasing stimulus frequency could indicate the presence of sharper mechanical tuning in the high frequencies (Dreisbach & Siegel, 2001). The change in the optimal frequency ratio with different stimulus frequencies provides evidence for the tonotopic organisation of the basilar membrane in the cochlea, and relates to critical band theory (Loven, 2009).

Animal studies have also produced evidence of considerable differences in cochlear mechanics in the base versus the apex in mammals like gerbils and guinea pigs (Cooper & Rhode, 1997; Ohlemiller & Siegel, 1994). So, for example, Ohlemiller and Siegel (1994) have demonstrated that different stimulus coding strategies are employed in the basal and apical cochlear regions of the Mongolian gerbil, which is evident by the differing suprathreshold response properties to tonebursts and the distribution of spontaneous firing rates.

In this study, f_2/f_1 of 1.20 and 1.22 which are the frequency ratios typically used in clinical settings, (Dhar & Hall, 2012), did not always elicit the highest DPOAE level at all frequencies, with reference to the highest frequencies tested specifically. Thus, it can be postulated that using a variable f_2/f_1 ratio during clinical testing might be desirable. This suggestion is feasible, as most commercial equipment for diagnostic DPOAEs allows for the manipulation of stimulus parameters and creating custom test sets (e.g., GSI Audera and MADSEN Capella[2]).

The results of the study showed that the DPOAE levels decreased as the stimulus frequencies increased. Therefore, in order to elicit the highest level DPOAEs, it is important to optimise the stimulus parameters. Based on the results of the current study, f_2/f_1 ratios of 1.20 and 1.22 are recommended for f_2 stimulus frequencies from 2000 to 4000 Hz, whereas at stimulus frequencies ≥ 5000 Hz, an f_2/f_1 ratio of 1.18 is recommended.

Regarding intensity, the current study's findings are in accordance with those that found the higher intensities of L_1/L_2 elicited the highest level DPOAEs (Beattie & Jones, 1998; Bian & Chen, 2008, Dhar et al, 1998). DPOAE levels are highest with L_1 greater than L_2, for L_1 of 65 dB SPL or lower (Gaskill & Brown, 1990; Whitehead, Stagner, et al., 1995) which was also the case in the current study. Thus, for clinical purposes, stimulus intensity levels of L_1/L_2 = 65/55 are

recommended to elicit high level DPOAEs, which are in accordance with the results of previous studies (Gaskill & Brown, 1990; Vento et al., 2004)

In terms of reliability, none of the stimulus parameters yielded any more or less reliable results than the other, which is in accordance with previous findings (Dreisbach 2006, Stuart, 2009, Wagner et al, 2008). However, Keppler found L_1/L_2 = 75/70 dB SPL to be the most reliable. Franklin (1992) also found 75/75 dB best in the short term, and 65/55 best in the long term. A possible reason for the difference is that Franklin (1992) and Keppler (2010) both used stimulus parameters ≥ 75 dB SPL, whereas the other studies, including the current one, used stimulus intensity levels ≤ 70 dB SPL. In addition, when higher level stimulus intensities are used, artifacts are generated in addition to the DPOAEs. These passive elements are not influenced by physiological changes in the ear and could also explain the higher repeatability of DPOAEs elicited by stimulus intensities of L_1/L_2 > 65 dB SPL (Keppler et al., 2010).

Conclusion

Based on the results of the current study, it is proposed that stimulus intensity levels of L_1/L_2 = 65/55 or 65/65 dB SPL should be used in clinical settings. In addition, it seems advisable to use frequency ratios of 1.20 or 1.22 at stimulus frequencies ≤ 4000 Hz, and 1.18 at higher frequencies up to 8000 Hz.

The current study only included normal hearing participants. In future studies, participants with hearing loss acquired through exposure to ototoxic medication may be used to determine whether these stimulus parameters yield the most sensitive and specific results in ears with damaged outer hair cells.

CHAPTER 9: STUDY WITH PARTICIPANTS WITH MDR-TB RECEIVING OTOTOXIC MEDICATION

This chapter presents phase 4 of the thesis as a paper that will be submitted to the *International Journal of Audiology*, with the methods section expanded to provide a full discussion of the methods used. For completeness, some repetition of the methodology was inevitable.

Towards the preferred stimulus parameters for distortion product otoacoustic emissions in adults with MDR-TB receiving ototoxic medication

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List of abbreviations:

dB – decibel, DPOAE – Distortion Product Otoacoustic Emission, Expt – experiment, F – female, ICC – intraclass correlation coefficient, HL – hearing level, L – left, M – male, MMA – mixed model analysis, MDR-TB – multi-drug resistant tuberculosis, n – number, NH – normal hearing, R – right, SD – standard deviation, SEM – standard error of the mean, SPL – sound pressure level.

Abstract

Objectives: In adults with multi-drug resistant tuberculosis (MDR-TB) receiving ototoxic medication to determine which stimulus parameters elicit the (1) largest distortion product otoacoustic emissions (DPOAEs) and (2) the most sensitive and specific DPOAEs. *Design:* A single group, repeated measures design. *Study Sample:* The study consisted of a convenience sample of 21 participants with MDR-TB receiving kanamycin, i.e., 15 females and 6 males, aged 18 to 46 years with conventional pure tone hearing thresholds within normal limits. *Method:* With a

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repeated measures design, participants were tested on two occasions with tympanometry, standard pure tone audiometry, high frequency pure tone audiometry (up to 16 kHz) and DPOAEs, 10 to 14 days apart. DPOAE stimulus parameters included f_2/f_1 ratios of 1.18, 1.20 and 1.22 and L_1/L_2 settings of 65/65, 65/55, and 60/53 dB SPL at f_2 frequencies between 2 to 8 kHz. *Results:* Descriptive statistics and mixed model analysis showed stimulus intensity levels L_1/L_2 of 65/55 dB SPL and 65/65 dB SPL, and f_2/f_1 ratios of 1.18 and 1.20, elicited the largest DPOAEs. The stimulus combination of L_1/L_2 = 65/55 dB SPL f_2/f_1 = 1.18 showed the largest reduction in DPOAE levels between baseline and the second test interval. *Conclusion:* The study concluded that for ototoxicity monitoring, it is advisable to use stimulus parameters of L_1/L_2 = 65/55 dB SPL and f_2/f_1 = 1.18 to detect outer hair cell damage the soonest, in conjunction with high frequency audiometry.

Keywords

Distortion product otoacoustic emissions (DPOAEs), stimulus parameters, validity, sensitivity, Multi-drug resistant tuberculosis (MDR-TB), ototoxicity

Introduction

Multidrug-resistant tuberculosis (MDR-TB) continues to pose a serious health challenge worldwide (Asgedom, Teweldemedhin, & Gebreyesus, 2018). The World Health Organization estimates that 558 000 new MDR-TB cases were detected in 2018 worldwide (World Health Organization, 2018a).

MDR-TB is more difficult to treat than drug-susceptible TB (World Health Organization, 2019b). Until as recent as 2017, aminoglycosides like kanamycin and amikacin, as well as capreomycin (a polypeptide antibiotic) were included in the treatment regimen for most MDR-TB patients in South Africa. These drugs are also used routinely in other countries like India, China, and Russia (Ministry of Health with Family Welfare, 2017; World Health Organization, 2019b; Yunusbaeva et al., 2019), which carry the largest share of the global burden of MDR-TB (World Health Organization, 2019a).

These drugs are known to be ototoxic and cause irreversible damage to the hearing structures within the cochlea (Duggal & Sarkar, 2007; Reavis et al., 2011). These ototoxic drugs enter the cochlear fluids via the bloodstream and result in intracellular morphological and biochemical changes in the cochlea, first affecting the basal outer hair cells and supporting cells (Henley et al., 1996; Schellack & Naude, 2013). The basal end of the cochlea is responsible for processing high frequency information. With prolonged use of ototoxic drugs, damage progresses toward the apical section of the cochlea responsible for transducing low frequency information (Barclay & Begg, 1994; Hashino, TinHan, & Salvi, 1995).

Changes occurring in cochlear function due to outer hair cell (OHC) damage in humans are most likely permanent since it has not been unequivocally determined that the OHCs are able to regenerate (Konrad-Martin et al., 2005). Since the damage starts in the high frequencies, the OHC damage might not be noticed by the individual, especially in the early stages (Fausti et al., 1984). When the person detects a hearing loss, considerable OHC damage has already occurred and communication ability may be impaired (Fausti et al., 1994).

To minimise the effect of OHC damage on a person's quality of life, early detection is key. Currently, serial testing of high frequency behavioural pure tone thresholds is the gold standard for ototoxicity monitoring (ASHA, 1994). However, patients with MDR-TB are often too ill to participate reliably when active participation is required, as is the case with pure tone threshold testing (Reavis et al., 2011).

An objective test, distortion product otoacoustic emissions (DPOAEs), is known to be sensitive and specific to damage to the outer hair cells of the cochlea (Lonsbury-Martin & Martin, 2002). It is, therefore, potentially a useful tool in the monitoring of ototoxicity, specifically when medication known to damage the cochlea is used.

It is known that the DPOAE stimulus parameters influence the test's ability to detect OHC damage. For example, Hall (2000) reported DPOAE sensitivity for cochlear lesions is improved when stimulus intensity levels are decreased. On the other hand, when the stimulus intensity levels are increased, DPOAE specificity is improved (Hall, 2000). Numerous authors such as Dhar

and Hall (2018) have indicated that stimulus intensities of L_1/L_2 = 65/55 dB SPL have the dual advantage of eliciting higher DPOAE levels and enhanced sensitivity to detect OHC damage. These two authors also discourage the use of L_1/L_2 combinations greater than 70/70 dB SPL to avoid possible response artifacts that can be misinterpreted as DPOAEs. Similarly, Dreisbach and Siegel (2001) reported the optimal f_2/f_1 ratio varies as a function of frequency, with lower f_2/f_1 values yielding higher DPOAE levels at high frequencies, and vice versa.

Recommendations for stimulus parameters have also varied depending on the clinical application. For general diagnostic purposes, parameters of L_1/L_2 = 65/55 and f_2/f_1 = 1.20 or 1.22 are often recommended (Dhar & Hall, 2018; Dorn et al., 2001). For ototoxicity monitoring, Dhar and Hall (2018) suggest f_2/f_1 ratios of 1.20 to 1.22 and L_1/L_2 combinations of 45/35 dB, 55/45 and 65/55 dB SPL.

The study described in Chapter 8 set out to determine the stimulus parameter combinations (L_1/L_2 and f_2/f_1 ratios) that yielded the highest level DPOAEs found L_1/L_2 = 65/55 and f_2/f_1 = 1.18 to be the preferred stimulus parameter combinations for f_2 frequencies ≥ 5000 Hz. However, this study was conducted on healthy participants with normal hearing and conclusions for ototoxicity monitoring cannot necessarily be drawn from the results.

Research in patients with MDR-TB receiving ototoxic medication yielded conflicting evidence regarding DPOAEs' ability to detect outer hair cell dysfunction compared to standard pure tone audiometry, i.e., ≤ 8 kHz and/or high frequency audiometry. South African research conducted by Appana, Joseph, and Paken (2016) found, in a longitudinal study including 52 adult participants with MDR-TB (15-56 years; mean age 34 years) receiving kanamycin, that DPOAEs detected ototoxic outer hair cell damage in more ears than standard or high frequency pure tone audiometry up to 12 kHz. In this study, screening DPOAEs were used. The pass/fail criteria applied in their study are unclear and specified criteria to determine significant change in DPOAE levels were not mentioned. In addition, their study only tested high frequencies up to 12 kHz, which would limit the sensitivity of high frequency audiometry as higher frequencies were not included.

On the contrary, a study by Vasconcelos, Frota, Ruffino-Netto, and Kritski (2018) found no significant differences in DPOAEs (DPgrams) in their longitudinal study in 10 patients (mean age 49 years; 18-69 years; 7 men, 3 women;) with MDR-TB receiving amikacin, whilst both standard and high frequency thresholds decreased significantly. Both studies, i.e., Appana et al. (2016) and Vasconcelos et al. (2018) used DPOAE stimulus parameters fixed at L_1/L_2 = 65/55 dB SPL and f_2/f_1 ratio = 1.22, thus neither study manipulated the stimulus parameter combinations to determine whether DPOAEs would be more or less sensitive or specific when intensity and frequency ratios were varied simultaneously.

In contrast to DPOAE research in patients with MDR-TB, the general consensus in the literature on ototoxicity in patients with cystic fibrosis, cancer and chemical solvent exposure is that DPOAEs detect outer hair cell dysfunction sooner than conventional pure tone audiometry but later than hfPTA (Al-Malky, Suri, Dawson, Sirimanna, & Kemp, 2011; Govender, Govender, & Matthews, 2013; Knight, Kraemer, Winter, & Neuwelt, 2007; Reavis et al., 2008; Sisto et al., 2013). These studies all employed fixed DPOAE stimulus parameters of L_1/L_2 = 65/55 dB SPL and an f_2/f_1 ratio = 1.22, except for Reavis et al. (2008) who used L_1/L_2 = 65/59 dB SPL and an f_2/f_1 ratio = 1.2.

While DPOAEs in the general ototoxicity literature detect outer hair cell damage after hfPTA, this test could potentially not be a viable option for very ill individuals like patients with MDR-TB as they might be unable to yield reliable and valid pure tone thresholds (Fausti et al., 1999). As DPOAEs do not rely on the active participation of the person being tested, it would be prudent to attempt to maximise the sensitivity of this test for ototoxicity to detect outer hair cell damage as soon as possible.

In addition, while it is known that DPOAEs can detect outer hair cell damage sooner than conventional audiometry, it is worthwhile exploring whether DPOAEs can detect this damage earlier with stimulus parameters that are different to the ones currently used in clinics. Although DPOAEs can detect outer hair cell damage prior to sfPTA, it is known that outer hair cell damage can still occur up to six months after treatment with ototoxic medication was stopped. Thus, early identification of outer hair cell damage could assist in preventing permanent damage that is

noticeable by the individual. In turn, preventing or minimising permanent outer hair cell damage could maximise quality of life (Konrad-Martin et al., 2005).

For ototoxicity monitoring it would be ideal to test high frequency DPOAEs to detect outer hair cell damage even earlier than emissions up to 8 kHz. Unfortunately, high frequency DPOAEs currently cannot be acquired reliably and validly with commercial equipment. In the absence of commercially available high frequency DPOAE equipment it would be prudent to determine optimal stimulus parameters for early detection of high frequency damage caused by ototoxic medication like aminoglycosides for MDR-TB treatment. By doing this, stimulus parameter sets can be customised for this population, rather than using a standard set of parameters (e.g., L1/L2 = 65/55 dB SPL and an f2/f1 ratio = 1.22) used for general diagnostic purposes.

Thus, the aim of this study was to test a range of recommended DPOAE stimulus parameters that manipulated both stimulus level and frequency ratio to determine which parameters elicit the (1) largest and (2) most sensitive and specific DPOAEs in a sample of adults with multi-drug resistant tuberculosis (MDR-TB) receiving ototoxic medication.

Method

Phase 4

With the optimal stimulus parameters defined in phase 2, participants with MDR-TB receiving ototoxic medication were assessed using DPOAEs to determine:

Aim 1

Which stimulus parameters yielded the largest DPOAE levels.

Aim 2

The concurrent validity of DPOAEs as a measure of auditory function in this population compared to pure tone audiometry.

Aim 2 was achieved by the following:

(1) Determining which DPOAE stimulus parameters generated the highest decrease in DPOAE level.
(2) Comparing the identification of outer hair cell damage through DPOAEs vs. pure tone audiometry.

Research design

A single group, repeated measures design was used for this study (Maxwell & Satake, 2006). This design enabled the examination of the influence of intensity and frequency ratio stimulus parameters on DPOAE levels. With a repeated measures design, it was possible to establish the highest level DPOAEs in the same participants, where the participants acted as their own controls. In addition, the change between DPOAEs and pure tone audiometry could be determined between the two test intervals 10 to 14 days apart. The time interval of a minimum of 10 days was chosen based on the study by Fausti, Henry, et al. (1992), who found high frequency hearing loss developed within an average of nine days after treatment with aminoglycosides started (n = 53). In their study, high frequency hearing loss was detected in the first participants after four days of aminoglycoside treatment.

A known disadvantage of using a repeated measures design is the possibility of a learning effect (Howell, 1999). However, learning effects do not influence the DPOAE test, as it is an objective measure that does not require active participation of the participant.

Participants

Inclusion criteria

All participants:

(a) were between 18 to 49 years of age, to minimise age-related deterioration of the cochlea (Gates & Mills, 2005);

(b) had normal non-diagnostic otoscopic results, to rule out possible outer or middle ear problems that could obscure measurement of cochlear outer hair cell function (Hall, 2000);

(c) had hearing thresholds ≤ 25 dB HL for the frequency range from 250 to 8000 Hz;

(d) had normal middle ear functioning, which is defined as a Type A tympanogram, with compliance between 0.3 and 1.4 cm^3, middle ear pressure ranging from -150 daPa to +100 daPa and ear canal size between 1.0 and 2.0 cm^3 (GSI 38 manual, 2003), because OAEs cannot be reliably measured in the presence of a middle ear abnormality (Hall, 2000);

(e) had no self-reported history of hereditary hearing loss, significant ear disease, ear surgery or long-term noise exposure;

(f) had no other medical conditions that could negatively influence hearing, e.g., diabetes or HIV-positive

(g) passed a DPOAE screening assessment with L_1/L_2 = 65/55 and f_2/f_1 ratio = 1.20 at f_2 = 2, 4 and 6 kHz; and

(h) were diagnosed with MDR-TB by a medical doctor and were receiving ototoxic medication (kanamycin, amikacin or capreomycin) as part of their treatment for 72 hours or less prior to the baseline audiological assessment.

Participant description

Twenty-one adult participants (15 females, 6 males) aged 18 to 46 years (mean = 30, SD = 7) receiving ototoxic medication (kanamycin, amikacin or capreomycin) as part of their MDR-TB treatment, were conveniently sampled from Brooklyn Chest Hospital, a specialised TB hospital in Cape Town, South Africa. All participants were on kanamycin for at least five days during this study (range: 5 to 14 days). Please see Table 9.1 for information on drug dosage and duration of kanamycin treatment (counted from first day of administration to the day of the second test interval).

Table 9.1 MDR-TB participant information

Participant	Age	Sex	Dosage (mg)	Treatment duration (days)
1	41	Male	1000	14
2	33	Male	750	7
3	20	Female	750	9
4	33	Male	750	14
5	29	Female	750	11
6	34	Female	1000	10
7	38	Female	750	8
8	30	Female	750	14
9	33	Female	1000	14
10	21	Female	1000	10
11	37	Female	750	14
12	43	Female	1000	11
13	18	Female	750	12
14	46	Female	1000	5
15	21	Female	750	7
16	19	Female	750	7
17	27	Female	1000	14
18	27	Male	750	10
19	30	Male	1000	12
20	36	Male	1000	14
21	32	Female	750	14

Recruitment and sampling

The resident audiologists at Brooklyn Chest hospital identified and recruited in- and outpatients meeting the inclusion criteria. The participants were conveniently sampled over a period of 13 months by accepting each individual who met the selection criteria and was willing to participate in the study.

Sample size

A priori sample size calculation with G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) indicated a sample size of 44 participants would be needed to reach power of 80%, with an alpha level of 0.05. The current study only managed to include 21 participants due to the following reasons:

(i) A long recruitment period, due to the strict inclusion criteria. One of the inclusion criteria was that the participants could not have medical conditions that could negatively influence hearing, e.g., diabetes or HIV-positive. As a result, more than half the potential participants were excluded due to them being HIV-positive.

(iii) In addition, at the time of data collection, the hospital where data was collected, mostly admitted patients with XDR-TB and patients with MDR-TB were mostly treated at TB facilities in/close to their community. Also, at the time of data collection, most patients were receiving bedaquiline and not aminoglycosides as part of their MDR-TB patients, which reduced the available pool of patients even further.

Data collection

Instrumentation

A Welch Allyn otoscope was used for non-diagnostic otoscopic examinations. A GSI Tympstar Version 2 tympanometer was used for tympanometry. Pure tone audiometry (conventional and high frequency) was conducted with an Interacoustics AC40 dual channel clinical audiometer and Sennheiser HDA 300 circumaural headphones. DPOAEs were collected with the GSI Audera Version C, a commercially available piece of equipment, which can reliably evaluate emissions up to 8 kHz.

All equipment was calibrated according to the relevant specifications, prior to data collection. In addition, a biological check of all the equipment was done prior to testing. All testing was conducted in a sound treated booth meeting South African National Standards (2006).

Procedure

Once ethics approval and written permission from the relevant authorities had been obtained, written informed consent was obtained from each potential participant (See Appendix F). Biographical information obtained included date of birth, sex, medical history, noise exposure and type of ototoxic medication.

The researcher verbally conveyed information regarding the purpose, protocol and the requirements of the study in the participant's language (English or Afrikaans), which was also shared with each participant in written form. If a participant preferred isiXhosa, a mother-tongue isiXhosa research assistant explained the study to the patient. IsiXhosa-speaking participants received written documentation in isiXhosa (See Appendix G). All discrepancies and uncertainties were addressed.

Participants were initially screened for inclusion in the study using a live voice interview, non-diagnostic otoscopy, tympanometry, conventional frequency pure tone audiometry (250- to 8000 Hz) and DPOAE screening. To pass the DPOAE screening, the participants had to show $2f_1$-f_2 DPOAEs at least 3 dB above the noise floor at f_2 frequencies 2, 4 and 6 kHz to tonal stimuli with an f_2/f_1 ratio of 1.2 and an L_1/L_2 setting of 65/55 dB SPL. If the potential participant's hearing was ≤ 25 dB HL, with normal otoscopy and tympanometry, and he/she passed the DPOAE screening, he/she continued with the rest of the study. If a hearing loss or ear abnormality was detected, the researcher explained the results to the participant. In addition, the researcher also provided information and emotional counselling to the participant, who was referred to the resident audiologists for management, where necessary.

Before commencing the test protocol, the researcher explained the nature of all test procedures to participants to familiarise them with the session. Participants were then given an opportunity to seek clarification and also reminded that questions could be asked at any stage during data collection. Participants were reminded that they could withdraw at any point in the study.

Participants were seated in a comfortable chair for the duration of the tests. A glass of water was provided. Testing was stopped in the case of coughing or sneezing and continued after coughing/sneezing ceased. Breaks in testing were provided for between tests.

Clear instructions were given before each procedure and repeated during the test if necessary. The same instructions were given to all participants to improve reliability.

The instructions used for pure tone testing were: "You will hear soft sounds. Each time you hear a sound, please raise your hand immediately. Even if the sound is very soft and you can barely

hear it, you must still raise your hand. We will start with your right ear." If it was deemed necessary, the instructions were repeated during testing.

For pure tone audiometry, the modified Hughson-Westlake technique was used to obtain thresholds from 250 to 16 000 Hz.

Before DPOAE measurement, participants were instructed as follows: "I am going to place a probe in your ear. It will not be painful. Your ear will just feel blocked up. Please remain quiet and still while the probe is in your ear. You do not have to do anything, even if you hear a sound. You can sit back and relax."

Baseline pure tone audiometry and DPOAE testing were conducted within 72 hours of commencing treatment with ototoxic medication, as recommended by ASHA (1994). Audiometry (conventional and high frequency) was conducted with an Interacoustics AC40 dual channel audiometer. The frequencies tested were 250, 500, 1000, 2000, 4000, 8000, 10 000, 12 000, 14 000 and 16 000 Hz.

Both pure tone audiometry and DPOAE testing were conducted in a sound treated booth with measured background noise levels < 55 dBA (Lee & Kim, 1999), as measured using a Brüel & Kjær 2238 class 1 hand-held sound level meter. The $2f_1$-f_2 DPOAE measurements were obtained from each ear of each participant using the following stimulus parameters: f_2/f_1 ratios of 1.18, 1.20, and 1.22; L_1/L_2 settings of 65/65, 65/55 and 60/53 dB SPL; and f_2 frequencies of 2003, 2519, 3178, 3996, 5000, 6996 and 8003 Hz. The intensity and ratio parameters used are based on the findings of phase 2, which were published by Petersen et al. (2018). In-ear calibration was completed prior to each test. To mitigate potential order effects, a single sequence of stimulus parameters was set and each participant was started at a different point in this sequence. The order of ear testing was reversed for each sequential participant. The $2f_1$-f_2 DPOAEs were sampled until one of two stopping rules was met: a) the noise floor at the distortion product frequency was less than -10 dB SPL, or b) until 32 seconds of artifact-free sampling had been averaged (Dille et al., 2010). DPOAEs were recorded at least twice per stimulus parameter combination, to check

whether the responses and the noise floor could be replicated. DPOAEs were accepted as present if they were ≥ 6 dB above the noise floor.

Participants were seated in a comfortable chair and were instructed to remain still and quiet during the DPOAE test procedure with breaks provided as required. The DPOAE test time per participant was approximately 60 to 90 minutes per test occasion. The uninterrupted power supply was placed outside the sound-treated room, to minimise machine-generated noise. Each participant underwent pure tone testing (conventional and high frequency pure tone audiometry) and DPOAE testing on two occasions: within 72 hours of the start of ototoxicity treatment and within 10 days to two weeks thereafter, by the same researcher.

The following data was recorded from each participant to each set of stimulus parameters at each f_2 frequency on each test occasion, i.e., at baseline, and 10 to 14 days later: conventional and high frequency audiometry thresholds, absolute level of DPOAE, absolute level of the noise floor and the DPOAE signal-to-noise ratio (SNR), calculated as the absolute level of the DPOAE minus the level of the noise floor.

Reliability and validity of data collection

Possible sources of error in measurement in this study include the instrument, tester, participant and the environment.

To enhance reliability in instrumentation, the necessary system calibration was done prior to data collection. In addition, daily calibration was done by inserting the DPOAE probe into a hard-walled cavity and measuring the response according to manufacturer specifications. Biological checks were conducted before each data collection session, and a visual probe inspection was done prior to testing each participant.

The way of measuring each participant was standardised so that the measurements took place in the same manner across participants and test intervals. Sufficient training reduced the error variance (Streiner & Norman, 2008). By following the same measurement procedure with each participant, the intent was to enhance intra-tester and inter-tester reliability. The researcher and

research assistants underwent a training period prior to data collection to become familiar with the equipment and the measurement procedure.

To minimise participant variability, the test time was kept as short as possible. Data collection took place at the same time of the day for participants, as far as possible.

The ambient noise level was controlled by conducting all testing in a soundproof booth. A possible source of equipment noise is the computer used to collect the data, which was therefore placed as far as possible from the participant.

Face validity refers to whether the instrument appears to assess the aspect of interest (Streiner & Norman, 2008). Experts like Dreisbach and Siegel (2001) believe that DPOAEs measure outer hair cell function of the cochlea.

Content validity implies that the instrument covers all domains of the phenomenon under investigation (Maxwell & Satake, 2006). With DPOAEs, two signals are sent into the ear at an intensity ≤ 70 dB SPL that elicits active cochlear activity. This activity is measured in the ear canal via a sensitive microphone. The stimulus intensities were monitored throughout the test procedure while DPOAEs were recorded, to ensure that the intended L_1/L_2 were delivered into the ear canal.

Criterion validity of DPOAEs has been evaluated and determined in patient populations (patients with cancer, cystic fibrosis, etc.) receiving ototoxic medication, e.g., cisplatin and aminoglycosides.

Data management

All data was entered into Excel spreadsheets. A research assistant checked 10% of the data to assure that data had been entered accurately. If data entry errors were detected, the whole dataset was rechecked. Spreadsheets were uploaded to Microsoft OneDrive, an online storage space, to a password protected account. Only the researcher had access to the password. The data will be kept for a minimum of five years, as per the South African Medical Research Council's recommendations (SAMRC, 2018).

Data analysis

All DPOAE data were found to meet parametric assumptions based on the values for skewness and kurtosis. Descriptive statistics were calculated for all DPOAE measures.

A mixed model analysis was conducted to identify the presence of any main effects of the combined level (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) settings on the DPOAE data, and for the interaction between stimulus intensity level, frequency ratio settings and f_2. Post-hoc pairwise comparisons were conducted to determine whether there were statistically significant differences between the different stimulus parameters used.

In addition, a separate mixed model analysis was conducted to determine main effects of changes between the DPOAE levels at baseline and 10–14 days later related to stimulus parameter combinations. In this instance, post-hoc comparisons were also done.

To compare DPOAE levels and high frequency pure tone thresholds (pure tone average for 10–16 kHz), Pearson correlation coefficients were calculated. Finally, sensitivity and specificity were determined with contingency tables (see Table 9.2), applying the ASHA criteria (1994) for ototoxicity monitoring to the pure tone thresholds. Shifts in pure tone thresholds were regarded as significant if there was (a) 20 dB decrease at one frequency, (b) 10 dB decrease at any two adjacent frequencies, or (c) loss of response at three consecutive test frequencies where responses were previously obtained. To compare DPOAE changes between baseline and the second test occasion, criteria published by Reavis et al. (2015) were used (see Appendix H). Sensitivity was defined as the DPOAEs' ability to detect outer hair cell dysfunction when this dysfunction was actually present, as indicated by high frequency pure tone thresholds (Streiner & Norman, 2008). This value, also referred to as the true positive rate, was calculated as follows: [True positive/(True positive + False negative)] x 100 (Riegelman, 2005). Specificity was defined as the DPOAEs' ability to indicate a true negative result, i.e. that OHC dysfunction is not present, as indicated by high frequency pure tone thresholds (Streiner & Norman, 2008). This value was calculated by [True negative/(True negative + False positive)] x 100 (Riegelman, 2005).

Table 9.2: Template for the contingency tables comparing the performance of DPOAEs to PTA in detecting significant hearing threshold shifts

	PTA positive = significant threshold shifts	PTA negative = no significant threshold shifts
DPOAE positive	A. True positives	B. False positives
DPOAE negative	C. False negative	D. True negatives
	A + C = Total with hearing loss	B + D = Total normal hearing

All statistical analyses were conducted using IBM SPSS Statistics (Version 25).

Ethical considerations

Unconditional ethical clearance was granted to conduct the study by the Faculty of Health Sciences Human Research Ethics Committee (HREC/REF: 512/2013; See Appendix A). Permission was obtained from the Western Cape Department of Health and the superintendent of Brooklyn Chest hospital prior to data collection. This study complied with the principles set out by the Declaration of Helsinki (World Medical Association, 2013).

Autonomy refers to the competent individual's right to self-determination (Katzenellenbogen et al., 1997). Providing the participants with adequate information to give informed consent to participate in the study adhered to this principle of autonomy and safeguarded their freedom of choice.

To facilitate understanding of the study, information was presented in the participant's first language (Afrikaans, English, Xhosa). Participants were given the opportunity to ask for clarification at any time during the study. It was also explained to participants that participation in the study was voluntary and that they could withdraw at any stage of data collection. The participants were informed that refusal to participate in or withdrawal from the study would not affect their management at Brooklyn Chest hospital. Participants were required to sign a consent form if they agreed to take part in the study (See Appendix F).

The ethical principle of beneficence requires that the actions of the researcher are aimed at improving the well-being of the participant (Katzenellenbogen et al., 1997). If any hearing or auditory abnormalities were detected, the participant was referred to the resident audiologists at Brooklyn Chest for further management.

Non-maleficence is the principle that refers to the researcher's obligation to not do harm (Katzenellenbogen et al., 1997). No procedures were used that could harm the participants, and all tests were non-invasive. All necessary equipment, e.g., probe tips and earphones, was cleaned with alcohol swabs after use with each participant to ensure that bacteria were not spread from one participant to the next. The researcher washed her hands with an antiseptic agent for the same reason. In addition, the researcher wore an N95 mask when collecting data from participants with MDR-TB, for protection of both parties. The researcher took all reasonable steps to ensure the participants' comfort.

The ethics principle of justice can be interpreted to mean a fair distribution of benefits of the research (Katzenellenbogen et al., 1997). Therefore, all eligible individuals were included in the study, provided that they met the selection criteria.

There were no direct benefits for the participants of the study, other than detecting possible hearing or auditory damage. There were no known risks for participants. Participants received compensation of R150 per session for travel costs and inconvenience (each test session took 60 to 90 minutes).

Confidentiality was respected at all times and all participants' details and results were kept confidential and anonymous by assigning a number to each participant, which corresponded with the order of enrolment in the study. This number was used on all documentation pertaining to the study. Identifying information was only known to the researcher and was kept separate from collected data in a locked filing cabinet in the researcher's office. The researcher also assured the participants that no identifying information would appear in any publication or presentation forthcoming from this study.

Researcher experience implies that the researcher is competent to conduct the research (World Medical Association, 2013). The researcher is professionally qualified as an audiologist and has sufficient theoretical and clinical expertise to conduct the research. The researcher acted in an accountable and responsible manner and upheld professional standards.

Results

Figure 9.1 shows the DPOAE mean absolute levels in the left ear for all combinations of f_2/f_1 ratio and L_1/L_2 at each f_2 frequency for the participants at the first assessment occasion (the right ear showed similar results). The figure illustrates that participants were more likely to show DPOAEs of higher intensity at the lower f_2 frequencies, i.e., 2006–3175 Hz, regardless of the primaries and f_2/f_1 ratios used. Descriptively, the stimulus combination of L_1/L_2 = 60/53 and f_2/f_1 ratio = 1.22 consistently yielded the lowest DPOAE levels, except at 6996 Hz.

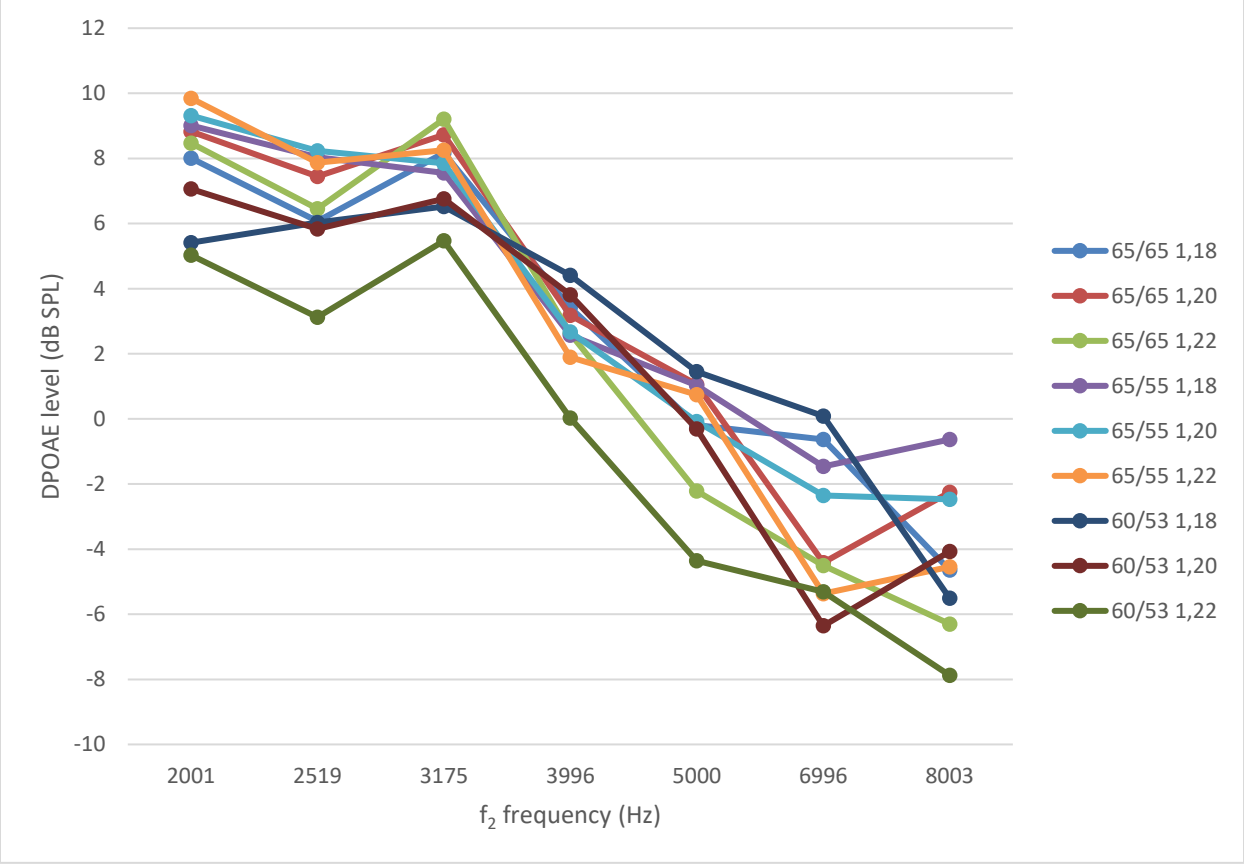

Figure 9.1: Mean DPOAE absolute levels per intensity and frequency ratio combinations for each frequency (f_2)

Table 9.3 shows the statistically significant results of the linear mixed model analyses for main effects of level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) settings. For all f_2 values and in both ears, these analyses showed that the 65/55 and 65/65 level settings consistently resulted in higher DPOAE levels across all f_2/f_1 settings ($p < 0.05$). The 1.18 f_2/f_1 setting resulted in higher DPOAE levels across all L_1/L_2 settings in the higher stimulus frequencies (≥ 5000 Hz and above). In a few instances, f_2/f_1 of 1.20 yielded the second highest DPOAE levels.

Table 9.3: Results of statistically significant results for the mixed model analyses for main effects ($p < 0.05$) of level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) settings.

f_2 (Hz)	Ear	Best L1/L2 (dB SPL)	Best f_2/f_1
2003	L	65/55, 65/65	
	R		1.18, 1.20
2519	L	65/55, 65/65	
	R	65/55	
3175	L	65/65, 65/55	
	R		
3996	L	65/65, 65/55	
	R	65/65	
5000	L	65/55, 65/65	1.18
	R	65/65, 65/55	1.18, 1.20
6996	L	65/65, 65/55	1.18
	R	65/55	1.18
8003	L	65/55	1.18, 1.20
	R	65/55, 65/65	1.18

Table 9.4 shows the statistically significant results of the mixed model analyses of all level and frequency settings combined. For all f_2 values and in both ears, these analyses showed that the intensity (dB SPL) and frequency ratio settings of 65/55 and 1.18 and 65/65 and 1.18 regularly resulted in higher DPOAE levels compared to other level and frequency ratio combinations, with stimulus frequencies ≥ 5000 Hz.

Table 9.4: Statistically significant results of the mixed model analyses of all level and frequency settings combined

f_2 (Hz)	Ear	Best combinations of L1/L2 (dB SPL) & f_2/f_1
2003	L	65/55 1.22
	R	
2519	L	
	R	
3175	L	
	R	
3996	L	
	R	
5000	L	65/55 1.18
	R	65/65 1.18; 65/55 1.18; 65/55 1.20; 65/65 1.20
6996	L	65/65 1.18
	R	65/55 1.18
8003	L	65/55 1.18
	R	65/55 1.18

Sensitivity and specificity

Table 9.5 shows the results of the linear mixed model analyses for main effects of stimulus intensity (L_1/L_2 in dB SPL) and frequency ratio (f_2/f_1) settings on the change in DPOAE levels between the two test occasions. For all f_2 values and in both ears, these analyses showed that, between test 1 and test 2, the 65/55 and 65/65 level settings consistently resulted in the greatest reduction of DPOAE levels across all f_2/f_1 settings. The f_2/f_1 ratio of 1.18 regularly resulted in the highest decrease of DPOAE levels across all L_1/L_2 settings, followed by 1.20.

Table 9.5: Results of the mixed model analyses for main effects ($p < 0.05$) of level (L_1/L_2 in dB SPL) and frequency (f_2/f_1) settings for test 1 vs test 2

f_2 (Hz)	Ear	Best L1/L2 (dB SPL)	Best f_2/f_1
2003	L	65/55, 65/65	
	R		1.18, 1.20
2519	L	65/55, 65/65	
	R	65/55	
3175	L	65/55, 65/65	
	R		
3996	L	65/55, 65/65	
	R	65/65	
5000	L	65/55, 65/65	1.18
	R	65/65, 65/55	1.18, 1.20
6996	L	65/55, 65/65	1.18
	R	65/55	1.18, 1.20
8003	L	65/55	1.18, 1.20
	R	65/55, 65/65	1.18

Table 9.6 shows the results of the mixed model analyses of all intensity and frequency settings on the DPOAE level changes between the two test intervals combined. When comparing the results of test 1 vs test 2, the L_1/L_2 and f_2/f_1 stimulus combinations of 65/55 and 1.18 yielded the most significant reduction between the two test occasions, followed by 65/65 and 1.18, 65/55 1.20 and 65/65 1.20 (see table 4). The L_1/L_2 pair of 60/53 consistently yielded the least difference between the two test occasions, regardless of the f_2/f_1 ratio used.

Table 9.6: Results of the mixed model analyses of all level and frequency settings combined comparing DPOAE changes between the two test occasions.

f_2 (Hz)	Ear	Best combinations of L_1/L_2 and f_2/f_1 (only combinations showing a best result on at least one occasion are shown)								
		65/65			65/55			60/53		
		1.22	1.20	1.18	1.22	1.20	1.18	1.22	1.20	1.18
2003	L	2	2	1	3	2	2			
	R		1	1		1	1			1
2519	L	1	1	1	1	1	1		1	1
	R		1		1	1				
3175	L	1	1		1					
	R									
3996	L	1	1	1	1	1	1			1
	R			1						
5039	L	1	1	1	1	1	3			1
	R		3	6		3	3			
6351	L			6		2	2			
	R		2			2	3			
8003	L		1			2	6			
	R		1			1	3			
Counts	-	6	15	18	8	17	25	0	1	4

Note: Within each f_2 and ear combination, only L_1/L_2 and f_2/f_1 stimulus combinations that produced significantly higher ($p < 0.05$) DPOAE levels than other stimulus combinations in that row, were counted

L, left; R, right.

The average change in pure tone thresholds between test 1 and 2 ranged from a decrease of -6.56 dB (meaning that the threshold improved from test 1 to test 2) to an increase of 3.42 dB (see Table 9.7). These mean changes are not clinically significant.

Table 9.7: Average changes in pure tone thresholds between test 1 and 2 (dB HL).

	Average *Test 1 thresholds		Average ^Test 2 thresholds		Average threshold change (dB HL)	
Hz	L	R	L	R	L	R
250	15,0	15,0	16,9	18,6	-1,9	-3,6
500	12,9	11,2	13,6	13,1	-0,7	-1,9
1000	7,6	9,5	8,6	10,2	-1,0	-0,7
2000	7,4	9,8	6,2	9,0	1,2	0,7
4000	10,5	13,3	8,3	12,4	2,1	1,0
8000	16,2	18,6	18,8	21,7	-2,6	-3,1
10000	12,9	15,5	9,5	14,5	3,3	1,0
12000	15,2	16,7	16,4	16,0	-1,2	0,7
14000	22,9	20,0	23,3	24,2	-0,4	-4,2
16000	30,3	28,1	26,9	34,7	3,4	-6,6

Key: *Test 1 thresholds refer to baseline; ^Test 2 thresholds refer to thresholds obtained 10-14 days after the baseline test

When comparing the DPOAEs to pure tone audiometry, the correlations between DPOAEs at each stimulus parameter combination at each test occasion and the pure tone average for 10–16 kHz was calculated. Significant correlations are displayed in Table 9.8. The majority of DPOAEs did not show a significant correlation with the pure tone average for thresholds at 10–16 kHz.

Table 9.8: Correlations between DPOAE levels and hfPTA (10–16 kHz)

Ear	Test interval	f_2 Hz	Stimulus parameter combination (L_1/L_2; f_2/f_1)	r	df	p
Left	2	5000	60/53; 1.22	-0.647	14	0.009
	2	2003	65/55; 1.18	-0.587	17	0.010
	2	6996	65/55; 1.18	-0.677	11	0.016
	2	8003	65/55; 1.20	-0.605	12	0.029
	2	6996	65/65; 1.18	-0.532	17	0.028
Right	1	2003	60/53; 1.18	0.478	17	0.045
	1	8003	65/55; 1.18	-0.562	12	0.046

In terms of change for DPOAE levels between the two test intervals, most changes did not significantly correlate with high frequency pure tone threshold change at 10–16 kHz. Only the DPOAE change in the left ear at f_2 = 2519 with L_1/L_2 of 65/55 dB SPL and f_2/f_1 = 1.18 yielded a significant correlation (r[16] = 0.499, p = 0.041)

When examining individual changes in pure tone thresholds from 10–16 kHz (using ASHA, 1994), out of 42 ears, thresholds in nine ears deteriorated, 25 ears' thresholds stayed the same, and in eight ears, the thresholds decreased (meaning that the hearing had improved). With DPOAEs across all stimulus parameters, out of 42 ears, on average, the level decreased in 14 ears, stayed the same in 21 ears and increased in seven ears when using Reavis et al.'s criteria for significant change in DPOAEs (2015) (see Table 9.9). In the nine ears that had poorer high frequency hearing thresholds, only two ears showed a decline in DPOAEs (See Table 9.10)

Table 9.9: DPOAE level changes for all stimulus parameter combinations (n=42 ears).

DPOAE level changes	65/65, 1.18*	65/65, 1.20	65/65, 1.22	65/55, 1.18	65/55, 1.20	65/55, 1.22	60/53, 1.18	60/53, 1.20	60/53, 1.22
Unchanged	21	17	20	19	20	25	25	22	22
Increased	7	13	8	7	9	4	2	6	5
Decreased	14	12	14	16	13	13	15	14	15

*L_1/L_2, f_2/f_1 ratio

When comparing changes of pure tone thresholds between 1–8 kHz to those at 10–16 kHz, high frequency pure tones detected clinically significant changes in four more participants than standard frequency audiometry (see Table 9.10). In participants where pure tone audiometry indicated significant threshold changes, only one or two participants had clinically significant changes in DPOAEs. Only the comparison with DPOAEs elicited by L_1/L_2 of 65/55 dB SPL and f_2/f_1 = 1.18 are shown in Table 9.10, but the results were similar for the remaining DPOAE stimulus parameters.

On average, in more than half the ears (54%) there was correspondence between the DPOAE results and high frequency pure tone thresholds indicating no clinically significant changes. DPOAEs decreased significantly between the two test sessions for 11 to 15 participants (depending on the f_2/f_1 ratio), where pure tone thresholds remained the same or improved (see Table 9.10).

For raw data on DPOAE level changes (including the noise floor and signal to noise ratio) in participants with significant changes in pure tone audiometry, see Appendixes I and J for the left and right ear, respectively.

Table 9.10: Correspondence between DPOAEs and pure tone audiometry to detect ototoxic changes (n=42)

		PT 1-8 kHz						PT 1-8 kHz						PT 1-8 kHz		
		*Positive	^Negative	Total				Positive	Negative	Total				Positive	Negative	Total
65/55 1.18	*Positive	1	15	16		65/55 1.20	Positive	2	11	13		65/55 1.22	Positive	1	12	13
	^Negative	4	22	26			Negative	3	26	29			Negative	4	25	29
	Total	5	37	42			Total	5	37	42			Total	5	37	42
		PT 10-16 kHz						PT 10-16 kHz						PT 10-16 kHz		
		Positive	Negative	Total				Positive	Negative	Total				Positive	Negative	Total
65/55 1.18	Positive	2	14	16		65/55 1.20	Positive	2	11	13		65/55 1.22	Positive	2	11	13
	Negative	7	19	26			Negative	7	22	29			Negative	7	22	29
	Total	9	33	42			Total	9	33	42			Total	9	33	42

Key: *Positive refers to the test detecting ototoxic changes. ^Negative refers to the test not detecting ototoxic changes

The sensitivity and specificity values of DPOAEs in relation to pure tone thresholds between 1 to 8 kHz and 10 to 16 kHz are displayed in Table 9.11. Sensitivity values ranged between 20 to 40% and specificity values from 58 to 70%, with f_2/f_1 = 1.20 yielding the highest values.

Table 9.11 Sensitivity and specificity of DPOAEs compared to pure tone thresholds

	PT 1-8 kHz		PT 10-16 kHz	
L_1/L_2; f_2/f_1	Sensitivity %	Specificity %	Sensitivity %	Specificity %
65/55 1.18	20	59	22	58
65/55 1.20	40	70	22	67
65/55 1.22	20	68	22	67

Discussion

Intensity

In the current study, L_1/L_2 = 65/55 dB SPL elicited the largest DPOAEs at most f_2 values, followed by an L_1/L_2 = 65/65 dB SPL. The stimulus intensity pair L_1/L_2 = 60/53 dB SPL consistently yielded the lowest DPOAE levels at all f_2 values. This finding in participants with MDR-TB receiving ototoxic medication agree with previous research in participants with normal hearing that found the higher intensities of L_1/L_2 elicited the highest level DPOAEs (Beattie & Jones, 1998; Bian & Chen, 2008; Dhar et al., 1998; Vento et al., 2004). In addition, previous research found the DPOAE levels are highest with L_1 greater than L_2 , for L_1 of 65 dB SPL or lower (Gaskill & Brown, 1990; Whitehead, Stagner, et al., 1995), which was also the case in the current study.

Ratio

Regarding frequency ratio, the study results show that typically an f_2/f_1 of 1.18 elicited the highest DPOAE levels at the higher frequencies (i.e., 5039–8003 Hz), with 1.20 the second highest. At the lower frequencies, on most occasions there was not a significant difference between the different frequency ratios. These results were in line with studies by Dreisbach and Siegel (2001), Londero (2002) and Moulin (2000b) who found that ratios at or close to 1.18 elicit the highest level DPOAEs at the higher frequencies up to 8 kHz. However, Gaskill and Brown (1990) found a ratio of 1.225 to yield the optimal DPOAE levels across f_1 frequencies ranging from 1–8 kHz. This finding was based on descriptive statistics, as no inferential statistics were done to arrive at this conclusion. Additionally, this experiment included only 11 ears. Similarly, another seminal study conducted by Harris et al. (1989) concluded that an f_2/f_1 ratio of 1.2 on average yielded the highest level DPOAEs. This study employed no inferential statistics and had a sample size of five participants.

In this study of participants with MDR-TB receiving ototoxic medication, the frequency ratio 1.22 typically used in clinical settings (Dhar & Hall, 2018), did not elicit the highest DPOAE level at any f_2 frequency. The reason could be that the current study did not use stimulus frequencies less

than 2000 Hz, as it is known that with lower f_2 frequency, the optimal f_2/f_1 ratio increases (Dreisbach & Siegel, 2001).

The stimulus combinations of L_1/L_2 = 65/55, f_2/f_1 = 1.18, and L_1/L_2 = 65/65, f_2/f_1 = 1.18 overall yielded the highest level DPOAEs, especially at f_2 frequencies ≥ 5000 Hz. These results are in accordance with the findings of Petersen et al. (2018). Direct comparison with other studies was not possible, due to the different stimulus parameters used.

Sensitivity and specificity

When examining the sensitivity and specificity rates in the current study for DPOAEs when using high frequency audiometry as the gold standard, the sensitivity of DPOAEs seemed poor (22%) and did not differ for the different stimulus parameters. These results are similar to those obtained by Vasconcelos et al. (2018), who found a 0% sensitivity rate for DPOAEs detecting ototoxic damage in relation to high frequency audiometry up to 16 kHz in a longitudinal study in 10 patients with MDR-TB receiving amikacin. Vasconcelos et al. (2018) used the ASHA criteria for ototoxic changes in pure tone thresholds and for DPOAEs, level reductions of 4 dB or more at a minimum of two adjacent frequencies, as specified by Reavis et al. (2011).

However, Al-Malky et al. (2011) found the sensitivity of DPOAEs to detect abnormal OHC function in eight participants with cystic fibrosis (CF) receiving aminoglycosides with high frequency sensorineural hearing loss (9-20 kHz) was 100% when using high frequency audiometry as the gold standard. These authors used a fixed f_2/f_1 ratio = 1.22 and L_1/L_2 = 65/55 dB SPL. One possible reason for the differing findings of the current study and that of Al-Malky et al. (2011) could be attributed to the different criteria used for OHC damage: Al-Malky et al. (2011) used statistical differences between DPOAEs of participants with CF receiving aminoglycosides who had high frequency hearing loss vs. participants with CF with no aminoglycoside exposure who had normal hearing, whereas the current study with MDR-TB participants used the standard error of measurement with 90% reference limits to determine significant shifts in DPOAEs to indicate OHC dysfunction.

The current study found specificity was highest for f_2/f_1 settings of 1.20 and 1.22, i.e., 67%, in comparison to 58% for f_2/f_1 = 1.18 when using high frequency pure tone audiometry as the gold standard. The specificity values were similar regardless of the stimulus intensity levels used. These results are in contrast with Bonfils and Avan (1992) who found that higher stimulus intensity levels yielded higher specificity values. So, for example in their study L_1/L_2 = 42/42 dB SPL resulted in lower specificity values (26-62%) than L_1/L_2 = 62/62 dB SPL with a specificity range of 84-100% (Bonfils & Avan, 1992). One plausible reason for the difference in results could be that Bonfils and Avan (1992) used standard pure tone audiometry up to 8000 Hz as the gold standard and not high frequency audiometry. Another possible reason for the discrepancy could be the difference in the number of participants with OHC damage determined through pure tone audiometry between the two studies. Bonfils and Avan (1992) included 50 ears with confirmed sensorineural hearing loss and the current study had nine ears that developed hearing loss, as indicated by high frequency audiometry. Specificity values with changes in f_2/f_1 could not be compared to other research due to a lack of information provided in relevant studies with high frequency audiometry as the gold standard.

The textbook definitions of sensitivity and specificity hinge on the use of a gold standard (Maxwell & Satake, 2006), in this instance high frequency pure tone audiometry (hfPTA) to detect ototoxicity. However, the gold standard, hfPTA, has its own limitations. The hfPTA in itself may be less sensitive to outer hair cell damage than the OAEs, especially in sick patients who are not able to concentrate for long periods of time (Vasquez & Mattucci, 2003). For valid pure tone audiometry results, one has to rely on people to provide active responses to the stimuli that are presented. Thus, in patients that are ill, valid results might not be obtained (Konrad-Martin et al., 2016). However, DPOAEs only require passive participation, making it ideal to use in ill patients or others who cannot participate in behavioural testing.

It is now widely accepted that DPOAEs are more sensitive than standard pure tone audiometry (≤ 8 kHz) to detect ototoxic damage in humans. In the absence of an acceptable gold standard at the time in humans, this knowledge was created by studies using a 4–8 dB decline in DPOAE levels at two adjacent frequencies to determine the number of ears/participants that exhibit ototoxic

damage (Knight et al., 2007). In addition, other studies compared the timing of DPOAEs vs. standard pure tone audiometry to detect ototoxicity (Mulheran & Degg, 1997; Ozturan, Jerger, Lew, & Lynch, 1996; Stavroulaki et al., 2002). The following discussion will use the same criteria to compare the ability of DPOAEs and high frequency audiometry to detect ototoxicity.

When comparing the DPOAE levels and pure tone thresholds at both test occasions statistically, there was no significant correlation between the two tests for the majority of DPOAEs, regardless of the stimulus parameters used. In addition, there was no statistical correlation between changes in DPOAE level and high frequency pure tones.

However, when examining clinically significant changes, on average, in more than half the ears (54%) there was correspondence between the DPOAE results and pure tone thresholds (standard and high frequencies) indicating no clinically significant changes. DPOAEs decreased significantly between the two test sessions for 11 to 15 participants (depending on the f_2/f_1 ratio used), where pure tone thresholds remained the same or improved. The frequency ratio of 1.18 detected the highest number of significant changes, regardless of the stimulus intensity pairs used. This result indicates that any of the stimulus intensity pairs could potentially be used to detect OHC damage, when combined with a frequency ratio of 1.18. Reavis et al. (2008) found a roughly equal proportion of ears experiencing DPOAE changes before, during and after behavioural hearing changes in a sample of 53 adults receiving either chemotherapy or ototoxic antibiotics. Thus, in a third of instances DPOAE changes occurred prior to pure tone threshold change, a third at the same time and a third after behavioural hearing changes in their study sample. These authors used stimulus parameters of L_1/L_2 = 65/59 dB SPL and an f_2/f_1 ratio of 1.20. Seeing that cochlear damage first occurs in the basal area of the organ of Corti, this ratio of 1.20 might not have been sensitive enough to detect early changes in outer hair cell function.

When examining individual changes in pure tone thresholds from 10–16 kHz (using ASHA, 1994), out of 42 ears, thresholds in 9 ears deteriorated, 25 ears' thresholds stayed the same, and in 8 ears, the thresholds decreased (meaning that the hearing improved). The improvement in pure tone thresholds between the baseline and 10-14 days later could be due to the participants being too ill to reliably participate in behavioural testing at baseline, but after receiving medication for

a few days, their health could have improved, which, in turn, could have led to more reliable pure tone thresholds. Another possible reason for improved pure tone thresholds could relate to the learning effect of pure tone testing, seeing that it requires active participation.

With DPOAEs across all stimulus parameters, out of 42 ears, on average, the level decreased in 14 ears, stayed the same in 21 ears and increased in 7 ears. Thus, DPOAEs detected OHC damage in more ears than high frequency pure tone audiometry, based on DPOAE level shift criteria provided by Reavis et al. (2015). This result is plausible, based on the findings of Arnold et al. (1999) that suggest DPOAEs may be sensitive to ototoxic damage to OHCs occurring at basal locations responsible for coding frequencies higher than the DPOAE eliciting tones. The results of the current study are in in agreement with those of Appana et al. (2016) who found that DPOAEs detected OHC damage in more ears than high frequency audiometry. Geyer et al. (2015) found more participants with abnormal DPOAEs than high frequency hearing loss in children and adolescents with cystic fibrosis (age range 7 – 20 years). In addition, Ress et al. (1999) and Yu et al. (2014) found that DPOAEs detected ototoxicity in as many ears as high frequency audiometry in their respective studies.

However, in contrast to the current study and abovementioned research, Abujamra et al. (2013) found high frequency audiometry detected hearing loss in the highest number of participants, i.e. 36 of 42 participants (86%) treated with cisplatin (age range 4- 37 years). DPOAEs detected changes in OHC function in 27 participants (64%), followed by standard pure tone audiometry, which detected the lowest number of participants with hearing loss, namely 24 (57%). A possible explanation for this difference could be the different criteria used by Abujamra et al. (2013) and the current study to determine ototoxic damage via high frequency hearing thresholds and DPOAE levels. For hearing loss, Abujamra et al. (2013) used normative data provided by three studies (Carvallo, Koga, Carvalho, & Ishida, 2002; Pedalini et al., 2000; Sahyeb, Costa Filho, & Alvarenga, 2003) and for DPOAEs, the emissions were regarded as present if the signal to noise ratio was between 0 to 10 dB and the DPOAE was 3 dB above the noise floor. The current study with MDR-TB patients used participants as their own controls through repeated measures rather than normative data to mitigate the known high intersubject variability of high frequency

audiometry. In addition, the current study used DPOAE change criteria determined through a meta-analysis of 10 studies (Reavis et al., 2015), which provides for a more robust measure of DPOAE change than present/abnormal/absent criteria used by Abujamra et al. (2013). It is also noted that the DPOAE criteria cited by Abujamra et al. (2013) contains an error, as it is not possible to have the acceptable signal to noise ratio as between 0 to 10 dB dB and regard as present DPOAEs if 3 dB above the noise floor.

Where DPOAEs increased over time, it could have been due to possible subclinical middle ear problems not detected by tympanometry or otoscopy. Based on the reference limits for DPOAE level shifts provided by Reavis et al. (2015), it is unlikely that the increases in DPOAE levels were due to chance.

When comparing the absolute DPOAE levels obtained at baseline to the second test occasion between 10–14 days later, the stimulus combinations of L_1/L_2 = 65/55 dB SPL with an f_2/f_1 of 1.18 showed the highest reduction in DPOAE level, using the reference limits constructed by Reavis et al. (2015) through meta-analysis to determine DPOAE level changes that signify true change in ear function not due to test-retest variability. This combination was followed by L_1/L_2 = 65/65 dB SPL and f_2/f_1 1.18, then L_1/L_2 = 65/55 dB SPL with f_2/f_1 = 1.20 and 65/65 1.20. The stimulus combination of L_1/L_2 60/53 consistently showed the least reduction in DPOAE level, regardless of the f_2/f_1 ratio used. These results are in contrast to recommendations by Dhar and Hall (2018), who suggest that parameter combinations of L_1/L_2 = 65/55, 55/45 and 45/35 dB SPL with an f_2/f_1 ratio of 1.22 be used for ototoxicity monitoring, suggesting that lower stimulus intensities are more suitable for ototoxicity monitoring. The source of their information was not provided and thus the reason for their recommendations could not be scrutinised. Based on the findings of a systematic review (Petersen et al., 2017), it appears that no other study manipulated both intensity and ratio stimulus parameters at the same time in a single study with participants receiving ototoxic medication. Thus, no direct comparison with other studies could be made.

These results illustrate the importance of a test battery approach for ototoxicity monitoring. If only high frequency pure tones were used, up to a third of participants who developed OHC

damage would have been missed. OHC damage in seven ears would potentially have gone unnoticed after two weeks if relying on DPOAEs only.

However, the current study of participants with MDR-TB only included 21 participants (42 ears). It is advised that future studies should include a larger sample size to determine whether these stimulus parameters yield the most sensitive and specific results in MDR-TB patients to diagnose ears with damaged outer hair cells. In addition, it is advisable to include conduct testing over a longer period of time, for example three to six months, until high frequency hearing loss is evident in more participants. In addition, shorter test intervals than 10–14 days (for example once daily or once every two days) can be recommended to determine when DPOAEs detect OHC damage due to ototoxic medication, in comparison to high frequency audiometry. In addition, a more representative sample of participants with MDR-TB should be studied. For the purpose of this study, potential participants were excluded if they were HIV-positive. However, in South Africa, up to 57% of patients with MDR-TB are HIV-positive (Cox et al., 2010; Harris et al., 2012) and it would be prudent to include these patients to have a realistic clinical picture.

As was noted in the Method section, the recruitment period was extensive as the stringent inclusion criteria excluded a large number of potential participants. Additionally, during the data collection period, the recommended WHO MDR-TB treatment guidelines with bedaquiline as the preferred treatment were implemented at Brooklyn Chest hospital, further reducing the number of eligible individuals for the study. Furthermore, data collection took place at a facility where mainly patients for extensively drug-resistant TB and only patients with complicated MDR-TB were hospitalised. For future research pertaining to patients with MDR-TB, it is recommended to conduct a similar study at decentralised treatment facilities where the testing can be conducted in the community to increase the pool of potential participants.

Conclusion

Based on the results of the current study, it is proposed that a frequency ratio 1.18, especially between f_2 frequencies from 5000 to 8000 Hz, are used in clinical settings when evaluating cochlear OHC function in adults for ototoxicity monitoring purposes. In addition, it seems advisable to use stimulus intensity levels of L_1/L_2 = 65/55 dB SPL when monitoring cochlear OHC function in adult MDR-TB patients.

This study of participants with MDR-TB was the first to manipulate DPOAE stimulus parameters in participants with MDR-TB receiving ototoxic medication to determine the best parameters for DPOAE level, sensitivity and specificity. Thus, it offers a useful starting point to explore the refinement of DPOAE stimulus parameters for ototoxicity monitoring purposes. However, further research with a larger sample size over a longer treatment period is recommended to determine the DPOAE stimulus parameters that yield the most sensitive and specific results in MDR-TB patients for ototoxic damage.

CHAPTER 10: DISCUSSION

In this chapter, the discussion of the results of the four studies presented in Chapters 6 to 9 will be revisited. In addition, this chapter offers a broader, more integrated discussion of the thesis findings before discussing the limitations of the study and its suggestions for future research.

Overall aim of the thesis

The overall aim of this thesis was to systematically determine the best stimulus parameters for eliciting the highest level and most reliable DPOAEs in young adults, and to systematically investigate the concurrent validity of DPOAEs as an early indication of ototoxicity in adults receiving ototoxic medication as part of their treatment for MDR-TB.

DPOAE level

Stimulus intensity pairs

The investigation of DPOAE stimulus parameters in healthy, normally hearing young adults reported in Chapters 7 and 8, as well as in participants with MDR-TB receiving ototoxic medication reported in Chapter 9, found the L_1/L_2 intensity levels of 65/55 dB SPL elicited the highest and most reliable DPOAE levels across all f_2/f_1 settings, followed by 65/65 dB SPL. These results correspond with previous findings where studies used similar stimulus parameters (Beattie & Jones, 1998; Vento et al., 2004). As per the systematic review in Chapter 6, studies that used higher L_1/L_2 settings generally found these higher intensity levels yielded the highest DPOAE levels (Chida, Fukuda, Satoh, Kashiwamura, et al., 2001; Dhar et al., 1998; Hauser & Probst, 1991; Lonsbury-Martin et al., 1990; Vinck, De Vel, Xu, & Cauwenberge, 1996). These higher L_1/L_2 levels were avoided in the present thesis studies because of their higher likelihood of eliciting passive cochlear activity, false negative results and artifacts (Dhar & Hall, 2018).

Regarding stimulus intensity combinations, the function of the cochlear amplifier (Harris et al., 1989) can be used to explain why the moderate stimulus intensities of L_1/L_2 = 65/55 and 65/65 dB SPL elicited larger DPOAEs than stimuli with lower L_1/L_2 levels of 60/53, 60/45 and 55/40 dB

SPL). The cochlear amplifier is dependent on the stimulus intensity levels and functions efficiently at low to moderate stimulus intensity levels (Abdala, 2000) and reaches saturation at stimulus intensity levels > 65 dB SPL (Zelle, Thiericke, Dalhoff, & Gummer, 2015)

Based on the findings of the studies reported in chapters 7, 8 and 9, it can be recommended that L_1/L_2 = 65/55 dB SPL continued to be used if the aim is to obtain the highest level DPOAEs without eliciting passive cochlear activity and/or artifacts in individuals with normal hearing and people with MDR-TB.

Stimulus frequency ratios

Through a systematic review of research determining the stimulus parameters that yield the highest level DPOAEs (Chapter 6), it was found that f_2/f_1 values between 1.20 and 1.22 and L_1/L_2 levels of 75/75 dB SPL yield the highest level DPOAEs (Petersen et al., 2017). However, the L_1/L_2 levels of 75/75 dB SPL are known to elicit DPOAEs from passive cochlear sources resulting in response artifacts (Dhar & Hall, 2018). Thus, stimulus intensities of $L_1/L_2 \geq 70$ dB SPL are not ideal to use in a clinical setting when seeking to determine the presence of OHC damage.

For the healthy normally hearing young adults reported in Chapters 7 and 8, as well as the participants with MDR-TB receiving ototoxic medication in Chapter 9, the f_2/f_1 ratios of 1.18, and 1.20 appeared to elicit the largest DPOAEs at most f_2 values. These results support previous findings relating to the f_2/f_1 ratios that are more probable to evoke larger DPOAEs from human adults (Abdala, 1996; Brown & Gaskill, 1990; Dreisbach & Siegel, 2001; Harris et al., 1989; Johnson et al., 2006; Moulin, 2000b).

In addition, the higher the stimulus frequencies, the smaller the ideal ratio for eliciting larger DPOAEs, i.e. at f_2 = < 5000 Hz ratios of 1.20 and 1.22 yielded the highest level DPOAEs, whereas at ≥ 5000 Hz, the f_2/f_1 ratios of 1.18 consistently resulted in higher DPOAE levels across all L_1/L_2 settings (Petersen et al., 2018). These findings support previous reports that the f_2/f_1 ratio that yields the highest DPOAE levels appears to decrease as f_2 increases and vice versa (Abdala & Sininger, 1996; Baiduc & Dhar, 2018; Dreisbach & Siegel, 2001; Londero et al., 2002; Meinke et al., 2013). Gaskill and Brown (1990) found that the optimal ratio was 1.225 for a stimulus

frequency range of f_2 = 500–8000 Hz. However, this finding was based on 11 ears and descriptive statistics only (mean and standard deviation).

The larger DPOAEs elicited by f_2/f_1 ratios of 1.18, or 1.20 could reflect cochlear frequency selectivity and bandpass filter function or properties (Allen & Fahey, 1993). These findings suggest that the changing frequency ratio is representative of the bandpass function in the human cochlea (Harris et al., 1989) and give an indication of this organ's frequency selectivity (Brown et al., 1993). Thus, the decreasing f_2/f_1 ratio with increasing stimulus frequency could indicate the presence of sharper mechanical tuning in the high frequencies (Dreisbach & Siegel, 2001). The change in the optimal frequency ratio with different stimulus frequencies provides evidence for the tonotopic organisation of the basilar membrane in the cochlea and relates to critical band theory (Loven, 2009). This suggestion would be consistent with Harris et al. (1989) who found that the highest level DPOAEs were evoked with f_2/f_1 ratios of 1.22 and 1.25, respectively, with a decline with lower or higher f_2/f_1 ratios. Stover, Neely and Gorga (1999) suggested that the lower DPOAE levels at f_2/f_1 ratios higher than 1.25 could be caused by the greater separation of the stimulus frequencies that reduce the interaction of their travelling waves on the basilar membrane and hence provide less stimulus interaction to generate distortion (Gaskill & Brown, 1990). In contrast, the declines of DPOAE levels at lower f_2/f_1 ratios could be the consequence of less separation of the stimulus frequencies that cause more cancellation of their travelling waves on the basilar membrane (Stover, Neely & Gorga, 1999).

The results of this thesis suggest it is desirable to use different f_2/f_1 ratios for different stimulus frequencies to elicit the highest level DPOAEs, i.e., to use 1.20 for frequencies lower than 5000 Hz, and 1.18 for frequencies ≥ 5000 Hz. The implementation of different ratios in the clinic is feasible as most commercially available DPOAE devices offer the option of creating customised stimulus parameter sets for testing, for example the GSI Audera and Otodynamics Echoport 292.

Reliability

Although, for the healthy, normally hearing young adults reported in Chapters 7 and 8, some L_1/L_2 combinations and f_2/f_1 ratios clearly elicited larger DPOAEs, there was no difference in the

reliability of elicited DPOAEs, regardless of the L_1/L_2 combinations and f_2/f_1 ratios used. These findings were consistent with previous reports that examined the influence of a variety of stimulus parameter combinations on DPOAE test-retest reliability (Stuart, Passmore, Culbertson, & Jones, 2009; Wagner, Heppelmann, Vonthein, & Zenner, 2008). Their studies yielded similar reliability values as the current study (Stuart, Passmore, Culbertson, & Jones, 2009; Wagner, Heppelmann, Vonthein, & Zenner, 2008). However, the current thesis' findings are contrary to reports finding higher L_1/L_2 combinations to evoke more reliable DPOAEs (Franklin, McCoy, Martin, & Lonsbury-Martin, 1992; Keppler et al., 2010; Roede, Harris, Probst, & Xu, 1993). All of the mentioned studies included stimulus parameters > 65 dB SPL, which could explain the discrepancy.

It must be noted that DPOAEs for f_2 = 8003 Hz in this study were most likely to be unreliable. This result is in agreement with Keppler et al. (2010), who also found the DPOAEs at 8000 Hz unreliable. This finding might be due to the in-ear calibration (Siegel, 2002) used in both studies reported in Chapters 7 and 8 and Keppler et al. (2010). The use of a forward pressure calibration method can be suggested to improve the test-retest reliability at high frequencies, as this type of calibration is less susceptible to standing waves than in-ear calibration (Burke et al., 2010). However, additional hard- and software is needed to conduct forward pressure calibration (Rasetshwane & Neely, 2011). Therefore, until this equipment is readily available commercially, it would not be feasible in a typical clinical setup.

Choice of statistical tests to evaluate reliability of a diagnostic tool

By using inferential statistics, one is able to determine in quantitative terms how confidently the findings of the study can be generalised to larger groups of the population, based on the sample included in the current thesis (Maxwell & Satake, 2006). Therefore, the choice of appropriate statistical tests is crucial. As mentioned earlier, this thesis employed the two-way mixed model ICC for agreement to make inferences about the findings on test-retest reliability of the DPOAEs elicited by various stimulus parameter combinations. Some studies examining test-retest reliability used different statistics for this purpose. Beattie et al. (2003) and Beattie et al. (2004), for example, used the non-inferential standard error of measurement (SEM). Wagner et al. (2008)

also used the SEM, together with the Cronbach alpha analyses. The Cronbach alpha can be likened to the two-way mixed effects ICC for consistency. While this statistical test is useful in certain contexts, it might not be the most appropriate for determining test-retest reliability of DPOAEs where one wants to have absolute agreement of means, rather than the means being different but in a consistent manner (McGraw & Wong, 1996). Similarly, Keppler et al. (2010) used the SEM, ANOVA and the ICC for consistency to analyse their results to determine the test-retest reliability of DPOAEs.

In two seminal articles, namely Gaskill and Brown (1990) and Harris et al. (1989), no inferential statistics were used to determine the optimal f_2/f_1 ratio and the L_1/L_2 combination that elicit the highest level DPOAEs. With the use of no inferential statistics, coupled with small samples, there is a risk that the results could have occurred by chance. Yet, these two articles are commonly cited in peer-reviewed journal papers and textbooks as a rationale for the choice of stimulus parameters.

Sensitivity and specificity of DPOAEs to ototoxicity during treatment for MDR-TB

The results of participants with MDR-TB reported in Chapter 9 for DPOAEs indicated poor sensitivity for DPOAEs (22%) for all stimulus parameters when using high frequency audiometry as the gold standard. Similarly, Vasconcelos et al. (2018) found a 0% sensitivity rate for DPOAEs detecting ototoxic damage compared to high frequency audiometry up to 16 kHz in a longitudinal study in 10 patients with MDR-TB receiving amikacin. Vasconcelos et al. (2018) used similar criteria as the current study with MDR-TB participants: the ASHA criteria for ototoxic changes in pure tone thresholds and for DPOAEs, level reductions of 4 dB or more at a minimum of two adjacent frequencies, as specified by Reavis et al. (2011).

In contrast, Al-Malky et al. (2011) found a sensitivity rate of 100% for DPOAEs to detect abnormal OHC function in eight participants with cystic fibrosis (CF) receiving aminoglycosides with high frequency sensorineural hearing loss (9-20 kHz) when using high frequency audiometry as the gold standard. These authors used a fixed f_2/f_1 ratio = 1.22 and L_1/L_2 = 65/55 dB SPL. A possible

explanation for the differing findings of the current study and that of Al-Malky et al. (2011) could be the different criteria used for OHC damage. Al-Malky et al. (2011) used statistical differences between DPOAEs of participants with CF receiving aminoglycosides who had high frequency hearing loss vs. participants with CF with no aminoglycoside exposure who had normal hearing to determine the presence or absence of OHC damage indicated by DPOAEs. Conversely the current study with MDR-TB participants (Chapter 9) used the standard error of measurement with 90% reference limits (Reavis et al., 2015) to determine significant shifts in DPOAEs to indicate OHC damage.

The study with MDR-TB participants reported in Chapter 9 found the highest specificity values of 67% for f_2/f_1 settings of 1.20 and 1.22, in comparison to 58% for f_2/f_1 = 1.18 when using high frequency pure tone audiometry as the gold standard. The specificity values did not differ with stimulus intensity levels. These findings are contradictory to Bonfils and Avan (1992) who found that specificity values increased with higher stimulus intensity levels. So, for example in their study Bonfils and Avan (1992) found that L_1/L_2 = 42/42 dB SPL resulted in lower specificity values (26-62%) than L_1/L_2 = 62/62 dB SPL with a specificity range of 84-100%. This contradictory finding could be due to the fact that Bonfils and Avan (1992) used standard pure tone audiometry up to 8000 Hz as the gold standard and not high frequency audiometry. It is known that standard pure tone audiometry is less sensitive to ototoxic damage than high frequency audiometry. Additionally, another possible reason for the discrepancy could be due to Bonfils and Avan's larger sample size of 50 ears with confirmed sensorineural hearing loss versus the current study's nine ears that developed hearing loss, as determined by high frequency audiometry. The influence of changes in the frequency ratio on specificity values could not be compared to other research due to a lack of information provided in relevant studies with high frequency audiometry as the gold standard.

The textbook definitions of sensitivity and specificity hinge on the use of a gold standard (Maxwell & Satake, 2006). For ototoxicity, temporal bone studies are viewed as the gold standard in animal studies. For obvious reasons, this technique is not feasible for clinical practice in humans. As a proxy for OHC damage, deterioration in high frequency pure tone thresholds serves as the gold

standard in humans (ASHA, 1994). However, the gold standard, hfPTA, has its own limitations. Certain populations like sick patients or children might not be able to concentrate for long periods of time (Vasquez & Mattucci, 2003) or be unable to yield reliable behavioural responses (Knight et al., 2007) as required by hfPTA. For valid pure tone audiometry results, one has to rely on people to provide active responses to the stimuli that are presented. Thus, in patients that are ill, valid results might not be obtained (Konrad-Martin et al., 2016). In addition, the participants with MDR-TB reported in Chapter 9 could have experienced depression, anxiety and/or distress, due to the disease itself or due to hospitalisation (Dos Santos, Lazzari, & Silva, 2017; Yilmaz & Dedeli, 2016), which could have negatively influenced performance on pure tone audiometry. Therefore, in these populations, hfPTA might not be the most sensitive test to detect OHC damage. As DPOAEs only require passive participation, it would be a more suitable test for OHC dysfunction in ill patients or others who cannot participate optimally in behavioural testing.

The higher sensitivity of DPOAEs to detect ototoxic damage in humans in comparison to standard pure tone audiometry (≤ 8 kHz) is widely accepted (Knight et al., 2007; Konrad-Martin et al., 2016; Stavroulaki et al., 2002). Due to the lack of an objective gold standard like temporal bone histopathology in humans, this knowledge was created by clinical studies using a 4 - 8 dB decline in DPOAE levels at a minimum of two adjacent frequencies to determine the number of ears/participants that exhibit OHC damage due to ototoxicity (Knight et al., 2007). Furthermore, other studies compared the timing of DPOAEs vs. standard pure tone audiometry to detect ototoxicity (Mulheran & Degg, 1997; Ozturan et al., 1996; Stavroulaki et al., 2002) and found DPOAEs to detect ototoxic damage first. The following discussion will use the same approach to compare the ability of DPOAEs and high frequency audiometry to detect ototoxicity.

In the participants receiving ototoxic medication as part of their treatment for MDR-TB (Chapter 9), the largest reductions in DPOAE levels from baseline to the second test occasion 10 to 14 days later were observed for the stimulus combinations of L_1/L_2 = 65/55 dB SPL with an f_2/f_1 of 1.18. The next largest reductions were observed for L_1/L_2 = 65/65 dB SPL and f_2/f_1 1.18, followed by L_1/L_2 = 65/55 dB SPL with f_2/f_1 = 1.20 and L_1/L_2 = 65/65 with f_2/f_1 = 1.20. The stimulus combination of L_1/L_2 60/53 consistently showed the least reduction in DPOAE level, regardless of the f_2/f_1 ratio

used. The reference limits used in Chapter 9 were constructed by Reavis et al. (2015) through meta-analysis to determine DPOAE level changes that signify true change in ear function not due to test-retest variability. These results differ from recommendations by Dhar and Hall (2018), who suggest stimulus parameter combinations of L_1/L_2 = 65/55, 55/45 and 45/35 dB SPL with an f_2/f_1 ratio of 1.22 for ototoxicity monitoring. This recommendation implies that lower stimulus intensities are more suitable for ototoxicity monitoring. The source of their information was not provided and thus the reason for their recommendations could not be examined. Based on the findings of the systematic review reported in Chapter 6, it appears that no other study manipulated both intensity and ratio stimulus parameters at the same time in a single study with MDR-TB participants receiving ototoxic medication (Petersen et al., 2017). Thus, the results of the current study could not be directly compared with other studies.

Based on the results of the current study with MDR-TB participants, it is proposed that stimulus parameters of L_1/L_2 = 65/55 dB SPL with an f_2/f_1 of 1.18 at $f_2 \geq 5000$ Hz be used for ototoxicity monitoring. As these stimulus parameters showed the highest reduction in DPOAE levels it could signal the early onset of OHC damage and thus enable early detection of ototoxicity.

When comparing the DPOAE levels and pure tone thresholds at both test occasions statistically, no significant correlation was found between the results of the two tests regardless of the stimulus parameters used. In addition, there was no correlation between changes in DPOAE level and high frequency pure tones. Some disagreement between OAE and behavioural threshold shifts should be anticipated due to (i) OAEs' greater sensitivity to cochlear OHC function, (ii) the fact that hearing loss > 50 dB HL can impede OAEs from being measured and (iii) hearing loss in some ears will not exclusively be due to OHC dysfunction (Ewert et al., 2012; Konrad-Martin et al., 2016; Trautwein, Hofstetter, Wang, Salvi, & Nostrant, 1996).

However, when examining clinically significant changes in the participants with MDR-TB (Chapter 9), using the ASHA criteria for ototoxic changes for pure tone audiometry (ASHA, 1994) and the criteria for DPOAEs published by Reavis et al. (2015), there was correspondence between the DPOAE results and pure tone thresholds (standard and high frequencies) indicating no clinically significant changes in more than half the ears (54%). In 11 to 15 participants (depending on the

f_2/f_1 ratio used), DPOAEs decreased significantly between the two test sessions. Pure tone thresholds for these participants remained the same or improved. It is evident that DPOAEs showed a larger number of ears with OHC damage than hfPTA (9 ears). The frequency ratio of 1.18 detected the highest number of significant changes, regardless of the stimulus intensity pairs used. This result indicates that the stimulus intensity pairs L_1/L_2 = 65/55 dB SPL and L_1/L_2 = 65/65 dB SPL could potentially be used to detect OHC damage when combined with a frequency ratio of 1.18. It is important to note that the lowest stimulus intensity pair L_1/L_2 = 60/53 dB SPL decreased the least between the baseline and second test and might not be as sensitive to determine early OHC damage as the other two stimulus intensity pairs. This finding needs to be confirmed with a larger sample size over a longer test period, though. Reavis et al. (2008) found a roughly equal proportion of ears experiencing DPOAE changes before, during and after behavioural hearing changes in a sample of 53 adults receiving either chemotherapy or ototoxic antibiotics. These authors used stimulus parameters of L_1/L_2 = 65/59 dB SPL and an f_2/f_1 ratio of 1.2. Seeing that cochlear damage first occurs in the basal area of the organ of Corti, this ratio might not have been sensitive enough to detect early changes in OHC function.

Upon investigating individual changes in high frequency pure tone thresholds from 10–16 kHz, using the ASHA criteria for ototoxicity (1994), thresholds in 9 out of 42 ears deteriorated, 25 ears' thresholds stayed the same, and the thresholds improved in 8 ears (meaning that the hearing improved). The improvement in pure tone thresholds between the baseline and 10–14 days later could be due to the participants being too ill to reliably participate optimally in behavioural testing. Fausti, Frey, Henry, Robertson, and Hertert (1992) reported when patients are critically ill, as is the case with MDR-TB, up to 33% might be unable to actively participate in behavioural testing. Additionally, the current study reported in Chapter 9 included patients with MDR-TB that were hospitalised, which could have led to increased anxiety and distress (Dos Santos et al., 2017; Yilmaz & Dedeli, 2016), which in turn could influence the validity of pure tone audiometry. After being hospitalised for a few days and receiving medication, participants' physical and/or mental health could have improved, which could also explain improved pure tone thresholds. Another

possible reason for improved pure tone thresholds could relate to the learning effect of pure tone testing, seeing that it requires active participation.

In MDR-TB participants reported in Chapter 9, with DPOAEs across all stimulus parameters, on average, the level decreased in 14 out of 42 ears, ears, remained the same in 21 ears and increased in seven ears. Based on these findings, DPOAEs detected OHC damage in more ears than high frequency pure tone audiometry (14 ears vs 9 ears) based on DPOAE level shift criteria provided by Reavis et al. (2015). This result concurs with the findings of Arnold et al. (1999) that suggest DPOAEs can be sensitive to OHC damage occurring at basal locations that are responsible for coding frequencies higher than the tones eliciting the DPOAEs. The results of the current study are in in agreement with those of Appana et al. (2016) and Geyer, Barreto, Weigert, and Teixeira (2015) who found that DPOAEs detected OHC damage in more ears than high frequency audiometry in patients with MDR-TB and cystic fibrosis, respectively. In addition, Ress et al. (1999) and Yu et al. (2014) found that DPOAEs detected ototoxicity in as many ears as high frequency audiometry in their respective studies, which also partially supports the current study's findings.

However, in contrast to the current study and abovementioned research, Abujamra et al. (2013) found high frequency audiometry detected hearing loss in the highest number of participants, i.e. 36 of 42 participants (86%) treated with cisplatin. In their study, DPOAEs detected the second highest number of participants with decreased OHC function, that is 27 participants (64%). Lastly, standard pure tone audiometry detected the lowest number of participants with hearing loss, i.e., 24 (57%). The two studies used different criteria to determine OHC damage, which could explain the discrepant results. For hearing loss, Abujamra et al. (2013) used normative data provided by three studies (Carvallo et al., 2002; Pedalini et al., 2000; Sahyeb et al., 2003). In contrast, the current study with MDR-TB patients (Chapter 9) used participants as their own controls through repeated measures rather than normative data to mitigate the known high intersubject variability of high frequency audiometry. In addition, the current study used the ASHA criteria (1994) to determine significant pure tone threshold shifts. For DPOAEs, Abujamra et al. (2013) regarded the emissions as present if the signal to noise ratio was between 0 to 10

dB and the DPOAE was 3 dB above the noise floor. The current study (Chapter 9) used DPOAE change criteria determined through a meta-analysis of 10 studies (Reavis et al., 2015), which provides for a more robust measure of DPOAE change than present/abnormal/absent criteria used by Abujamra et al. (2013). It is also noted that the DPOAE criteria cited by Abujamra et al. (2013) likely contains an error, as it is not possible to have DPOAEs 3 dB above the noise floor if the acceptable signal to noise ratio is 0 to 10 dB.

Where DPOAEs increased over time, it could have been due to possible subclinical middle ear problems not detected by tympanometry or otoscopy. Based on the reference limits for DPOAE level shifts provided by Reavis et al. (2015), it is unlikely that the increases in DPOAE levels were due to chance.

Based on the results of the study with MDR-TB patients (Chapter 9), if only high frequency pure tone audiometry was used, up to a third of participants who developed outer hair cell damage would have been missed. Cochlear outer hair cell damage in seven ears would potentially have gone unnoticed after two weeks if relying on DPOAEs only. These results of the current study and previous studies illustrate the importance of a test battery approach for ototoxicity monitoring, including both high frequency audiometry and DPOAEs. However, if only one test can be chosen for ototoxicity monitoring in patients with MDR-TB, it is recommended that DPOAEs be used with $L_1/L_2 = 65/55$, $f_2/f_1 = 1.18$ (for $f_2 \geq 5000$ Hz) and $f_2/f_1 = 1.20$ for $f_2 = < 5000$ Hz. The recommendation to use DPOAEs is supported by (i) the higher number of ears identified with OHC damage, (ii) it being an objective test that does not require active participation and (iii) DPOAE test time being shorter than high frequency pure tone audiometry. This shorter test time will enable more patients to be tested, which is especially useful in resource-constrained settings like South Africa.

This thesis focused on adults. However, children might not reliably respond to pure tone audiometry due to limited attention span or being too ill (Knight et al., 2007). DPOAEs with optimal stimulus parameters might be more ideal to use in this population but has to be confirmed by future studies.

Early identification of cochlear OHC damage in patients receiving ototoxic medication can prevent or minimise potential hearing loss that could impact negatively on quality of life. In addition, early identification and prevention of hearing loss can lessen the economic impact as Jiang, Karasawa, and Steyger (2017) indicated that the socioeconomic burden for each adult acquiring a hearing loss is > $35000 in 2015 dollars over their remaining lifespan. In the South African context early identification and management of hearing loss becomes crucial to reduce economic strain on already limited resources.

Various criteria exist for DPOAE change (Beattie & Bleech, 2000; Beattie et al., 2003; Reavis et al., 2011; Reavis et al., 2015). The current thesis used Reavis et al. (2015) as it is the best evidence currently available. However, universal criteria to determine clinically significant DPOAE change constituting cochlear damage would be welcomed to aid comparisons between studies (Konrad-Martin et al., 2016).

Broad discussion of the thesis

The current study set out to systematically investigate DPOAE stimulus parameters to determine the optimal parameter combination to obtain the highest level, most reliable and valid DPOAEs. The DPOAE stimulus parameters currently used clinically seemed well-established and largely unquestioned. However, the present thesis revealed that the widely-accepted stimulus parameters were not derived from the most stringent research, with this criticism extending to even seminal articles such as Gaskill and Brown (1990) and Harris et al. (1989). Through a rigorous sequential research process, the present thesis showed that, for the most part, the "well-established and largely unquestioned" DPOAE stimulus parameters used clinically are suitable for eliciting the largest and most reliable DPOAEs in healthy, normally hearing young adults, and for monitoring of ototoxicity in adult patients being treated for MDR-TB.

The sequential research design enabled the researcher to interrogate the question of ideal DPOAE stimulus parameters through a sequence of studies. These studies progressively narrowed the range of stimulus parameters under investigation in healthy normally hearing adults before finally examining the most promising of these parameters on the final target of

patients with MDR-TB receiving ototoxic medication. If, for example, the thesis only consisted of the systematic review to determine the recommended stimulus parameters for the highest level, most reliable and valid DPOAEs, the stimulus intensity levels of L_1/L_2 = 75/75 dB SPL would have been suggested for clinical use. However, it is well known that such high intensity stimuli would elicit passive responses from the cochlea, which could result in a high number of false negative DPOAE results when testing for ototoxicity as an example of outer hair cell damage.

This sequential study design allowed for the results of the thesis to be replicated in three studies following the same data collection protocol, i.e., a large number of stimulus parameter combinations were evaluated in the preliminary study to eliminate the stimulus parameters with the worst performance, and these parameters were subsequently confirmed in the larger sample of healthy participants and the patient population. The study with normal-hearing, healthy participants reported in Chapter 7 allowed the researcher to eliminate stimulus parameters that did not elicit high DPOAE levels in a small sample before proceeding to examine stimulus parameters in a larger sample (Chapter 8). This practice is aligned to the ethical principle of non-maleficence to not subject a large number of participants to long periods of data collection that is not going to render useful information. If the patients with MDR-TB were not included, the concurrent validity of DPOAEs with a variety of stimulus parameters could not have been determined.

In this thesis, the framework for validity included test-retest reliability and concurrent validity. This framework enabled the researcher to examine DPOAE stimulus parameters in a comprehensive manner and to obtain empirical data in a systematic way. Knowledge generation is cyclical and an iterative process, as demonstrated in Figure 10.1.

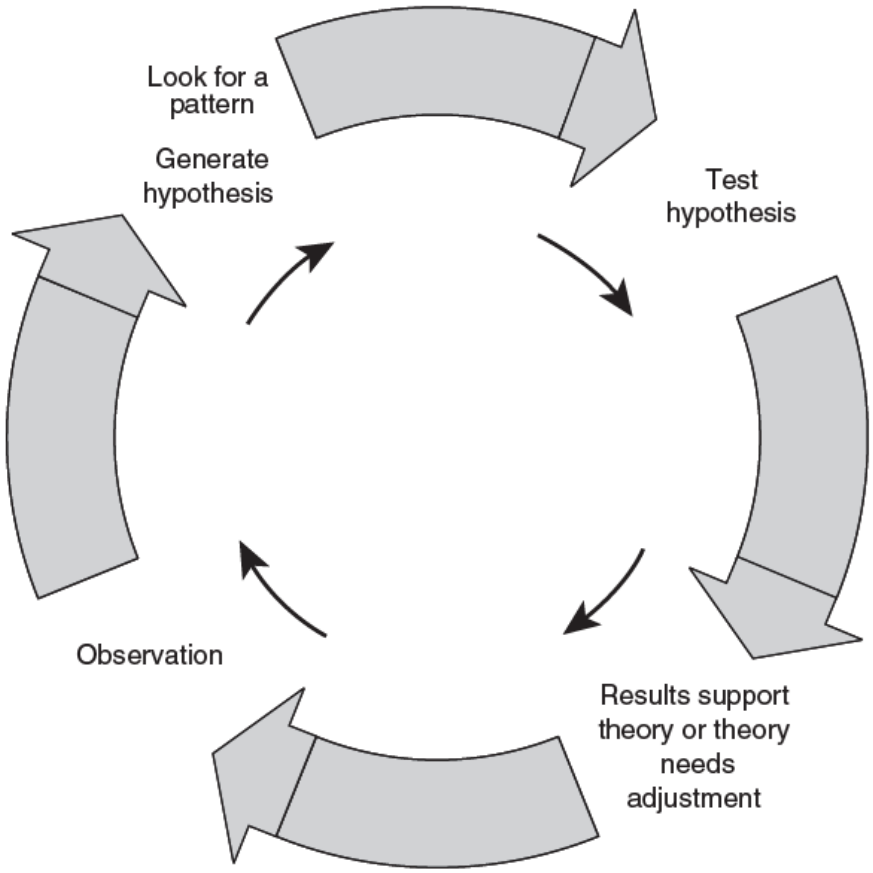

Figure 10.1 The research wheel. Source: Mukherji and Albon (2014)

With this iterative process in mind, overall, L_1/L_2 = 65/55 with an f_2/f_1 ratio of 1.18 consistently yielded the largest magnitude DPOAEs across the different participant groups. However, it was also evident that all the stimulus parameters evaluated (L_1/L_2 = 65/65, 65/55, 60/53 dB SPL and f_2/f_1 ratios of 1.18, 1.20 and 1.22) performed equally well when comparing them to the change in high frequency pure tone thresholds over time (based on the results of the study with MDR-TB participants). Seeing that a range of stimulus parameter combinations is available for ototoxicity monitoring, it could be time to focus on making the technology more accessible rather than refining the stimulus parameters further.

Patients suffering from MDR-TB are mostly likely to be from a developing country (World Health Organization, 2019a) where access to healthcare is compromised due to limited resources. Due to the burden of this disease, large numbers of patients require routine ototoxicity monitoring up to 6 months after MDR-treatment has been stopped. Seeing that DPOAEs are quick and easy

to administer, with good reliability and high sensitivity and specificity, the hearing healthcare community ought now to consider innovative ways to broaden ototoxicity monitoring so that it can be universal.

To broaden access to ototoxicity monitoring, personnel like nursing assistants, community health workers and volunteers could be trained to conduct the testing, similar to the implementation of neonatal and infant hearing screening. With the decentralised treatment of MDR-TB recommended by the World Health Organization, primary management does not take place at a large secondary or tertiary institution, but rather closer to home, at clinics in the community (World Health Organization, 2019b). This decentralisation also means that audiology services need to be more widespread than before with centralised TB-treatment.

Apart from training personnel, more cost-effective ototoxicity monitoring strategies could be implemented with DPOAEs. With rapid advances in technology, through options like tele-Audiology, DPOAE applications for cell phones or even self-testing do not sound so far-fetched anymore. An example of applying cell phone technology to broaden access to hearing healthcare is the applications of the hearX group (a South African company) which enables otoscopy, hearing screening and diagnostic hearing testing without the need of a soundproof booth (hearX, 2020). With such an approach, earlier detection of ototoxic damage could be realised, thereby facilitating early and timely management. In turn, such an approach could minimise the extent of ototoxic damage, which could potentially lessen the impact on quality of life for individuals exposed to ototoxic medication.

Quantitative research strives to determine whether a hypothesis is true or false, and it can assist in refining a test like DPOAEs. Obviously, it is important to know that a test has been optimised in terms of reliability and validity, but questions about accessibility and acceptability to patients also need to be answered for successful implementation. Thus, it is also important to consider context and need. In this instance, MDR-TB patients need to be kept in mind when developing/refining a test like DPOAEs. Therefore, it would be ideal to employ a post-positivist research paradigm that would enable the use of quantitative and qualitative research methodologies to gather information about facilitators and barriers to the successful

implementation of new strategies and technologies with DPOAEs. Such an approach has the transformative potential to change practice and make a real impact on hearing healthcare related to ototoxicity monitoring.

Limitations of the thesis

One of the limitations of the thesis is the small sample sizes of the studies reported in Chapters 7 and 9, that is, the preliminary studies with (a) the normally hearing, healthy adults to determine the stimulus parameters used in the rest of the thesis, and (b) the participants with MDR-TB receiving ototoxic medication. Thus, the results of these studies cannot be generalised to the population as a whole. It is recommended that a study with MDR-TB participants receiving ototoxic medication be replicated with a larger sample size. In addition, the constraints of clinical research in developing country contexts need to be taken into account when embarking on such a study. As was noted in Chapter 9, the recruitment period for participants with MDR-TB was extensive as the stringent inclusion criteria excluded potential participants with existing hearing loss or middle ear dysfunction and individuals with other medical conditions that could negatively influence hearing, e.g., diabetes or being HIV-positive. Thus, the pool of potential participants was significantly reduced. Furthermore, data collection took place at a facility where mainly patients for extensively drug-resistant TB and only patients with complicated MDR-TB were hospitalised. For future research pertaining to patients with MDR-TB, it is recommended to conduct a similar study at decentralised treatment facilities where the testing can be conducted in the community to increase the pool of potential participants. It would, however, be challenging for future research to find alternative data collection sites in the Western Cape with a soundproof booth or facilities where data collection would not interrupt routine clinical services. Additionally, during the data collection period, the recommended WHO MDR-TB treatment guidelines with bedaquiline as the preferred treatment were implemented at Brooklyn Chest hospital, further reducing the number of eligible individuals for the study.

Another limitation of the thesis is not including f_2/f_1 ratios less than 1.18. As this thesis and previous research showed, lower frequency ratios elicit higher level DPOAEs at higher frequencies, it is possible that ratios less than 1.18 could have yielded improved DPOAEs. Thus, it is recommended that future research with DPOAEs up to 8 kHz includes f_2/f_1 ratios less than 1.18.

The use of in-ear calibration, utilising sound pressure level, instead of calibration with forward pressure level can also be viewed as a limitation of the study. Forward pressure level accounts for ear canal acoustics and can deliver more accurately calibrated stimuli to the ear than the traditional calibration method (Burke et al., 2010).

Conclusion

The cochlea is a highly complex but vulnerable, organ. Ototoxic drugs damage the OHCs of the cochlea, and the resulting hearing loss can have a negative impact on the individual's quality of life.

To detect outer hair cell damage as early as possible, it is crucial to refine diagnostic tests to improve their sensitivity and specificity for ototoxicity. Otoacoustic emissions measure the functioning of the OHCs in a non-invasive and objective manner and does not need active cooperation from patients.

This study identified L_1/L_2 of 65/55, together with an f_2/f_1 ratio of 1.18 to yield the highest level DPOAEs. In addition, it seems that DPOAEs using these stimulus parameters are most sensitive to ototoxic damage, especially for stimulus frequencies ≥ 5000 Hz, when compared to high frequency pure tone audiometry.

Future attempts to determine a clearly defined set of ideal stimulus parameters to evoke DPOAEs of higher level and/or greater reliability in healthy adults and higher sensitivity and specificity in adults with cochlear lesions could involve larger study samples of participants who receive ototoxic medication as part of their treatment. While this research continues, clinicians should turn to meta-analyses of stimulus parameters for eliciting DPOAEs from adult humans, as was

done by Carter et al. (2015) and Reavis et al. (2015). This approach could allow the clinician to obtain DPOAEs with the highest absolute levels, best test-retest reliability and most sensitive and specificity to ototoxic damage of DPOAEs when the best current evidence for sets stimulus parameters are incorporated into clinical practice. In addition, research could focus on making this DPOAE technology more accessible, especially for populations in developing countries.

References

AAA. (2009). *Position statement and clinical practice guidelines: Ototoxicity management*. Retrieved from https://www.audiology.org/publications-resources/document-library/ototoxicity-monitoring

Abdala, C. (1996). Distortion product otoacoustic emission (2f1-2f2) amplitude as a function of f2/f1 frequency ratio and primary tone level separation in human adults and neonates. *Journal of the Acoustical Society of America, 100*(6), 3726-3740.

Abdala, C. (2000). Distortion product otoacoustic emission (2f1-f2) amplitude growth in human adults and neonates. *The Journal of the Acoustical Society of America, 107*(1), 446-456.

Abdala, C., & Kalluri, R. (2017). Towards a joint reflection-distortion otoacoustic emission profile: Results in normal and impaired ears. *J Acoust Soc Am, 142*(2), 812. doi:10.1121/1.4996859

Abdala, C., & Sininger, Y. S. (1996). The development of cochlear frequency resolution in the human auditory system. *Ear and hearing, 17*(5), 374-385.

Abujamra, A. L., Escosteguy, J. R., Dall'Igna, C., Manica, D., Cigana, L. F., Coradini, P., . . . Gregianin, L. J. (2013). The use of high-frequency audiometry increases the diagnosis of asymptomatic hearing loss in pediatric patients treated with cisplatin-based chemotherapy. *Pediatric blood & cancer, 60*(3), 474-478.

Al-Malky, G., Suri, R., Dawson, S. J., Sirimanna, T., & Kemp, D. (2011). Aminoglycoside antibiotics cochleotoxicity in paediatric cystic fibrosis (cf) patients: a study using extended high-frequency audiometry and distortion product otoacoustic emissions. *International Journal of Audiology, 50*(2), 112-122. doi:http://dx.doi.org/10.3109/14992027.2010.524253

Allen, J., & Fahey, P. (1993). A second cochlear-frequency map that correlates distortion product and neural tuning measurements. *The Journal of the Acoustical Society of America, 94*(2), 809-816.

Appana, D., Joseph, L., & Paken, J. (2016). An audiological profile of patients infected with multi-drug resistant tuberculosis at a district hospital in KwaZulu-Natal. *South African Journal of Communication Disorders, 63*(1), 1-12.

Arnold, A., Cooke, G. S., Kon, O. M., Dedicoat, M., Lipman, M., Loyse, A., . . . Harrison, T. S. (2017). Adverse effects and choice between the injectable agents amikacin and Capreomycin in multidrug-resistant tuberculosis. *Antimicrobial agents and chemotherapy, 61*(9), e02586-02516.

Arnold, D. J., Lonsbury-Martin, B. L., & Martin, G. K. (1999). High-frequency hearing influences lower-frequency distortion-product otoacoustic emissions. *Archives of Otolaryngology - Head & Neck Surgery, 125*(2), 215-222. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=1999024306&site=ehost-live

Asgedom, S. W., Teweldemedhin, M., & Gebreyesus, H. (2018). Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review. *Journal of pathogens, 2018*.

ASHA. (1994). Audiologic management of individuals receiving cochleotoxic drug therapy.

Baiduc, R. R., & Dhar, S. (2018). Exploring Optimal Stimulus Frequency Ratio for Measurement of the Quadratic f2–f1 Distortion Product Otoacoustic Emission in Humans. *Journal of Speech, Language, and Hearing Research, 61*(7), 1794-1806. doi:10.1044/2018_JSLHR-H-17-0349

Barclay, M., & Begg, E. (1994). Aminoglycoside toxicity and relation to dose regimen. *Adverse drug reactions and toxicological reviews, 13*(4), 207-234.

Beattie, R. C., & Bleech, J. (2000). Effects of sample size on the reliability of noise floor and DPOAE. *British journal of audiology, 34*(5), 305-309. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0033768449&partnerID=40&md5=0a0ffd23f151272c05d5bed62de9fd68

Beattie, R. C., Caldwell, S., & Kenworthy, O. T. (2005). Variability in DPOAE measurements and its relation to the magnitude and frequency of occurrence of peaks in the DPOAE-gram. *The Australian and New Zealand Journal of Audiology, 27*(2), 113-130.

Beattie, R. C., & Ireland, A. (2000). Effects of sample size on the noise floor and distortion product otoacoustic emissions. *Scandinavian Audiology, 29*(2), 93-102. doi:10.1080/010503900424507

Beattie, R. C., & Jones, R. L. (1998). Effects of relative levels of the primary tones on distortion product otoacoustic emissions in normal-hearing subjects. *Audiology, 37*(4), 187-197.

Beattie, R. C., Kenworthy, O., & Neal-Johnson, C. M. (2004). Distortion product otoacoustic emissions: Input-output functions for primary-tone pairs at 1000, 2000 and 4000 Hz in normal-hearing females. *Australian and New Zealand Journal of Audiology, The, 26*(2), 116.

Beattie, R. C., Kenworthy, O. T., & Luna, C. A. (2003). Immediate and short-term reliability of distortion-product otoacoustic emissions. *International Journal of Audiology, 42*(6), 348-354.

Bennett, C. C. (1996). The aminoglycosides. *Primary Care Update for OB/GYNS, 3*(6), 186-191.

Berlin, C. I. (1998). *Otoacoustic emissions: basic science and clinical applications*: Singular Publishing Group.

Bian, L., & Chen, S. (2008). Comparing the optimal signal conditions for recording cubic and quadratic distortion product otoacoustic emissions. *The Journal of the Acoustical Society of America, 124*(6), 3739-3750. doi:10.1121/1.3001706

Bilger, R. C., Matthies, M. L., Hammel, D. R., & Demorest, M. E. (1990). Genetic implications of gender differences in the prevalence of spontaneous otoacoustic emissions. *Journal of Speech, Language, and Hearing Research, 33*(3), 418-432.

Bland, J., & Altman, D. (1990). A note on the use of the intraclass correlation coefficient in the evaluation of agreement between two methods of measurement. *Computers in biology and medicine, 20*(5), 337-340.

Bonfils, P. (1989). Spontaneous otoacoustic emissions: clinical interest. *The Laryngoscope, 99*(7), 752-756.

Bonfils, P., & Avan, P. (1992). Distortion-product otoacoustic emissions: Values for clinical use. *Archives of Otolaryngology–Head & Neck Surgery, 118*(10), 1069-1076.

Bonfils, P., Avan, P., Londero, A., Trotoux, J., & Narcy, P. (1991). Objective low-frequency audiometry by distortion-product acoustic emissions. *Archives of Otolaryngology–Head & Neck Surgery, 117*(10), 1167-1171.

Bright, K. (2002). Spontaneous otoacoustic emissions. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic emissions: Clinical applications* (2nd ed., pp. 74-94). New York: Thieme Medical Publishers.

Brown, A. M., & Gaskill, S. A. (1990). Measurement of acoustic distortion reveals underlying similarities between human and rodent mechanical responses. *The Journal of the Acoustical Society of America, 88*(2), 840-849. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=2212309&site=ehost-live

Brown, A. M., Gaskill, S. A., Carlyon, R. P., & Williams, D. M. (1993). Acoustic distortion as a measure of frequency selectivity: relation to psychophysical equivalent rectangular bandwidth. *The Journal of the Acoustical Society of America, 93*(6), 3291-3297.

Brown, A. M., Sheppard, S. L., & Russell, P. T. (1994). Acoustic distortion products (ADP) from the ears of term infants and young adults using low stimulus levels. *British journal of audiology, 28*(4-5), 273-280.

Brown, S. J. (2014). *Evidence-based nursing: The research-practice connection* (3rd ed.): Jones & Bartlett Publishers.

Burke, S. R., Rogers, A. R., Neely, S. T., Kopun, J. G., Tan, H., & Gorga, M. P. (2010). Influence of Calibration Method on Distortion-Product Otoacoustic Emission Measurements: I. Test Performance. *Ear & Hearing, 31*(4), 533-545. doi:10.1097/AUD.0b013e3181d86b3d

Burns, E. M., Arehart, K. H., & Campbell, S. L. (1992). Prevalence of spontaneous otoacoustic emissions in neonates. *The Journal of the Acoustical Society of America, 91*(3), 1571-1575.

Campbell, K., & Durrant, J. (1993). Audiologic monitoring for ototoxicity. *Otolaryngologic Clinics of North America, 26*(5), 903-914.

Carter, L., Williams, W., & Seeto, M. (2015). TE and DP otoacoustic emission data from an Australian cross-sectional hearing study. *International Journal of Audiology, 54*(11), 806-817.

Carvallo, R. M. M., Koga, M. C., Carvalho, M. d., & Ishida, I. M. (2002). Limiares auditivos para altas frequências em adultos sem queixa auditiva. *Acta AWHO, 21*(1), 16.

Castillo, M. P., & Roland, P. S. (2007). Disorders of the auditory system. In R. J. Roeser, M. Valente, & H. Hosford-Dunn (Eds.), *Audiology diagnosis* (2nd ed.). New York: Thieme.

Chida, E., Fukuda, S., Satoh, N., Kashiwamukra, M., Matsumura, M., Ohwatari, R., . . . Inuyama, Y. (2001). Optimal stimulus level conditions for measurements of distortion product otoacoustic emissions. *Auris, Nasus, Larynx, 28 Suppl*, S19-22.

Chida, E., Fukuda, S., Satoh, N., Kashiwamura, M., Matsumura, M., Ohwatari, R., . . . Inuyama, Y. (2001). Optimal stimulus level conditions for measurements of distortion product otoacoustic emissions. *Auris Nasus Larynx, 28*, S19-S22.

Clarke, J. (2011). What is a systematic review? *Evidence-based nursing, 14*(3), 64-64.

Cohen, S. M., Labadie, R. F., Dietrich, M. S., & Haynes, D. S. (2004). Quality of life in hearing-impaired adults: the role of cochlear implants and hearing aids. *Otolaryngology-Head and Neck Surgery, 131*(4), 413-422.

Coolican, H. (2017). *Research methods and statistics in psychology* (7th ed.): Psychology Press.

Cooper, N., & Rhode, W. (1997). Mechanical responses to two-tone distortion products in the apical and basal turns of the mammalian cochlea. *Journal of neurophysiology, 78*(1), 261-270.

Cox, H. S., McDermid, C., Azevedo, V., Muller, O., Coetzee, D., Simpson, J., . . . Goemaere, E. (2010). Epidemic levels of drug resistant tuberculosis (MDR and XDR-TB) in a high HIV prevalence setting in Khayelitsha, South Africa. *PloS one, 5*(11), e13901.

Cox, R. M. (2005). Evidence-based practice in provision of amplification. *Journal of the American Academy of Audiology, 16*(7), 419-438.

De Jager, P., & Van Altena, R. (2002). Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. *The International journal of tuberculosis and lung disease, 6*(7), 622-627.

Department of Health. (2019). *Multi-drug resistant tuberculosis: A Policy Framework on Decentralised and Deinstitutionalised Management for South Africa*. Pretoria: Department of Health

Dhar, S., & Hall, J. W. (2012). *Otoacoustic emissions: Principles, procedures and protocols*. San Diego: Plural Publishing.

Dhar, S., & Hall, J. W. (2018). *Otoacoustic emissions: principles, procedures, and protocols* (2nd ed.). San Diego: Plural.

Dhar, S., Long, G. R., & Culpepper, N. B. (1998). The Dependence of the Distortion Product 2f1–f2 on Primary Levels in Non-Impaired Human Ears. *Journal of Speech, Language, and Hearing Research, 41*(6), 1307-1318.

Dhar, S., Long, G. R., Talmadge, C. L., & Tubis, A. (2005). The effect of stimulus-frequency ratio on distortion product otoacoustic emission components. *The Journal of the Acoustical Society of America, 117*(6), 3766-3776.

Dille, M. F., McMillan, G. P., Reavis, K. M., Jacobs, P., Fausti, S. A., & Konrad-Martin, D. (2010). Ototoxicity risk assessment combining distortion product otoacoustic emissions with a cisplatin dose model a. *The Journal of the Acoustical Society of America, 128*(3), 1163-1174.

Dorn, P. A., Konrad-Martin, D., Neely, S. T., Keefe, D. H., Cyr, E., & Gorga, M. P. (2001). Distortion product otoacoustic emission input/output functions in normal-hearing and hearing-impaired human ears. *The Journal of the Acoustical Society of America, 110*(6), 3119-3131.

Dos Santos, A. P. C., Lazzari, T. K., & Silva, D. R. (2017). Health-related quality of life, depression and anxiety in hospitalized patients with tuberculosis. *Tuberculosis and respiratory diseases, 80*(1), 69-76.

Dreisbach, L. E., & Siegel, J. H. (2001). Distortion-product otoacoustic emissions measured at high frequencies in humans. *The Journal of the Acoustical Society of America, 110*(5), 2456-2469.

Dreisbach, L. E., & Siegel, J. H. (2005). Level dependence of distortion-product otoacoustic emissions measured at high frequencies in humans. *The Journal of the Acoustical Society of America, 117*(5), 2980-2988.

Duggal, P., & Sarkar, M. (2007). Audiologic monitoring of multi-drug resistant tuberculosis patients on aminoglycoside treatment with long term follow-up. *BMC Ear, Nose and Throat Disorders, 7*(1), 5.

Ewert, D. L., Lu, J., Li, W., Du, X., Floyd, R., & Kopke, R. (2012). Antioxidant treatment reduces blast-induced cochlear damage and hearing loss. *Hearing research, 285*(1-2), 29-39.

Fätkenheuer, G., Taelman, H., Lepage, P., Schwenk, A., & Wenzel, R. (1999). The return of tuberculosis. *Diagnostic microbiology and infectious disease, 34*(2), 139-146.

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods, 39*(2), 175-191.

Fausti, S., Helt, W., Gordon, J., Reavis, K., Phillips, D., & Konrad-Martin, D. (2007). Audiologic monitoring for ototoxicity and patient management. In K. Campbell (Ed.), *Pharmacology and ototoxicity for audiologists.* (pp. 230-251). New York: Thomson Delmar Learning.

Fausti, S. A., Frey, R., Henry, J., Robertson, P., & Hertert, R. (1992). Portable stimulus generator for obtaining high-frequency (8–14 kHz) auditory brainstem responses. *J Am Acad Audiol, 3*, 166-175.

Fausti, S. A., Henry, J. A., Helt, W. J., Phillips, D. S., Frey, R. H., Noffsinger, D., . . . Fowler, C. G. (1999). An individualized, sensitive frequency range for early detection of ototoxicity. *Ear and hearing, 20*(6), 497-505.

Fausti, S. A., Henry, J. A., Schaffer, H. I., Olson, D. J., Frey, R. H., & McDonald, W. J. (1992). High-frequency audiometric monitoring for early detection of aminoglycoside ototoxicity. *Journal of infectious diseases, 165*(6), 1026-1032.

Fausti, S. A., Larson, V. D., Noffsinger, D., Wilson, R. H., Phillips, D. S., & Fowler, C. G. (1994). High-frequency audiometric monitoring strategies for early detection of ototoxicity. *Ear and hearing, 15*(3), 232-239.

Fausti, S. A., Rappaport, B. Z., Schechter, M. A., Frey, R. H., Ward, T. T., & Brummett, R. E. (1984). Detection of aminoglycoside ototoxicity by high-frequency auditory evaluation: selected case studies. *American Journal of Otolaryngology, 5*(3), 177-182.

Field, A. (2013). *Discovering statistics using IBM SPSS statistics* (3rd ed.): SAGE.

Fletcher, H. (1940). Auditory patterns. *Reviews of modern physics, 12*(1), 47.

Franklin, D. J., McCoy, M. J., Martin, G. K., & Lonsbury-Martin, B. L. (1992). Test/Retest Reliability of Distortion-Product and Transiently Evoked Otoacoustic Emissions. *Ear and hearing, 13*(6), 417-429.

Gaskill, S. A., & Brown, A. M. (1990). The behavior of the acoustic distortion product, 2f1-f2, from the human ear and its relation to auditory sensitivity. *Journal of the Acoustical Society of America, 88*(2), 821-839.

Gates, G. A., & Mills, J. H. (2005). Presbycusis. *The Lancet, 366*(9491), 1111-1120.

Geyer, L. B., Barreto, S. S. M., Weigert, L. L., & Teixeira, A. R. (2015). High frequency hearing thresholds and product distortion otoacoustic emissions in cystic fibrosis patients. *Brazilian journal of otorhinolaryngology, 81*(6), 589-597.

Ghafari, N., Court, R., Chirehwa, M. T., Wiesner, L., Petersen, L., Maartens, G., . . . Ramma, L. (2019). Pharmacokinetics and other risk factors for kanamycin-induced hearing loss in patients with multi-drug resistant tuberculosis. *International Journal of Audiology*, 1-5.

Goodman, S. S., Fitzpatrick, D. F., Ellison, J. C., Jesteadt, W., & Keefe, D. H. (2009). High-frequency click-evoked otoacoustic emissions and behavioral thresholds in humans. *The Journal of the Acoustical Society of America, 125*(2), 1014-1032.

Gorga, M. P., Neely, S. T., & Dorn, P. A. (2002). Distortion product otoacoustic emissions in relation to hearing loss. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic emissions* (2nd ed., pp. 243–272). New York: Thieme.

Gorga, M. P., Nelson, K., Davis, T., Dorn, P. A., & Neely, S. T. (2000). Distortion product otoacoustic emission test performance when both 2f1–f2 and 2f2–f1 are used to predict auditory status. *Journal of the Acoustical Society of America, 107*(4), 2128-2135.

Govender, S. M., Govender, C. D., & Matthews, G. (2013). Cochlear function in patients with chronic kidney disease. *South African Journal of Communication Disorders, 60*(1), 44-49.

Grason-Stadler. (2003). GSI 38 manual. In. Madison: Author.

Hall, J. W. (2000). *Handbook of otoacoustic emissions*. San Diego: Cengage Learning.

Harris, F. P., Lonsbury-Martin, B. L., Stagner, B. B., Coats, A. C., & Martin, G. K. (1989). Acoustic distortion products in humans: Systematic changes in amplitude as a function of f2/f1 ratio. *Journal of the Acoustical Society of America, 85*(1), 220-229.

Harris, T., Bardien, S., Schaaf, H. S., Petersen, L., De Jong, G., & Fagan, J. J. (2012). Aminoglycoside: induced hearing loss in HIV-positive and HIV-negative multidrug-resistant tuberculosis patients. *SAMJ: South African Medical Journal, 102*(6), 363-365.

Hashino, E., TinHan, E. K., & Salvi, R. J. (1995). Base-to-apex gradient of cell proliferation in the chick cochlea following kanamycin-induced hair cell loss. *Hearing research, 88*(1), 156-168.

Hauser, R., & Probst, R. (1991). The influence of systematic primary-tone level variation L 2− L 1 on the acoustic distortion product emission 2 f 1− f 2 in normal human ears. *The Journal of the Acoustical Society of America, 89*(1), 280-286.

hearX. (2020). World-first digital hearing health solutions. Retrieved from https://www.hearxgroup.com/

Henley, C. M., Weatherly, R. A., Martin, G. K., & Lonsbury-Martin, B. (1996). Sensitive developmental periods for kanamycin ototoxic effects on distortion-product otoacoustic emissions. *Hearing research, 98*(1), 93-103.

Herselman, S. (2002). 'An Epidemic Within an Epidemic': The Case of Multidrug-Resistant Tuberculosis. *Anthropology Southern Africa, 25*(3-4), 71-77.

Hong, Q. N., Pluye, P., Bujold, M., & Wassef, M. (2017). Convergent and sequential synthesis designs: implications for conducting and reporting systematic reviews of qualitative and quantitative evidence. *Systematic reviews, 6*(1), 61.

Hood, L. J., & Berlin, C. I. (2002). Clinical applications of otoacoustic emissions. In C. I. Berlin, L. J. Hood, & A. Ricci (Eds.), *Hair cell micromechanics and otoacoustic emissions*. New York: Thomson Delmar Learning.

Hoth, S., Gudmundsdottir, K., & Plinkert, P. (2010). Age dependence of otoacoustic emissions: the loss of amplitude is primarily caused by age-related hearing loss and not by aging alone. *European Archives of Oto-Rhino-Laryngology, 267*(5), 679-690. doi:10.1007/s00405-009-1106-5

Howell, D. C. (1999). *Fundamental statistics for the behavioural sciences* (4th ed.). California: Brooks/Cole Publishing.

Ismail, N. A., Mvusi, L., Nanoo, A., Dreyer, A., Omar, S. V., Babatunde, S., . . . Deyde, V. (2018). Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. *The Lancet Infectious Diseases, 18*(7), 779-787.

Janssen, T., Kummer, P., & Arnold, W. (1995a). Wachstumsverhalten der Distorsionsproduktemissionen bei kochleären Hörstörungen. *Oto-Rhino-Laryngologia Nova, 5*(1), 34-46.

Janssen, T., Kummer, P., & Arnold, W. (1995b). Wachstumsverhalten der Distorsionsproduktemissionen bei normaler Hörfunction. *Oto-Rhino-Laryngologia Nova, 5*(5), 211-221.

Jiang, M., Karasawa, T., & Steyger, P. S. (2017). Aminoglycoside-induced cochleotoxicity: a review. *Frontiers in cellular neuroscience, 11*, 308. doi:doi: 10.3389/fncel.2017.00308

Johnson, T. A., Neely, S. T., Garner, C. A., & Gorga, M. P. (2006). Influence of primary-level and primary-frequency ratios on human distortion product otoacoustic emissions. *The Journal of the Acoustical Society of America, 119*(1), 418-428.

Katz, J. (2002). *Handbook of Audiology* (5th ed.). Philadelphia: Lippincott Williams & Wilkins.

Katzenellenbogen, J. M., Joubert, G., & Abdool Karim, S. S. (1997). *Epidemiology: A manual for South Africa*. Cape Town: Oxford University Press.

Keefe, D. H., & Abdala, C. (2007). Theory of forward and reverse middle-ear transmission applied to otoacoustic emissions in infant and adult ears. *The Journal of the Acoustical Society of America, 121*(2), 978-993.

Keilson, S., Khanna, S., Ulfendahl, M., & Teich, M. (1993). Spontaneous cellular vibrations in the guinea-pig cochlea. *Acta Oto-Laryngologica, 113*(5), 591-597.

Kemp, D. (2002). Exploring cochlear status with otoacoustic emissions. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic emissions: Clinical applications* (2nd ed., pp. 1-47). New York: Thieme Medical Publishers.

Kemp, D. T. (1998). Otoacoustic emissions: Distorted echos of the cochlea's traveling wave. In C. I. Berlin (Ed.), *Otoacoustic emissions: basic science and clinical applications* (pp. 1-60). SanDiego: Singular Publishing.

Keppler, H., Dhooge, I., Maes, L., D'Haenens, W., Bockstael, A., Philips, B., . . . Vinck, B. (2010). Transient-evoked and distortion product otoacoustic emissions: A short-term test-retest reliability study. *International Journal of Audiology, 49*(2), 99-109. doi:10.3109/14992020903300431

Kironde, S. (2000). Tuberculosis in South Africa : chapter 17. *South African Health Review, 2000*(1), 335-349. Retrieved from https://journals.co.za/content/healthr/2000/1/AJA10251715_50

Knight, K. R., Kraemer, D. F., Winter, C., & Neuwelt, E. A. (2007). Early changes in auditory function as a result of platinum chemotherapy: use of extended high-frequency audiometry and evoked distortion product otoacoustic emissions. *Journal of Clinical Oncology, 25*(10), 1190-1195.

Konrad-Martin, D., Helt, W. J., Reavis, K. M., Gordon, J. S., Coleman, L. L., Bratt, G. W., & Fausti, S. A. (2005). Ototoxicity: Early detection and monitoring. *The ASHA Leader, 10*(7), 1-14.

Konrad-Martin, D., Poling, G. L., Dreisbach, L. E., Reavis, K. M., McMillan, G. P., Miller, J. A. L., & Marshall, L. (2016). Serial monitoring of otoacoustic emissions in clinical trials. *Otology & Neurotology, 37*(8), e286-e294.

Koo, T., & Li, M. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *Journal of Chiropractic Medicine, 15*(2), 155-163. doi:https://doi.org/10.1016/j.jcm.2016.02.012

Kummer, P., Janssen, T., & Arnold, W. (1998). The level and growth behavior of the 2 f12f2 distortion product otoacoustic emission and its relationship to auditory sensitivity in normal hearing and cochlear hearing loss. *Journal of the Acoustical Society of America, 103*(6), 3431-3444.

Kummer, P., Janssen, T., Hulin, P., & Arnold, W. (2000). Optimal L 1− L 2 primary tone level separation remains independent of test frequency in humans. *Hearing research, 146*(1), 47-56.

Kuroda, T. (2007). Clinical investigation on spontaneous otoacoustic emission (SOAE) in 447 ears. *Auris, Nasus, Larynx, 34*(1), 29-38. doi:10.1016/j.anl.2006.09.023

Lasky, R. E. (1998). Distortion product otoacoustic emissions in human newborns and adults. II. Level effects. *The Journal of the Acoustical Society of America, 103*(2), 992-1000.

Lee, J., & Kim, J. (1999). The Maximum Permissible Ambient Noise and Frequency-specific Averaging Time on the Measurement of Distortion Product Otoacoustic Emissions. *International Journal of Audiology, 38*(1), 19-23. doi:10.3109/00206099909072998

Londero, A., Bonfils, P., & Avan, P. (2002). Magnitudes and phases of human distortion-product otoacoustic emissions at 2f(1)-f(2) against f(2)/f(1): effects of an audiometric notch. *Hearing research, 167*(1-2), 46-56.

Long, G. R., & Tubis, A. (1988). Modification of spontaneous and evoked otoacoustic emissions and associated psychoacoustic microstructure by aspirin consumption. *The Journal of the Acoustical Society of America, 84*(4), 1343-1353.

Lonsbury-Martin, B. L., Harris, F. P., Hawkins, M. D., Stagner, B. B., & Martin, G. K. (1990). Distortion product emissions in humans: I. Basic properties in normally hearing subjects. *Annals of Otology, Rhinology & Laryngology, 99*(5_suppl), 3-14.

Lonsbury-Martin, B. L., & Martin, G. K. (2001). Evoked otoacoustic emissions as objective screeners for ototoxicity. *Seminars in Hearing, 22*(4), 377-391.

Lonsbury-Martin, B. L., & Martin, G. K. (2002). Distortion product otoacoustic emissions. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic emissions: Clinical applications* (2nd ed., pp. 116-142). New York: Thieme.

Loven, F. (2009). *Introduction to Normal Auditory Perception*. New York: Delmar Cengage Learning.

Marcrum, S. C., Kummer, P., Kreitmayer, C., & Steffens, T. (2016). Average optimal DPOAE primary tone levels in normal-hearing adults. *International Journal of Audiology, 55*(6), 325-332.

Martin, G. K., Stagner, B. B., Jassir, D., Telischi, F. F., & Lonsbury-Martin, B. L. (1999). Suppression and enhancement of distortion-product otoacoustic emissions by interference tones above f2. I. Basic findings in rabbits. *Hearing research, 136*(1-2), 105-123.

Max, L., & Onghena, P. (1999). Some issues in the statistical analysis of completely randomized and repeated measures designs for speech, language, and hearing research. *Journal of Speech, Language, and Hearing Research, 42*(2), 261-270.

Maxwell, D. L., & Satake, E. (2006). *Research and statistical methods in communication sciences and disorders*: Singular Publishing Group.

McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological methods, 1*(1), 30.

Meinke, D. K., Clavier, O. H., Norris, J., Kline-Schoder, R., Allen, L., & Buckey, J. C. (2013). Distortion product otoacoustic emission level maps from normal and noise-damaged cochleae. *Noise and Health, 15*(66), 315.

Mills, D. M., Feeney, M. P., & Gates, G. A. (2007). Evaluation of cochlear hearing disorders: normative distortion product otoacoustic emission measurements. *Ear and hearing, 28*(6), 778-792.

Ministry of Health and Family Welfare. (2017). *National strategic plan for tuberculosis: 2017-25. Elimination by 2025*. New Delhi: Ministry of Health and Family Welfare

Ministry of Health with Family Welfare. (2017). *National strategic plan for tuberculosis: 2017-2025*. New Delhi

Moore, R. D., Smith, C. R., & Lietman, P. S. (1984). Risk factors for the development of auditory toxicity in patients receiving aminoglycosides. *Journal of infectious diseases, 149*(1), 23-30.

Morlet, T., Lapillonne, A., Ferber, C., Duclaux, R., Sann, L., Putet, G., . . . Collet, L. (1995). Spontaneous otoacoustic emissions in preterm neonates: prevalence and gender effects. *Hearing research, 90*(1-2), 44-54.

Moulin, A. (2000a). Influence of primary frequencies ratio on distortion product otoacoustic emissions amplitude. I. Intersubject variability and consequences on the DPOAE-gram. *The Journal of the Acoustical Society of America, 107*(3), 1460-1470.

Moulin, A. (2000b). Influence of primary frequencies ratio on distortion product otoacoustic emissions amplitude. II. Interrelations between multicomponent DPOAEs, tone-burst-evoked OAEs, and

spontaneous OAEs. *The Journal of the Acoustical Society of America, 107*(3), 1471-1486. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=cmedm&AN=10738802&site=ehost-live

Moulin, A., Bera, J.-C., & Collet, L. (1994). Distortion product otoacoustic emissions and sensorineural hearing loss. *Audiology, 33*(6), 305-326.

Moulin, A., Jourdain, F., & Collet, L. (1999). Using distortion product otoacoustic emissions in clinical applications: Influence of f2/f1 on DPOAEgram. *British journal of audiology, 33*, 89.

Mukherjee, J. S., Rich, M. L., Socci, A. R., Joseph, J. K., Virú, F. A., Shin, S. S., . . . Kim, J. Y. (2004). Programmes and principles in treatment of multidrug-resistant tuberculosis. *The Lancet, 363*(9407), 474-481.

Mukherji, P., & Albon, D. (2014). *Research methods in early childhood: An introductory guide* (2nd ed.): Sage.

Mulheran, M., & Degg, C. (1997). Comparison of distortion product OAE generation between a patient group requiring frequent gentamicin therapy and control subjects. *British journal of audiology, 31*(1), 5-9.

National Department of Health. (2019). *Multi-Drug Resistant Tuberculosis: A Policy Framework on Decentralised and Deinstitutionalised Management for South Africa*. Pretoria: National Department of Health

Neely, S. T., Johnson, T. A., Kopun, J., Dierking, D. M., & Gorga, M. P. (2009). Distortion-product otoacoustic emission input/output characteristics in normal-hearing and hearing-impaired human ears. *The Journal of the Acoustical Society of America, 126*(2), 728-738. doi:10.1121/1.3158859

Ng, I. H., & McPherson, B. (2005). Test-retest reliability of distortion product otoacoustic emissions in the 1 to 7 kHz range. *Audiological Medicine, 3*(2), 108-115.

O'Sullivan, M. E., Perez, A., Lin, R., Sajjadi, A., Ricci, A. J., & Cheng, A. G. (2017). Towards the prevention of aminoglycoside-related hearing loss. *Frontiers in cellular neuroscience, 11*, 1-14.

Ohlemiller, K. K., & Siegel, J. H. (1994). Cochlear basal and apical differences reflected in the effects of cooling on responses of single auditory nerve fibers. *Hearing research, 80*(2), 174-190. doi:https://doi.org/10.1016/0378-5955(94)90109-0

Ozturan, O., Jerger, J., Lew, H., & Lynch, G. R. (1996). Monitoring of cisplatin ototoxicity by distortion-product otoacoustic emissions. *Auris Nasus Larynx, 23*(1), 147-151.

Pedalini, M. E. B., Sanchez, T. G., D'Antonio, A., D'Antonio, W., Balbani, A., Hachiya, A., . . . Bento, R. F. (2000). Média dos limiares tonais na audiometria de alta frequência em indivíduos normais de 4 a 60 anos. *Pró-fono, 12*(2), 17-20.

Peloquin, C. A., Berning, S. E., Nitta, A. T., Simone, P. M., Goble, M., Huitt, G. A., . . . Curran-Everett, D. (2004). Aminoglycoside toxicity: daily versus thrice-weekly dosing for treatment of mycobacterial diseases. *Clinical Infectious Diseases, 38*(11), 1538-1544.

Penner, M. (1996). The emergence and disappearance of one subject's spontaneous otoacoustic emissions. *Ear and hearing, 17*(2), 116-119.

Petersen, L. (2005). *A comparison of pure tone thresholds and distortion product otoacoustic emissions in patients with tuberculosis receiving aminoglycosides.* (MSc Audiology). University of Cape Town,

Petersen, L., & Rogers, C. (2015). Aminoglycoside-induced hearing deficits–a review of cochlear ototoxicity. *South African Family Practice, 57*(2), 77-82.

Petersen, L., Wilson, W. J., & Kathard, H. (2017). A systematic review of stimulus parameters for eliciting distortion product otoacoustic emissions from adult humans. *International Journal of Audiology*, 1-10.

Petersen, L., Wilson, W. J., & Kathard, H. (2018). Towards the preferred stimulus parameters for distortion product otoacoustic emissions in adults: A preliminary study. *South African Journal of Communication Disorders, 65*(1), 1-10. doi:doi:10.4102/sajcd.v65i1.585

Pillay-van Wyk, V., Msemburi, W., Laubscher, R., Dorrington, R. E., Groenewald, P., Glass, T., . . . Prinsloo, M. (2016). Mortality trends and differentials in South Africa from 1997 to 2012: second National Burden of Disease Study. *The Lancet Global Health, 4*(9), e642-e653.

Prieve, B., & Fitzgerald, T. (2015). Otoacoustic emissions. In J. Katz, M. Chasin, K. English, L. J. Hood, & K. Tillery (Eds.), *Handbook of clinical audiology* (Seventh edition ed., pp. 357-379). Philadelphia: Wolters Kluwer Health.

Probst, R., Harris, F., & Hauser, R. (1993). Clinical monitoring using otoacoustic emissions. *British journal of audiology, 27*(2), 85-90.

Probst, R., Lonsbury-Martin, B. L., & Martin, G. K. (1991). A review of otoacoustic emissions. *The Journal of the Acoustical Society of America, 89*(5), 2027-2067.

Puddy, R. W., & Wilkins, N. (2011). Understanding Evidence Part 1: Best Available Research Evidence. A Guide to the Continuum of Evidence of Effectiveness. In. Atlanta, Georgia: Centers for Disease Control and Prevention.

Ramma, L., & Ibekwe, T. S. (2012). Cochleo-vestibular clinical findings among drug resistant tuberculosis patients on therapy-a pilot study. *International archives of medicine, 5*(1), 3.

Rasetshwane, D. M., Bosen, E. C., Kopun, J. G., & Neely, S. T. (2019). Comparison of distortion-product otoacoustic emission and stimulus-frequency otoacoustic emission two-tone suppression in humans. *The Journal of the Acoustical Society of America, 146*(6), 4481-4492.

Rasetshwane, D. M., & Neely, S. T. (2011). Calibration of otoacoustic emission probe microphones. *Journal of the Acoustical Society of America, 130*(4), EL238-EL243. doi:10.1121/1.3632047

Rasmussen, A. N., Popelka, G. R., Osterhammel, P. A., & Nielsen, L. H. (1993). Clinical significance of relative probe-tone levels on distortion product otoacoustic emissions. *Scandinavian Audiology, 22*(4), 223-229.

Reavis, K. M., McMillan, G., Austin, D., Gallun, F., Fausti, S. A., Gordon, J. S., . . . Konrad-Martin, D. (2011). Distortion-product otoacoustic emission test performance for ototoxicity monitoring. *Ear & Hearing (01960202), 32*(1), 61-74. doi:10.1097/AUD.0b013e3181e8b6a7

Reavis, K. M., McMillan, G. P., Dille, M. F., & Konrad-Martin, D. (2015). Meta-analysis of distortion product otoacoustic emission retest variability for serial monitoring of cochlear function in adults. *Ear and hearing, 36*(5), e251.

Reavis, K. M., Phillips, D. S., Fausti, S. A., Gordon, J. S., Helt, W. J., Wilmington, D., . . . Konrad-Martin, D. (2008). Factors affecting sensitivity of distortion-product otoacoustic emissions to ototoxic hearing loss. *Ear and hearing, 29*(6), 875-893.

Ress, B. D., Sridhar, K. S., Balkany, T. J., Waxman, G. M., Stagner, B. B., & Lonsbury-Martin, B. L. (1999). Effects of cis-platinum chemotherapy on otoacoustic emissions: The development of an objective screening protocol. *Otolaryngology-Head and Neck Surgery, 121*(6), 693-701.

Riegelman, R. K. (2005). *Studying a study and testing a test: how to read the medical evidence* (4th ed.): Lippincott Williams & Wilkins.

Robles, L., & Ruggero, M. A. (2001). Mechanics of the mammalian cochlea. *Physiological reviews, 81*(3), 1305-1352.

Roede, J., Harris, F. P., Probst, R., & Xu, L. (1993). Repeatability of distortion product otoacoustic emissions in humans. *Audiology, 32*, 273-281.

Ruben, R. J. (2000). Redefining the survival of the fittest: communication disorders in the 21st century. *The Laryngoscope, 110*(2), 241-241.

Ruggero, M. A., Rich, N., & Freyman, R. (1983). Spontaneous and impulsively evoked otoacoustic emission: indicators of cochlear pathology? *Hearing research, 10*(3), 283-300.

Rybak, L., & Ramkumar, V. (2007). Ototoxicity. *Kidney international, 72*(8), 931-935.

Sagwa, E. L., Souverein, P. C., Ribeiro, I., Leufkens, H. G., & Mantel-Teeuwisse, A. K. (2017). Differences in VigiBase® reporting of aminoglycoside and capreomycin-suspected ototoxicity during tuberculosis treatment. *Pharmacoepidemiology and drug safety, 26*(1), 1-8.

Sahyeb, D. R., Costa Filho, O. A., & Alvarenga, K. d. F. (2003). Audiometria de alta freqüência: estudo com indivíduos audiologicamente normais. *Revista Brasileira de Otorrinolaringologia, 69*(1), 93-99.

SAMRC. (2018). The South African Medical Research Council Guidelines on the responsible conduct of research

SANS. (2006). The measurement and assessment of acoustic environments for audiometric tests. In (Vol. 10182:2006 Edition 2.2). Pretoria: SABS.

Schacht, J. (1999). Antioxidant therapy attenuates aminoglycoside-induced hearing loss. *Annals of the New York Academy of Sciences, 884*, 125-130.

Schellack, N., & Naude, A. (2013). An overview of pharmacotherapy-induced ototoxicity. *South African Family Practice, 55*(4), 357-365.

Schmidt, N., & Brown, J. (2015). *Evidence-Based Practice for Nurses: Appraisal and Application of Research* (3rd ed.). Burlington: Jones & Bartlett Learning.

Schuknecht, H. F. (1955). Presbycusis. *The Laryngoscope, 65*(6), 402-419.

Seidman, M. D., Quirk, W. S., & Shirwany, N. A. (1999). Mechanisms of alterations in the microcirculation of the cochlea. *Annals of the New York Academy of Sciences, 884*(1), 226-232.

Selimoglu, E. (2007). Aminoglycoside-induced ototoxicity. *Current pharmaceutical design, 13*(1), 119-126.

Sha, S.-H., & Schacht, J. (1997). Prevention of aminoglycoside-induced hearing loss. *The Keio journal of medicine, 46*(3), 115-119.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., . . . Stewart, L. A. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *British Medical Journal, 349*, g7647.

Shera, C. A., & Guinan Jr, J. J. (1999). Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs. *The Journal of the Acoustical Society of America, 105*(2), 782-798.

Siegel, J. H. (2002). Calibrating otoacoustic emission probes. In M. S. Robinette & T. J. Glattke (Eds.), *Otoacoustic emissions: Clinical applications* (2nd ed., pp. 416-441). New York: Thieme.

Sisto, R., Cerini, L., Gatto, M., Gherardi, M., Gordiani, A., Sanjust, F., . . . Moleti, A. (2013). Otoacoustic emission sensitivity to exposure to styrene and noise. *The Journal of the Acoustical Society of America, 134*(5), 3739-3748.

Stavroulaki, P., Apostolopoulos, N., Segas, J., Tsakanikos, M., & Adamopoulos, G. (2001). Evoked otoacoustic emissions--an approach for monitoring cisplatin induced ototoxicity in children. *International journal of pediatric otorhinolaryngology, 59*(1), 47-57.

Stavroulaki, P., Vossinakis, I. C., Dinopoulou, D., Doudounakis, S., Adamopoulos, G., & Apostolopoulos, N. (2002). Otoacoustic emissions for monitoring aminoglycoside-induced ototoxicity in children with cystic fibrosis. *Archives of Otolaryngology–Head & Neck Surgery, 128*(2), 150-155.

Stover, L., Gorga, M. P., Neely, S. T., & Montoya, D. (1996). Toward optimizing the clinical utility of distortion product otoacoustic emission measurements. *Journal of the Acoustical Society of America, 100*(2), 956-967.

Stover, L. J., Neely, S. T., & Gorga, M. P. (1999). Cochlear generation of intermodulation distortion revealed by DPOAE frequency functions in normal and impaired ears. *Journal of the Acoustical Society of America, 106*(5), 2669-2678. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10573884

Streiner, D. L., & Norman, G. R. (2008). *Health measurement scales: a practical guide to their development and use* (4th ed.). New York: Oxford University Press.

Strickland, E. A., Burns, E. M., & Tubis, A. (1985). Incidence of spontaneous otoacoustic emissions in children and infants. *The Journal of the Acoustical Society of America, 78*(3), 931-935.

Stuart, A., Passmore, A. L., Culbertson, D. S., & Jones, S. M. (2009). Test-retest reliability of low-level evoked distortion product otoacoustic emissions. *Journal of Speech, Language, and Hearing Research, 52*(3), 671-681. doi:10.1044/1092-4388(2008/08-0118)

Sturdy, A., Goodman, A., José, R. J., Loyse, A., O'Donoghue, M., Kon, O. M., . . . Lipman, M. (2011). Multidrug-resistant tuberculosis (MDR-TB) treatment in the UK: a study of injectable use and toxicity in practice. *Journal of antimicrobial chemotherapy, 66*(8), 1815-1820.

Sun, X.-M., Jung, M. D., Kim, D. O., & Randolph, K. J. (1996). Distortion product otoacoustic emission test of sensorineural hearing loss in humans: comparison of unequal-and equal-level stimuli. *Annals of Otology, Rhinology & Laryngology, 105*(12), 982-990.

Sutton, L. A., Lonsbury-Martin, B. L., Martin, G. K., & Whitehead, M. L. (1994). Sensitivity of distortion-product otoacoustic emissions in humans to tonal over-exposure: time course of recovery and effects of lowering L2. *Hearing research, 75*(1-2), 161-174.

Taylor, P. C., & Medina, M. (2011). Educational research paradigms: From positivism to pluralism. *College Research Journal, 1*(1), 1-16.

Trautwein, P., Hofstetter, P., Wang, J., Salvi, R., & Nostrant, A. (1996). Selective inner hair cell loss does not alter distortion product otoacoustic emissions. *Hearing research, 96*(1-2), 71-82.

Tubis, A., & Talmadge, C. L. (1998). Ear canal reflectance in the presence of spontaneous otoacoustic emissions. I. Limit-cycle oscillator model. *The Journal of the Acoustical Society of America, 103*(1), 454-461.

van Dijk, P., Wit, H. P., Tubis, A., Talmadge, C. L., & Long, G. R. (1994). Correlation between amplitude and frequency fluctuations of spontaneous otoacoustic emissions. *The Journal of the Acoustical Society of America, 96*(1), 163-169.

Vasconcelos, K. A., Frota, S., Ruffino-Netto, A., & Kritski, A. L. (2018). Sequential analysis as a tool for detection of amikacin ototoxicity in the treatment of multidrug-resistant tuberculosis. *J Bras Pneumol, 44*(2), 85-92. doi:10.1590/s1806-37562016000000312

Vasquez, R., & Mattucci, K. F. (2003). A proposed protocol for monitoring ototoxicity in patients who take cochleo-or vestibulotoxic drugs. *Ear, nose & throat journal, 82*(3), 181-184.

Vento, B. A., Durrant, J. D., Sabo, D. L., & Boston, J. R. (2004). Development of f2/f1 ratio functions in humans. *The Journal of the Acoustical Society of America, 115*(5 Pt 1), 2138-2147.

Vinck, B., De Vel, E., Xu, Z.-M., & Cauwenberge, P. B. J. (1996). Distortion Product Otoacoustic Emissions: A Normative Study. *Audiology, 35*(5), 231-245.

Wagner, W., Heppelmann, G., Vonthein, R., & Zenner, H. P. (2008). Test-retest repeatability of distortion product otoacoustic emissions. *Ear and hearing, 29*(3), 378-391. doi:10.1097/AUD.0b013e31816906e7

Whitehead, M., McCoy, M., Lonsbury-Martin, B., & Martin, G. (1995). Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. I. Effects of decreasing L 2 below L 1. *The Journal of the Acoustical Society of America, 97*(4), 2346-2358.

Whitehead, M., Stagner, B., McCoy, M., Lonsbury-Martin, B., & Martin, G. (1995). Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. II. Asymmetry in L 1, L 2 space. *The Journal of the Acoustical Society of America, 97*(4), 2359-2377.

World Health Organization. (2018a). Multidrug-resistant tuberculosis (MDR-TB) 2018 Update. Retrieved from https://www.who.int/tb/areas-of-work/drug-resistant-tb/MDR-RR_TB_factsheet_2018_Apr2019.pdf?ua=1

World Health Organization. (2018b). *Rapid communication: key changes to treatment of multidrug-and rifampicin-resistant tuberculosis (MDR/RR-TB)*. Retrieved from

World Health Organization. (2019a). *Global tuberculosis report 2019*. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf?ua=1

World Health Organization. (2019b). *WHO consolidated guidelines on drug-resistant tuberculosis treatment*. Retrieved from https://www.who.int/tb/publications/2019/consolidated-guidelines-drug-resistant-TB-treatment/en/:

World Medical Association. (2013). World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. *Journal of the American Medical Association, 310*(20), 2191-2194. doi:https://doi.org/10.1001/jama.2013.281053

Yilmaz, A., & Dedeli, O. (2016). Assessment of anxiety, depression, loneliness and stigmatization in patients with tuberculosis. *Acta Paulista de Enfermagem, 29*(5), 549-557.

Yu, K. K., Choi, C. H., An, Y.-H., Kwak, M. Y., Gong, S. J., Yoon, S. W., & Shim, H. J. (2014). Comparison of the effectiveness of monitoring cisplatin-induced ototoxicity with extended high-frequency pure-tone audiometry or distortion-product otoacoustic emission. *Korean journal of audiology, 18*(2), 58.

Yunusbaeva, M., Borodina, L., Alekseev, P., Davydov, R., Yunusbaev, U., Sharipov, R., . . . Yunusbayev, B. (2019). Treatment efficacy of drug-resistant tuberculosis in Bashkortostan, Russia: A retrospective cohort study. *International Journal of Infectious Diseases, 81*, 203-209.

Zelle, D., Thiericke, J. P., Dalhoff, E., & Gummer, A. W. (2015). Level dependence of the nonlinear-distortion component of distortion-product otoacoustic emissions in humans. *The Journal of the Acoustical Society of America, 138*(6), 3475-3490.

Zhao, F., & Stephens, D. (1999). Test-retest variability of distortion-product otoacoustic emissions in human ears with normal hearing. *Scandinavian Audiology, 28*, 171-178.

Appendix A: Ethics approval

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Form FHS006 Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)		
☑ Approved	☑ Type of review: Expedited	☐ Full committee
This serves as notification that all changes and documentation described below are approved.		
Signature Chairperson of the HREC: Signed by candidate	Date: 2/11/2014	

Note: All major amendments should include a PI Synopsis justifying the changes for the amendment (please see notice dated 23 April 2012)

Principal Investigator to complete the following:

1. Protocol Information

Date (when submitting this form)	28 October 2014
HREC REF Number	HREC/REF: 512/2013
Protocol title	High frequency distortion product otoacoustic emissions: Toward reliable and valid early identification and monitoring of hearing in patients receiving ototoxic medication
Protocol number (if applicable)	
Principal Investigator	Harsha Kathard (supervisor) Lucretia Petersen (PhD student)
Department / Office Internal Mail Address	Department of Health and Rehabilitation Sciences, F45 Old Main Building, Groote Schuur Hospital

1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	☐ Major	✓☐ Minor
1.2 Does this protocol receive US Federal funding?	☐ Yes	✓☐ No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval?	☐ Yes	✓☐ No

2. List of Proposed Amendments with Revised Version Numbers and Dates

Please itemise on the page below, all amendments with revised version numbers and dates, which need approval.
This page will be detached, signed and returned to the PI as notification of approval. Please add extra pages if necessary.

The stimulus parameters for the study that will be used in the data collection process have to change due to equipment difficulties. Instead of studying high frequency distortion product otoacoustic emissions, the frequency range will span from 2 to 8 kHz, which constitute the standard frequency range.

Thus the aims and objectives of the study had to change:

Appendix B: Advertisement for preliminary study

Are you between 18–30 years, and think you have normal hearing?

You are needed for a study evaluating the reliability and validity of a test that evaluates inner ear function (distortion product otoacoustic emissions).

Study outline

I am a PhD student at the University of Cape Town, and I am investigating the reliability and validity of distortion product otoacoustic emissions. If this test can measure inner ear function in a reliable and valid manner, it could assist with early identification of hearing loss in people receiving medication that damages their ears and hearing.

You will be required to **attend two sessions** at the Audiology Research Laboratory, **E48 Room 12**, **Old Main Building**, Groote Schuur Hospital. At the **first session** your hearing and middle ear function will be assessed. Various distortion product measurements (measurement of inner ear function) will be made with different intensities and frequency pairs. This session will take **90 minutes**. The **second session** will be scheduled approximately **24-48 hours after the first one**, where the distortion product emission measurements will be repeated. The duration of this session will be a maximum of **60 minutes**. The hearing test requires that you indicate when you've heard sounds played to you. All that is required of you for the rest of the tests is that you remain quiet. **None of the tests are painful or invasive.** You will be compensated **R100** for any costs and inconvenience.

Those interested in participating should:

- be between 18-30 years of age
- think they have normal hearing,
- have no self-reported history of hereditary hearing loss, significant ear disease, ear operations or long-term noise exposure and
- have no medical condition that can affect the hearing test results negatively

A benefit of taking part in the study includes having hearing and ear function tests done.

Deadline for signing up: ….

If you are interested in participating in this study, and require additional information, please contact:

Lucretia Petersen

E-mail: Lucretia.petersen@uct.ac.za

The study has received clearance from the Faculty of Health Sciences Research Ethics Committee, and permission from UCT's Student Affairs.

Appendix C: Informed consent letter for the preliminary study

Department of Health and Rehabilitation Sciences
Faculty of Health Sciences
Divisions of Communications Sciences and Disorders, Nursing and Midwifery, Occupational Therapy, Physiotherapy
F45 Old Main Building, Groote Schuur Hospital,
Observatory 7925
Tel: +27 (0) 21 406 6401 Fax: +27 (0) 21 406 6323
Internet: www.uct.ac.za

Dear Participant

What is this study about?

I am a part-time PhD student at the University of Cape Town and this study is for degree purposes. The title of my study is "*Distortion product otoacoustic emissions: towards reliable and valid early identification and monitoring of hearing in patients receiving ototoxic medication*".

Hearing loss can occur in the inner ear. My study wants to find out whether a tool that tests how the inner ear works gives the same results when used repeatedly. Therefore, I need to do this test more than once on the same person. I also want to find out whether it makes a difference if different testing parameters are used. If the test is found to be reliable and valid, it could help with early detection of hearing loss. If hearing loss can be detected early, correct management can start before the person notices that he/she has a hearing loss.

Why is this study important?

This test involves sounds that the healthy ear produces when two sounds of different frequencies (pitch) are sent into the ear. These sounds that the healthy ear sends out are measured in the ear canal with a sensitive microphone.

In order to determine the reliability and validity of the test, I first need to present sounds at different intensities and frequencies to your ear to determine which combination provides the best results to use for this test in healthy, normal-hearing individuals.

What will you have to do?

If you agree to participate in the study you will be **required to attend two sessions**, one at the start of the study and another session within 24 to 48 hours of the initial session. The first session will take approximately **90 minutes**, and the second one **60 minutes**.

What will happen during the study?

At the start of the study (first session), I will conduct the following tests to **see whether your hearing is normal**:

- I will look into your ear with a light to check for outer ear abnormalities or excess wax (otoscopy).
- I will place a probe into your ear that will measure how well your middle ear is working. You will not have to do anything but remain quiet for the test duration (tympanometry).
- Hearing test: The aim of this test is to see what the softest sounds are that you can hear. After the earphones have been placed on your ears you will be alone in the sound-treated room. You will still be able to communicate with me, though. You will hear sounds through the earphones and one ear at a time will be tested. Most of the sounds will be very soft, but you will have to raise your hand whenever you hear the sound. None of the above tests will be uncomfortable. If your hearing is found to be normal with this test, we can proceed with the rest of the study.
- If your hearing is normal, we will **conduct the test** (distortion product otoacoustic emissions: DPOAEs): This test involves putting a soft plastic/foam probe into your ear canal, which might be mildly uncomfortable but not painful. The probe sends sounds into the ear and then measures the responses of the inner ear. You do not have to do anything but remain quiet

for the duration of the test. This test will be repeated twice during the session. Your hearing will not be damaged by any of the tests.

- At the **second session**, I will conduct the DPOAE test **twice**. This session will last a maximum of 60 minutes.
- Before the start of each test, I will give you instructions and explain the test to you. You can request a break at any stage during the session. If you are unsure of what to do, you can ask a question at any stage.

"What happens if I get hurt taking part in this study?"

None of these tests are harmful to you in any way. If, however, you get hurt because of taking part in this study, you can claim compensation from the University of Cape Town No Faults Compensation Insurance For Clinical Trials and/or Human Volunteers Studies (Policy number: SPRGL1300443). The University of Cape Town has taken out insurance from Lockton Companies in the event of a research/trial-related injury, i.e., harm suffered as a result of participation in the trial. The principal investigator agrees to pay all reasonable medical costs in accordance with the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side-effect resulting directly from your participation in the study. The ABPI guidelines recommend that the sponsor of the study should compensate you, without you having to prove that the sponsor is at fault, for any injury resulting from you getting the study medication or other procedures carried out in accordance with the protocol for this study. I, the principal investigator, will not be liable for any loss, injuries and/or harm that you may sustain where the loss is caused by:

- any injury that results from you not following the protocol requirements or the instructions that the study personnel may give you
- an injury that results from negligence on your part.

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence. In particular, your right to purse such a claim in a South African court in terms of South African law must be ensured.

You will receive no direct benefits from taking part in this study, except for finding out what your hearing is like. If any abnormalities in hearing or ear function are detected, I will explain what these results mean and provide information and emotional counselling. I will also refer you to the appropriate health practitioner for management. All the tests will be done in the audiology booths in E48, Old Main Building, Groote Schuur Hospital, Observatory.

You are free to ask questions at any time during the study and have them answered by the researcher. In addition, you are free to withdraw from the study at any stage, without being negatively affected in any way. You can also contact the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee if you have any questions about your rights and welfare while taking part in this research (see contact details below).

All the information obtained will remain confidential and you will not be identified in any publications from this study. As mentioned before, there are no direct benefits or risks to you for taking part in the study.

There will be food and drinks available for your consumption before or after the testing. You will be compensated R100 for transport costs and inconvenience for each of the two sessions.

Researcher: Lucretia Petersen

Contact telephone number: (021) 406 6993

Cell phone number: 083 556 3327

Address: Division of Communication Sciences and Disorders, F45 Old Main Building, Groote Schuur Hospital, Observatory, 7925

Research supervisor: Associate prof. H. Kathard

Contact telephone number: (021) 406 6041

Address: Division of Communication Sciences and Disorders, F45 Old Main Building, Groote Schuur Hospital, Observatory, 7925

UCT Faculty of Health Sciences Human Research Ethics Committee Contact Details:

Contact person: Prof. M. Blockman

Address: E52.24, Old Main Building, Groote Schuur Hospital

Tel: 021 406 6492

Fax: 021 406 6411

Consent form

I hereby acknowledge that the purpose and procedures of the study has been fully explained to me. I also understand what is expected of me. I am aware that I can withdraw from the study at any stage without being affected in any way. I have the contact details of the UCT Faculty of Health Sciences Human Research Ethics Committee if I have any queries about taking part in this study. There was an opportunity for me to ask questions, and there will be during testing.

Please tick the appropriate box.

I hereby give my consent to participate in the study

I do not give my consent to participate in the study

Signed: _____ (Participant)

_____ (Researcher)

Date: _____

Appendix D:

Are you between 18-30 years, and think you have normal hearing?

You are needed for a study evaluating the reliability and validity of a test that evaluates inner ear function (distortion product otoacoustic emissions).

Study outline

I am a PhD student at the University of Cape Town, and I am investigating the reliability and validity of distortion product otoacoustic emissions. If this test can measure inner ear function in a reliable and valid manner, it could assist with early identification of hearing loss in people receiving medication that damages their ears and hearing.

You will be required to **attend two sessions** at the Audiology Research Laboratory, **E48 Room 12**, **Old Main Building**, Groote Schuur Hospital. At the **first session** your hearing and middle ear function will be assessed. Various distortion product measurements (measurement of inner ear function) will be made with different intensities and frequency pairs. This session will take **60 minutes**. The **second session** will be scheduled approximately **10 to 14 days after the first one**, where the distortion product emission measurements will be repeated. The duration of this session will be a maximum of **60 minutes**. The hearing test requires that you indicate when you've heard sounds played to you. All that is required of you for the rest of the tests is that you remain quiet. **None of the tests are painful or invasive.** You will be compensated **R100** for any costs and inconvenience.

Those interested in participating should:

- be between 18-30 years of age
- think they have normal hearing,
- have no self-reported history of hereditary hearing loss, significant ear disease, ear operations or long-term noise exposure and
- have no medical condition that can affect the hearing test results negatively

A benefit of taking part in the study includes having hearing and ear function tests done.

Deadline for signing up: ….

If you are interested in participating in this study, and require additional information, please contact:

Lucretia Petersen

E-mail: Lucretia.petersen@uct.ac.za

The study has received clearance from the Faculty of Health Sciences Research Ethics Committee, and permission from UCT's Student Affairs and Human Resources Department.

Appendix E: Informed consent letter for the phase 3 study

Department of Health and Rehabilitation Sciences
Faculty of Health Sciences
Divisions of Communications Sciences and Disorders, Nursing and Midwifery, Occupational Therapy, Physiotherapy
F45 Old Main Building, Groote Schuur Hospital,
Observatory 7925
Tel: +27 (0) 21 406 6401 Fax: +27 (0) 21 406 6323
Internet: www.uct.ac.za

Dear Participant

What is this study about?

I am a part-time PhD student at the University of Cape Town and this study is for degree purposes. The title of my study is "*Distortion product otoacoustic emissions: towards reliable and valid early identification and monitoring of hearing in patients receiving ototoxic medication*".

Hearing loss can occur in the inner ear. My study wants to find out whether a tool that tests how the inner ear works gives the same results when used repeatedly. Therefore, I need to do this test more than once on the same person. I also want to find out whether it makes a difference if different testing parameters are used. If the test is found to be reliable and valid, it could help with early detection of hearing loss. If hearing loss can be detected early, correct management can start before the person notices that he/she has a hearing loss.

Why is this study important?

This test involves sounds that the healthy ear produces when two sounds of different frequencies (pitch) are sent into the ear. These sounds that the healthy ear sends out are measured in the ear canal with a sensitive microphone.

In order to determine the reliability and validity of the test, I first need to present sounds at different intensities and frequencies to your ear to determine which combination provides the best results to use for this test in healthy, normal-hearing individuals.

What will you have to do?

If you agree to participate in the study you will be **required to attend two sessions**, one at the start of the study and another session within 24 to 48 hours of the initial session. Both sessions will take approximately **60 minutes** each.

What will happen during the study?

At the start of the study (first session), I will conduct the following tests to **see whether your hearing is normal**:

- I will look into your ear with a light to check for outer ear abnormalities or excess wax (otoscopy).
- I will place a probe into your ear that will measure how well your middle ear is working. You will not have to do anything but remain quiet for the test duration (tympanometry).
- Hearing test: The aim of this test is to see what the softest sounds are that you can hear. After the earphones have been placed on your ears you will be alone in the sound-treated room. You will still be able to communicate with me, though. You will hear sounds through the earphones and one ear at a time will be tested. Most of the sounds will be very soft, but you will have to raise your hand whenever you hear the sound. None of the above tests will be uncomfortable. If your hearing is found to be normal with this test, we can proceed with the rest of the study.
- If your hearing is normal, we will **conduct the test** (distortion product otoacoustic emissions: DPOAEs): This test involves putting a soft plastic/foam probe into your ear canal, which might be mildly uncomfortable but not painful. The probe sends sounds into the ear and then measures the responses of the inner ear. You do not have to do anything but remain quiet

for the duration of the test. This test will be repeated twice during the session. Your hearing will not be damaged by any of the tests.

- At the **second session**, I will conduct the DPOAE test **twice**. This session will last a maximum of 60 minutes.
- Before the start of each test, I will give you instructions and explain the test to you. You can request a break at any stage during the session. If you are unsure of what to do, you can ask a question at any stage.

"What happens if I get hurt taking part in this study?"

None of these tests are harmful to you in any way. If, however, you get hurt because of taking part in this study, you can claim compensation from the University of Cape Town No Faults Compensation Insurance For Clinical Trials and/or Human Volunteers Studies (Policy number: SPRGL1300443). The University of Cape Town has taken out insurance from Lockton Companies in the event of a research/trial-related injury, i.e., harm suffered as a result of participation in the trial. The principal investigator agrees to pay all reasonable medical costs in accordance with the Association of the British Pharmaceutical Industry Guidelines (ABPI) in the event of an injury or side-effect resulting directly from your participation in the study. The ABPI guidelines recommend that the sponsor of the study should compensate you, without you having to prove that the sponsor is at fault, for any injury resulting from you getting the study medication or other procedures carried out in accordance with the protocol for this study. I, the principal investigator, will not be liable for any loss, injuries and/or harm that you may sustain where the loss is caused by:

- any injury that results from you not following the protocol requirements or the instructions that the study personnel may give you
- an injury that results from negligence on your part.

By agreeing to participate in this study, you do not give up your right to claim compensation for injury where you can prove negligence. In particular, your right to purse such a claim in a South African court in terms of South African law must be ensured.

You will receive no direct benefits from taking part in this study, except for finding out what your hearing is like. If any abnormalities in hearing or ear function are detected, I will explain what these results mean and provide information and emotional counselling. I will also refer you to the appropriate health practitioner for management. All the tests will be done in the audiology booths in E48, Old Main Building, Groote Schuur Hospital, Observatory.

You are free to ask questions at any time during the study and have them answered by the researcher. In addition, you are free to withdraw from the study at any stage, without being negatively affected in any way. You can also contact the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee if you have any questions about your rights and welfare while taking part in this research (see contact details below).

All the information obtained will remain confidential and you will not be identified in any publications from this study. As mentioned before, there are no direct benefits or risks to you for taking part in the study.

There will be food and drinks available for your consumption before or after the testing. You will be compensated R100 for transport costs and inconvenience for each of the two sessions.

Researcher:	Lucretia Petersen
Contact telephone number:	(021) 406 6993
Cell phone number:	083 556 3327
Address:	Division of Communication Sciences and Disorders, F45 Old Main Building, Groote Schuur Hospital, Observatory, 7925
Research supervisor:	Associate prof. H. Kathard
Contact telephone number:	(021) 406 6041
Address:	Division of Communication Sciences and Disorders, F45 Old Main Building, Groote Schuur Hospital, Observatory, 7925

UCT Faculty of Health Sciences Human Research Ethics Committee Contact Details:

Contact person: Prof. M. Blockman

Address: E52.24, Old Main Building, Groote Schuur Hospital

Tel: 021 406 6492

Fax: 021 406 6411

Consent form

I hereby acknowledge that the purpose and procedures of the study has been fully explained to me. I also understand what is expected of me. I am aware that I can withdraw from the study at any stage without being affected in any way. I have the contact details of the UCT Faculty of Health Sciences Human Research Ethics Committee if I have any queries about taking part in this study. There was an opportunity for me to ask questions, and there will be during testing.

Please tick the appropriate box.

I hereby give my consent to participate in the study

I do not give my consent to participate in the study

Signed: _____ (Participant)

_____ (Researcher)

Date: _____

Appendix F: Informed consent form for the phase 4 study with participants with MDR-TB - English

School of Health and Rehabilitation Sciences

Faculty of Health Sciences

Divisions of Communications Sciences and Disorders, Nursing and Midwifery, Occupational Therapy, Physiotherapy

Dear Participant

I am a part-time PhD student at the University of Cape Town and this study is for degree purposes. The title of my study is "Towards the reliability and validity of distortion product otoacoustic emissions in healthy individuals and patients with drug-resistant tuberculosis". My study intends to determine whether a tool that tests the function of the inner ear (distortion product otoacoustic emissions-DPOAEs) is reliable and valid in healthy individuals and patients with drug-resistant tuberculosis. If the test is found to be reliable and valid, it could enable early detection of hearing loss.

DPOAEs are sounds that the ear produces when two sounds of different frequencies (pitch) are sent into the ear. These DPOAEs are measured in the ear canal with a sensitive microphone.

I need to determine whether the DPOAE test gives the same results when used repeatedly. Therefore, I need to do this DPOAE test more than once in the same person during the same session. In addition, I also need to find out whether the DPOAE test can detect ear problems in the same way as a hearing test. Thus, I need to conduct the DPOAE test again two weeks later.

If you agree to take part in the study you will need to attend two sessions of approximately 90 minutes each. At the sessions I will conduct the following tests:

- Otoscopy: I will look into your ear with a light to look for outer ear abnormalities or excess wax
- Tympanometry: I will place a probe into your ear that will measure how well your middle ear is working. You will not have to do anything but remain quiet for the test duration.
- Hearing test: The aim of this test is to see what the softest sounds are that you can hear. After the earphones have been placed on your ears you will be alone in the sound-treated room. You will still be able see me and communicate with me, though. You will hear sounds through the earphones and one

ear at a time will be tested. Most of the sounds will be very soft, but you will have to raise your hand whenever you hear the sound. None of the above tests will be uncomfortable.

- DPOAEs: This test involves putting a soft plastic/foam probe into your ear canal, which is not painful. The probe sends sounds into the ear and then measures the responses of the inner ear. You do not have to do anything but remain quiet for the duration of the test.

Before the start of each test, I will give you instructions and explain the test to you. You can ask for a break at any stage during the session. If you are unsure of what to do, you can ask a question at any stage.

None of these tests are harmful to you in any way. Your hearing will not be damaged by any of the tests. You will receive no direct benefits from taking part in this study.

If necessary, you will be referred to the appropriate health practitioner. All the tests for the study will be done in the audiology booths at your TB treatment facility.

You are free to ask questions at any time during the study and have them answered by the researcher. In addition, you are free to withdraw from the study at any stage, without being negatively affected in any way.

All the information obtained will remain confidential and you will not be identified in any publications from this study.

You will be compensated R150 for transport costs and inconvenience for the two sessions, which you will receive at the second session.

If you have any questions, you are free to ask them now or at any time during the study.

Researcher:	Lucretia Petersen
Contact telephone number:	(021) 406 6993
Cellphone number:	083 556 3327
Address:	Division of Communication Sciences and Disorders, F45 Old Main Building, Groote Schuur Hospital, Observatory, 7925

Human Research Ethics Committee Contact Details:

Contact person:	Prof. M. Blockman
Address:	E52.24, Old Main Building, Groote Schuur Hospital
Tel: 021 406 6492	Fax: 021 406 6411

I hereby acknowledge that the purpose and procedures of the study has been fully explained to me. I also understand what is expected of me. I am aware that I can withdraw from the study at any stage without being affected in any way. There was an opportunity for me to ask questions, and there will be during testing.

Please tick the appropriate box.

- ☐ I hereby give my consent to participate in the study
- ☐ I do not give my consent to participate in the study

Signed: _____ (Participant)

_____ (Researcher)

Date: _____

Appendix G: Informed consent form for the phase 4 study with participants with MDR-TB – isiXhosa

School of Health and Rehabilitation Sciences

Faculty of Health Sciences

Divisions of Communications Sciences and Disorders, Nursing and Midwifery, Occupational Therapy, Physiotherapy

Mthathi nxhaxheba obekekileyo

Ngingum'fundi wethutyana owenza izifundo zenzulu-lwazi kwi Dyunivesithi yaseKapa kwaye oluphando kumalunga nemfundo ephakamileyo. Isihloko soluphando simalunga "nokubekiseleleke ekuthembekeni ngokuphelelisileyo ihigh frequency distortion product Otoacoustic emissions kubantu abaphilileyo nabantu amaphila nesifo semiphunga esinganyangeki ngokulula (DR-TB). Oluphando lujonge ukufumanisa ukuba ngaba isixhobo esijonga ukusebenza kwendlebe engaphakathi ithembekile na kwaye ingundoqo kubantu abaphilileyo nabantu amaphila nesifo semiphunga esinganyangeki ngokulula (DR-TB). Okokubangaba okukuqondisisa kuthembekile kwaye kuphelelisile, kuyawu kwazeka ukufumanekisa ngethuba ukulahlekana ngokuva izandi eziphakame butswina.

iDPOAEs zizandi ezithi indlebe izivelise ngelixa izandi ezimbini ezohlukeneyo ngokutswina ziyezithunyelwe endlebeni. Ezi DPOAEs ziyezilinganiswe kwimbobo ye ndlebe ngemayikrofoni ebuthathaka.

Ndifuna ukwazi ukuba oku kuphononongа kwe DPOAEs inika iziphumo izifanayo xa isetyenziswe ngama thuba aphinda phindeneyo. Kungoko ke ndifuna ukwenza uphononongo lwe DPOAEs amathuba angaphezulu kune sinye kumntu ngamnye kwisihlandlo esinye. Ukwengeza, ndikwafuna ukufumanisa ukuba uphononongo lwe DPOAEs lungazi fumana iingxaki ze ndlebe ngohlobo olunye lokufumana uphononongo lokuva. Kunje ngoko ndifuna ukuwenza uphononongo lwe DPOAES kwakhona emva kweveki ezimbini ezilandelayo.

Ukuba ngaba uyavuma uthatha inxaxheba koluphando kuzakufuneka uhambe izihlandlo ezimbini eziya kuthabatha imizuzu elingamashumi alithoba sisinye. Kwezizinhlandlo ndiyakuqhuba oluphononongo lulandelayo:

- I-otoskopi: ndiyawu ku jonga ngaphakathi endlebeni ngoku khanyisa ukukhangela izinto ezingaqhelekanga kwi ndlebe engaphandle okanye incindi yendlebe ophuphumayo.
- I-thimphanometri: ndiyawu ku beka iprobu ngaphakathi kwendlebe yakho ezawuku linganisa ukuba isebenza kakuhle na indlebe yakho engaphakathi. Awusayi kwenza nto ngaphandle kokuthula ngethuba lophononongo.
- Uphononongo lokuva: Injongo yoluphononongo kukubona zezi phi izandi eziphantsi onokuziva. Emveni kokuba iearphone zibekiwe ezindlebeni zakho uyawukuba wedwa egumbini elinyangelwe ukuva. Uyawu kukwazi uku ndibona kwaye sinako uku thetha sobabini, kananjalo. Uzawukuva izandi kwiearphone kwaye indlebe nganye uyakhuphonongwa ngethuba. Ubuninzi bezandi buyakuba phantsi kakhulu, kodwa uyawu nyanzeleka uphakamise isandla sakho thuba ngalinya usiva isandi. Akukho nalunye kweliphononongo lungasentla oluza kwenza ungaziva kakuhle.
- iDPOAEs: oluphononongo lubandakanya ukufaka iplastik yeprobu okanye isiponji seprobi kwimbobo yendlebe yakho, engena buhlungu. Iprobu ithumela izandi kwindlebe kwaye iye ilanginise iimpendulo zendlebe engaphakathi. Awusayi kwenza nto ngaphandle kokuthula ngethuba lophononongo.

Ngaphambi kokuqala kophononongo ngalunye ndiyaku kunika imiyolelo nenchazelo yophononongo. Unganakho ukufuna ikhefu nangaliphi na ithuba kwisihlandlo. Ukuba awuqinisekanga ukuba wenze ntoni unakho ukubuza umbuzo na nini na.

Akukho nalunye oluphononongo elino buzaza kuwe nangayiphi indlela. Ukuva kwakho akuyi konakala nangaluphi na uphononongo. Awuzu fumana mbuyekezo ingqalileyo ngoku thatha inxaxheba koluphando.

Ukuba kuya funeka, uyawuku thunyelwa kwincaphephe yezempilo efanelekileyo. Lonke oluphononongo loluphando liyawukwenzelwa kwigumbi lophononongo lokuva kwindawo yakho lwe nyango lwe-TB.

Uvumelekile ukubuza imibuzo nangaliphi ithuba, kwixesha ngexesha lophando, kwaye kufeneka iphendulwe ngumphandi. Uko ngeza, unakho urhoxisa imvume yakho eluphandweni nangaliphi ithuba ngaphandle kokuba ubandakaneke ngokunga fanelekanga nangaluphi uhlobo. Yonke incazelo eyawufumaneka uyawuku gcineka ilihlebo kwaye ayiku chazeka upapasho lophando.

Kuyawu kubakho ukutya neziselo ezifumanekayo zokutyiwa phambi okanye emveni kophononongo. Uzakunikwa imbuyekezo elikhulu leranti kwisi hlandlo ngasinye ngencitho nangethuba olu nokulisebenzisa mhlobo lumbi kuso ngasinye isihlandlo siphononongo.

Ukuba unayo nayiphi imibuzo ukhululekile ukubuza ngelithuba okanye elinye ithuba ekuqhubekeni kophando.

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Ndiyavuma ukuba injongo kwakunye inqubo yophando iye yakucaciswa ngokupheleleyo kum. Kwaye ndiyakuqonda okulindelekileyo kum. Ndiyazi ukuba ndinokurhoxa koluphando nangaliphi ixesha ndingakhange ndibe ndinokuchaphazeleka nangaluphi na uhlobo. Ndiye ndalifumana ithuba lokubuza imibuzo, kwaye liyawukuba khona nangexesha lophononongo.

Nceda ubonakalise nge √ kwi bokisi efanelekileyo.

- ▫ ndiyayibika imvume yam yokuthatha inxhaxheba koluphando
- ▫ andiyiniki imvume yam yokuthatha inxhaxheba koluphando

Kutyikitywe: _____(umthathi nxhaxheba)

_____ (umphandi)

_____(umhla)

Appendix H: DPOAE mean SEM

TABLE 3. Table of mean SEM with upper and lower 90% reference limits of each f_2 primary frequency by days since baseline test

	f_2 Frequency							
	1000		2000		4000		6000	
Days From Baseline	SEM	90% Reference Limits	SEM	90% Reference Limits	SEM	90% Reference Limits	SEM	90% Reference Limits
1	1.7	±3.95	1.7	±3.98	1.8	±4.16	1.6	±3.76
2	1.7	±3.98	1.7	±4.03	1.8	±4.23	1.7	±3.85
3	1.7	±4.02	1.7	±4.07	1.9	±4.31	1.7	±3.93
4	1.7	±4.05	1.8	±4.11	1.9	±4.39	1.7	±4.02
5	1.8	±4.08	1.8	±4.15	1.9	±4.47	1.8	±4.11
6	1.8	±4.11	1.8	±4.19	2.0	±4.54	1.8	±4.20
7	1.8	±4.15	1.8	±4.23	2.0	±4.62	1.8	±4.29
8	1.8	±4.18	1.8	±4.27	2.0	±4.70	1.9	±4.37
9	1.8	±4.21	1.9	±4.31	2.1	±4.78	1.9	±4.46
10	1.8	±4.24	1.9	±4.35	2.1	±4.85	2.0	±4.55
15	1.9	±4.41	2.0	±4.56	2.3	±5.24	2.1	±4.99
20	2.0	±4.57	2.0	±4.76	2.4	±5.63	2.3	±5.43

The 90% reference limits can be computed at other days not shown using the equation $\pm 1.645 \cdot \sqrt{2} \cdot \widehat{SEM}(D)$. However, results beyond about 15 days need to be extrapolated and should be used with caution. Results at 20 days, shown below, are extrapolated from the fitted model.
SEM, standard error of the measurement.

Source: Reavis, K. M., McMillan, G. P., Dille, M. F., & Konrad-Martin, D. (2015). Meta-analysis of distortion product otoacoustic emission retest variability for serial monitoring of cochlear function in adults. *Ear and hearing, 36*(5), e251.

Appendix I: Left ear changes in DPOAE level for L1/L2 = 65/55 and f2/f1 ratio of 1.18 for participants with significant pure tone threshold changes

	Participant #	3	7	8	9	10	14	15	16	18	20
Frequency	HL change	R (16 kHz)	R (8 kHz)	Both (16 kHz)	R (8 kHz)	Both (8 & 16 kHz)	L (8 kHz)	R (16 kHz)	L (16 kHz)	L (8 kHz)	R (16 kHz)
2003	DPOAE 1	19.7	10.1	16	10	9.2	4.8	17.5	11.3	2.4	12
	Noise level 1	-12.3	-15.4	-10.9	-11.1	-15	-12.1	-10.1	-12.5	-16.4	-9.6
	SNR 1	32	25.5	26.9	21.1	24.2	16.9	27.6	23.8	18.8	21.6
	DPOAE 2	19	10.9	3.5	NR	9.2	-9.6	15.8	11.7	13.7	NR
	Noise level 2	-11.6	-15.8	-12.4	-19.9	-15	-19.6	-16.6	-13.3	-13.5	-17.6
	SNR 2	30.6	26.7	15.9	NA	24.2	10	32.4	25	27.2	NA
	Change	0.7	-0.8	**12.5**	**SC**	0	**14.4**	1.7	-0.4	-11.3	**SC**
2519	DPOAE 1	14.9	13.8	11.3	9.1	8.4	0.7	14.8	10.3	-17.9	3.4
	Noise level 1	-15.4	-17.5	-14.3	-15.4	-18	-13.9	-16.3	-16.2	-22.1	-12
	SNR 1	30.3	31.3	25.6	24.5	26.4	14.6	31.1	26.5	4.2	15.4
	DPOAE 2	15.3	11.4	-9.7	-11.2	4.6	13.8	10.3	18	3.3	NR
	Noise level 2	-15.4	-17	-20	-21.2	-18	-12.8	-15.5	-18.4	-21.8	18.2
	SNR 2	30.7	28.4	10.3	10	22.6	26.6	25.8	36.4	25.1	NA
	Change	-0.4	2.4	**21**	**20.3**	3.8	-13.1	4.5	-7.7	-21.2	**SC**
3175	DPOAE 1	15.4	11.6	19.1	10.1	4.6	13.2	11.9	17.3	-4.3	12.8
	Noise level 1	-22.2	-19	-13.8	-14.3	-22.4	-18.1	-22.1	-23.8	-28	-15.4
	SNR 1	37.6	30.6	32.9	24.4	27	31.3	34	41.1	23.7	28.2
	DPOAE 2	16.2	15.2	-0.4	-2	8.4	11.6	12.4	16.3	2.8	NR
	Noise level 2	-16.2	-20.5	-20.5	-12.5	-22.4	-23.2	-17.8	-20.6	-23.1	-19.7
	SNR 2	32.4	35.7	20.1	10.5	30.8	34.8	30.2	36.9	25.9	NA
	Change	-0.8	-3.6	**19.5**	**12.1**	-3.8	1.6	-0.5	1	-7.1	**SC**
3996	DPOAE 1	9.8	10.1	11.7	3.2	7.1	-10.1	10.7	8.3	-27.9	-3.1
	Noise level 1	-21.3	-25.1	-18.6	-19.6	-22.4	-20.6	-16.7	-21.6	-37.9	-18.9
	SNR 1	31.1	35.2	30.3	22.8	29.5	10.5	27.4	29.9	10	15.8
	DPOAE 2	10.2	8	-9.7	-4.2	7.1	4	11.3	6.6	-0.1	NR
	Noise level 2	-22.2	-21.7	-21.3	-14.7	-22.4	-22.9	-17.2	-21.8	-28.6	-17.3
	SNR 2	32.4	29.7	11.6	10.5	29.5	26.9	28.5	28.4	28.5	NA
	Change	-0.4	2.1	**21.4**	**7.4**	0	-14.1	-0.6	1.7	-27.8	**SC**
5000	DPOAE 1	12.6	3.6	13.8	7.6	1.6	-14.3	9	-1.3	-20.8	-14.1
	Noise level 1	-14	-13.4	-10.7	-11.8	-13.4	-24.4	-9.4	-14	-28.8	-24.1
	SNR 1	26.6	17	24.5	19.4	15	10.1	18.4	12.7	8	10
	DPOAE 2	10.3	7.5	1	6.7	1.6	-6.7	11.7	-12.9	-16.3	NR
	Noise level 2	-12.3	-14.3	-14	-12.4	-13.4	-17.3	-20.2	-22.9	-26.6	-18.3
	SNR 2	22.6	21.8	15	19.1	15	10.6	31.9	10	10.3	NA
	Change	2.3	-3.9	**12.8**	0.9	0	-7.6	-2.7	**11.6**	-4.5	**SC**
6996	DPOAE 1	4.2	-14.1	10.6	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9	-8.9
	Noise level 1	-11.5	-22	-19.8	-17.9	-13.5	-18.5	-11.3	-20.8	-26.3	-13.3
	SNR 1	15.7	7.9	30.4	9	4.6	9.6	2.4	11.9	17.4	4.4
	DPOAE 2	0.2	-15	-3.3	NR	-3.1	NR	10.5	NR	1.6	NR
	Noise level 2	-10.3	-21.8	-13.4	-19.1	-13.5	-22.1	-14.3	-19	-20	-16.5
	SNR 2	10.5	6.8	10.1	NA	10.4	NA	24.8	NA	21.6	NA
	Change	4	0.9	**13.9**	**SC**	-5.8	**SC**	-19.4	**SC**	-10.5	**SC**
8003	DPOAE 1	8.8	-1	9.2	-13.7	1.7	NR	9	NR	-11.9	NR
	Noise level 1	-8.7	-11	-10.1	-19.7	-13	-19.4	-9.2	-20.8	-22	-18.9
	SNR 1	17.5	10	19.3	6	14.7	NA	18.2	NA	10.1	NA
	DPOAE 2	2.2	-7.6	-2.2	-3.3	1.7	6.1	12.9	NR	-1.5	NR
	Noise level 2	-13.5	-17.6	-12.4	-13.5	-13	-16.2	-15.2	-21.1	-16.4	-22.9
	SNR 2	15.7	10	10.2	10.2	14.7	22.3	28.1	NA	14.9	NA
	Change	**6.6**	**6.6**	**11.4**	-10.4	0	IMP	-3.9	IMP	-10.4	IMP

DPOAE 1: Baseline DPOAE; DPOAE 2: Test conducted 10-14 days after baseline; SC: significant change, as per Reavis et al. (2015); NC: no change; NA: where signal to noise ratio could not be calculated; IMP: DPOAE improved, but change value could not be calculated; Significant reduction in DPOAE level **in bold**

Appendix J: Right ear changes in DPOAE level for L1/L2 = 65/55 and f2/f1 ratio of 1.18 for participants with significant pure tone threshold changes

	Participant #	3	7	8	9	10	14	15	16	18	20
Frequency	HL change	R (16 kHz)	R (8 kHz)	Both (16 kHz)	R (8 kHz)	Both (8 & 16 kHz)	L (8 kHz)	R (16 kHz)	L (16 kHz)	L (8 kHz)	R (16 kHz)
2003	DPOAE 1	NR	NR	5.9	14.3	10.5	NR	18.6	11.9	12.8	7.3
	Noise level 1	-11.7	-16.7	-16	-11.5	-11.4	-19.7	-12.8	-11.2	-13.6	-10.9
	SNR 1	NA	NA	21.9	25.8	21.9	NA	31.4	23.1	26.4	18.2
	DPOAE 2	10	NR	2.3	NR	8.2	18.9	16.9	12.3	9.5	4.7
	Noise level 2	-11.7	-19.8	-17.3	-13.4	-11.4	-9.3	-11.2	-10.8	-14.8	-11.9
	SNR 2	21.7	NA	19.6	NA	19.6	28.2	28.1	23.1	24.3	16.6
	Change	IMP	NC	3.6	**SC**	2.3	IMP	1.7	-0.4	3.3	2.6
2519	DPOAE 1	NR	NR	-0.1	11.6	11.8	NR	10.8	17.3	4.3	7.2
	Noise level 1	-20.9	-16.8	-16.9	-10.5	-17.5	-16.1	-16	-21.7	-19.3	-13.8
	SNR 1	NA	NA	16.8	22.1	29.3	NA	26.8	39	23.6	21
	DPOAE 2	2.7	NR	3.4	NR	11	14.9	10.9	16.8	6.5	-12.2
	Noise level 2	-13.2	-19.1	-22.4	-14.4	-17.5	-12.1	-16.7	-16.1	-16.7	-22.9
	SNR 2	15.9	NA	25.8	NA	28.5	27	27.6	32.9	23.2	10.7
	Change	IMP	NC	-3.5	**SC**	0.8	IMP	-0.1	0.5	-2.2	**19.4**
3175	DPOAE 1	NR	NR	-8.5	8.3	14.2	NR	18.2	20.5	3.3	-3.8
	Noise level 1	-21.8	-19.1	-24.9	-18.9	-23.3	-18.3	-22.6	-20.1	-20.3	-13.8
	SNR 1	NA	NA	16.4	27.2	37.5	NA	40.8	40.6	23.6	10
	DPOAE 2	-16.6	NR	-6.4	NR	13.8	8.1	17.1	15.6	0.6	-22
	Noise level 2	-26.6	-19.3	-23.2	-17.6	-23.3	-17.4	-18.7	-17.3	-20.1	-32
	SNR 2	10	NA	16.8	NA	37.1	25.5	35.8	32.9	20.7	10
	Change	IMP	NC	-2.1	**SC**	0.4	IMP	1.1	**4.9**	2.7	**18.2**
3996	DPOAE 1	NR	NR	-8.1	6	12.2	NR	12.6	7.7	-14.1	-5
	Noise level 1	-16.9	-25.3	-23.8	-18.7	-20.3	-22.9	-17.3	-20.9	-24.4	-17.8
	SNR 1	NA	NA	15.7	24.7	32.5	NA	29.9	28.6	10.3	12.8
	DPOAE 2	-6.5	NR	1.5	NR	9.7	2.1	12.9	12.9	-10.5	-12.5
	Noise level 2	-20.1	-22.7	-24	-18.9	-20.3	-17.7	-15.8	-20.2	-21.2	-22.8
	SNR 2	13.6	NA	25.5	NA	30	19.8	28.7	33.1	10.7	10.3
	Change	IMP	NC	-9.6	**SC**	2.5	IMP	-0.3	-5.2	-3.6	**7.5**
5000	DPOAE 1	NR	NR	-2	-2.2	9.5	NR	13.4	3.7	-19.2	-13.3
	Noise level 1	-21.5	-22.4	-15.7	-14.7	-14.6	-22.3	-11.3	-13	-26.1	-23.4
	SNR 1	NA	NA	13.7	12.5	24.1	NA	24.7	16.7	6.9	10.1
	DPOAE 2	3.5	NR	6.6	NR	6.3	-10.1	14.8	-0.5	-8	-10.2
	Noise level 2	-12.1	-20.1	-19.1	-17.8	-14.6	-20.3	-17.2	-13.4	-18.1	-20.3
	SNR 2	15.6	NA	25.7	NA	20.9	10.2	32	12.9	10.1	10.1
	Change	IMP	IMP	-8.6	**SC**	3.2	IMP	-1.4	4.2	-11.2	-3.1
6996	DPOAE 1	NR	NR	1.5	-10.4	10.6	NR	3.2	-10.9	-8.2	-10.9
	Noise level 1	-19.6	-21.4	-10	-20.5	-10	-22.5	-10	-19	-18.6	-18.6
	SNR 1	NA	NA	11.5	10.1	20.6	NA	13.2	8.1	10.4	7.7
	DPOAE 2	6.4	NR	5.2	NR	7.1	-10.8	2.2	NR	-6.6	NR
	Noise level 2	-7.9	-22.4	-14.6	-17.8	-10	-18.6	-10.8	-21.9	-16.6	-22.6
	SNR 2	14.3	NA	19.8	NA	17.1	7.8	13	NA	10	NA
	Change	IMP	NC	-3.7	**SC**	3.5	IMP	1	**SC**	-1.6	**SC**
8003	DPOAE 1	NR	NR	-1.4	-11.6	5.1	NR	4.9	NR	-10.1	NR
	Noise level 1	-18.7	-22.6	-11.9	-21.6	-12	-19.9	-9.7	-21.8	-20.1	-21.9
	SNR 1	NA	NA	10.5	10	17.1	NA	14.6	NA	10	NA
	DPOAE 2	2.1	NR	0.3	NR	2.2	-20.4	8.5	NR	-0.5	NR
	Noise level 2	-10.2	-21.2	-15.4	-19.4	-12	-23.4	-9.3	-21.8	-10.8	-22.7
	SNR 2	12.3	NA	15.7	NA	14.2	3	17.8	NA	10.3	NA
	Change	IMP	IMP	-1.7	**SC**	2.9	IMP	-3.6	NC	-9.6	NC

DPOAE 1: Baseline DPOAE; DPOAE 2: Test conducted 10-14 days after baseline; SC: significant change, as per Reavis et al. (2015); NC: no change; NA: where signal to noise ratio could not be calculated; IMP: DPOAE improved, but change value could not be calculated; Significant reduction in DPOAE level **in bold**