FELASA Working Group Standardization of Enrichment

Working Group Report

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1. Preamble

In laboratory animals, environmental enrichment was originally used as an experimental tool in neurobehavioral research as early as in the 1940s (Hebb 1947). At that time it was considered to be *any modification of a captive animal's environment by providing physical or social stimuli.* Environmental enrichment was also developed for zoo enclosures in response to the abnormal behaviours shown by animals in environments that did not meet their needs. (Hediger 1950).
Subsequent to the emergence of laboratory science as a scientific discipline of its own in the 1960ies environmental enrichment was introduced as a concept in laboratory animal care in the 1980s, i.e. with a delay of about two decades. The definition of environmental enrichment became more specific and included explicitly the well-being of animals as its major goal, e.g.: *"Environmental enrichment is any modification in the environment of the captive animals that seeks to enhance its physical and psychological well-being by providing stimuli meeting the animals' species-specific needs"* (Baumans 2000). Even though enrichment was not a topic when Russel and Burch (1959) formulated their 3R-principles, it is obvious that the essence of enrichment in relation to animal welfare is refinement.

During the past twenty years considerable progress has been made concerning the knowledge about and the introduction of environmental enrichment as part of laboratory animal care and husbandry. This is reflected by an ever increasing body of literature related to this topic and – last but not least – by the inclusion of environmental enrichment into the draft revision of Appendix A of the *Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* (ETS 123). Special paragraphs have been dedicated to environmental enrichment in the general section as well as in the species specific provisions of Appendix A and associated part B of this official European document.

The practical application of environmental enrichment led to a considerable variability of enrichment procedures. However, a number of enrichment procedures were not subjected to scientific scrutiny prior to their introduction. Instead, they became popular because of the commercialization of enrichment items or an anthropomorphic approach to the animal's situation rather than being based on sound evidence of the given procedure's welfare benefit.

The FELASA-working group on "Standardisation of Enrichment" was established with the goal to provide guidance how to standardize enrichment in laboratory animal enclosures such that essential species-specific needs and individual needs of gender and life stage are fulfilled to guarantee animal welfare to minimise interference with experimental results. In other words, the working group was required to create guidance on how to establish "good"/effective enrichment programs and eliminate "bad"/ineffective ones.

Because the development of environmental enrichment programs is an ongoing process, it was the consensus within the working group that future environmental enrichment innovation should not be hampered by the present effort. Thus, the working group prefers the term "harmonisation" instead of "standardisation" of enrichment procedures. Also, it was decided **not** to attempt yet another literature review that would become outdated within a few years (although the list of references became quite long). Emphasis was rather put on the formulation of principles – principles of environmental enrichment *per se*, principles of the evaluation of enrichment etc. These principles are intended as guidance, as a navigation tool for the future development in this particular area of animal welfare.

The structure of the present document emerged from the above considerations. In the first chapter, "**Principles of Environmental Enrichment and their Harmonisation**", key concepts are presented for a general understanding of the topic, of how enrichment can benefit animal welfare and what needs to be kept in mind when aiming at the harmonisation of enrichment. As the working group is convinced that environmental enrichment needs thorough evaluation before it is put into broader practice, "**Principles of the Evaluation of Environmental Enrichment**" are provided in the following chapter. These principles are intended as food for thought to ensure that enrichment programmes are science-based and serve their purpose, i.e. improve animal welfare, as effectively as possible. One key concept of welfare-oriented enrichment is offering choices for the animal, and this necessarily involves diversification of its environment. Although it seems logical to expect that improving the animal's homeostasis would reduce variability, a number of researchers are concerned that enrichment instead might increase data variability or even compromise the experimental outcome. These aspects are addressed in the chapter "**Validity and Variability**". Environmental enrichment programmes should not only follow certain principles, but they should be evaluated before introduction and should support the validity of animal experiments instead of compromising them, they also encompass a wide variety of practical solutions, depending on the animal species and various other circumstances. This is the topic of a particular chapter entitled "**Species Related Types of Environmental Enrichment**". The principles of environmental enrichment will only become effective when put into practice in a way which is compatible with the technicalities and aims of the experimental design, including ergonomics and – if relevant – formal quality assurance requirements, known as GLP (Good Laboratory Practice). These problems are discussed in a "**Points to consider**"-chapter, followed by "**Conclusions & Recommendations**".

It is hoped that the principles and considerations presented will contribute to a more harmonised evolution of future enrichment programmes and will help to abandon invalid concepts. Time will show whether this document is sufficiently general to survive the ongoing development in this field and at the same time is being specific enough to be a useful tool of guidance.

2. Principles of Environmental Enrichment and their harmonization

2.1 Animal needs and their relationship with Environmental Enrichment

The 'needs' concept has been incorporated into the European Directive (86/609/EEC), which states that "any restriction on the extent to which an experimental animal can satisfy its physiological and ethological needs shall be limited to an absolute minimum" (European Commission 1986; Article 5, paragraph b).

It is often assumed that the choice of a particular environmental enrichment is dictated by the will to satisfy a particular need of a particular animal held in captivity. When discussing animal welfare, the term "need" presents different characterizations. Curtis, for example, has proposed that animal welfare depends on a "hierarchy of needs": i) "physiological needs"; ii) "safety needs"; iii) "behavioural needs" (Curtis 1987). Later, other authors have identified "life-sustaining needs" as the most basic, followed by "health-sustaining needs" and "comfort sustaining needs" (Hurnik & Lehman 1988). However, it has been argued that the "catalogue approach" in relation to animal needs is not very effective, intended as being too simplistic (Jensen & Toates 1993). For example, nest building of domestic sows (*Sus scrofa*) and dust-bathing in hens (*Gallus gallus*) have been described as very composite behaviours, in terms of the relationship between internal and external factors, that makes it difficult to categorise them as "needs" opposed to other items in their behavioural repertoire (see Jensen & Toates 1993).

It can be argued that the distinction between physiological needs and behavioural needs is, to say the least, blurred. Once we accept that animals have the possibility to suffer in psychological terms, not only physiological ones, then it follows that a bad mental state has its effects also on the physiology of that particular individual. Many examples of the influence of psychological stressors on the immune response of different species can be found in the literature. For example, in the classic work by Gaertner *et al.* (1980) it was shown that rats exhibited alterations in different physiological parameters (such as hormones concentration, heart rate, serum glucose) after being removed for one minute from their home cage. Furthermore, social stress has been observed to have various effects on the CNS in rodents, such as an increase in the number of mast cells (Cirulli *et al.* 1998), an increase in the level of NGF in plasma and hypothalamus (Alleva & Santucci 2001) (see also Claassen 1994, Bartolomucci *et al.* 2005, for a review). It can be proposed here that the attention to the behavioural needs of animals held in captivity can be a good strategy in order to ensure also the satisfaction of life-sustaining or health-sustaining needs.

Dawkins (1983) has linked an ethological or behavioural need with causal factors that lead the animal to perform a particular behaviour. When an animal is prevented from performing a certain behaviour it is highly motivated for, it can show stereotypical behaviours (Mason 1991a,b). The careful use of environmental enrichment can prevent the occurrence of abnormal behaviours, providing the animal with a physical or social context in which it can satisfy a certain behavioural need. For example, gerbils kept in a captive environment where they are not provided with an artificial burrow develop stereotyped digging. Such behavioural abnormality can be limited or prevented by enriching the cage with an opaque artificial nest-box (Waiblinger & Konig 2004, for review see also Shepherdson *et al.* 1999, Young 2003).

However, it is not necessarily true that an environmental enrichment strategy must be aimed at letting the animal express as much as possible of its natural behavioural repertoire. Animals in the wild experience a series of stressful events (shortage of food, the presence of predators), which clearly would not be beneficial for them in captivity. As has been suggested by Veasey *et al.* (1996), we must instead focus on the possibility for the animal to respond in species-typical manner to a series of stimuli provided in captivity. The series of stimuli proposed, for example as a series of enrichments, must take into account the natural history of that particular species. In this context, a basic principle to be taken in to account is that the behaviour of the species used in laboratory settings evolved in complex environments, characterized by a certain degree of predictability, and on which they can exert a certain degree of control. Therefore, complexity, predictability and control are three concepts to bear in mind when applying an environmental enrichment strategy.

2.2 Complexity, control, predictability

Unless used in breeding colonies, for laboratory animals the only "option" in their life often is to be subjects in experiments. They are bred for this purpose, in many cases, their life ends with the termination of the experiment. So, what seems to be absent (or seriously compromised) in the life of the animals used for experimentation is the capacity to "control" their life. Complex animals, such as mammals, exercise their cognitive capacities operating behavioural choices based on their previous experience in a similar context, and based on a somehow limited ability to predict the consequences of an action (see Tomasello & Call 1997). Control and choice, when providing environmental enrichment, should be two key concepts. The animal should be given the possibility to control some features of its environment, and to choose which feature to exploit at a particular time.

With respect to control, Snowdon and Savage (1989) have rightly stated that: "animals cannot passively receive environmental events; they must be able to act on the environment and consequences must result from their actions". The possibility to control features of the environment can have a positive effect on the welfare of animals in the long term. For

example, studies on macaques demonstrated that individuals who had control on their feeding schedules, showed less fear of novel objects in subsequent tests. It must also pointed out that sometimes loss of control can be even more stressful than never having control in the first place (Hanson *et al.* 1976).
However, a balance must be found between control and lack of control (Sambrook & Buchanan-Smith 1997). These two authors argued that there is an optimum degree of predictability of events: that total predictability may result in behavioural frustration and boredom whilst complete unpredictability results in stress. The same view was supported by the observation that an apparatus responsive to manipulation was more attractive than an apparatus that would not respond (Markowitz & Line 1989). Again, for example, Chamove (1989) has shown that captive chimpanzees favoured objects that responded with a certain degree of unpredictability, over inanimate objects.

We could think of some steps concerning the introduction of environmental enrichment and its relationship with the well-being of an animal:

1) The researcher must familiarise himself with the natural history of the species in question;
2) In relation to the information gathered, an appropriate social stimulation or an object is introduced;
3) This new level of complexity gives the animal the possibility to increase the choice of selecting a behaviour in its repertoire, this could already represent an increase in the level of welfare;
4) by adding a possibility of control on the frequency and way of use of the introduced enrichment, we could further increase the level of welfare.

2.3 Harmonisation of different features

The ideal captive environment for an animal should try to harmonise, with the help of environmental enrichment, different features. Having said this, some priorities need to be made. Generally speaking, mammals are social animals, and sociality is a fundamental component of their biological being, i.e. social housing should be considered the default condition and a priority. Especially in the case of non-human primates, no methods of enrichment has proven to be more effective than the presence of preferred conspecifics. Sociality can also help animals to better adjust to stressful laboratory events such as moving from a cage to another (Smith *et al.* 1998). The concept of control applies also to social enrichment. Although social housing of primates is extremely beneficial it should be noted that negative interactions are part of social relationships. Therefore, the cage must be arranged in a way that a subordinate individual can hide or escape from a dominant one, when this necessity arises. Space, visual and physical barriers must be provided, and these features help to better regulate the social life of an individual in captivity (Reinhardt & Reinhardt 2000, Bloomsmith *et al.* 2001). Different individuals can then have some degree of control over their social life. In territorial rodents, for example, the housing style should be “strain” and “sex” specific (Alleva 1993). A shelter should be provided in cages housing mice groups, to allow those subjects which are repeatedly attacked to protect themselves. Female mice show aggressive behaviour only during pregnancy and lactation to protect their pups (Ostermeyer 1983). If females with offspring cohabit with males, nest-defence should be favoured by presenting the cage with a protected nest.
The effects of social relationships must also be taken into consideration when providing physical enrichments. For example, shelters and objects can be used differently by individuals of different social position (e.g. Galef & Sorge 2000), or feeding enrichments can be

monopolized by dominant individuals, such as in chimpanzees (Bloomstrand *et al.* 1986), or in rodents (Howard 2002).
If animals have to be housed individually, for example for particular experimental purposes, it is important to provide at least some forms of indirect sociality. For example, the possibility to observe other singly-housed individuals can be beneficial (Brinkman 1996).
In most enrichment studies physical and social enrichments are used at the same time, so it is difficult to disentangle the exact contribution of each component determining a specific outcome. For example, in a study on CD-1 mice social or physical enrichment during periadolescence exerts distinct long-term effects on behavioural responses (Pietropaolo *et al.* 2004). In particular, subjects exposed to a physically-enriched environment showed decreased explorative activity, reduced interactions with unfamiliar objects and low level of aggressiveness. Social enrichment did not affect exploratory behaviour, but appeared sufficient to modify the quality of the agonistic response. Mice kept in groups during adolescence showed a more affiliative and less aggressive social interaction strategy, suggesting that social experiences at critical developmental stages are more relevant than physical complexity for the development of adequate strategies to deal with social challenges.
Finally, enriching environments with physical, social and sensory stimuli are now established to be beneficial to brain development and ageing (e.g. Meaney *et al.* 1992, Cotman & Berchtold 2002), and has also led to ideas for new approaches to ameliorate disease states (Clausing *et al.* 2000, Hockly 2002).

3. Principles of the evaluation of environmental enrichment

It is fundamental to good husbandry that laboratory environments should meet the needs of the animals housed within them and enrichment to standard laboratory cages or environments is usually needed to achieve this. Animals housed in unenriched laboratory animal housing often develop abnormal behaviour, such as stereotypies, excessive aggression or self-injurious behaviour and these are usually signs that the housing is not meeting the animals' needs. Housing enrichment has become an integral part of laboratory animal care and there is a growing body of evidence on a variety of species demonstrating the beneficial effects of various types of enrichment (eg, Olsson & Dahlborn 2002 Bayne *et al.* 2002 , Garner *et al.* 2003, Patterson-Kane 2004, Sørensen *et al.* 2004, see also Reinhardt's Laboratory Animal Database http://www.animalwelfare.com/lab_animals/biblio/refine.htm and the background information collected for the draft revision of Appendix A to Council of Europe Convention ETS 123 (http://www.coe.int/T/E/Legal_affairs/Legal_co-operation/Biological_safety,_use_of_animals/Laboratory_animals/draft%20revision%20of%20Appendix%20A.asp#TopOfPage). However, not all so-called enrichments or changes to husbandry achieve their aim of benefiting the animals. Ideally, enrichment devices or protocols should be validated to show that they are beneficial to the animals, that they have no unexpected adverse effects on them, and that the enrichment does not jeopardize experimental outcomes (see Section 4 Validity and Variability). This last is important, not only because of the potential damage to the research, but also because animals might be wasted in experiments that have little or no value. Doing nothing, however, is not an option as environments that do not meet the animals' needs can also jeopardise experimental results (eg Poole 1997, Sherwin 2004a).

Studies on enrichment can broadly be divided into those carried out by scientists with a research interest in the area of animal welfare or in the area of neuroscience, the latter using enrichment as a tool e.g. in order to induce changes in the brain, and those carried out ad-hoc by animal care staff with the aim of validating a particular enrichment idea or regimen. While the latter can be of value, animal care staff are not trained in welfare research, and may lack the ability or time to carry out well designed studies. Ideally, hypotheses about potential enrichment should be tested by scientists qualified in the area such as applied ethologists. This is because the assessment of welfare and enrichment techniques is often not a simple process. Those involved in welfare assessment need to have an understanding of behaviour and physiology and relevant neurobiological studies on enrichment.

The process of developing an environmental enrichment regime should begin with a thorough understanding of species-specific behaviour and hypotheses regarding likely enrichment should be based on this. Scientists developing enrichment techniques often begin by studying the behaviour of the animals in different types of environment which may differ somewhat from the standard laboratory enclosure. Preferences by the animal for particular features are then used to derive hypotheses that are then further tested and refined before being validated for effectiveness and practicality in the standard laboratory setting. Preference tests by themselves do not, however, inform us about the basis of a preference. For example, they may be constrained by evolutionary factors, they may measure the animal's desire to minimise deprivation, maximise pleasure or to monitor an unwanted problem. Moreover, some choices made by the animal can actually be bad for its welfare for example, marshmallow sweets are highly motivating for marmosets and yet in excess could cause dental caries or obesity. Once a preference for a resource has been demonstrated, the extent of the animal's motivation for that resource can be assessed using techniques such as consumer demand studies that measure the elasticity of demand for a particular resource (eg Dawkins 1983, Sherwin & Nicol 1995, Van de Weerd *et al.* 1995, Sherwin 1998). Measures and experimental design are critical for the validity of these tests, (see eg Mason *et al.* 1998 and subsequent commentaries pp 1076-1083 *ibid*, Ladewig *et al.* 2002).

The effect of environmental enrichment on animal welfare can be assessed using a variety of different measures. Often behaviour in an enriched cage is compared with baseline measures of behaviour in the home cage. But it is important to assess whether changes in behaviour are short or long-term effects (see for example Van de Weerd and Baumans 1995). Other measures of the effects of enrichment on the welfare of the animal include behavioural tests such as open field, hole board or elevated plus mazes to measure affective state. As previously described, information from preference tests and other experiments may be used to measure the strength of motivation for an enrichment option. Physiological measures of welfare include measures of hormones (eg corticosteroids, catecholamines) heart rate, blood pressure, immune function (eg lymphocyte/neutrophil ratio), body condition, reproduction, post mortem parameters, (eg adrenal weight, ulcers etc) (Baumans *et al.* 1994). Whatever methods or measures are used, expertise is needed to interpret the results in terms of animal welfare. The assessment of welfare is often not straightforward (eg stereotypies can persist as a "behavioural scar" (Mason 1991b), and are thus not always a good indicator of current welfare (Mason & Latham 2004)) and the results of welfare studies can be dependent on the welfare measure used, strain, sex, age, social status and interactions with housing (Mason & Mendl 1993). Animal welfare studies are often complex, involving different measures and methods so that interpretation can be problematic (eg Warburton & Nicol 2001, Warburton & Mason 2003). For these reasons animal welfare studies are often best carried out by specialists in the area.

Concerns are sometimes expressed by researchers that enrichment may affect their experimental results (see section 4 Validity and Variability). These concerns should be addressed because if they are valid, animals, time and money may all be wasted. The likely impact of enrichment on experimental results depends on the type of enrichment used, the parameter studied, and strain of the animal (Van de Weerd *et al.*, 2002). Any assessment of the possible effects of enrichment on experimental outcome should also take into account the possible impact of an unenriched environment on experimental results. It should be remembered that enrichment of laboratory environments is now well established, that there are many instances of enrichment being successfully used in parallel with scientific research, and therefore objections to enrichment should be critically assessed to ensure that they are based on valid reasons. If there are reasonable concerns that a particular enrichment might influence the outcome of an experiment then ideally studies should be carried out to determine whether an effect truly exists or consideration should be given to a different type of enrichment. It is often possible to deduce on the basis of existing evidence whether a particular enrichment will have an effect on a particular experimental outcome. For this reason enrichment items with certificates of analysis, or if they are food items of human food standard may be used even within the regulatory toxicology environment (Dean 1999).

Practical development of enrichment for animals used in research

Many establishments make some attempt to validate new enrichment ideas, however, in practice it is usually far too time consuming and expensive to evaluate all enrichment variants in the context of different species, strains, husbandry systems and experimental protocols. Indeed, in many cases it may not be necessary as a new enrichment idea is often a variation on an existing theme, eg a foraging device for primates. In such circumstances a full validation of the animals' need for the type of enrichment is not required as there is abundant evidence indicating that foraging devices can be useful. So in these cases an ad-hoc assessment by local animal care staff can be sufficient to indicate that the device has no practical problems. Even this might not be necessary if the "new" enrichment has been used successfully in similar studies by others.

If formal studies are not carried out, new enrichments should be assessed at the design stage with respect to the likely benefit to the animals and the likelihood of their influencing experimental results. For example, a foraging device might be incompatible with a nutritional study but might be acceptable in a study on neurophysiology. If the item is truly new and has not been used elsewhere, preliminary data within a sample of the animals should be collected to show that the animals make use of it and that the device does not pose a danger to the animals or adversely affect the animals, for example, by increasing aggression. Data on use might be collected very simply by recording the consumption of forage material. Casual or subjective observations during normal husbandry on use and incidence of abnormal behaviour can also be useful as long as their limitations are recognised. Young (2003) also suggests that each institution should maintain an environmental enrichment book describing the types of enrichment used by that institution. Such a book could include headings such as device, species, purpose, etc.

When evaluating any new enrichment it is important to remember that factors such as species, strain, sex, age and social rank can all be important. For example nesting material is generally accepted to be a useful addition for mice, but as nude mice lack eyelashes sawdust can irritate the eyes of this strain.

For the development of an enrichment program and the assessment of new enrichment ideas the following figures may be helpful as guidance.

Figure 1: Flow diagram for developing an enrichment program

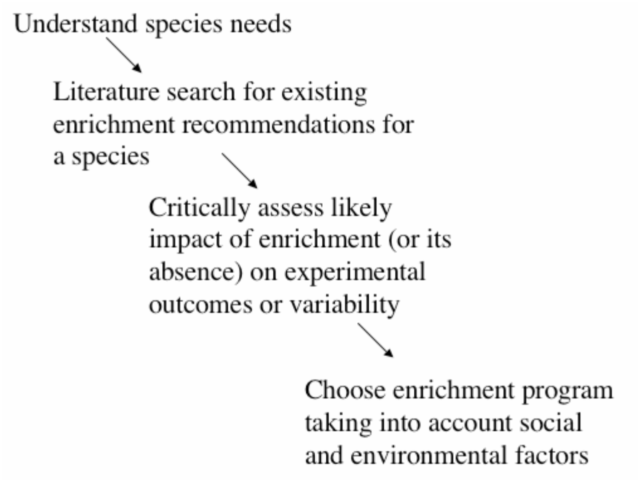
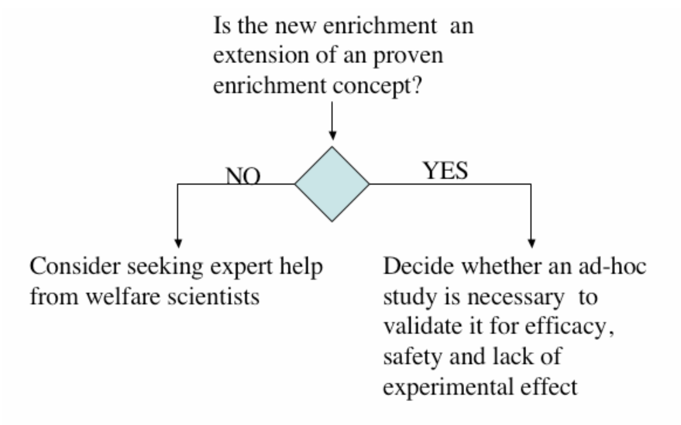


Figure 2: Decision tree for assessment of new enrichment ideas



4. Validity and variability

It is usually the experimenter's aim to reduce variation to a minimum, but inter-individual variation is a fundamental biological property which arises through genetic variation and as a result of differing environmental experiences. Genetic variation can be minimised by choosing to use inbred strains where these are both available and appropriate, whilst environmental causes of variation can be minimised by standardising laboratory environments. However, variation can never be completely eliminated and even before birth, environmental influences can be important factors. For example, the position of pig foetuses in the uterine horns results in different growth rates (Hartsock *et al.* 1976), and these differences are subsequently amplified when the largest littermate monopolises the most productive teat (Hartsock *et al.* 1976). Similarly, in mice the numbers of adjacent foetuses of the same or different gender affects adult sexual and aggressive behaviour (Vom Saal 1989).

Codes of practice for the housing and husbandry of laboratory animals such as Annex 1 of the European Directive 86/609 or National Codes of Practice exist for two purposes: First, for animal welfare reasons to ensure that at least minimum standards of housing and husbandry are maintained; Second, to help standardise conditions between laboratories so as to improve replicability. The directive 86/609 requires that any restriction on the extent to which an animal can satisfy its physiological and ethological needs shall be limited to a minimum, but there is growing evidence that unenriched minimum conditions as laid down in the Directive are not always adequate to meet animals needs. One line of evidence for this is the fact that in standard unenriched housing conditions animals often develop behavioural abnormalities such as stereotypies (eg Larsson *et al.* 2002, Powell *et al.* 1999, Bourgeon *et al.* 2004). Stereotypies are invariant, repetitive and apparently purposeless motor activity (rhesus monkey: Paulk *et al.* 1977, chimpanzee: Berkson *et al.* 1963, mice: Baumgardner *et al.* 1980), and they have been correlated with an alteration of brain function (Garner *et al.*, 2003). It is clear that conditions leading to fundamental changes such as these may increase variation and act as a source of bias on experimental results.

4.1 Enrichment and Variability

As enrichment is designed to improve welfare it is not surprising that it has effects on the animals' physiology and behaviour and therefore in some circumstances that it could affect experimental outcomes. For example, enriched animals may habituate faster, perform better, and possess improved motor coordination (rat: Larsson *et al.* 2002, mice: Caston *et al.* 1999). In mice, anxiety and stress responses are attenuated by enrichment whereas the immunity reaction is increased (Benaroya-Milshtein *et al.* 2004). There have been a number of studies evaluating the effect of enrichment on variability but, as yet, the results are inconclusive, showing that enrichment either increases variation, reduces variation or has no effect. The differences probably being due to the different variables measured. However, variability is not the only factor affecting statistical power of group comparisons. When the consequences of enrichment are assessed, one should look at variability **and** the extent of mean differences. For instance, the loss of statistical power caused by an enrichment-induced increase of variability could be re-gained, when the experimental effect is more pronounced under enriched conditions. This could occur when baseline values under enriched conditions are "better" - e.g. lower in a study where an increase of the measured values is expected.

4.2 Enrichment and Experimental Bias

When animals are exposed to an enriched environment there may be transient changes such as an increase in corticoid levels and aggressive behavior (Marashi *et al.* 2003). Other changes over a longer period may also occur. For example, functional and behavioral adaptation to the more extensive and varied stimulation can result in multiple morphological changes in the recipient brain structures and a modulation of the genomic expression of receptors in the hippocampus (Bredy *et al.* 2004). Enrichment has been shown to promote neurogenesis and to protect against CNS insults (reviewed in Lewis 2004). The extent and the complexity of the changes wrought by enrichment when compared with unenriched environments may give cause for concern. However, as animals, and indeed humans, in a natural environment are exposed to much more complicated and stimulating environments than a standard unenriched laboratory cage, it can be argued that enrichment results in a more normal animal and in many cases in better science.

Enrichment should be designed with the species' needs in mind taking into account strain, age (pups or senescent animal) and health of the animals including sensorimotor capability and the history of the animal. The choice of enrichment strategy should also take into account the fact that different enrichments have different stimulatory effects on different nervous pathways. For example, social or inanimate environmental stimuli activate dissociable neural pathways (Laviola *et al.* 2004), whilst the movements of the whole visual environment or of a small target activate retinopretectal and retinofugal pathways respectively (Reber *et al.* 1991).

The natural environment provides a wide range of stimulation to the brain and so the captive environment should provide a similar range of stimulation. This is particularly important during ontogenesis when there is a risk that concentrating on one form of enrichment might result in differential development of the brain. For example, the primary somatosensory cortex is especially developed in rats reared in an enriched environment (Coq & Xerri 1998). Similarly, if only running wheels are provided there is a risk that there could be preferential changes in the visual and vestibular systems, as these are extremely adaptable (Berthoz *et al.* 1981, Keller & Precht 1981).

In summary, enrichment during development and during the study should be both standardized, and designed to stimulate a wide range of natural behaviours, so to reduce the two risks of bias and increased variation. However, variation is intrinsic to all animals and cannot be totally eliminated by standard housing conditions. The likely effects of enrichment on experimental outcomes always needs to be considered but, in most cases, animals raised and kept in well designed laboratory housing with a range of enrichments are likely to be more normal animals and better experimental subjects with somatosensory systems and cognitive capabilities that have not been stunted by inadequate housing.

5. Species related types of environmental enrichment

Environmental enrichment should not be a process of randomly applying objects which we consider attractive for the animals, but it should be a well designed and critically evaluated programme in terms of use by and benefit for the animals and impact on experimental procedures and data (see Section 4.). Various categories of enrichment can be identified (Van de Weerd & Baumans 1995, Young 2003):

5.1. *Social enrichment*, which can be divided in contact and in non-contact with either conspecifics and/ or other species, including humans.

5.1.1 *Social contact enrichment.* Gregarious species should be group or pair housed with conspecifics. However, the group composition should be stable and harmonious (Love 1994, Morton *et al.* 1993, Stauffacher 1997; Turner *et al.* 1997), and visual barriers or hiding places may be necessary to minimise aggression, especially in males (Stauffacher 2000, Van de Weerd & Baumans 1995, Van Loo *et al.* 2002). Even in harmonious groups, it is necessary to allow individuals to initiate contact by approach, or avoid contact by withdrawal out of sight. For animals living socially, a social partner is the most challenging enrichment factor. Whereas enrichment objects are static and of interest for specific activities only, a social partner always creates new and unpredictable situations to which the animal must react. A social partner leads to an increase of alertness and exploratory behaviour and it provides diversion, occupation and probably also some feelings of "security" in stable harmonious groups (Stauffacher 2000). Procedure induced stress like responses are less frequent and of shorter duration in group-housed rats than in those housed singly (Sharp *et al.* 2002, 2003). In 1997, the Multilateral Consultation of the Council of Europe adopted a resolution on the accommodation and care of laboratory animals, which specified that “group housing, even pair housing, is preferable to individual housing for all gregarious species normally manifesting social behaviour, as long as the groups are stable and harmonious.”

The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources (ILAR), 1996, states: "Animals should be housed with a goal of maximizing species-specific behaviours and minimizing stress-induced behaviours. For social species, this normally requires housing in compatible pairs or groups."
Contact with humans, such as handling, training and socialising, will usually benefit both the animals and the outcome of experiments as it engages the animal on a cognitive level and allows positive interaction with animal caretakers, technicians and scientists (Baumans 2004, Shepherdson 1998, Van de Weerd & Baumans 1995).

5.1.2 *Social non-contact enrichment* includes visual, auditory and olfactory communication with conspecifics or contraspecifics, e.g. through bars or mesh. The Council of Europe's Resolution (1997) on the accommodation and care of laboratory animals states that when group housing is not possible, " consideration should be given to accommodating conspecifics within sight, sound or smell of one another".

5.2 *Physical enrichment*, including complexity of the enclosure, sensory and nutritional stimuli.
5.2.1 *Complexity.* Usually appropriate structuring of the cage/pen environment is more beneficial than provision of a larger floor area; however a minimum floor area is needed to provide a structured space. Minimum floor areas are indicated in Appendix A of the European Convention on the accommodation and care of animals used for experimental purposes. Except for locomotor activity, such as playing, animals do not use space *per se*; they use resources and structures within an area for specific behaviours. Most rodents and rabbits attempt to divide their living space into separate areas for feeding, resting and excretion. Structures within the cage may facilitate these divisions such as shelters, nest boxes, nesting material, tubes, and platforms providing withdrawal areas and look out possibilities and allow the animals to control their environment, including light levels (Baumans 1997, 1999, Blom 1993, Manser 1998, Sherwin 1997, Stauffacher 1997, Townsend 1997). Providing nesting material has been shown to enhance breeding results in mice and rats. It can lead to a reduction in pre-weaning mortality, so a higher number of pups survive (Porter & Lane-Petter 1965, Nolen & Alexander 1966, Norris & Adams 1976). But not only breeding animals use nesting material. It was shown that laboratory mice will readily use nesting material by performing nest building behaviour (Van de Weerd *et al.* 1997a; Van de Weerd *et al.* 1998) and spent 10-20% of their time-budget on manipulating nesting material (Van de Weerd *et al.* 1997b). Toys can have a beneficial effect on the animals in developing exploratory behaviour, locomotor and visual performance. One of the reasons for animal play is that the animal can practice the behavioural skills, needed for survival (Young 2003). However, toys have a limited period of time that they are attractive, typically one day (Young 2003). A certain level of exploration can be maintained by changing or rotating toys.

5.2.2 *Sensory enrichment*, including visual, auditory, olfactory, tactile and taste stimuli.
Visual, auditory, olfactory and tactile communication with conspecifics or contraspecifics either direct or through bars might be the most satisfying enrichment for rodents and rabbits. Mirrors provided in mouse cages did not seem to be an enrichment item, such as in primates (Sherwin 2004b).
It has been suggested that a constant background noise, such as radio music has some benefits in facilitating breeding and making animals less excited by reducing the startle effect of sudden noises. Behavioural results suggest that new age music has an overall calming effect on mice, compared to classical, pop or no music. However, mice still showed a disturbance reaction (freeze, flight) during exposure to loud noise, irrespective of background music (Van Loo *et*

al. 2004). However radios in animal facilities may benefit the animal staff, which could have beneficial consequences for the animals in turn (Sherwin 2002, Van Loo *et al.* 2004).
Cage cleaning is a necessary routine procedure in laboratory animal facilities. However, removal of the olfactory cues disturbs the social hierarchy of the animals in the cage, often resulting in a peak in aggression among male mice. It has been shown that olfactory cues from nesting and bedding material affect aggression in a different way: transfer of nesting material reduces aggression, whereas sawdust containing urine/ faeces intensifies aggression (Hurst *et al.* 1994; Van Loo *et al.* 2000).
Providing the animals with different food items such as carrots and seeds for rabbits and rodents, respectively may act as taste stimuli (see Nutritional enrichment).
Tactile stimulation can be achieved by providing e.g. nesting material, shelter and the possibility to dig.

5.2.3 *Nutritional enrichment.* Animals tend to be highly motivated to make use of enrichment based on food items. Frequency and schedule have an impact on the animal. Krohn *et al.* (1999) showed that feeding rabbits just before dark, in their active period, instead of in the morning, reduced stereotypic behaviour remarkably. Presentation of food, giving the animal the opportunity to forage, e.g. food scattered in the bedding, will prevent boredom as in nature a large part of the time-budget is spent on this activity. Animals will preferentially search for food even when it is readily available as this gives information about the location and quality of potential foraging sites (Mench 1998). Additional food items such as hay, straw or grass cubes can satisfy the need for roughage and for chewing in guinea pigs and rabbits (Baumans 1997). Soft wood sticks are used for gnawing in rodents and rabbits. Rats gnaw on aspen blocks, especially when housed without bedding (Eskola *et al.* 1999, Kaliste-Korhonen *et al.* 1995). Hamsters (Niethammer 1988) and gerbils (Brain 1999) routinely store food and should be provided with food pellets inside the cage. Primates frequently receive a varied diet to improve the interest value. When toys are related to food such as balls containing food pellets or puzzle feeders for primates, they will keep their attraction longer.

5.3 *Species related EE programs.* In this part some examples of species related EE are given. More information can be found in the literature (see Morton *et al.* 1993; Jennings *et al.* 1998, Olsson & Dahlborn 2002, Reinhardt & Reinhardt 2002, 2004, Young 2003).
Suitable enrichment for *rabbits* includes at least roughage, hay blocks or chew sticks as well as an area for withdrawal and look out such as a platform. For breeding does, nesting material and a nest box or another refuge should be provided. In floor pens for group housing visual barriers should be provided.
Nesting material is important for *rats, mice, hamsters and gerbils* as it enables them to create appropriate microenvironments for resting and breeding. Nest boxes or other refuges should be provided for guinea pigs and rats. *Guinea pigs* are cursorial rodents which do not burrow, but which in the wild may live in burrows made by other animals. Refuges such as tubes or shelters should be provided within the cage or pen to allow the animal to climb onto or hide under them. Hay will satisfy the need for roughage and wood sticks for chewing and gnawing can be used. In the wild, *gerbils* build extensive tunnel systems, and in the laboratory they often develop stereotypic digging behaviour unless provided with adequate facilities. For this reason gerbils need comparatively more space in order to allow them to build or use burrows of sufficient size and they require a thick layer of litter for digging and nesting and/or a burrow substitute, which may need to be up to 20 cm long. Nesting material (hay, straw, etc.) and wood sticks can be used for chewing and gnawing. The wild ancestors of the *hamster* were largely solitary. Group housing is possible but special care should be taken in forming socially harmonious groups and aggressive animals, especially females, should be separated.

Minimum enrichment should include nesting material, refuge area (e.g. tube, hut), roughage and gnawing objects.
In general, complexity will allow all animal species to structure their environment.
For all gregarious species social housing should be provided and should only be denied in exceptional cases.

Being part of a compatible group provides a sense of security for the vast majority of *non-human primates*. It also provides opportunities for a whole range of species-specific social activities such as grooming, embracing, huddling, patting and kissing Singly housed primates are particularly prone to show abnormal behaviour, whereas keeping them in groups reduces the incidence of this behaviour (Reinhardt 2002).
For most species, the best way to produce behaviourally and physiologically normal monkeys, suitable for breeding and long term study, is to ensure, wherever practicable, that they remain in the natal group for as long as possible. Juveniles separated from their mothers for whatever reason should be reared in social, preferably well organised groups.
Primates should be housed in enriched environments, which allow them to carry out a normal behavioural repertoire, showing species-typical, complete and well-balanced behaviour. Enclosures for primates should enable them to fully utilise the vertical dimension (see Poole 1998, Rose 1994; Reinhardt *et al.* 1996, Reinhardt 2002).
A complex environment, which includes swings, perches and branches, allows the animals to display a wide locomotor repertoire. Captive rhesus macaques were observed to walk, gallop, leap, climb, swim and hang from climbing structures. Long tailed macaques, rhesus monkeys and vervets are good swimmers, thus providing the opportunity to swim might be useful. Leaping is a common mode of locomotion for arboreal species, such as callitrichids, squirrel monkeys and long tailed macaques.
Although puzzle feeders and foraging boxes are more effective in reducing stereotypic behaviour and increasing activity in rhesus monkeys, watching videos and manipulating video game joysticks, can also be beneficial (Platt & Novak 1997). Marmosets should have wooden perches, which they can mark and scent-mark, and a refuge for sleeping. Mirrors might be useful to allow viewing events outside their cages.

Whenever possible, *pigs and minipigs* should be purchased in groups already formed of familiar or socially compatible animals. Commission Directive 91/630/EEC specifies that pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities such as rooting. It identifies suitable substrates as including straw, hay, wood, sawdust, mushroom compost and peat. Hay is the supplement of choice for mini-pigs, as it prevents gastric mucosal hyperkeratosis (Svendsen, unpublished data) in addition to providing environmental enrichment. Food balls will be used for play and foraging.

Dogs should be held in socially harmonious groups or in pairs.
Dog treats and toys afford welfare benefits to the animals, providing these are used sensibly and adequately monitored. As chewing is an important behaviour, items should be provided which meet this need. Dogs will make extensive use of items, particularly if they are food-flavoured. Proper presentation, for example by suspending chews a few centimetres from the floor of the pen on a spring, can help to minimise cleaning and possessive aggression problems whilst also allowing the animals to chew in a species-specific manner. Dogs extensively use a platform to play and rest on and to allow easy viewing of events outside their enclosures (Hubrecht 1993, 1995).

6. Points to consider when implementing environmental enrichment programmes

At this point it is taken for granted that an enrichment programme has been evaluated before implementation (see Section 3) and therefore, is serving its purpose by effectively contributing to animal welfare. However, besides this there are other points to consider which will be addressed in the following paragraphs.

6.1 Interaction with the experiment

Before establishing environmental enrichment programmes, careful assessment is necessary concerning possible impacts on the experimental outcome and the comparability of the results with previous (and future) studies. This is especially significant when historical data are used to validate current results. However, the assessment prior to introducing an enrichment program should also include an evaluation whether it is really necessary to continue with certain experimental procedures when they are in conflict with enrichment-related improvements of animal welfare.

Of course, it is not advisable to introduce new enrichment procedures during an ongoing study. Standardizing the enrichment plan for an established group of animals, prior to the initiation of the study is the least thing to do to mitigate the confounding effects of enrichment (Stewart & Bayne, 2004).

Group housing and foraging to obtain food (using special devices) are two widely accepted enrichment procedures. In both cases, measurement of food consumption may become less accurate or even compromised. In addition, it is likely that growth curves and certain metabolic parameters are affected, and hormone data which may shift to a different base level. Therefore, the introduction of an enrichment program needs to be carefully planned. Nevertheless it is not acceptable to neglect the improvement of animal welfare by environmental enrichment just because this might create a one time-shift in background data.

Many toxicologists consider the measurement of food intake as indispensable. But it has been questioned whether this parameter is necessary under all circumstances, particularly when food is provided in restricted amounts (as often for dogs and minipigs) and body weight, a closely correlated parameter, is recorded simultaneously (Dean, 1999). In other cases, e.g. when the test substance is incorporated into the food and the achieved dose has to be calculated using food consumption data, such data will be absolutely essential.

Traditionally, certain experimental situations such as telemetry studies or animals carrying catheters or brain electrodes are considered incompatible with group housing. However, it has been demonstrated that group housing is possible under such conditions, when the necessary precautions are taken or suitable techniques are used (cf. Coelho & Carey, 1990; Schaefer & Michael, 1991; Hawkins *et al.* 2004).

Once environmental enrichment becomes standardized within an animal facility and codified as an established component of the daily care of the animals, concerns regarding the impact of that enrichment procedure on scientific results are alleviated (Stewart & Bayne, 2004).

6.2 Practical considerations for staff of implementing enrichment

The introduction of environmental enrichment in an animal facility can create more workload and thus may require more personnel. However, animal caretakers and biotechnicians typically are highly motivated to improve animal welfare. Thus, staff is often willing to put extra efforts into implementing and running an enrichment programme which then results in the satisfaction from the improved animal welfare standards obtained.

An increased workload can occur for two reasons. First, additional time is needed to provide, remove and clean enrichment devices. Some of the enrichment objects have to be changed or re-introduced frequently, others are more permanently implemented and therefore may consume less time once they are installed. Second, extra time may be needed for animal handling either as part of the enrichment procedure *per se* (e.g. dog socialisation) or because animals use the opportunity to hide in a more structured cage environment. However, results indicate that environmental enrichment in the form of shelter (e.g. pipes) does not complicate catching or handling of mice, and providing enrichment does not interfere with the management or cost of laboratory animals (Moons *et al.*, 2004).

Finally, it should be taken into consideration that enrichment has also the potential to save time, e.g. because the animals may be less timid and, therefore may be more easy to handle.

6.3 Occupational health and safety aspects

Prevention of injuries and elimination of occupational hazards is always an important issue for laboratory animal facilities. Therefore, reasonable precautions should be taken to ensure that enrichment strategies and devices are safe for personnel. Environmental enrichment programs in general do not require more precautions than those normally in place in laboratory animal facilities. No occupational health recommendations particularly related to enrichment were found in the literature. Sharp or cutting edges should, of course, be avoided (e.g. cage dividers and other metal devices), and just as normal bedding, nesting material should, as far as possible, be dust-free to diminish the risk of allergy or infection by airborn microorganisms.
Enrichment can involve more frequent lifting of heavier objects (e.g. larger structured cages) which may pose an ergonomic problem and could induce back injuries. This can be prevented by constructing enrichment objects either on a “module” basis or in a way that they can easily be disassembled. In any event, appropriate training of the personnel and careful implementation of the enrichment programs is the best solution to these problems.

6.4 Financial aspects

Financial aspects of environmental enrichment are twofold: Funds for environmental enrichment-related research and financial resources to implement and conduct enrichment programmes *per se*.

The 3R-principles are part of the European animal welfare policy and of the AAALAC accreditation process. Introducing environmental enrichment in the care and husbandry program of animal facilities is an important way of refining experimentation so as to reduce unnecessary suffering. Therefore, European and national institutions, private foundations and animal welfare charities should feel encouraged to promote quality enrichment programmes by offering more grants for peer-reviewed enrichment-related research. An example is given by the Centre for the 3Rs in the UK. In the light of research results that contribute to proper and effective implementation of enrichment-based welfare improvement, the importance of supporting such investigations can hardly be overestimated.

Implementation and conduct of valid environmental enrichment programs needs funding too. This has to be budgeted and allocated with due regard to scientific merits, ethics and animal welfare legislation. Cost saving tips for environmental enrichment programs (Stewart & Bayne, 2004) can reduce the financial burden at least outside an GLP-environment. This includes the use of commonly available materials, such as plastic pipes, which can be cut to a length fitting to their use as shelters for a variety of species. Small pipette-tip boxes can be easily cut open, autoclaved and used as shelters for mice. Implementation of environmental enrichment does not necessarily translate into undue financial burden, although the costs of the devices are not only determined by the initial purchase-price, but also by the frequency of replacement due to damage, consumption or soiling.

6.5 Good laboratory practice (GLP) aspects

Although GLP is a clearly defined area within pre-clinical research and development (pharmaceuticals) and pre-marketing safety assessments (pesticides, chemicals), the large number of animals used in this field as required by law makes it an important area for animal welfare considerations.

It should be kept in mind that GLP is not about what to do (this is determined by science), but how to do it. This means that animal studies conducted under GLP have to be thoroughly described – either in standard operating procedures (SOPs) or in the study protocol – and materials, equipment and animals used in such studies have to be certified/specified and/or validated. From this it becomes clear that GLP does not prohibit enrichment. But it sets the stage for certain conditions under which environmental enrichment is to be implemented in this type of studies.

In essence, the GLP-aspects of environmental enrichment are not complicated and can be categorized into procedural and material elements. With regard to the procedural elements, enrichment procedures

(a) should be validated concerning their impact on the outcome of the study, either by a separate validation study or based on sufficient evidence from published enrichment-research;
(b) should either be standardized and always carried out in the same way (hence SOPs) or thoroughly described in the study protocol.

With regard to the material elements of environmental enrichment – as with other materials used in GLP-regulated studies (diet, bedding, water, disinfectants etc.), enrichment materials and devices have to be certified and/or specified to make sure that they do not interfere with the desired outcome of the study (e.g. determination of the no-observed-adverse effect level of the compound under study). This means, within defined ranges, enrichment materials have always to be of the same composition and free of hazardous components (i.e. known possible contaminants have to be below specified limits etc.). It is desirable, although not an unconditional prerequisite that this is substantiated by Certificates of Analysis for the given batch of material used. Under such conditions environmental enrichment and GLP are compatible without problems.

Under certain conditions it is even possible to leave the strictly regulated territory of GLP in GLP-regulated studies. This, however, has to be carefully considered and agreed upon with the authorities on a case-by-case basis. One example is the use of hay for minipigs. Hay is an important nutritive enrichment component for this species, and, although it is practically impossible to have a “batch”-specification for hay, its use is widely accepted by regulatory authorities base on the assurance that no pesticides or fecal slurry were used on the meadows where the hay came from.

6.6 Training of staff and communication with researchers

Good interaction between the staff of the animal facility and the researchers are very important, because - understandably - researchers are concerned about any changes or additional variables of their study protocols (Gärtner, 1998). It is the combination of the progressive investigator and animal care staff to prove to other investigators that animal-friendly changes will have no disadvantage for the outcome of their studies (Dean, 1999).

An important issue is the training of the personnel involved in environmental enrichment. This includes the staff of animal caretakers, biotechnicians and investigators. Every educational program on laboratory animal science should include reference to environmental enrichment. It seems to be a neglected issue to translate results of enrichment-research into textbook-knowledge.

6.7 Unwanted side effects of enrichment on animal welfare

The possible adverse consequences to an enrichment programme should be fully explored. This can be done for example by literature-search or consultation of competent persons, e.g. veterinarians or ethologists (Poole, 1998, see also Section 3). This is particular true for the introduction of new, innovative enrichment devices in the daily care of laboratory animals.

For instance, creating passageways may often be useful but under certain circumstances they may increase territorial and aggressive behaviour, for instance in DBA/2J and CBA/J mice (Haemisch & Gaertner 1994). Following up on this, more recent research provided knowledge how to control aggressive behaviour in group-housed male mice using environmental enrichment as a tool (Van Loo *et al.* 2003). This is an excellent example how enrichment-research translates into science-based welfare improvements for laboratory animals.

Attention should be given to the possibility that the objects may cause competition for coming into their possession. This applies especially for dogs and primates. Care must be taken that this does not increase aggression. By providing enrichment items as hanging objects facilitates floor cleaning and helps to avoid that things are monopolised by individual animals. In addition, this minimises the risk of swallowing (e.g. by dogs), and thus causing gastrointestinal complications. Such precautionary measures make this type of complications a highly unlikely event, so that the welfare benefits of enrichment heavily outweigh this potential risk.

Another issue is the inadvertent exposure to substances present in enrichment devices, possibly leading to interferences with the experimental result. This should be kept in mind when selecting the materials for enrichment. It can be concluded that proven suitability of enrichment devices is always important, not only for GLP-regulated studies.

6.8 Observability of the animals

When using nesting-material or offering refuge for the animals, observing the animals is less easy. However, the proven welfare-benefits of this type of enrichment outbalance the additional time that has to be spent to observe the animals in experimentation and during the normal check-up in breeding programs.

6.9 Sudden changes of environmental enrichment when animals are transferred

Laboratory animals come from breeding colonies and will be used for different kinds of research (behavioural studies, toxicology, metabolic research etc.) each with specific requirements or limitations concerning animal husbandry and care. Besides special requirements, general housing practice can just differ between breeding stock and experimental units which raises concerns about welfare impacts of changed or abandoned enrichment programs. Such concerns have been repeatedly voiced, but up to date they seem to be insufficiently addressed in enrichment-research.

Complete information about housing conditions and the "environmental enrichment history" should be available to enable continuation of enrichment programs or at least allow a qualified assessment of the effects of discontinuation. A good liaison of breeders with users of laboratory animals will facilitate the arrangement of compatible enrichment programmes.

6.10 Encourage EE description in the "methods section" of journal publications

The "materials and methods" section in scientific publications, often reveals a lack of information about the animals and their environmental conditions, contrary to that on instruments and chemicals, which are always carefully described (Öbrinck & Rehbinder, 1999). Investigators do not always realize the possible influence of environmental variables and animal characteristics on experimental results. As a result, animals and their living conditions including environmental enrichment are described inadequately or superficially (Davis *et al.* 1973, Lang & Vessel 1976, Clough 1982, Reinhardt 2004). Having to repeat experiments or hampering the reproducibility of results simply because they were poorly documented is time consuming, expensive and unjustified from an animal welfare perspective (Öbrinck & Rehbinder, 1999).

If journal editors make an adequate description of environmental enrichment (and other aspects of animal care) a mandatory condition for the acceptance of manuscripts, it will contribute to the comparability of the results of different studies and facilitate harmonization of environmental enrichment, and thus provide reviewers the opportunity to comment on it. Eventually this will help to eliminate "bad" and promote "good" (i.e. validated) enrichment.

7. Conclusions & Recommendations

(1) Currently environmental enrichment is one of the most complex challenges for laboratory animal science and welfare. It is used more broadly and systematically only since recent years and its ultimate goal is to improve animal welfare **and** to enhance the outcome of animal experiments, or, at least to improve animal welfare without negatively affecting the scientific results. The complexity of this issue arises from the fact that a tremendous number of permutations of enrichment possibilities (social, physical) can be applied to various species/age groups/sexes. This makes it very difficult to establish general rules for the implementation of environmental enrichment and at the same time generates fears of compromising scientific results or harming instead of benefiting the animals.

(2) The concept of environmental enrichment is based on a set of principles derived from different life science disciplines. These principles have to be taken into account to ensure that environmental enrichment programmes have a scientifically sound basis. Thus, the concept of environmental enrichment is rooted in the following:

   a. Animals have physiological and behavioural needs. These needs are inter-related and their satisfaction affects physiological as well as behavioural responses, and thus well-being and experimental results.
   b. Complexity, control and predictability are essential elements for the successful establishment of an environmental enrichment programme, i.e. specific consideration of each of these elements (including their mutual influence) is necessary when introducing environmental enrichment.
   c. For a valid design of a (new) enrichment programme it is of key importance to be familiar with the natural history of the species in question and to be aware of "sensitive" periods during development which may be exploited for an effective enrichment strategy.

(3) To ensure that enrichment achieves the aim of benefiting the animals it needs scientific evaluation. Ideally this evaluation would be a combination of hypothesis-driven studies carried out by qualified scientists (e.g. applied ethologists) and supervised "clinical trials" performed under field conditions in several animal facilities by animal care staff. Likewise it is important to enhance communication on ad-hoc enrichment ideas between animal care staff, animal welfare scientists and researchers for an in-depth assessment of the benefit for the particular species or group of animals.

(4) Concerns about an enrichment-induced increase in variability exist since a number of years. Even in mice, probably the species most extensively investigated in this regard, results seem to be contradictory. Concerns were raised as early as 1998 by Gaertner and supported by findings of Tsai *et al.* (2002, 2003). Others, e.g. van de Weerd *et al.* (2002), Augustsson *et al.* (2003) and most recently Wuerbel *et al.* (2005) described no effect or even a reduction of variability. Here we should also remember that an increased variability in some specific studies should be put in balance with the housing of thousands of animals. In other words, if as a result of the enrichment one researcher needs to increase his/her sample sizes by 20%, but associated with a 5% decrease of stereotypies in 10.000 mice housed in the facility, implementation of the enrichment may still be preferable. 3Rs are valid in experimentation AND in housing etc. (cost/benefit analysis). In addition, validity needs to be kept in mind. Validity refers to animals with proper baseline values, i.e. "more normal", non-biased animals in terms of their physiology and behaviour. Bias or deviation from normality can be caused by barren housing as well as by inadequate forms of enrichment. It is concluded that in the foreseeable future there will be no general answer to the issues of variablity and validity. Rather, both issues can mostly be addressed by cost/benefit analyses on a case-by case basis.

(5) A systematic approach may be helpful for the practical design of an enrichment programme. This includes:

   a. always to consider how to implement the species' social structure, as this is certainly the best way to enrich the housing, than look at the physical enrichment (complexity of accommodations and furniture, sensory and nutritional enrichment)
   b. practical aspects summarised in the "Points to Consider" section.

(6) Environmental enrichment is a particularly complex component of animal husbandry and welfare. It was the working group's intention to clarify that it is unrealistic if not wrong to expect that the rather nascent process of implementing environmental enrichment could be standardised in an "engineer-type" manner. With the present document it was attempted to raise awareness that the only way to adress this problem are "performance standards", i.e. application of the principles of environmental enrichment and a scientific evaluation of the planned enrichment (including the assessment of variability and validity) under due consideration of practical aspects. Through such a process "performance standards" (=”look at the effects”-approach) will evolve which will contribute to the harmonization of enrichment. In fact this evolution has already begun.

(7) The working group recommends:

a. to establish databases of environmental enrichment programmes currently in place;
b. to perform surveys of enrichment programmes currently in place;
c. to strongly support a systematic scientific evaluation of important enrichment components already in use or intended for implementation;
d. to identify new types of enrichment based on a scientific assessment of the animals needs;
e. to encourage a description of the environmental enrichment used in the Materials and Methods" section of publications.

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