

**REPORT ON THE USE OF ANIMALES IN EXPERIMENTATION AND OTHER
SCIENTIFICA PURPOSES, INCLUDING TEACHING AT THE BIOLOGICAL
RESEARCH CENTER MARGARITA SALAS IN 2022**

Roysa Decree 53/2013, of February 1st, which establishes the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching, establishes that Member States of the European Union will collect annual information on the use of animales in experimental procedures, which will be published annually by the Ministry of Agriculture, Food and Fisheries.

In order to comply with mentioned regulations, the Biological Research Center Margarita Salas annually notifies the Community of Madrid of the number of uses of animals that have been carried out in the experimental projects that are authorized at the center.

The information contained in this report constitutes a summary of that sent for mentioned notification and is collected according to data existing in the center on the use of animals in different projects that were active in the year 2022, data compared with those notified during years 2020 and 2021 in order to analyze the evolution and existing trends in the center regarding the use of experimental animals.

This report quantifies how many times animals have been used in procedures, and does not represent the total number of animals used, as it is possible for the same animal to be used in several procedures as long as appropriate welfare requirements are met.

1. Number of uses of the species used.

At the Biological Research Center Margarita Salas there are various experimental projects approved by the competent authority. Specifically, in 2020, 33 projects were active, and they were increased to a total of 47 projects in 2021, producing a slight decrease in the number of active projects in 2022, which amounted to a total of 38. These variations can be explained due to the completion of various experimental projects during the aforementioned year (Table 1).

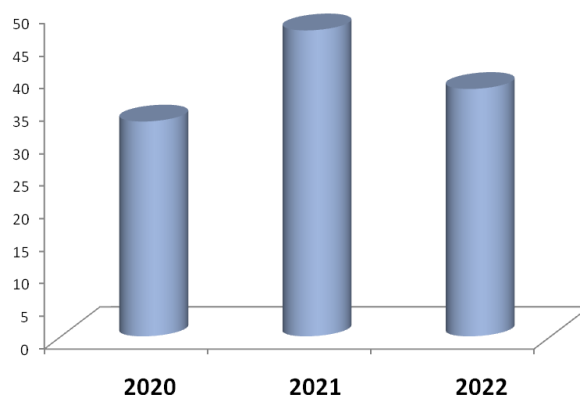


TABLE 1. Numbers of annual animal projects.

Analyzing data of species used (Table 2), it can be observed that in 2021 and 2022 only animales on the murine species (mouse, *Mus musculus*) were used, while in 2020 5 rabbits (*Oryctolagus cuniculus*) were used in an authorized procedure for the production of polyclonal antibody, which accounted for 99.83% of the uses made of animal that year. After the completion of this experimental Project, this animal specie (rabbit) has not been used in our center.

ESPECIE	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Ratón (<i>Mus musculus</i>)	2977	99,83	4580	100	1700	100
Conejo (<i>Oryctolagus cuniculus</i>)	5	0,17	0	0	0	0
TOTAL	2982	100	4580	100	1700	100

TABLE 2. Number of uses according to animal species.

In reference to the use of mice, in 2021 there was a significant increase (53.58%) compared to 2020 in the use of animales, due to the increment in the number of active projects during that year. When we compared the years 2021 and 2022, we can observe that the number of uses decreased drastically (65.89%) in the last year since 9 active projects were completed.

1. Number of uses of animals according to the leve of severity of the procedures to which the animals are subjected.

Data presented refers to the number of uses of animals according to the severity of the procedures to which they are subjected, which is determined by the degree of pain, anguish, suffering or lasting damage that experimental techniques produce on the animal.

According to Royal Decree 53/2013, experimental procedures can be classified as:

- ✓ **Whitout recovery:** they are performed entirely under general anesthesia and the animal is sacrificed without regaining consciousness.
- ✓ **Mild:** procedures that produce pain, suffering or anguish of a mild nature and of short duration, as well as those procedures in which there is no significant alteration of well-being or general conditions.

- ✓ **Moderate:** procedures that produce moderate pain, suffering or anguish in the animal of short duration, or mild but long-lasting, as well as those that could cause a moderate alteration of the well-being or general condition of the animals.
- ✓ **Severe:** procedures that probably produce intense or moderate pain, suffering or anguish in the animal but lasting, as well as those that can cause a serious alteration in the well-being or general condition of the animal.

Data collected (Table 3) indicates that there was a reduction in the number of uses of animals in procedures classified as “without recovery” between the years 2020 and 2021. If we compare data according to severity of procedures between years 2021 and 2022, it can be observed that in latter there was a reduction in the number of procedures classifies as “withouth recovery” and “mild”, while the number of uses of “moderate” procedures was maintained. On the contrary, 63 uses of “severe” procedures were reported for the firs time in the center, consituting 3.7% of total procedures carried out.

These severe procedures were performed in projects aimed at oncological research that produced severe health alterations.

Severidad de los procedimientos	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Sin recuperación	1546	51,84	1141	24,91	66	3,9
Leve	0	0	2296	50,14	611	35,94
Moderado	1436	48,16	1143	24,95	960	56,46
Severo	0	0	0	0	63	3,7

TABLE 3. Uses according to severity of procedures.

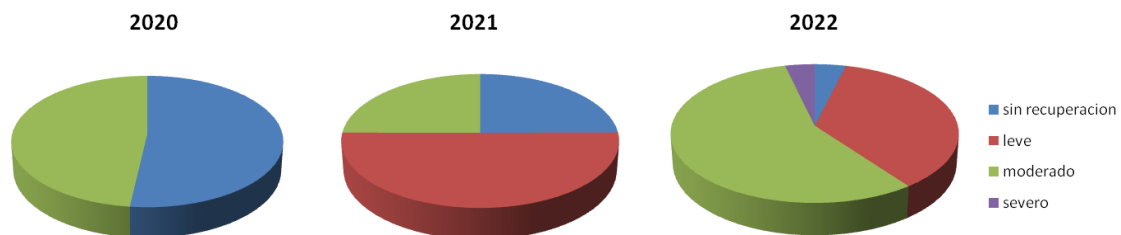


FIGURE 1. Procedures according to their severity classification

2. Number of animals used according to their genetic status.

An animal is considered to be genetically modified if it has been subjected to any manipulation that involves a modification of its genotype or has suffered as spontaneous mutation that produces an altered genotype. Such genetic modification may produce alterations in the general or health status of the animal (considered a pathological phenotype) or may not produce such modifications (considered a non-pathological phenotype).

According to data shown (Table 4), it can be observed that genetically modified animals are mostly used at the center.

Estado genético de los animales	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Animales no alterados genéticamente	145	4,86	457	9,99	368	21,64
Animales alterados genéticamente SIN fenotipo dañino	2721	91,25	4053	88,5	1210	71,18
Animales alterados genéticamente CON fenotipo dañino	116	3,89	70	1,52	122	7,18

TABLE 4. Number of uses according to genetic status of animal.

It can be observed between 2020 and 2021 that use of non-genetically altered animals increased since experimental projects were carried out in which this type of animals were used. However, due to the completion of some of these projects, in 2022 the use of animals that do not carry any genetic modification was moderately reduced, despite the fact that they represented 21.64% of total animals used in the aforementioned year.

In reference to genetically modified animals, it can be observed that in the years 2020, 2021 and 2022, those that do not present a harmful phenotype represent the majority of animals used (91.25%, 88.5% and 71.18%, respectively), compared to those that have a harmful phenotype, which are a minority (3.89%, 1.52% and 7.18%, respectively). An increase in the use of animals of the latter type was detected in 2022, due to the implementation of projects based on the use of immunodeficient animals.

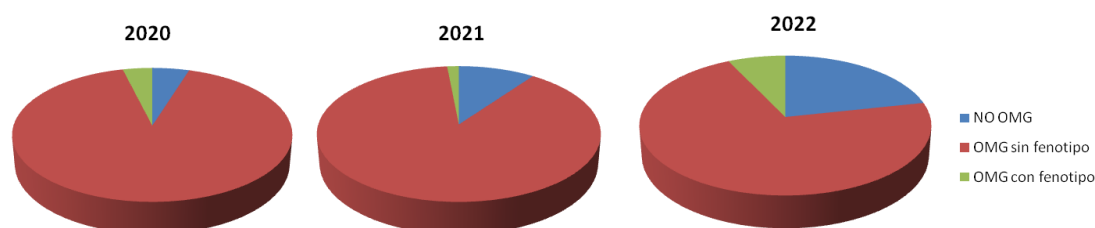


FIGURE 2. Number of uses according to genetic status of the animal

3. Number of animal reuses

According to Royal Decree 53/2013, animals can be reused in different procedures as long as the requirements are respected:

- ✓ That actual severity of the procedure to which animal has previously been subjected is mild or moderate.
- ✓ That full recovery of the state of health and well-being has been demonstrated.
- ✓ That new procedure to be performed is classified as mild, moderate or without recovery.
- ✓ That favorable veterinary advice is available.

On the other hand, legislation recommends that animals not be reused when another animal that has not previously undergone the procedure can be used in their place. Following this recommendation, at Biological Research Center Margarita Salas, 100% of uses of animals that were carried out in years 2020, 2021 and 2022 were considered “first use”, thus avoiding reuse (Table 5).

Reutilización de animales	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Primer uso	2982	100	4580	100	1700	100
Animales reutilizados	0	0	0	0	0	0

TABLE 5. Number of animal reuses.

4. Number of uses according to the origin of animals.

From legislative point of view, the origin of the animals used in experimental procedures is of great relevance, especially in higher species such as non-human primates. According to Royal Decree 53/2013, only animals that have been bred for experimental purposes that come from centers registered as breeders or suppliers of animals can be used.

In Table 6 it can be observed that all animals used in the Biological Research Center Margarita Salas in years 2020, 2021 and 2022 come from establishments registered in the European Union.

Lugar de nacimiento	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Nacidos UE en establecimiento registrado	2982	100	4580	100	1700	100
Nacidos UE en establecimiento no registrado	0	0	0	0	0	0
Nacidos en el resto de Europa	0	0	0	0	0	0
Nacidos en el resto del mundo	0	0	0	0	0	0

TABLE 6. Number of uses depending on the place of birth of animals.

5. Number of uses according to the purpose to which animals are dedicated

If thematic research areas to which the use of animals is dedicated in the Biological Research Center Margarita Salas are analyzed, it can be observed (Table 7) that in years 2020 and 2021, 100% of the animals used were dedicated to basic research projects, while in 2022 the 15.3% of uses were for applied research, due to various studies on vaccine candidates for Sars-Cov-2.

Fines de utilización de animales	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Investigación básica	2982	100	3879	84,7	1700	100
Investigación traslacional y aplicada	0	0	701	15,3	0	0
Utilización reglamentaria y producción rutinaria	0	0	0	0	0	0
Protección del medio ambiente	0	0	0	0	0	0
Enseñanza superior	0	0	0	0	0	0
Investigaciones forenses	0	0	0	0	0	0
Mantenimiento de colonias de OMGs	0	0	0	0	0	0

TABLE 7. Number of uses dependind on the purpose.

If we focus on the different areas of study that are included within the field of basic research, it can be observed (Table 8) that between 2020 and 2021 there was an increase in the number of uses in oncology trials, which were significantly reduced in 2022. Moreover, the use of animals dedicated to cardiovascular and immune system studies and trials focused on immune system and multisystem research remained constant between 2021 and 2022.

On the other hand, a slight reduction was observed in the percentage of uses of animals dedicated to the study of sensory organs during 2022.

In contrast, during 2022, no studies focused on the nervous, musculoskeletal or reproductive sistemas, nor trials related to developmental biology, were carried out, but two studies were performed within the field of the gastrointestinal system.

Investigación básica	2020		2021		2022	
	Nº Usos	%	Nº Usos	%	Nº Usos	%
Oncología	776	26,02	1376	35,29	397	23,35
Sistema cardiovascular	0	0	85	2,18	66	3,88
Sistema nervioso	357	11,97	57	1,46	0	0
Sistema gastrointestinal	0	0	0	0	197	11,58
Sistema musculoesquelético	116	3,89	0	0	0	0
Sistema inmunitario	1064	35,34	810	20,77	485	28,52
Sistema reproductor	109	3,65	0	0	0	0
Órganos sensoriales	510	17,1	1268	32,52	402	23,64
Multisistémico	60	2,01	287	7,36	75	4,41
Biología del desarrollo	0	0	16	0,41	0	0