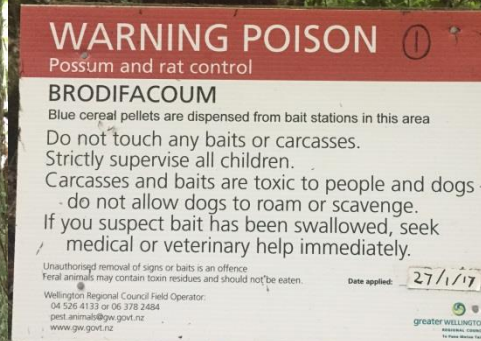


ASSESSING THE SUSTAINABILITY OF ANTICOAGULANT-BASED RODENT CONTROL FOR WILDLIFE CONSERVATION IN NEW ZEALAND



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INTRODUCTION

- Anticoagulants - Substance that prevents or delays blood clotting
- First generation anticoagulant rodenticides
Eg: Warfarin, Pindone
 - 1940s-1960s
 - Effective
 - Multi-dose



MODE OF ACTION

- ❑ All anticoagulant poisons - Same mode of action

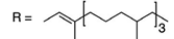
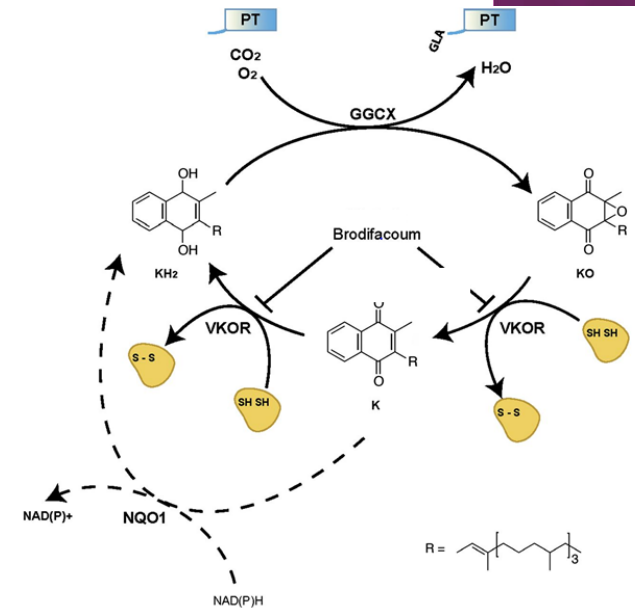
Antagonists of Vitamin K Epoxide Reductase (VKOR) enzyme

Hinder Vitamin K recycling

Depletion of Vitamin K Dependent Clotting factors

Gradual Increase Blood clotting time

Fatal Haemorrhages



FAILURE OF FIRST GENERATION ANTICOAGULANTS - RESISTANCE

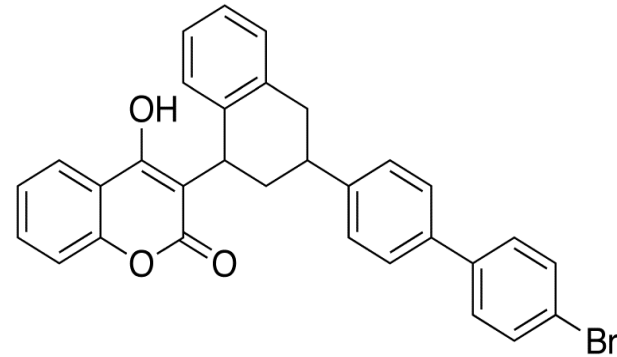
- ◉ **Resistance:** a heritable factor that allows an animal to survive a dose of an anticoagulant that would kill $\geq 99\%$ of normal individuals
- ◉ Selected by continuous use of anticoagulant that kills non-resistant individuals, allowing resistant animals a greater genetic input
- ◉ Resistance reported for most first-generation anticoagulants

SECOND GENERATION ANTICOAGULANTS

- ❑ Second-generation anticoagulant rodenticides
 - Early 1970s-1980s
 - Effective against first-generation anticoagulant-resistant rodents
 - More potent – single dose effective
- ⊙ Less toxic – e.g. Bromadiolone, Difenacoum
 - Rapid decline in effectiveness due to Cross-Resistance
- ⊙ More toxic – e.g. Brodifacoum, Flocomafen
 - Low-grade resistance to brodifacoum in Norway rats

BRODIFACOUM

- Registered in 1981
 - Pestoff®, Talon®
- Highly toxic and persistent
 - Half life > 80 days
- Extensively used for rodent and possum control
 - Bait stations on mainland
 - Aerial application on offshore islands
- DOC stopped use in 2002 on mainland
- Extensively used by Regional Councils, Community groups, Private Landowners



ANTICOAGULANT RESISTANCE IN NZ

- Rat tail samples collected from across New Zealand in 2010-12
- 2 mutations in VKORC1 gene found in ship rats:
 - Tyr25Phe - Associated with resistance to anticoagulants in ship rats in Spain
 - Ala26Val - not evaluated yet
- BCR testing or feeding trials needed to show resistance
- Resistance to which anticoagulant/s



MECHANISM OF RESISTANCE

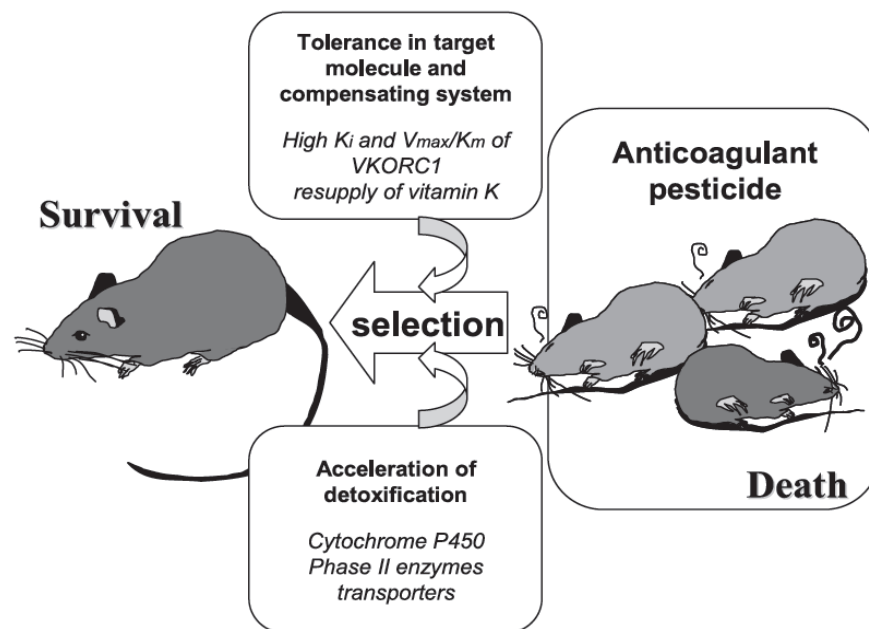
- ◉ **Pharmacodynamic resistance**

- Mutation in VKORC1 gene - Alters structure of VKOR enzyme so less sensitive to anticoagulant inhibition

- ◉ **Pharmacokinetic-based resistance**

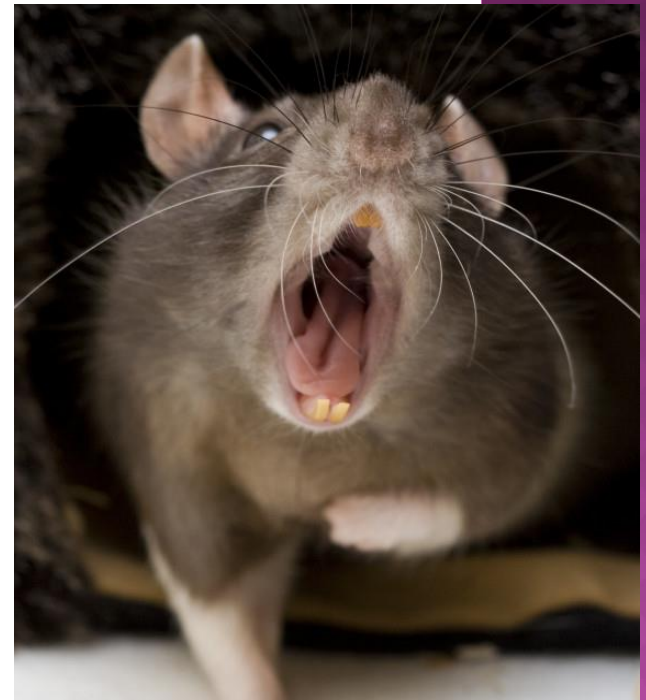
- Metabolise & excrete
- Cytochrome P450 complex

- ◉ **May be multi-factorial**



OBJECTIVES...

1. Determine effective dose of brodifacoum in *Rattus rattus*
2. Compare blood-clotting response times in *R. rattus* and *M. musculus* from areas of low, medium and high brodifacoum use
3. Genetic analysis of VKORC1 (Vitamin K epoxide reductase complex 1) gene
4. If resistance is found, find pathway of resistance
5. Develop recommendations for efficient pest control operations in New Zealand



1. DETERMINING THE EFFECTIVE DOSE OF BRODIFACOU M IN *Rattus rattus* (SHIP RAT)

- ◉ Blood-Clotting Response (BCR) Testing

Comparison of blood-clotting times before and after administration of a sub-lethal dose of anticoagulant

- ◉ Effective dose:

Sub-lethal dose that produces a measurable response in blood-clotting time

- ◉ Effective dose of brodifacoum for *M. musculus*

- Male mice = 0.39 mg/kg bw
- Female mice = 0.35 mg/kg bw



METHODS

- ◉ *Rattus rattus* captured from Akatarawa forest - No brodifacoum history
- ◉ No traps placed within 1 km of forest boundaries



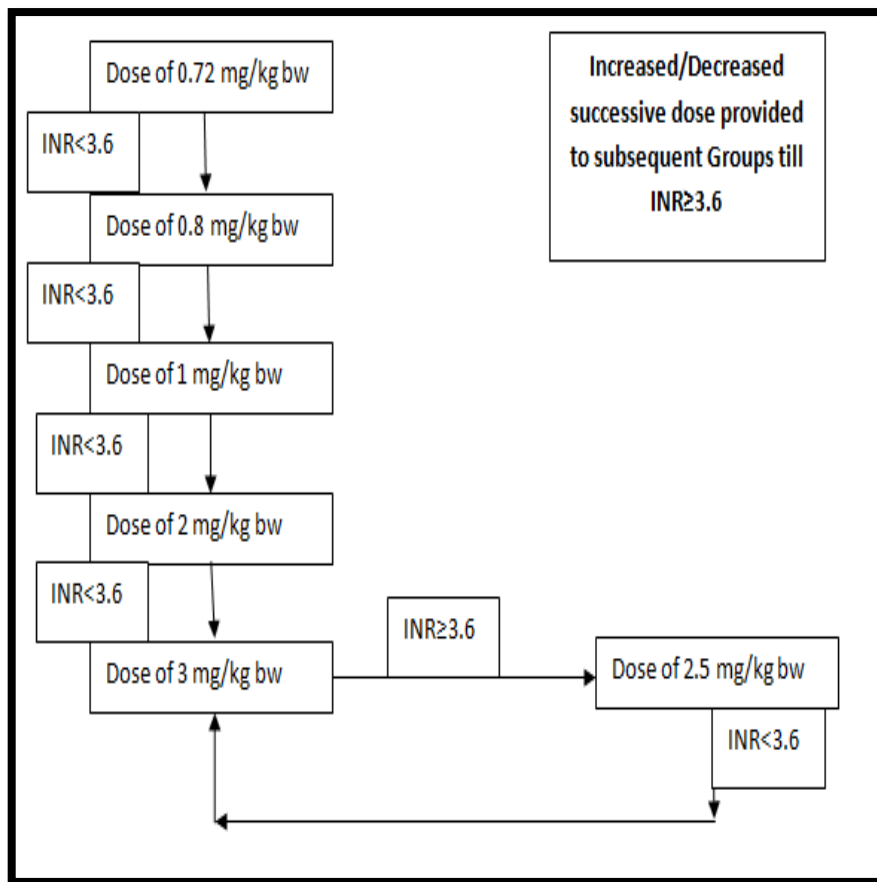
METHODS

- Day 14
 - Acclimatisation
- DAY 15
 - Pre-treatment blood sampling from Saphenous vein
 - Dose administration by Subcutaneous injection (Brodifacoum dissolved in Polyethylene glycol)
- DAY 16
 - Post-treatment blood sampling by cardiac puncture
 - Euthanasea
- International Normalised Ratio
 - $INR = \frac{\text{Post-treatment blood clotting time}}{\text{Pre-treatment blood clotting time}}$
- Ship rats considered to be responder if $INR \geq 3.6$ (Garg and Singla 2015)

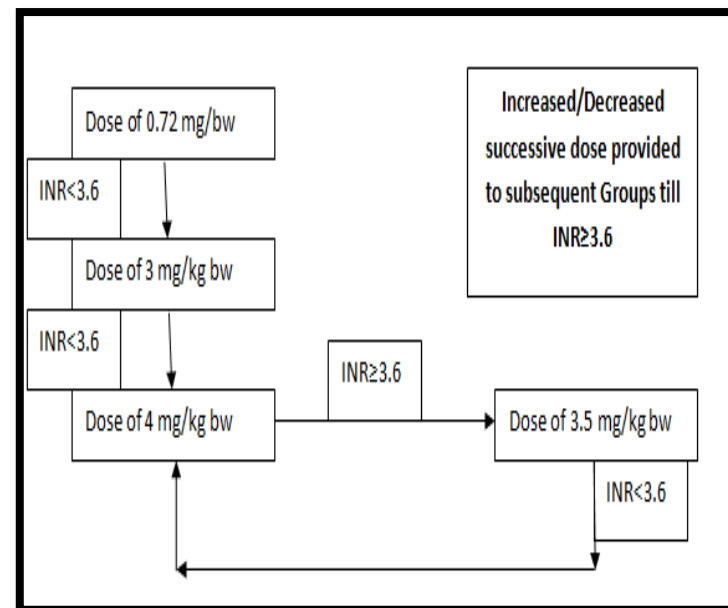


METHODS

Males

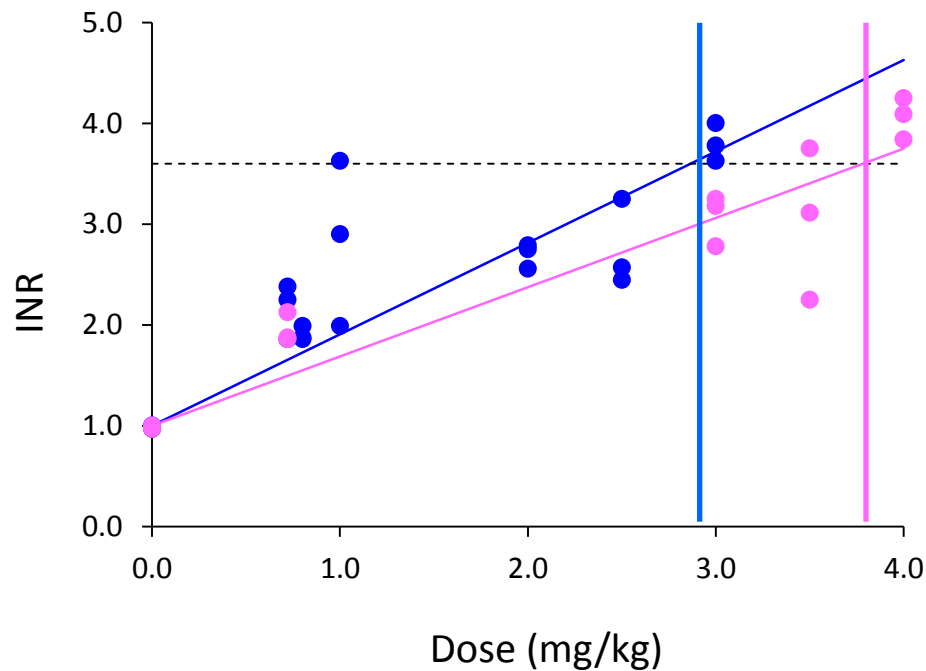


Females



RESULTS

- ED₅₀ value for females = 3.8 mg/kg bw
- ED₅₀ value for males = 2.9 mg/kg bw



LD₅₀ VS ED₅₀

- ◉ LD₅₀ - Concentration expected to kill 50%
 - ◉ 0.46 mg/kg bw (O'Connor and Booth 2001)
- ◉ ED₅₀ - Lower concentration that does not kill the animal but produces a measurable response in blood clotting time
- ◉ ED₅₀ females = 3.8 mg/kg bw = 6 x published LD₅₀
- ◉ ED₅₀ males = 2.9 mg/kg bw = 8 x published LD₅₀

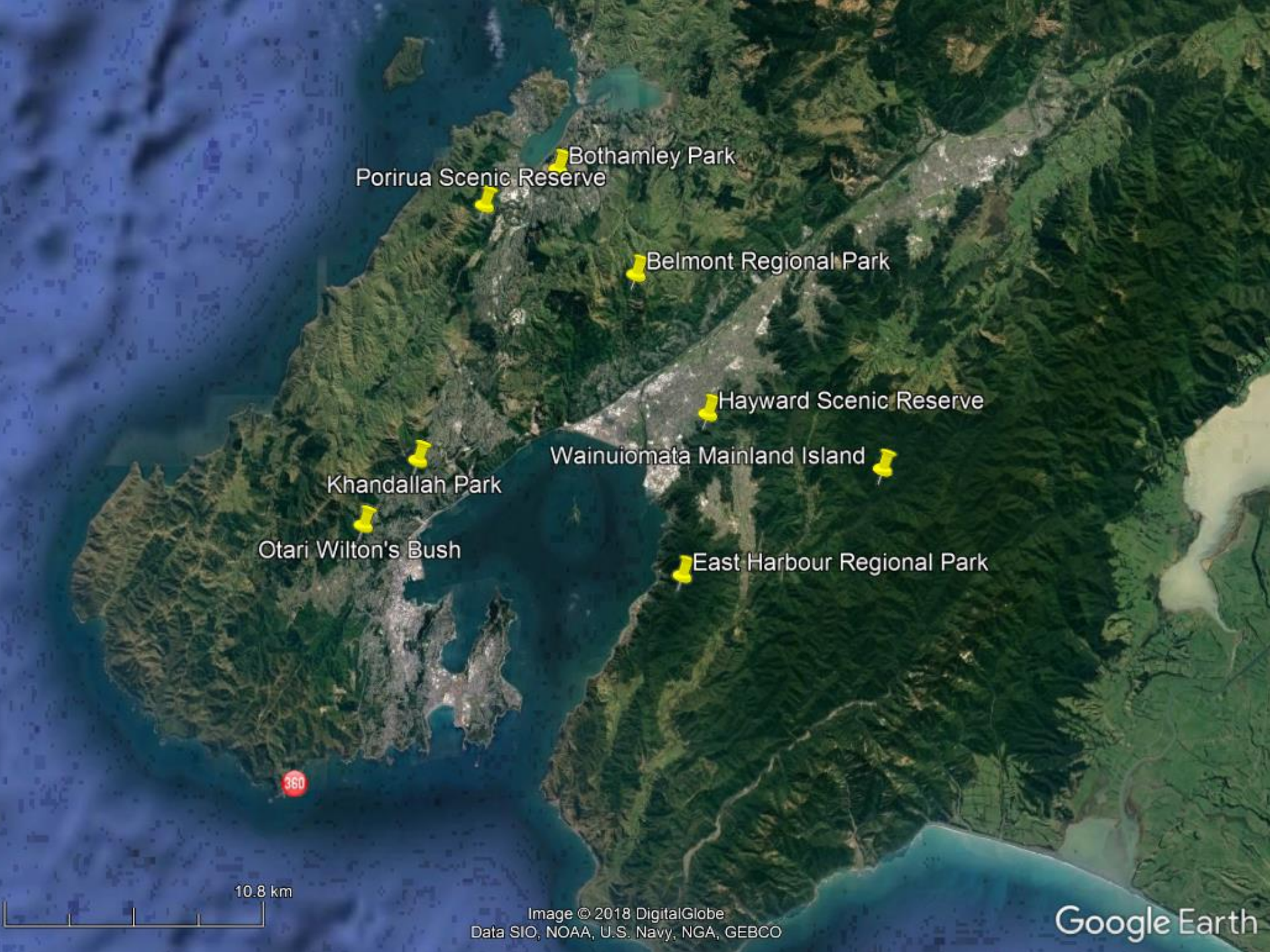
Species of rodent	LD ₅₀ value	ED ₅₀ value
Norway rat	0.17-0.56 (Redfern et al 1976, O'Connor and Booth 2001, Jacob and Buckle 2018)	0.22 (Prescott et al 2007)
House mouse	0.40-0.52 (Redfern et al 1976, O'Connor and Booth 2001)	0.35-0.39 (Prescott et al 2007)
Ship rat	0.46-0.77 (O'Connor and Booth 2001, Prakash and Mathur 1981)	2.88-3.81 (Present Study)

Why are *R. rattus* highly tolerant at Akatarawa?

- ◉ Brodifacoum used in Neighbouring areas
 - ❖ Private Landowners
 - ❖ GWRC
- ◉ Pest management by 1080 in Maungakotukutuku region of Akatarawa Forest

2. COMPARING BLOOD-CLOTTING RESPONSES IN *RATTUS RATTUS* AND *MUS MUSCULUS* FROM AREAS OF LOW, MEDIUM AND HIGH BRODIFACOUM USE





Bothamley Park
Porirua Scenic Reserve

Belmont Regional Park

Hayward Scenic Reserve

Wainuiomata Mainland Island

Khandallah Park

Otari Wilton's Bush

East Harbour Regional Park

360

10.8 km

Image © 2018 DigitalGlobe
Data SIO, NOAA, U.S. Navy, NGA, GEBCO

Google Earth

Turites Reserve

Porirua Scenic Reserve

Belmont Regional Park

Hayward Scenic Reserve

Otari Wilton's Bush

Wainuiomata Mainland Island

Khandallah Park

East Harbour Regional Park

360

31 km

Image Landsat / Copernicus
Data LDEO-Columbia, NSF, NOAA
Data SIO, NOAA, U.S. Navy, NGA, GEBCO

Google Earth

BCR TESTS FOR SHIP RATS

- ◉ BCR tests run using evaluated ED₅₀ values
- ◉ Failure to respond to this dose is evidence of resistance
- ◉ INR values analyzed to compare susceptibility in populations with three exposure levels

Site	Brodifacoum use history at time of trapping	Number of ship rats captured	Resistant / Non respondent rats	Range of INR
Otari-Wilton's Bush	23 years	4	4	1.96 - 2.75
Porirua Scenic Reserve	20 years	1	1	2.75
Khandallah Park	19 years	0	-	-
Belmont regional Park	13 years	7	3	2.75-5.12
Turitea Reserve	12 years	13	10	1.87-4.12
Wainui Mainland Island	12 years	7	5	1.62 - 4
East Harbor Regional Park	12 years	5	3	2.12 – 4.25
Bothamley Park	9 years	16	4	3 - 8.5
Hayward Scenic Reserve	2 years	1	0	4.87

RESISTANCE IN SHIP RATS TO BRODIFACOUM

Area based on Brodifacoum use history	Total number of rats	Number of rats resistant
High	5	5
Moderate	48	28
Low	1	0

BCR TESTS FOR HOUSE MICE

- ◉ ED₅₀ value of brodifacoum for males = 0.35 mg/kg bw
- ◉ ED₅₀ value of brodifacoum for females = 0.39 mg/kg bw

- ◉ For non-resistant house mouse
 - ❖ ED₉₉ value of brodifacoum = 0.46 - 0.52 mg/kg

- ◉ House mice considered to be responder if INR ≥ 5

(Prescott *et al.* 2007)

HOUSE MICE

- Blood collection difficult
 - Control group consisting of 6 mice to calculate average normal blood clotting time = 9.6 s
- Only post-treatment blood clotting time measured to test for resistance

<i>Site</i>	<i>Brodifacoum use history at time of trapping</i>	<i>Number of mice captured</i>	<i>Resistant mice</i>	<i>Range of INR</i>
Otari-Wilton's Bush	23 years	12	9	1 – 7.75
Porirua Scenic Reserve	20 years	1	1	1
Khandallah Park	19 years	0	-	-
Belmont regional Park	13 years	0	0	0
Turitea Reserve	12 years	0	0	0
Wainui Mainland Island	12 years	0	0	0
East Harbor Regional Park	12 years	3	2	1.12-9.25
Bothamley Park	9 years	5	4	1 - 9
Hayward Scenic Reserve	2 years	1	1	3.25
Akatarawa Forest	0 years	2	2	1

RESISTANCE IN HOUSE MICE TO BRODIFACOUM

◉ LD50 IN NZ COMPARE:

Area based on Brodifacoum use history	Total number of mice	Number of resistant mice
High	13	10
Moderate	8	6
Low	3	2

DISCUSSION

- ◉ Resistance to brodifacoum seems widespread in Wellington area and Turitea reserve for both ship rats and house mice
- ◉ Brodifacoum is most toxic anticoagulant
 - Other anticoagulants not an option
- ◉ Increasing concentration of brodifacoum in baits
 - Non-target poisoning
 - Least favourable option in terms of animal welfare (Beausoleil et al. 2016)

Genetic analysis

- ❑ Tail samples are being analysed – All rats and mice captured
- ❑ Gene sequencing – identify any mutations in VKORC1 gene



WHAT NEXT?

- ◉ Liver sections will be removed after perfusion through hepatic vein
- ◉ Liver microsomes will be prepared
 - ◉ *In vitro* VKOR enzyme activity (Thijssen 1987)
 - ◉ *In vitro* Cytochrome P450 activity (Ishizuka *et al.* 2007)

DEVELOPMENT OF RECOMMENDATIONS FOR EFFICIENT PEST CONTROL OPERATIONS IN NEW ZEALAND

Sustainable rodent control

- Increased efficiency of pest-control operations
- Limit development of resistance to anticoagulant rodenticides

*THANK
YOU*

Questions?

