Home Office

Guidance

Supplementary instructions for completion of annual returns of procedures in the UK

December 2022

Version 2

Contents

Introduction

From 4 October 2021, you need to complete your return of procedures online via the Animals in Science Procedures e-Licensing (ASPeL) system by completing the return of procedures forms. Guidance on completing the return of procedures is provided in the ASPeL system and these supplementary instructions provide additional examples of how to classify procedures for the return.

Completing the preliminary questions

The initial questions in ASPeL will tailor the return to your project. For example, if you only use wild type animals you will not be asked about genetic status again.

General

- Data should be provided on each procedure, i.e., each use of an animal. In most cases this means a single protocol.
- Do not count animals unless used on **regulated procedures** (these are procedures authorised on a project licence). Animals killed by Schedule 1 (or other PEL-permitted) methods of killing, for example, for tissue collection, are not counted unless they were genetically altered and bred under project licence authorities. Surplus animals that are killed are not included unless they have been produced under project licence authority, for example, genetically altered animals.
- Mammals, birds, and reptiles should be counted from when they are born or hatched. Fish, amphibians, and cephalopods should be counted from the stage at which the animal becomes capable of independent feeding. This will be immediately post-hatching for octopus, squid and Medaka, from around five days post-fertilisation for Zebra fish, and from around seven days post-hatching for cuttlefish.

Please note: Returns should be submitted every year. In exceptional cases where a single study involving a large group of animals extends over two calendar years, and data collection is not complete until the end of the entire study (as opposed to at the time of death of each individual subject) it is acceptable to count all procedures in the year in which the last procedure ends, i.e., at the end of the study. This must be agreed with the Home Office in advance.

Number of procedures

- This is the number of uses, i.e., the number of times animals were used in a particular experiment or study.
- If an animal has been used in more than one study or experiment, i.e., re-used, provide details of each use in a separate row (see below for guidance on re-use).
- If an animal has been used multiple times, the number of procedures is the number of times it was used.
- In the case of very small animals, such as fish larvae, an estimate of the total numbers used is acceptable.

Example: PROCEDURE

10 rats were used in a study involving administration of a drug, then 5 separate blood samples and a final surgical intervention, before being killed by a Schedule 1 method.

Number of procedures = 10
Re-use = No

Note: If an animal is used on a study extending over more than one calendar year, it should not be counted until the procedure ends.

Example: PROCEDURE OVER TWO CALENDAR YEARS

In November 2019, 10 rats were used in a study that ended when all of the rats were killed in March 2020.

Number of procedures reported in the 2019 return = 0. All procedures will be returned in the 2020 return.

Re-use

Each animal should be reported at the end of each procedure for which it was used. Re-use will normally have been authorised in the project licence.

Example A: RE-USE

10 rats were cannulated and used in a study involving administration of a drug then 7 separate blood samples.

At the end of that study those same rats had a wash out period then were used again to test a separate drug. There was no need to use the same rats for the second study, therefore the second study constitutes “Re-use”.

First Row:
Number of procedures = 10 and Re-use = No,
THEN IN A SECOND ROW
Number of procedures = 10 and Re-use = Yes (the place of birth for these reuse procedures is not required).

Example B: RE-USE

100 sheep were used to supply normal blood by being bled repeatedly at approximately monthly intervals.
90 had been used in previous years. 10 were bought in during this reporting year.
Each bleed constitutes a separate procedure, therefore all except the first bleed constitutes “re-use”.
Each sheep was bled 10 times, therefore the total number of procedures was 1000 and should be reported in 3 (or possibly 2) separate rows of data, as follows:

Row 1.
The previously used sheep.
Number of procedures = 900, Re-use = Yes and the place of birth is not required.

Row 2.
The new sheep, first bleed.
Number of procedures = 10, Re-use = No and the place of birth is required.

Row 3 (or added to Row 1)
The second and subsequent bleeds of the new sheep.
Number of procedures = 90, Re-use = Yes and the place of birth is not required.

Note: For the purpose of statistical reporting **a single procedure, or use of an animal, extends from the time when the first technique was applied to the animal until the completion of data collection, observations, or achievement of the particular purpose**. In most cases this means a single protocol.

Continued use

- Continued use is when a single experiment or study extends over more than one licence or protocol and constitutes a single use; it is **not re-use**. In this case the end user should report the entire procedure, even if it began on another project licence, and the initiator of the study does not report such procedures.
- Continued use includes when genetically altered animals are bred under one licence then transferred to a second licence (possibly at a different establishment) for the remainder of the study; the breeder would not report these animals, they would be returned under the end user's project licence return.
- If in any doubt as to which classification is correct, contact: ASRULicensing@homeoffice.gov.uk

Example: CONTINUED USE

10 rats were surgically prepared under Project Licence PAB123456. This had actual severity of Moderate because it involved surgery.

These rats were then moved onto a different Project Licence PCD654321 for use in a PK (pharmacokinetics) study. This part of the study has an actual severity of Mild.

PPL PAB123456 does not report any of these rats.

PPL PCD654321 reports all 10 rats when the PK study is completed, and the Actual Severity is reported as Moderate to take account of the severity of the entire procedure which started on a different licence (or protocol).

Note: Any subsequent PK studies using the same rats, reported as "Re-use" should have Actual Severity of Mild (if this is what happened on the re-use)

Place of birth: all species except non-human primates

The place of birth, not the source of the animal, is required. A registered breeder can be any breeder within the EU who is registered under Article 20 of Directive 2010/63 EU. In the UK licensed establishments are registered breeders. Animals born in your own establishment should be entered as "Animals born in the UK at a licensed establishment".

- In the case of eggs of birds, reptiles, amphibia and fish the "place of birth" should be the place where the eggs hatched, if this is different from where the eggs were produced.
- In the case of mammals where source of embryos is different from where the embryos are implanted or animals are born, the place of birth is the place where they were born, not the source of the embryos.
- The 'Rest of Europe' means Council of Europe countries[1] and Israel.

Example: PLACE OF BIRTH

1. **Transgenic mice bred in house and used in a study.**
   Place of birth "Animals born in the UK at a licensed establishment"
2. **Transgenic mice bred at one university in the UK, licensed under Animals Scientific Procedures Act 1986 (ASPA), then moved to a different project licence at a second university for use in an experiment.**
   - The PPL holder at the first university who supplied the mice does not report them at all. The PPL holder who received and used the mice at the second university reports them all.
   - If 50 mice were supplied but only 40 were used, with the remaining 10 culled as surplus, the return would be as follows:
     - 40 Mice, "Animals born in the UK at a licensed establishment" purpose as appropriate e.g., "Basic research; Immune system"; and
     - 10 mice "Animals born in the UK at a licensed establishment" purpose "Maintenance of established lines of GA animals", because the only procedure the surplus 10 were subjected to was being born with a genetic alteration (even if this PPL does not authorise B&M).
3. **Mice were bought from supplier, licensed under the EU Directive, in Germany.**
   Place of birth "Animals born in the EU (non-UK) at a registered breeder"
4. **Mice were bought from supplier in the USA.**
   Place of birth "Animals born in the rest of the world"
5. **Cattle were sourced from a commercial dairy farm.**
   Place of birth "Animals born in the UK but not at a licensed establishment"
6. **Wild caught animals.**
   Place of birth "Animals born in the UK but not at a licensed establishment"

[1] Council of Europe countries are Albania, Andorra, Armenia, Azerbaijan, Bosnia & Herzegovina, Georgia, Iceland, Liechtenstein, Macedonia, Moldova, Monaco, Montenegro, Norway, Russian Federation, San Marino, Serbia, Switzerland, Turkey, and Ukraine.

Genetic status

The genetic status of the animals should be described as either 'Not genetically altered', 'genetically altered without a harmful phenotype' or genetically altered with a harmful phenotype'.

Genetic Altered Animals (GAAs) without a harmful phenotype

- GAAs without a harmful phenotype includes all GAAs that do not exhibit a harmful phenotype during the procedure.

Examples: GAA WITHOUT A HARMFUL PHENOTYPE

- Green fluorescent protein (GFP) expressing lines of mice or fish.
- Cre expressing lines of mice.
- Conditional genetic alterations without induction of conditional gene expression (assuming it is induction of expression that leads to harm).
- Transgenic and knockout mice which appear overtly normal
- Strains of mice prone to disease, e.g., tumour development but used or killed prior to the onset of tumour development.

Genetically altered animals with a harmful phenotype.

- 'GAAs with a harmful phenotype' includes all GAAs that exhibit an overtly or potentially harmful phenotype at some time during the procedure. This category can apply to any purpose. It includes animals used for the creation of new strains, animals used in further procedures and animals used for maintenance of established colonies, but only if a harmful phenotype manifests.
- If the strain is known to have a harmful phenotype but some individuals do not exhibit that phenotype, then do not use this category for those individuals, use 'Genetically altered animals without a harmful phenotype'.

Example: GAA WITH A HARMFUL PHENOTYPE

Immunocompromised mice, e.g., Nude, SCID, Rag KO. Although all of this type of strain have potentially harmful phenotypes and must be reported as such, the actual severity is likely to be "Sub-threshold" (if not used in further experiments)

EXAMPLE: Nude mice bred but not used in further studies and culled as surplus. All will be reported under "'Breeding/maintenance of colonies of established genetically altered animals, not used in other procedures".

Heterozygous offspring: "Genetically altered without a harmful phenotype", Actual severity "Subthreshold".

Homozygous Nude offspring: Genetically altered with a harmful phenotype", Actual severity "Sub-threshold".

Wild type offspring. Not reported (unless genotyped by a regulated method).

Creation of a new genetically altered animal line

Creation of a new line of GA animals should be selected for only those procedures performed to produce a new GA line. Rederivation and archiving of established lines is reported under the purpose of 'breeding/maintenance of colonies of established genetically altered animals, not used in other procedures'. Any animals used for the creation of a new genetically altered animal line (including the crossing of two established lines) intended to be used for the purposes of basic research or translational and applied research should be recorded according to the purpose for which they were created.

Purposes

Choose the best fit for the purpose of the study. This will generally be the purpose given in the project licence. Please check that no other option is suitable before selecting "Other" as the sub-purpose.

1. **Basic research** includes studies of a fundamental nature, including physiology.

Studies that are designed to add knowledge about the normal and abnormal structure, functioning and behaviour of living organisms and the environment. These include fundamental studies in toxicology. Investigation and analysis focused on a better or fuller understanding of a subject, phenomenon, or a basic law of nature instead of on a specific practical application of the results.

Further guidance on basic research categories:

- 'Oncology'. Any research studying oncology regardless of target system.
- 'Nervous system'. Includes neuroscience, peripheral or central nervous system, psychology.
- 'Sensory organs' (skin, eyes, ears).
- 'Multisystemic' should only include research where the aim is to study multiple systems for example, some infectious diseases. However, if there is a primary target system, please report the primary target system as the sub-purpose. This category excludes oncology.
- 'Ethology/animal behaviour/animal biology' covers both animals in the wild and in captivity with the primary goal of learning more about that specific species.
- Dentistry should be reported under 'dentistry' not 'musculoskeletal system'.
- 'Other'. Research not related to an organ/system listed above or is not organ/system specific.

Animals used for the production and maintenance of infectious agents, vectors and neoplasms or other biological material, and animals used for the production of antibodies, but excluding production of monoclonal antibodies by ascites method (which is covered under purpose "Regulatory use" and sub-purpose "Routine production …"), should be reported under "Basic research" or 'Translational/applied research". Where the purpose could be reported under the two categories you should only report the main purpose.

2. Translational / applied research includes discovery toxicology, investigations prior to formal regulatory studies and method development. It includes efficacy testing during the development of new medicinal products. It **does not** include studies required for regulatory submissions.

Additional guidance on translational/applied research categories:

- "Human cancer". You should include any applied research studying human cancer, regardless of the target.
- "Human infectious disorders". You should include any applied research studying human infectious disorders, regardless of the target.
- Any regulatory use of animals is to be excluded, such as regulatory carcinogenicity studies.
- You should report studies on disorders of the nose under "Human respiratory disorders" and those of the tongue under "Human gastrointestinal disorders including liver".
- Human dentistry should be reported under 'human dentistry' not 'musculoskeletal system'.
- Renal disease should be reported under "Human urogenital/reproductive disorders".
- "Diagnosis of diseases" includes animals used in direct diagnosis of diseases such as rabies, botulism, but excludes those covered under regulatory use.
- Non-regulatory toxicology covers discovery toxicology and investigations prior to formalising the regulatory studies and method development. This category does not include studies required for regulatory submissions (preliminary studies, maximum tolerated dose).
- Animal welfare should include studies as per Article 5(b)(iii) of Directive 2010/63 EU i.e., "the welfare of animals and the improvement of the production conditions for animals reared for agricultural purposes"

3. Protection of the natural environment in the interests of the health or welfare of human beings or animals

This includes studies aimed at investigating and understanding phenomena such as environmental pollution, loss of biodiversity and epidemiology studies in wild animals. This excludes the regulatory use of animals used for ecotoxicology purposes.

4. Preservation of species.

This includes research where the primary purpose is the preservation of a species e.g., vulnerable, or endangered wild animals.

5. Higher education

Education in the tertiary setting e.g., university.

6. Training for the acquisition, maintenance, or improvement of vocational skills

e.g., training in microsurgery, modular training for PIL applicants. This includes training to acquire and maintain practical competence in techniques as required under Article 23(2) of Directive 2010/63 EU.

7. **Forensic enquiries**.

This includes tests as part of forensic investigations and the production of materials, for example, antisera, for use in forensic investigations where this is not being carried out to meet a regulatory requirement.

8. **Breeding / maintenance of colonies of established genetically altered animals, not used in other procedures.**

Includes the animals required for the maintenance of colonies of genetically altered animals (GAAs); the intended purpose for which the line is being bred is not recorded (in contrast to “creation of new genetic lines”).

It includes genetically altered breeding stock and surplus animals unless killed for use of tissues post-mortem, i.e., **all GAA that are bred but not used for a further scientific purpose, whether regulated or not.**

This category should be used for established or long-standing strains of GAAs, i.e., those that have had a welfare assessment carried out, or those that are generating animals being used in experimental procedures. The latter can be considered effectively “established”. You should report the creation of new strains under the purpose for which they are being created.

Breeding / maintenance of colonies of established genetically altered animals, not used in other procedures **excludes**:

- Genetically altered animals bred under project authorisation but killed using Schedule 1 listed methods whose tissues are then used for research: these should be reported under the purpose for which their tissues were used.
- Live animals that go on to be used in further regulated procedures.

Examples: How to return breeding and maintenance (B&M) of colonies of established lines of GAA

If the phenotype of offspring of a newly created line is not yet known, return under “Creation of new genetic line” then under appropriate purpose for which the new line was created, e.g., Basic Research Oncology. **Do not record as B&M**.

If the line has been bred for more than two generations and its phenotype is known, further breeding should be reported under purpose as B&M. “Creation of new genetic line” = NO.

Example: The line has no phenotype when heterozygous, but homozygotes show paralysis from 6 months of age. A heterozygous transgenic mouse is mated with a wild type of mouse, and produces offspring:

- The transgenic parent is reported when it is culled under: purpose B&M, genetic status “Genetically altered without a harmful phenotype” and actual severity “Sub-threshold”.
- The wild type of parent is not reported

- All heterozygous offspring's (F1) genetic status are reported as "Genetically altered without a harmful phenotype" and their actual severity are reported as "Sub-threshold".
- Wild type offspring are not reported (unless see below)
- Rarely, offspring have a second biopsy to confirm genotype that is not considered identification and must be included in the severity assessment. These will be reported as actual severity = "Mild", reflecting the biopsy procedure, whether transgenic or wild type.

The next generation is bred by crossing 2 heterozygous offspring:

- Parents (F1) are returned under genetic status "Genetically altered without a harmful phenotype", under purpose as B&M and under actual severity as "Sub-threshold", when they are culled.
- Homozygous offspring (F2) culled at 3 months of age, before appearance of phenotype: are recorded under genetic status as "Genetically altered without a harmful phenotype" and under actual severity as "Sub-threshold". If some of these were used for tissues following Schedule 1 killing, these should be reported under the purpose for which the tissues were used e.g., Basic Research, Nervous system, not under B&M.
- If offspring were kept and culled because they developed paralysis but were discarded and tissues not used then return as: purpose B&M, genetic status "Genetically altered with a harmful phenotype" and actual severity "Severe".

9. Regulatory use and routine production

Use of animals in procedures carried out with a view to satisfying legal requirements for producing, placing, and maintaining products/substances on the market, including safety and risk assessment for food and feed. For all Regulatory use, please provide the legislation name(s) and number(s) in the Comments field.

Regulatory use includes tests carried out on products/substances for which no regulatory submission is made i.e., tests performed on those products/substances (for which a regulatory submission was foreseen) that are ultimately deemed unsuitable for the market by the developer, and thus fail to reach the end of the development process.

This category also includes animals used in the manufacturing process of products if that manufacturing process requires regulatory approval (for example, animals used in the manufacturing of serum-based medicinal products should be included within this category). This includes quality assurance and potency testing of biologicals.

Efficacy testing during the development of new medicinal products is excluded and you should report this under "Translational/applied research".

Additional guidance on regulatory use and routine production categories:

- **Routine production**. Legislative requirement not required.

This category should **not be used** for immunisations etc. when done for basic or applied research. That should be reported under the appropriate sub-purpose of applied or basic research and not under routine production, except when using ascites, see below.

Routine production / blood-based products e.g., serum, whole blood:

- Any use of Ascites to generate monoclonal antibodies should be reported here, regardless of purpose and also report this as a technique of special interest
- Routine production of polyclonal antisera and immunisation for monoclonal antibody production, but only for commercial purposes e.g., for manufacture of test kits, is reported under this category
- Other forms of production of biological materials that use live animals.

- **Quality control (including batch safety and potency testing)**
  Quality control includes animals used in the testing of purity, stability, efficacy, potency and other quality control parameters of the final product and its constituents. It also includes any controls carried out during the manufacturing process for registration purposes, to satisfy any other national or international regulatory requirements or to satisfy the in-house policy of the manufacturer. This includes pyrogenicity testing.
  - PR61 (Quality control) Batch safety testing (excludes pyrogenicity testing)
  - PR62 (Quality control) Pyrogenicity testing
  - PR63 (Quality control) Batch potency testing
  - PR64 (Quality control) Other quality controls

- **PR71 (Regulatory use) Other efficacy and tolerance testing**
  - Efficacy testing of biocides and pesticides is covered under this category as well as the tolerance testing of additives in animal nutrition.
  - Combined tolerance/efficacy studies, dose range finding studies and maximum tolerated dose studies **when being carried out to support regulatory submissions** should be reported under this category.

- **Toxicity and other safety testing including pharmacology by test type -**
  Includes safety evaluation of products and devices for human medicine and dentistry and veterinary medicine. This covers studies carried out on any product or substance to determine its potential to cause any dangerous or undesirable effects in humans or animals as a result of its intended or abnormal use, as a result of its manufacture or as a potential or actual contaminant in the environment.
  Please choose the most appropriate test description:
  - Immunotoxicology studies should be reported under “Repeated dose toxicity”.
  - Kinetics (pharmacokinetics, toxicokinetic, residue depletion): If toxicokinetic is performed as part of the regulatory repeat dose toxicity study, you should report it under ‘Repeated dose toxicity’.
  - Safety testing in the food and feed area includes testing of drinking water (including target animal safety testing).
  - Target animal safety: This is testing to ensure that a product for a specific animal can be used safely on that species (excluding batch safety testing, which is covered under “Quality control”).

10. Other purpose

If you have chosen any of the “Other” sub-purpose categories, you should provide details.

Testing by legislation

- Information should only be entered in this field if '[PR] Regulatory use’ was listed as the purpose.
- The legislative requirement should be entered as per the intended **primary** use. For example, in relation to water quality, if it is concerning tap water for drinking you should report it under “Food legislation”.
- Note that legislative requirement is not required for Routine production of e.g., Blood products.

Other testing by legislation

- If you have entered “Other” provide details.

Legislative requirements (origin of the legislation)

- Information should only be entered in this field if '[PR] Regulatory use’ was listed as the purpose and it was not Routine production.
- This category allows identification of the level of harmonisation between different legislative requirements. The determining factor is not who requests the test to be carried out but which legislation is satisfied, giving priority to the widest level of harmonisation.
- Where national legislation is derived from EU legislation, only ‘Legislation satisfying EU requirements’ should be chosen. ‘Legislation satisfying EU requirements’ also includes any international requirement that at the same time satisfies EU requirements (such as testing to the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), the Organisation for Economic Cooperation and Development (OECD), and European Pharmacopoeia monographs).
- ‘Legislation satisfying UK requirements only’ is to be chosen only when the test is carried out to satisfy UK requirements and there is no equivalent requirement in the EU.
- ‘Legislation satisfying non-EU requirements only’ is to be chosen when there is no equivalent requirement to carry out the test to satisfy EU requirements.

Severity

You should give the actual severity that animals used on the procedure experienced, **not** the severity classification or limit of the protocol.

Refer to the Home Office document “Advisory notes on recording and reporting the actual severity of regulated procedures” and the guidance for Severity classification of genetically altered animals under the Animals (Scientific Procedures) Act 1986:
Assign the severity to one of the categories:

- Sub-threshold
- [SV1] Non-recovery

- [SV2] Mild
- [SV3] Moderate
- [SV4] Severe

Sub-threshold severity is chosen when a procedure was regulated, and therefore it was considered prospectively that the procedure might have caused mild, moderate, or severe suffering, but which in retrospect did not. If “Sub-threshold” is reported in combination with an experimental study, please provide an explanatory comment. An experimental study is one where “No” was entered for “Creation of a new genetic line” and a purpose was entered **other than** “Breeding / maintenance of colonies of established genetically altered animals, not used in other procedures”.

Whenever the severe classification is exceeded (where an animal was suffering severe prolonged pain that was not alleviated) - whether preauthorised or not - you should report these animals and their use normally like any other use, and under the “Severe” category. You should add further details explaining:

- whether prior authority was obtained.
- the details of the use; and
- the reasons why the severe classification was exceeded.

If “Severe” is reported for over 999 procedures in combination with the purpose “Breeding/maintenance of colonies of established genetically altered animals, not used in other procedures”, please provide an explanatory comment.

If “Non-recovery” is reported in combination with either “Creation of a new genetic line” or “Breeding / maintenance of colonies of established genetically altered animals, not used in other procedures”, please provide an explanatory comment.

Reporting of wild animals used in procedures under ASPA

- Procedures should be reported, and severity assessed at the end of a procedure, this poses challenges for work in the wild.
- The procedures should be reported in the best way practicable, following guidance given in separate documents on “Working with animals taken from the Wild” and reporting on actual Severity.
- Where possible animals should be reported when the procedure ends, or the animal is known to have died. If this is not practicable then report either:
  - At the end of the study when attempts to recapture are no longer made or,
  - At the end of the relevant project licence when the work will not continue
- There will often be uncertainty as to the fate of animals in the wild.
- Refer to the above guidance and discuss this with your local inspector.

Comments for the attention of the Home Office

If more than 99 non-human primates or 999 of any other species are entered in a single cell, then you should add an explanatory note:

- If the large number applies to a single study, then briefly explain why so many animals were used.
- If multiple studies have been combined into one entry, and this is the reason for the large number, simply state e.g., ‘Combination of studies.

- If a large number of animals used on the same breeding protocol has been entered on one line, simply state “Breeding”.

In addition, please provide an explanatory comment when reporting any of the following:

- Schedule 2 species[2] (NOT born at a licensed establishment or at a registered breeder.
- “Not genetically altered” animals reported in combination with the purpose “Breeding/maintenance of colonies of established genetically altered animals, not used in other procedures”.
- “Sub-threshold” is reported in combination with an experimental study. An experimental study is one where “No” was entered for “Creation of a new genetic line” and a purpose was entered **other than** “Breeding /
- maintenance of colonies of established genetically altered animals, not used in other procedures”.
- “Severe” reported for over 999 procedures in combination with the purpose “Breeding/maintenance of colonies of established genetically altered animals, not used in other procedures”.
- “Non-recovery” reported in combination with either “Creation of a new genetic line” or “Breeding / maintenance of colonies of established genetically altered animals, not used in other procedures”.

[2] Schedule 2 species are mice, rats, guinea pigs, hamsters, gerbils, rabbits, cats, dogs, ferrets, non-human primates, pigs (if genetically modified), sheep (if genetically modified), common quail (Coturnix coturnix), amphibians (of the species Xenopus laevis, Xenopus tropicalis, Rana temporaria and Rana pipiens), and zebrafish.