

NSW 2016 Animal Use in Research Statistics

January 2018

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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 2016. There are 10 Purpose Categories (see *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 *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 2016. These charts provide a breakdown of the following species groups:
 - laboratory mammals,
 - domestic mammals,
 - birds,
 - primates.
- Lethality testing for 2016. 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 2016.
- 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 6) 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 *Major Physiological Challenge*.

2. General Charts

2.1 Number of animals used over time by species grouping

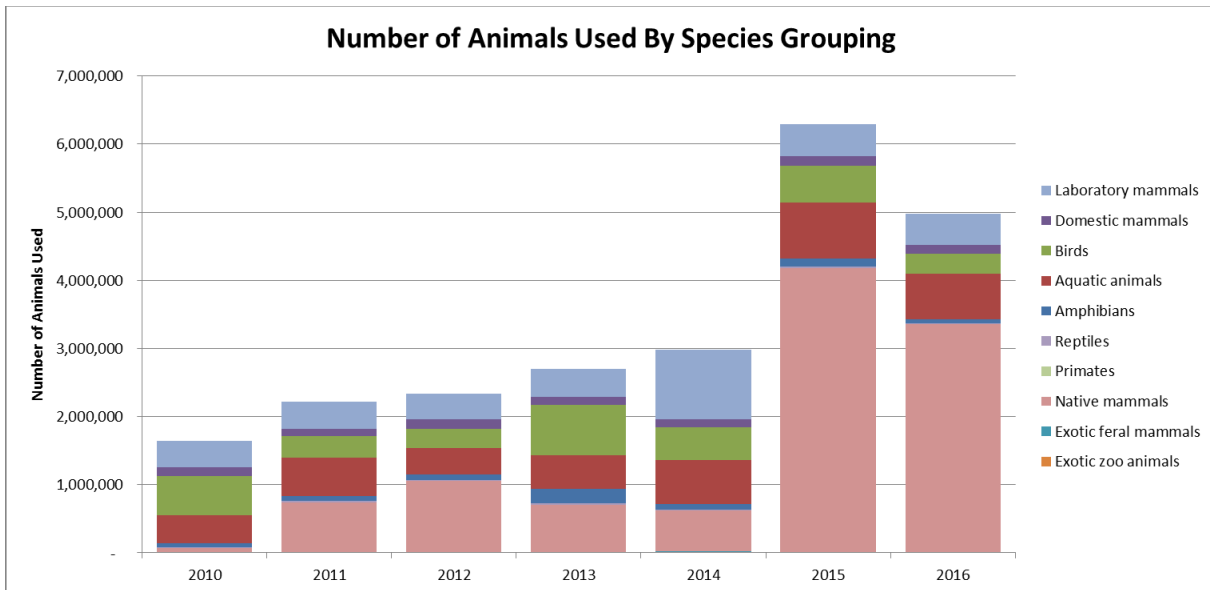


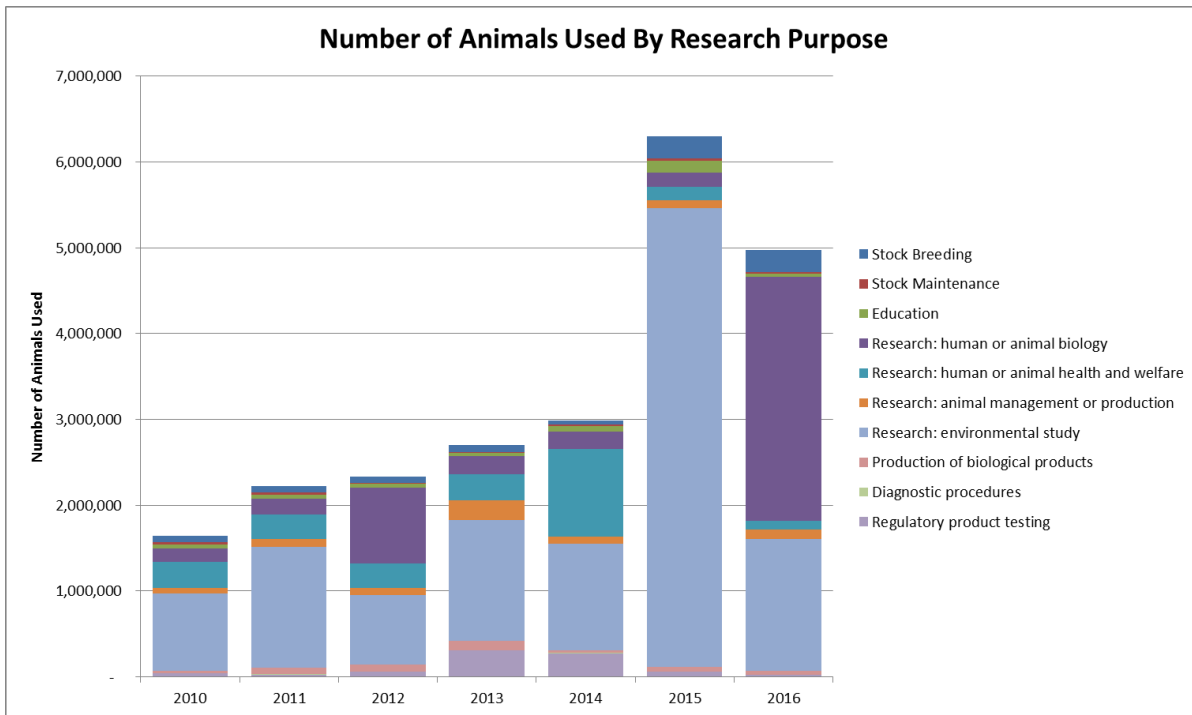
Chart 1: Number of animals used over time by species grouping

| | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | Grand Total |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-------------------|
| Amphibians | 54,992 | 79,446 | 87,417 | 214,616 | 75,424 | 118,721 | 49,008 | 679,624 |
| Aquatic animals | 409,917 | 562,356 | 386,102 | 491,114 | 652,902 | 830,769 | 670,498 | 4,003,658 |
| Birds | 576,787 | 311,690 | 283,461 | 739,293 | 478,754 | 534,812 | 292,834 | 3,217,631 |
| Domestic mammals | 127,468 | 114,511 | 141,288 | 114,914 | 120,239 | 145,685 | 133,537 | 897,642 |
| Exotic feral mammals | 5,318 | 5,195 | 6,525 | 9,411 | 23,200 | 12,541 | 15,351 | 77,541 |
| Exotic zoo animals | 27 | 32 | 71 | 72 | 155 | 83 | 32 | 472 |
| Laboratory mammals | 389,507 | 388,701 | 374,037 | 414,652 | 1,017,494 | 470,619 | 457,431 | 3,512,441 |
| Native mammals | 59,870 | 738,903 | 1,044,611 | 697,764 | 598,737 | 4,161,992 | 3,340,256 | 10,642,133 |
| Primates | 184 | 27 | 18 | 22 | 41 | 179 | 96 | 567 |
| Reptiles | 18,328 | 12,141 | 13,398 | 17,674 | 15,730 | 22,067 | 18,196 | 117,534 |
| #N/A | 195 | 5,460 | | | | | | 5,655 |
| Grand Total | 1,642,593 | 2,218,462 | 2,336,928 | 2,699,532 | 2,982,676 | 6,297,468 | 4,977,239 | 23,154,898 |

Note:

- 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 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.

2.2 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 | Grand Total |
| Stock Breeding | 75,867 | 65,936 | 75,488 | 80,774 | 47,116 | 259,464 | 263,601 | 868,246 |
| Stock Maintenance | 27,165 | 33,850 | 15,448 | 7,890 | 10,500 | 26,508 | 13,684 | 135,045 |
| Education | 43,344 | 41,230 | 40,904 | 34,960 | 68,717 | 141,366 | 39,301 | 409,822 |
| Research: human or animal biology | 158,880 | 189,450 | 882,024 | 218,541 | 201,636 | 161,990 | 2,839,472 | 4,651,993 |
| Research: human or animal health and welfare | 298,611 | 283,546 | 286,375 | 303,050 | 1,024,919 | 156,378 | 104,590 | 2,457,469 |
| Research: animal management or production | 71,722 | 94,019 | 81,831 | 227,769 | 76,422 | 91,603 | 111,880 | 755,246 |
| Research: environmental study | 901,504 | 1,402,726 | 813,500 | 1,411,046 | 1,247,301 | 5,341,812 | 1,539,475 | 12,657,364 |
| Production of biological products | 19,568 | 74,625 | 78,419 | 109,229 | 28,870 | 54,811 | 42,890 | 408,412 |
| Diagnostic procedures | 3,630 | 8,540 | 1,994 | 1,031 | 1,310 | 766 | 1,307 | 18,578 |
| Regulatory product testing | 42,302 | 24,540 | 60,945 | 305,242 | 275,885 | 62,770 | 21,039 | 792,723 |
| Grand Total | 1,642,593 | 2,218,462 | 2,336,928 | 2,699,532 | 2,982,676 | 6,297,468 | 4,977,239 | 23,154,898 |

2.3 Number of animals used over time by research procedure

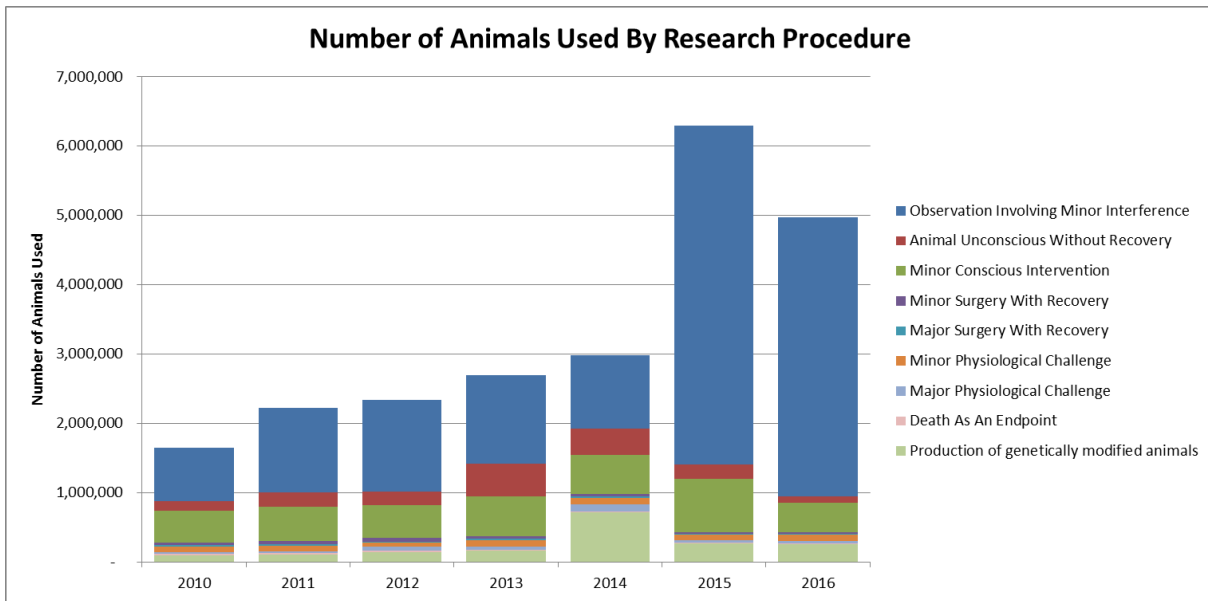
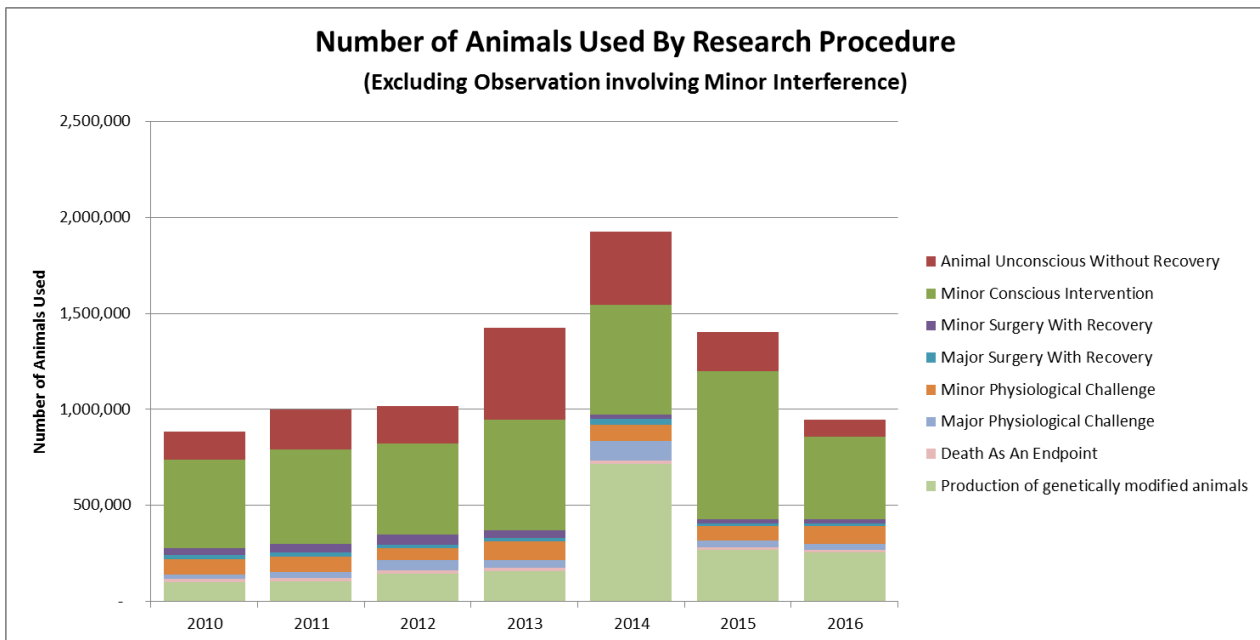


Chart 3: Number of animals used over time by research procedure

| | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | Grand Total |
|--|------------------|------------------|------------------|------------------|------------------|------------------|------------------|-------------------|
| Observation Involving Minor Interference | 760,592 | 1,217,773 | 1,320,108 | 1,276,501 | 1,054,859 | 4,895,641 | 4,030,182 | 14,555,656 |
| Animal Unconscious Without Recovery | 143,155 | 207,753 | 192,840 | 475,557 | 384,503 | 203,920 | 87,443 | 1,695,171 |
| Minor Conscious Intervention | 459,712 | 491,747 | 477,377 | 576,018 | 568,416 | 769,829 | 432,697 | 3,775,796 |
| Minor Surgery With Recovery | 35,765 | 46,839 | 50,552 | 40,145 | 24,794 | 20,530 | 19,838 | 238,463 |
| Major Surgery With Recovery | 25,823 | 19,643 | 19,514 | 18,105 | 28,592 | 16,722 | 16,082 | 144,481 |
| Minor Physiological Challenge | 79,070 | 82,309 | 60,350 | 96,384 | 85,842 | 73,304 | 92,516 | 569,775 |
| Major Physiological Challenge | 22,625 | 28,614 | 54,411 | 42,647 | 103,859 | 34,489 | 29,148 | 315,793 |
| Death As An Endpoint | 17,465 | 17,767 | 17,445 | 15,997 | 16,351 | 16,771 | 15,741 | 117,537 |
| Production of genetically modified animals | 98,386 | 106,017 | 144,331 | 158,178 | 715,460 | 266,262 | 253,592 | 1,742,226 |
| Grand Total | 1,642,593 | 2,218,462 | 2,336,928 | 2,699,532 | 2,982,676 | 6,297,468 | 4,977,239 | 23,154,898 |

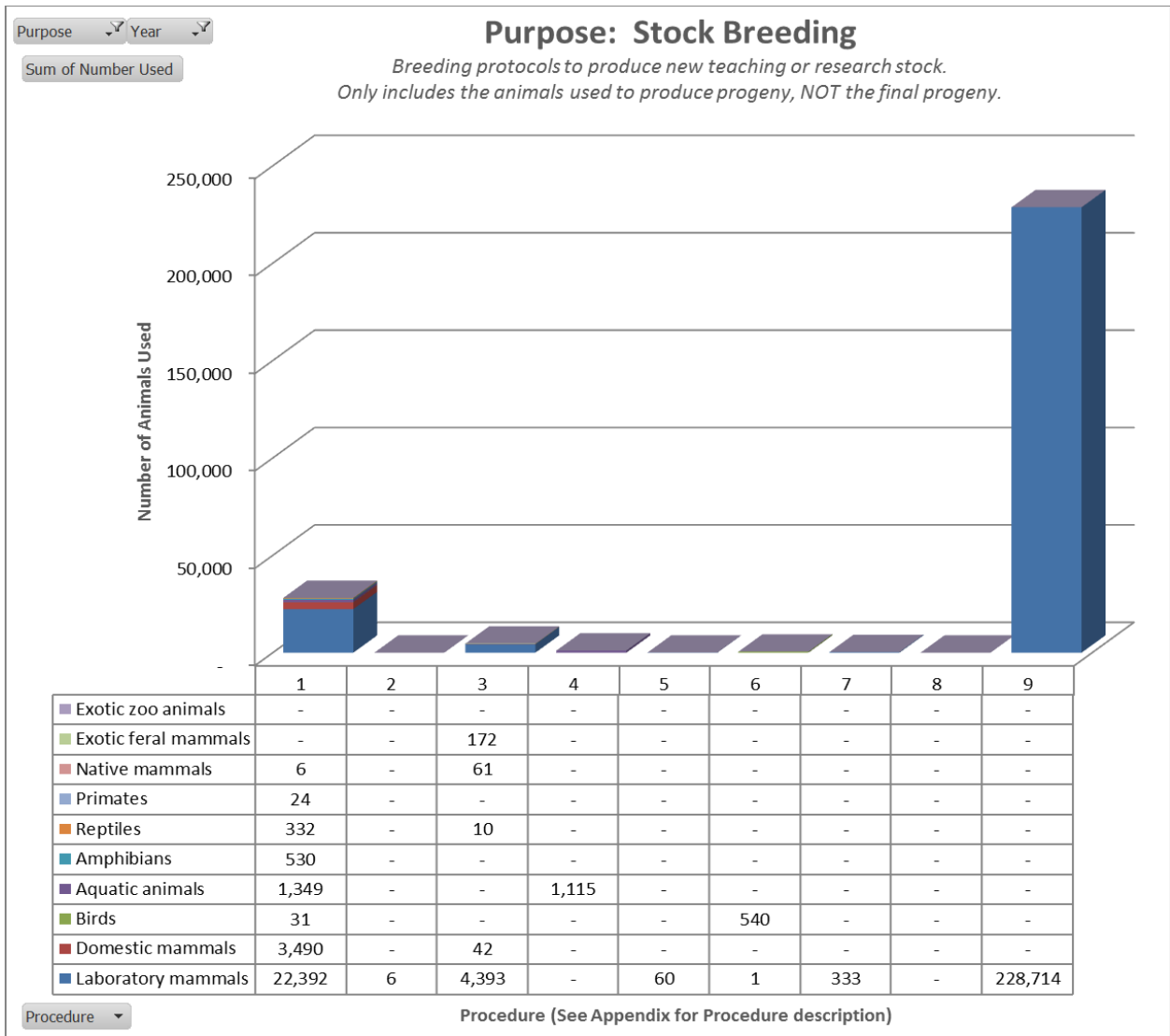
2.4 Number of animals used over time by research procedure excluding 'Observation involving minor interference'



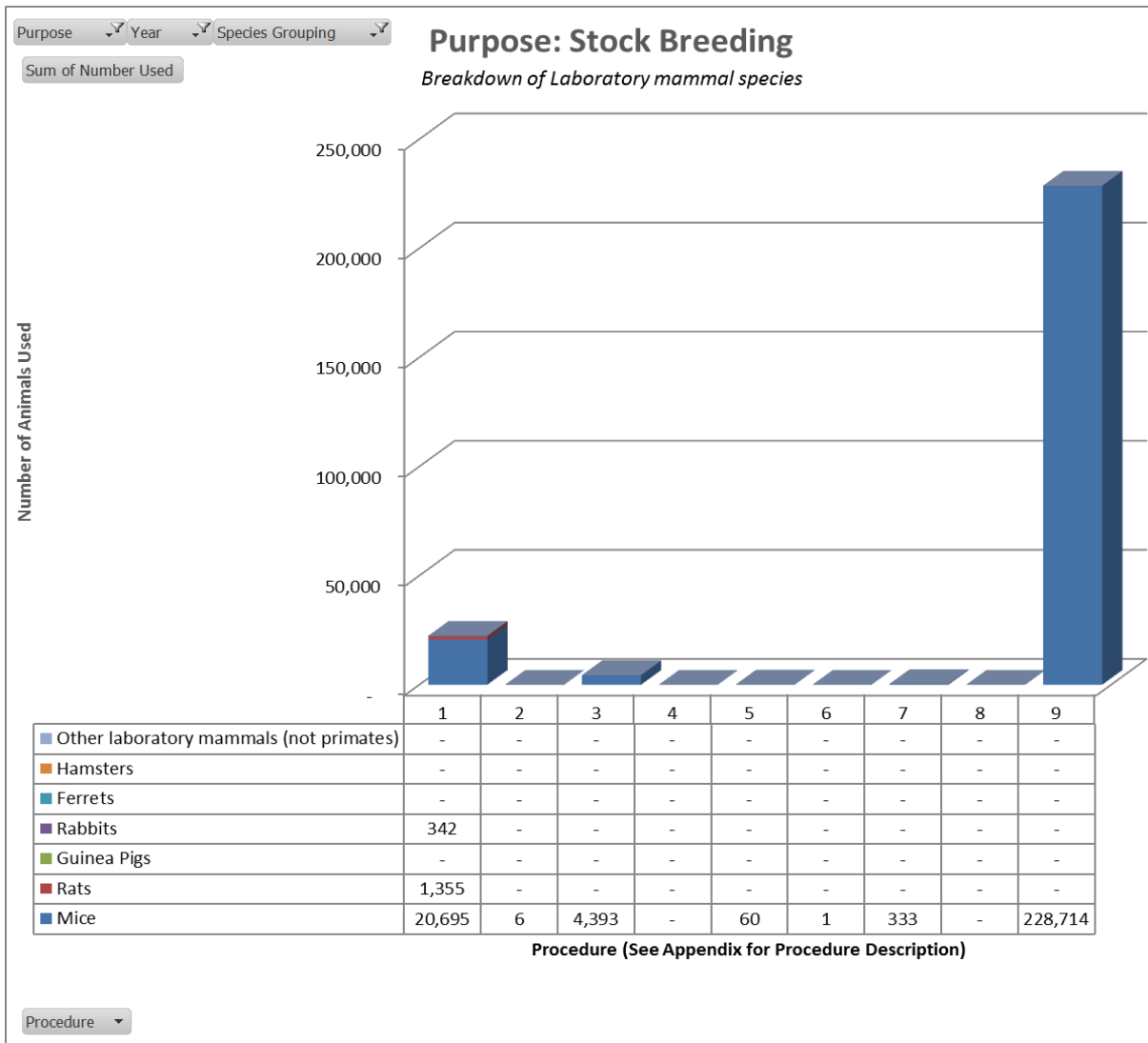
| Chart 4: Number of animals used over time by research procedure excluding "Observation with minor Interference procedure" | | | | | | | | |
|---|----------------|------------------|------------------|------------------|------------------|------------------|----------------|------------------|
| | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | Grand Total |
| Animal Unconscious Without Recovery | 143,155 | 207,753 | 192,840 | 475,557 | 384,503 | 203,920 | 87,443 | 1,695,171 |
| Minor Conscious Interference | 459,712 | 491,747 | 477,377 | 576,018 | 568,416 | 769,829 | 432,697 | 3,775,796 |
| Minor Surgery With Recovery | 35,765 | 46,839 | 50,552 | 40,145 | 24,794 | 20,530 | 19,838 | 238,463 |
| Major Surgery With Recovery | 25,823 | 19,643 | 19,514 | 18,105 | 28,592 | 16,722 | 16,082 | 144,481 |
| Minor Physiological Challenge | 79,070 | 82,309 | 60,350 | 96,384 | 85,842 | 73,304 | 92,516 | 569,775 |
| Major Physiological Challenge | 22,625 | 28,614 | 54,411 | 42,647 | 103,859 | 34,489 | 29,148 | 315,793 |
| Death As An Endpoint | 17,465 | 17,767 | 17,445 | 15,997 | 16,351 | 16,771 | 15,741 | 117,537 |
| Production of genetically modified animals | 98,386 | 106,017 | 144,331 | 158,178 | 715,460 | 266,262 | 253,592 | 1,742,226 |
| Grand Total | 882,001 | 1,000,689 | 1,016,820 | 1,423,031 | 1,927,817 | 1,401,827 | 693,465 | 8,599,242 |

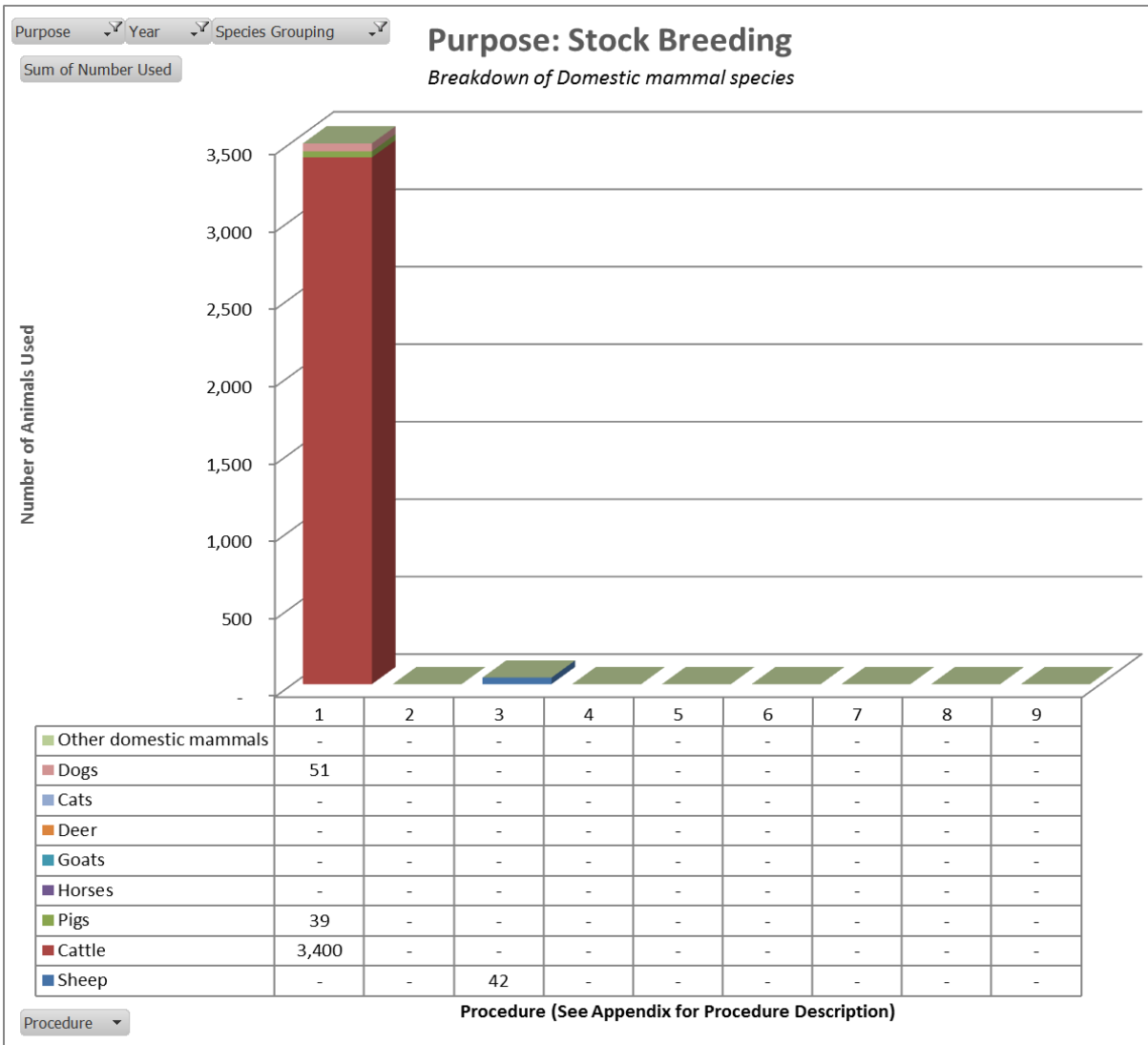
3. Purpose and Species charts

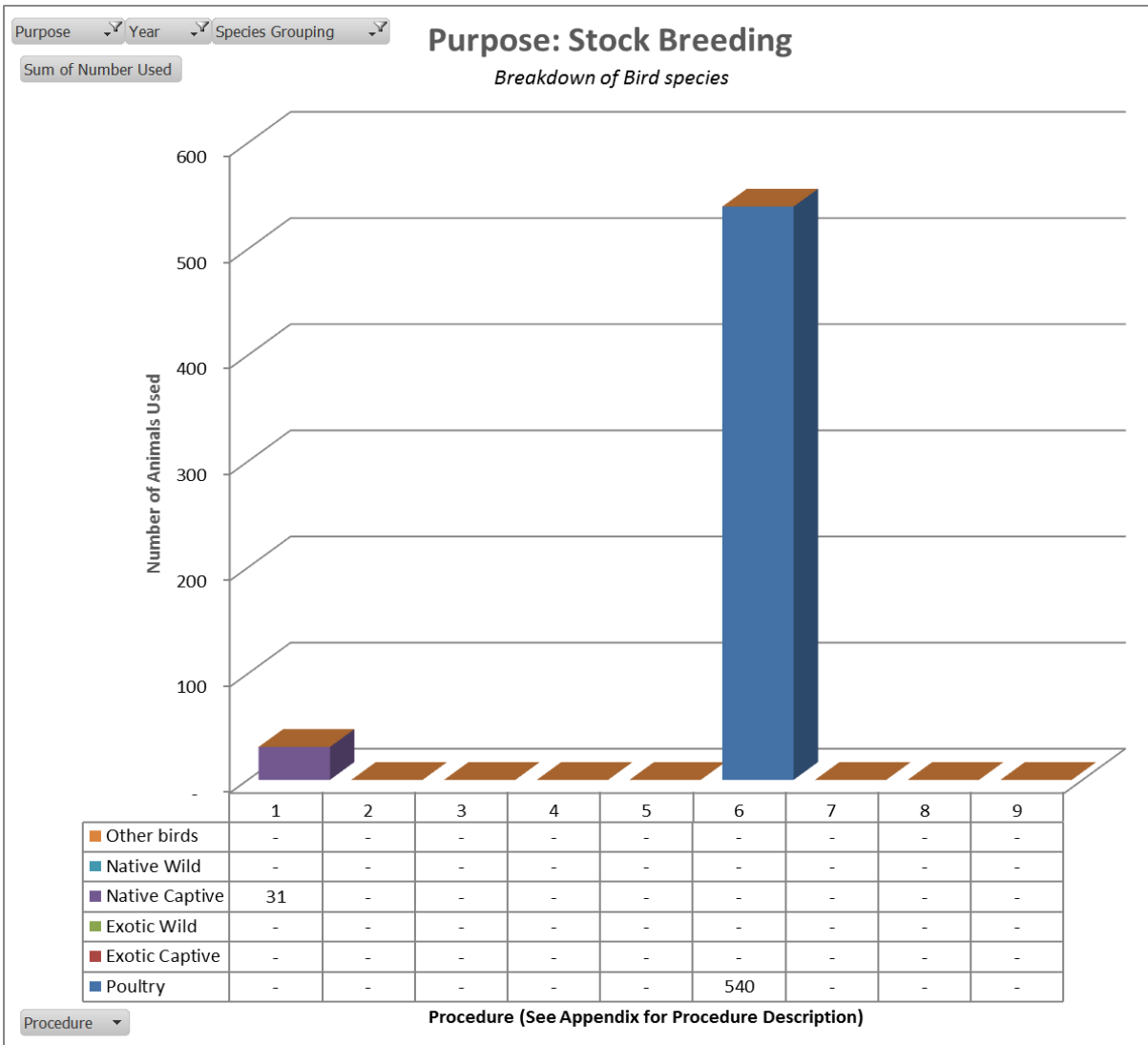
3.1 Stock Breeding

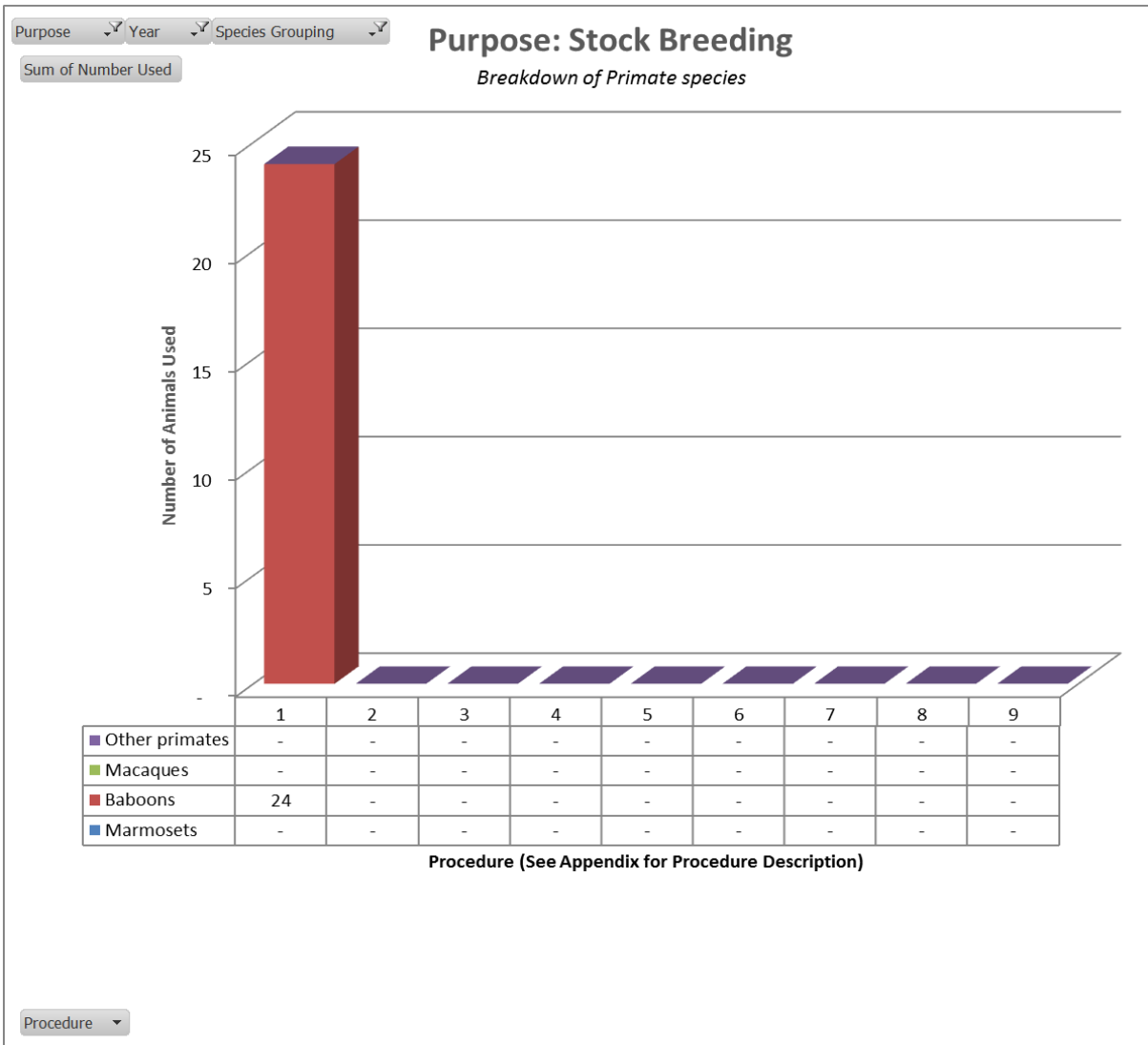


3.1.1 Species Charts for Stock Breeding

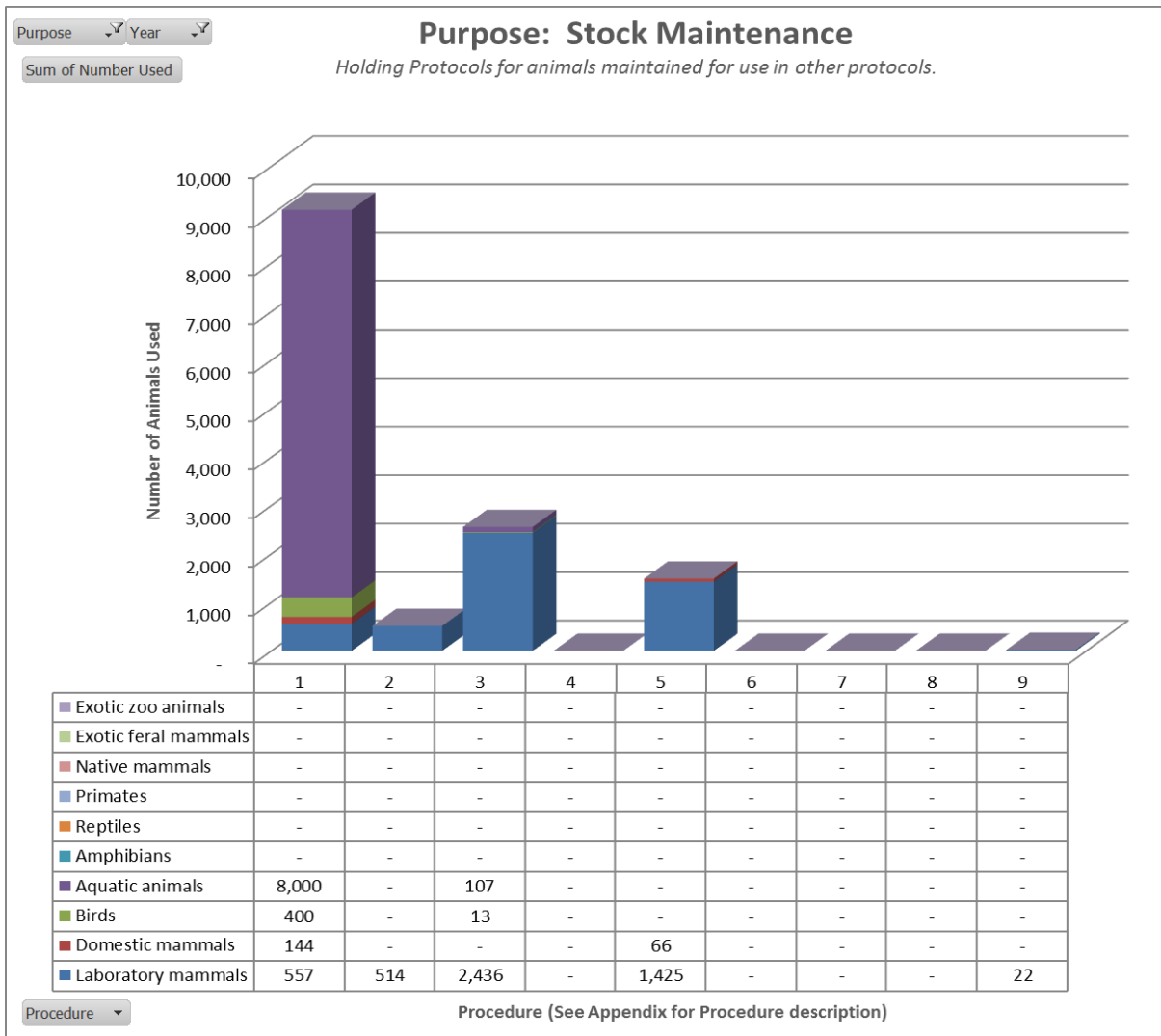




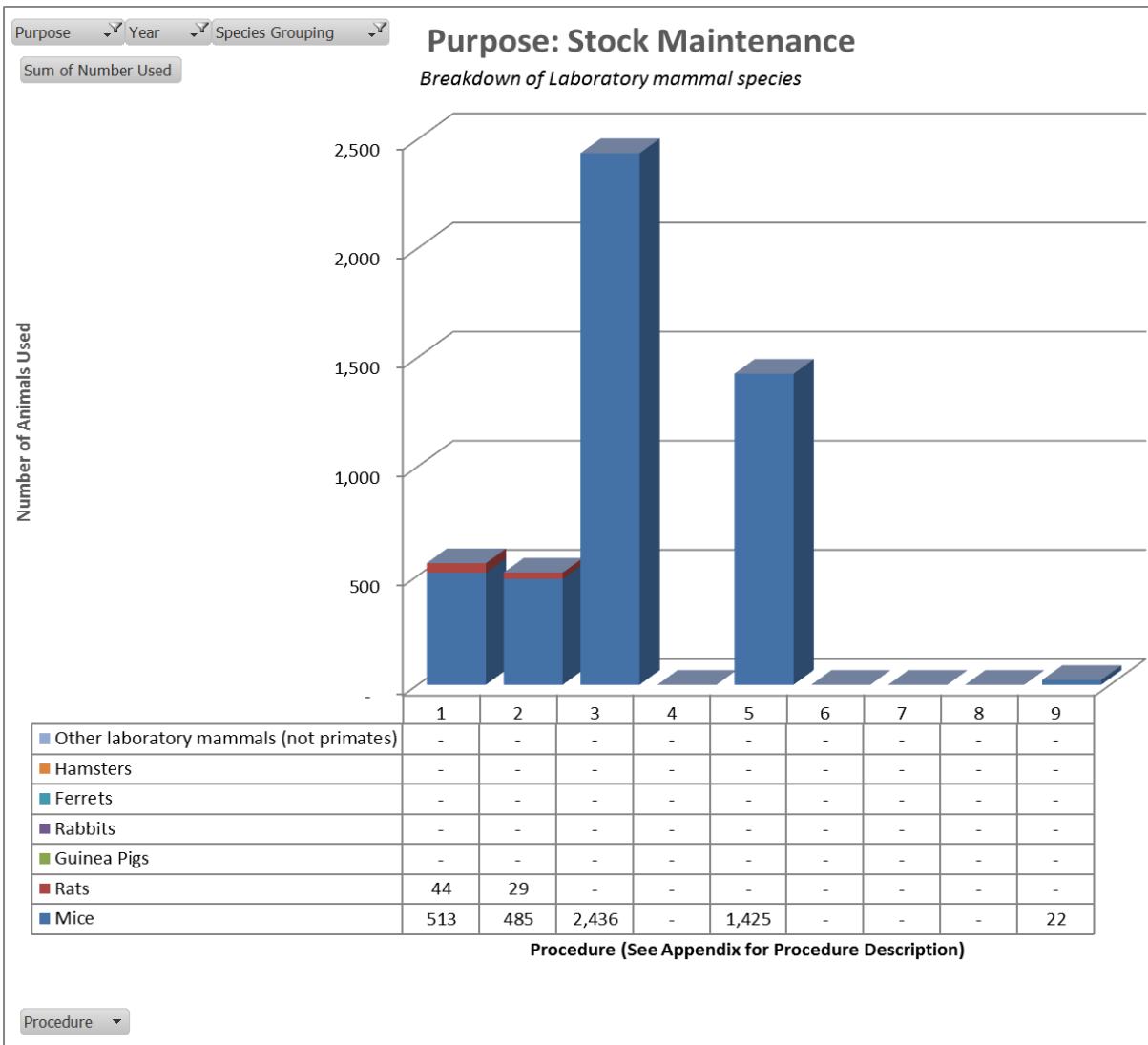


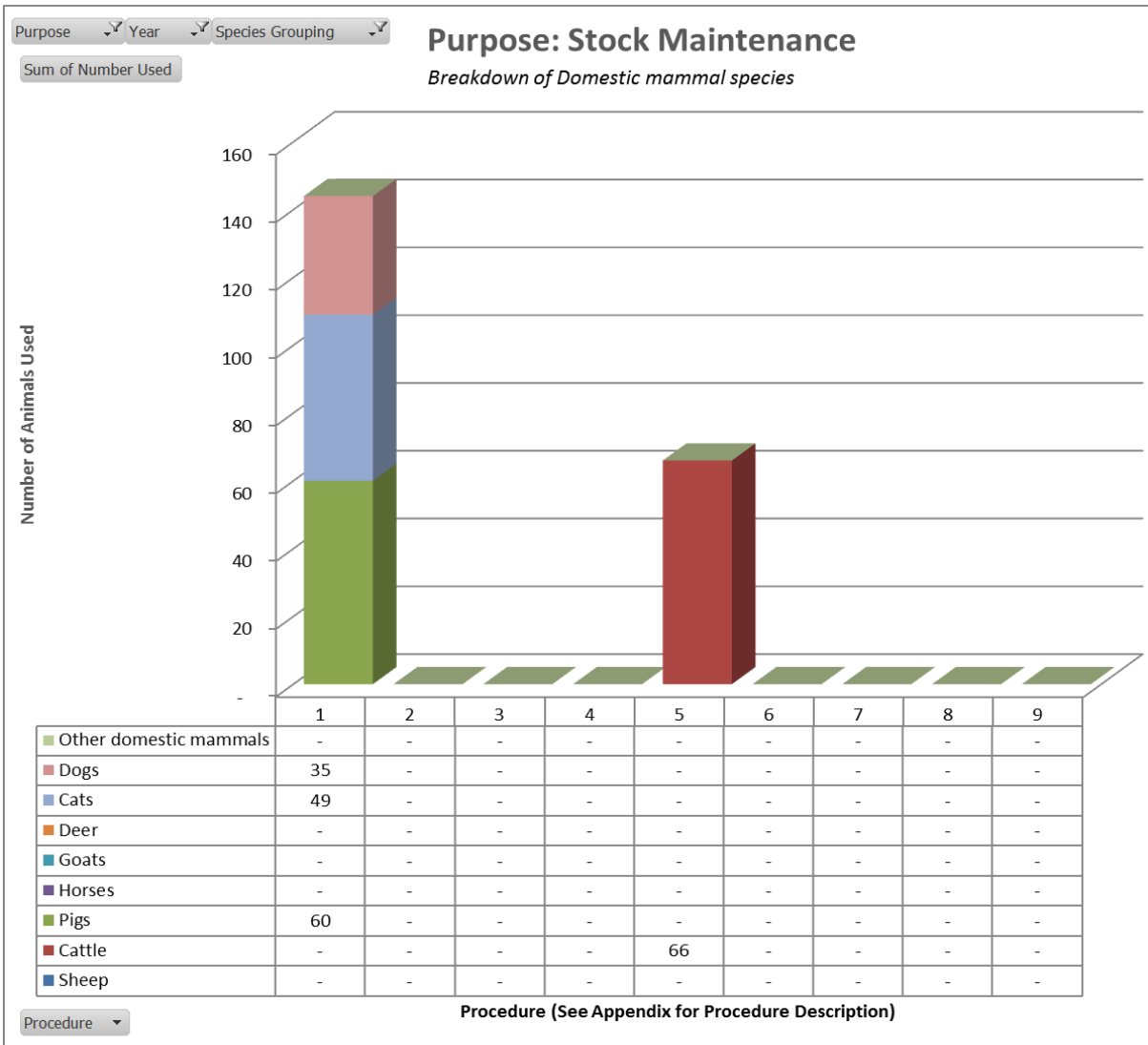


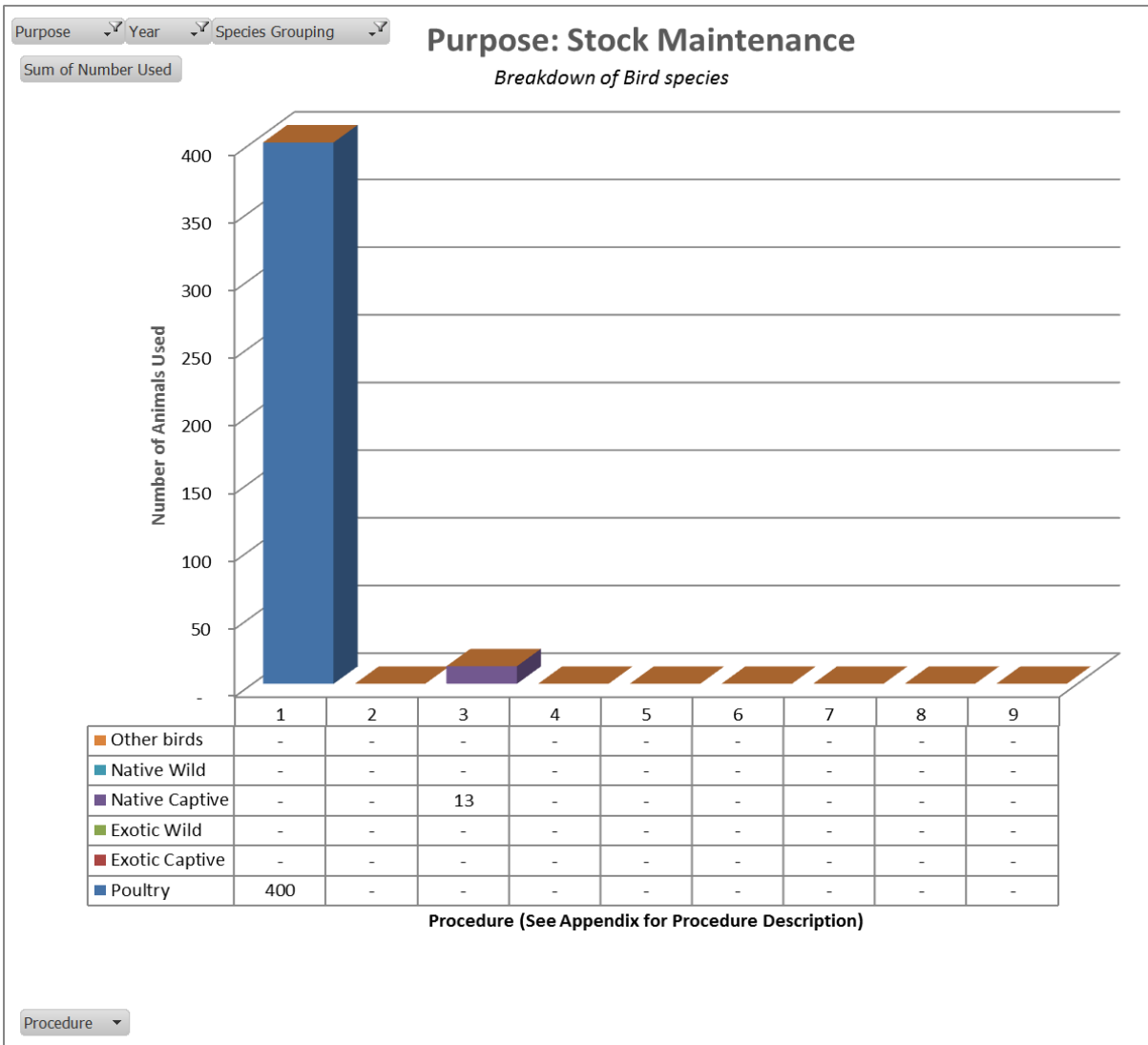
3.2 Stock Maintenance



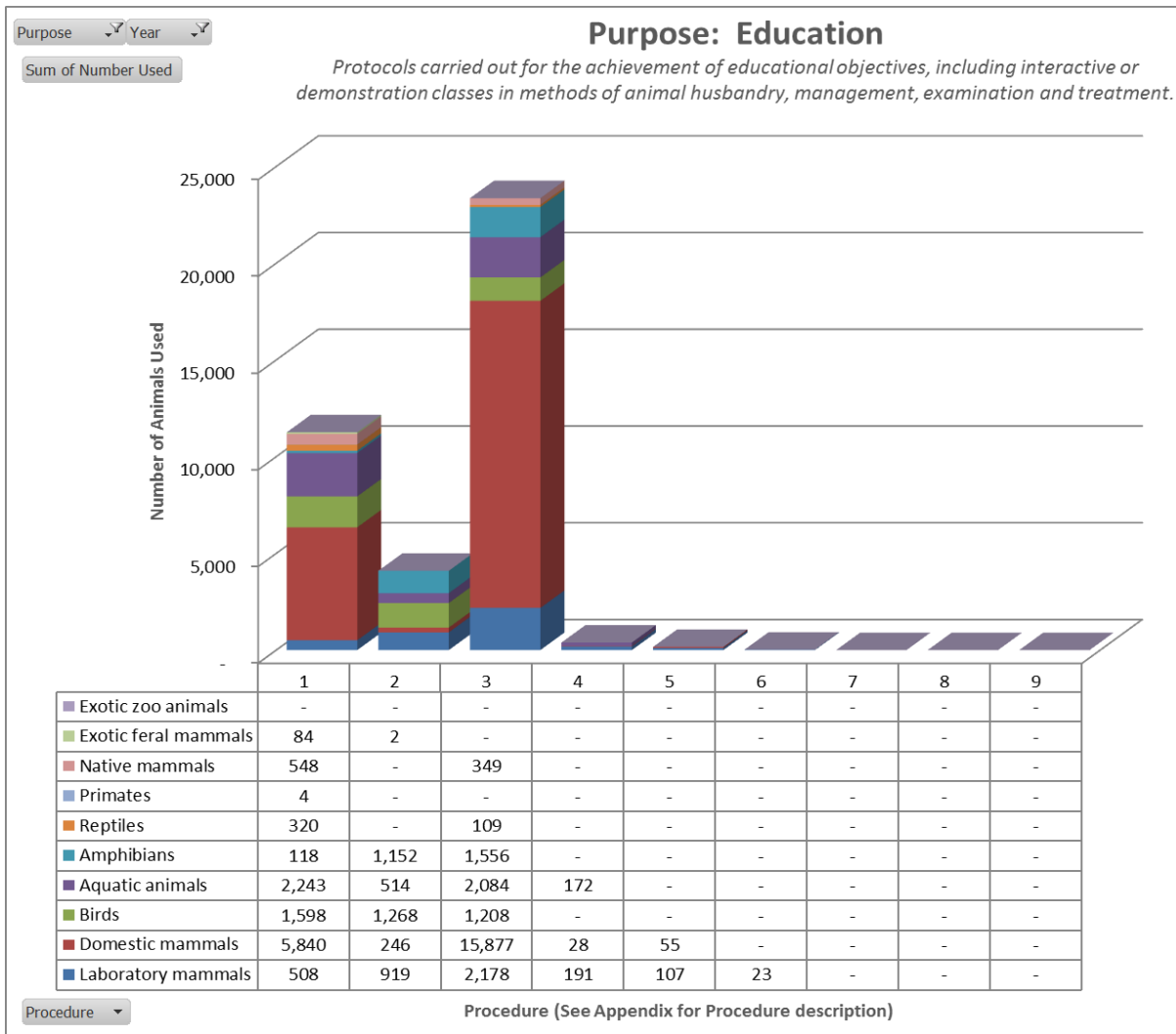
3.2.1 Species Charts for Stock Maintenance



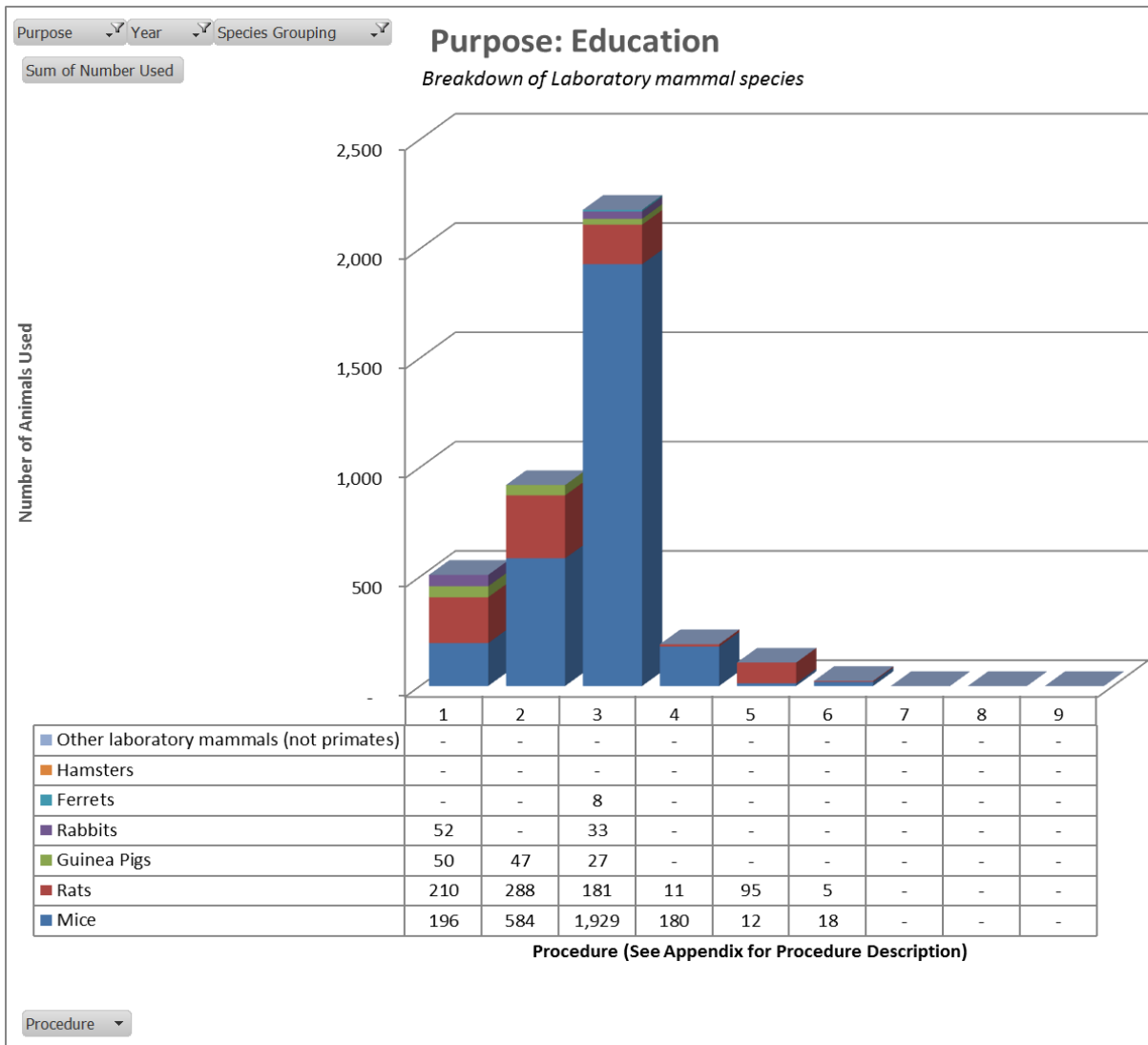


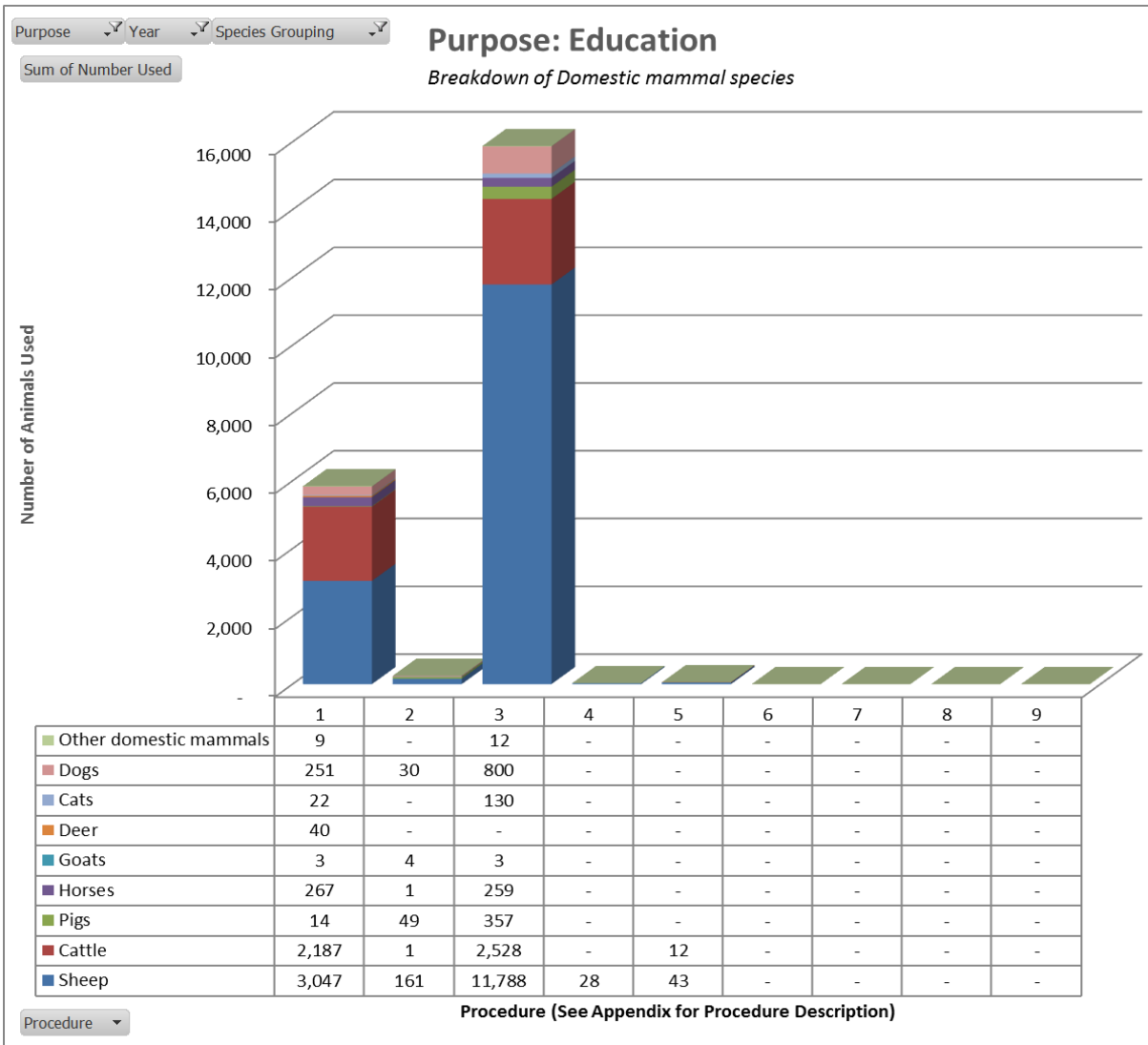


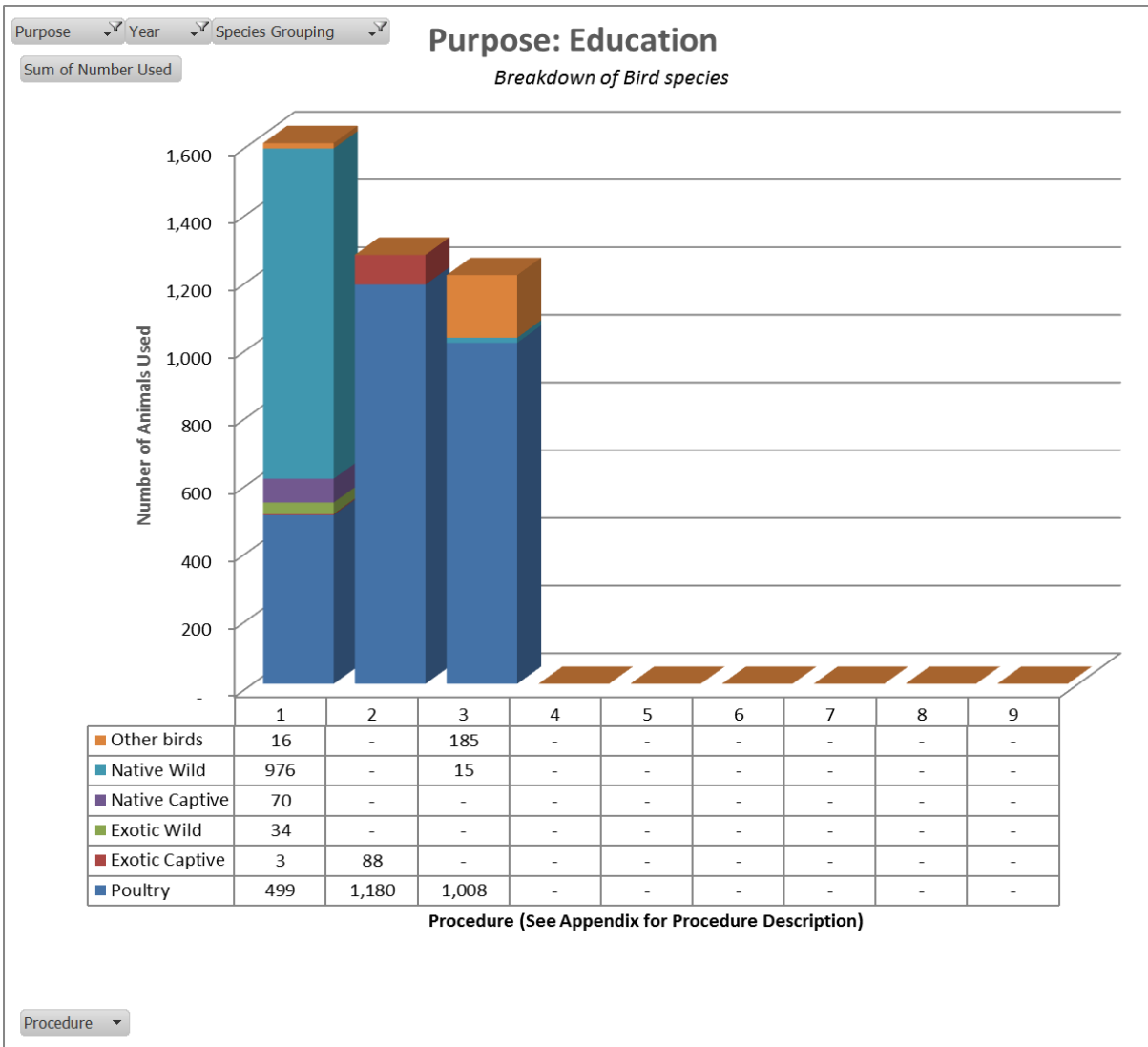
3.3 Education

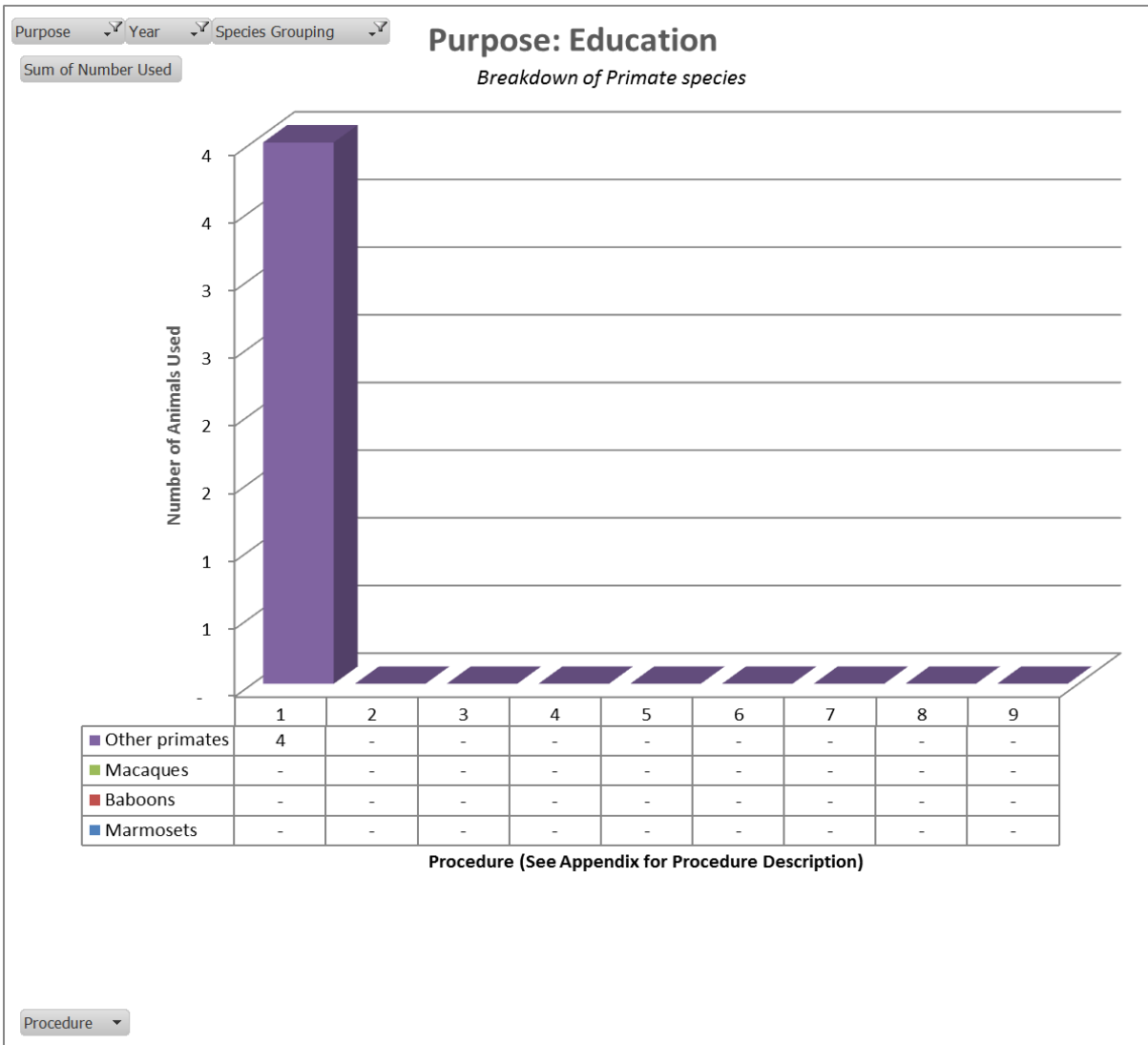


3.3.1 Species Charts for Education

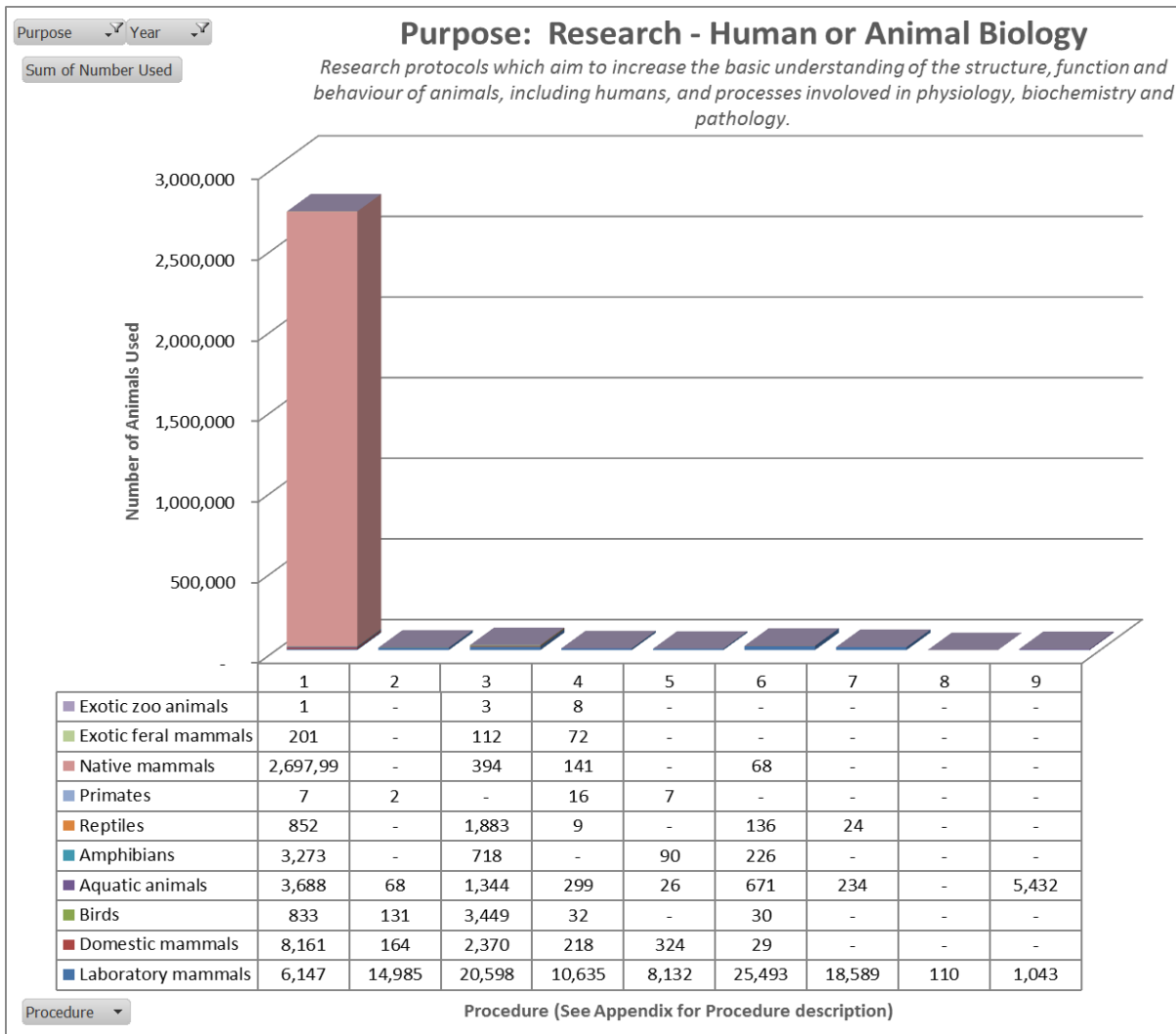




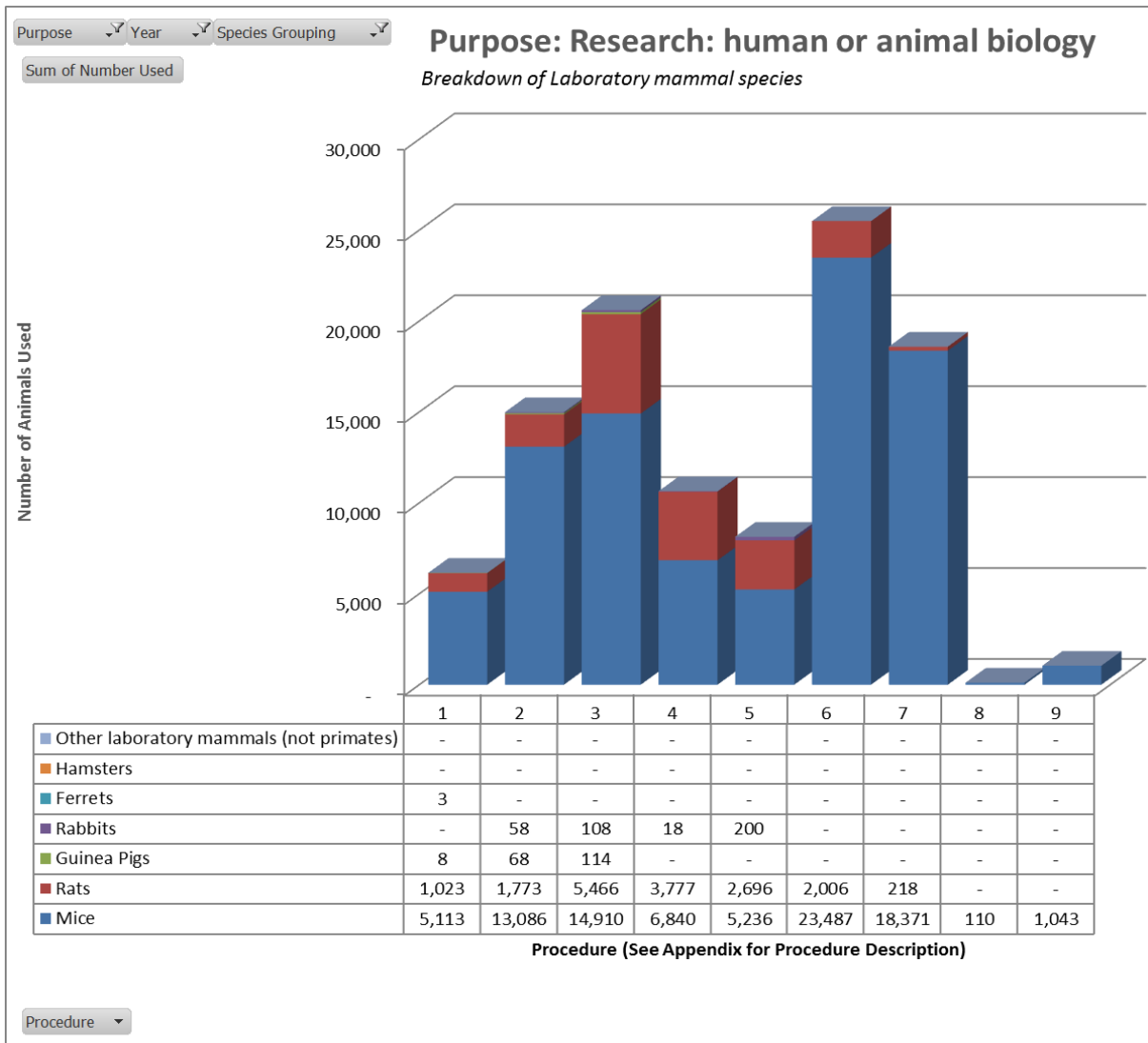


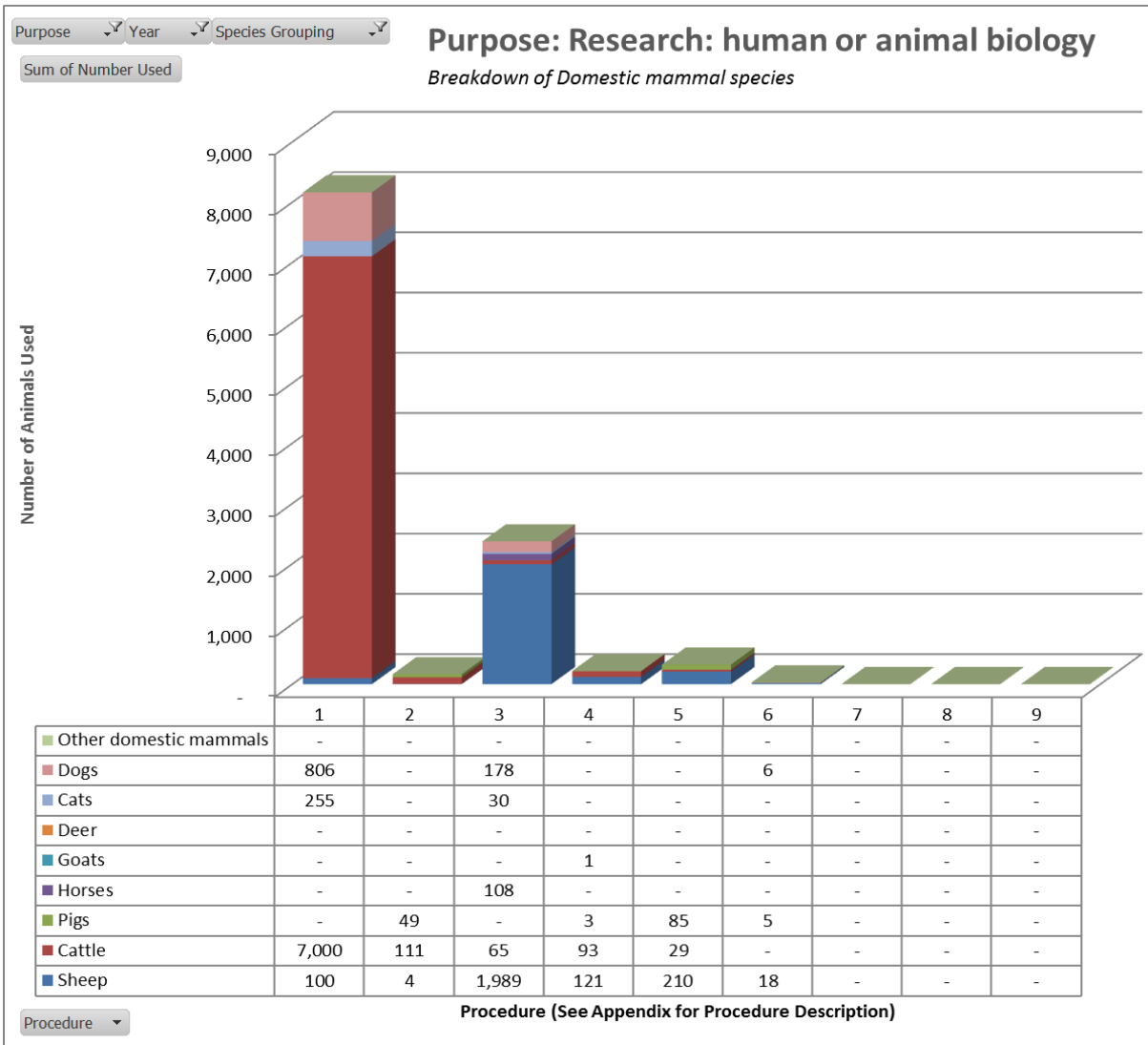


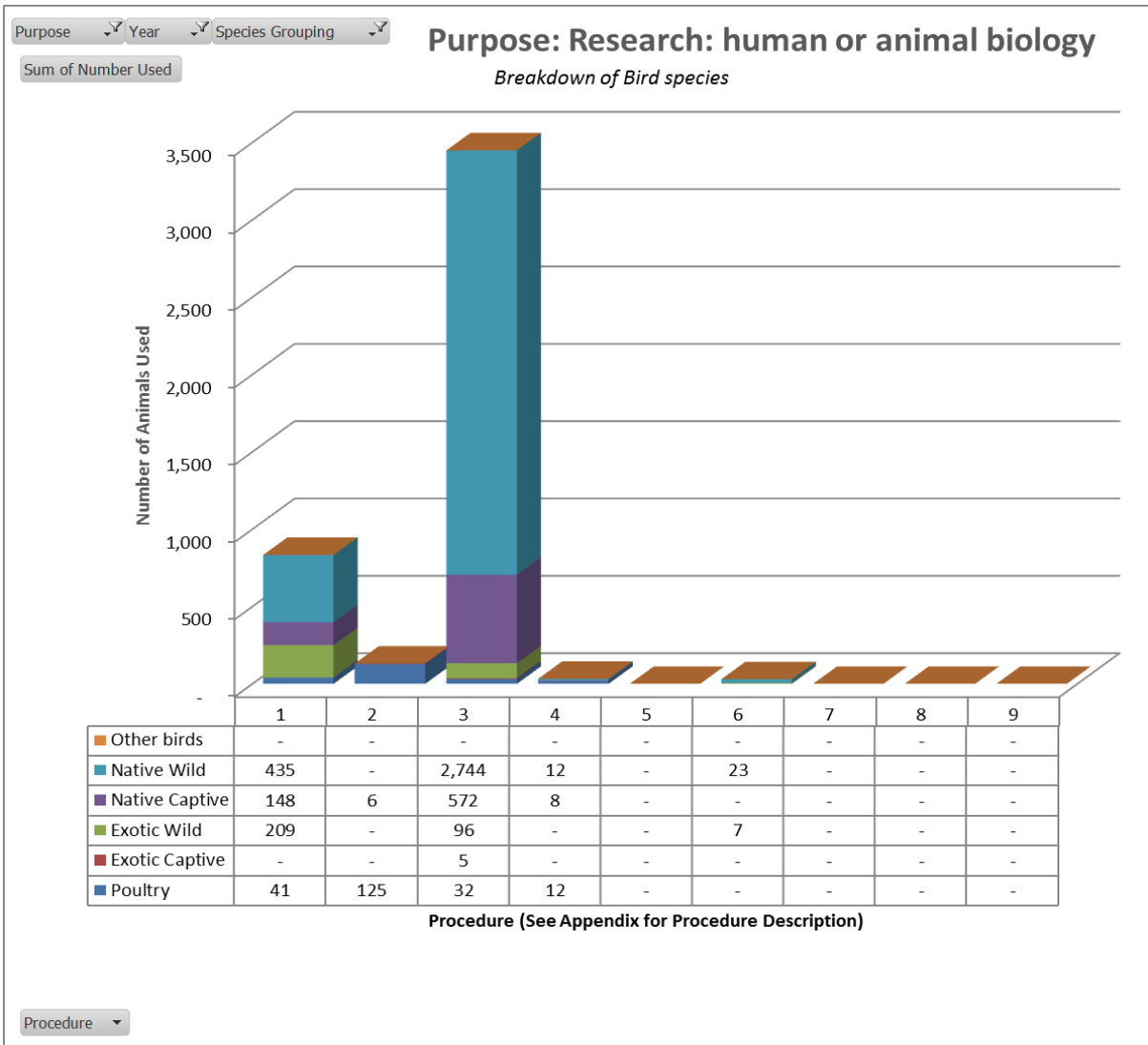
3.4 Research - Human or Animal Biology

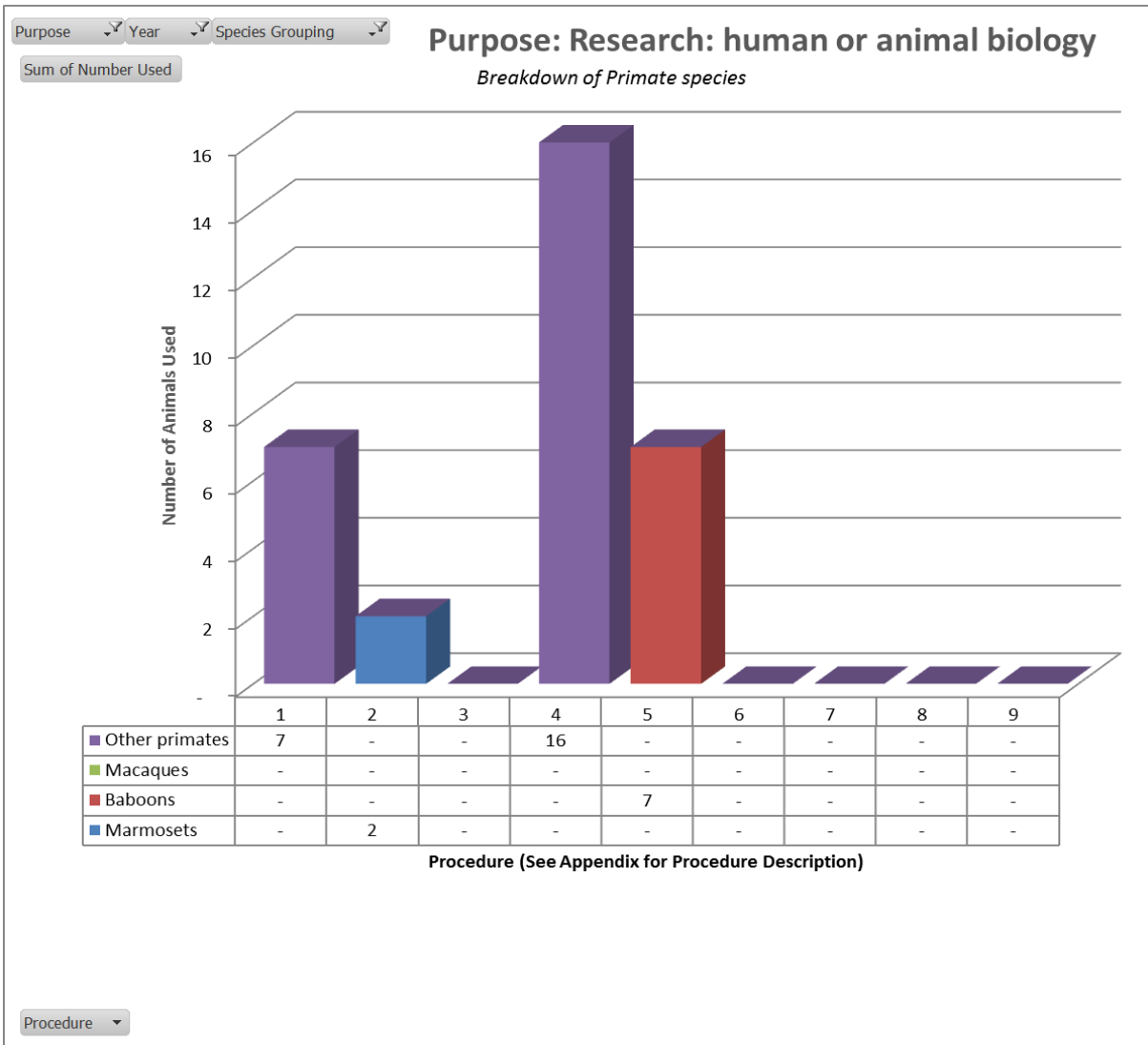


3.4.1 Species Charts for Research - Human or Animal Biology

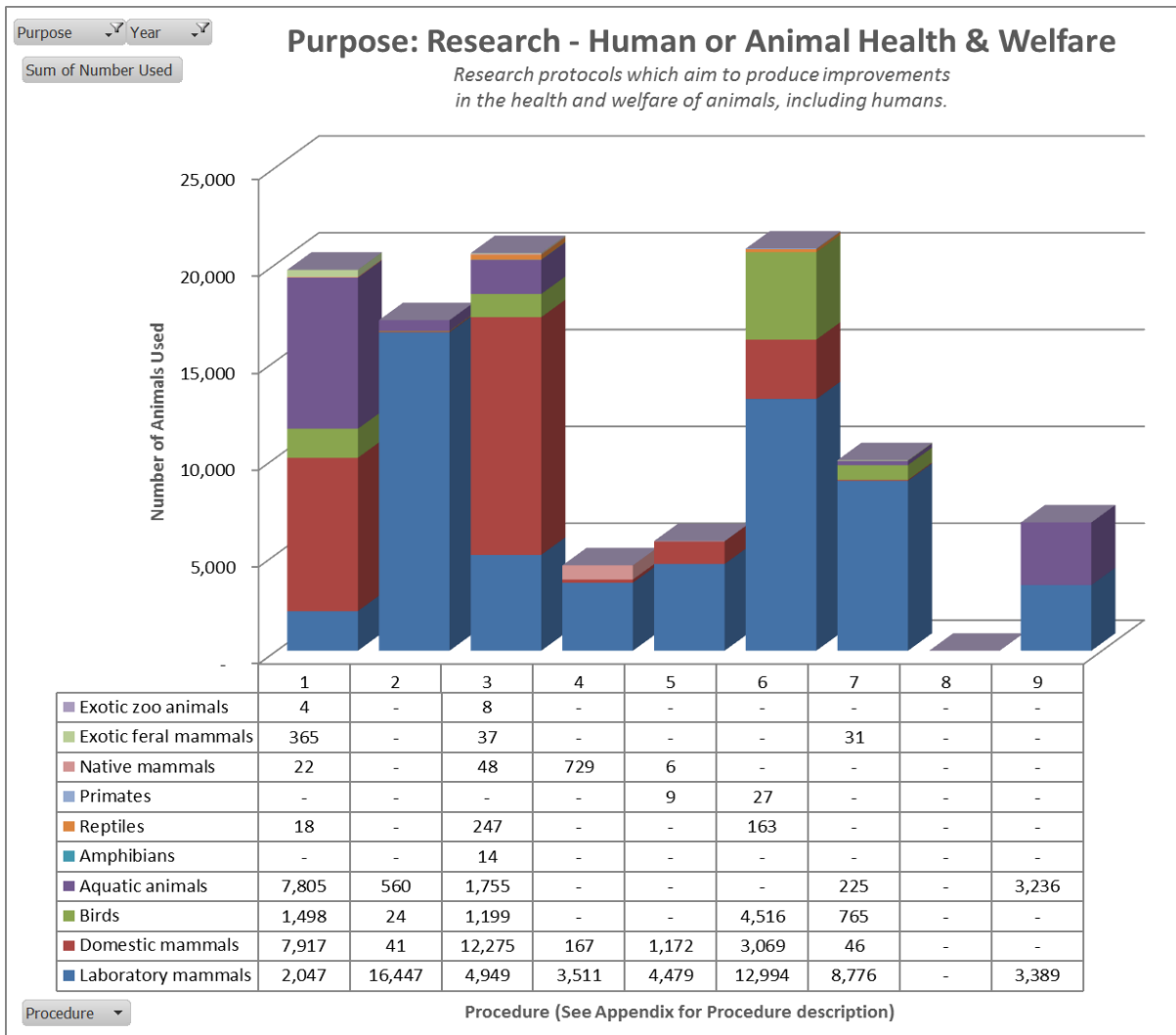




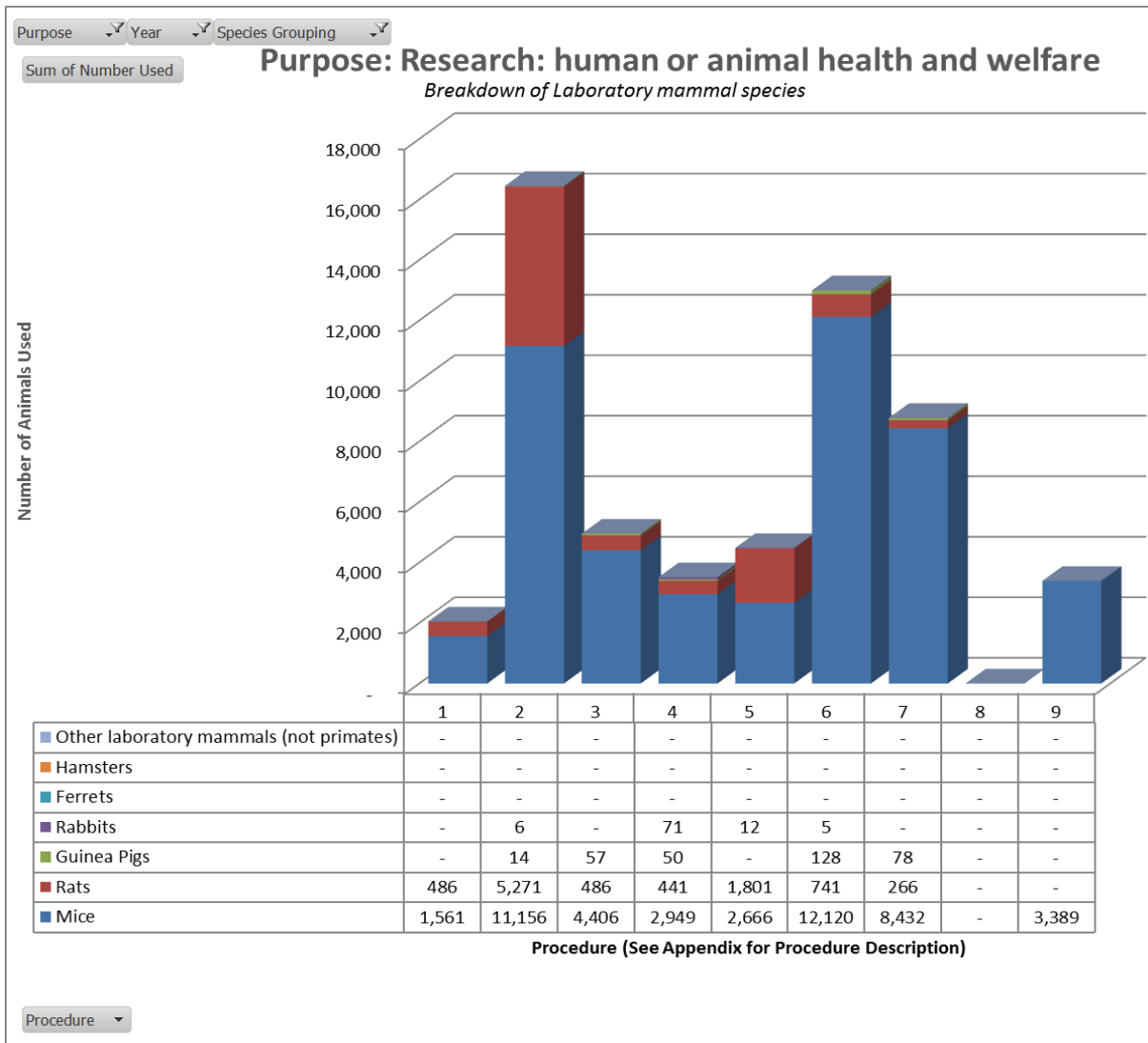


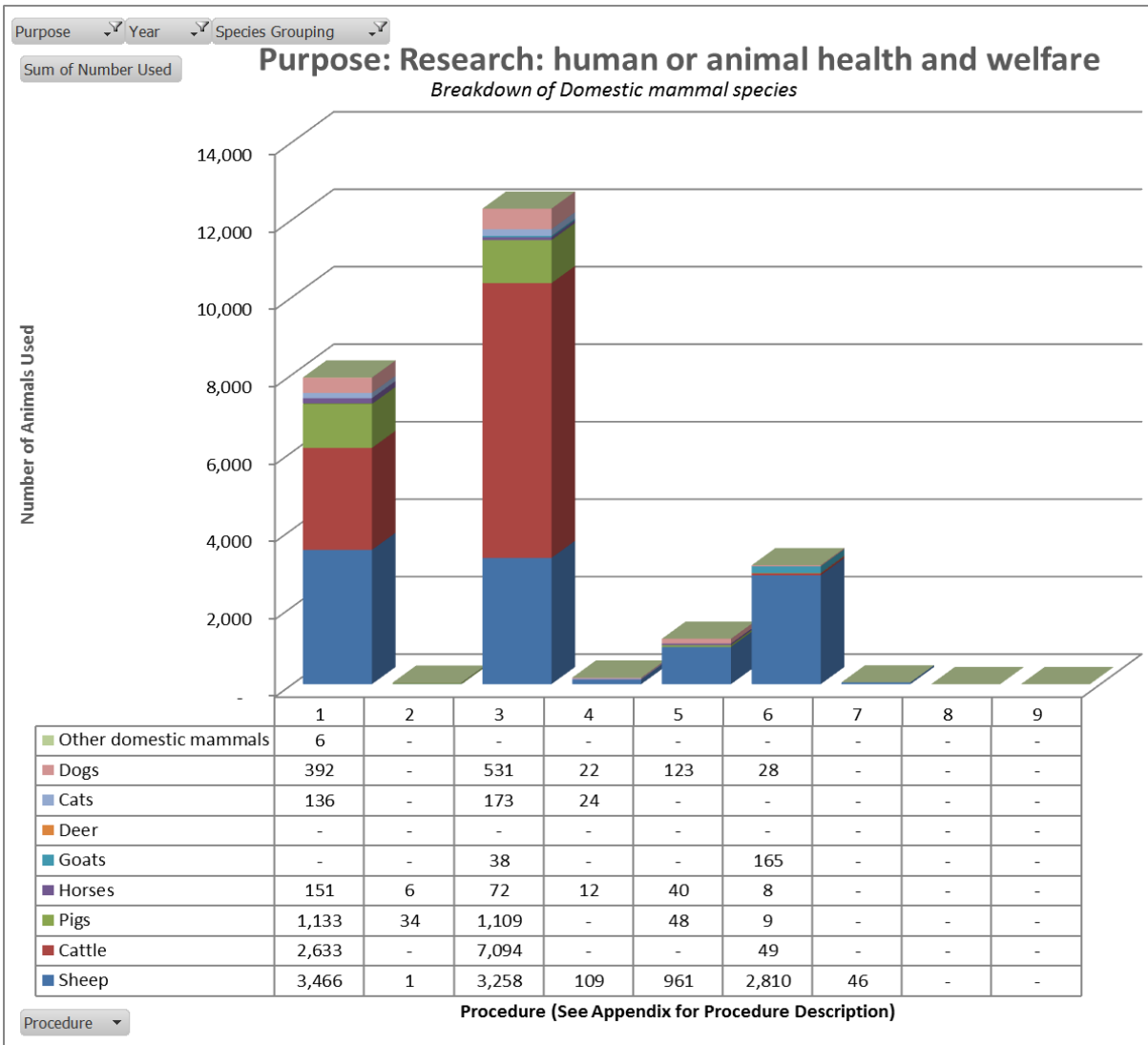


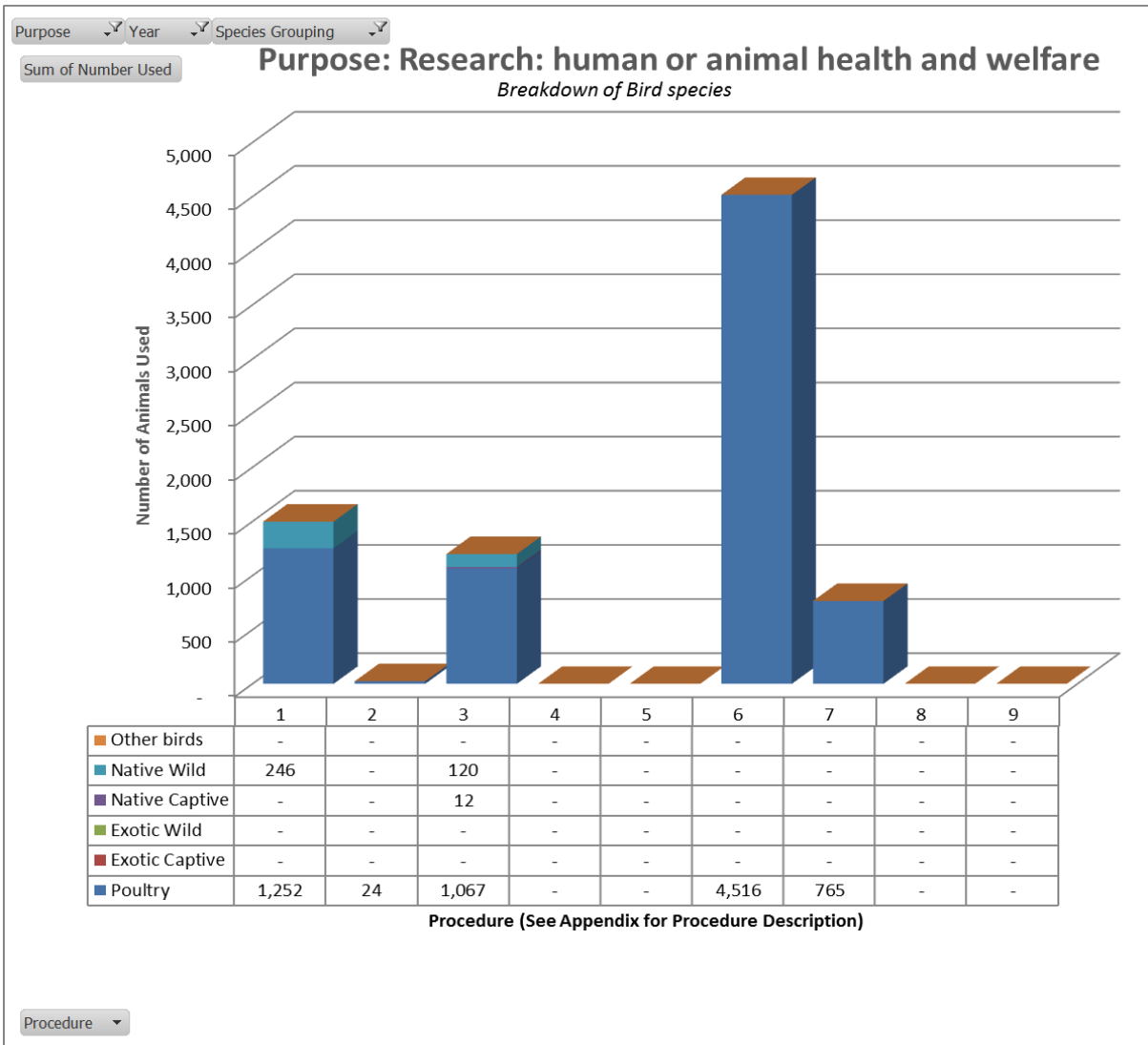
3.5 Research - Human or Animal Health and Welfare

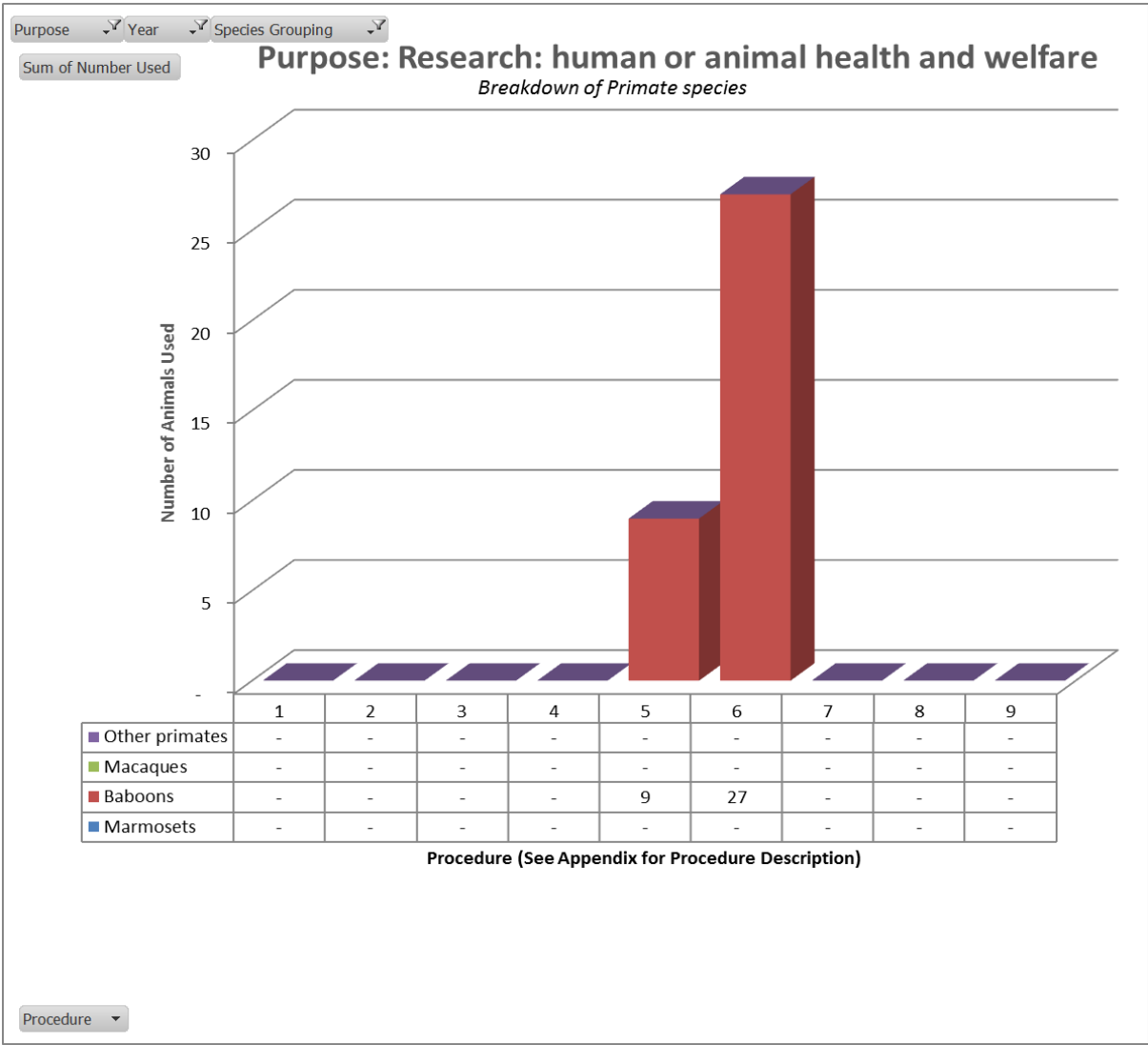


3.5.1 Species Charts for Research - Human or Animal Health and Welfare

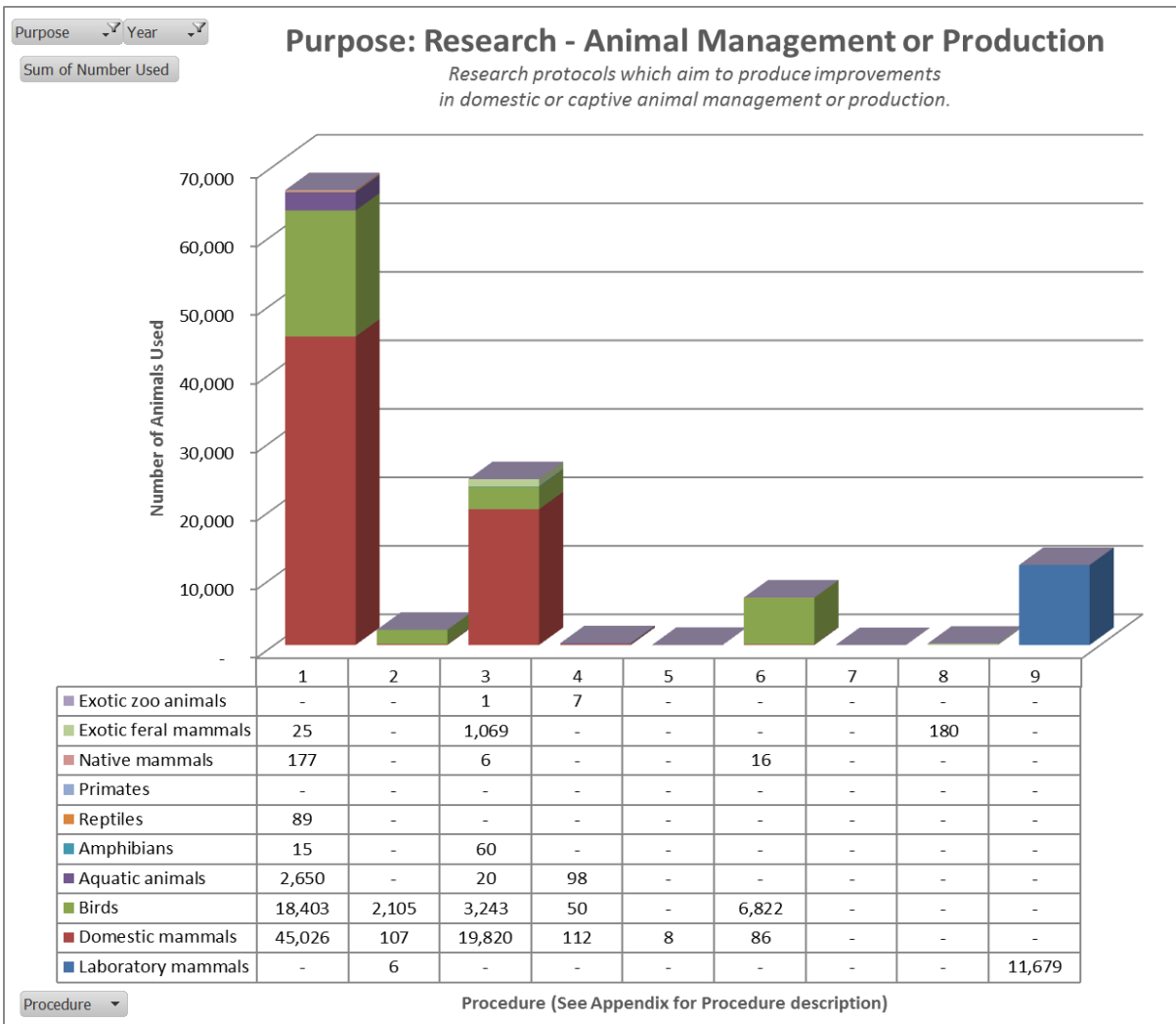




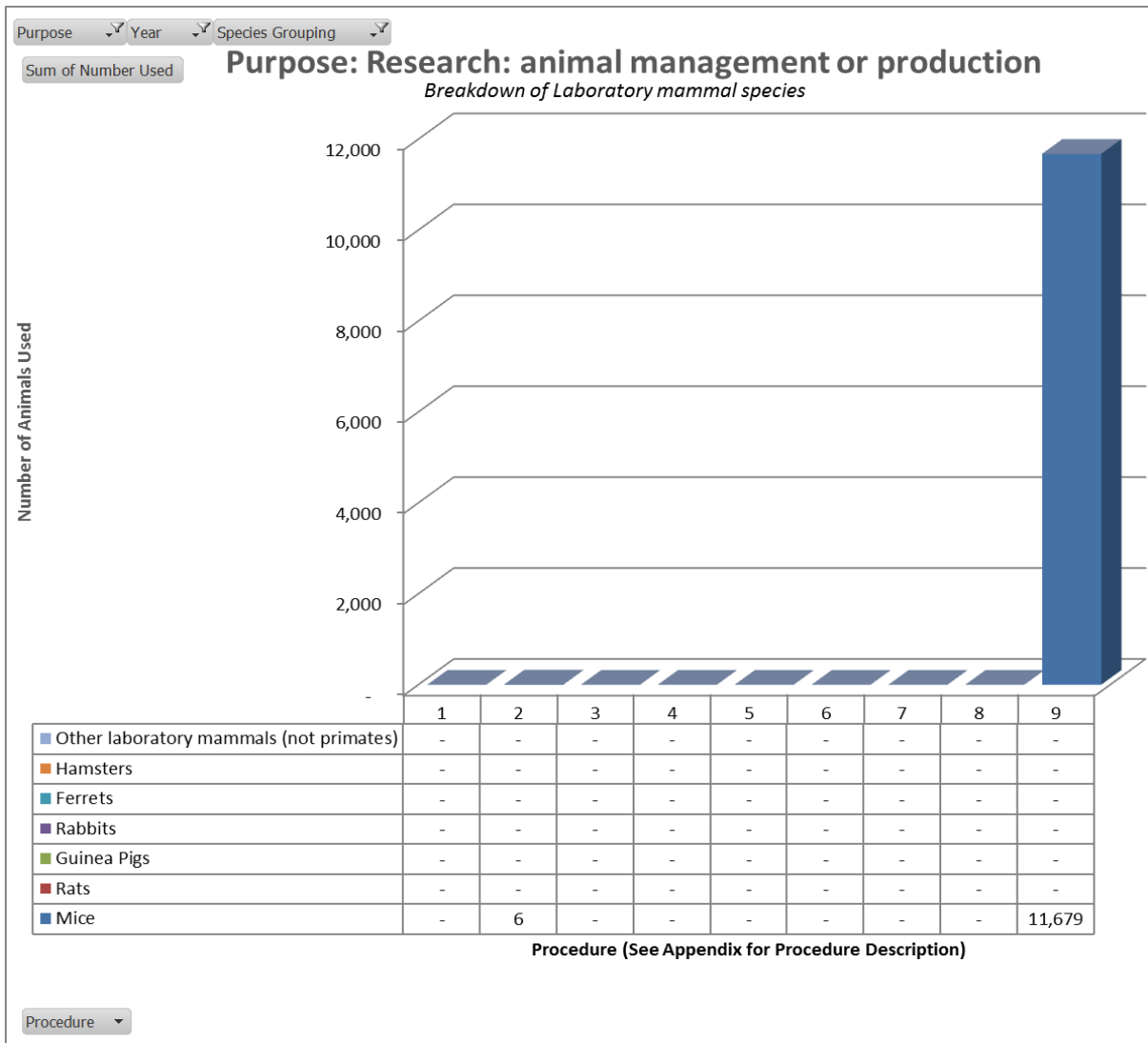


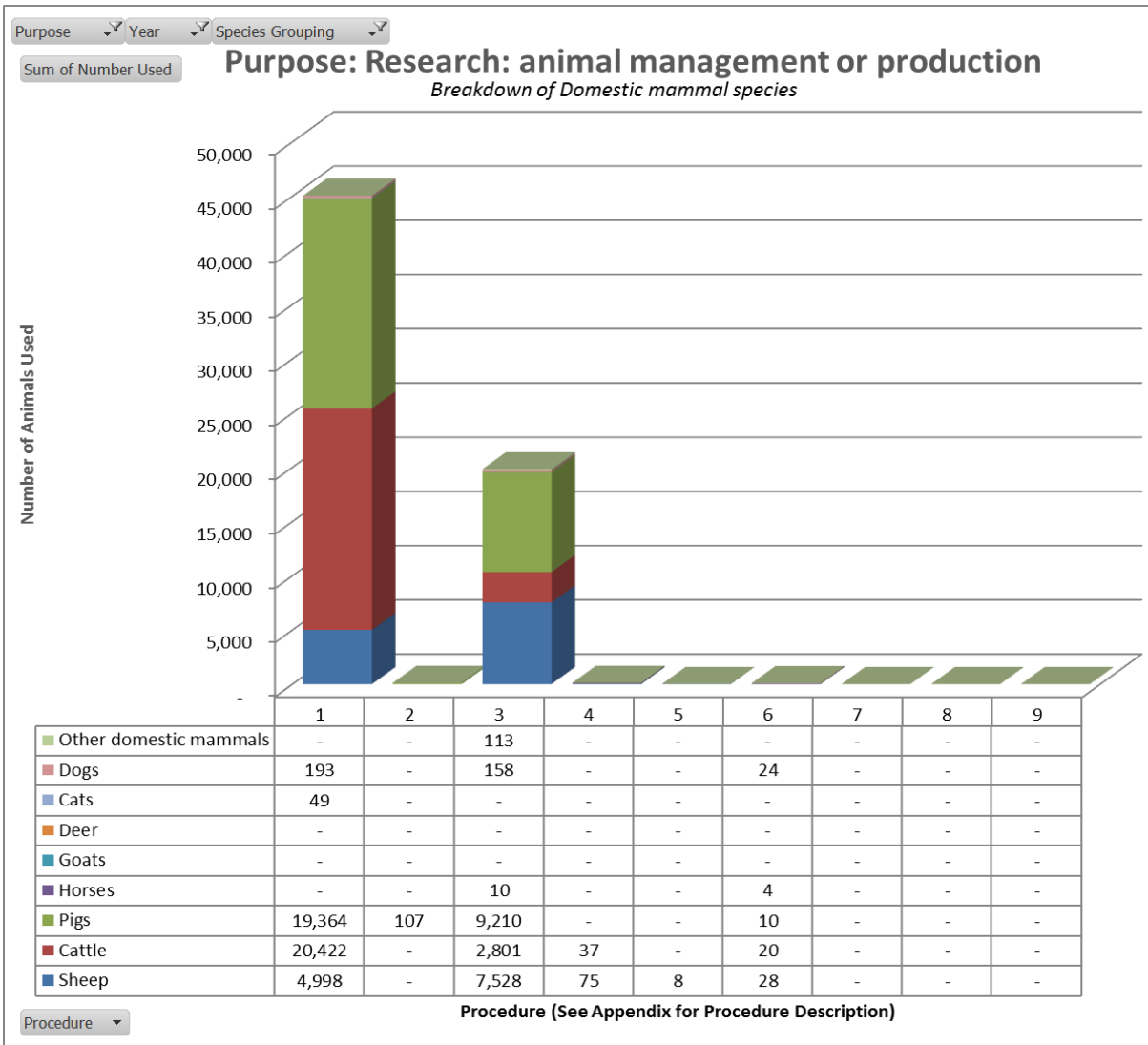


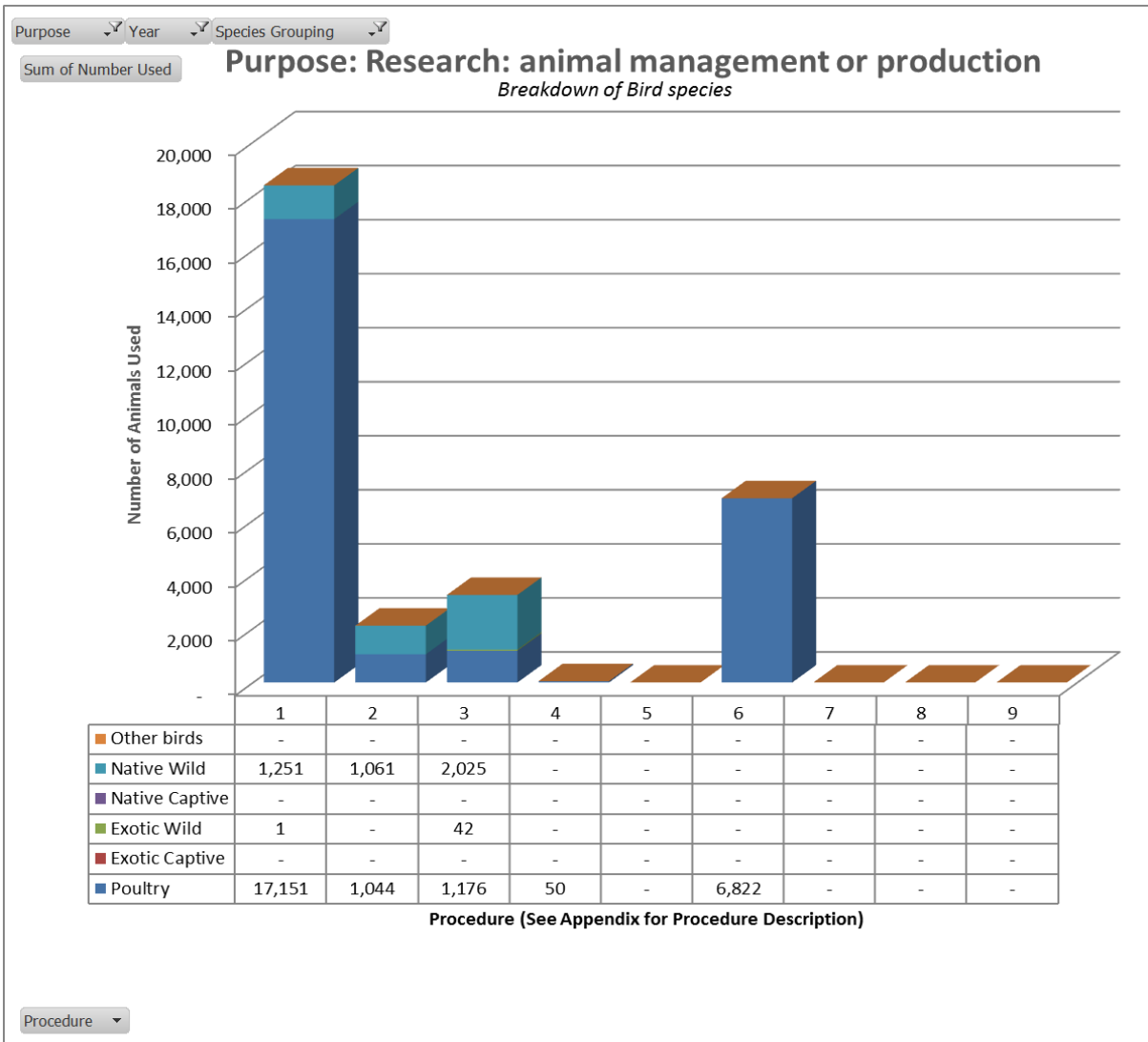
3.6 Research - Animal Management or Production



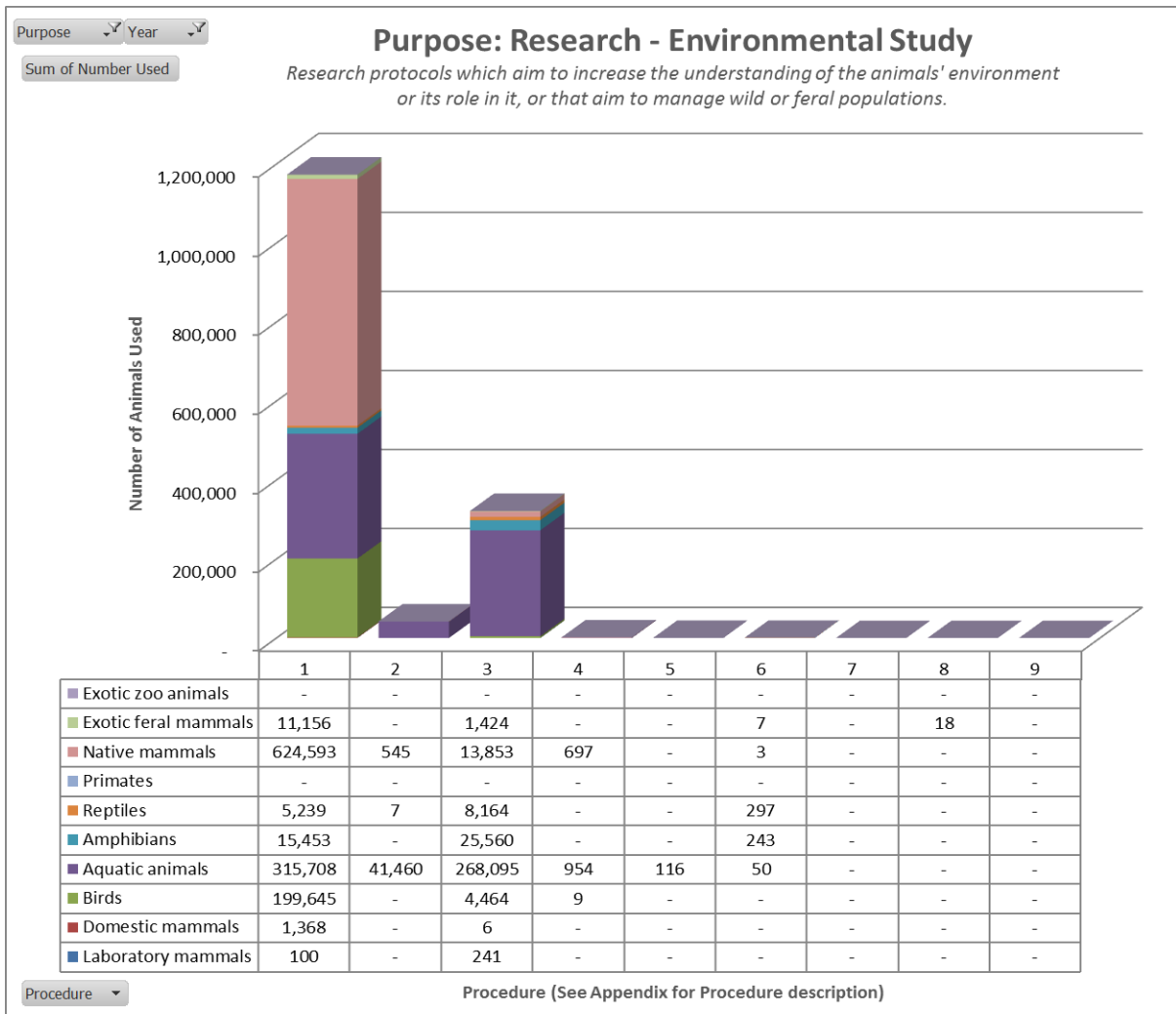
3.6.1 Species Charts for Research - Animal Management or Production



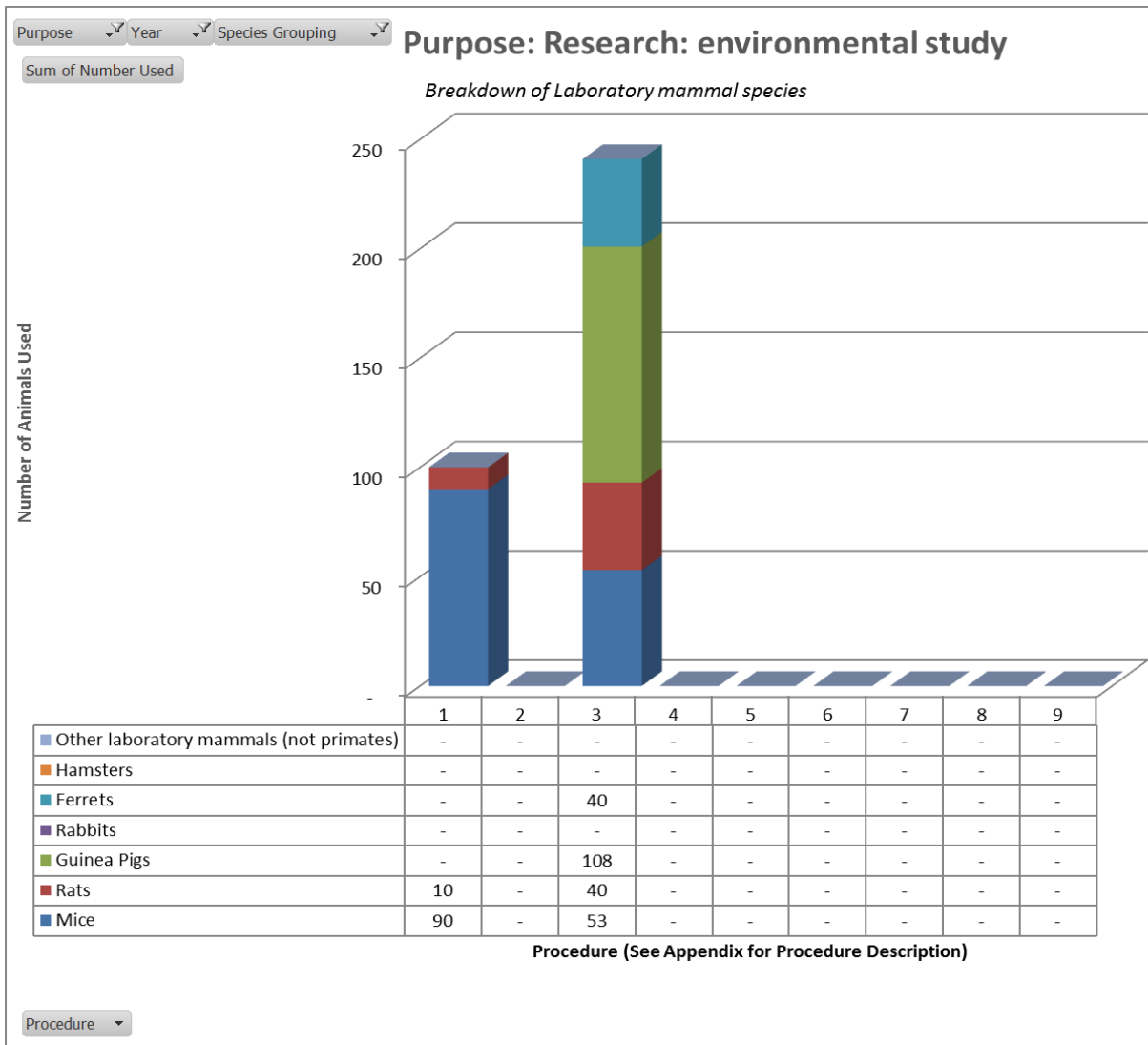


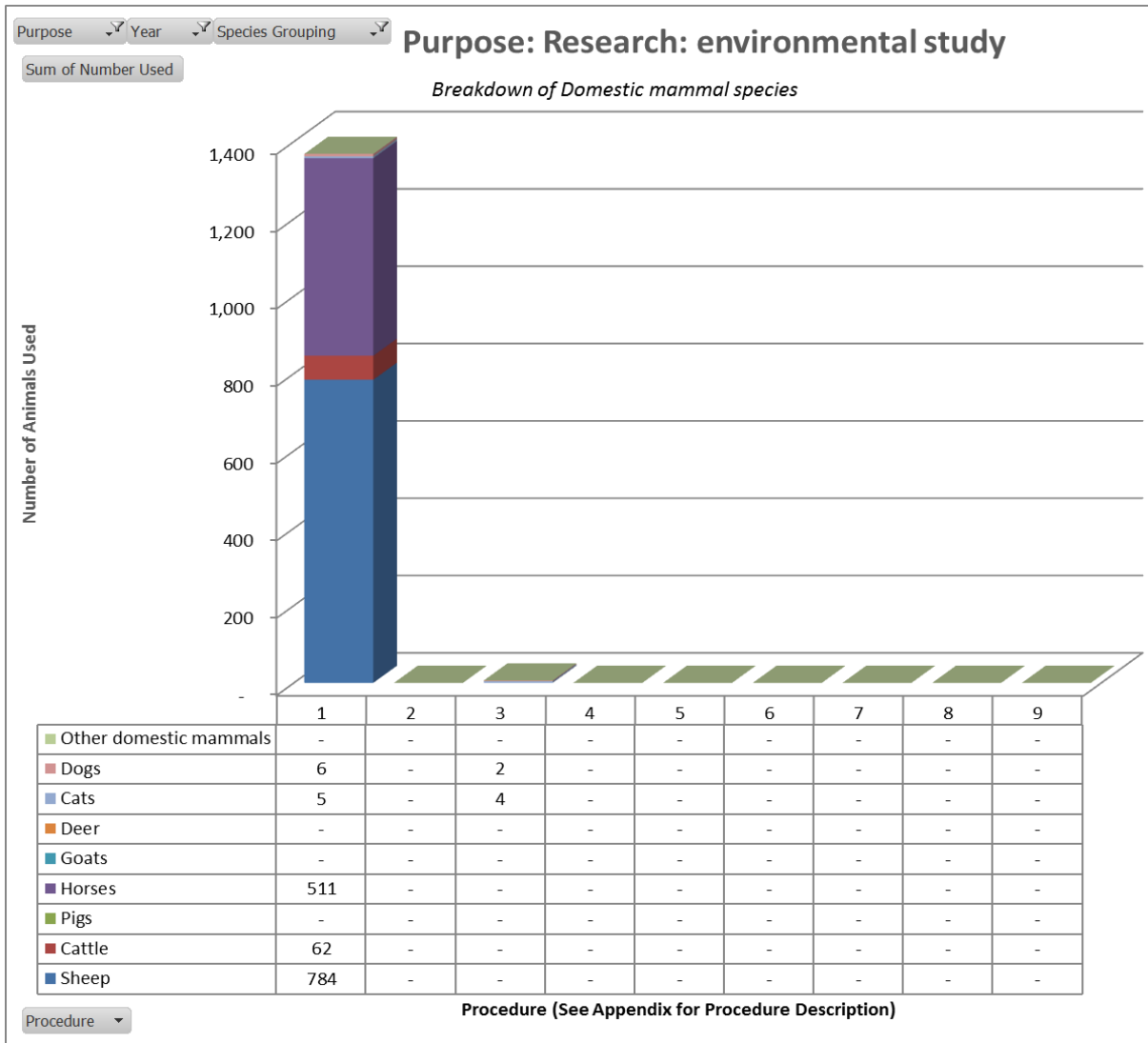


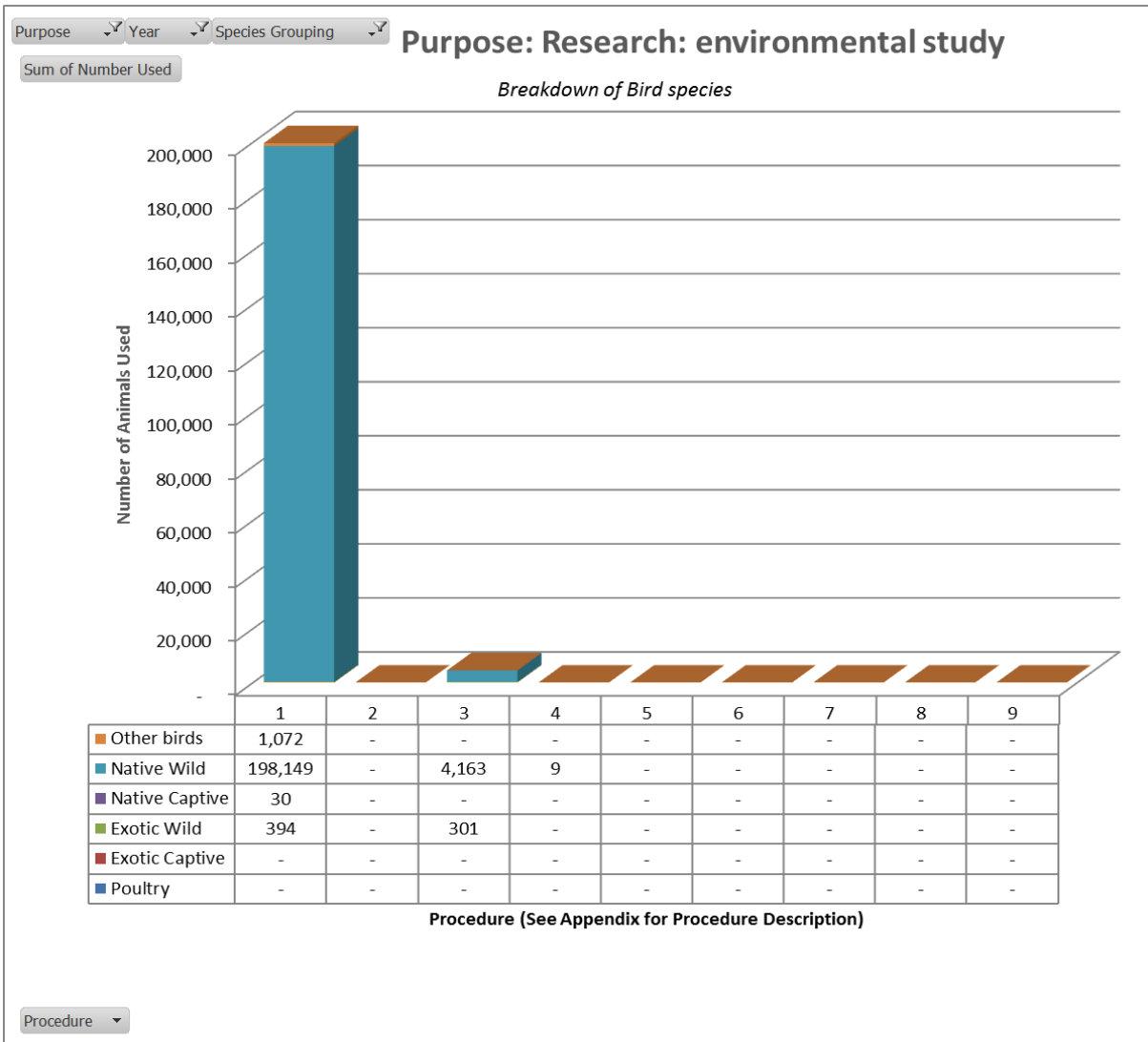
3.7 Research - Environmental Study



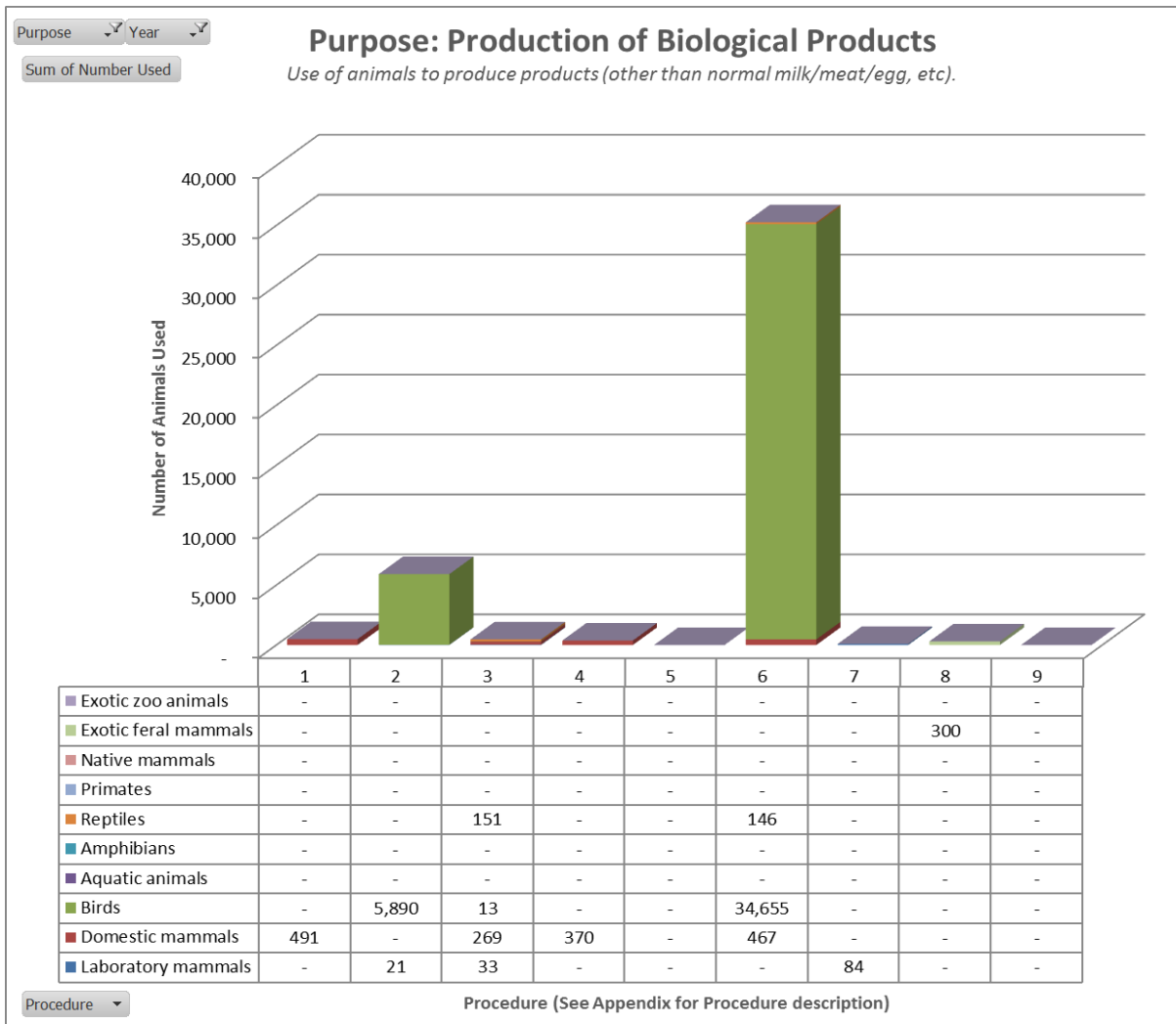
3.7.1 Species Charts for Research - Environmental Study



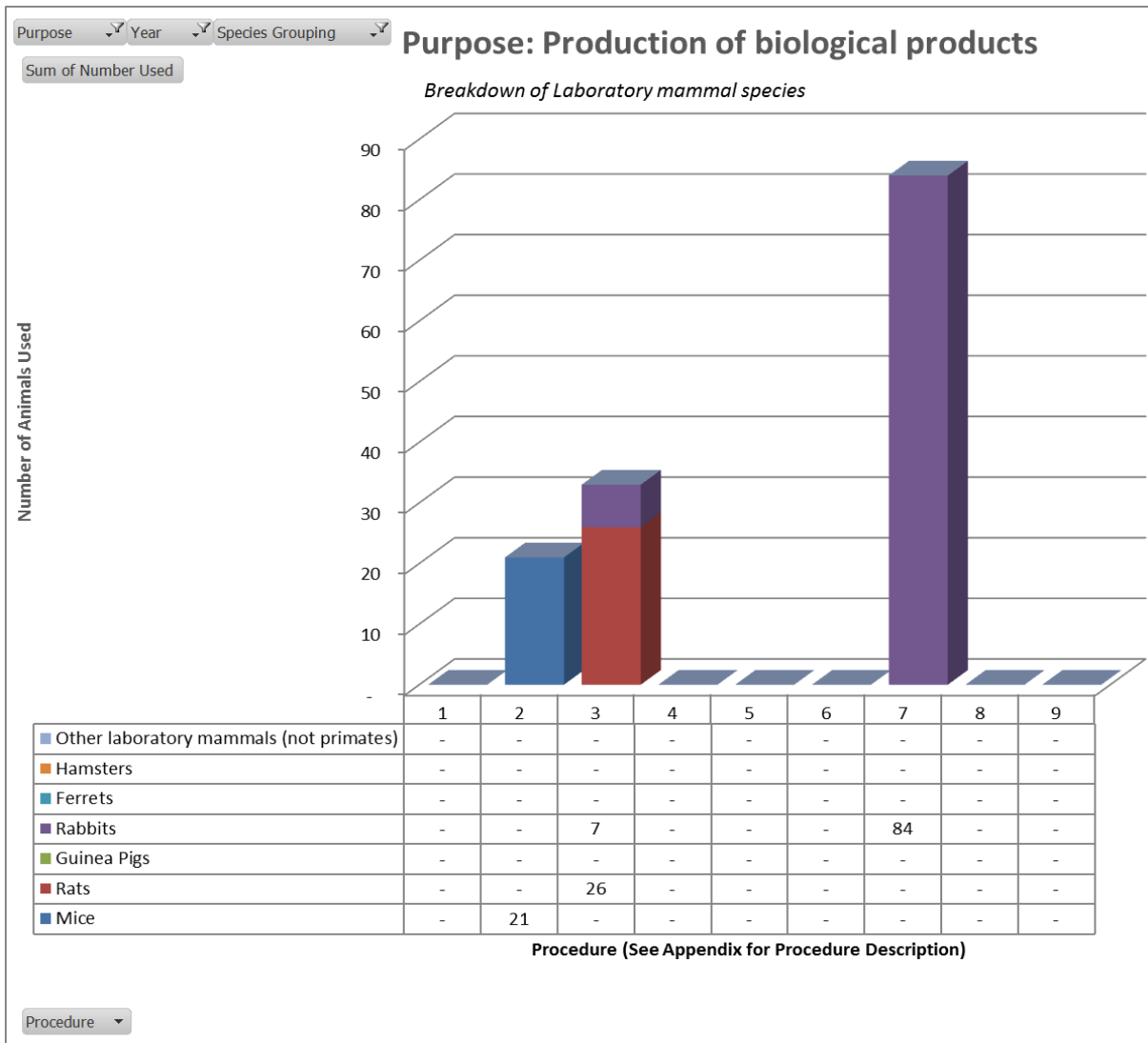


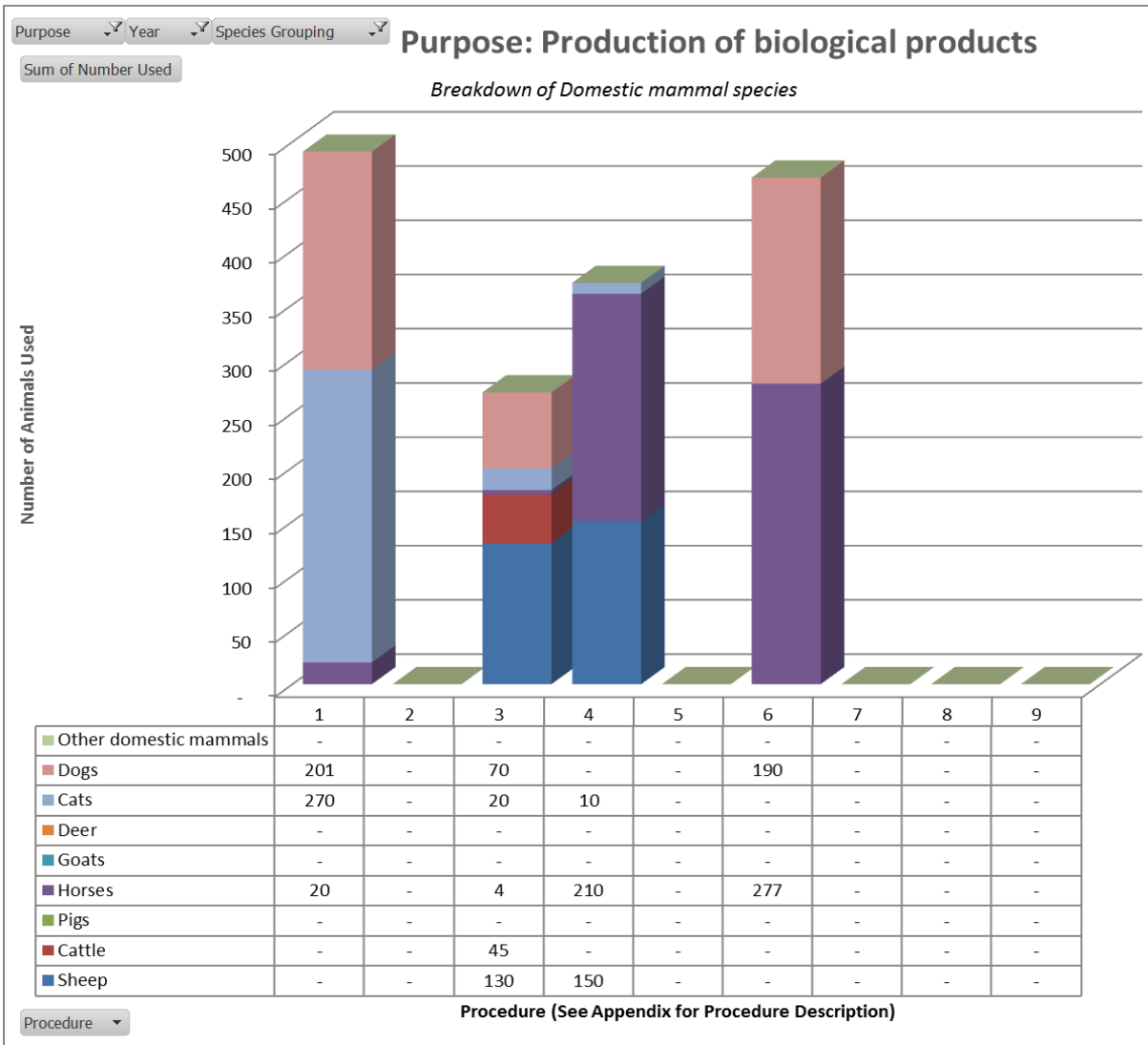


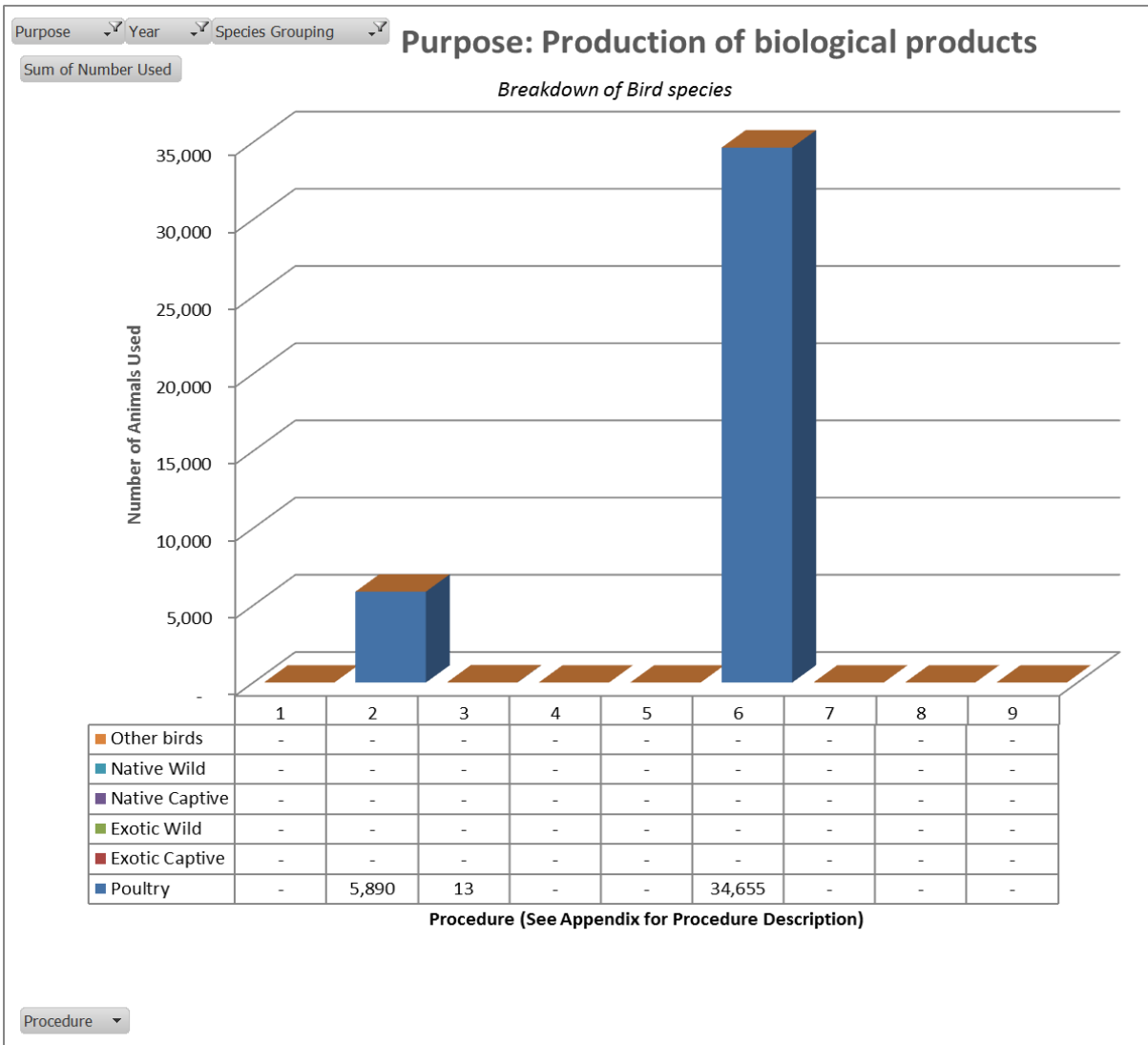
3.8 Production of Biological Products



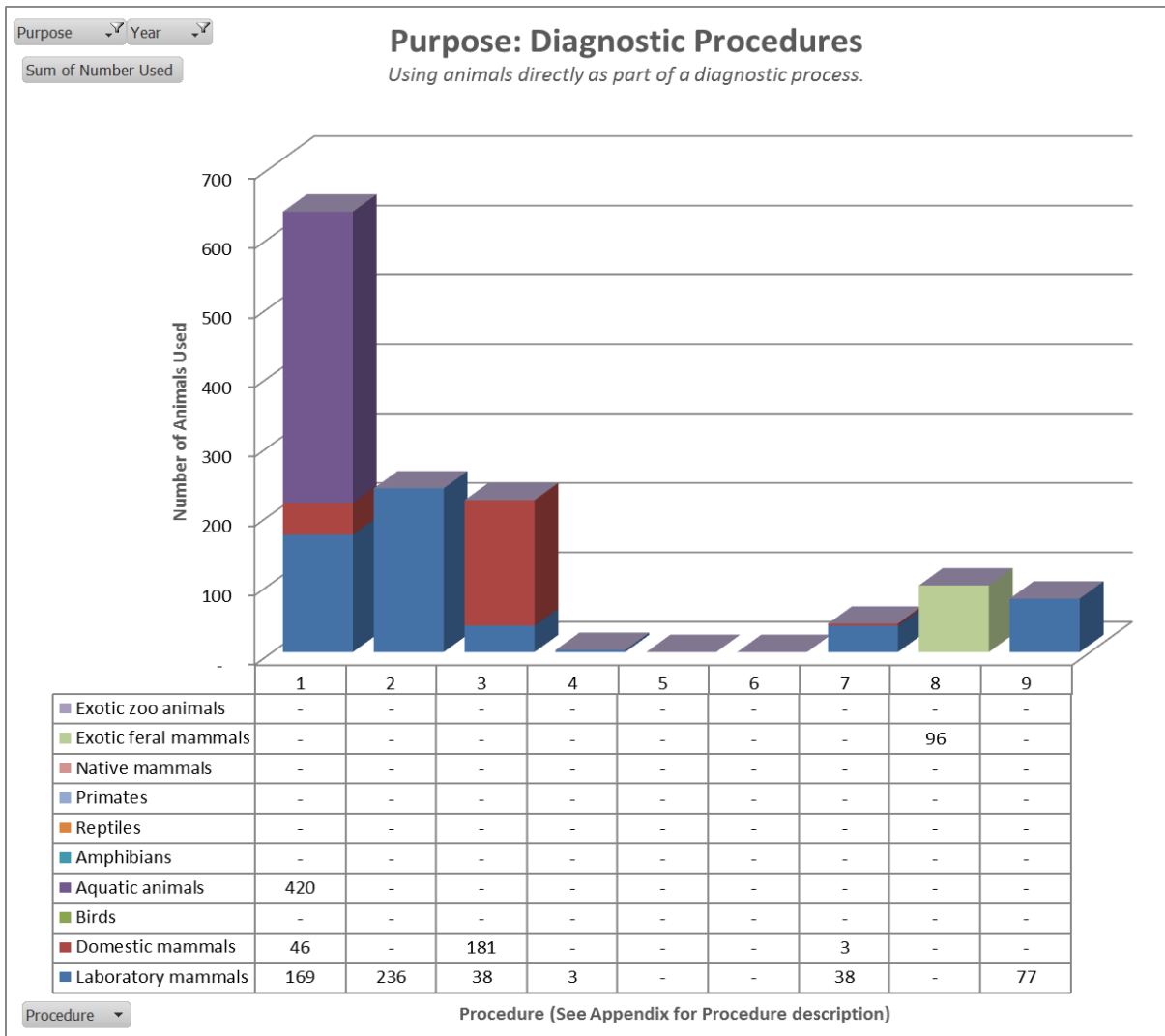
3.8.1 Species Charts for Production of Biological Products



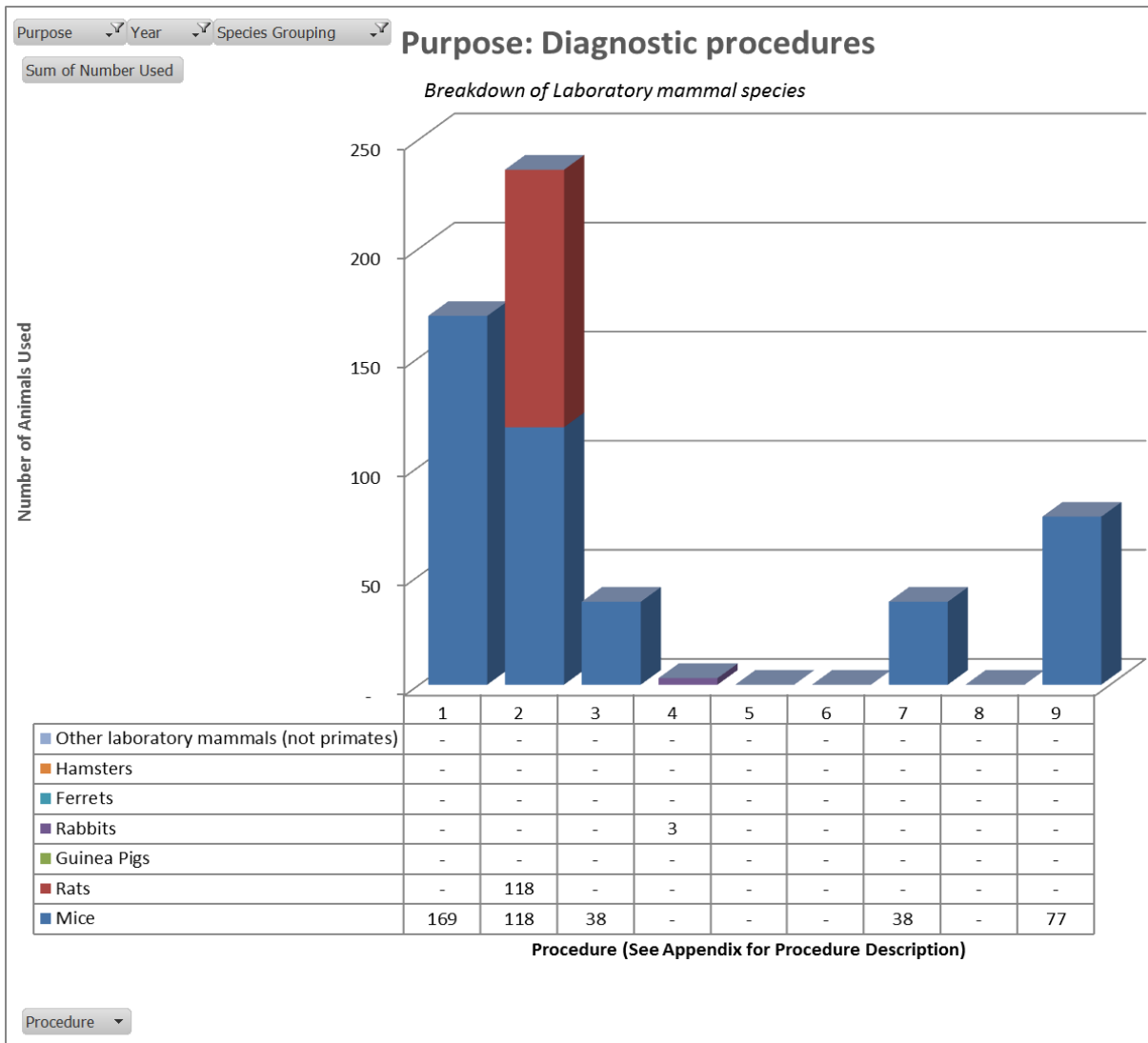


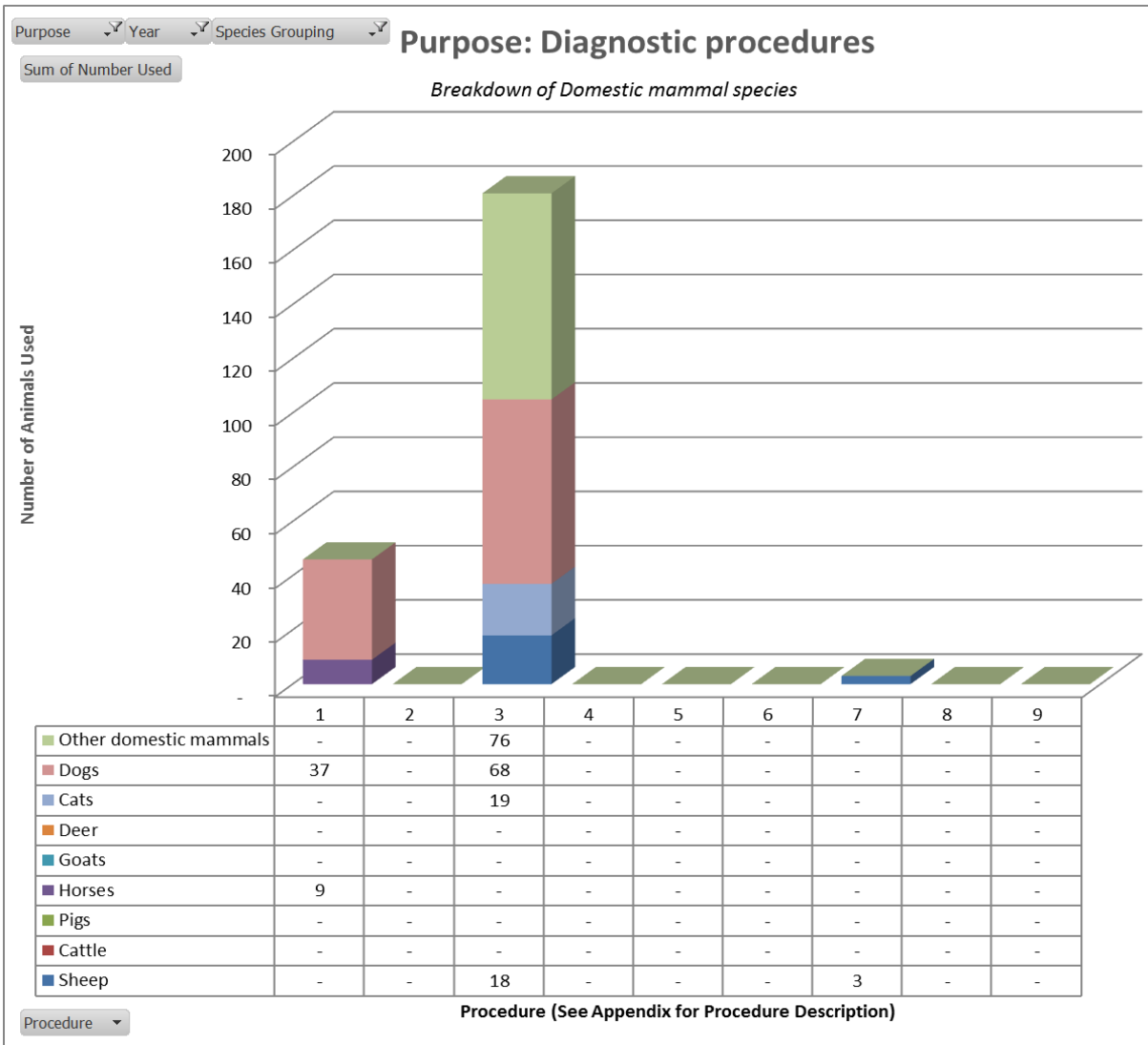


3.9 Diagnostic Procedures

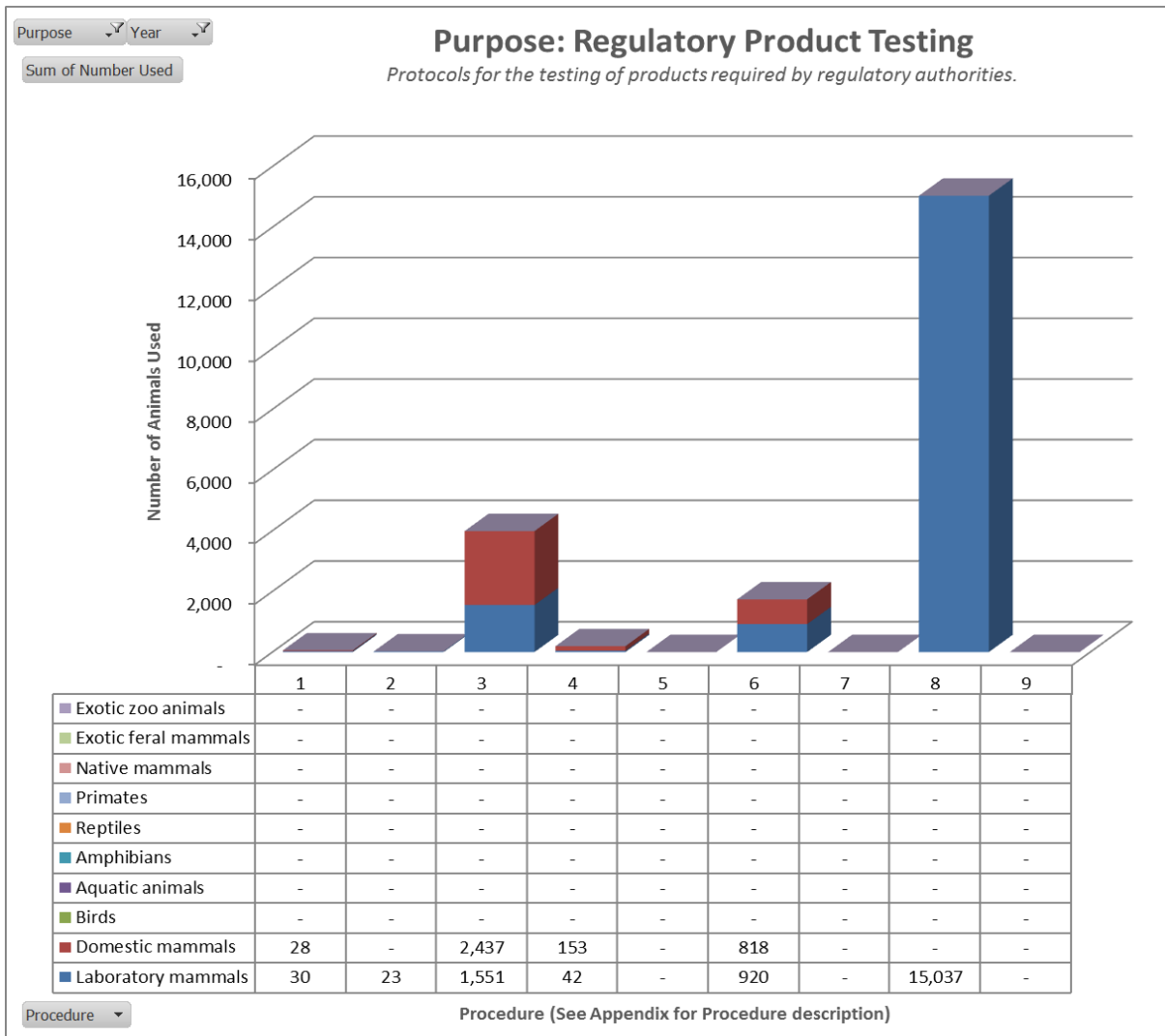


3.9.1 Species Charts for Diagnostic Procedures

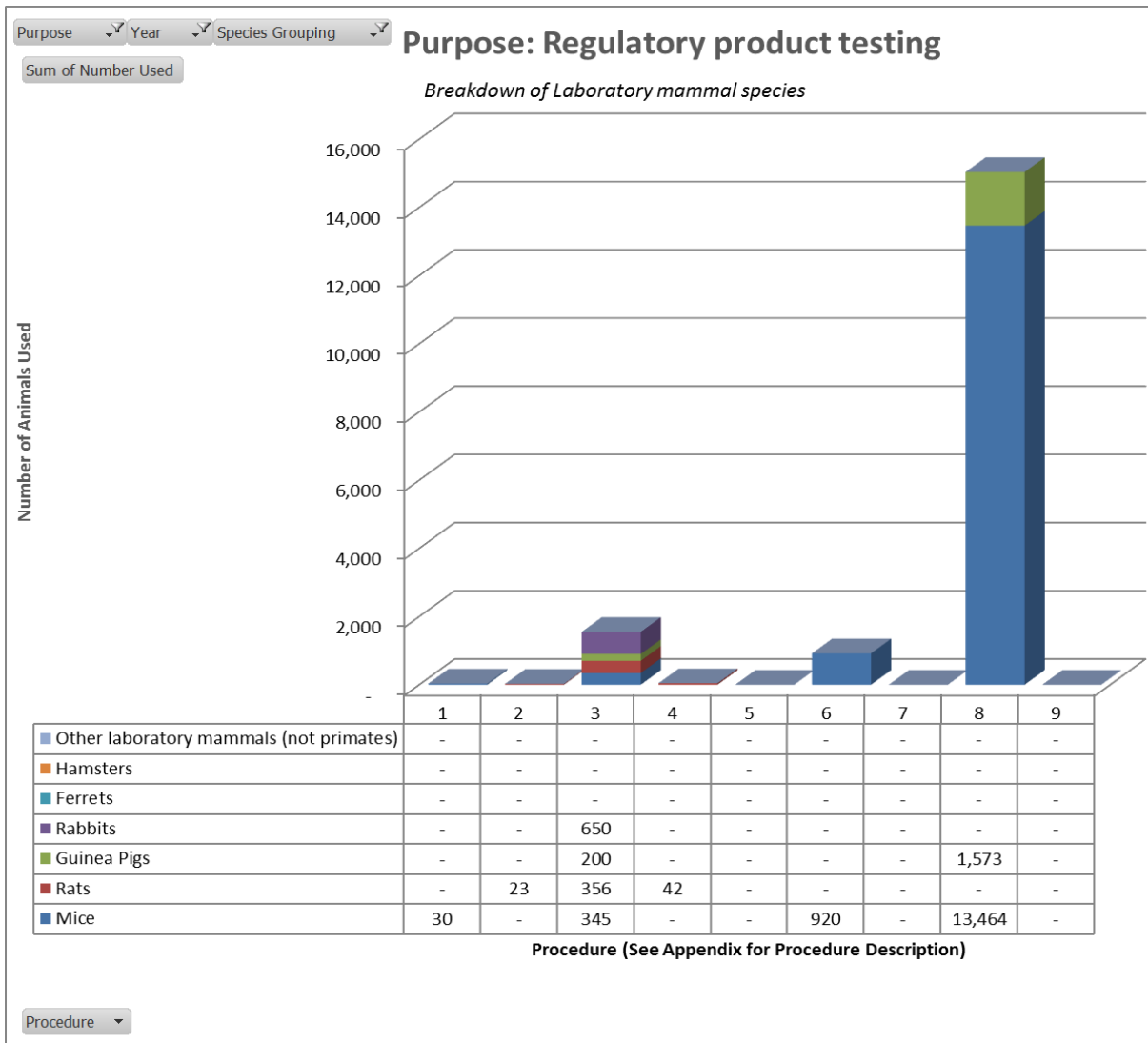


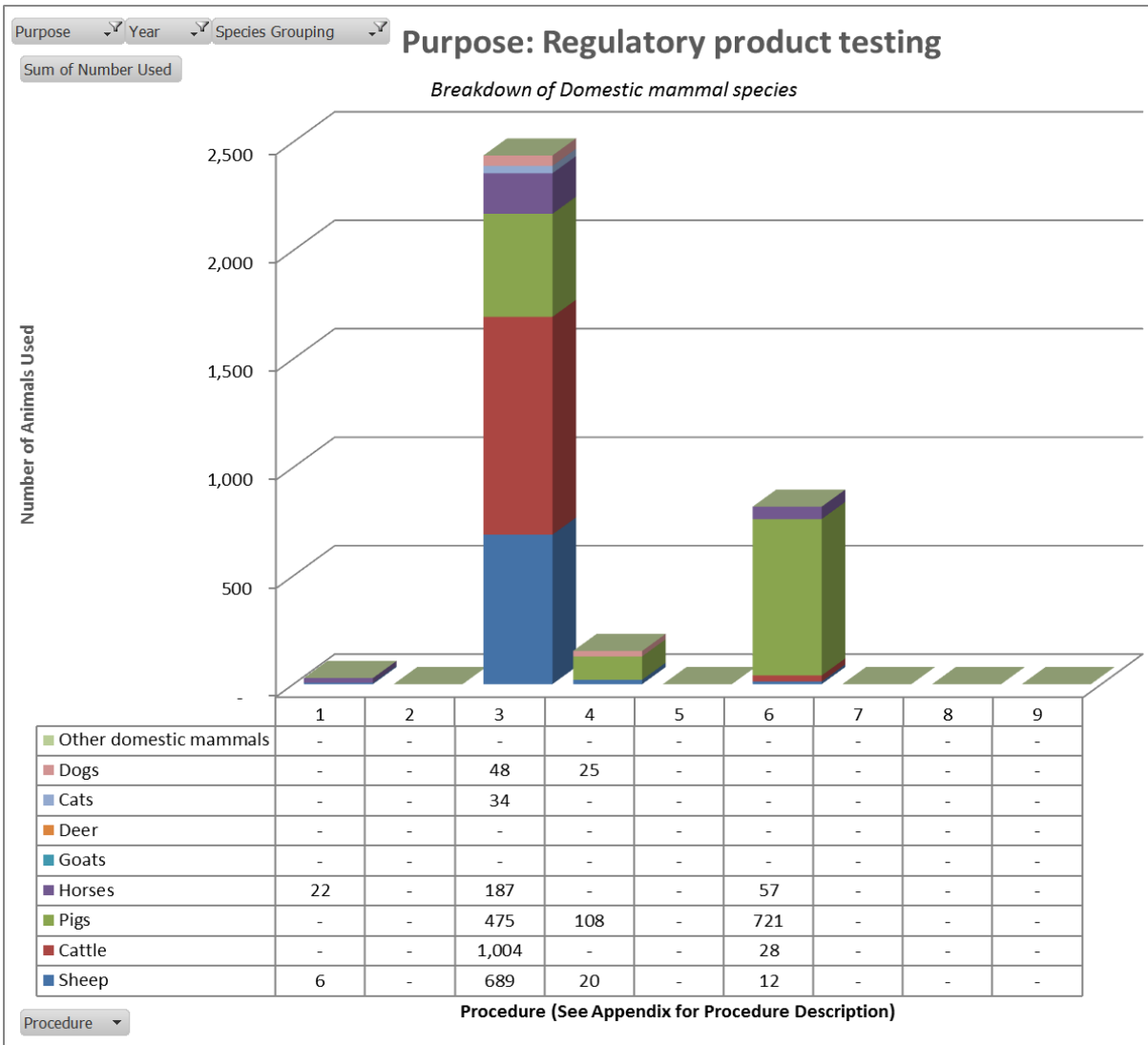


3.10 Regulatory Product Testing



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 2016.

| Species | Number used | Number died/ euthanased | Procedure | Justification | Alternatives |
|-------------|-------------|----------------------------------|---|---|---|
| Guinea Pigs | 1,573 | 343 | Vaccinated animals are challenged with test organism in order to demonstrate protection and hence vaccine efficacy. | Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. Assessment of in-process or development material to determine suitability for further manufacture. | This test is based upon regulatory guidelines. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop <i>in vitro</i> assays which may be used to replace existing <i>in vivo</i> assays subject to regulatory approval of these replacement assays. |
| Mice | 7,784 | 1,629 = died 405 = euthanized | 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 formulation. | 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 existing <i>in vivo</i> assays subject to regulatory approval of these replacement assays. |
| Mice | 4,034 | 1,830 = died 221 = euthanized | 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 dilutions to determine potency of antigen preparations. | This test is based upon regulatory requirements for the assessment of in-process products. There are no alternatives available at this time however the establishment has embarked on a long-term program to develop <i>in vitro</i> assays which may be used to replace existing <i>in vivo</i> assays subject to regulatory approval of these replacement assays. |

| | | | | | |
|-----------------------------------|-------|-------------------------------|--|--|---|
| Mice | 0 | 0 | Challenge of vaccinated mice with target organisms to demonstrate efficacy of vaccine. | Regulatory testing required to demonstrate efficacy (potency) of vaccines prior to release. | No alternatives at this time. |
| Mice | 1,328 | 371 = died 54 = euthanized | L+titration in mice: Susceptible animals are challenged with test toxin in order to determine potency of antigen preparations. | 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 existing <i>in vivo</i> assays subject to regulatory approval of these replacement assays. |
| <i>Mus musculus</i> – wild caught | 96 | Nil | Feeding trial | The eradication of ships rats and house mice is required to protect the biodiversity of the local ecosystem and remove identified threats to a number of threatened species on the island. The success of an eradication programme depends upon 100% of target individuals consuming toxic baits and dying as a result. Extensive domestic use of brodifacoum has resulted in the possibility that a proportion of the rodent population on the islands are resistant. Feeding trials using toxic bait and wild-caught rats and mice were carried out (and reported on) in 2013. The results of that work showed that a much higher dose than the accepted mouse LD50 was required to kill the mice. The 2016 experiment was necessary to confirm the operational efficacy | The alternatives—blood clotting response tests and genetic testing—would not supply the necessary information. |

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| | | | | of the rodenticide on the specific population of mice being targeted for eradication. | |
| Feral goats and feral deer | Unknown (depends on attendance of free-living animals at a feeding structure where a toxic bait may be delivered) | 18 (Feral goats) | Field trials using a targeted feeding structure and non-lethal or lethal bait types. | Negative impacts associated with overabundant pest herbivore species are legally well accepted, with feral goats <i>Capra hircus</i> listed as a key threatening process under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999. Additionally, herbivory and environmental degradation caused by feral goats and deer are listed as Key Threatening Processes in New South Wales under Schedule 3 of the Threatened Species Conservation Act 1995. 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. |

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 2016 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 analyses for statistics instituted to prevent under or over use of animals.
- Where possible, the number of animals used has been reduced, and in some projects opportunistically acquired tissues, and/or the use of remote camera surveys have replaced the need to directly work with live animals.
- 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.
- 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 are conducted to condition animals to study procedures.
- We make available samples that are collected opportunistically (under AEC approval) from animals under our care. Access to this important material reduces the need for additional interference with animals and has benefited many collaborative researchers through the years.
- Electronic weigh scales have been installed on each of the 3 farm sites for 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.
- Installed mineral dispensers on farms 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.
- 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.
- Selected a farm for project X that has a walk over weigh scale to reduce the handling of animals.
- Refined herd testing procedures from two milk collections down to one.
- Combining vaccinations with other routine management in to reduce the amount of handling of sheep.
- We will generate transgenic constructs of which expression is monitored by fluorescence protein expression. This technique will enable us to enrich embryos that possibly carry integrated transgenes, contributing to reduce the number of fish that will be raised as founder candidates.
- Since zebrafish are externally fertilised and embryos can be easily collected outside the mother, the proposed studies do not require interventions that would be otherwise necessary if mammals were used to perform similar studies.
- There will also be opportunities for the sharing of tissue from the pigs with other laboratories to maximise the scientific output from the animals.
- In order to reduce the number of animals used, the native heart removed from the recipient will be made available for studies extending from other protocols.
- The training and skill of technicians is important in minimising stress on the animals. The work of the technicians is constantly being reviewed under regular checks made as part of the quality control programme.
- We are using a mouse model in which the obesity/diabetes phenotype and the epigenotype of the responsible allele are tightly linked to the mouse coat-colour phenotype and thus can be assessed noninvasively, and in which obesity occurs spontaneously and does not require any dietary, pharmacological or genetic manipulation. In assessing the transgenerational effects of

parental obesity on offspring, we have chosen procedures that are as non-invasive as possible, and predominately involve post-mortem analysis of blood/serum and tissues.

- Animal usage is reduced by determining key time points during immune responses to focus studies rather than analysing at many different time points. Impact on animals is refined by examining immune responses over the shortest times required.
- We have proposed to use prostate cancer cells transduced with luciferase to induce tumours. We will be able to monitor the tumour growth at different time points by measuring bioluminescence. So same animal will be imaged at different time points without sacrificing the animal and this will reduce the number of animals four times (four time points).
- Use of audio-visual material such as videos, slides, interactive computer programs.
- Use of training models to teach techniques (e.g. latex rat for injections).
- Use of abattoir specimens and cadavers.
- Use of plant tissue as a replacement for animal tissue for certain enzymatic assays.
- Routine husbandry procedures to be performed on animals are coordinated with teaching activities.
- Use of animals killed in road accidents.
- Researchers moving away from primary cultures and using stem cell differentiation.
- Sharing of tissue among researchers.
- Use of pilot projects with reduced animal numbers.
- Obtaining more data from the use of fewer animals by combining objectives.
- Close scrutiny of the numbers of animals requested in applications and progress reports to the Committee.
- Incorporating animals from one project as breeding stock for a subsequent project, rather than discarding.
- Use of a device invented by a researcher to extend the life of neuronal tissue for electrophysiology and imaging which has resulted in less animals being used.
- Use of organs collected from culled mice for in-vitro testing (e.g. gastrointestinal studies).
- Improvements to animal housing and management (e.g. introduction of "buddy cages" to avoid single housing of mice).
- Training of researchers (animal monitoring and administration of injections).
- Working with researchers to develop better project specific monitoring checklists to identify, action and report adverse events and the development of an adverse event form.
- The use of less invasive procedures in wildlife studies e.g. sand pads rather than trapping.
- Developing competency assessment procedures.
- Providing alternative procedures to minimise impact.
- Program to develop alternatives to the use of guinea pigs and mice for vaccine production. The probability of success and estimated timelines are difficult to estimate, however a best case scenario for guinea pigs being replaced would be 3 years, and twice this time would be a pessimistic estimate.
- Continued to re-home excess guinea pigs during 2016. 92 guinea pigs were given to the non-profit organization Research Animal Re-homing Service in 2016. All 92 were in excess of needs and had not had any scientific procedures performed on them. By giving them to the re-homing organization, given homes rather than being euthanized.
- Made an enquiry to the APVMA to request written permission to cease the batch safety testing of new and improved formulations in the target species. This test was previously ceased for registered products but had been ongoing for any batches of vaccines that were under development. The establishment provided argument as to why it should not be required, including the fact that Europe no longer required this test. The APVMA responded and confirmed that the establishment could remove the requirement for Target Animal Batch Safety testing for any batch of vaccine including those in development. The AEC were pleased to see this reduction in the need for cattle and sheep. The application for Target Batch Safety Testing in both sheep and cattle was therefore not renewed as it is no longer a requirement of the regulator.
- Used a 'dummy' cow model to assess rain fastness and UV stability. The cattle hide was collected from a local abattoir.
- Used a fistulated cow model to measure rumen capsule payout rates. This reduced the need to sacrifice cattle each time the payout needs to be measured.

- Many studies involved a cross over design. This reduced the number of animals required in the study.
- A study where a number of objectives were combined in a study. This was done on a number of studies in 2016.
- House dust mite sensitized dogs are used for studies other than allergic disease. This reduces the number of individuals required to run the varied studies.
- Capsule measurement intervals were increased from weekly to fortnightly to reduce the impact on animals.
- 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.
- Dogs are trained to walk up a set of portable stairs onto a table to assist in blood collection and external parasite assessment.
- Suitable pain relief is always used for any intensive or surgical procedure.
- An artificial intelligence deterrent system uses lights and noise to deter animals considered as pests from crops and eliminates the need to shoot or poison them – the project itself is an example of the 3Rs.
- Collecting samples from recently dead flying foxes found in camps to reduce the need to capture live animals.
- The team have no interaction with the animals; camera technology allows them to monitor birds from significant distances.
- Reduce the duration of observation periods; look for evidence of presence rather than seek to observe the animals (ie. evidence of eating).
- Increased focus on avoiding trapping in adverse weather – this involved the postponing of a number of scheduled field trips.
- Reduced (from 14 to 1) the number of sites using snap-traps (kill-traps). Capture and release traps used instead.
- Uses a mob of fistulated sheep to allow connection of rumen fluid. Maintaining these animals for periods of years allows the continued collection of this fluid without the need to perform this surgery on additional animals.
- Undertaking observational studies or the use of motion detection cameras, rather than capturing animals, has proved useful in a number of instances.
- Where applicable in vitro studies are used. Results from previous studies are used to reduce the number of animals required for some experiments.
- Video recording of experiments, especially behavioural experiments, enables future use without the need to repeat the experiment.
- Where suitable, non-animal models are employed in training e.g. neoprene/cork to train in suturing techniques, latex (Koken) rats for IV tail injection or gavage. While these techniques are training aids, ultimately live animals are required in order to train to and assess competency.
- Studies are co-ordinated, where possible, to enable the sharing of tissues and surplus animals by other investigators or as a teaching resource.
- The establishment maintains and, whenever possible, generates a tissue bank, so that tissues not required for immediate use can be preserved for future use.
- Where animals are used in teaching, animals are allocated to small groups of students rather than to individual students.
- When designing a project, investigators often refer to previous or similar studies to ensure that work is not being unnecessarily duplicated.
- A pilot study or proof of concept study is employed by an investigator before proceeding further.
- Investigators seek the advice of a biostatistician, where appropriate.
- Experiments are sometimes run in parallel using just one control group.
- Pooling of samples is undertaken whenever possible.
- The Committee requires studies to be concluded when sufficient data has been collected and not at the duration of the time approved or when the full allocation of approved animals has been exhausted.
- The minimum number of animals are used at each stage to provided statistical significance.
- It is common to undertake a literature review or use previous research data during the planning stage of the project.
- Animals not required are allocated to other projects, where possible.

- Animals are sometimes used as their own control, resulting in a greater statistical power and decreasing the number of animals required.
- Wildlife studies use traps that are appropriate for the size of the animal being studied, thereby reducing unwanted by-catch.
- Investigators share research information, results, models and methods to avoid repeating work and to ensure they are using the most appropriate methods that will lead to valid results while minimising the number of animals required.
- Some research projects are undertaken collaboratively with other institutions.
- Surgical procedures are performed under general anaesthesia, with appropriate peri- and post-operative analgesia and antibiotics (where appropriate).
- Refinement of experiments is based on previous experiments and research outcomes.
- The capture of native animals and aquatic life is always undertaken with minimal interference to the animals. Animals are released as soon as data has been collected, at the point of capture, and at a time when environmental conditions are most suitable.
- All procedures are carried out by competent, qualified technicians or experienced investigators/staff, following AEC-approved SOPs and industry best practice.
- Inexperienced personnel work under the direct supervision of experienced personnel until competent.
- Pitfall traps are provided with shelter materials (sand, leaf litter, cloth and PVC piping) for animals awaiting release. Traps are checked early each morning and each evening to avoid animals spending more time than necessary in captivity.
- Insect surface sprays are used around pitfall traps to reduce possible irritation from ants, for example.
- A number of experiments have been designed to measure physiological parameters employing minimal, if no, handling of animals, while others are purely observational or use infra-red cameras.
- Where appropriate, animal behaviour is captured using motion detection cameras placed at a distance so as not to disturb animals.
- Exposure times for testing behavioural responses in experiments are limited to as short a time as possible.
- Attention is paid to good housing, care, feeding, handling, transport, and monitoring of each species at all times.
- Wherever possible, social isolation of animals undergoing experimentation is avoided.
- Proper attention is paid to monitoring all animals during and after all procedures.
- The AEC always tries to ensure that projects are designed to reduce the need for repeat procedures, and stress on animals.
- Data loggers are used for data collection to avoid strict confinement of animals.
- Use of captive wildlife is preferred to use of wild specimens as they are accustomed to human presence.
- Invasive techniques are revised as new information becomes available.
- Mannequins, audio-visual materials, photographs, taxidermised and preserved specimens were used as substitutes for live animals where the learning outcomes were able to be met by substitute means.
- Injection and ear-tagging of sheep practiced on cardboard and leather.
- Use of mechanical horse for horse riding and racing training.
- Bandaging and health care procedures are performed on dummies.
- Life size fibreglass replica of horse used for demonstrations.
- Use of photos and wool samples to identify breeds of sheep.
- Use of dog and cat cadavers for anatomy studies and injection practice.
- Visit to zoos, aquariums and museums to familiarise students with a range of native animals, eliminating the need for field visits or trapping.
- Use of case study data to eliminate need for live animal data.
- Co-enrolment and work on distance learning methodology, so that there is no need for live animal use.
- During shearing training the learner shearer begins by shearing only part of the sheep, with the professional shearer completing the task, to reduce handling time, injury and stress.
- Horses are monitored for behavioural changes and replaced regularly. Horse usage is rotated to prevent overuse.
- Using treats for medication.
- Reduction of lamp size to less intense light; use of red light covers for spotlighting activities.

- For native animals, handling is conducted by the licensed person only, with students observing the techniques.
- Animals are given appropriate rest periods.
- Designated animal carers attend class with animals to ensure the animals are not unduly stressed or over-used.
- Use of instructional activities that maximise students' competence in handling animals.
- Professional development for teachers to improve skills and knowledge.
- Use of industry sites where animals are housed to minimise stress.
- All fauna survey techniques follow guidelines approved by National Parks and Wildlife and Local Land Services.
- Miniature pony used in place of foals for handling and health care procedures.
- Uncomfortable procedures e.g. temperature taking only done once.
- Students are referred to Standard Operating Procedures prior to animal use.
- Following animal welfare procedural guidelines.
- Training of teachers in recognition of signs of stress and distress in animals.
- Simulated penning of sheep by demonstration.
- Minimum number of animals used in teaching activities.
- Use of ultrasound equipment to capture images (cattle) for replay to students.
- The number of occasions that an animal is used is minimised e.g. lambs are tagged and drenched at the same time as normal management schedule.
- Working with other establishments on native animal projects rather than duplicating own projects.
- Timetabling of classes is coordinated so that activities are spread over the semester, to avoid over-use of the same animal.
- Appropriate animal to student ratio.
- Native and feral fauna are only involved in limited field surveys under natural settings and no repeated exposure of any individual animal to survey techniques.
- Cattle and sheep are used on farm in industry environments. Animals are divided into groups and these groups are not reused for health applications and not more than twice for drafting.
- Students attend various workplaces to reduce the use of a particular mob of animals.
- Animals are placed on a rotation roster so they are not overused.
- Weighing and husbandry of cattle are carried out as part of their normal, regular commercial schedule.
- Use of a booking system and individual animal records to record animal use.
- Field work involving the trapping of animals is minimised and related to other contact with animals such as zoo visits.
- Maximum amount of data and student/animal contact is derived from each trapping in order to minimise trapping requirement.
- Activities carried out as part of animals' routine farm husbandry schedule.
- Using a radiograph based end point for retention meant that the teat sealant could be viewed in situ in the teat sinus, and may mean that comparison with the Reference Item is more acceptable to regulators without having to conduct large scale field studies. The difference in viscosity specifications for the investigational product was tested in this shortened study.
- Additional published data on the use of progesterone will be used to augment the application, so fewer repeats of the studies will be required. In addition comparative PK data will be used to demonstrate progesterone release in adequate amounts to result in clinical effects required to gain claims.
- As the study progressed it became apparent one of the investigational products would not proceed to market, and investigation of this product ceased. The conduct of the study, communication with co-operators and monitoring of sites was continually improved over the 18 enrolled sites. This ensured the procedures ran smoothly, all personnel were adequately trained and that co-operators and monitors were reporting any potential issues regarding animal welfare as soon as possible. An amendment for three sites was written to reduce the study period.
- This pilot study ensured that the WOPA crush used to handle the cows for radiography in subsequent studies was suitable and did not cause distress to the animals.
- An electronic scanner was used to read the identification of each heifer, so the heifers were less distressed with handling. For example, most heifers did not need to have their heads caught in the crush bail or their heads or ears handled, procedures cattle dislike. In addition, procedures of identification and GnRH injection were conducted in the pre crush race area prior to the FTAI which means that the cattle were less distressed during the FTAI procedure. This meant the

procedure was safer for the cattle and veterinarians performing the artificial insemination procedure.

- The use of experienced horse handlers (those which usually handle the animals) during study procedures, and the conduct of procedures in an environment in which the animal is familiar with, has reduced any pain or distress that the animals might otherwise have experienced.
- Animals were restrained appropriately and were handled by trained and experienced personnel. This minimised the pain or distress experienced by the animals prior to sample collection.
- Day 0 to Day 56 showed that both the Control product and the investigational product had adequately demonstrated the knock down effect on lice. The knock down effect is the effective removal of at least two life cycles post treatment represented by 0 lice counts at Day 56 according to the APVMA guidance. It was deemed unnecessary for the sheep in the Untreated Control (UTC) Group to remain untreated. The UTC Group was removed from the study and each animal was treated with Extinosad®. The removal and treatment reduced any distress or pain caused by lice the sheep may have experienced if they were left untreated for the remainder of the study.
- Animals were handled daily to acclimatise them to handling and reduce their anxiety. Animals were anaesthetised during tail vein injection to reduce pain and increase speed and safety of the injection, and the animal's tails were removed from the warm water after 10 seconds if they did not react.
- Animals were handled daily to acclimatise them to handling and reduce anxiety. Animals were anaesthetised during hind paw injection to reduce pain and increase speed and safety of the injection. An amendment was added to the study to halve the dose of the formulations in the two phases of the study. It was determined that this would reduce pain and distress to the animals without impacting on the scientific outcomes of the study.
- The method for handling sheep was calm and normal farm practice. Placing sheep in the crate in groups of two or three (depending on size of sheep) and then releasing them at once was helpful because the sheep were not alone during that procedure and we could work through all the processes e.g. local anaesthetic needs time to work so if you inject all three and then start back at the beginning the anaesthetic had taken effect.
- The use of experienced cattle handlers and appropriate restraint facilities in an environment familiar to the animals helped to reduce any anxiety, pain or distress that the animals might otherwise have experienced.
- Animals were handled daily to acclimatise them to handling and reduce anxiety. Animals were restrained only briefly when injections were carried out, and animals were monitored closely throughout sedation for signs of distress.
- With the removal of one investigational product during the study, subsequent sites enrolled had a total of 300, rather than the previous 400, animals enrolled. This also meant that there were only 100, rather than 200, animals treated with an unregistered veterinary product at any one site. Approval was granted to use 7100 animals throughout the study, however, in 2016 the accumulation of data provided by 18 sites and 6431 animals was considered sufficient to achieve the study objectives. The study was concluded at this point, therefore reducing the potential number of animals to be used in the study by 669.
- A small number of cows (n = 12) were used to confirm basic physical characteristics of the investigational product and compared to the Reference product before conducting larger field studies.
- Once 10 horses (per protocol) had been included in the investigational group and at least six horses included in the control groups (as per VICH GL 7), efficacy against ascarids in young horses had been demonstrated and the study ascarid population shown to include ML-resistant ascarids, the decision was made to end the study because study objectives had been adequately met. This meant that, despite the approval to use up to 75 animals in this study, only 62 horses were 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.
- On Day 56 the Untreated Control Group (10 animals) was removed from the study reducing the number of animals used for the remainder of the study.
- The number of animals recommended in the guideline VICH GL 43 for the untreated control group was reduced from eight to four based on the justification that sheep had pre-treatment biopsies and therefore acted as their own control.

- The minimum number of animals required to achieve suitable power for statistical comparisons was calculated prior to the start of this study. The minimum animal number determined by this calculation (i.e. 12) was used in this study. The study design (a crossover) reduced the number of animals required in comparison to other study designs (e.g. parallel).
- Ongoing support of a mailing list to facilitate tissue sharing among researchers, including researchers from other institutions requesting tissue.
- Training of researchers in current best practice techniques.
- 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.
- Transfer of animals (undesired genotype/sex) from one project to another as approved by AEC.
- Re-use of animals among multiple projects when ethically justified and as approved by AEC.
- Improved peri- and post-operative analgesia to reduce pain from surgery.
- Development of *in vitro* techniques to replace the use of animals.
- Use of *ex vivo* assays to minimise adverse impact on animals.
- 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 modern trapping techniques and equipment to minimise potential for animal injury. Use of smaller, less invasive tags for identification.
- 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.
- Increased awareness and use of environmental enrichment.
- Experimental results used for computational modelling.
- Combination of experiments so that only a single “control” group is used.
- “Single Mouse Trials” where the efficacy of anti-cancer drugs were assessed. Such trials resulted in a 16-fold reduction in the number of mice used, resulting in 2400 fewer mice that would have otherwise been used.
- The hatchlings were initially going to be euthanized, however, following AEC advice after approval, an attempt had been made to rear hatchlings at completion of the project.
- Examination of the stable carbon and nitrogen isotopic composition of feathers to investigate trophic structure of migrating shorebird communities replace blood sampling requirements.
- Reduce fish stocking rates to lower stress.
- Large photo cards have been used in practical classes instead of live animals to teach students how to identify freshwater fish species.
- Fish are released within 20 minutes of capture at the same location as capture. Digital cameras are used to record images of fish in photo identification tanks that are then used to identify specimens to reduce time in captivity.
- For pen and field studies the APVMA guidelines stipulate the minimum number of animals per treatment group. This minimum was always the number used. Wherever possible in pen studies control groups were shared between studies to reduce the number of animals required.
- Accommodation of research horses in a large paddock on a professional horse spelling/pre-training farm.
- Rehoming of retired research horses to suitable new owners.
- Spontaneous collection of naturally voided urine for the purpose of drug analyses.
- In-vitro simulation of the equine metabolism of designer anabolic steroids using horse liver.
- By maximising the number of samples from each rabbit, using one ear on each rabbit to test a vehicle control and the other to test a treatment, experimental variability and animal numbers were minimised.
- Using a bilateral (instead of unilateral) spinal fusion model.
- Aiming to test the response to escalating doses of the fungal toxin in sequential rather than concurrent treatment groups until a statistically significant difference to the control group was identified, sparing animals which would have been tested with higher doses.
- Structuring experiments with escalating doses and three time points within each dose so any *in vivo* effects of the fungal toxin could be observed using the lowest dose for the shortest time period.
- Most of the animals to be used in the study were already collected and used in previous, non-invasive experiments, thus reducing the need for new toads.
- The birds will be held in a cage that is of suitable size for them to go about their daily routine without any distress. No pain will be inflicted at all due to refinement. There will be a rest day in

between each feeding trial where the birds will return to their original aviary in order to rest and return to a familiar and normal environment in order to reduce distress.

- Where possible *in vitro* testing is considered/undertaken prior to application for use of mice.
- Where mice are used, care is taken to have the procedures undertaken by skilled staff.
- Minimum numbers of animals to obtain statistically valid data have been used for projects.
- 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.
- Replacement methods, such as ADInstruments, videos and earthworms for teaching projects that previously used toads, rabbits or crustaceans
- The establishment continues to encourage researchers to harvest and share tissues in instances where animals have been humanely killed.
- The AEC continues to encourage investigators to perform pilot studies to improve project design and reduce the total number of animals used in projects
- The AEC has continued to encourage investigators to consult biostatisticians when designing projects to reduce animal numbers and wastage, and refine experimental design
- Animal services have continued to provide resources at multiple animal facilities to reduce the number of animals required for experimental procedures
- Introduction of institution wide policies on animals used in teaching, training of new investigators, post approval monitoring and animal breeding to refine the use of animals in research.
- The AEC has continued to request conditions of approval to include the presence of either the Animal Welfare Officer or Animal Facility Managers to oversee high impact or new scientific procedures.
- The AEC has requested conditions of approval to include the Animal Welfare Officer in oversight of welfare and monitoring conditions of externally based or field based research
- The AEC has encouraged researchers to perform pilot studies to investigate the use of alternative oral dosing methods that minimise the welfare impact on animals compared to oral gavage
- The AEC has encouraged investigators to refine the use of metabolic cages in experimental protocols by reducing the time animals are required to spend in metabolic cages and provide environmental enrichment for animals during the procedures
- The Animal Welfare Officer and Wildlife and Aviary Manager have worked with investigators to improve the emergency euthanasia plans for research using wild animals in the field.
- A rat pain assessment protocol was refined to improve the monitoring of welfare of the animals post-surgery.
- Introduced new measures to improve aseptic handling of immunocompromised mice to reduce the risk of introducing pathogens into the animal colonies during routine handling.
- The Animal Welfare Officer has continued training for all new and existing staff and students in the role of ethics and welfare in animal research.
- Researchers are encouraged to share tissue samples wherever possible.
- Breeding programs are designed and maintained to produce stock to order, to reduce numbers and overproduction.
- Genetically modified animals are bred for the desired genotype as far as possible to reduce numbers.
- Animals used for courses are shared between multiple participants to achieve the best learning outcome whilst reducing overall numbers of animals used.
- Training courses and induction programs refine techniques to ensure the most meaningful results are achieved with the minimum number of animals.
- Introduction of protocols involving the use of animals across facilities of both institutions. By streamlining processes for projects which utilise imaging equipment there is a reduction in the administrative burden on researchers and allow for a reduction in animal use and refinement of experimental procedures carried out.
- 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.
- Expansion of secure outdoor enclosures furnished with native habitat for rearing endangered frogs in the location of their natural range instead of the artificial environment of indoor tanks at a breeding facility.
- Adjustment to monitoring schedules for seasonally breeding frogs to improve detection of changes to survivorship.
- External expert reviewer appointed to assess statistical validity of a study with high welfare impacts.

- Rehoming of fish to private tanks when no longer suitable for experimental purposes.
- Merging of project applications/documentation for related projects to improve accuracy of reporting and AEC tracking of individual welfare outcomes.
- Calculation of calorie content of different bait compositions used in small mammal traps to ensure provision of adequate calories to animals trapped overnight.
- Full revision of AEC guidelines for wildlife survey and monitoring with referencing to published literature.
- Investigation into temperature changes in harp traps exposed to early morning sunshine (Ellis, M.V. (2016) Australasian Bat Society Newsletter 47, 23-27). Further studies into harp trap microclimate in press 2017.
- The Company has replaced the standard guinea pig skin sensitising maximisation test with the local lymph node assay, which represents a significant improvement in terms of animal welfare. The advantages of LLNA over the guinea pig skin sensitising method are:
 - It greatly reduces animal trauma and discomfort, being considerably less invasive than the maximisation test that involves repetitive shaving, dermal application and intradermal injection of Freund's adjuvant.
 - It has a shorter test duration i.e. of 12 days, which includes 5 days acclimatisation period and 6 days of actual treatment.
 - It uses fewer animals.
- Sighting studies using small numbers of animals are routinely carried out to determine the optimal dose levels of test items before a main study involving greater numbers of animals is initiated.
- The substitution of mice with in vitro models of infection in airway epithelial cells.
- The development of a method to grow mouse uterine epithelial cells in culture which replaces the need to extract these cells from mice.
- The testing of regimens in cell culture systems before being tested in animal models.
- Use of preserved reptile specimens from the Australian Museum.
- The use of cell culture to test cell lines and fresh melanoma isolates to test the hypothesis prior to applying the knowledge gathered from in vitro studies in animal models.
- Making use of isolated protein channels that are studied in lipid bilayers. These proteins are extracted from either human or animal tissue but once collected from a single tissue can be used for 5 to 20 experiments.
- The utilisation of cell culture work in place of animal work.
- The performance of studies on nanoliposome binding mechanisms using immortalised human uterine cell lines, rather than primary cells obtained from mouse uterine tissue.
- A change to the route of administration of the drug to subcutaneous injections rather than the surgical implantation of an osmotic pump in mice.
- Calorie restriction through alternate day feeding improved both the health and life spans of mice in an ageing protocol.
- The modification of intrauterine growth restriction surgery from using diathermy to silicon tubes. Silicon tubing was used at a large enough internal diameter to minimise immediate blood flow to foetuses, however this allowed gradual restriction of blood flow as the vessels expand throughout pregnancy. This improved foetal viability and limited the possibility of hypoxic stress to foetuses due to immediate restrictions in blood flow induced by artery ablation from diathermy.
- The introduction of a number of measures such as bedding and wet weather covers for traps resulted in no trap deaths from a study. This was a positive outcome based on previous research and the number of animals captured.
- A reduction in the volume of drug being delivered and very lightly sedating animals during injections.
- Current best practice techniques were taught in training sessions in order to reduce the impact on the animals used for training and research, when these techniques are then utilised in research projects.

- The use of 9 mm wound clips to close the skin layer of mice instead of 7 mm wound clips as wounds were considered less likely to reopen with the slightly larger clips.
- Control and treatment group mice received a saccharin water solution during the week prior to the introduction of caffeine to the treatment group to prevent over-drinking and the possible over-consumption of caffeine by the treated mice.
- The utilisation of an adjuvant (TitreMax Gold) as an alternative to Complete Freund's Adjuvant (CFA) as CFA can be associated with granulomatous inflammation, focal necrosis, ulceration of skin and hypersensitivity reactions.
- A digital balance was purchased to assist in weighing individual animals which improved the monitoring of distress/weight loss experienced by an animal.
- The inclusion of additional neurological tests to assist in assessing the wellbeing of animals following the induction of glioma tumours.
- A delay in the exposure of female mice to cigarette smoke for several days after giving birth gave the mice time to adjust to their babies and establish feeding routines. This resulted in less stress for both the mothers and their pups.
- The use of a pipette for milking small snakes rather than a plastic beaker/vial minimised damage to the posterior maxillary, palatal and dentary teeth.
- The use of a small pilot study to determine if mice could tolerate anaesthesia before proceeding towards full experiments.
- The incorporation of anti-LAG-3 treatment improved animal welfare due to decreased tumour burden.
- The addition of a small rubber O-ring which covered the metal edge of the smoking tubes to prevent a specific strain of mice from suffering cuts on their nose.
- Weighing mice beginning a diet daily during the first week of the diet to detect any abnormalities sooner.
- The use of both Azithromycin and Salmeterol as anti-inflammatory treatments to reduce allergic airways disease in obese mice.
- The implantation of small pumps to progressively deliver drugs to mice to overcome complex problems associated with repeated handling and repeated injections over time and reduced the risk of the interventions stressing the animals.
- The design and use of a thread occlusion apparatus to induce experimental stroke by temporarily occluding blood flow in one middle cerebral artery produced more consistent occlusion and fewer animal deaths resulting in a reduction in the number of animals required.
- Determining the dosage of ethanol in a preliminary study due to concerns about ethanol causing behavioural or neurotoxic effects in older mice.
- The development of a new method to assess tissue strength in-vitro so that tissue can be collected earlier with less in-vivo measures.
- A reduction in the volume of blood collection to approximately 1.25% of the Total Blood Volume (TBV) of a 20g mouse to ensure that the frequency of blood collection did not have an adverse impact on the well-being of the animals.
- The use of a micropipette to collect blood rather than a capillary tube.
- The implementation of measures recommended by an expert in murine cardiac surgery (LAD ligation) improved animal recovery by:
 - ventilating with medical air rather than oxygen (improved lung function and reduced risk of lung collapse following thoracotomy);
 - reducing stress caused by frequent handling (reduced risk of cardiac rupture); and
 - only administering additional analgesics as required (to reduce the risk of myocardial depression, cardiac dysrhythmias or cardiotoxicity).
- Treating mice with macrolides to suppress bacterial or viral-induced exacerbations as macrolides have anti-inflammatory properties, therefore treatment with macrolides have beneficial effects on the mice.

- The development of a mealworm fishing method of capture for *P. entrecasteauxii* and *Lampropholis* species, and the hand-capture method for *S. equalis*. This is a more humane method than trapping as animals are not stuck in a trap with limited access to water or escape from high temperatures for any period of time. This method also reduces the potential risk of injuring a lizard when noosing is utilised. The hand capture method relies on turning a rock and gently catching the lizard underneath with bare hands and therefore there is no risk of injury or stress aside from handling.
- Improved surgical techniques were learnt when visiting a specialist centre.
- The use of fyke nets provided an opportunity for animals needing to come to the surface to do so as a portion of the net is held above the water.
- The use of an endoscope to guide the delivery of substances via a needle as a new route of administration for treatments as the administration of substances has the potential to damage reproductive organs if not done correctly. This technique was piloted on a small number of animals where a dye was used and the mice were sacrificed prior to recovery from anaesthesia. This ensured that the researchers were capable of safely performing this technique and that the route of administration allowed for the delivery of substances to the correct area.
- A previous pilot study allowed procedures to be optimised and staff to be trained thereby ensuring that the most information possible was obtained from each animal in the subsequent protocol.
- The improvement of data collection methods so that more tissue was harvested and investigated in each experiment thus providing more information and hence value adding to the experiments.
- A change in the strain of rat reduced the number of animals required to achieve the project aims.
- Experimental replicates were conducted on the minimum number of animals necessary to achieve statistical significance.
- The transfer of methods developed for one species of lizard to other species reduced the ultimate number of animals required for the development of reproductive technologies and cryopreservation protocols.
- The use of frozen sperm and tissue samples for archiving purposes at museums and other institutions and making tissue samples from euthanized animals available to other researchers.
- The use of the minimum number of mice necessary to ensure reliable and reproducible results.
- The harvesting of brains, livers, colons, spleens, hearts and limbs at each endpoint for use in secondary research projects by other research groups.
- Drugs being administered to one eye and the use of the fellow eye as a matched untreated control.
- Statistical analyses being performed using one and two-way analysis of variance (ANOVA) with repeated measures to partition the between animal variability and examine the effect of multiple treatments within an animal increased the power of a study and decreased the numbers of rabbits needed for the research.
- Rabbits or tissue suitable for use in another project being made available to other researchers.
- Melanoma cells being xenografted into each flank of mice minimise the number of animals required.
- The use of invertebrates reduced the number of vertebrate subjects used by students.
- The collection of multiple tissues from individual animals minimised the numbers of animals required to collect sufficient replicate data for all parameters to be tested.
- The use of tissues from animals used in previous projects as controls reduced the number of animals required.
- The use of control and stress animals from another approved protocol minimised the number of animals required to complete the aim of a study.
- The use of pilot data allowed power calculations to be performed to calculate the number of animals required for the new study in order to gather statistically significant data for publication.
- Tissue was archived and made available to other research groups with an appropriate research question. Peripheral tissue was also made available for other investigations.
- The collection of as many samples from the same animal, and the use of techniques which required a reduced amount of biological material, such as qRT-PCR, made it possible to reduce the number of animals used.
- Biological material from different animals was mixed in order to reach the amount of material necessary without compromising the development of the study.
- The adopting of a 'within-subjects' statistical design enabled each animal to receive a dosage of experimental drug, be tested, and then receive a dosage of a control drug and be tested. Testing each animal with the control drug and the experimental drug over several days allowed the number of animals required for the study to be reduced.

- Where a single compound dose was found to be efficacious, subsequent dose response or multiple dose experiments were assessed to determine if a reduction in the number of animals was possible.
- Running experiments at the same time and sharing control groups between experiments to reduce animal usage.
- The calculation of the number of animals necessary to provide sufficient statistical power while minimising the number of animals to be included in a project where possible.
- Xenografting tumour cells into each flank of mice reduced the number of mice used by around 50%.
- Performing in vivo studies
- Close monitoring of animals and development of monitoring checklists to identify adverse reactions in animals. The AEC will place conditions on projects at the approval stage to ensure that any pain or distress to animals is alleviated quickly in projects where it is impossible to eliminate this completely.
- Use of experienced veterinarians and other staff.
- Restraint time and dose rates kept to a minimum.
- Adoption of less stressful methodologies.
- Suitable housing provided and maintained including controlled environment facility.
- Use of adjuvants known not to produce adverse reactions.
- Procedures used routinely so that animals become accustomed.
- Procedures performed under anaesthesia or sedation when appropriate.
- 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.
- Reduction in number of animals used - researchers in have moved to PCR to reduce the number of animals used.
- On termination of the protocol, goats were re-homed to a suitable new owner.
- Animals used in teaching are assigned to a group of students rather than a single individual.
- Pilot studies are often undertaken by investigators before proceeding further.
- Close scrutiny of the volume of blood collected with respect to the weight of the animal.
- 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.
- Similar studies have shared the same control animals.
- Environment enrichment continues to be used for pigs and rabbits.
- Minimise movement and activity around the colonies as much as is practicable. Any activity that requires entry into or near the colony will be conducted in such a way that minimal stress and disturbance is caused to the birds. Field technicians are highly trained so as to work quickly and effectively, consistent with a calm and humane approach.
- 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
- 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. 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.
- Cameras and ultrasonic recording devices have been used in place of box trapping and harp (bat) trapping.
- Acoustic recorders are being deployed to collect calls from birds, koalas and frogs. The development of call recognisers for these groups is aimed at reducing the need for call playback and/or spotlighting.
- 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 euthanized.
- Rehoming and re-use of 350 animals (lizards) with a long-term known pedigree from another tertiary Institution which would have otherwise been euthanized. 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.
- 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.
- 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.

- With the increased availability of computer simulations, the university is moving away from the traditional model of having students individually dissect an animal. The current model is for undergraduate students to access a computer/video demonstration, with a hands-on aspect of learning how to correctly handle an animal.
- Actively encouraging 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.
- Researchers are asked to provide power analysis to demonstrate an understanding of how to ensure that the minimal number of animal replicates is used.
- Observation of rabbit culls to audit the process and provide recommendations to improve animal welfare.
- Collection of biopsy samples from fish that are being caught as by-catch by commercial fisherman.
- Assessment of the impact of housing and husbandry on the development of clinical syndrome lumpy jaw in captive macropods.
- Assessment on farm of the effect of flock size and stocking rate on lamb survival.
- Where possible, staff will replace the use of live animals with video or synthetic / cadaver models, or by creating computer models.
- Power analyses are frequently submitted as part of the application which demonstrate how researchers and teachers calculate the most suitable numbers of animals required to give valid data.
- Applications include justification by applicants for appropriate handling techniques where necessary, avoidance of pain and distress to the animals and how pain or distress will be alleviated if the animal shows signs of displaying either state.
- An increase in pilot studies for field work minimising the impact on larger groups of wild animals until critical information has been gathered and methods trialled in the pilot.
- Minimise the number of rodents used by increased use of bioinformatics and cell lines.
- Actively advocate the use of alternative methods of DNA sampling such as plucking hair or feathers or buccal swabbing over blood sampling, ear biopsy etc, this has led to increased use of these methods.
- Provision of veterinary advice and support 24/7.
- Minimise the impact on wildlife in their natural habitat by increasing the use of camera traps.
- Continued use of species appropriate environment enrichment.
- Conception of 3Rs award to formally recognise the exceptional application of the 3Rs.
- Researchers are required to apply the 3Rs (replacement, reduction and refinement) at all stages of their research. Some researchers report on field work that involves mainly observation and low impact to animals within the study. Where research involves housing animals and performing procedures, researchers report the use of extensive in vitro experiments prior to the use of animal models, conducting power analyses to identify the least amount of animals required for experimentation to achieve statistical power, the use of highly skilled personnel and housing of animals in a safe, stress free environment where they can socialise. Animals are provided with clean food and water as well as environmental enrichment. Any procedures performed on the animals are conducted away from the other animals to minimise any distress, and strategies addressing the minimisation of pain are incorporated into protocols reviewed by the AEC.
- We use a transplantation technique that enables us to generate multiple recombined mammary glands from a single donor. In addition we have utilized a number of in vitro based approaches to investigate specific experimental questions where we can. Examples of these approaches include cell migration and invasion assays performed on Incucyte and xCELLigence apparatus.
- We have sought to record from neurons from different receptor regions within the one animal, in order to reduce the need for separate recording experiments. We have also sought to record and stain as many neurons as possible within each animal, again to minimize animal numbers.
- Embryonic stem cell lines are used whenever possible to optimise experimental protocols and therefore reduce animals needed.
- Although the 3Rs were applied during the original design of these experiments, we have continued to refine our experiments by proposing to inject a B cell depleting anti-CD20 monoclonal antibody (instead of breeding KO mice for this purpose). We successfully submitted a modification application for this. Not only was this a better approach, it significantly reduced the number of mice required to test our central hypothesis that B cells were responsible for UV-protection from EAE (this is now published).

- We have developed an in-vitro microsome model to reduce the requirement to medicate live animals.
- We reduce the numbers of animals required, maximising the amount of heart tissue acquired from every procedure. We also reduce by harvesting as many diverse tissue types as possible. We have collated an extensive tissue bank that is available to our collaborators to ensure no additional animals are sacrificed.
- We have developed a technique called fundus fluorescein angiography to non-invasively monitor retinal vascular changes in mice. This technique has significantly reduced the number of animals required for studies of retinal vascular changes in Akita diabetic mice and Muller cell knockout mice.
- During this study we have been able to refine procedures to minimise the impact on the animals. Initially, the study compared blood hormone profiles to the physical changes in sows over oestrus. The blood collection process was replaced by a faecal collection that is considerably less invasive and less distressing for the sow while also allowing for an accurate biological comparison.
- We have begun measuring blood pressure using radio-transmitters in the conscious freely moving rat. This avoids the need for chronic indwelling arterial catheters which present a potential for discomfort and infection to the animal. The signal also permits heart rate measurements and so I have been able to remove the need of chronic electrocardiogram recording via subcutaneous placed stainless steel wires. In addition, this technique provides much more robust measures of blood pressure and cardiovascular status than traditional techniques such as tail cuff, or recordings made under anaesthesia. In addition, it is possible to make more observation using fewer animals and therefore reduce the number of animals required to identify causes and/or mechanisms of hypertension.
- In terms of refinement, rats will be handled for at least 4 days prior to the beginning of the experiment so that they become familiar with the investigator.
- We refined our thermogenic seizure testing technique so mice spend less time exposed to uncomfortable temperatures and WT mice don't experience any seizures. This alteration has reduced the negative impact on mice and yields more reliable results.
- A pilot study was conducted in order to optimise the study protocol, including the optimal time for blood sampling, placement of intravenous catheters and infusion of caspofungin. Due to the housing, sampling techniques, and sedation protocol for jugular catheter placement, stress and pain was significantly minimised.
- In 2016, the Breed'n Betsy reproduction model will be introduced into the curriculum. The Breed'n Betsy is an artificial reproduction training tool that allows for introduction to rectal palpation, pregnancy diagnose and artificial insemination training. Results suggest that Breed'n Betsy cannot fully replace training in live cows, but may be a valuable addition to the classical teaching method (Bossaert, et al, Journal of Veterinary Medical Education Vol 42 issue 5). The first pregnancy diagnosis (PD) practical sessions for the BVSc degree will be replaced with the Breed'n Betsy training sessions which will reduce the number of PD practicals by 6 sessions and refine the teaching process to improve student experience.
- No animals were sampled as we used existing biobanks.
- We replaced some in vivo tumour experiments with in vitro cultured lines to test our antibodies to MUC1. This proved highly effective and reproducible.
- All students undertook two one-day workshops in preparation for the actual handling classes. In these workshops when introducing students to and demonstrating procedures, live animals were replaced by mannequins, stuffed toys and other inanimate models.
- We have not used any mouse tissue in this study, finding that our cell-culture-based experiments produced adequate results.
- Prior to carrying out any animal work we will have validated our system in a range of in vitro work aimed at clarifying cellular processes pertinent to the disease issues of interest in the efficacy of the system in glucose utilization and insulin production. For example, we employed pseudo-islets of MIN6 cells (which have been shown to be more efficient in insulin production than monolayers of cells). Other approaches included titrating the number of MIN6 pseudo-islets needed for optimizing, insulin secretion to make calculated decision on conditions that needs to be used to get physiologically relevant responses.
- We always use cell culture / in vitro and alternate tools wherever possible to reduce the use of animals in experimentation. We continued to implement these alternates for the entire duration of present study.
- We have replaced the need for capturing, sedating and causing any harm to animals by collecting faeces from which we extract DNA and conduct important ecological research.

- Prior to carrying out any animal work we will have validated our system in a range of in-vitro work aimed at clarifying cellular processes pertinent to the disease issues of interest in the efficacy of the system in glucose utilization and insulin production. The stem cells have undergone extensive molecular biological testing to ensure the cells we are transplanting are on the correct differentiation path.
- The project interventions are minimal and involve periodic animal vaccination, weighing, measuring girth, taking faecal samples. These interventions are for the purpose of improving animal welfare and health.
- The number of animals required was actively reduced by the re-use of already collected tissue samples across multiple trials. The AEC was reassured that the re-use of the tissue did not affect the quality of the data, or impact on the project findings; the AEC also saw fit to limit the number of samples that could be collected per animal, and imposed restrictions on re-testing.
- The research team introduced the use of a new tool to collect tissue samples that reduced the sample size of tissue that was required.
- 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. Furthermore, the AEC has approved a number of research projects that will assess the impacts of environmental enrichment.
- Weaning can be a stressful time for piglets as they are removed from the sow and placed in new accommodation, need to adjust to new feed etc. Previously we have weighed all piglets prior to the day of weaning which may be an additional stressor for them. Piglets are now weighed a few days prior to weaning to allow them to settle prior to their physical weaning from the sow.
- Piglet carriers have been purchased to facilitate carrying piglets for more intensive type experiments. They carriers have hessian in the bottom for bedding and can fit 2 piglets to allow for social company.
- A SOP was developed for the use of Lethobarb to euthanize healthy viable piglets under research protocol. Whilst blunt force trauma is permissible on younger piglets (according to Model Code of Practice for the welfare of Animals-Pigs), concerns were raised about the use of this technique on piglets that are healthy under a research protocol. Our AEC and staff are much more comfortable using this SOP for healthy piglets under a research protocol.
- Samples collected under an ARA are stored and made available to other researchers.
- Using control data across related protocols.
- All tissue used in one protocol came from tissue sharing arrangements, whereby samples were collected upon euthanasia of animals by other researchers based at the establishment and by collaborators in Berlin
- Immortalised cell lines were used instead of animals.
- Monitoring record sheets are tailored to meet the requirements of individual protocols.
- A minimum of 2 personnel are listed for all protocols where animal monitoring is required.
- Companionship provided by vasectomised or ovariectomised animals.
- Wildlife enclosures are provided with a range of natural and man-made hides and basking spots.
- Live insect prey/food provided to encourage natural behaviors.
- Wildlife is rotated through indoor and outdoor enclosures whenever possible.
- Animals individually identified e.g. with microchips or visually implanted elastomer to allow group housing.
- Animals that are scheduled for approved euthanasia are made available for tissue harvest, new technique training or post mortem technique training.
- At the conclusion of appropriate research protocols, some animals are retained for animal handling training.
- Researchers are encouraged to consult with statisticians to determine the minimum number of animals required for statistically valid and relevant results.
- A protocol investigating novel synthetic bone substitutes conducted a pilot study first, and modified their procedures to refine their techniques.

- A protocol on Alzheimer's disease has minimised the number of animals needed to perform the experiments. Known statistical variance from previous studies was used to arrive at the smallest sample size that will still permit for the study aims to be achieved. The researchers have also utilised state of the art retinal measurement equipment to ensure the highest accuracy and least amount of error in order to increase the statistical power of the analysis and thereby minimize required animal numbers.
- A protocol on canines used paw prints and video footage as replacement tools for animals. The design of the data acquisition device was refined to suit the animals used.
- A protocol investigating asthma used male breeders from another protocol.
- A protocol on glaucoma used a pilot study to assess the feasibility of the proposed method and approach.
- A protocol on kangaroo-human interactions has selected as few sites as possible and conducted as few replications while ensuring the findings can be statistically valid. Animals are observed from a distance and the researchers move slowly and calmly throughout the animals' habitat to avoid unintended disturbance.
- A protocol investigating predator foraging behaviour has used non-invasive survey methods such as camera traps, urine collection from sanctuaries, and direct observation.
- A protocol investigating the distribution and abundance of fish around an artificial reef has used baited remote underwater video stations to record the numbers of fish. To reduce the disturbance arising from this technique the researchers have very slowly lowered the video station from a boat so that it touches the seabed lightly, used only fish-based bait that is purchased from a supplier to commercial fishers (thereby eliminating the introduction of unnatural products into the environment, and restricting the attractiveness of the bait to carnivorous fishes), deployed the camera at either the top of high tide or the bottom of low tide (when tidal water movements cease for approximately 1 hr) to limit and standardize the area over which the bait spreads, and deployed the camera for no longer than 35 mins. The deployment time is based on pilot studies that showed sightings of additional new species are not substantially increased with longer deployment times.
- A protocol investigating renal development has reused females to mate to reduce the total number of animals used. A protocol on asthma has transferred unused animals to a training protocol.
- A protocol on asthma used a pilot study before conducting the main study. The results from the pilot study will be used for power calculations of animals for the main study.
- A protocol on diabetes will use the minimum number of animals to obtain statistically significant results.
- A protocol on shark detection technology is using a pilot study to determine the recording time needed for 25 separate detections of sharks. The average of the three times will be used as the optimal recording time.
- The operation of the backpack electrofisher only by highly trained and experienced researchers. The researcher involved has over 3 years of professional experience using this method. Also, in order to avoid any risk to non-target vertebrates, if any of these are seen in the sampling reach, electrofishing will cease until the area is clear of non-target animals.
- In terms of reduction, the number of fish identified, temporarily held, and returned to the stream is dependent upon both the abundance of fish at the survey sites, and the degree of fishing effort that is required to determine (with a certain level of statistical confidence) the species present and their relative abundance. To minimise the impact of the research on fish, no more than the necessary degree of fishing effort to achieve robust results will be used.
- 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.
- Application forms are designed to ensure that researchers consider how they might replace, refine and reduce animal numbers in their research.
- The AEC 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 set out by the AEC. For instance, the AEC considered a domestic cat tracking project and assisted researchers to find a more appropriate harness with a safety release buckle to reduce the risk of snag injuries.

- Pilot studies are often undertaken by investigators before proceeding further, which assists researchers to refine future protocols.
- Control groups are shared whenever logistically possible and negative control groups are reused to reduce the number of chickens used.
- Continue to use surplus SPF males from the breeding farm for *Eimeria* oocyst production. The increased yields from these older surplus birds have reduced chick requirements by 2 to 10 times.
- Implementation of Continuous Flow Centrifuge (CFC) will reduce waste and increase efficiency, targeting higher yields out of birds resulting in fewer birds required.
- PI's to list what will happen to any excess animals as part of their application process. Every effort is made to utilise excess birds rather than euthanize.
- Excess eggs in QC testing are used for training purposes.
- Continuing experience in handling poultry which are particularly prone to stress. Staff enter bird houses and communicate in a quiet manner for the welfare of chickens.
- 24hr alarm monitoring including industrial temperatures within all bird houses, hatches and Grow Out sheds, power to industrial equipment and building security. Alarms can now be monitored in the office.
- Video surveillance in bird houses and laboratories to monitor activity within these areas.
- The AEC 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 establishment's Animal Ethics Online Portal (which includes a mandatory test for all staff and students).
- We are continuing to identify alternative methods that reduce animal use numbers particularly use of remote cameras for field surveys. Project design for field sampling also provides the opportunity to identify alternative methods for survey.
- There has been a greater focus from the AEC on using field sampling design processes to inform minimum number of individuals that may need to be tagged to inform environmental population studies. This has focussed on projects in New South Wales (pit tagging of Green and Golden Bell Frog) and Queensland (radio tracking of turtles). This process provides a stronger scientific basis to minimise overall animal use.
- Standard operating procedures are formally reviewed annually and regularly updated as new information is received. Examples include incorporation of the American Veterinary Medical Association Guidelines for Euthanasia (2013) into the standard operating procedures which provide more up to date information on humane euthanasia. The driver of these changes has been the requirement to assess the potential off site impacts of firefighting chemicals on fish by chemical analysis of fish flesh.
- Refinements include improvements to turtle tagging processes, fish sampling and holding processes.
- 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 and agistment property to supplement physical monitoring and increase the frequency of monitoring that occurs out-of-hours and on weekends.
- Launch of the online animal ethics theory modules and the practical training module for work with mice, rats and rabbits.
- Animals used in handling or dissection training were utilised from other research protocols. This practice enabled the establishment to reduce the number of animals necessary for training purposes and to reinforce the 3Rs mindset.
- The Committee approved an amendment to Animal Breeding Protocol for Zebra Fish with no projects envisaged the remaining three fish were rehomed at the facility.
- Continuing from a Protocol where an initial pilot study enabled refinement of techniques, a similar approach and pilot trials was conducted for amphibians under another protocol. This approach enabled the Principal Investigator to refine animal holding techniques and reduce the number of animals required.

- Investigators required to statistically confirm the number of animals requested and validate statistical data.
- To improve bird welfare, the trial facility has undergone major renovations including: installation of new fully insulated roof, erection of blacked out curtains along the southern end of the shed, installation of free range areas covered by shade clothes, improved lighting and fogging systems.
- Weighing only a portion of birds to determine pen weight, to reduce handling and still provides the same statistically significant result
- Use half pens to reduce bird numbers, but maintain target stocking density.
- Perform small-scale trials on 5 birds per treatment group to test effectiveness of sero-conversion of treatments (vaccines) before larger scale trials
- Addition of microtracers into feed to ensure correct feed is administered to correct treatment group, ie. for QA purposes. Reduces risk of repeating trial if error occurs with administration of incorrect feed.
- Use of video training tools and injection techniques.
- Use of museum specimens, skeletons including skulls and toad dissections (online).
- Use of invertebrates in biology course - snail metabolic activity and heart rate.
- Use of cadavers for initial and progression to training competent on the cadavers.
- Use of anatomical models for blood collection.
- Use of remote underwater video instead of trapping and releasing fish as a less intrusive research method.
- Uses of pilot studies to refine techniques before large numbers of animals are used.
- Rather than teaching researchers all techniques for handling and injection only training them in the procedures they will need.
- Sharing tissue from deceased rats and mice with other researchers eg blood, skin, brains, lenses, livers and hearts.
- Transfer of unused animals between protocols instead of ordering additional animals.
- 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.
- 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 tissues sharing throughout Australia.
- The Committee continues to require a pilot study if the study interventions on animal 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.
- 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 minimising the numbers of female mice used for fostering purposes.
- Animal facilities use mice for training purposes that were identified with an undesired genotype (hence would have been euthanised regardless).
- Where possible, mouse lines are shared between different research groups to avoid unnecessary breeding.
- Researchers are required to investigate all alternatives before resorting to the use of live animals. Alternatives may include using established cell lines or tissues (eyes, bone, muscle, abdominal organs) obtained from animals humanely killed from other sources including rodent breeding colonies (retired breeders/aged stock, wrong genotype) and tissues from animals derived from other experimental protocols. Researchers are encouraged to utilise animals

euthanased (as per above) for dissection to familiarise the anatomy for surgical procedures, pathology specimens etc. Recent examples include the use of euthanased rats to perfect renal artery cannulation as part of a study on reperfusion injury in donor kidneys and for the familiarisation of cervical anatomy for electrode placement in sleep apnoea studies.

- When developing an experimental protocol, the Committee requires that researchers consult a biostatistician to ensure that the appropriate number of animals are factored into the experimental plan to enable statistically significant data to be collected to achieve the desired objectives.
- Where breeding colonies are maintained for the production of animals to supply experimental protocol requirements, the researchers must ensure they plan appropriately and only breed sufficient animals to satisfy experimental needs (including continued maintenance of colonies) or otherwise source animals directly from external suppliers.
- The Committee reviews all submissions thoroughly to ensure that researchers are aware of their obligations and responsibilities and to take every action to minimise pain suffering and distress (or lasting harm) to the animals in their care. Ensuring close monitoring of all animals is the responsibility of the Principal Investigator (or a delegated senior researcher) and all efforts must be made to alleviate pain and suffering if such clinical signs are detected. New and inexperienced staff must be supervised and guided by competent and experienced staff.
- Providing environmental enrichment appropriate to the species is essential to meet physical and psychological needs, allowing the expression of species specific behaviours and hopefully mitigating stress.
- Continue to conduct routine animal health monitoring through Opti-Spot® sample collection (or dried blood spot testing). Animals do not need to be terminally bled and there is no need to anaesthetise mice during sampling, therefore less stress involved.
- Continue to apply using environmental enrichment materials, such as crinkle nest paper and cardboard paper rolls for mice and rats that are singly housed and for breeder cages. Allow for expression of species-specific behaviours, such as nesting opportunities and less aggression.
- Continue to use analgesics and anaesthetics for painful procedures and surgeries.
- Improved rodent husbandry practices in the form of provision of sipper sack watering system instead of water bottles.
- Score sheets for all monitoring during approved surgical procedures have been introduced to refine the process and appropriately identify and manage pain and distress in the animals.
- Use of less sentinels – surveillance animals placed in environment via direct contact or dirty bedding, used to monitor colonies for presence of pathogens. Sentinel mice will now only be required for the immunodeficient mice room and quarantine rooms.
- Sharing data and resources e.g. animals, tissue samples and equipment between research groups and neighboring institutions.
- Use of imaging and related technologies to enable longitudinal studies in the same animals.
- Fewer breeder pairs can be set up for strains of immunodeficient mice due to improved housing with individually ventilated caging system, environment and health conditions.
- Regular stock take by researchers to ensure animals being maintained are only what is needed.
- Animal tissue sharing between research groups whenever possible.
- Reduction of animals used for training purposes – use of animals that are due to be culled only; also use of dead animals for suturing practice.
- Application of pilot studies for projects where appropriate.
- Meeting with research groups routinely for colony management to ensure breeding is optimised for experimental or maintenance production only.
- Cell-based experiments whenever possible; use of alternatives to animal-based research e.g. mathematical and computer models.

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 3: PURPOSE

Enter the **most appropriate** numerical code (1-10) from those listed below to describe the **primary** purpose of the project (one purpose only for each project should be entered).

| Purpose Number: | Description: |
|-----------------|---|
| 1 | <p>Stock breeding</p> <p>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> |
| 2 | <p>Stock maintenance</p> <p>Holding projects for animals maintained for use in other projects. These animals may be maintained under an ethics 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> |
| 3 | <p>Education</p> <p>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> |
| 4 | <p>Research: human or animal biology</p> <p>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> |
| 5 | <p>Research: human or animal health and welfare</p> <p>Research projects which aim to produce improvements in the health and welfare of animals, including humans.</p> |
| 6 | <p>Research: animal management or production</p> <p>Research projects which aim to produce improvements in domestic or captive animal management or production.</p> |
| 7 | <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> <p><i>Pre-logging or pre-development fauna surveys</i></p> |
| 8 | <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"> • Use of a sheep flock to donate blood to produce microbiological media • Production of commercial anti-serum • Production of products, such as hormones or drugs, in milk or eggs from genetically modified animals • Quality Assurance testing of drugs but do not include animals which come under Purpose 10, below. |
| 9 | <p><i>Diagnostic procedures</i></p> <p>Using animals directly as part of a diagnostic process.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • Inoculation of day old chicks with ND Virus to determine virulence • Blue-green algae toxicity testing • Water supply testing using fish |
| 10 | <p><i>Regulatory product testing</i></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 normally be category 8 in the case of QA testing.)</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • Pre-registration efficacy or toxicity testing of drugs and vaccines |

Column 4: PROCEDURE

Enter the **highest appropriate** numerical code (**1-9**) 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 (8 or 9) rather than any others which might also appear appropriate.

| Procedure Number: | Description: |
|--------------------------|--|
| 1 | <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> |
| 2 | <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> |
| 3 | <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> |
| 4 | <p>Minor Surgery With Recovery</p> <p>Animal is rendered unconscious 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 post-operative 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> • <i>Sedation/anaesthesia for relocation, examination or injections/blood sampling</i> |
| 5 | <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"> • Orthopaedic surgery • Abdominal or thoracic surgery • Transplant surgery |
| 6 | <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"> • Minor infection • Minor or moderate phenotypic modification • Early oncogenesis • Arthritis studies with pain alleviation • Induction of metabolic disease • Prolonged deficient diets • Polyclonal antibody production • Antiserum production |
| 7 | <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"> • Major infection • Major phenotypic modification • Oncogenesis without pain alleviation • Arthritis studies with no pain alleviation • Uncontrolled metabolic disease • Isolation or environmental deprivation for extended periods • Monoclonal antibody raising in mice |
| 8 | <p>Death As An Endpoint</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 6 or 7.</p> <p><i>Examples</i></p> <ul style="list-style-type: none"> • Lethality testing (including LD50, LC50) <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> |
| 9 | <p>Production of genetically modified animals</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"> • Initial breeding animals for GM production • Animals culled as part of the GM production process |

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 |
| | 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 |

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