Department of Primary Industries

NSW 2018 Animal Use in Research Statistics

Table of Contents

1. Summary

Information on the use of animals in research in NSW is collected by animal research establishments on a calendar-year basis and submitted to the NSW Department of Primary Industries (DPI) for reporting.

The following information is included in this report:

- General charts to show the trend of animal use since 2010.

- Purpose charts for 2018. There are ten Purpose Categories (see Appendix: *Guide to the categories of reporting*) and these charts show the numbers of animals used, in species groups, for each purpose against the nine categories of procedures (see Appendix: *Guide to the categories of reporting*). The categorisation of procedures aims to give some indication of the 'invasiveness' or 'impact' of the research being undertaken on the animals involved.

- Species charts for each purpose for 2018. These charts provide a breakdown of the following species groups:

  - laboratory mammals,

  - domestic mammals,

  - birds,

  - primates.

- Lethality testing for 2018. 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 provided by animal research establishments of the implementation of the 3Rs (Replacement, Reduction and Refinement in animal use) in 2018.

- Appendix - Guide to the categories of reporting.

Information provided by research establishments each year also includes the collection of statistics 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 procedure category of 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 (see page 8) which excludes this procedure category from the total number of animals used.

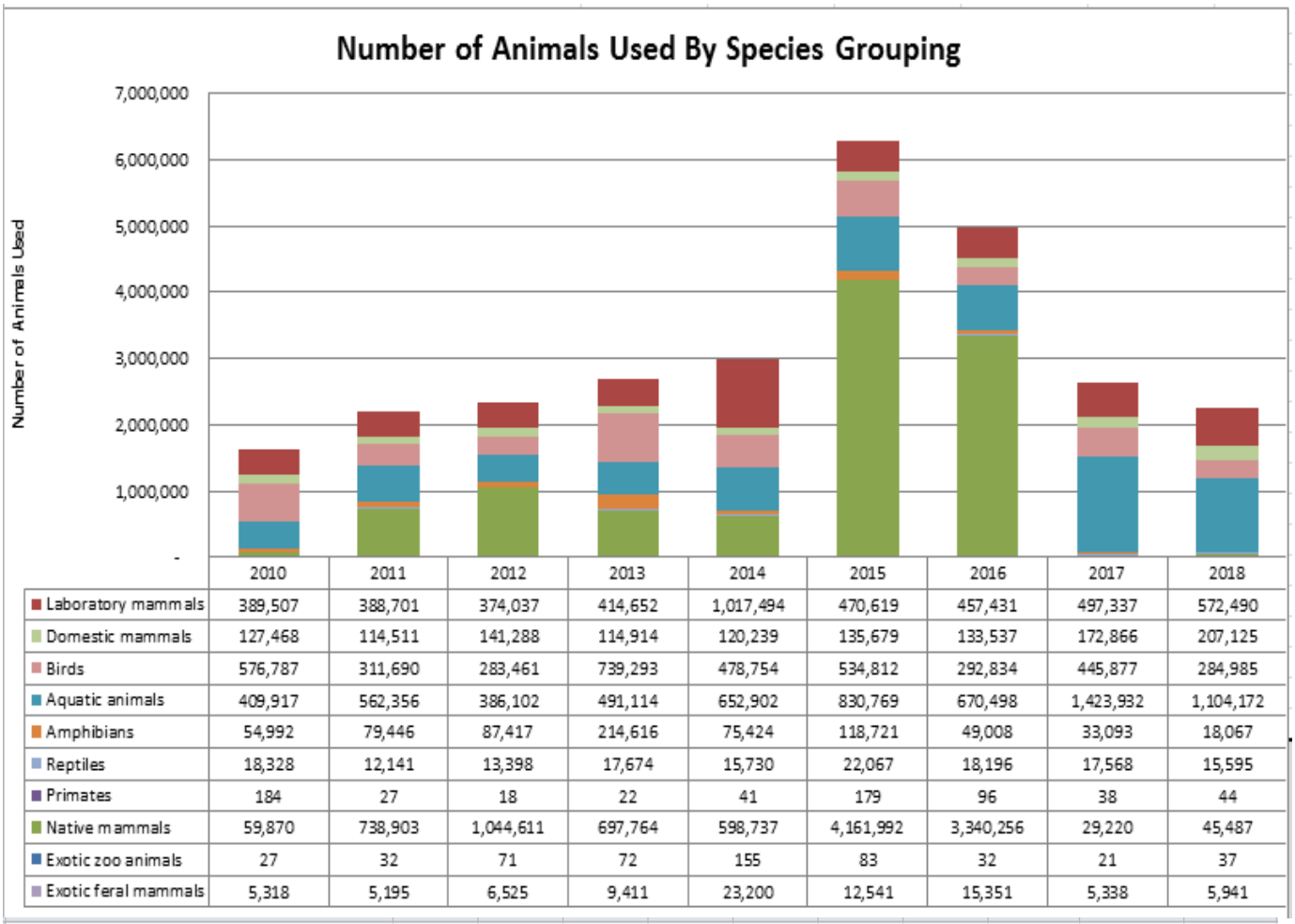
Collation and reporting of statistics is reliant upon the information provided by animal research establishments. This means there can be minor differences in the interpretation of which Purpose and Procedure categories of use are the most appropriate. The magnitude of the information submitted by research establishments means that NSW DPI is not able to verify the data within each individual research project – this is 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*.

Notes:

- For the 2018 reporting year, some establishments voluntarily reported on a new category – Fate of animals. For this reporting, all animals were in the Purpose category A7 - *Research: environmental study*, and the Fate of Animals category F10 - *Remain free living in the wild or released to the wild*. From the 2019 reporting year onwards, reporting on this category will be mandatory for the use of domestic cats and dogs, and voluntary for other species.
- 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 the 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 "*#N/A*" because an incorrect species code was used. The impact of this on the charts is negligible.

2. General Charts

2.1 Number of animals used over time by species grouping

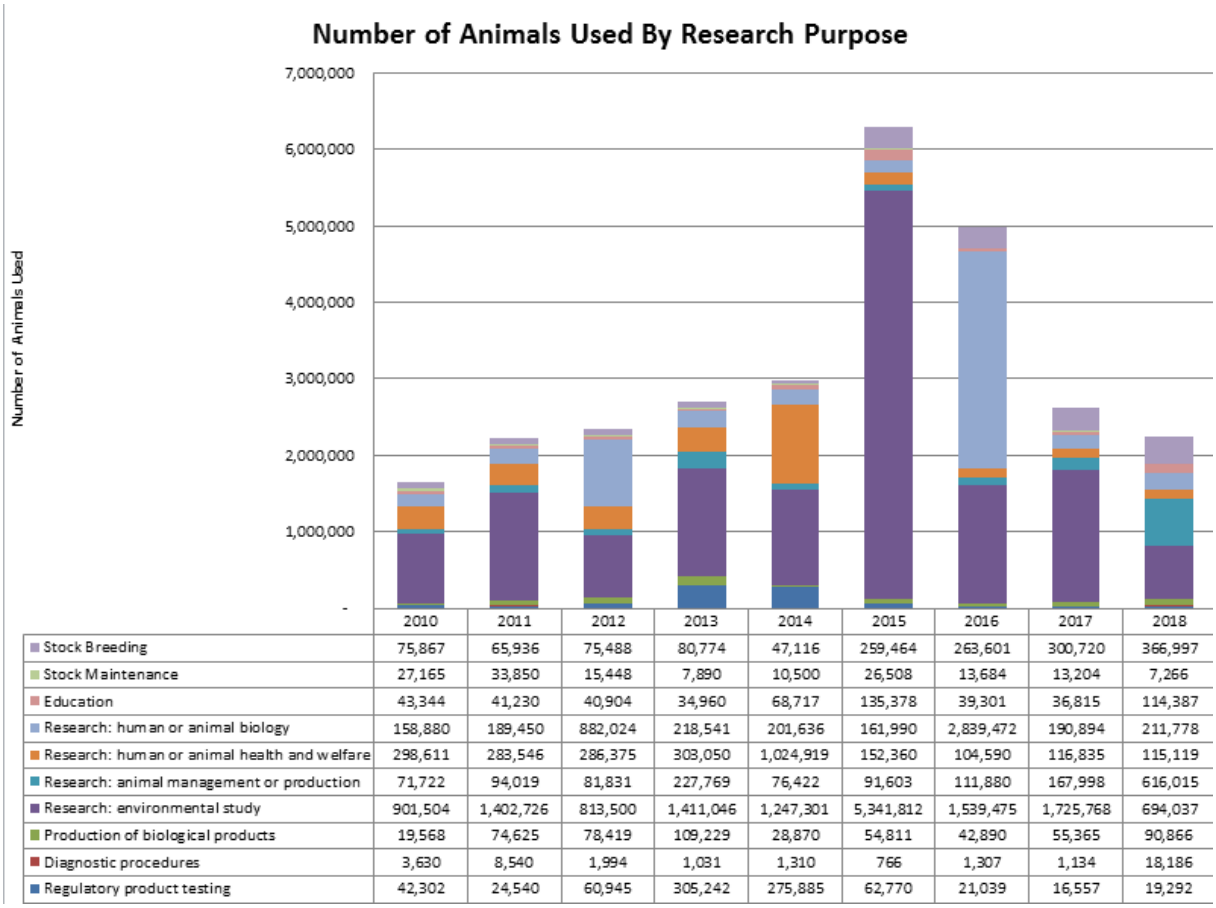


	2010	2011	2012	2013	2014	2015	2016	2017	2018
Laboratory mammals	389,507	388,701	374,037	414,652	1,017,494	470,619	457,431	497,337	572,490
Domestic mammals	127,468	114,511	141,288	114,914	120,239	135,679	133,537	172,866	207,125
Birds	576,787	311,690	283,461	739,293	478,754	534,812	292,834	445,877	284,985
Aquatic animals	409,917	562,356	386,102	491,114	652,902	830,769	670,498	1,423,932	1,104,172
Amphibians	54,992	79,446	87,417	214,616	75,424	118,721	49,008	33,093	18,067
Reptiles	18,328	12,141	13,398	17,674	15,730	22,067	18,196	17,568	15,595
Primates	184	27	18	22	41	179	96	38	44
Native mammals	59,870	738,903	1,044,611	697,764	598,737	4,161,992	3,340,256	29,220	45,487
Exotic zoo animals	27	32	71	72	155	83	32	21	37
Exotic feral mammals	5,318	5,195	6,525	9,411	23,200	12,541	15,351	5,338	5,941

Chart 1: Number of animals used over time by species grouping

	2010	2011	2012	2013	2014	2015	2016	2017	2018	Grand Total
Amphibians	54,992	79,446	87,417	214,616	75,424	118,721	49,008	33,093	18,067	730,784
Aquatic animals	409,917	562,356	386,102	491,114	652,902	830,769	670,498	1,423,932	1,104,172	6,531,762
Birds	576,787	311,690	283,461	739,293	478,754	534,812	292,834	445,877	284,985	3,948,493
Domestic mammals	127,468	114,511	141,288	114,914	120,239	135,679	133,537	172,866	207,125	1,267,627
Exotic feral mammals	5,318	5,195	6,525	9,411	23,200	12,541	15,351	5,338	5,941	88,820
Exotic zoo animals	27	32	71	72	155	83	32	21	37	530
Laboratory mammals	389,507	388,701	374,037	414,652	1,017,494	470,619	457,431	497,337	572,490	4,582,268
Native mammals	59,870	738,903	1,044,611	697,764	598,737	4,161,992	3,340,256	29,220	45,487	10,716,840
Primates	184	27	18	22	41	179	96	38	44	649
Reptiles	18,328	12,141	13,398	17,674	15,730	22,067	18,196	17,568	15,595	150,697
#N/A	195	5,460								5,655
Grand Total	**1,642,593**	**2,218,462**	**2,336,928**	**2,699,532**	**2,982,676**	**6,287,462**	**4,977,239**	**2,625,290**	**2,253,943**	**28,024,125**

2.2 Number of animals used over time by research purpose

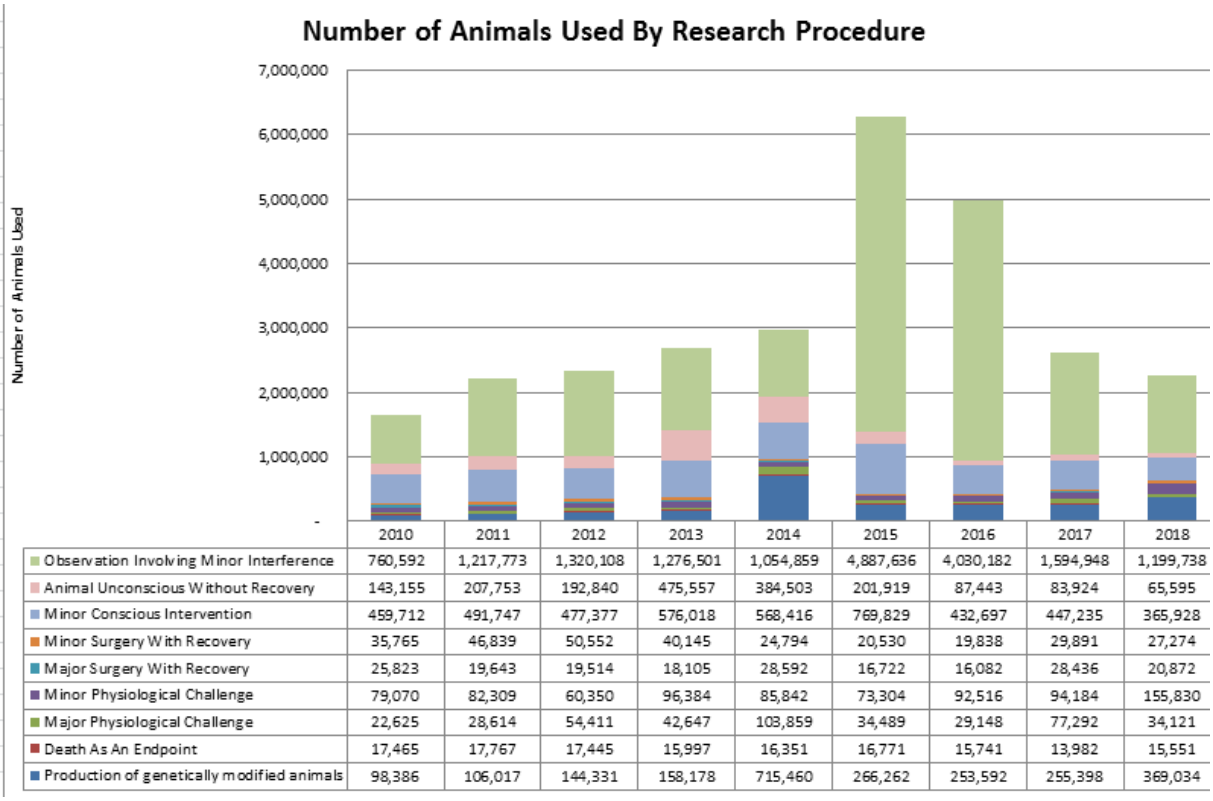


	2010	2011	2012	2013	2014	2015	2016	2017	2018
Stock Breeding	75,867	65,936	75,488	80,774	47,116	259,464	263,601	300,720	366,997
Stock Maintenance	27,165	33,850	15,448	7,890	10,500	26,508	13,684	13,204	7,266
Education	43,344	41,230	40,904	34,960	68,717	135,378	39,301	36,815	114,387
Research: human or animal biology	158,880	189,450	882,024	218,541	201,636	161,990	2,839,472	190,894	211,778
Research: human or animal health and welfare	298,611	283,546	286,375	303,050	1,024,919	152,360	104,590	116,835	115,119
Research: animal management or production	71,722	94,019	81,831	227,769	76,422	91,603	111,880	167,998	616,015
Research: environmental study	901,504	1,402,726	813,500	1,411,046	1,247,301	5,341,812	1,539,475	1,725,768	694,037
Production of biological products	19,568	74,625	78,419	109,229	28,870	54,811	42,890	55,365	90,866
Diagnostic procedures	3,630	8,540	1,994	1,031	1,310	766	1,307	1,134	18,186
Regulatory product testing	42,302	24,540	60,945	305,242	275,885	62,770	21,039	16,557	19,292

Chart 2: Number of animals used over time by research purpose

	2010	2011	2012	2013	2014	2015	2016	2017	2018	Grand Total
Stock Breeding	75,867	65,936	75,488	80,774	47,116	259,464	263,601	300,720	366,997	1,535,963
Stock Maintenance	27,165	33,850	15,448	7,890	10,500	26,508	13,684	13,204	7,266	155,515
Education	43,344	41,230	40,904	34,960	68,717	135,378	39,301	36,815	114,387	555,036
Research: human or animal biology	158,880	189,450	882,024	218,541	201,636	161,990	2,839,472	190,894	211,778	5,054,665
Research: human or animal health and welfare	298,611	283,546	286,375	303,050	1,024,919	152,360	104,590	116,835	115,119	2,685,405
Research: animal management or production	71,722	94,019	81,831	227,769	76,422	91,603	111,880	167,998	616,015	1,539,259
Research: environmental study	901,504	1,402,726	813,500	1,411,046	1,247,301	5,341,812	1,539,475	1,725,768	694,037	15,077,169
Production of biological products	19,568	74,625	78,419	109,229	28,870	54,811	42,890	55,365	90,866	554,643
Diagnostic procedures	3,630	8,540	1,994	1,031	1,310	766	1,307	1,134	18,186	37,898
Regulatory product testing	42,302	24,540	60,945	305,242	275,885	62,770	21,039	16,557	19,292	828,572
Grand Total	1,642,593	2,218,462	2,336,928	2,699,532	2,982,676	6,287,462	4,977,239	2,625,290	2,253,943	28,024,125

2.3 Number of animals used over time by research procedure

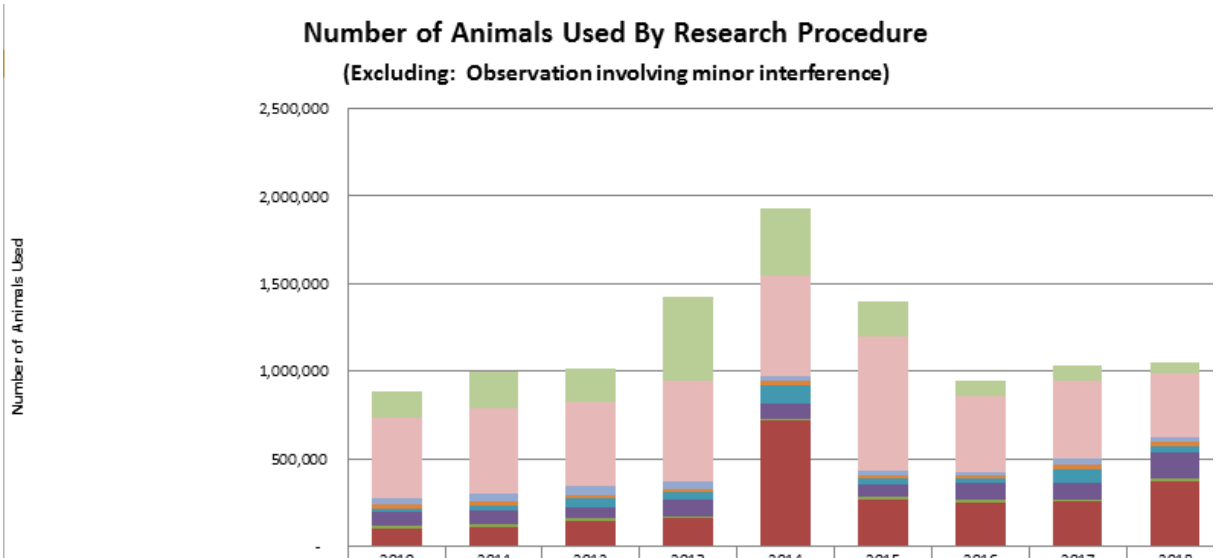


	2010	2011	2012	2013	2014	2015	2016	2017	2018
Observation Involving Minor Interference	760,592	1,217,773	1,320,108	1,276,501	1,054,859	4,887,636	4,030,182	1,594,948	1,199,738
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	365,928
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	155,830
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	369,034

Chart 3: Number of animals used over time by research procedure

	2010	2011	2012	2013	2014	2015	2016	2017	2018	Grand 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	1,594,948	1,199,738	17,342,337
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595	1,842,689
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	365,928	4,588,959
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274	295,628
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872	193,789
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	155,830	819,789
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121	427,206
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551	147,070
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	369,034	2,366,658
Grand Total	**1,642,593**	**2,218,462**	**2,336,928**	**2,699,532**	**2,982,676**	**6,287,462**	**4,977,239**	**2,625,290**	**2,253,943**	**28,024,125**

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



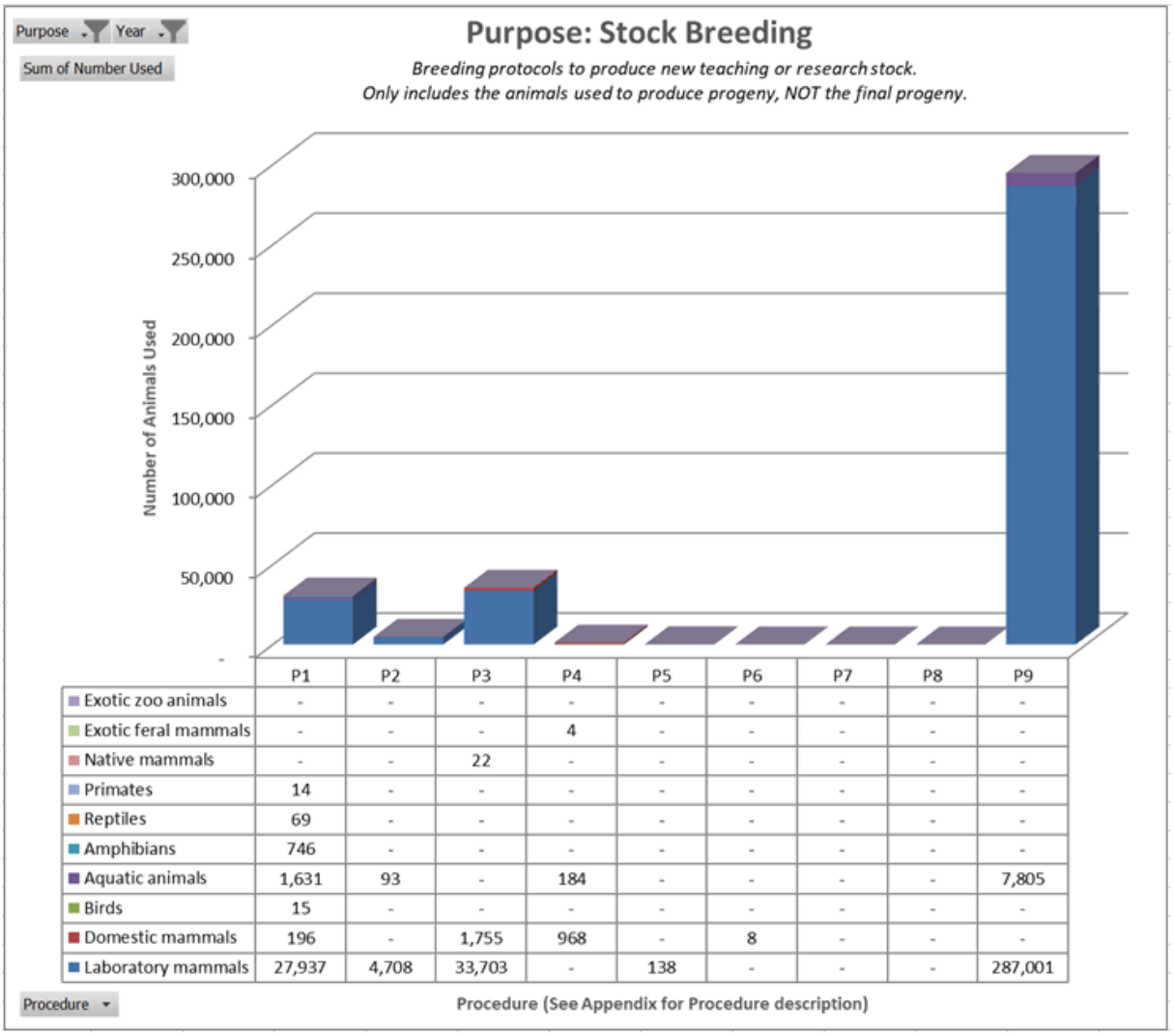
	2010	2011	2012	2013	2014	2015	2016	2017	2018
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	365,928
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	155,830
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	369,034

Chart 4: Number of animals used over time by research procedure excluding "Observation involving minor interference" procedure

	2010	2011	2012	2013	2014	2015	2016	2017	2018	Grand Total
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	65,595	1,842,689
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	365,928	4,588,959
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	27,274	295,628
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	20,872	193,789
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	34,121	427,206
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	155,830	819,789
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	15,551	147,070
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	369,034	2,366,658
Grand Total	**882,001**	**1,000,689**	**1,016,820**	**1,423,031**	**1,927,817**	**1,399,826**	**947,057**	**1,030,342**	**1,054,205**	**10,681,788**

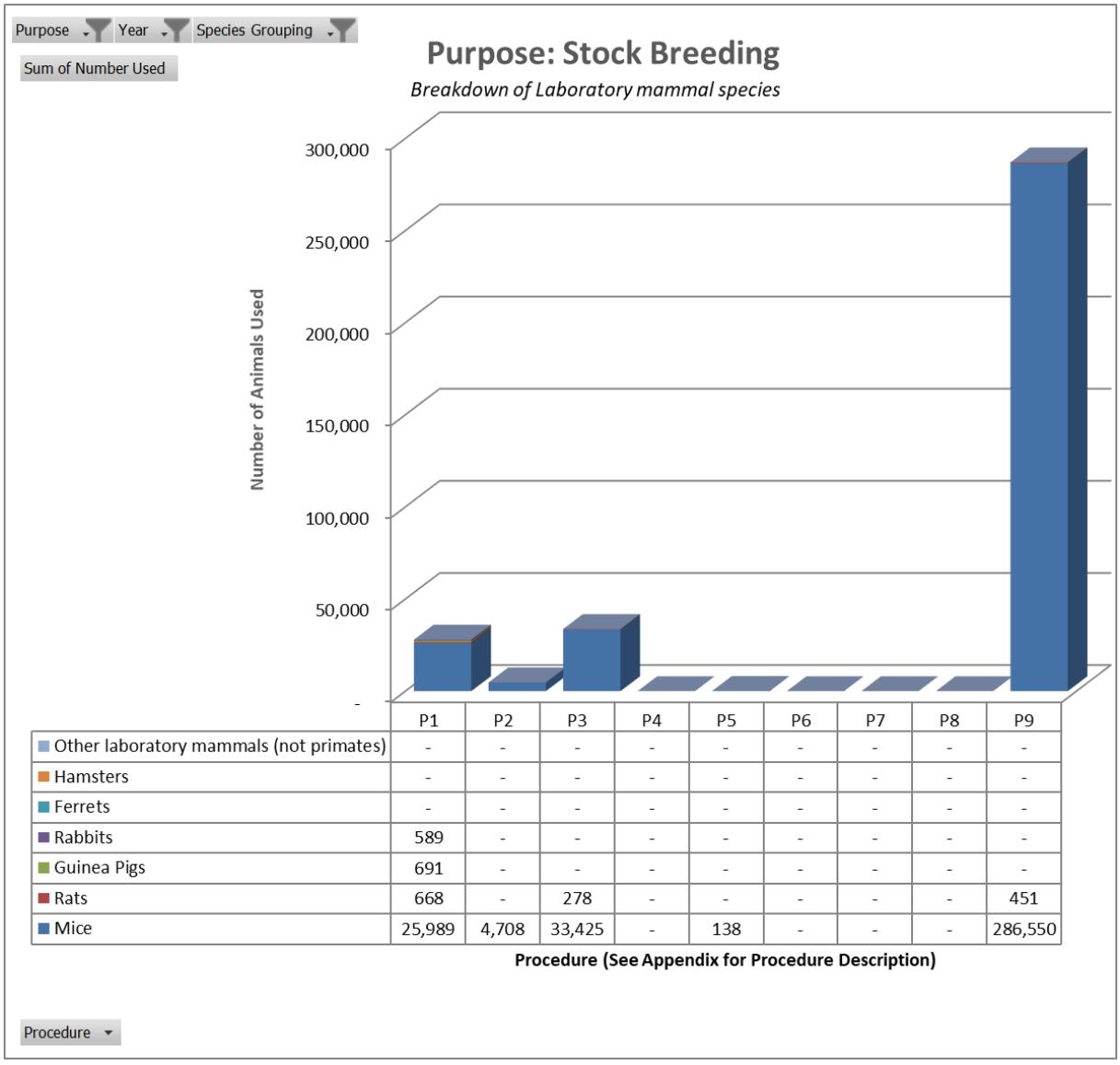
3. Purpose and Species Charts

3.1 Stock Breeding



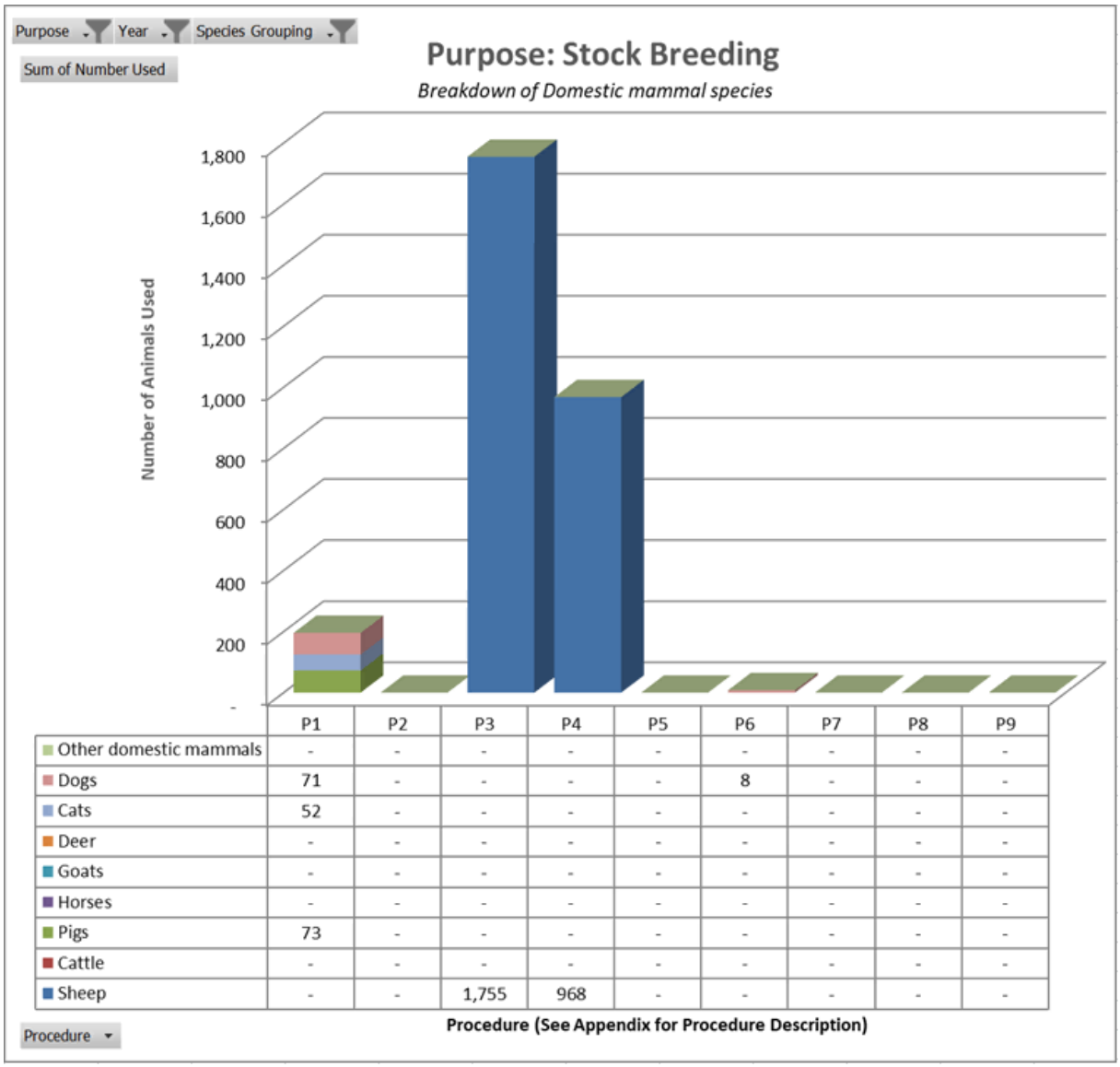
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	-	-	-	4	-	-	-	-	-
Native mammals	-	-	22	-	-	-	-	-	-
Primates	14	-	-	-	-	-	-	-	-
Reptiles	69	-	-	-	-	-	-	-	-
Amphibians	746	-	-	-	-	-	-	-	-
Aquatic animals	1,631	93	-	184	-	-	-	-	7,805
Birds	15	-	-	-	-	-	-	-	-
Domestic mammals	196	-	1,755	968	-	8	-	-	-
Laboratory mammals	27,937	4,708	33,703	-	138	-	-	-	287,001

3.1.1 Species Charts for Stock Breeding

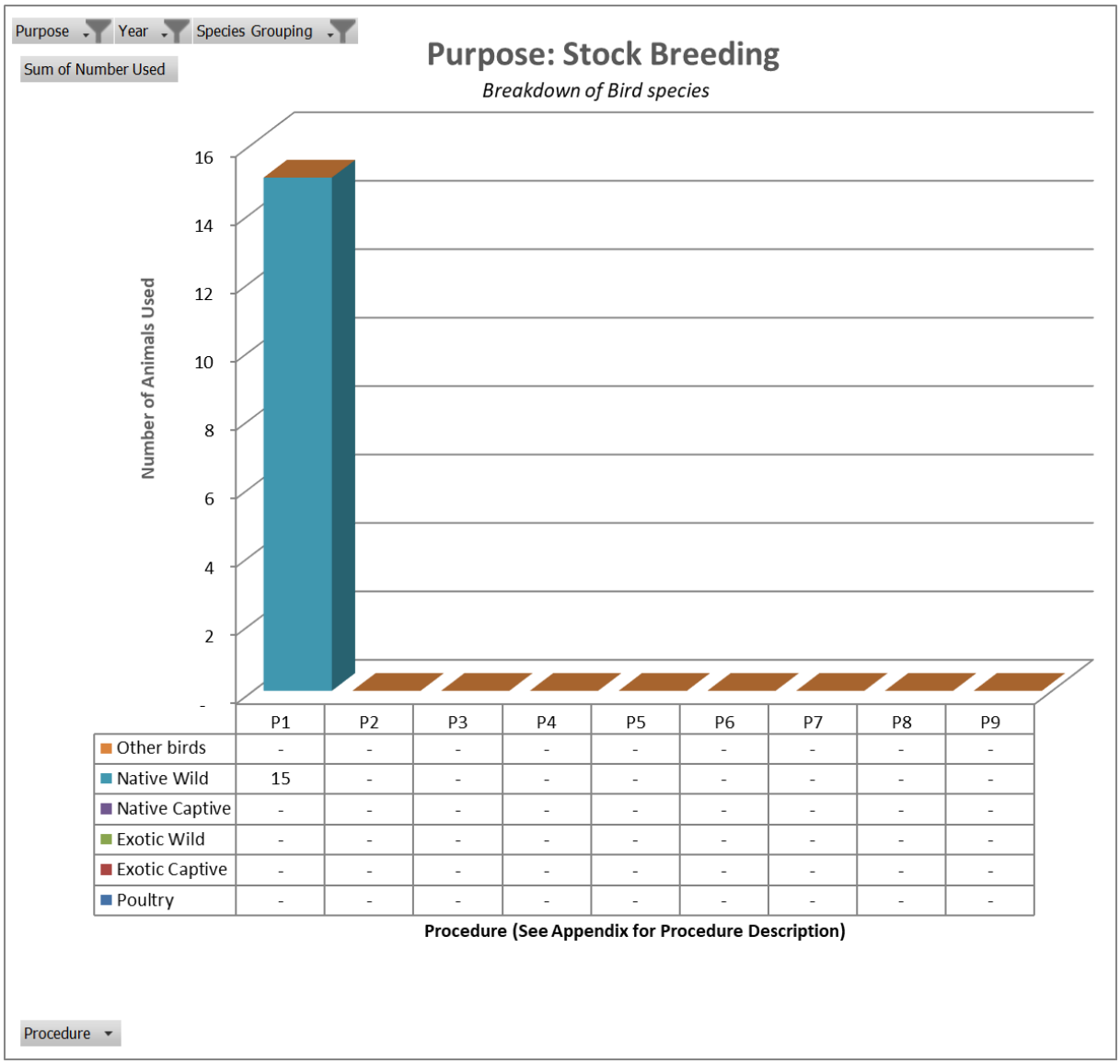


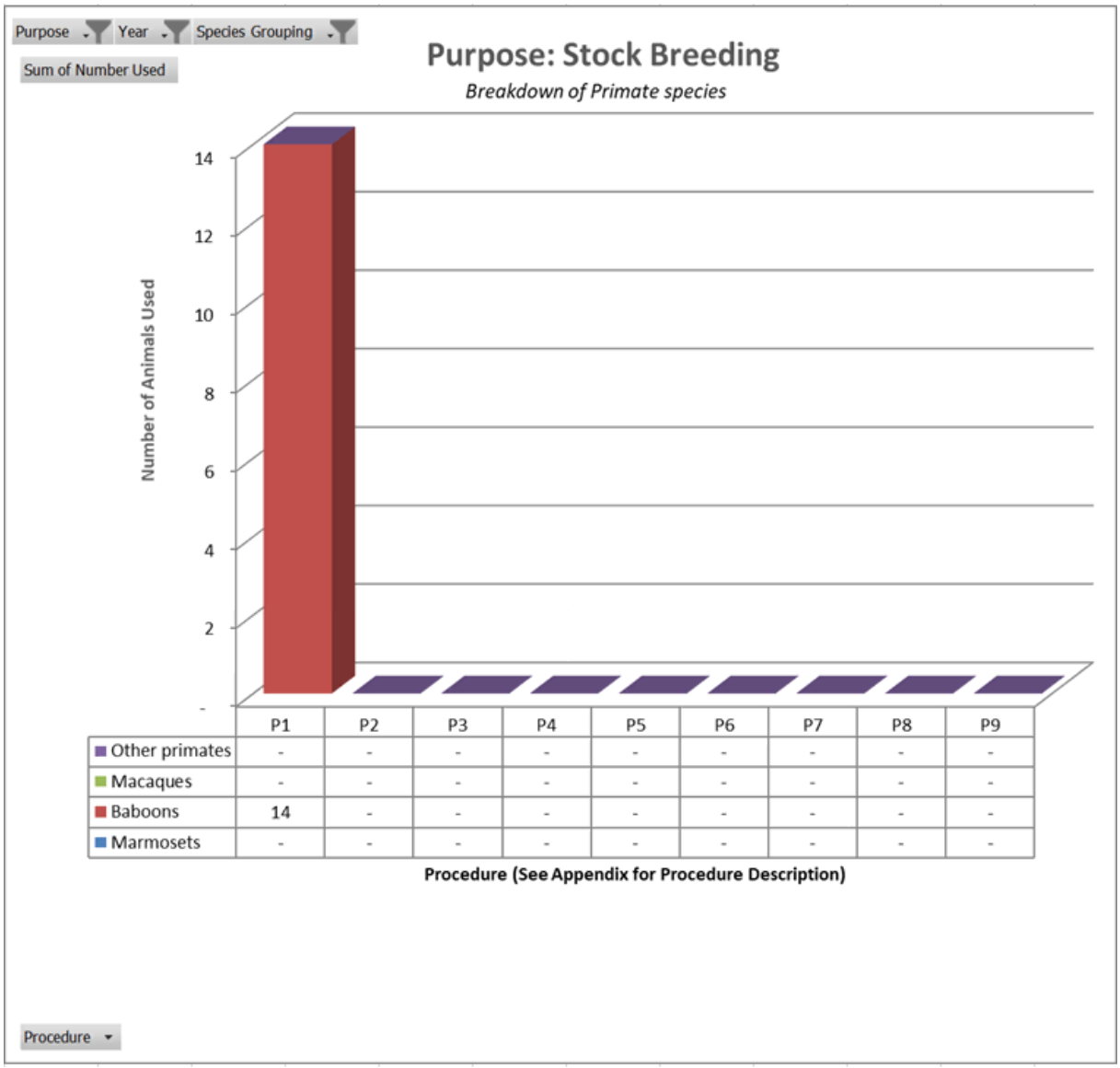
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	-	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	-	-	-	-	-	-	-
Rabbits	589	-	-	-	-	-	-	-	-
Guinea Pigs	691	-	-	-	-	-	-	-	-
Rats	668	-	278	-	-	-	-	-	451
Mice	25,989	4,708	33,425	-	138	-	-	-	286,550

Procedure (See Appendix for Procedure Description)

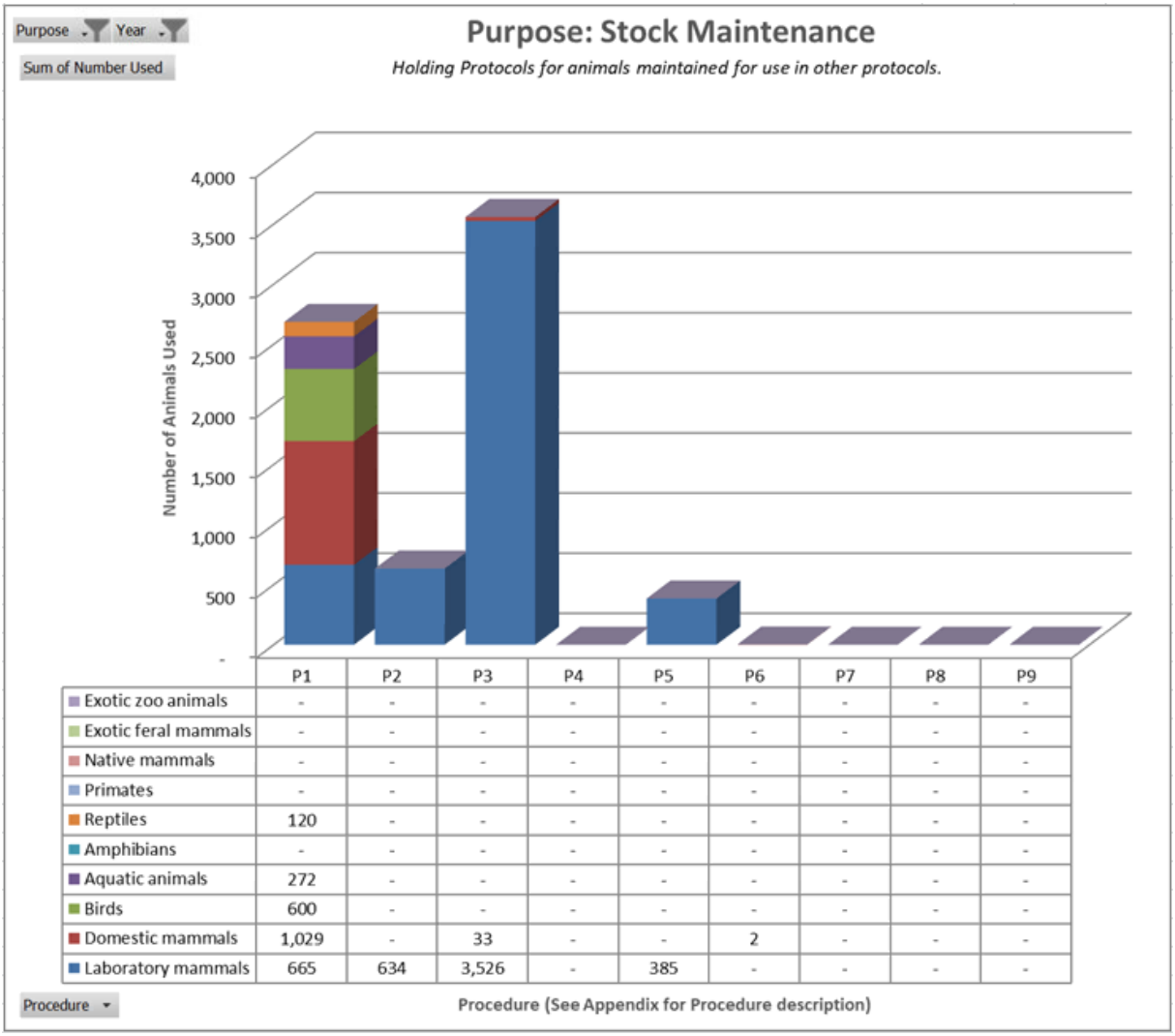


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	71	-	-	-	-	8	-	-	-
Cats	52	-	-	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	-	-	-	-	-	-	-
Pigs	73	-	-	-	-	-	-	-	-
Cattle	-	-	-	-	-	-	-	-	-
Sheep	-	-	1,755	968	-	-	-	-	-


Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Stock Breeding
Breakdown of Bird species
Number of Animals Used
16
14
12
10
8
6
4
2
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other birds - - - - - - - - -
Native Wild 15 - - - - - - - -
Native Captive - - - - - - - - -
Exotic Wild - - - - - - - - -
Exotic Captive - - - - - - - - -
Poultry - - - - - - - - -
Procedure (See Appendix for Procedure Description)
Procedure

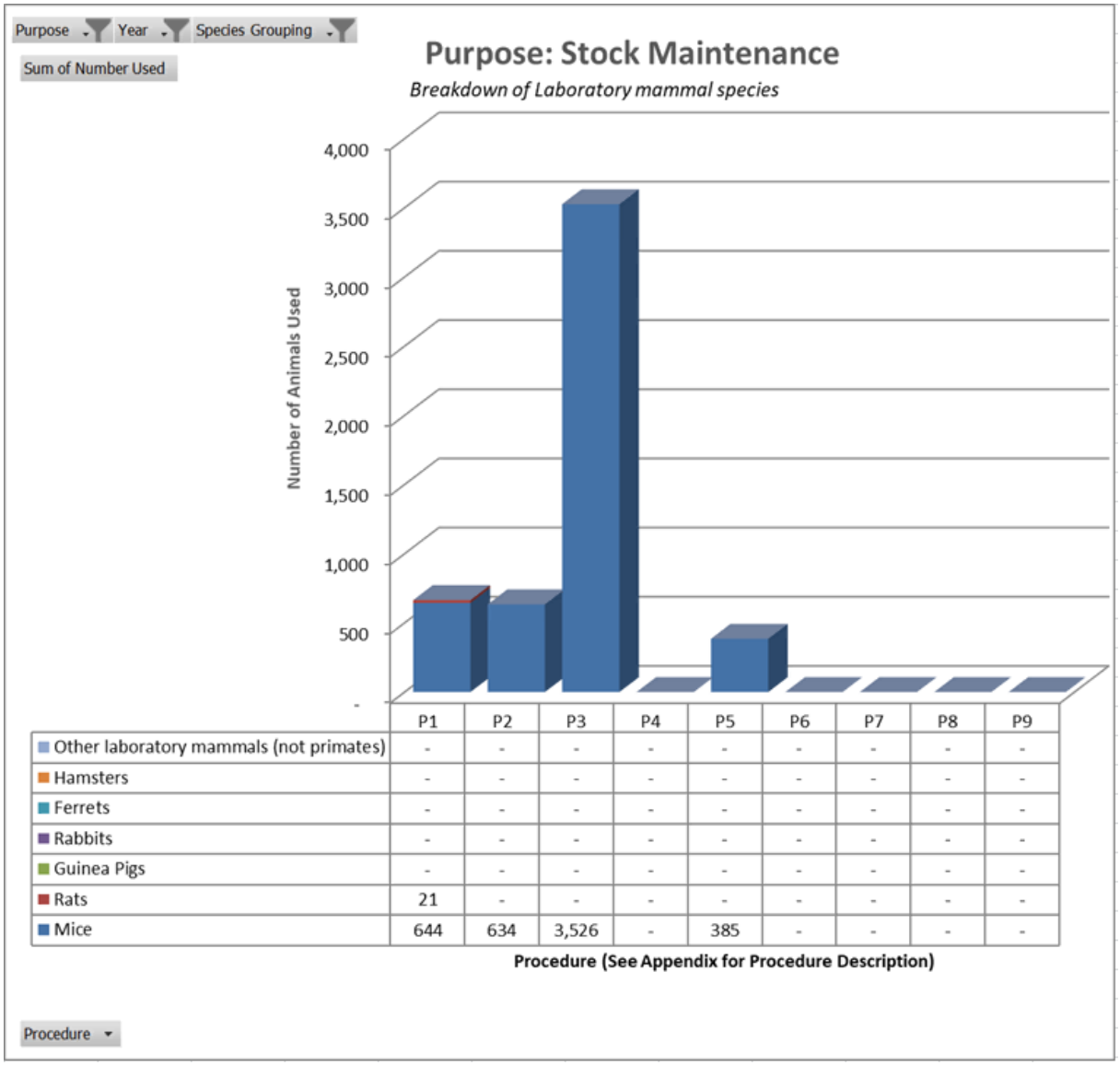

Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Stock Breeding
Breakdown of Primate species
Number of Animals Used
14
12
10
8
6
4
2
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other primates - - - - - - - - -
Macaques - - - - - - - - -
Baboons 14 - - - - - - - -
Marmosets - - - - - - - - -
Procedure (See Appendix for Procedure Description)
Procedure

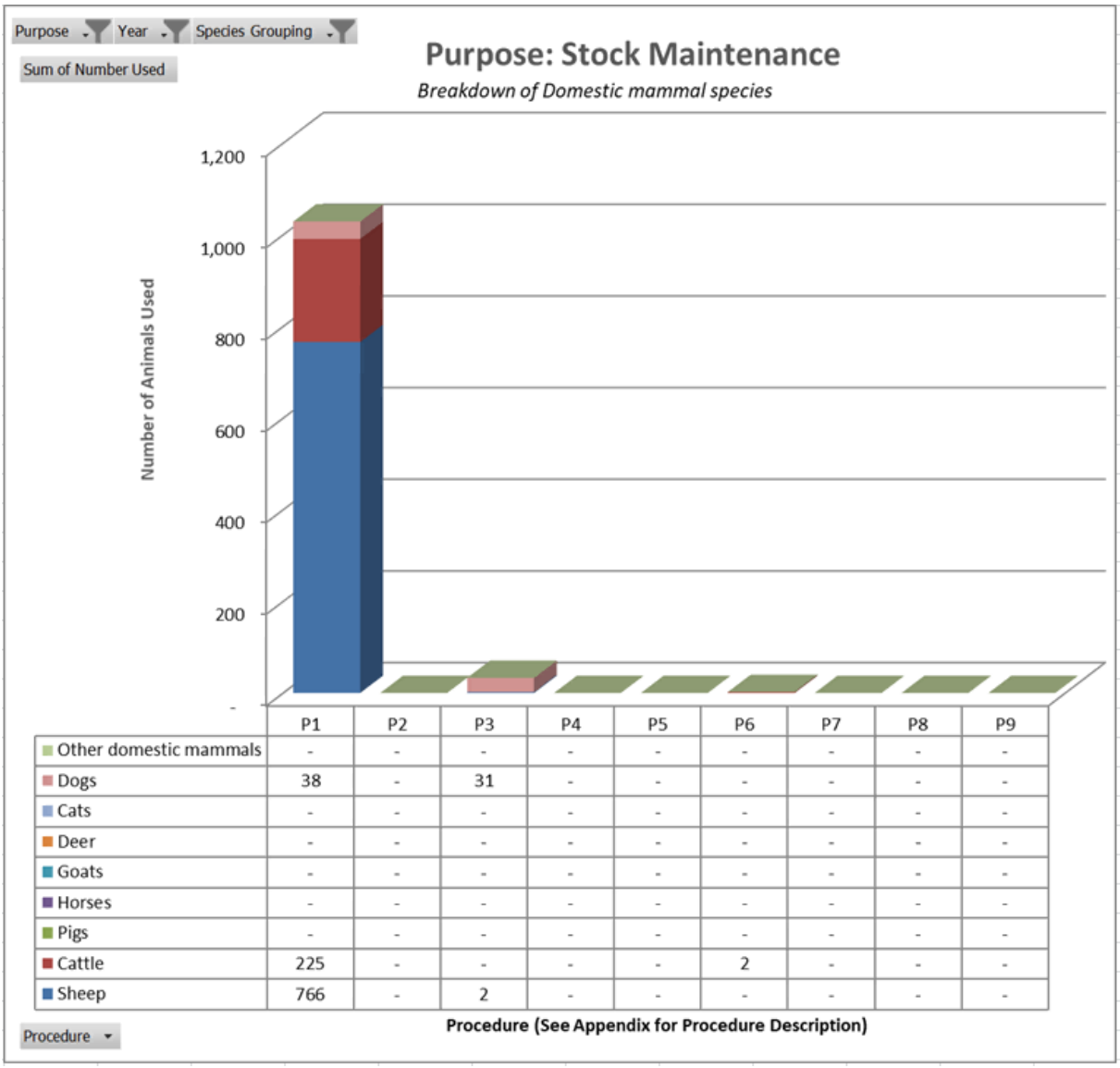
3.2 Stock Maintenance



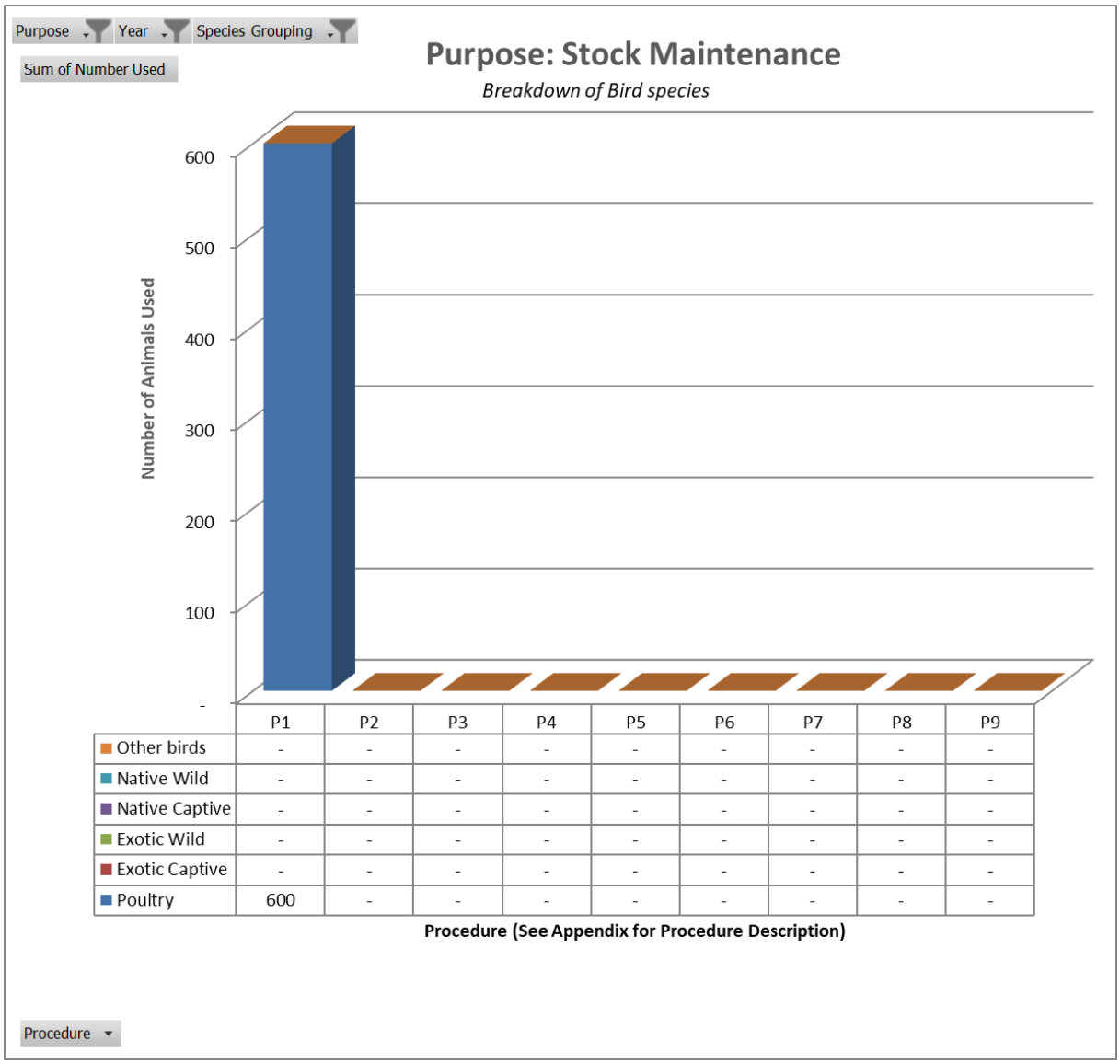
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	-	-	-	-	-	-	-	-	-
Native mammals	-	-	-	-	-	-	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	120	-	-	-	-	-	-	-	-
Amphibians	-	-	-	-	-	-	-	-	-
Aquatic animals	272	-	-	-	-	-	-	-	-
Birds	600	-	-	-	-	-	-	-	-
Domestic mammals	1,029	-	33	-	-	2	-	-	-
Laboratory mammals	665	634	3,526	-	385	-	-	-	-

3.2.1 Species Charts for Stock Maintenance

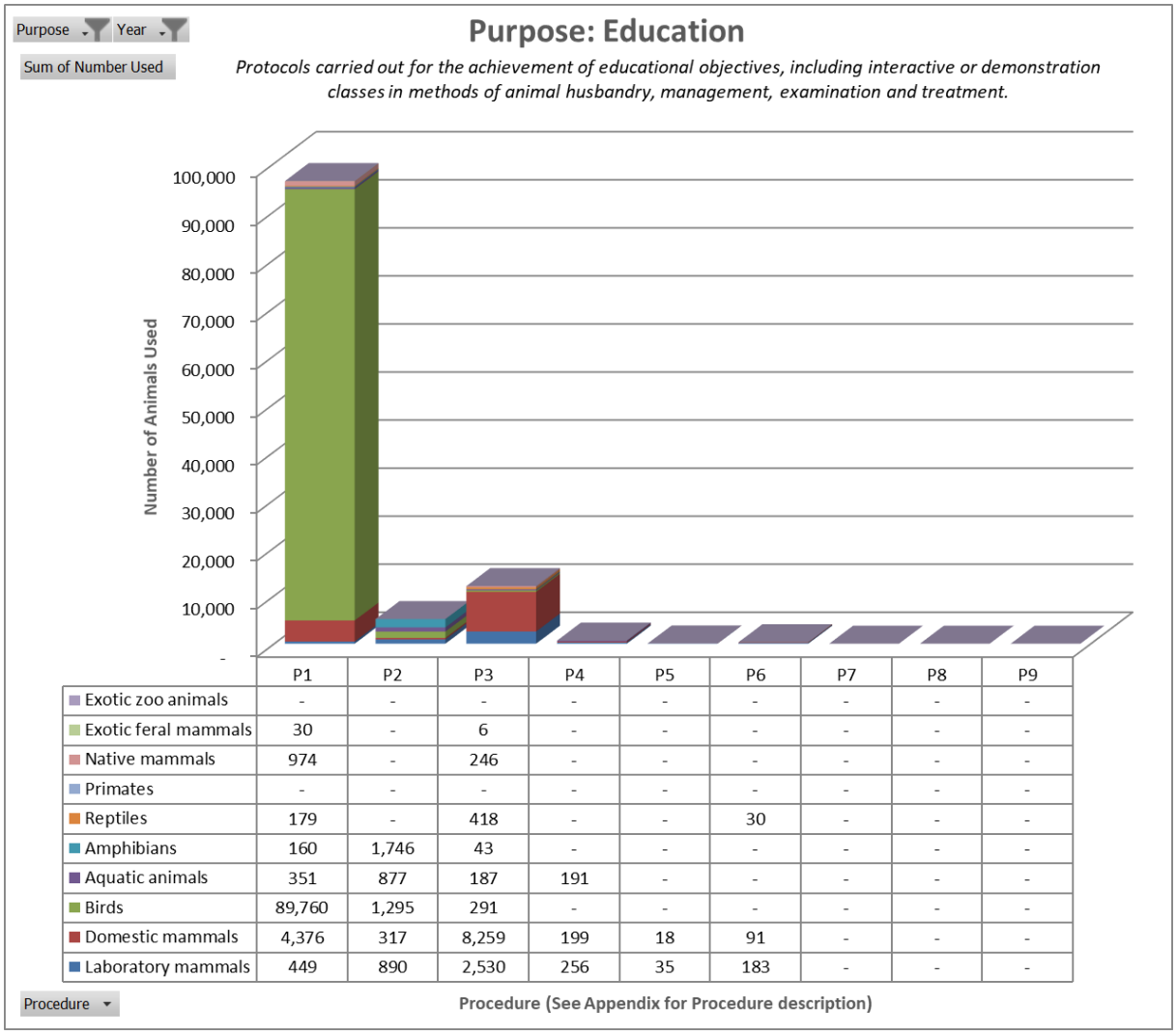




	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	38	-	31	-	-	-	-	-	-
Cats	-	-	-	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	-	-	-	-	-	-	-
Pigs	-	-	-	-	-	-	-	-	-
Cattle	225	-	-	-	-	2	-	-	-
Sheep	766	-	2	-	-	-	-	-	-

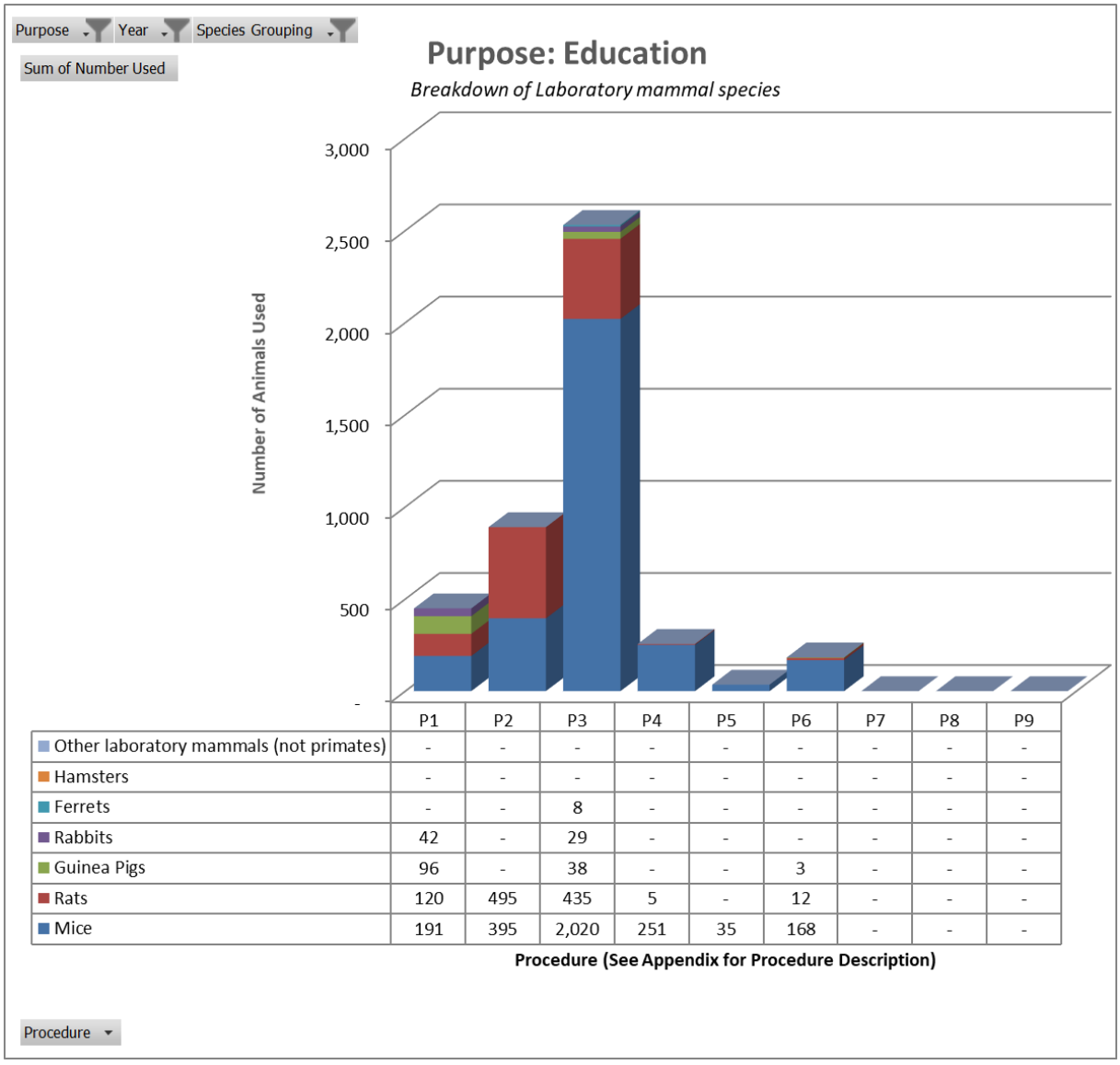

Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Stock Maintenance
Breakdown of Bird species
Number of Animals Used
600
500
400
300
200
100
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other birds - - - - - - - - -
Native Wild - - - - - - - - -
Native Captive - - - - - - - - -
Exotic Wild - - - - - - - - -
Exotic Captive - - - - - - - - -
Poultry 600 - - - - - - - -
Procedure (See Appendix for Procedure Description)
Procedure

3.3 Education

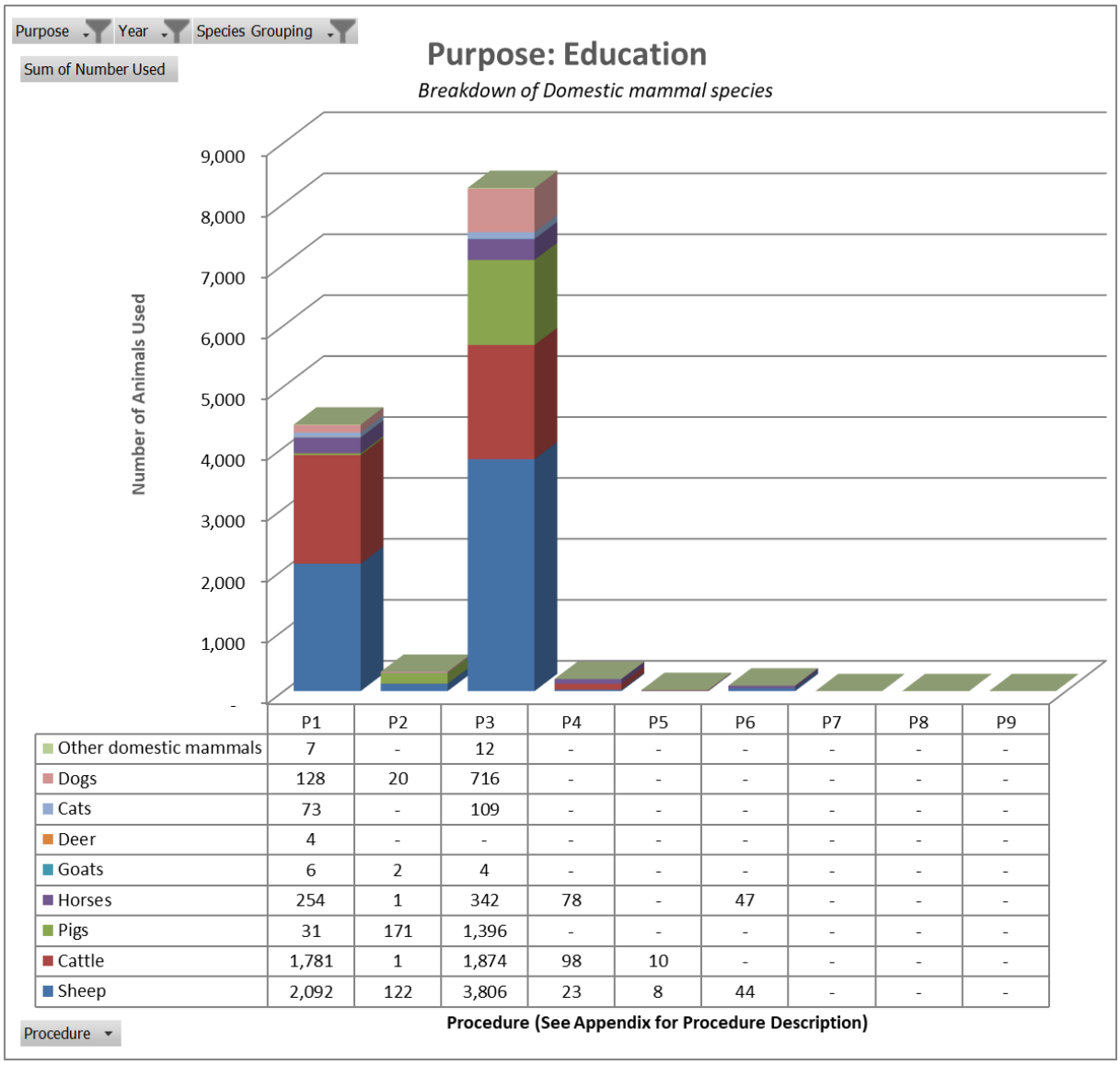


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	30	-	6	-	-	-	-	-	-
Native mammals	974	-	246	-	-	-	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	179	-	418	-	-	30	-	-	-
Amphibians	160	1,746	43	-	-	-	-	-	-
Aquatic animals	351	877	187	191	-	-	-	-	-
Birds	89,760	1,295	291	-	-	-	-	-	-
Domestic mammals	4,376	317	8,259	199	18	91	-	-	-
Laboratory mammals	449	890	2,530	256	35	183	-	-	-

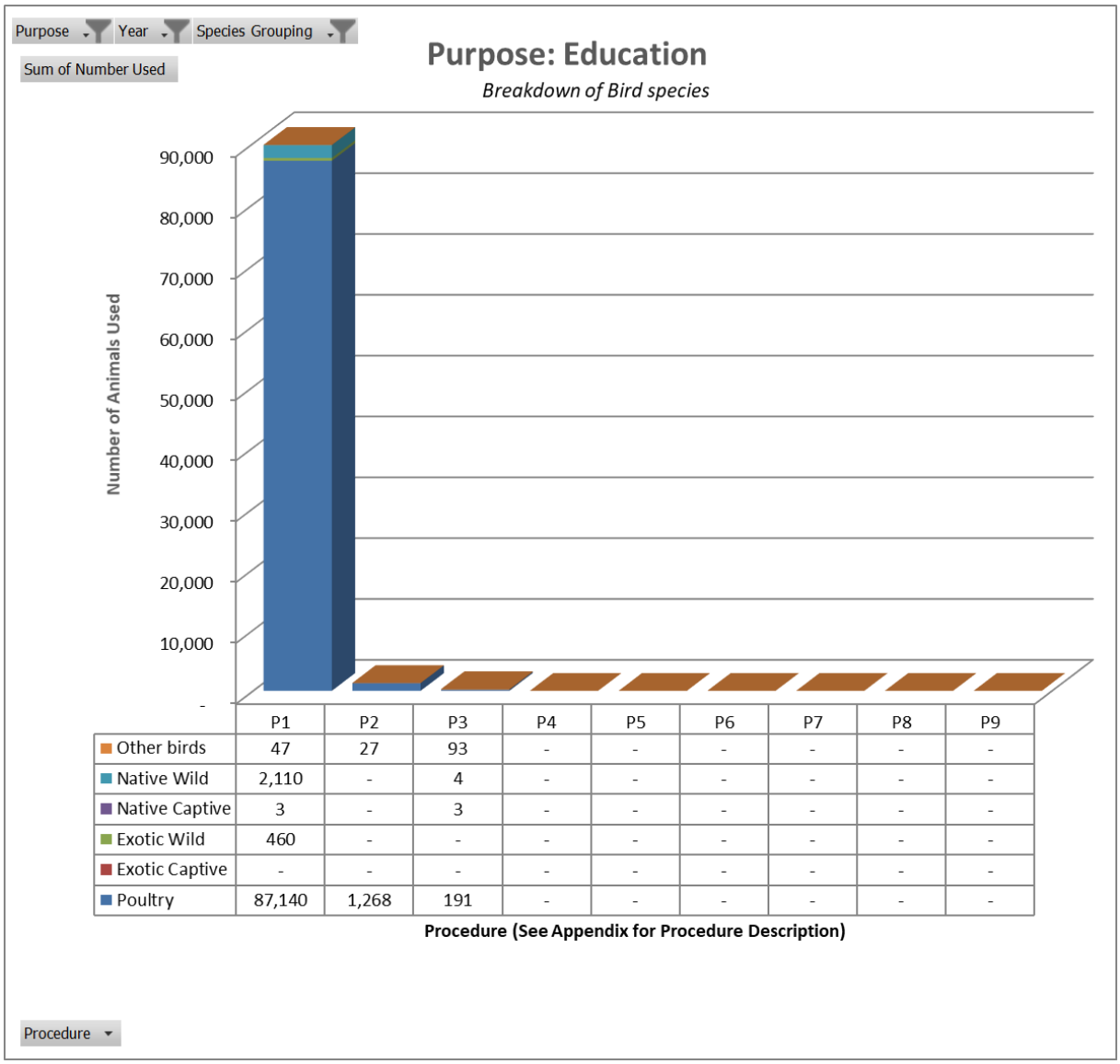
3.3.1 Species Charts for Education



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	-	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	8	-	-	-	-	-	-
Rabbits	42	-	29	-	-	-	-	-	-
Guinea Pigs	96	-	38	-	-	3	-	-	-
Rats	120	495	435	5	-	12	-	-	-
Mice	191	395	2,020	251	35	168	-	-	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	7	-	12	-	-	-	-	-	-
Dogs	128	20	716	-	-	-	-	-	-
Cats	73	-	109	-	-	-	-	-	-
Deer	4	-	-	-	-	-	-	-	-
Goats	6	2	4	-	-	-	-	-	-
Horses	254	1	342	78	-	47	-	-	-
Pigs	31	171	1,396	-	-	-	-	-	-
Cattle	1,781	1	1,874	98	10	-	-	-	-
Sheep	2,092	122	3,806	23	8	44	-	-	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other birds	47	27	93	-	-	-	-	-	-
Native Wild	2,110	-	4	-	-	-	-	-	-
Native Captive	3	-	3	-	-	-	-	-	-
Exotic Wild	460	-	-	-	-	-	-	-	-
Exotic Captive	-	-	-	-	-	-	-	-	-
Poultry	87,140	1,268	191	-	-	-	-	-	-

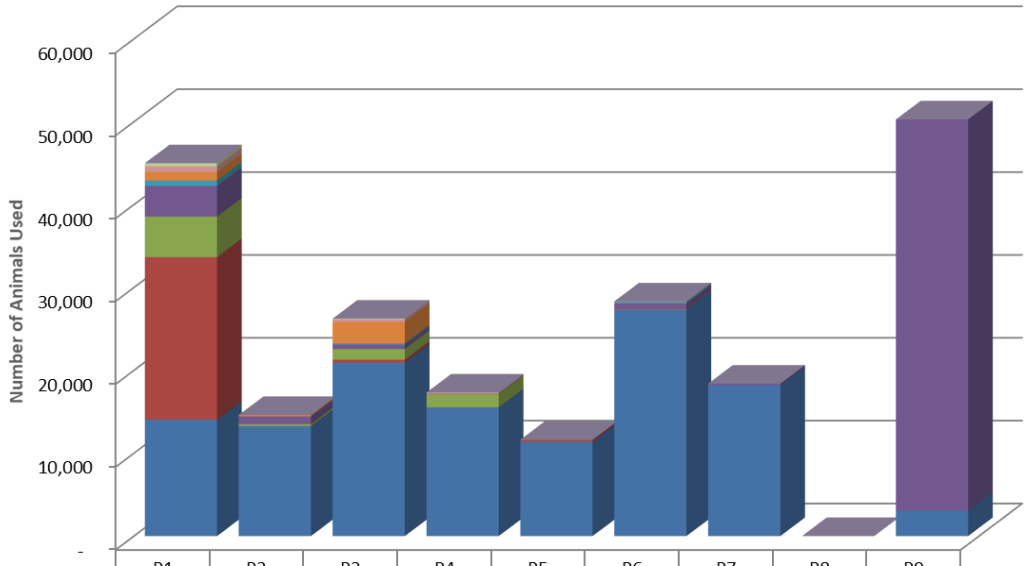
3.4 Research: Human or Animal Biology

Purpose | Year

Sum of Number Used

Purpose: Research: human or animal biology

Research protocols which aim to increase the basic understanding of the structure, function and behaviour of animals, including humans, and processes involoved in physiology, biochemistry and pathology.

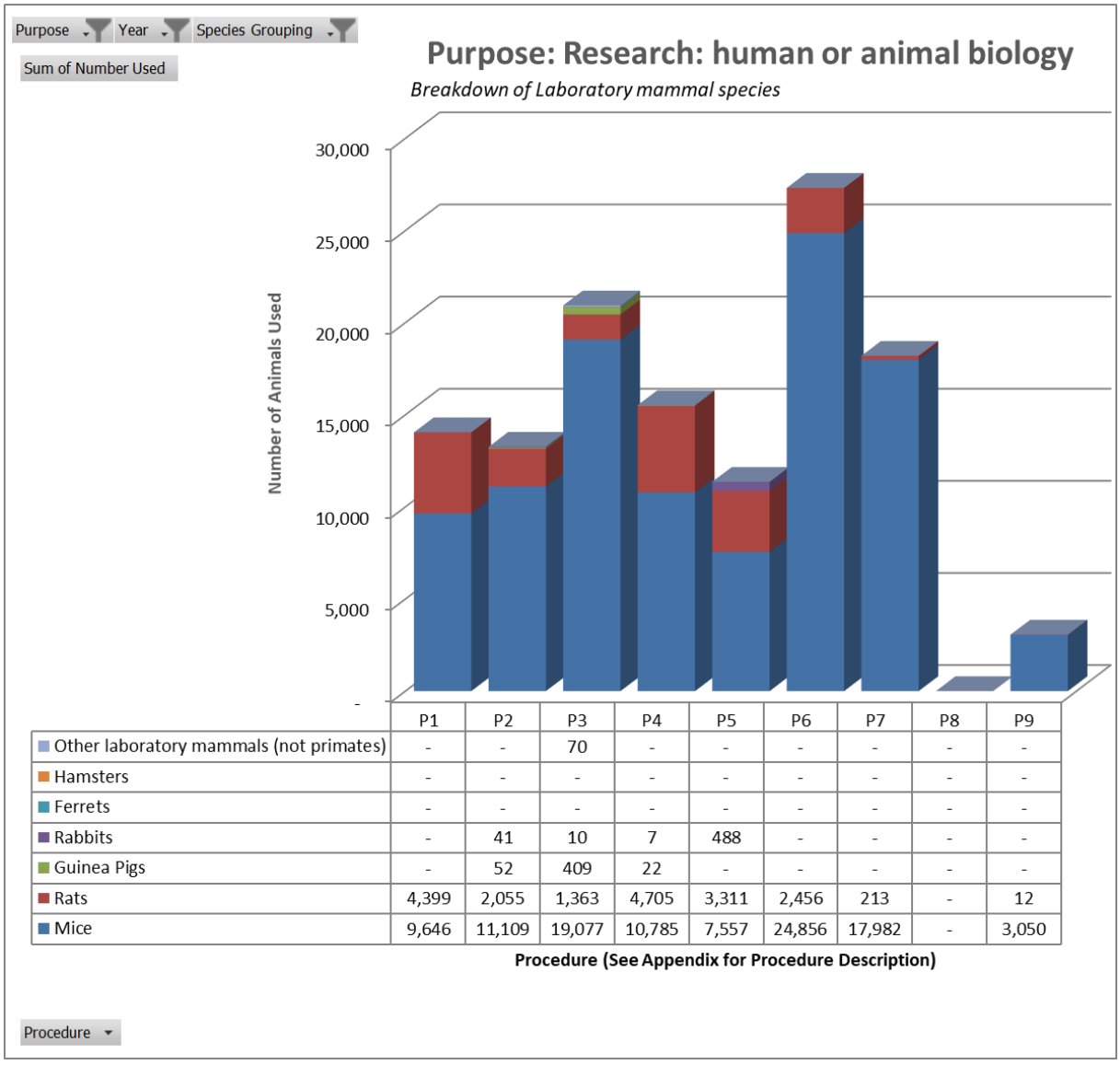


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	6	-	-	-	-	-
Exotic feral mammals	371	-	83	26	-	-	-	-	-
Native mammals	684	1	326	122	1	21	-	-	-
Primates	8	-	2	-	2	-	-	-	-
Reptiles	1,017	153	2,561	-	-	39	-	-	-
Amphibians	650	-	152	-	38	200	-	-	-
Aquatic animals	3,705	946	540	55	-	680	198	-	47,216
Birds	4,896	243	1,292	1,582	-	32	-	-	-
Domestic mammals	19,614	8	351	14	233	35	-	-	-
Laboratory mammals	14,045	13,257	20,929	15,519	11,356	27,312	18,195	-	3,062

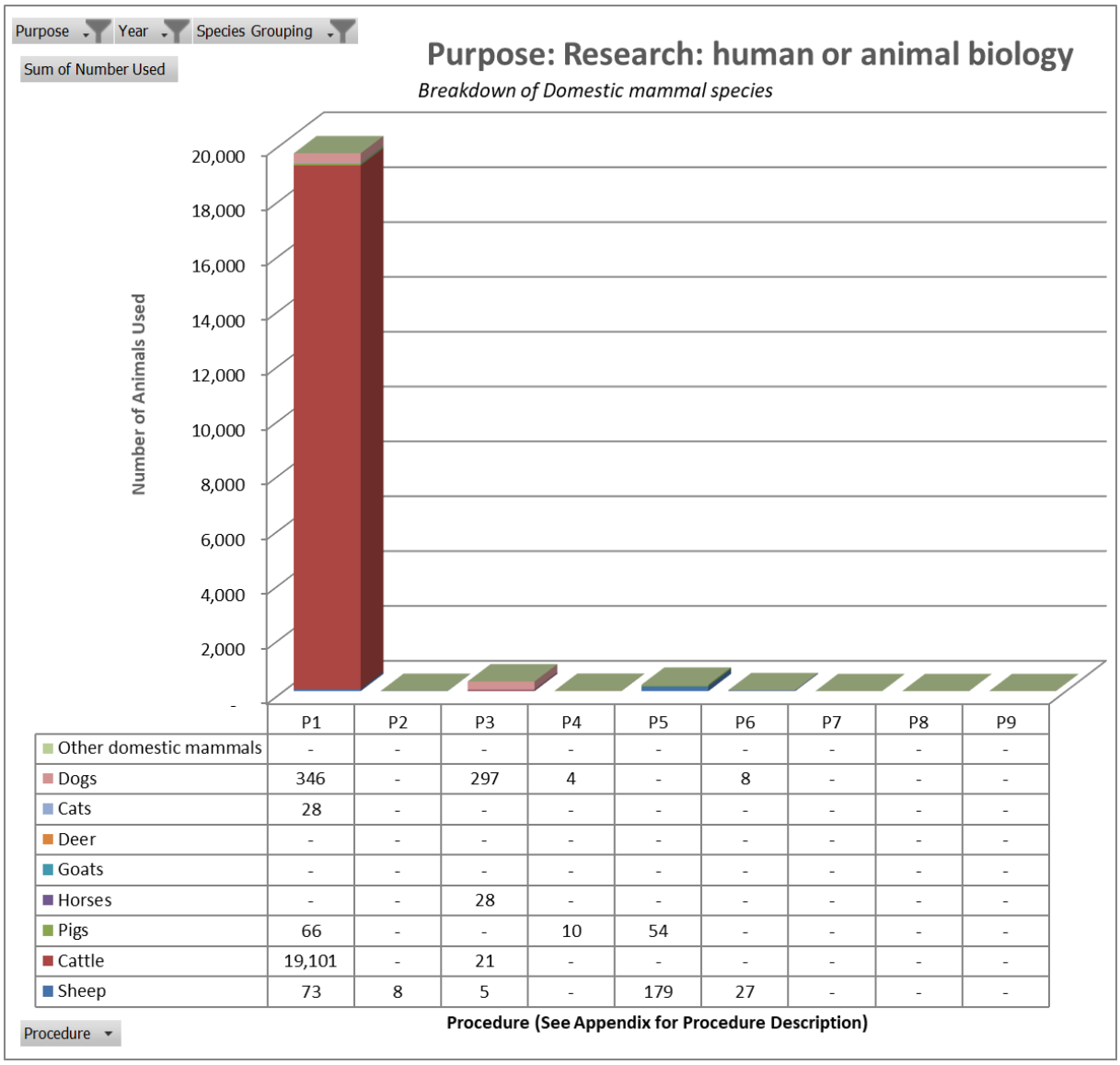
Procedure

Procedure (See Appendix for Procedure description)

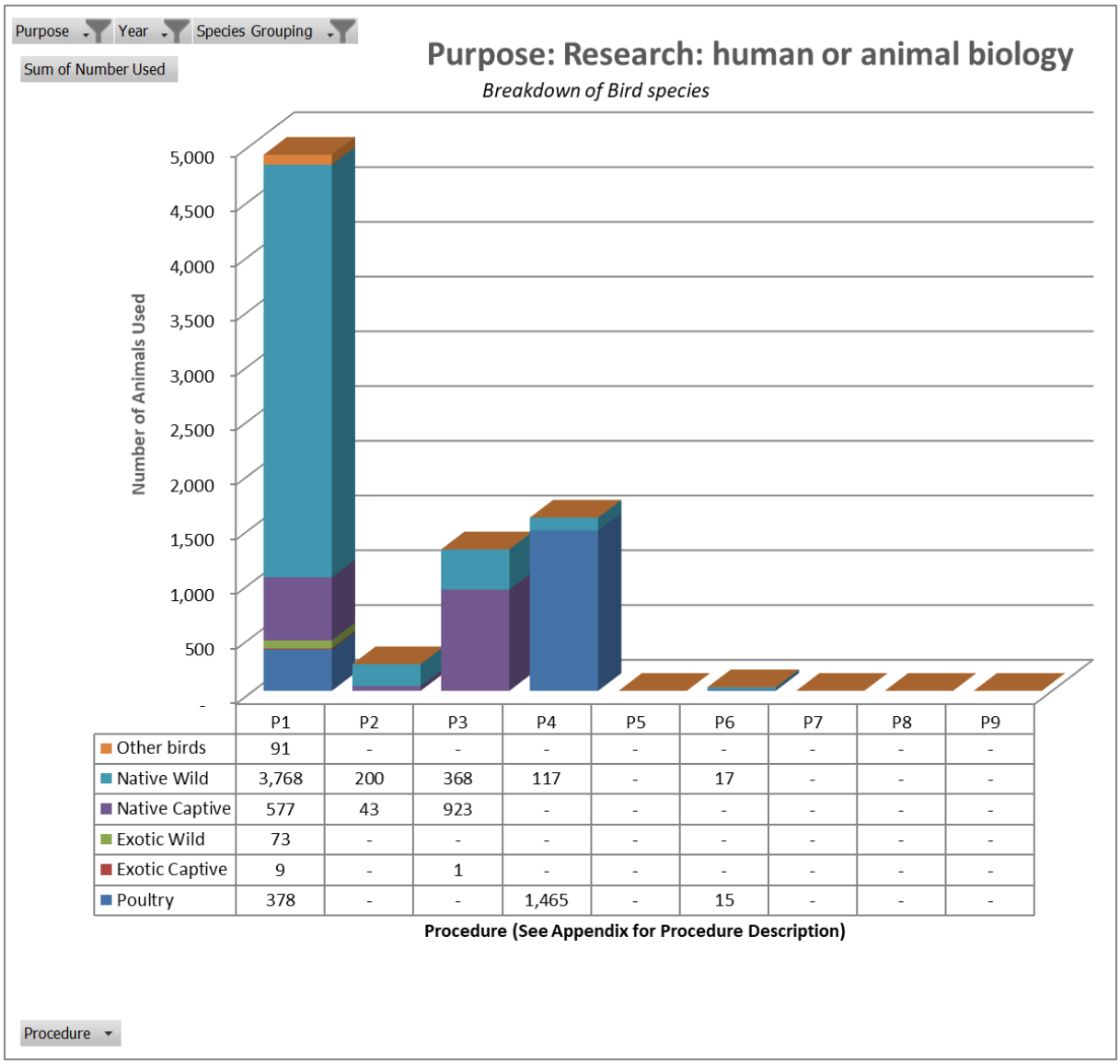
3.4.1 Species Charts for Research: Human or Animal Biology



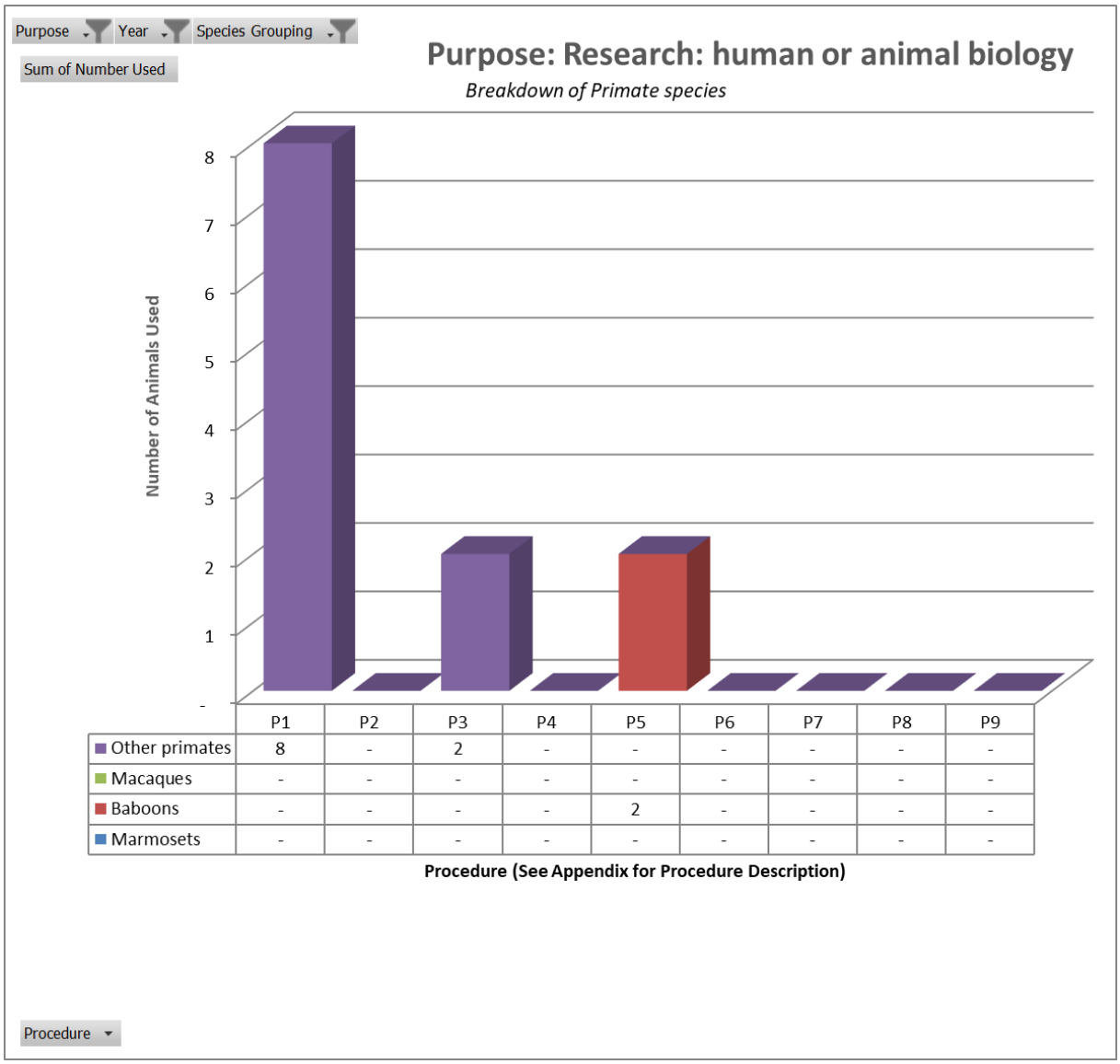
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	70	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	-	-	-	-	-	-	-
Rabbits	-	41	10	7	488	-	-	-	-
Guinea Pigs	-	52	409	22	-	-	-	-	-
Rats	4,399	2,055	1,363	4,705	3,311	2,456	213	-	12
Mice	9,646	11,109	19,077	10,785	7,557	24,856	17,982	-	3,050



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	346	-	297	4	-	8	-	-	-
Cats	28	-	-	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	28	-	-	-	-	-	-
Pigs	66	-	-	10	54	-	-	-	-
Cattle	19,101	-	21	-	-	-	-	-	-
Sheep	73	8	5	-	179	27	-	-	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other birds	91	-	-	-	-	-	-	-	-
Native Wild	3,768	200	368	117	-	17	-	-	-
Native Captive	577	43	923	-	-	-	-	-	-
Exotic Wild	73	-	-	-	-	-	-	-	-
Exotic Captive	9	-	1	-	-	-	-	-	-
Poultry	378	-	-	1,465	-	15	-	-	-


Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Research: human or animal biology
Breakdown of Primate species
Number of Animals Used
8
7
6
5
4
3
2
1
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other primates 8 - 2 - - - - - -
Macaques - - - - - - - - -
Baboons - - - - 2 - - - -
Marmosets - - - - - - - - -
Procedure (See Appendix for Procedure Description)
Procedure

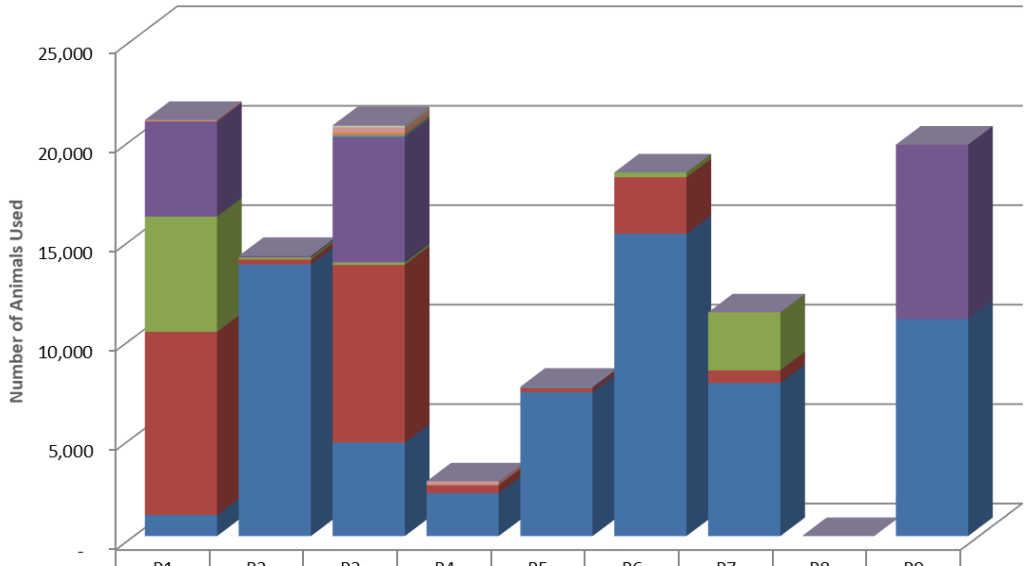
3.5 Research: Human or Animal Health and Welfare

Purpose | Year

Sum of Number Used

Purpose: Research: human or animal health and welfare

Research protocols which aim to produce improvements in the health and welfare of animals, including humans.

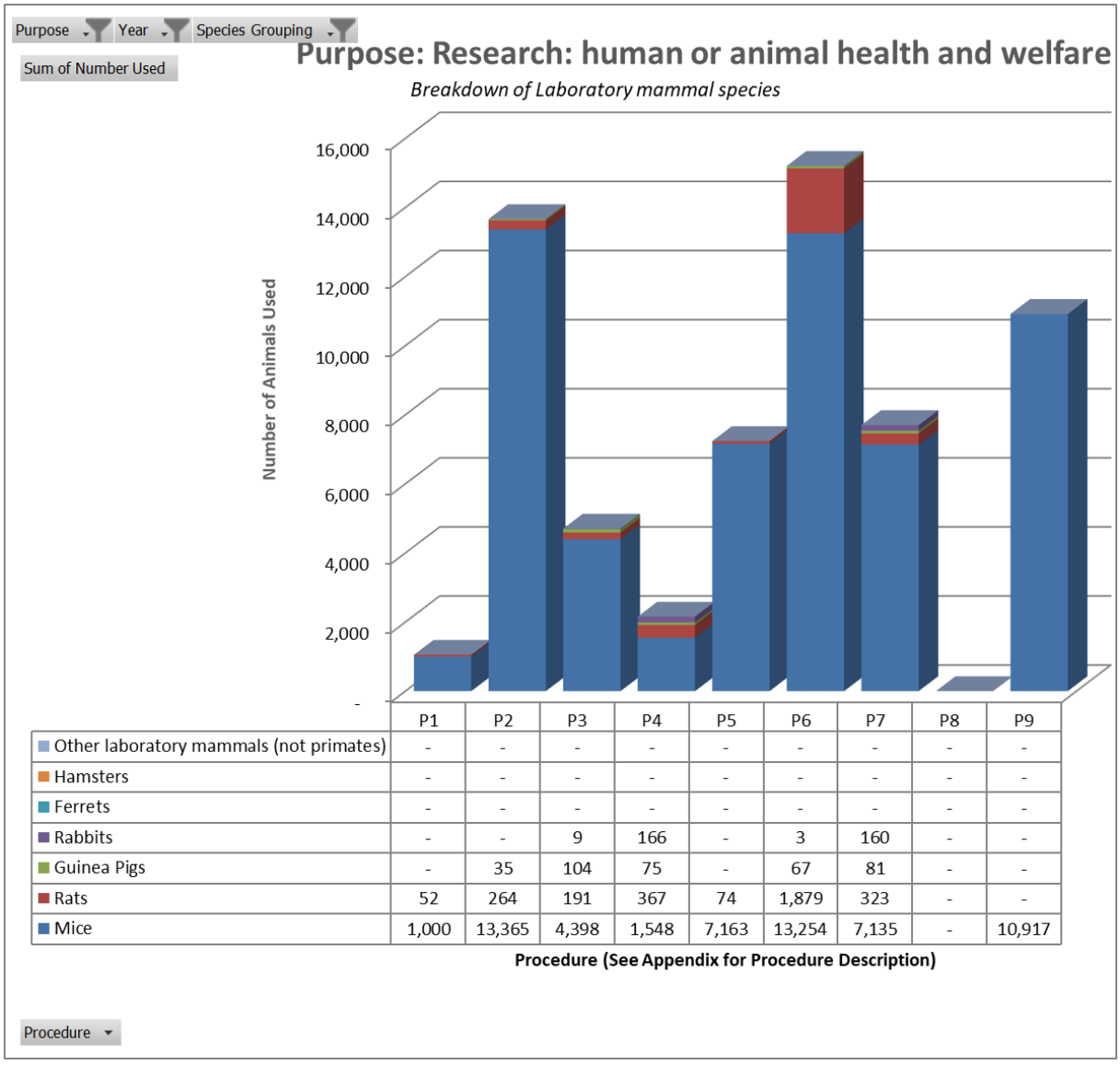


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	2	-	29	-	-	-	-	-	-
Exotic feral mammals	-	-	74	1	-	-	-	-	-
Native mammals	45	-	274	169	4	-	-	-	-
Primates	-	-	-	11	7	-	-	-	-
Reptiles	29	-	123	10	-	-	12	-	-
Amphibians	-	-	80	-	47	-	-	-	-
Aquatic animals	4,782	75	6,306	-	-	-	-	-	8,763
Birds	5,796	110	125	-	-	244	2,905	-	-
Domestic mammals	9,216	235	8,934	402	213	2,836	630	-	-
Laboratory mammals	1,052	13,664	4,702	2,156	7,237	15,203	7,699	-	10,917

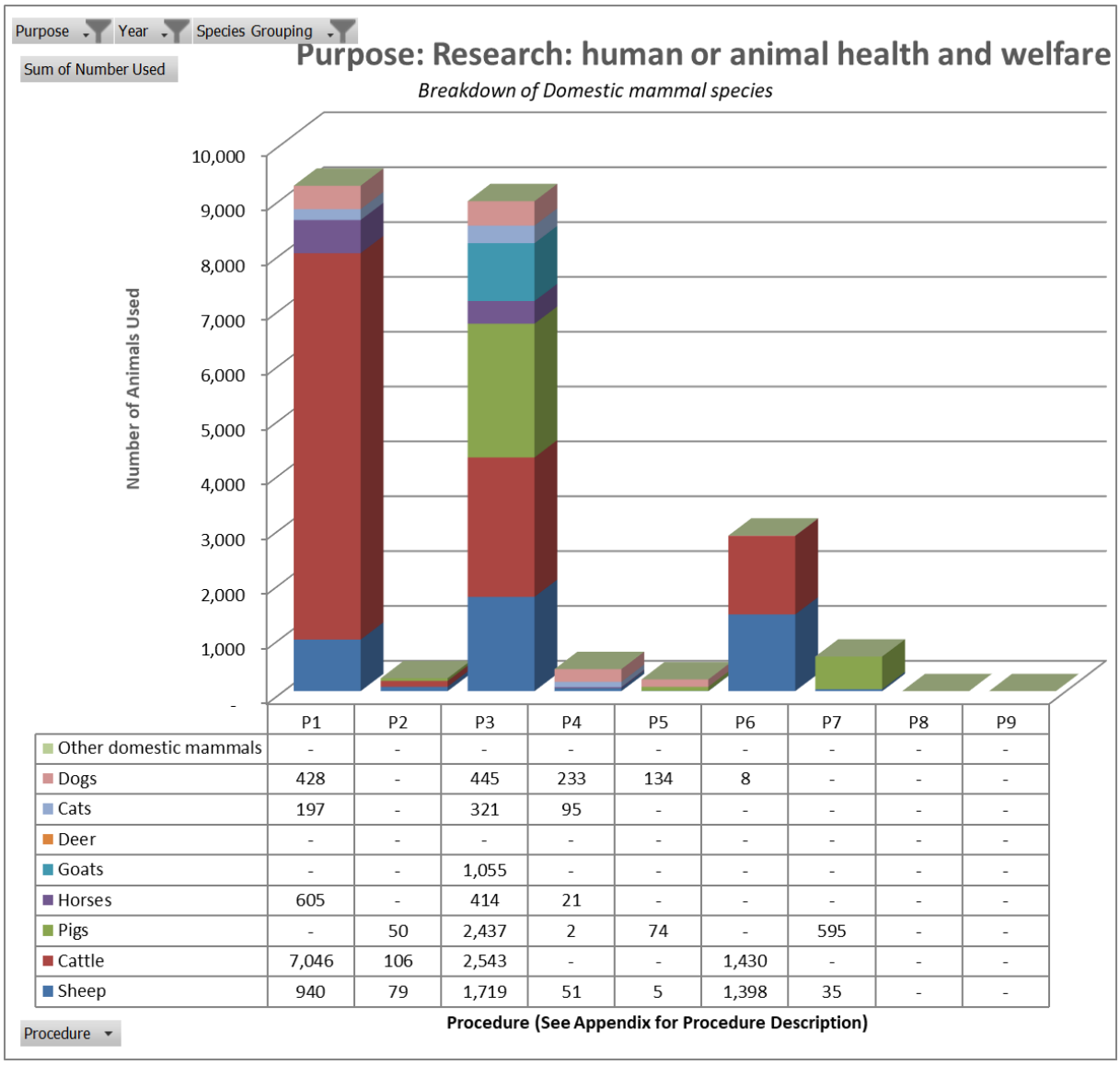
Procedure

Procedure (See Appendix for Procedure description)

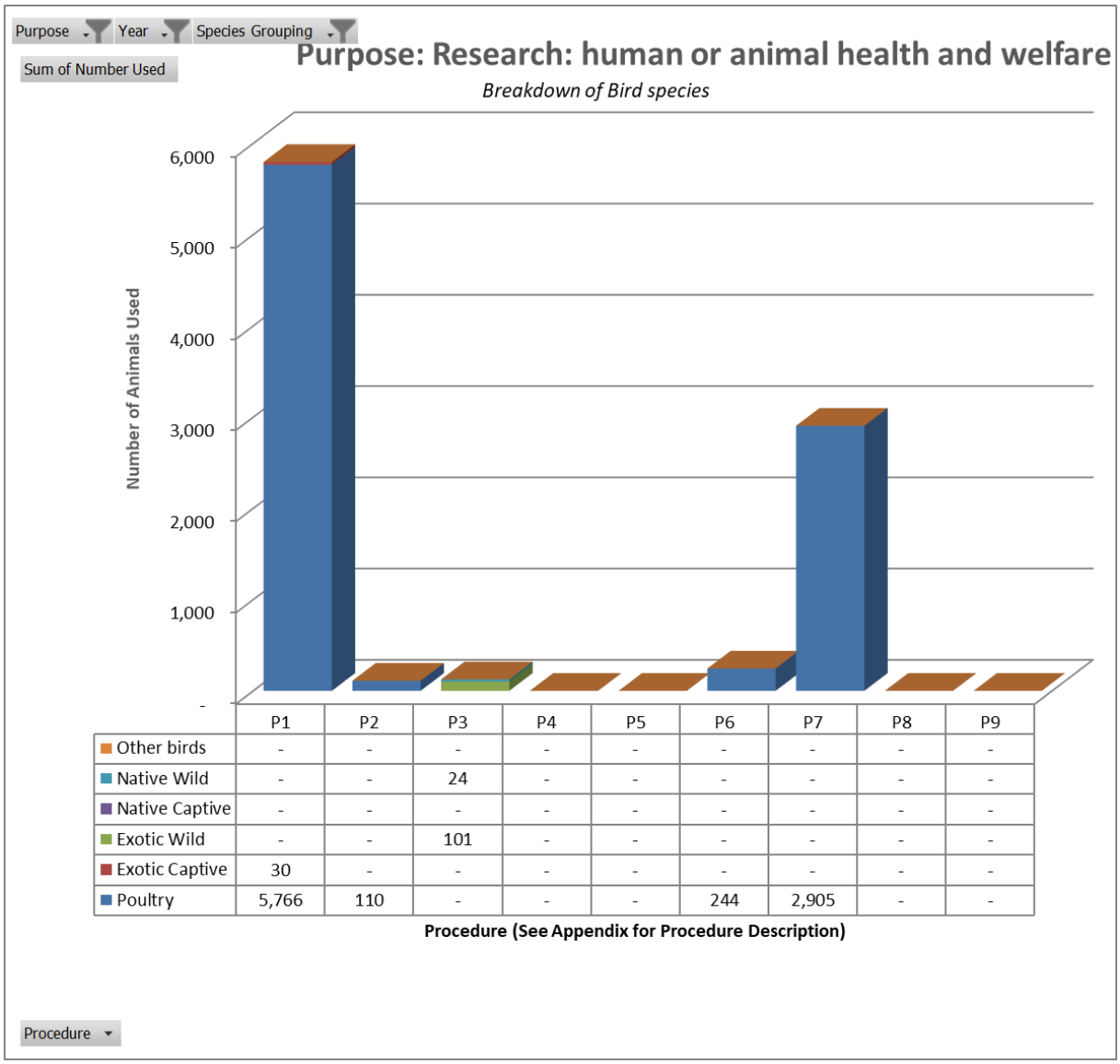
3.5.1 Species Charts for Research: Human or Animal Health and Welfare



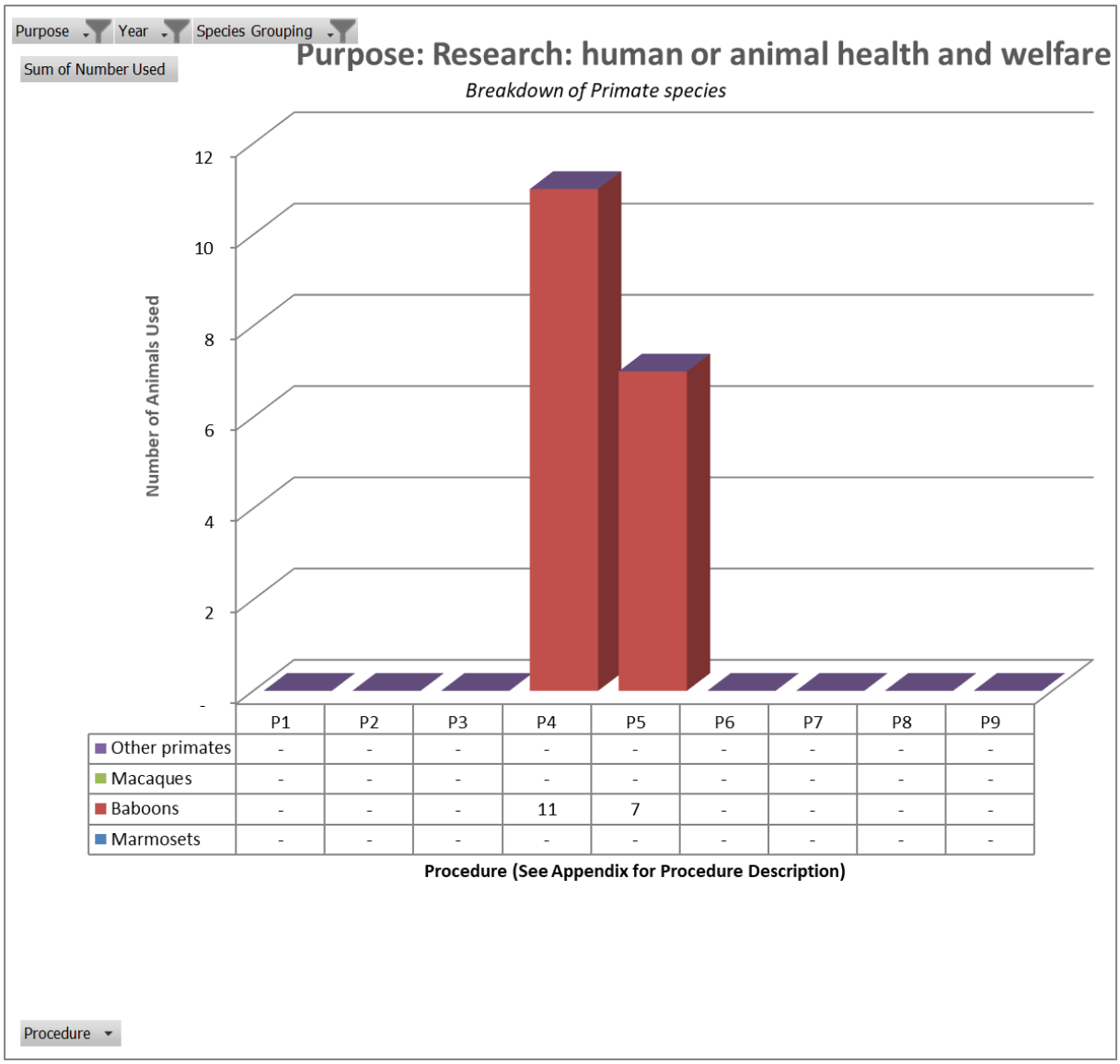
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	-	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	-	-	-	-	-	-	-
Rabbits	-	-	9	166	-	3	160	-	-
Guinea Pigs	-	35	104	75	-	67	81	-	-
Rats	52	264	191	367	74	1,879	323	-	-
Mice	1,000	13,365	4,398	1,548	7,163	13,254	7,135	-	10,917



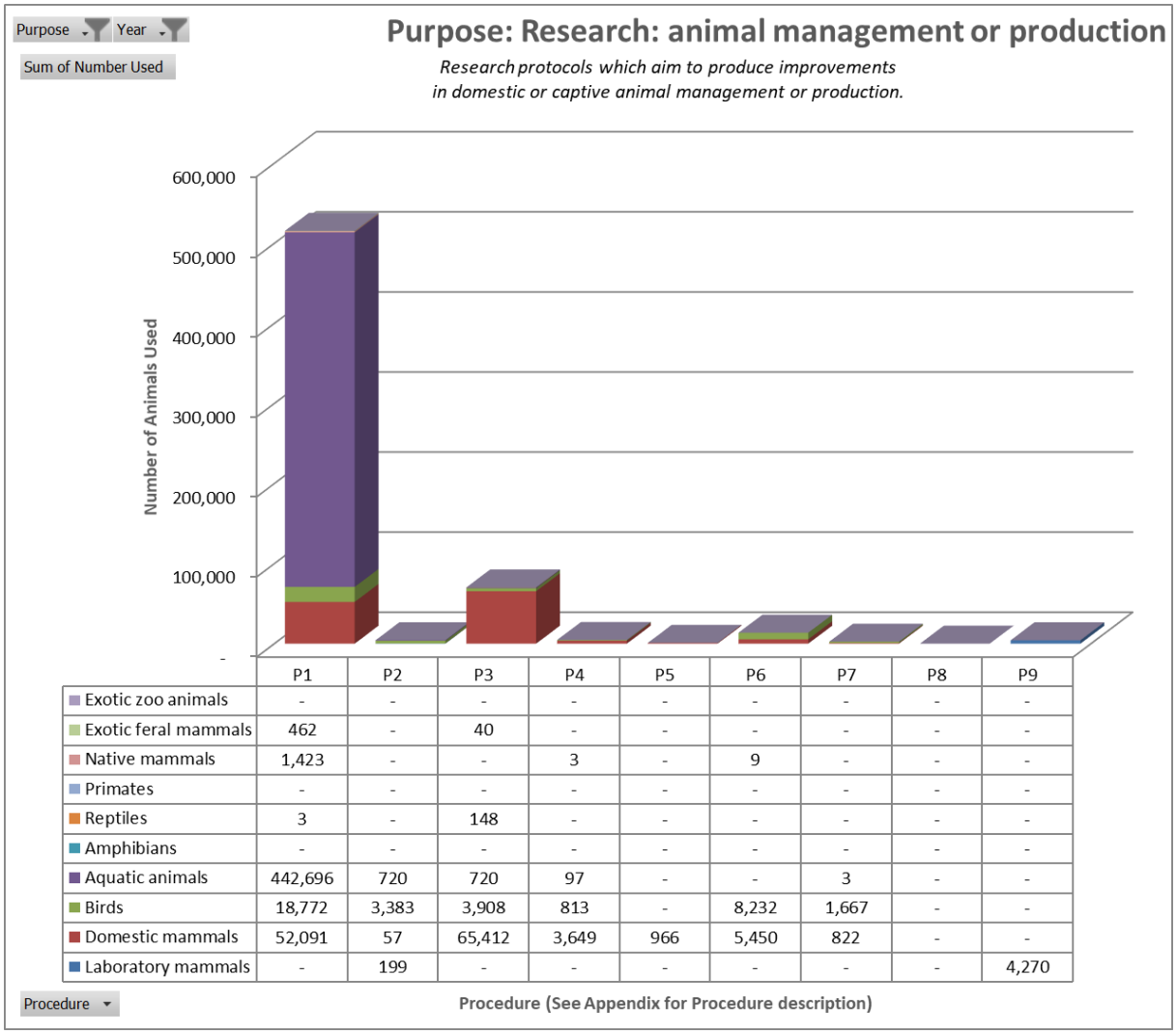
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	428	-	445	233	134	8	-	-	-
Cats	197	-	321	95	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	1,055	-	-	-	-	-	-
Horses	605	-	414	21	-	-	-	-	-
Pigs	-	50	2,437	2	74	-	595	-	-
Cattle	7,046	106	2,543	-	-	1,430	-	-	-
Sheep	940	79	1,719	51	5	1,398	35	-	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other birds	-	-	-	-	-	-	-	-	-
Native Wild	-	-	24	-	-	-	-	-	-
Native Captive	-	-	-	-	-	-	-	-	-
Exotic Wild	-	-	101	-	-	-	-	-	-
Exotic Captive	30	-	-	-	-	-	-	-	-
Poultry	5,766	110	-	-	-	244	2,905	-	-

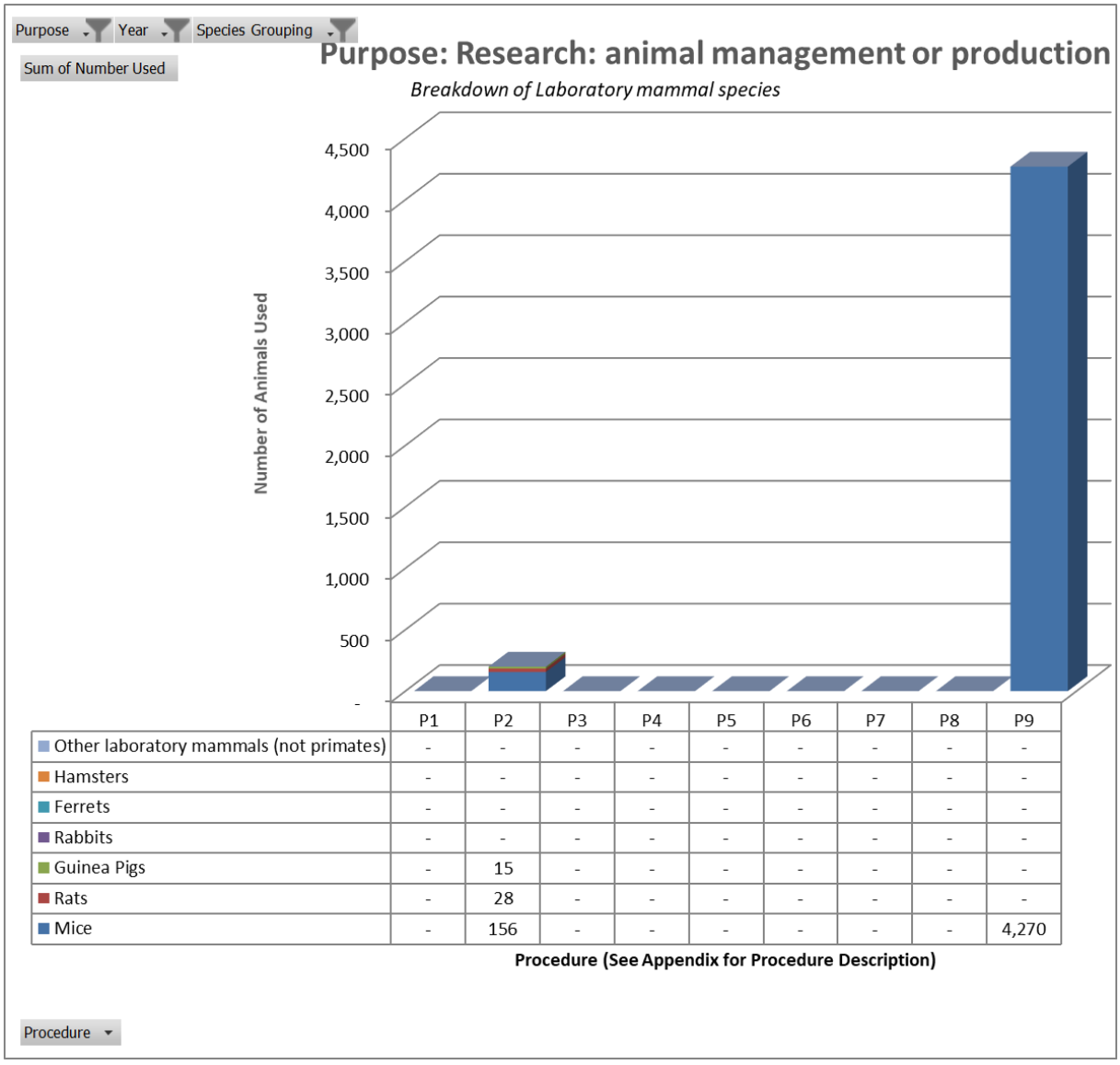

Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Research: human or animal health and welfare
Breakdown of Primate species
Number of Animals Used
12
10
8
6
4
2
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other primates - - - - - - - - -
Macaques - - - - - - - - -
Baboons - - - 11 7 - - - -
Marmosets - - - - - - - - -
Procedure (See Appendix for Procedure Description)
Procedure

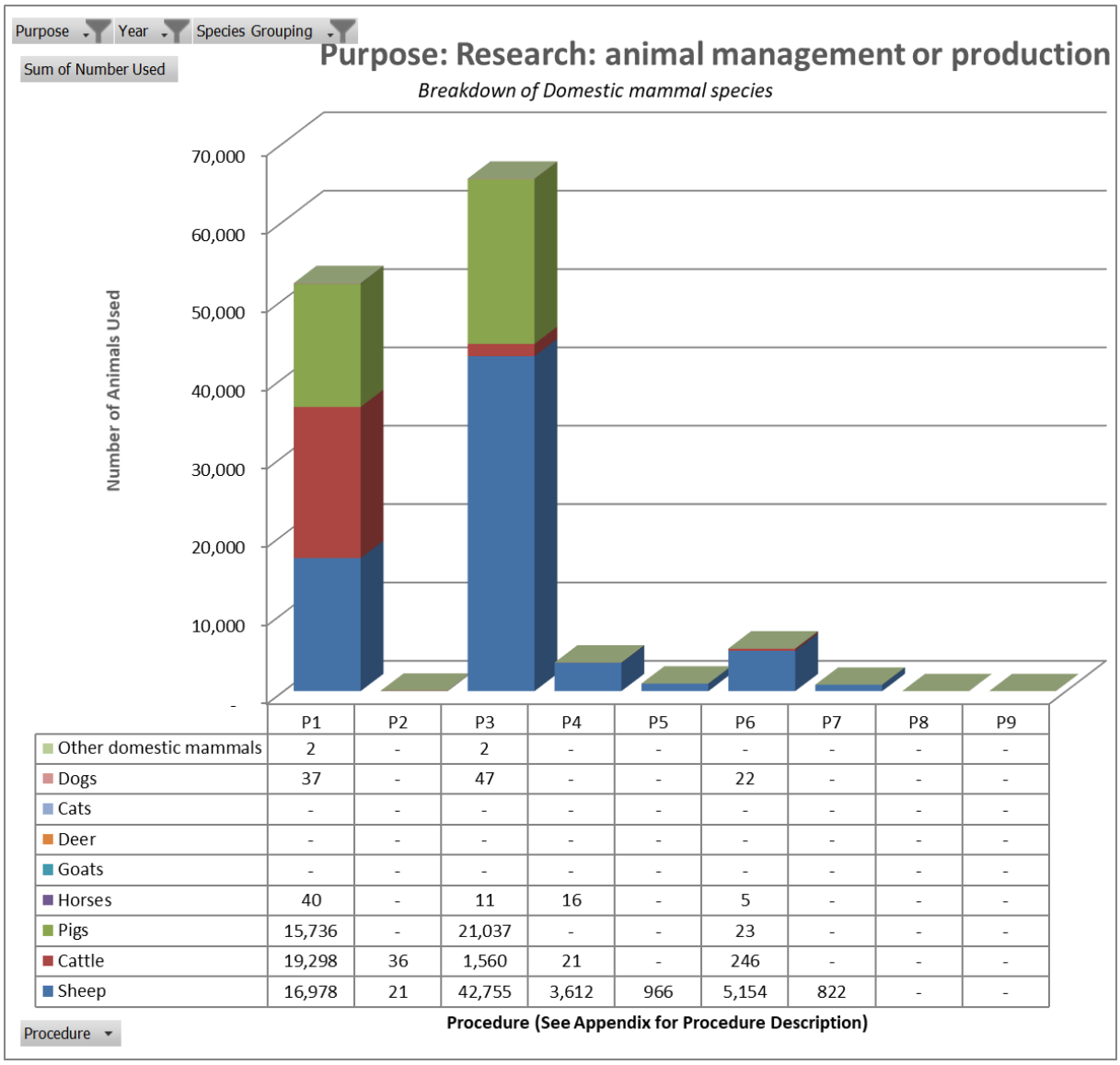
3.6 Research: Animal Management or Production



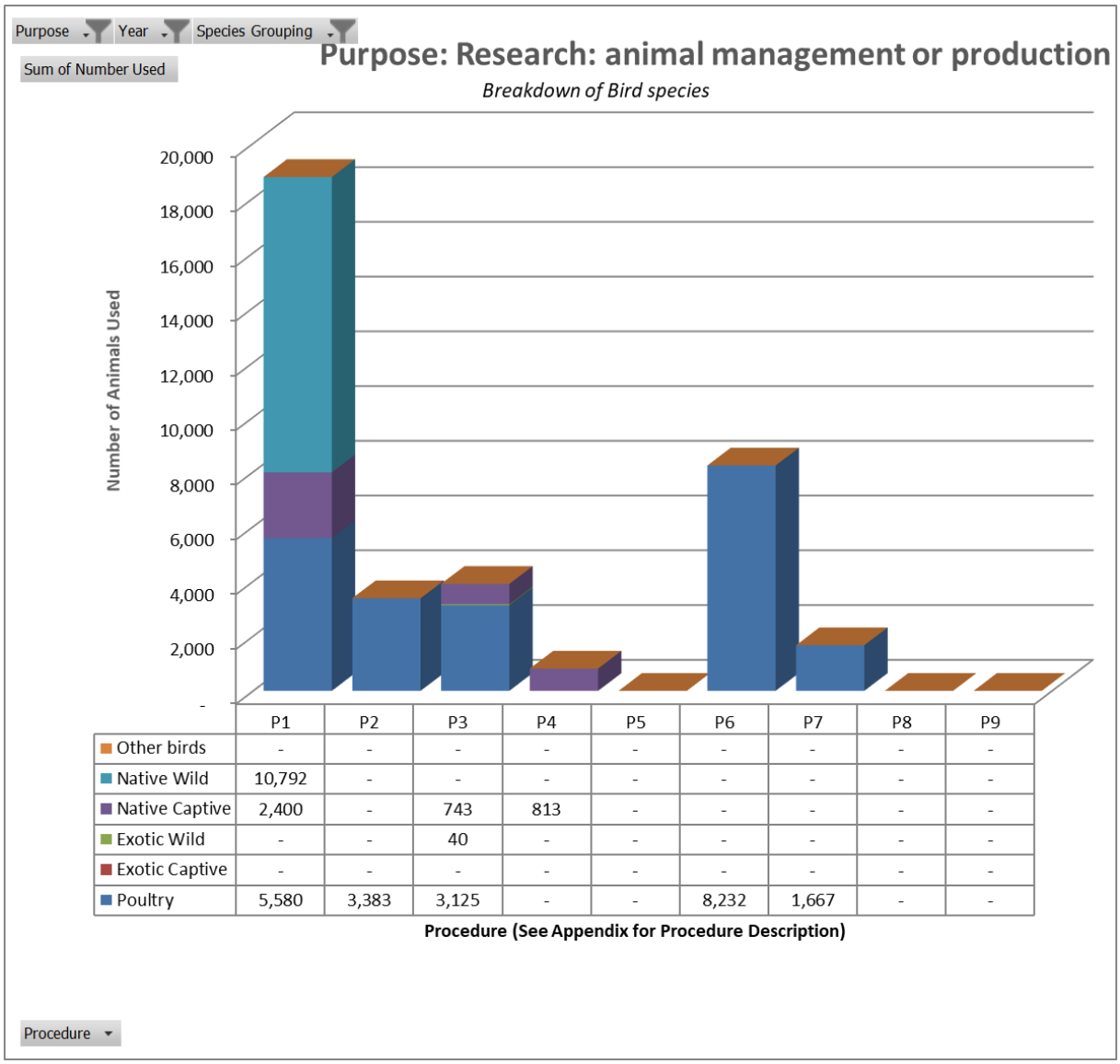
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	462	-	40	-	-	-	-	-	-
Native mammals	1,423	-	-	3	-	9	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	3	-	148	-	-	-	-	-	-
Amphibians	-	-	-	-	-	-	-	-	-
Aquatic animals	442,696	720	720	97	-	-	3	-	-
Birds	18,772	3,383	3,908	813	-	8,232	1,667	-	-
Domestic mammals	52,091	57	65,412	3,649	966	5,450	822	-	-
Laboratory mammals	-	199	-	-	-	-	-	-	4,270

3.6.1 Species Charts for Research: Animal Management or Production



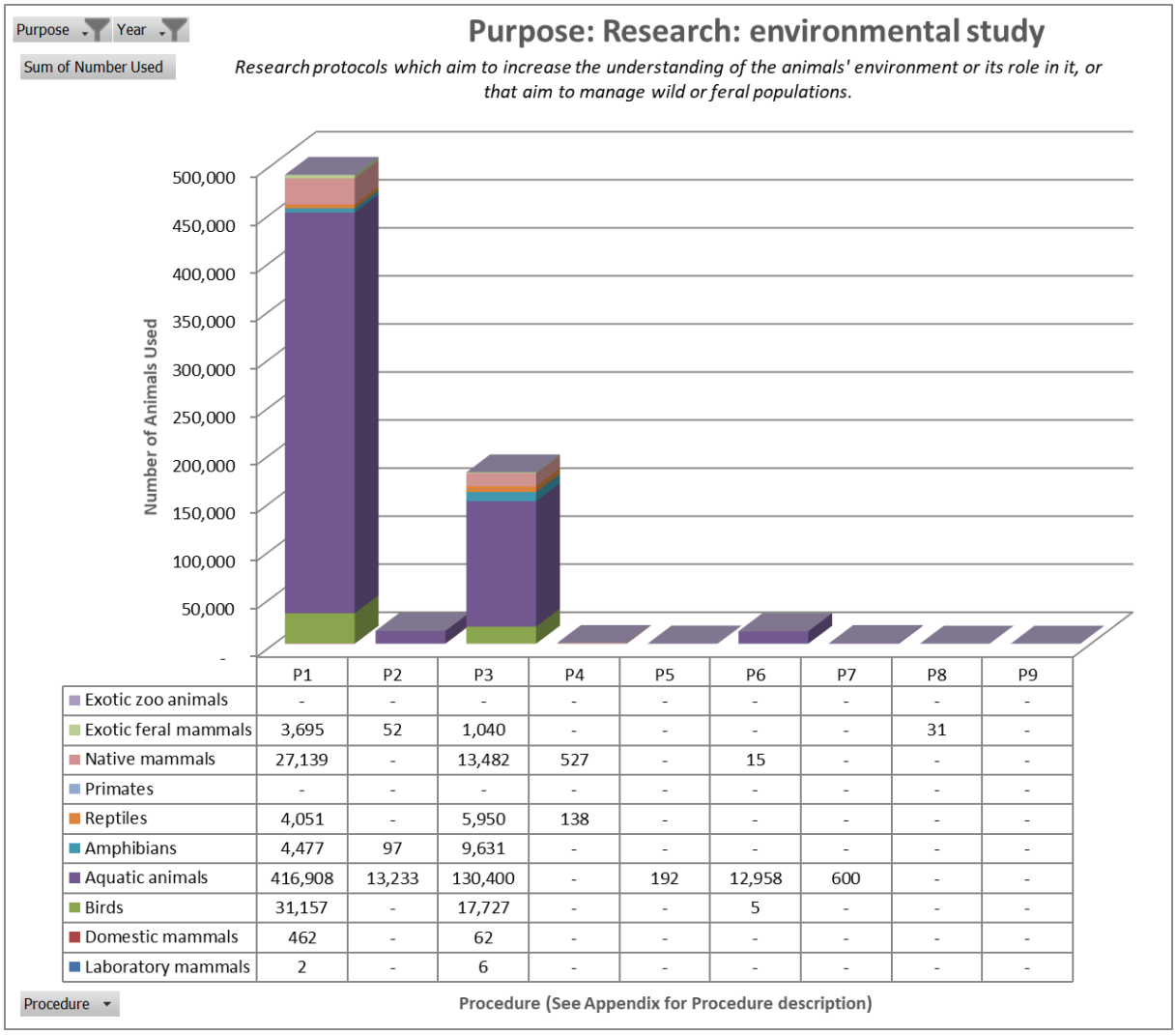


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	2	-	2	-	-	-	-	-	-
Dogs	37	-	47	-	-	22	-	-	-
Cats	-	-	-	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	40	-	11	16	-	5	-	-	-
Pigs	15,736	-	21,037	-	-	23	-	-	-
Cattle	19,298	36	1,560	21	-	246	-	-	-
Sheep	16,978	21	42,755	3,612	966	5,154	822	-	-



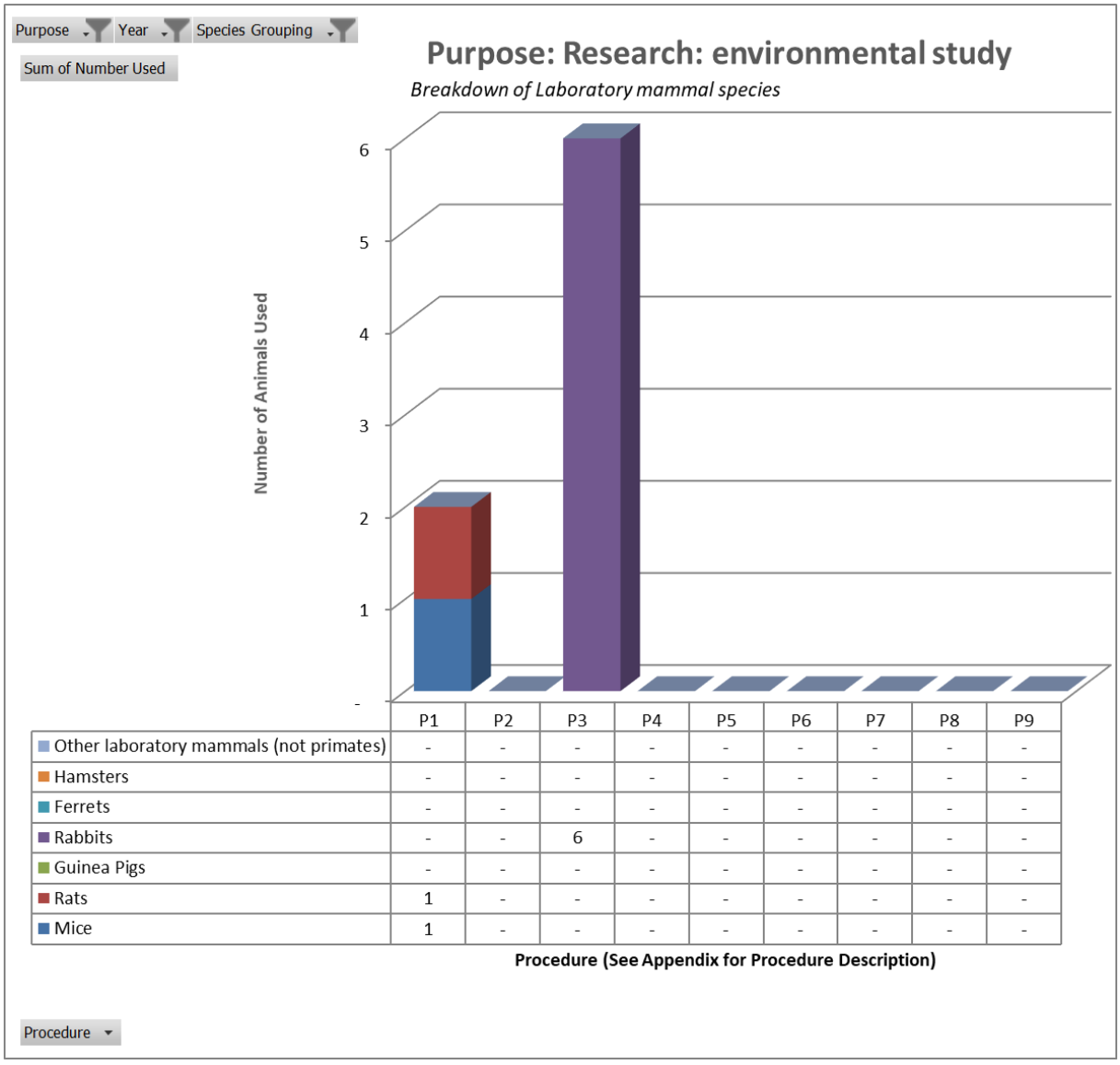
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other birds	-	-	-	-	-	-	-	-	-
Native Wild	10,792	-	-	-	-	-	-	-	-
Native Captive	2,400	-	743	813	-	-	-	-	-
Exotic Wild	-	-	40	-	-	-	-	-	-
Exotic Captive	-	-	-	-	-	-	-	-	-
Poultry	5,580	3,383	3,125	-	-	8,232	1,667	-	-

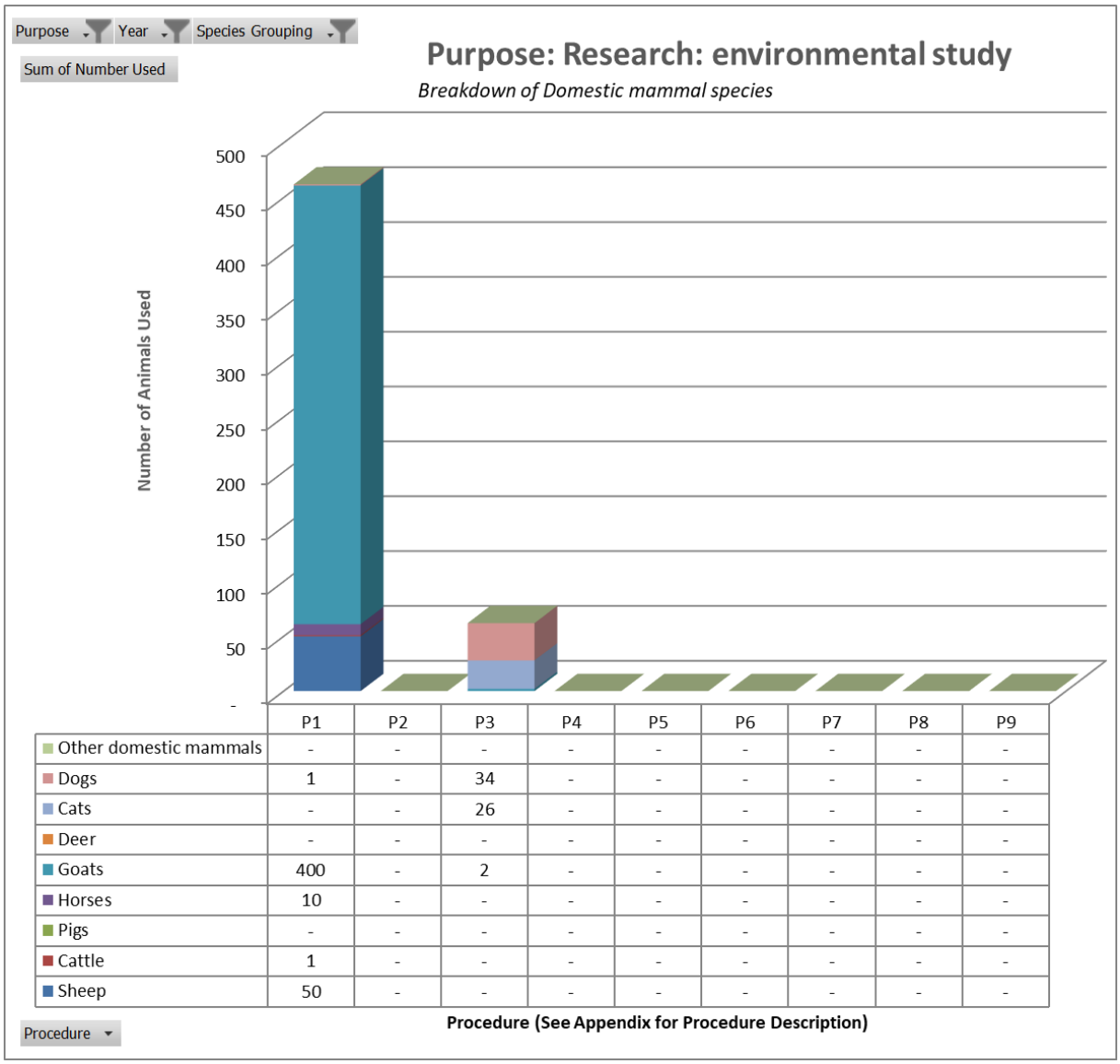
3.7 Research: Environmental Study



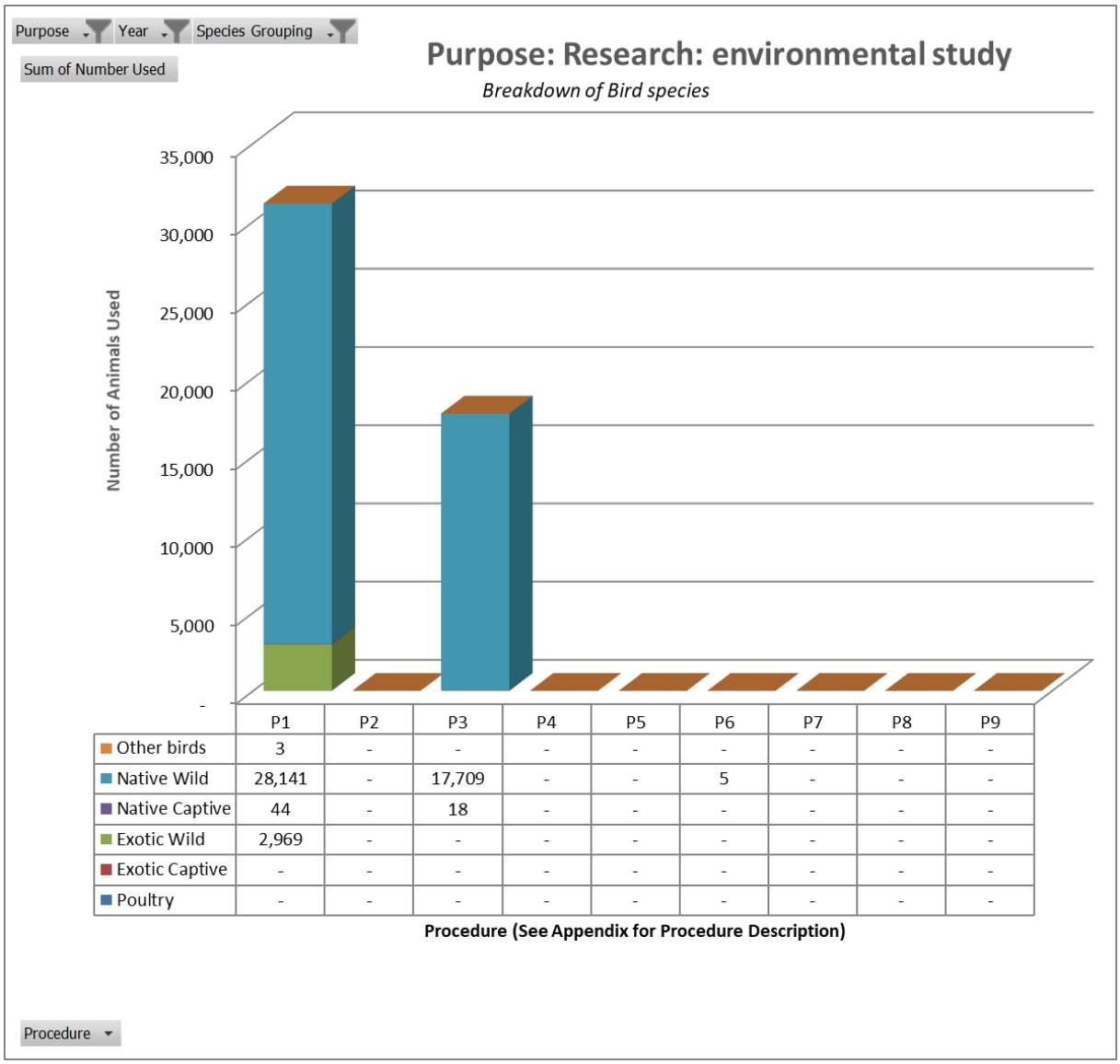
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	3,695	52	1,040	-	-	-	-	31	-
Native mammals	27,139	-	13,482	527	-	15	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	4,051	-	5,950	138	-	-	-	-	-
Amphibians	4,477	97	9,631	-	-	-	-	-	-
Aquatic animals	416,908	13,233	130,400	-	192	12,958	600	-	-
Birds	31,157	-	17,727	-	-	5	-	-	-
Domestic mammals	462	-	62	-	-	-	-	-	-
Laboratory mammals	2	-	6	-	-	-	-	-	-

3.7.1 Species Charts for Research: Environmental Study



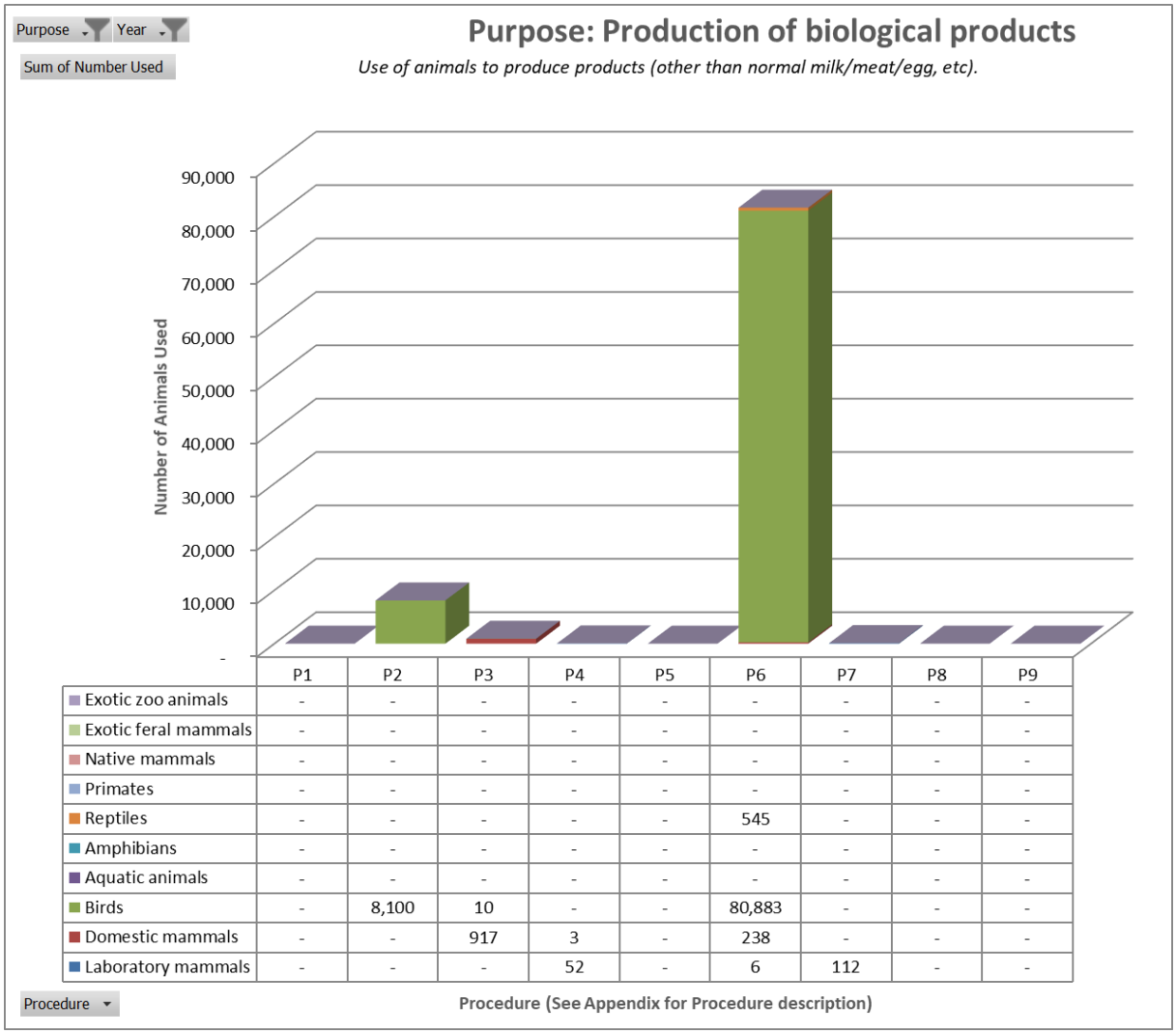


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	1	-	34	-	-	-	-	-	-
Cats	-	-	26	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	400	-	2	-	-	-	-	-	-
Horses	10	-	-	-	-	-	-	-	-
Pigs	-	-	-	-	-	-	-	-	-
Cattle	1	-	-	-	-	-	-	-	-
Sheep	50	-	-	-	-	-	-	-	-



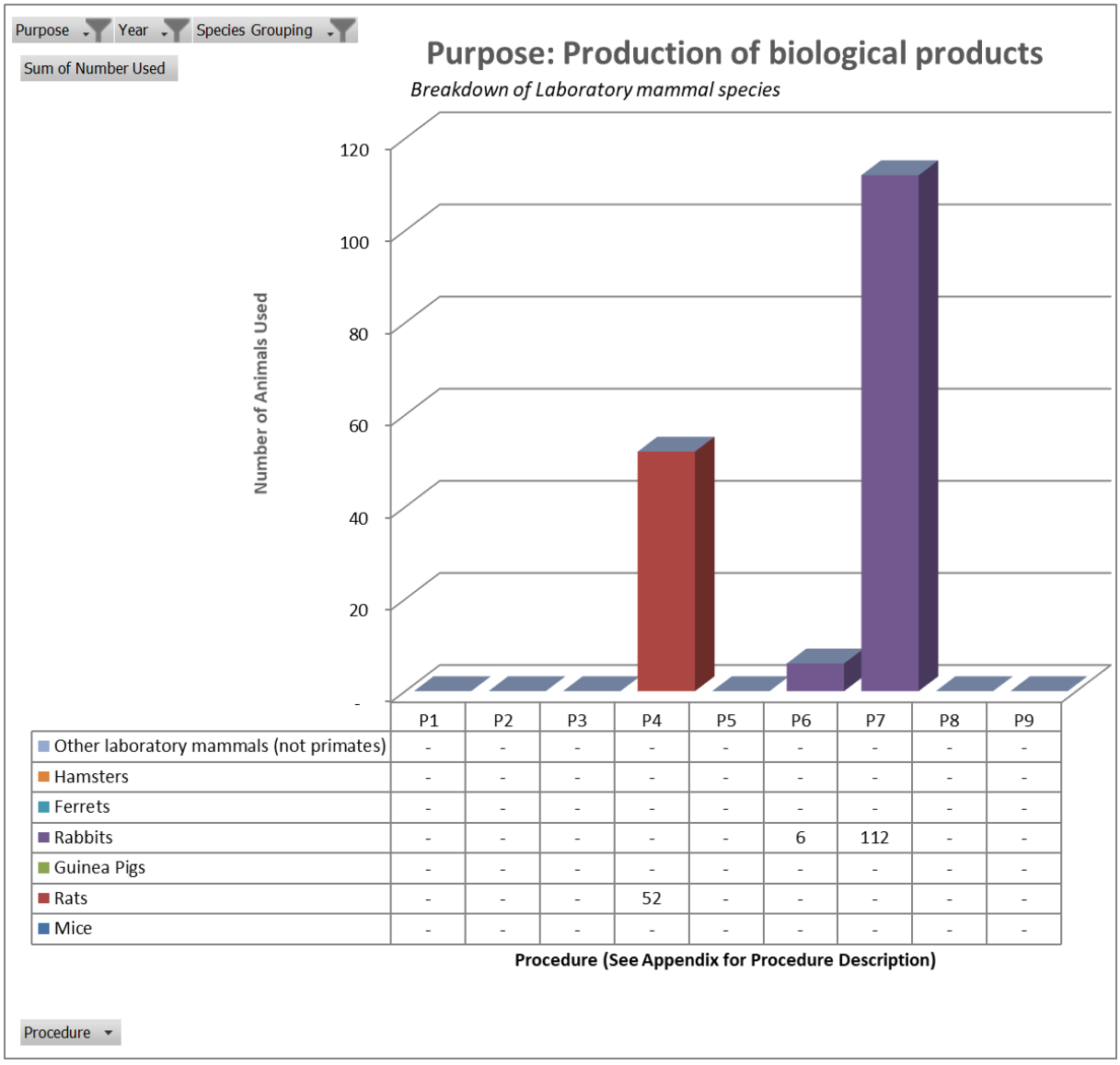
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other birds	3	-	-	-	-	-	-	-	-
Native Wild	28,141	-	17,709	-	-	5	-	-	-
Native Captive	44	-	18	-	-	-	-	-	-
Exotic Wild	2,969	-	-	-	-	-	-	-	-
Exotic Captive	-	-	-	-	-	-	-	-	-
Poultry	-	-	-	-	-	-	-	-	-

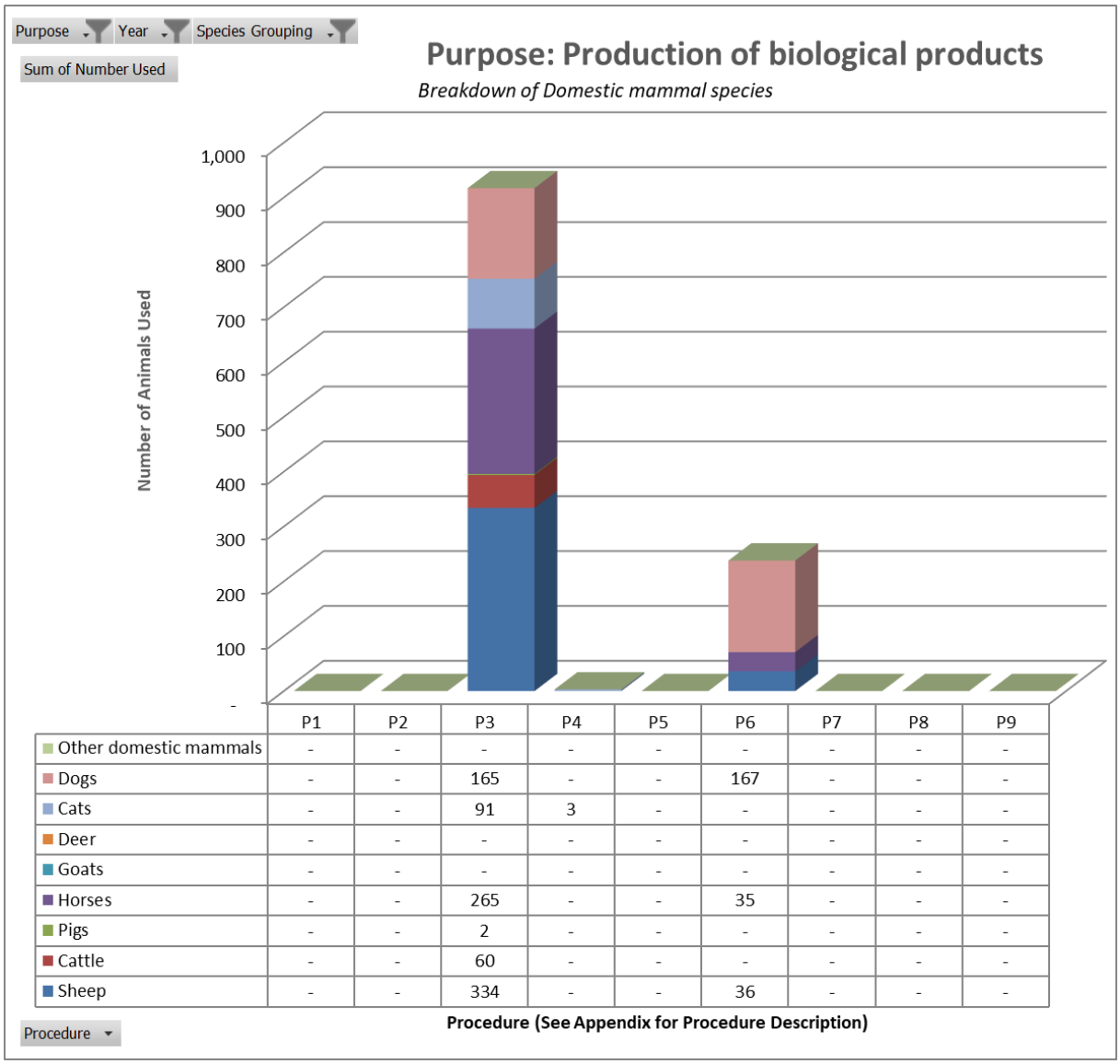
3.8 Production of Biological Products



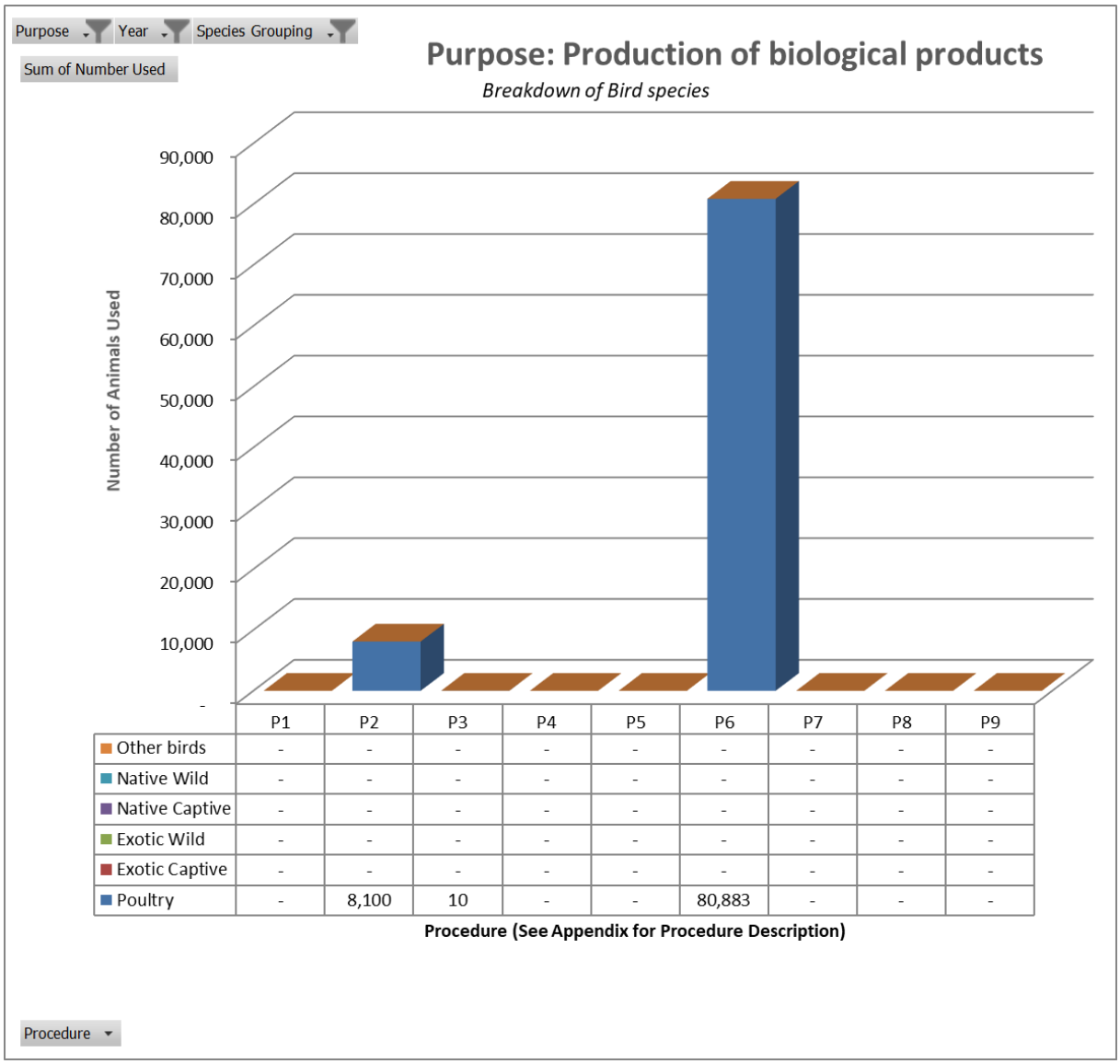
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	-	-	-	-	-	-	-	-	-
Native mammals	-	-	-	-	-	-	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	-	-	-	-	-	545	-	-	-
Amphibians	-	-	-	-	-	-	-	-	-
Aquatic animals	-	-	-	-	-	-	-	-	-
Birds	-	8,100	10	-	-	80,883	-	-	-
Domestic mammals	-	-	917	3	-	238	-	-	-
Laboratory mammals	-	-	-	52	-	6	112	-	-

3.8.1 Species Charts for Production of Biological Products

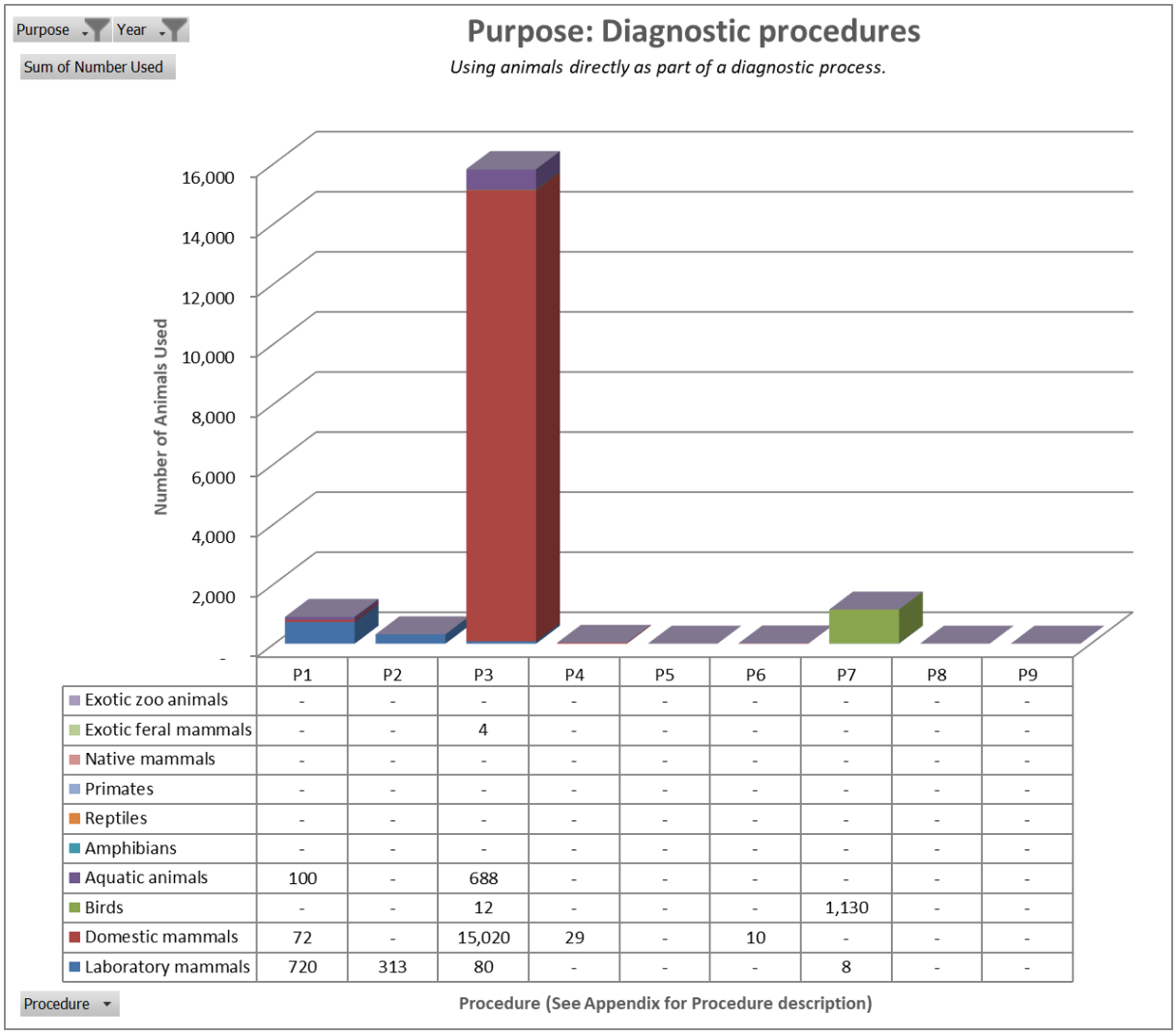




	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	-	-	165	-	-	167	-	-	-
Cats	-	-	91	3	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	265	-	-	35	-	-	-
Pigs	-	-	2	-	-	-	-	-	-
Cattle	-	-	60	-	-	-	-	-	-
Sheep	-	-	334	-	-	36	-	-	-

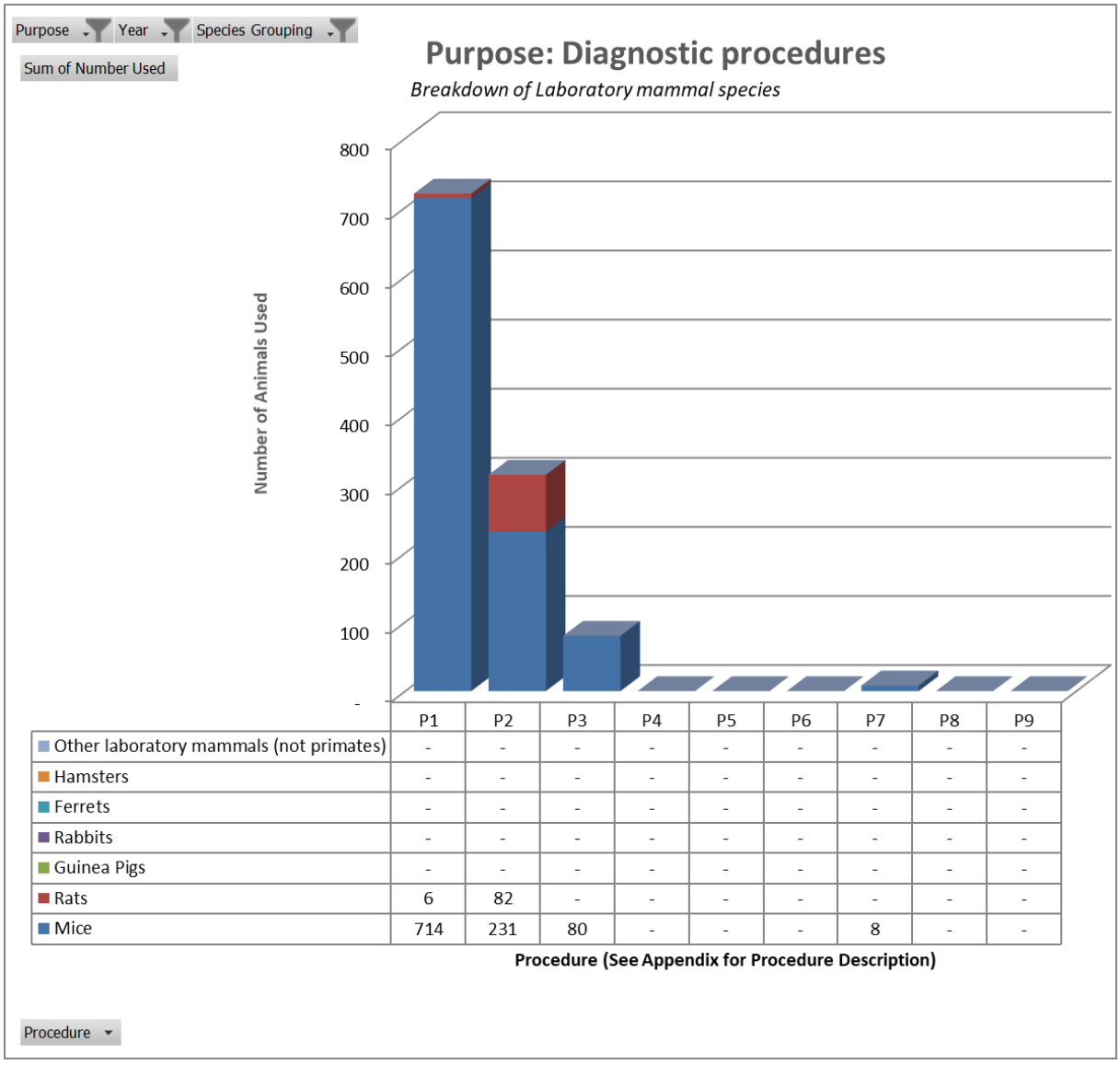

Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Production of biological products
Breakdown of Bird species
Number of Animals Used
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other birds - - - - - - - - -
Native Wild - - - - - - - - -
Native Captive - - - - - - - - -
Exotic Wild - - - - - - - - -
Exotic Captive - - - - - - - - -
Poultry - 8,100 10 - - 80,883 - - -
Procedure (See Appendix for Procedure Description)
Procedure

3.9 Diagnostic Procedures

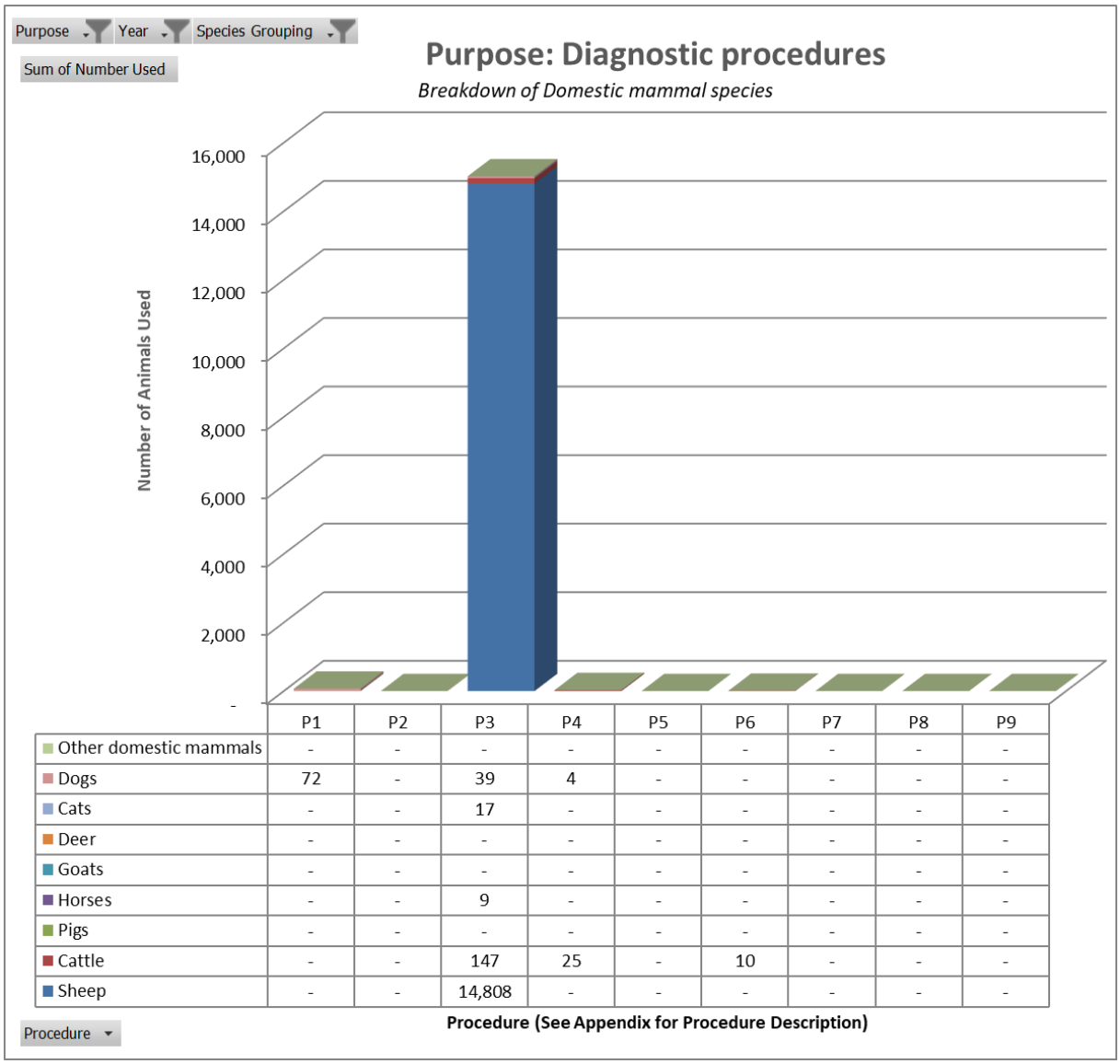


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	-	-	4	-	-	-	-	-	-
Native mammals	-	-	-	-	-	-	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	-	-	-	-	-	-	-	-	-
Amphibians	-	-	-	-	-	-	-	-	-
Aquatic animals	100	-	688	-	-	-	-	-	-
Birds	-	-	12	-	-	-	1,130	-	-
Domestic mammals	72	-	15,020	29	-	10	-	-	-
Laboratory mammals	720	313	80	-	-	-	8	-	-

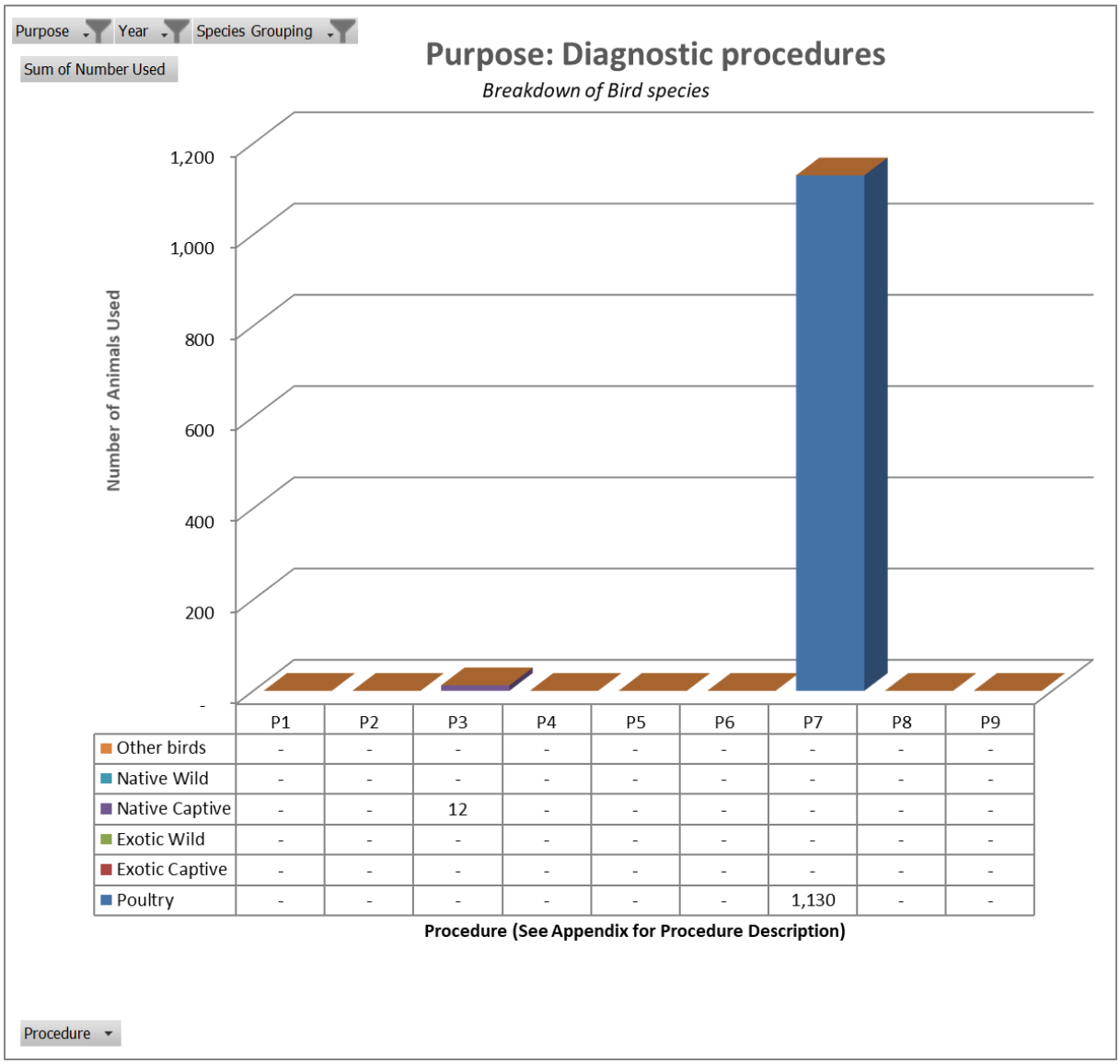
3.9.1 Species Charts for Diagnostic Procedures



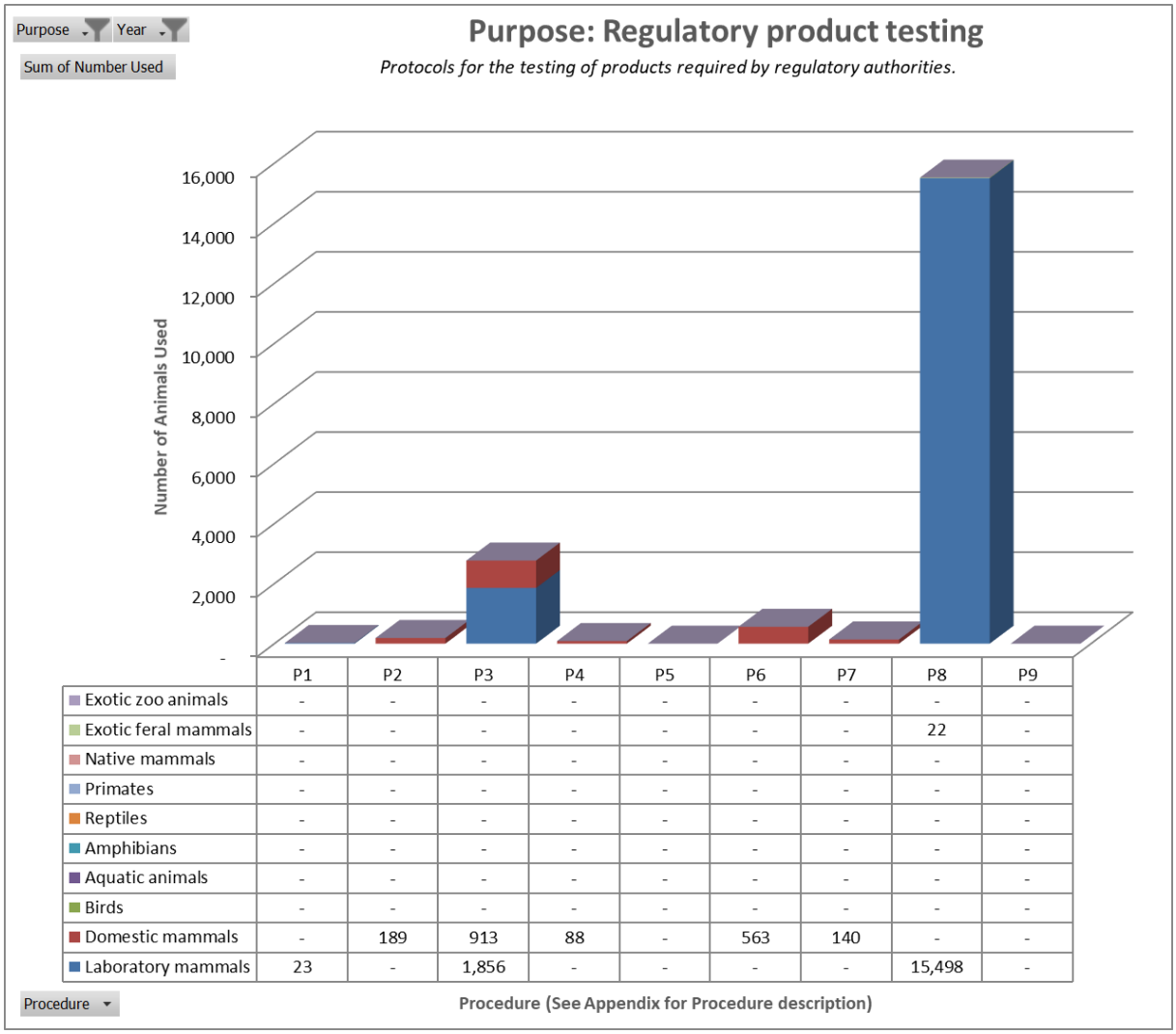
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	-	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	-	-	-	-	-	-	-
Rabbits	-	-	-	-	-	-	-	-	-
Guinea Pigs	-	-	-	-	-	-	-	-	-
Rats	6	82	-	-	-	-	-	-	-
Mice	714	231	80	-	-	-	8	-	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	72	-	39	4	-	-	-	-	-
Cats	-	-	17	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	9	-	-	-	-	-	-
Pigs	-	-	-	-	-	-	-	-	-
Cattle	-	-	147	25	-	10	-	-	-
Sheep	-	-	14,808	-	-	-	-	-	-

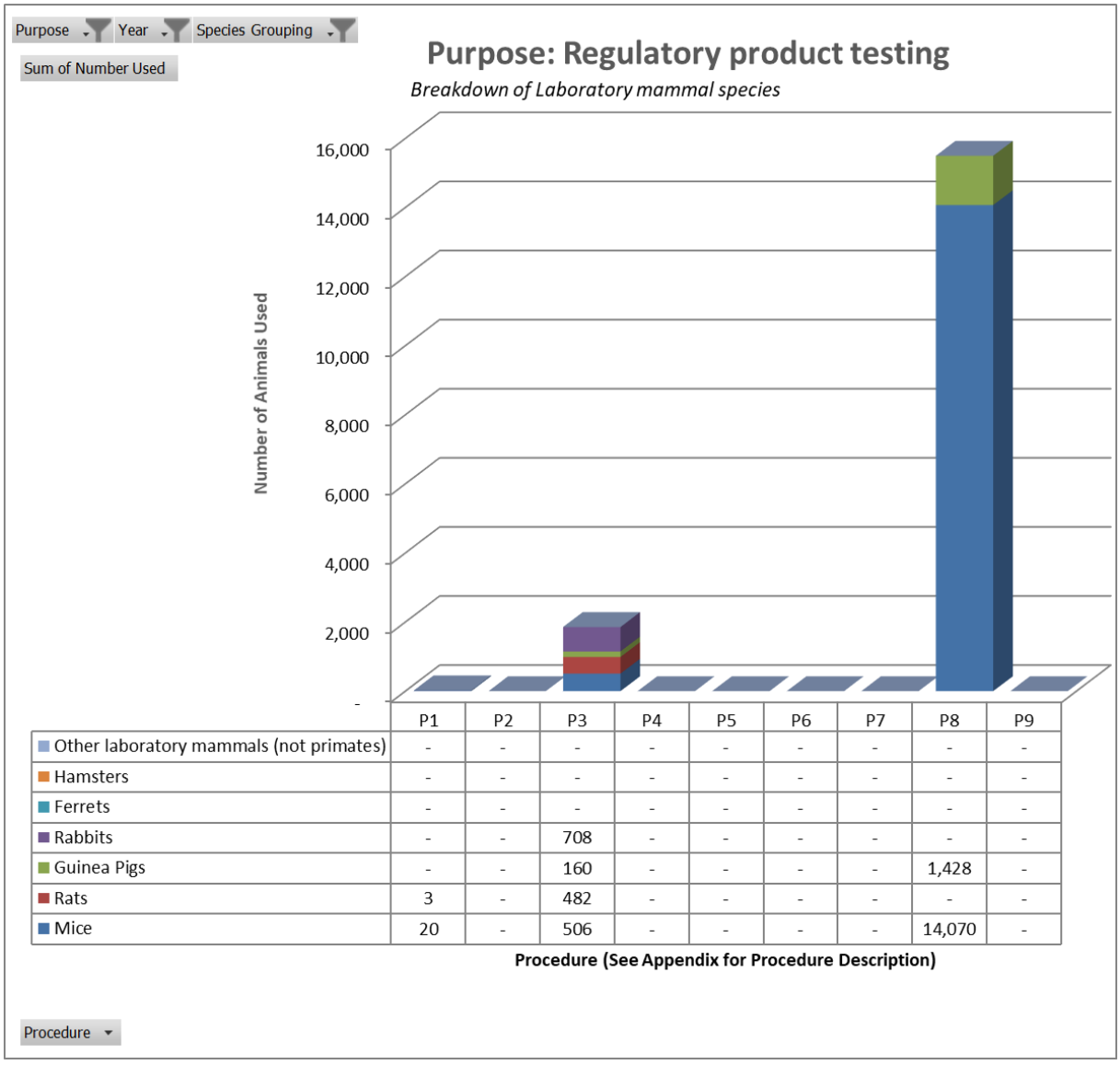

Purpose
Year
Species Grouping
Sum of Number Used
Purpose: Diagnostic procedures
Breakdown of Bird species
Number of Animals Used
1,200
1,000
800
600
400
200
-
P1 P2 P3 P4 P5 P6 P7 P8 P9
Other birds - - - - - - - - -
Native Wild - - - - - - - - -
Native Captive - - 12 - - - - - -
Exotic Wild - - - - - - - - -
Exotic Captive - - - - - - - - -
Poultry - - - - - - 1,130 - -
Procedure (See Appendix for Procedure Description)
Procedure

3.10 Regulatory Product Testing

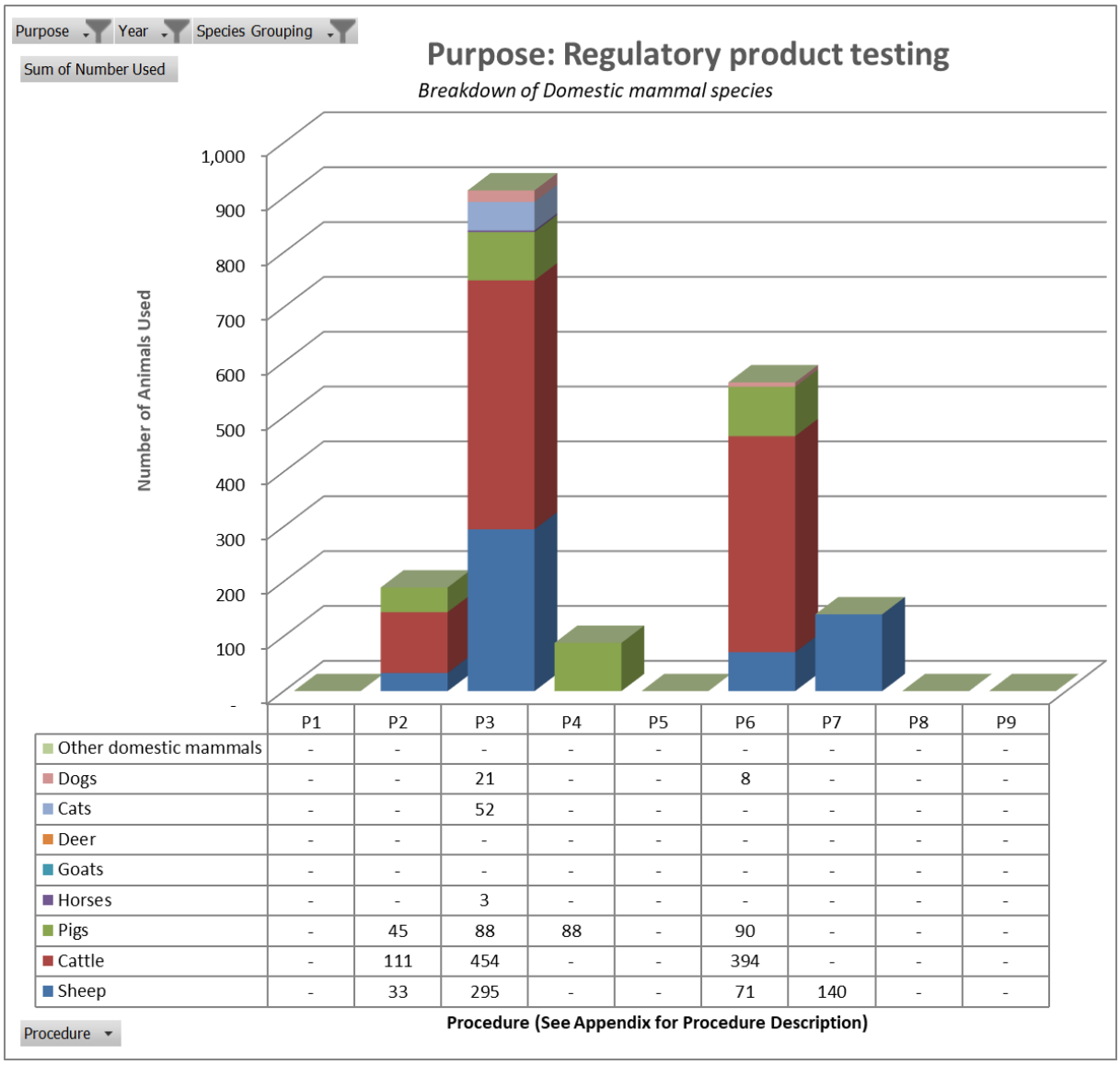


	P1	P2	P3	P4	P5	P6	P7	P8	P9
Exotic zoo animals	-	-	-	-	-	-	-	-	-
Exotic feral mammals	-	-	-	-	-	-	-	22	-
Native mammals	-	-	-	-	-	-	-	-	-
Primates	-	-	-	-	-	-	-	-	-
Reptiles	-	-	-	-	-	-	-	-	-
Amphibians	-	-	-	-	-	-	-	-	-
Aquatic animals	-	-	-	-	-	-	-	-	-
Birds	-	-	-	-	-	-	-	-	-
Domestic mammals	-	189	913	88	-	563	140	-	-
Laboratory mammals	23	-	1,856	-	-	-	-	15,498	-

3.10.1 Species Charts for Regulatory Product Testing



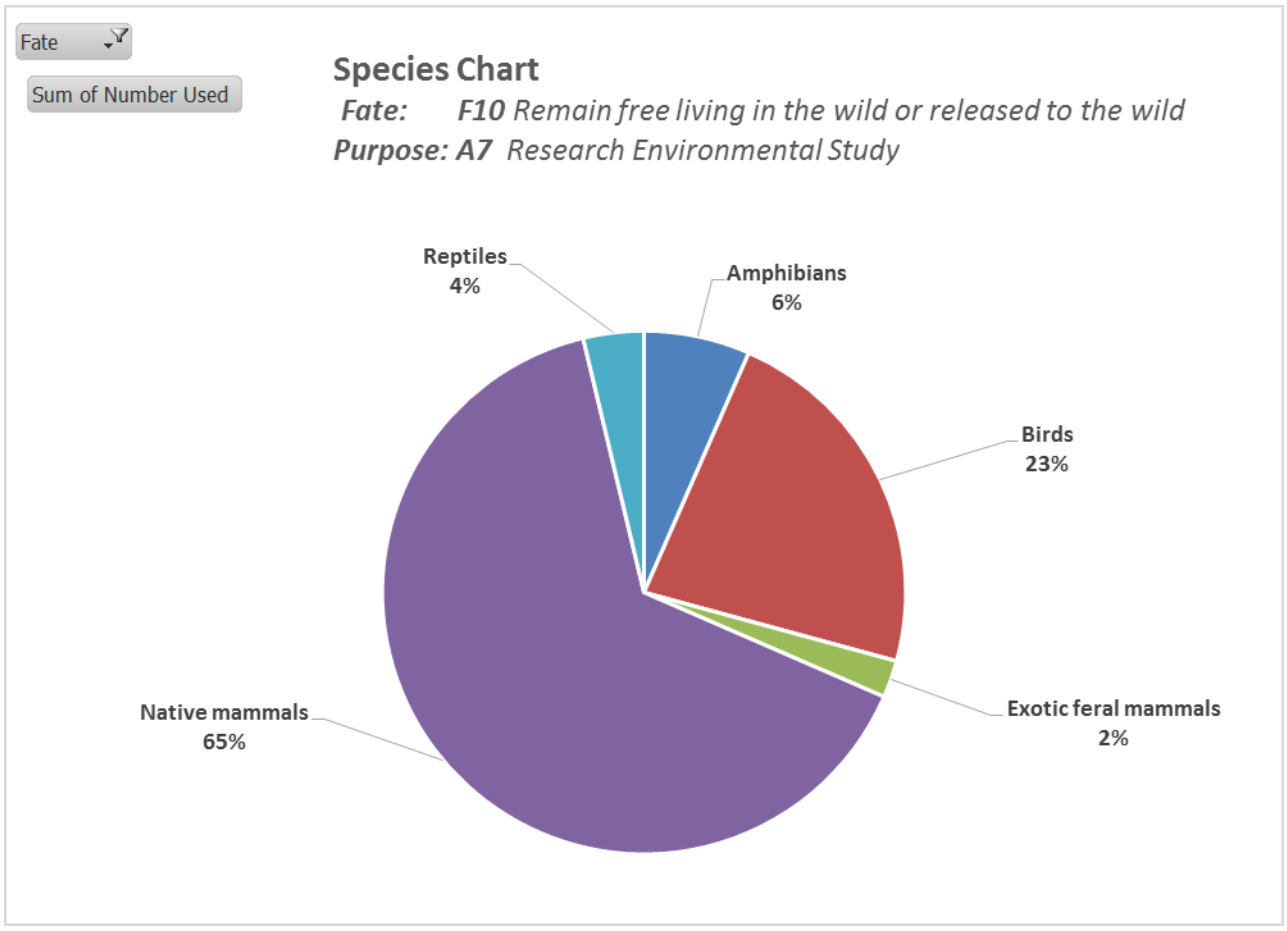
	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other laboratory mammals (not primates)	-	-	-	-	-	-	-	-	-
Hamsters	-	-	-	-	-	-	-	-	-
Ferrets	-	-	-	-	-	-	-	-	-
Rabbits	-	-	708	-	-	-	-	-	-
Guinea Pigs	-	-	160	-	-	-	-	1,428	-
Rats	3	-	482	-	-	-	-	-	-
Mice	20	-	506	-	-	-	-	14,070	-



	P1	P2	P3	P4	P5	P6	P7	P8	P9
Other domestic mammals	-	-	-	-	-	-	-	-	-
Dogs	-	-	21	-	-	8	-	-	-
Cats	-	-	52	-	-	-	-	-	-
Deer	-	-	-	-	-	-	-	-	-
Goats	-	-	-	-	-	-	-	-	-
Horses	-	-	3	-	-	-	-	-	-
Pigs	-	45	88	88	-	90	-	-	-
Cattle	-	111	454	-	-	394	-	-	-
Sheep	-	33	295	-	-	71	140	-	-

4. Fate of animals

For the 2018 reporting year, some establishments voluntarily reported on a new category – Fate of animals. For this reporting, all animals were in the Purpose category A7 - *Research: environmental study*, and the Fate of Animals category F10 - *Remain free living in the wild or released to the wild*. From the 2019 reporting year onwards, reporting on this category will be mandatory for the use of domestic cats and dogs, and voluntary for other species.



Fate F10: Remain free living in the wild or released to the wild

2018 - Fate of Animal By Species & Species Groupings Charts	
Fate	F10
Row Labels	**Sum of Number Used**
Amphibians	**986**
Amphibians	986
Birds	**3406**
Native Wild	3406
Exotic feral mammals	**346**
Cats	126
Cattle	2
Dingo/Wild Dogs	20
Foxes	28
Goats	42
Horses	44
Mice	44
Other exotic feral mammals	16
Pigs	2
Rabbits	12
Rats	10
Native mammals	**9728**
Bandicoots	1174
Bats	1248
Dasyurids	1398
Koalas	16
Macropods	1666
Monotremes	180
Native rats and mice	2212
Possums and gliders	1342
Wombats	492
Reptiles	**562**
Lizards	530
Snakes	32
Grand Total	**15028**

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 2018.

Species	Number used	Number died	Number euthanased as early endpoint	Procedure	Justification	Alternatives
Guinea Pigs	1428	162	16	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.	This test is based upon regulatory guidelines. 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 assavs.
Mice	9298	2421	562	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	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

Species	Number used	Number died	Number euthanased as early endpoint	Procedure	Justification	Alternatives
					stability batches and new product formulations.	existing in vivo assays subject to regulatorv approval of these replacement assavs
Mice	3794	1676	295	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 guidelines. 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 assavs.
Mice	978	244	39	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 guidelines. 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

Species	Number used	Number died	Number euthanased as early endpoint	Procedure	Justification	Alternatives
						existing in vivo assays subject to requlatorv approval of these replacement assays.
Feral Goats and Feral Deer	Unknown (depends on attendance of free-living animals at a feeding structure where a toxic bait may be delivered)	22	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	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	Number euthanased as early endpoint	Procedure	Justification	Alternatives
					Wales under Schedule 4 of the Biodiversity Conservation Act 2016. Despite these listings, control techniques for these species appear limited, particularly when compared to the number of techniques available for other pest species. Ongoing field trials continue to identify the potential for further development of a target selective, humane and cost-efficient method as an additional technique for controlling overabundant herbivore pest species.	

6. Examples of methods used to implement the '3Rs'

The following are practical 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 2018 reporting year. Examples relate to 'Replacement' (of animals with other methods), 'Reduction' (in the number of animals used in specific protocols) and 'Refinement' (of techniques used to reduce the impact on animals).

6.1 Replacement

- The projects on development of collections benefit from donations and exchanges with other institutions, thereby minimising the need to collect live animals in the field.
- An increasing number of projects are using tissue samples only (often donated from other institutions - e.g. zoos that collect samples during routine health inspections of animals).
- One new project is investigating the potential use of iDNA (invertebrate-derived DNA) to detect amphibians (frogs). In particular, blood-feeding frog parasites (e.g. mosquitos) are collected and their blood meals investigated for traces of frog DNA. This would enable researchers to detect presence of rare frog species in nature more effectively than using traditional surveys, and simultaneously potentially minimise (eliminate) the impacts of direct handling of live amphibians in future studies.
- The establishment conducts a number of meta-analyses which allows research questions to be addressed using existing data replacing the use of live animals and is more powerful than individual studies
- We regard the use of sheep to be an important aspect of testing forage species since it simulates the effects of conditions on a working farm, including soil trampling, and the deposition of faeces and saliva that facilitate nutrient cycling and stimulate compensatory growth, respectively. It is our intention to use a mower to sample the plots when possible and on occasions we may be able to remove all herbage on the experiment using this means and eliminate the need to graze, e.g. when pasture is very short in winter, when sheep are not available.
- Encouragement of researchers to undertake literature and systematic reviews.
- Increased availability of in vitro technology such as tissue culture to address scientific questions without the use of animals.
- The use of ADinstruments, videos and invertebrates for teaching projects as an alternative to live animals.
- Tissue culture models are used as far as possible for mechanistic studies.
- We have utilized in vitro methods to optimize techniques, such as cell labelling, to ensure in vivo experiments are well designed.

- Peripheral blood from human patients will be analysed for their response to bacterial challenge in vitro. This will minimize the reliance on mouse models and enhance the translational significance of the work.
- We will establish cell lines from the bone tumours that arise in the lung; these cell lines will be used for future in vitro and in vivo work.
- Replacing some sample collection from animals with rack exhaust or cage filter screening.
- Where possible, we have replaced the in vivo mouse model with in vitro experiments using isolated cells and cell lines (e.g. in vitro adhesion and phagocytosis assays).
- Alternatives such as suturing pads, in house training videos are used in training to optimise the preparation of students before they handle live mice.
- The in-vitro project progressed significantly in 2018 and included the addition of a second employee to work on the project and the outsourcing of validation to expedite progress towards ELISA animal replacement tests. By the end of 2018, ELISAs for finished product potency tests were developed and validated for 4 tests. The Change Control document to implement 3 tests was initiated to begin the documentation and process changes that are necessary before the tests can be used for product release. The change control process for these three tests is expected to be completed during 2019 which will replace the lethal mice component for these 3 antigens. It has not yet been determined whether in-vitro tests can immediately replace animal tests or if they need to be run in parallel for 10 batches as per European requirements. It is anticipated the quinea piq challenge test will be able to cease in mid 2020, subject to APVMA approval.
- Models for anatomical and clinical examinations are being reviewed constantly. Where appropriate these are used to replace the use of animals.
- The use of cadavers for model and surgical procedure development and staff training.
- Promoting the use of audio-visual or computer model methods in teaching projects wherever possible.
- The AEC continues to encourage investigators to thoroughly investigate all possible alternatives to using live animals when planning their proposed project. Whenever possible, in vitro methods/techniques are utilised and if possible, tissue sharing options are encouraged. The use of live animals can only be justified if all other apposite options have been exhausted. Examples include: Utilisation of tissue from small animals humanely killed for other reasons (retired breeders, aged stock, tissue sharing from animals humanely killed from other research projects). Body parts (limbs/joints) and organs (kidneys) from large animals (sheep, pigs) humanely killed from other projects may also be used for anatomical and surgical training and for isolated organ (kidney) perfusion studies. For diagnostic purposes, in vitro assays to identity agents obviating the need to use animals. Investigators to undertake

literature reviews when planning studies to avoid repeating research work that may have already been performed.

- The establishment hosted a workshop on the Systematic Review of Animal Studies: Improving transparency on quality and translatability of animal studies. The workshop was delivered by Dr Judith van Luijk and Dr Rob de Vries, experts in the field of systematic review from Department of Health Evidence, Radboud University Medical Centre.
- On a regular basis researchers are implementing methods that partially replace the use of animals in their projects. Often the establishment gets to hear about these through annual reports or expiry reports submitted by researchers.
- An expert on alternatives remains an advisor to the AEC on alternatives to the use of animals and his services and advice are available to all research staff and students undertaking animal based research at the establishment.
- Increased use in 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.
- The establishment is moving towards an increased use of computer simulations, and/or the use of deceased animals which have been professionally preserved. Only advanced level teaching activities now require the use of animals. During 2018 we used a total of 12 live animals (cane toads) for laboratory based teaching activities.
- Large photo cards have been developed in practical classes instead of live animals to teach students how to identify freshwater fish species.
- Samples will only be taken from dead marine turtles.
- A protocol investigating whether parasite molecules alter metabolic activity of macrophages performed extensive work in in vitro to characterise the biological activity of the proteins with macrophage cell lines.
- Our Laboratory works on the replacement of model organisms such as mice with less sentient organisms, in our case we use drosophila melanogaster (fruit fly) and human stem cells to validate work before working in mice.
- Animal experiments have been reduced by over half in Pharmacology and computer based experiments are also made available to reinforce the learning experience when using animal tissue.
- Extensive in vitro characterization of actions of each peptide on a range of ion channel targets expressed in cultured immortalized cell lines and animal cells (Xenopus laevis oocytes) is completed prior to in vivo use.
- We are working, where possible, on in vitro models of secretory function and disease. This includes work with cell lines (we use the MIN6 mouse insulinoma cell line) and work to culture animal cells which extends the lifetime of our experiments and means

we can use fewer animals. We are also beginning to work with human stem cells which, at some time in the future, could provide a better model for human disease.

- We have, where appropriate, conducted some aspects of our research in mammalian cell line models, thus replacing and/or reducing our use of animals.
- Mock surgery performed on animal tissue for training purposes or refinement of techniques.
- In vitro cell culture experiments are used.
- Four protocols all aim to develop diagnostic assays to measure animal disease and welfare and ultimately to replace the use of live animals with laboratory tests.
- Assessment or use of cell lines as non-animal alternatives projects.
- Non-animal alternatives were incorporated to partially replace the use of animals: the use of human ovary cell lines, donated and purchased human ovary tissues and the use of donated human oocytes.
- Developed in vitro (cell culture) models based on rat brain cells to replace animals in some experiments.
- Collected mare ovaries from an abattoir to recover equine oocytes for in vitro maturation studies, and to develop the procedures used to examine the good quality oocytes collected from fertile mares.
- Used human and mouse cell lines to assess the effects of cigarette smoke extract/corticosteroid functioning and performed co-culture experiments with specific bacterial species of interest.
- Utilised in vitro models (suspension cultures, patient blasts ex vivo) to test the effectiveness of a compound, both on its own and in combination with chemotherapeutics, reducing the number of animals required for study.
- Employed primary cell culture at air liquid interface to differentiate epithelial cells to be similar to those lining the airway wall as replacement of mice in mechanistic studies.
- In vitro cell culture and immunohistochemistry was conducted to confirm results. Tumours that were collected from the mice have been stored so that researchers can continue to test on these preparations rather than using additional mice.
- Used in vitro models to pre-screen reagents.
- Used in vitro cell model systems to study cell to cell transmission.
- Used isolated protein channels extracted from either human or animal tissues. A single tissue can be used for 5- 20 experiments.
- Cell specific targeting strategies were undertaken in vitro.
- The assessment of the role of Leydig cells and identification of mechanisms was undertaken in vitro.

- The in silico model for the hypothalamus-pituitary and testis axis regulation of the androgen production was used to test the hypothesis prior to animal usage.
- In vitro studies were conducted to minimize the use of animals.
- Used human sperm collected with human ethics approval.
- Used cell lines to test the hypothesis.
- The validation of the nanoparticles and targeted mutagenesis was carried out in vitro.
- Dog Abdominal Surrogate for Instructional Exercise are used for suture training.
- Using bio-models in surgical training workshops.
- Conduct in-vitro assays, replacing the use of animals in early product development.
- Attempts were made to develop a reliable acaricidal assay by feeding ticks on an artificial membrane. This was unsuccessful.
- The Committee continues to maintain a Biological Non-Human Tissue Database and in December 2018 a new tissue 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.
- Video and images for demonstration of fish disease management.
- Generation of anatomical 3D models for the zebrafish to replace use of live animals.
- Use of archival footage from documentaries and tour operators available on the web for behavioural studies on foraging behaviours of leopard seals.
- Development of Eco-App for student use on smart phones for bird identification.
- Replacement of direct observation of animals with camera trapping.
- Ongoing support of a mailing list to facilitate tissue sharing among researchers, including researchers from other institutions requesting tissue.
- Use of artificial models, e.g. Koken/Curvet rat, knitted mouse models for training.
- Use of videos and on-line resources for training.
- Use of animals that were humanely killed under another approved project for training and assay/in vitro testing.
- Experimental results used for computational modelling
- Refinement of bat survey techniques through adoption of new technologies to refine bat acoustic recording capability and use of infrared cameras at cave entrances to conduct counts, replacing the need for extensive harp trapping.
- Mannequins, audio-visual materials, photographs, taxidermised 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.
- Training in 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.
- 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.
- In vitro work before application in live animal models.
- Cell-based in-vitro experiments whenever possible.
- Animals used for multiple tests where possible.
- Use of audio-visual material such as videos, slides, interactive computer programs.
- Use of training models to teach techniques (e.g. latex rat for injections).
- Use of abattoir specimens and cadavers.
- Use of plant tissue as a replacement for animal tissue for certain enzymatic assays.
- Routine husbandry procedures to be performed on animals are coordinated with teaching activities.
- Use of animals killed in road accidents.
- Researchers moving away from primary cultures and using stem cell differentiation.

6.2 Reduction

- Power analysis has been used to detect minimal differences between treatments and the number of animals required to detect a required response.
- Re-use of fistulated cattle across multiple studies
- Spare birds from a project were utilized for gavage training instead of purchasing birds specifically for this task
- Spare pigs from a study were used to collect control blood for development of bioanalytical methods and also for training of staff in pig handling and blood collection techniques, meaning that additional pigs did not need to be purchased.
- Use of cattle from a study into another study
- General questioning of project teams on the numbers of animals for inclusion in each group — often the team asks for higher numbers of animals to be included and we question the validity of this whilst still being confident of meeting the aims of the study.

- Conduct of endoscopy in study, which was initially unplanned, was able to provide pivotal data to the project team which ultimately result in a "no-go" decision for the project
- When the projects involve directly contributing to collections, efforts are made to only capture minimal number of animals from taxa that are poorly represented in the collections. For example Collection staff collected only 4 of the 39 captured Large Forest Bats (Vespadelus darlingtoni), to add to the collection. However, before their release, 16 captured bats had a tissue sample taken, and deposited in the tissue collection to be used in future genetic studies, and all 39 animals had their basic measurements.
- The establishment co-ordinated one of their rumen fluid collections from a project with a researcher at another establishment to allow this researcher to utilise a portion of the rumen fluid collected for in vitro analysis. This replaced the need for this researcher to collect rumen fluid from other cattle.
- Investigated the possibility of collecting additional data from a current project to reduce the requirement for further animal studies in this area.
- The study on flying foxes has used where possible animals which have died of natural causes.
- 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.
- Where possible carcass samples (liver) will be collected at the abattoir from animals that have been sampled (such as blood, rumen fluid or faeces) on properties. The abattoir samples will be used to correlate with samples collected on properties to ascertain whether abattoir samples could be used as an alternative diagnostic for some nutrient deficiencies.
- Informed experimental design. Our aim is to have a reasonable number of sheep (between 5-10) to keep the sheep behaving normally, and have them moved off the plots in the shortest possible timeframe. This is efficient in terms of human labour also to safely manage the water and husbandry aspects of the grazing period.
- Statistical analysis has been performed (epidemiological calculator EpiTools) to estimate a sample size of 20-25 is sufficient for detecting presence of antibodies and virus in the population with 95% confidence. These animals are being salvaged from broad scale rabbit control programs that remove many more than we are seeking to use.
- Samples from previous experiments (stored at -80 C) from animals grazing in the proposed areas will be analysed (app. 180 animals). This initiative has reduced the number of properties to visit and the number of animals to sample as this area (where the 180 animals were grazing) will not be sampled again.

- We have minimised the number of animals required to observe changes in population dynamics as much as possible by reducing the number of live and snap traps set, and for the three key periods each year. This enables us to measure changes in mouse population dynamics. We have been successful at achieving this through our previous AEC approvals. We will be setting two lines of 10 snap traps (20 traps) on the benchmark site only, which is a minimum replication of the long-term monitoring that has been undertaken by various Government researchers over the past ~25 years. In the past, the various departments were using snap traps at 14 sites. We have reduced this to one site only. This information is the minimum required to run the forecast models at this site. Similarly, the live trapping data collected at two sites in South Australia and Victoria is also the minimum required to run the forecast models, and to compare to the historic data (~30 year long-term data sets) to look for trends in population dynamics to inform potential mouse damage situations. We collect trapping information from the 3 Benchmark sites only, and utilise the rapid assessment techniques (mouse chew cards and active burrow counts) on all other sites across the 5 states. We are building a large body of data which will be analysed to look to further refine the techniques used. This analysis requires situations when mouse populations have been moderate and high, and unfortunately, during the period of 12-09 and 2015-19, there have only been one or two instances of high populations, so the analysis is dominated by zero or very low captures, and at the moment does not really reveal very much. Further analysis of these data are planned.
- Biostatistician consultation by investigators for assistance with experimental design and sample size calculations when preparing an AEC application is required to ensure animal numbers and wastage are kept to a minimum.
- Pilot studies are to be conducted to assess the feasibility of project design and perform a cost benefit analysist prior to continuing to a main study.
- An 'Animal Tissue Sharing Program' is available to all researchers where animals have been humanely killed.
- Fluorescence protein expression - This technique will enable us to enrich embryos that possibly carry integrated transgenes, contributing to reduce the number of fish that will be raised as founder candidates.
- We have optimised our end-point analysis, aiming to perform several analyses using the same explanted heart sample, rather than needing one heart sample per analysis: Specifically, we have established a multimodal pipeline, where explanted whole hearts are images using micro-CT, then sectioned for histology and lastly ultra-thin sectioned for electron microscopy. Similarly, we have optimised our DNA and RNA extraction methods to achieve higher yields, allowing sufficient DNA and RNA recovery from a single heart rather than pooled heart samples.

- By accessing to a multi-electrode array system is used to study electrical and chemical properties of explanted adult zebrafish hearts (ECG, conduction velocity, electrical stimulation, calcium transients), and which furthermore allows treatment with different drugs, allows numerous different parameters to be measured and tested consecutively using the same heart, reducing the numbers of hearts/ animals which would be needed to gain the same amount on information from in vivo experiments.
- Where possible, we also reduce the number of animals used by first conducting in vitro experiments to investigate dosing regimes, targeted therapies, gene knock-down or overexpression studies, such that only experiments with proven efficacy in vitro are investigated in vivo.
- Tissue culture techniques will be used in this project wherever possible to reduce the numbers of animals used. We will also seek to share tissues with other researchers at harvest and we will endeavour to donate excess mice (mostly males) that have resulted from breeding to other users.
- We have used in vitro models wherever possible and use cell lines and tissue culture wherever possible.
- We will perfuse organs within these mouse studies to enable us to come back to different tissue samples and we will make a tissue library for other researchers to investigate proteins of interest if required.
- The animals used in the setting and testing of the aquatic system will be made available for researchers and the breeding program.
- Studies are designed to optimize control groups. Tissues can be shared with other researchers.
- We have aimed to reduce the number of mice used by designing experiments that maximise the amount of data generated (e.g. by analysing several parameters within the same sample by twophoton microscopy, micro-CT, histology and serum markers of osteoclastic activity) as well as optimizing the quality of the data (to avoid unnecessary repetition of experiments).
- We have reduced mouse numbers used by first optimising our experimental protocols and time points to be assessed in a previous ARA.
- The use of mixed bone marrow chimeras instead of creating double lineage reports for our osteoclast imaging work reduces our animal usage dramatically.
- Developing recovery procedures with our intravital imaging such that we can achieve longitudinal data from the same mouse over 3 time points. We have also developed our ability to obtain longitudinal serum samples through retro-orbital bleeds, again achieving data at multiple time points from the same mouse.
- Ultraovulation will be used if possible to replace superovulation and reduce the number of donor mice.

- Use of sperm freezing and IVF for rederivation whenever possible as less mice are required.
- Promoting cryopreservation instead of keeping a maintenance colony of mice.
- Use of direct, non-terminal health screening of imported mice to replace sentinels
- The animal numbers allotted for each experimental and control group represent the absolute minimum number of animals required to produce enough tissue, data and replication for satisfactory statistical analysis of data.
- Females that fail to become pregnant in the timed mating approach (typically 2 of the 4 setup) are re-used in subsequent rounds of timed matings.
- Experienced males (successful breeders) are re-used for subsequent rounds of timed matings
- The animal numbers allotted for each experimental and control group represent the minimum number of animals required to produce enough tissue, data and replication for satisfactory statistical analysis of data. For the characterisation of the new mouse line we have proposed performing the behavioural tests on mice being used for immunohistochemistry/sequencing analysis to reduce total number of animals used.
- For short-term pharmacodynamics analysis, tumour expansions or explant studies, animals may receive bilateral MFP tumour implant to reduce the number of animals needed.
- The experimental designs used in this project have been calculated to minimise the number of animals used whilst maintaining adequate statistical power. Where possible, we will reduce the number of animals used by first conducting in vitro experiments to investigate dosing regimens, targeted therapies, gene knock-down or overexpression studies, such that only experiments with efficacy in vitro are investigated in vivo.
- We will assign mice that refuse to exercise to non-exercising groups where possible to ensure we do not waste any animals.
- For the characterisation of the new mouse lines we have proposed performing the behavioural tests on mice being used for immunohistochemistry/sequencing analysis to reduce total number of animals used.
- Use of hormone synchronization for time mating of females should minimise the number of females that need to be mated.
- Ultrasound diagnosis of pregnancy at > 9 days gestation minimises wastage associated with issuing non pregnant females.
- Cryopreservation is encouraged to minimise the practice of maintaining mouse line using minimal breeding.
- Enhanced identification using tail tattooing results in fewer mice wasted from mistakes during issuing and mating.

- Where feasible we will collect tissues for biochemistry and histology from the same animal that has undergone surgery to allow a reduction in the total number of animals needed for these studies.
- Some biomethodology training allows mice to be reutilised for further training after a rest period of 1 -2 weeks.
- Survival sampling of mice has minimised the number of mice used in health monitoring. The health status of immunocompetent mice can be assessed using a single drop of blood, an oropharyngeal swab (culture), faecal swab (culture), pelage swab (PCRs) and a sample of faeces (PCRs).
- We will be using contralateral limbs as a control for the limb undergoing exercise intervention, reducing animals required to serve as controls.
- We have aimed to reduce the number of mice used by designing experiments that reuses mice whenever possible such as for two-photon microscopy at several timepoints and maximises the type of data generated (eg by analysing several parameters simultaneously by two-photon microscopy, flow cytometry and quantitative histology) and the quality of the data (to avoid unnecessary repetition of experiments).
- A minimum sample of 30 Wedge-tailed Shearwater fledglings per year was identified as necessary to adequately quantify variation in fledgling body condition such that differences due to the ingestion of plastic can be detected. However, for Little Shearwater fledglings, sample sizes have been reduced to minimise disturbance to this small population. As a result, it may be difficult to identify trends over time, however, the information generated in regards to ingestion rates and metal concentrations is still very relevant and useful (knowledge of any aspect of this species' biology is extremely limited, worldwide).
- A maximum total sample size of 480 yellowtail kingfish from the waters adjacent to southeast Australia is the number animals required to achieve this project's aims with statistical validity, thus providing results that are robust to the peer-reviewed publication process. This sample size can be considered as 60 fish per location per sampling season. This number has been adopted from the results of two power analyses. All biological information that can be practically attained from each fish will be in order to maximise the value of killing these animals for scientific purposes. This information may be used to create convergent lines of evidence to bolster the results of the present study, used in a subsequent follow-on study, or contributed to colleagues also studying aspects of the biology and ecology of yellowtail kingfish. These include; tissue samples for stable isotope analysis that can help to determine feeding position of this species within the ecosystem, blood samples (10 ml) to measure various aspects of body condition, gonad samples that can indicate the

animal's state of sexual maturity, otoliths (ear bones) that can be used to age the fish and fin clips that DNA can be extracted from to determine the genetic connectivity of different population of this species.

- Variation in fledgling body condition (e.g., mass) can be due to factors other than ingested plastic. For example, adult birds may provision their chicks with food at different rates based on the availability of prey. To try and account for natural variation, a statistician and associate investigator on this project is regularly consulted to ensure the sample sizes and methods being used are appropriate to answer the question(s) being asked. A number of years ago, we identified a minimum sample of 40 fledglings per year to adequately quantify variation in fledgling body condition such that differences due to the ingestion of plastic can be detected. This sampling schedule has also been adopted by one of the longest running seabird-plastic monitoring programs in the world ('Save the North Sea') which also utilises 40-50 Northern Fulmars as the minimum number of birds that should be sampled each year to monitor plastic pollution in the North Sea (van Franeker et al. 2005).
- Excess guinea pigs were re-homed through a non-profit in 2018. All 173 that were re-homed were in excess of the institution's needs and had not had any scientific procedures performed on them. By giving them to the re-homing organization, they were able to be placed as pets in suitable homes rather than being euthanased.
- Vaccine potency tests in rabbits for new formulations in development have had a reduction in animals from the standard 10 vaccinates to 5 vaccinates. This halves the number of rabbits being used for formulations in development and is a great reduction particularly given they are the subject of unknown formulations and may have undesirable results such as injection site reactions. The reduction is unlikely to jeopardise the scientific validity of the study as the tests are not registered for proof of concept work and rabbit blood samples are pooled for testing.
- All animals at this site underwent a health check prior to inclusion in the study. Some animals were excluded from the study based on their mastitis or general health status. This process ensured that only healthy animals were enrolled in the study, as a way of reducing the number of animals used.
- Since it was expected that there would be no blowfly pressure at two study sites, it was decided that only pain-relief study variables would be assessed at these sites. Therefore, only a maximum of 80 lambs were used at each study site, instead of a maximum of 400 lambs.
- The study was originally approved for 10 investigator sites, however after the lambs at the second site displayed signs of stinging when the novel formulation was applied to their mules wound the decision was made to not proceed with further sites. This resulted in a reduction of the animals used in this project.

- This pilot study was designed to use minimal numbers of animals to achieve the study objectives. Ten animals were included in each of the four treatment groups at both sites, which was considered sufficient to demonstrate pain on application and safety & efficacy of the formulations.
- A maximum of six animals was used per group. This number was the minimum required to give sufficient power for statistical analysis whilst still ensuring maximum reduction of the animals used.
- Animals were re-used between phases of the study and were reused in a total of 3 studies to ensure a reduction in the total number of animals used throughout all studies.
- Animals were injected in one hindpaw one week, and after one week's washout, they were injected in the other paw the following week. This strategy allowed four groups of twenty animals, whilst only using a total of forty animals instead of eighty.
- Blood samples are used from clinical cases that have been cleared of being infectious.
- 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.
- Protocols are constantly being reviewed. Students are placed within small groups where animals are used for surgical or other training. This reduces animal use.
- Requiring investigators to provide a sound statistical basis for animal numbers, especially in medical research projects.
- Promoting the use of pilot studies, particularly where models or methods are being used for the first time.
- The AEC expects investigators to consult a biostatistician when planning a project as to the number of animals required to ensure that any study will deliver appropriate statistically significant data relating to the stated project objectives. Thus, the overall aim is to minimise the number of animals needed to obtain statistically reliable results. However it is also essential that sufficient animals are used to achieve a statistical valid result and not have to possibly repeat studies and use more animals.
- Proof of concept and pilot studies may be recommended by the Committee where concerns are raised and based on the preliminary results presented, the Committee will decide if the project will proceed or be terminated.
- Where ever possible, investigators are asked (with input from a biostatistician) to determine the minimum number of controls required within experimental groups.
- In regards to breeding colony management, investigators are advised to breed sufficient numbers only to supply their experimental project needs and to provide future breeders to maintain the colony/s.

- Using retired breeder rats as a terminal blood feed source for mosquitoes and bed bug colonies.
- The establishment has a statistical consulting unit that is dedicated to providing free advice, support and assistance in experimental design to ensure animal group numbers are correct and will provided meaningful and statistically valid results, minimising animal waste.
- A reduction on impact on some species in the wild through the development of partnerships with other organisations, consolidating monitoring and trapping of species and sharing data.
- The use of museum specimens wherever possible to replace the use of live animals.
- A formal notification from the AEC to the senior managers of research schools using animals reiterating the requirements of the Code and the AEC's expectations re: demonstrating good statistical design of animal studies and the need to provide evidence with applications.
- Increased promotion and requirement of pilot studies, well designed so that pilot results can contribute to a larger study if it goes ahead based on pilot results.
- Where possible, once experiments were completed cadavers were shared by other researchers for training in dissections and stereotaxic procedures. Animal tissues are frozen to be shared by other researchers for protein extraction rather than purchasing additional animals for the same task.
- A biostatistician continued to support the AEC and researchers throughout 2018. The biostatistician provided valuable advice and challenged investigators to statistically confirm the number of animals requested and to validate statistical data obtained from animal experimentation.
- Researchers are encouraged to share tissue samples wherever possible.
- Breeding programs are designed and maintained to produce stock to order, to reduce numbers and overproduction.
- Genetically modified animals are bred for the desired genotype as far as possible to reduce numbers.
- Animals used for courses are shared between multiple participants to achieve the best learning outcome whilst reducing overall numbers of animals used.
- Training courses and induction programs refine techniques to ensure the most meaningful results are achieved with the minimum number of animals.
- The establishment actively encourages researchers working together to develop projects that can be run in parallel, which uses different tissues of the same animals in order to reduce the overall number of animals.

- Established a tissue bank where unused samples can be stored for future projects by students. Collected tissues from different disease models are also being shared across research disciplines in laboratory based projects.
- The project will involve drone surveys over the minimum number of plots that will still result in accurate and useful results.
- Moderate size herds from representative production environments have been included to increase accuracy and reduce the need for further replication.
- The algorithm and sensors already reduce the number of non-target animals affected.
- Moderate size herds from representative production environments have been included to increase accuracy and reduce the need for further replication.
- 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.
- Introducing students working in groups where each student dissects a different species, students gain an idea of the diversity of fish anatomy without having to dissect multiple specimens themselves.
- Less animals were collected than originally planned, so replicate numbers for each treatment were reduced.
- A protocol investigating a new application of liposome in metabolic disorders used a pilot study to test the efficacy of the dose regime.
- A protocol investigating whether parasite molecules alter metabolic activity of macrophages used primary cells instead of whole animals to significantly reduce the number of animals required for the study.
- A protocol modelling third-hand e-vaping used minimum numbers of animals in a pilot study to determine the power calculation for follow-on studies.
- A protocol determining how thermal pollution in Australian rivers affects native freshwater fish removed one species from their experiments as research had already been conducted on this species, and used animal numbers which were statistically significant and which had been used in similar studies on the same species.
- A protocol investigating the impact of maternal exposure to low-nicotine cigarette smoke on foetal outcomes and long-term health risks in offspring compared to maternal exposure to normal-nicotine cigarette smoke conducted power calculations using data from a previous study which used the same model. The left and right brain hemisphere, lungs and kidneys will be used for different procedures (histology and mRNA assays), halving the number of animals used.
- A comprehensive program of monitoring and excluding pathogens in the Animal Facility is using excess animals from existing protocols.

- A protocol investigating the asthma and Chronic Obstructive Pulmonary Disease will carry out multiple measures on one tissue by diving it into two halves so that the number of animals for endpoint measurements is not doubled.
- A protocol establishing a dose-response curve of e-cigarette exposure has made their data available to other users of the same model for power calculation purposes.
- A protocol developing a set of methods for an ECG analysis process, tailored to the detection and identification of the normal and abnormal morphology of the canine sinus rhythm reduced the number of animals for their protocol based on existing data sets in the literature.
- By using the same animals for more than one project. A reduction was made in animal numbers by using the same ewes from one project to produce lambs for another project.
- The AEC continues to reduce the numbers of animals in experiments. Extensive statistical analysis and justification is required in every application (including consultation with a statistician or statistical referencing) to ensure only the minimum number of animals are used, while remaining statistically useful and able to demonstrate significant difference. Furthermore, Research Scientists are continuing to use advanced statistical software packages that utilise more precise sample power calculations to determine appropriate sample size for experiments. There was a reduction in pig numbers used in 2018 (compared to 2017), despite an increase in approved projects.
- Several experiments have adopted staged approaches where a smaller number of animals are exposed to treatments and if there are no adverse effects, then the remainder of the animals undergo treatment.
- We collect various organs from all mice that are euthanased according to the approved procedures. With these we have built up a "tissue bank", which means that we do not have to carry out some experiments because we already have the necessary samples.
- Our local manipulation of gene expression using a single intramuscular delivery of AAV (as opposed to systemic manipulation of all the muscles in a mouse) mean that control and treated muscles are derived from a single mouse. This reduces the number of animals used by 50%.
- To reduce and refine our animal experiments, we analyse results of an experiment before starting the next experiment. This analysis includes statistical testing to determine the minimum number of mice required to achieve statistical significance in follow up experiments.
- Mammary transplantation techniques were undertaken when possible which enables a single donor mouse to be utilised over twenty subsequent host mice. This results in

less mice being used as there is not the wastage associated the wrong genotypes being produced.

- Multiple procedures are performed in each sheep (i.e. three valve insertions in each sheep) with multivariate statistical analysis performed for each procedure, thereby reducing the number of sheep required.
- Tissue samples are collected and stored for future use or use by other researchers
- Surplus animals are used by other researchers or as a teaching resource.
- In teaching practicums, where two experiments are to be performed by students, half the class will perform one experiment and the other half will perform the other and share the resulting data.
- Sourcing of invertebrate tissue for use in class practicums.
- Experience from previous studies or information from similar studies is utilised to ensure appropriate numbers of animals are used in experiments.
- Where feasible, observational studies are conducted and/or recorded for future use and analysis.
- Where possible, animals are sourced from other approved projects where they would otherwise not be required.
- Researchers are asked to use longstanding and well-established procedures in their research to ensure the minimum number of animals are used.
- The AEC promotes the use of cadavers and maintains a register of their use by researchers and teachers.
- A statistician is appointed to the AEC and attends the meetings to ensure that the minimum number of animals required for research or teaching are approved.
- The trial farm used by the projects approved by the AEC is a miniaturised commercial facility with small pens, thereby significantly reducing the number of animals used if full sized commercial facilities were used for similar experiments.
- Re-use of animals – dairy cattle were transferred to a project for education and teaching purposes.
- Animals used in teaching are assigned to a group of students rather than a single individual.
- Pilot studies are often undertaken by investigators before proceeding further.
- Close scrutiny of the number of animals requested and Biometrician's comments reviewed to ensure numbers are adequate to obtain the desired statistical outcomes, to minimise the number of animals involved in trials and to ensure that trials do not have to be repeated unnecessarily.
- Similar studies have shared the same control animals.
- The Animal Ethics Committee requires that all projects are signed off by a biometrician. This, along with close scrutiny by Committee members, ensures

numbers are adequate to obtain the desired statistical outcomes, but to still minimise the number of animals used, and also to ensure trials do not have to be repeated unnecessarily. The Chair is actively working with the Biometrics and Data Analytics team to improve collaboration and understanding of AEC requirements of biometricians.

- The establishment has purchased a combined CT scanner and bioluminescence imager (the 'IVIS Spectrum CT animal imager'). This technology is expected to reduce animal usage by allowing monitoring of a single cohort of animals over the full course of an experiment rather than having to kill multiple cohorts of animals at set time points.
- Proof-of-concept studies for a chemotherapeutic trial found that certain candidate compounds could be excluded due to lack of efficacy. This meant that the number of animal cohorts could be reduced.
- The study design has permitted a slight reduction in the number of rats that were originally anticipated for this study, and efforts are being made to reduce animal numbers further.
- It is planned that future studies in cancer chemotherapy will require a reduced number of cohorts because of implementation of monitoring with the IVIS Spectrum CT animal imager.
- It was possible to conclude from early data that a smaller number of lizards needed to be captured as part of a trial to assess feeding.
- Use of invertebrates by some student projects meant less vertebrates were studied.
- Control groups were shared amongst projects by varying the projects to add investigational drugs using the same administration route and vehicle.
- Multiple procedures on physiological variables within each animal reduced the required sample size, and a lower number of animals compared to the initial plan were used.
- Procedures were repeated in the same mare in subsequent reproductive cycles, minimising the number of animals required.
- Tumours were grown on both flanks to reduce the overall numbers of mice required.
- Utilised spare tissue from previous experiments to determine the number of mice needed for the experimental endpoints to yield useful data, avoiding receiving insufficient statistical power or using excess mice.
- Multiple tissues were collected from individual animals to enable analysis of as many parameters as possible from each animal. Group sizes represented the minimum number of animals required to obtain statistically relevant data.
- Multiple samples were taken from each mouse to reduce the number of mice required. Control groups for multiple compounds were conserved and combined to eliminate the need for more groups to control for each intervention.

- Ran experiments simultaneously and shared controls across experiments to reduce mouse numbers.
- Adopted a technique for culturing colon sections in the 24 well plates, which produces 6-8 sections per mouse. This significantly reduced the number of mice that were required.
- Only those compounds that showed efficacy in vitro were subsequently tested in vivo, thus reducing the animal numbers being used.
- The number of mice used in the project was reduced as the number of tumour cells required were less than expected.
- Used in vitro cell model systems to study cell to cell transmission and pre-screen reagents.
- Reused males for multiple breeding experiments.
- All groups were run at the same time so the same controls can be used without the need for repetition.
- Consulted with statistician to reduce the number of animals needed to reach statistical significance and reduce redundancy.
- Ran a pilot study to ensure infection is successful before proceeding with more extensive experiments, and multiple analyses were performed on the same animal.
- Tissues from animals in one project were reused in other experiments to obtain preliminary results.
- The tissues collected in one study were used as additional control tissues for another project.
- All samples (blood, serum, harvested cells, tissue samples and homogenates) in excess of experimental needs were stored to create a mini biobank of samples. The availability of these stored samples should reduce animal numbers required in future studies.
- Control groups were run in tandem to reduce the number of mice required.
- The breeding strategy for mice was adjusted so that the genotype of the offspring is known and no animals were wasted due to a wrong genotype.
- Where there were multiple pups of one sex in a litter, researchers ensured that each pup was usable in a group by allocating different treatments and/or age group collections.
- Collected both endocrine and peripheral tissue from the same cohort.
- Collected a bank of tissue to assess both reproductive and peripheral tissue from the same cohort.
- Experimental validation of the nanoparticle efficiency of both targeting and editing was carried out in vitro, to allow the selection of an efficient combination for the in vivo experiment

- Collected multiple tissues/cells from one mouse.
- Reduced numbers by testing each rat on more than one test and by using tissue from same animal to perform as many molecular experiments as possible.
- The other eye was used as a matched untreated control, which added power to the design as inter-ocular differences are less than inter-subject variability. This reduced the number of animals required.
- Multiple tissues were collected from individual animals, minimising the number of animals required to collect sufficient replicate data for all parameters to be tested.
- Mice were shared amongst groups by co-ordinating experimental days. From the same mouse, one group used the spinal cord and the other used the inner ears.
- Once the researchers were able to establish the model, the number of animals were reduced to the minimal necessary for statistical differences between groups involved in the experiment.
- Collected a bank of tissue to assess both reproductive and peripheral tissue from the same cohort. The development and use of viral vectors reduced the need to breed and maintain transgenic mouse colonies
- Shared tissues amongst other experimental groups.
- Used teaching microscopes with live camera attached so new students and investigators can watch extraction of sperm and/or eggs from the tissue in real time to minimise use of mice for teaching purposes.
- Used both sexes to minimise animal wastage as well as ensuring results have the widest applicability. In vitro studies were undertaken first to provide a mechanistic understanding, ensuring that in vivo studies efficiently capture all relevant data and minimised the number of animals required.
- Utilised Xenogen bioluminescent imaging to reduce the number of mice that would normally be used. Using sequential bioluminescent imaging, the same mouse can be followed during the course of treatment.
- Timed the breeding of mice to produce a greater pregnancy rate and reduce the number of females used to attain the same number of pups.
- Careful scrutiny of the numbers of animals requested to ensure that sufficient numbers are used to provide a statistically valid result, thus preventing the need for repeat experiments and use of additional animals.
- Approval of new techniques for embryo freezing rather than continuous breeding to maintain lines.
- Re-use of animals, where appropriate, after extended recovery interval.
- Making surplus tissue available through a Tissue Database and seeking prior agreement from investigators to make surplus tissue available.

- Consolidating breeding protocols to ensure no over-breeding which in turn reduces the need for culling.
- Rederivation: Animal facilities optimise fostering process and thereby minimise the numbers of female mice used for fostering purposes.
- Training: Animal facilities use mice for training purposes that were identified with an undesired genotype (hence would have been euthanased regardless).
- Sharing: Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.
- Continuous review of data obtained during experiments to refine our estimates of group variability and repeat power analysis to determine if sample size may be reduced in subsequent experiments.
- A number of researchers are utilising pilot studies to optimise animal numbers – often statistically significant results can be obtained with smaller numbers of animals.
- Data from previous studies are utilised to reduce the number of animals required.
- The establishment continues to encourage researchers to harvest and share tissues. In instances where animals have been humanely killed specimens are donated to the museum or other researchers.
- Sharing of tissues or storage of samples for re-use in future protocols where possible.
- Re-use of animals for research that would have been already in the laboratory for other research in order to reduce the number captured from the wild.
- Collection of samples such as hair, mouth/sternal gland/pouch swabs and blood from animals captured for other routine health checks minimising handling and use of wild animals.
- Blood samples collected from animals brought to the wildlife animal hospital by members of the public. In instances where a blood sample would normally be taken as part of standard prognosis and treatment procedures for wildlife.
- Re-use of animals from other previously approved projects where the animals are not allowed to be released back into wild and would otherwise be euthanased.
- Rehoming and re-use of 350 animals (lizards) with a long-term known pedigree from another tertiary Institution which would have otherwise been euthanased. These lizards have a long-term known pedigree and will provide researchers with access to both the parents and offspring for behavioural experiments. The level of background information provided is extremely rare and often takes years to obtain.
- Use of captive animals for observation and filming of feeding behaviours reducing use of wild animals.
- Transfer of animals (undesired genotype/sex) from one project to another as approved by AEC.

- Re-use of animals among multiple projects when ethically justified and as approved by AEC.
- Pilot studies to ensure the least number of animals are used to obtain statistically valid data.
- Use of in vivo imaging technology to perform repeated measurements on the same animals over the course of an experiment, resulting in a significant reduction of animals used.
- Combination of experiments so that only a single "control" group is used.
- Researchers continue to focus on monitoring design for trapping projects to identify the minimum sample size for survey implementation. Reductions in sample sizes have been achieved through greater attention to design.
- Continued review by principal researchers and AEC members of the numbers of animal required to conduct field surveys. This has been applied to field work requirements for green and golden bell frog and annual review of ongoing fish surveys using gill nets.
- Simulated penning of sheep by demonstration.
- Minimum number of animals used in teaching activities.
- The number of occasions that an animal is used is minimised e.g. lambs are tagged and drenched at the same time as normal management schedule.
- Working with university researchers and National Parks on native animal projects rather than duplicating own projects.
- The minimum number of animals are used to execute the training and development of student skill levels. Where possible photographs, taxidermised and preserved specimens are used.
- Appropriate animal to student ratio.
- Students attend various workplaces to reduce the use of a particular mob of animals.
- Use normal scheduled animal health husbandry routines for teaching activities.
- Use of a booking system and individual animal records to record animal use.
- 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.
- Power analysis for statistics instituted to prevent under or over use of animals.
- Regular stocktake of animals so as only maintaining those required.

- More frequent communication with researchers so wildtype animals are culled when no longer needed and soon after genotyping is performed.
- Tissue sharing.
- Using only minimum number of animals – discussion with researchers about their proposed AEC project.
- Practising technical procedures on recently culled animals to gain experience in new techniques prior to working with live mice.
- Culling unwanted male stock and keeping only the outbred females e.g. with ARC/s mice.
- Routine meetings with research groups for colony management to ensure breeding is optimised for experimental or maintenance production only, thereby minimising or eliminating the generation of unrequired animals through breeding strategies used.
- Application of pilot studies for AEC projects where appropriate and improved experimental design and statistical analysis.
- Statistical analysis to ensure appropriate numbers of animals are used (not too many, not too few).
- Sharing of tissue among researchers.
- Use of pilot projects with reduced animal numbers.
- Obtaining more data from the use of fewer animals by combining objectives.
- Close scrutiny of the numbers of animals requested in applications and progress reports to the Committee.
- Incorporating animals from one project as breeding stock for a subsequent project, rather than discarding.
- 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.
- For rodent studies for registration purposes the APVMA states the minimum number of animals required, and only the minimum requirement is used.
- Preliminary experiments to assess variability -> power calculations to use minimum numbers of rats & mice to give statistically valid results.

6.3 Refinement

- SOPs are used, modified and reviewed as required.
- As part of an ongoing training commitment to the AEC committee members participated in 3R's survey conducted
- Provision of enrichment options to all Sponsor's 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

- Staggered dosing of animals in research studies where little is known about the active to reduce the likelihood of multiple adverse events.
- 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; these include rope toys, Kongs with treats inside.
- 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.
- Suitable pain relief is always used for any intensive or surgical procedure.
- A project investigating the host-pathogen dynamics in amphibian chytrid fungus disease and 'reservoir species' (frog species less susceptible to it) is exploring the use of photographic identification method for individual frogs. The aim of this method is to decrease the need for more invasive marking techniques in amphibian research in future.
- Installed additional silos and feed heads on farms participating in a project to minimise human intervention in administering the treatment product.
- GoPro, footage is being utilised in a project to monitor feed residuals and reduce human intervention.
- Trained personnel only administer treatments and collect samples on commercial farms to reduce adverse impacts on animals.
- The project with flying foxes has improved their field protocol for holding animals after capture while being processed to improve animal welfare.
- The use of cameras and audio equipment (acoustic and ultrasonic) reduces the need to trap animals. 13 of the 17 approved projects used the remote recording devices, along with other survey techniques, including trapping.
- The use of the Animal Welfare Unit survey guidelines and the establishment's fauna survey protocols, provides information on correct, current trapping and handling techniques for fauna. The latter also provides information on the ethical considerations for different species and survey techniques.
- Steps have been enacted to ensure the safeguard of the livestock. Using more sheep for shorter durations fulfils the requirements of the experiment and reduces the length of time for both humans and animals. Sourcing the sheep from a location close to the research experiment assists to reduce transport time from the collaborating farmer. After consultation with the farmer and the manager, the sheep (either DRY ewes or large lambs) will be moved to the neighbouring paddock to graze either the wheat or canola and negate the potential issue of the negative effects of grazing

canola by ewes in the early stage of pregnancy. While the sheep are grazing, they will be continually monitored to ensure the wellbeing of the animals.

- Our plan includes steps to safeguard the animals in our study. Sheep are sourced from the existing flock on each property and therefore transportation to the experimental site is minimal. We have also identified the possible symptoms of ill health in sheep and will monitor wellbeing on a daily basis, including weekends.
- The sampling of the animals will be done when animals are mustered (moved) by the farmers for other purposes (e.g. when farmers are doing the preg-test, control of parasites, moving animals to other paddocks, etc). Doing it will reduce the number of times mustering the animals.
- Our sampling methods will minimise pain, suffering, and distress and are expected to exhibit no lasting harm. The animals will be in a cattle crush for approximately 5-10 minutes which would not be considered prolonged.
- Through the development of the rapid assessment techniques (mouse chew cards and active burrow counts) we have refined the techniques to measure mouse activity. These techniques allow us to monitor a large number of sites reasonably quickly so we can gain a picture of mouse populations which informs our understanding of the potential for mouse populations to cause economic damage to crops. All live trapping is conducted by experienced staff, and the welfare of these animals is the highest priority. All traps are checked within 2 hours of dawn, and adequate food (wheat grains) and dacron for nesting material are provided in the traps. The handling time of mice when checking traps is about 2 minutes to reduce stress. We note that we often obtain recaptures of mice on subsequent days, and in all cases these animals have been active and healthy. If the weather becomes too hot, we will pick up all the traps and put them in the shade and provide animals with a piece of apple or potato for moisture and release mice into mouse burrows near capture location when conditions are cooler. We collect the minimum amount of information for us to understand the mouse population dynamics (population size, breeding condition, overall condition, population dynamics etc).
- Research using organisms with no or limited sentience such as zebra fish embryos instead of mammals.
- Observation only studies for wildlife research including the use of field surveys, scat surveys, camera traps and drones.
- Direct access to experienced staff in Animal Ethics, Welfare and Services and a full time veterinarian for the entire duration of an AEC project.
- Researchers are encouraged to consult the 'Animal Ethics Review Support Group' for assistance in writing an AEC application.

- Conditions of approval are applied to projects for the Animal Welfare Officer or Animal Facility Supervisor to oversee an initial procedure conducted or competent Animal Services Technicians to conduct the procedure on behalf of the project investigator both within animal facilities and in the field.
- Adoption of an online database for use by both Animal Services and researchers to monitor animal usage and avoid potential excess breeding of animals.
- Ongoing training and upskilling of Animal Services staff involved in the care and use of animals for scientific purposes.
- The use of zebrafish (embryos) in general can reduce the impact on sentient animals because much research is conducted on embryos before their nervous system has fully developed pathways for perception of pain or distress.
- The experiments in this project simply require the generation of embryos by natural breeding methods, which generate little or no stress to animals. Microinjections will be performed with one cell stage embryos, which unlikely sense any pain or stress during the procedure since there are no neurons present at this stage of development. We will generate transgenic constructs of which expression is monitored by fluorescence protein expression. This technique will enable us to enrich embryos that possibly carry integrated transgenes, contributing to reduce the number of fish that will be raised as founder candidates.
- The use of zebrafish as a vertebrate model system can be defined as an act of replacement. Since zebrafish are externally fertilized and embryos can be easily collected outside the mother, the proposed studies do not require interventions that would be otherwise necessary if mammals were used to perform similar studies.
- Microinjections will be performed with one-cell stage embryos, which unlikely sense any pain or stress during the procedure since there are no neurons present at this stage of development.
- Shipping frozen embryos of sperm instead of live mice prevents the animal welfare problems sometimes associated with the transport of live mice (especially on long haul international flights).
- The training and skill of technicians is important in minimising stress on the animals. All staff working with zebrafish will undergo a training program including the online AALAS Zebrafish husbandry course as well as one-on-one training within the facility to ensure that the animals are managed well.
- All researchers are appropriately trained and experienced in the procedures to be performed and mice are handled regularly to minimize stress during experimental analyses.
- Inhalational anesthesia obviates any pain caused by subcutaneous injections or retro-orbital bleeds or injections and replacing tail vein injections without anesthesia with retro-orbital injections. In addition, mice undergoing non-survival surgery for

intravital microscopy experiments will have continuous monitoring to ensure surgical plane anesthesia and regular clinical assessments of response to painful stimuli during the experiment.

- We will conduct a pilot trial under the control of a veterinarian to establish clear endpoints that minimise the total amount of pain and or distress caused to the animals.
- Bactrim will be added to sterile drinking water (0.032mg/mL Trimethoprim, 0.16mg/mLSulfamethoxazole, with an estimated daily dose of 30mg/kg Bactrim) for 1 week following surgery to minimise the likelihood of contracting an infection from xenografted tissue.
- 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
- A protocol has been modified to eliminate a second adjuvant vaccination. Additionally, pertussis toxin is given IP to avoid tail intravenous injection.
- Experienced males (successful breeders) are re-used but must be single housed (to avoid fighting with other males) so these males are housed with OVX females as companions.
- House pregnant females in pairs after timed mating.
- Longitudinal imaging of mice after recovery from surgery will allow us to perform more powerful analyses by comparing changes within the same animal thereby reducing the number of animals used. We have also refined our experimental procedures to increase the efficiency of the imaging and minimise any trauma from surgery. Adoption of the Mouse Grimace Scale has also improved our ability to detect post-operative pain and provide adequate analgesia. We have also refined our photoconversion method such that we only make a small incision overlying the lymph node. This avoids making a large skin flap and imposes a far less physiological insult to the animal. There is consequently much less discomfort and pain and the mice are less likely to remove the fewer smaller sutures. Thus this refinement has also significantly reduced our mouse use by increasing the success rates of photoconversion experiments.
- Use of analgesic after surgery.
- Aseptic surgical techniques.
- To reduce pain in post-transplant animals, we have referred to literature for optimal pain control regimens. All animals will be closely monitored for the first 6 hours post-surgery and then checked twice daily in the first 48 hours or more if any signs of pain are noted. Pain score charts and analgesia charts will be kept for each animal for the 7 days post transplants. Food and water will be provided at foot level to minimize stretching that can cause abdominal wound pain.

- In vivo imaging also reduces stress to the mice by allowing careful monitoring of tumour burden and metastasis and termination of experiments before mice become moribund.
- 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.
- Use of extra enrichment or companion mice where possible.
- Pair housing post embryo transfer surgery.
- Where xenografts or drug treatments results in weight loss, saline (SC)/soggy food/gel will be provided to animals.
- Animals will be kept in recovery cages individually (half the cage on heating pad to allow thermoregulation) overnight post- surgery with careful monitoring.
- All researchers undergo specific training, which will assist them with recognition of pain and distress in laboratory animals. Internet resources, such as http://www.ahwla.org.uk and http://www.procedureswithcare.org.uk are utilised to assist with training.
- We have adopted the use of companion fish to avoid social isolation of fish required to be held in single tanks or single-sex tanks and prevent females from becoming egg-bound. For this purpose we use the transgenic line "mzkrt", which, due to its red, glowing body, is easily distinguished from experimental fish sharing the same tank.
- The transport protocol involves the use of transport cages inside darkened trolleys and then 30min to settle before handling for IP injection.
- All procedures (except for surgical procedures required to be performed in PC2) will be performed on level 1 so as to minimise stress from transport.
- 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.
- Mice undergoing intravital microscopy experiments will have continuous monitoring of physiological parameters (such as respiratory rate and amplitude) to ensure surgical plane anaesthesia and regular clinical assessments of response to painful stimuli during the course of the experiment.
- By using ear tumours for photoconversion and imaging experiments we have reduced the amount of pain and stress to the animals since our approach avoids the need for surgical procedures to access tumours.
- We will also administer anaesthesia prior to injecting tumor cells. This has the double advantage of sparing animals any unnecessary stress and fear, and delivering accurate tumor cell injections, which are comparable in each animal. Reduced variations and reproducibility of tumor growth is the key to keeping low numbers of animal per group.

- We have established an ultrasound protocol requiring no contact between ultrasound probe and animal and allowing the fish to remain submerged in water at all times. For ECG recordings, we have replaced needle electrodes with non-invasive button electrodes that require minimal/no direct contact during ECG recordings.
- Expanded on-line training for technicians to include the recognition of pain and distress, assessment and monitoring of wound healing, anaesthesia, and aseptic technique.
- Because the study is specifically designed to evaluate feral horse numbers, there is no possible alternative to replace the use of live animals. The number of animals in the area is being recorded, so there is also no option to reduce the number of horses. However, the impact on the horses is being reduced by conducting the study completely non-invasively, using observations taken at a distance from the horses, and using remote cameras.
- 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.
- Only experienced and trained people will be allowed to perform any procedure with minimal numbers in attendance to reduce stress to the colony and to reduce the likelihood of burrow collapses or damage to the vegetation.
- The GLS devices weigh a maximum of 3 grams less than 1% of an 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). 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.
- Individuals will only be handled once per year for three years in a row reducing the number of times each bird is handled. 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, we will identify burrows for the GLS device group that are easily accessible from walkways to reduce the time spent in the area and the likelihood of burrow collapses or vegetation damage.
- The feather analysis will be undertaken during the day to avoid disturbing adults returning to feed chicks. The chicks will be handled by experienced and trained people with a maximum of two people required for each visit.
- Stomach contents will be obtained during the night with one to two trained and experienced people in attendance. The area chosen will be away from the GLS and feather group in an area that is easily accessible at night and has the most stable substrate to reduce burrow collapses and damage to vegetation.
- The productivity study will be in an area away from the GLS device and diet analysis groups and will be conducted during the day when adults are foraging for food and only chicks are present in the burrows. This will ensure adults are not disturbed and

chicks are handled quickly and efficiently ensuring they are placed back in situ within five minutes of the disturbance. Areas of potential burrow collapse will be avoided where possible, areas will be identified in consultation with NPWS field officers who are knowledgeable of the Reserve.

- Disturbance to the colony: birds will be taken to a 'processing station' adjacent to the colony where burrow density is lower (soil is more stable) and disturbance to the rest of the colony is minimal. Impacts to individual birds: birds will be placed in a breathable cloth bag to prevent injury to the wings when being weighed and measured. The dark colour of the bag offers protection to the bird's eyes which are sensitive to bright light. When working at night, field technicians are required to use red light filters for their head-lamps which are less disruptive to the bird's vision. Handling time will be strictly monitored and kept to a minimum by collecting only a small number of (important) measurements and by sampling feathers rather than blood because it is quicker and less invasive.
- Fish will be caught using best possible fishing practices that minimise the angling duration time and subsequently reducing the time between initial capture and death. Additionally, this fishing method (hook-and-line) will catch only a single fish at any one time, and as a result there is no risk of accidentally exceeding the number of fish requested in this application, which could be the case if using trap or gillnet fishing methods that can catch multiple fish simultaneous.
- The anticipated impact on horses is not greater than experienced daily. We are minimizing impacts by avoiding treatments on foals that may become separated from their mothers (<1 week old), or heavily pregnant mares that might be impacted by running. All observations are behavioural, and we only approach horses until the first horse runs. We do not approach bands with very young foals (<1 week old) or heavily pregnant mares.
- Visual census techniques of trophic interactions in the field are a form of replacement for more destructive and intrusive methods such as sacrificing individuals for gut contents analysis.
- Seine nets will be operated by hand-hauling so that the fish are sampled and returned to their environment within 5 minutes of trapping. Seine nets will not be pulled out of water onto shore, a procedure which allows more efficient counting of individuals but can result in higher rates of mortality. Handling of fish will be the minimal required for identification, and in a manner to minimise scale and mucal damage.
- Lethal Animal Collections: Spear and hand-netting collection is an ethical form of collecting fish because there is no by-catch. Collection will only be carried out by experienced and trained spear fishers/hand-netters that are familiar with the fish of Australia's East coast to reduce miss-hits. The fish will then be immediately euthanised

by pithing or immersion in a clove-oil (or artificial substitute) ice bath. Although angling is a less selective form of fishing, the process can be refined by using selective lures, circle hooks where possible and following best practise by-catch release. Circle hooks have been found to increase the frequency of mouth-hooking (as opposed to deep-hooking; Cooke and Suski 2004), up to 100% in some New South Wales fish species. Like spearfishing, angling will be performed by experienced fishers in order to minimise the time fish are out of the water. 'Deep-hooked' fish will have the line cut-off close to/inside their mouth, as leaving hooks in-place has been shown to increase short-term survival of deep-hooked fish from 12% to >85% (with 76% of the deep-hooked fish studied shedding their hooks within three weeks; Grixti et al. 2007). Shallow-hooked fish will have hooks removed using needle-nosed pliers whilst the fish is under the water to reduce the amount of time fish are out of the water. Needle-nosed pliers for removing hook from mouth of shallow-hooked fish, best performed whilst fish is held underwater where feasible.

- The biopsy procedure will not involve sharks being removed from the water or restrained, furthermore the use of anaesthetics will be avoided to assure alertness of the shark post-procedure.
- Continuing on from 2017, the institution further investigated reducing the time from the guinea pig challenge to the first monitoring timepoint in order to be able to capture control guinea pigs that are moribund and conduct euthanasia as an early end point. It was confirmed that the optimum time for intervention and early end-point euthanasia was 17 hours post challenge. In order to implement this change it became necessary to perform the challenge later in the day (ie. 3pm rather than 10 am) so that the period of time until the first monitoring is reduced from 24 hours to 17 hours. This important refinement was implemented in 04/2018.
- Mice that are on test that do not have death as an end point have had white pipes added to their cages for environmental enrichment.
- Work was carried out in 2018 to refine the euthanasia of guinea pigs as the existing IP 1 ml injection of lethabarb per guinea pig took several minutes and resulted in squirming and discomfort prior to death. After reviewing literature and trialling different dose rates, the dose rate was increased to 2ml per guinea pig which results in a significant improvement in the time it takes for guinea pigs to become comatose, that is, 1.5 minutes, which is 1-2 minutes faster than the 1 ml dose. Death does not occur until 4 minutes which is similar to the 1 ml dose, however, the refinement is 1-2 minutes less of suffering per guinea pig during euthanasia.
- Rabbits have historically always been identified with ear tags, but these have been problematic in that they regularly would get caught, cause ear irritation and fall out and subsequently need repeat application and treatment of ear wounds. In 2018

alternatives to ear tags were investigated and it was decided to replace ear tags with tattooing. Rabbits receive a topical anaesthetic/numbing cream and after several minutes receive a tattoo of a unique identifying number on their ear. These tattoos have proven to be successful. Rabbits do not appear to be disturbed by the tattoo, even immediately after application, and no further application is required which requires less handling and treatment so is a successful refinement.

- Animals were housed on-site in their familiar environment. They were handled by personnel with cattle handling experience, and treated by staff skilled and efficient in the procedures. Prior to treatment animals were kept together in the holding yards, then moved and treated together in groups. Upon release they were free to roam and graze in their regular paddocks. These methods reduced any pain and distress the animals may have experienced.
- The formulations applied in this study all contained active ingredients for local anaesthesia, therefore reducing pain experienced by the animals after mulesing and tail docking. The animals were closely monitored from the time of mulesing and during the first 24 hours after mulesing to observe for signs of pain and distress.
- Animals were handled daily to acclimatise them to handling and reduce their anxiety. Animals were restrained only briefly when injections were carried out and ultrafine gauge needles (31G) were used to minimise pain. Animals were monitored closely throughout sedation for signs of distress. Mice were placed in a warmed cage throughout the anaesthesia observation period to mitigate any reductions in core temperature.
- Reversal agents such as Atipamazole and Naloxone were on hand during the study to help reverse the anaesthesia of the mice if any adverse effects were observed.
- Work from a previous study found that a pain observation period of 5 minutes was sufficient to collect accurate data, thus minimising the disturbance to the animal.
- Ensuring that animals receive anaesthesia and adequate pain relief following painful procedures.
- Ensuring that environmental enrichment is provided wherever possible.
- Requiring an acclimation period of at least 7 days after arrival in new surroundings, before they are used in a project.
- Acclimating animals to handling and research procedures before being used in a research project.
- A research project was carried out to determine the best environmental enrichment for sea turtles being held in captivity.

- Investigators are asked to consider all procedures and actions when developing their submissions and to identify ways in which pain and stress can be minimised and whenever possible, the wellbeing of the animal is impacted least.
- Close monitoring of animals post procedure to identify any evidence of discomfort/suffering and the administration of pain relief promptly is essential to maintaining the wellbeing of the animal.
- New and/or inexperienced staff must be supervised by senior experienced personnel. New staff must be educated in observing behaviours indicative of distress, pain and suffering.
- Providing easy access to food and water for rodents that have had major surgery and may have difficulty reaching food hoppers and water bottles can in association with analgesia, enhance recovery.
- Utilising environmental enrichment appropriate to the species may help to meet their physical and psychological needs.
- Returning sheep to grassed paddocks post procedure as soon as they are active and safe to do so is preferred to them being confined in pens.
- The use of toys and rewards to encourage and stimulate positive interactions between the animals and their carers certainly provides entertainment and beneficial behavioural activities for Non Human Primates.
- Specially designed excluders for wildlife traps have decreased bycatch of non-target species.
- Use of captive populations where possible to replace using wild animals or to pilot particular techniques or equipment in a controlled environment before going into the field.
- Increased use in camera traps and ink pad tunnels has decreased the use of live trapping for survey work or determining species presence in an area.
- Training on animal ethics is compulsory for anyone listed on animal ethics protocols, this includes a session delivered by an expert on alternatives and the application of the 3Rs.
- Increased use in less invasive sampling for DNA, such as plucking hair or feathers or buccal swabbing over blood sampling, ear biopsy etc
- Expert veterinary advice is available 24/7 to all staff and students working with animals, including those in the field.
- Ongoing refinement on a case by case basis for the use of analgesia, anaesthetics, and tailored monitoring of any animals undergoing higher impact procedures. Direct veterinary input and in some cases direct oversight or periodical reports to vets and the AEC are implemented on a regular basis.

- Increased promotion and requirement of pilot studies, well designed so that pilot results can contribute to a larger study if it goes ahead based on pilot results.
- Investigation, trial and implementation of newer technology that improves welfare outcomes, i.e. smaller microchip sizes, small and lighter GPS trackers, improved surveillance equipment for camera and video trapping.
- Dosing regimens for analgesics continued to be improved with the expert advice received by the Category A member. Researchers and AEC members were educated in how to modify doses effectively to provide continual and effective pain relief following surgical procedures.
- Researchers modified procedures to minimise potential stress impact on the animal by lightly anaesthetising rats prior to oral gavage in rats that had a jugular cannula inserted.
- The establishment maintains a watching brief for best practice examples that can be adopted to refine our procedures. We have recently appointed an Animal Welfare Officer who has commenced a review of all standard operating protocols in use. Example - Precision livestock management research – general farm monitoring systems (behavioural, activity, weights) both allowing for less invasive and stressful monitoring of livestock herds in research and production settings.
- Blood samples are being taken as part of routine health checks anyway. The only impact on these animals is that a slightly higher volume of blood is being collected (estimated at 3 - 4 ml per animal, which is very small given the size and blood volume of a cow).
- Test flights to determine optimal flight altitude will be performed prior to surveying to ensure maximal distance between plantation canopy and drone is maintained, whilst still ensuring optimal image quality for analysis. Experienced local researchers and ground counters will be present throughout all surveys to observe and assist in monitoring the condition of koalas within the study site. At the first sign of any notable distress drone pilots will be advised, and if necessary, missions ceased.
- Blood sampling (via the tail vein, and associated restraint) will be conducted by Veterinarian staff from the Veterinary Centre as per normal routine. This will minimise any distress and discomfort. Collection of a blood sample from a cow, takes approximately 2 minutes (including restraint). This degree of pain / discomfort is small, and for a very short period of time.
- Grooming traps have been purposefully designed to be the most humane, unobtrusive and least painful technique of controlling feral cats. Importantly, unlike existing control techniques, grooming traps do not expose non-target animals to being held in cage or leg-hold traps or poison baits. RSPCA supports the development of Grooming Traps as a humane option for necessary feral cat control.

Target pests are typically controlled using approved but less humane/targeted techniques in established management programs - so no additional welfare cost to target animals and reduced costs to non-targets.

- To reduce adverse impacts on animals, the AEC reviews each procedure carefully and may require more information to be provided (e.g. What type of adhesive is used to attach the trackers (aqueous or solvent based). Bandicoots (in our backyard) or techniques to be justified (e.g.
- The maximum attachment time of the trackers needed to be clarified and justified in reference to past studies for a project.
- Invasive mammals must only be kept for a maximum of 12 hours in the traps, amended from 24 hours for a project.
- 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.
- The amount of time was limited over dolphins at altitudes less than 60 m because the research shows that dolphins may detect drones at these altitudes.
- The addition of a three lead ECG was recommended by a veterinary cardiologist and will be implemented in future scans. The ECG panels are adhesive and are not expected to cause any additional distress to the animal.
- Processing times have been greatly reduced by removing the number of morphometric measures taken.
- Snakes are identified based on their unique head markings which means that subcutaneous injection of a microchip is not required.
- A protocol investigating whether the restoration of degraded sandstone rock outcrops can help to conserve the endangered broad-headed snake used standard techniques used by reptile ecologists to ensure minimal suffering to animals.
- A protocol investigating asthma and Chronic Obstructive Pulmonary Disease will use a lower dose treatment than what has been used in existing literature.
- A protocol developing a set of methods for an ECG analysis process, tailored to the detection and identification of the normal and abnormal morphology of the canine sinus rhythm used trained professional veterinarians to perform the procedures to minimise suffering on animals, and allowed the animals' caretakers to be present throughout the experiment to help relax the animal.
- Refining a protocol were by an additional welfare check has been added.
- A refinement was identified in a project whereby the project team have added an additional welfare check by collecting body temperature of the sheep on two occasions.
- Refining at study to show that the use of analgesia had benefits at lamb marking. The study showed that there was proof and benefits of using the analgesia treatment at

lamb marking, it also showed that the project had been used as an opportunity to conduct the study on the basis which otherwise would have been routine farm work.

- Environmental enrichment is an important component of housing to provide an opportunity for enhanced welfare. The establishment has committed to 100% of their group housed sows having enrichment for part of their breeding cycle from 2020. Our AEC application template includes a section "Are animals provided with a solid floored area with bedding material for rooting and resting?". Whilst it is not a requirement in the Model Code of Practice for the Welfare of Animals-Piqs to provide enrichment, where the accommodation allows, environmental enrichment is being used in research projects-ranging from chew to daily provision of straw enrichment blocks. Our Research unit is also trialling a pig 'Treat' program where stock people and Technical Officers can hand feed a treat to pigs-enhance human animal bond. AEC members participated in this program during a site visit in 2018 and members enjoyed the interaction with our pigs.
- Experiment investigated using large cubes for creep feed for piglets (to enhance welfare and mimic natural foraging behaviours of pigs in the pre-weaning period). Under natural conditions, piglets will forage under the canopy and consume acorns or other large seeds. These large creep pellets are similar in size to the acorns and may be an efficient enrichment material for piglets in the future.
- In 2018, as a direct result of positive outcomes from the establishment's research (Developing ways to measure and improve sow contentment in farrowing crates), nest building material is now provided to all sows pre-farrowing. This environmental enrichment promotes nesting behaviour pre-farrowing and improves sow welfare and contentment and reduces the number of stillborn piglets. This a significant advancement in animal welfare and will provide opportunity for experimental sows to experience enhanced welfare.
- Remodelling of our individual weaner housing facility is in progress. This new state of the art research facility will allow for increased floor space allowance, social companions, enrichment, and improved safety features for technicians when handling the pigs. This is a significant advancement for animal welfare.
- The Grower Discovery Centre (GDC) ventilation and environmental system has improved significantly. This has been achieved by removing an old shed adjacent to the building (as per recommendation of the AEC) to improve inlet air flow into the system. Furthermore, we have implemented a comprehensive preventative maintenance program in that shed and as a result the environmental system was successful at reducing the ambient temperature by 20°C (over the 2018/2019 Summer reached external temperatures of 45°C+ and pigs in the GDC were at a comfortable 25°C during that time).

- The establishment continues to investigate and refine novel technology to assess physiology and behaviour of animals. Novel, non-invasive techniques to investigate body temperature, heart rate and respiration rate were planned to be investigated in novel ear tag technology for measuring heart rate and body temperature, however the technology was not developed within a reasonable time frame and the experiment was cancelled. There has been some progress in this area (including additional research funding) and new applications will be submitted to the AEC in 2019/2020 to develop this technology.
- In trial Heat stress in maternal and F1 sows- indicators and production impacts used infra-red camera to assess body temperature. These technologies are advancing quickly, and the infra-red camera was able to detect changes in body temperature before clinical disease occurred. The establishment continues to investigate these technologies - particularly for early identification and intervention for disease management. This technology will be further developed and assessed in project Developing remote monitoring methods for early detection of respiratory disease in pigs.
- A project was modified to eliminate the need for blood sampling of piglets. At that stage the investigators were not confident in the use of Brain-derived neurotrophic factor as a marker for positive emotional state. Given that the project was focused on piglet curiosity the behavioural tests were the major focus in the experiment.
- In trial Human enrichment program for breeding sows: proof of concept investigated novel ways to assess positive affective state in pigs - particularly the use of a factor in the blood that is released when animals are in a positive emotional/affective state. This measure of the factor showed promise and will be investigated further in the future welfare experiments.
- A project investigated use of a novel injector that may have been more effective to use and marked the pigs with ink to allow for quick efficient identification
- The research team have implemented and are using comprehensive monitoring sheets for daily monitoring/recording of animals under research.
- SOP Method for blood sampling from umbilical vein and measuring placental efficiency was reviewed and will include procedure to ensure that increased activity around pigs during key physiological data collection periods will not negatively impact pigs, and ensure extra trained staff are available during these periods to help reduce this stress.
- An estimate of expected baseline mortality is now a requirement of all AEC applications.
- The establishment management continues to enforce the requirement of AEC applications being written in 'lay terms' and including definitions of medications,

including active ingredient(s), if the medication and/or use is outside the current scope of the Herd Health Plan. This allows the researchers to more actively justify the need for the research, as well as allowing the discussion surrounding applications to be in non-technical language understandable by all Committee members, especially Category D.

- In the course of this pilot protocol, we have introduced a number of additional refinements which include the use of local anaesthetic infiltration of the abdominal incision site to reduce postoperative pain and discomfort, use of a mouse warming cabinet in the first 24 hours after surgery and provision of a high-energy soft diet (DietGel boost) in the post-operative period to assist recovery.
- This protocol aims to improve competency and consistency of common mouse procedures within the establishment by offering professional and hands-on practical training by experienced staff. Refinement of animal research and improved validity of data are thereby encouraged overall by this protocol.
- We have used highly sensitive thermal cameras that allow us to detect wallabies from a distance, rather than just at feeding stations. This may allow us to answer questions we could otherwise only answer by catching wallabies, something that is feasible but we would prefer to avoid if we can.
- We undertook refinement to housing conditions (new bedding, nesting material, cage change frequency) to ensure the well being of the mice undergoing photo carcinogenesis.
- We have purchased some of the latest, high-tech, low- weight GPS collars to minimise the impact of the collars on individual animals. The collars we are using are less than 1/3rd of the weight of most comparable collars on the market (100g compared with >300g for units from the leading competitor).
- Use of remote video camera monitoring in all large animal recovery rooms at the facility and its agistment property to supplement physical monitoring and increase the frequency of monitoring that occurs out-of-hours and on weekends.
- Video camera installed in the trailer to allow animal monitoring during transportation.
- Extension of the Animal House to include an outdoor exercise area for pigs.
- Installation of surgical gas lines into the Animal House for enhanced animal welfare during induction of anaesthesia and post-operative recovery.
- Continual fine-tuning of post-operative care including enhanced design of the sheep supportive 'sling' and pig jackets (for post-operative wound protection)
- Educational posters installed in the rat and mice housing rooms on "Recognition of the signs of pain and distress" in each species.
- Online animal ethics theory modules and the practical training module for work with mice and rats. Practical training in the use of large animals

- Monitoring is an important requirement within the Scientific Use Code. To recognise this, a formal program of project reviews continued in 2018. The purpose of these reviews is to ensure that a project is conducted in accordance with the approved proposal and to provide an educative opportunity for researcher/s and the Committee on research methods and ethical conduct. During 2018, several projects were reviewed. The monitoring program will continue and increase in 2019.
- Continued emphasis on environmental enrichment.
- Direct observation and camera recording in the field for wildlife studies.
- The Principles of 3Rs have been emphasized during the Animal Ethics training workshops as one of the key concepts that researchers need to keep in mind when drafting ethics application and progress reports.
- The AEC granted permission for pilot studies to be undertaken for projects using invasive procedures (case-by-case), with strict condition that alternative procedures/designs must be sought via amendment request where possible, depending on the outcome of the pilot.
- Procedures are carried out on animals according to AEC approved Standard Operating Procedures.
- All procedures are performed or supervised by highly trained personnel.
- All animal housing is designed and maintained to meet species-specific needs.
- Appropriate anaesthetic and analgesic regimes are implemented as required.
- All animals are closely monitored on a daily basis and especially following any procedure. Should an adverse outcome be observed, veterinary advice is sought.
- Designs of field based apparatus minimise the negative impact on animals in the study.
- Amendment on the Guidelines for Using Weight Loss in Animal Welfare Assessment and Humane Endpoint Determination was reviewed and approved by the AEC.
- 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.
- Experimental design is critically evaluated by the committee so that the best probable outcome can occur. For example the design of a project was significantly alternated after advice of the committee's expert nutritionist and veterinarians.
- Close monitoring of animals and development of monitoring checklists to identify adverse reactions in animals.
- The AEC has placed conditions on projects at the approval stage to ensure that any pain or distress to animals is alleviated quickly in projects where it is impossible to eliminate this completely.
- Use of experienced veterinarians and other staff.
- Restraint time and dose rates kept to a minimum.

- Suitable housing provided and maintained including controlled environment facility.
- Use of adjuvants known not to produce adverse reactions.
- Procedures performed under anaesthesia or sedation when appropriate.
- Close scrutiny of the volume of blood collected.
- Use of the saphenous vein method as the standard technique for blood collection in rodents.
- A number of studies conducted on animals at the owner's property to minimise any possible stress.
- Pilot studies are often undertaken by investigators before proceeding further, which assists researchers to refine future protocols.
- Establishment's 3Rs grant program: The establishment trialled an internal grant program initiated by the Animal Welfare Officer in which competitive funding would be offered for small-scale projects or pilot studies (up to $2,500, 12 months duration) which specifically address one of the 3Rs. Two projects were awarded grants, both of which involved the assessment of analgesics to refine animal models where scientific convention is not currently supportive of analgesia: 1) Investigating the use of analgesia in a common rodent model of rheumatoid arthritis. 2) Assessing the effects of buprenorphine on inflammation during Group A Streptococcus infection. Final results are pending, but it is anticipated that these projects will provide examples of models where analgesia may in fact be implemented without adversely affecting scientific outcomes.
- The Animal Welfare Officer and Animal Technician developed the lateral saphenous route for blood collection in dunnarts to replace the retro-orbital route which had become established in the literature). It is hoped that this method will be published to encourage wider use.
- Test drug was administered to mice in Nutella paste instead of oral gavage. Animal handling has also been refined through the use of jars or pipes to pick up the mice rather than grasping them by the tail or scruff. Subjective assessment suggests that the mice are considerably more calm using these techniques. Also, an earlier endpoint was implemented in consultation with the Animal Welfare Officer and Animal Technicians.
- At the request of the AEC, an amended scoring system for the DSS model of colitis was evaluated. This showed that the required scientific endpoints could be achieved by a reduced dose of DSS thereby reducing the severity of the condition.
- A pilot study found that in a model of metastatic cancer, it is possible to achieve the required scientific endpoint after 3 weeks rather than the conventional period of 4 weeks which is described in the literature.

- The PI was awarded a 3Rs grant from the establishment to evaluate the use of analgesia in a widely used model of rheumatoid arthritis. The results of this evaluation (if successful) will be incorporated into the PI's current project.
- A lab-based aquarium study was undertaken to assist the selection of acoustic tags for a marine wildlife study to help ensure the tags had minimal impact on fish in the wild.
- Analysis of preliminary data showed that the proportion of animals undergoing a surgical procedure could be reduced substantially.
- When investigating a natural disease outbreak in a colony of frogs, clinical observations suggested that euthanasia using benzocaine gel avoided the signs of distress and aversion that were usually observed with the existing method of euthanasia (i.e. immersion in a solution of MS222).
- Sedation has been found to reduce or eliminate the risk of post-treatment seizuring in rats in a study that is showing promise as a means of improving the management of brain tumours which would be translatable to humans. This study has also shown the potential benefits of a reduced but more focused regime of radiotherapy.
- Reduction in tail bleeding procedures appears to have reduced the risk of disease in immune deficient mice.
- In a genetic study of native frogs, an attempt was made to perform genotyping using a sample of the gel capsule surrounding eggs rather than collecting tissue from tadpoles. Unfortunately this was not successful.
- Added camera traps to partially replace spotlighting.
- Used Fyke netting to capture tadpoles and fish. This method is a more effective form of sampling.
- The technique of intrahepatic injection was refined through practice on cadavers so that the peritoneum did not need to be opened surgically. Only a skin incision was used without further surgical intervention or externalization of the liver.
- Used a pilot study to optimise the anaesthetic agent/delivery method.
- Advances in surgical techniques and using well establish methods led to shortened anaesthesia and reduced surgical trauma.
- Tested a dose response so as to elicit the greatest immune response but minimal impact on the animal wellbeing.
- Modified partition used in behavioural experiments. Modified dose of drug given following an adverse reaction.
- Changed from intra-tracheal to intranasal administration to reduce the welfare impact on animals.
- Further developed in vitro techniques to shorten the time that animals need to be maintained after treatment.

- Investigators on the project practiced all techniques on culled animals.
- Trained stallions to use a collection phantom so a mare is not required for semen collections.
- Timed the breeding of mice so that females give birth over a weekend and are not exposed to the intervention of the smoke machine and any associated stresses.
- Altered surgical/recovery location, the analgesics being administered, and post-operative recovery procedures to increase survival rates and reduce the likelihood of post-operative cardiac rupture.
- The use of Unmanned Aerial Vehicles (UAVs, Drones) to collect biological samples from whales, reducing the need for a close approach in a large vessel.
- Use of Remotely Operated Vehicles (ROV) and UAV to observe marine animals resulting in less disturbance.
- Limiting the length of time wildlife is held, allowing them to be released after completion of the research.
- Collaboration with other institutions when/where wildlife is studied to reduce impacts on populations and reduce environmental disturbance.
- Eight Foxhounds were donated to Beagle Freedom, as they were no longer required or suitable for research. There are ongoing efforts to reduce the colony by rehoming surplus animals (dogs and cats), or those that can no longer be used in research due to illness or old age.
- Husbandry and care practices are modified to minimise pain, suffering and distress . Enrichment and training programmes have been initiated to condition animals to study procedures.
- Companion animal field studies are designed to reflect normal veterinary practices, such that study activities are what would be performed anyway, e.g. only necessary blood samples and x-rays are taken.
- A training programme was introduced to enable staff to develop expertise in the use of an otoscope camera, prior to commencement of study activities.
- The Committee continues to encourage researchers to undertake a pilot study if the impact of the proposed study interventions on animal health and well-being is unknown.
- Animal House veterinary managers review protocols with researchers in order to optimise anaesthesia protocols (including monitoring) and analgesia.
- 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.
- Use of Observational only applications.

- Longer periods of acclimation for wild caught animals in facilities post capture and prior to performing experimental trials thus providing animals with the opportunity to rehydrate and increase body conditioning prior to experiments being conducted.
- Non-invasive monitoring techniques such as use of cameras for identifying habitat use by animals in preference to traditional trapping methods this has minimised the need for animal handling.
- Increasing use of targeted remote infra-red cameras to replace/supplement trapping for wildlife surveys and monitoring.
- Utilisation of advanced technology which reduces size and weight of tracking devices.
- Utilisation of remotely operated Unmanned Aerial Vehicles (UAV's) mounted with automatic camera system for use in seabird and shorebird surveys. These techniques allow birds to be counted from a distance and altitude that do not cause flushing so that the likelihood of disturbance is greatly reduced.
- Location and sexing of free swimming adult turtles using unmanned aerial vehicles (drone copters) with cameras providing faster and less intrusive means of observation.
- Individually housing wild caught animals collected from different sites to prevent negative interactions between animals from different locations/harems (Permit requirement).
- Improvements to housing and segregation of animals captured from the wild, reducing the risk of introduction of potential pathogens to natural populations when animals are released back into the wild (respective government authorities permitting).
- Refinement of anaesthetic agents used and dosage rates reducing recovery times and the risk of complications such as respiratory depression for animals where surgical procedures are conducted.
- Blood sampling and analysis for refinement of anaesthetic doses on turtle hatchlings.
- Upgrades to animal housing facilities: Upgrades to Animal House Facility - use of a BAS system (Building automation system). The BAS system will be used for monitoring temperature set-points and other variables such as humidity within the rooms. Alarm notifications will be improved. Installation of Ro water system to overcome issues with water quality and in particular issues with copper in the tap water.
- Donation/Rehoming of animals unable to be returned to the wild to suitable organisations such as Zoos, Wildlife and Conservation Parks.
- Identification tags attached to sharks and rays to eliminate the need to recapture animals.
- Training of researchers in current best practise techniques.
- Improved peri- and post- operative analgesia to reduce pain from surgery.
- Use of ex vivo assays to minimise adverse impact on animals.

- Use of modern trapping techniques and equipment to minimise potential for animal injury.
- Use of smaller, less invasive tags for identification.
- Increased awareness and use of environmental enrichment.
- Encouragement of researchers to report near misses where an adverse incident did not occur but the issue identified that further opportunities for further refinements to field methods.
- Purchase of smaller fyke traps for use in shallow waters to address issues with extended periods of low flows.
- Further refinement of turtle trapping and acoustic monitoring techniques.
- There have been no animals used in any projects in 2018, other than animals with real disease.
- The current ongoing project has animals monitored by qualified veterinarians or vet nurses. Any animal showing any signs of distress would be treated with pain medication and treatments accordingly.
- 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 e.g. 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 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.
- Weighing and husbandry of cattle are carried out as part of their normal, regular commercial schedule.
- Development of adverse Reactions Log to monitor action, outcome and preventative measures taken.
- Use of R1-129 mice instead of ARC/s mice for lab animal handling and training, more docile, easier for new research staff to handle.
- Continue to make use of Opti-Spot test strips for rodent animal health monitoring, no need to anaesthetise animal, sentinels no longer required as we sample stock animals instead.
- Minimum amount of blood required from animals during health testing.
- Proper use of analgesics and anaesthetics.
- Use of EMLA cream as topical anaesthetic ointment with painful procedures prior to incision or injection.
- Surgical training – individual training session with researchers who will be performing mice surgical procedures for the first time so techniques get refined.
- Ensuring only the right sized surgical instruments are used that is suitable for animal species.
- Smaller incisions for faster wound healing.
- New environmental enrichment introduced e.g. double mouse swing for mice, PVC tunnels for rats.
- Promote and encourage the use of tissue sharing throughout the facility as well as external bodies.
- Score sheets for all monitoring during approved surgical procedures have been introduced to refine the process and appropriately identify and manage any pain and distress in animals.
- Continued use of analgesics and anaesthetics for painful procedures and surgeries.
- A rich supply of free environmental enrichment has been sourced for our animals allowing us to accommodate budget to further training resources for refinement of techniques in line with best practice in the industry. We have also been able to supply five external facilities with free enrichment.
- Rehoming of fish to private tanks when no longer suitable for experimental purposes.

- Continued use of remote controlled infrared digital cameras, acoustic recording devices and drones instead of, or in addition to, other techniques (low impact but more disruptive to fauna or environment) to detect species presence or absence.
- Improvements to animal housing and management (e.g. introduction of "buddy cages" to avoid single housing of mice).
- Training of researchers (animal monitoring and administration of injections).
- 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.
- The use of less invasive procedures in wildlife studies e.g. sand pads rather than trapping.
- Use of an Observational Only - Field Research Form (No Trapping, Handling or Spotlighting).
- Developing competency assessment procedures.
- Providing alternative procedures to minimise impact.
- Non-stressful memory tests.

7. Appendix – 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

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*

INT20/7627