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**METHIMAZOLE ADMINISTRATION TO CATS:**  
***IN VIVO AND IN VITRO* STUDIES**  
**OF TRANSDERMAL ABSORPTION**

A thesis presented in partial fulfilment  
of the requirements for the degree of

Doctor or Philosophy  
in  
Veterinary Science

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New Zealand.

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***Dedication:***

*To Mum and Dad,*

*Thank-you for instilling the love of education and science,*

*Thank-you for instilling the love of life,*

*Thank- you for your love and patience,*

*Mum, I miss you each day.*

*Dad, I love you and love our Skype chats.*



## ABSTRACT

The use of medications formulated as ointments or gels and applied to the inner pinna of cats has become popular in veterinary medicine due to the ease of administration by this route compared to oral administration. Benign hyperplasia of the thyroid is a very common condition in cats older than ten years of age. Medical therapy with anti-thyroid drugs such as methimazole or the pro-drug of methimazole, carbimazole, is one of the treatment options for cats with hyperthyroidism. All previous studies of methimazole applied to the inner ear of cats have used Pluronic<sup>®</sup> lecithin organogel as the vehicle, however carbimazole and methimazole are lipophilic drugs, and PLO gel might not be the most suitable vehicle for a lipid soluble drug.

A series of studies were designed to test a new, lipophilic formulation of carbimazole and subsequently a new formulation of methimazole for transdermal application to the inner ear of cats for the therapy of hyperthyroidism. Two pharmacokinetic studies in healthy cats, one pilot trial and one clinical trial in client owned hyperthyroid cats, established that the lipophilic formulation could be absorbed from the ear and was a safe and efficacious therapy for hyperthyroidism in cats. A drug company (Bomac Ltd, now Bayer NZ Ltd) was interested in the novel formulation and the product was patented (International Application Number PCT/NZ2008/000011). The commercial product containing the drug and vehicle was sold in New Zealand as Hyper-T<sup>™</sup> Earspot. Finally, a series of three *in vitro* studies were performed to determine that methimazole in the lipophilic vehicle was: a) absorbed across the pinna skin; b) absorbed more completely at that site than the same formulation applied to the neck, groin or thoracic skin; and c) able to penetrate from the inner to the outer ear of cats in an *in vitro* model.

These studies represent the most extensive studies to date of a drug applied to the inner pinna of cats. The results from these studies suggest that methimazole in a lipophilic vehicle can be absorbed across the skin of cats and is an efficacious therapy for the treatment of hyperthyroidism in cats.



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## LIST OF ABBREVIATIONS

<sup>131</sup> I	Radioactive iodine	fT4	free T4
ALP	Alkaline phosphatase	h	Hour
ALT	Alanine transaminase	HPLC	High performance liquid chromatography
ANA	Antinuclear antibodies	I <sup>-</sup>	Iodide
AST	Aspartate transaminase	INN	International Nonproprietary Name
AUC	Area under the curve	LOD	Limit of detection
BID	Twice a day	LOQ	Limit of quantification
BSA	Bovine Serum Albumin	Ltd	Limited
CBC	Complete blood cell count	MHC	Major Histocompatibility
CBZ	Carbimazole	MIT	Mono-iodotyrosine
CI	Confidence interval	MMI	Methimazole
C <sub>max</sub>	Maximum concentration	Na <sup>+</sup>	Sodium
DIT	Diiodotyrosine	NZ	New Zealand
FDA	Federal Drug Agency	OECD	Organisation for Economic Co-operation and Development

PBS	Phosphate buffered saline	TBG	Thyroid binding globulin
PCAA	Professional Compounding Association of America	TBPA	Thyroxine binding pre- albumin
PLO	Pluronic <sup>®</sup> lecithin organogel	TD	Transdermal
PO	per os	t <sub>max</sub>	Time at maximum concentration
PTU	Propylthiouracil	TRH	Thyroid releasing hormone
rT3	reverse T3	TSH	Thyroid-stimulating hormone
SD	Standard deviation	TT4	Total thyroxine concentrations
SID	Once a day	UA	Urinalysis
t <sub>1/2</sub>	Elimination half-life	USA	United States of America
T3	Tri-iodothyronine	USAN	United States Adopted Name
T4	Thyroxine	USG	Urine-specific gravity

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discs of two older intact male cats and four young cats using an *in vitro* Franz cell model. The error bars represent 95% confidence intervals.

## Chapter 6

**Figure 1:** Cumulative methimazole (MMI) with vertical error bars representing 95% confidence intervals in six cats after application of 10 mg of methimazole to skin of the ear, neck, groin or thorax over 36 hours. Each data point represents the geometric mean of two replicates from six cats. The cumulative amount of methimazole per  $\mu\text{g/mL}$  is on the y axis. There was a significant difference ( $p<0.001$ ) of region. 245

**Figure 2:** Mean methimazole absorption (flux) (MMI) with vertical error bars representing 95% confidence intervals in six cats after application of 10 mg of methimazole to skin of the ear, neck, groin or thorax using a concentrations over 36 hours. Each data point represents the geometric mean of two replicates from six cats. There was a significant difference ( $p<0.001$ ) of region. 246

**Figure 3:** Haematoxylin and eosin (H&E) (1000x) stained images of the groin (A), neck (B), thorax (C) and left ear inner epidermis (D) of cats in the study on the effect of region on the absorption of transdermal methimazole. The stratum corneum layer (SC) and epidermal (E) layers are labelled in B. A significant difference in region was found for the epidermis ( $p<0.001$ ). The epidermis was thinner for the groin compared to the ear ( $p=0.001$ ) and the ear and thorax ( $p=0.006$ ). There was no difference in skin region for the thickness of the stratum corneum ( $p=0.601$ ). 249

## Chapter 7

**Figure 1:** Two chambered Franz type diffusion cell used in the study on percutaneous absorption of methimazole in the cat. The skin is clamped between the upper donor compartment and lower receptor compartment. The receptor compartment solution simulates the physical conditions surrounding the subcutaneous tissues. All cells were mounted in a diffusion apparatus, and placed in a water bath set to maintain the temperature of the skin in the diffusion cell at approximately  $32^{\circ}\text{C}$ . The receptor compartment solution is magnetically stirred. Samples of the receptor solution are removed via the sampling port at set time points, with equal volumes of fresh solution being replaced into the port. 265

**Figure 2:** In an *in vitro* study showing that methimazole can cross from the inner to outer pinna of cats, one cat had a neuter tattoo that penetrated the cartilage. After application of 10 mg of methimazole to the inner pinna and the whole ear placed in an *in vitro* Franz cell for 30 h, the right ear of this cat had 3.3 mg of methimazole in the cartilage, the highest of all the cats in the study. 269  
A = Cartilage, B= inner ear.

**Figure 3:** The mean amount (mg/g) of methimazole recovered from six cat whole ear samples (11 ears) after application of 10 mg of methimazole in a lipophilic vehicle to the inner pinna and the whole ear placed in an *in vitro* 271

Franz cell for 30 h, stratified by site (the right ear of one cat was excluded due to abnormally high concentrations of methimazole). The y-axis shows the amount of methimazole (mg/g), the standard deviation is shown with bars. After adjusting for the effect of ear (left or right), no significant difference was found in methimazole concentration between the different sites of the ear (inner ear, cartilage, outer ear) ( $p=0.47$ ). A difference was found between the total methimazole concentration between the left and right ear ( $*p < 0.001$ ).

## Appendix A

**Figure 1:** The round biopsy punch and mallet (A) used to cut a circular section measuring 2 cm<sup>2</sup> from each ear, as shown for the whole ear studies in **Chapter 7** (B). 307

**Figure 2:** Cumulative methimazole (MMI) concentration using an *in vitro* Franz cell model, after the application of 10 mg of MMI in a lipophilic vehicle to the right inner pinna skin of six cats (■) (**Chapter 5**) or six pairs of inner pinnal skin discs (●) (**Chapter 6**) and the data from both **Chapter 5** and **6** combined (▲). Data shown as mean and the error bars represent standard deviation. There was no difference between the two studies ( $p = 0.88$ ). 319

**Figure 3:** Cumulative methimazole (MMI) concentration using an *in vitro* Franz cell model, after the application of 10 mg of MMI in a lipophilic vehicle to the right inner pinna skin of six cats (■) (**Chapter 5**) or six pairs of inner pinnal skin discs (●) (**Chapter 6**). Data shown as geometric mean and the error bars represent 95% confidence intervals. There was no difference between the two studies ( $p = 0.81$ ). 319

**Figure 4:** Cumulative methimazole (MMI) concentration using an *in vitro* Franz cell model, after the application of 10 mg of MMI in a lipophilic vehicle to the inner pinna skin of four male cats (●) and eight female cats (■). No difference was found between the two sexes ( $p = 0.94$ ). Data shown as mean and the error bars represent standard deviation. 320

**Figure 5:** Cumulative methimazole (MMI) concentration using an *in vitro* Franz cell model, after the application of 10 mg of MMI in a lipophilic vehicle to the inner pinna skin of four male cats (●) and eight female cats (■). No difference was found between the two sexes ( $p = 0.76$ ). Data shown as geometric mean and the error bars represent 95% confidence intervals. 320

## Appendix B

**Figure 1:** Haematoxylin and eosin (H&E) stain of cat pinna (40 x magnification). A = the outer pinna, B = the inner pinna, C = the cartilage of the ear and D = the dermis. To measure thickness of the epidermis, dermis, cartilage and stratum corneum, three areas were measured and then averaged. 325

**Figure 2:** Haematoxylin and eosin (H&E) stain of cat neck skin (40x). To measure each skin region, three regions were measured in microns (µm) and 326

averaged. (Monteiro-Riviere *et al.* 1990). An example of three measured regions of stratum corneum (cornified, non viable epidermis) (SC 1–3) and viable epidermis (E 1–3), are shown in this figure.

**Figure 3:** Haematoxylin and eosin (H&E) stain of cat inner pinna skin (100x). To measure each skin region, three regions were measured in microns ( $\mu\text{m}$ ) and averaged. (Monteiro-Riviere *et al.* 1990). An example of three measured regions of stratum corneum (cornified, non-viable epidermis) (SC 1–3) and viable epidermis (E 1–3), are shown in this figure. 327

**Figure 4:** Haematoxylin and eosin (H&E) stain the inner pinnae of cats. Considerable variation was seen between 18 cats where H&E stain was performed on the inner pinnae. (A) and (B) show the inner pinna of the left ear of cat 9 (A = 100x) (B = 1000x) in the study and (C) and (D) the inner pinna of the right ear of cat 12 (C = 100x, D = 1000x) demonstrating that cat 12 had a thinner epidermis compared to cat 9 and a very thin stratum corneum (indicated by the arrow). 331

## Appendix C

**Figure 1:** Examples of checking the linearity by running 6 different concentrations of methimazole (1.25–40 ng/mL) in mobile phase and in serum from cats. 341

**Figure 2:** To determine the specificity of the HPLC, blank plasma from 3 different cats was analysed to check that no other peaks eluted at the same time as methimazole. No interfering peaks were detected in blank samples (a). A methimazole peak is usually seen between 7 to 8 minutes (b). 342