Department of Primary Industries

NSW 2020 Animal Use in Research Statistics

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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 2020 report:

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

Purpose tables for 2020: 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 2020: 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 2020: 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 2020.

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*.

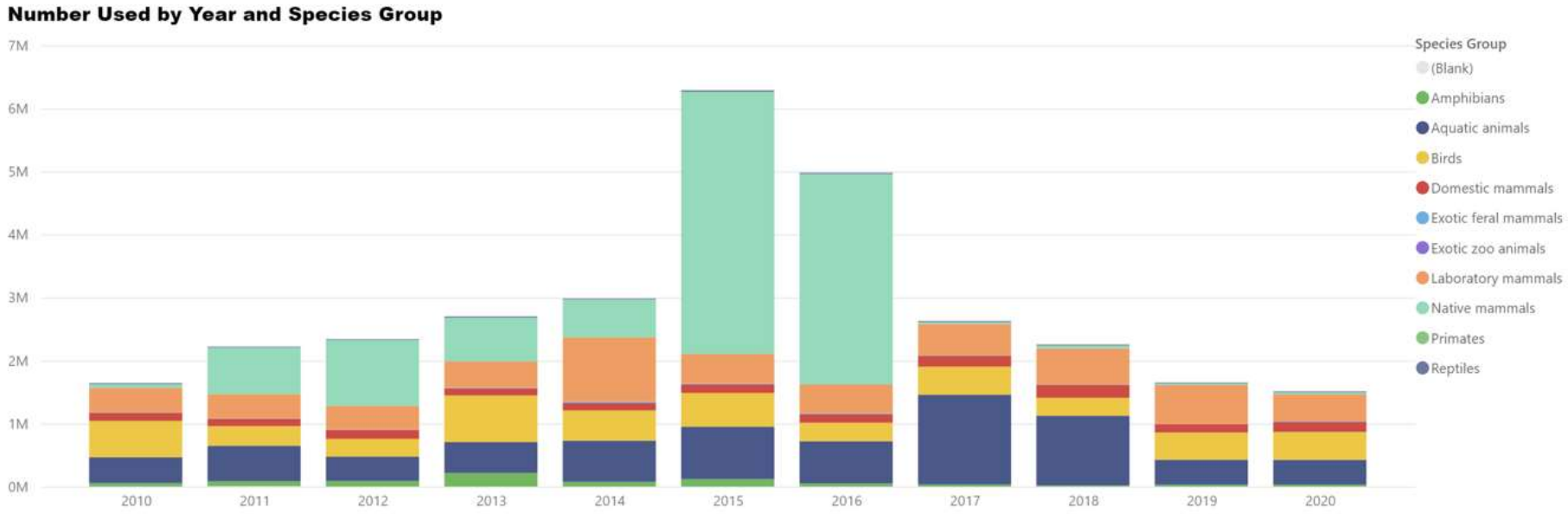
Reports are published each year at https://www.animalethics.org.au/animal-use-statistics

2. General Charts

2.1 Introduction to general charts

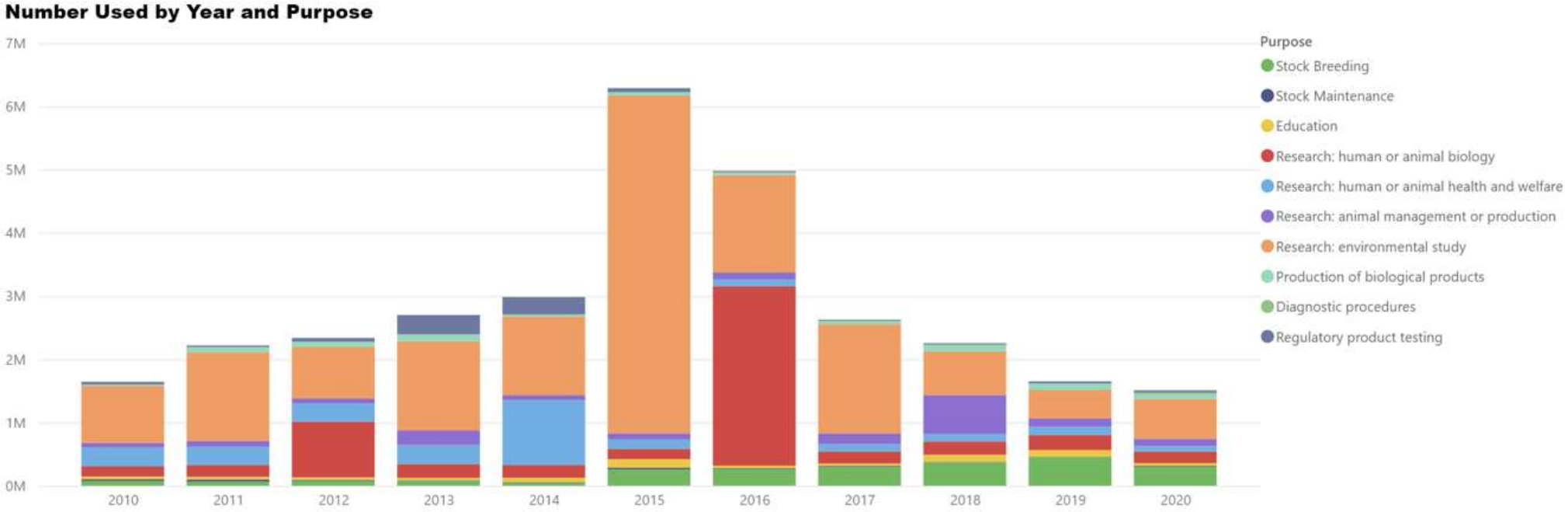
- Animals are counted for each year in which they are used. This means, for example, that animals in a project that runs for a period of more than one calendar year will be counted again for each year in which they are used. Therefore, a year to year comparison of animal numbers includes individual animals that may be the same across two or more years.
- 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



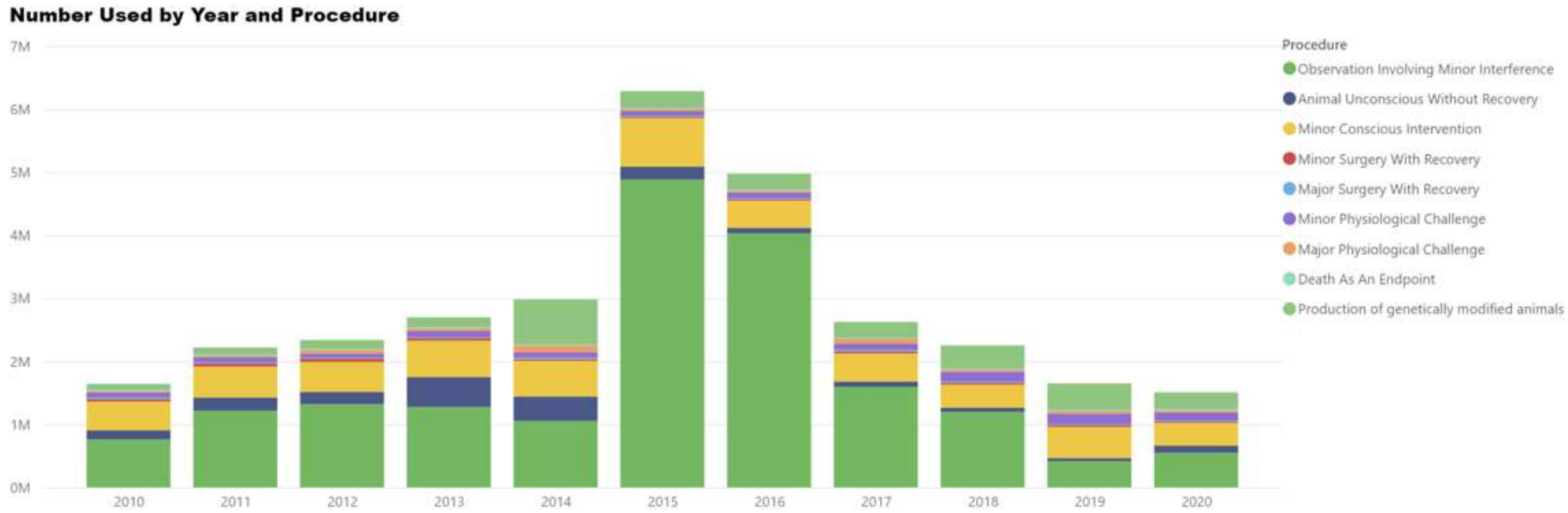
Species group	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	**Total**
	195	5,460										**5,655**
Amphibians	54,992	79,446	87,417	214,616	75,424	118,721	49,008	33,093	18,067	28,413	30,814	**790,011**
Aquatic animals	409,917	562,356	386,102	491,114	652,902	830,769	670,514	1,424,101	1,104,172	397,139	393,127	**7,322,213**
Birds	576,787	311,690	283,461	739,293	478,754	534,812	292,834	445,877	284,985	430,573	441,729	**4,820,795**
Domestic mammals	127,468	114,511	141,288	114,914	120,239	135,679	133,537	172,866	207,125	133,515	161,886	**1,563,028**
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	**101,243**
Exotic zoo animals	27	32	71	72	155	83	32	21	37	140	66	**736**
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	**5,623,265**
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	**10,774,958**
Primates	184	27	18	22	41	179	96	38	44	53	217	**919**
Reptiles	18,328	12,141	13,398	17,674	15,730	22,067	18,196	17,568	15,595	14,749	14,756	**180,202**
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**	**31,183,025**

2.3 Number of animals used over time by research purpose



Purpose	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	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	**2,292,875**
Stock Maintenance	27,165	33,850	15,448	7,890	10,500	26,508	13,684	13,204	7,266	5,875	9,899	**171,289**
Education	43,344	41,230	40,904	34,960	68,717	135,378	39,301	36,904	114,387	105,110	43,484	**703,719**
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	**5,477,138**
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	**2,899,268**
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	**1,788,766**
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	**16,157,018**
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	**731,626**
Diagnostic procedures	3,630	8,540	1,994	1,031	1,310	766	1,307	1,134	18,186	2,959	24,415	**65,272**
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	**896,054**
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**	**31,183,025**

2.4 Number of animals used over time by research procedure



Procedure	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	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	**18,309,712**
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	**2,000,295**
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	**5,448,716**
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	**344,052**
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	**227,150**
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	**1,116,420**
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	**496,323**
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	**173,521**
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	**3,066,836**
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**	**31,183,025**

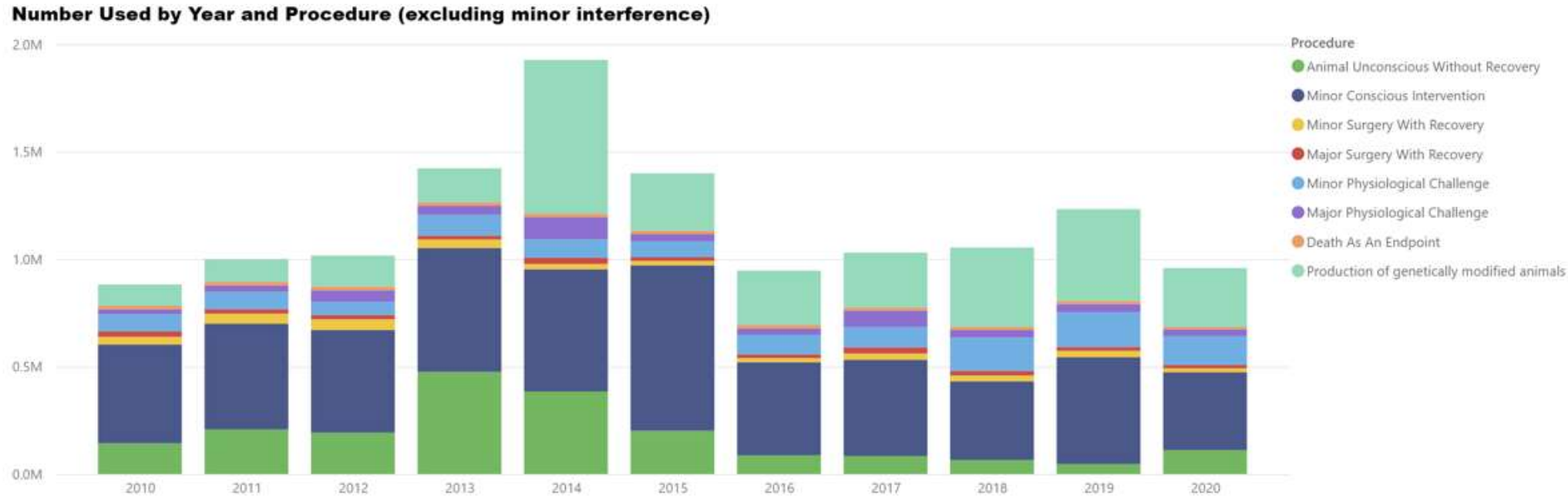
2.5 Number of animals used over time by research procedure excluding "Observation involving minor interference"

Information provided by research establishments each year includes the collection of data on animals used in the procedure category of "*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.

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



Procedure	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	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	**2,000,295**
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	**5,448,716**
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	**344,052**
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	**227,150**
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	**1,116,420**
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	**496,323**
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	**173,521**
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	**3,066,836**
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**	**12,873,313**

3. Purpose, Procedure and Species Charts 2020

3.1 Numbers of animals by Purpose and Procedure categories 2020

Purpose by Procedure 2020	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	27,879	423	14,826	1,128	3				260,395	304,654
Regulatory product testing	178	93	3,192			19,908		10,920		34,291
Stock Maintenance	3,632	156	3,005		115				2,991	9,899
Education	31,123	2,407	7,845	1,865	38	206				43,484
Research: human or animal biology	91,749	12,201	21,273	10,187	10,622	17,965	14,497		4,733	183,227
Research: human or animal health and welfare	18,759	4,540	27,015	2,056	5,347	10,750	10,063		5,741	84,271
Research: animal management or production	62,693	7,649	35,626	2,224	130	5,359	1,912			115,593
Research: environmental study	313,528	83,767	233,057	574	14	126		6		631,072
Production of biological products			325	78		76,987	96			77,486
Diagnostic procedures	156	315	16,090			3,185	4,669			24,415
Total	549,697	111,551	362,254	18,112	16,269	134,486	31,237	10,926	273,860	1,508,392

3.2 Number of animals and species used for Purpose: Stock Breeding 2020

Number of animals used for Purpose: Stock Breeding in 2020

	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										**760**
Amphibians	760									**760**
Aquatic animals										**18,302**
Fish	567								17,735	**18,302**
Domestic mammals										**2,756**
Cats	49									**49**
Dogs	46									**46**
Sheep	768		769	1,124						**2,661**
Laboratory mammals										**282,361**
Guinea Pigs	240									**240**
Mice	21,851	391	14,005	4	3				241,726	**277,980**
Rabbits	68									**68**
Rats	3,107	32							934	**4,073**
Native mammals										**352**
Native rats and mice			52							**52**
Whales and dolphins	300									**300**
Primates										**15**
Baboons	15									**15**
Reptiles										**108**
Turtles and Tortoises	108									**108**
Purpose Total	**27,879**	**423**	**14,826**	**1,128**	**3**				**260,395**	**304,654**

3.3 Number of animals and species used for Purpose: Stock Maintenance 2020

Number of animals used for Purpose: Stock Maintenance in 2020

	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										**4**
Fish	4									**4**
Birds										**768**
Native Wild	8		160							**168**
Poultry	600									**600**
Domestic mammals										**2,011**
Cattle			9							**9**
Dogs			83							**83**
Sheep	1,855		64							**1,919**
Laboratory mammals										**6,949**
Mice	889	150	2,689		115				2,991	**6,834**
Rats	109	6								**115**
Primates										**167**
Baboons	167									**167**
Purpose Total	**3,632**	**156**	**3,005**		**115**				**2,991**	**9,899**

3.4 Number of animals and species used for Purpose: Education 2020

Number of animals used for Purpose: Education in 2020

	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										**395**
Amphibians	60	335								**395**
Aquatic animals										**1,261**
Crustaceans (reporting not mandatory)	15									**15**
Fish	429	524	1	292						**1,246**
Birds										**24,968**
Exotic Captive	5		13							**18**
Exotic Wild	25									**25**
Native Captive	119									**119**
Native Wild	7,813									**7,813**
Other birds	15,915									**15,915**
Poultry	273	517	288							**1,078**
Domestic mammals										**10,566**
Cats	110		18							**128**
Cattle	370	1	1,044	95		188				**1,698**
Dogs	135		416	8						**559**
Goats	12	4	2							**18**
Horses	117	1	212	202						**532**
Other domestic mammals	13		8							**21**
Pigs	39	54	182							**275**
Sheep	1,921	88	4,780	490	38	18				**7,335**
Exotic feral mammals										**70**
Cats	12									**12**
Dingo/Wild Dogs	6									**6**
Foxes	17									**17**
Mice	28		7							**35**
Exotic zoo animals										**52**
Exotic zoo animals	52									**52**

	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
Laboratory mammals										**2,576**
Ferrets	15									**15**
Guinea Pigs	52	11	5							**68**
Mice	97	606	521	752						**1,976**
Rabbits	41		10							**51**
Rats	151	266	43	6						**466**
Native mammals										**3,074**
Bandicoots	14									**14**
Bats	40									**40**
Dasyurids	1,244		41							**1,285**
Koalas	3									**3**
Macropods	124									**124**
Monotremes	27									**27**
Native rats and mice	74									**74**
Possums and gliders	1,356		1							**1,357**
Whales and dolphins	150									**150**
Reptiles										**522**
Lizards	93		103	20						**216**
Other reptiles	20		2							**22**
Snakes	103		148							**251**
Turtles and Tortoises	33									**33**
Purpose Total	**31,123**	**2,407**	**7,845**	**1,865**	**38**	**206**				**43,484**

3.5 Number of animals and species used for Purpose: Research: Human or Animal Biology 2020

Number of animals used for Purpose: Research: human or animal biology in 2020

	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										**4,517**
Amphibians	3,178		1,101		96	4	138			**4,517**
Aquatic animals										**10,612**
Cephalopods (reporting not mandatory)	20									**20**
Fish	4,092	196	604	105		939	300		4,356	**10,592**
Birds										**73,772**
Exotic Captive			2							**2**
Exotic Wild	4,033	5	620							**4,658**
Native Captive	897	36	910			9				**1,852**
Native Wild	64,978		741			24				**65,743**
Poultry	19	60	1,438							**1,517**
Domestic mammals										**672**
Cats	37									**37**
Cattle	2		32							**34**
Dogs	34		176			4				**214**
Goats	30									**30**
Horses	11		19							**30**
Other domestic mammals		19								**19**
Pigs					13					**13**
Sheep		1	71		149	74				**295**
Exotic feral mammals										**60**
Dingo/Wild Dogs	4									**4**
Foxes	1									**1**
Mice	31		8							**39**
Other exotic feral mammals	2									**2**
Rats			14							**14**
Exotic zoo animals										**2**
Exotic zoo animals			2							**2**

	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
Laboratory mammals										**88,192**
Guinea Pigs		53	5			56				**114**
Mice	6,191	10,323	11,659	7,278	8,317	15,630	13,825		377	**73,600**
Rabbits		9		12	422					**443**
Rats	5,762	1,499	1,109	2,701	1,623	1,107	234			**14,035**
Native mammals										**1,568**
Bats	805		7							**812**
Dasyurids	16									**16**
Koalas			2	82						**84**
Macropods	67									**67**
Monotremes			6	5						**11**
Possums and gliders	155					7				**162**
Whales and dolphins	416									**416**
Primates										**3**
Baboons					2					**2**
Other primates				1						**1**
Reptiles										**3,829**
Lizards	804		1,361	3		111				**2,279**
Other reptiles			20							**20**
Snakes	31		50							**81**
Turtles and Tortoises	133		1,316							**1,449**
Purpose Total	**91,749**	**12,201**	**21,273**	**10,187**	**10,622**	**17,965**	**14,497**		**4,733**	**183,227**

3.6 Number of animals and species used for Purpose: Research: Human or Animal Health and Welfare 2020

Number of animals used for Purpose: Research: human or animal health and welfare in 2020

	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										**51**
Amphibians	18				33					**51**
Aquatic animals										**7,941**
Fish		1,314	1,266			1,022			4,339	**7,941**
Birds										**9,450**
Exotic Wild			9				8			**17**
Native Captive			6	7						**13**
Native Wild			30							**30**
Poultry	4,501	856	1,881	210		40	1,902			**9,390**
Domestic mammals										**35,676**
Cats	170		332		17					**519**
Cattle	896		1,733			1,815				**4,444**
Dogs	374		431	107	223	31				**1,166**
Horses	50		429	21						**500**
Pigs	5,600	3	14,593		86	21	7			**20,310**
Sheep	6,803	17	835		475	607				**8,737**
Exotic feral mammals										**332**
Dingo/Wild Dogs			12							**12**
Pigs	208									**208**
Rats				94			18			**112**
Exotic zoo animals										**7**
Exotic zoo animals			7							**7**

	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
Laboratory mammals										**30,188**
Guinea Pigs			150	56		32				**238**
Mice	70	2,231	4,674	1,258	2,491	7,067	7,659		1,402	**26,852**
Rabbits				16			186			**202**
Rats	2	119	362	108	2,019	115	171			**2,896**
Native mammals										**315**
Bandicoots			24	63						**87**
Bats			1							**1**
Dasyurids			10	17						**27**
Koalas			26	7						**33**
Macropods			38	1			18			**57**
Native rats and mice			49	17						**66**
Other native mammals	10									**10**
Possums and gliders			16	18						**34**
Primates										**32**
Baboons			25	4	3					**32**
Reptiles										**279**
Lizards	52		28	52			21			**153**
Snakes							2			**2**
Turtles and Tortoises	5		48				71			**124**
Purpose Total	**18,759**	**4,540**	**27,015**	**2,056**	**5,347**	**10,750**	**10,063**		**5,741**	**84,271**

3.7 Number of animals and species used for Purpose: Research: Animal Management or Production 2020

Number of animals used for Purpose: Research: animal management or production in 2020

	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										**2,919**
Fish	1,330	780	720	89						**2,919**
Birds										**19,573**
Native Captive	32		53							**85**
Native Wild		47								**47**
Poultry	8,613	6,818	874			1,232	1,904			**19,441**
Domestic mammals										**90,331**
Cats	36									**36**
Cattle	2,957		2,925	60		59				**6,001**
Dogs	60		53			30				**143**
Goats	1,325		80	50		588				**2,043**
Horses						27				**27**
Other domestic mammals	24		48							**72**
Pigs	36,072		1,728			31				**37,831**
Sheep	10,026	2	28,733	2,025		3,392				**44,178**
Exotic feral mammals										**2,218**
Mice	34									**34**
Rats	2,184									**2,184**
Exotic zoo animals										**5**
Exotic zoo animals			5							**5**
Laboratory mammals										**132**
Guinea Pigs		2								**2**
Mice					130					**130**
Native mammals										**81**
Dasyurids			1							**1**
Macropods			76				4			**80**
Reptiles										**334**
Lizards			330				4			**334**
Purpose Total	**62,693**	**7,649**	**35,626**	**2,224**	**130**	**5,359**	**1,912**			**115,593**

3.8 Numbers of animals and species used for Purpose: Research: Environmental Study 2020

Number of animals used for Purpose: Research: environmental study in 2020

	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										**25,091**
Amphibians	11,216	74	13,801							**25,091**
Aquatic animals										**344,929**
Crustaceans (reporting not mandatory)	85		406							**491**
Fish	68,322	83,414	192,047	515	14	126				**344,438**
Birds										**216,649**
Exotic Wild	257		6,428							**6,685**
Native Captive	72									**72**
Native Wild	207,276		2,493							**209,769**
Other birds	28		95							**123**
Domestic mammals										**1,260**
Cattle	50									**50**
Deer	1,040	18	34							**1,092**
Dogs	14		1							**15**
Goats			65							**65**
Sheep	38									**38**
Exotic feral mammals										**7,783**
Cats	56		44							**100**
Cattle	3									**3**
Dingo/Wild Dogs	179		30							**209**
Foxes	2,110		28							**2,138**
Goats	1,125									**1,125**
Hares	189									**189**
Horses	86									**86**
Mice	1,395		1,000							**2,395**
Other exotic feral mammals	47		8							**55**
Pigs	139		69							**208**
Rabbits	936	60						6		**1,002**
Rats			273							**273**

	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
Laboratory mammals										**47**
Mice	15	32								**47**
Native mammals										**25,774**
Bandicoots	61		261							**322**
Bats	2,694		4,084							**6,778**
Dasyurids	648		1,413							**2,061**
Koalas	548		65							**613**
Macropods	7,319	160	275	44						**7,798**
Monotremes	87		40							**127**
Native rats and mice	235		1,720							**1,955**
Other native mammals	2,304		290							**2,594**
Possums and gliders	1,967		1,206	15						**3,188**
Whales and dolphins	20									**20**
Wombats	316		2							**318**
Reptiles										**9,539**
Lizards	2,231	9	3,171							**5,411**
Other reptiles	3									**3**
Snakes	171		184							**355**
Turtles and Tortoises	246		3,524							**3,770**
Purpose Total	**313,528**	**83,767**	**233,057**	**574**	**14**	**126**		**6**		**631,072**

3.9 Number of animals and species used for Purpose: Production of Biological Products 2020

Number of animals used for Purpose: Production of biological products in 2020

	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										**76,853**
Poultry			42			76,811				**76,853**
Domestic mammals										**322**
Cats				8		30				**38**
Cattle						11				**11**
Dogs			96			95				**191**
Horses			14							**14**
Other domestic mammals			2							**2**
Sheep			26			40				**66**
Laboratory mammals										**166**
Rabbits							96			**96**
Rats				70						**70**
Reptiles										**145**
Snakes			145							**145**
Purpose Total			**325**	**78**		**76,987**	**96**			**77,486**

3.10 Number of animals and species used for Purpose: Diagnostic Procedures 2020

Number of animals used for Purpose: Diagnostic procedures in 2020

	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										**7,159**
Fish						3,159	4,000			**7,159**
Birds										**689**
Native Captive	24									**24**
Poultry							665			**665**
Domestic mammals										**16,145**
Horses	55		3							**58**
Sheep			16,087							**16,087**
Laboratory mammals										**422**
Mice	57	295					4			**356**
Rabbits						26				**26**
Rats	20	20								**40**
Purpose Total	**156**	**315**	**16,090**			**3,185**	**4,669**			**24,415**

3.11 Number of animals and species used for Purpose: Regulatory Product Testing 2020

Number of animals used for Purpose: Regulatory product testing in 2020

	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										**19,007**
Poultry						19,007				**19,007**
Domestic mammals										**2,147**
Cats			51			26				**77**
Cattle	74	33	92			159				**358**
Dogs			116			20				**136**
Horses			38			44				**82**
Pigs		2	2							**4**
Sheep	104	58	676			652				**1,490**
Laboratory mammals										**13,137**
Guinea Pigs			700					569		**1,269**
Mice			437					10,351		**10,788**
Rabbits			712							**712**
Rats			368							**368**
Purpose Total	**178**	**93**	**3,192**			**19,908**		**10,920**		**34,291**

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.

In 2020 for domestic dogs and cats, data reported shows:

Domestic dogs:

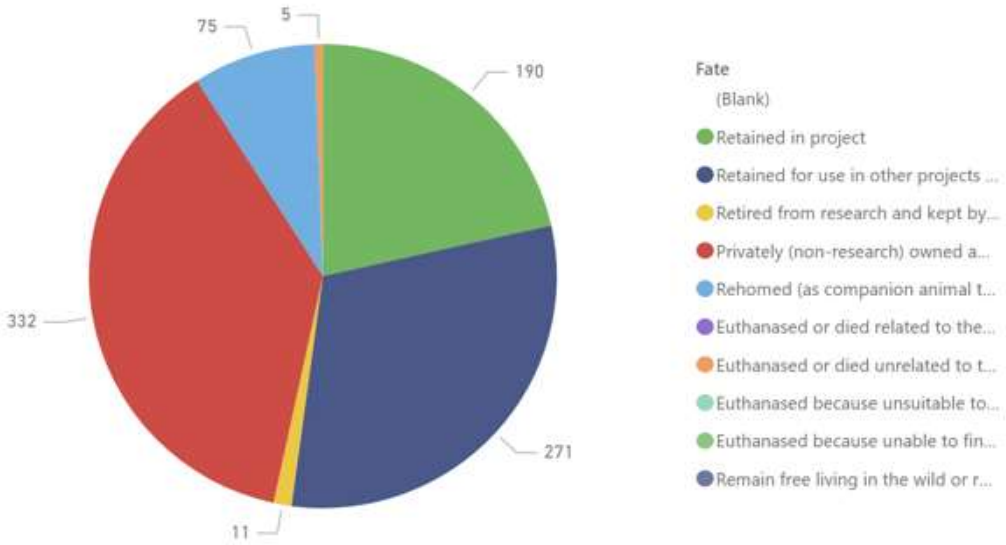
- 1,575 (62%) were privately (non-research) owned and remained with their owners. Examples of this type of research are:
    - *Animal presented to veterinary clinic for treatment and participates in clinical trial*
    - *Behavioural study with privately owned companion animals*
- 957 (37%) were retained for use in research (and will go on to be counted in each year in which they are used).
- 12 were retired from research and kept by the establishment/individual.
- 9 were euthanased or died unrelated to the project.
- No domestic dogs were euthanased because they were unable to be rehomed.

Domestic cats:

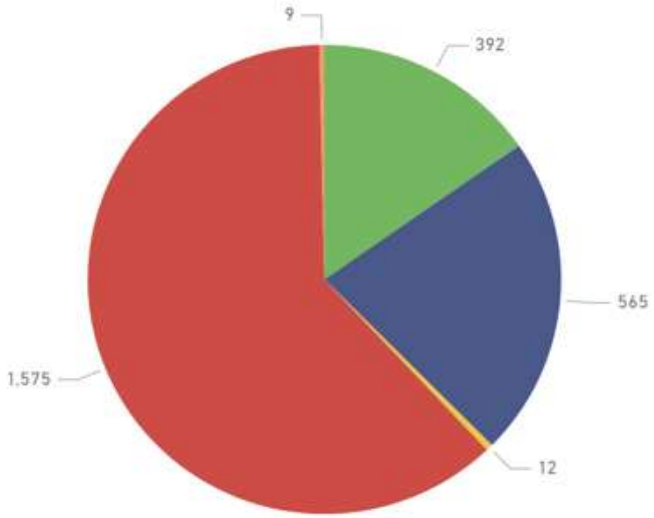
- 332 (38%) were privately (non-research) owned and remained with their owners. Examples of this type of research are:
    - *Animal presented to veterinary clinic for treatment and participates in clinical trial*
    - *Behavioural study with privately owned companion animals*
- 461 (52%) were retained for use in research (and will go on to be counted in each year in which they are used).
- 75 were rehomed and an additional 11 were retired from research and kept by the establishment/individual.
- 5 were euthanased or died unrelated to the project.
- No domestic cats were euthanased because they were unable to be rehomed.

4.1 Number of domestic cats and domestic dogs used by category: Fate of animals 2020

Number Domestic Cats Used by Fate 2020

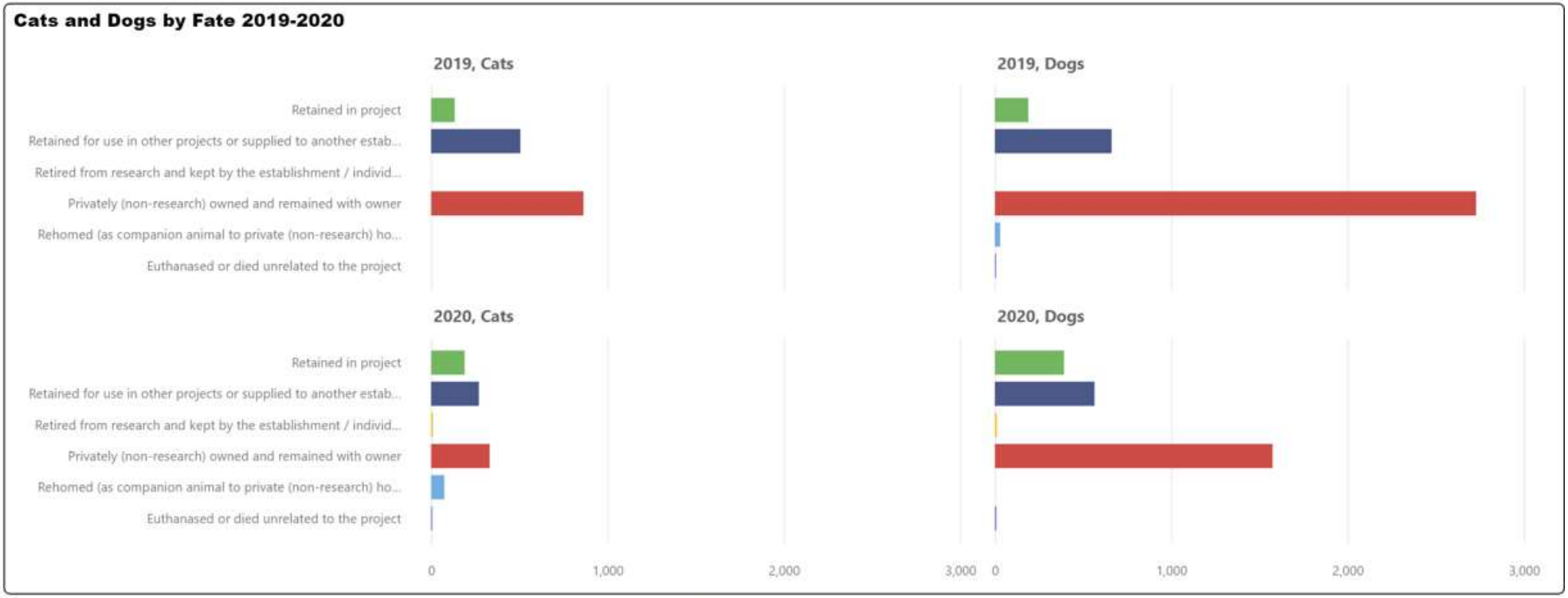


Number Domestic Dogs Used by Fate 2020



Fate	Cats	Dogs
Retained in project	190	392
Retained for use in other projects or supplied to another establishment / individual for research	271	565
Retired from research and kept by the establishment / individual	11	12
Privately (non-research) owned and remained with owner	332	1,575
Rehomed (as companion animal to private (non-research) home or rehoming organisation)	75	
Euthanased or died related to the project		
Euthanased or died unrelated to the project	5	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	**884**	**2,553**

4.2 Fate of domestic cats and domestic dogs 2019 – 2020



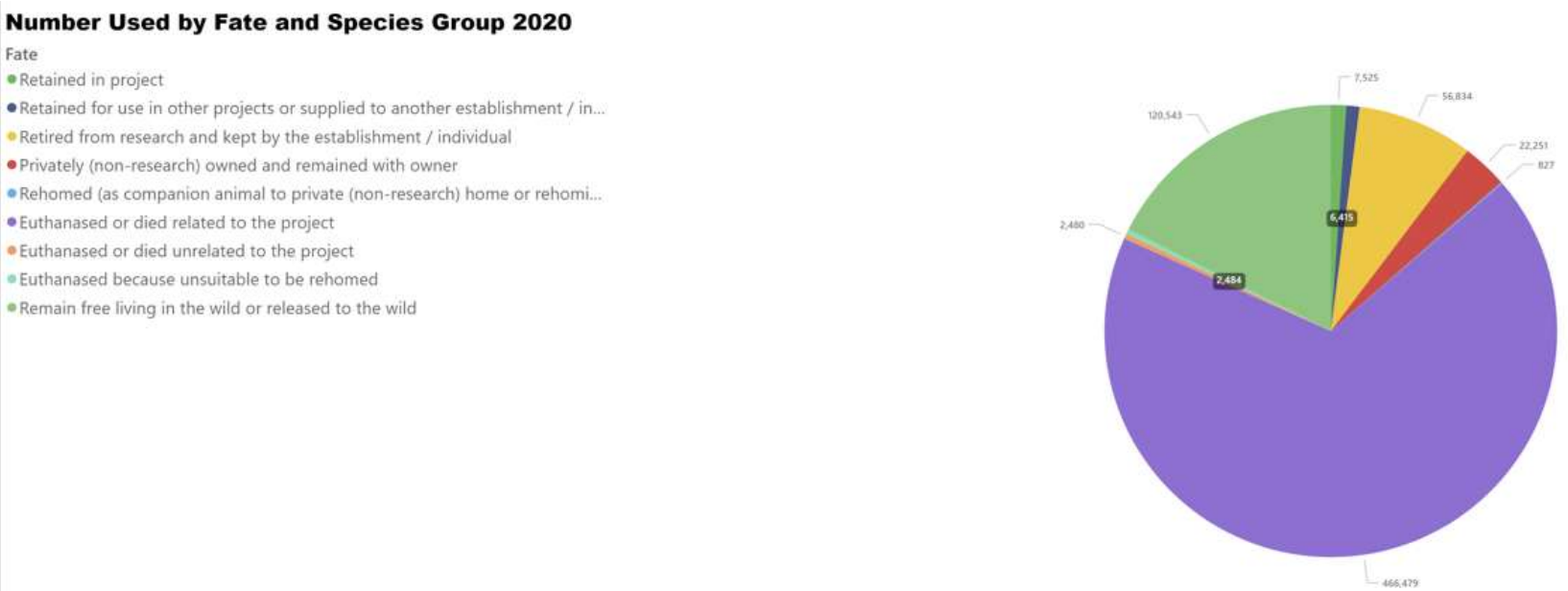
Fate of Cats	2019	2020
Retained in project	134	190
Retained for use in other projects or supplied to another establishment / individual for research	506	271
Retired from research and kept by the establishment / individual		11
Privately (non-research) owned and remained with owner	864	332
Rehomed (as companion animal to private (non-research) home or rehoming organisation)		75
Euthanased or died related to the project		
Euthanased or died unrelated to the project		5
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		

Fate of Dogs	2019	2020
Retained in project	190	392
Retained for use in other projects or supplied to another establishment / individual for research	662	565
Retired from research and kept by the establishment / individual		12
Privately (non-research) owned and remained with owner	2,729	1,575
Rehomed (as companion animal to private (non-research) home or rehoming organisation)	30	
Euthanased or died related to the project		
Euthanased or died unrelated to the project	5	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		

4.3 Number of species groups used by Category: Fate of animals 2020

(Voluntary reporting for species other than domestic cats and dogs)

Number Used by Fate and Species Group 2020



Fate by Species Group 2020	Amphibian	Aquatic	Birds	Domestic	Exotic feral	Exotic zoo	Laboratory	Native	Primate	Reptile	Total
Retained in project	884	248	181	5,345	2		182	359		324	**7,525**
Retained for use in other projects or supplied to another establishment / individual for research	7	18	53	4,537			1,729			71	**6,415**
Retired from research and kept by the establishment / individual			7	56,810		12		5			**56,834**
Privately (non-research) owned and remained with owner			763	21,221	127	54	1	22	1	62	**22,251**
Rehomed (as companion animal to private (non-research) home or rehoming organisation)		2	69	615			28	2		111	**827**
Euthanased or died related to the project	279	95,156	113,365	3,077	48		254,311	75	2	166	**466,479**
Euthanased or died unrelated to the project	78	214	65	86	14		1,854	169			**2,480**
Euthanased because unsuitable to be rehomed		217	2,208				58	1			**2,484**
Remain free living in the wild or released to the wild	10,270	36,672	38,603	3,909	7,858			16,725		6,506	**120,543**
Total	**11,518**	**132,527**	**155,314**	**95,600**	**8,049**	**66**	**258,163**	**17,358**	**3**	**7,240**	**685,838**

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 2020.

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
Guinea Pigs	569	38	63	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.
Mice	2,918	1,672	408	Serum neutralisation test in mice: Susceptible animals are challenged with test toxin/antibody dilutions to determine antibody titre.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Testing of stability batches and new product formulations.	An in-vitro project is currently in place which has progressed significantly with ELISAs for finished product potency testing of, C. septicum, C. perfringens Type D and C. tetani having been developed transferred into the QC Laboratories for routine testing during the second half of 2020.

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
						The remaining replacement assays for C. novyi Type B; C. botulinum Type C and C. botulinum Type D are currently under development with the objective of implementation during 2021/22.
Mice	2,730	792	152	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 assays subject to requlatory approval of these replacement assays.
Mice	2,720	561	167	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

Species	Number used	Number died (not euthanased)	Number euthanased as early endpoint	Procedure	Justification	Alternatives
						vitro assays which may be used to replace existing in vivo assays subject to requlatorv approval of these replacement assays.
Feral Deer	Individual animals cannot be accurately identified at camera trap sites therefore the number of camera events are recorded. For the relevant reporting period camera events recorded per species were: Fallow deer (523) Red deer (8) Sambar deer (125).	3	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 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	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
					cost-efficient method as an additional technique for controlling overabundant herbivore pest species.	
Feral Goats	Individual animals cannot be accurately identified at camera trap sites therefore the number of camera events are recorded. For the relevant reporting period 328 feral goat camera events were recorded.)	2	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 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-	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
					efficient method as an additional technique for controlling overabundant herbivore pest species.	

6. General examples of methods used to implement the 3Rs in 2020

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 2020 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 in this section.

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 were reported by accredited animal research establishments for the 2020 reporting year. Additional specific examples of replacement methods reported in 2020 are contained in Appendix A.

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.

In-vitro technology

5. Increased availability of in-vitro technology such the use of established cell lines.
6. Researchers moving away from primary cultures and using stem cell differentiation.
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 plant tissue as a replacement for animal tissue for certain enzymatic assays.

Mathematical / computer modelling

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

11. Increasingly over recent years, including 2020, some projects have allowed for replacement of animals due to advances in statistical modelling.

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 were reported by accredited animal research establishments for the 2020 reporting year. Additional specific examples of reduction methods reported in 2020 are contained in Appendix A.

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.

Review of existing information

3. 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.
4. We relied heavily on the existing literature to ensure that we are not repeating any studies for which the outcome is known, and rather here are focussed on obtaining only that new information that is of specific interest and value to the conservation management of threatened species.
5. 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.

Statistical / study design

6. 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.
7. 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.
8. Obtaining more data from the use of fewer animals by combining objectives.
9. The use of both sexes to minimise animal wastage and ensure that results had the widest applicability.

10. The use of a 'within-study' design allowed one animal to be used for multiple testing parameters which reduced the total number of animals required.

Shared animals / tissue

11. 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.
12. Shared control groups between studies are used when possible.
13. Multiple clinical parameters may be obtained from single groups of animals rather than parallel groups of animals.
14. 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".
15. Sharing of data and resources (animals, tissues and equipment) between research groups.
16. Tissue samples are collected and stored for future use or use by other researchers.
17. An increasing number of projects are using tissue samples only (often collected during routine health inspections of animals).
18. 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.
19. Routine husbandry procedures to be performed on animals are coordinated with teaching activities.
20. Re-use of animals, where appropriate, after extended recovery interval.
21. Training animals used are only those identified for culling or use of dead animals to gain experience in new technical procedures as practicable.
22. Opportunities to combine experimental protocols is encouraged where this means no or minimal additional procedural load on animals.
23. 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.
24. 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.
25. Mock surgery performed on animal tissue for training purposes or refinement of techniques.

Breeding

26. 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.

27. Breeding programs are designed and maintained to produce stock to order, to reduce numbers and overproduction.
28. Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.
29. 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 were reported by accredited animal research establishments for the 2020 reporting year. Additional specific examples of refinement methods reported in 2020 are contained in Appendix A.

Environmental enrichment / housing

1. Provision of enrichment options to all sponsors during planning phase of all studies to ensure cats and dogs can continue to get as much enrichment during study periods as allowed within the constraints of study requirements.
2. 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.
3. A rich supply of cost-free environmental enrichment has been sourced for our animals, allowing us to divert resources for further training in refinement of techniques in line with best practice in the industry. We have also been able to supply two external facilities with free enrichment.

Anaesthesia / analgesia

4. Suitable pain relief is always used for any intensive or surgical procedure.
5. 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.

Education, veterinary input, and monitoring of research

6. The AEC has continued to educate researchers on the 3Rs, and in particular, methods to minimise potential pain and distress and procedures to enhance animal welfare. The application forms (revised late 2019) provide researchers with greater opportunity to outline methods that they implementing to minimise the impact on animals.
7. Launch of revised Animal Ethics Online training.
8. Introduced a system of monitoring for remote studies.
9. The Animal Welfare and Ethics Coordinator served as a fulltime Animal Welfare Officer resulting in increased monitoring of projects and the launch of an education series involving presentations on the 3Rs for AEC members.
10. Development of Animal Ethics guidance and the implementation of the 3Rs to align with the NHMRC Information paper: 'The implementation of the 3Rs in Australia, 2019'.
11. A formal program of project monitoring conducted by the establishment.
12. An Experimental Technique Procedure template was developed 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.
13. The Animal Care team continued training of researchers in current best practice techniques.
14. Direct access to experienced staff in Animal Ethics and Welfare and two full time veterinarians.

Wildlife studies

15. The use of non-capture methods for wildlife research and teaching including the use of field surveys, scat surveys, camera traps and drones.
16. The use of less invasive procedures in wildlife studies e.g. sand pads rather than trapping.
17. Use of an Observational Only - Field Research Form (No Trapping, Handling or Spotlighting).

Rehoming

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

7. Appendix A – Specific examples of methods used to implement the 3Rs in 2020

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 2020 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

Simulation

1. Use of cadavers and dummies for training, where applicable became important during tail vein injection training. Researchers were first trained on artificial rat tails developed by the Animal Welfare Officer as well as a commercial model tail before practising on live animals. The artificial rat tails are considerably cheaper than commercial tails and therefore offer scope for replacement of animals in the early stages of training.
2. Large photo cards have been used in practical classes instead of live animals to teach students how to identify freshwater fish species.
3. This year the class was replaced with a Zoom tutorial and no animals were used.
4. Teaching procedures:
    - Mannequins, audio-visual materials, photographs, taxidermized and preserved specimens were used as substitutes for live animals.
    - Use of instructional aids for general identification and research of native animals.
    - Use of mechanical horse.
    - Bandaging and health care procedures are performed on dummies.
    - Life size fibreglass replica of horse used for demonstrations.
    - Use of photos and wool samples to identify breeds of sheep.
    - Use industry sites where animals are normally kept.
    - Visit to zoos, aquariums and museums to familiarise students with a range of native animals, eliminating the need for field visits or trapping.
    - Use of horse legs from knackery for hoof trimming and basic shoeing.
    - 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 case study data to replace need for capturing live animal data.
- Use of identification tags without live sheep for simulation.

5. Application form updates, reported in the 2019 report, have greatly improved the AEC's ability to assess alternatives that have been considered and or/adopted. Where possible, use of animals in teaching has been replaced with computer modelling, sharing of tissues and in-vitro models. For example, one particular teaching unit removed the use of live animals entirely. Sheep were replaced with previously collected urine samples, cane toads were replaced with computer simulations and live poultry were replaced with poultry carcasses from previous research.
6. Artificial animal models such as the Koken/Curvet rat and knitted mouse models were used for training purposes, replacing live animals where possible.
7. 2020 saw the development and roll-out of the new comprehensive online Animal Care and Ethics Course, replacing the previous introductory hands-on module and no longer using live animals for this purpose.

In-vitro technology

8. Researchers developed in-vitro culture technique for Chlamydia, replacing embryonated eggs.
9. Generation of cell lines from turtle tissues to examine the effect of contaminants, to replace the requirement to capture animals for tissues.
10. The team have identified the potential development of a world's first organotypic human 3D skin model to study GAS-host interactions. This model involves the use of human cell lines to establish human 3D skin and have immune cells incorporated in order to closely mimic GAS-infected skin in a clinical setting.
11. Development of a high-throughput screening cell culture-based assay to find new drugs that deplete proliferating immune cells that mediate Graft versus host disease. This would reduce the need to use mice for drug screening.
12. The use of in-vitro kits to study the metabolism of novel radiotracers continued to be implemented. The kits provide an alternative to characterising metabolic fate of drugs in live animals. Furthermore, numerous studies have been carried out using cell-based models of disease.
13. Established mouse cell line culture techniques were used for the assessment of chemically modified gonocyte specific phage clone ability to induce cell death. This replaced the need to utilise cultured primary cells isolated from animals.

14. The use of an air-liquid interface culture of human bronchial epithelial cells to produce a differentiated epithelium allowed multiple mechanisms of viral infection and inflammation to be studied without requiring an intact immune system in a whole animal.
15. Donated human spermatozoa allowed human cells to be used as a live animal replacement model.
16. An in-vitro system was used to demonstrate and compare the effects of factors identified in mouse models, replacing the need to perform additional experiments using mice.
17. Cell culture models replicated what was happening in a mouse to show how obesity and hormones interact.
18. Mice were substituted with in-vitro models of infection in airway epithelial cells.
19. In-silico modelling was used to refine a hypothesis to test the impact of gene modification, which was then confirmed using cells in culture.
20. Treatments were examined in cell culture to identify their efficacy in altering responses to infection.
21. Experiments have been done in culture wherever possible and applicable with a collaborator by infecting human monocyte-derived macrophages, as well as mouse bone marrow-derived macrophages with Influenza A virus as well as other viruses such as Zika and West Nile virus.
22. We are in the process of establishing novel models using human donor tissue that would reduce the number of rats we may need in future studies
23. A novel methodology was pioneered that enabled the quantification of miRNA in samples from human infants, which allowed for some investigations to be performed in a clinical setting rather than an animal model.
24. In-vitro techniques to test new drugs and ascertain safety and efficacy data ensured that only drugs showing potential proceeded to live animal experiments, reducing potentially non-efficacious drugs being tested on animals.
25. The ELISA for Clostridium chauvoei was approved by the APVMA in May 2020. This means that vaccinated and control guinea pigs will no longer be subjected to lethal challenge. The test was implemented in Q4 2020 saving a total of 76 guinea pigs in the last 2 months of 2020. This new test should save approximately 700 guinea pigs per year in the future.
26. ELISA for Clostridium perfringens type D, C. septicum and C. tetani were approved, which replace the mice serum neutralisation test for these antigens. The ELISAs were implemented in Q4 2020 saving 360 mice in the last months of 2020. These 3 new assays will save approximately 2,000 mice annually.
27. Work has continued in 2020 to develop an ELISA test to replace the Serum Neutralisation mice test for C. novyi.

Mathematical / computer modelling

28. Unfortunately, to answer the questions posed in this project alternative techniques (such as cell culture) are not possible. However, data collected can be used to produce subsequent mathematical models that have the potential for replacement of animals.

7.2 Reduction – specific examples

Pilot studies

1. To ensure the minimum number of animals are used to obtain statistically valid data, several pilot studies were approved in 2020, including for efficacy and tolerability of new drugs and optimisation of new experimental models. One such study aimed to optimise a single animal model to assess the different stages of development of liver disease, enabling several research questions to be addressed in a single model. Related to this, conditions of approval to submit reports on the results of the pilot studies were applied to several projects to provide oversight by the AECs, ensuring animal welfare and justification for use of further animals in these studies.
2. A pilot study to determine the effects on blood flow prior to measuring infarct volume changes allowed researchers to conclude an activity early and avoided the use of additional animals.

Shared animals / tissue / data

3. The Committee continues to maintain a Biological Non-Human Tissue Database and in December 2018 a new database was created. This database allows researchers to share excess tissue, thus replacing the use of live animals with the use of stored tissue. In addition, to make these tissues more widely available, the Committee has joined the Ethitex tissue sharing database which facilitates tissue sharing throughout Australia.
4. Frozen cadavers were used for training in more invasive procedures. For example, investigators used frozen cadavers for training in stereotaxic surgery, prior to progressing to live animal surgery.
5. Collecting DNA from discarded feathers rather than capturing and directly sampling birds.
6. In 2020, 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 animals for faecal swabs.
7. The projects are conducted within a commercial piggery setting on existing production animals, rather than being purpose bred for research.
8. Blood samples are used from clinical cases that have been cleared of being infectious.

9. Maintenance of a mailing list facilitating tissue sharing among researchers, including researchers from other institutions requesting tissue from the establishment. In 2020, a total of 2,749 animals were used for tissue sharing, which consisted of rats (n=2,626), mice (n=117), rabbits (n=4) and sheep (n=2).
10. 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 2020, a total of 827 animals were transferred between projects, including: mice (n=397), rabbits (n=300), rats (n=107) and sheep (n=23). 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, hands-on training sourced all required animals via transfer from other projects.
11. The re-use of animals when it is strongly justified and the wellbeing of the animal(s) is not compromised. For example, Atlantic salmon were transferred from one AEC 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 AEC application that were to be humanely killed at the end of the experiment, were instead administered a terminal anaesthesia in order to practice a procedure under another approved protocol.
12. We design studies to optimize control groups and to allow for harvesting of multiple tissues (eg pancreatic islets, peritoneal macrophages, adipose tissues). In particular, by freezing peritoneal exudates for later analyses, we can now use the same mice for islet isolation. There is the possibility for tissue sharing with other researchers.
13. Diabetic retinopathy is a consequence of type II diabetes. After our mice have undergone hindlimb ischemia and are ready for collection at the desired timepoints, we will also collect the retinas from these mice. This will eliminate the need for extra mice to specifically study diabetic retinopathy.
14. Cells and tissues will be isolated from excess mice as a result of our breeding.
15. To reduce the size or our breeding colony, we will begin to cryopreserve strains that are not currently required for our work.
16. Wherever possible, mice used as studs and embryo donors for cryopreservation are to be sourced from stocks indicated as unusable by researchers. Although less efficient in terms of production of embryo number from females (older females don't produce high numbers of embryos), and optimal collection of mice (numbers of embryos with the desired genotype may accumulate more slowly), this avoids the need for extra breeding for this activity and gives a purpose to mice that have been unavoidably produced in breeding schemes, but aren't useful for experimental procedures.
17. Stud males can be used for several purposes, such as timed matings for generating timed embryo stages, or for continuing the breeding for a line, as well as used for rederivation or cryopreservation purposes. This ensures the males have as much contact with females as possible and reduces the total number required.

18. When a genetic modified mouse line can be cryopreserved with sperm, (it is not always possible because it preserves only one half of the genome), it will reduce the number of mice needed. Sperm cryopreservation requires only 2-5 males to archive the line.
19. Incorporating animals from one project as breeding stock or experimental animals for a subsequent project, rather than euthanasing.
20. Animals used in more than one research trial with ongoing monitoring to avoid unnecessary stress on the animals. Blood collection from sheep for diagnostic laboratory tests and for laboratory bioassays.
21. Researchers are required to describe what will happen to any excess birds, which may occur when hatched chick numbers are higher than expected. These excess chicks will most often be transferred to another approved project and it is rare that birds are euthanased due wholly to being excess to forecast needs.
22. Excess eggs in testing may be used for training purposes.
23. Surplus SPF males from the breeding farm are preferentially used for Eimeria oocyst production rather than being culled at the time of sex determination.
24. Training: Animal facilities use mice for training purposes that were identified with an undesired genotype (hence would have been euthanased regardless).
25. The establishment makes samples available that are collected opportunistically (under AEC approval) from collection animals and wildlife under our care or that have died. Access to this important material reduces the need for additional interference with animals and has benefited many collaborative researchers through the years.
26. Animals that had completed their purpose under other research/breeding protocols were subsequently used for handling or dissection training where possible. This practice enabled the establishment to reduce the number of animals necessary for training purposes and to reinforce the 3Rs mindset when students or new personnel require training in these techniques.
27. Building strong networks with conservation organisations to replace wild caught animals with animals from captive bred populations for species reintroduction projects.
28. A reduction on some species in the wild through the continuation of partnerships with other organisations, consolidating monitoring and trapping of species and sharing data.
29. Sentinels no longer required (except in immunodeficient mouse rooms) as we sample stock animals instead.
30. Less sentinel animals are utilised with animal health monitoring being done on stock animals via dried blood spot, fur and oral swab sampling as survival procedures.
31. The nature of organotypic spinal cord slice preparations mean several experiments could be undertaken from each animal.

32. 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 be utilised for future embryo/neonate production. This reduced the need to establish larger breeding colonies to supply adult mice of the required sex.
33. The use of protein channels isolated from cells where tissue from one animal can produce enough material for up to 20 experiments.
34. The collection of multiple tissue / cells from different regions of the reproductive tract of the same mouse reduced mice numbers and treating.
35. Understanding population genetic structure and gene flow in mouse populations to improve management outcomes We utilise ear clippings previously collected from concurrent mouse studies for baseline genetic comparisons.
36. We reduced the number of animals used in research by reusing rats from previous protocols. We altered our ethics submission to allow rats of an older age to be considered which was essential if they came from another protocol. They were chosen from a compatible behavioural study that had no impact on the results of our study. The rats would have been euthanased if not reused.
37. Cattle in a study were sourced from the existing herd. These animals could not be sold as they were discarded from another unrelated study and needed to be held onsite for 12 months or euthanased. This meant these cattle were able to be used and new animals did not need to be purchased.
38. At the conclusion of a study chickens were used to collect control tissues for bioanalytical method development therefore avoiding the use of additional chickens for this purpose. This reduced the need for euthanasia of over 60 birds. In addition a number of birds were also used for training purposes for a new process not previously conducted at the facility.
39. Re-use of fistulated cattle across multiple studies. A suitable washout period between studies is observed.
40. Genome sequences were entered into an international repository and can be widely used and accessed in future studies.

Statistical / study design

41. When the projects involve directly contributing to collections, efforts are made to only capture a minimal number of animals from taxa or locations that are poorly represented in the collections.
42. Project: Generation of boars that produce all female litters using a novel sex selection technique. This project has a stop/go measure in place at day 28 of gestation when the sows will be pregnancy tested. This will provide the researchers and the AEC with information to determine if techniques being used allow for implantation and successful pregnancy. Once this information is known a decision will then be made as to the continuance of the project.

43. Due to teaching requirements a set number of animals are kept on campus and used. These though are rotated between classes to minimise individual use. Furthermore, minimal numbers, taking in welfare considerations, are used for classes.
44. Students are placed within small groups where animals are used for surgical or other training. This reduces animal use.
45. 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. The bushfires of 2019/20 have increased the requirements for surveys in fire-affected forest areas. Monitoring, using cameras and acoustic and ultrasonic recorders will reduce some of the trapping previously required.
46. The revised application form (2019), requires greater emphasis from researchers regarding animal numbers. This includes the recommendation that a biostatistician is consulted with, and justification of the number of animals used is appropriate for the level of significance required. During 2020, researchers answers to reduction questions were scrutinised at a much higher level. This resulted in less animals being used, or in some circumstances more animals being requested. An additional question has been added to the application form regarding animal mortality, this aids the Committee in ensuring the animal numbers are sufficient considering expected mortality, where appropriate.
47. 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.
48. 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.
49. Where possible, we will seek to reduce the numbers of animals used by exploring computational modelling approaches and by re-using publicly available genome scale datasets related to the study.
50. The number of animals requested has been calculated to provide the minimum number with which we can adequately compensate for the inter-experiment variability inherent in a fairly complicated longitudinal study, for the inter-animal variability which is unavoidable with all animal studies, and for any losses due to age/surgery/missed injections/failed tissue processing etc. Furthermore, using a combination of experienced personnel and careful planning, we will endeavour to avoid having to use animals in our 'buffer' allocation.
51. A pancreas perfusion method optimizes islet yield beyond simple pancreas removal and digestion.

52. We will reduce the overall number of animals used by conducting control cohort studies to compare responses in GM and non-GM C57BL/6 strains. If baseline responses are similar between these control groups we can eliminate the need for one of the control groups when using GM and non-GM lines. This approach has the potential make a substantial impact on usage.
53. The introduction of new hyper-superovulation reagent CARD Hyper Ova combined with in-vitro fertilisation will reduce the amount of embryo donor female mice needed to obtain a sufficient amount of frozen embryos as stock. The hyper-superovulation reagent will induce ovulation at a higher efficiency with a 3 to 4 times more ova per mouse which can reduce the number of females by a third or a quarter.
54. Use of the Thunder microscope may extend our studies by allowing imaging of additional organs or tissues than that obtained by tissue harvest.
55. Decreased animal production to maintain and manage essential animal lines during COVID-19 induced shutdown periods, and decreasing non-essential research to successfully avoid the need to euthanase a large number of animals in response to the pandemic.
56. Use of the Braincubator (device invented by a researcher), to extend the life of neuronal tissue for electrophysiology and imaging which has resulted in less animals being used.
57. The trial farm is a miniaturised version of a commercial poultry shed. The small pen sizes allow the same stocking density used in large commercial operations but significantly reduces the number of birds required for a project from thousands of animals to hundreds of animals.
58. The AEC actively promotes the Biometrics and Data Analytics team to ensure engagement at the project concept phase. The Chair is actively working with the Biometrics and Data Analytics team to improve collaboration and understanding of AEC requirements of biometricians.
59. Acoustic recordings of bats have been used to maximise data sets and reduce the number of field trips.
60. Egg and bird numbers are continually monitored, with reports versus 'standards' being provided and reviewed at each quarterly AEC meeting. Significant deviations versus expectation may give rise to an Adverse Event report so that immediate action can be taken.
61. Continued use of technology such as 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.
62. All researchers are required to review sampling sizes and trapping efforts to minimise handling and capture of native animals. Particular attention is made to where there may be the requirement for relatively large numbers of individuals to be surveyed. Key projects that have carefully considered opportunities for reduction include reducing the number of microbats tagged for

radio-tracking for Pilbara mining projects, limiting the number of tagged bats to the minimum necessary to obtain useful data in addition to reducing the number of frogs that are required for genetic samples.

63. Minimise use of sentinels which are only used to sample rats and mice in IVC caging.
64. A protocol investigating advance therapeutics for diabetes used mice which failed to develop diabetes as negative controls, to reduce the use of mice in the control group.
65. Students in a teaching project chose to study invertebrates resulting in lower vertebrate animal use.
66. Food uptake and smoke exposure were performed on animals to negate the requirement for two separate sets of experiments and reduced animal numbers.
67. Skin biopsies performed when a frog was initially captured replaced the need for a second capture.
68. Selecting only a subset of tadpoles per pond for inclusion in a project.
69. Limiting the use of compounds to those that show efficacy in-vitro before subsequently being tested in-vivo reduced the number of animals being used.
70. Performing in-vitro assays on secondary cancer cell lines and on primary human cancer cells isolated from patient samples reduced the number of animals required.
71. The introduction of two control groups to determine if the changes observed in the microbiome were a result of surgery or diet change. These controls aimed to minimise the need to duplicate experiments or increase animal numbers for each group.
72. The adoption of a replacement of live animal model provided a targeted and selective platform for utilising isolated mouse germ cells which reduced the number of animals required in characterising the molecular interaction of mobile phone and Wi-Fi fields on spermatozoa.
73. Adrenal targeting peptides were first tested in cell culture which allowed the identification of two out of the five peptide derivatives that were not specific to adrenal cells.
74. When using pregnant dams, the male pups were used for studies involving bleomycin and female pups for studies using IL-33, with both experiments being running simultaneously. This removed the potential for animal wastage as animals of either sex could be utilised.
75. The development of an in-vitro organoid culture system provided information that should reduce the number of animals required.
76. The use of a more effective mouse model, in comparison to a rat model, reduced the number of animals being used overall.
77. The selection of time points for analyses in mouse models were informed by data collected from a cell culture experiment and were confirmed as suitable time points from pilot studies in-vivo.

78. The implementation of a new lung function testing apparatus (FEV extension of flexivent apparatus) improved the reproducibility and reduced the variability of testing. As a result, experimental power was increased, and some experiments may require fewer animals.
79. Extended Commercial Trials of NUMNUTS®. We initially considered asking producers to use the Numnuts® tool on 50% of their flock, which means that the remaining 50% would be marked without local anaesthetic. The current protocol uses a smaller number of animals (n=60) to gather a more detailed evaluation of the producer-observed outcomes of using Numnuts® on their farm.
80. We are actively developing software methods of analysing multiparametric data as part of all of our projects. This also helps to reduce the number of animals needed, without reducing the statistical power of the experimental results.
81. A statistician was consulted during the study and as a result of the consistency of test results with the first 12 cats, the number of visits was able to be reduced from 8 to 4, and the total number of cats recruited from 30 to 12.
82. At all times, we analysed results from one experiment before commencing the next. This allowed us to determine number and genotypes needed for the next study. As a consequence, several mouse lines were not used in this study resulting in a significant lower number of mice used in total compared with applied for.
83. We purchased a special incubator (BraincubatorTM) that can keep tissue viable for up to 48 hours. We are going to use it in future experiments to prolong tissue lifespan and thus reduce the number of experimental animals.
84. Since the initiation of this protocol, a new methodology for the detection of T cells which recognise a particular peptide antigen (peptide-exchange tetramers) has become commercially available. As a result, the number of mice of each recipient strain required to screen a given number of peptides has been reduced significantly.
85. In this project, the team were collecting blood for in-vitro analyses when the animals were being euthanased. To collect this amount of blood from living animals it would take a larger number of animals to produce the required sample volume.
86. A modification allowed the collection of additional rumen samples from fistulated cattle at the originally planned time of collection. Hence more value was achieved from the experiment without the need for more collections or additional animals.
87. A modification to the protocol allowed using different groups of animals for a two part experiment, without an overall increase in numbers.
88. Teaching procedures:
    - Simulated penning of sheep by demonstration.
    - Working with university researchers and National Parks on native animal projects rather than duplicating own projects.
    - Where possible photographs, taxidermized and preserved specimens are used.
    - Appropriate animal to student ratio.

- Use normal scheduled animal health husbandry routines for teaching activities.
- Opportunistic field observations of native fauna.
- Maximum amount of data and student/animal contact is derived from each trapping in order to minimise trapping requirement.
- Only one animal brought in for a demonstration.
- Use of ultrasound machine to replay images from live animal and 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.

89. The use of thermal imaging cameras for determining presence/absence and quantitative data continues to reduce the need to trap animals.
90. To reduce the number of animals required, larger animals were used to obtain the required quantity of tissues.
91. Disintegrating tablet in dogs: as each dog will be offered the same tablet on five occasions, this reduces the total number of dogs required.
92. Assessment of a new design of intra-ruminal controlled release capsule in rumen fistulated cattle. Five capsules were placed in each animal to maximize the amount of data collected per animal, minimizing the number of animals required.
93. Comparative Efficacy and Pharmacokinetic Study: Administration of Lufenuron Tablet and Novel Chew Formulations in Cats. At the stage in development, pharmacokinetic and efficacy studies are required to demonstrate that the dose and formulation are appropriate. By combining both objectives the maximum data is able to be obtained from one group of cats rather than repeating treatments in a second study. The number of cats included in each treatment group is the minimum recommended in the applicable guidelines.
94. Study design allowed the same untreated control group to be used to calculate product efficacy across treatment groups. This allows the total number of untreated control animals to be minimized without compromising animal welfare outcomes.
95. Oral and Subcutaneous Dose Escalation Study in Dogs: Only one animal of each sex is used per dose level group for each route of administration (oral and subcutaneous). By administering a placebo first, each dog acts as its own control. This removes the need for using additional control dogs.

Breeding

96. Approval of new techniques for embryo freezing rather than continuous breeding to maintain lines.
97. Rederivation: Animal facilities optimise the fostering process and thereby minimise the numbers of female mice used for fostering purposes.

98. In 2020, the AEC continued to assess the requirement for under-utilised mouse breeding colonies. The AEC set deadlines for researchers to submit animal ethics applications or provide proof of project approval for experiments that would utilise these mouse models. The AEC's persistence on this issue resulted in the institute closing one unused breeding colony with a second colony set to close early 2021.

7.3 Refinement – specific examples

Environmental enrichment / housing

1. Dogs are trained to walk up a set of portable stairs onto a table to assist in blood collection and external parasite assessment. All dogs are routinely leash walked around the facility.
2. A group of researchers was the recipient of a grant that focuses on developing a publicly available website that provides information on enrichment options for a range of species and includes the effect that these enrichments may have. This website is due to be launched in early 2021.
3. Modifications were made to the facility to improve each of bird placement, security from bush fires, general biosecurity from diseases, and potential for cross contamination of antigens. The Company also installed dedicated building and plant facilities to contain security of the new incubator and hatcher.
4. Modifications to floor grids in the Grow-Out sheds and to fencing in the Bird Houses was done to improve bird welfare.
5. Placement of LED lights along the drinking line did help 'start-off chicks' to better identify the drinkers, and then the feeders, during their first week in the Grow-Out shed.
6. The introduction of music in the Grow-Out sheds has been in place for a number of years and does help to acclimatise birds to noise from outside the sheds, aiming to ensure the birds are not easily startled.
7. Installation of air conditioning units during 2020 assisted to improve the environment for chickens in summer. Improved air distribution for the brooding period (day-old to 2 weeks) improved and reduced temperature variation for the chicks.
8. High density polyethylene perches were introduced into Grow-Out sheds and cages, which have been used selectively.
9. There was continued 'alarm monitoring', including particular emphasis on temperatures within all Bird Houses, egg incubators, and Grow-Out sheds, power supply to industrial equipment, and building security via external video surveillance of the buildings.
10. Refinement of procedures extended to the design of the housing set-up for experiments involving fish. Small modifications to various aspects of the tank design greatly improved animal welfare outcomes which will be utilised in future experiments with this species.
11. Accommodation of research horses in a large paddock at a professional horse retirement farm.

12. Sheep enrichment - best achieved via social contact with conspecifics, gentle, quiet and confident human handlers (the same people where possible), provision of lucerne/oaten chaff and poplar branches (need to be regularly replaced as they really strip these) and lengths of suspended chain (zip locked onto the pen).
13. Canine facility refinement: Enrichment was provided by daily social interactions with the dog handlers/carers and other dogs (facilitated by mesh between cages), and free-running in the exercise yard. Extra enrichment was provided during the trial with the construction of 2 large exercise yards with sandpits.
14. Improvement in animal housing and management such as use of extra enrichment or companion mice where possible.
15. Improvements to animal housing and management (e.g. introduction of "buddy cages" to avoid single housing of mice, provision of environmental enrichment).
16. Environmental enrichment for single caged rabbits –hay filled paper bags. Restriction placed in the period of time rabbits can be individually caged. Group housing facility for rabbits containing hide spots, gnawing logs and paper bags to provide both environmental enrichment and to minimise adverse social interaction between dominant and submissive rabbits.
17. With a focus on environment enrichment, there was the introduction of wood shavings to the floor in the Grow-Out sheds and sustained minimal light intensity through the use of coloured bulbs/tubes.
18. Providing extra nesting material to nude/hairless mice for warmth.

Reduced impact of procedures (including anaesthesia / analgesia)

19. All animals that undergo surgery will receive Bupivicaine drops on the cut surface of their skin immediately before suturing and will also receive a Carprofen injection immediately after surgery.
20. Use of analgesia (4mg/kg ketoprofen by SC injection) upon signs of discomfort after adjuvant injections.
21. Use of EMLA cream as topical anaesthetic ointment or instillation of local anaesthetic substances in combination with injectable anaesthetics for any painful procedures prior to incision or injection.
22. Infiltration of the abdominal wall around the incision site with the long-acting local anaesthetic bupivacaine to reduce postoperative discomfort has now been added to the protocol. As a result of this change, mice appear more active at the time of the first post-op buprenorphine injection.
23. Commencement of a pilot study using the Tecniplast Digital Ventilated Cage System (DVC) to non-invasively monitor behavioural changes arising from basic procedures in mice and changes in bedding status over time. The DVC is a device that has the ability to monitor animal cages around-the-clock to facilitate assessment of animal wellbeing (Animal Locomotion Index) and optimise cage management (Bedding Status Index).

24. 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, and hearts for sino-atrial node electrophysiology recordings.
25. Methodology is being developed to conduct non-terminal anthelmintic studies in dogs.
26. We routinely use eye lubricant to avoid keratitis, even in short duration anaesthesia, and we place our animals on clean heating pads with a supplementary oxygen supply to aid surgical recovery.
27. Use of heating pads, saline injections, and easy access to food to maintain healthy hydration and body weight and reduce mortality associated with neurotoxin use.
28. Animal pain is reduced by reducing the frequency of immunisations with HTV (from one injection a week to one injection every two weeks) and monitoring the animal's serum antibody titres frequently, preventing further unnecessary immunisation procedures from occurring.
29. Use of the recipient mother as sentinel for the rederived pups spares the offspring from blood collection for health screening.
30. Use of a subcutaneous implant to replace the ovariectomy procedure, which is less invasive.
31. Mice (C57BL/6) are treated with 110 mg/kg of the drug Alloxan thereby rendering a mouse diabetic. With this drug we do not see any mortality (100% survival) and diabetes is brought on in a robust and reproducible fashion (within 2-3 days) compared to STZ. Thus by using Alloxan it is easier to manage animal health and it appears to have no adverse reactions and importantly no unpredicted mortality.
32. Decontamination of equipment to be used in direct contact with animals to prevent potential spread of pathogens.
33. 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.
34. Requiring an acclimation period of at least 7 days after arrival in new surroundings, before they are used in a project.
35. Acclimating animals to handling and research procedures before being used in a research project.
36. In an application to study the physiological response of lizards to changes in environmental temperature and its ecological implications, the researchers proposed to use mouth gaping as an endpoint rather than loss of righting ability which has been used elsewhere.
37. A trial of a quick, humane method of euthanasing large lizards (captive bolt) was incorporated into the protocol.
38. The AEC implemented its requirement that the Forced Swim Test could only be used to validate a lower impact alternative.
39. The AEC required researchers to assess alternatives to the intraperitoneal route of injection which has been shown to be a significant potential confounder due to undetected mis-injection.

40. 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.
41. We are making use of privately-owned sheep that have already undergone one pregnancy scanning measurement, as part of normal husbandry procedures. Further, the sheep will stay on their normal property, thus avoiding any distress associated with transport to another site.
42. Mouse testing for Yersiniavax now includes pain relief.
43. Introduction of a new dietary supplement that is a high calorie, protein and Omega 3 fatty acids for lactating and breeding rodents.
44. Providing a high energy nutritional supplement that provides caloric support for pre-weanlings, weanlings, post-surgical, debilitated and aging animals.
45. Non-terminal sampling methods are used during routine blood collection for health monitoring. Minimum amount of blood taken from animals during health testing.
46. Smaller incisions during rodent surgery for faster wound healing.
47. Minimum dose of injectable anaesthetics administered for shorter procedures (e.g. for imaging purposes) enabling faster recovery time.
48. Allowing animals to acclimatise to the specific experimental setting and handle them with care to minimise fear and distress.
49. Continue using anaesthetised mice when practising subcutaneous/intraperitoneal injections, tail vein bleeding/injections and submandibular bleeding during practical orientation sessions with new researchers and staff (minimise harm to animal).
50. Continue to make use of Opti-Spot test strips for rodent animal health monitoring, no need to anaesthetise animal.
51. Ensuring only the right sized surgical instruments are used that is suitable for animal species.
52. Frequent communication with researchers for prompting of quicker turnover of genotyping results as practicable, to ensure unneeded animals are not held unnecessarily after genotyping.
53. A protocol investigating volatile organic compounds produced by the decomposition of human remains made use of several teams of cadaver detection dogs to ensure the work and time required for each animal was kept to a minimum. Dog teams were monitored by their handlers and training was ceased if they displayed any signs of stress or fatigue.
54. Restraint stress (also known as immobilization) was produced by confining a naive animal inside a 50 mL Falcon tube. This technique replaced the use of repeated social defeat which is a relatively severe stressor in rodents based on social hierarchy and dominance.

55. Steps were taken to reduce the dosages of drugs, infectious inoculations or inflammatory compounds to prevent or reduce weight loss which had been observed with these inoculations. This meant that mice which lost weight after treatment gained that weight back quickly and the extent of weight loss has been quite small.
56. A protocol was amended to allow the administration of two ovalbumin sensitisations with half the dose of alugel (aluminum hydroxide adjuvant) which reduced the weight loss observed after a single dose.
57. The on-going improvement of surgical techniques refined experiments and reduced adverse impacts on animals.
58. Implanting a remote temperature logger (thermistor) subcutaneously rather than intraperitoneally made surgery far less invasive for an animal and meant a quicker recovery time post-surgery.
59. The development of a sophisticated in-vitro assay to rapidly detect beneficial biomechanical changes reduced the need to maintain animals for extended periods in-vivo.
60. Non-Surgical Embryo Transfer (NSET) facilitated mouse uterine embryo transfer and eliminated the need for the anaesthesia and recovery usually required for conventional surgical transfer methods. NSET provides an effective procedure reducing potential pain or distress on the recipient female.
61. The use of ultrasound for frogs was a non-invasive technique that generated extensive additional data to existing protocols, improved overall monitoring of the health and well-being of animals in a project, and resulted in the use of fewer animals.
62. Performing an ultrasound to confirm pregnancy rather than performing a caesarean procedure to collect foetal tissue without verifying pregnancy.
63. Extended Commercial Trials of NUMNUTS®. Collection of video footage allows us to conduct an offline scoring, using a standardised and trained observer team, which will reduce the standard deviation around the scores assigned as compared to the 'citizen science' type approach using untrained observers on farm. Furthermore, although not formally part of the current study, the video footage will be made available to the collaborating universities for student training purposes, and it is envisaged that student projects may focus on detailed behavioural observations of each lamb, with the individual lamb as the experimental unit.
64. Testing a non-invasive remote mouse monitoring system. This project aims to develop a non-invasive remote mouse monitoring network. The development of the device network will allow for a remote monitoring technique with minimal disturbance to animals. Data collected from the remote monitoring devices will add rigor to current monitoring strategies at a broader scale than conventional monitoring strategies.
65. We targeted our photographing days to the same day as grading so fish did not have to be handled and anesthetized twice.
66. 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).

67. New holding yards were set up with better footing, drainage and access to water. Yards have also allowed for better cow flow into the practical class area which reduces stress during the loading into the crushes.
68. We use an in-house developed awake animal imaging technique called Open-Field PET which avoids the use of anaesthesia. This mitigates the morbidity and mortality associated with the use of anaesthesia in small animals while optimising scientific outcomes from the experiments.
69. Project investigating alternative applications or virtual fencing stimuli in sheep: The team improved/refined the design of an experimental device which allowed one device instead of two different devices being used in the experiment, hence reducing the number of procedures performed and also increasing the certainty of the conclusions drawn from the experiment.
70. Teaching procedures:
    - Students attend various workplaces to reduce the use of a particular mob of animals.
    - Horses are monitored for behavioural changes and replaced regularly. Horse usage is rotated to prevent overuse.
    - Using treats and water as substitution for medication.
    - 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.
    - Horse usage recording system to rotate horses and minimise over use of horses.
    - 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.
    - Keeping a diary of on-farm activities.
    - Students are referred to Standard Operating Procedures prior to animal use.
    - Following animal welfare procedural guidelines.
    - 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.

- Timetabling of classes is coordinated so that activities are spread over the semester, to avoid over-use of the same animal.

71. Spontaneous collection of naturally voided urine from horses for the purpose of drug analyses.
72. Research was done to ensure that both the drugs being tested, and the paint added to make the water opaque in the Morris maze, were nontoxic.

Education, veterinary input, and monitoring of research

73. Providing mandatory training to use a mobile anaesthetic induction unit (Stinger anaesthetic machine) and ensuring researchers do not use obsolete anaesthetic techniques.
74. Mandatory routine mortality report for super genomics project. This is closely monitored in collaboration with the AEC.
75. Veterinarian involvement in assessment of livestock for teaching and field days.
76. Ongoing training and on-going experience in handling poultry which are particularly prone to stress, is standard practice for the Company.
77. 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.
78. The Animal Welfare Officer had assisted researchers to implement a range of refinements in complex protocols. Particularly noteworthy were a mouse model of graft versus host disease, a rat model of brain tumours, and procedures for transporting tropical fish.
79. A new invasive procedure involving cannula implantation into the ventricle of the brain was undertaken. The investigators trained extensively in cadavers initially. The procedure was performed in live animals in the presence of a veterinarian who was able to provide immediate advice for unexpected impacts, and refine the procedure for future experiments.
80. Veterinary pre-review process for applications – help designing and planning procedures.
81. Expert veterinary advice is available 24/7 to all staff and students working with animals, including those in the field.
82. Encouragement of researchers to consult the Animal Welfare Officer and the Animal Ethics Office for assistance in writing an Animal Ethics application.
83. 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.
84. Ongoing training and upskilling of staff involved in the care and use of animals for scientific purposes.
85. Compulsory online training for new staff and students involved in the care and use of animals, in addition to hands-on training and competency assessment.

86. Proposed adoption of an online database to improve record keeping and to monitor animal use and experience, avoiding potential excess breeding of animals.
87. Developing competency assessment procedures.
88. 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.
89. Working with researchers to update any obsolete techniques to improve animal welfare and reduce risk of adverse events (e.g. use of isoflurane instead of injectable anaesthetic drugs).
90. Promotion of refinement strategies, including new ARRIVE 2.0 Guidelines, through quarterly Animal Ethics Newsletter which goes out to all animal users, animal care staff and facility managers. It contains information on alternatives to animal use, partial replacement options, information from regulators, new technology, veterinary updates etc.
91. Research Services in conjunction with the AEC continued the development of key SOPs/Guidelines for researchers to use. These help ensure best practice methodology is implemented and the standards and expectations are clear.
92. Creating an online Lab Animal User course for new animal care staff and researchers that is available throughout most of the year.

Animal monitoring / technology

93. We will implement the use of Physiosuite to measure physiological parameters such as temperature, heart rate, blood pressure and spO2.
94. The investment in a new Jamesway incubator and hatcher, has resulted in improved hatch rates and healthier day-old chicks through 2019 and 2020. The use of a dedicated incubator room reduced the need to transport incubated eggs from one incubation area to another, which has helped to maintain egg temperature and avoid stressing the embryos at transfer.
95. Animal care staff and researchers working together to complete post-procedural monitoring of research animals.
96. We have purchased additional surgical monitoring equipment for this protocol. These include an oxygen saturation monitor and a heat pad/temperature monitor that self-adjusts to maintain optimal animal temperature. With this additional equipment we are able to respond faster and more accurately to any fluctuations during surgery. This likely increased animal stability throughout surgeries and enabled a faster recovery period.
97. 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.

98. Working with researchers to develop better project specific monitoring checklists to identify, action and report adverse events and the development of an adverse event form.
99. 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.
100. In 2020, the committee challenged investigators to improve the quality of their monitoring sheets. The committee noted that on many occasions, the monitoring sheets lacked adequate interventions and scored criteria. The committee provided support to investigators for developing improved monitoring sheets.
101. Score sheets for monitoring approved surgical procedures have been introduced to enhance the process and appropriately identify and manage any pain and distress of animals.

Wildlife studies

102. One project has introduced, and successfully implemented, using 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.
103. A method of visual surveys of fish was introduced, which has no/minimal effects on animal welfare.
104. Improvements made to trapping equipment and datalogging equipment used in wildlife research to minimise risk of adverse events.
105. Refinements reported for projects involving flying foxes include adding knots to the holding ropes to prevent holding bags from sagging together, administering subcutaneous fluids instead of providing a juice supplement and limiting the amount of animals processed each day to ensure holding times are adhered to.
106. Anaesthesia is used to collect samples from trapped wildlife where the AEC considers that it is appropriate.
107. The AEC assesses each project as to the impact on the animals to ensure pain, suffering or other harm is minimised as much as possible within the project. An example has been to close pitfall traps during rains and extreme heat, clearing traps more frequently than originally proposed and designing a technique that minimises ant attack.
108. For protocol Wildlife forensics: developing a tool for the illegal wildlife trade – the Principal Investigators were asked by the Committee to consider the need for future radiographs prior to the use of an animal from the Wildlife Hospital, to ensure radiation thresholds are not exceeded.
109. Reduction in impact on wild animals by running pilots on captive populations before moving on to wild animals (for example transmitter trials).
110. Continued use of specially designed excluders for wildlife traps have decreased bycatch of non-target species.

111. Limiting the amount of time for drones over dolphins at altitudes less than 60 m because our research shows that dolphins may detect drones at these altitudes.
112. Selection of sites where students can work on the river bank to minimise time fish are held in captivity.
113. Mobile phones are used to record images of fish in photo identification tanks that are then used to identify specimens to reduce time in captivity.
114. In a project that surveys and monitors fish, where possible, more invasive techniques such as gill netting have been replaced with electrofishing (Backpack and boat). They also developed a number of practices to minimise suffering and reduce handling time and stress including use of plumbed holding tanks on electrofishing boats.
115. In 2020 the NSW permitting agency allowed us to return skinks back to their natural environment, we were able to update our ethics application to incorporate their return.
116. Mark-recapture practices allow researchers to only collect genetic material from an individual frog once, even if that frog was encountered on multiple occasions.
117. The use of genetic techniques to investigate dispersal and reproductive effort reduced the need for techniques requiring capture-mark-recapture. This meant that there was a reduction in the handling of frogs.
118. Molecular investigation of novel bacteria, viruses, and protozoa in Australian ticks associated with human illness. Light anaesthesia with isoflurane will be used to minimise animal distress and pain during sampling and humane killing. Only trained personnel will perform animal handling, anaesthesia, and sampling procedures to minimise the risk of harm to animals. To improve the post-release survival of animals, they will be closely monitored during recovery to ensure they are alert and active when released and marked with a small fur clipping to ensure we don't repeatedly handle and sample the same individuals.
119. The project specifically compares birds from two colonies; one located in a subtropical area with low productivity and the other, temperate with higher productivity. No previous studies on this species have specifically studied these aspects. This study is focussed on retrieving already deployed loggers to reduce the potential welfare impacts of carrying devices long-term.
    Previous studies that have attached GLS devices have had mixed return rates. Cecere et al. (2013) attached 14 devices and recovered ten (71%), Catry et al. (2009) attached 16 devices with seven failing (44%), Carey et al. (2014) attached 27 devices to Short-tailed shearwaters (species) and recovered 20 (74%). By attaching 45 GLS devices annually to individuals it takes into consideration that a percentage will either fail or not be recovered during the life of the project. There will however be opportunities to recover all of the devices at a later stage. More specifically the Velcro attachment is known to breakdown after two to three years ensuring there is no long-term impact to individuals from the GLS group. The GLS devices that have been attached weigh less than 1% of an average adults body weight, with the maximum weight recommended being 3% or less (Phillips et al. 2003). The devices have been used in prior studies and found not to impact on the birds ability to forage (Carey et

al. 2014). Individuals will only be handled once to specifically remove loggers already attached in prior years. There will only be between one and two trained and experienced people completing the procedure each visit. In order to minimise disturbance to each colony, the birds to be targeted have had their burrows identified and were chosen due to their ease of access from walkways to reduce the time spent in the area and the likelihood of burrows collapsing or vegetation being damaged.

120. 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.
121. Development of a 'selfie trap' (a device that uses facial imaging software to individually identify gliders/quolls photographed with an automatic camera) as an alternative and refinement to physical trapping methods of wildlife.
122. Continued use of camera traps and ink pad tunnels has decreased the use of live trapping for survey work or determining species presence in an area.
123. Reliance on camera surveys so impact to species is negligible.
124. Use of audio recording for long term monitoring and our transect counts involve minimal disturbance to frogs (call broadcasts and counts of responses).
125. We have introduced a drone standard operating procedure that will enable non-trap techniques such as use of infrared cameras to detect koalas using drones, animal census using drone imagery.
126. Researchers are currently trying to understand the impact of urbanisation on frogs and turtles in the ACT and surrounding regions. For their frog surveys, researchers have replaced the need to capture or mark animals. An alternative method to be used here is to record their calls, which is useful, and researchers are able to know presence/absence of different species.
127. Initial trial drone runs performed to refine survey techniques and minimise stress to detected animals. Minimal use of spotlighting during animal detection.
128. Testing a non-invasive remote mouse monitoring system: The devices will be placed on current trapping grids and monitoring sites with known population sizes or activity estimates. By placing the devices on established trapping and monitoring sites we remove the need for capture and handling of new animals.
129. Monitoring floodplain vegetation and wildlife with trail cameras. By using camera imagery we have replaced other methods of assessing wildlife abundance and diversity, such as trapping. Cameras have been selected that can record time-lapse and motion-sense images at the same time, thus refining the methodology and reducing the numbers of cameras to be deployed.
130. Most recently we have used highly sensitive thermal cameras that allow us to detect wallabies from a distance, rather than just at feeding stations, and we aim to test the use of computer software to identify individual animals based on their features. This may allow us to answer questions we could otherwise only answer by catching wallabies.

131. Refinement for field studies employed modern trapping and sampling techniques and equipment to minimise potential for animal injury as well as use of smaller, less invasive identification methods in wildlife studies. Indirect sampling via drone/arial surveys and underwater cameras, to replace trapping and baited underwater surveys, were used to provide population data. Further, new methods were utilised in 2020 to study predator-prey interactions, using prey species in a separate transparent enclosure and observing predator interactions while mitigating potential for predator species to come into direct contact with prey species.

Invertebrates

132. Invertebrates such as Drosophila or nematode worms for teaching projects.
133. Our laboratory regularly uses less sentient species - drosophila flies, to validate this kind of research. Drosophila pain research currently has a limited number of assays which we are currently working to improve and we hope in future to replace parts of mouse pain research with drosophila.

Rehoming / retirement

134. Two research horses have been retired from research duties but remain with the two younger horses still involved with research activities.
135. Rehoming of animals when they are no longer required. In 2020, guppy fish and cichlids were able to be rehomed upon completion of a project.
136. Three dogs and eleven cats were donated to Beagle Freedom during the reporting years, as they were no longer required or suitable for research.
137. 86 excess guinea pigs were re-homed to animal rescue organisations, so they were able to be placed as pets in suitable homes rather than being euthanased.

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*
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
	S29	Turtles and Tortoises
	S30	Other reptiles

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

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*

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