

19 March 2010

Supplementary statement on toeclipping in rodents

1) Recent publications

After Norecopa published its position statement on toeclipping in December 2008, three scientific papers of relevance to this issue have been published:

1. Castelhana-Carlos MJ *et al.*, (2009): Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation
2. Schaefer DC *et al.*, (2009): Analysis of physiological and behavioural parameters in mice after toeclipping as newborns
3. Trygg J (2009): Evaluation of identification methods in mice (thesis at the Swedish Agricultural University, supervisor Elin Spangenberg)

Castelhana-Carlos *et al.* compared three methods of identification (toeclipping, tattooing and the use of transponders) on mice on day 5 after birth. They concluded that toeclipping was the least invasive of the three methods, and that toeclipping was possibly the recommended method when it is necessary to genotype very young animals.

Schaefer *et al.* studied the effects of toeclipping at days 3 and 7 after birth, but did not compare this to other methods. They concluded that amputation should be performed on day 7 in order to take into account the development of the toes, and that the procedure when performed at that age did not lead to hyperalgesia or reduction in grip strength when the mice were 12 weeks old. The study concluded that the greatest stress factor for the animals was the handling, not the toe clipping itself.

Trygg compared three methods of identification in mice (toetip clipping on day 7/8, and earmarking (hole or tag) on day 19). On the animals that were given tags, 2mm of the tip of the tail was removed on day 14. There were no detectable differences in behaviour between the mice that were subjected to toe clipping or tail clipping. The toes that were clipped were swollen for 3 days after the procedure, but no similar reaction was seen following tail clipping. The author cites another study (Borgens, 1982, Science 217: 747-750) which pointed out that the toe tip has a tendency to grow out again if the cut is placed in the joint between the two distal phalangeal bones, and one should therefore make cut through the outermost bone but one. Trygg pointed out that it is difficult to place the cut exactly at the same place in mice at that age.

Anaesthesia or analgesia were not used in any of these studies.

2) Norecopa's evaluation of toetip clipping compared to tail clipping

Norecopa has considered the following issues when making its conclusion:

1. Previous studies (Hankenson *et al.*, 2008) have shown that there is cartilage in the outermost tip of the tail of the mouse. There is therefore reason to believe that there is little difference between toetip clipping and tail clipping as regards pain perception and the risk of chronic pain (phantom pains) after the procedures. The question is whether it is functionally worse for a mouse to lose a toetip (or several of these) than it is to lose the tip of the tail.
2. The tail is an important organ for balance and thermoregulation. Loss of significant lengths of the tail result undeniably in reduced function. There is, however, reason to believe that the loss of the tip of the tail (3-5mm) has little functional significance. The tip of the tail is not used when a mouse grasps objects while climbing, neither is it in continual contact with the cage floor. Toes, in comparison, are important for the animal's finer motor skills and grasping ability, and they are in continuous contact with the cage floor, which is probably more unpleasant if they experience chronic pain after amputation of cartilage.
3. Norecopa is only aware of one study where toe clipping and tail clipping have been compared directly (Trygg, 2009). This study reported swelling for 3 days after toeclipping, but no mention was made of similar swelling after tail clipping.
4. In those cases where toetip clipping is used as a combined marking and genotyping method, some mice in a litter will lose several toe tips, with correspondingly greater functional loss.
5. It is probably easier to standardise the site of the amputation and the length of the piece that is removed when performing tail clipping (by means of an immobilisation device and ruler), compared to toetip clipping in very young mice.

Norecopas conclusion

Norecopa is still of the opinion that the use of amputations should be very restrictive, in agreement with Norwegian law. Such procedures should be limited to those situations where there is a well documented requirement and/or welfare grounds for acquisition of sufficient DNA to be able to perform quantitative genotyping in rodents before weaning. They should never be used as marking methods alone.

Norecopa wishes to see:

1. more studies where toetip clipping and tail clipping are compared directly
2. studies where the use of anaesthesia and analgesia are evaluated
3. more detailed studies of methods for non-invasive genotyping and marking

In the light of this evaluation of toetip clipping compared to tail clipping, Norecopa maintains, for the time being, its earlier conclusion:

Norecopa's Board is therefore of the opinion that toetip clipping should not be permitted. In those cases where it is absolutely necessary to undertake quantitative genotyping, tail clipping should be used (3-5 mm performed only once per animal) under anaesthesia, and with post-operative analgesia as long as this does not in itself create a greater burden for the animal.

In cases where qualitative genotyping is adequate, less invasive methods should be employed, such as the collection of saliva, blood, faecal or hair samples, or (in larger animals) the material removed by ear punching.

Further studies should be performed to identify the optimal anaesthesia and analgesia for the various methods of identification and tissue sampling.

A minority of the Board (the representative for academia) has dissented and has the following comments:

All the studies presented here strengthen the arguments for toetip amputation as being the best method where marking is combined with biopsy. The two published studies come from research environments that are recognised for their work within animal welfare.

Perforation of the ear is the only alternative if a biopsy and identification method are to be combined in the same procedure. The third of the studies referred to above strengthens the arguments for this method being a greater burden on the animal, because handling itself imposes the greatest stress. This also supports the case for using just one method which combines both functions, rather than two separate methods.

Tail clipping has the advantage that it can be carried out at all ages (but is often limited to 28 days of age), and must be considered to have a low degree of invasiveness and stress. The disadvantage of tail clipping is that it must be combined with a marking method, which lengthens the handling time and increases the burden on the animals. Neither blood, saliva, faeces nor hair samples can be taken from 7 day-old animals, and the use of transponders is not possible. Tattooing is technically speaking more complicated and more stressful.

Anaesthesia for toeclipping is simple to perform, whereas the proximity to the ear makes it difficult to anaesthetise animals for earmarking. Perforation of the ear at 15-18 days of age is painful and does not give the same possibility for analgesia that toe clipping at 7 days of age has.

Reduced function has not been observed with toe clipping, whereas the effect of ear perforation on hearing and thermoregulation is probable but unknown.

Inaccurate marking and mistakes during reading are more common with ear marking than with toe clipping. Toe clipping is also the easiest procedure to perform from a technical point of view.

Early marking/biopsy increases survival rates in many experiments with inbred, mutant and genetically modified strains of mice, because litter size can be reduced. This also reduces the length of time under which the mice live under experimental conditions.

A central theme in animal welfare is the balance between the severity and painfulness of a procedure on the one hand, and the benefit of the procedure on the other. Toe clipping on one

hindtoe under anaesthesia is a refinement compared to today's methods for marking and biopsy techniques.

The studies mentioned above were performed because toe clipping meets with resistance from lay persons, but the technique has obvious advantages compared to other methods. Toe clipping satisfies the conditions mentioned in §9 of the Norwegian Animal Welfare Act, and it should be allowed in well-planned animal experiments. One of the above-mentioned studies concludes that choice of identification method should be the result of deliberation and tailored to the experiment, rather than be a decision based on traditional thinking.