Department of Primary Industries
Department of Regional NSW

NSW GOVERNMENT

NSW 2021 Animal Use in Research Statistics

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Table of Contents

1. Introduction

In NSW, information on the use of animals in research is collected by animal research establishments on a calendar-year basis. It is a requirement under the *Animal Research Act 1985* that this information is submitted to the NSW Department of Primary Industries (DPI). The information is then collated and published in the annual NSW Animal use in research statistics reports.

The following information is included in this 2021 report:

General charts: Show the trend of animal use in NSW since 2010.

Purpose tables for 2021: There are 10 Purpose Categories (see Appendix B: *Guide to the categories of reporting*). The purpose tables show the numbers of animals used, in species groups, for each purpose plotted against the 9 categories of procedures (see Appendix B: *Guide to the categories of reporting*). Sorting procedures into categories aims to give some indication of the 'invasiveness' or 'impact' of the research being undertaken on the animals involved.

Fate of animals graphs for 2021: Includes mandatory reporting data on the fate of all domestic cats and dogs, and voluntary reporting data on the fate of other animals (see Appendix B: *Guide to the categories of reporting* for the categories of Fate of animals).

Lethality testing data for 2021: The *Animal Research Act 1985* defines a 'lethality test' as *'an animal research procedure in which any material or substance is administered to animals for the purpose of determining whether any animals will die or how many animals will die'*.

Examples of the 3Rs: Examples provided by animal research establishments on the implementation of the 3Rs (Replacement, Reduction and Refinement in animal use) in 2021.

Appendix B: Guide to the categories of reporting.

Collation and reporting of data is reliant on the information provided by animal research establishments. This means there can be minor differences in the interpretation of which Purpose and Procedure categories are the most appropriate.

The magnitude of the information submitted by research establishments means that NSW DPI is not able to verify the data within each individual research project – this is instead the responsibility of the reporting establishments. However, NSW DPI does, where necessary, undertake verification of information submitted to the level of individual projects at each research establishment. For example, NSW DPI seeks to resolve apparent discrepancies reported in category combinations, such as Purpose category *Education* reported with a corresponding Procedure category *Death As An Endpoint*.

Reports are published each year at https://www.animalethics.org.au/animal-use-statistics

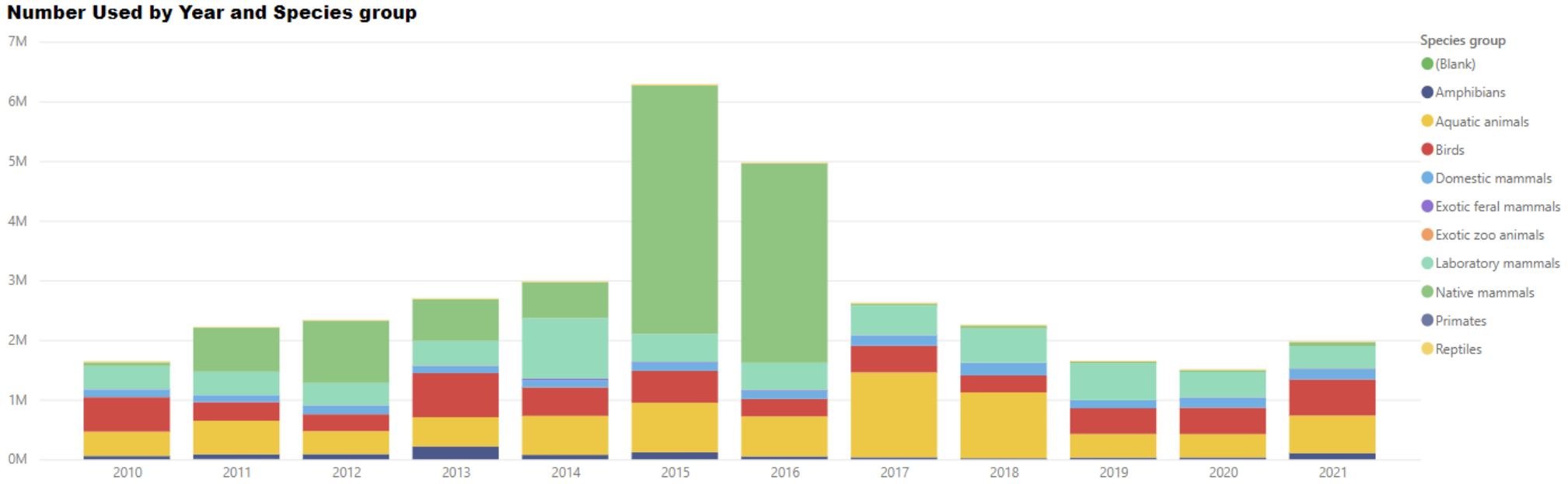
2. General Charts

2.1 Introduction to general charts

- Animals are counted for each year in which they are used. This means, for example, that animals in a project that runs for a period of more than one calendar year will be counted again for each year in which they are used. Therefore, a year to year comparison of animal numbers includes individual animals that may be the same across two or more years.
- Animals are counted in each project in which they are used in a reporting year. This means the same animal will be counted more than once if they are used in more than one project. This reporting is consistent with reporting in other Australian jurisdictions.
- Animals that are held at research establishments, but not used in a research project in a reporting year, will not be included in statistics reported for that year.
- For the 2021 reporting year there were three research projects which used a total of 644,792 animals, which is 33% of the total number of animals used.
    - One project used 327,960 poultry. The Procedure category (level of impact on the animals) for this project was *P1 Observation involving minor interference*. These were chickens in commercial facilities, where the only intervention was feeding one of two alternative feeds.
    - One project used 202,639 fish. The Procedure category (level of impact on the animals) for this project was *P3 Minor conscious intervention.* The project involved research carried out on commercial fishing vessels, assessing methods to reduce bycatch, and included all fish caught as part of the commercial fishing operations.
    - One project used 114,193 fish. The Procedure category (level of impact on the animals) for this project was *P3 Minor conscious intervention.* The project involved observations and tagging.
- For the 2020 reporting year there were 167 baboons reported in the Purpose category A2 Stock maintenance. The establishment that reported this subsequently advised this had been an error in reporting and the correct figure was 153. This data has not been corrected as it was already reported on for 2020.
- For the 2019 reporting year, 32 domestic cats were reported by an establishment in the high impact category of *Major physiological challenge* and in the Fate category *Privately (non-research) owned and remained with owner*. These figures are recorded in the 2019 data. However, in July 2021, advice was provided by the establishment that both the categories reported for these 32 cats were incorrect. The establishment clarified the correct reporting should have been the lower impact category of "*Minor physiological challenge*" and the Fate category should have been "*Retained for use in other projects or supplied to another establishment/ individual for research*". This data has not been corrected as it was already reported on for 2019.
- For the 2017 reporting year, there was a large increase in the number of aquatic animals used. This was primarily due to two projects which used almost 775,000 fish in the procedure category "Observation Involving Minor Interference". One of these studies involved counting over 500,000 fish by camera recordings.

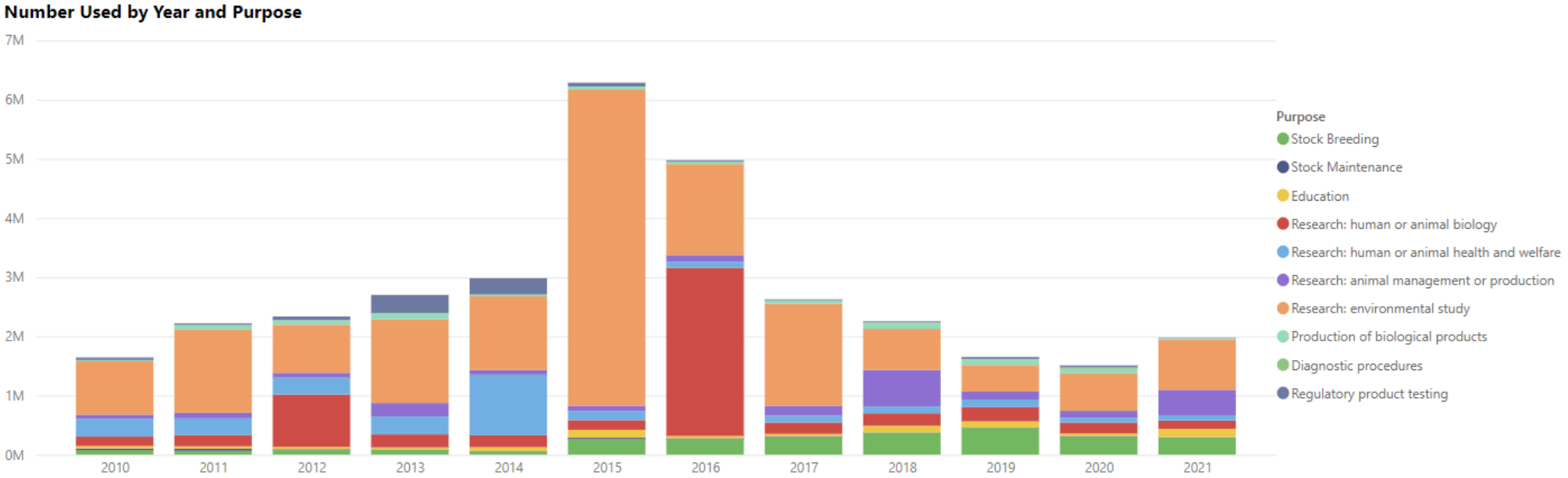
- For the 2015 and 2016 reporting years, there was a large increase in the numbers of animals used. This was primarily due to two projects which involved the aerial counting of bats throughout NSW. There was no interaction with the majority of animals in these projects and these accounted for the reporting of approximately three million animals for each reporting year.
- For the 2016 Animal Use in Statistics Report there were some errors in item 2 General Charts for the entries for the 2015 year (over-reporting of numbers), and the total for Chart 4 for 2016 (final line not included in the total). These errors have been corrected for subsequent Animal Use in Statistics reports.
- For the 2010 and 2011 reporting years, there are species recorded as blank species categories because an incorrect species code was used. The impact of this on the charts is negligible.

2.2 Number of animals used over time by species grouping



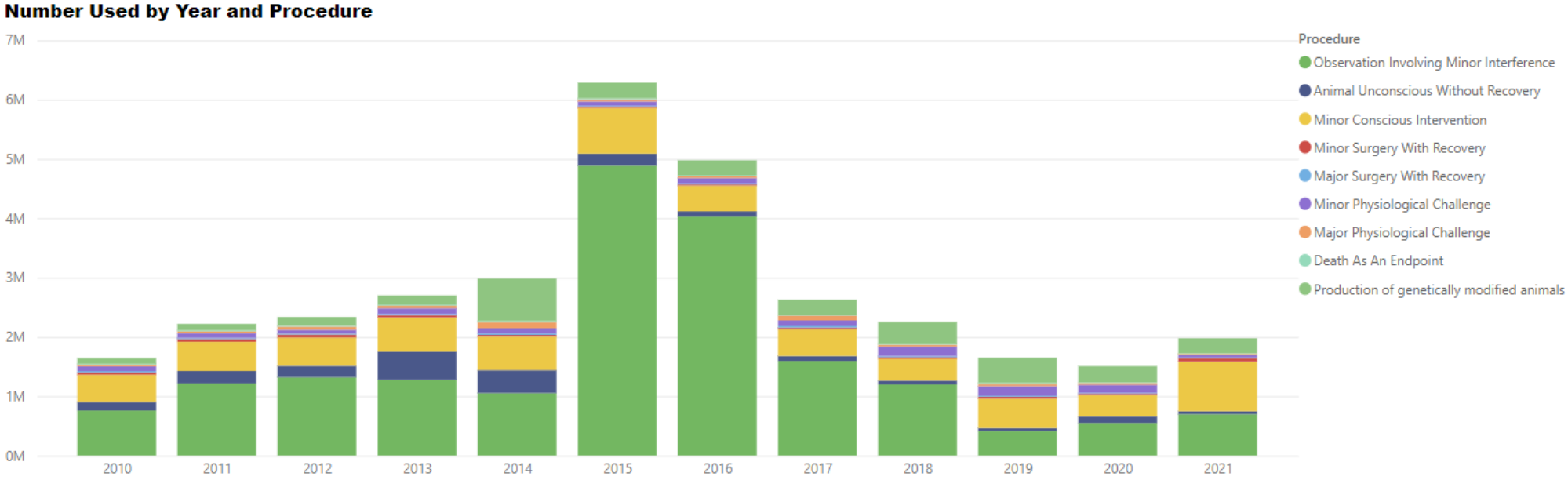
Species group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
	195	5,460											**5,655**
Amphibians	54,992	79,446	87,417	214,616	75,424	118,721	49,008	33,093	18,067	28,413	30,814	103,430	**893,441**
Aquatic animals	409,917	562,356	386,102	491,114	652,902	830,769	670,514	1,424,101	1,104,172	397,139	393,127	631,196	**7,953,409**
Birds	576,787	311,690	283,461	739,293	478,754	534,812	292,834	445,877	284,985	430,573	441,729	605,293	**5,426,088**
Domestic mammals	127,468	114,511	141,288	114,914	120,239	135,679	133,537	172,866	207,125	133,515	161,886	167,318	**1,730,346**
Exotic feral mammals	5,318	5,195	6,525	9,411	23,200	12,541	15,351	5,338	5,941	1,960	10,463	12,202	**113,445**
Exotic zoo animals	27	32	71	72	155	83	32	21	37	140	66	74	**810**
Laboratory mammals	389,507	388,701	374,037	414,652	1,017,494	470,634	457,431	497,337	572,490	616,812	424,170	377,838	**6,001,103**
Native mammals	59,870	738,903	1,044,611	697,764	598,737	4,161,992	3,340,256	29,220	45,487	26,954	31,164	65,645	**10,840,603**
Primates	184	27	18	22	41	179	96	38	44	53	217	162	**1,081**
Reptiles	18,328	12,141	13,398	17,674	15,730	22,067	18,196	17,568	15,595	14,749	14,756	14,117	**194,319**
Total	**1,642,593**	**2,218,462**	**2,336,928**	**2,699,532**	**2,982,676**	**6,287,477**	**4,977,255**	**2,625,459**	**2,253,943**	**1,650,308**	**1,508,392**	**1,977,275**	**33,160,300**

2.3 Number of animals used over time by research purpose



Purpose	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	**Total**
Stock Breeding	75,867	65,936	75,488	80,774	47,116	259,464	263,601	300,720	366,997	452,258	304,654	286,602	**2,579,477**
Stock Maintenance	27,165	33,850	15,448	7,890	10,500	26,508	13,684	13,204	7,266	5,875	9,899	7,936	**179,225**
Education	43,344	41,230	40,904	34,960	68,717	135,378	39,301	36,904	114,387	105,110	43,484	142,170	**845,889**
Research: human or animal biology	158,880	189,450	882,024	218,541	201,636	161,990	2,839,488	190,934	211,778	239,190	183,227	139,996	**5,617,134**
Research: human or animal health and welfare	298,611	283,546	286,375	303,050	1,024,919	152,375	104,590	116,835	115,119	129,577	84,271	90,289	**2,989,557**
Research: animal management or production	71,722	94,019	81,831	227,769	76,422	91,603	111,880	167,998	616,015	133,914	115,593	429,291	**2,218,057**
Research: environmental study	901,504	1,402,726	813,500	1,411,046	1,247,301	5,341,812	1,539,475	1,725,808	694,037	448,737	631,072	847,344	**17,004,362**
Production of biological products	19,568	74,625	78,419	109,229	28,870	54,811	42,890	55,365	90,866	99,497	77,486	18,579	**750,205**
Diagnostic procedures	3,630	8,540	1,994	1,031	1,310	766	1,307	1,134	18,186	2,959	24,415	3,884	**69,156**
Regulatory product testing	42,302	24,540	60,945	305,242	275,885	62,770	21,039	16,557	19,292	33,191	34,291	11,184	**907,238**
Total	**1,642,593**	**2,218,462**	**2,336,928**	**2,699,532**	**2,982,676**	**6,287,477**	**4,977,255**	**2,625,459**	**2,253,943**	**1,650,308**	**1,508,392**	**1,977,275**	**33,160,300**

2.4 Number of animals used over time by research procedure



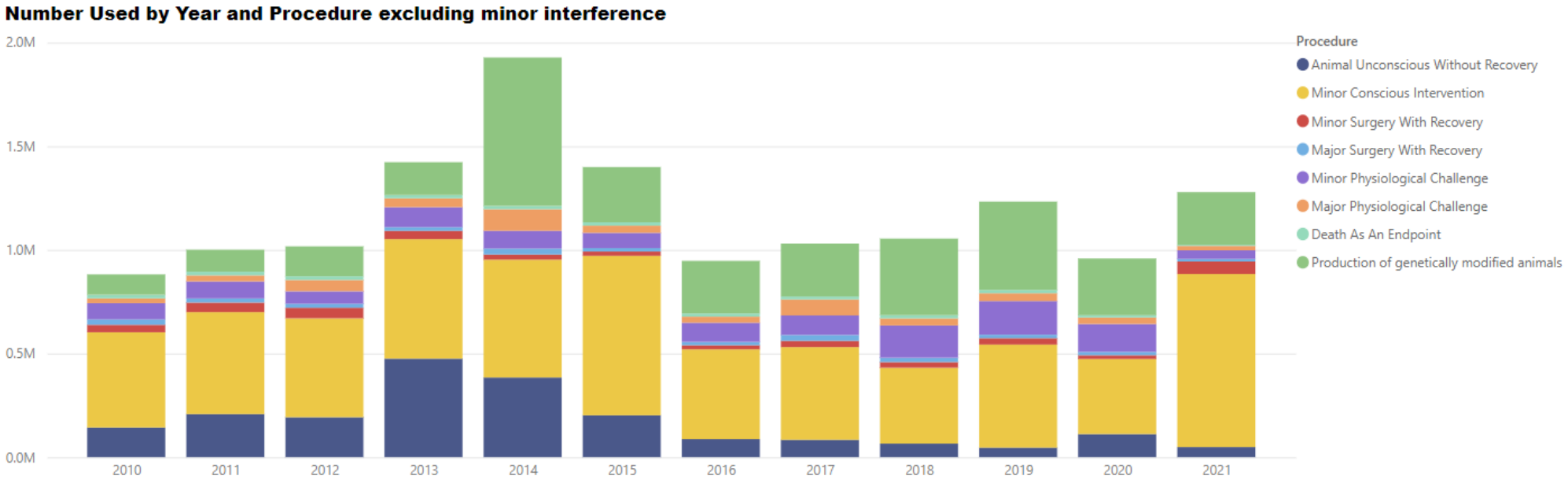
Procedure	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	**Total**
Observation Involving Minor Interference	760,592	1,217,773	1,320,108	1,276,501	1,054,859	4,887,636	4,030,182	1,595,028	1,199,738	417,598	549,697	698,542	**19,008,254**
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595	46,055	111,551	49,172	**2,049,467**
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,324	365,928	497,414	362,254	834,944	**6,283,660**
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274	30,312	18,112	59,736	**403,788**
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872	17,092	16,269	12,377	**239,527**
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,319	92,516	94,184	155,830	162,130	134,486	41,741	**1,158,161**
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121	37,880	31,237	20,844	**517,167**
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551	15,525	10,926	5,443	**178,964**
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,608	255,398	369,034	426,302	273,860	254,476	**3,321,312**
Total	**1,642,593**	**2,218,462**	**2,336,928**	**2,699,532**	**2,982,676**	**6,287,477**	**4,977,255**	**2,625,459**	**2,253,943**	**1,650,308**	**1,508,392**	**1,977,275**	**33,160,300**

2.5 Number of animals used over time by research procedure excluding "Observation involving minor interference"

Information provided by research establishments each year includes the collection of data on animals used in the procedure category of P1: "*Observation Involving Minor Interference*".

The guidance for inclusion of animals in this procedure category is: *"Animals are not interacted with or, where there is interaction, it would not be expected to compromise the animal's welfare any more than normal handling, feeding, etc. There is no pain or suffering involved."*

This category of procedure use potentially includes large numbers of animals. For example, it includes the observation of free-living animals – such as where an aerial survey of birds could record thousands of animals. A chart has therefore been included (chart 2.5) which excludes this procedure category from the total number of animals used.



Procedure	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	Total
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595	46,055	111,551	49,172	**2,049,467**
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,324	365,928	497,414	362,254	834,944	**6,283,660**
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274	30,312	18,112	59,736	**403,788**
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872	17,092	16,269	12,377	**239,527**
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,319	92,516	94,184	155,830	162,130	134,486	41,741	**1,158,161**
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121	37,880	31,237	20,844	**517,167**
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551	15,525	10,926	5,443	**178,964**
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,608	255,398	369,034	426,302	273,860	254,476	**3,321,312**
Total	**882,001**	**1,000,689**	**1,016,820**	**1,423,031**	**1,927,817**	**1,399,841**	**947,073**	**1,030,431**	**1,054,205**	**1,232,710**	**958,695**	**1,278,733**	**14,152,046**

3. Purpose, Procedure and Species Charts 2021

3.1 Numbers of animals by Purpose and Procedure categories 2021

Purpose by Procedure 2021	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Stock Breeding	34,056	5,953	8,296	49	1				238,247	286,602
Stock Maintenance	830	215	3,756						3,135	7,936
Education	33,571	1,924	105,680	914	17	64				142,170
Research: human or animal biology	48,532	12,912	22,429	11,040	9,698	17,112	12,473		5,800	139,996
Research: human or animal health and welfare	29,258	5,448	24,203	4,481	1,955	9,744	7,906		7,294	90,289
Research: animal management or production	366,613	8,808	48,918	2,541		2,191	220			429,291
Research: environmental study	184,582	13,485	605,224	40,574	706	2,726	47			847,344
Production of biological products	730	194	10,562	89		6,834	170			18,579
Diagnostic procedures	70	104	3,036			646	28			3,884
Regulatory product testing	300	129	2,840	48		2,424		5,443		11,184
Total	698,542	49,172	834,944	59,736	12,377	41,741	20,844	5,443	254,476	1,977,275

3.2 Number of animals and species used for Purpose: Stock Breeding 2021

Number of animals used for Purpose: Stock Breeding in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**552**
Amphibians	552									**552**
Aquatic animals										**11,796**
Fish	759			20					11,017	**11,796**
Birds										**21**
Native Captive			21							**21**
Domestic mammals										**15,116**
Pigs	8									**8**
Sheep	14,087		1,000	21						**15,108**
Laboratory mammals										**258,766**
Guinea Pigs	717									**717**
Mice	14,464	5,953	7,213	8	1				227,101	**254,740**
Rabbits	21									**21**
Rats	3,159								129	**3,288**
Native mammals										**62**
Native rats and mice			62							**62**
Primates										**17**
Baboons	17									**17**
Reptiles										**272**
Snakes	121									**121**
Turtles and Tortoises	151									**151**
Purpose Total	**34,056**	**5,953**	**8,296**	**49**	**1**				**238,247**	**286,602**

3.3 Number of animals and species used for Purpose: Stock Maintenance 2021

Number of animals used for Purpose: Stock Maintenance in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**2,221**
Amphibians			2,221							**2,221**
Aquatic animals										**109**
Fish									109	**109**
Birds										**80**
Poultry	80									**80**
Domestic mammals										**52**
Sheep			52							**52**
Laboratory mammals										**5,001**
Mice	371	215	1,267						3,026	**4,879**
Rabbits			82							**82**
Rats	40									**40**
Native mammals										**40**
Dasyurids	33									**33**
Possums and gliders	7									**7**
Primates										**139**
Baboons	139									**139**
Reptiles										**294**
Lizards	160		134							**294**
Purpose Total	**830**	**215**	**3,756**						**3,135**	**7,936**

3.4 Number of animals and species used for Purpose: Education 2021

Number of animals used for Purpose: Education in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**881**
Amphibians	337	544								**881**
Aquatic animals										**949**
Crustaceans (reporting not mandatory)	11									**11**
Fish	572	150	12	204						**938**
Birds										**116,653**
Exotic Captive	1		13							**14**
Exotic Wild	6,204									**6,204**
Native Captive	113									**113**
Native Wild	21,340									**21,340**
Poultry	357	639	87,986							**88,982**
Domestic mammals										**18,680**
Cats	140		58							**198**
Cattle	686	1	4,661	46	9					**5,403**
Dogs	132		510							**642**
Goats	6		1							**7**
Horses	195	1	975	55						**1,226**
Other domestic mammals	2		6							**8**
Pigs	24	45	1,338							**1,407**
Sheep	685	44	9,048		8	4				**9,789**
Exotic feral mammals										**388**
Camels			4							**4**
Cats	6									**6**
Dingo/Wild Dogs	8									**8**
Foxes	94									**94**
Goats	124									**124**
Mice	4		1							**5**
Other exotic feral mammals	50									**50**
Pigs	9									**9**
Rabbits	88									**88**

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint	Production of genetically modified animals	Total
Exotic zoo animals										**56**
Exotic zoo animals	56									**56**
Laboratory mammals										**2,222**
Ferrets	2									**2**
Guinea Pigs	49		7							**56**
Mice	54	164	776	602		60				**1,656**
Rabbits	50		13							**63**
Rats	9	336	93	7						**445**
Native mammals										**1,886**
Bandicoots	32									**32**
Bats	94									**94**
Dasyurids	92		18							**110**
Koalas	6									**6**
Macropods	483									**483**
Monotremes	42									**42**
Native rats and mice	409		22							**431**
Possums and gliders	406									**406**
Whales and dolphins	150									**150**
Wombats	132									**132**
Reptiles										**455**
Lizards	223		24							**247**
Other reptiles	34									**34**
Snakes	41		114							**155**
Turtles and Tortoises	19									**19**
Purpose Total	**33,571**	**1,924**	**105,680**	**914**	**17**	**64**				**142,170**

3.5 Number of animals and species used for Purpose: Research: Human or Animal Biology 2021

Number of animals used for Purpose: Research: human or animal biology in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**5,819**
Amphibians	4,425		1,086		70		238			**5,819**
Aquatic animals										**27,114**
Fish	18,230	57	511	68		1,999	2,406		3,843	**27,114**
Birds										**4,223**
Exotic Wild	264		58							**322**
Native Captive	301	549	594	61						**1,505**
Native Wild	810	338	207			367				**1,722**
Poultry		80	594							**674**
Domestic mammals										**689**
Cats	51		5							**56**
Cattle	3		195							**198**
Dogs	74		25							**99**
Horses	1		147							**148**
Other domestic mammals		12								**12**
Pigs					12					**12**
Sheep		8	11		145					**164**
Exotic feral mammals										**49**
Cattle					6					**6**
Mice			19							**19**
Other exotic feral mammals			24							**24**

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint	Production of genetically modified animals	Total
Exotic zoo animals										**4**
Exotic zoo animals	4									**4**
Laboratory mammals										**75,684**
Guinea Pigs		83								**83**
Mice	1,630	7,906	12,396	7,285	6,186	13,904	9,829		1,404	**60,540**
Rabbits			16	5	435					**456**
Rats	876	3,824	2,182	3,490	2,841	839			553	**14,605**
Native mammals										**22,768**
Bats	20,541		1,834	12						**22,387**
Dasyurids	17		36	1						**54**
Koalas	2		2	101	2					**107**
Macropods	80									**80**
Monotremes	7			14						**21**
Native rats and mice			10							**10**
Possums and gliders	35									**35**
Seals	5									**5**
Whales and dolphins	68									**68**
Wombats				1						**1**
Primates										**3**
Baboons					1					**1**
Other primates				2						**2**
Reptiles										**3,643**
Lizards	1	55	836							**892**
Other reptiles			1,464							**1,464**
Turtles and Tortoises	1,107		177			3				**1,287**
Purpose Total	**48,532**	**12,912**	**22,429**	**11,040**	**9,698**	**17,112**	**12,473**		**5,800**	**139,996**

3.6 Number of animals and species used for Purpose: Research: Human or Animal Health and Welfare 2021

Number of animals used for Purpose: Research: human or animal health and welfare in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**69**
Amphibians	16				20	20	13			**69**
Aquatic animals										**11,901**
Fish	896		3,907						7,098	**11,901**
Birds										**3,849**
Native Captive			35							**35**
Native Wild		17	132							**149**
Poultry		808	927	870		575	485			**3,665**
Domestic mammals										**45,528**
Cats	74		453	16						**543**
Cattle	17,317	125	3,840	37	40	48				**21,407**
Dogs	193		445	108		19				**765**
Horses	263		112		20	260				**655**
Pigs	4,165	20	7,867	2	48	13				**12,115**
Sheep	5,249	5	642	1,200	13	2,934				**10,043**
Exotic feral mammals										**38**
Rats							38			**38**

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint	Production of genetically modified animals	Total
Exotic zoo animals										**5**
Exotic zoo animals			5							**5**
Laboratory mammals										**28,727**
Guinea Pigs		15	352	102	4	3	22			**498**
Mice	952	4,373	5,367	2,006	1,646	5,472	7,005		196	**27,017**
Rabbits				23			23			**46**
Rats	85	85	88	95	164	400	249			**1,166**
Native mammals										**152**
Bats				10			15			**25**
Dasyurids				1						**1**
Koalas	48		19	2			4			**73**
Macropods			9				38			**47**
Monotremes				4						**4**
Wombats				2						**2**
Primates										**3**
Baboons				3						**3**
Reptiles										**17**
Lizards			3				14			**17**
Purpose Total	**29,258**	**5,448**	**24,203**	**4,481**	**1,955**	**9,744**	**7,906**		**7,294**	**90,289**

3.7 Number of animals and species used for Purpose: Research: Animal Management or Production 2021

Number of animals used for Purpose: Research: animal management or production in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**70**
Amphibians			70							**70**
Aquatic animals										**5,180**
Fish	3,420	750	720	38		32	220			**5,180**
Birds										**347,027**
Native Captive	112			113						**225**
Poultry	337,790	6,831	1,845			336				**346,802**
Domestic mammals										**75,371**
Cats	3									**3**
Cattle	1,625	1,200	6,623	13		135				**9,596**
Dogs	1									**1**
Goats			75			70				**145**
Horses						7				**7**
Other domestic mammals			12							**12**
Pigs	10,041		3,302							**13,343**
Sheep	13,481	8	35,295	2,209		1,271				**52,264**
Exotic feral mammals										**1,375**
Foxes						340				**340**
Goats			70							**70**
Mice			720							**720**
Rats	88	19	138							**245**
Exotic zoo animals										**4**
Exotic zoo animals				4						**4**
Laboratory mammals										**164**
Mice				164						**164**
Native mammals										**100**
Other native mammals			48							**48**
Possums and gliders	52									**52**
Purpose Total	**366,613**	**8,808**	**48,918**	**2,541**		**2,191**	**220**			**429,291**

3.8 Numbers of animals and species used for Purpose: Research: Environmental Study 2021

Number of animals used for Purpose: Research: environmental study in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Amphibians										**93,818**
Amphibians	10,058		83,661			70	29			**93,818**
Aquatic animals										**571,287**
Crustaceans (reporting not mandatory)		25	29,110							**29,135**
Fish	18,113	13,411	466,752	40,514	706	2,656				**542,152**
Birds										**124,817**
Exotic Wild	251									**251**
Native Captive			58							**58**
Native Wild	87,131		3,653	3						**90,787**
Other birds	33,586									**33,586**
Poultry			135							**135**
Domestic mammals										**79**
Cats	3									**3**
Cattle			6							**6**
Deer			5							**5**
Dogs	56		1							**57**
Sheep	8									**8**
Exotic feral mammals										**7,300**
Cats	344		85							**429**
Dingo/Wild Dogs	328		66	4						**398**
Foxes	398		87							**485**
Goats	21									**21**
Hares	22		2							**24**
Horses	77									**77**
Mice	482		3,546							**4,028**
Other exotic feral mammals	283		140							**423**
Pigs	978		161							**1,139**
Rabbits	19	18	22							**59**
Rats	27	31	159							**217**

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint	Production of genetically modified animals	Total
Exotic zoo animals										**5**
Exotic zoo animals	5									**5**
Laboratory mammals										**136**
Mice	113									**113**
Rats	11		12							**23**
Native mammals										**40,636**
Bandicoots	405		431							**836**
Bats	21,512		3,931							**25,443**
Dasyurids	646		1,399							**2,045**
Koalas	45		21	6						**72**
Macropods	3,211		1,015	41						**4,267**
Monotremes	125		81							**206**
Native rats and mice	969		2,878	6						**3,853**
Other native mammals	57		1,425							**1,482**
Possums and gliders	1,243		819							**2,062**
Whales and dolphins	31									**31**
Wombats	118		221							**339**
Reptiles										**9,266**
Lizards	1,967		2,615				18			**4,600**
Other reptiles	13									**13**
Snakes	1,520		207							**1,727**
Turtles and Tortoises	406		2,520							**2,926**
Purpose Total	**184,582**	**13,485**	**605,224**	**40,574**	**706**	**2,726**	**47**			**847,344**

3.9 Number of animals and species used for Purpose: Production of Biological Products 2021

Number of animals used for Purpose: Production of biological products in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Birds										**7,995**
Native Captive	12									**12**
Native Wild	20		7,450							**7,470**
Poultry	480	20	13							**513**
Domestic mammals										**7,136**
Cats			30	10		1				**41**
Cattle				27		223				**250**
Dogs			98			172				**270**
Horses			18							**18**
Sheep	57		65			6,435				**6,557**
Exotic feral mammals										**3,052**
Hares		1								**1**
Mice	160	173	2,718							**3,051**
Laboratory mammals										**225**
Mice						3				**3**
Rabbits							170			**170**
Rats				52						**52**
Native mammals										**1**
Monotremes	1									**1**
Reptiles										**170**
Snakes			165							**165**
Turtles and Tortoises			5							**5**
Purpose Total	**730**	**194**	**10,562**	**89**		**6,834**	**170**			**18,579**

3.10 Number of animals and species used for Purpose: Diagnostic Procedures 2021

Number of animals used for Purpose: Diagnostic procedures in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Aquatic animals										**2,860**
Fish			2,860							**2,860**
Birds										**610**
Poultry						610				**610**
Domestic mammals										**31**
Horses	19		12							**31**
Laboratory mammals										**383**
Mice	51	84	163				28			**326**
Rabbits						36				**36**
Rats		20	1							**21**
Purpose Total	**70**	**104**	**3,036**			**646**	**28**			**3,884**

3.11 Number of animals and species used for Purpose: Regulatory Product Testing 2021

Number of animals used for Purpose: Regulatory product testing in 2021

	Observation Involving Minor Interference	Animal Unconscious Without Recovery	Minor Conscious Intervention	Minor Surgery With Recovery	Major Surgery With Recovery	Minor Physiological Challenge	Major Physiological Challenge	Death As An Endpoint **	Production of genetically modified animals	Total
Birds										**18**
Poultry						18				**18**
Domestic mammals										**4,636**
Cattle		3	595			713				**1,311**
Dogs	15		215							**230**
Horses						64				**64**
Pigs	245		48	48		194				**535**
Sheep	40	126	895			1,435				**2,496**
Laboratory mammals										**6,530**
Guinea Pigs			167					65		**232**
Mice			282					5,378		**5,660**
Rabbits			488							**488**
Rats			150							**150**
Purpose Total	**300**	**129**	**2,840**	**48**		**2,424**		**5,443**		**11,184**

4. Fate of animals

From the 2019 reporting year onwards, reporting on the Fate of animals category is mandatory for the use of domestic dogs and cats, and voluntary for other species.

Fate is reported on for each year in which an animal is used in a research project. This means, for example, that if an animal is not used in a research project in a reporting year but is rehomed during that year, the rehoming will not be captured in the reported statistics. This could lead to differences between reported rehoming numbers (contained within this report) and actual numbers of domestic dogs and cats rehomed in a reporting year.

In 2021 for domestic dogs and cats, data reported shows:

Domestic dogs:

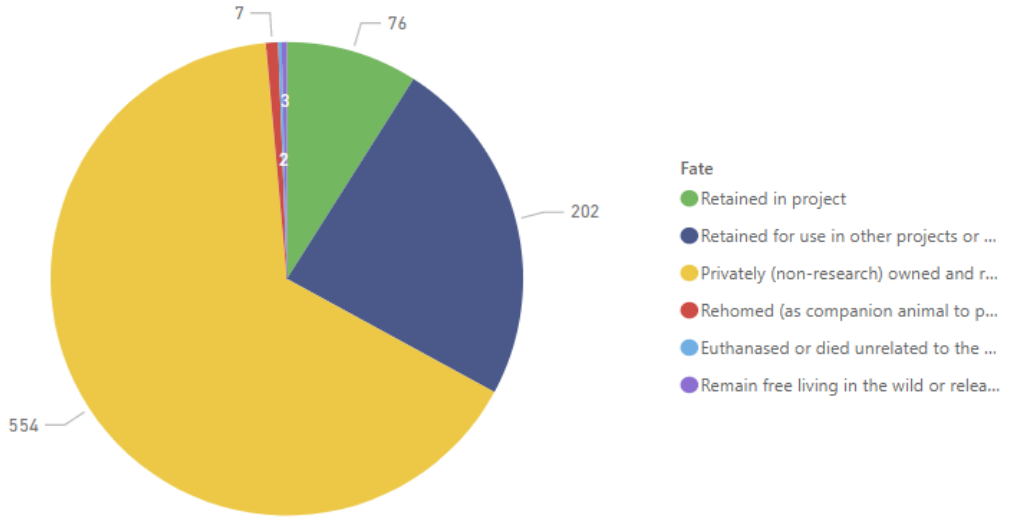
- 1,153 (56%) were privately (non-research) owned and remained with their owners. Examples of this type of research are:
    - *Animal presented to veterinary clinic for treatment and participates in clinical trial*
    - *Behavioural study with privately owned companion animals*
- 886 (43%) were retained for use in research (and will go on to be counted in each year in which they are used).
- 16 were rehomed.
- 9 were euthanased or died unrelated to the project.
- No domestic dogs used in research were euthanased because they were unable to be rehomed.

Domestic cats:

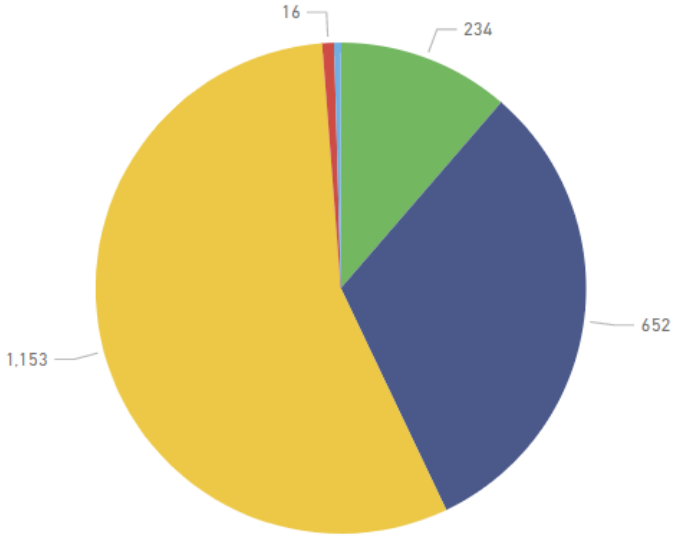
- 554 (66%) were privately (non-research) owned and remained with their owners. Examples of this type of research are:
    - *Animal presented to veterinary clinic for treatment and participates in clinical trial*
    - *Behavioural study with privately owned companion animals*
- 278 (33%) were retained for use in research (and will go on to be counted in each year in which they are used).
- 7 were rehomed.
- 2 were euthanased or died unrelated to the project.
- 3 remained free living in the wild.
- No domestic cats used in research were euthanased because they were unable to be rehomed.

4.1 Number of domestic cats and domestic dogs used by category: Fate of animals 2021

Number Domestic Cats Used by Fate 2021

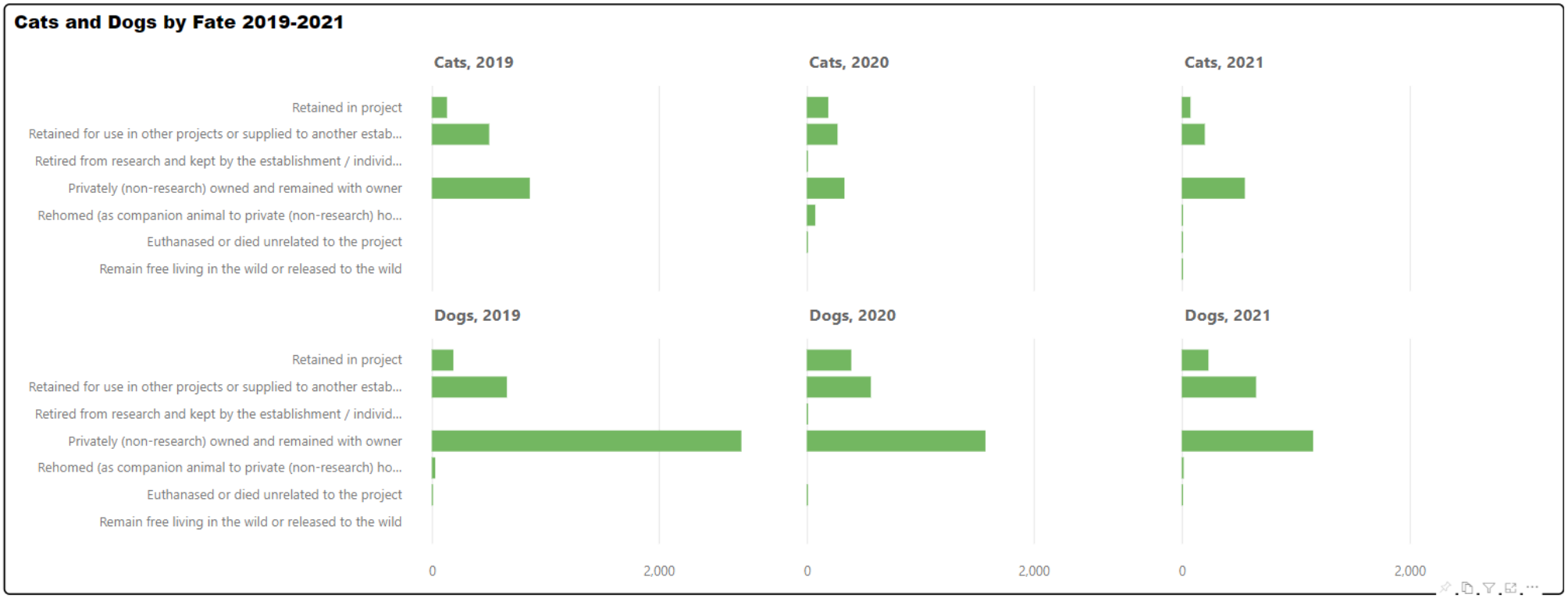


Number Domestic Dogs Used by Fate 2021



Fate	Cats	Dogs
Retained in project	76	234
Retained for use in other projects or supplied to another establishment / individual for research	202	652
Retired from research and kept by the establishment / individual		
Privately (non-research) owned and remained with owner	554	1,153
Rehomed (as companion animal to private (non-research) home or rehoming organisation)	7	16
Euthanased or died related to the project		
Euthanased or died unrelated to the project	2	9
Euthanased because unsuitable to be rehomed		
Euthanased because unable to find a suitable home		
Remain free living in the wild or released to the wild	3	
Total	**844**	**2,064**

4.2 Fate of domestic cats and domestic dogs 2019 – 2021

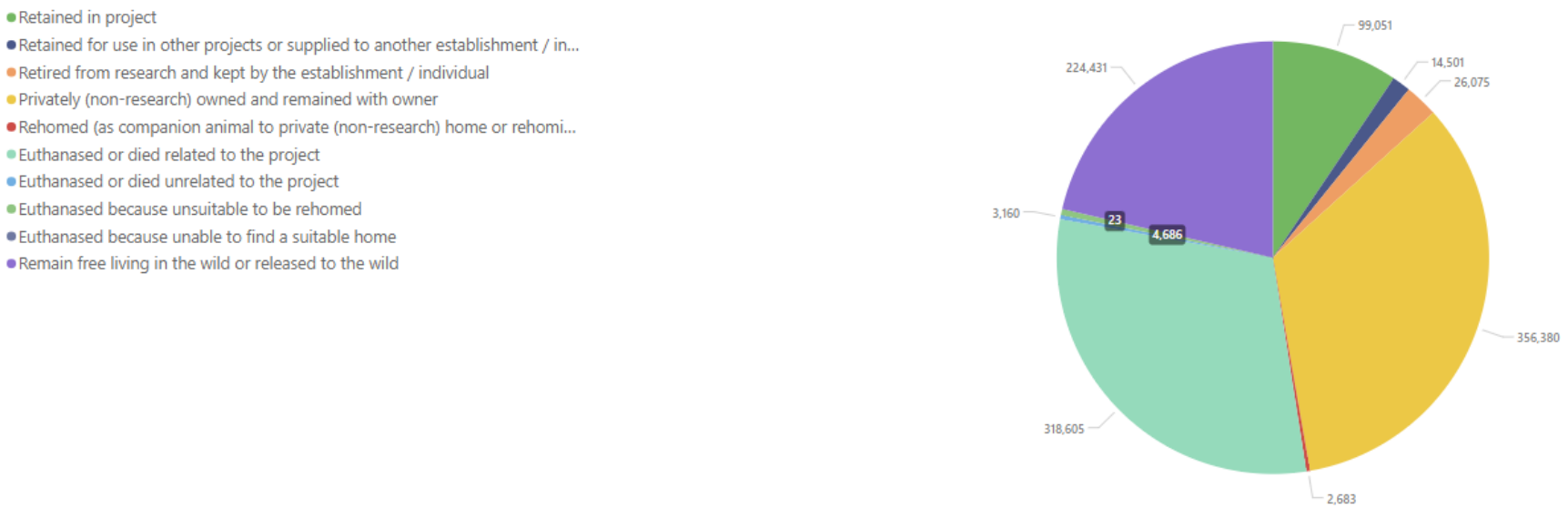


Fate of Cats	2019	2020	2021
Retained in project	134	190	76
Retained for use in other projects or supplied to another establishment / individual for research	506	271	202
Retired from research and kept by the establishment / individual		11	
Privately (non-research) owned and remained with owner	864	332	554
Rehomed (as companion animal to private (non-research) home or rehoming organisation)		75	7
Euthanased or died related to the project			
Euthanased or died unrelated to the project		5	2
Euthanased because unsuitable to be rehomed			
Euthanased because unable to find a suitable home			
Remain free living in the wild or released to the wild			3
Total	**1,504**	**884**	**844**

Fate of Dogs	2019	2020	2021
Retained in project	190	392	234
Retained for use in other projects or supplied to another establishment / individual for research	662	565	652
Retired from research and kept by the establishment / individual		12	
Privately (non-research) owned and remained with owner	2,729	1,575	1,153
Rehomed (as companion animal to private (non-research) home or rehoming organisation)	30		16
Euthanased or died related to the project			
Euthanased or died unrelated to the project	5	9	9
Euthanased because unsuitable to be rehomed			
Euthanased because unable to find a suitable home			
Remain free living in the wild or released to the wild			
Total	**3,616**	**2,553**	**2,064**

4.3 Number of species groups used by Category: Fate of animals 2021

Number Used by Fate and Species Group 2021



Fate	Amphibians	Aquatic animals	Birds	Domestic mammals	Exotic feral mammals	Exotic zoo animals	Laboratory mammals	Native mammals	Primates	Reptiles
Retained in project	791	2,078	88,252	6,977	4	2	360	172	17	398
Retained for use in other projects or supplied to another establishment / individual for research	2		437	6,522		56	7,302	44	130	8
Retired from research and kept by the establishment / individual			113	25,950		9		3		
Privately (non-research) owned and remained with owner	70	13	328,066	28,199		2		28	2	
Rehomed (as companion animal to private (non-research) home or rehoming organisation)		100	2,366	200			15			2
Euthanased or died related to the project	401	24,994	10,759	2,671	275		279,417	27	1	60
Euthanased or died unrelated to the project	20	1,293	143	85			1,567	39	12	1
Euthanased because unsuitable to be rehomed	89	2,104	2,280				213			
Euthanased because unable to find a suitable home							23			
Remain free living in the wild or released to the wild	17,775	126,753	58,486	758	5,795	5		9,415		5,444

5. Lethality testing

The *Animal Research Act 1985* defines a 'lethality test' as *'an animal research procedure in which any material or substance is administered to animals for the purpose of determining whether any animals will die or how many animals will die'*. Lethality tests include, but are not limited to, LD50 tests. The following are the figures reported on animal use for lethality testing in 2021.

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
Guinea Pigs	65	20	7	Clostridium chauvoei Potency by Challenge in guinea pigs. Vaccinated animals are challenged with test organism in order to demonstrate protection and hence vaccine efficacy.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Assessment of in-process or development material to determine suitability for further manufacture.	An in vitro replacement assay for the Clostridium chauvoei challenge assay has been implemented and is currently in use as a routine. The use of these guinea pigs was prior to the full implementation of the in vitro test. The use of this test ceased mid 2021, and is only currently approved as a contingency in the event that problems are encountered with the replacement in vitro test.
Mice	2,668	832	119	Serum neutralisation test in mice: Susceptible animals	Regulatory testing required to demonstrate efficacy	An in vitro project is currently in place with the objective of

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
				are challenged with test toxin/antibody dilutions to determine antibody titre.	(potency) of vaccines prior to release. Testing of stability batches and new product formulations.	replacing animal tests with in-vitro tests. Three SN replacement in vitro assays for C. septicum, C. perfringens Type D and C. tetani were transferred to Quality Control Laboratory late in 2020 and are in routine use. The remaining replacement assays for C. novyi Type B; C. botulinum Type C and C. botulinum Type D are currently under development.
Mice	1,894	1070	135	Total Combining Power test in mice: Susceptible animals are challenged with test antigen/toxin/antibody dilutions to determine potency of antigen preparations.	In-process testing of vaccine constituents to allow evaluation of suitability for further manufacture.	This test is based upon regulatory requirements for the assessment of in-process products. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop in vitro assays which may be used to replace existing in vivo

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
						assays subject to requlatory approval of these replacement assays.
Mice	816	263	45	L + titration in mice: Susceptible animals are challenged with test toxin in order to determine potency of antigen preparation.	In-process testing of production and development antigen growths to allow stop/go decision during manufacturing process.	This test is based upon regulatory requirements for the assessment of in-process products. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop in vitro assays which may be used to replace existing in vivo assays subject to requlatorv approval of these replacement assays.
Feral Deer Goats	736 224	2 0	0 0	Field trials using a targeted feeding structure and non-lethal or lethal bait types	Negative impacts associated with overabundant pest herbivore species are well accepted, with feral goats *Capra hircus* listed as a key threatening process under the	The purpose of this research is to devise a humane method for killing free-living feral species. There are no alternatives to lethality testing.

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
					Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. Additionally, competition and habitat degradation by feral goats and herbivory and environmental degradation caused by feral deer are listed as Key Threatening Processes in New South Wales under Schedule 4 of the *Biodiversity Conservation Act 2016*. Despite these listings, control techniques for these species appear limited, particularly when compared to the number of techniques available for other pest species. Ongoing field trials continue to identify the potential for further development of a target selective, humane and cost-efficient method as an	

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
					additional technique for controlling overabundant herbivore pest species.	

6. General examples of methods used to implement the 3Rs in 2021

The following are general examples of strategies used to implement the 3Rs (Replacement, Reduction and Refinement in animal use). These examples have all been directly reported by accredited animal research establishments for the 2021 reporting year. Examples relate to 'Replacement' (of animals with other methods), 'Reduction' (in the number of animals used) and 'Refinement' (the use of methods that alleviate or minimise potential pain and distress and enhance animal welfare). Reported information has been divided into 'general' and 'specific' examples, to help improve the accessibility of information.

6.1 Replacement – general examples

Replacement relates to methods which avoid or replace the use of animals in research and teaching. Below are general examples of replacement that have been reported by accredited animal research establishments. Additional specific examples of replacement methods reported in 2021 are contained in Appendix A.

6.1.1 Simulation

1. Models for anatomical and clinical examinations.
2. Use of training models to teach techniques (e.g. latex rat for injections).
3. Use of audio-visual material such as videos, slides, and interactive computer programs.
4. Promoting the use of software-based simulations to assist with teaching.

6.1.2 In-vitro technology

5. Increased availability of in-vitro technology such as the use of established cell lines.
6. Researchers moving away from primary cultures and using stem cell differentiation or commercially available human cell lines, for more translatability to medical research.
7. Continued development and implementation of ELISA testing to replace animal testing.
8. Cancer cell lines and human tissue samples partially replaced the use of animals.
9. Use of established cell lines.
10. Use of plant tissue as a replacement for animal tissue for certain enzymatic assays.

6.1.3 Mathematical / computer modelling

11. The use of mathematical and computer models, videos.

12. Some projects have allowed for replacement of animals due to advances in statistical modelling and use of relevant historical data.

6.2 Reduction – general examples

Reduction relates to methods to minimise the number of animals used. This includes obtaining comparable information from the use of fewer animals and obtaining more information from the same number of animals. Below are general examples of reduction that have been reported by accredited animal research establishments. Additional specific examples of reduction methods reported in 2021 are contained in Appendix A.

6.2.1 Pilot studies

1. Pilot trials and the implementation of stop/go measures are in place for some projects to allow review before more animals are recruited into the study.
2. Use of pilot studies to refine techniques and reduce animal numbers.

6.2.2 Shared animals / tissue

3. Animals used for courses are shared amongst multiple participants in order to achieve the best learning outcomes whilst reducing the overall number of animals used.
4. Shared control groups between studies are used when possible.
5. Multiple clinical parameters may be obtained from single groups of animals rather than parallel groups of animals.
6. Performing “sighting studies” in reduced numbers of animals to determine the toxicity potential of novel or unknown test compounds prior to experiments in “full numbers”.
7. Sharing of data and resources (animals, tissues and equipment) between research groups.
8. Tissue samples are collected and stored for future use or use by other researchers.
9. An increasing number of projects are using tissue samples only (often collected during routine health inspections of animals).
10. During most AEC approved fieldwork, staff will collect remains of individuals that have already died from unrelated causes (e.g. roadkill, skeletal remains of dead animals), reducing the use of live individuals of the collected species.
11. Routine husbandry procedures to be performed on animals are coordinated with teaching activities.
12. Re-use of animals, where appropriate, after extended recovery interval.
13. Training animals used are only those identified for culling or use of dead animals to gain experience in new technical procedures as practicable.
14. Opportunities to combine experimental protocols is encouraged where this means no or minimal additional procedural load on animals.

15. The use of tissues or cells sourced from dead animals, such as roadkill or animals that were humanely killed as part of another AEC application.
16. Use of animals that died of natural causes rather than using samples from live animals, such as animals in wildlife rehabilitation centres which are humanely euthanased.
17. Mock surgery performed on animal tissue for training purposes or refinement of techniques.
18. Use of museum specimens.
19. Encourages researchers to collaborate to develop projects where more information can be obtained from the same number of animals, maximising the use of animals and avoiding additional animal use.
20. A tissue bank has been established where unused samples can be stored for future projects by pharmacology students. Collected tissues from different disease models are also being shared across research disciplines in laboratory-based projects.

6.2.3 Statistical / study design

21. Projects are statistically designed with animal numbers determined by using power analysis for all projects. This ensures that an appropriate number of animals are used.
22. Consultation with a biostatistician for assistance with experimental design, sample size calculations and data analysis when preparing an AEC application to ensure animal numbers and wastage are kept to a minimum.
23. Power calculations have been used to detect the correct number of mice needed to detect a statistical significance and to avoid overuse of animals.
24. Obtaining more data from the use of fewer animals by combining objectives.
25. The minimum number of timepoints to produce significant results was used in each model to further reduce the number of mice required.

6.2.4 Review of existing information

26. Avoid performing unnecessary experimental work with animals by conducting a comprehensive review of existing data, available models and alternative experimental approaches, before renewing existing, or commencing novel, research projects.
27. Literature reviews and screening of treatments at both the theoretical and benchtop level are undertaken to ensure projects are valid prior to moving to the in-vivo testing stage.
28. Continued use of technology such as Geographic Information Systems (GIS) and programs such as the Atlas of Living Australia assists in targeting areas or species, reducing the need to undertake some work and in some cases totally replacing it.

6.2.5 Breeding

29. Avoiding breeding of animals to reduce the number of animals and wastage by sourcing animals (or tissues) from external suppliers or other researchers and reviewing opportunities to use a similar, suitable model to avoid the breeding of animals. Where breeding of animals cannot be avoided, efficient colony management and monitoring is essential to ensure production is as minimal as possible.
30. Breeding programs are designed and maintained to produce stock to order, to reduce numbers and overproduction.
31. Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.
32. Genetically modified animals are bred for the desired genotype as far as possible to reduce numbers.

6.3 Refinement – general examples

Refinement relates to methods which alleviate or minimise potential pain and distress and enhance animal welfare. Below are general examples of refinement that have been reported by accredited animal research establishments. Additional specific examples of refinement methods reported in 2021 are contained in Appendix A.

6.3.1 Environmental enrichment / housing

1. Behavioural enrichment tools are included in studies where deemed appropriate and functional. These may include food scattering, play with handlers, stumps for sheep to climb on, balls hung from the roof for sheep to play with, bones for dogs and cats to chew. Additional play items were introduced this year which include rope toys and Kongs with treats inside.
2. Ensuring animals are provided with best practice care, housing and environmental enrichment that allows the expression of species-specific behaviours and social structures.

6.3.2 Reduced impact of procedures (including anaesthesia / analgesia)

3. Refinements to analgesia and anaesthesia were employed to improve intra- and post-operative recovery, pain management and reduce surgical complications. Through consultation with the AECs and Animal Care team, several projects underwent revision and refinement prior and post approval, optimising the anaesthetic and analgesic doses and combinations as well as frequency of administration and ability of researchers to recognise signs of pain and distress, triggering intervention in the form of pain relief.
4. Use of invertebrates such as Drosophila or nematode worms for teaching projects.
5. Requiring an acclimation period of at least 7 days after arrival in new surroundings, before they are used in a project
6. Acclimating animals to handling and research procedures before being used in a research project.

6.3.3 Technology

7. A three-year duration limit, with the opportunity of no more than a one year extension on all projects, ensures advances in procedures are regularly incorporated in renewed projects.

6.3.4 Education, veterinary input, and monitoring of research

8. The Animal Care team continued training of researchers in current best practice techniques.
9. Direct access to experienced staff in Animal Ethics and Welfare and veterinarians.
10. Continued compulsory training and competency assessment for all animal users in animal ethics and practical techniques.
11. Principal investigator, associates and animal handling staff with appropriate training and/or experience in handling animal species used in research projects.
12. Requiring new researchers to undertake a mandatory animal ethics course prior to starting work and requesting existing researchers to undertake a follow-up refresher course every 5 years thereafter.

6.3.5 Wildlife studies

13. The use of non-capture methods for wildlife research and teaching including the use of field surveys, scat surveys, camera traps and drones.
14. The use of less invasive procedures in wildlife studies e.g. sand pads rather than trapping.
15. Use of an Observational Only - Field Research Form (No Trapping, Handling or Spotlighting).
16. The use of thermal imaging cameras for determining presence/absence and quantitative data continues to reduce the need to trap animals.
17. The use of cameras and audio equipment (acoustic and ultrasonic) where permitted by regulators, reduces the need to trap animals.
18. Biosecurity risk management continues to be well implemented.

6.3.6 Rehoming

19. AEC review and investigation into possible rehoming of research animals.
20. Rehoming of fish to private tanks when no longer suitable for experimental purposes.

6.3.7 Funding

21. As part of the establishment's commitment to the 3R principles, it awarded a total of $250,000 to support research into the 3Rs through the 3Rs Grant scheme which was established in 2019. The objective of this grant is to support science-based projects with a primary goal of replacing, reducing, or refining the use of animals in biomedical research. Two applications were funded in full and a third was funded partially with the remaining funds:
    - Reducing animal use by demonstrating the viability of animal re-use in behavioural experiments (funded in full).
    - Using the 3Rs to improve animal welfare outcomes in teaching and research (funded in full).
    - In-vitro, non-animal-based technologies that involve the use of spheroids or organoids.

7. Appendix A – Specific examples of methods used to implement the 3Rs in 2021

The following are specific examples of strategies used to implement the 3Rs (Replacement, Reduction and Refinement in animal use). These examples have all been directly reported by accredited animal research establishments for the 2021 reporting year. Examples relate to 'Replacement' (of animals with other methods), 'Reduction' (in the number of animals used) and 'Refinement' (the use of methods that alleviate or minimise potential pain and distress and enhance animal welfare).

7.1 Replacement – specific examples

For the 2021 reporting year there are 51 examples of Replacement compared to 28 in the 2020 reporting year.

7.1.1 Simulation

1. Mannequins, audio-visual materials, virtual reality (VR) prototypes, photographs, taxidermized and preserved specimens were used as substitutes for live animals.
2. Teaching procedures:
    - Use of a mechanical horse.
    - Bandaging and health care procedures are performed on dummies.
    - Life size fibreglass replica of a horse used for demonstrations.
    - Use of photos and wool samples to identify breeds of sheep.
    - Use of cardboards and leather to practise injections and ear tagging.
    - Use of condition scoring mannequins to replace the use of live sheep.
    - Co-enrolments with and use of distance education methodology.
    - Use of identification tags without live sheep for simulation.
3. We developed a teaching video with the assistance of the Versatile Vet that demonstrates appropriate techniques for performing a post mortem in a sheep.
4. The use of Micro-CT scanning and digital dissections of organisms for educational purposes providing anatomical data, and promoting the conservation of endangered species.
5. Alternative to running Poultry trials simulating in-vivo conditions using in-vitro techniques, such as artificial gut models, allows for reduction and replacement of animals when testing products and concepts.

6. Threatened and introduced mammal species' ecology in fragmented and remnant landscapes in New South Wales and the Australian Capital Territory: This protocol utilised animal cadavers donated by members of the public from road kill or natural senescence to teach researchers about general anatomy and also how to collect samples for research purposes.
7. Protocol Selectively targeting 9L gliosarcomas in the brain of a Fisher 344 rat using lanthanum mangnatite nanoparticles and Protocol Investigating in-vivo the radiosensitivity of 9L gliosarcomas in the brain of fisher 344 rat using thulium nanoparticles: These protocols made extensive use of a commercial rat model with fake tail , as well as inhouse Jelly/latex tails to train researchers in IV catheter insertion prior to practicing on live animals. In addition these protocols utilised a gelatine rat model for in-vitro radiation modelling prior to undertaking live animal experiments.
8. Excised cow teats were utilised to investigate viscosity effects on a teat sealant product, replacing live animals.
9. Use of abattoir specimens prior to students learning pregnancy testing in cattle.
10. For project Workshop for veterinary professional training in wildlife treatment and care, the Principal Investigators replaced live native animals with cadavers and soft toys to achieve their aims.

7.1.2 In-vitro technology

11. Where possible, we have replaced animal models with complex 2D and 3D tissue culture systems to partially mimic in-vivo models. These assays have helped to inform in-vivo models, thereby replacing the animals used at the early stages of the study.
12. We have utilized in-vitro methods to optimize techniques, such as cell labelling and drug dosing, to ensure in-vivo experiments are well designed.
13. We have developed a number of in-vitro analyses which utilise yeast cells or mammalian cell cultures, so that we only use mice when absolutely necessary.
14. Dust and environmental sampling is used where appropriate to eliminate the need for direct animal sampling.
15. Replacing some sample collection from animals with rack exhaust or cage filter screening.
16. A project established that environmental samples could be used for viral prevalence studies in their target species, meaning that they no longer need to trap animal for faecal swabs.
17. One study aimed to try to bridge in-vivo data to laboratory dissolution data. If this is achieved it may be possible to replace animals in a study for some changes to formulation in future.
18. Laboratory equipment was used to determine resistance to deformation by a medical device. This allowed researchers to identify materials suitable for use in animals without subjecting animals to this deformation testing.

19. A staff member was among the winners of a competition to promote the 3Rs run by the Australian and New Zealand Laboratory Animal Association (ANZLAA) relating to the use of 3D printing to create models to replace the use of animals for training researchers in the administration of IV injections.
20. A novel 3D bioprinter is being developed to prepare 3D spheroids embedded in a tissue-like matrix in an automated manner. If successful, the bioprinter will be able to generate both adult and paediatric direct-patient derived tumoroids that have previously been difficult or unable to be grown both by in-vitro and in-vivo methods. This would significantly reduce animal usage in the future as it would allow for an alternative 3D in-vitro method to be used instead.
21. One project successfully used scats rather than live trapping to determine occupancy.
22. An in-vitro system was used to demonstrate and compare the effects of factors identified in a mouse model of the function of NK cells. This meant that additional experiments requiring mice were not needed.
23. A group was able to utilise established mouse cell line culture techniques to assess the ability of modified gonocyte specific phage clone to induce cell death within a non-specific cell type. This replaced the need to utilise cultured primary cells isolated from animals.
24. A variety of in-vitro models were developed (including neurosphere models) to identify doses and the best models to be used, which led to a reduction in the number of mice required for this project.
25. In-vitro human models of oxidative stress (cell lines and human sperm cells) have been used to characterise the importance of ALOX15 in the catalysis of lipid peroxidation and cell death. These studies have led to a defined mechanism whereby ALOX15 function can be inhibited and so prevent cell death and oxidative damage in-vitro. Additionally, in silico drug docking has been used to model the effect of ALOX15 inhibitors on the human enzyme.
26. Cell lines and 3D cell culture were used to evaluate if the DNA-damaging therapy, doxorubicin, could be improved by targeting Δ40p53.
27. Some experiments were performed using isolated proteins (e.g. ryanodine receptors) supplied by another laboratory. Some modelling was also performed.
28. HEK293 cells were used to understand phosphorylation kinetics in the testis.
29. MeCap cells, a caput epithelial cell line, were used to optimize protocols and study work at an in-vitro level.
30. Experimental work was begun with 'organoids', which are stem cells grown in a rotatory chamber so that they develop into a 3-dimensional cluster, rather than a flat, monolayer of cells that usually constitutes cell culture. These stem cells are 'encouraged' (using various transcription and growth factors) to become primitive hair cells. This technique is in its early stages but may be a promising alternative for some experiments in the future.

31. One group has been the first lab in Australia to establish an organoid cell culture system for oesophageal cells. Using the organoids generated from eosinophilic oesophagitis participants, the group are screening for those who are responsive to the novel treatment compound. This work will complement the initial data generated in mouse models and tailor future treatment strategies minimising the need for further in-vivo studies to elucidate the epithelial component TNFSF10 signalling in the pathogenesis of eosinophilic oesophagitis.
32. Air-liquid interface culture of human bronchial epithelial cells was used to produce a differentiated epithelium. In this way the group have been able to study many of the mechanisms of viral infection and inflammation which do not require an intact immune system in a whole animal.
33. Bulk tank milk (BTM) was chosen as the initial overall herd screening sample to test for the presence of C. burnetii DNA and antibody at the herd level in order to identify farms in which coxiellosis (Q fever) is endemic and which were suitable for obtaining individual cow samples to isolate the bacterium from.
34. Use of a Poultry modelling program that incorporates decades of research to model broiler growth in response to diet composition. This demonstrates the impact of variable diets, and the ability of stochastic and max-profit feed formulation strategies without the use of animals.
35. Over the past 12 months, the AEC has implemented an exemption process. If the teaching or research project meets a set of criteria, it may be eligible for exemption. The AEC Executive, Risk and Biosafety must review all exemption requests, ensuring researchers have not self-assessed incorrectly. This process has encouraged more researchers and teachers to consider conducting projects that do not require the "use" of live animals. For example, the following exemptions have been approved:
    - Obtaining animal cadavers from a local council. These animals were euthanised from a pest management process.
    - Obtaining animal organs from animals euthanised as part of routine commercial food and fibre production.
    - Using computer models & fake rats to train research students in injections etc. prior to handling any live animals.
    - Accessing existing animal tag/ GPS data collected by researchers under previous USQ AEC approved projects.
    - Recording bird sounds in a park etc.
36. A protocol investigating how a substance can impact the behaviour of cells within bone marrow was first conducted with macrophages in-vitro to understand how parasite peptides work.
37. The in-vitro project delivered 4 ELISAs in late 2020. These were used in 2021 to good effect. The C.chauvoei ELISA has resulted in a significant reduction in the use of guinea pigs and the removal of a very unpleasant challenge test. The ELISA for C.tetani has also been a success. The ELISAs for C.septicum and C.perfingens D had some problems in 2021. During the 3rd quarter of the year Covid impacted on the number of days staff could work in the laboratory as well as the number of days available for work. These and other factors slowed the

problem-solving of these two ELISAs. At the very end of December it appears a resolution for the C.septicum ELISA was found. However the C.perfingens D ELISA is still being worked on.

38. Replacement tests to the mice Serum Neutralisation tests for C. novyi B, C. botulinum C and D are currently being developed.
39. In-vitro testing and analysis to establish the veracity of suspected toxins before using mice (e.g. botulism)

7.1.3 Human studies / tissue

40. Decitabine and Decitabine/Cedazuridine have been shown to have comparable efficacy in the clinic, and therefore no longer need to be compared here.
41. Samples from human clinical trials demonstrated that miR122 is elevated in bronchiolitis induced by rhinovirus and were associated with a longer time on oxygen treatment. These data replaced the need for further mouse experiments exploring the kinetics of miR122 in rhinovirus infection.
42. Analysis of human faecal samples and bloods as well as prospective dietary surveys have been conducted however a mouse model is necessary to determine the underpinning mechanisms of the associations observed. Understanding these mechanisms is particularly important because this may allow for the design of more targeted or personalised dietary interventions. An in-vivo model of the human gastrointestinal tract is required to study systemic changes in immune populations, intestinal permeability and inflammation as well as to replicate the digestion of dietary fibre and breakdown by microbes.
43. In a study on Epigenetics of sex determination involving reptiles, researchers will be using an organ culture to replace manipulations on whole embryos.
44. Although in-vitro and in-vivo animal models of Motor Neuron Disease are critical to understanding disease cause, progression and treatment we are concomitantly investigating animal alternatives where possible, specifically:
    - We have initiated studies involving patient- and control- derived induced pluripotent stem cells for the derivation human 'brain in a dish' models of Motor Neuron Disease.
    - Run concomitant studies in donated human tissue to validate our findings from in-vitro and in-vivo models.
45. The team does a significant amount of in-vitro efficacy testing on a range of human cancer cell lines before progressing to in-vivo efficacy studies, has established 3D tumour spheroid models, in which tumour cells grow in a spheroidal shape, mimicking many of the properties of in-vivo tumours (e.g. nutrient, metabolite and waste gradients, necrotic core).

7.1.4 Mathematical / computer modelling / available data

46. Conduct surveys and a workshop with producers to assess feasibility of recording certain welfare measures on farm and eliminating unfeasible measures prior to a validation study. These surveys replaced the use of animals and reduced the total number measures actually recorded on animals.
47. Use of case study data to replace need for capturing live animal data.
48. Computational modelling was used to inform on stimulation paradigms for activation of retinal neurons prior to proceeding to live animal experiments in the development of a visual prothesis.
49. We will use in-vivo physiological data obtained from mice to perform numerical simulations and mathematical modelling of the vestibular dynamic response, ultimately reducing the number of animals needed to explain an effect.
50. A protocol investigating neurosystems determined, through revision of data and literature, the minimum number of animals that would be required to produce enough tissue, data and replication for statistical analysis.
51. A protocol investigating canine arrhythmias utilised retrospective data collected from animals that had undergone ECGs as part of their routine clinical care, rather than prospectively collecting this data from the animals for this research study.

7.2 Reduction – specific examples

7.2.1 Pilot studies

1. Through the use of a pilot study, data analysed indicated that a significant result had been obtained with a clear difference between groups observed. Through this approval format, the number of animals per group required to achieve experimental significance reduced from 8 to 5. This example further evidences the committee's commitment in this area, with the intention to expand this approval framework for all new and/or novel application of treatment/intervention procedure in the future.
2. Small cohorts of mouse numbers can be used when first conducting in-vitro experiments to investigate dosing regimens, targeted therapies, gene knock-down or overexpression studies, such that only experiments with efficacy in-vitro are investigated in-vivo.
3. Pilot Study: Validation of embryo transfer technique for IVF pig embryos has been approved as precursor to a larger project Generation of boars that produce all female litters using a novel sex selection technique but has not yet commenced. This pilot study is to be undertaken to ensure that the techniques being used in both the IVF of the embryos and the surgical transfer of the embryos will allow for the success of the larger more involved project. Up to 5 sows can be used for this pilot study.

7.2.2 Shared animals / tissue / data

4. The use of deceased animals which have been professionally preserved in teaching.

5. Toads previously caught and euthanized as part of a pest eradication program are used in teaching.
6. Stools naturally expelled by turtles and collected by the collaborators at the partnering organisations are used in research.
7. Blood samples used from clinical cases that have been cleared of being infectious.
8. Due to teaching requirements a set number of animals are kept on campus and used. These though are rotated between classes to minimal numbers, taking in welfare considerations.
9. Students are placed within small groups where animals are used for surgical or other training. This reduces animal use.
10. Using/purchasing less sentinel animals for conducting health monitoring testing. Colony animals in immunocompetent rooms are used in place of sentinel rodents. Sentinel animals are replaced every six months in the immunodeficient rodent rooms.
11. Project "DNA-based approaches to commercial cow herd fertility – assessing bull power", DNA sampling is being conducted on skin samples that come from the animals during the standard practice of ear tagging. Therefore no additional procedures are being conducted on the animals.
12. The re-use of animals when it is strongly justified and the wellbeing of the animal(s) is not compromised. For example, Atlantic salmon may be transferred from one Animal Ethics application to another, where the experience of the animals involved feeding of diets that did not impact on the health or behaviour of the individuals. Another example is where mice from one Animal Ethics application that were to be humanely killed at the end of an experiment, were instead administered a terminal anaesthesia in order to practice a procedure under another approved protocol.
13. All the cattle in a study were sourced from the existing herd, which were required to remain on site for 12 months or be euthanized as per the small scales trial permit. This maintained the herd on site, and reduced the need for additional animals.
14. The animals in a study were previously used in another study. Animals were kept on site for an extended washout period, to allow for use in the study.
15. Reduction in animal use has been achieved by collaboration between research establishments and between approved projects, where animals trapped for one purpose have been used for another project e.g. tissue collection, bat banding. This has occurred with appropriate approvals and collaborative agreements.
16. When mice are euthanized tissues are halved and used for both flow cytometric and histological analysis, and blood is collected after death for serum antibody analysis. Other tissues such as bone marrow or lymph nodes may be shared between researchers such that the maximum amount of data / information can be obtained from a single mouse.
17. Ultraovulation will be used when possible to replace superovulation and reduce the number of donor mice. Ultraovulation is most effective if used to produce oocytes for use in IVF. It is not as effective if time mating is being performed to produce embryos.

18. Use of sperm freezing and IVF for rederivation and embryo freezing whenever possible as less mice are required.
19. Promoting cryopreservation instead of keeping a maintenance colony of mice.
20. Use of direct, non-terminal health screening of imported mice to replace sentinels.
21. Multiple tissues are harvested from animals to ensure as much data is collected from each animal as possible. Experiments are limited to the collection of data required to decide whether progression to human trials is warranted.
22. Sharing the same PDX model and common vehicle or treatment arms will be combined wherever possible to significantly reduce animal use. When possible tumour tissue and organs will be shared between research groups.
23. To reduce mouse numbers some experiments allow for bilateral MFP tumour implantation. Common experiments that can occasionally permit bilateral implantation include short term pharmacodynamics analysis, tumour expansion or organoid/explant studies.
24. Survival sampling of mice has minimised the number of mice used in health monitoring. The health status of immunocompetent mice can be assessed using a single drop of blood, an oropharygeal swab (culture), faecal swab (culture), pelage swab (PCRs) and a sample of faeces (PCRs).
25. Some biomethodology training allows mice to be reutilised for further training after a rest period of 1 -2 weeks.
26. Euthanized mice will be placed in the freezer and re-used for training wherever possible.
27. We will perfuse organs within these mouse studies to enable us to come back to different tissue samples and we will make a tissue library for other researchers to investigate proteins of interest if required. We have performed power analysis and will use the minimum number of mice possible. We will assign mice that refuse to exercise to non-exercising groups where possible to ensure we do not waste any animals.
28. Study will use previously collected data from previous sampling undertaken before bushfire periods, thereby reducing the amount of sampling of non-burnt out area required and reduction overall of animals.
29. Using testes after castration of calves in project M21/11 for project M19/13, where the teste cells are required for cell culture use. Previously this would have involved using additional calves.
30. Teaching procedures:
    - Use of horse legs from knackery for hoof trimming and basic shoeing.
    - Simulated penning of sheep by demonstration.
    - Working with university researchers and National Parks on native animal projects rather than duplicating our own projects.
    - Opportunistic field observations of native fauna.

- Maximum amount of data and student/animal contact is derived from each trapping to minimise trapping requirement.
- Only one animal is brought in for a demonstration.
- Use of an ultrasound machine to replay images from a live animal allows more students to observe procedures used on an animal.
- Use of work placements to reduce the need for students to use animals on campus.

31. Researchers assess if measures can be obtained from data that is routinely recorded on farm as part of usual animal husbandry, without additional experimental intervention including the use of Big Data analysis from the establishment's herd management system. One protocol which was approved by the AEC in 2021 and starting in 2022 (21-057- Eliminating pig tail removal to improve welfare and industry sustainability) will minimise the number of additional data collection points on individual pigs by utilising a range of standard commercial measures to create a comprehensive data set for analysis of potential tail biting triggers.
32. The number of animals used in an individual experiment can also be reduced through individual tracking, enabling a reduced sample size with the individual pig as the experimental unit. Alternatively, the same animals may be followed through multiple life stages (ie. 21-014 Raising pigs with tails: Evaluation of the cost benefit of tail docking and alternative measures to prevent tail-biting and 21-034- Early life stress and subsequent emotional and neurophysiological indicators of stress resilience) reducing the total number of animals required for experiments.
33. A workshop that was supposed to use greyhounds in swabbing and first aid chose not to use live animals in class training sessions but instead have a third party assessment involving swabbing greyhounds under supervision of a Greyhound NSW steward when required at races, as the final part of the training.
34. The establishment has a number of collaborations with Institutions throughout the world and where practical aims to combine research objectives and design studies that maximise the amount of scientific output from studies, reducing the number of studies conducted and therefore the number of animals used. An example is in both 2020 and 2021 the establishment reduced the need to conduct two additional studies by collaborating with another establishment and sought amendment approvals to obtain rumen fluid samples from animals enrolled in two of their large, approved projects. Approval was also sought from a third establishment. Rumen fluid sample collection was not part of the third establishment's project aims but the findings of the rumen results will provide further insights into their study.
35. In order to minimise the number of animals used, histology experiments requiring various fixatives were conducted in the same animals, when possible. Animal tissue was also shared with other investigators. The investigators also optimised their perfusion procedure to allow the collection of both fresh tissue for molecular experiments and OCT embedding as well as fixed tissue for immunohistochemistry to be collected from the same animal.
36. Moderate size herds from representative production environments have been included to increase accuracy and reduce the need for further replication.

37. Animal Care continued their ongoing support in facilitating tissue sharing among researchers, including researchers from other institutions requesting tissue. In 2021, a total of 2409 animals were used for tissue sharing, which consisted of rats (n=2383), mice (n=23), rabbits (n=3).

38. Re-use of animals among multiple projects when ethically justified and taking into consideration cumulative usage was approved by the AECs allowing transfer of animals from one project to another. In 2021, a total of 808 animals were transferred between projects, including: mice (n=244), rabbits (n=223), rats (n=207), sheep (n=44), fish (n=88) and guinea pigs (n=2). Reasons for transfer were varied, however primarily due to COVID-19 impacts such as issues with supply of animals and other resources resulting in researchers reallocating animals post-delivery. In addition, all animals used for Animal Care hands-on competency training were sourced from other projects.

39. The identification of mTOR (a protein kinase) was derived from archived material from a previous application.

40. Primary cultures are derived in parallel to address mechanistic questions and get the most information out of animal models.

41. Other researchers with an interest in diabetes were able to harvest tissue from animals sacrificed for a study.

42. An in-vitro drug screening panel on a number of DIPG cell lines is carried out prior to selection of efficacious drug combinations to ensure that only those that are highly efficacious are moved to in-vivo studies. This reduces the number of animals required for studies.

43. Fewer than the expected number of animals were used by dividing tissue collected to be processed in different ways.

44. Multiple samples were taken from each mouse to reduce the number of mice required to collect all necessary sample types. Control groups for different experiments were conserved and combined to eliminate the need for more groups to control for each intervention. For experiments with overlapping design which each assessed a different compound, they could sometimes be combined into one experiment, so that the control groups (which would need to be run for each experiment) would only be run once.

45. Brain tissue from animals collected for additional analyses of gene expression was used. Tissue from these brains remains archived.

46. Research outcomes from one group have provided a targeted and selective platform for utilising isolated mouse germ cells, hence reducing the number of animals required in characterising the molecular interaction of mobile phone and Wi-Fi fields on spermatozoa. Furthermore, the number of animals required to collect adequate material for experimental analysis was successfully reduced by utilising validated and established laboratory techniques.

47. A TLR2 agonist / corticosteroid synergy experiment was combined with a lactoferrin experiment to conserve the baseline control groups (share them) between the two studies to reduce the number of mice used overall, while supplying each experiment with age-matched batch-specific control mice. The minimum two timepoints were selected, rather than the whole-time course to initially identify any benefits before proceeding with the other time points.

48. Neural tissue, such as spinal cords was provided for other projects. After euthanasing mice and removing the inner ears and brainstem, the rest of the mouse was usually donated to other researchers for use in their preliminary experiment to see if they can get neural tissue to grow on organic semiconductors, and if the two can be electrically coupled.

49. Adult tissues were used for some preliminary in-vitro screening, meaning more efficient use of animals and less pups being required.
50. Harvesting techniques were improved so that both peripheral and central tissue could be used from the same mouse. This meant, at times, that researchers were able to halve the number of animals needed (or alternatively, they now get twice the information from one mouse).
51. Imaging of dead animals and their temporal bones will help improve resolution and maximise the results obtained from any one animal, thus reducing animals in this and future studies.
52. We have reduced the number of animals required to fulfill this project by refining our islet isolation procedure so that we will get the maximum number of islets from a single mouse. We have also refined the subsequent in-vitro downstream experimental procedures so that we will be able to perform a few analyses from the number of islets we will be able to get from one isolation. Whenever possible, tissues from these mice once the pancreas is harvested can be used for other projects and be shared with other investigators to reduce animal use.
53. The non-invasive nature of ultrasound allows for a longitudinal study without increasing animal numbers.
54. In a study on Epigenetics of sex determination involving reptiles, the number of animals needed for this experiment is reduced by using existing developmental data for P. vitticeps to precisely target required developmental stages.
55. Within a teaching course, students are required to undertake activities in the field, i.e. trapping, recording animal details, soil plots, etc. As a part of the teaching application, the researchers/ teachers have included a research component. Allowing for researchers to use the data collected by students and teachers to be used for research purposes. Reducing the need for researchers to repeat these activities for research purposes.
56. The establishment is both a funder and participant in the Dairy UP grant that is funded through the NSW Bushfire Industry Grant. This grant has facilitated the collaboration of many organisations within the NSW dairy industry, allowing the sharing of data and samples, thus reducing the need for animal studies.
57. Recovery procedures with our intravital imaging such that we can achieve longitudinal data from the same mouse over 3 time points. We have developed our ability to obtain longitudinal serum samples through retro-orbital bleeds, again achieving data at multiple time points from the same mouse.
58. Cadaver mice were used for training of staff on correct injection and cervical dislocation training, replacing 48 live animals.
59. Where possible we continue to strive to reduce animal numbers and the need for animals altogether. In this regard we have:
    - Performed small viability pilot studies to test drug analog efficacy for the NPY studies.
    - Incorporated both male and female animals into our studies where possible, with the advantage of reducing animal wastage as well as avoiding gender bias in our studies.

- Re-established the Thy1-YFP colony with homozygous breeders, effectively omitting all animals that would otherwise be culled due to a negative/wild type genotype.
- Shared tissue between researchers where possible (with regards to tissue derived for biochemical and histological analysis, as well as primary cell culture).
- Organised to have colonies 'frozen down' to keep breeding for the maintenance of colonies to a minimum.

7.2.3 Statistical / study design

60. The AEC liaises with investigators to refine projects that use invasive monitoring techniques. One outcome is the reduction of projects to pilot studies if a high risk of insufficient data exists, thus ensuring the validity of a future, advanced study.
61. The majority of projects approved by this committee uses a miniaturised poultry shed that allows the use of commercial bird densities but the reduces the number birds used from tens of thousands to hundreds.
62. Statistical analysis was used (epidemiological calculator EpiTools) to estimate a sample size and determined that 20-25 samples is sufficient for detecting the presence of antibodies and virus in the rabbit population with 95% confidence. All animals required are salvaged from broad-scale rabbit control programs.
63. Used previous experiments and consultant statisticians to identify the absolute minimum numbers of plots and animals within plots for each measurement. Power analysis of this design was conducted using measured variation from a similar small plot experiment where 2 groups of 4 plots were compared with 3 sheep per plot. The results from the power analysis indicated we could detect a 50 g/day difference in liveweight gain and a 15% difference in wool growth at the $P<0.05$ level with a power of 80%.
64. A study used a combination of multiple compounds in one dosing which allowed for nine compounds to be evaluated in one single study. This is a massive reduction in animal numbers.
65. The cassette of 5 compounds in a study reduced the number of animals required five fold.
66. The study combined two objectives, that is, the evaluation of the dose linearity of the compound when administered to fed dogs, and then at an additional dose rate in both the fed and fasted states. This additional phase prevented a second study from being required, and reduced animal numbers.
67. Substance administered orally at four dose rates (10, 5, 2.5, 0.5 mg/kg), in comparison to a positive and negative control, to control skin lesions and pruritus induced in House Dust Mite sensitized dogs. The well utilized cross-over design reduced the number of animals required for the study.
68. Feedlot pen study assessing the payout of commercially manufactured monensin controlled release capsules in rumen fistulated cattle. Two capsules were utilized per animal to maximize the amount of data collected per animal, therefore reducing the number of animals required.

69. A study combined safety and PK end points, reducing the need for two separate studies.

70. At an early stage of a study, statistical significance was not required so the group size was minimized to three dogs per mode of administration. This was adequate to allow for early characterization of the compounds. In addition, the compounds were administered in a single formulation reducing the number of studies or groups required.

71. Each dog was provided tablets twice, to halve the group size needed.

72. A minimal group size (4) was deemed sufficient for a pilot. PK time points were also added to eliminate the need for a separate study.

73. An improvement in modelling techniques has reduced the need for some types of surveys, and careful survey design optimises the number of trapping events/nights.

74. Although the establishment must undertake pre-harvest surveys for some species as part of their licensing conditions, new conditions agreed have reduced the number of species requiring trapping.

75. The animals used in the setting and testing of the aquatic system will be made available for researchers and the breeding program.

76. The use of sighting studies as a way to measure study endpoints is a critical tool to assessment of toxicity without the large use of animals. This class of studies was high in the calendar period, indicating the use of these types of studies to reduce the numbers of animals used.

77. We reduced the number of animals used in our experiments because the results of the first phase indicated that the second phase of the experiment could begin. We therefore ended the first phase before we had collected the approved maximum number of animals. We collected fewer animals than anticipated without adversely affecting the outcome of the experiment.

78. Where possible, the same control pigs are used across multiple experiments to reduce animal numbers used i.e. Two experiments (21-010-Improved feed efficiency and control backfat and maintenance of pork quality in finishing pigs fed bitter extracts and 21-022-Ammonium chloride to reduce feed intake in finishing pigs) utilised the same control pigs which meant that less pigs were required overall.

79. Focal animals out of the larger group are used for invasive measures such as blood sampling, to reduce the number of pigs blood sampled in the experiment (i.e. 19R049- Investigating the cumulative impact of inclusion of gilt progeny in an Australian pig breeding program sampled a subset of piglets for blood sampling) and when possible multiple laboratory tests are carried out on a single blood sample per pig (ie. 21-016-Use of a targeted DSM Immunity Booster Solution to improve piglet survivability and sow lactation performance).

80. The investigators amended the existing protocol to include the more reliably immunodeficient strains NOD/SCID and NSG animals using the existing Nude mice numbers, so that the best chance is given to produce meaningful data without any unnecessary animal usage.

81. Fewer than the expected number of animals were able to be used by recording oscillations at the same time as MMN was being recorded.

82. During initial experiments, exact time points of stone formation were identified, and this reduced the number of mice used in further experiments. It was also identified that cystine stones were more common in Garfield mutant male mice than females, which also reduced the number of mice used in these experiments.

83. One group found that mice and rats have very similar phosphorylation patterns of Izumo1. As such, they choose to focus on mice as they could make transgenic animals, so fewer rats were used.

84. A group observed that once estrogen release ceased, tumours stopped growing. Thus, the mice would not reach the survival endpoint under current experimental conditions and the analysis was removed, reducing the number of animals required.

85. Previous pilot investigations plus a new innovation with a high sensitivity fluorescent video imaging allowed optimisation of experimental design so that each experiment could be performed using a minimum number of animals to achieve statistical significance.

86. The technique allows the valid use of the embryo as the statistical unit of study rather than the dam. As such, statistically valid results can be obtained from the use of 10-fold fewer animals (the average litter size).

87. The ultrasound study is longitudinal and therefore reduced the number of animals as multiple timepoint data can be collected from each animal.

88. All poultry nutrition trials are now using a power analysis tool developed by Gene Pesti to accurately calculate the numbers of chickens and number of replicated required reducing the number of poultry used.

89. The Residual Toxicity in Mice test was reduced from 8 to 5 mice per test. Previously 8 were used to be in line with guidelines such as the US 9CFR but given the European Pharmacopoeia (EP) only specifies 5 mice per test then we are able to reduce to 5 mice per test with no regulatory implication. This reduction could save more than 200 mice per year.

90. A stability trial requiring sheep vaccination and blood sampling was reduced from 340 to 170 sheep after negotiations with the APVMA.

7.2.4 Technology

91. The acquisition of new imaging equipment led to the upskilling and enhancement of imaging capabilities – with the intention to both reduce the number of animals required due to improvements in resolution and accuracy, and refine the imaging based procedures to reduce the impact on animal welfare from the longer duration under anaesthesia that these activities require.

92. Access to a multi-electrode array system is used to study electrical and chemical properties of explanted adult zebrafish hearts (ECG, conduction velocity, electrical stimulation, calcium transients), which furthermore allows treatment with different drugs. This allows numerous different parameters to be measured and tested consecutively using the same heart, reducing the numbers of hearts/ animals which would be needed to gain the same amount on information from in-vivo experiments

93. The use of mixed bone marrow chimeras instead of creating double lineage reports for our osteoclast imaging work reduces our animal usage dramatically. Through the development of our in-vivo models we will have improved understanding of the variability produced, helping us refine our study designs to reduce animal use.

94. Sentinel testing coupled with targeted hygiene practices has allowed the investigators to keep pathogens restricted to specific rooms and racks within the facility. The use of virus free mice might reduce variability in some projects and potentially lead to smaller numbers of mice being used.

95. Since the commencement of this project 4 years ago, more than 160 staff members have been trained in the various techniques listed in the protocol. Refining their technique has allowed investigators to maintain consistency in their experiments, thereby reducing wastage due to inaccurate dosing or animal injury.

96. Four mice can provide mast cells for eight recipient mice i.e. by culturing the cells they are able to expand the number of mast cells. The investigators were able to obtain larger numbers of cells from smaller numbers of animals by culturing bone marrow from B6 mice with flt-3L in order to obtain large numbers of purified dendritic cells, avoiding the need to euthanase larger numbers of animals to purify cells from spleens.

97. Significant reductions in animal numbers as well as refinements in experimental and humane endpoint criteria were enabled by in-vivo imaging technology which allows repeated measurements on the same animals over the course of an experiment. For example, drug kinetics or biological reactions to treatments can be studied using one experimental group across multiple timepoints. Further, a number of cancer studies utilise repeated imaging in order to monitor tumour growth and determine optimum timing for treatment, this is particularly important where the tumour location is not directly palpable e.g. colorectal cancer.

98. Several laboratory methods were designed to reduce the numbers of animals required. One of these was the extraction of RNA, protein and assessment of bacterial load in a single sample. This allowed the numbers of animals required to achieve the same outcomes to be greatly reduced.

99. Only those compounds that showed efficacy in-vitro were subsequently tested in-vivo, thus reducing the animal numbers being used. Development and optimisation of the targeting peptides in-vitro was conducted on cell lines derived from Sertoli and Leydig cells from mouse testes and granulosa cells of the ovaries. This was done after confirmation in the lab that these cell lines express the targeted receptors. Additionally, cell ablation agents, both targeted and nontargeted, were initially tested using these cell lines.

100. Ca2+ imaging allows for within subject comparisons and therefore results in a lower number of total animals used.

101. Sophisticated in-vitro assays have been developed to rapidly detect beneficial biomechanical changes which allow multiple testing of tissues, reducing the number of live animals required.

102. An in-vitro assay was developed which allows drug screening. This allowed less drug doses to be required to be tested in-vivo.

103. Use of the Braincubator to extend the life of neuronal tissue for electrophysiology and imaging which has resulted in less animals being used (Buskila, Y. et al, 2014. Extending the viability of acute brain slices. Scientific Reports. 4: 5309.)

7.2.5 Breeding

104. The AEC continued to assess the requirement for under-utilised mouse breeding colonies. The AEC is awaiting portal and ethics applications within the coming 6 months. If no such applications are made within the agreed timeframe, further exploration into freezing the line down, with management of breeding activities and gamete production, are to be undertaken to reduce the number of animals used to support the animal line.

105. The AEC approved and published an improved set of standards on breeding of laboratory rodents. This was designed to improve conditions for breeding animals, set minimum standards, and reduce the wastage and overproduction of laboratory rodents.

106. Use of cryopreservation of sperm, embryos and ova from mice ensuring preservation of mouse strains not currently in use, reducing unnecessary breeding.

107. Routine room stocktakes are conducted monthly. Helps maintain breeder and stock animals at suitable levels avoiding over production.

108. Implementation of the new animal management program, Tick@Lab for use by Animal Services and researchers to improve record keeping, monitor animal use and experience and assist in avoiding potential excess breeding of animals.

109. Routine meetings with research groups for colony management to ensure breeding is optimised for experimental or maintenance production only, thereby minimising or eliminating the generation of unrequired animals through breeding strategies used.

110. Rederivation: Animal facilities optimise fostering process and thereby minimise the numbers of female mice used for fostering purposes.

111. Approval of new techniques for embryo freezing rather than continuous breeding to maintain lines.

112. Females that fail to become pregnant in the timed mating approach (typically 2 of the 4 setup) are re-used in subsequent rounds of timed matings. Experienced males (successful breeders) are re-used for subsequent rounds of timed matings.

113. During the production of neonates for spermatogonia isolation, both male and female mice were born, however only male mice were required for cell isolation. The females were weaned and aged to sexual maturity to utilise for future embryo/neonate production. This reduced the need to set up larger breeding colonies to supply adult female mice at the MSB animal facility.

7.3 Refinement – specific examples

7.3.1 Environmental enrichment / housing

1. To minimise distress and suffering and enhance well-being, rabbits are kept in climate-controlled rooms with visual, auditory, and olfactory contact with other rabbits. They are housed in accordance with the 'Guidelines for the Housing of Rabbits in Scientific Institutions' (ARRP, 2003). They receive enrichment such as hides, 3D space (provided via platforms), hay for foraging in, and newspaper balls to play with. Animals are not kept for longer than necessary and experiments are terminated as soon as practicable.
2. Keep sheep in groups, use low stress handling techniques, give the animals ample time to explore and adapt to the sheds before putting them in pens, and ensure procedures are performed for the minimum time possible and by highly competent staff.
3. Three facilities have been upgraded to provide improved climate control, ventilation, and security. Block AZ was designed with extended runs, ensuring outdoor and indoor capabilities.
4. Use of companion mice where possible.
5. Pair housing post embryo transfer surgery.
6. We have adopted the use of companion fish to avoid social isolation of fish required to be held in single tanks or single-sex tanks and prevent females from becoming egg-bound. For this purpose we use the transgenic line "mzkrt", which, due to its red, glowing body, is easily distinguished from experimental fish sharing the same tank.
7. Environmental enrichment for single caged rabbits – hay filled paper bags. Restriction on time rabbits can be individually caged. Group housing facility for rabbits contains hide spots, gnawing logs and paper bags.
8. Accommodation of research horses to roam free and graze in a large paddock at a professional horse retirement/post-training farm.
9. Environmental enrichment is an important component of housing and to provide an opportunity for enhanced welfare, the establishment has committed to 100% of their group housed sows having enrichment for part of their breeding cycle from 2020. Our AEC application template includes a section "Are animals provided with a solid floored area with bedding material for rooting and resting?". Whilst it is not a requirement in the Model Code of Practice for the Welfare of Animals, Pigs to provide enrichment, where the accommodation allows for environmental enrichment, it is being used in research projects. These range from chew toys to daily provision of wood shavings, enrichment blocks and cotton rope as per recommendation in the European Convention.
10. Environmental improvement of the commercial grower/finisher shed was conducted in 2021. This included improved ventilation and fans installed to improve air flow. This is a significant advancement for animal welfare and the quality and capacity for research conducted at the establishment.

11. Constantly improving larval rearing techniques. Focusing on optimising the concentrations of live feeds that are used to give better water quality. It appears that this has improved survival.
12. Enhanced husbandry practices were used to increase the lifespan of Dunnarts by including natural photoperiod/ lighting, larger boxes, many nest boxes, tunnels, and bridges. Animals were fed with cat food, mince mix, mealworms, and crickets seven days a week. Data show that when enhanced enrichment is added to the husbandry practices of two species of Dunnart, their lifespan increases from 2-3 years to approximately five years of age.
13. Rodents now have access to greater environmental enrichment, and several schemes have been introduced and are under investigation to improve environmental enrichment in poultry floor pens and conventional cages, including provision of dust bathing and scratch pads.
14. Improvements to animal housing and management (e.g. introduction of “buddy cages” to avoid single housing of mice, provision of environmental enrichment).

7.3.2 Reduced impact of procedures (including anaesthesia / analgesia)

15. The committee performed an SOP review, with the intention to update and align procedures with best practice. Multiple modifications and improvements have been made to various SOPs, further refining procedures and experimentation.
16. Continued use and promotion of less invasive sampling for DNA, such as plucking hair or feathers or buccal swabbing over blood sampling, ear biopsy etc.
17. The establishment has increased the number of paddocks in rotation: this assists with the welfare of livestock by resting paddocks to minimise worm infestations, and also provides enrichment in the form of exploratory behaviour.
18. Sample animals during mustering by farmers for other purposes (e.g. when farmers are doing the preg-test, control of parasites, moving animals to other paddocks, etc). Use sampling methods which minimise pain, suffering, and distress and are expected to exhibit no lasting harm. The animals will be in a cattle crush for approximately 5-10 minutes which would not be considered prolonged.
19. Research using organisms such as zebrafish embryos instead of mammals.
20. Changes to routes of administration for injection and blood collection techniques with the lowest impact and using appropriate anaesthesia and analgesia to minimise pain and distress where appropriate.
21. Implantation of a subcutaneous osmotic pump for vehicle/drug delivery specifically designed for use in mice, instead of conducting daily oral gavage to reduce the negative impact on animal welfare associated with daily restraint and discomfort associated with daily insertion of a stomach tube.
22. The decision to not approve a proposal to conduct the Forced Swim Test, directing investigators to consider alternative behavioural testing methods.

23. In all studies utilizing novel compounds, staggered dosing is carried out to reduce possible severity and the incidence of adverse events.
24. Real time analysis of the samples allowed for adaptation of sampling time points, which closed off the studies prior to planned end.
25. An interim bioanalytical analysis occurred following the 1344 hr blood collection, which shortened the study.
26. The protocol allowed animals early exit from the study and minimized the number of lice assessments at the point of efficacy loss.
27. To minimize animal handling, ruminal fluid sample collection and cattle weighing procedures were performed at the same time points as capsule retrieval.
28. Animals were housed individually for 8 hr rather than for the extent of the study, to minimize stress and ensure socialization.
29. Two studies examined novel approaches to monitoring key variables in cats, in a non invasive manner.
30. Mice are given diet gel (Tecniplast) and sunflower seeds to increase food intake after treatments.
31. Post-surgery care will be provided including saline injection and gel food to avoid dehydration.
32. The use of zebrafish in general can reduce the impact because much research is conducted on embryos before their nervous system has fully developed pathways for perception of pain or distress.
33. Shipping frozen embryos or sperm instead of live mice prevents the animal welfare problems sometimes associated with the transport of live mice (especially on long haul international flights).
34. Close monitoring of post-surgical mice and newly generated GM mouse lines to detect pain or discomfort at an early stage and treat or cull mice as required.
35. During the experimental timeline, animals will be handled as little as possible to reduce any unnecessary stress. Mice will undergo training on the blood pressure measurement machine to minimize stress associated with the machine
36. Bactrim will be added to sterile drinking water (0.032mg/mL Trimethoprim, 0.16mg/mL Sulfamethoxazole, with an estimated daily dose of 30mg/kg Bactrim) for 1 week following surgery to minimise the likelihood of contracting an infection from xenografted tissue (although handled in a sterile manner, tumour tissue is exposed to open air for ~15min during collection and preparation for its implantation into recipient mice).
37. Buprenorphine (0.075mg/kg; s.c.) and Marcaine (8.0mg/kg in 100µl; s.c. at incision site) will be administered during surgery to provide pain relief. If the animal is assessed to have pain during handling for weighing, inspection of the incision and abdominal palpation, Buprenorphine will be administered at 0.075mg/kg s.c
38. Where xenografts or drug treatments results in weight loss, saline (SC)/soggy food/gel will be provided to animals.

39. Analgesics are to be administered following major and minor surgeries to prevent pain during recovery. Feeding arrangement will also be changed with food being available at floor of the cage to prevent pain caused to animals due to stretching.
40. Routine use of eye lubricant to avoid keratitis, even in short duration anaesthesia; we place our animals on clean heating pads with a supplementary oxygen supply to aid surgical recovery; and we have switched to administer injectable analgesic at the beginning of the surgery rather than at the conclusion, under advice given by current and former veterinary staff.
41. Analgesia (ketoprofen, bupivacaine, buprenorphine) and optimal surgical technique will be used to minimise the pain and stress caused by surgical procedures.
42. Adoption of the Mouse Grimace Scale has also improved our ability to detect post-operative pain and provide adequate analgesia.
43. We have refined our photoconversion method such that we only make a small incision overlying the lymph node. This avoids making a large skin flap and imposes a far less physiological insult to the animal. There is consequently much less discomfort and pain and the mice are less likely to remove the fewer smaller sutures. Thus this refinement has also significantly reduced our mouse use by increasing the success rates of photoconversion experiments.
44. We will irradiate them only once with half the customary dose used in other mouse strains to minimise morbidity.
45. The use of scavenged tissue for training purposes meant that researchers could refine injection and euthanasia techniques before using live animals.
46. The impact of procedures on animals was minimised through the use of a head halter and blind so animals could not see blood sampling personnel; walking through races and crush at end of sampling so that animals get accustomed to process and walk through more easily; clipping hair off the jugular groove allowed better visualisation of the vein during blood collection; alternating between the left and right jugular veins each time during blood collection so that the maximum rest of the vein between blood collections occurred; using an experienced cattle handler on days when number order was important and when multiple blood collections were taken.
47. The AEC requests that investigators required to handle sheep as part of their research are appropriately skilled in low stress handling techniques to ensure that the impact of any procedures being carried out on them is minimal.
48. Addition of sedation, local anaesthetic and pain relief to calf castration and disbudding procedure.
49. Introduction of recommendation by the AEC that animals are sedated prior to euthanasia when this is required in a project. Researchers have adopted this recommendation.
50. Rotating sheep and sides of neck to be used for blood collection for laboratory diagnostic purposes under a project to increase recovery time.

51. Blood sampling and resampling of animals based on scientific protocols outlining acceptable blood harvesting based on animal's age, total blood volume and recovery period before next sampling.
52. Visits to zoos, aquariums and museums to familiarise students with a range of native animals, eliminating the need for field visits or trapping.
53. Teaching procedures:
    - The number of occasions that an animal is used is minimised by scheduled animal health and husbandry routines for teaching activities. For example, lambs are tagged and drenched as part of normal management.
    - Timetabling of classes is coordinated so that activities are spread over the semester, to avoid over-use of the same animal.
    - Students attend various workplaces to reduce the use of a particular mob of animals.
    - Weighing and husbandry of cattle for training are carried out as part of their normal, regular commercial schedule.
    - Horses are monitored for behavioural changes and replaced regularly. Horse usage is rotated to prevent overuse.
    - Horse usage recording system to rotate horses and minimise over-use of horses.
    - Reduction of lamp size to less intense light; use of red light covers for spotlighting activities.
    - For native animals, handling is conducted by the licensed person only, with students observing the techniques.
    - Animals are given appropriate rest periods.
    - Use of instructional activities that maximise students' competence in handling animals.
    - Professional development for teachers to improve skills and knowledge.
    - Use of industry sites where animals are housed to minimise stress.
    - Uncomfortable procedures such as temperature taking only done once.
    - Students are referred to Standard Operating Procedures prior to animal use.
    - Rotation of locations to minimise repeated exposure to the same native animal colonies.
    - Use of non-painful and non-invasive procedures for student activities.
    - Animals are monitored closely for signs of stress and distress and are removed from class if under undue stress.
    - Cattle and sheep used on-farm are divided into groups so that they are not re-used for health applications and not more than twice for drafting.

- Animal use monitoring forms identify the number of times an animal has been used.
- Simulations are used to practise and refine techniques before contact with live animals.

54. Spontaneous collection of naturally voided urine for the purpose of drug analyses.

55. In some experiments it is possible to replace some existing standard measurements with less invasive measures or smaller sample sizes. For example, in experiment Use of a targeted DSM Immunity Booster Solution to improve piglet survivability and sow lactation performance a Brix refractometer was used to measure colostrum with a few drops rather than through the standard laboratory tests which would require a considerably larger sample taking colostrum away from newborn piglets.

56. Experiment 21-014-Raising pigs with tails made use of a handheld thermal camera to measure temperature of the wound site after tail removal. The establishment continues to develop this technology. The outcomes from previous research in this area concluded that this technology may be a useful tool to allow early and minimally invasive identification of welfare and health challenges. In 2022 the potential use of technology for early disease detection will be further investigated through studying feed and water intake changes in individual pigs over time, combined with standard health monitoring data (ie. 21-056- Detect sick pigs using frequent feed and water intake monitoring-Experiment 1 (Individual pigs).

57. Experiments looking at the refinement of standard production procedures and handling of pigs are conducted. For example experiment 19R049- Investigating the cumulative impact of inclusion of gilt progeny in an Australian pig breeding program, looked at the impact of breeding gilt-progeny boars with gilt-progeny sows on the lifetime health and well-being of the piglets produced the results from which could help with optimising mating practices across the herd. Experiment 21-034- Early life stress and subsequent emotional and neurophysiological indicators of stress resilience, looked at how early life stressors may impact pigs throughout their life and possible methods of improving stress resilience leading to improved health and welfare.

58. The establishment continues to investigate and refine novel technology to assess physiology, welfare and behaviour of animals. Novel, non-invasive techniques to monitor body temperature, heart rate and respiration rate are planned to be explored during 2022 including experiments investigating the use of a Biopolymer vaccine. If successful this new vaccine technology will potentially reduce the number of vaccines required throughout the lifetime of pigs significantly with a slow release design decreasing the need for boosters.

59. The use of the cylinder mouse restrainer was replaced by a 20cm 5-side acrylic box with a slit on one of the sides so that mice do not feel restrained/distressed during intravenous injections. The box was visualised by Zoom imaging.

60. The investigators are generating a fluorescing liver cancer cell line that is syngeneic to C57BL/6 mice and could be injected where tumours produce a superior near-infrared tissue-penetrating signal reliably measured by an in-vivo imaging device. This new tumour model permits non-invasive, longitudinal tumour monitoring and allows the direct visualization of therapeutic responses.

61. The project was completed early with the use of embryos instead of adult fish through Year 2 of the approval.

62. In 2019 one project has introduced, and successfully implemented, the use toe-webbing biopsies in amphibians. This is deemed a less invasive technique, compared to toe-tipping in species that have large webbing on the hind feet.

63. A project has introduced a method of visual surveys of fish, which has no/minimal effects on animal welfare.

64. The Tube Fishway Project uses electronic sensor fish to measure the conditions within the transport mechanism prior to using live animals.

65. Impacts on animal wellbeing were minimised through use of ex vivo assays, where animals were used only for tissue collection, including brain tissue for neurochemical studies, cochlea explants for culture, hearts for sino-atrial node electrophysiology recordings etc.

66. One project successfully implemented a fertilised chicken egg model in the lab for studying cancer cell growth, invasion and metastasis. Therefore, the NOD/SCID mice approved under the protocol were no longer required.

67. A new cryopreservation protocol was developed that allows the cryopreservation of sperm collected in urine from males in a simple liquid nitrogen dry shipper. This can be used in the laboratory, but its main benefit will be to allow the cryopreservation of sperm collected in the field, without the need to bring males back to the laboratory.

68. After implementing changes to surgical/recovery location, analgesics being administered, and post-operative recovery procedures, one group has observed improvements in animal impact and survival and have continued these practices throughout all animal experiments.

69. Monitoring of mice after smoke exposure has been improved by specifying that researchers responsible for monitoring of mice while on the heater must only be involved in this task (i.e. the person operating the smoking machine is not responsible for monitoring mice during recovery). A section has been added to the monitoring checklist to record the room temperature to avoid overheating.

70. One group has made improvements in surgical technique to minimize loss of dental cement caps, implemented a reduction in HU-210 dose in females to abolish stereotyped behaviour seen in some animals after repeated injections, and introduced new social interaction apparatus.

71. The dose of agonists given to mice was able to be titrated down based on cell culture results, from micro-molar doses to nano- or pico- molar doses.

72. The administration route of luciferin was changed to subcutaneous injection, which is less stressful for the animals than intraperitoneal injections and provided a more reproducible luminescence signal.

73. Experiments were performed on tissues harvested from humanely euthanized rodents.

74. Methods of generating bushfire smoke materials in liquid suspension for intranasal administration to mice were designed to avoid the use of direct exposure of animals to bushfire smoke in air, as well as improve consistency and reproducibility. This minimises potential stressors to the mice as intranasal administrations are delivered under light anaesthesia and minimises factors that may contribute to adverse events as experienced in other smoke exposure models.

75. Restraint stress (also known as immobilization) was used by confining a naive animal inside a 50 mL Falcon tube. This is a commonly used stressor that stimulates the hypothalamo-pituitary-adrenal axis (i.e. stress). This technique was used rather than repeated social defeat, which is a relatively severe stressor in rodents based on social hierarchy and dominance.
76. One group continued to develop their myopia model, including the use of contact lenses and hoods, which provided better welfare outcomes for animals.
77. The oral administration of drugs by pipette was developed by a researcher. This reduced the number of anaesthesia exposures to mice over the course of the 3-week eosinophilic oesophagitis model from 30 to 9.
78. Sophisticated in-vitro assays have been developed to rapidly detect beneficial biomechanical changes, which reduced the need to maintain animals for extended periods in-vivo.
79. By using ultrasound guided injection to administer our therapy directly into the heart wall, we were able to avoid an entire thoracotomy surgery. Thoracotomy surgery is highly invasive and requires intubation and analgesics.
80. The UAV was only flown to chase the birds if they had shown clear intention to feed on vineyard grapes. Hence the impact on non-targeted species was reduced, birds that migrated past the vineyard were not impacted. The distance between the UAV and any bird was closely monitored to ensure no collisions.
81. We obtained funding to purchase a warming cabinet for use in postoperative recovery.
82. We have continued the practice of checking for and removing urethral plugs following skin graft surgery. To share our experience with the management of this problem, a manuscript describing the phenomenon of urethral plugging and our strategy to prevent associated morbidity has been prepared. It is now with co-authors for comment prior to resubmission.
83. Studies from post-mortem human brain tissue showed that Nix level reached to twice amount in the brain region resistant to Parkinsons disease (PD) pathology, indicating that Nix may play a neuroprotective role in PD. This result gave us confidence to over-express Nix in the brain of mice as a treatment for PD. We will analyse data after each in-vitro experiment to provide real-time feedback to our proposed dosage/time points for safer and accurate range to be used in mouse studies, which may further reduce the number of mice proposed to be used for dosage/timepoint trial studies.
84. Cadavers were used to determine the most appropriately sized cannulae prior to initiation of studies with live animals.
85. We have refined the thermally-induced seizure model to limit the number of seizures experienced to one (other protocols induce multiple seizures in the same animal).
86. We are closing the protocol early as we have enough data to be able to narrow our focus and reduce the number of diets used in future research.

87. A protocol investigating spinal cord injuries has amended the dosage levels of anaesthesia and pain medications to reduce complications during and post-surgery, has increased monitoring times, and has made changes to post-surgery recovery procedures.

88. Data taken from a series of unexpected adverse events were published in an open access, high impact journal to reduce the chance of similar problems arising in future work of this nature.

89. Delivery method of medication via wafers, eliminated stress caused by injections and to mimic a human scenario where the drug is taken orally. The team also implemented voluntary exercise on the running wheel rather than forced.

90. Protocol AE21/15 combination therapy to improve on CuATSM outcomes in motor neurone disease: A condition of approval of this project was for a toxicity and PK study to be performed prior to ordering experimental mice to ensure appropriate dosing and avoid the potential for toxic side-effects. This protocol is also only approved to use flexible gavage needles rather than rigid metal needles to decrease the chance of oesophageal trauma and perforation with the daily administration of medications. Changes have been made to the monitoring criteria to refine the endpoints – originally mice were sacrificed at either a 20% loss of body weight OR stage 4 ALS with a loss of righting reflex with 30 s allowed to right. This has been reduced to a 10 s time to right. Changes have also been made to the bedding and enrichment to ensure mice with weakness/paralysis can move easily and access food and water.

91. The establishment has installed an additional silo and feed head on the farm participating in project 0519-1220 to minimise human intervention in administering the treatment product.

92. Providing alternative procedures to minimise impact to the welfare of the animals (e.g. replacement of the Morris Water maze with a more relevant and less stressful dry-land alternative for mice as they are not natural swimmers).

93. An establishment using dogs and cats for research into effectiveness of various pharmaceutical tick prevention products has established a project to specifically acclimatize the animals to tick infestation in order to reduce impact of larger infestations used during pharmaceutical trials.

94. This teaching protocol replaced some vertebrate observational studies with invertebrate studies.

95. A workshop teaching equine reproduction related procedures has a highly experienced veterinarian demonstrate ultrasound and vaginal speculum procedures on mares rather than letting each of the 16 students (veterinarians) practice the techniques on mares themselves.

7.2.3 Animal monitoring / technology

96. The committee challenged investigators to improve the quality of their monitoring and clinical scoring sheets. Multiple approved protocols were directed to modify and improve monitoring and clinical sheets as a condition of approval – with the updated sheets rereviewed by the committee. Given the diverse experience and capability represented on the AEC, this led to measurable improvement in the detail and data captured. The longitudinal intention is to further develop post approval monitoring and compliance assessment as well as assist with investigation in response to an adverse event if one occurs during animal research activities.

97. The general farm monitoring systems (behavioural, activity, weights) utilised in the establishment's Precision livestock management research, allows for less invasive monitoring of livestock herds, decreasing potential stress. All animals at the partner research property have been acclimated to the use of the technology for several seasons now, meaning most cattle on the property were acclimated in a low stress manner through following their mother as a calf.

98. Score sheets for all monitoring during approved surgical procedures have been introduced to refine the process and appropriately identify and manage any pain and distress in animals.

99. Indwelling telemetry devices collecting real-time blood pressure/heart rate in rats, reducing the amount of handling.

100. Development of guidelines for researchers around unexpected adverse event reporting.

101. Daily monitoring of research animals with observations documented.

102. Activity meter data that was collected from activity meters that were secured around each cow's ankle in project 0519-1220. This allowed detection of oestrus without the need to disturb the cows or have staff manually monitoring for signs of oestrus or applying other forms of oestrus detection to the cows such as Kamar Heat Mount Detectors.

103. The use of CCTV footage in project 0519-1220 to film feed residuals has removed the need to disrupt and extend milking, hence cows are not held off feed for additional time.

104. Scoring systems for monitoring of experimental animals have been developed and refined, with the aim of minimising potential pain and distress that animals may experience as part of certain research related procedures.

105. Protocol Combinational therapies to prevent graft versus host disease: This protocol is subject to ongoing refinement of monitoring criteria to ensure animal are sacrificed as at an early endpoint. This has involved the implementation of decreased % allowance for weight loss, implementation of acute weight loss monitoring with triggers for increased frequency of monitoring and interventions based on weight loss, activity, gait, posture, skin integrity and pruritis. It is now uncommon for animals to be found dead on this protocol.

106. Earlier intervention points have been implemented along with additional monitoring for locomotion/gait analysis to ensure animals developing spinal cord lesions are detected early and humanely sacrificed.

107. Protocol AE21/19 Selectively targeting 9L gliosarcomas in the brain of a Fisher 344 rat using lanthanum mangnatite nanoparticles and Protocol AE20/02 Investigating in-vivo the radiosensitivity of 9L gliosarcomas in the brain of fisher 344 rat using thulium nanoparticles: The monitoring criteria and intervention points have been modified for both of these protocols to ensure animals with recurring tumours following radiation therapy are detected early and humanely killed. In addition the seizure management protocol has been refined further with the addition of Intramuscular methylprednisolone just prior to radiation treatment which has reduced the incidence of brain oedema and post radiation seizure activity.

7.2.4 Education, veterinary input, and monitoring of research

108. Animals in excess of breeding line maintenance requirements continue to be used on P311 for the purpose of practical training in animal research procedures. While animals that have been used for instruction in handling and dissection are usually utilised under existing approved research protocols this protocol is available should specialist training and assessment be required. This allowed multiple personnel to acquire formal competency attainment in various procedures. The continued drive towards robust and effective training and competency regime continues to be a focus, supporting researchers producing reliable and robust data, with minimal impact to animal welfare though the expertise and skill obtained through training in the animal research space.

109. Continued publication and promotion of guidelines and standards (commenced 2019) to improve practices and provide a central resource for animal-based research including:

- Laboratory Rodent Husbandry and Care
- Mouse Breeding Standards
- Xenopus Husbandry and Breeding Standards
- Blood Collection from Rodents
- Oral Gavage Standard Score Sheet.

110. A review on laboratory animal anaesthesia training with improved process to ensure full competency assessment by the veterinary team (note this was delayed by the COVID lockdown and the project will continue into 2022).

111. Continued support to researchers with option available of a veterinary pre-review by the Vet Services Team for new animal ethics protocol (particularly high impact) applications.

112. Continuation of 24/7 veterinary advice availability to all researchers.

113. The Animal Welfare Officer is undertaking a review of all standard operating protocols in use and maintains a watching brief for best practice examples that can be adopted to refine our procedures.

114. The adverse event flow chart has been developed to ensure prompt and appropriate action is taken in the event of an adverse event, upholding the welfare of animals.

115. Constant review and update of forms to ensure thorough and accurate information is provided for rigorous review by the AECs, in addition to standard monitoring sheets and the creation of project specific monitoring sheets with clinical signs clearly described, to ensure that intervention and humane endpoints are at the earliest time points and align with the Animal Ethics applications.

116. Ongoing training and upskilling of Animal Services staff involved in the care and use of animals for scientific purposes.

117. Compulsory online training for new staff and students involved in the care and use of animals for scientific, in addition to hands-on training and competency assessment.

118. Conditions of approval may be applied to projects for the Animal Welfare Officer, Animal Facility Supervisor or competent animal care staff to oversee an initial procedure conducted or to conduct the procedure on behalf of the project investigator both within animal facilities and in the field.

119. The training and skill of technicians is important in minimising stress on the animals. All staff working with zebrafish will undergo a training program including the online AALAS Zebrafish husbandry course as well as one-on-one training within the facility to ensure that the animals are managed well.

120. Expanded on-line training for technicians to include the recognition of pain and distress, assessment and monitoring of wound healing, anaesthesia, and aseptic techniques.

121. Animal House veterinary managers review protocols with researchers in order to optimise anaesthesia protocols (including monitoring) and analgesia.

122. The establishment employs two Veterinarians/Animal Welfare Officers to support, and promote, the ethical use of animals in biomedical research. These veterinarians contribute to enhancement of animal welfare and ethics at this institution by actively liaising with animal care facility staff, regularly meeting with researchers and veterinarians from other institutions, pre-screening AEC applications, providing veterinary care and treatment when required, and keeping abreast of current animal welfare issues.

123. An Experimental Technique Procedure (ETP) template was developed by Animal Ethics in close collaboration with researchers to assist researchers to standardise and refine procedures in line with current and best practice for common experimental techniques involving animals. In 2021, three ETP's were approved by the AEC and subsequently have been incorporated into several approved projects.

124. We continue to work closely with the establishment Vets throughout the optimisation phase of our protocols to ensure all procedures are performed as optimally and least invasively as possible. In this regard we have:

- Carefully optimised all surgical protocols.
- Reduced the invasiveness of osmotic pump application to minimise animal stress while allowing the pump to perform correctly and as optimally as possible.
- Refined the intranasal dosing protocol to omit the need for anaesthesia, achieved with a careful animal training regime.
- Incorporated all best practice recommendations outline by the Vet/AEC, for example with regard to CO2 asphyxiation protocols, the use of an EVAC accessory for the isoflurane anaesthesia machine, etc.

7.2.5 Wildlife studies

125. Increasingly stringent trapping and handling protocols for wildlife continue to reduce the impact on individual animals, including reduced time between trap capture and return to the natural environment.

126. Reduction in impact on wild animals by running pilots on captive populations before moving on to wild animals (for example use of marking systems on New Holland Mice in captivity prior to roll out in the field).

127. Use of zoos and captive animals to minimise the impact on wild animals.

128. Continued use of specially designed excluders for wildlife traps have decreased bycatch of non-target species.

129. Bird netting only during daytime ensures non-target species such as micro-bats are not caught. Recaptured birds are processed at the capture site where possible (rather than taken back to the banding station) which means reduced holding times and faster release times. Nets closed during adverse weather conditions or in the presence of known predators of birds in nets, such as Pied Currawongs.

130. Working with other research teams assessing the potential use of remote sensing approaches including weather radar and airborne drones to assess the size of bat populations to replace ground-based surveys.

131. Audio recording of vocalising species is being implemented for monitoring projects; the development of species recognition software is furthering the uptake of these techniques.

132. The development of the koala recogniser has led to the use of audio recorders for surveying koalas in NSW by not only researchers associated with this committee but also by researchers and consultants in the broader research community.

133. For aquatic surveys teams are increasingly using eDNA techniques for qualitative fish and platypus surveys. Results from eDNA have had strong correlation with fish surveys conducted by a government research institute for the same waters.

134. Use of drones for Koala surveys (through engaging night time operators) in NSW has yielded positive results and provided important information for the conservation of the species.

135. A study on environmental contaminants preferentially uses larval forms of frogs, rather than adult forms.

136. Flying foxes sampled under anaesthetic in the field, to eliminate any impact or distress associated with collection, handling, and sampling. Flying foxes will only be held for the amount of time required for processing.

137. Using Unmanned Aerial Systems to collect biological samples from free-swimming whales, reducing the need to get close in a boat, and thus reducing the amount of disturbance.

138. Rather than use visual implant elastomer tags (VIE) to mark individuals, we are making note of already existing unique features of the individuals (e.g. scars, size, tail regeneration length, etc). This passive method of animal identification results in less stress to the animal than if we used VIE marking.

139. The NSW government adjusted the rules regarding the release of lizards (caught in the field) back into the field after being held in captivity, therefore we were able to release animals that would have previously been humanely killed.

140. Projects attempt to utilise non-invasive survey and monitoring methods wherever possible. A majority of projects refine methods to include only passive survey techniques, such as camera trapping, sound recording, or scat collections. Additionally, these non-invasive methods are often refined further to prevent impacts on animal welfare. For example, many projects use black flash on camera traps rather than white flash; or conduct scat surveys at times when the target animal is unlikely to be present.

141. Wildlife surveys are conducted at times when young are not in the pouch/nest.

142. The end of the Elliot traps are covered in plastic bags to provide additional warmth for any trapped animals. Shredded coconut husk fibre is placed inside Elliott traps for animals to use as bedding and provide warmth.

143. Traps are set as close as possible to dusk and checked at sunrise to ensure animals are released as quickly as possible.

144. Using detection dogs to find species (via scat scent) rather than capturing animals.

145. Limiting the amount of time over dolphins at altitudes less than 60 m because the research shows that dolphins may detect drones at these altitudes.

146. Utilising audio recording for long term monitoring and our transect counts involve minimal disturbance to frogs (call broadcasts and counts of responses).

147. The minimum number of animals required for a study of assessing food-web responses to streamflow has been carefully considered and based on the power analysis. Further reducing the number of animals increases the risk that possible variation in food webs due to changes in streamflow are not detected. This will jeopardise future management decisions that aim to benefit river food webs. Careful consideration has been given to all approaches to minimise the impact on breeding individuals. For this reason, researchers propose for long-lived large bodied native fish to be fin clipped rather than euthanised. In the same study, all possible measures are being taken to minimise the suffering of animals, including the appropriate use of analgesic on target animals. The CI has experience in all techniques being performed, and will train and supervise the research students. This includes fish and yabby handling, fin clipping, and euthanasia. The potential distress of non-target animals (e.g. turtles, platypus) is minimised by setting the traps close to dusk and retrieving soon after dawn. The CI has experience in setting these nets to ensure that animals have access to air for breathing. It is not possible to monitor the traps all night and enter the rivers during the dark due to concerns for safety of human participants. However, the catch of non-target species is rare, and does not cause undue distress or drowning when equipment is used correctly.

148. In trapping projects researchers are required to check traps a minimum of twice a day. The frequency of checking reduces the time an animal is held in a trap, minimises the potential for pain and distress.

149. A researcher is conducting a project that involves experts (external to the establishment) in the field also as researchers. The experts will be handling animals with the establishment researcher for support or observation. This is to ensure the wellbeing of the animals is maintained.

150. To reduce adverse impacts on animals, the AEC reviews each procedure carefully and may require the project to be refined. For example, for project Uluru-Kata Tjuta Parks mala census – the AEC requested the Principal Investigators to make several changes to the protocol following three unexpected adverse events involving 7 individuals. The changes made included reducing animal handling time, ensuring an experienced veterinarian is present during the census, increased monitoring of animals while they're held in bags, changing the bags to hessian sacks to improve ventilation, heat exchange and visualisation, reducing the number of traps and modifying trap size to reduce the risk of injury. The PIs have also expanded the census to assess whether drone based thermal imagery is as effective as traditional capture-mark-recapture techniques. If this technique provides reliable for population estimates, trapping would only be required to assess population structure and animal body condition, so the number of traps and trapping nights could be reduced.

151. Researchers found when reviewing camera data that there was a potential for predation from other animals - when using pitfall traps with amphibians. The project has since closed but if research is to be conducted in the future using pitfall traps with amphibians the researchers will need to justify this approach and provide details on how predation will be further minimised/ prevented.

152. A protocol investigating koala fingerprinting for the purposes of conservation will pilot the efficacy of photographing the animals' paws as it is the least invasive strategy, before assessing whether physical ink-printing is required. This will also be carried out while the animals are most relaxed (i.e. when sleeping or eating).

153. A protocol investigating whether animals will use artificial hollows, is observing animals through camera trap images at some sites to reduce unnecessary handling and other stressors for the animals.

154. Improvements made to trapping equipment and datalogging equipment used in wildlife research to minimise risk of adverse events (e.g. refining the placement of trapping equipment to preclude access to the trap by predators of the target species, and reducing the length of stock logger antennas to decrease chances of the antenna becoming caught).

7.2.6 Rehoming / retirement

155. Juvenile barramundi were purchased from a commercial hatchery; five fish were surplus and kept in a spare aquarium during the experiment, and then rehomed as pets.

8. Appendix B – Guide to the categories of reporting

The following is the guidance provided in Form L – Animal use statistics on categories for Purpose, Procedure, Species and Fate of Animal.

Column C: PURPOSE

Please note – Purpose Codes now have an A (for Activity) in front of the existing purpose number code in order to help improve accuracy of data entry.

Enter the **most appropriate** numerical code **(A1-A10)** from those listed below to describe the **primary** purpose of the project (one purpose only for each project should be entered).

Purpose Code:	Description:
A1	*Stock breeding* Breeding projects to produce new teaching or research stock. Include the animals used to produce progeny and any breeders or progeny culled in the process, NOT the final progeny themselves (as these will be counted under the project in which they go on to be used).
A2	*Stock maintenance* Holding projects for animals maintained for use in other projects. These animals may be maintained under an Animal Research Authority because they require special management. If they are not held under an Authority, (e.g. normal stock animals kept mainly for commercial production, but occasionally used in research) then they are only counted in the project where they are used for teaching/research. *Examples* • *Fistulated ruminants which are maintained under a holding project, for use in other short term feeding trial projects* • *Non-breeding colony of diabetic rats held for research in other projects*

A3	*Education* Projects carried out for the achievement of educational objectives. The purpose of the project is not to acquire new knowledge, rather to pass on established knowledge to others. This would include interactive or demonstration classes in methods of animal husbandry, management, examination and treatment. *Examples* • *Animals used by veterinary schools to teach examination procedures such as pregnancy diagnosis* • *Sheep used in shearing demonstration classes for students; Dogs used to teach animal care to TAFE students*
A4	*Research: human or animal biology* Research projects which aim to increase the basic understanding of the structure, function and behaviour of animals, including humans, and processes involved in physiology, biochemistry and pathology.
A5	*Research: human or animal health and welfare* Research projects which aim to produce improvements in the health and welfare of animals, including humans.
A6	*Research: animal management or production* Research projects which aim to produce improvements in domestic or captive animal management or production.
A7	*Research: environmental study* Research projects which aim to increase the understanding of animals' environment or their role in it. These will include studies to determine population levels and diversity and may involve techniques such as observation, radio tracking or capture and release. *Examples* • *Pre-logging or pre-development fauna surveys*
A8	*Production of biological products* Using animals to produce products other than milk, meat, eggs, leather, fur, etc. *Examples* • *Use of a sheep flock to donate blood to produce microbiological media* • *Production of commercial anti-serum* • *Production of products, such as hormones or drugs, in milk or eggs from genetically modified animals*

	• *Quality Assurance testing of drugs* **but do not include animals which come under Purpose A10, below.**
A9	***Diagnostic procedures*** Using animals directly as part of a diagnostic process. *Examples* • *Inoculation of day old chicks with ND Virus to determine virulence* • *Water supply testing using fish*
A10	***Regulatory product testing*** Projects for the testing of products required by regulatory authorities, such as the APVMA. **If the product testing is not a regulatory requirement, eg it is part of a quality assurance system only, those animals should be included in the appropriate category selected from above.** (This would be normally be Purpose A8 (Production of biological products) in the case of QA testing.) *Examples* • *Pre-registration efficacy or toxicity testing of drugs and vaccines*

Column D: PROCEDURE

Please note – Procedure codes now have a P (for Procedure) in front of the existing procedure number code in order to help improve accuracy of data entry.

Enter the **highest appropriate** alphanumerical code **(P1-P9)** from those listed below to describe the type of procedures carried out on the animals in the project. The descriptions given are a guide only. **Note:** for each project include additional lines for each procedure category where different animals within the same project are subjected to different procedure categories.

Where 'Death as an endpoint' or 'Production of genetically modified animals ' applies, animals must be placed in these categories (P8 or P9) rather than any others which might also appear appropriate.

Procedure Code:	Description:
P1	*Observation Involving Minor Interference*

	Animals are not interacted with or, where there is interaction, it would not be expected to compromise the animal's welfare any more than normal handling, feeding, etc. There is no pain or suffering involved. *Examples* • *Observational study only* • *Breeding animals for supply, where only normal husbandry procedures are used* • *Breeding or reproductive study with no detriment to the animal* • *Feeding trial, such as Digestible Energy determination of feed in a balanced diet* • *Behavioural study with minor environmental manipulation* • *Teaching of normal, non-invasive husbandry such as handling and grooming*
P2	*Animal Unconscious Without Recovery* Animal is rendered unconscious under controlled circumstances with little or no pain or distress. Capture methods are not required. Any pain is minor and brief and does not require analgesia. Procedures are carried out on the unconscious animal which is then killed without regaining consciousness. *Examples* • *Laboratory animals killed painlessly for dissection, biochemical analysis, etc* • *Teaching surgical techniques on live, anaesthetised patients which are not allowed to recover following the procedure*
P3	*Minor Conscious Intervention* Animal is subjected to minor procedures which would normally not require anaesthesia or analgesia. Any pain is minor and analgesia is usually unnecessary, although some distress may occur as a result of trapping or handling. *Examples* • *Injections, blood sampling in conscious animal* • *Minor dietary or environmental deprivation or manipulation, such as feeding nutrient-deficient diets for short periods* • *Trapping and release as used in species impact studies*

	• *Trapping and humane euthanasia for collection of specimens* • *Stomach tubing, shearing*
P4	*Minor Surgery With Recovery* Animal is given appropriate regional or general anaesthesia with as little pain or distress as possible. A minor procedure such as cannulation or skin biopsy is carried out and the animal allowed to recover. Depending on the procedure, pain may be minor or moderate and postoperative analgesia may be appropriate. Field capture using chemical restraint methods is also included here. *Examples* • *Biopsies* • *Cannulations* • *Sedation/anaesthesia for relocation, examination or injections/blood sampling* • *Castration with regional or general anaesthesia and post-operative analgesia*
P5	*Major Surgery With Recovery* Animal is rendered unconscious with as little pain or distress as possible. A major procedure such as abdominal or orthopaedic surgery is carried out and the animal allowed to recover. Post operative pain is usually considerable and at a level requiring analgesia. *Examples* • *Orthopaedic surgery* • *Abdominal or thoracic surgery* • *Transplant surgery*

P6	*Minor Physiological Challenge* Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge may cause only a small degree of pain/distress or any pain/distress is quickly and effectively alleviated. *Examples* • *Minor infection* • *Minor or moderate phenotypic modification* • *Early oncogenesis* • *Arthritis studies with pain alleviation* • *Induction of metabolic disease* • *Prolonged deficient diets* • *Polyclonal antibody production* • *Antiserum production*
P7	*Major Physiological Challenge* Animal remains conscious for some or all of the procedure. There is interference with the animal's physiological or psychological processes. The challenge causes a moderate or large degree of pain/distress which is not quickly or effectively alleviated. *Examples* • *Major infection* • *Major phenotypic modification* • *Oncogenesis without pain alleviation* • *Arthritis studies with no pain alleviation* • *Uncontrolled metabolic disease*

	• *Isolation or environmental deprivation for extended periods* • *Monoclonal antibody raising in mice* • *Forced swim test* • *Nose-only smoke exposure*
P8	*Death As An Endpoint* This category only applies in those rare cases where the death of the animal is a planned part of the procedures and animals die but are not euthanased. Where predictive signs of death have been determined *and* euthanasia is carried out before significant suffering occurs, they may be placed in category P6 or P7. *Examples* • *Lethality testing (including LD50, LC50)* It does not include: death by natural causes; animals which are euthanased as part of the project; animals which are euthanased if something goes wrong; animals euthanased for dissection or for use as museum specimens; or accidental deaths.
P9	*Production of genetically modified animals* This category is intended to allow for the variety of procedures which occur during the **production** of genetically modified animals. As animals in this category may be subjected to both minor *and* major physiological challenges *and* surgical procedures, this category reflects the varied nature of the procedures carried out. It effectively includes ALL animals used in GM production other than the final progeny which are used in a different category of procedure. *Examples* • *Initial breeding animals for GM production* • *Animals culled as part of the GM production process*

Column E: SPECIES

Please note – the species codes now have an S (for Species) in front of the existing numerical species code in order to help improve accuracy of data entry.

- Enter the alphanumerical code from those listed below to describe the species or species group used in the project.

- The alphanumerical code is not sequential - for each species used select the appropriate numerical code as listed in the table below.
- There are no species codes S15, S19, S22, S25, S26, S44 or S55, and the highest number is S56.
- In filling out the spreadsheet include additional lines for each species where more than one species is used in a project.

Laboratory mammals	S1	Mice
	S2	Rats
	S3	Guinea Pigs
	S4	Rabbits
	S5	Hamsters
	S6	Ferrets
	S7	Other laboratory mammals (not primates)
Domestic mammals	S8	Sheep
	S9	Cattle
	S10	Pigs
	S11	Horses
	S12	Goats
	S14	Deer
	S31	Cats
	S32	Dogs
	S33	Other domestic mammals
Birds	S13	Poultry
	S16	Exotic Captive
	S17	Exotic Wild
	S18	Native Captive
	S20	Native Wild
	S21	Other birds
Aquatic animals	S23	Fish
	S23A	Cephalopods (reporting not mandatory)
	S23B	Crustaceans (reporting not mandatory)
Amphibians	S24	Amphibians
Reptiles	S27	Lizards
	S28	Snakes

Primates	S34	Marmosets
	S35	Macaques
	S36	Baboons
	S37	Other primates
Native mammals	S38	Macropods
	S39	Possums and gliders
	S40	Native rats and mice
	S41	Dasyurids
	S42	Wombats
	S43	Koalas
	S44A	Monotremes
	S44B	Bandicoots
	S44C	Bats
	S44D	Other native mammals
	S44E	Seals
	S44F	Whales and dolphins
Exotic feral mammals	S45	Camels
	S46	Cats
	S47	Cattle
	S48	Goats
	S49	Hares
	S50	Horses
	S51	Mice
	S52	Pigs
	S53	Rabbits
	S54	Rats
	S55A	Dingo/Wild Dogs
	S55B	Foxes
	S55C	Other exotic feral mammals
Exotic zoo animals	S56	Exotic zoo animals

	S29	Turtles and Tortoises			
	S30	Other reptiles			

Column F: FATE OF ANIMAL

This column **MUST** be completed where species S31 Domestic cats or S32 Domestic dogs have been used.

This column may also be completed where other species are used and it is recommended that this information be provided.

For each project, include additional lines where there are different fates of animals within the same project.

Fate Code	Description
F1	**Retained in project** This is where the project is ongoing and the animal will remain in the project in the next reporting year.
F2	**Retained for use in other projects or supplied to another establishment / individual for research** This is where the animal is kept by the establishment / individual for use in other research projects or supplied to another establishment / individual for use in research.
F3	**Retired from research and kept by the establishment / individual** This is where the animal is kept by the establishment / individual in retirement with no further plans for use in research.
F4	**Privately (non-research) owned and remained with owner** This is where the animal is privately owned and remains with the owner. *Examples:* • *Animal presented to veterinary clinic for treatment and participates in clinical trial* • *Behavioural study with privately owned companion animals*
F5	**Rehomed (as companion animal to private (non-research) home or rehoming organisation)** This is where the animal is rehomed as a companion animal to a private (non-research) home or to a rehoming organisation with the consent of the rehoming organisation.
F6	**Euthanased or died related to the project** This is where the animal is required to be euthanased as an integral part of the research project, or is euthanased or dies during the project as a consequence of the project procedures.

F7	**Euthanased or died unrelated to the project** This is where the animal is euthanased or dies during the project for reasons unrelated to the project. *Example:* • *Animal in long-term food palatability trial euthanased due to unmanageable osteoarthritis*
F8	**Euthanased because unsuitable to be rehomed** This is where the animal is no longer required for research and is euthanased on the basis of an assessment that the animal is unsuitable for rehoming. Reasons the animal is unsuitable for rehoming may include physical, behavioural and biosecurity factors. *Examples:* • *Animals with unmanageable health conditions causing discomfort or distress* • *Animals that have problem behaviours that are unable to be addressed through rehabilitation* • *Animals that could pose a biosecurity risk to other animals, people or the environment* • *Animals that are genetically modified*
F9	**Euthanased because unable to find a suitable home** This is where the animal is no longer required for research and is assessed as suitable for rehoming, but is euthanased because a suitable home is unable to be found.
F10	**Remain free living in the wild or released to the wild** This is where the animal is free living and remains in the wild (including where the animal is captured and released) and where the animal is released to the wild. *Examples:* • *Wildlife fauna surveys* • *Native animal captive breeding and monitored release programs*