ANIMAL PROCEDURES COMMITTEE

June 2009

Supplementary Review of Schedule 1 of the Animals (Scientific Procedures) Act 1986

Appropriate methods of humane killing for fish

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1. Background

In December 2006 the Animal Procedures Committee published its review of Schedule 1 of the Animals (Scientific Procedures) Act 1986: Appropriate methods of humane killing[1]. This report noted that there was a need for further consideration of fish euthanasia. Specifically, the Working Group was aware that further research is required into the humane killing of fish and the humane killing of embryonated eggs; and that these are areas of continuing debate and active ongoing research. The Housing and Husbandry subcommittee was asked by the APC to provide a supplementary report on the humane killing of fish.

To inform the sub-committee about the use of current techniques we sent out a questionnaire to 20 establishments. Four responses were received (see appendix) for which we were very grateful, but the sample was too small to provide a useful overview. The subcommittee also recruited a group of scientists involved in fish biology and welfare research to: assess the evidence relating to the techniques currently in use, identify where evidence is lacking and where further research is needed, and advise on other techniques that should be considered. These experts provided opinions on best practice when using current techniques that we have included in this report in the interests of promoting refinement.

As noted in the previous Schedule 1 report, both the method of killing and the handling techniques associated with the killing process should be humane. The previous report also pointed out that killing is not humane unless either the animal loses sensibility instantaneously, or very rapidly (over one or a few seconds), or where loss of consciousness takes longer but the technique does not result in poor welfare (in this context we take *poor welfare* to mean - pain, suffering or other strong unpleasant feelings during induction of unconsciousness).

1.1 Practical factors that affect the choice of humane killing technique

As in the previous report, when considering our recommendations, we took account of the handling techniques involved in each procedure as well as the actual killing method. We also considered other important factors including

[1] http://www.apc.gov.uk/reference/schedule-1-report.pdf

equipment, labour and other costs, aesthetic concerns[2] and the safety risk to personnel of using the method.

Due to the range of species, sizes and developmental stages of fish used for research, it is unlikely that a single method will be suitable for humane killing in all circumstances. Specific issues that need to be considered include the resistance of the species to anaesthetics or hypoxia (e.g. eels), and any anatomical adaptations that may impact on the process. Practical issues include the numbers of animals to be euthanased, which may be thousands of individuals in the case of some studies involving the use of larval or immature fish forms. There may be practical difficulties in the use of some methods where procedures are carried out at places other than a designated scientific establishment (e.g. field sites), as well as the need to consider potential risks to the environment arising from inadvertent release of chemical compounds. The choice of euthanasia technique may also be influenced by specific housing conditions. For example, the use of recirculation as opposed to flow through tank systems could restrict the application of some chemical killing methods. The requirement to confirm death may be problematic when killing large numbers of animals. Moreover, if large numbers of animals are stunned at the same time there is the possibility that some animals might recover before death has been confirmed. Experimental design is another factor, for instance in longitudinal studies subgroups of fish are taken from the same tank for euthanasia at different times so that killing *in situ* is not possible.

Finally, even for commonly used euthanasia techniques in fish, there is often only very limited evidence of their humaneness within the laboratory setting - hence the recommendation in the main APC report[1] that there is a need for further research. In drawing up the current document it became apparent, from the experts' evidence, that much of the recent research activity relating to humane killing of fish has been carried out with respect to fish farming. We considered this to be valuable information that could be used to inform this document. However, there are obvious differences between the sectors, which need to be taken into account. For example, some species of fish used in research are smaller than commonly farmed fish, and some methods such as overdose of anaesthesia would not be appropriate for animals destined to enter the food chain but are an option under the ASPA.

2. Assessment of current Schedule 1 techniques

Schedule 1 lists the following methods as suitable for fish:

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[2] We gave the welfare of the animal priority over the aesthetic concerns for the staff during our considerations. However, expecting people to use techniques that they are not comfortable with can have a negative impact on staff morale and the culture of care. This can affect people's ability to empathise with animals, which has consequences for animal welfare.

i. Concussion of the brain by striking the cranium with destruction of the brain before the return of consciousness.

ii. Overdose of an anaesthetic using a route and an anaesthetic agent appropriate for the size and species of animal.

Schedule 1 indicates that these methods are only appropriate subject to completion by methods that aim to ensure confirmation of death, specified in subparagraph 1 a. to f. of the Schedule.

2.1 Concussion

Concussion is a technique for killing fish that is commonly used in field studies but is not confined to this area of research. The major source of stress arising from this technique is likely to come from capture and handling (see section below on handling). As noted in paragraph 3.4 of the *Code of Practice for the Humane Killing of Animals under Schedule 1 to the Animals (Scientific Procedures) Act 1986*, physical methods of killing animals can be quick and humane if carried out competently and appropriate to the species and size of animal. It is worth noting here that some species may be difficult to kill by concussion (e.g. certain catfish which have bony plates protecting the brain, Those carrying out the technique should be trained, prepared to use a physical method and fully confident that they can perform the technique competently.

2.2 Anaesthetic overdose

Currently the most favoured method of euthanasia for fish is anaesthetic overdose using a suitable agent and route. Only MS222 is licensed as an anaesthetic agent for use with fish in the UK and European Union, however Schedule 1 does not limit the agents that can be used for killing. This flexible approach allows better and more effective agents to be adopted as scientific knowledge and drug development progress. The most common agents used for killing fish by anaesthetic overdose in the UK are benzocaine, benzocaine hydrochloride (ethyl aminobenzoate) and MS222 (tricaine methane sulphonate), although there are a number of other anaesthetic agents, such as quinaldine sulphate and 2-phenoxyethanol that may also be used.

Factors that need to be taken into account when choosing anaesthetic agents include speed of induction in the species of fish concerned, together with the likely aversiveness of the agent at the concentration used. The physical properties of the agent need to be fully considered (JWGR 2001), for example: the potential for irritancy, whether it will dissolve correctly in the temperature of water in which the fish are held and the need to buffer acidic compounds such as MS222. Where the agent has to be dissolved in a solvent other than water, then any impact of the solvent on the fish also needs to be taken into account. Disposal of the agent after use is also an important factor.

For humane killing, anaesthetic agents must be used correctly. A concentration of anaesthetic solution which induces anaesthesia quickly will help to decrease the time exposed to the anaesthetic solution, and the overall handling time; both of which lower the overall levels of stress (Fox *et al.* 1997). However, a balance needs to be struck between the time taken to induce anaesthesia and the irritancy of the agent. With some agents it is not easy to reliably achieve correct concentrations. For example, 2-phenoxyethanol is not readily miscible in water so that, over time, the concentration of the anaesthetic in the water can change giving unpredictable results. Sudden changes in water quality and temperature can generate stress responses in fish (Wedemeyer 1997). Therefore care should be taken to minimise any temperature difference between the anaesthetic solution and the tank water. It was also suggested to us that dim lighting or even a darkened tank (with a dark plastic cover) may help to calm fish during anaesthesia or euthanasia, but we are not aware of data to support this.

Anaesthetics do not take effect instantly and some agents may be aversive during induction. The previous Schedule 1 review drew attention to the aversive nature of gases used for killing rodents, but it appeared to us that there might be a shortage of research on the humaneness of anaesthetic agents commonly used for killing fish, and on the most humane protocols for using these agents. For example, Close *et al.* (1996) in their discussion on the use of MS222 for euthanasia refer to only one paper by Brown (1988), which describes appropriate buffering of the agent. However, neither Close *et al.* (1996), nor Brown (1988) refer to the aversiveness, or otherwise of MS222. This is, perhaps not surprising as the issue has only become topical relatively recently for other taxa. We, therefore, asked whether there was evidence for anaesthetic agents commonly used for killing fish regarding aversiveness; and if they were aversive, how much, and for how long? Additionally, we asked whether there was any evidence regarding differing reactions to these agents by fish species commonly used in research. No literature on the subject was reported, but our advisory experts told us of anecdotal accounts that anaesthetics may, at times, cause aversive reactions in fish. These include: aversive reactions in trout when they are introduced to water containing the agent at the full recommended dose; aversive reactions in salmon when the agent is gradually introduced to their water; and catfish attempting to leave the water once the anaesthetic has been introduced. However, it is not known whether these responses were to the anaesthetics themselves or to variations in the effective buffering of anaesthetic solutions, especially when more concentrated solutions are used for euthanasia.

2.3 Stress prior to killing

In addition to stress caused by the killing methods themselves, it is important to consider stress caused by procedures leading up to the killing method. Many studies have demonstrated that fish are stressed by capture and handling (Billard *et al.* 1981; Barton & Iwama, 1991; Pickering 1992; Wendelaar Bonga 1997), and that this results in a biochemical and physiological stress response, which occurs almost immediately and can be evident for a prolonged time afterwards (Mazeaud *et al*. 1977). Handling stress, although short term, can impact on feed intake and immunology (e.g. Pickering *et al.* 1982), thereby causing further metabolic consequences. Alterations to the social environment can also induce increased cortisol production as an indicator of stress responses (Fox *et al.* 1997).

Emersion stress (stress resulting from removal from water) is a common paradigm in published studies on fish stress and is known to cause an acute stress response, including that of the hypothalamic-pituitary-interrenal (HPI) axis and elevated circulating cortisol (e.g. Sloman *et al.* 2001; Lankford *et al.* 2006). Fish typically display a period of intense activity during air exposure, which has been termed a maximal emergency response, and it has been suggested that capture and handling may cause psychological stress such as fear (Schreck, 1981; Yue *et al* .2004) - although the capacity for fish to be aware of such emotions is still debated (Braithwaite & Boulcott 2007). Fish also experience oxygen debt associated with the intense exertion and the restriction on normal ventilation (e.g. Ferguson & Tufts 1992; Davis & Schreck 1997) and an acute endocrinological stress response (commonly known as handling stress) follows, presumably owing to the combination of psychological and respiratory stressors (see Portz *et al.* 2006 for a review).

There are, however, considerable differences between species in terms of their ability to cope with handling, with some showing more extreme reactions than others (Brydges *et al.* in press). Certain species are very sensitive to handling, for example, most species of salmonid are very stressed by handling and direct exposure to air, but other species appear to be less stressed (e.g. three spined sticklebacks, *Clarius* spp. of catfish).

2.3.1 Handling stress and experimental outcomes

Handling stress not only affects welfare but can also influence the outcome of experiments and trials. For example, Pottinger and Calder (1995) showed that the results of toxicological trials were affected by the degree of disturbance and handling experienced by the fish. Similarly, behavioural trials can also be compromised by stress; Pickering *et al.* (1982) found that brown trout *Salmo trutta* did not feed for 3 days after handling. Devising handling techniques that minimize distress would therefore help to improve the quality of data collected in experimental research.

However, as noted above, species vary in their responses to handling, and, as yet, there is no formal, standardised comparison of the effects of routine handling procedures across fish species. Age and size can also influence the ease with which fish are caught within a tank. For small fish, capture and handling is usually much easier than large fish over 500g which can have faster swimming speed and be difficult to handle due to their large size and strength. The longer it takes to catch the fish the more stressed they can become owing to prolonged chasing (Fox *et al.* 1997).

2.3.2 Stress from netting

Most laboratory and ornamental fish are still routinely caught using hand-nets. The fish are usually removed from the water and briefly held in the air. Netting affects both fish behaviour and physiology. Physical abrasion from the net can disrupt the mucous coating and scales, potentially increasing susceptibility to pathogens (FSBI 2002; Conte 2004). Additionally, impact with the net, conspecifics or other surfaces can cause physical damage to protruding structures such as the fins and eyes (Barthel *et al*. 2003). Prolonged chasing with a net causes stress, not only to the pursued fish but to conspecifics if they are present in the same tank (Barnett & Pankhurst 1998). In addition, there may be stress from exposure to air.

From the above, it is clear that netting, carried out for any husbandry process including euthanasia, should be done as gently and efficiently as possible. Stress from exposure to air should be minimised by reducing to a minimum the time taken to transfer the fish between the home tank and anaesthesia vessel. If conducted in a quick and efficient manner netting and handling can only take a few seconds, therefore, it is vital that researchers are properly trained to do so and we understand that this is covered in personal license training.

2.3.3 Stress from catching by hand

Fish are occasionally caught directly by hand. If fish are to be held directly this should only be done with wet hands, and preferably with soft, wet gloves (e.g. unpowdered latex gloves) to avoid damaging the mucous layer. However, many of the fish species used under the ASPA are too small to be effectively caught by hand or there may be other reasons why the technique is unsuitable (e.g. design of enclosure, swimming speed of the species involved, etc.).

2.3.4 Reducing stress by transferring fish in water containers

Methods of handling that allow fish to remain submerged in water are likely to reduce exertion, anoxia, stress and physical damage. An alternative to netting is to transfer fish in a vessel containing water. This can range in size from a small beaker to a large container on wheels dependent upon fish size. Transferring fish in water rather than in air is believed to be better practice but research is limited. Recent observations on a variety of fish species have shown that the time fish take to recover from handling and transfer between tanks can be reduced if the fish are transferred within a darkened, water-filled scoop rather than a dip-net (Brydges *et al.* in press).

For long transfers (>1 min), transfer in water is the preferable method. The water should be continuously aerated for all except the shortest of transfers as levels of oxygen may become depleted this can occur in as little as one minute, depending on factors such as fish size and water volume. It is advisable to cover the vessel with a lid so that the fish is not disturbed visually. The fish should have enough space to be able to turn around and be completely straight, so that this does not impose a confinement stress (Trenzado *et al.* 2003). On the other hand, too much space may allow fish to achieve speeds that could result in injuries. For heavier containers (~5+ kilograms), a trolley should be used for health and safety reasons. A trolley would also reduce any human induced motion changes that may cause stress. There are no comparable studies that enable us to definitively state a maximum acceptable transport time, but current knowledge relating to stressors in fish and common sense both dictate that transport times should be as short as possible.

2.3.5 Reducing stress by killing in home tank using an overdose of anaesthetic

Killing in the home tank negates the need for netting, capture, handling and transport and exposure to the air. If several fish are to be euthanased at once, it may be possible to add anaesthetic directly to a tank so that handling is minimised, but care should be taken to calculate the appropriate concentration of anaesthetic for: the volume of water in the tank, the number of fish in the water and to add it in a way that ensures rapid even dispersion. One way of achieving this is to take water from the tank, mix it with the agent and then slowly add it back to the tank. Assuming that the anaesthetic is not overly aversive, this would seem the quickest, most humane way of killing the fish and would presumably minimise stress although there is no scientific evidence for this

However, killing in the home tank is not a common method as there are a number of disadvantages and practical issues. The technique can only be used with isolated tanks as opposed to tanks linked to a common filter system where the anaesthesia would contaminate the system and affect other fish. After applying the anaesthetic to an isolated tank, the whole tank and its contents would have to be rinsed to remove the anaesthetic. Under current legislation, the tank would also have to be visually isolated so that other fish did not witness the death. Moreover, when killing single fish, the fish would have to be held individually rather than in groups. The welfare impact of this on gregarious fish such as common carp, stickleback and zebrafish would need to be taken into account. In summary, this method could be difficult to implement without extra technical support or research time.

2.4 Conclusions

There appears to be an urgent need for research into humaneness of chemical methods of euthanasia. Research is needed both on the aversiveness or otherwise of the agents and on the methods of their use.

Netting prior to euthanasia can be humane provided that the capture time and time to euthanasia is short and that prolonged chasing and air emersion are avoided. There may, however, be more humane techniques that should always be considered first. For example, capture and transfer in water containers is likely to be more humane than netting and transport in air. The welfare benefits of different types of transport and capture should be taken into account when these techniques are used.

When killing fish, consideration should be given to any welfare benefits and the feasibility of euthanasia in the home tank.

3. Consideration of alternatives to current Schedule 1 techniques

There are a number of reviews available on the humane killing of fish. Notable amongst these are Close *et al.* (1996, 1997); CCAC (2005); AVMA (2007). These reviews list some alternative techniques that are not currently included in Schedule 1.

3.1 Maceration

Maceration is usually recommended as a method of killing for embryonic and larval forms or neonatal animals and involves the use of a mechanical device, with rotating blades or projections that cause immediate fragmentation and death. It has been suggested that maceration could be used for very small fish (<2cm) Close *et al.* 1996), although Close *et al.* (1997) recommended against whole body crushing. There appears to be little literature on the subject for fish, but maceration is used in agricultural practice to kill chicks aged up to 72 hours (HSA 2004, The Welfare of Animals (Slaughter or Killing) Regulations 1995 (as amended)). Maceration requires specialised equipment, which should be kept in excellent working order. Moreover, the rate at which animals are introduced should not be such that it causes the equipment to jam or animals to rebound from the blades. As death should be almost instantaneous in a properly designed macerator, the technique could be more humane than overdose of some anaesthetics, although the fish would still have to be caught and handled. The macerators would, however, have to be specifically designed for each fish size, and would need to be kept in good order. As a secondary consideration, the process is likely to be aesthetically unpleasant for operators. Moreover, the technique would only be viable where there was no further need for the individual body tissues of the fish.

3.*2* Microwave radiation

Microwave radiation, using specially designed equipment to focus the radiation on the brain, is likely to result in rapid death of small animals (Close *et al.* 1997; AVMA 2007). However the technique would denature proteins limiting its value for some research projects, and we are not aware of the existence of specially designed microwave equipment suitable for euthanasing fish. Also, water is likely to reduce microwave penetration so the use of this method in aquatic animals would be likely to require their removal from water, with potential stress related to handling.

3.3 Chilling to stun or kill

If fish are slowly chilled they become less active. There is also the appearance of reduced reaction to stimulation, but true anaesthesia is unlikely to be achieved and there will still be some level of consciousness and sensation (Ross & Ross 2008).

It has been suggested that for farmed fish destined for slaughter, slurry ice might be used to desensitise the fish[3]. One of our experts noted that:

> "I have observed this method of killing carried out with many different species. On each occasion the fish showed extreme aversive reaction and our studies have shown that they take a protracted amount of time to die (see for example, Robb & Kestin 2002; van de Vis *et al.* 2003). Immersion in ice slurry or ice should never be considered as a practical method by itself, however, our work has shown that this can be a suitable method of ensuring that fish do not recover from an electric stun as long as a stun of sufficient duration has been induced".

Moreover, Roth *et al.* (2009) report that exposing turbot to subzero temperatures results muscle contractions which can be associated with severe pain in addition to primary and secondary stress responses.

AVMA (2007) does not support the use of hypothermia or freezing because of concerns that crystal formation might cause pain. AVMA (2007) does, however, note that quick freezing may be acceptable as a method of killing previously deeply anaesthetised animals.

Some users have claimed that chilling tropical fish such as zebrafish by immersion in water at 4^{o}C causes rapid cessation of gill movement and immobility (within seconds), They also report that even after short exposures animals cannot recover if placed back into their standard water temperature. However, we are not aware of any research on this issue for tropical and other non-temperate species.

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[3] http://www.fsbi.org.uk/phpbb/viewtopic.php?t=13

Cooling of foetuses followed by immersion in cold tissue fixative is allowed as a method of killing under Schedule 1 for mice, rats and rabbits. The use of similar methods for larval fish or immersion in liquid nitrogen has been suggested by some. Due to the Leidenfrost effect (where any liquid in contact with an object significantly hotter than its boiling-point boils immediately, enveloping the object in insulating nitrogen gas in the case of liquid nitrogen) it is important to consider carefully how tissue preservation is performed in order to ensure death is instantaneous. The use of pre-chilled surfaces, such as a copper plate, or pre-cooling can help to reduce potential problems by increasing the speed at which the core temperature of the animal will drop when using liquid nitrogen or similar methods. The size and surface area of the animal is also likely to be important in how rapidly cooling, and therefore death, occurs.

3.4 Electrical stunning

One technique that has received recent attention for farmed fish has been the use of electrical stunning. The use of electrical stunning as a method of sedation or anaesthesia is a logical extension of electro-fishing. Electro-fishing[4] as a technique has been effectively established in fish for many years and relevant literature extends over decades. Field sampling methods using this technique are very well known and an extensive literature exists on this well-researched technique (Cowx *et al.* 1990).

Electric stunners, in contrast to electro fishing, deliver a very much stronger pulse-field over a relatively short time period (usually only seconds). The field is sufficient to depolarise the brain and induce epileptiform activity during which it is presumed that the fish are not conscious. Electrical stunning has been reasonably well researched and a modest literature on its efficacy and effects is available, (see Ross & Ross 2008 for summary). Evidence for a stun can be gained either from EEG measurements, or from behavioural indicators (Kestin *et al.* 2002; Robb & Kestin 2002). Given a suitably strong electric field some fish have been shown to be stunned within half a second. Moreover, there is reason to expect that during this short time window, even if a self sustaining stun is not achieved, the brain is incapable of functioning in a way that would enable the fish to be considered sensible.

It is known that the duration that insensibility lasts is related to the fish species and the strength of the electrical field experienced by the fish (this is a function of

[4] Electro-fishing does not necessarily stun the fish. It usually uses pulsed DC and can be tuned to a particular waveform, amplitude and frequency. Once tuned, fish of a particular size (and species) are selectively affected at a particular setting, so a biased sample is captured. Small fish generally require a higher voltage and frequency than larger fish. The muscles of the fish are stimulated by the electric current and this causes some fish to swim towards the anode. The fish are captured either because of muscle exhaustion or because they are unable to coordinate an escape. It is possible that fish very close to the field are truly stunned, but usually, given the current involved, only for a very short time. However, if too high a current is used and fish are too close to each other, it is possible to kill fish.

the electric field in the water, the water conductivity and the fish orientation). It is also determined by the duration of exposure to the electric field and the frequency and type of waveform of the electric field. In addition, evidence from electric fishing suggests that larger fish are more susceptible than smaller fish to being affected by an electric field in the water.

3.4.1 Adverse effects from the use of electrical stunning

When sufficient current is supplied to achieve a stun, there is evidence that this can be associated with physical damage in fish (Lines & Kestin 2005). The type and extent of damage is believed to be species specific. For example, haemorrhage and broken bones are particularly common in salmonids (see for example (Robb & Roth 2003; Roth *et al.* 2003). It is reported that there is an apparent trade off between the duration of stun achieved, or the ability to kill, and the prevalence of carcass damage caused by the current.

In order to achieve a reliable stun there will always be the possibility of some form of damage to the animal as a result of the strong muscular contractions associated with a current great enough to induce a stun. Post stun damage will probably also exist that may not be readily visible upon dissection.

There are further disadvantages to the use of electrical stunning as a means of euthanasia. Lines (pers. comm.) points out that if a voltage insufficient to cause a stun is applied for a sufficient amount of time, total muscle exhaustion takes place and that this can be confused with anaesthesia; by all behavioural measures the fish appear unconscious and it is only by means of EEG that its true conscious state can be determined.

3.4.2 Electrical stunning prior to euthanasia by immersion in an overdose of anaesthetic

This particular combination is most unlikely to be a reliable approach to euthanasing fish. When fish are stunned, opercular movement ceases. The heart may also stop though it may resume as the electric stun wears off if the animal is not killed by the stun. It is presumed that the slowing or cessation of opercular movement and the fall or cessation of circulation would prevent the anaesthetic agent from working effectively. Therefore in the intervening period between recovery from the stun and succumbing to the anaesthetic agent the welfare of the animal could be compromised, and additionally, the state of welfare would be difficult to determine.

Further, electric stunning should be carried out only in a purpose-designed environment where the geometry and the electrode size facilitate the generation of a uniform electric field so that, regardless of fish location and orientation, it is properly stunned. Therefore, home tanks would have to be designed to ensure reliable electric stunning without the risk that the fish might be exposed to an electric field that causes pain but not insensibility.

3.4.3 Electrical stunning prior to killing by destruction of the brain

This could be a suitable method of euthanasia provided that the destruction of the brain took place immediately after the stun. Electrical stunning is thought to produce an instantaneous stun and, with the correct set up it could be carried out without prior disturbance to the fish. The stun duration would have to be sufficient to prevent recovery during handling prior to killing by concussion or destruction of the brain.

There are clearly practical problems associated with this approach including, for group housed fish, finding a way to protect other fish from the effect of shocks. It is may be worth considering the use of a wet brail (net) or a water flow system to transport the fish into a purpose designed stunning tank or tube.

However, it should be possible to produce a current in a given environment that would be sufficient to reliably produce a simultaneous stun and kill, without recovery (see section below on electrical killing), thus obviating the need to carry out a concussion or other physical insult to the brain.

3.5 Electrical killing

Trials with electrical stunning have shown that fish can be made unconscious immediately by application of an electrical voltage or current at a range of frequencies. Maintenance of the applied voltage and current for some tens of seconds, depending upon species and size, results in death. Longer periods of exposure (several minutes) are capable of killing otherwise very resilient fish such as turbot, while a shorter exposure (for example 20 seconds) is capable of ensuring fish such as trout and salmon remain insensible and without the breathing reflex until death by asphyxiation. However, data are only available for a few farmed species of fish. The event itself may well be totally stress-free as stunning is immediate.

Recent work on electrical stunning of farmed fish has given good results (Lambooij *et al.* 2008 a,b). This work should provide a base from which to move forward and to thoroughly investigate the method for large and small scale euthanasia. Work would be needed to determine how best to administer the killing shock (see stunning above) and to address concerns about operator safety, ensuring that each fish was killed and not just immobilised.

3.5 Conclusions

Maceration using appropriate equipment can be a humane method of euthanasia, and it seems appropriate that consideration should be given to adding it to Schedule 1, subject to the use of appropriate equipment.

Whilst microwave radiation, using appropriate equipment, could be a humane method of euthanasia it should not be added to Schedule 1 methods at present because of the practical difficulties.

There is clear evidence that chilling is not appropriate as a method of stunning or killing for temperate fish species used for food. Research is, however, needed on the humaneness of chilling techniques for small tropical species such as zebrafish.

Although freezing following stunning would be humane, there is no particular benefit in including this as a Schedule 1 technique.

Because of the technical difficulties involved with using liquid nitrogen killing with liquid nitrogen should not be added to Schedule 1 as a technique to be used with conscious fish.

Electrical killing is an area where further research on species commonly used in research is justified.

4. Recommendations

1. Research is needed into the aversiveness or otherwise of anaesthetic agents such as MS222 when used for the euthanasia of fish, and on the most humane methods of using these agents. The APC asks that funding bodies should give consideration to developing this knowledge.

2. This report draws attention to the need to minimise the stressful effects of handling, for example by considering alternatives to netting, exposure to air and avoidable handling when euthanasing fish. Section 6 of the Code of Practice to Schedule 1 should emphasise, for fish, the requirement to refine the entire euthanasia process, including handling. Consideration should be given to providing specific guidance, (e.g. regarding transfer in water-filled containers, or killing in the home tank where these are appropriate).

3. Subject to the use of appropriate equipment, there may be a case for including maceration as a technique in Schedule 1.

4. Schedule 1 should not permit chilling before euthanasia or killing by freezing unless evidence of humaneness is forthcoming for particular fish species and life stages.

5. A series of well-designed trials of electrical euthanasia of a range of fish species would be timely and may result in a humane killing method that could be used in research facilities. The APC asks that funding bodies should give consideration to developing this knowledge.

These recommendations have been made with full consideration of the Terms of Reference and responsibilities of the Animal Procedures Committee, including regard to both the legitimate requirements of science and industry and to the protection of animals against avoidable suffering.

The recommendations have also been made in the context of the better regulation agenda with the aim of not increasing and where possible reducing administrative burdens on business and the public sector.

Acknowledgements

The subcommittee is very grateful to the following for their substantial contribution to this document;

Lindsay Ross
Lynne Sneddon
Toby Knowles
David Robb
Jeff Lines
Jimmy Turnbull
Victoria Braithwaite
Nichola Brydges

We are also grateful to members of the Home Office Inspectorate who provided technical advice.

References

AVMA Guidelines on Euthanasia June 2007
http://www.avma.org/issues/animal_welfare/euthanasia.pdf

Barthel BL, Cooke SJ, Suski CD, Philipp DP (2003). Effects of landing mesh type on injury and mortality in a freshwater recreational fishery. Fisheries Research 63: pg 275-282.

Braithwaite VA, Boulcott, P (2007). Pain perception, aversion and fear in fish. Dis. Aquat. Org. 75: pg131-138.

Barton, BA and Iwama, GK (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Diseases **1**: pg 3–26.

Barnett CW and Pankhurst NW (1998) The effects of common laboratory and husbandry practices on the stress response of greenback flounder Rhombosolea tapirina (Günther, 1862). Aquaculture 162: pg 313-329.

Billard R, Bry C and Gillet C (1981). Stress, environment and reproduction in teleost fish. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London: pg 85–108.

Brown LA. Anesthesia in fish. Veterinary Clinics of North America: Small Animal Practice 18 (2): pg 317-330.

Brydges NM, Boulcott P, Ellis T. & Braithwaite VA. (in press) Quantifying stress responses induced by different handling methods in three species of fish. Appl. Anim. Behav. Sci.

CCAC 2005 Canadian Council on Animal Care guidelines on: the care and use of fish in research, teaching and testing.
http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Fish/Fish_Guidelines_English.pdf

Close B, Banister K, Baumans V, Bernoth E-M, et al, (1996). Recommendations for euthanasia of experimental animals Part 1 Report of a Working Party. Laboratory Animals 30: pg 293-316.

Close, B, BanisterK, Baumans V, Bernoth E-M, et al, (1997). Recommendations for euthanasia of experimental animals Part 2 Report of a Working Party. Laboratory Animals 31: pg 1-32.

Conte FS, (2004). Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86: pg 205-223.

Cowx, I.G. and Lamarque, P. (1990). Fishing with Electricity. Applications in freshwater fishery management. Fishing news Books. p248.

Davis LE, Schreck CB, (1997). The energetic response to handling stress in juvenile coho salmon. *Trans.* Am Fish Soc. 126: pg 248-258.

Ferguson RA, Tufts BL, (1992). Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*) – implications for catch and release fisheries. Can J Fish Aquat Sci. 49: pg 1157-1162.

Fox HE, White SA, Kao MHF, Fernald RD (1997). Stress and dominance in a social fish. J. Neurosci. 17: pg 6463-6469.

FSBI. 2002. Briefing paper 2: Fish welfare. Cambridge: FSBI*:* pg 1-25.

Gregory NG (2005). "Recent concerns about stunning and slaughter." Meat Science 70(3): pg 481-491.

HSA Code of practice for the disposal of chicks in hatcheries 2nd ed 2004. Humane Slaughter Association, Wheathampstead, Herts

JWGR (2001) Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. Laboratory Animals 35 pg 1-41.

Kestin SC, van de Vis JW, et al. (2002). "Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them." Veterinary Record 150 (10): pg 302.

Lankford SE, Adams BM, Adams TE, Cech JJ (2006) Using specific antisera to neutralize ACTH in sturgeon: A method for manipulating the interrenal response during stress. General And Comparative Endocrinology 147: pg 384-390.

Lambooij E, Gerritzen MA, et al. (2008a) A humane protocol for electro-stunning and killing of Nile tilapia in fresh water. Aquaculture 275: 88–95

Lambooij E, Gerritzen MA, et al. (2008b) Evaluation of electrical stunning of sea bass (Dicentrarchus labrax) in seawater and killing by chilling: welfare aspects, product quality and possibilities for implementation. Aquaculture Research 39: 50-58.

Lines J and S Kestin (2005). Electric stunning of trout: power reduction using a two-stage stun. Aquacultural Engineering 32(3-4): pg 483-491.

Mazeaud MM, Mazeaud F and Donaldson EM, (1977). Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish Soc*.* **106**: pg. 201–212.

Pickering AD, (1992), Rainbow trout husbandry: management of the stress response. Aquaculture **100**: pg. 125–139.

Pickering AD, Pottinger TG, Christie P. (1982). Recovery of brown trout, *Salmo trutta* L., from acute handling stress: a time course study. J. Fish Biol*.* 20: pg 229-244.

Portz DE, Woodley CM, Cech Jr JJ. (2006). Stress-associated impacts of short-term holding on fishes. Rev. Fish Biol. Fish 16: pg 125-170.

Pottinger TG, Calder GM. (1995). Physiological stress in fish during toxicological procedures: A potentially confounding factor. *Environ. Toxicol. Water Qual*. 10: 135-146.

Robb & Kestin (2002). Methods Used To Kill Fish: Field Observations And Literature

Reviewed. Animal Welfare 11**:** 269-282

Robb DHF and Roth B (2003). "Brain activity of Atlantic salmon (Salmo salar) following electrical stunning using various field strengths and pulse durations." Aquaculture 216(1-4): 363-369.

Ross LG and Ross B (2008). Anaesthetic and Sedative Techniques for Aquatic Animals. Oxford, Blackwell Publishing.

Roth B, Imsland A, et al. (2003). "Effect of electric field strength and current duration on stunning and injuries in market-sized Atlantic salmon held in seawater." North American Journal of Aquaculture 65(1): pg 8-13.

Roth B, Imsland AK and Foss A (2009) "Live chilling of turbot and subsequent effect on behaviour, muscle stiffness, muscle quality, blood gases and chemistry." Animal Welfare 9: pg 33-41.

Schreck CB. (1981). Stress and compensation in teleostean fishes: response to social and physical factors. pg. 295-321 in Pickering, A.D. (ed) Stress and Fish, Academic Press, London.

Sloman KA, Taylor AC, Metcalfe NB, Gilmour KM (2001) Stress from air emersion fails to alter chloride cell numbers in the gills of rainbow trout. Journal of Fish Biology 59: pg186-190.

Trenzado CE, Carrick TR, Pottinger TG. (2003) Divergence of endocrine and metabolic responses to stress in two rainbow trout lines selected for differing cortisol responsiveness to stress. General and Comparative Endocrinology, 133: pg 332-340.

Wedemeyer GA. (1997). Effects of rearing conditions on the health and physiological quality of fish in intensive culture. in, Iwama GK, Pickering AD, Sumpter JP, Schreck CB (Eds.), Fish Stress and Health in Aquaculture. Soc. Exp. Biol. Semin. Ser. 62. Cambridge University Press, UK: pp. 35–71.

van de Vis H, Kestin S et al. (2003). "Is humane slaughter of fish possible for industry?" Aquaculture Research 34(3): pg 211-220.

Wendelaar Bonga SE, (1997),The stress response in fish. Physiol. Rev. **77**: pg 591–625.

Yue S, Moccia RD, Duncan IJH. (2004) Investigating fear in domestic rainbow trout Oncorhynchus mykiss using an avoidance learning task. Appl Anim Behav Sci, 87:pg 343-54.

Appendix APC fish euthanasia survey – summary of results

The summary results below are from two universities and two professional bodies.

University 1

If already cannulated, injection of an anaesthetic such as Safan.

If not cannulated:

- submersion in an excessive concentration of anaesthetic such as MS222.
- Sharp blow to the top of the head and, if necessary, subsequent transection of spinal cord followed by disruption of the brain or decapitation.

Species: rainbow and brown trout and chub.

University 2

Concussion followed by brain destruction. Larger fish such as salmon trout, carp, cod, whiting (>50g) can be hit on the head with an instrument such as a trout priest. Smaller fish such as stickleback and zebrafish can be concussed by the use of blunt tweezers which also causes pithing, or a sharp blow to the top of the skull.

In neuroanatomical studies, sometimes an overdose of anaesthetic followed by exsanguination - at least 10 times the normal dose should be used such that fish lose equilibrium in the first 15-30 seconds. Since fish can recover from anaesthesia they should be left in the anaesthetic bucket for at least 15 minutes. However, if following this by exsanguination anaesthesia should still be maintained until gill tissues turn a light pink indicating loss of blood such that no recovery is possible.

Fish from <1g up to 1kilo in size.

Professional body 1

Overdose of anaesthetic (MS222 buffered to pH7). Fish are left for 20 minutes and when death is confirmed the animals are then decapitated to exsanguinate.

Species: zebra fish.

Professional body 2

Cited work at Bristol University that led to development of an electric stunning system which is now installed on many trout farms.