GV-SOLAS
Gesellschaft für Versuchstierkunde

Specialist information

From the Committee for Genetics and Laboratory Animal Breeding

Recommendations for transporting genetically engineered mice and rats of risk group 1

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1. Introduction

Genetically engineered animals are invaluable in vivo models for biomedical research. To an increasing extent, they are also used for studies of isolated organs and tissues (in vitro). The number of genetically engineered lines/strains worldwide has grown exponentially in the last few years, especially in mice, but also in other animals. Yet, genetically engineered animals are no longer produced solely in research laboratories of universities and large research institutions; they are increasingly produced also by specialized commercial suppliers. Whereas, in the early days of their use, individual or just a few genetically engineered animals were occasionally exchanged between institutes engaged in scientific cooperation, today we increasingly often see ever larger contingents of animals being transported for an even wider group of users.

2. Genetically engineered (GE) animals as per GenTG versus genetically modified (GM) animals as per TierSchG

According to § 3 of Germany's Gene Technology Act (GenTG) a genetically engineered organism is "an organism, with the exception of humans, whose genetic material has been changed in a way that does not occur under natural conditions through crossbreeding or natural recombination; a genetically engineered organism is also an organism that has arisen through interbreeding or natural recombination between genetically engineered organisms or with one or more genetically engineered organisms or through other kinds of reproduction of a genetically engineered organism, provided the genetic material of the organism shows characteristics that are attributable to genetic engineering procedures". This definition in the GenTG naturally applies also to genetically engineered animals. All animals generated on the basis of homologous recombination (constitutive or conditional knock-out, knock-in) or additive gene transfer (e.g. pronuclear injection of DNA constructs, viral gene transfer or reproductive clones) are thus genetically engineered (GE) animals according to GenTG (GE animals). Mutations that occurred spontaneously or were induced physically by radiation or chemically by mutagenic substances are not per se genetically engineered modifications within the meaning of GenTG. Mutations induced only through the action of zinc finger nucleases (ZFNs) are also regarded as genetically engineered modifications within the meaning of GenTG concerning the use of zinc finger nuclease technology 1 (ZFN-1)[1]. A final legal assessment of the extent to which transgenic animals produced using new methods such as CRISPR/Cas9 fall within the scope of the GenTG. This Question was addressed and decided (albeit with regard to the breeding of plants) by the European Court of Justice. In the amendment to Germany's Animal Welfare Act (TierSchG) of 2013, the term "genetically modified animal" is introduced under § 7a (5). Genetically modified animals according to TierSchG are all animals that carry specific mutations of individual gene loci, regardless of whether these mutations are the result of genetically engineered modifications according to GenTG, such as transgenic or homologous recombination, or of other factors, such as radiation or chemical mutagenesis. The definition of genetically modified animals according to TierSchG thus also includes genetically engineered animals according to GenTG, but also extends well beyond this.

[1] General position statement of the ZKBS on the use of zinc finger nuclease-1 technique (ZFN-1): http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/ZKBS/02_Allgemeine_Stellungnahmen_englisch/01_general_subjects/zkbs_general_ZFN_1.pdf%3F__blob=publicationFile%26v=3

3. Legal framework for transporting genetically engineered animals

3.1. Gene Technology Act (GenTG)

Genetically engineered animals (GM animals) are genetically modified organisms (GMOs) within the meaning of the German GenTG. The production, housing and/or breeding of such animals and their use in animal experiments are deemed to be genetic engineering procedures that can only be performed in genetic engineering facilities approved by the authorities. The overwhelming majority of genetically engineered lines/strains are classified as Risk Group 1 animals, i.e. they pose no risk to human health and the environment according to the current state of scientific knowledge.

According to § 7 of the German regulation on the Safety of Gene Technology (GenTSV), animals as recipient organisms are assigned to Risk Group 1 if they are not expected to have any harmful effects on humans, the environment, animals, and plants. In the case of genetic engineering procedures with animals, therefore, care must also be taken to ensure that any viral vector used is not horizontally transmissible and that the genetic modification can thus only be transmitted to the offspring of the animal. Furthermore, the transferred genetic information must not lead to any added risk; it must therefore be well characterized and have no pathogenic potential for other organisms than the GMOs themselves (for an example, see also statement Az.: 6790-10-06 of the ZKBS on the mouse line Big BlueTM). Criteria for risk assessment are listed in Appendix I of GenTSV.

The in-house transport of GM animals is also a genetic engineering procedure according to GenTG (§ 3 no. 2 GenTG). However, the term “in-house” is currently interpreted in different ways. A comment on the German Gene Technology Act defines “in-house transport” as transport within a gene technology facility. Therefore, transport between several different facilities on the same business premises does not meet this definition (Eberbach, Lange, Ronellenfitsch: Recht der Gentechnik und der Biomedizin, Kommentar, Materialien, 79. Aktualisierung September 2012).

If animals are transported across areas that do not belong to a gene technology facility, the transport container must satisfy the requirements of closed containment according to that of a gene technology facility. It must therefore be sufficiently secure to prevent animals escaping (see 5.2. and 5.3). The animal cages must be provided with cards showing the strain and genotype of the animals. The external transport of GE animals is not covered by German gene technology legislation. An external transport is regarded as such even if the animals have a stopover during the transport. This stopover does not have to be in an animal facility licensed under gene technology legislation, as long as the animals do not spend more than three working days at the location of the stopover. If the animals remain in one place for longer, this constitutes storage according to Germany’s Gene Technology Act, which requires the animals to be accommodated in a gene technology facility (State Working Group Decision on Gene Technology)[2].

[2] Gentechnik-Beschluss 11/1992,
http://www.laggentechnik.de/dokumente/EndfassungLAGBeschlusssammlung.pdf

For the transport of GE animals, the GV-SOLAS Committee for Genetics and Breeding of Laboratory Animals urgently recommends the use of lockable and unbreakable containers that also guarantee hygienic isolation of the animals. When transporting between facilities, the sender must also make sure before shipment that the receiving laboratory meets the personnel and spatial requirements laid down in gene technology legislation. This is regulated by the rules of the ADR (for road transport), IATA (for air transport), the German Animal Welfare Act and the German Regulation on animal welfare during transport (Tierschutztransport-verordnung), as outlined below.

3.2. European Agreement on the International Carriage of Dangerous Goods by Road (ADR) and classification according to dangerous goods law

According to 2.2.9.1.11 of the ADR, genetically engineered organisms are essentially assigned to Class 9 (packaging norm UN No. 3245) “if they do not meet the definition of toxic substances or of infectious substances, but are capable of altering animals, plants or microbiological substances in a way not normally the result of natural reproduction”. With the amendment of the ADR in 2017, however, the following remark was included under 2.2.9.1.11: “Genetically modified live animals which, in accordance with the current state of scientific knowledge, have no known pathogenic effect on humans, animals and plants and are carried in receptacles that are suitable for safely preventing both the escape of the animals and unauthorized access to them, are not subject to the provisions of ADR. The provisions specified by the International Air Transport Association (IATA) for air transport "Live Animals Regulations, LAR" can be drawn on as guidelines for suitable receptacles for the transport of live animals.” The use of appropriate containers in keeping with IATA rules for the transport of genetically engineered live animals in Risk Group 1 is thus sufficient, and there are no further labelling requirements according to the ADR regulations.

3.3. International Air Transport Association (IATA)

For air transport, the provisions specified by the IATA (Live Animals Regulations, LAR) must be observed. It is specified in the IATA regulations under 3.9.2.5.2 that “genetically engineered organisms and microorganisms which do not meet the definition of toxic or infectious substances must be assigned to UN 3245.” However, this is qualified by the further provision, under 3.9.2.5.4 of the IATA regulations, that “genetically engineered live animals must be transported under terms and conditions of the appropriate national authorities of the States of origin and destination.” There are currently no rules, however, stating which authority is responsible for specifying these conditions and how decisions according to GenTG are made with regard to classifying under dangerous goods law the transport of GE animals on Safety Level 1 that are not experimentally infected or treated with dangerous goods.

IATA regulations lay down extensive requirements regarding transport containers for live (GE) animals, which are discussed under section 6.

3.4. German Animal Welfare Act (TierSchG)

Before the shipment of animals, including GE animals in Risk Group 1, it must be ensured that the receiving laboratory meets the requirements laid down in the German Animal Welfare Act (TierSchG). For example, the recipient must have a licence for animal husbandry in

accordance with § 11 TierSchG. In addition, it must be borne in mind that simply breeding and housing animals that are proven to have a constraint as the result of a genetically engineered modification or are not yet characterized in this respect constitutes an animal experiment that is either notifiable or requires regulatory approval. Animals can only be imported in such cases if the recipient has a duly notified and approved procedure in place in which the breeding or housing of the strain concerned is explicitly named. In the case of imports from third countries, an application for “approval under animal welfare law to import vertebrates from third countries for use as laboratory animals” must be submitted to the responsible authority according to §11a (4), sentence 1 TierSchG.

3.5. German Regulation on animal welfare during transport (TierSchTrV)

The German Regulation for the protection of animals during transport and for the implementation of the European Regulation (EC) No 1/2005 was enacted on 11 February 2009 based on §§ 2a and 12 of the German Animal Welfare Act. For the sender and recipient of GE animals, sections 2 “Transport in containers” and 5 “Cross-border transport” are especially relevant. Regardless of the provisions of European Union Community Law Regulations, senders must, in the case of domestic transport, satisfy themselves that the recipient’s address is correct and inform the recipient before shipment of when the consignment will be shipped, the estimated time of arrival, the destination and the mode of transport. The sender must further ensure that the animals are protected against foreseeable adverse climatic events by the transport containers or by other means offering equivalent protection. Since it is difficult to ensure protection against the influence of the weather with the usual transport containers, it is recommended that a qualified carrier be commissioned who specializes in animal transport and has appropriately air-conditioned business premises and vehicles and offers “direct transport” or “door-to-door transport”, in contrast to general freight carriers, which might involve unregulated stops and reloading with practically no possibility for checking in terms of weather. The animals must be provided with sufficient food and water for double the transport time, so that there are no shortages even in the event of a possible return transport. When setting the day of transport, it must be ensured that a return transport can be completed by the end of a working week on Friday or before public holidays. The time of packing and the time (in days) during which the animals are guaranteed a reliable supply of food and water by the quantities provided are usually to be shown as a rule in the accompanying documents.

Transportation across EU borders can only be handled via certain customs and border inspection posts known to the veterinary authorities. One of the duties of the sender or recipient of animal consignments is to inform the responsible local veterinary authority in advance about "Traces" (Commission Decision 2003/623/EC regarding the European Council Directive 90/438/EEC) and the relevant border inspection post concerned in advance (at least one day before) (this is usually done by the qualified carrier commissioned with the transport). The border inspection post conducts an import inspection, which involves not only checking the animals (through suitable openings or partially transparent packaging; see 6. Suitable transport containers), but also checking the accompanying documents and the identity for compliance with animal welfare regulations. Customs regulations are not discussed here. In view of the large number of requirements to be met by the sender and the recipient, particularly when it comes to the cross-border transport of animals, it is advisable to commission a specialist carrier.

3.6. Special cases

Examples of animals that are formally assigned to Risk Group 1 but nevertheless require special attention are transgenic animals that express receptors for germs pathogenic to humans and thus represent a new artificial (HIV, Browning et al., 1997; measles virus, Horvat et al., 1996; polio virus, Koike et al., 1993) or better (Tseng et al., 2007) host organism for these pathogens. There are also receptor transgenic animal lines that show new susceptibilities for animal pathogens (avian leucosis, Federspiel et al., 1994; porcine retroviruses, Martina et al, 2006). The housing and breeding of such transgenic animals that are not infected with the corresponding pathogen constitutes a Safety Level 1 genetic engineering procedure. However, escaped animals that are susceptible to these pathogens could create new pathogen reservoirs in the environment, which could jeopardize the corresponding epidemic control programs of the WHO (WHO Committee 1993). Although animals that express receptors for human pathogens as a result of their genetic engineered modification are only assigned to Risk Group 1, the WHO (WHO Committee 1993) justifiably and urgently recommends the use of a “box-in-box” system for such animals, in which the inner container must have a germ-proof filter and the outer container must be particularly stable and secured against unauthorized opening (padlock). For GM animals with transgenic receptors for human infectious pathogens, the WHO also recommends labelling them “potentially biohazardous” and castrating these animals before transport.

4. Practical aspects and alternatives for the transport of GE animals in Risk Group 1

4.1. Transport of genetically engineered live animals

According to the assessment of GV-SOLAS, it is paramount that the escape of GE animals in Risk Group 1 during transport, like conventional laboratory animals classed under Safety Level 1 (BioStoffV), be prevented, for the reasons mentioned above. This can be achieved using of the escape-proof transport containers described in section 6. This also corresponds standard international assessments and practice (White et al. 2010).

4.2. Transport of GE animals in the form of cryopreserved embryos or sperm

An alternative to the live transport of GE animals is the transfer of cryopreserved embryos/sperm. This has several advantages: an accidental escape of animals is a priori ruled out; the risk of introducing pathogenic microorganisms of the species concerned is minimized and so quarantine is usually unnecessary; and transport-induced stress in the animals can be avoided. However, transport in the form of such cryopreserved material is only possible/useful if the sender has the techniques to cryopreserve embryos and sperm and if the recipient is skilled in the methods of IVF and embryo transfer. Depending on the genetic background of the transgene variant, the low efficiency of embryo collection means that it can take several weeks before a sufficient number of embryos have been cryopreserved. Finally, after successful reimplantation of the embryos in mice, for example, a further two months may pass until transgenic animals are available that are ready for breeding.

5. Transport risks

5.1. Risk to transport staff

In the event of damage to the transport container, there is a possibility that the transport staff will come into direct contact with GE animals or be injured by these animals. In the case of GE animals classed in Risk Group 1, no risk is to be expected here as a result of the genetically engineered modification, because the genetically engineered genes can only be passed on vertically to offspring and not transmitted horizontally (e.g., in the saliva) to other species. The risk of infection from conventional microorganisms as a result of rodent bites remains unchanged but is low with specific pathogen-free (SPF) animals.

5.2. Escape of genetically engineered animals

Laboratory mice or laboratory rats are usually domesticated and heavily inbred animals that are not adapted to survival in the wild as a result of being kept under artificial or experimental conditions. The strains often used as genetic background for the production of transgenic animals thus have selection disadvantages, such as age-related hearing loss (C57BL/6), retinal degeneration (C57BL/6N, C3H, CBA, etc.) or albinism (Wistar rat, BALB/c mice), as well as a reduced reproduction rate. Based on current knowledge, it is extremely unlikely that GE animals which escape during transport will reproduce among themselves or mate with wild forms and thus transfer the genetically engineered modification into the environment. These laboratory animals would most likely die without reproducing while in the environment. However, since the fate of escaped laboratory animals in the environment has not yet been experimentally studied, the transfer of the genetically engineered modification into the environment cannot be ruled out with complete certainty. If GE animals of Risk Group 1 are involved, accidental escape cannot be expected to pose a direct risk either to humans or to the environment. Nevertheless, the transport routes and containers must be checked with particular care to avoid accidental escape, as the authorities may consider it necessary to undertake costly measures to capture and kill escaped animals or otherwise prevent the transgene from spreading into natural populations. These measures could result in further legal (e.g., liability) claims.

5.3. Legal assessment of the escape of genetically engineered animals during transport

The current practice for transporting GE animals from Safety Level 1 in sealed and unbreakable transport containers (without classification according to UN 3245) has not met with objections at international level in the past. Accordingly, there is to our knowledge no experience to date concerning the legal assessment of GE animals escaping in the context of a transport accident. The problem is complex, because the specific circumstances of the escape may differ, and this has to be taken into account. It can be assumed that, if necessary, the situation would be examined to establish whether there has been a breach of the law on the transport of dangerous goods. The authority responsible for gene technology law will also consider whether there has been a breach of the Gene Technology Act (GenTG).

In 2012, a ruling by the German Federal Administrative Court on the unauthorized introduction of genetically engineered rapeseed into the environment was published[3]. In this legal dispute, the court came to the conclusion that the criminally relevant offence of illegal targeted release can also exist if the presence of genetically engineered organisms in the environment is merely the result of a wilful act. It is unclear whether this broad legal interpretation can be applied to the unintentional escape of GE laboratory animals during transport. Nevertheless, it has to be expected that the competent authority would launch an investigation to determine whether there had been a breach of the law if the animals escaped.

6. Suitable transport containers

The dimensions of the transport container must be adequate, and the animals protected from environmental influences and infectious pathogens. An adequate ventilation of the container, which must be constructed in such a way that it is not obstructed or occluded even when the transport containers are stacked, ensures that the animals remain supplied with oxygen while noxious gases and heat are discharged. As regards the shipment of SPF animals, transport containers must be capable of decontamination, e.g., autoclavable or resistant to gaseous or liquid chemicals.

In view of the possible legal consequences of animals escaping during transport, as described under section 5.3., the containers must be mechanically stable, impact-resistant, stackable, weatherproof, disinfectable (ideally by steam sterilization) and escape-proof. For this reason, packaging made entirely of cardboard is only suitable to a limited extent. Possible transport containers should be made of plastic (see Figures 1 and 2). The material of the boxes and the box lining must be such that all areas accessible to the animal are protected from gnawing. This also applies to the ventilation openings. If these are closed with wire mesh, the mesh size must be such that the animals cannot stick their paws or snouts through them or injure themselves on the wire mesh. The containers must also provide adequate protection against moisture from the outside. In order to minimize the risk to the microbiological status of the animals, the ventilation openings should also be protected by filters. It should be borne in mind that, depending on pore size, this can greatly reduce the ventilation rate. It should also be ensured that the ventilation openings remain free of obstruction even when the cages are stacked, which is usually necessary. To allow the animals to be inspected during transport, a window should be provided, or the container should be partially transparent.

The lid of the container must be secured in such a way that it cannot be opened accidentally or capable of jumping open by bumping into it. This can be achieved, for example, with the additional safeguard of plastic clamping components (see Figure 1). Opening by non-authorized persons should be prevented (e.g., by notices, fasteners).

[3] Ruling of the German Federal Administrative Court on 29.02.2012 (http://www.bverwg.de/entscheidungen/entscheidung.php?az=7+C+8.11&datum=29.02.2012); item 19:
When genetically engineered organisms enter into the environment, however, this alone does not constitute release. It is rather the case that the adjective “targeted” expresses a final element. Accordingly, the presence of the genetically engineered organisms in the environment must be the result of targeted action. This can be understood to mean that the aim must consist in releasing the genetically engineered organisms. But it is not necessarily so, for the word “targeted” in the sense of intentional (alone) may also refer to the presence of genetically engineered organisms in the environment being the result of a wilful action. This interpretation is also covered by the possible meaning of the word.

In the case of air transport, the guidelines laid down by IATA (Live Animals Regulations, LAR) must be observed. Two different transport containers are described here, one for small mammals, including mice and rats, and the other specifically for laboratory rodents (gerbils, guinea pigs, hamsters, rats, and mice) with SPF status.

Also, for safety reasons, the transport containers must be visible from the outside according to IATA regulations. If the transport containers deviate from the above-mentioned requirements, this can lead to delays, to adverse effects impacting on animal welfare or to the consignment being turned back. Visibility is also important to ensure that the animals can be counted during transport and during border inspection by veterinarians at the border inspection posts and their state of health assessed without having to open the packaging.

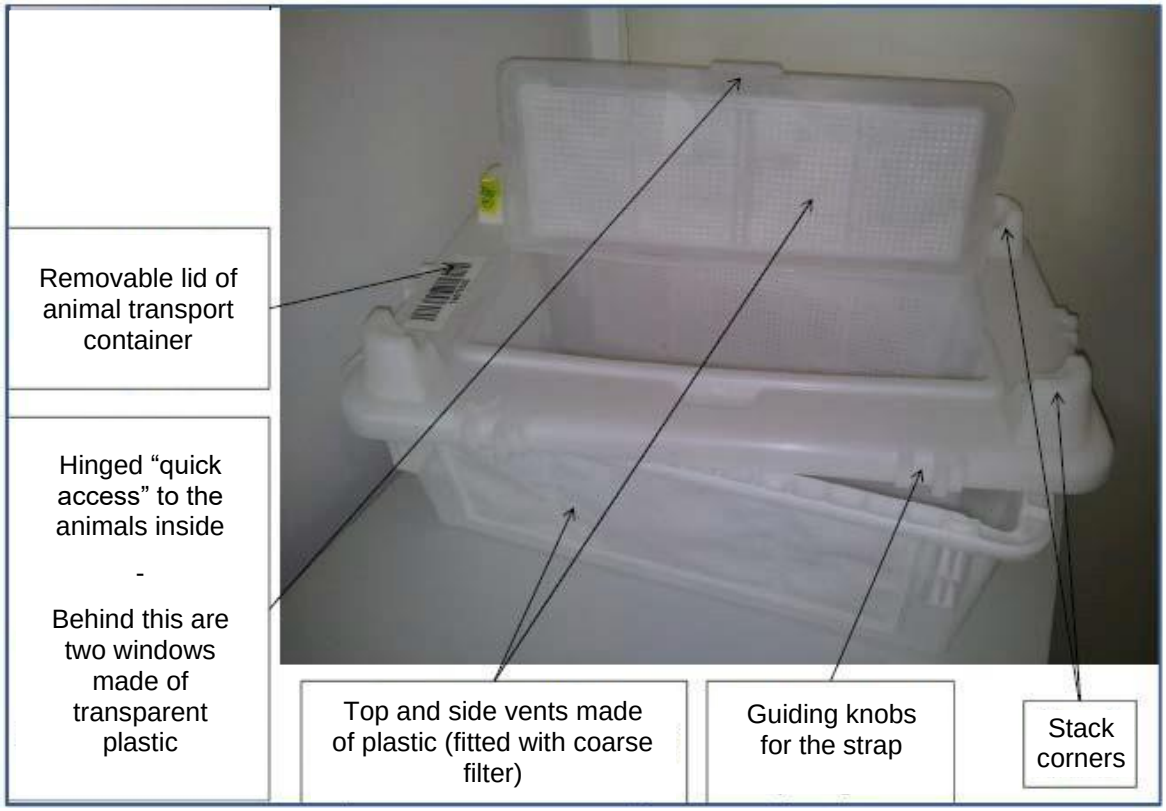

Figure 1: Autoclavable, single-walled plastic box for transporting mice. It is stackable and has vents on the top and at the sides. The entire lid and the hinged “quick access” is first fixed by locking knobs, but then additionally secured by means of two straps. The inlet in the upper left corner shows the finished and packed container. Transport documents are affixed to the side walls of the container.

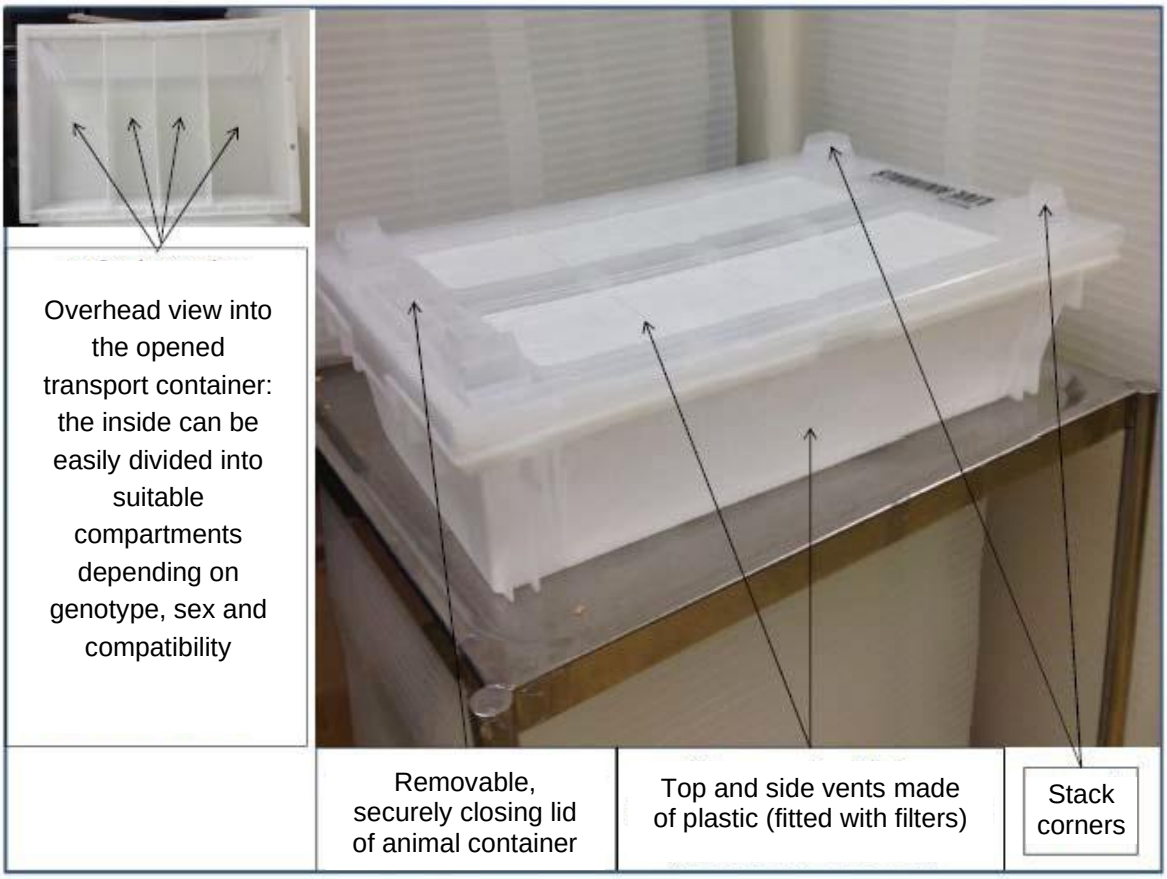

Figure 2: Stackable, single-walled plastic transport box

7. Declaration recommendations

Apart from the required information “Live animals” and, if applicable, a note on the side indicating which side up the container must stand “Top ↑”, a note should also be affixed to the transport containers stating: “Only to be opened after consultation with the sender or recipient”.

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