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Original article

Welfare of the minipig with special reference to use in regulatory toxicology studies☆

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A B S T R A C T

This paper reviews the animal welfare challenges associated with the use of minipigs in toxicology testing, and compares these to published knowledge on the other widely used non-rodent species (dogs and non-human primates).
Welfare challenges arise from housing and management of populations under laboratory conditions, and from the procedures carried out for product evaluation. Welfare assessment requires a multidisciplinary approach: cardiovascular parameters, adrenocortical hormones and behaviour are well known parameters. However, reliable non-invasive methods to assess welfare and species-specific benchmarks need further development in minipigs. Husbandry of minipigs (housing, diet, and socialisation needs) to promote good welfare is described in the revised Appendix A of the European Convention (ETS 123). This has been supplemented by knowledge of species biology and expert opinion from experienced minipig users.
Challenges when using minipigs in toxicity testing have been reviewed in detail. Although deeper location of the peripheral blood vessels makes blood sampling more challenging, samples can be taken with minimal distress when staff members are well trained. Temporary and chronic vascular catheters can also be used for frequent sampling, and are likely to improve the welfare of the animals. Available training courses with a focus on stress free handling and dosing, as well as surgical placement of temporary and chronic vascular catheters, should be utilised to improve welfare during these procedures. Humane endpoints have been described, mainly based on current industry practices, but further scientific investigations are required.
From an animal welfare perspective there are no basic restrictions to using minipigs in toxicity testing that are unique to this species. We conclude that it is easier to keep minipigs to a good standard of welfare under laboratory conditions than it is for dogs or non-human primates, since minipigs are not athletic (like dogs) or arboreal (like non-human primates).

1. Introduction

In this article we review animal welfare issues relating to the use of minipigs in regulatory toxicity testing, as a contribution to the *RETHINK* project (Forster, Bode, Ellegaard, van der Laan, 2010a,b-this issue). While there is a long experience in the use of dogs and monkeys as laboratory animals, and understanding of their animal welfare needs is well developed, experience with minipigs as laboratory animals is more limited. This article first defines animal welfare (and animal needs) and discusses welfare indicators for a miniature swine, i.e. the Göttingen minipig (Section 2). Available information regarding animal welfare as related to the husbandry of minipigs (e.g. housing, diet, socialisation needs *etc.*) and their use in procedures utilised in toxicology testing (e.g. blood sampling, administration of substances, anaesthesia *etc.*) is given in Sections 3 and 4 respectively. Section 5 presents (in table format) a comparative view of the potential animal welfare issues arising from the use of minipigs, dogs, marmosets and macaque monkeys in toxicity testing.

☆ The opinions expressed in this paper are those of the authors and not necessarily those of the organizations they represent.

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Finally, Section 6 of this article presents the conclusions and recommendations, documenting gaps in the available knowledge relating to minipig welfare and identifying corresponding needs for further research.

2. Animal welfare definition and indicators for the Göttingen minipig

Animal welfare is a complex subject which has both scientific and ethical components. As highlighted by Fraser (2003), a person's view on the welfare of animals can be greatly influenced by their ethical perspective. Three different perspectives on welfare have been identified: which focus on natural living, biological function and affective state respectively. The relative priority which is given to each of these aspects may result in different conclusions about the relative acceptability of different animal housing and management practices.

2.1. Perspectives on welfare

The *concept of natural living* implies that animals should be maintained in conditions similar to those experienced by their wild counterparts or ancestors, and given the opportunity to perform the range of behaviours commonly observed under such conditions. Whilst re-creation of the natural habitat may not be necessary for animal welfare, meeting inherent behavioural or physiological needs by appropriate environmental provision is a welfare requirement (Edwards, 2007a,b).

The *concept of biological functioning* takes a much more practical view, and is commonly the perspective of animal production scientists and veterinarians. It implies that animals which show good survival, health, growth and reproductive performance are having their welfare needs met.

The *concept of affective state* probably comes closest to welfare as subjectively perceived for themselves by humans (Duncan, 1993), but is difficult to assess objectively in other species. It ascribes to animals the feeling of pleasant and unpleasant emotions, about which it is only possible to speculate. However, their presence can be inferred both by homology with human biology, and by observations of physiological and behavioural response to situations where such states might be predicted to occur.

2.2. Definitions of welfare

Arising from these considerations, it is apparent that any definition of overall welfare must encompass physical, physiological and psychological aspects. Whilst many definitions have been proposed, a few can be highlighted as being widely cited and generally accepted:

- “an individual's state as regards its attempts to cope with its environment” (Broom, 1986)
- “prevention of the experience of an unpleasant mental state” (Duncan & Petherick, 1991)
- “the capacity of an animal to sustain physical fitness and avoid mental suffering” (Webster, 1994).

2.3. Defining needs

A need has been defined as “a requirement, which is a consequence of the biology of the animal, to obtain a particular resource or respond to a particular environment or bodily stimulus” (Broom & Johnson, 1993). Needs must be distinguished from preferences, which may be of little importance to the animal, and will be characterized by measurable welfare impairment if they are not adequately met.

The needs of minipigs can be considered as closely related to those of other types of pig, since a common evolutionary history forms the context under which such needs have developed. However, it is important to identify the extent to which selective breeding may have modified needs in the minipig. Relatively few scientific data exist on this topic and this is a research need. However, studies in the domestic farm pig indicate that, despite many generations of selective breeding under controlled farm conditions, the motivations and consequent behavioural needs have remained little changed from their wild ancestors. The needs of pigs may be broadly classified in the following general areas (after EFSA 2007):

- To breathe air of appropriate quality which will support respiration and not impair lung health.
- To have appropriate sensory input which will provide adequate stimulation without causing visual, auditory, tactile or olfactory discomfort.
- To rest and sleep in comfort in order to recuperate, and to show normal biological cycles.
- To exercise and maintain normal development of bone, muscle and joints.
- To obtain food and water in a quantity and quality which is satisfying and which will not cause digestive or metabolic problems.
- To exhibit foraging and exploratory behaviour.
- To have appropriate harmonious social interactions with other pigs, caretakers and technicians.
- To experience adequate maternal care, minimal aggression and competition for resources.
- To avoid fear from perceived predation or unavoidable danger.
- To thermoregulate and maintain themselves within their thermoneutral zone by expressing appropriate behavioural strategies for warmth and cooling if environmentally challenged.
- To maintain themselves free of harmful substances or organisms by skin care, and maintenance of good hygiene.
- To express reproductive and maternal functions when strongly motivated to do so, including nest building in the peri-parturient period.
- To avoid pain and disease.

2.4. Welfare indicators

There are two general types of welfare indicators; those indicating failure to cope, and those assessing the effort involved and its effectiveness (Broom, 1986). Productivity is not a good measurement of welfare, yet it can be used as one measure in a battery of measures until more reliable indicators are found. Coping attempts with short, difficult or long situations may be assessed with cardiovascular parameters, adrenocortical hormones and behaviour (Broom, 1986). A common feature for all these indicators is the necessity to sample with a non-invasive method or observe without the animal noticing the observer. Unfortunately, there are only a few methods available to reliably assess animal welfare non-invasively. Individual coping responses vary, and any single indicators may show poor welfare, while the converse can only be proven with a wide battery of indicators (Duncan & Filshie, 1979).

There are no welfare assessment schemes for minipigs, and hence we have to suffice with the work done with swine in pork production. Several monitoring systems exist in Europe, such as the Animal Needs Index used in Austria and Germany (Bartussek, 1999) and the decision-support system in the Netherlands (Bracke, Metz, & Dijkhuizen, 2001; Bracke, Metz, & Spruijt, 2001). These assessment schemes focus on housing parameters and on selected animal observations.

Unfortunately no single golden standard exists for welfare assessment. Key elements of any welfare scheme are validity, reliability and sensitivity of the ‘instruments’, and first pig-specific welfare assessment schemes, combining several direct and indirect measurements, have now surfaced (Smulders, Verbeke, Mormede, & Geers, 2006). Performance records, behavioural, physiological and clinical parameters can be a good basis for assessing welfare at the level of an animal. Pig welfare assessment should include both resource-based and animal-based measures.

2.4.1. Body condition scoring

There are basic health and welfare assessment schemes applicable to all laboratory animals (AHWLA, n.d.), which briefly discuss following items, at the level of an animal, to observe: body condition, body shape, posture, fur, facial expression, skin, mucous membranes, eyes, ears, nose, mouth, tail, and perineum. There is also a pig-specific body condition scoring system for farm pigs described by MAFF (1998).

2.4.2. Cardiovascular responses

In stress situations the autonomic nervous system (ANS) and hypothalamic–pituitary–adrenal (HPA) axis are activated, leading to secretion of catecholamines and corticotropin, respectively. These will change heart rate, heart rate variability and blood pressure, which can also be used to assess both short and long-term stress at the level of an individual. Successful use of these cardiovascular parameters requires instrumented animals with telemetric transponders. Diurnal variation in the level of hydrocortisone was seen by Killian, Garverick, and Day (1973).

2.4.3. Behaviour

Behavioural assessments may be structured observation of normal and abnormal behaviours, or specific tests designed to assess general or specific welfare indicators. Smulders et al. (2006) reports a behavioural test consisting of a 5-min observation of the animals in their confinement, hence the observation level is (mostly) the pen. A range of behaviours is observed, either based on literature or their own studies.

2.4.4. Linking hormonal data with behavioural related scores

The HPA axis and the ANS are markedly activated during stress, resulting in the release of glucocorticoids and catecholamines, respectively. There is considerable work done on the relation between acute environmental and psychological challenges in farm animals and levels of these hormones (Dantzer & Mormede, 1983). The long-term effect of stressors on neuroendocrine activity has been studied less, but hypertrophy and hyperactivity of the adrenal gland can occur and be measured histologically *post mortem* or by response to ACTH challenge in the live animal.

2.4.5. Hormones

Non-invasive sampling methods, based on quantification of stress sensitive molecules, are important in objective assessment of animal welfare as an alternative to quantification of such molecules in blood. Blood sampling usually involves restraint of the animal and may often be perceived as painful, which is likely to stress the animal. Analyses of the concentration of stress sensitive compounds in normal secretions, including saliva, urine and faeces, are increasingly used with the aim to monitor stress of short and long duration, respectively (Mostl & Palme, 2002).

2.4.6. Blood hormones in minipigs

Kissinger et al. (submitted for publication) evaluated the use of an indwelling jugular catheter in farm swine and Göttingen minipigs of both genders for several days up to 2 weeks. Blood samples were collected automatically from conscious and freely-moving pigs or manually from the same catheter while the pigs were physically restrained. The adrenal response of the pigs to handling was immediate and impressive with plasma catecholamine concentrations more than an order of magnitude higher than the baseline monitored in blood collected while the animals were unperturbed. More significantly, pigs responded with an increase in plasma catecholamines when other pigs were restrained and manually sampled in the same room, although not to the same extent as seen in the manually-sampled subjects.

2.5. Conclusion

It can be concluded from this review that no single parameter is adequate to assess welfare state. Triangulation when testing stress is recommended by Webster (1994). No physiological or behavioural indicators have been specifically validated in minipigs, but measurements validated in farm pigs should be appropriate. However, since the extent to which behavioural and physiological coping strategies are adopted may differ between breeds, it will be important to characterize the stress responses of minipigs and to assess both types of measure in any welfare study. There is a research need to develop and validate simple welfare assessment tools for minipigs which can be used under practical conditions.

3. Minipig husbandry

The housing and husbandry of animals used for experimental purposes should ensure that animals experience as little stress as possible under the given conditions for animal welfare and scientific reasons. In order to achieve this for minipigs, the general trend is to create an environment which is as natural as possible (e.g. use of bedding to satisfy rooting behaviour). Scientific, practical and economic factors influence what can be achieved and it should be stressed that it is not possible to create a completely natural environment in a biomedical research facility and that the housing used in production facilities is not always adaptable or desirable for minipigs used in research.

Guidelines for the accommodation and care of laboratory animals are detailed in the revised Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS 123). This document details the main aspects of housing design for minipigs, including minimum space requirements and the environmental conditions to be maintained, so the present article will not cover these general requirements. Instead it highlights areas of best practice or examples where consideration should be given to enhancing housing. A recommended best practice minipig pen for toxicology is given by Ellegaard Göttingen Minipigs in 2007 (Ellegaard, 2007). Several factors need to be taken into account when deciding on appropriate housing for minipigs used in research. It must be remembered, that it is necessary to handle the minipigs during a study so positive animal–human interaction is very important and housing needs to be designed to accommodate this. If the animal–human interaction is negative, the minipigs may react strongly to the procedures performed, which may be an indication of stress. Besides the negative impact on the welfare of the minipig, poor handling may influence the results obtained in a study since the stress response may interfere with parameters such as insulin levels. It is very important that the staff working with minipigs have a good knowledge of minipig behaviour to allow abnormal behaviours to be identified and the causative factors eliminated.

3.1. Physical environment

3.1.1. Temperature

Pigs are highly sensitive to environmental temperature and place a high behavioural priority on thermoregulation. Pigs may be kept in a uniform, temperature controlled environment, in which case the whole room should be maintained within the thermoneutral zone. Alternatively, they may be kept in an enclosure with different microclimates, by providing localised heating or kennelling of the lying area and provision of adequate bedding material. A temperature gradient within the enclosure is considered beneficial, since it allows the animals a choice of climate.

According to Georgiev, Georgieva, Kehrer, and Weil (1977) a broad thermoneutral zone does not exist for the Göttingen minipig, but a metabolic neutral temperature was identified. The metabolic neutral temperature was shown to be 29 °C for Göttingen minipig piglets 6–8 weeks old, 24 °C for 14–16 week old Göttingen minipigs and 17.4 °C for Göttingen minipigs 34–36 weeks of age.

Piglets of low body weight are very sensitive to environmental temperature and should be provided with higher temperatures. Newborn piglets should be offered a lying area minimum of 32 °C, decreasing to 26 °C at the age of 4 weeks. For farrowing/lactation rooms, the minimum room temperature necessary is that required to allow an adequate temperature to be maintained in the piglet lying area, taking account of any local heat supply. Since excessive room temperature may result in heat stress of the lactating sow, local heating in the piglet resting area within a cooler room of 20–22 °C is recommended.

3.1.2. Humidity

As described by Morrison et al. (1969), relative humidity between 45% and 90% does not seem to influence growth rate and food utilisation of pigs. Georgiev et al. (1977) found the relationship between temperature and relative humidity to be true also for Göttingen Minipigs, where relative humidity does not seem to influence energy conversion of minipigs as long as they are housed in accordance with their thermoregulatory neutral temperature. As long as the humidity is not extremely low or high (45–75%), controlling the temperature is more important than controlling the humidity in minipig units. Since a relative humidity of about 45% is also recommended for humans, it can be believed that the same relative humidity is suitable for both the care staff and the animals.

3.1.3. Lighting

Pigs evolved as diurnal and crepuscular animals, with activity peaks typically occurring at twilight. Less intense light (10 lx) is preferred to intense light (110 lx) (Baldwin & Start, 1985). A diurnal cycle of light and dark with a minimum of 8 h light should be provided. This light period should provide a minimum light level of 50 lx for the animals, but a higher level of at least 250 lx should be available at times of inspection to enable all animals and pen facilities to be clearly seen, and any problems detected. Pigs prefer a low light intensity for rest and sleep, with 2.4 lx being preferred to 4, 40 or 400 lx, and spent approximately 7 h per day in the compartment with lowest illumination (Taylor et al., 2006).

3.1.4. Bedding

Bedding contributes to pig welfare in many ways. It enhances physical and thermal comfort (except in hot environmental conditions), can be eaten to provide gut fill and enhance satiety, and provides a substrate for foraging and nest building behaviours. The extent to which each of these different benefits can be provided will depend on the nature of the bedding, with long straw providing the best overall material but alternatives such as chopped straw, sawdust, wood shavings and shredded paper conferring some benefits. Bedding should be non-toxic and, where possible, provide structural diversity to stimulate exploratory behaviour. Bedding should be provided for all pigs, unless precluded for experimental reasons, and is particularly important for farrowing sows, which have a strong motivation to perform nest building behaviour, and for pigs on restricted feeding regimes, which have a strong motivation to express foraging behaviour.

3.1.5. Space

The space allowance for pigs should be adequate for both their physical exercise needs and to permit adequate social behaviour (see ETS 123 and below). Close individual confinement should be avoided except when necessary for veterinary treatment or as part of designated procedures. In contrast to farm pigs, it has not been found necessary to use farrowing crates for minipigs to enhance neonatal piglet survival, and well designed loose farrowing pens allowing the sow to express pre-parturient activity and the natural repertoire of nest building behaviours should be provided at this time.

3.2. Social environment

3.2.1. Group vs. single housing

Single housing has, in the past, been the preferred way of housing minipigs used in research as it was considered to be a way to exclude possible variables that may occur as a result of group housing. It has been documented that isolated pigs experience a chronic stress situation compared to group housed pigs (Kanitz, Tuchscherer, Puppe, Tuchscherer & Stabenow, 2004; Ruis et al., 2001). Tethered pigs or pigs kept in stalls also show changed or redirected behaviours, which are believed to be an expression of reduced welfare, and tethering should not be used when housing minipigs. Group housing is therefore recommended, unless there are specific experimental, veterinary or animal welfare reasons for single housing to be utilised.

How to group minipigs and the optimal group size under specific conditions has not been specifically investigated, although some work has been performed on the impact of stocking density on the behaviour of group housed minipigs (Krohn & Ellegaard, 2000). In this study the minipigs were divided into groups consisting of six animals and no difference in behaviour between groups of low and high density stocking was seen. It has been suggested that enrichment and pen design play a larger role in determining pig behaviour than does the floor space allowance.

The natural social hierarchy of minipig groups should be considered when making decisions on housing type. The social organization of feral pigs is that sows live in groups with a stable hierarchy, whilst young boars establish bachelor groups and older boars live solitarily. Keeping sows in groups would therefore be in concordance with the natural establishment. Since older boars are solitary in the wild, and because of the risk of fierce fighting between the boars, group housing of older boars has to be questioned. Minipig boars can be group housed when young, but breeding animals should be housed singly, with visual, tactile and olfactory contact to other minipigs. Often boars less than one year of age can be housed in groups, but it must be considered that, when boars mature, they may suddenly start to fight even if they have been housed together from a young age. It is therefore not possible to give exact age ranges where it can be regarded as safe to group house boars.

Sexually mature boars show dominant behaviour by mounting each other and it is necessary to closely evaluate the lowest ranking minipig, since this animal will be mounted most often. This may create a chronic stress situation, such that it is necessary to take this animal out of the group. In a research facility, it is worth considering single housing of the boar, provided that it can be housed with tactile, auditory and visual contact to other minipigs. Many of the studies on the effects of single housing vs. group housing use single housing where no tactile or visual contact is possible. Studies should be performed to see if there are fewer welfare concerns if the singly housed minipig has visual, olfactory and tactile contact with other minipigs.

3.2.2. Dominance

Pigs living in groups, establish a social hierarchy, which serves to maintain stability in the group and makes it unnecessary for the group members to fight every time they encounter each other (Beilharz & Cox, 1967). The dominant animal in a group will almost always be the larger pig. It has been described that pigs of uneven size establish social ranking quicker than pigs of even weight and strength. If the same situation applies to minipigs, then it is worth considering this arrangement when housing groups of animals used in research and testing.

In laboratory facilities, minipigs are usually not bred in-house but obtained from a commercial source and, because of this circumstance, animals bought may not have been previously housed together. When grouping unfamiliar minipigs of the same size, which is very often the case in study protocols, establishment of a hierarchy could result in fierce fighting between the minipigs simply because they are more even in size and strength. It is therefore preferable, where possible, to obtain animals that have been reared in the same pen at the breeder thereby reducing the risk of fighting. Regrouping should be minimised and avoided if possible. If regrouping is necessary, it may be helpful to introduce all of the minipigs to each other simultaneously and in a novel pen, so that none of animals feels that the pen is their territory. It can also be helpful to provide greater space allowance for the first 24–48 h and the possibility for defeated pigs to hide behind temporary curtains from aggressors (Edwards et al., 1993). Minipigs can be aggressive toward the lowest ranking minipig and it is necessary to evaluate if the minipigs create a harmonious group, or if one or more of the pigs show signs of being in a chronic stress situation. If a harmonious group is not formed relatively quickly, i.e. within days, splitting the group should be considered.

3.3. Feeding and watering

Conventional pigs are typically fed *ad libitum* until approaching maturity, after which restricted feeding practices are necessary to avoid obesity and associated health problems with skeletal and cardiovascular function. The Göttingen minipig, especially the sow, unlike production pigs, has a tendency to become obese and they are usually kept on a restricted diet unless being used as models for obesity. The food used for minipigs is a high fibre diet compared to that used for production pigs. Bollen and Skydsgaard (2006) showed that feeding the Göttingen minipig a diet specifically designed for minipigs is essential to ensure that restricted fed animals are provided with enough nutrients. Diets not specifically designed for minipigs, and fed in a restricted manner, may cause serious fat atrophy in the males.

With restricted feeding practices, young growing animals should be fed at least twice daily whereas mature animals should be fed once daily as an adequate meal size is important for the animal to reach satiety and will minimise aggression. Where feeding is restricted, all individuals within the social group should have access to food without the need to compete for the food; this is a common cause for aggressive behaviour. Where troughs are used for feeding, adequate space should be provided to ensure that all animals can feed simultaneously. If floor feeding is adopted, it is important to ensure that hygiene is maintained and that feed is scattered over a sufficiently large area to allow access of all individuals. Close observation by staff is necessary to ensure that all minipigs receive enough food.

When feed restriction is necessary, pigs will show increased foraging motivation which can be expressed as increased activity, aggression and development of stereotyped oral behaviours. To avoid these problems it is important to modify diets to enhance gut fill and satiety, for example by provision of increased dietary fibre, and to provide an appropriate foraging substrate such as straw.

Minipigs should have continuous access to clean fresh water. Water should be readily accessible to all individuals within the social group to prevent dominant animals impeding access to the drinking point. Drinkers (nipples or bowls) should be at the correct height (nipples should be level with shoulder) and of the correct size to give easy access, should provide an adequate flow rate and should be regularly checked and maintained.

3.4. Enrichment

Rooting, grazing and browsing are feeding behaviours in pigs and, under natural conditions, occupy a large amount of their time. According to Horell et al. (2001) rooting is a need in its own right in pigs. Pigs are also strongly motivated to explore environments; rooting, chewing and checking scent are exploratory behaviours and pigs will also spend a lot of time on these activities.

3.4.1. Bedding

To satisfy the rooting behaviour of pigs the use of bedding is recommended. The use of bedding serves as a nutritional substrate, as well as providing environmental enrichment, especially in experimental units where the possibility to move around is limited. Different suitable bedding materials exist and Appendix A to ETS No. 123 describes long straw as being the best overall material but also mentions chopped straw, sawdust, wood shavings and shredded paper as alternatives. The positive role of straw in enhancing pig welfare has been researched as it improves the physical comfort of pens and also allows pigs to exhibit exploratory, rooting, chewing and foraging behaviours but there are some practical implications with the provision of straw that need to be overcome (Tuyttens, 2005).

The use of bedding may not be possible in certain areas of biomedical research and it may be necessary to consider that softwood bedding materials such as pine, spruce, aspen and cedar may have an impact on the activity of liver microsomal enzymes and may even exert a carcinogenic effect. In an *in vitro* assay on aspen and pine, both show a cytotoxic effect (Odynets, Sanchez, & Donnelly, 1993). Breeders of barrier-bred minipigs for experimental research also face a considerable challenge when it comes to satisfying pig needs in terms of bedding materials and roughage. If bedding is not used, it is important to consider how to satisfy the pigs' needs in other ways. Different types of environmental enrichment can be used, but need careful consideration of the properties necessary to provide for the exploratory needs of the pig (van de Weerd, Docking, Day, Avery, & Edwards, 2003). Studies are underway to investigate whether the amount of straw can be reduced yet still satisfy the pigs' investigatory needs and rooting behaviour. In farm pigs, relatively small amounts of recreational straw have been shown to provide much of the occupational value of full straw bedding (Day et al., 2002).

3.5. Health

When using minipigs for biomedical research it is very important to use healthy animals. Clinical disease may pose a risk to biomedical research, but so may subclinical and latent infections, as these can cause physiological changes that can alter the minipigs' response to a treatment and thereby make evaluation of reactions difficult or impossible. Disease states are also, in themselves, a cause of reduced welfare for the animal.

Göttingen Minipigs are bred in barriered units and are monitored for a wide list of infectious agents, based on FELASA guidelines (www.felasa.org). The minipigs are free of many agents and they should not be housed near animals that may carry agents transferable to the minipigs, especially other pigs of a different health status. Otherwise vaccination should be considered. Appendix A to ETS 123 (Council of Europe, 2006) states that preventive medicine programmes should be developed. It is advisable that these programmes are developed together with a veterinarian who is knowledgeable of the specific minipig breed and under which conditions it has been bred.

It is most important that technicians/caretakers have a good knowledge and understanding of normal minipig behaviour and that they can detect changes in the behaviour of individual pigs. Subtle changes in behaviour are often the first signs of disease. A veterinarian experienced in pig diseases should be contacted at the first sign of disease.

3.6. Animal–human interactions

It has been shown that a pig's response towards humans is influenced by its previous experiences with humans — be they negative, neutral or positive. An extensive programme of research on this subject has been carried out in farm pigs but knowledge in minipigs is based on experience rather than experimentation. During a toxicology study, handling of the minipigs is necessary and positive contact with the technician and training of the minipig are crucial to minimising stress and experimental variability. Lots of positive contact with humans, especially during rearing, can markedly reduce the level of fearfulness shown towards humans. A socialising scheme should therefore be implemented at breeding facilities and positive interactions with humans should continue throughout their lives. An example of a proven socialisation and training programme is given below:

Step 1 (Day 0): The new arrivals are left for a 2–5 h to settle; the technicians have minimal interaction with the new arrivals as they tend to be nervous.
Step 2 (Days 0–5): The technicians sit in the pen with a food reward (diet/apples) and wait until the pigs come to them for the reward. Food reward must be used with caution. Once the pigs take their reward the technicians can start to touch the pigs to accustom them to physical contact with humans.
Step 3 (Days 2–8): The pigs are trained to be handled by picking them up; this is carried out in the pen and performed a couple of times a day — this gets the pig used to human interaction, touch and being handled. Each time the pig is handled it should be given a verbal and/or patting reward, and can, after a full procedure, be given a food reward.
The animals are trained to walk up and down the corridors in the pig bays and, to step onto a balance in the procedure room. A verbal and/or patting reward should be given on performance of the required behaviour; alternatively a food reward can be given.
Step 4: Training for blood sampling can be started as soon as the pig is comfortable being picked up and carried. The pig is walked to the procedure room and placed on its back on the table using the 'V' shaped restrainer to mimic restraining for bleeds. The pigs receive a lot of positive physical contact and verbal praise. A food reward is given each time after having been to the procedure room (if the research protocol allows it).
Training for oral dosing: The pigs are walked to the procedure room and held in a dosing chair (or a chair suitable for the handler) to get the pig used to this type of handling.
Training for anaesthesia: The pigs are walked to the procedure room and held in a dosing chair (or a chair suitable for the handler). A second person places a suitably sized anaesthetic mask, rubbed inside with wet/moist diet (or apple, provided the animal knows apples) so that the pig can associate it with food or treat, over the pig's nose.
Sling training: If sling training is required for certain procedures, it can be started in the home pen (or just outside the pen) and then change to the procedure room. Ideally it is performed every day for 3–5 days before the procedure.

Minipigs have been trained to co-operate with husbandry and scientific procedures using positive reinforcement (a form of operant conditioning whereby performance of a required behaviour is increased by providing a reward whenever the behaviour is performed). Clicker training has been found to be a powerful and effective use of limited training time when compared with other training methods (Bertelsen, Nielsen, Lund, & Gade, 2008; Blye, Burke, James, Fitzgerald, & Cox, 2006). The clicker is a small plastic box with a metal interior which clicks when it is flexed with thumb or finger. The clicker noise is initially paired with a primary reinforcer such as food and eventually becomes itself reinforcing for the animal (secondary conditioning). The clicker sound can be delivered immediately on performance of the required behaviour and is consistent over time. Furthermore, use of the clicker does not require the animal to be near or looking at the trainer. Being 'hands free', clicker training has the advantage of being a powerful tool in relationship building with animals that have little previous experience of handling or training as it avoids the stress associated with handling in other training methods. It also avoids repeated use of food treats, which can cause obesity in minipigs.

3.7. Transport

Generally the same principles of transport container design and construction and general care can be applied to dogs and minipigs. Temperatures and ventilation must be monitored and alarm parameters set, as minipigs can be prone to heat stress. A proportion of food (around 10–20% of the daily ration) should be offered in the morning, with the remaining proportion offered in the evening (either when parked overnight or on arrival at their destination). It is important to offer water every 4–6 h depending on external conditions and length of journey. Minipigs are more accepting of confinement than dogs or primates but if housed singly during transportation social isolation may be stressful. They can be housed in compatible groups during transportation but it is essential that they can lie down and be able to stretch out fully. During transportation they can lose 6–8% of bodyweight but they will regain this within 3 days. Transportation from the breeder(s) can be achieved throughout Europe within the 24 h stipulated under EU legislation on the protection of animals during transport. As with most species the greatest impact of transportation is psychological, with loading and unloading being probably the most stressful stages of transport, but there is very little published material regarding this area so more research is required. In farm pigs physiological values have been known to return to baseline very rapidly (within 2 days) and this could, if proven in minipigs, allow a shorter acclimatisation period prior to study start than with the other non-rodent species.

3.8. Acquisition and supply

Within Europe there is one main commercial breeder for which availability of animals is currently good. Minipigs can be transported from this breeder throughout Europe although there could be concerns regarding continuity of supply in the event of outbreaks within the farming/agricultural community that could result in movement restrictions of swine being imposed.

4. Challenges when using the minipigs in biomedical research

Pharmacologically active substances have desirable therapeutic effects and undesirable side effects. Adverse effects which may be health-threatening are investigated in toxicological studies. These studies are largely conducted in animals and aim to assess the balance between the benefits and the risks of a (potential) medicine. Most safety studies are conducted according to fixed study protocols which are designed for the identification of potential negative, hazardous effects of the treatment. For the different types of test items (pharmaceuticals, biologicals, chemicals *etc.*) and different areas of safety testing where the minipig has already found applications (general toxicity, reproductive toxicity *etc.*) see van der Laan et al., 2010-this issue and Bode et al. (2010-this issue). In general, in any toxicity study the experimental animals are subjected to procedures related to:

- the administration of compounds,
- observations and clinical examinations,
- the collection of blood samples or other biospecimens, and
- euthanasia.

In this section, the most common procedures and techniques used in toxicity testing are listed and the challenges when using minipigs are briefly described.

4.1. Administration of compounds, dosing routes

4.1.1. Oral route

4.1.1.1. Oral (PO) in food. Dosing in food causes little stress but poor palatability can restrict the intake by the animal. Taste aversion may develop if minipigs associate the intake of the food with pain or distress occurring after the meal. It may be necessary to mix the compound with preferred food (canned, wet, preferred tastes). Knowledge of the feeding behaviour and preferences of minipigs are important. In general this method is preferred over oral dosing by gavage, but specific properties of the test item may necessitate recourse to other techniques.

4.1.1.2. Oral in water. This way of oral dosing is not reliable, because it is not sure how much drug has been taken unless fluid intake is restricted. The procedure is rarely used in the practice of toxicity testing in non-rodent species.

4.1.1.3. Capsules and tablets. The mouth should be kept closed until the capsule is swallowed; some water syringed into the throat may elicit swallowing. This procedure may cause distress (minipigs vocalize). Training of staff and minipigs minimises the stress caused by restraining the animal.

4.1.1.4. Oral by gavage. Accurate placing of the tube requires care because of the morphology of the caudal part of the nasopharynx of the minipig. This procedure is regarded as stressful (struggling and vocalization of the animals). In one study the minipigs showed elevated cortisol concentrations (Jensen, 2004). In this investigation, the minipigs were not habituated to the gavaging. Training of staff and habituation of the minipigs to this procedure may reduce the stress, and there is a need for parallel physiological data to evaluate this.

4.1.2. Parenteral route (injections)

4.1.2.1. Intradermal injections. The animal is manually restrained. The injection itself may be aversive, depending on volume and pH of the formulation and staff competency. Local anaesthesia can be used (e.g. EMLA, 45 min.) If this is the intended route in the clinic the minipig is a suitable model.

4.1.2.2. Subcutaneous injections. Manual restraint is required. In pigs limited injection sites are suitable for subcutaneous injections. Commonly used injection sites are the area behind the ears and the flanks (thin skin). Subcutaneous injections are rarely painful. It can be helpful to change or rotate injection sites with successive doses.

4.1.2.3. Intramuscular injections. Manual restraint is required. In adult pigs, injections are given into the neck muscles, but a long needle is required to penetrate the fat layer. Care must be taken to avoid injection into the subcutaneous fat. The hind legs are also frequently used for intramuscular injections. It can be helpful to change or rotate injection sites with successive doses. The light pigmented skin with little hair of the Göttingen minipig facilitates inspection of the injection sites. Injection of large volumes has to be avoided because distension of muscle fibres is painful. Care is needed with the injection of adjuvants or other poorly tolerated compounds because tissue damage is hidden. Intramuscular injection needs trained and competent technical staff.

4.1.2.4. Intravenous injections. Manual restraint is required. Injections may be difficult, especially in piglets, because of the small size and deep location of the peripheral blood vessels in the surrounding tissue. The ear vein is usually used for both single dose and multiple dose studies. The Göttingen minipig has relatively small ears and ear veins compared to many other pig- and minipig breeds. Training of staff may be necessary to overcome difficulties in dosing via the ear vein in the Göttingen minipig.

Less pain is caused if flexible cannulae are used in preference to rigid needles. The use of surgically implanted catheters or vascular access ports (VAP) is recommended for multiple injections in adult minipigs. Intravenous injection needs trained and competent technical staff.

4.1.2.5. Intraperitoneal injections. Manual restraint is required. It is easy to misplace the injection into an organ. This dosing route is not recommended for animals larger than rodents. It is generally not used in toxicity studies, because this route is not used in humans.

4.1.2.6. Intranasal injections. Manual restraint is required. Intranasal injections of liquids or suspensions have rarely been used in the Göttingen minipig. It appears to cause discomfort to pigs, which may sneeze so that it is not easy to ensure that the complete volume is in the nostril(s).

4.1.2.7. Intratracheal injections. This route has rarely been used in the Göttingen minipig in toxicity testing. Anaesthesia is recommended to prevent restraining the animal and the use of a sling.

4.1.3. Topical dermal applications, patches and occlusive dressings

The (mini)pig is one of the species of choice for dermal studies because of the close resemblance of the pig skin to the human skin. Up to 10% of body surface is treated. Dressings, collars or jackets are used. Manual restraint is required to change dressings. Animals need to be trained to accept these or other restraints. Group housing may be difficult because pen mates try and remove or destroy dressings. In practice dosed animals are housed singly. Removal of adhesive dressings can be painful. It is recommended to train pigs to enter a transport crate for food and change dressing as the pig is distracted whilst feeding. Half of the food can be given on return to the pen as reward after completion of the whole procedure.

4.1.4. Inhalation

Restraint chairs or slings are required for the application of inhalation masks (cone). Animals can be exposed for up to 3–4 h. This long period of restraint is stressful. Training and habituation of the minipig is essential. Intubation of the trachea is different from human, dogs and non-human primates because of the specific anatomy of the upper airway system of the minipig and requires instruction. In some inhalation studies anaesthesia of the pigs is used. Inhalation chambers have been used successfully for whole body exposure to drugs. Issues are isolation distress and confined space. Habituation before study onset is likely to be of benefit (Koch & Clausing, 2001; Windt, 2010).

4.1.5. Osmotic pumps

Implanted osmotic pumps, or new programmable micro infusion pumps (Iprecio, n.d.) delivering the test drug can be used to avoid multiple injections and restraint or anaesthesia. Osmotic pumps have been used successfully in minipigs.

4.2. Observations and clinical examinations

4.2.1. Clinical signs

Various protocols exist for the observation of clinical signs or symptoms in pigs and minipigs (Morton & Townsend, 1992). Trained staff can easily observe the behaviour and judge the physical condition of minipigs without disturbing the animals in their pen. Usually the following issues are addressed: eating, drinking, vomiting, diarrhoea, body posture, activity, behaviour, dehydration, colour of skin and mucus membranes (pigment), and hair coat.

The development of a body condition scoring protocol especially for minipigs is recommended, because of their different conformation and adiposity in comparison with farm pigs.

4.2.2. Clinical examinations

4.2.2.1. Body weight (gain/loss). Slight manual restraint is required. Portable/movable scales can be used for the measurement of minipig body weight in the pen. Alternatively, minipigs can be trained to walk to the balance located outside the pen or to enter a transport crate.

4.2.2.2. Body temperature. Body temperature is usually measured applying a rectal probe whilst the minipig remains in its pen. High quality thermometers allow rapid measurements (seconds) and minimise the duration of the manual restraint of the animals. Electronic ear thermometers are also available. In the case of frequent, short interval measurements, the application of implanted temperature responders or other telemetric devices is recommended.

4.2.2.3. Cardiovascular measurements

4.2.2.3.1. Heart rate. The pulse of minipigs can be measured easily with a belt. Minimal manual restraint is required. Habituation of the animals to the procedure and trained technical staff are prerequisites for the stress free measurement of the heart rate. Telemetry equipment for heart rate measurements in minipigs is available.

4.2.2.3.2. Blood pressure. The minipig is not anatomically suited to the use of cuff techniques for the measurement of blood pressure. Invasive techniques are possible, but with a welfare penalty in terms of surgery and restraint. For long-term studies the use of telemetry systems is recommended.

4.2.2.3.3. Electrocardiogram (ECG). Pigs are frequently used for the measurement of drug effects on the electrocardiography. Restraint of the animals is required. Trained technical staff and habituation of the animals to the procedure are prerequisites for undisturbed measurements. Limb leads and Nöebb Spori lead schemes are both being used. Telemetry systems for ECG are available (long-term studies) (Stubhan et al., 2008).

4.2.2.3.4. Respiratory rate. The use of minipigs in inhalation studies has been rather limited. Denac, Spörri, and Beglinger (1977) measured an average value of 5.1 L/min for minipigs with mean weight of 22.8 kg. In an inhalation study made by Koch and Clausing (2001) animals of 10 kg were used; this study provided an estimate for the minute volume of 2.1 L/min and visual observations of the motion of the breathing valves showed a smooth breathing pattern with a cycle length of about 2 s. The correlation between body weight and respiratory minute volume is given by Guyton (1947).

4.2.2.3.5. Neurological examination. Manual restraint is required. No standard protocol for neurological examinations is available. The development of neurological tests in the minipig is recommended, also with respect to the definition of humane endpoints.

4.2.2.3.6. Ophthalmology. Manual restraint is required. Habituation to the procedure and skilled technical staff reduce the immobilization stress.

4.2.2.3.7. Telemetry/remote monitoring. Telemetry devices exist for monitoring the body temperature, heart rate, blood pressure, ECG and EEG in (mini)pigs. The stress and trauma of surgery vs. reduction or elimination in handling have to be weighed against each other. The further refinement of telemetry technologies will allow group housing of instrumented animals. A broader application of these technologies offers opportunities for refinement and reduction within toxicity testing.

4.3. Humane endpoints

Humane endpoints provide an animal welfare safeguard in the conduct of research and should contribute to the achievement of sound and reliable scientific results. There are no agreed humane endpoints specific to the use of minipigs in toxicology studies, but there is ample literature dealing with humane endpoints in general, including guidance documents from organizations such as the OECD (2000). A plethora of documents describe how individual research institutions manage and supervise humane endpoints in experiments at their individual facilities (e.g. University of Illinois, 2007; University of Virginia, 2007). While these documents deal predominantly with rodents (rats and mice) the general considerations are applicable to minipigs, and species-specific approaches may be adapted to the specificities of minipigs. Recognition and scoring of animal pain, distress and discomfort are fundamental for the development and implementation of humane endpoints (Morton, 1990; Morton & Griffiths, 1985).

Understanding normal porcine behaviour and social behaviour is essential in order to make an accurate clinical evaluation of a minipig. While minipigs have been bred specifically for biomedical research their behaviour is still rooted in the wild pig (rooting and chewing behaviour, curiosity and exploration) and the social behaviour of the wild pig (herd behaviour, dominance and territoriality).

Minipigs are fond of food to such an extent that this can reasonably form the basis of a humane endpoint. For example if minipigs do not eat for 24–36 h, remedial measures should immediately be taken to permit recovery of the animal. Body weight loss (muscle/fat mass) over a defined period of time (e.g. >10% or 10–20% over a fixed number of days) may also be considered as the basis for a minipig humane endpoint. Further signs that something is not right with the minipig include (but are not limited to):

- locomotory or obvious signs of discomfort
- not wagging the tail or the tail is held between the legs (look for tail bite)
- the animal is crouched in a corner (and does not react to people entering)
- ears and head are hanging
- lacks normal curiosity
- displays a change in the normal vocalization pattern upon touch.

Clinical signs and behaviour which may indicate pain in minipigs are listed below. The different signs are not to be seen as an indication of severity of pain but as a list of possible clinical presentations of suffering. The response of individual animals to pain varies significantly and hence familiarisation and experience with individual minipigs are important when assessing pain. Consideration of these clinical signs could form the basis of further minipig specific humane endpoints.

4.4. Behavioural signs of pain in the minipig

4.4.1. Social

- Separation from the social group.
- Lack of interest in the immediate surroundings.

4.4.2. Human interaction

- Avoidance of being touched.
- Excessive/panicked flight reflex.
- Increased vocalization when touched.
- Aggressive behaviour towards humans.

4.4.3. Posture

- Limbs held tightly under the body, hunched back.
- Head held low (below shoulder height).
- Hanging ears.
- Sitting like a dog (minimises pressure on abdominal muscles after laparotomy).
- Ventral recumbency (with legs pointing forward).
- Lateral recumbency with legs under the body (indication of colic).

4.4.4. Activity

- Decreased activity.
- Slow/lethargic movement.
- Restlessness.
- Resistance toward any movement.
- Remaining lying down despite human presence in the pen (can be normal).
- Has to be chased to stand up.
- Total apathy.

4.4.5. Eating/drinking

- Decreased or absent appetite.
- No appetite; nor drinking.

4.4.6. Other physiological changes

- Increased heart rate (tachycardia).
- Changed respiratory pattern (fast superficial respiration, tachypnea).
- Weight loss (emaciation due to loss of muscle mass and/or fat).
- Grinding teeth (bruxism).
- Scruffy hair.

4.5. Collection of blood samples and other biospecimens

4.5.1. Blood samples

The collection of blood samples is required for the measurement of haematology and biochemistry parameters (Fig. 1). Ear veins of minipigs are small and in most cases they are not suitable for blood sampling. Sedation or anaesthesia is most often necessary if the ear veins are to be used for blood sampling and it is not possible to withdraw large amounts of blood. The cranial vena cava is feasible for blood sampling, also in non-sedated minipigs. It is possible to take out large samples of blood. The technique is blind and training of staff is necessary, but otherwise the technique does not present any specific problems.

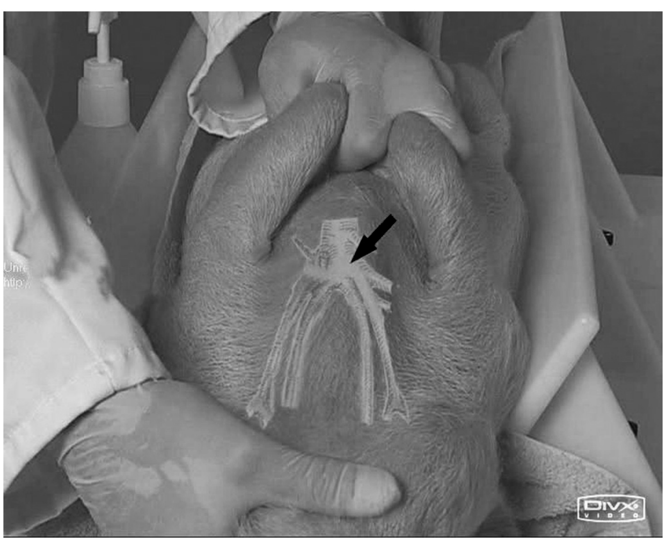

Fig. 1. Anatomic areas from where to bleed minipigs. The throat region with vena cava and jugular veins are visualized.

Manual restraint, restraint chairs or slings are required for successful sampling. Technical staff should not be under time pressure when sampling since this appears to affect the pig's response to handling and procedures. Restraint stress can further be reduced significantly by habituation of the animal to the procedure. Skilled technical staff is a prerequisite to reduce the risk of haemorrhages or other injuries. For frequent and/or repeated sampling, temporary vascular catheters, permanent (surgical) catheters and vascular access ports (VAP) are available. Automated sampling systems have been developed and successfully applied in pigs. However, application of devices for the stress free collection of blood samples requires anaesthesia and surgery. The number of blood samples to be collected, the frequency of sampling and sample volume determine the optimal sampling procedure to be used.

4.5.2. Urine and faeces

Urine and faeces samples can be collected in the home pen of minipigs. Restraint is required for the collection of uncontaminated urine. In toxicokinetic studies pigs may be housed in metabolic cages. The single housing and confined environment affect the welfare of the animals however. Habituation to the metabolic cage and positive human–animal interactions are likely to reduce the stress of single housing and confinement.

4.5.3. Cerebrospinal fluid (CSF) and bone marrow

In rare cases cerebrospinal fluid or bone marrow samples are collected during the in-life phase of a toxicity study. This is usually done under anaesthesia (Swindle, 2007).

4.5.4. Saliva

Saliva is easily obtained from the minipig and may be a useful matrix for the determination of drug exposure and biochemical health monitoring parameters. It has been little exploited to date.

4.6. Anaesthesia and euthanasia

Protocols exist for the pre- and post operative care and anaesthesia of pigs and minipigs. Usually pigs are sedated via intramuscular injection. General anaesthesia can be induced using a face mask with isoflurane in oxygen followed by endotracheal intubation. Minipigs can be euthanized by means of an intravenous injection with a lethal dose of barbiturate. Other euthanasia protocols are sometimes used (Olsen, 2010).

4.7. In summary

All usual routes of substance administration in the practice of toxicity testing and routine clinical examinations can be applied in the minipig, although there are some practical challenges. Habituation of the animals to the procedures and training of the technical staff can minimise the stress of the restrained animals that are subjected to dosing. A further technical development and broader application of telemetry equipment can contribute to refinement and reduction within toxicity testing. The peripheral blood vessels of the minipig are deeply located in the surrounding tissues. The technique is blind and training of staff is necessary, but otherwise the technique does not present any specific problems.

Temporary and chronic vascular catheters can be used for frequent sampling, and are likely to improve the welfare of the animals. Skilled staff and habituating of the animals to procedures for the collection of blood samples and other biospecimens are prerequisites for a minimal challenge of the animals. It is recommended to (further) develop protocols for the handling and training of minipigs subjected to procedures used in toxicity testing as well as training procedures for the technical staff. A DVD to assist with training is available (Ellegaard Göttingen Minipigs, 2007).

5. Impact on animal welfare of use of minipig, dog, marmoset or macaque in toxicology studies

This table presents comparative features of four animal species used in toxicology studies, with emphasis on the potential impact of this use on their welfare.

	Minipig	Dog	Marmoset	Macaque
Basic data				
Species/breed	Göttingen minipig* (Pig, *Sus scrofa*) *Other breeds include Minnesota, Hanford, Sinclair, Yucatan and Micro-Yucatan. In this table, data from the same breed is used to permit readily comparison.	Beagle* (Domestic dog, *Canis familiaris*) *Other purpose-bred breeds are available and used in smaller numbers. In this table, data from the same breed is used to permit readily comparison.	Common marmoset (*Callithrix jacchus*)	Cynomolgus monkey/Long-tailed macaque (*Macaca fascicularis*) or Rhesus macaque (*Macaca mulatta*).* *In this table, data from the same species is used to permit readily comparison.
Natural life span (years)	15+	Up to 15	10–16	Cynomolgus: 15–25 Rhesus: 20–30
Adult body weight (kg)	35 (at 24 months of age with restricted feeding)	Males: 20–25 (at 10–16 months of age) Females: 24–28 (at 12–16 months of age)	0.35–0.5 (at 18–24 months of age)	Cynomolgus males: 4.5–8.0 (at age >48 months) Cynomolgus females: 3.5–5.5 (at age >48 months) Rhesus males: 6–11 (at age 48–60 months) Rhesus females: 4–9 (at age 36–60 months)
Birth weight (g)	450 g (300 to 550 g). Neonates can be handled easily. For juvenile studies they can be used from day two after birth.	250 g. Neonates can be handled easily.	25–35 g. Neonates carried by family members and therefore not easy handled.	Cynomolgus: 330–350 Rhesus: 400–550. Neonates carried by mother and therefore not easy handled.
Growth	Linear/steady rapid growth on a restricted feeding regimen; <12 months of age: gain 2 kg per month; >12 months: gain 1 kg per month; >24 months: Gain fat only if fed unrestricted.	Adult weight reached at 10–12 months of age.	Maximum body weight reached at c. 20 months of age.	Both sexes show an adolescent growth spurt, which is more pronounced in males, causing a peak in weight gain between 30 and 48 months.
Sexual maturity (months)	3–4 months (males), 4–5 (females); age to breed 5–9. Quick to mature.	7–8 months (males), 8–14 (females); age to breed 12–24.	16 months (males), 14 (females); age to breed 18–24.	Cynomolgus: 36–48 months; age to breed 48–60. Rhesus: 36–48 (males), 24–36. (females); age to breed 48–60 (males), 36–48 (females). Very slow to mature.
Age (months)/weight (kg) when first used for toxicology studies (varies between facilities/i nstitutes — some have a policy to use mature animals)	c. 3–4/7–8 (males); 4–5/9–11 (females), thus animals are sexually mature for general and reproductive toxicology studies	c. 5–10/6–12, thus animals may not be mature for long-term studies, which can cause problems with interpretation of results (eg testicular toxicity)	c. 14–24 months/0.3–0.4, thus animals are mature for most studies	c. 18–24 months/2.5–4, thus animals are immature for all studies, which can complicate interpretation of results (e.g. testicular toxicity). Prolonged lead-time; animals must be maintained in captivity for a long time before use
Breeding and rearing				
Rate of reproduction	Polyoestrus with short cycle every 19–21 days. Gestation period 114 days (+/−4 days) Synchronisation of oestrus is possible for scheduling of studies.	Monoestrous (22 days) with interval of 7–8 months. Gestation period 59–67 days.	Polyoestrous with cycles every 14–28 days. Gestation period 140–148 days. Inter-birth interval 154–178 days.	Cynomolgus: Polyoestrus with cycle every 31±1 days. Gestation period 153–179 days. Rhesus: Polyoestrus with cycle every 28 days. Gestation period 146–180 days. Rhesus breeding is seasonal (summer months).
Litter size	Large — average of 5 offspring for primiparous females and 6.5 for multiparous females. Able to form reasonably sized groups of homogenous weight from large litters. Related animals available for forming stable social groups. Can cross-foster relatively easily for juvenile toxicology studies	Large — 5–7 offspring. Able to form reasonably sized groups of homogenous weight from large litters. Also related animals available for forming stable social groups. Possible to cross-foster for juvenile toxicology studies.	Small — multiple births, most frequently (dizygotic) twins. Usually unable to raise triplets or quadruplets without supplementary feeding of infants.	Very small — usually singletons. Plus higher rate of spontaneous abortion in captivity than for other second species.
Litters per year	2.1–2.3	1–2	1–2	1
Average offspring per	6.5 offspring × 2.2 litters per	6 offspring × 1.5 litters per	2 offspring × 1.5 litters per	1 offspring × 1 litter per year =

	Minipig	Dog	Marmoset	Macaque
breeding female per year	year = >14 offspring. Short oestrus and greater fecundity means fewer breeding animals are required than for other species. Also more information can be obtained more quickly in reproductive toxicology studies than for other second species	year = 9 offspring. Greater fecundity means fewer breeding animals are required than for non-human primates. Also more information can be obtained more quickly in reproductive toxicology studies than for non-human primates.	year = 3 offspring.	1 offspring.
Breeding system	Harem groups. Sows can be kept in groups until 1 week before farrowing and then moved to a farrowing pen where the sow can move freely with fender protection of the piglets.	Harem groups or observed mating (i.e. male and female housed together only during oestrus cycle).	Breeding pairs in family groups.	Generally harem groups.
Male:female ratio	1:10 (although a pool of breeding males may be maintained for genetic management).	1:4–12 (although a pool of breeding males may be maintained for genetic management).	1:1	1:4 to 1:12.
Weaning age for research use (weeks)	4 weeks; food is introduced from 10 to 14 days. Short lead-in time for juvenile toxicology studies	4–6 weeks. Short lead-in time for juvenile toxicology studies.	24–32 weeks; will suckle for longer and remain dependent on family members for psychological support for some time afterwards.	24–56 weeks; will suckle for longer and remain dependent on mothers for psychological and social support for some time afterwards. Prolonged period of development prior to adulthood means early weaning (e.g. 24–32 weeks) is likely to be detrimental to animal welfare
Health status	Göttingen minipigs are supplied meeting the “Microbiologically defined status” standard.	Conventional.	Conventional.	Conventional. Not all colonies are Herpes B virus free; Herpes B virus is a serious health threat to animal handling staff
Acquisition and supply				
Source of animals	Good availability and localised supply; EU demand met from breeders within EU (Denmark and Germany); also bred in USA. The use of AAALAC accredited suppliers is recommended.	Good availability and localised supply; EU demand met from breeders within EU, with some imports from USA. The use of AAALAC accredited suppliers is recommended.	Fair availability and localised supply; EU demand can generally be met from breeders within EU, with some imports from USA. The use of AAALAC accredited suppliers is recommended.Brazil and other South.American countries have banned the export of wild caught marmosets.	Fair availability; EU (and World) demand exceeds supply. Imported from third countries, such as Mauritius, China and Vietnam. Breeder's standards of animal welfare may be variable and the 2use of AAALAC accredited suppliers is recommended.
Filial generation	Purpose-bred in closed colonies, >F30 (partly inbred, but not more than conventional pigs)	Purpose-bred in closed colonies for many years, >F2	Purpose-bred in closed colonies for many years, >F2	Most breeding stock at suppliers consists of wild caught animals, and many supplied animals are therefore F1.
Quarantine and shipping restrictions	Quarantine required for imports from outside EU, plus Finland. No CITES permit is required (Convention on International Trade in Endangered Species).	Quarantine required for imports from outside EU. No CITES permit required	Quarantine required for imports from outside EU. CITES permit is required for shipping of marmosets or samples/materials derived from them.	Quarantine required for imports from outside EU. CITES permit is required for shipping of macaque monkeys or samples/materials derived from the monkeys.
Risk to supply/transportation interrupted	Potential risk in practice low.	Potential risk in practice low.	Potential risk in practice low.	Potential risk in practice low.
Transport				
Journey duration	EU travel achieved within 24 h for most countries. EU legislation on the protection of animals during transport imposes a maximum journey time of 24 h for pigs in high standard vehicles.	EU travel achieved within 24 h for most countries.	EU travel achieved within 24 h for most countries.	Import from third world countries means long, complex journeys, with loading/unloading. International journeys commonly 30–70 h therefore minimising journey duration is very important.
Response to transport	Transported most often in compatible pairs; social	May not willingly accept confinement; habituation to transport container and short	Do not willingly accept confinement; habituation to transport container and short	Do not willingly accept confinement; habituation to transport container and short

(continued on next page)

	Minipig	Dog	Marmoset	Macaque
	isolation may be stressful. More prone to heat stress than other second species; monitor temperature and ventilation within the cargo area. Can lose 4–8% of body weight during long transport (as result of stress and reduced food intake) but regain weight within 3 days. Figures on mortality during transport for farm pigs are not applicable to minipigs. For Gottingen Minipigs 0.01% mortality during 15 years.	confinement may be beneficial. Usually transported singly; social isolation may be stressful. Prone to motion sickness; do not feed within 4 h of the start of a journey. Can be lead-walked for on-site transport, if appropriately trained.	periods of confinement may be beneficial. Social separation is stressful; transport as family group or compatible pairs.	periods of confinement may be beneficial. Social separation is stressful; transport juveniles (and adults if possible) as compatible pairs.
Response to relocation/acclimatisation	Generally 7–10 days (range 4–28) is allowed for acclimatisation following transport. Physiological values return to baseline in farm pigs rapidly (e.g. after 2 days) (Brown, Knowles, Edwards & Warriss, 1999; Warris et al., 1992).	Generally 7–10 days is allowed for acclimatisation following transport involving a journey in a vehicle and between sites (not based on published data).	7–28 days is allowed for acclimatisation following transport. Physiological and behavioural changes can persist for >56 days post-relocation (Schaffner & Smith, 2005).	7–28 days is allowed for acclimatisation following transport. Behavioural changes reflecting stress can persist for >28 days post-relocation (Honess, Johnson & Wolfensohn, 2004).
Grouping				
Social organization	Under unrestricted conditions, small groups of related females and their offspring, plus solitary boars; sometimes all-male groups.	Feral dogs form loose and changeable social groups of males and females, influenced by food supply.	Family groups of 3 to 15 individuals; mainly a breeding pair and their offspring. Groups are stable with little immigration. Usually monogamous but flexible social system. Co-operative breeding with juveniles helping to raise younger siblings.	10–50 animals in a group; sometimes over 100. Composed of matrilines (female kin groups) and immigrant males. Strict dominance hierarchies in both sexes (organized around matrilines in females).
Typical grouping in the laboratory	Weaned into same sex stock groups of c. 15–20 animals. Experimental animals housed in groups of 2–6; i.e. smaller than for natural groups of pigs.	Weaned into same sex stock groups of c. 10–25 animals. Experimental animals housed in groups of 2–10.	Weaned into same sex stock groups of up to 100 animals (unknown welfare implications). Experimental animals housed in same sex or mixed-sex pairs; i.e. smaller than for natural groups of marmosets.	Weaned into same sex stock groups of up to 50 animals. Experimental animals housed in pairs or groups of 3–12; i.e. smaller than for natural groups of macaques.
Social needs in housing and response to grouping	Unfamiliar pigs may fight until a dominance hierarchy is established, especially if similar in size/weight. Fighting is less in a neutral and enriched environment and in large pens. Females are easier to keep in groups than males. Young boars may show a high degree of mounting behaviour when kept in groups; older boars (>1 year) may fight fiercely	Unfamiliar dogs may fight until a dominance hierarchy is established, especially if similar in size/weight.	Removal from close-knit family group is stressful. Reproductive adults are hostile to same sex intruders. Mixed-sex pairs (with sterilisation) are more stable than single-sex pairs; siblings may be more compatible than non-siblings.	Adapt more readily than marmosets to being paired or grouped, but may fight until dominance hierarchy is established. Fighting may result in mortality. Singly housed animals show a poor behavioural repertoire with abnormal, stereotypic and/or self-harming behaviours and do not have well-developed coping skills.
Frequency of aggression	Linear dominance hierarchy predominates. Serious incidents are rare in stable groups in well-managed environments. Usually re-establish the hierarchy after overt aggression within 2–5 days. Since minipigs mature early, sexual aggression between mature males can be an animal welfare problem (especially for the lowest ranking pig).	Aggressive incidents leading to physical injury are rare in stable groups in well-managed environments, but aggression can occur around times of arousal, e.g. cleaning, feeding, grouping, oestrus. Good management strategies are needed to minimise aggression.	Low in family groups, but highly territorial and aggressive to intruders. Generally low in compatible pairs, although pairs can breakdown, often leading to single housing, which is stressful.	Aggression quite common, especially after separation or regrouping, leading to changes in the dominance hierarchy. Good management strategies are needed to minimise aggression.
Feeding				
Diet	Omnivorous.	Carnivorous, but can eat an omnivorous diet.	Omnivorous and varied diet.	Omnivorous and varied diet.
Regimen	Prone to obesity on conventional pig diets;	Restricted feeding may necessary in some cases to prevent obesity	Generally eat to meet their energy requirements. Varied diet	Generally eat to meet their energy requirements, but

	Minipig	Dog	Marmoset	Macaque
	minipig formulated diet (= reduced calories [10 MJ/kg] and high [13–14%] fibre). Restricted feeding necessary to prevent obesity and results in a more homogeneous population; males get 10–20% more than females (Bollen, 2001; Bollen & Skydsgaard, 2006) to avoid atrophy; typically fed to 50–70% appetite. Older animals (>7 months) animals are fed only once a day to satisfy the appetite.	(Prescott & Jennings, 2004)	and opportunity to forage important for psychological well-being (Joint Working Group on Refinement, 2009)	restricted feeding may be necessary in some cases to prevent obesity. Varied diet and opportunity to forage important for psychological well-being (Joint Working Group on Refinement, 2009).
Feeding competition	High where feed is restricted; separate temporarily for feeding or ensure an adequate trough space for all animals to feed simultaneously.	High; separate temporarily for feeding or ensure an adequate number of bowls for all animals to feed simultaneously.	Low within pairs and family groups.	High. Position in hierarchy may determine access to food. Scatter feed or ensure an adequate number of foraging stations.
Housing and husbandry				
Use of space	Terrestrial, inquisitive.	Terrestrial, inquisitive and active.	Arboreal (3 dimensional), inquisitive and highly active.	Arboreal (3 dimensional), inquisitive and highly active.
Habitat	Forest and field.	Forested areas or shrub lands in the vicinity of human habitation.	Rain forest.	Tropical, dry deciduous, mixed deciduous, bamboo and temperate forests and mangrove swamps.
Natural home range	Varies for wild boar depending on, e.g. food availability, sex, human disturbance; >400 ha in the UK.	0.1 to 60 ha, depending on food availability.	Small home ranges of 0.5 to 6 ha. Defend small territories (up to 2 ha) around gum-bearing trees.	0.01 to 400 ha, depending on food availability.
Typical laboratory housing	Indoor pens.	Indoor pens, usually with exercise areas and sometimes with outdoor runs. Cages are unsuitable for long-term housing.	Cages.	Cages or customised rooms.
Handling and restraint				
Tractability	Fair; selected for docility, but strong. Tractability is influenced by age and socialisation with humans during infancy.	Good; docile and amenable socialised to humans — better suited to studies requiring close monitoring and frequent sampling than other second species.	Poor; highly agile and active.	Poor; highly agile, active and strong. Risk of injury to handler from aggressive adult animals.
Socialisation with humans	Socialisation with humans is important. A socialisation scheme is presented in Section 3 of this article.	Essential to socialise dogs with humans during a critical period of 3–8 weeks of age, otherwise they may be unapproachable by 14 weeks (Freedman, King, & Elliot, 1961; Prescott et al., 2004). Socialisation schemes exist; intensive socialisation and training reduces behavioural variability (Boxall, Heath, Bate, & Brautigam, 2004; Vanderlip, Vanderlip, & Myles, 1985).	Socialisation with humans is beneficial; it can enhance animal well-being and facilitate health monitoring. Formal socialisation programmes are rare (Prescott & Buchanan-Smith, 2007; Rennie & Buchanan-Smith, 2006)	Socialisation with humans is beneficial; it can enhance animal well-being, reduce abnormal behaviour, facilitate health monitoring, and improve staff safety and morale. Formal socialisation programmes are rare (Prescott & Buchanan-Smith, 2007; Rennie & Buchanan-Smith, 2006).
Domestication, and amenability to human contact	Domesticated species. Moderately amenable to human contact, depending on management system.	Domesticated species; special relationship with man. Highly amenable to human contact. Interact with humans naturally, which facilitates close monitoring, (e.g. post-dose observations and clinical effects). Can be comforted by familiar caregivers during procedures, e.g. blood sampling.	Non-domesticated species. Moderately amenable to human contact, depending on management system.	Non-domesticated species. Moderately amenable to human contact, depending on management system.
Response to handling/restraint	Do not like being caught, handled or restrained	Potentially stressful, especially without training. Breed	Prey species; do not like being caught, handled or restrained;	Repeated capture and physical restraint in the animal house can

(continued on next page)

	Minipig	Dog	Marmoset	Macaque
	(usually struggle and vocalize which can be stressful for staff). 10 fold increase in catecholamines in response to restraint relative to unrestrained baseline. Restraint stress can be avoided, e.g. via handling courses, clicker training and telemetry.	differences exist.	this can be avoided via training or telemetry	be stressful; this can be reduced or avoided via training or telemetry.
Human health and safety	Low risk of zoonoses.	Low risk of zoonoses (technicians should be vaccinated against rabies). Bite wounds can be severe.	Low risk of zoonoses.	Low risk of zoonoses but bite and scratch wounds can be severe. Exclude the use of Herpes B positive monkeys. Staff should be vaccinated against Hepatitis B.
Training				
Techniques used	Positive reinforcement is recommended; motivated by food (can bite if taking food from the hand). Use of clicker (as secondary conditioning) is recommended to avoid handling and over-feeding of treats.	Positive reinforcement is recommended; motivated by food, verbal praise and human contact.	Positive reinforcement is recommended; motivated by preferred food. Use of clicker (as secondary conditioning) is recommended to avoid handling.	Positive reinforcement is recommended; motivated by preferred food. Use of clicker (as secondary conditioning) is recommended to avoid handling and over-feeding of treats.
Availability of protocols for training of animals to co-operate with procedures	Low, but have been trained, e.g. to enter a transport crate on demand for relocation, weighing and restraint.	Low, but have been trained, e.g. lead walking, calm behaviour on table, co-operation with minor procedures. Behaviour scoring systems are increasingly used to evaluate progress with socialisation and training and to select suitable dogs for studies.	Low, but have been trained, e.g. to enter a transport crate on demand for relocation and weighing, for urine collection.	High for some procedures, e.g. 10 articles deal with co-operation with venepuncture.
Scientific procedures				
Experienced animal care staff	May be difficult to find. Training is needed.	Staff commonly more familiar with dog behaviours than behaviours of other second species, due to their status as companion animals.	May be difficult to find. Training is needed.	May be difficult to find. Training is needed.
Blood sampling	Ear veins of minipigs are small and in most cases they are not suitable for blood sampling. Sedation or anaesthesia is most often necessary if the ear veins are to be used for blood sampling and it is not possible to withdraw large amounts of blood. The cranial vena cava is feasible for blood sampling, also in non-sedated minipigs. It is possible to take out large samples of blood. The technique is blind and training of staff is necessary, but otherwise the technique does not present any specific problems. Since minipigs have relatively few superficial accessible veins in comparison with dogs, vascular access ports or chronic catheterisation are used, particularly for repeated sampling. This requires surgery and anaesthesia (with attendant risks) and recovery period. Seldinger technique using an Arrow catheter requires anaesthesia but no surgery, with recovery time of only one day.	Blood readily obtained; jugular or cephalic veins are used.	Training and manual dexterity required; femoral vein is the best method. Coccygeal vein can also be used. Marmosets are too small to allow a large series of samples to be taken from the same individual, in which case sequential samples are taken from different groups of animals. Thus, their small size may influence the number of animals required for some studies. Where haematology and clinical chemistry samples are also required, blood volume, in addition to the number of samples, becomes a problem, leading to use of additional animals.	Femoral vein is the best method. Cephalic or jugular veins can also be used. Microcapillary tube method can be used to collect small samples from the heel without anaesthetic. Large size makes it easier for staff to take blood from macaques than from marmosets; though macaque blood vessels are more difficult to access than those of dogs. Can be trained to present a limb for blood sampling.

	Minipig	Dog	Marmoset	Macaque
Welfare assessment				
Validated welfare indicators/assessment schemes	Vocalizations, position of the eyes, body posture, movement of the tail. No schemes for miniature swine but DEFRA pig-specific body condition scoring scheme for production pigs is applicable (DEFRA, 1998).	Schemes are widely available for measuring pain in clinical settings and welfare in kennels.	Lack of published schemes.	Several schemes are available, e.g. abnormal behaviour, body condition, alopecia.
Stereotypic/abnormal behaviours	E.g. ear/tail biting, flank sucking, bar biting in inadequate, barren environments. Stereotyped oral behaviours can occur due to increased foraging motivation on restricted feeding regimes; modify diets to enhance gut fill and satiety (e.g. increased dietary fibre plus forage such as straw).	Occur in small or barren environments, including laboratory kennels.	–	Associated with single housing, impoverished environments and nursery rearing.

6. Conclusions and recommendations

In this article, as a contribution to the *RETHINK* project, we have reviewed animal welfare indicators for the minipig, animal welfare considerations in minipig husbandry, welfare issues arising from the use of minipigs in biomedical research, and welfare issues (in comparison with other commonly used species) relating to the use of minipigs in regulatory toxicology studies. This review allows us to draw some general conclusions. Animal welfare is concerned with the fulfilment of animal needs. As regards minipigs, what can be said about the definition of those needs? Minipigs have been selectively bred for small size, pale skin and docility in order to make them ideal animal models for laboratory biomedical research. Relatively little scientific data exists addressing the extent to which selective breeding may have modified needs in the minipig, but it is expected that the motivations and consequent behavioural needs of the minipig have remained unchanged or little changed from those of their wild ancestors. Specific studies on the refinement of minipig housing and procedures are very limited in number. Studies on the welfare needs of production pigs probably represent a useful source of this guidance, and this article makes numerous references to the literature on production pigs but there is an element of uncertainty in any extrapolation from production (farm) pig studies.

Regardless of the definition of the needs of minipigs, can we assess welfare directly? It emerges from the available data that no single parameter is adequate to assess welfare state, and we support the concept of triangulation in which measures of welfare from different perspectives are integrated. To date, no physiological or behavioural indicators have been specifically validated in minipigs, but indicators that have been validated in farm pigs should be appropriate. However, since the extent to which behavioural and physiological coping strategies are adopted may differ between breeds, it will be important to characterize the stress responses of minipigs and to assess both types of measure in any welfare study.

In terms of housing, it is recommended that all minipigs, with the exception of mature boars, are kept in groups when housed for biomedical research purposes. How to group minipigs and the optimal group size under specific conditions has not been specifically investigated and should be the subject of further research (see below). More research is needed to identify if single housing with close and positive social contact with other animals and technical staff is better than group housing where a strong hierarchy may bring detrimental consequences.

During the use of minipigs in toxicology studies, handling of the minipigs is necessary. In order to minimise the stress associated with handling (and consequent impact on experimental variability), we consider that positively-reinforced socialisation to human contact and training of the minipig for procedures that will be performed are essential. It is also essential that animal handling staff have a high standard of training for working with minipigs. Minipig handling and dosing programmes should be adopted in all laboratories working with these animals.

Various schemes and protocols are available for the recording and scoring of clinical condition and clinical signs or symptoms in minipigs. The development of a body condition scoring protocol specifically for minipigs is needed because of their different conformation and adiposity in comparison with farm pigs. All commonly used routes of administration of test items in the performance of toxicity testing can be quite readily applied in the minipig. Routine clinical examinations can also be performed readily in minipigs. Further technical development and broader application of telemetry equipment can contribute to refinement and reduction within toxicity testing. The peripheral blood vessels of the minipig are deeply located in the surrounding tissues. Therefore the collection of blood samples from the ear vein or other veins is demanding, both for the animal and the technician. Temporary and chronic vascular catheters can be used for frequent sampling, and are likely to improve the welfare of the animals.

On the basis of the various considerations outlined above, we consider that it would be an important contribution to the welfare of minipigs to prepare and disseminate a “Code of Practice” for the optimal housing and management of minipigs whilst being used in research. It is recommended to further develop detailed protocols for the handling and training of minipigs subjected to procedures used in toxicity testing as well as training procedures for the technical staff.

In conclusion, from an animal welfare perspective welfare needs appear to be addressed and there are no areas where significant restrictions or limitations to the use of minipigs in toxicity testing are required. The potential (welfare) advantages and disadvantages of selection of the minipig as the toxicology model will be dependent on the specific study type and study requirements. Opportunities exist for refinement and reduction. We consider that the welfare of minipigs before and during use in toxicology studies will be strongly dependent on the skills, knowledge and empathy of the handling staff.

6.1. Areas for research

There are numerous issues where our understanding of minipig welfare, and our ability to optimise welfare, would be enhanced by focussed research. This research falls into different areas.

6.2. Identification of welfare needs

- Research is needed to identify the extent to which selective breeding may have modified needs in the minipig, in comparison with other porcine breeds and strains, and how these basic needs translate into welfare needs.

6.3. Assessment of welfare

- There are no welfare assessment schemes for minipigs. Monitoring schemes for pork production pigs exist such as the Animal Needs Index used in Austria and Germany (Bartussek, 1999) and the decision-support system in the Netherlands (Bracke, Metz, Dijkhuizen, et al., 2001). These assessment schemes focus on housing parameters and on selected animal observations and are not oriented to the specifics of laboratory housing and biomedical experimentation. Research is needed to develop and validate simple welfare assessment tools for minipigs which can be used under practical conditions. The triangulation test of stress and welfare, described by Webster (1994), provides a useful principle on which an overall assessment tool could be based.
- Development of blood biochemistry assays (for welfare indicators such as hormone levels) based on saliva samples rather than blood samples would be of general interest for animal welfare. In the minipig this issue is of special relevance, in view of the difficulties (real or imagined) in obtaining blood samples. Collection of saliva (or swabs) rather than blood would place less physiological and interventional stress on experimental animals. Saliva sampling could also be achieved in settings where trained laboratory technicians may not be available for blood sample collection (e.g. during transport), permitting a broader vision of welfare during the animal's life-cycle. The saliva technology could subsequently contribute to non-invasive sampling during toxicology studies or other experimental work (for example in drug level determinations).

6.4. Minipig specific husbandry

There are numerous issues surrounding social needs and housing

- How to group minipigs and the optimal group size under specific conditions has not been the subject of focussed research, although some work has been performed on the impact of stocking density on the behaviour of group housed minipigs (Krohn & Ellegaard, 2000). Research is needed to define the optimal group size for minipigs housed in groups. This research should take account of the different social needs of immature and mature male and female minipigs. Appropriate methods should be developed to ensure socialisation to human contact for individual minipigs within these groups.
- Single (individual) housing has traditionally meant social isolation for minipigs since little provision was made in housing units for tactile or visual contact between animals. Research is needed to establish the benefits of providing singly housed minipigs with visual, olfactory and tactile contact with other minipigs. This research should be extended to evaluate whether singly housed minipigs, with a strong and positive socialisation with animal handlers and with visual, tactile and olfactory contact to other pigs, enjoy a greater (or lower) level of welfare (or reduced stress) in comparison with minipigs kept in different configurations of group housing.
- Research is needed to assess the amount and type of bedding/enrichment which is necessary for minipigs, particularly under the constraints of barrier rearing.
- More research is needed to define the welfare consequences of minipig transport. Loading and unloading is probably the most stressful stage of transport. In farm pigs physiological values of stress indicators have been known to return to baseline very rapidly (within 2 days). Relevant data is not available to evaluate the stress involved in minipig transport (and the measures required to minimise any impact on the quality and reliability of toxicology and biomedical research subsequently undertaken with these animals).

6.5. Use of minipigs in toxicology

Further research needs relating to the use of minipigs in toxicology are detailed in Bode et al. (2010-this issue).

- Individual housing of minipigs is necessary for some kinds of safety studies, in order to avoid interference by pen mates. Examples include continuous infusion studies (in order to protect catheterised animals) and many kinds of cutaneous administration (in order to protect administration sites and materials). In order to meet the welfare needs of these individually housed animals, better understanding of the welfare impact of single vs. group housing is required to cover these situations.
- The unsatisfactory use of cuff approaches in measuring blood pressure leads to the use of invasive approaches for the determination of this parameter. The development of non-invasive or minimally invasive approaches would be welcomed.
- The development of a panel of simple neurological tests in the minipig is required. This panel should contribute to the definition of neurological and behavioural humane endpoints for the minipig.

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