

NSW 2017 Animal Use in Research Statistics

February 2019

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1. Summary

Information on animal use in research in NSW is collected on a calendar-year basis.

The following is included in this report:

- General charts which show the trend of animal use since 2010.
- Purpose charts for 2017. There are 10 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 9 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 work on the animals involved.
- Species charts for each purpose for 2017. These charts provide a breakdown of the following species groups:
 - laboratory mammals,
 - domestic mammals,
 - birds,
 - primates.
- Lethality testing for 2017. 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 2017.
- Appendix - Guide to the categories of reporting.

The system 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 7) which excludes this procedure category from the totals of animals used.

The system relies on the reporting by the animal research establishments. This means there can be minor differences in the interpretation of which Purpose and Procedure categories of use are most appropriate. The magnitude of the information submitted means NSW Department of Primary Industries is not able to verify each submission by individual research project – this is the responsibility of the reporting establishments. However, the system does allow checking, down to the level of individual projects at each research establishment, where necessary – for example where the category combinations entered are questionable, such as Purpose category *Education* with Procedure category *Death As An Endpoint*.

Notes:

- For the 2015 and 2016 reporting years there was a large increase in the numbers of animals used. This was mainly 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 3 million animals for each reporting year.
- For the 2017 reporting year there was a large increase in the number of aquatic animals used. This was mainly due to two projects which used almost 775,000 fish in the procedure category "*Observation Involving Minor Interference*". One of these studies involved the counting of over 500,000 fish by camera recordings.

- 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 in this 2017 Animal Use in Statistics report.
- 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

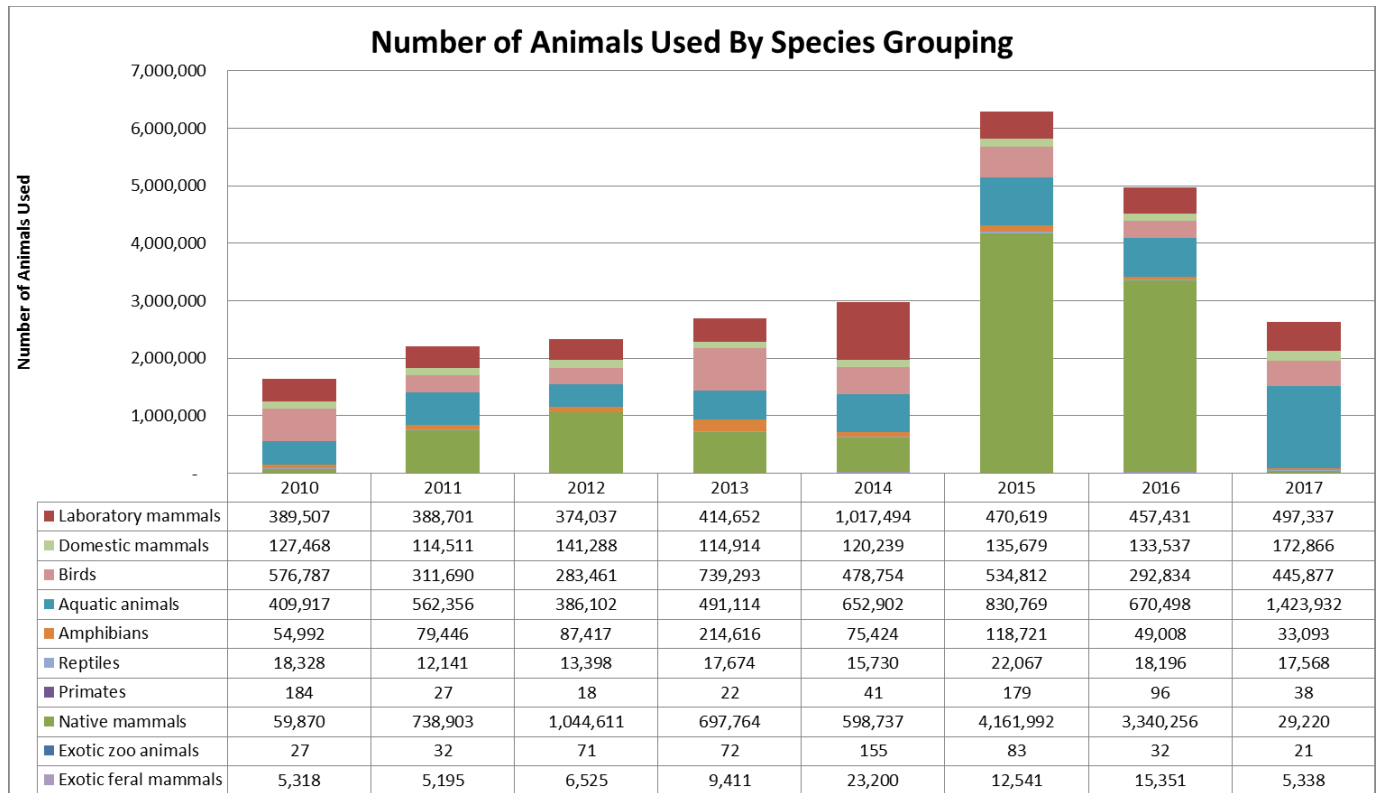


Chart 1: Number of animals used over time by species grouping

	2010	2011	2012	2013	2014	2015	2016	2017	Grand Total
Amphibians	54,992	79,446	87,417	214,616	75,424	118,721	49,008	33,093	712,717
Aquatic animals	409,917	562,356	386,102	491,114	652,902	830,769	670,498	1,423,932	5,427,590
Birds	576,787	311,690	283,461	739,293	478,754	534,812	292,834	445,877	3,663,508
Domestic mammals	127,468	114,511	141,288	114,914	120,239	135,679	133,537	172,866	1,060,502
Exotic feral mammals	5,318	5,195	6,525	9,411	23,200	12,541	15,351	5,338	82,879
Exotic zoo animals	27	32	71	72	155	83	32	21	493
Laboratory mammals	389,507	388,701	374,037	414,652	1,017,494	470,619	457,431	497,337	4,009,778
Native mammals	59,870	738,903	1,044,611	697,764	598,737	4,161,992	3,340,256	29,220	10,671,353
Primates	184	27	18	22	41	179	96	38	605
Reptiles	18,328	12,141	13,398	17,674	15,730	22,067	18,196	17,568	135,102
#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	25,770,182

2.2 Number of animals used over time by research purpose

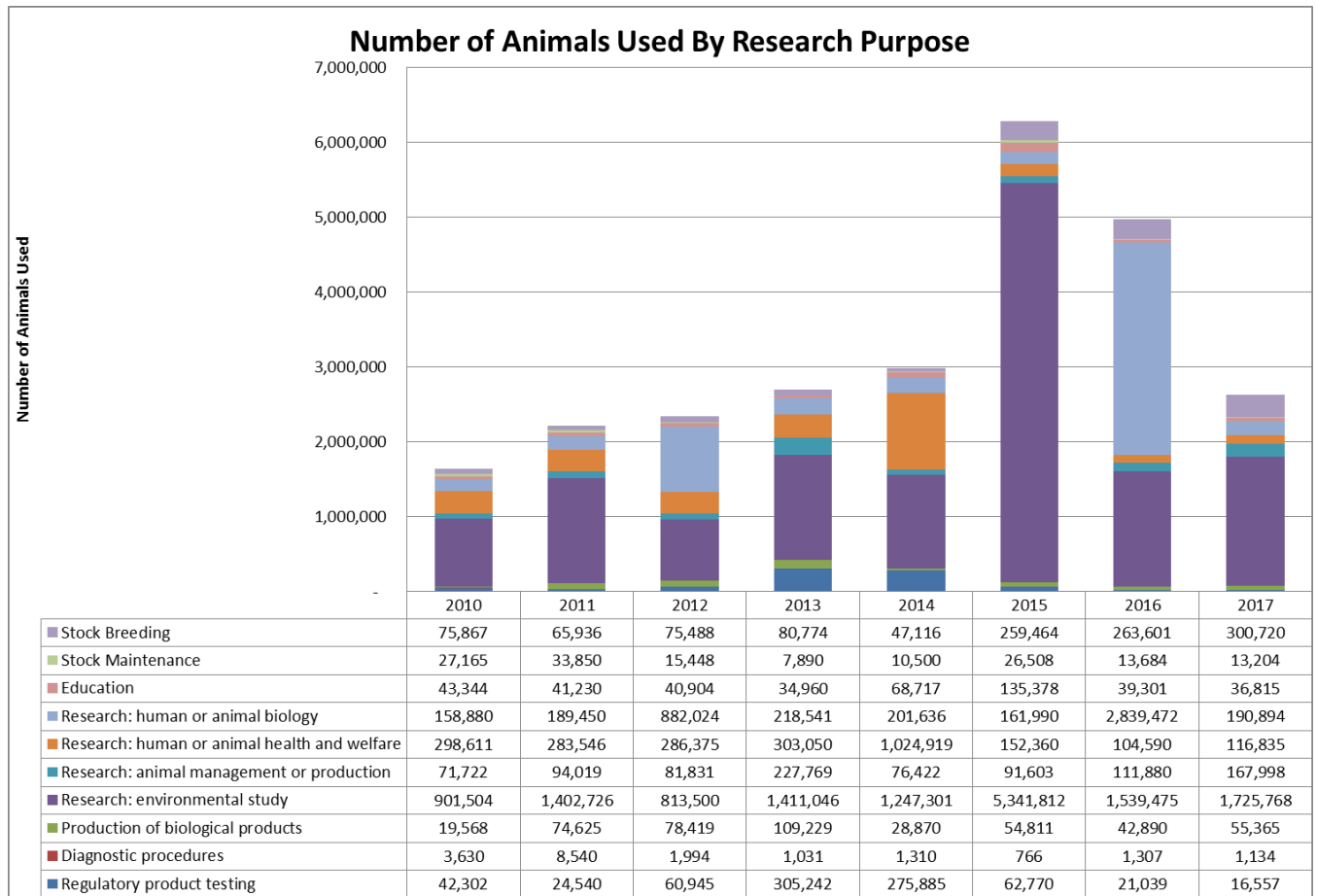


Chart 2: Number of animals used over time by research purpose

	2010	2011	2012	2013	2014	2015	2016	2017	Grand Total
Stock Breeding	75,867	65,936	75,488	80,774	47,116	259,464	263,601	300,720	1,168,966
Stock Maintenance	27,165	33,850	15,448	7,890	10,500	26,508	13,684	13,204	148,249
Education	43,344	41,230	40,904	34,960	68,717	135,378	39,301	36,815	440,649
Research: human or animal biology	158,880	189,450	882,024	218,541	201,636	161,990	2,839,472	190,894	4,842,887
Research: human or animal health and welfare	298,611	283,546	286,375	303,050	1,024,919	152,360	104,590	116,835	2,570,286
Research: animal management or production	71,722	94,019	81,831	227,769	76,422	91,603	111,880	167,998	923,244
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	14,383,132
Production of biological products	19,568	74,625	78,419	109,229	28,870	54,811	42,890	55,365	463,777
Diagnostic procedures	3,630	8,540	1,994	1,031	1,310	766	1,307	1,134	19,712
Regulatory product testing	42,302	24,540	60,945	305,242	275,885	62,770	21,039	16,557	809,280
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	25,770,182

2.3 Number of animals used over time by research procedure

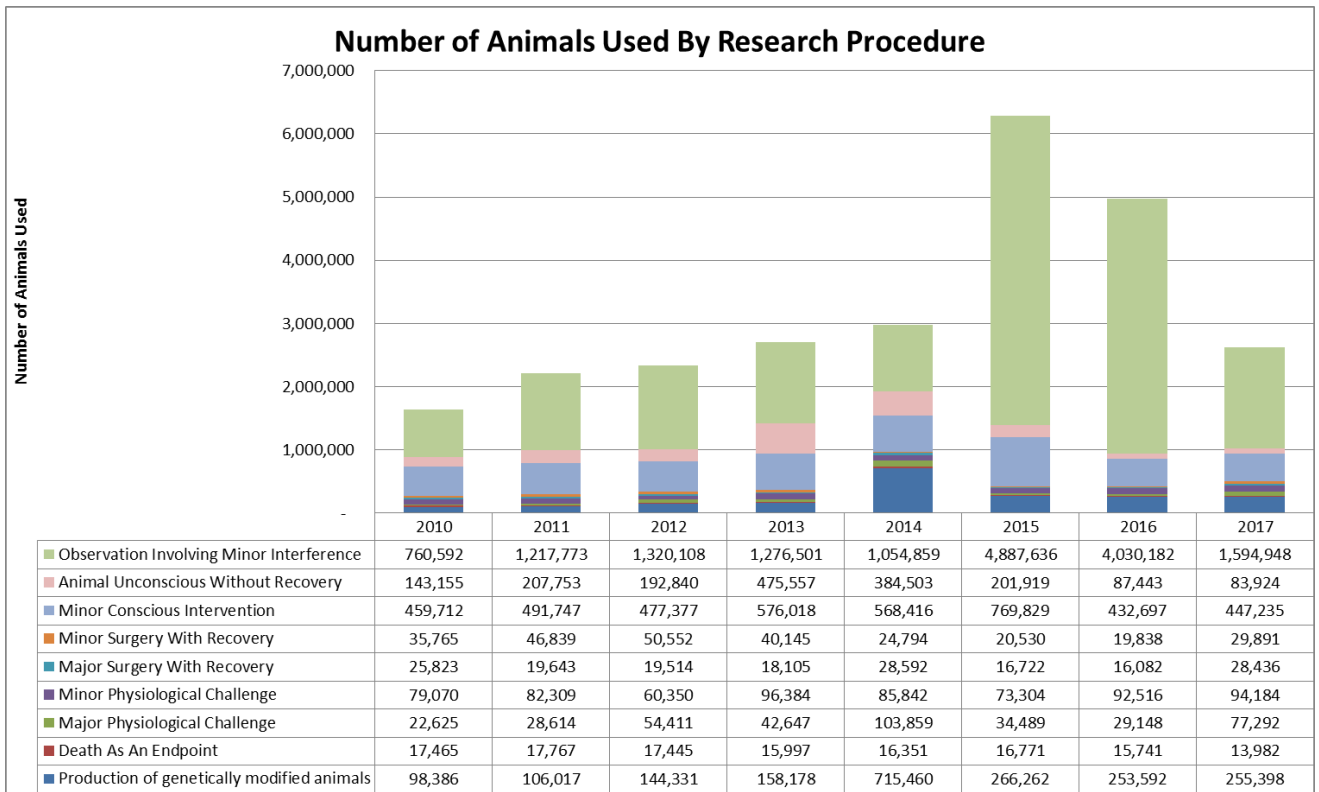


Chart 3: Number of animals used over time by research procedure

	2010	2011	2012	2013	2014	2015	2016	2017	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	16,142,599
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	1,777,062
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	4,223,031
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	268,354
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	172,917
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	663,959
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	393,085
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	131,551
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	1,997,624
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	25,770,182

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

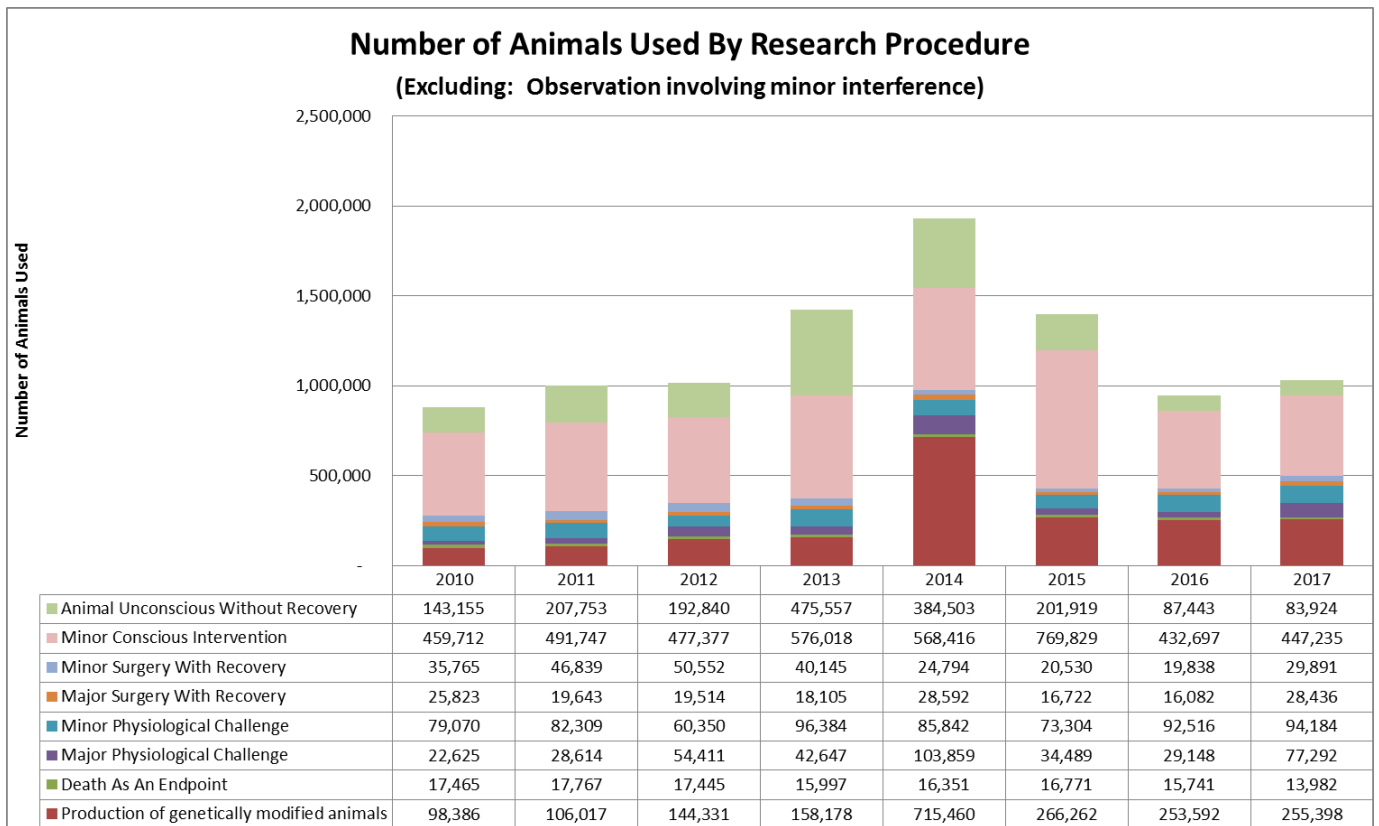
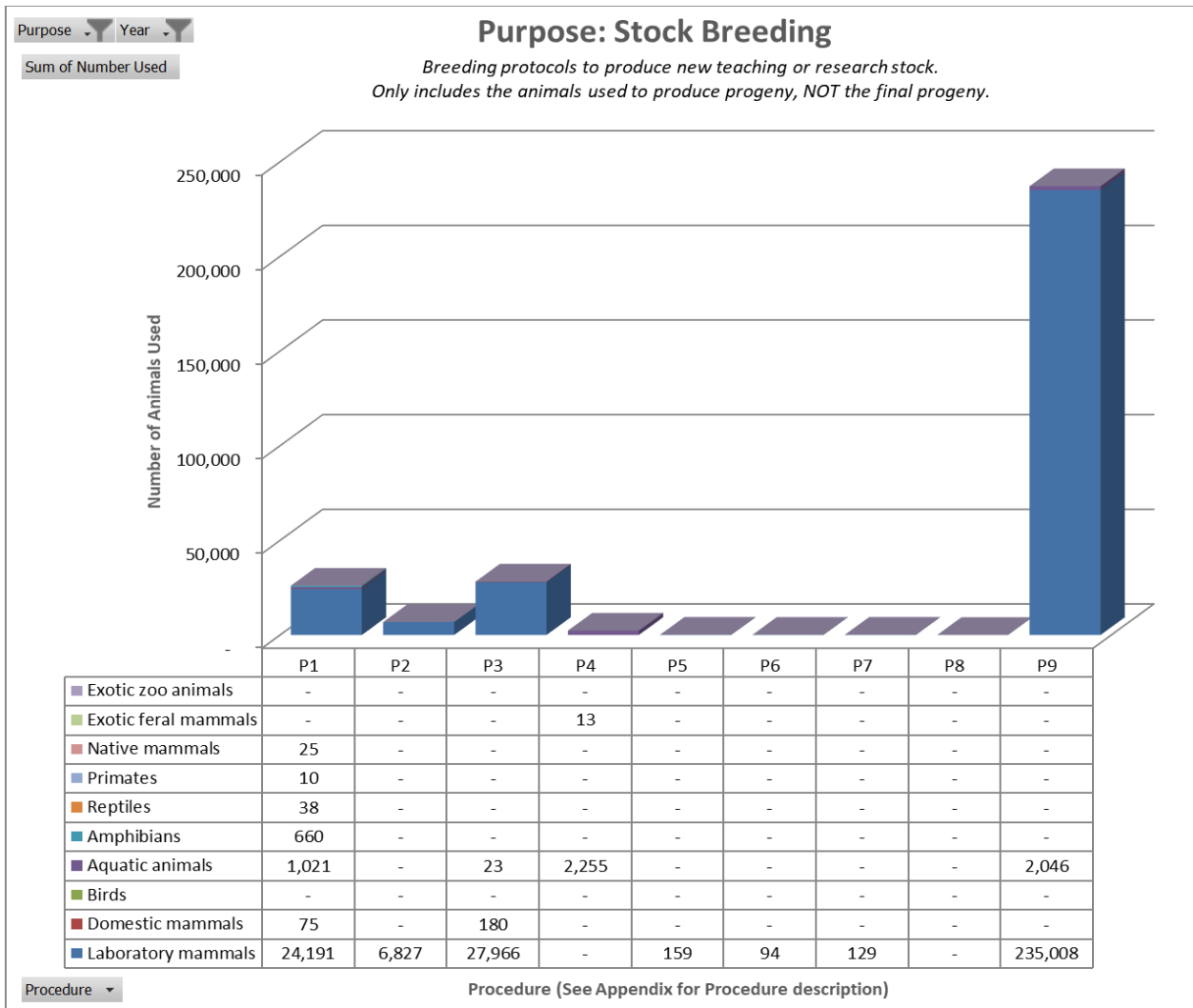


Chart 4: Number of animals used over time by research procedure excluding "Observation involving minor interference" procedure

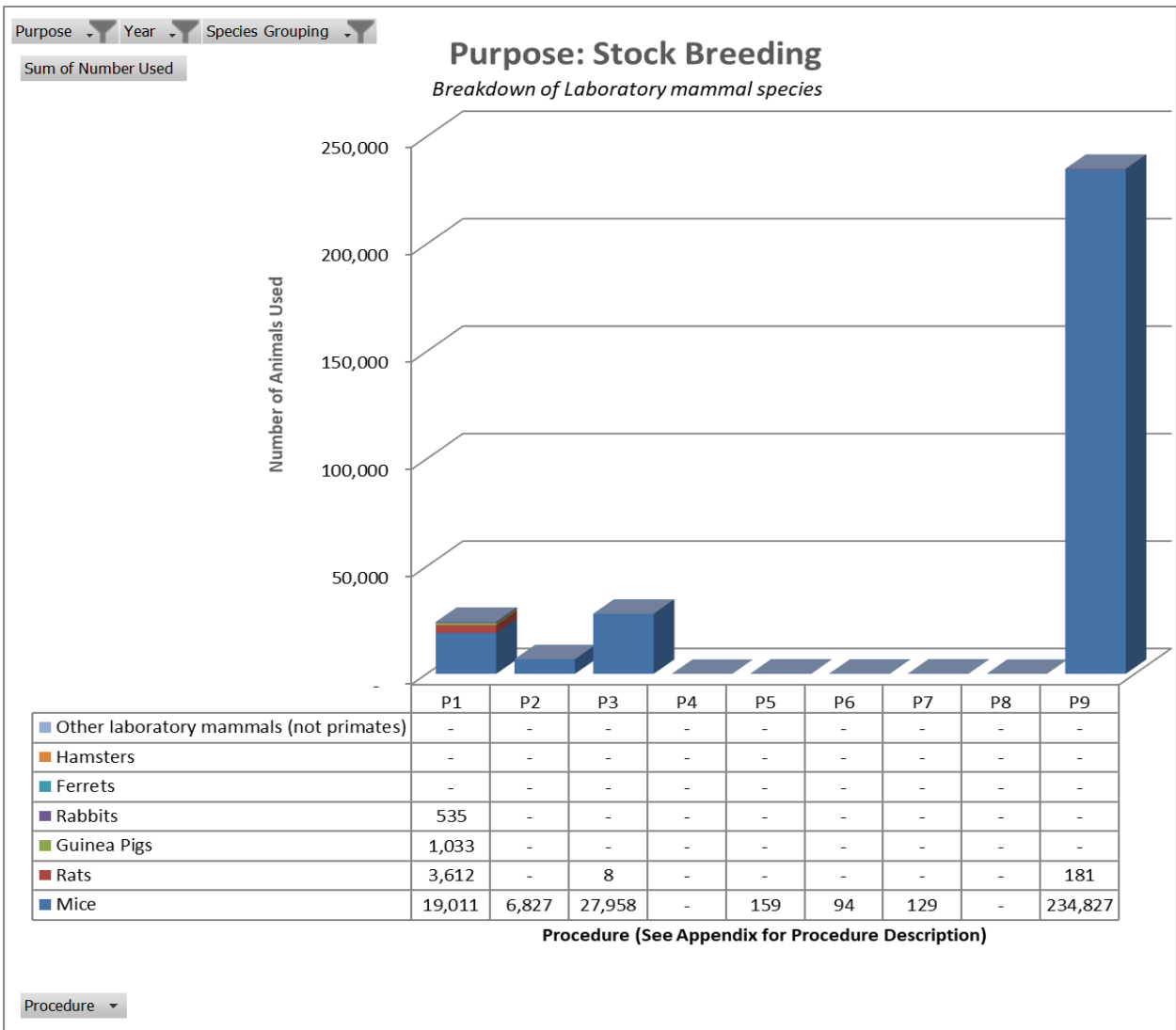
	2010	2011	2012	2013	2014	2015	2016	2017	Grand Total
Animal Unconscious Without Recovery	143,155	207,753	192,840	475,557	384,503	201,919	87,443	83,924	1,777,062
Minor Conscious Intervention	459,712	491,747	477,377	576,018	568,416	769,829	432,697	447,235	4,223,031
Minor Surgery With Recovery	35,765	46,839	50,552	40,145	24,794	20,530	19,838	29,891	268,354
Major Surgery With Recovery	25,823	19,643	19,514	18,105	28,592	16,722	16,082	28,436	172,917
Minor Physiological Challenge	79,070	82,309	60,350	96,384	85,842	73,304	92,516	94,184	663,959
Major Physiological Challenge	22,625	28,614	54,411	42,647	103,859	34,489	29,148	77,292	393,085
Death As An Endpoint	17,465	17,767	17,445	15,997	16,351	16,771	15,741	13,982	131,551
Production of genetically modified animals	98,386	106,017	144,331	158,178	715,460	266,262	253,592	255,398	1,997,624
Grand Total	882,001	1,000,689	1,016,820	1,423,031	1,927,817	1,399,826	947,057	1,030,342	9,627,583

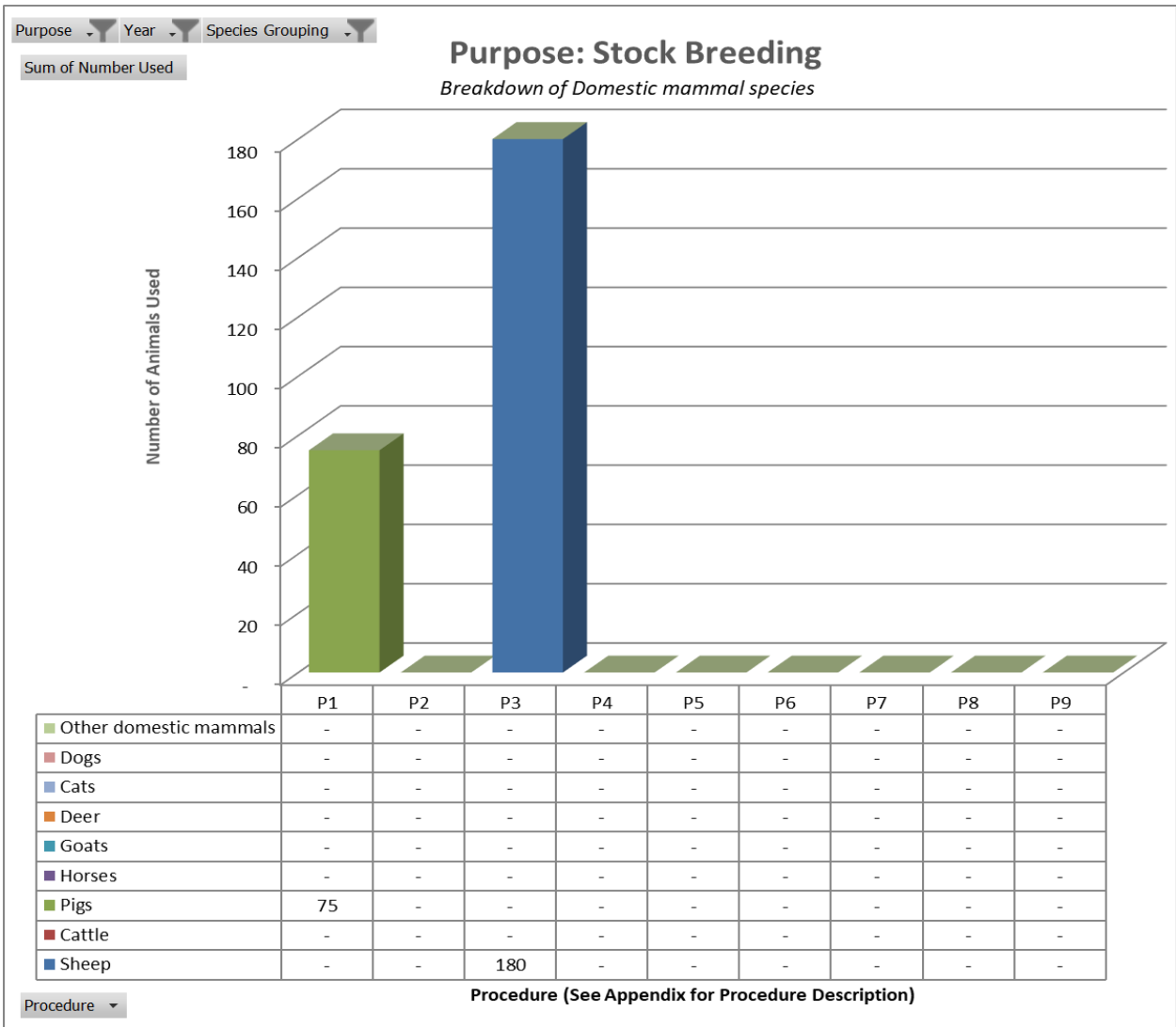
3. Purpose and Species charts

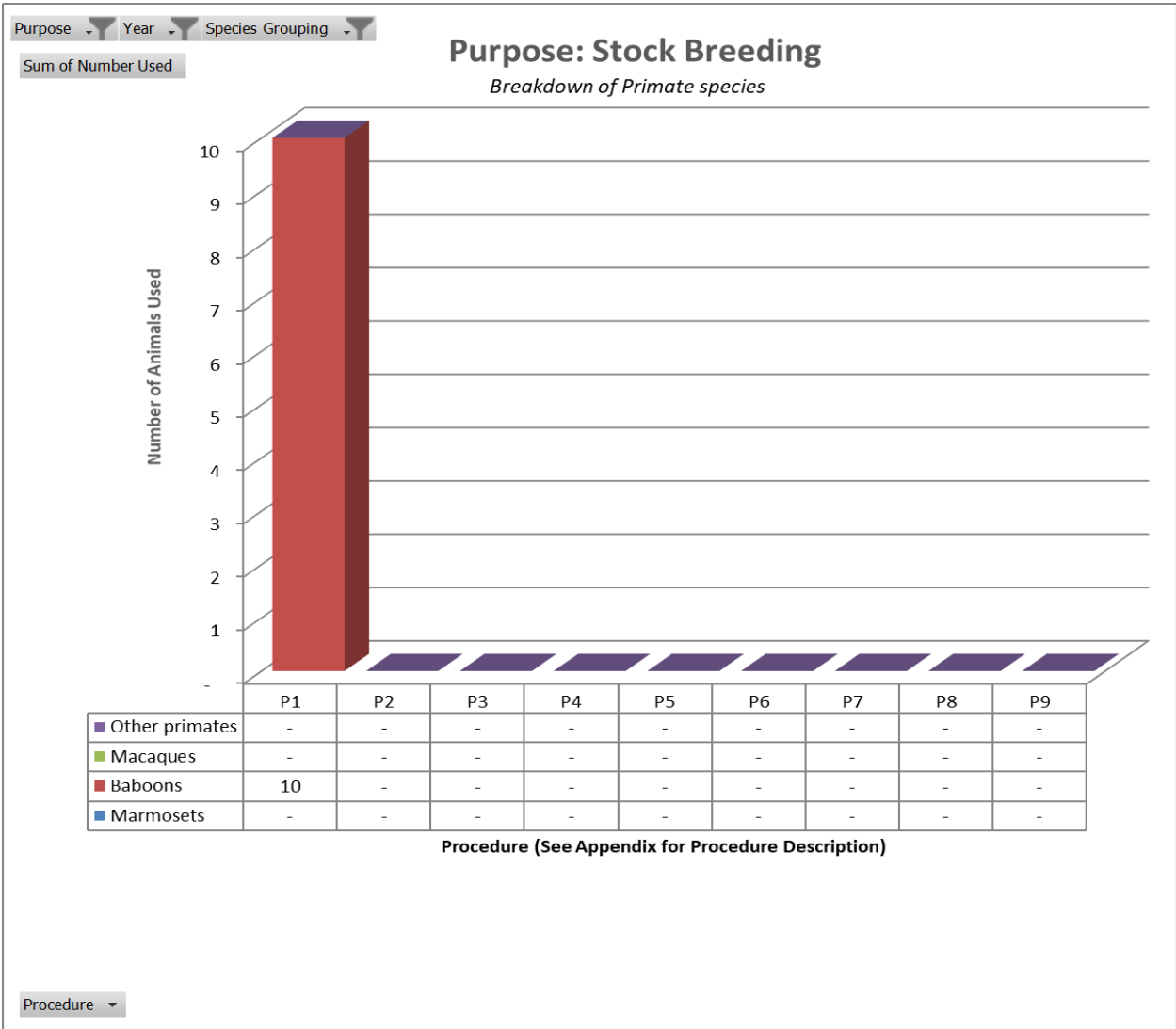
3.1 Stock Breeding



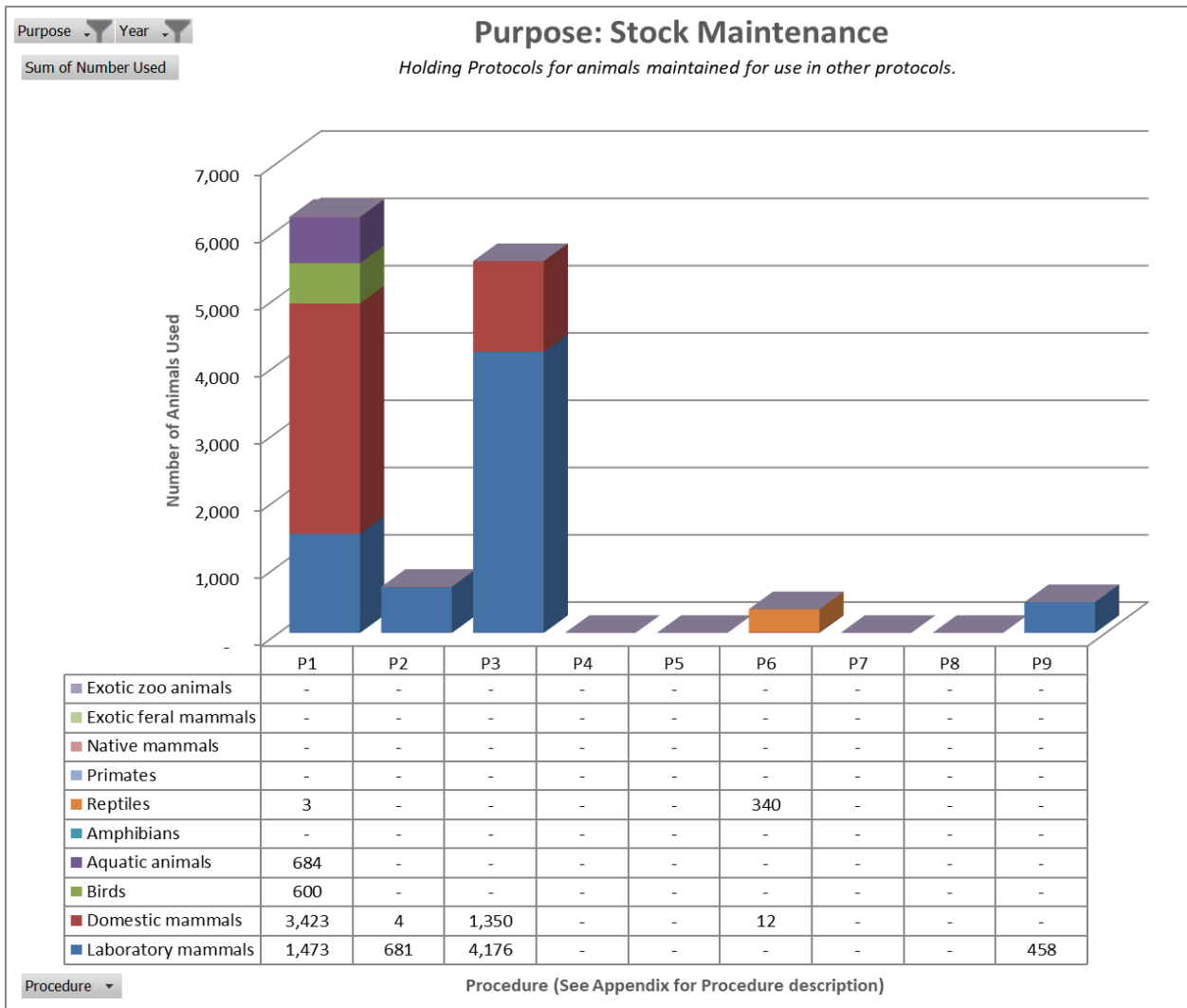
3.1.1 Species Charts for Stock Breeding



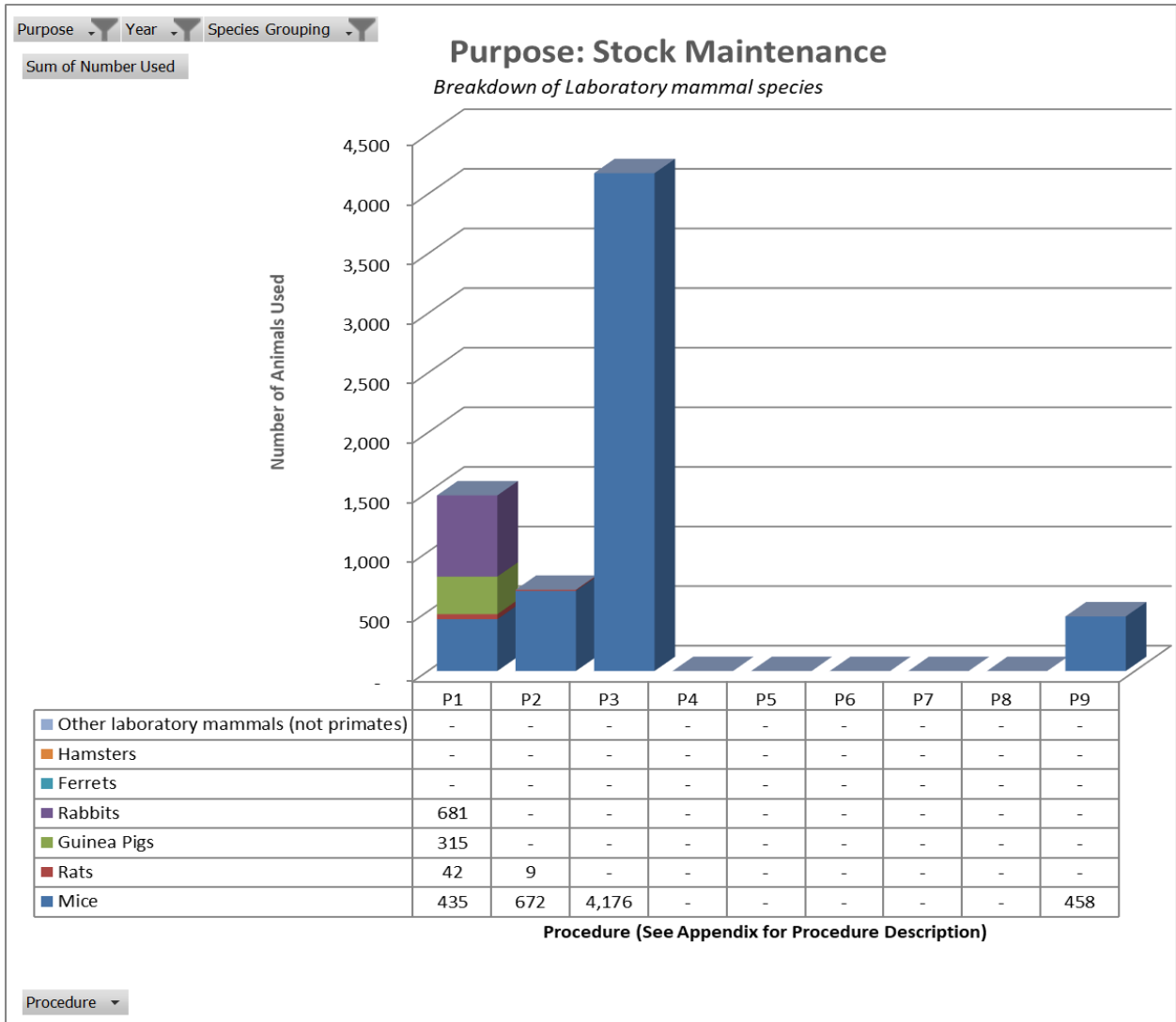


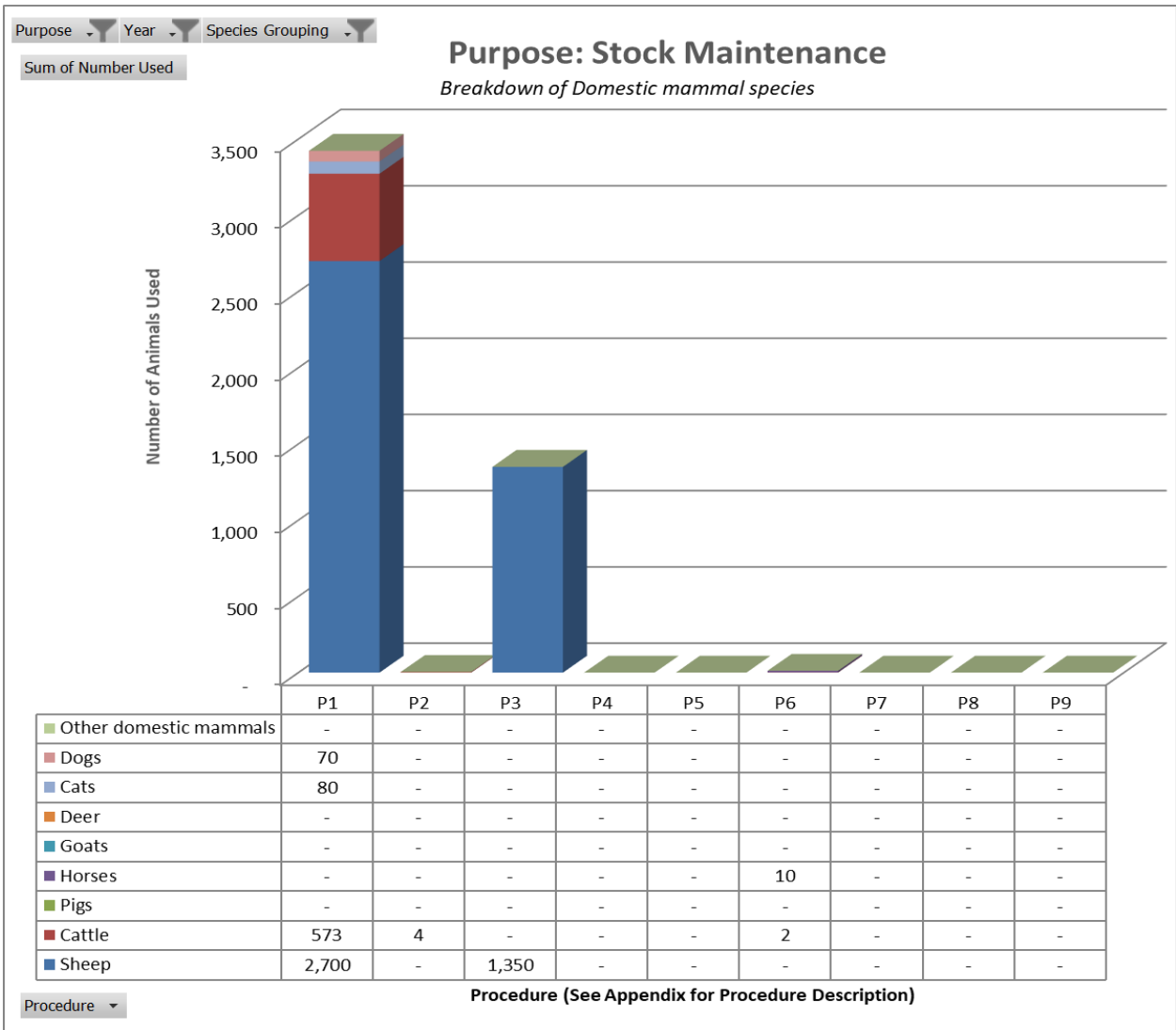


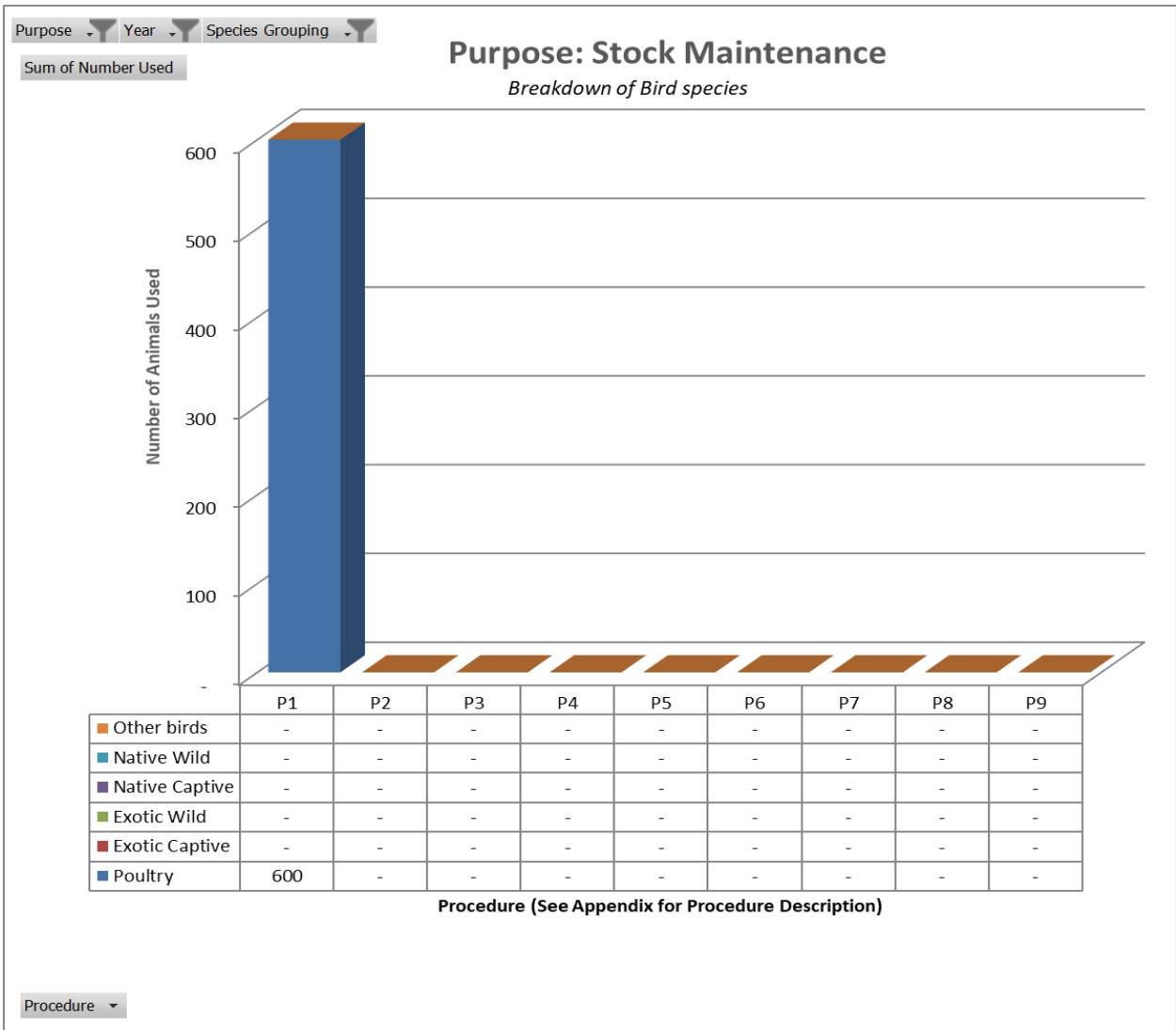
3.2 Stock Maintenance



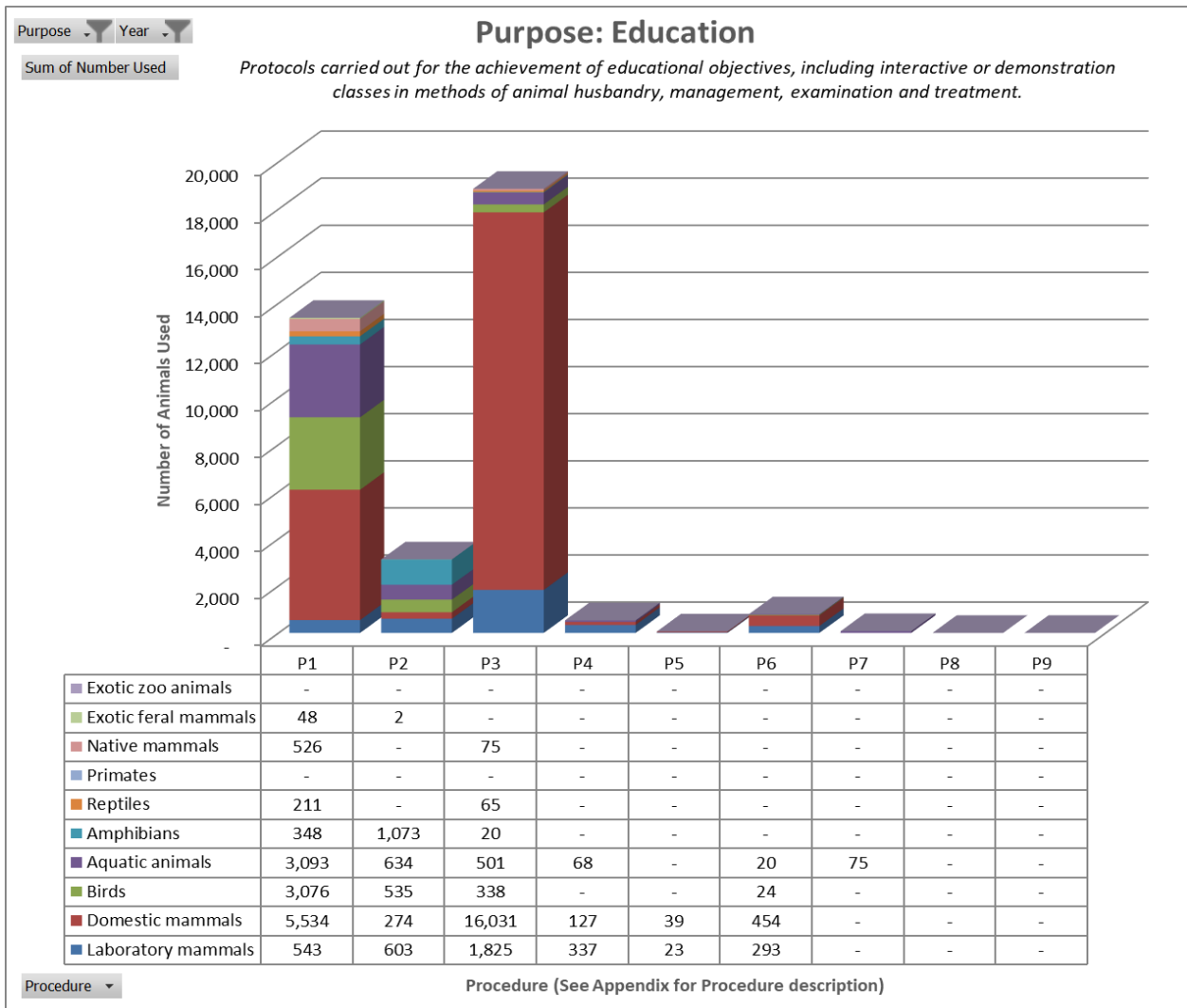
3.2.1 Species Charts for Stock Maintenance



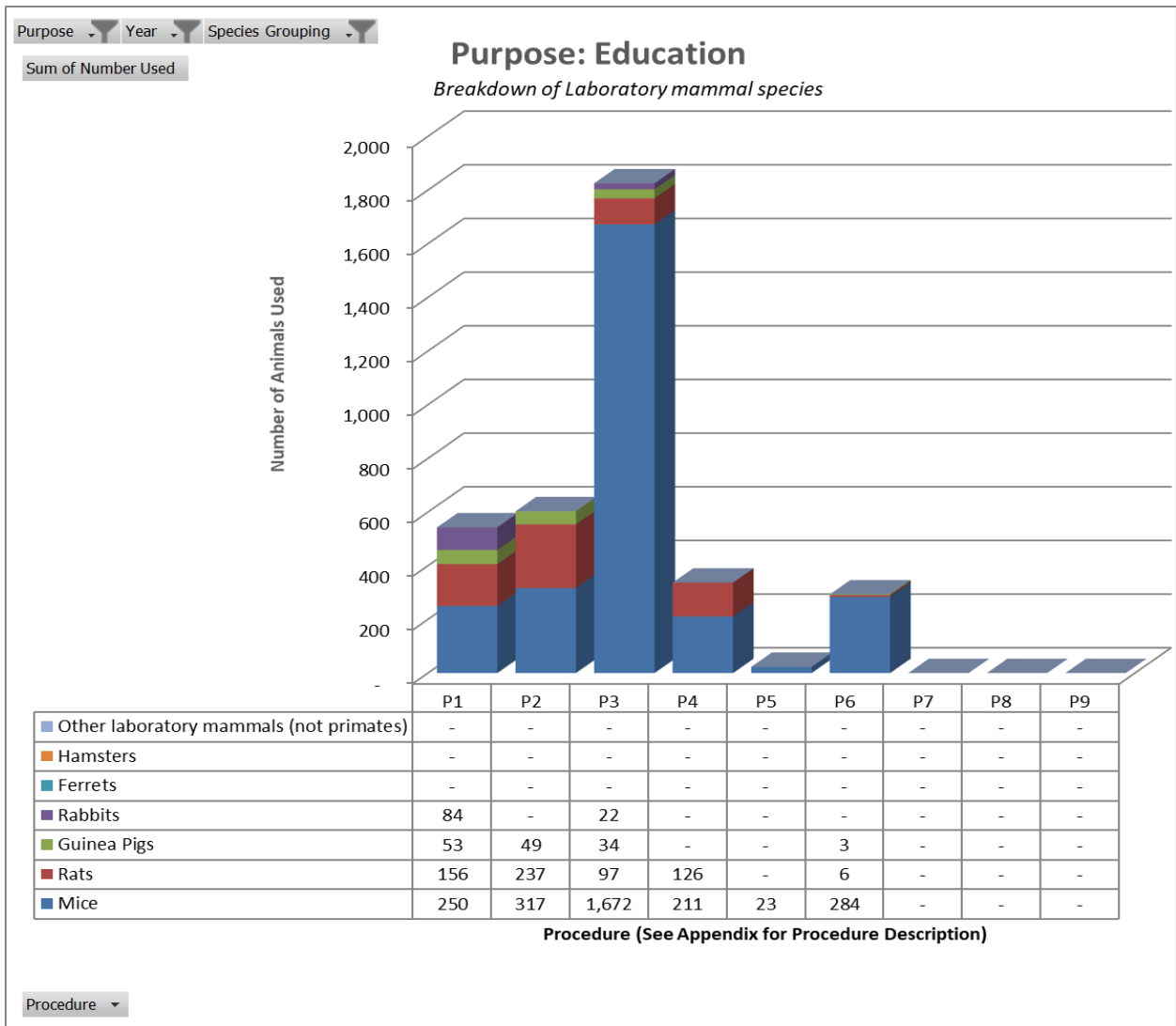


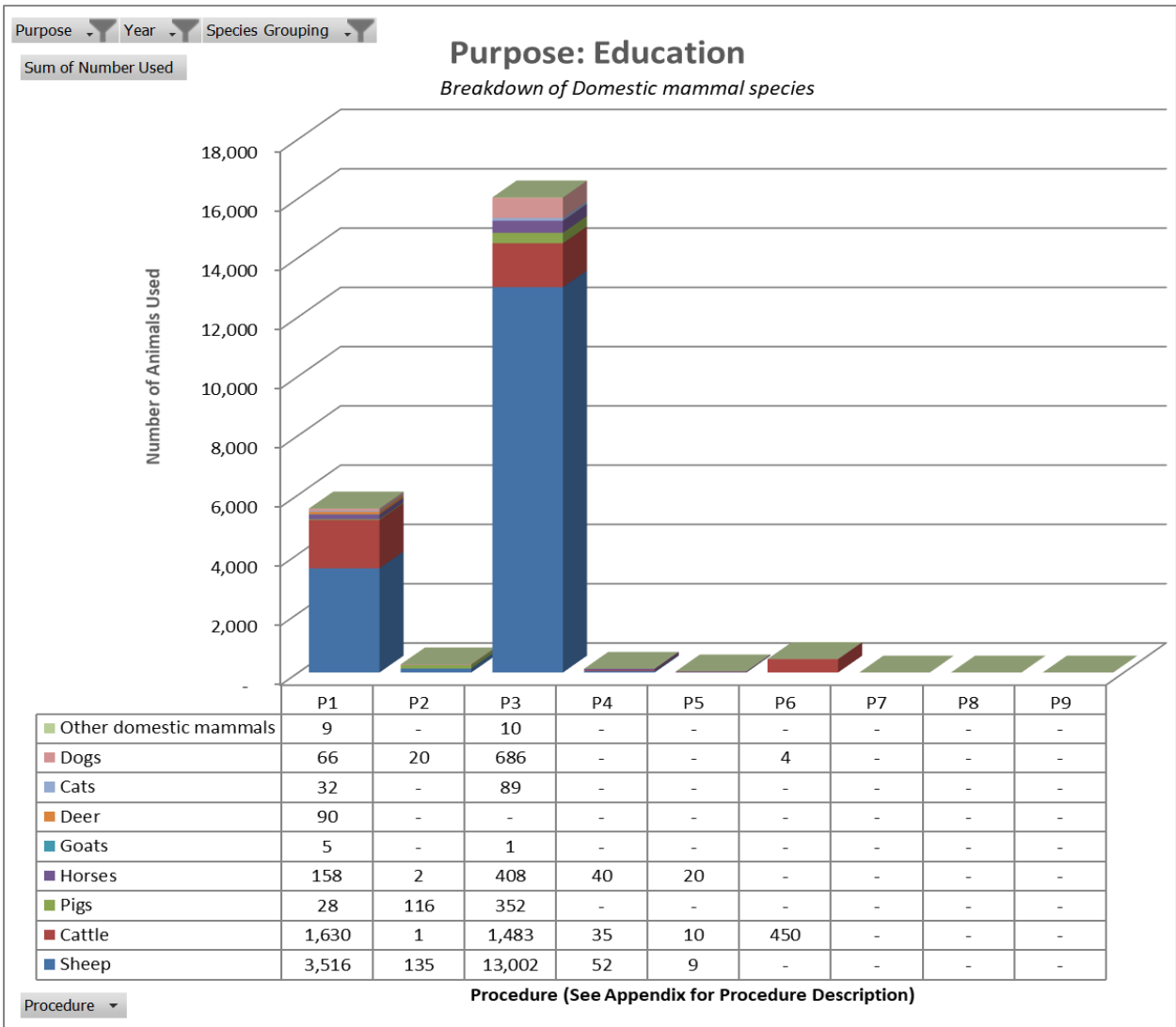


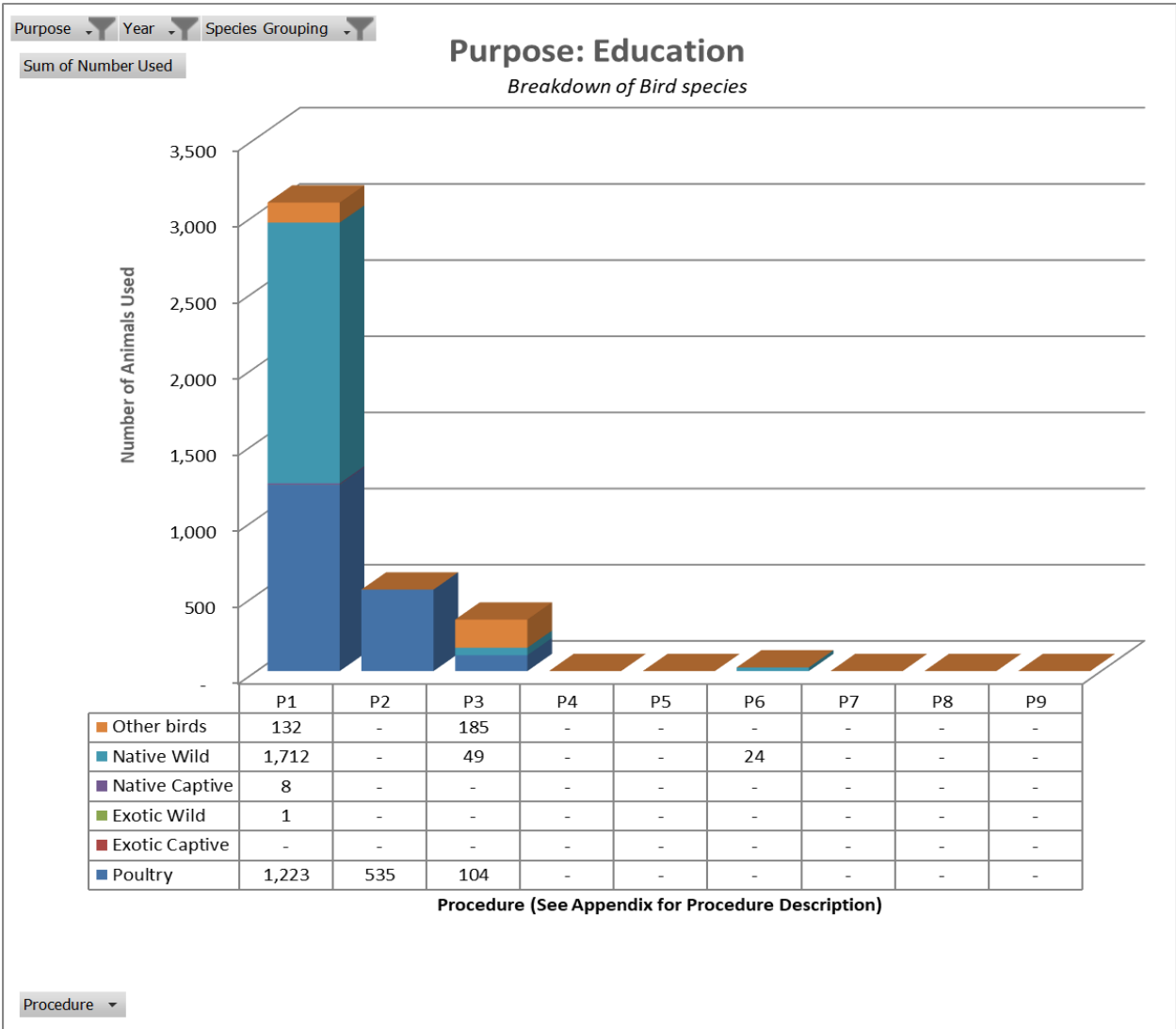
3.3 Education



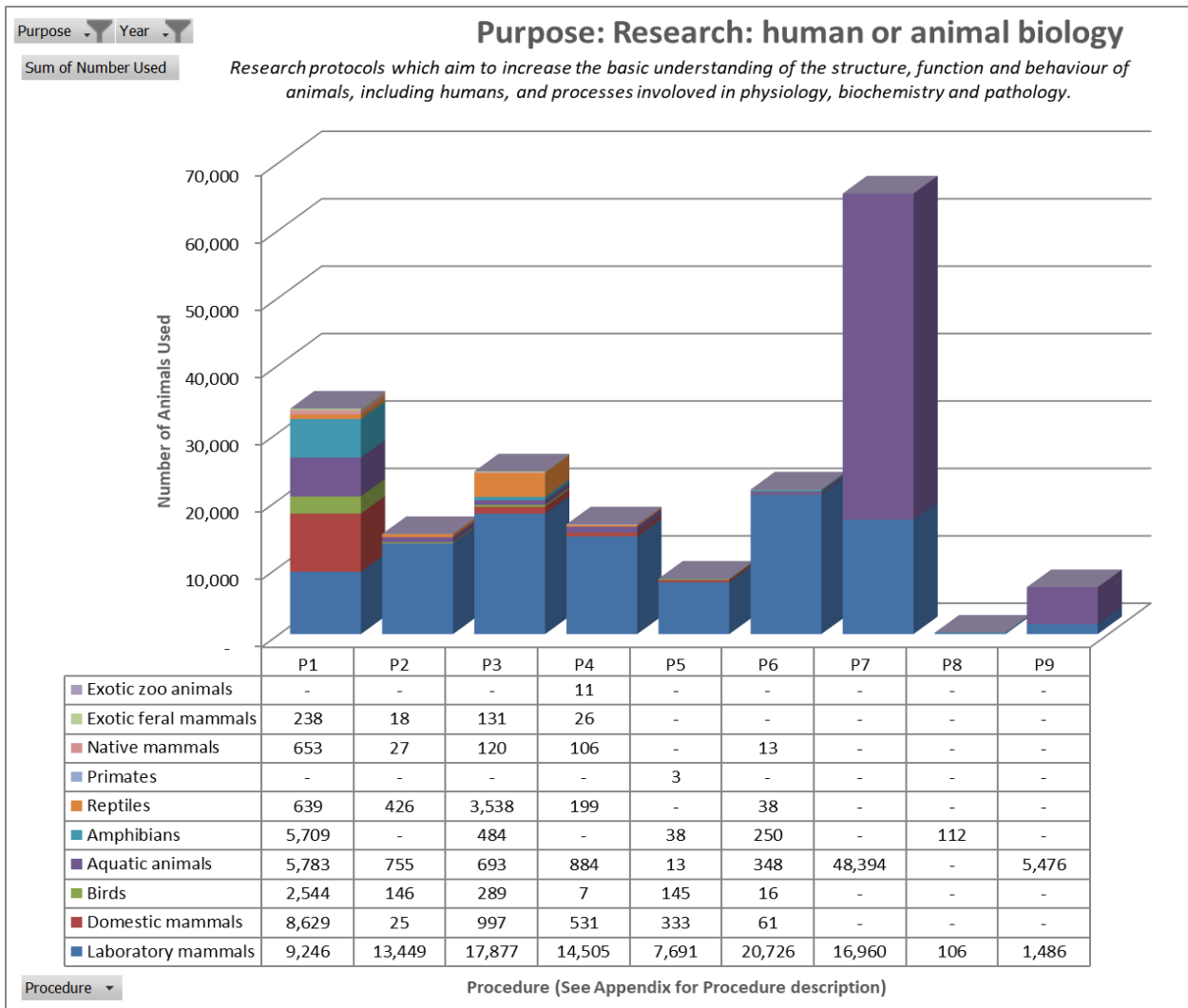
3.3.1 Species Charts for Education



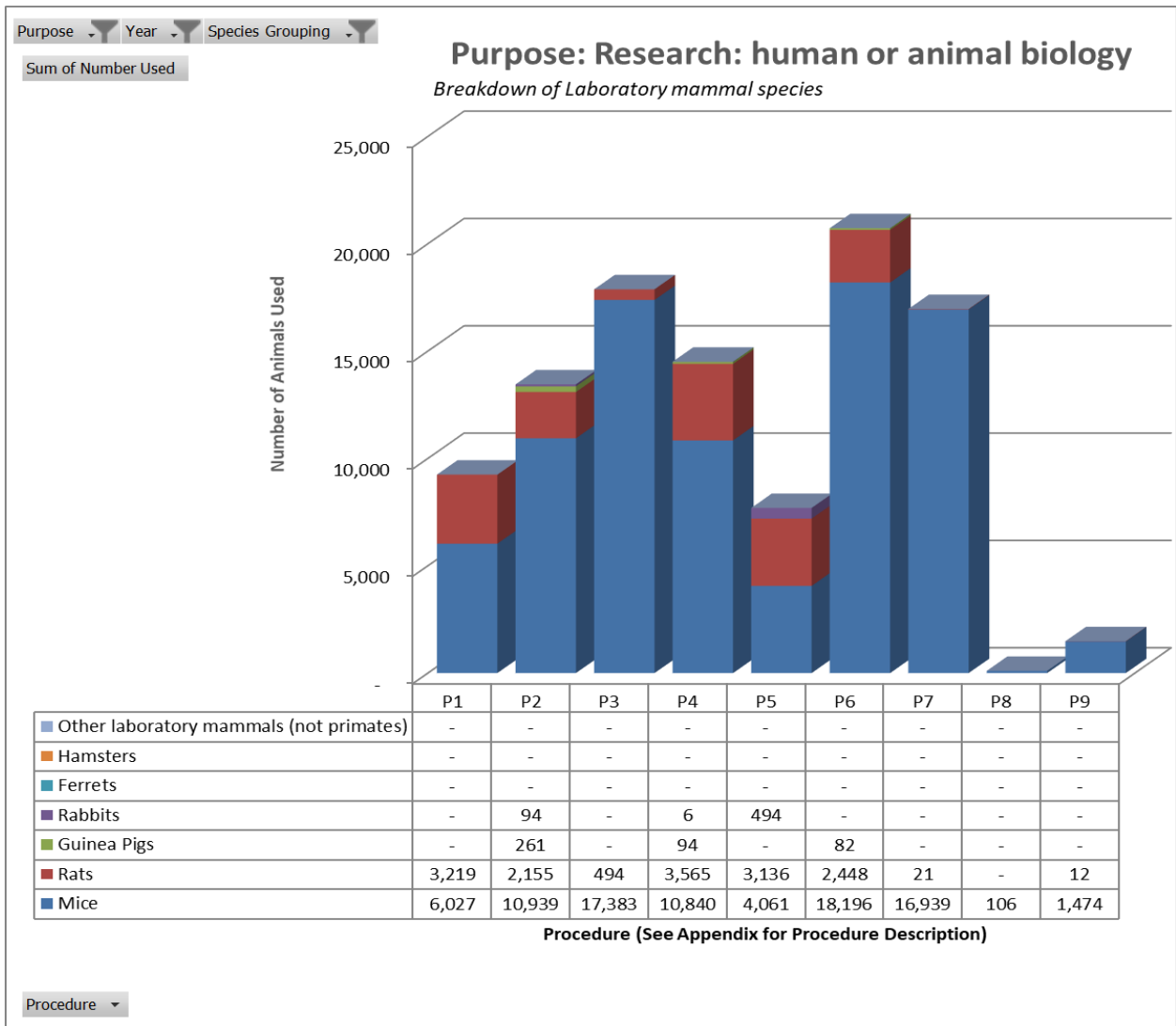


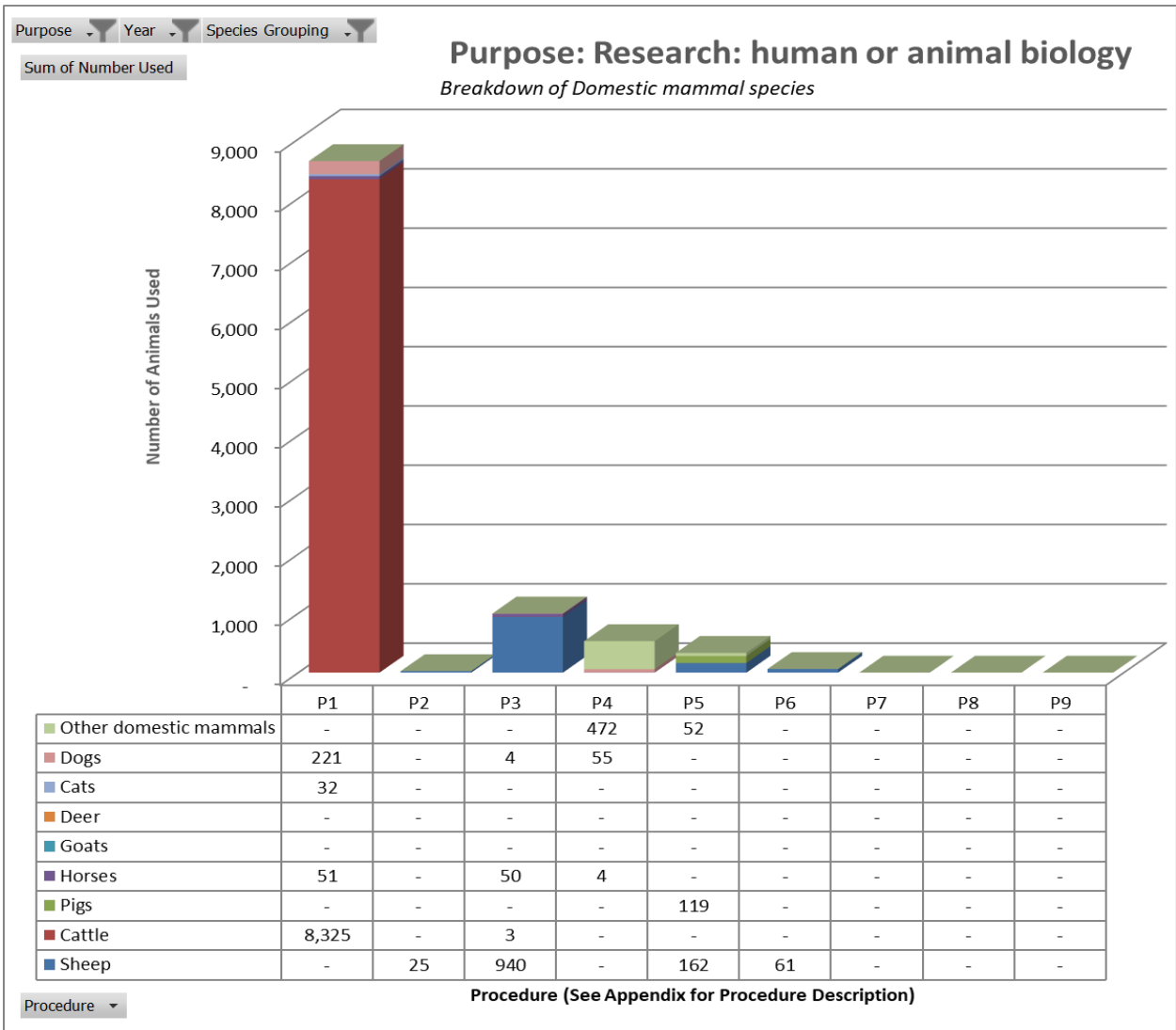


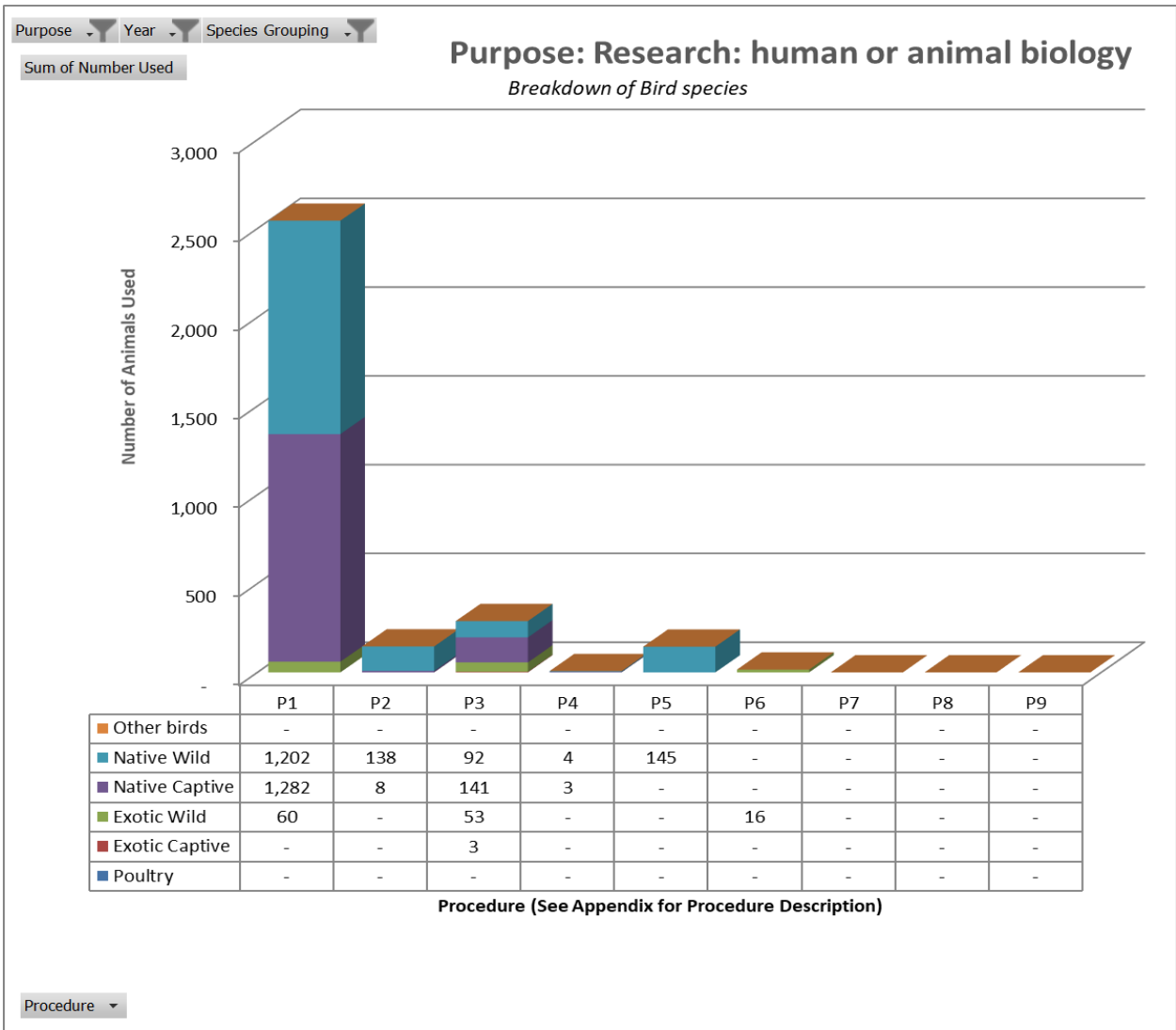
3.4 Research - Human or Animal Biology

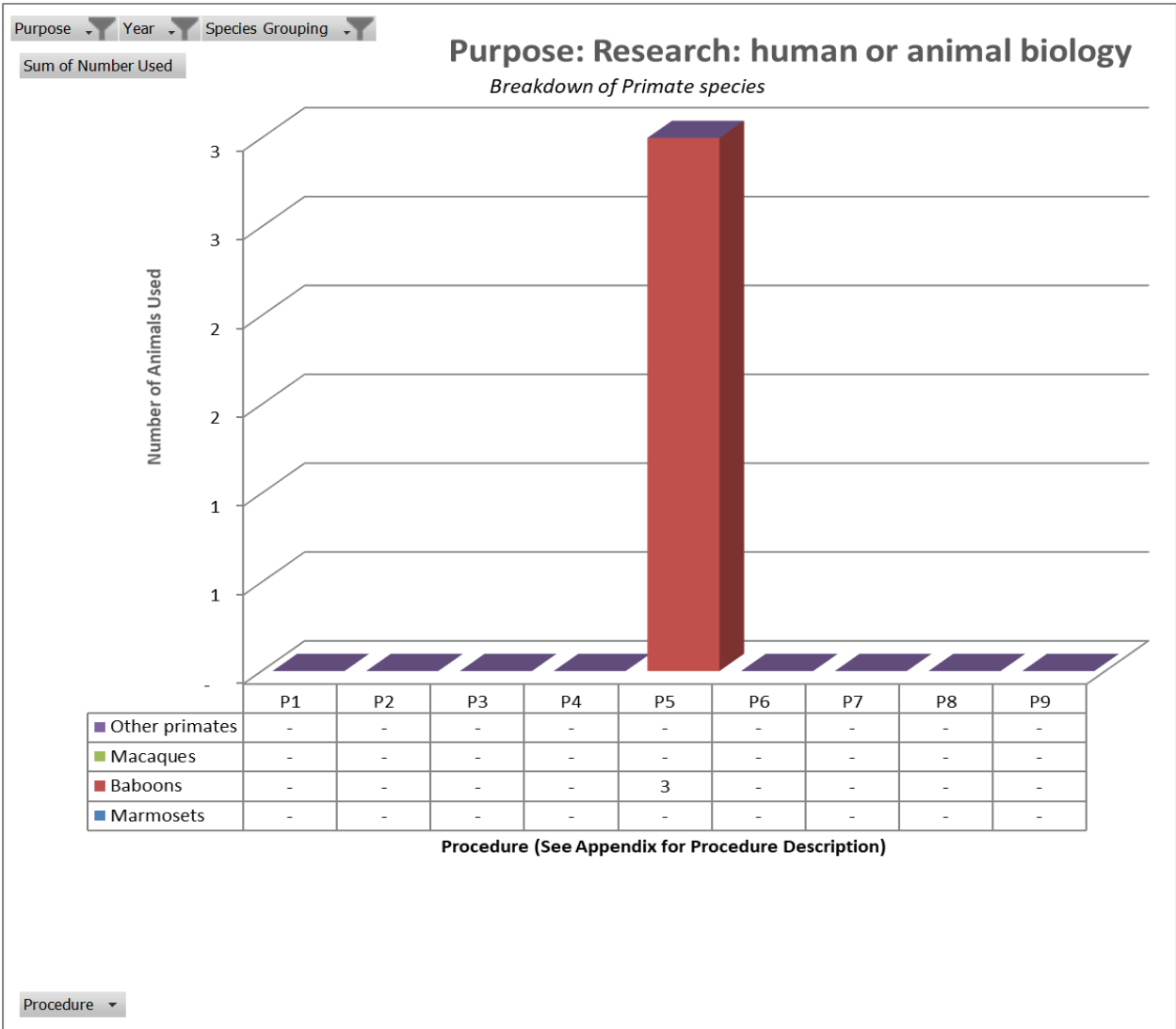


3.4.1 Species Charts for Research - Human or Animal Biology

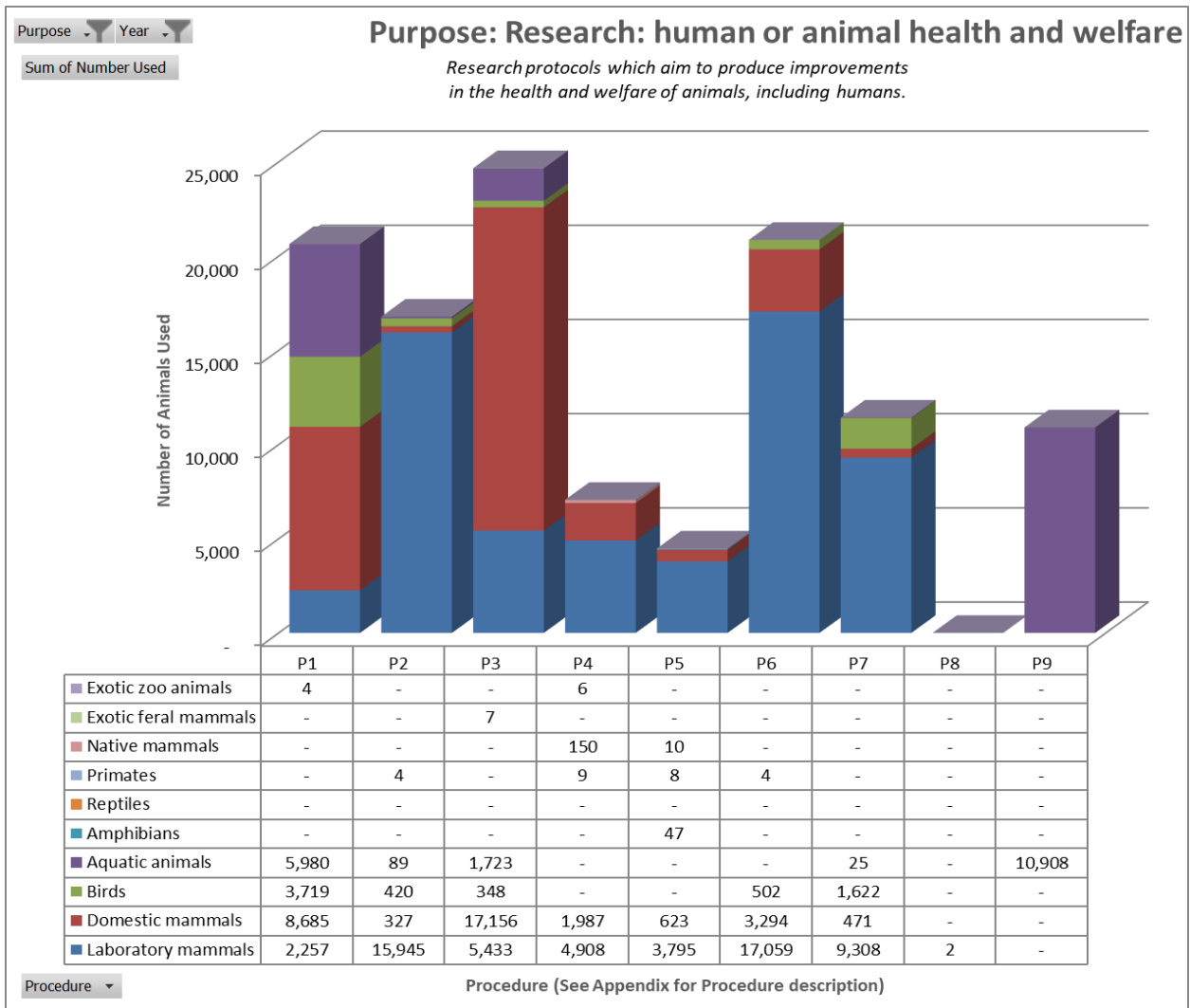




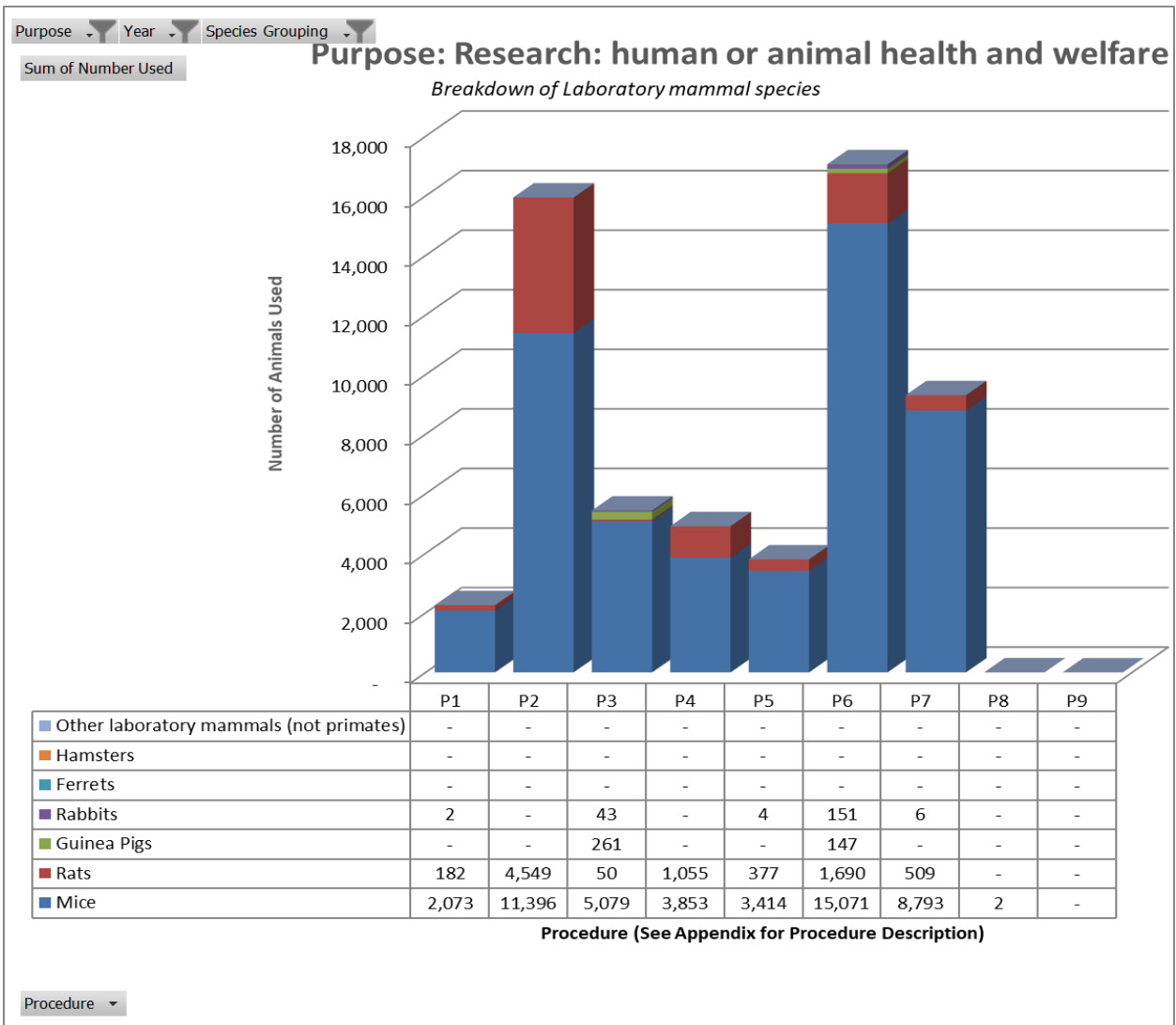


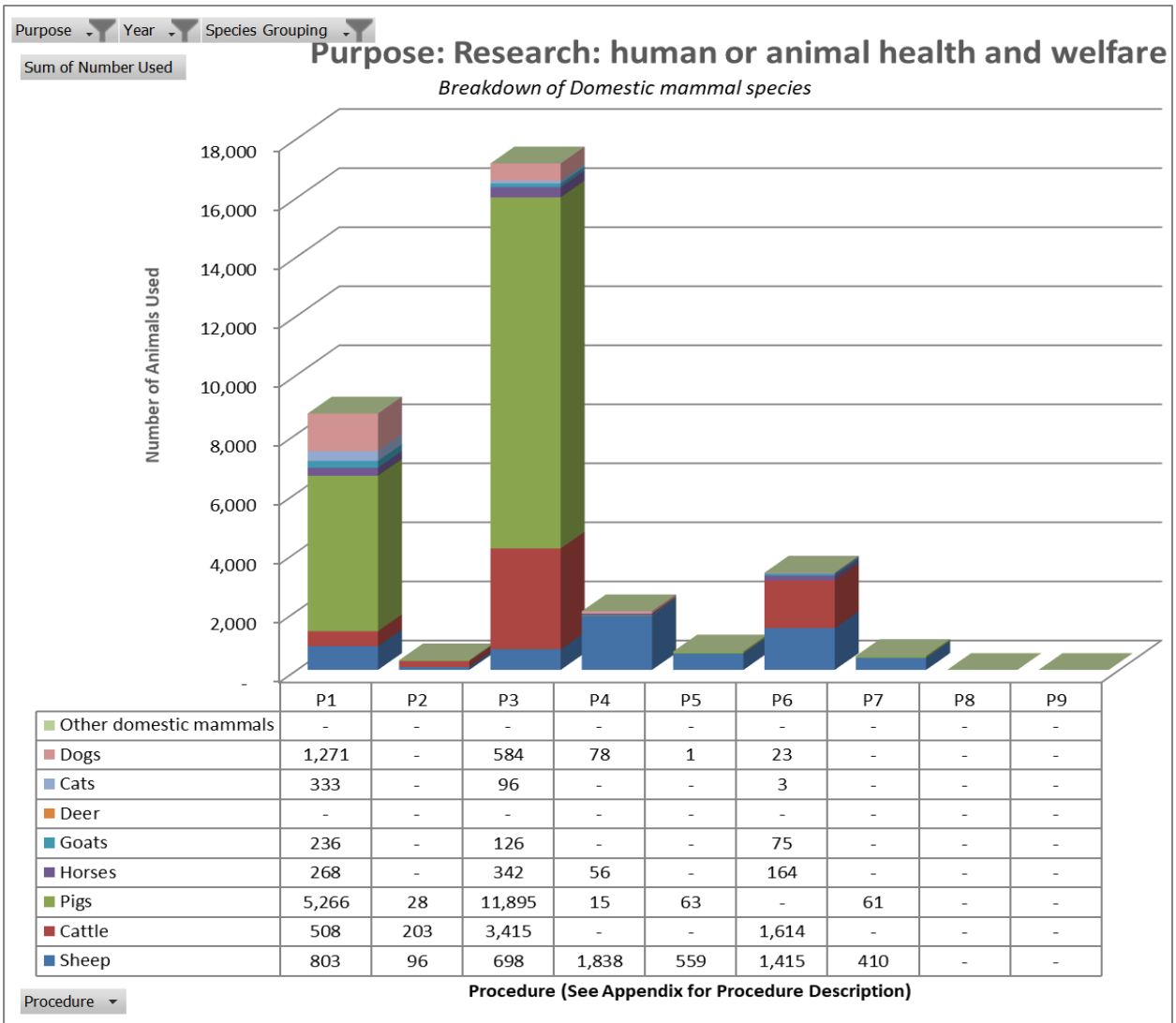


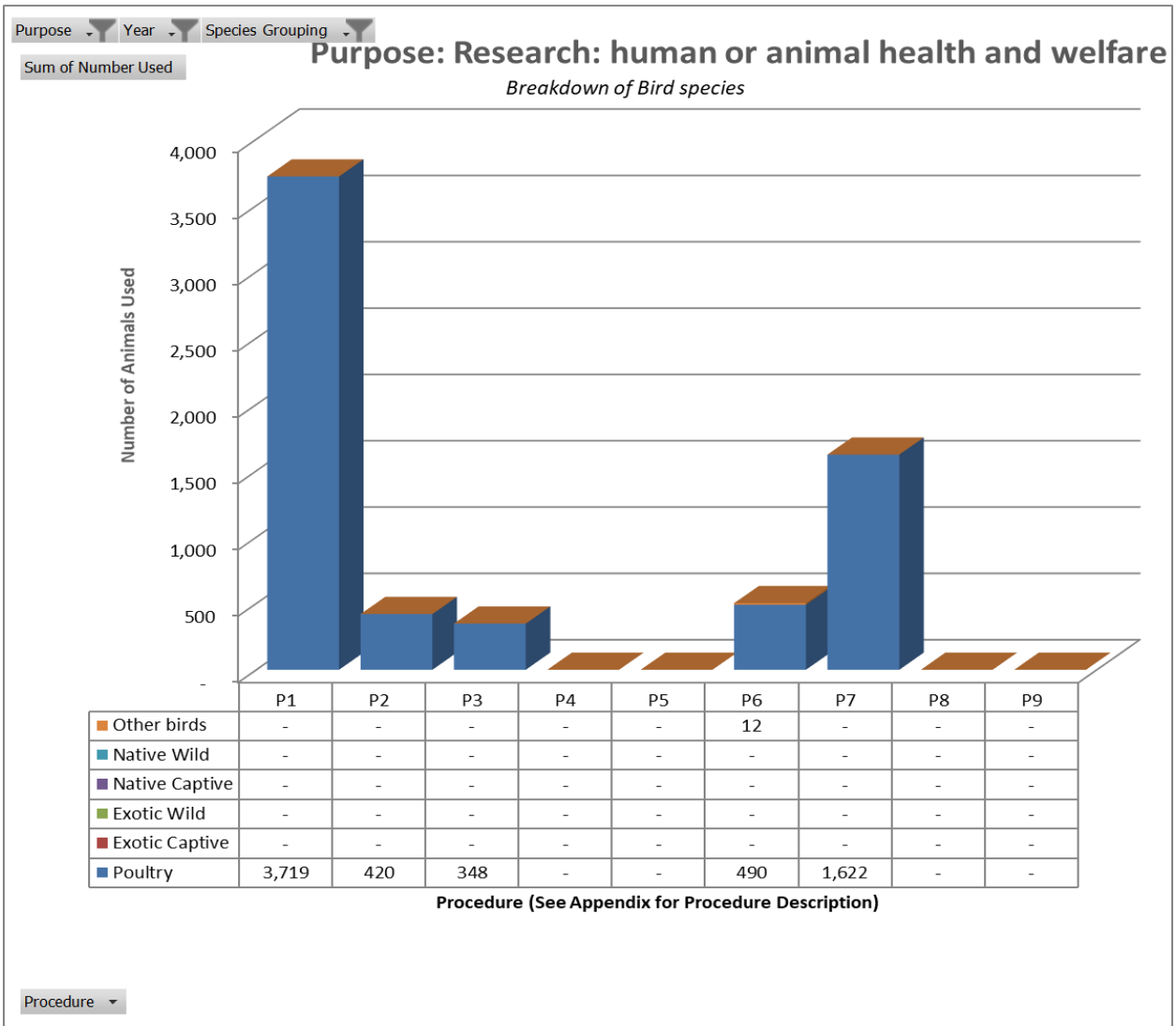
3.5 Research - Human or Animal Health and Welfare

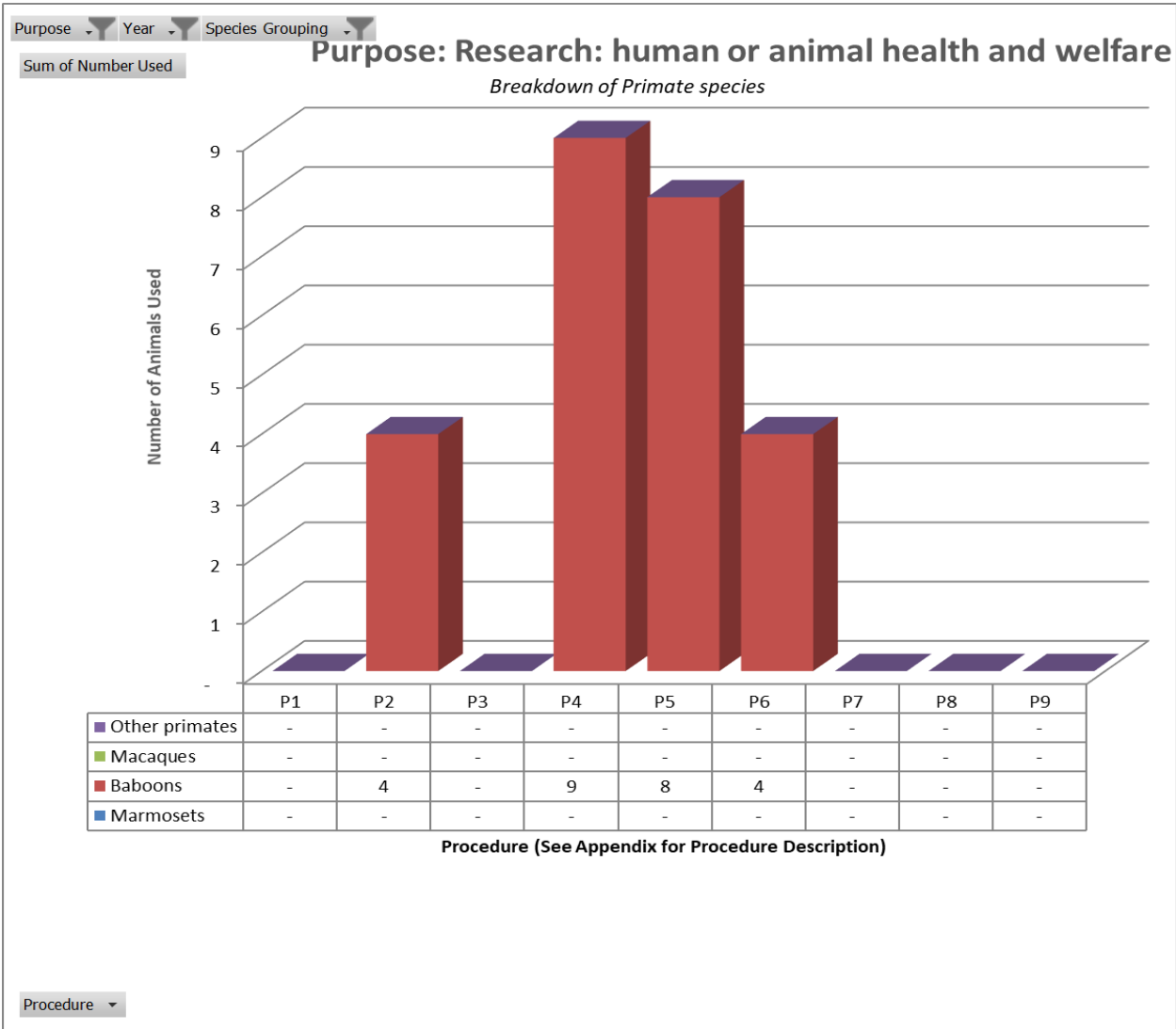


3.5.1 Species Charts for Research - Human or Animal Health and Welfare

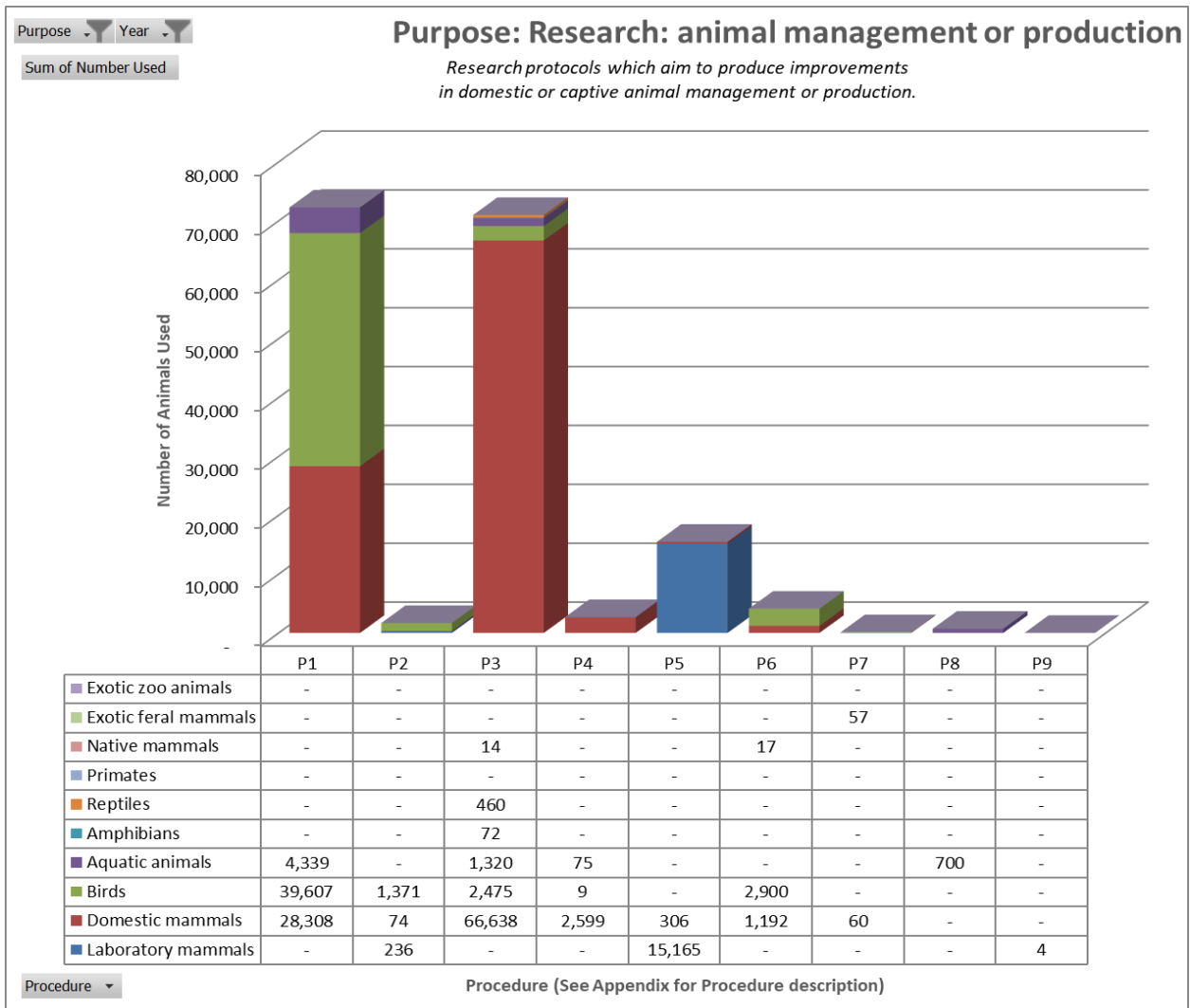




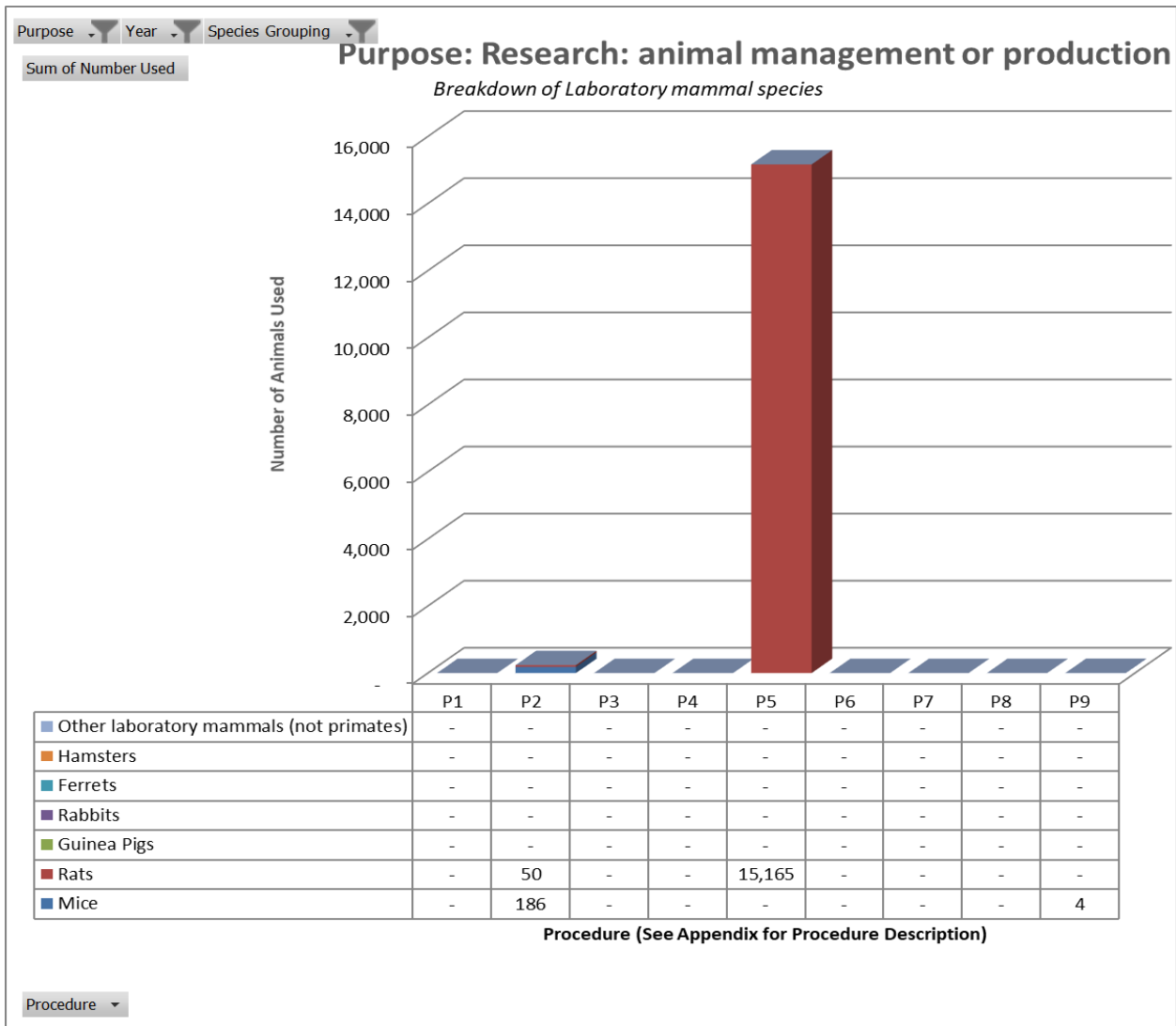


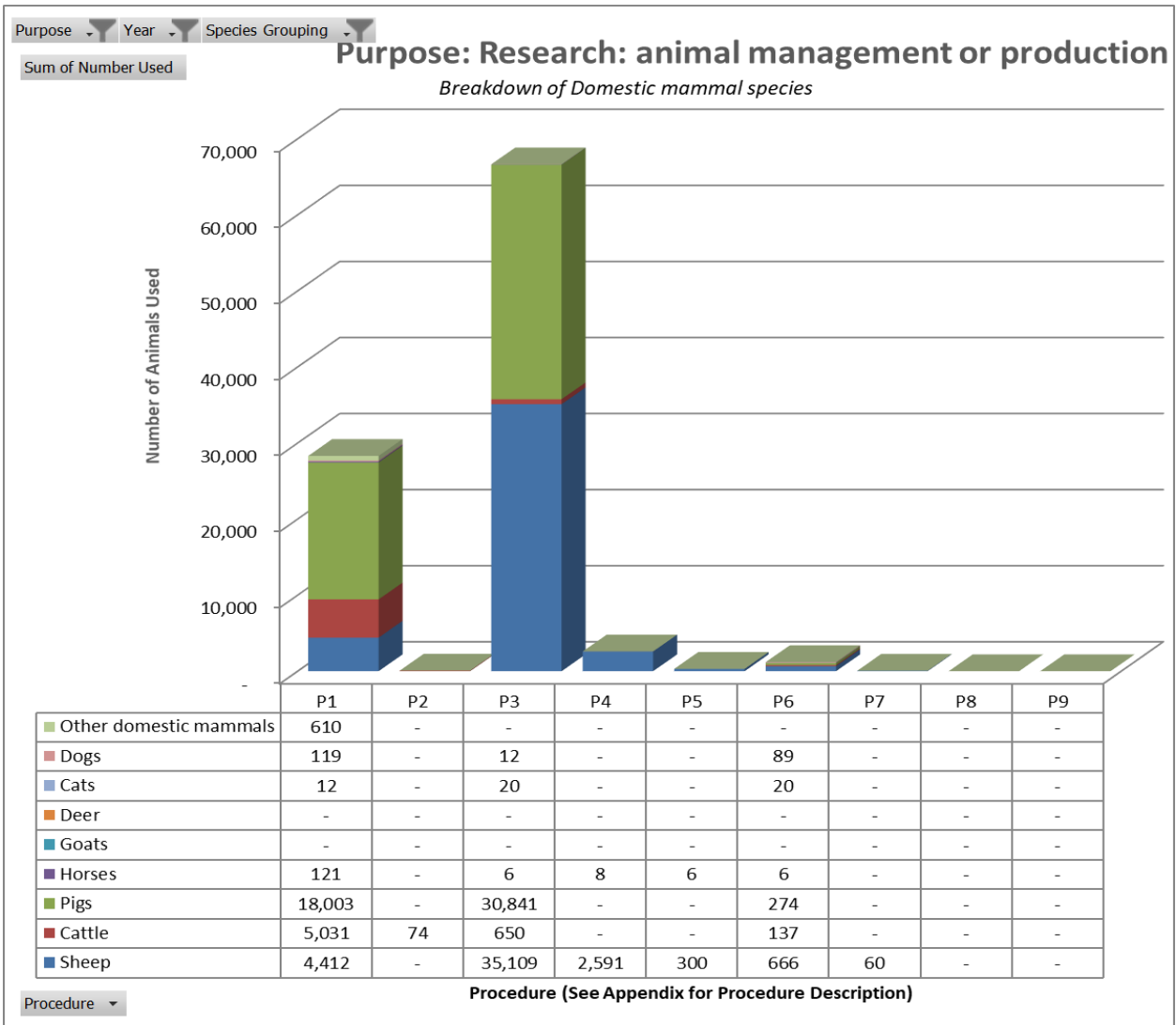


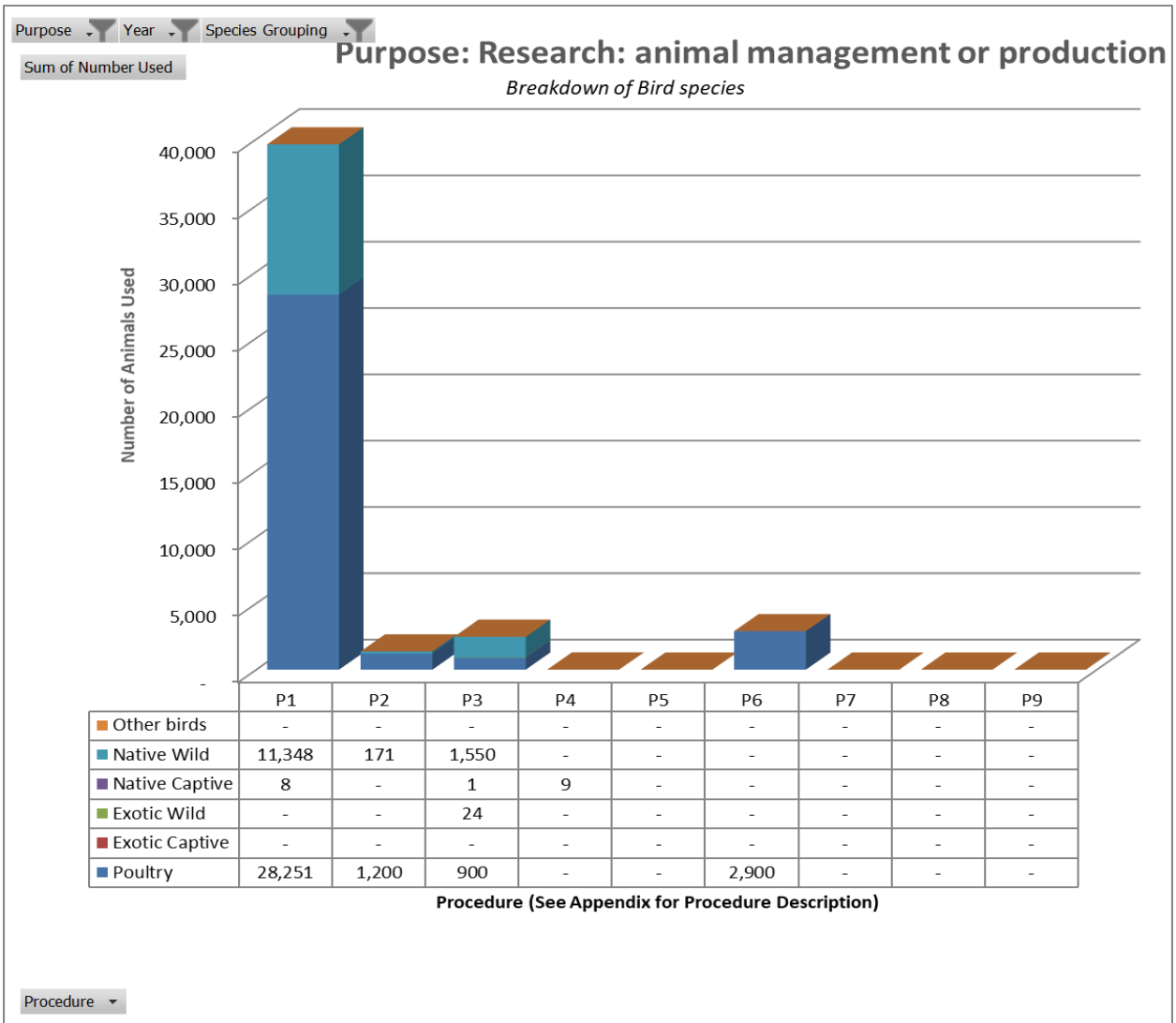
3.6 Research - Animal Management or Production



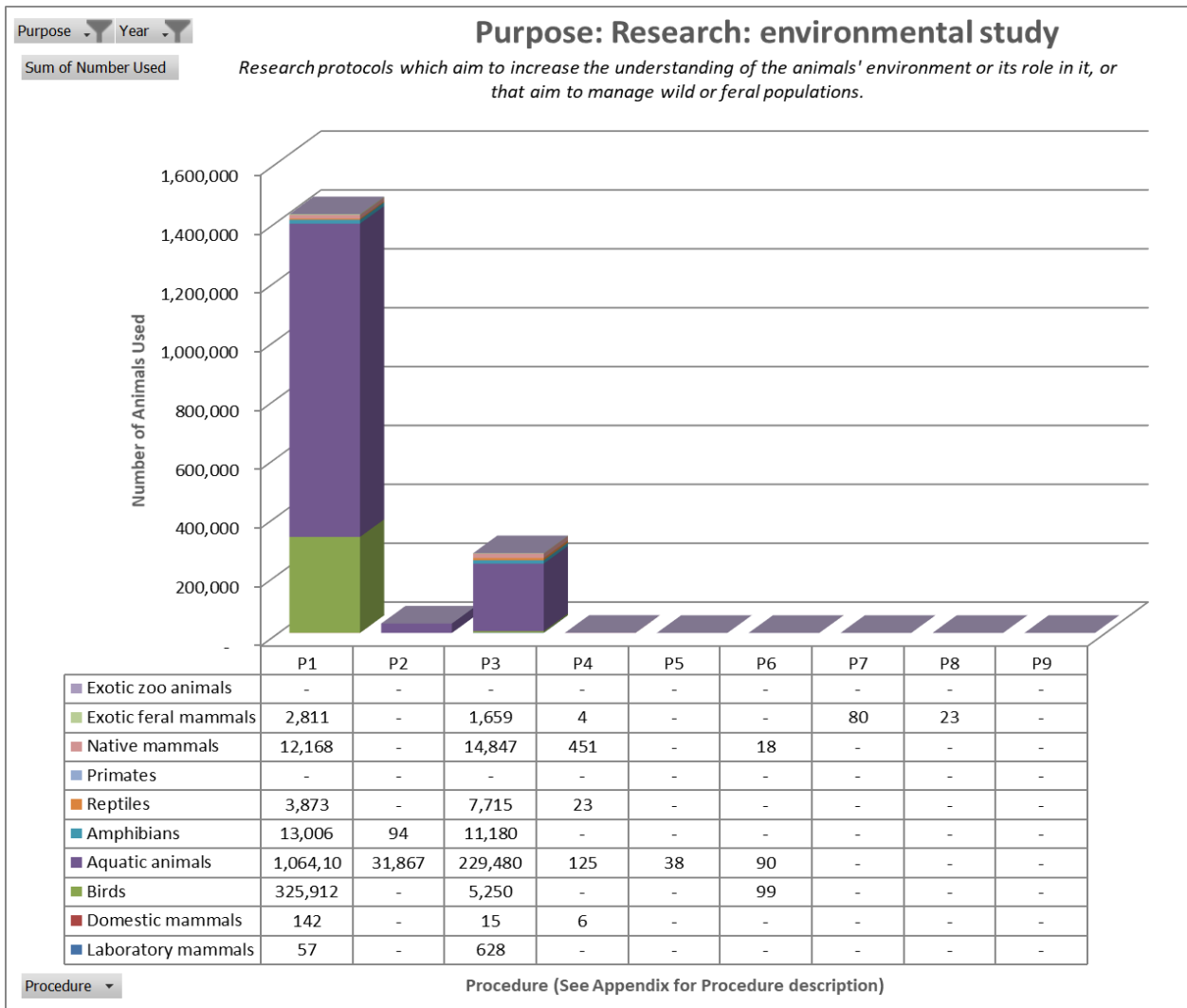
3.6.1 Species Charts for Research - Animal Management or Production



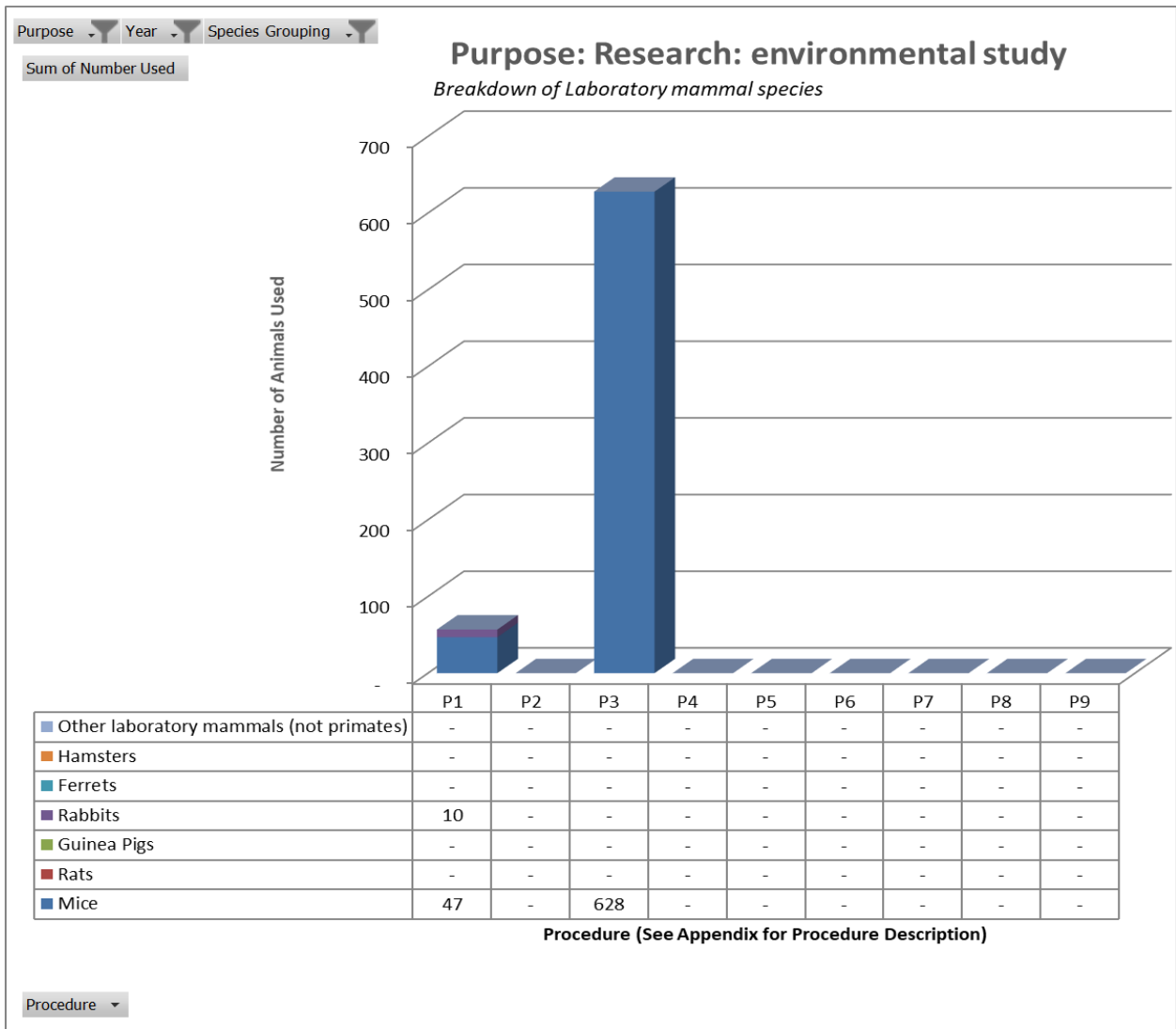


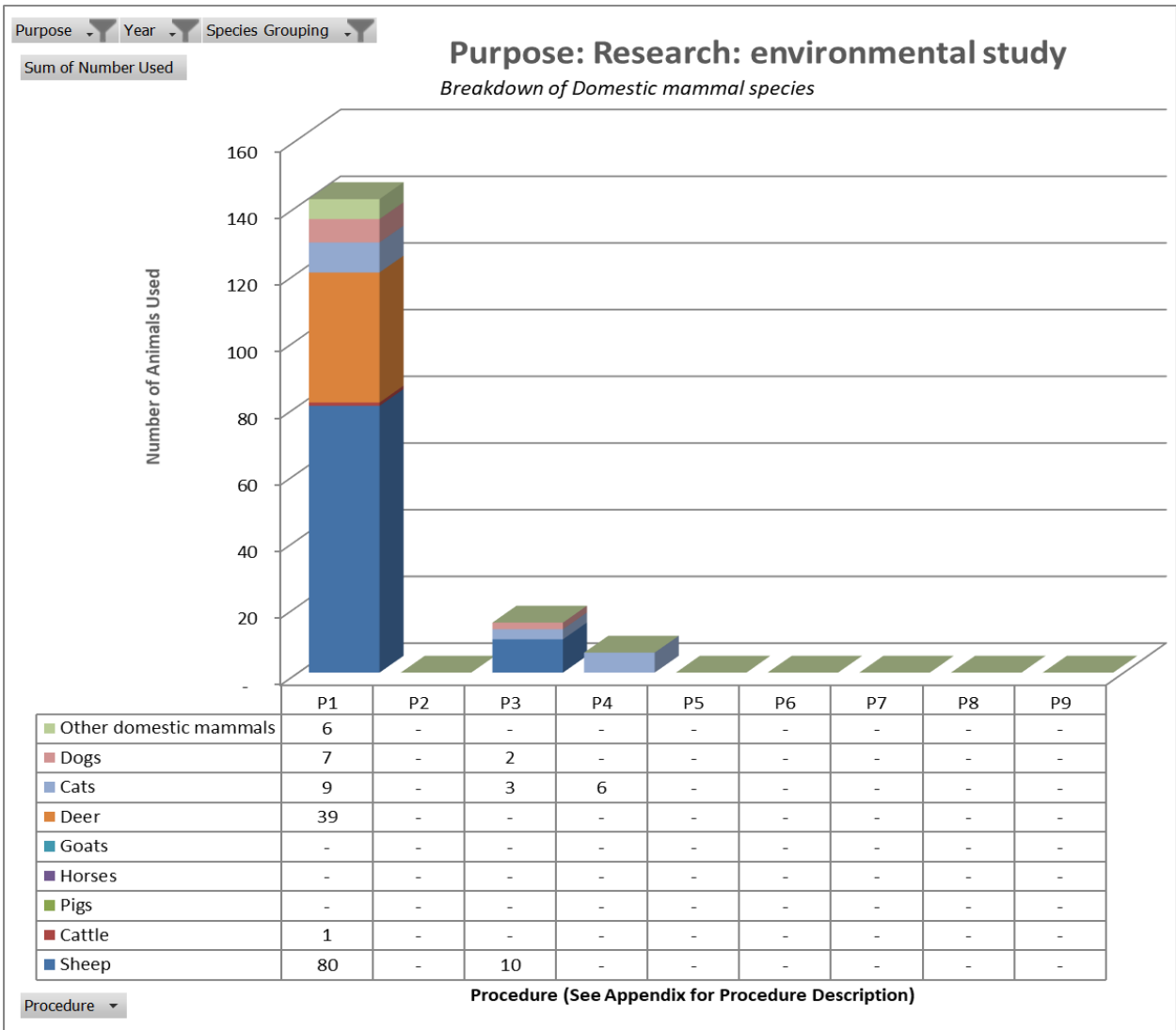


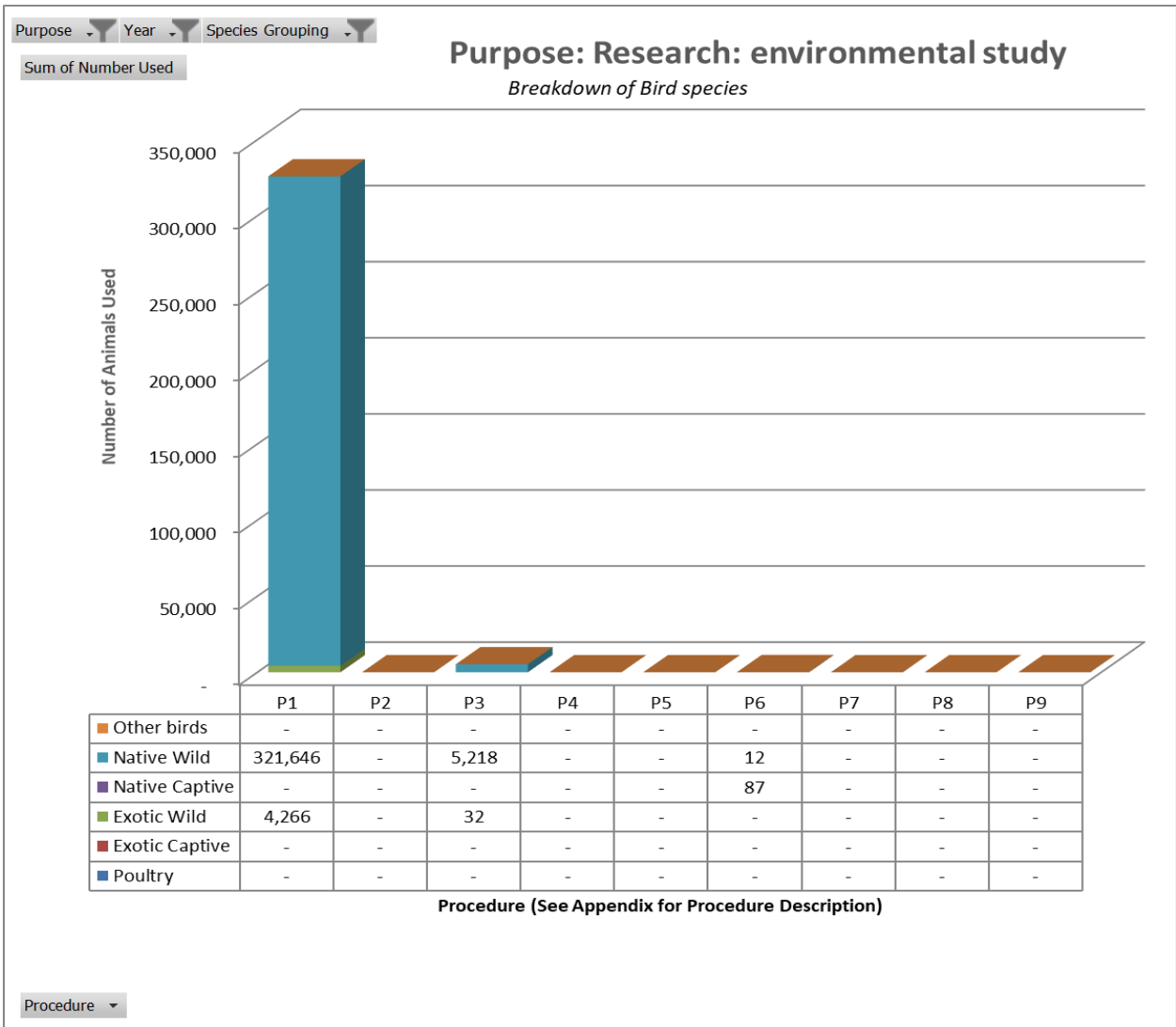
3.7 Research - Environmental Study



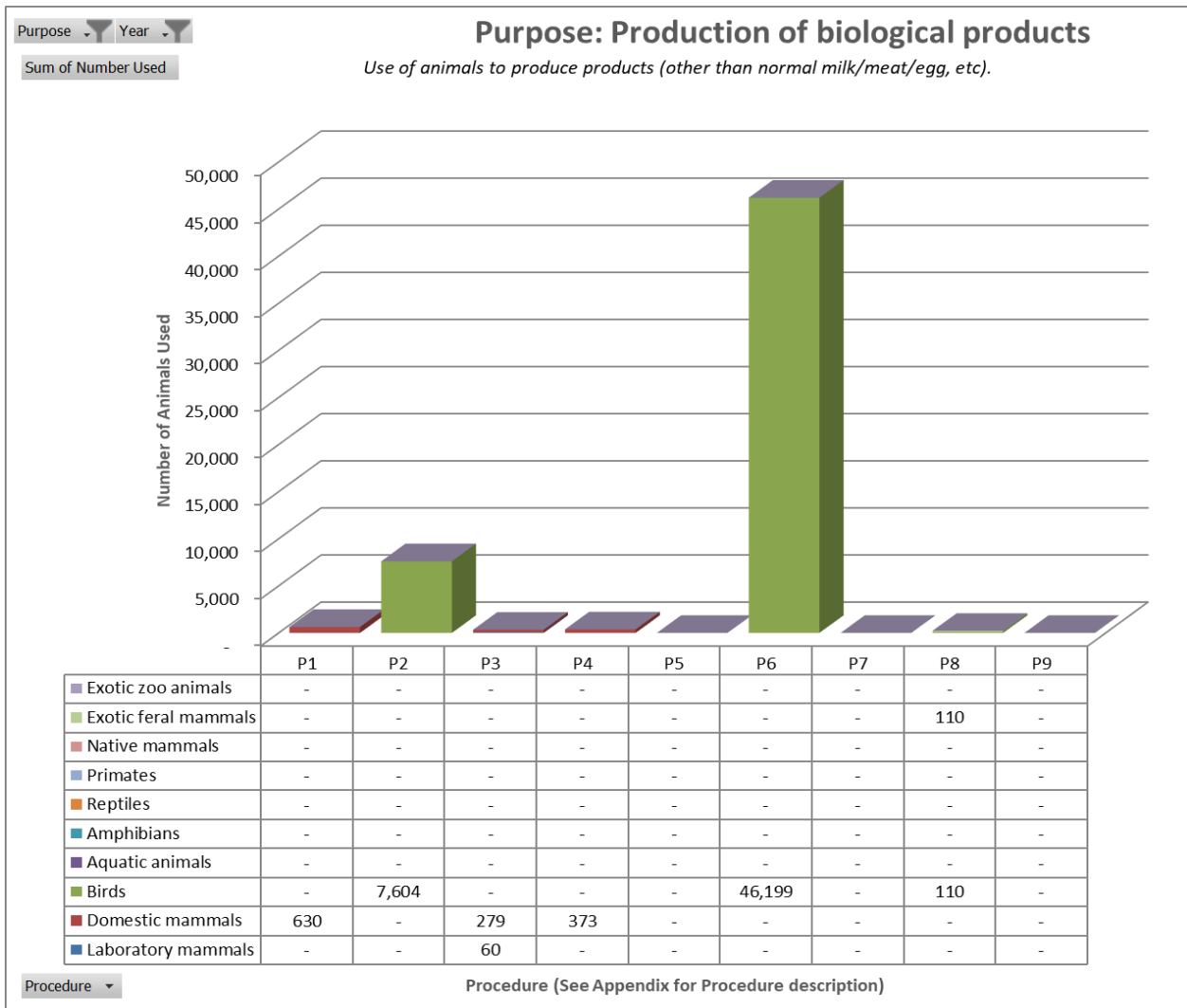
3.7.1 Species Charts for Research - Environmental Study



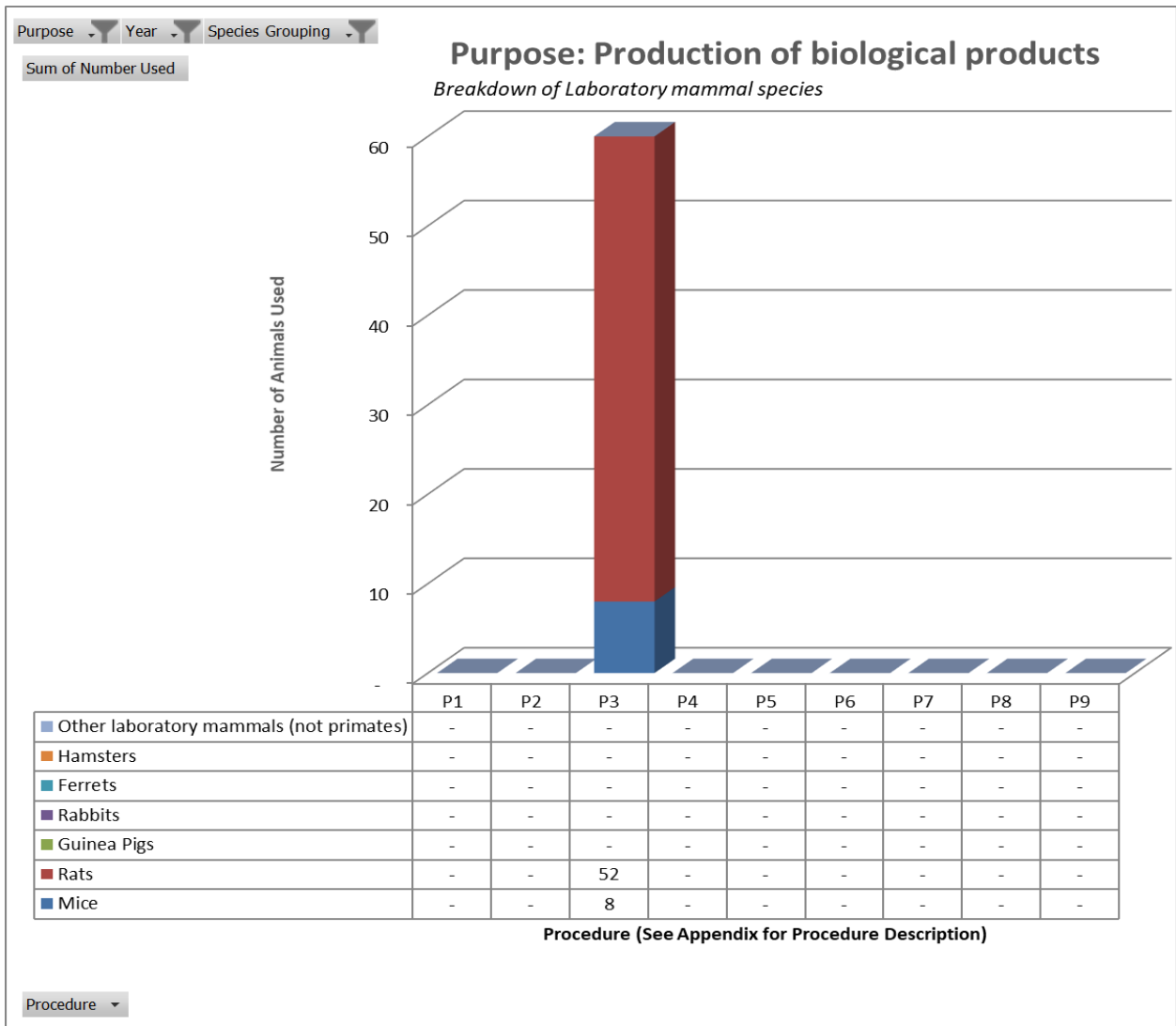


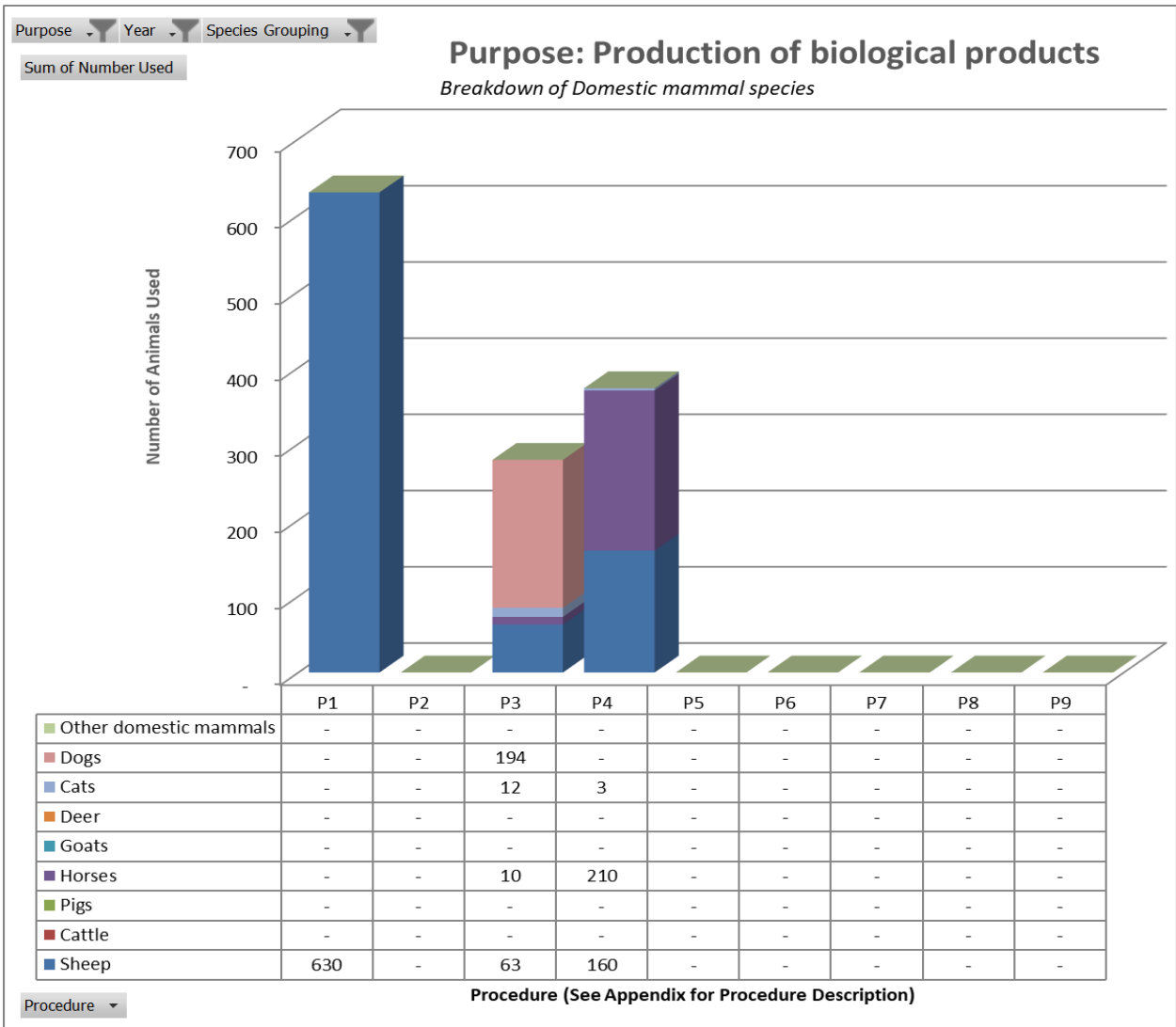


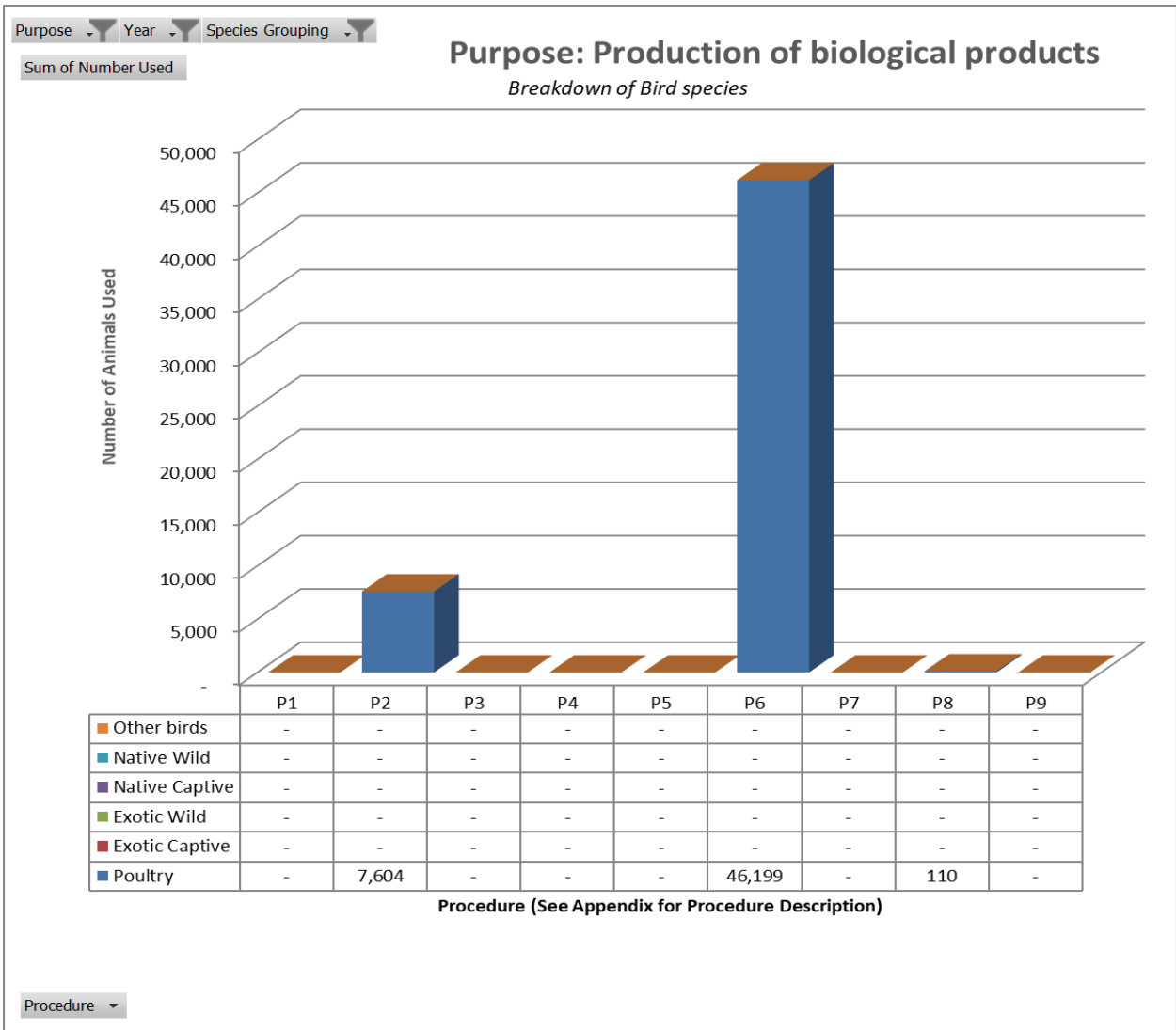
3.8 Production of Biological Products



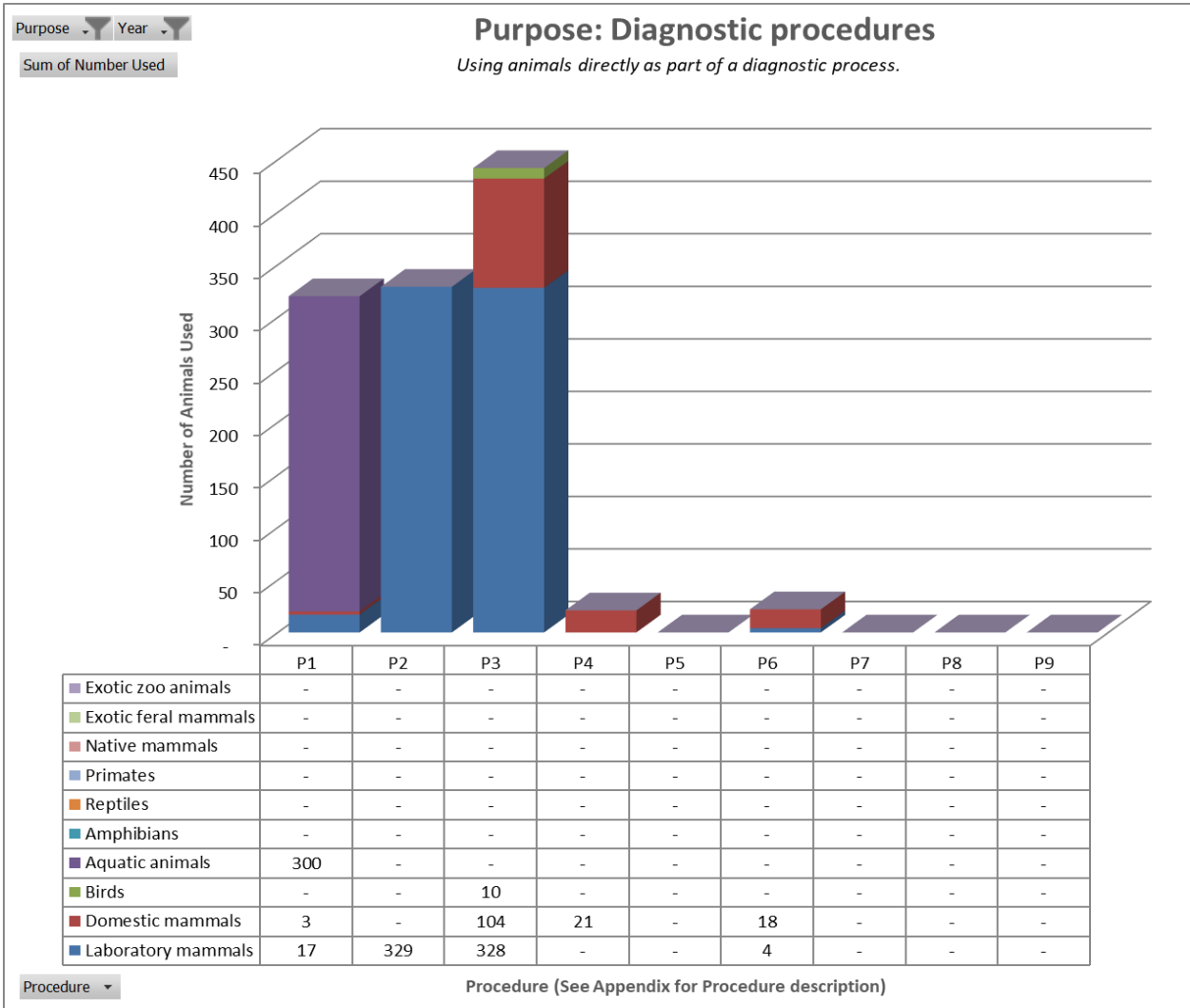
3.8.1 Species Charts for Production of Biological Products



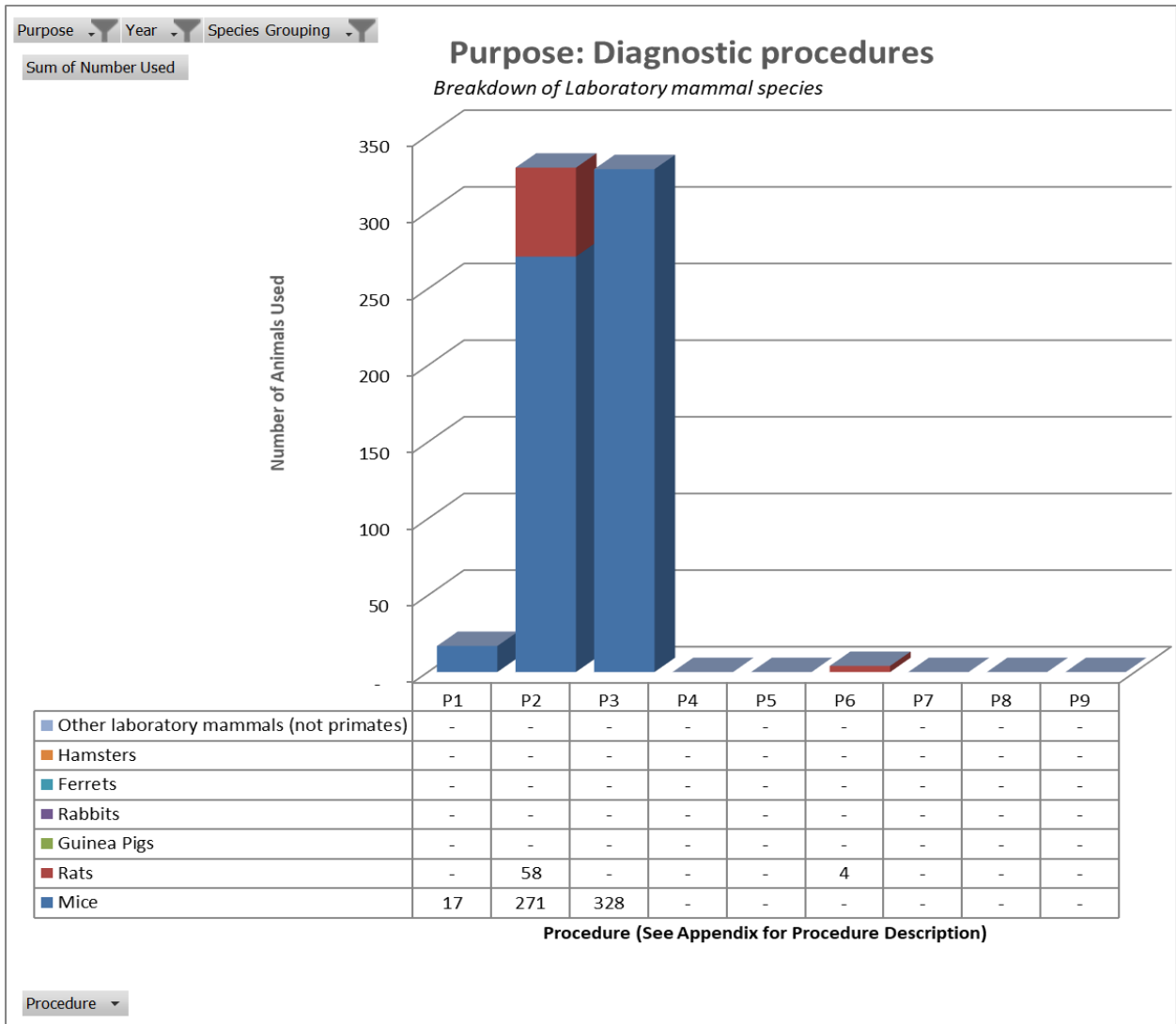


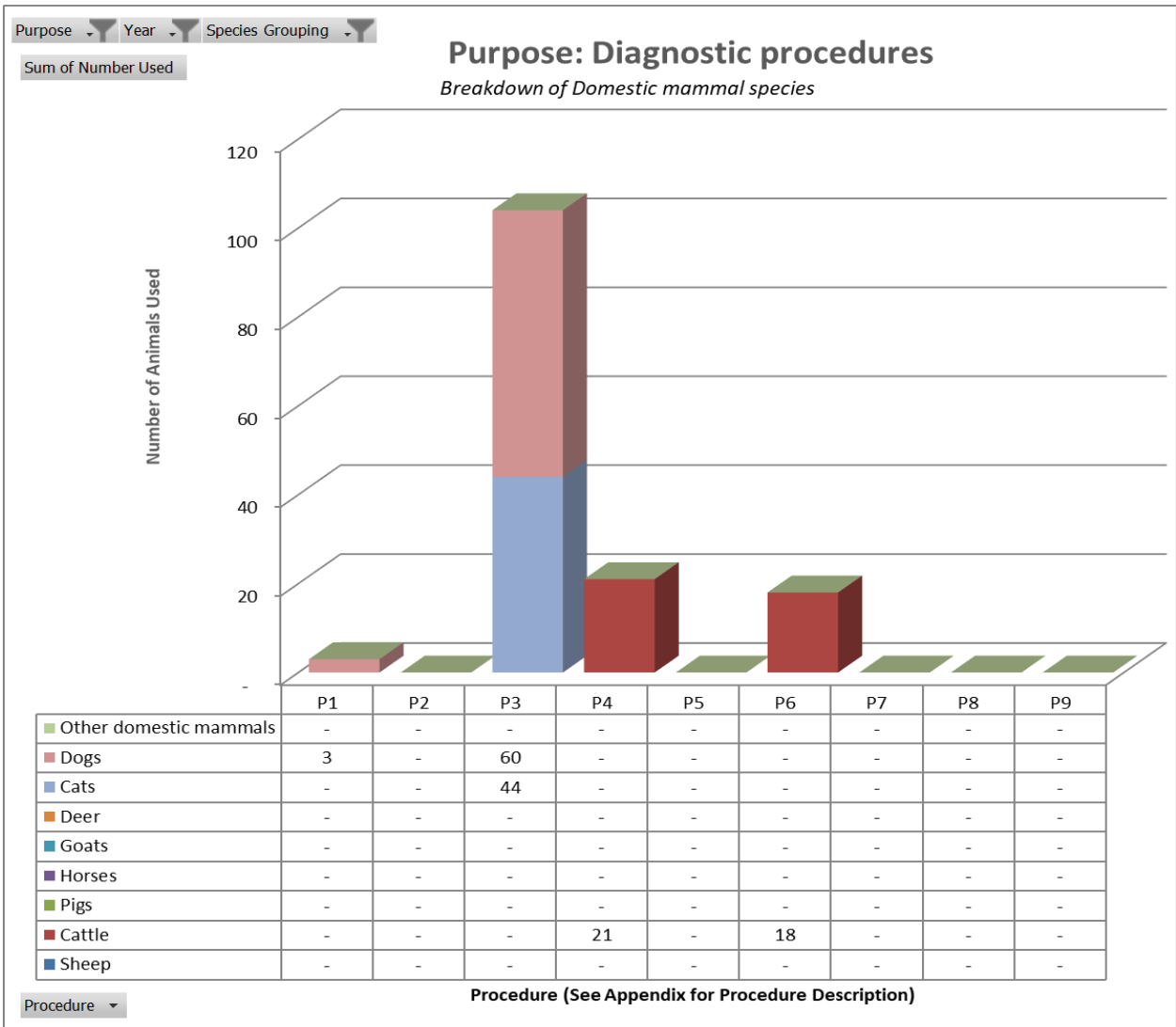


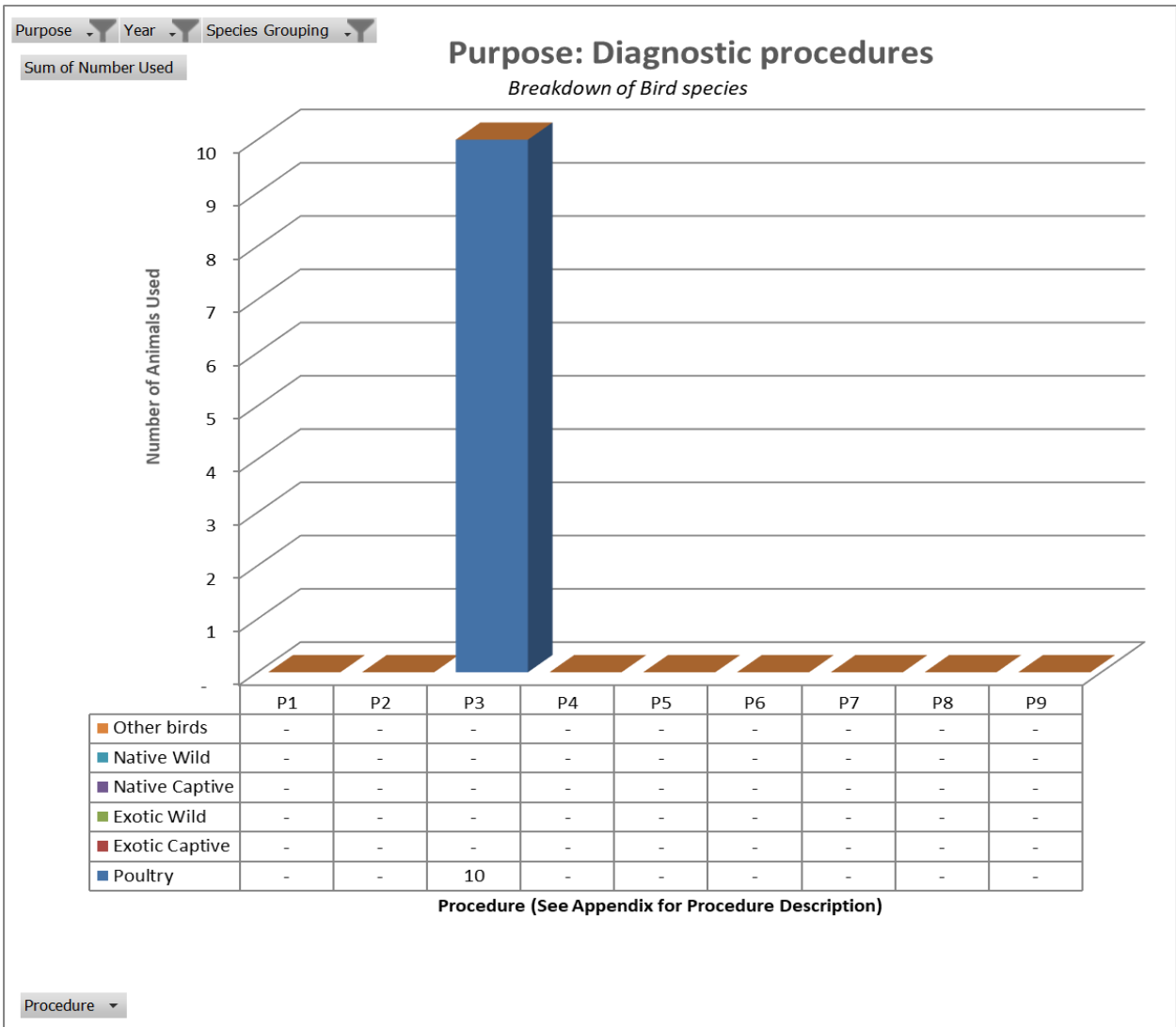
3.9 Diagnostic Procedures



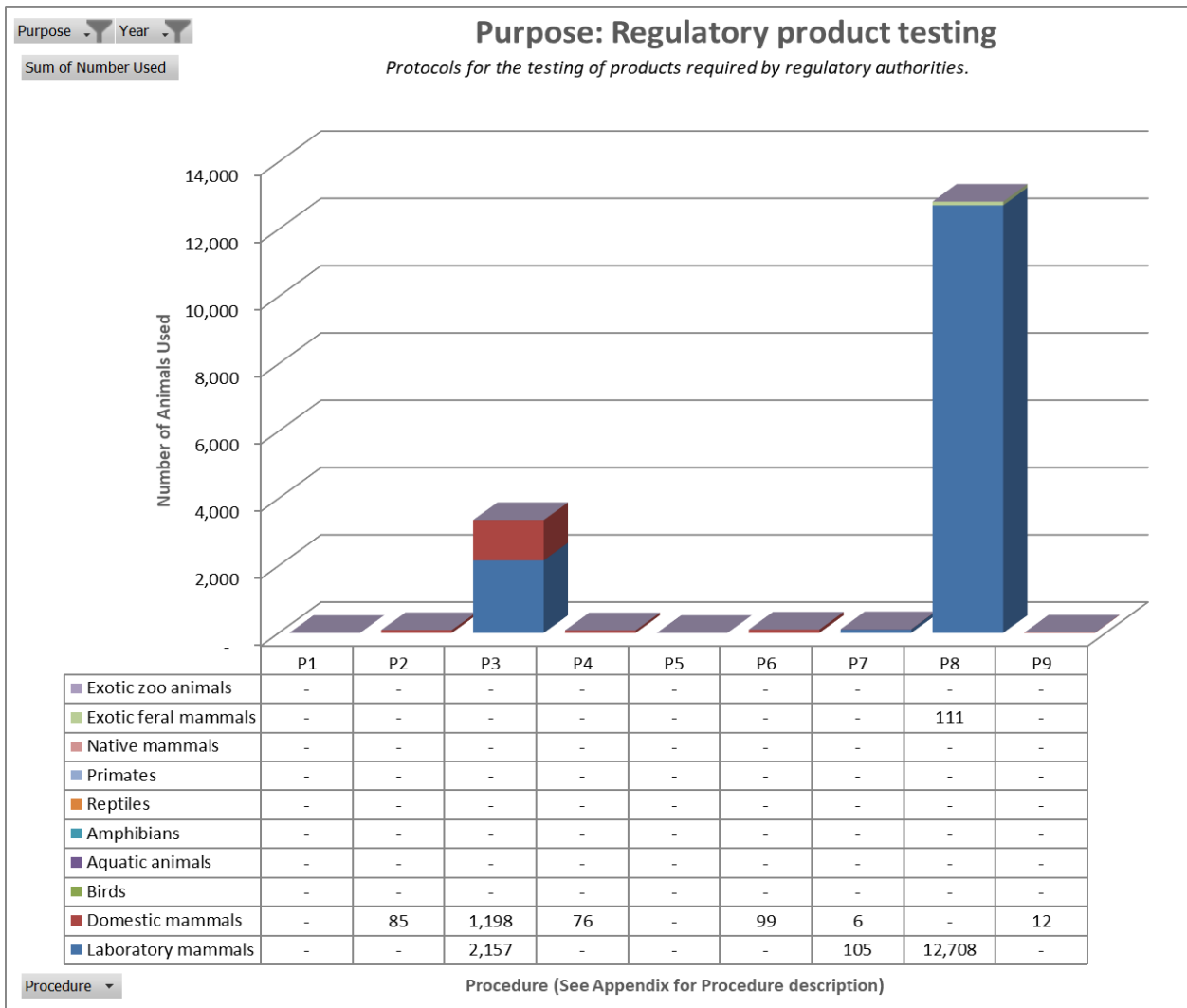
3.9.1 Species Charts for Diagnostic Procedures



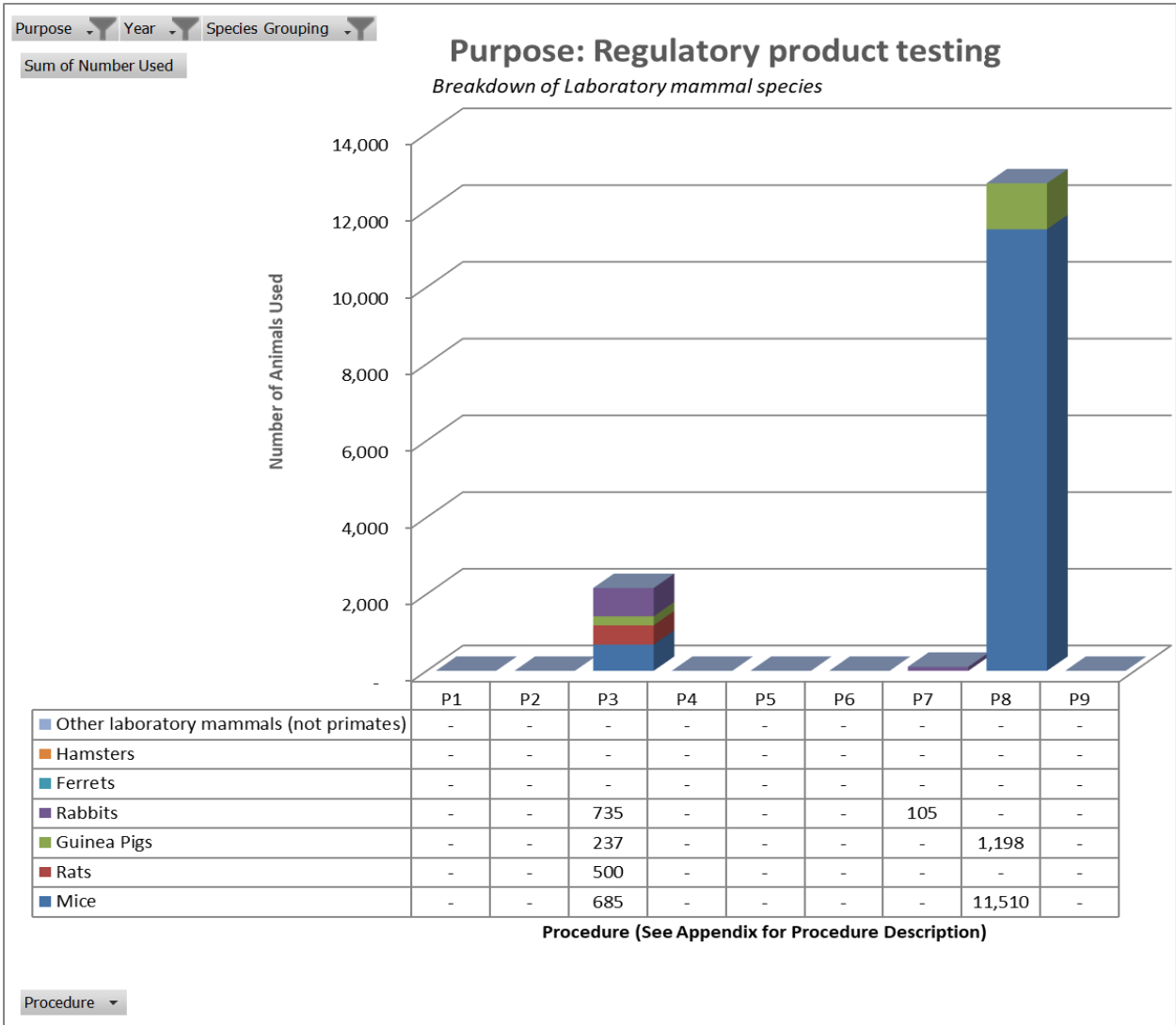


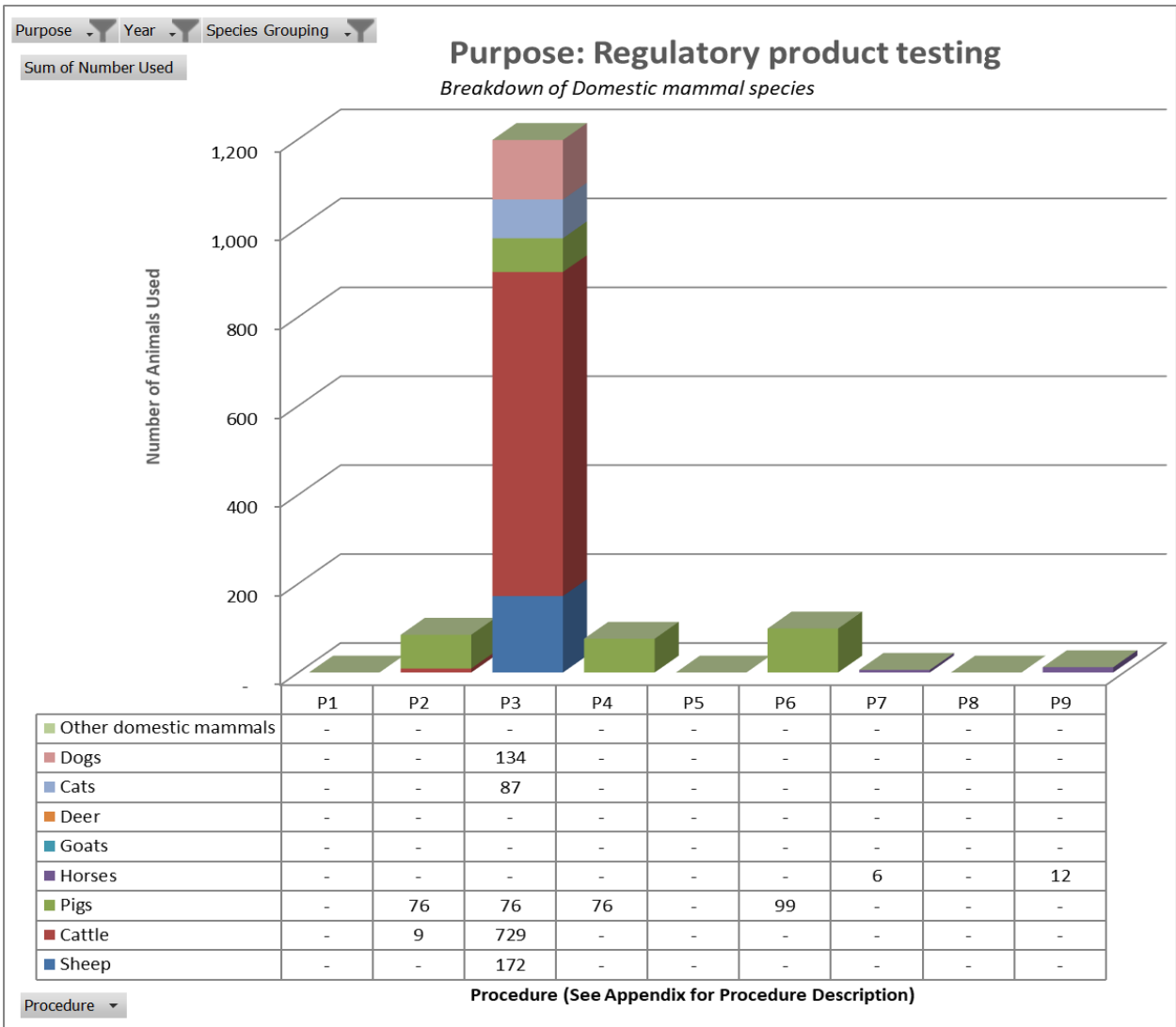


3.10 Regulatory Product Testing



3.10.1 Species Charts for Regulatory Product Testing





4. 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 2017.

Species	Number used	Number died	Number euthanased	Procedure	Justification	Alternatives
Feral Goats	Unknown	23		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 <i>Capra hircus</i> listed as a key threatening process under the Commonwealth <i>Environment Protection and Biodiversity Conservation Act 1999</i> . Additionally, competition and habitat degradation by feral goats and herbivory and environmental degradation caused by feral deer are listed as Key Threatening Processes in New South Wales under Schedule 4 of the <i>Biodiversity Conservation Act 2016</i> . 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.	The purpose of this research is to devise a humane method for killing free-living feral species. There are no alternatives to lethality testing.
Guinea	1,171	242		Vaccinated animals	Regulatory testing	This test is based

pigs				are challenged with test organism in order to demonstrate protection and hence vaccine efficacy.	required to demonstrate efficacy (potency) of vaccines prior to release. Assessment of in-process or development material to determine suitability for further manufacture.	upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop <i>in vitro</i> assays which may be used to replace exiting <i>in vivo</i> assays subject to regulatory approval of these replacement assays.
Mice	8,353	1,774	381	Serum neutralisation test in mice: Susceptible animals are challenged with test toxin / antibody dilutions to determine antibody titre.	Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Testing of stability batches and new product formulations.	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 <i>in vitro</i> assays which may be used to replace exiting <i>in vivo</i> assays subject to regulatory approval of these replacement assays.
Mice	2,197	863	111	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 <i>in vitro</i> assays which may be used to replace exiting <i>in vivo</i> assays subject to regulatory approval of these

						replacement assays.
Mice	960	299	12	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 <i>in vitro</i> assays which may be used to replace exiting <i>in vivo</i> assays subject to regulatory approval of these replacement assays.

5. 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 reported by accredited animal research establishments for the 2017 reporting year. They deal with '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).

Replacement, Reduction and Refinement

- Power analysis has been used to detect minimal difference treatments and the number of animals required to detect a required response.
- The Committee assess each project to ensure the project keeps the number of animals used to a minimum necessary but not too few which may result in insignificant or inconclusive results. Wherever possible, applications should be endorsed by a statistician/biometrician, or reference to an appropriate statistical text must be provided.
- Encouragement and use of animal tissue and tissue sharing.
- In-vitro studies are used, where applicable.
- Scrutiny of the numbers of animals requested in applications to the Committee.
- Use of pilot studies to refine techniques and reduce animal numbers.
- Judicious use of control groups across similar studies.
- Use of remote video camera monitoring in all large animal recovery rooms to supplement physical monitoring and increase the frequency of monitoring that occurs out-of-hours and on weekends.
- Online animal ethics theory modules and the practical training module for work with mice, rats and rabbits.
- Continued emphasis on environmental enrichment.

- 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.
- 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 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.
- 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.
- Reduction by using the same animals for more than one project - by using the same ewes to produce lambs for two projects.
- A modification was submitted for a project to use sensors on animals. The researcher has taken the opportunity to use animals available in the project rather than setting up a new project for collecting sensor data.
- A project required a horse to be euthanased for a dissection workshop, the animal that was euthanised for the project had existing health problems and was destined to be euthanased anyway. By using the horse with health problems saved a healthy animal.
- 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.
- Sourcing and utilising data sets from previous field studies conducted outside of the establishment (under approved permits) has greatly reduced the need to further impact upon native birds.
- The establishment has formally implemented a 3Rs award to recognise the exceptional application of the 3Rs by a staff member or student involved in animal based research.
- 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 provide 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.
- 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 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.
- The use of cameras and audio equipment (acoustic and ultrasonic) where permitted by regulators, reduces the need to trap animals. 14 of the 17 approved projects used the remote recording devices, along with other survey techniques, including trapping.
- An improvement in modelling techniques has reduced the need for some types of surveys, and careful survey design optimises the number of trapping events/nights.
- The 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.
- Encouragement of researchers to undertake literature and systemic 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.
- Research using organisms with no or limited sentience such as zebra fish embryos instead of mammals.

- 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 analysis prior to continuing to a main study.
- An 'Animal Tissue Sharing Program' is available to all researchers where animals have been humanely killed.
- The Animal Management and Governance Committee enforce institution wide policies for animals used for research and teaching purposes including compliance, training and post approval monitoring.
- Researchers are encouraged to consult the 'Animal Ethics Review Support Group' for assistance in writing an AEC application, which consists of the Animal Welfare Officer, Animal Facility Managers and Veterinarian.
- Conditions of approval are applied to projects to include the presence of either the Animal Welfare Officer or Animal Facility Managers to oversee new or high impact scientific procedures conducted 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.
- Training for investigators on necropsy procedures and reporting of Unexpected Adverse Events in the field.
- Ongoing upskilling of Animal Services staff and full time veterinarian access available to ensure care of animals is paramount.
- Regular seminars held for all new and existing staff and students on Animal Ethics and Welfare.
- 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 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
- Experimental results used for computational modelling
- Transfer of animals (undesired genotype/sex) from one project to another as approved by ACEC
- Re-use of animals among multiple projects when ethically justified and as approved by ACEC
- Teaching projects utilising computer practicals to reduce the number of animals used
- 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
- 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
- The Committee continues to maintain a Biological Non-Human Tissue Database through which researchers are able 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.
- 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 Biological Non-Human 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 euthanised regardless).
- Sharing: Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.
- The Committee continues to encourage researchers to undertake a pilot study if the impact of the proposed study interventions on animal health and wellbeing 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.
- Rehoming of fish to private tanks when no longer suitable for experimental purposes.
- Statistical analysis to ensure appropriate numbers of animals are used (not too many, not too few).
- Finalised development of a compact carbon dioxide delivery unit for euthanasia of small mammals in the field to replace the use of mechanical methods which require training and skill to perform humanely.
- Continued use of remote controlled infrared digital cameras and acoustic recording devices instead of, or in addition to, trapping to detect species presence or absence.
- Trials of unmanned aerial vehicles (drones) for aerial surveys and population counts of waterbirds and rockwallabies.
- The establishment is still committed to the development of in-vitro testing to reduce the use of animals for our vaccine testing. The plan has progressed significantly since the AEC was last presented with an in-vitro update 18 months prior. Most of the required materials for the test development have been sourced and good progress has been made on the test development of the finished product potency ELISAs for *C. Chauvoei*, *C. Septicum* and *C. Perfringens* D. It is pleasing to see that validation of these tests will commence in the next 12-24 months and that the priority is being placed on replacing the *C. Chauvoei* challenge test in guinea pigs.
- The establishment continued to re-home excess guinea pigs during 2017 which meets the criteria of reduction. 25 guinea pigs were given to the non-profit organization Research Animal Rehoming Service in 2017. All 25 were in excess of 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.
- The AEC created 3 new inspection checklists based on the ARRPs housing guidelines for mice, guinea pigs and rabbits. A subset of the AEC performed facility inspections using these checklists and these were subsequently presented to the wider committee. Several recommendations were made as a result of the inspection and the institution worked proactively to implement them. One such improvement was replacing the bedding of mice that are on test from sawdust to wood shavings. Sawdust was not recommended by the AEC as it was very fine substrate and did not provide adequate opportunity to burrow. The AEC is pleased with this improvement for test mice and it is more consistent with the bedding used for breeding mice and weaners.
- The AEC noticed a trend of birth defects occurring in the guinea pig breeding unit. Each Veterinary Report presented to the AEC recorded several instances of pure white guinea pigs being born with maleruption of teeth. These pups are unable to suckle and are euthanased. The issue was researched by a subset of the AEC and the likely cause was determined as a genetic defect occurring from the mating of guinea pigs with roan or dalmatian coats (which results in a 25% chance of "white lethals" being born). The AEC recommended that any guinea pig with a Roan or Dalmatian coat is not mated with another guinea pig with a Roan or Dalmatian coat. The institution agreed to remove all roan or dalmatian males from the breeding colony over a period of approximately 12 months. This action has resulted in a significant reduction of the deformed guinea pigs being born.
- The AEC asked the institution to investigate the euthanasia technique for guinea pigs to determine if the IP route of administration could be refined in order to reduce the discomfort and quicken time to death. A laboratory trial was conducted, diluting Lethobarb and monitoring the pH of the formulation. No major reduction in pH was found. This investigation will be continued in 2018.
- The AEC discussed the issue of guinea pigs suffering from Moxidectin toxicity when being vaccinated with the Eweguard vaccine (6in1 plus Moxidectin). Various options were explored including reducing the vaccine dose or using a different international challenge model, however

these were deemed as inappropriate due to the large amount of validation that would be required (and subsequently more animals used). An improvement was identified, however, by the Industrial Operations department whereby they will use a different vaccine formulation whenever possible for their vaccine prototypes. Historically Eweguard was used as it was the most complex formulation, but it has been agreed to use the plain 6in1 vaccine without Moxidectin if appropriate for the prototype test and thus reduce the number of samples containing Moxidectin used in guinea pigs.

- In general, the nature of the establishment's scientific work precludes replacement of animal use. Projects are focused on animal health, nutrition, behaviour/welfare, reproduction and genetics. Outcomes such as animal behaviour, stress physiology, growth rate, incidence of mortality and morbidity and reproductive performance are all common measures in the experiments which necessitate the use of live animals. Furthermore, a number of our experiments investigate alternative housing and management systems for animals, which also require the use of live animals to assess the impacts of the housing or management system on their welfare and performance.
- The AEC continue to place particular focus on this area. Extensive statistical analysis/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 now using advanced statistical software packages that utilise more precise sample power calculations to determine appropriate sample size for experiments. In a number of experiments, we have been able to use fewer sows in group housing experiments by utilising groups of 15 sows rather than usual larger groups of 40-80 sows. Experimental design allows for individual pen of sows to be an experimental unit.
- We are conscious of continued improvement in the techniques of handling and treating animals on trial. Most experiments conducted do not involve any procedure more invasive than blood sampling. Other minimally invasive procedures conducted include snaring/restraining for the purpose of blood sampling and weighing animals. A small number of veterinary/health immune challenge studies were approved in 2017 (conducted in NSW); the AEC was confident that the number of animals to be compromised was kept as low as possible, and the potential welfare benefit to the herd and herds across the Australian pig industry was very high. In the reporting period the following modifications were made to improve handling animal and reduce impact on welfare: -Environmental enrichment is an important component of housing to provide an opportunity for enhanced welfare. Our AEC application template now includes a section "Are animals provided with a solid floored area with bedding material for rooting and resting?". Whilst it is not a requirement in the Model Code of Practice for the Welfare of Animals-Pigs to provide enrichment, where the accommodation allows, environmental enrichment is being used in research projects-ranging from chew toys to daily provision of straw, lucerne, enrichment blocks or silage. The establishment has committed to 100% of their group housed sows having enrichment for part of their breeding cycle from 2020. This is a significant advancement in animal welfare and will provide opportunity for sows to experience enhanced welfare.
- Prior to administration of the challenge, a sedative (Stresnil) was given to pigs to ensure they were calm during the procedure. This is an improvement on previous challenge.
- The AEC continues to encourage researchers to thoroughly consider what they are planning to do to ensure that all in vitro methods have been examined and utilised if suitable to produce the desired outcome and that they only resort to the use of animals if all other options have been exhausted. Examples include: Researchers utilise small animals humanely killed for other reasons (retired breeders, wrong genotype, and aged stock] wherever possible. Such animals are then used of a range of purposes including surgical procedures training (portal vein and renal artery cannulation), anatomical dissection and tissue collection for in vitro cell studies.
- Body parts (limbs/joints) and organs (kidneys) derived from large animals (sheep, pigs) humanely killed from other studies are also used for anatomical and surgical training purposes.
- To minimise the number of animals required but at the same time, ensure sufficient numbers of animals are included in study to obtain statistically valid results, consultation with biostatistician is recommended to ensure the study will deliver appropriate statistically significant data relating to the study objectives.
- Reduction applies to breeding colony management where researchers are advised to breed only sufficient numbers to supply experimental protocol needs and to provide future breeders to maintain the colony.

- Close monitoring of animals post procedure is crucial to ensure any evidence of discomfort and pain is recognised and relieved appropriately. New and/or inexperienced staff must be supervised by senior experienced staff.
- Providing environment enrichment appropriate for each species held within the facility helps to meet physical and psychological needs and hopefully adds to the overall wellbeing of the animals used for biomedical research.
- This study required cardiac bleeding from a sample of recently euthanised chickens. The training for his procedure was carried out on chickens enrolled in the study rather than acquiring extra chickens solely for the training sessions.
- Twenty chickens that were from a previous study were used for training purposes. We did not need to purchase an extra 20 for training.
- This study involved trialling different types of resins for an intra-ruminal capsule. Rather than using one capsule per cow, 5 capsules were placed in each fistulated cow dramatically reducing the number of animals required for the study
- These two studies involve breeding brown ticks and fleas for studies. A more efficient technique of harvesting fleas and ticks was developed which reduced the use of dogs by more than 50%.
- This was a safety study specifically targeting adverse events, each dog would normally be individually housed and would not be able to go out and exercise unless exercising as an individual dog so that all adverse events can be attributed. This was a 3.5 month study and it would have been unethical to have the dogs housed individually indoors with no exercise for the entire study. To overcome this we used indoor cameras so the dogs could be housed in compatible pairs and we put up GoPros in the outdoor exercise yards so the dogs could run in groups of up to 4 dogs. We were able to monitor the group housed dogs and attribute any adverse events to individual dogs.
- 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.
- Power analyses are frequently submitted as part of the application (sometimes at the request of the Animal Care and Ethics Committee) which demonstrate how researchers and teachers calculate the most suitable numbers of animals required to give valid data.
- Wherever possible the number of animals is reduced in a study, while maintaining statistical significance. The use of excessive animals is avoided. Pilot studies are conducted with a small number of animals to test unknown hypotheses, avoiding the use of large numbers of animals when outcomes are unknown or not guaranteed.
- Entomology conducts in-vitro assays, replacing the use of animals in early product development.
- 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 reflect what would be performed anyway, e.g. only necessary blood samples and x-rays are taken.
- Large photo flip cards have been developed and used in practical classes instead of live animals to teach students how to identify freshwater fish species.
- Less animals were collected than originally planned, so replicate numbers for each treatment were reduced.
- Rotating the field activities among student groups during field trips that helps reduce not only the number of animals caught, but the impact on species communities.
- Using Go Pro Cameras with extension poles or drones to observe and minimise the disturbance on marine species.
- Using bird netting to ensure fish are kept safely in fish tanks and aquaria.
- Using of custom-designed fish aquaria out in the field to reduce handling whilst identifying species.
- Teaching about Animal Care and Ethics as part of the curriculum in some Wildlife, Fisheries and Aquaculture Undergraduate Units.
- Developing fishing gear protocols to increase likelihood of target species and reducing by-catch.

- The establishment makes available samples 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.
- To reduce adverse impacts on animals, the AEC reviews each procedure carefully and may require that more information is given (e.g. precautions taken by the PI to prevent overheating of trapped animals for a project) or techniques are justified (e.g. method of euthanasia for a project) or alternative methods are proposed and adopted by the PI (e.g. emergency procedure for injured animals for a project).
- Dog Abdominal Surrogate for Instructional Exercise (DASIE) are used for suture training
- 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.
- 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
- In-vitro cell cultures were employed as a predictor of cellular responses, and used wherever possible in place of in vivo work.
- Conducted research on primary cell culture from human alveolar macrophages, isolated from clinical studies of asthma.
- Human cancer cell lines and human tissue samples sourced from the Cancer Biobank (approval granted) reduced the number of animals required.
- Generated preliminary data using cell lines to examine the role of an intracellular pathway in granulosa cell function and examined human samples.
- Used cell culture experiments to minimise the number of animal experiments required on a protocol.
- Conducted experiments in parallel to minimise the number of control mice required.
- Conducted preliminary studies in vitro. Literature reviews were utilised to uncover optimal dosages prior to administration.
- Selected specific targets from human cohorts to remove the need for animals in early stages of the experiment. Where specific cellular pathways were known, each of the selected interventions was administered in vitro to replace further animal studies.
- Undertook initial cytotoxicity screening in cell lines before moving to coupling of peptides to xenobiotic.
- Substituted the use of animals with human sperm donations.
- Replaced mice with embryonated chicken eggs.
- Adopted a "within-subjects" statistical design for some experiments, meaning each animal received a dose of the experimental drug, was tested, then received a dose of the control drug and was tested again. This allowed reduction in the number of animals required.
- Accessed a licensed captive collection of an endangered snake species for venom milking, rather than removing from the wild.
- After two types of administration proved ineffective at treating pancreatic cancer at the given dose, ACEC approval was granted to use the animals to investigate intratumoural treatment. This saved using another 36 mice for the study.
- Moving to aviary experiments meant animals were no longer required for cage studies, reducing the total number of animals required for the project.
- Non-obese control groups were shared with other experiments, reducing the required number of mice.
- Animals required for the project reduced due to the small amount of tumour cells required for the phage panning.
- Only compounds and dosages that showed efficacy in vitro were subsequently tested in vivo, thus reducing the number of animals used.
- Multiple tissue samples were taken from every mouse used in studies, to reduce mouse numbers.
- Running experiments simultaneously and sharing controls across experiments cut down on mouse numbers. Some animals also acted as their own controls, reducing the number of animals used.

- In order to reduce the total number of animals used, females were allowed to carry litters to parturition, after which they continued to participate in the fertility trials rather than being culled after each mating trial.
- Used many less female mice than expected (-450), as the project is male reproduction orientated, and less sperm-egg binding experiments were required than predicted.
- The number of PP2A activators was narrowed based on preliminary results. This reduced the number of experimental groups and therefore the total number of animals.
- Statistical methods to account for multiple pups from one dam were adopted to limit the need for a larger number of pregnant dams.
- Proteins extracted from a single tissue were used for 5-20 experiments.
- Utilised a proven rabbit model of aortic valve stenosis, minimising the amount of animals required.
- As the project proceeded and the results of differentiation were examined, less mice were required.
- In consultation with a statistician, the project was designed to enable the use of multiple neonates from within litters. This reduced the total number of pregnant dams required.
- Used the same animals to examine whether multiple periods of egg-sperm production can occur during a single breeding season, and for experiments to study multiple egg-sperm production.
- Small pilot studies were used to ensure there was minimal impact on animal welfare from interventions, and that the projects remain viable.
- Performed multiple analyses from the one pool of generated samples.
- Tested the peptide-gadolinium conjugate in vitro using testes and ovaries from mice that had been used as control mice for a different study.
- Reduced the number of mice by half when two tumours (one tumour on each flank) were grown on each mouse.
- Used human spermatozoa instead of animals as much as possible, saving a considerable amount of animals compared with the original forecast.
- Minimised the number of animals required by studying pregnant glands from the same animals from which embryos are studied.
- Improvements in phage display technologies reduced numbers of oocytes necessary for analysis, so less mice were required to undertake library screening.
- Planning and immediate analysis of initial experiments demonstrated that male offspring testes and sperm production were not affected in this msi deficient mouse. Thus, the number of animals used in this study was significantly reduced.
- Introduced in vivo xenogen imaging to obtain more information about tumour growth and follow treatment strategies.
- Practised and refined techniques on culled animals before transitioning to live animals.
- Advances in surgical techniques led to shortened anaesthesia and reduced surgical trauma.
- Allowed animals to recover undisturbed for the first 24 hours post-surgery. After this animals were moved to a holding room where they were left undisturbed (no handling unless necessary) for the next seven days as a means of reducing the risk of cardiac rupture. The introduction of these steps resulted in a significant decrease in the incidence of cardiac ruptures post MI surgery, and thus post-operative mortality rates.
- Incorporated trials on culled animals into blood collection work where inexperienced personnel or novel species were involved.
- Combined multiple techniques to minimise mouse handling, for example weighing each mouse, then administering a compound. This reduced overall handling, stress and risk.
- Monitoring and frequency of weighing were changed to minimise risk, as animals are particularly sensitive to stress caused by handling and noise in the first week post procedure.
- Substituted the type of needle used to reduce risk of damage to the brain parenchyma.
- Replacement of medetomidine/ketamine anaesthesia with isoflurane.
- Changed from injectable intraperitoneal anaesthesia to isoflurane.
- Replaced intravitreal injections with eye drops.
- Administered drugs to mice in the morning so any adverse effects could be immediately acted upon.
- Halted the collection of blood from the tail vein or saphenous vein of the mouse.
- Addition of visual environmental enrichment, to improve the welfare of the animals and shorten the myopia induction time (less time with lens wear).

- Substituted mice for a line that successfully grows tumor xenografts without the need to implant beta-estradiol pellets. This benefited the welfare of the mice and simplified the experiment.
- New method to assess tissue strength in-vitro was developed. Tissue can be collected earlier with less in-vivo measures.
- Collecting saliva via the cheek pouch as a non-invasive alternative to blood collection.
- Ran a dose-dependency study first, to identify the optimal dose of progesterone for delaying the onset of labour without causing any adverse effects (zero maternal deaths).
- Choice of diet and administration of the compound in drinking water reduced the invasiveness of treatment (less handling, no need for anaesthesia, less invasive than gavage or intranasal administration).
- Trained stallions to use a collection phantom so a mare is not required for semen collections.
- Variations to procedures enabled the reduction of risk of cardiac rupture post-operatively from approximately 16% to 12%, falling below the expected 20%.
- Minimum numbers of animals to obtain statistically valid data have been used for projects.
- Animals are reused where possible. Tissues are harvested from surplus breeding stock that arises as a result of maintaining mouse colonies
- For some projects, only post mortem tissue from already culled animals is used.
- The Committee maintains a website which provides detailed information and links to external websites and databases that promote alternatives to the use of animals in research and/or teaching. The 3Rs are also discussed during Animal Ethics workshops and mentioned in detail in the Animal Ethics Online Portal (which includes a mandatory test for all staff and students) and the Standard Operating Procedures.
- In-vitro simulation of equine metabolism using horse liver, horse liver microsomes and the equine S9 fraction.
- Single-dose administration studies where possible.
- Accommodation of research horses in a large paddock on a professional horse spelling/pre-training farm.
- Spontaneous collection of naturally voided urine for the purposes of drug analyses.
- Where possible, tissues from an abattoir are used.
- The number of animals used for each blood collection is the minimum required to validate the analytical testing methods for the analytes of interest. All samples are frozen and used as necessary; fresh samples are only taken if absolutely required.
- The minimum number of animals (12) required to achieve suitable power for statistical analysis was determined prior to the conduct of the study; the design of this study was replicated for another study and therefore 12 animals were used. The crossover study design allowed for a smaller number of required animals compared to other study designs, such as a parallel study.
- The study plan states that at least 200 maiden heifers will be used in the study. At the current site 183 heifers were enrolled as this was the number of animals presented and considered suitable for inclusion on treatment day. As more sites are enrolled and data analysis is undertaken, statistically significant and clinically relevant results may be achieved therefore reducing the total amount of animals required for the study.
- 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 restrained appropriately and were handled and bled by trained personnel. This minimised the pain or distress experienced by the animals during blood collection.
- Pain and distress to the animals was reduced by maintaining them in a familiar environment, using appropriate facilities for restraint and utilising study personnel trained and experienced in cattle handling. The animals used in this study were the same as those used in the previous study and were therefore better conditioned to handling and restraint than if novel animals had been used.
- During the conduct of this study thus far, animals were on-site in their familiar environment. They were handled by personnel with cattle handling experience, and treated by veterinary staff skilled and efficient in the procedures. Prior to treatment heifers were kept together in the holding yards, then moved and treated together in groups of 5 to 6 animals. Upon release they were free to roam and graze in their regular paddock. These methods reduced any pain and distress which may have been experienced by the animals.

- 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. Doses were administered in a manner where the lowest doses were administered first and subsequent higher doses were only administered if the sedation period did not exceed 60 minutes and the Study Director felt it was safe to do so. Mice were placed in a warmed cage throughout the anaesthesia observation period to mitigate any reductions in core temperature
- We replaced the need to handle domestic animals by collecting only voided faecal samples. Therefore no domestic animals were handled or directly involved in this study.
- Dogs are not handled in any way, rather photos are taken of their presence and activity and scats collected. By doing these, the use of animals (in terms of handling, procedures or effects on behaviour) has been entirely eliminated. This represents a non-invasive approach to studying the distribution and density of wild dogs.
- We have conducted extensive in vitro experiments prior to the use of animal models to examine the efficacy of our novel anti-cancer agents. These include MTT studies with human cancer and normal cells to assess the cytotoxicity of these agents and determine their selectivity. In addition, we have also performed extensive studies to examine the molecular effects of these using human cancer cells through techniques such as western blotting and RT-PCR.
- Research has been done into yielding microparticles from red blood cells activated in vitro instead of using Plasmodium-infected mice as the source, with success providing future prospects of replacement
- The project has used existing stored blood samples, to avoid further sampling of animals.
- In order to largely replace the number of animals, all of our studies are based on work undertaken in a less sentient species *Drosophila melanogaster*. This has in most cases proven a very successful strategy and as a real system it is often more reliable than cell culture.
- We used fundus fluorescein angiography to non-invasively monitor retinal vascular changes. This technique has significantly reduced the number of mice required for this project. The procedures we employed conform to current best practice.
- We have started to video record some relevant pig husbandry procedures or behaviours that are relevant for students to observe. For example, semen collection and suckling behaviour, respectively. Due to the increase in class numbers and class sizes, it is not always practical to demonstrate collection of semen. Similarly, if classes coincide at times when there are no suckling pigs available, then we can use video clips to demonstrate suckling behaviour and milk let down.
- We constantly attempt to reduce the number of animals used to the absolute minimum. We have changed the flow cytometer we use. Previously our analysis was performed on a flow cytometer with a limited number of fluorescent detectors. We are now analysing with a 10-colour flow cytometer which allows us to more immediately answer our questions. This results in a lower number of animals needing to be used to obtain more informative results.
- The collection of blood from mice and the subsequent isolation of platelets have undergone extensive refinement over 10 years to yield the current maximum yield of 60% total blood platelets, thereby reducing the number of mice required.
- We have established a large archive of measures of minerals in the yolks of eggs and the retained yolk sacs of chicks giving us a strong database to estimate standard deviation in expected levels in eggs from meat chicken breeder hens from the field. This baseline information will allow researchers worldwide to select future experimental numbers for this type of study to minimise requirements of animal use. Once fully published, this information will be of great value to the research community and to chicken producers worldwide.
- We have been refining our sample preparation methods for mass spectrometry, in particular phospho-proteomics to reduce animal usage. Due to the advances in technology as well as our own efforts, we have been able to optimise the system so that a single animal is able to be utilised instead of needing 2 or 3 animals for the same quality of data.
- In terms of GPS collaring, 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 1/3 of the weight of most comparable collars on the market.
- I replaced trapping bats with simply recording them to ensure the least invasive approach.
- Wherever possible we utilise synthetic replicas which have the same anatomical features as real birds to allow the students to practice their handling techniques prior to handling live birds. Once the students appear to have grasped these principles, we then allow them to demonstrate their

ability on live birds. This reduces the time that the birds are handled and they only ever handle the live birds with one on one supervision.

- We have also refined and perfected our portal cannulation surgery, resulting in better post surgery recovering and a higher cannula patency rate, meaning improved animal wellbeing and fewer animals excluded from studies.
- We introduced the use of the Breed'n Betsy Artificial Cow to the course which is used to introduce pregnancy diagnosis procedures in cattle to students as well as artificial insemination techniques. This allows students to learn about safety of rectal palpation and AI in cattle prior to conducting these procedures in a live animal.
- Incompetent handling of mice may introduce preventable or unintended stressors that in turn disturb the physical and psychological well-being of the animal. This protocol aims to improve competency and consistency of common mouse procedures 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.
- Protocol Training of Personnel in Animal Procedures was minimally used during 2017. Rather, animals that had completed their purpose in other research protocols were subsequently used in handling or dissection training where possible
- The ACEC required that tumour mapping studies were completed and assessed for all new tumour models prior to proceeding with further experiments using that model. This is to ensure that only the minimal numbers of animals were being requested and used for studies involving tumoured animals. This is particularly relevant to the establishment as studies involving novel radiotracers require more deliberate and careful timing to ensure the molecules for testing are available (due to half-life and isotope availability limitations) at the right time during tumour development.
- Recommendations made by the Category A member concerning the use of analgesics pre and post-operative procedures were implemented. These recommendations were specific to the type of experiment, ensuring that the right analgesic was used for the type of studies to not affect the results and that the dosing regime was effective in reducing adverse impacts on animal welfare. The biostatistician and advisor continued to support the ACEC and researchers throughout 2017. She provided valuable advice and challenged investigators to statistically confirm the number of animals requested and to validate statistical data obtained from animal experimentation.
- 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
- The establishment is conducting two separate projects which are testing 2 different products with different modes of action and hypotheses on the same cows to reduce the total number of cattle required. Milk, bodyweight, health, and disease data are being used for both projects.
- The establishment selected a farm for a project that has a walk over weigh scale to reduce the handling of animals.
- The establishment has installed electronic weigh scales on each of the 3 farm sites for a project which refines the way animal handled. There will now be minimal handling of sheep during weighing and the time animals will be handled or kept in a yard will be reduced as there will be no need for manual identification of sheep.
- Trained personnel only administered treatments on commercial farms to reduce adverse impacts on animals.
- The establishment has installed mineral dispensers on farms involved in a project that allow treatment pellets to be automatically dispensed to individual cows using the farm's existing herd management software. This removes the need for any additional contact with the cattle when the treatment pellets are being dispensed.
- The establishment has refined herd testing procedures from two milk collections down to one.
- A protocol investigating the effects of a protein in the prevention of diabetes is using an islet cell line in vitro to perform a number of mechanistic studies to compliment the in vivo findings. The minimum number of animals is also being used, together with protocols that are considered best practice.
- A protocol for the training of researchers within the animal facility is ensuring that during the initial stages of training, non-animal alternatives are used. The Chief Investigator is developing a series of training videos for use by trainees before they proceed on to live animals, to reduce the numbers of animals required to achieve competency. The number of animals have been minimised by implementing workshops with groups of people, only using animals sourced as

excess stock from existing protocols that are no longer required and would have been culled, and implementing training and competency assessment to standardise techniques using best practice.

- A protocol seeking to characterise a new class of anticancer drugs is basing their in vivo study on a significant body of data gathered using in vitro cellular assays which support the therapeutic potential of these compounds. The researchers are using the minimum number of animals possible.
- A protocol investigating the responses of marine and estuarine fish to climate change on the east Australian coast is using minimal numbers of key species.
- A protocol investigating the mechanism of action of proteins in the treatment of diabetes has characterised the biological activity of the proteins in vitro with macrophage cell lines. The assays were all refined and the minimum numbers of cells required to achieve a significant outcome is known. Therefore, the researchers are able to use the smallest number of animals possible to achieve sufficient power in their statistical analysis.
- A mouse breeding protocol is keeping breeding to the minimum numbers required to maintain the three strains involved in experimental work for five separate protocols.
- A protocol investigating the effects of different molecules in glaucoma is basing numbers of previous studies known statistical variance to use the smallest sample size to achieve the study aims. It is also using state of the art retinal measurement equipment to ensure the highest accuracy and statistical power for the analysis.
- A protocol seeking to establish a link between Alzheimer's disease (AD) and air-pollutant magnetic magnetite particles is using minimum numbers based on sample size calculation and previous studies in the literature, as well as using both males and females to reduce wastage of animals during breeding.
- A protocol investigating whether sleep fragmentation (SF), a highly prevalent condition and a hallmark of sleep apnoea, has any impact on both development and worsening of allergic asthma is using power calculations taking from previous studies to use the minimum number of animals to allow the identification of statistically significant changes in the model being used. The protocol also uses a specialised chamber which is well-validated in sleep research.
- A protocol investigating the impact of eCig vaping in obese subjects on their lung function is using the minimum number of animals to generate significant results in tissue analysis using multiple functional assessments.
- A protocol investigating the behaviour of key inshore fish species is using minimal numbers of key species as well as using more observation.
- A protocol aiming to understand how behavioural traits have changed during the invasion of the invasive Asian house gecko in Australia is only using species that are biologically suitable for the study, and is ensuring the number of animals is statistically and scientifically valid.
- A protocol investigating how maternal effects and incubation temperatures influence offspring morphology, physiology, antipredator behaviour, and cognitive abilities of geckos is only using species that are biologically suitable for the study, and is ensuring the number of animals is statistically and scientifically valid. The researchers are also exploring the use of alternative marking methods as a means of identifying individual hatchling geckos.
- A protocol investigating the role of retinal degeneration in Alzheimer's disease is using both male and female mice that have been re-derived through IVF, as well as using state of the art retinal imaging.
- A protocol aiming to test the effect of immune modifying microparticles is using a power calculation to ensure the minimum number of mice are being used to achieve statistical significance for the tissue analysis.
- A protocol aiming to develop an accurate survey methodology for collecting remote data about large herbivores are using imagery collected from unmanned aerial vehicle (UAV) mounted cameras to minimise the impact on animals being observed in the study.
- A protocol investigating a new strategy for the treatment of severe allergic asthma using a successfully tested mouse model of severe asthma from a previous approved protocol are applying well established and routinely used mouse models. The researchers have refined the experimental procedures in six different ways to minimise suffering of the mice.
- A teaching protocol to understand and promote coral reef ecology and management and feeding and behavioural responses of coral reef fish is using in situ observations only.
- A teaching protocol on fish assemblage structure in estuarine habitats is using the minimal number of fish and releasing fish at the site of collection, and is ensuring the proper training of students prior to the fieldwork component.

- A protocol investigating the use of artificial hollows by mammals and birds is only using species that are biologically suitable for the study, using non-invasive methods (camera traps) when possible, and the minimum number of animals to ensure statistical and scientific validity. The researchers are also deploying PIT tag data loggers outside hollows. This method allows PIT tags to be read automatically, and tag numbers are stored in a memory file. This means that the researchers do not need to capture animals to fit them with expensive (and bulkier) GPS data loggers.
- A protocol that seeks to establish the mechanism by which a parasite derived peptide regulates the immune response to switch off inflammation in asthma is using refined experimental procedures to minimise the suffering of the animals and reduce the duration of the study.
- A protocol aiming to assess the behaviour and psychological rehabilitation of Asian elephants that have been transitioned from captive, working conditions to native forest habitat is only employing non-invasive methodologies such as observation, camera traps, tracking, vegetation surveys and dung collection.
- A protocol that aims to design a clinically applicable protocol for the reversal of diabetes is using refined i.p injection techniques and refined surgical procedures, as well as the minimum number of animals to obtain statistically significant results.
- A protocol on examining the ecology of seadragons' feeding, aging, reproductive behaviours and genetic diversity is using photo-identified. The molecular analysis of seadragons has been designed to sample in situ as few animals as possible while still providing the project with sufficient data to analyse the population structure and genetic diversity of the species and therefore expand on the ecological understanding of these fish. As members of family Syngnathidae are protected, the researchers are utilising the most efficient manner of sampling, which is to take a very small appendage clip. This minimally invasive process in situ without removing the animal from its habitat requires no animals be sacrificed for muscle tissue, and is quicker and faster than taking a fin clip.
- A protocol studying the outcomes of potential new therapies for conditions such as pulmonary fibrosis is using an in vitro screening program in which candidate drugs are first tested in numerous in vitro cellular assays. Only drugs which fit a particular pattern of responses are then tested for specific outcomes in animals. Oral administration of compounds is being done via drinking water rather than via stressful invasive procedures such as gavage. Animals are being conditioned to all handling and weighing procedures and the number of animal handlers is restricted to minimise stress.
- A protocol studying the outcomes of potential new therapies for conditions such as hepatic fibrosis and cirrhosis is using an in vitro screening program in which candidate drugs are first tested in numerous in vitro cellular assays. Only drugs which fit a particular response profile when tested in vitro are analysed for anti-fibrotic effects in animal models. Oral administration of compounds is being done via drinking water rather than via stressful invasive procedures such as gavage. Animals are being conditioned to all handling and weighing procedures and the number of animal handlers is restricted to minimise stress.
- Direct observation and camera recording in the field for wildlife studies in place of capture and release methods
- The use of computer simulations where practicable.
- 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 vitro cell culture experiments are used.
- In teaching practicums, a number of students will work together to utilise animal tissues.
- Sourcing of invertebrate tissue for use in class practicums.
- Where two experiments are to be performed by students, half the class will perform one experiment and the other half of the class will perform the other experiment and share the resulting data.
- A biostatistician analysis is undertaken to determine the option number of animals required to ensure statistical significance.
- Experience from previous studies or information from similar studies is utilised to ensure appropriate numbers of animals are used in experiments.
- A power analysis is conducted to determine the ideal number of animals required.
- A pilot study or proof of concept study is employed by an investigator before proceeding further.
- Where feasible, observational studies are conducted or observational studies are recorded for future use and analysis.

- Where possible, animals are sourced from other approved projects where they would otherwise not be required.
- Rather than maintain colonies of animals, animals are captured, photographed, and biological measurements are taken before they are released back into the wild. These animals are then monitored via camera surveillance.
- Researchers will use longstanding and well established procedures in their research to ensure the minimum number of animals are used.
- Infrared cameras and passive observation techniques are used to minimise the stress and impact on wildlife.
- All procedures are performed or supervised by highly trained personnel.
- All animal housing is designed and maintained to meet species-specific needs.
- Tissues are collected post mortem for analysis or storage.
- Drugs are administered via the drinking water, where appropriate.
- Designs of field based apparatus minimise the negative impact on animals in the study.
- All staff and students undertaking research involving animals must be deemed competent in animal handling and any procedures being performed.
- Remote monitoring of metabolic parameters in animals is undertaken in the field and in the laboratory.
- 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 2017 we used no live animals for laboratory based teaching activities.
- 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.
- A tissue bank has been established where unused samples can be stored for future projects by pharmacology students. Collected tissues from different disease models are also being shared across research disciplines in laboratory based projects.
- 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; modification of smaller and lighter monitoring collars for cattle; not only refine research projects but also refining livestock practice.
- Partial replacement involving the staged use of animal models, cadavers in developing animal models or in training is common and required by the AEC.
- Livestock: Use sick animals donated by farmers for pathology training of vet students (while not strictly Reduction, these animals were to be euthanised anyway)
- Freshwater fish: Collaboration and data sharing with colleagues allowed an overall reduction of fish sampled.
- Mice: Conducting large experiments between researchers allowed for control groups to be shared and so reduced the total number of mice used.
- Pale-yellow Robin: Preliminary results in the project meant that trapping the birds in mist nets was no longer necessary.
- Wildlife: Reduce handling time to less than 1 minute
- Sea turtles: Environmental enrichment projects trialling different types of EE were carried out to improve the experience of the turtles while in captivity
- We have trialed boat mounted remote cameras to conduct surveillance of bats in water supply tunnel.
- As projects are primarily ecological surveys that may require active sampling replacement techniques are often difficult to meet legislative requirements. We continue to review project design seeking opportunities to identify alternative methods for survey.
- All researchers are required to review sampling sizes and trapping efforts to minimise handling and capture of native animals. Particular attention is made to where there may be the requirement for relatively large numbers of individuals to be surveyed.
- Standard operating procedures are formally reviewed annually and regularly updated as new information is received. Examples include: Use of mesh screens to separate small and large bodied fish in fyke nets as well as prevent entry of birds and platypus, and modifying acoustic tag attachment to turtles.
- Use of cell lines as non-animal alternatives.

- Use of video training tools for training in animal handling and injection techniques.
- Use of cadavers for initial training in injection techniques and progression to training on live animals only once competent on the cadavers.
- Complementary methods that might ultimately replace animals in certain situations are being assessed in one protocol. These include quartz crystal microbalance with dissipation, neutron reflectometry, and electrical impedance spectroscopy.
- Use of remote underwater video instead of trapping and releasing fish.
- Development and use of in vitro cell culture systems prior to trialling with live animals
- Use of pilot studies to refine techniques.
- Modification of camera trapping design to enable its use in small mammal (terrestrial and arboreal) monitoring without the need for live trapping.
- Sharing tissues from deceased animals with other researchers.
- Use of video recordings to demonstrate imaging equipment to avoid duplication of live animal imaging.
- Scientific end point was reached earlier than expected so experiment did not need to use as many animals as expected.
- Use of transformed cell lines with the GABA receptor of interest to reduce use of live animals.
- Use of proof-of-concept pilot studies prior to commencing main study – resulted in substantial reduction in animal numbers (24 mice instead of 60).
- Use of additional non-animal based models for screening group A streptococcal virulence effectors and virulence control mechanisms. This has resulted in reduction of the total number of animals required for both breeding and experimental protocols.
- Training protocol makes use of excess rats and mice that have not been used for experiments to train researchers in various techniques, thus minimising number of animals required in their research applications and providing Certificates of Competency as needed.
- Development of a temperament assessment matrix for rats to determine suitability for rehoming.
- Rehoming of animals under a scheme developed in collaboration with the Research Animal Rehoming Service and supported by the establishment.
- Scoring system for animal welfare impact reviewed to refine humane endpoints during the course of a study.
- Successful completion of a pilot protocol to enable drug administration using voluntary ingestion of the drug in a flavoured paste (Nutella) without handling rather than direct oral dosing or gavage.
- Most of the animals to be used in this study were already collected and used in previous, non-invasive experiments, thus reducing the need for new toads.
- Use half pens to reduce bird numbers, but maintain target stocking density.
- Consideration to reducing overall bird stress from unnecessary handling has been made, the number of birds subjected to experimental procedures is reduced to the minimum need to reach statistical significance. For example in project only 60 of the 744 birds were euthanised for sample collection.
- 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.
- 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
- Careful consideration has been given to the sampling design of this study, and the results of the power analyses presented in section 3.2 of this application provide a strong quantitative basis for the number of fish required to complete this study. As a result, we have been able to identify the minimum number of fish required and have adopted this number as our desired sample size. 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 simultaneously.

- AEC promotes the use of Cadavers and is notified of their use by researchers.
- A Statistician regularly attends AEC meetings to ensure that the minimum number of animals required for a research project are approved.
- The AEC requests that investigators handling sheep are appropriately skilled to ensure that low stress handling occurs to reduce the impact of any procedures being carried out on them.
- Power analyses for statistics instituted to prevent under or over use of animals.
- Less sentinel animals are utilised with animal health monitoring being done on stock animals via dried blood spot sampling as survival procedure. Sentinels only used for blood sampling in immunodeficient mice models and quarantine mice (cMRI)
- Application of statistical analysis to validate animal studies, only using minimum animal numbers required.
- Tissue sharing when possible.
- Training animals used are only those identified for culling or use of dead animals to practice technical procedures as practicable.
- Avoid performing unnecessary experimental work with animals by conducting a comprehensive review of existing data, available models and alternative experimental approaches before renewing existing, or commencing novel research projects.
- Effective use of analgesics & anaesthetics for painful and invasive animal procedures following recommendations by the AEC and veterinarians.
- Use of softer bedding material for mice with rectal prolapse.
- New environmental enrichment devices for rodents - metal rings, crinkl' nest for breeding animals.
- Smaller incisions during surgery for faster wound healing.
- Treating the animals with empathy, allowing them to acclimatise to the specific experimental setting and handle them with care to minimise fear and distress.
- The ACEC 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 is using tissue samples only (often donated from other institutions - e.g. zoos, collected during routine health inspections of animals), and some projects are increasingly using camera surveys, thereby decreasing the direct negative impacts on live animals.
- Protocols aim to develop diagnostic assays to measure animal disease and welfare and ultimately to replace the use of live animals with laboratory tests .
- Re-use of animals - researchers were able to reuse rabbits instead of purchasing the same number of new animals. The rabbits were previously used in a trial testing suitability of temperature data loggers attached to a collar.
- The Committee agreed on the reuse of 4 sheep and considered the benefits of reducing the number of animals outweighed the increase to the length of their confinement.
- 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.
- 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.
- Similar studies have shared the same control animals.
- Use of experienced veterinarians and other staff. Restraint time and dose rates kept to a minimum. Adoption of less stressful methodologies.
- Use of adjuvants known not to produce adverse reactions.
- Procedures used routinely so that animals become accustomed.
- 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.
- 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 Committee also looked at the reuse of animals, where appropriate, to reduce the overall number of animals used by the organisation. An example was a protocol that used young rabbits sourced from a licenced supplier for a non-invasive procedure. These animals were then used in a protocol under the supervision of another of the establishment's AECs as the particular protocol sources animals from the same supplier.
- Application forms are designed to ensure that researchers consider how they might replace, refine and reduce animal numbers in their research. The AEC also developed a complementary "Guidelines" document that steps researchers through every question on the application form, outlining what they need to consider and where they can find additional information on the principles of replacement, refinement and reduction.
- All approved applications for Animal Research Authorities are considered to have properly considered the principles of replacement, reduction and refinement. Some projects were revised following guidelines and advice set out by the AEC. For example, a protocol that is looking measuring the pelvic dimensions of young sheep had conditions imposed on it to: ensure that a calibration component of the study was undertaken first; ensure early intervention for any animals with significant accidental injury; and increased monitoring of any animal suspected of having sustained minor accidental injury.
- Use of approved standard operating procedures for the use of animals in teaching, research and extension also aids in addressing these principles.
- Pilot studies are often undertaken by investigators before proceeding further, which assists researchers to refine future protocols.
- A new health screening process was trialled that involved the use of filtered media hung on racks, negating the use of sentinal mice. The media was sent to the USA for bacteria testing during the trial period.
- The committee encourages the use of tissue sharing amongst groups internally and following delivery of courses.
- Purchase of a euthanasia chamber that can accommodate the mouse caging. This allows the animal to stay in its familiar home cage during euthanasia in order to reduce stress on the animal.
- The Committee has implemented ethics training requirements for all people working with animals. The training is continued with facility specific inductions, including procedural training.

6. Appendix - Guide to the categories of reporting

The following is the guidance provided in [Form L – Animal use statistics](#) on categories for Purpose and Procedure and for species.

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	<p>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).</p>
A2	<p>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, (eg 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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Fistulated ruminants which are maintained under a holding project, for use in other short term feeding trial projects</i> • <i>Non-breeding colony of diabetic rats held for research in other projects</i>
A3	<p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Animals used by veterinary schools to teach examination procedures such as pregnancy diagnosis</i> • <i>Sheep used in shearing demonstration classes for students; Dogs used to teach animal care to TAFE students</i>
A4	<p>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.</p>
A5	<p>Research: human or animal health and welfare Research projects which aim to produce improvements in the health and welfare of animals, including humans.</p>
A6	<p>Research: animal management or production Research projects which aim to produce improvements in domestic or captive animal management</p>

	or production.
A7	<p>Research: environmental study</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Pre-logging or pre-development fauna surveys</i>
A8	<p>Production of biological products</p> <p>Using animals to produce products other than milk, meat, eggs, leather, fur, etc.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Use of a sheep flock to donate blood to produce microbiological media</i> • <i>Production of commercial anti-serum</i> • <i>Production of products, such as hormones or drugs, in milk or eggs from genetically modified animals</i> • <i>Quality Assurance testing of drugs but do not include animals which come under Purpose A10, below.</i>
A9	<p>Diagnostic procedures</p> <p>Using animals directly as part of a diagnostic process.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Inoculation of day old chicks with ND Virus to determine virulence</i> • <i>Water supply testing using fish</i>
A10	<p>Regulatory product testing</p> <p>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.)</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Pre-registration efficacy or toxicity testing of drugs and vaccines</i>

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	<p>Observation Involving Minor Interference</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Observational study only</i> • <i>Breeding animals for supply, where only normal husbandry procedures are used</i> • <i>Breeding or reproductive study with no detriment to the animal</i> • <i>Feeding trial, such as Digestible Energy determination of feed in a balanced diet</i> • <i>Behavioural study with minor environmental manipulation</i> • <i>Teaching of normal, non-invasive husbandry such as handling and grooming</i>
P2	<p>Animal Unconscious Without Recovery</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Laboratory animals killed painlessly for dissection, biochemical analysis, etc</i> • <i>Teaching surgical techniques on live, anaesthetised patients which are not allowed to recover following the procedure</i>
P3	<p>Minor Conscious Intervention</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Injections, blood sampling in conscious animal</i> • <i>Minor dietary or environmental deprivation or manipulation, such as feeding nutrient-deficient diets for short periods</i> • <i>Trapping and release as used in species impact studies</i> • <i>Trapping and humane euthanasia for collection of specimens</i> • <i>Stomach tubing, shearing</i>
P4	<p>Minor Surgery With Recovery</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Biopsies</i> • <i>Cannulations</i>

	<ul style="list-style-type: none"> • <i>Sedation/anaesthesia for relocation, examination or injections/blood sampling</i> • <i>Castration with regional or general anaesthesia and post-operative analgesia</i>
P5	<p>Major Surgery With Recovery</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Orthopaedic surgery</i> • <i>Abdominal or thoracic surgery</i> • <i>Transplant surgery</i>
P6	<p>Minor Physiological Challenge</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Minor infection</i> • <i>Minor or moderate phenotypic modification</i> • <i>Early oncogenesis</i> • <i>Arthritis studies with pain alleviation</i> • <i>Induction of metabolic disease</i> • <i>Prolonged deficient diets</i> • <i>Polyclonal antibody production</i> • <i>Antiserum production</i>
P7	<p>Major Physiological Challenge</p> <p>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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Major infection</i> • <i>Major phenotypic modification</i> • <i>Oncogenesis without pain alleviation</i> • <i>Arthritis studies with no pain alleviation</i> • <i>Uncontrolled metabolic disease</i> • <i>Isolation or environmental deprivation for extended periods</i> • <i>Monoclonal antibody raising in mice</i>

<p>P8</p>	<p><i>Death As An Endpoint</i></p> <p>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 <i>and</i> euthanasia is carried out before significant suffering occurs, they may be placed in category P6 or P7.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Lethality testing (including LD50, LC50)</i> <p>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.</p>
<p>P9</p>	<p><i>Production of genetically modified animals</i></p> <p>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 <i>and</i> major physiological challenges <i>and</i> 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.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • <i>Initial breeding animals for GM production</i> • <i>Animals culled as part of the GM production process</i>

Column 5: SPECIES

Laboratory mammals	Mice
	Rats
	Guinea Pigs
	Rabbits
	Hamsters
	Ferrets
	Other laboratory mammals (not primates)
Domestic mammals	Sheep
	Cattle
	Pigs
	Horses
	Goats
	Deer
	Cats
	Dogs
	Other domestic mammals
Birds	Poultry
	Exotic Captive
	Exotic Wild
	Native Captive

Primates	Marmosets
	Macaques
	Baboons
	Other primates
Native mammals	Macropods
	Possums and gliders
	Native rats and mice
	Dasyurids
	Wombats
	Koalas
	Monotremes
	Bandicoots
	Bats
	Other native mammals
	Seals
	Whales and dolphins
Exotic feral mammals	Camels
	Cats
	Cattle
	Goats
	Hares
	Horses

	Native Wild
	Other birds
Aquatic animals	Fish
	Cephalopods (reporting not mandatory)
	Crustaceans (reporting not mandatory)
Amphibians	Amphibians
Reptiles	Lizards
	Snakes
	Turtles and Tortoises
	Other reptiles

	Mice
	Pigs
	Rabbits
	Rats
	Dingo/Wild Dogs
	Foxes
	Other exotic feral mammals
Exotic zoo animals	Exotic zoo animals

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