Spring 2005 tecniplast quarterly news-sheet N°15

"We should kiss a frog"

What kinds of studies can be conducted on an animal like the Frog?
This might be a question that a lot of people not involved in this field would formulate. And yet this animal has been used for scientific purposes for over 150 years.

Let us summarize the story. The world of amphibia is divided into three main orders: Gymnophiona (or Apoda), Urodela (or Caudata) and Anura. The most widely used in research are Urodela and Anura with the latter being the more important.
A classification based on the habitat is also possible. We can recognize four different kinds of animal: Aquatic (e.g. Urodeles: Ambystoma mexicanum - Anura: Xenopus laevis), semiaquatic (e.g. Anura: Rana Temporaria), semiterrestrial (e.g.: Anura: Bufo marinus) and arboreal (e.g. Anura: Hyla cinerea).
Among all these amphibia, the most commonly used in research of all the 15 species in Genus Xenopus, is for sure Xenopus laevis (from the Greek meaning: Xenopus-"Strange foot" and laevis-"Smooth"): the first drawing representing this species was made by Francois Marie Daudin (1774 - 1804) in 1802.

Where does this animal come from? All Xenopus species are native only to sub-Saharan regions of Africa (Mainly: Cameroon, Central African Republic, Congo, Ethiopia, Gabon, Ghana, Kenya, Liberia, Malawi, Nigeria, Rwanda, Sierra Leone, South Africa, Tanzania, Uganda, Ivory Coast, Zaire and Zambia) and are Ectothermic (poikilothermic or cold-blooded) animals, with a body temperature that varies with that of the environment.

During the 19th century and at the beginning of the 20th, a lot of scientists started looking at Xenopus to try to better understand this new species, focusing mainly on its spawning.
But it was with Lancelot Hogben, a British biologist, that Xenopus laevis became a widely used lab animal. During the '30s Xenopus laevis became the model animal for endocrinological research and for early diagnosis of pregnancy. Hogben demonstrated how Xenopus laevis could be used as an indicator of the presence of gonadotrophins in the urine of pregnant women. Just one injection of urine containing gonadotrophinic hormone into the dorsal lymph sac induced egg laying 8-12 hours later. Strange though it may sound nowadays, pregnancy tests in the past were carried out exploiting frogs, but at that time the basements of British and American hospitals were full of these animals!
After the '50s Xenopus laevis became the main animal model for developmental biology and embriology, thanks to the reduced size of its easily obtainable eggs, with external fertilization and development. (The eggs, compared to those of other species used in embryology, are nonetheless quite large - enough so to allow embriologists to perform microsurgery and manipulate the embryos experimentally far more easily than in other vertebrate embryos).

Nowadays Xenopus laevis is normally used as a research animal and lots of Xenopus scientific communities are to be found throughout the world, involved again in embryology, developmental and cell biology.
Its exploitation as a laboratory research animal has also increased through the use of Xenopus (Silurana) tropicalis which allows to run better genetic studies on these amphibians.
The X.laevis genome is polyploid (tetraploid, 4 copies of each gene, 2n = 36. X.laevis underwent a genome duplication event), while X.tropicalis is diploid (2 copies of each gene 2n = 20).
Moreover, X. Tropicalis is smaller, (See table 2) the genome size is more or less half that of the X.laevis (about the same as zebrafish), and it has a short generation time.
To better answer the question posed at the beginning of this article, we can briefly summarize some of the main research fields that contemplate the use of Xenopus as follows:
Embryology (Ontogenesis - Biophysics); Developmental Biology; Biomedical Research; Cell Biology; Molecular Biology; Pharmacology; Physiology (osmoregulatory, muscle and other metabolism pathways); Immunology; Hormone Assay; Toxicity (Teratogen Susceptibility - Fetax Studies); Genetics.

Considering the increasing importance of Xenopus Spp. as an animal model in scientific research and always taking into maximum account the welfare of the animals, in Tecniplast we decided to create a dedicated system for housing Xenopus frogs.
Tecniplast paid great attention in designing a system that could provide the best housing conditions for this species: up to now, the only prescriptions about the housing of Xenopus are contained in the European draft guidelines (Draft Appendix A of the European convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes [ETS No. 123] - Guidelines for accommodation and care of animals [Article 5 of the Convention] to be approved by multilateral consultation).

Aquatic anurans, e.g., Xenopus spp: Minimum enclosure dimensions and space allowances:

Body length (cm) - measured from snout to vent	Minimum water surface area (cm^2)	Minimum water surface area for each additional animal in group-holding (cm^2)	Minimum water depth (cm)	XenopLus™ - Maximum number of animals per Tecniplast tank
Less than 6	160	40	6	48
From 6 to 9	300	75	8	24
Over 9 to 12	600	150	10	10
Over 12	920	230	12,5	6

Apart from the draft of this guideline, husbandry care parameters shared all over the world exist and are summarized in the following table:

Species	X. laevis	X. tropicalis
Ploidy	Tetraploid	Diploid
N	18 chromosomes	10 chromosomes
Genome size	3.1 x109 bp	1,7 x 109 bp
Optimal temp.	16 - 22°C	25 - 30°C
Average adult size	9 - 12 cm	4 - 5 cm
Average egg size	1 - 1.3 mm	0,7 - 0,8 mm
Eggs/spawn	300 - 1000	1000 - 3000
Average generation time	1 - 2 years	≤ 5 months
pH	6,5 - 8,5	6,5 - 8,5
Ammonia	≤ 0,2 mg/liter	≤ 0,2 mg/liter
Nitrates	≤ 0,3 mg/liter	≤ 0,3 mg/liter
Cond.	50 - 2000 µS	50 - 2000 µS
Chlorine and chloramine	(to be removed)	(to be removed)

Thanks to our experience in IVC Systems in the rodent research field, in Tecniplast we realized how important it would be to apply the same concept and fluid management to aquatic care systems. The result is **XenopLus™**, a system that can house both Xenopus laevis and tropicalis - like all Tecniplast products featuring a high degree of safety and flexibility.
The system can be set up both as a stand-alone rack or in a multilinking configuration, with up to four racks, allowing the researchers to work with the exact number of animals they need.

The system features automatic equalization of water pressure keeping water changes per hour (WCH) constant even while the valves are being closed, since fast flow seems to be stressful for these animals.

Focusing the attention on the micro-environment, the tank is made of polycarbonate (smoked brown makrolon) for better visibility, the brownish colour providing the animals with a correct environment in terms of light. The totally transparent polycarbonate lid, made up of two sliding sections, can be opened from either side. Opening is smooth and quiet to ensure that the animals are not exposed to stress due to noise. The water depth in the tank is 13 cm in order to allow the animal to breath easily above the surface of the water and even standing with the hind legs touching the bottom of the tank. Finally, the tank can be easily emptied by simply removing the inner pipe.

The system features also a central WTU (water treatment unit), which houses water filtration devices and a Monitor and Control Unit, which constantly monitor and display all the water parameters. Visual warnings help the user to manage the system properly. At the touch of a finger you can easily set chemical and physical water parameters to ensure the highest quality research.

A exciting new adventure now begins: Tecniplast is ready to break into the world of aquatic systems!

Marco Brocca, Aquatic System Product Manager

IN THIS ISSUE

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featuring an article about
RABBIT CAGE WASHING

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Jacqueline Marvel, PhD,
Director of PBES,
in Lyon, France

Telephone interview held with Jacqueline Marvel PhD, Director of PBES (Plateau de Biologie Expérimentale de la Souris) in Lyon, France on Monday 29 November 2004

Conference ALL

George Crowhurst,
Panorama Editor,
in Varese, Italy

G.C. : Firstly, please give us an overview of your research platform, PBES.

J.M.: We have a number of facilities inside one building. There is a breeding facility with restricted access limited to animal caretakers. Here we breed all the transgenic mice using exclusively IVCs.
Then there are experimental sections at three levels - A1, A2 and A3. In the level 3 section experiments are carried out using viruses and so we make use of isolators. We also have a department called ANIGENE that produces transgenics on demand.

G.C.: Are you involved in animal production only for your own use or do you supply other laboratories?

J.M.: PBES was set up initially to serve a number of labs of the ENS (École normale supérieure de Lyon) and the IFR128 (Institut fédératif de recherche-128). This is a consortium of research units that got together to build this facility.
The services are now open to other research labs in France. We were fortunate in that we had the chance to construct a facility that was quite diversified in terms of services provided, starting with a completely new building. We have around 1000m^2 dedicated to animals.
Then there is a technical floor as well as some space still available for further development, so we still have room for further expansion.

G.C.: You already house quite a number of animals, but I gather you are still below your potential.

J.M.: Yes, that's true. The animals which were originally housed in older buildings on the site have been gradually transferred over here. Here we're dealing with all the transgenic lines, so we had to ensure that we could upgrade the health status so that we now have all SPF (specific pathogen free) mice.
We have some 120 different transgenic strains all bred in this building. The transfer started in June 2003 and we now accommodate a total of roughly 6,000 mice. You know, it takes time to breed - even with an average of 2.5 to 3 months per generation you only get 4 generations a year (if you're lucky!), so it takes time.

G.C.: Tell us something about the equipment you have installed. Do you have exclusively IVCs?

J.M.: I wish we did! In the breeding facility we have all IVCs and we have double security. Staff shower and change clothes upon entry and then work on safe changing stations under protective hoods.
So the mice are pretty well protected from humans, who are always a potential vector of contamination - and remember, only 4 people have access to this area.

G.C.: What about the A3 area?

J.M.: We have low pressure isolators with DPTE doors and transfert isolators so that animals can be transferred to the hoods under perfectly safe conditions.
In the A2 area we have Tecniplast IVCs working at negative pressure, while the overall environment is itself positively protected and mice are handled only under bio-safety cabinets.
It's not so easy for the operators since these cabinets are really not so suitable for handling cages since they were originally designed for cell culture. They have a sliding front which allows you to open them just a little to get the cage in, though it is pretty tedious changing cages in these conditions. Anyway, they serve to ensure that our staff and users are protected in line with European legislation.
In the A1 area we have mostly conventional cages since the mice tend to stay there for just a short time. They are either SPF animals bought from suppliers, or in-house transgenic mice produced in the SPF zone and then transferred for a week or two, possibly up to one month, for relatively short-term experiments. However, we do also have some 250 IVCs for researchers who may have a particularly sensitive transgenic line, or for longer-term experiments where the status of the mice has to be safely maintained.

G.C.: Have you had any allergy problems?

J.M.: Fortunately not with our own staff - with modern equipment it is much better working with animals than in the past, with far less dust or smells in the environment. Some guest researchers have had allergy problems, but not our own personnel.

G.C.: What about washing, autoclaving etc.?

J.M.: As you will see from the drawings on this page, all our areas have been designed with totally separate clean and dirty sides.
Cages and bottles move out on the dirty side for changing and washing. We have a dedicated lift for the bins containing dirty material.
The facility is on two levels, with the A2/A3 zones at the top with their own cleaning and sterilizing equipment.
I believe that we have a good set-up here. We are well organized and we have relatively simple procedures which are easy to follow.
I believe it is better for people to have fairly easy routines to follow rather than going for a highly complicated fool-proof system which is, in fact, more likely to lead to human error.

G.C.: And what about the state of the art with regard to computer systems?

J.M.: We are particularly satisfied with our custom-made PHARE software which allows us to manage efficiently the whole unit. Except for one office, we have avoided cables and use a radio transmission system so that researchers and operators can input all relevant data from any one of the areas in real time - and this data is also accessible to the management of the facility so that it is possible to monitor the breeding and experimentation situation at any time.

AIR SUPPLY LOCATION AND AIR SPEED IN IVC SYSTEMS

Air distribution in IVC systems is a complex issue that involves specialists with different backgrounds. A number of parameters are considered during the design of the ventilation system, plenums, valves and cages. The combination of air changes per hour (ACH) in each cage, speed of the air and pressure consistency are established in relation to ventilation efficiency (in terms of temperature control within the cages and removal of pollutants) and animal welfare.

It is interesting to note that the same number of air changes per hour can be achieved in a cage as well as in a room in different ways. HEPA filtered air can be pushed and/or drawn to/from a cage from different positions leading to the same volume of air moved through the enclosure but with different results in terms of air speed, direct exposure of rodents to the air flow and removal of pollutants.

Environmental parameters in IVCs and static top filtered cages have been extensively studied over the years. Intra-cage temperature, relative humidity, ammonia, CO2 and noise are all parameters assessed with different strains, sex densities and room conditions (1,2,3,4,5). In general at least 60 air changes per hour are considered sufficient to provide a comfortable environment for mice and achieve the goal of gas removal etc.

Nevertheless, one of the major matters of concern is the exposure of rodents to draughts because of their potential impact on behaviour and health . Preference tests have demonstrated that mice prefer air supply in the cover of the cages, blowing the air at low speed horizontally and to the front, as against an air supply positioned directly in the back wall of the cage (6). Therefore, the speed of air and its direction within the cage have to be carefully considered. It is generally accepted that speed of air at animal level should be lower than 0.2 m/sec. (7) and, of course, not blown directly at animal level.

Different systems of air distribution within IVCs are graphically illustrated in Figs 1 to 6. It is interesting to note that, in a recent study, speed of air at animal level (4 cm above the bottom of cages) was considered among other parameters (8). The intra-cage air distribution configurations which represent the subject of the study were those shown in Figs. 1 and 6. By means of different nozzles the author was able to push the air at low or high speed maintaining the 60 ACH. Findings showed that mean air velocities at animal level for the high-and-low velocity cages were respectively 0.33 m/sec. and 0.21 m/sec.

The choice made by Tecniplast to work with a medium velocity air supply by means of a horizontal air flow positioned on the top rear of the cage in order to have a mean of 0.14 m/sec air speed at animal level (65 ACH) seems a reasonable and successful choice. (Fig.5)

Gianpaolo Milite, D.V.M. M.Sc.

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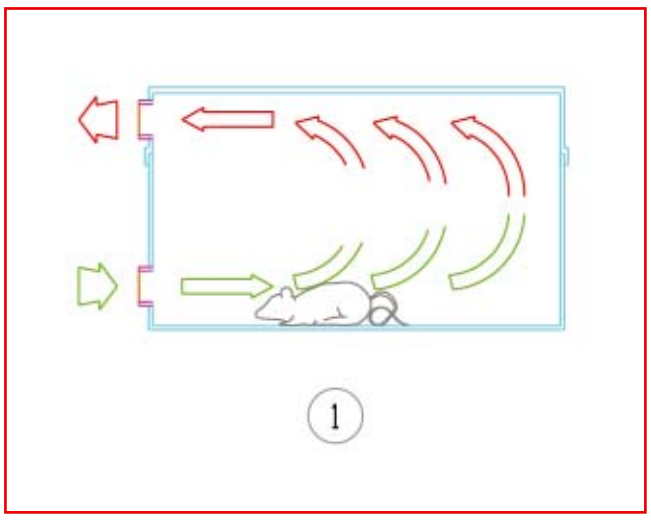
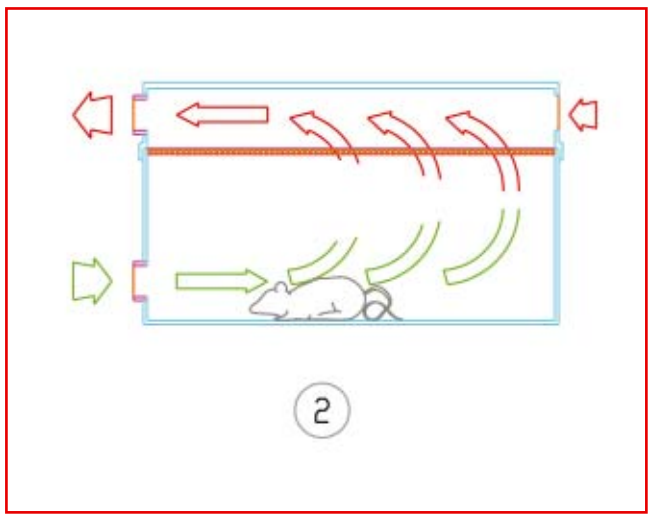
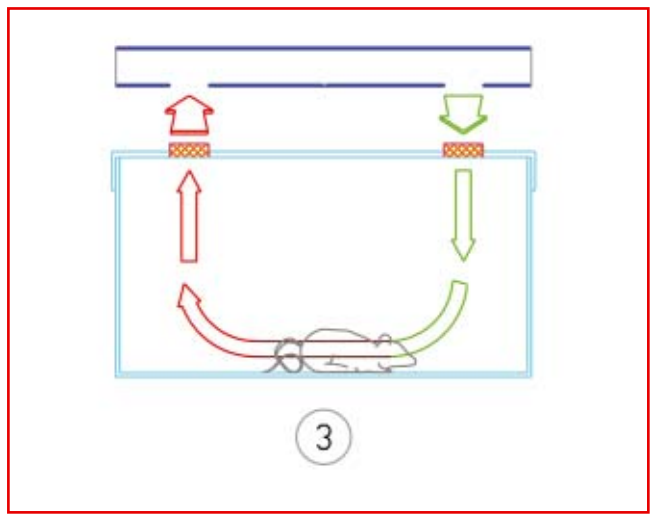


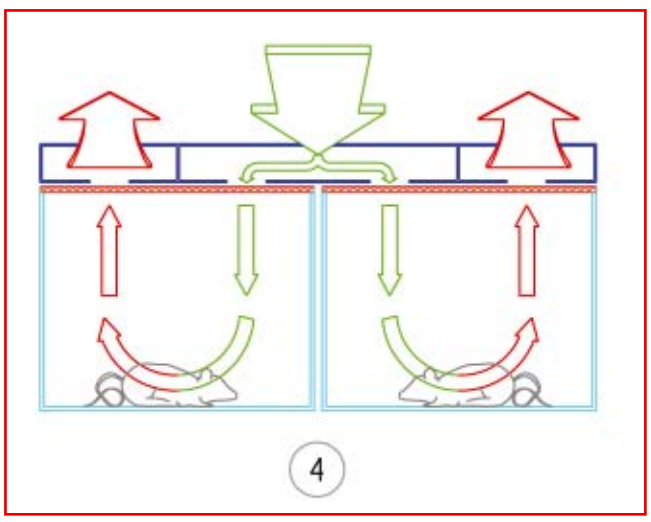
- Injection of air at animal level.
  Need for high speed to avoid shortcut with the above exhaust valve.



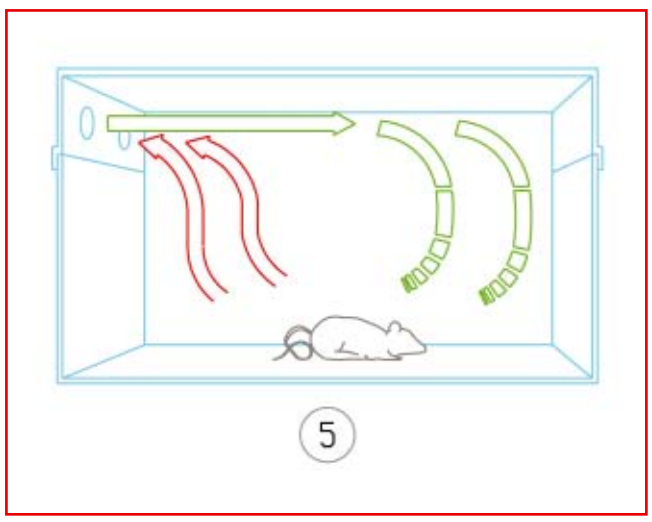
- Same as for Fig.1, but paper filter before the exhaust.



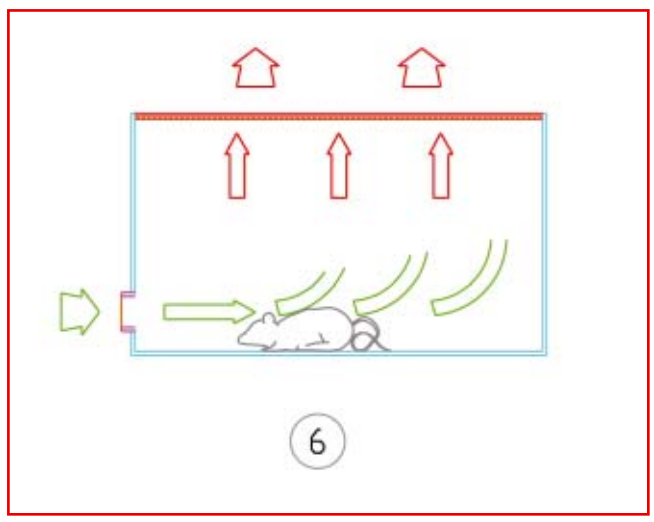
- Direct vertical injection of air in the cage.



- Same as for Fig. 3 but in a double cage system.



- Tecniplast cage. Horizontal flow of air far from the animals. No shortcut possibilities due to a plastic rib between supply and exhaust valves. Air received indirectly by the animals at low speed.



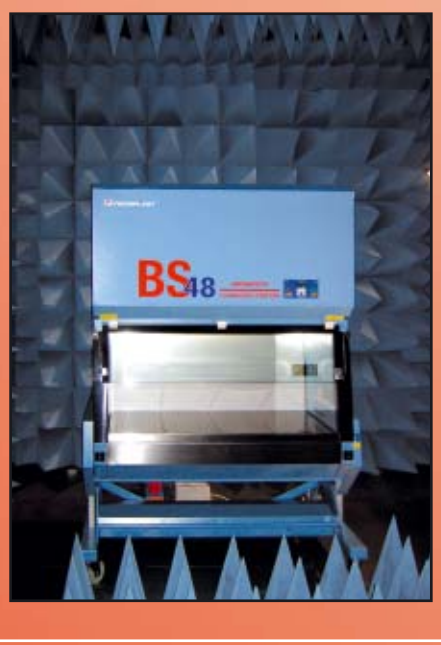
- Injection of air at animal level.
  Passive Exhaust through porous filter.

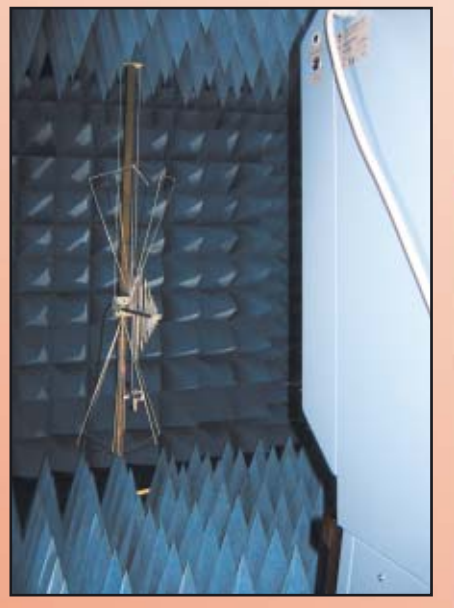
PRODUCT INNOVATION & ENHANCEMENT

EMC TEST FOR BS48

Another successful test has been carried out on one of our cage changing cabinets.

On 30th November 2004 an EMC (Electromagnetic Compatibility) test was carried out on our BS48 changing station by M. Masini Institute, a certified and independent body, using the BS48 Cabinet Station located in Rho, close to Milan.

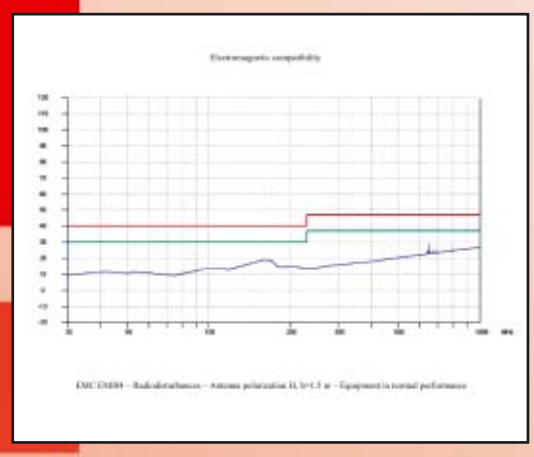




The test was conducted in an anechoic room which provided conditions of total absence of EM interferences: its purpose was to measure conducted disturbances on the power supply mains (terminals), radiation of radio-frequency disturbances from appliances (enclosure), of harmonic current, of voltage fluctuations and flicker, and of electrostatic discharge immunity.

The test is performed to avoid emissions of electromagnetic interferences from the cabinet to the power grid and the filtering of interferences of the power grid to the machine's circuitry.

As is clearly shown in the sample graph, the values of all the measurements of the circuitry on the BS48 (which features an electrical board installed on all our laminar flow machines) are below the parameters established by the norm.



Giovanni Tartaglia, Flow Division Manager

THE VET'S CORNER

Mice accomodation....

Cage size for all laboratory mice is defined in terms of square cm or inches per mouse, female with litter, singly housed mouse etc. Over the years the maximum number of mice that may be allocated within a cage has generally decreased and special consideration was taken when static top flow cages were developed (suggested reduction of 10% in animal density) due to poor ventilation (air changes per hour) and consequent rapid build-up of ammonia and relative humidity.

The coming of a new cage concept, individually ventilated cages, has inherited the acceptance of densities typical of open cages without taking into consideration the huge improvements made in terms of environment within this new highly technological enclosure. The question is, in our opinion, very simple: can the demonstrated, improved environmental condition in IVCs positively impact with the number of group-housed mice in the same cage?

At Tecniplast we are working on this issue to evaluate whether real differences exist both in terms of environmental parameters and of mice related physiological parameters when slightly higher densities are used in type two and type three ventilated cages.

It is extremely interesting to understand whether or not forced ventilation plays a role in terms of density vs animal welfare, as well as many other practical aspects already recognised for this special equipment.

Gianpaolo Milite, D.V.M. M.Sc.

TELEMETRY IVC RACK

Tecniplast introduces into its range of products a new ventilated Sealsafe rack arranged for telemetry, Cat. N. 2U16MAC20DSI.

The rack is a 2U16 model and can be retrofitted with DSI equipment: for each cage position a Faraday Cage type has been developed to avoid crosstalk between transmitter and receiver devices and between groups of transmitters.

Paolo Tamborini, IVCs Product Manager