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Species-specific welfare aspects of the main systems of stunning and killing of farmed fish: rainbow trout [1]

Scientific Opinion of the Panel on Animal Health and Welfare

(Question No EFSA-Q--2008-438)

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SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on welfare aspect of the main systems of stunning and killing (stun/kill) of farmed rainbow trout (*Oncorhynchus mykiss*) in the EU.

Within the EU two main production systems of trout exist: whole production in freshwater, and sea based production. In the latter system, larval and juvenile stages are grown in freshwater, and later growing for slaughter in sea water. This system is almost identical to Atlantic salmon production. In the other type, whole production in freshwater, in family businesses, most slaughter takes place on site in smaller numbers of fish and not in a large slaughterhouse.

Harvesting and processing of sea-farmed trout are the same as for farmed trout Atlantic salmon (*Salmo salar*). Therefore the Scientific Opinion on salmon[2] and its conclusions can be applied to the sea farmed trout production.

For freshwater farmed rainbow trout, a semi-quantitative risk assessment approach was used to rank the risks of poor welfare associated with the different commercially applied stunning and killing methods. The risk assessment was also used to elucidate other concerns and to provide guidance for future research. The risk assessment was mainly based on expert opinion, due to the limited amount of quantitative data and published peer-reviewed data. Pre-slaughter stages which have a direct impact on the welfare immediately before and during killing were included in the risk assessment. Stunning and killing methods that are not commercially used in Europe were also mentioned but not included in the risk assessment. The opportunity to develop new methods for slaughtering trout is considerable and should be encouraged.

The five stunning and killing methods assessed were: 1. Percussive stunning; 2. Electrical stunning; 3. Carbon dioxide; 4. Asphyxia; 5. and asphyxia in ice slurry. All methods are followed by evisceration (portion sized trout), or exsanguination and evisceration (large trout).

The most important hazards in the pre-slaughter phase were associated with crowding and transfer by pumping. Keeping trout in holding units may also be a matter of concern if the quality of water is poor. Feed deprivation can result in the utilisation of body fat reserves and then functional tissue; and should not exceed 50 degree day.

Pre-slaughter handling directly before stunning is presently unavoidable. Efforts should be made to minimise the stress caused by crowding. Exposing trout to air or shallow water which restricts the movements of the fish should be avoided. Transfer should preferably be performed by free-flowing water or small scale dip-netting. Vacuum pumping of fish should be avoided, as small scale netting and free flow are preferable and cause less harm. Transport of trout from farm to processing plant immediately prior to slaughter should be carried out as gently as possible.

Regarding the stunning and killing methods, percussive methods and electrical stunning were assessed to reliably cause unconsciousness in the vast majority of trout. The semi-automatic percussive stunning has a higher risk of resulting in poor welfare because fish are being handled in air on a table for some seconds or even minutes. This risk, however, may be mitigated by reducing or avoiding the time of exposure to air and by spraying the fish constantly with water.

Carbon dioxide, asphyxia on ice and asphyxia are the methods resulting in the poorest welfare. Carbon dioxide has the highest risk score because exposure to the gas causes a strong adverse reaction; in addition, it also does not reliably result in unconsciousness. Thus trout may be

[2] Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on welfare aspect of the main systems of stunning and killing of farmed Atlantic salmon. The EFSA Journal (2009) 1011

eviscerated or bled when conscious. Killing trout by asphyxia is judged to be a severe hazard. Asphyxia in ice slurry had a higher score since the temperature shock was an additional hazard.

To the experts' knowledge depopulation of trout for disease control occurs rather rarely. If a disease outbreak did require culling trout on a farm, killing may be performed either by normal stunning and killing procedures, or by an overdose of anaesthetic.

Currently is not possible to identify welfare indicators that could be used to monitor slaughter procedures for trout. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced. Validated, robust and practically feasible welfare indicators should be developed.

Key words: **fish, animal welfare, risk assessment, pre-slaughter, stunning, killing, slaughter, disease control, rainbow trout, *Oncorhynchus mykiss*, Atlantic salmon, *Salmo salar***

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Directive 93/119/EC provides conditions for the stunning and killing of farm animals. Fish are legally part of the scope of the EU legislation but no specific provisions were ever adopted. Following a previous request from the Commission, EFSA issued in 2004 a scientific opinion on the welfare aspects of the principal methods for stunning and killing the main commercial species of animals, including farmed fish. As regards farmed fish, this opinion concluded that *"Many existing commercial killing methods expose fish to substantial suffering over a prolonged period of time*". Furthermore, *'for many species, there is not a commercially acceptable method that can kill fish humanely"*. Moreover, the respective EFSA report highlighted that different methods for stunning and killing of farmed fish must be developed and optimised according to the species specific different needs and welfare aspects.

"Fish are often treated as one species when it comes to regulations and legislation governing welfare during farming or at slaughter. But, it is important to realise that a very wide number of species of fish are farmed, with an equally wide variety of ecological adaptations and evolutionary developments. These differences mean that different species fish reacts differently to similar situations. For example, at a given environmental temperature, some species like trout die relatively quickly when removed from water into air, whilst others like eels or marine flatfish can take several hours. Similarly, in electrical stunning situations, eels require a much larger amount of stunning current than trout or salmon to render them unconscious. Species differences need to be taken into account when adopting particular procedures. Processes must be developed and optimised with respect to welfare specifically for each species. For example, it would be as unreasonable to assume that a process developed for killing trout in freshwater would be suitable for killing tuna in the sea as it would be to assume that a system developed for quail would be effective on ostriches."

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In view of the above, the European Commission requests EFSA to issue a scientific opinion on the species-specific welfare aspects of the main systems of stunning and killing of farmed fish. The opinion should assess whether the general conclusions and recommendations of the 2004 opinion apply to the species of fish specified below. Furthermore, the above mentioned conclusions and recommendations should be updated in a species specific approach, integrating where possible reference to welfare indicators and to new scientific developments. Where relevant, the animal health and food safety aspects should be taken into account.

The following species should be considered Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), European eel (*Anguilla anguilla*), gilthead seabream (*Sparus auratus*), European seabass (*Dicentrarchus labrax)*, European turbot (*Psetta maxima*), common carp (*Cyprinus carpio*), and farmed tuna (*Thunnus* spp.).

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ASSESSMENT

1. SCOPE AND OBJECTIVES OF THE SCIENTIFIC OPINION

The scope of this report is the welfare aspects of stunning and killing methods applied to farmed rainbow trout, *Oncorhynchus mykiss,* which represents most of the European production of trout. These are produced either in fresh water or in sea based systems.

The objective of this report is to briefly describe the current practices for trout slaughter, to identify welfare hazards and to assess welfare risks associated with those practices. The aim is also to identify, as far as possible, suitable welfare indicators at slaughter where they may exist.

Most of the considerations in this report would also apply to brown trout (*Salmo trutta*), char species (*Salvelinus* spp.) and char hybrids. However, these species and their hybrids may show different reactions to stunning and killing methods. For example, during emergency killing, char was found to be more resistant than expected to application of electricity (Rösch, personal communication).

The harvesting system of sea farmed trout including transport, lairage, stunning and killing and processing are the same as for farmed Atlantic salmon. Therefore the existing salmon report and its conclusions can be mainly applied to the sea farmed trout production and only specific differences will be addressed in this report.

Freshwater production of trout is performed in a wide variety of production systems, and also in a wide variety of small and medium enterprises. Their annual production ranges from family businesses with less than 20 metric tons of portion-size trout, up to companies producing several thousands of tons. There are no statistics available that provide production figures for individual production unit within the EU[3].

A considerable amount of the trout produced in fresh water in the EU is marketed directly, i.e. the fish are killed on site and not in a processing plant. For example, in Germany it is estimated, that up to 50 % of the production are marketed at the door step.

As most of the current scientific information about stunning and killing relates to Atlantic salmon and there is an absence of similar information for rainbow trout, and because of the similarities between the Atlantic salmon and the rainbow trout, assumptions are made that some information are applicable to both species.

The pre-slaughter process is only considered where evidence exists for a direct impact on welfare at stunning and killing. Where fish welfare, immediately before and during killing or stunning and slaughter, is affected, it has also been considered as part of the slaughter process. Therefore, the welfare aspects of the farming phase of trout as well as the transport of trout are not included in this report.

During stunning and killing usually water temperature ranges between 2 and 20 C, which has a great influence on the physiological responses of trout. At higher temperatures trout are more easily stressed (EFSA, 2008).

[3] Eurostat, Statistics in focus, Agriculture and fisheries, http://epp.eurostat.ec.europa.eu/cache/ITY_OFFPUB/KS-NN-06-023/EN/KS-NN-06-023-EN.PDF

Emergency killing at production units for disease control or other reasons is included in the report. However, humane killing of individual fish, in the course of farming operations (i.e. sorting, grading, or background morbidity) is not included.

Meat quality and safety are not part of the assessment. Food safety issues are addressed by the BIOHAZ panel.

In drafting this Scientific Opinion, the panel did not take into consideration any ethical, socio-economic, human safety, cultural or religious or management issues, the emphasis has been to look at the scientific evidence and to interpret that in the light of the terms of reference. Nevertheless, it is acknowledged that such aspects can have an important impact on animal welfare.

2. Pre-slaughter process with direct implication in stunning

Pre-slaughter process considered in this report is the harvesting of trout directly followed by slaughter which may consist of different steps, and these mainly depend on the type of farming system, technical equipment used on the plant, and market demand. There are a lot of different methods for catching trout for stun/kill purposes. In some small and medium enterprises, these procedures are performed on site. In others, trout are transported alive to a separate processing plant. In production systems using fresh water cages pre-slaughter activities involve cage harvest followed by transport to a holding unit or a processing plant where stunning and killing take place, killing may occur also at the farming site.

Holding units are used for direct marketing, where fish often are kept in high densities in small ponds or tanks near the marketing unit. These units may be equipped with a high water flow through and/or aeration/oxygenation. From these facilities, trout are caught by a dip-net, the required number of fish is selected and killed on site.

2.1. Crowding

During harvest, the fish are usually crowded. Depending on the system, crowding lasts from a few minutes to several hours during which the welfare of the fish may be compromised. It is reasonable to assume that the longer the trout are crowded, the greater is the stress. The duration of crowding, the degree, speed, repeated crowding and the quality of water may have a direct impact on the welfare of trout and can cause injuries. Water quality may deteriorate during crowding. Rough handling during crowding can even cause injuries and increased incidence of skin lesions (Winfree et al., 1998; North et al., 2006), primarily due to physical contact (Abbott & Dill, 1985).

Net confinement or crowding can result in typical stress-related changes in blood chemistry and muscle biochemistry (Waring et al., 1992; Brown et al., 2008). If the crowding is too severe, this can lead to excessive swimming (escape behaviour). From sea water it is known that 6 min of chasing rainbow trout will lead to a considerable increase of stress hormones, and plasma ions, and a sharp drop in blood and muscle pH (Wood, 1991). In fresh water trout, plasma ions decrease (Wendelaar Bonga, 1997). Furthermore, similar considerable stress reactions can occur if rainbow trout are exposed to air for 60 sec (Ferguson and Tufts, 1992). Usually the transfer of rainbow trout through the air is much shorter (Rösch, person. communication). If trout is out of the water for longer than 10 seconds, microscopic pathological lesions may be observed in gills. This depends on water temperature.

The skill of the personnel is considered an essential factor to minimize handling stress during this operation.

There is a wide range of crowding techniques used in order to collect only the amount of fish to slaughter which will be put on the market. Such techniques include crowding by feed pellet distribution followed by dip netting, crowding with a seine net or a grid, by lowering the water level or emptying the pond. In fresh water cages crowding is carried out by raising the net.

Those methods however do not necessarily represent different levels of risk from a point of view of fish welfare provided that crowding is conducted under correct management and with careful handling.

Monitoring points are:

- Oxygen level in the outflow water should not be below 5 mg/l (EFSA, 2008)
- No excessive swimming activity, fight and flight behaviour
- No exposure to air longer than 10 seconds

2.2. Transfer

There are different types of systems of transfer depending on type of production. Trout are netted or pumped or flow freely (gravity), depending on the local situation, from the rearing unit or the truck to the place where they are killed. In the case of netting, care has to be taken not to overload the net. Fish can be netted in air or in water. Transfer systems can bring different types of hazards.

In freshwater farms, trout are harvested mainly by seine nets of different types of pumping systems: the vacuum pumps (single or twin), Venturi pumps, siphons, air lift or fish elevators (screw type). The pumping distances can range from few meters up to 200 meters. Lifting heights may vary between 0 and 5 meters. Gravity flow systems with or without initial pumping may be used.

Proper design of the transfer system (hoses, pump, strainer, chutes etc) to stunning unit is essential for good fish welfare as faulty constructions can result in injuries to the fish, such as excessive scale loss. Examples of improper pumping causing injuries are presence of inner pipe flanges, sharp bends and fish at high speed colliding with bulkheads, pipe walls etc. (Mejdell et al., 2009). It should be noted that pumping is also used in farms for sorting and grading.

Monitoring points:

- No fresh injuries post transfer;
- No exposure to air longer than 10 seconds.

2.3. Transport to slaughter site

When separate slaughter facilities are used, transport is usually carried out by truck. Transport by itself may be stressful and can have an impact on the welfare of the trout.

After arrival at the slaughter site, trout are either transferred from the vehicle directly to the slaughter line or to holding facilities. Transfer is carried out as described in previous section.

The trout may be kept in the holding facility for a few hours, up to a few days, before they are processed depending on market requirements (quantity and size). During holding, trout can recover from transport stress. They are usually not fed during this period. Feed deprivation is a common practice during the pre-slaughter period. Welfare aspects of feed deprivation have been addressed in EFSA (2008). Feed deprivation can result at first in the utilization of body fat reserves and then functional tissue: the latter is associated with poor welfare

Water quality, in particular temperature, oxygen, ammonia, CO_2 and pH levels, is important at this stage to ensure welfare of trout.

Monitoring points in transport and holding water:

- No level of oxygen lower than 60 % saturation
- No level of pH lower than 5.5 (EFSA, 2008)
- No abrupt temperature shifts
- No increased mortality[4]
- Normal swimming behaviour

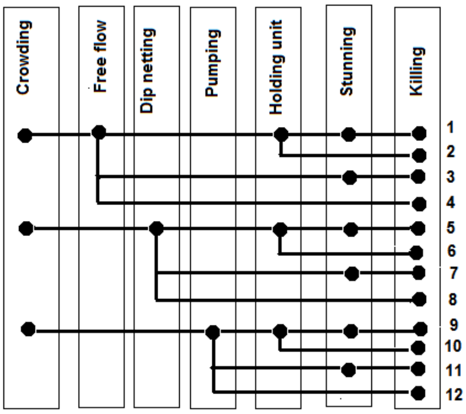

Figure 1. Pathways for pre-slaughter and slaughter on site. Dots represent major steps of the process occurring on a time line from left to right. Pathways 1 to 4 relate to the use of free flow (e.g. fish elevators) as transfer procedure, with or without holding unit, with or without stunning before killing. Pathways 5 to 12 represent the same but taking dip netting and pumping respectively as transfer method. From the holding unit, fish are crowded and transferred to stun/kill.

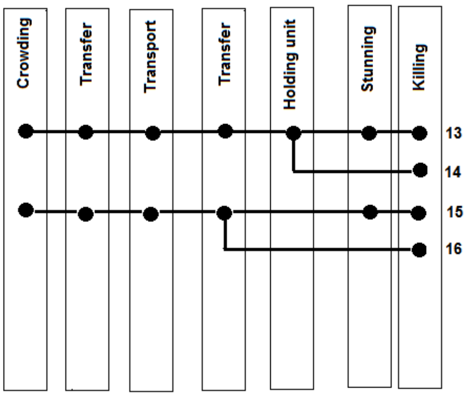

Figure 2. Pathways for pre-slaughter and slaughter including transport. Dots represent events occurring on a time line from left to right. Pathways may include holding unit and/or stunning before killing. From the holding unit, fish are

[4] This refers to Council Directive 2006/88/EC.

crowded and transferred to stun/kill. Transfer covers dip-netting, free-flow, pumping.

3. Stunning and killing methods for trout

3.1. Recognition of consciousness, unconsciousness and death

Stunning methods are supposed to induce immediate or rapid (less than 1 second) unconsciousness. It is important for people involved in fish slaughtering operations to be able to recognise whether a stunning operation has rendered a fish rapidly unconscious.

In trout, field recognition for consciousness in general would comprise gill movements, eye roll (VOR), and body movements. In experimental conditions, visual evoked responses (VER) may also be used. CO_2 method may induce loss of body/gill movements although fish remains conscious for approximately 6 minutes as shown for salmon by Robb et al. (2000a). Therefore, CO_2 is not considered as a stunning method. If no sign of consciousness, trout would be regarded as unconscious.

There is no simple indicator to assess death under field conditions except the duration of unconsciousness.

3.2. Purpose of stun/kill

3.2.1. Human consumption

For human consumption, the methods used presently for stunning and killing are: percussive stunning, electrical stunning, carbon dioxide, asphyxia and asphyxia in ice slurry. All followed by evisceration (portion sized trout) or exsanguination and evisceration (large trout). In addition, several combinations of these methods may be used.

Table 1. Methods to kill, stun and stun/kill used for trout in Europe for human consumption

Method	Percussion / exsanguination	Electricity stunning /exsanguination and or evisceration	Carbon dioxide[5] / exsanguination and or evisceration	Asphyxia on ice / Ice slurry	Asphyxia	Exsanguination / Evisceration
Type of action	*Stun or Stun/kill*	*Stun / kill*	*Sedation and kill*	*Sedation and kill*	*Kill*	*Kill*

3.2.2. Rejected fish

Fish not fit for human consumption (e.g. clinical signs of diseases, deformities, injuries, etc.) is sorted out. Rejected fish are usually killed with the methods listed in previous section, with possible addition of asphyxia. Practical experience shows that usually the percentage of rejected trout is less than 0.1% (Rösch, personal communication). Exception exists when triploidisation method is incorrectly applied (Loopstra *et al*., 2008).

[5] CO_2 will be banned in Norway from 2010.

3.2.3. Emergency killing on production site

Emergency killing may be recommended for disease control, extreme situations (e.g. water pollution, compromised welfare, etc.) or food safety reasons and can be part of contingency plans. Depending on the situation, whether it is a disease outbreak or destruction of a population due to food safety reasons, emergency killing is often carried out on site or the fish are transported to a designated slaughter facility.

Fish which are killed in case of emergency, but still suitable for human consumption, are killed following normal stun/kill procedures. During this emergency process fish may however be sorted in several steps and increase the number of rejected fish. Fish seen as unfit for human consumption are sorted out at harvest or stunning. There is no standard system and practices mainly depend on facilities and logistics.

Where the whole population is unfit for human consumption, an emergency killing may be carried out by using normal stun/kill procedures. In addition, anaesthetics applied alone (overdose), or in combination with methods such as electricity are also possible.

Various anaesthetics may be used for the purpose of emergency killing and stock destruction.

Fish can be anaesthetised by immersion in anaesthetic solutions. A large selection of anaesthetic agents is being used in fish, but in practise only isoeugenol, metacaine (MS-222) and benzocaine are used for trout. Isoeugenol is used in some countries (e.g. New Zealand, Chile) for stunning in combination with exsanguination of salmonids for human consumption. This anaesthetic is prohibited for such use in the EU. The method can be used for emergency killing only.

When anaesthetics are properly applied, fish show no conspicuous aversive reaction. However, blood chemistry (including release of stress hormones) can be affected (Bartol and Peter, 1982; Davidson et al., 2000) but large variations are observed in practice (Hille et al., 1982). Induction time seems to decrease with high concentration of anaesthetic, high water temperature and stress (Ross and Ross, 2008; Zahl et al., submitted).

Too low concentration of anaesthetic will result in a slow induction of unconsciousness. This may give time to the fish to sense the chemical which may also act as irritant to the skin. Furthermore, loss of balance during slow induction may also induce a stress response (Oyama, 1973; Oyama and Wakayama, 1988, Kiessling et al., 2009).

3.3. Specific stunning and killing methods for trout

This section describes the various methods applied for stunning and killing trout (see Table 1). Methods for stunning and killing are applied in combinations based on farming systems, size of the fish, and technical options at the slaughter plant.

3.3.1. Percussive methods

The principle of a percussive stunning is that one blow or repeated blows are delivered to the top of the head above the brain by a club or hammer with a force sufficient enough to stun or kill instantaneously, e.g. due to hemorrhaging in the brain (Kestin et al 2002; Robb and Kestin 2002; Roth et al 2007). The stunning effect depends on the force, the velocity, weight and shape of the hammer or club.

When the blow is correctly applied and is of adequate force, loss of movement and VERs is immediate and permanent in trout (Kestin, Wotton and Adams, 1995; Robb et al., 2000a). When applied incorrectly or with insufficient force, unconsciousness is not immediate or consciousness is recovered after a short period of unconsciousness (Kestin, Wotton and Adams, 1995; Robb et al., 2000b) and injuries to the fish can result such as eye popping, eye burst or hemorrhaging (Roth et al 2007).

Percussive stun kills instantaneously or renders the fish unconscious. One of the major challenges with percussive stunning machines is to get live fish into the machines and a correct hit to the skull. Restraining live fish may cause panic and escape reactions both affecting the welfare of the animal. In streamline systems dealing with relative low number of fish, manual feeding of percussive machines (semi-automatic system) is practiced in certain countries. Since the percussive machines have fixed positions of the cylinder, the major challenge is to hit all animals correctly, independent of size (Roth et al 2007). For manual feeding, this is solved by adjusting the machines for different sizes, where the fish based on size are fed into different machines.

Several commercial machines are available on the market, but they are not effective on trout of less than 1 kg of body weight (portion size trout). Technically there are differences between those machines. Examples are provided below to illustrate those differences.

All percussive stunning machines work on the same principle. A rapid blow to the top of the head causes sudden movement of the brain within the skull with consequent bruising, haemorrhage, tissue destruction and loss of consciousness. The machines are designed to allow a degree of movement of the head rather than holding the head rigid and this ensures the rapid movement of the brain within the skull and an effective stun. There is no penetration of the skull. All machines use a pneumatic device to deliver the blow.

The percussive stunners are either hand-fed, or, now more commonly, employ automatic flow-through systems. The hand-fed systems rely on personnel guiding the fish into the stunner to receive the blow, removing the fish from the stunner and passing it on to be exsanguinated.

3.3.2. Electrical methods

An electrical current is administered between electrodes, which are submerged in a fish holding tank or transportation system. While passing between the electrodes, using water and fish as a conductor, the current generates an electric field. Low field strength causes sufficient muscle stimulation for immobilisation of the fish, but does not render fish unconscious. This technique is used in electro fishing. A stimulation of higher nerve centres causes their dysfunction, either by induction of epileptic seizures or by complete cessation of function and renders fish insensible immediately (Kestin at al., 1995). Electro immobilisation and electro stunning is achieved by use of direct (DC). as well as alternating currents (AC) and different combinations of voltage, frequency and duration. The reactions of fish to electric currents depend on the intensity of the electric field, the duration of electric stimulation and the size of the fish. Assuming similar orientation to the field, larger fish intercept a greater potential difference and therefore are stunned more rapidly for a longer period of time (Ross & Ross, 2008). Furthermore, large electrodes which produce an electric field with uniform field strength were found to have the best anaesthetic effect. The use of rectangular tanks with full- width plate electrodes was found to be the best arrangement to avoid that fish "escape" during an electrotaxis phase to an area of low and ineffective field strength (Darroux, 1983).

The effectiveness of the stunning depends of the strength of the electric field applied, the density and distribution of fish in the tank. For portion size trout, field strength between 3 and 6 V/cm had to be applied for 30 to 60 sec in order to achieve permanent insensibility (Lines and Kestin, 2004). Fresh water conductivity may vary on a broad scale (20 to 1000 µS/cm) which influences the strength electric field and consequently the efficacy of the stun. This can be calculated by the formula: $P = 0.001\ E^2C$ (where: P: power in watts per liter of water, E: electric field strength in V/cm, C: water conductivity in µS/cm).

In consequence, at higher water conductivity, insensibility can be achieved at lower field strength (Lines and Kestin, 2004).

If trout are stunned and brain function is lost, the fish may enter a stage of mild tonic and clonic spasms that can last approximately 20 – 50 sec (Kestin, Wotton and Adams, 1995; Robb and Roth, 2003). This depends on water temperature: the higher the temperature, the shorter the spasms last. If trout are not killed by the process recovery of consciousness may happen within a few minutes depending on stunning parameters. Higher field strength, increased water conductivity and longer electrical application times may be associated with longer periods of unconsciousness and a larger proportion of fish killed by the process. Higher frequencies (up to 2000 Hz) are associated with shorter periods of unconsciousness and lower mortality (Roth, 2003).

Electrical stunning equipment: Because effective stunning mainly depends on electric field strength, numerous designs and parameters for electrical stunning exist in terms of duration, voltage, AC/DC and frequences. Some commercial machines are available and many farms have developed and build their own system in order to obtain good fillet quality. Because of safety reasons, several systems are operated at low Voltage such as 24 or 50 V. In most electrical stunning conditions, fish are stunned whilst in water. Typically, a tank with electrodes attached to opposite sides (or bottom and lid) is filled with water and fish before electricity is applied. A lead ensures the head of fish is maintained in the water during electricity is applied. A current is passed between the electrodes, using the water and the fish within as a conductor. Provided certain parameters are met, the fish are immediately stunned. After the current is turned off, the fish are removed from water. Semi automatic continuous throughput electrical stunning devises have also been developed as electric stunning tunnel. This device achieves stunning by pumping fish through a long electrified tube connected between the tank and the processing line. Fish are exposed to an electric field during their transfer through the pipe and arrive unconscious or dead into the harvest container.

3.3.3. Carbon dioxide

Carbon dioxide is highly soluble in water and has a narcotic effect on fish placed in water saturated with the gas. Under commercial slaughter conditions of trout, carbon dioxide is bubbled continuously into a tub, tank or bath of water. The pH of the water falls as it becomes saturated with carbon dioxide, and when it stabilizes at about pH 4.5, the water approaches saturation with the gas (Anon, 1995). Fish are then transferred into the water and are left in the bath until movement stops. They are then removed and further processed (exsanguinated and/or eviscerated). Modifications to the process outlined above include cooling the carbon dioxide saturated water to about 1 C, by the addition of ice. This has been found to result in a faster loss of physical activity although activity may still continue for about 1 min (Robb, pers. comm.). Carbon dioxide narcosis is an easy method to mechanize and requires little labor to manage. For this reason, it is popular in some countries where labor is expensive.

Trout show strong aversion for at least 30 seconds after immersion in carbon dioxide, although times over three minutes have been recorded (Robb et al., 2002). They swim very rapidly, making escape attempts (Kestin, Wotton and Adams, 1995). This behaviour can last for about 3 minutes (Robb *et al.*, 2000a; Kestin, Wotton and Adams, 1995). Loss of consciousness is not exactly known but brain functions are lost after 4.7 minutes (Robb et al., 2002). Trout are reported to show signs of increased mucus production during carbon dioxide narcosis (Marx *et al.*, 1997) which could be further indications that the process is irritating. The aversive reactions to carbon dioxide stunning and the resulting high activity have been reported to cause injury and scale loss (Robb et al., 2002; Akse and Midling, 1999; Roth et al., 2002) as well as gill haemorrhage (Robb and Kestin, pers. comm.). There is no evidence to show that carbon dioxide has any analgesic or anaesthetic effects.

Because fish become immobile before loss of consciousness (Robb et al., 2000a), there is a risk that fish could be exsanguinated or eviscerated while still conscious. Industry codes recommend that the fish should be left in the water for at least 4 to 5 min before exsanguination (Anon, 1995), and observations indicate that fish are often kept in the tank for about 5 min (Erikson, 2008).

Nitrogen was experimentally used in one study instead of carbon dioxide resulting in effective stunning with no strong aversive reactions in rainbow trout (Will et al., 2006).

3.3.4. Asphyxia in ice, ice slurry

Asphyxia in ice means transfer from water at ambient temperature into different water or slush ice at a significantly lower temperature (temperature differential is usually greater than 10 C), often followed by a draining of the water. The aim is to simultaneously chill, sedate and kill the fish by asphyxia. The lethal temperature for rainbow trout has been reported as -0.75 C (Fletcher et al., 1988).

Asphyxiation in ice does not result in immediate unconsciousness. For trout, it takes 9.6 minutes at 2 C compared to 3 minutes at 14 C (Robb and Kestin, 2002). It has been proposed that when the differential between the ambient temperature of the fish and the ice slurry is relatively great, thermal shock may shorten time to loss of brain function and elevated plasma cortisol levels have been reported (Donaldson, 1981). Fish transferred from iced water immediately after loss of VERs or SERs to water at normal temperatures quickly recovered brain function and subsequently muscular movement (Robb and Kestin, 2002).

3.3.5. Asphyxia

Fish are killed by this method simply by removing them from water and leaving them to die in air. This is a killing method and not a stunning method. Asphyxia is usually achieved by netting the fish from the water or pumping fish through a grid and placing them in free draining bins or boxes. No special equipment is required. Fish are left to die and when movement has ceased, they are further processed. The time required for the fish to die is temperature dependent (Robb and Kestin, 2002).

3.3.6. Exsanguination and evisceration

Exsanguination is commonly used for large trout which are exsanguinated immediately after stunning. Smaller trout are usually not exsanguinated but directly eviscerated after stunning. To achieve exsanguination, the gills are cut or pulled out, and the fish returned to water to bleed for a period of 10 to 15 min (Wardle, 1997). In some cases the isthmus is cut or the heart pierced with a knife. In commercial practice, exsanguination is the main cause of ultimate death. Robb and Roth (2003) both indicate that a functioning heart is not necessary for an efficient bleed-out and that provided major vessels like the gill arches, isthmus or heart are cut, there is little difference in the efficiency of exsanguination.

4. Risk Assessment

The general risk assessment guidelines used to assess the risk to welfare at the time of stunning/ killing of farmed fish is described in Appendix A. The risk assessment applied to the stunning and killing of farmed trout is described in the following section.

4.1. Application of the risk assessment approach

The risk assessment was applied to the stunning and slaughter of rainbow trout. The pre-slaughter events preceding stunning and slaughter start with crowding the fish, transfer and possible transport to the place of slaughter. The hazards associated with typical pre-slaughter management were assessed, in relation to their effect on stunning and killing in general.

The assumption that exposure to the hazard resulted in all the fish suffering the adverse effect held for all hazards.

Definitions of intensity of an adverse effect for hazards occurring pre- and post-stunning were defined (Table 2).

Table 2. Intensity categories of adverse effects arising from hazards associated with pre-slaughter and slaughter operations in rainbow trout

Evaluation	Score	Description of fish response in water	Description of fish response in air
MILD The animal is minimally affected as evidenced by minor changes in behaviour.	1	Slight increase in swimming pace. Schooling is preserved. Slightly increased gill movements.	Exposure to air for few seconds does not cause gasping. Fish are more excitable at higher temperature.
MODERATE	2	Not in the mild or severe category	Not in the mild or severe category
SEVERE The animal is affected greatly, as evidenced by marked changes from normal behaviour	3	Signs may include energetic escape behaviour, rapid and erratic swimming, flashing swimming, colliding with the net or tank walls, gasping at the surface.	Fish show excessive tail flapping, gasping and struggling. Fish are more excitable at higher temperature.

Different categorisation for duration of the adverse effect was used for pre-slaughter and slaughter / stunning hazards, as presented in Table 3 and Table 4.

Table 3. Duration categories for adverse effects arising from hazards associated with pre-slaughter handling of rainbow trout

Duration (minutes)	Score
<5min	1
>5-10 min	2
>10-30 min	3
>30 min	4

*adverse effects with a durations of less than one second are not scored

Table 4. Duration categories for adverse effects arising from hazards associated with slaughter of rainbow trout

Duration (minutes)	Score
>1 sec -1 min	1
>1-2 min	2

>2-6 min	3
>6 min	4

*adverse effects with a duration of less than one second are not scored

4.2. Results

4.2.1. Pre-slaughter hazards

A total of 12 pre-slaughter hazards were identified which were categorised by i) crowding, ii) transfer (pumping, free-flow or netting), iii) holding unit, and iv) post-transport. The hazards were scored for trout killed on farm and off-site, since the duration of the adverse effects of some hazards had a longer duration for trout farmed off-site. The risk scores for the hazards ranged from less than one to 67 (Table 5 and Table 6).

Table 5. Pre-slaughter hazards (off site slaughter)

	Hazard description	Risk score most likely	min	max	Magnitude[6]
	Crowding				
1	Increased density	15.00	12.50	18.75	25
2	Fish exposed to shallow water	7.50	2.50	10.00	25
3	Fish exposed to air (>15 seconds)	1.50	0.50	2.50	50
4	Poor water quality (O2, TOC, ammonia, suspended solids, temperature)	0.75	0.25	2.50	25
5	Contact with the physical structures	1.33	0.07	3.33	67
	Transfer				
6	Pumping	66.67	66.67	66.67	67
7	Poor equipment design (adjusted to the size of the fish)	0.33	0.01	0.67	67
8	Delay in pipe or screw	0.50	0.05	0.90	100
9	Dip-Netting	1.67	0.83	3.33	17
	Holding unit				
10o	Poor water quality (pH, TOC, DO, water temp, CO2, ammonia)	3.00	1.00	5.00	100
11	Dip-Netting in holding unit	1.67	0.83	3.33	17
	Post-transport condition				
12	Poorly performed transport	0.50	0.01	1.00	100

Table 6. Pre-slaughter hazards (on site slaughter)

	Hazard description	Risk score most likely	min	max	Magnitude[7]
	Crowding				
1	Increased density	15.00	12.50	18.75	25
2	Fish exposed to shallow water	7.50	2.50	10.00	25
3	Fish exposed to air (>15 seconds)	1.50	0.50	2.50	50

[6] Magnitude of the adverse effect given that the fish is exposed to the hazard
[7] Magnitude of the adverse effect given that the fish is exposed to the hazard

4	Poor water quality (O2, TOC, ammonia, suspended solids, temperature)	0.75	0.25	2.50	25
5	Contact with the physical structures	1.00	0.05	2.50	50
	Transfer				
6	Pumping	50.00	50.00	50.00	50
7	Poor equipment design (adjusted to the size of the fish)	0.25	0.01	0.50	50
8	Delay in pipe or screw	0.38	0.04	0.68	75
9	Dip-Netting	1.67	0.83	3.33	17
	Holding unit				
10o	Poor water quality (pH, TOC, DO, water temp, CO2, ammonia)	1.50	0.50	2.50	50
11	Dip-Netting in holding unit	1.67	0.83	3.33	17

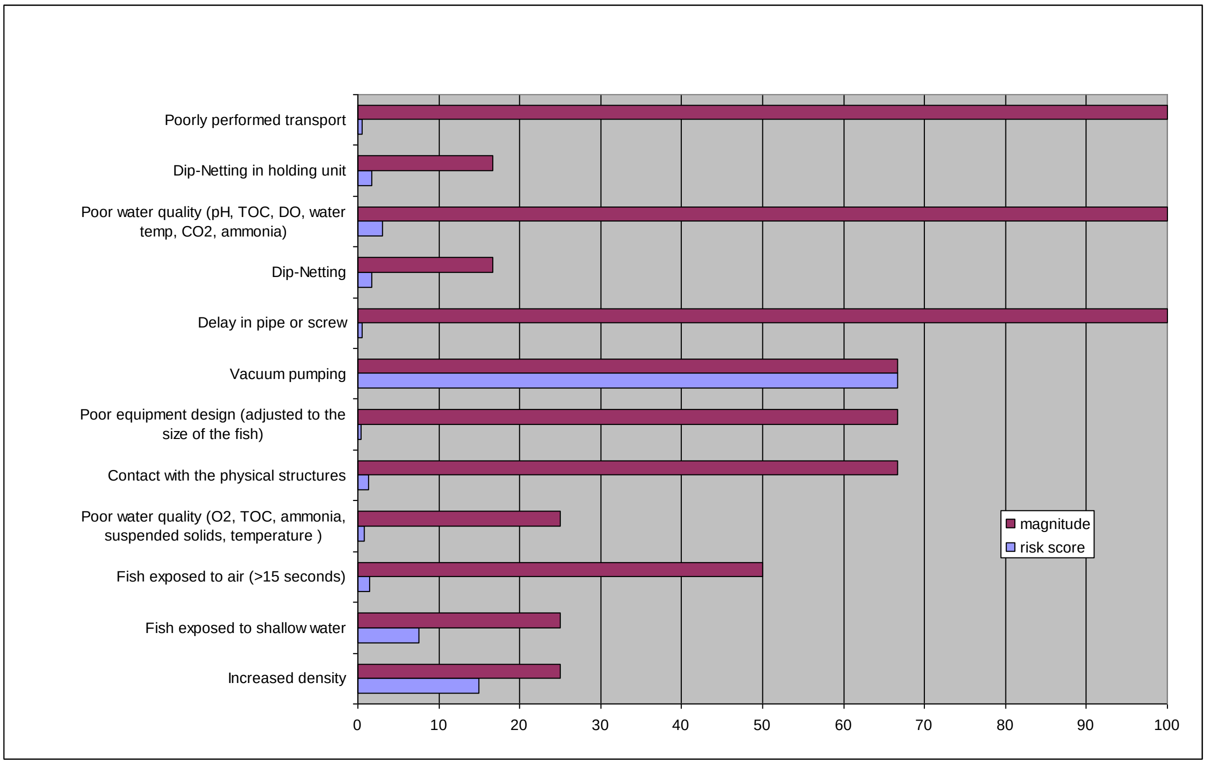

Figure 3. Magnitude and risk scores for pre-slaughter hazards (off site)

Of the twelve pre-slaughter hazards, pumping fish (to transfer fish e.g. out of production ponds into containers) stands out as the highest ranked risk (risk score 67). The second highest ranked risk is the increased density that occurs when fish are crowded at the start of the process of gathering fish. At the same time fish may be exposed to shallow water, causing stress which was the third highest ranked hazard.

A number of the hazards have very high magnitude scores (see Figure 3) indicating that those fish which are affected experience severe adverse effects with high duration scores (3 or 4). However, with the exception of pumping, these hazards have a low probability so have low risk scores.

The hazards were categorised by the stage in the pre-slaughter process and the risk scores summed for the hazards in each category.

Table 7. Sum risk scores for hazards categorised by phase in pre-slaughter period

	Number of hazards	*Sum of risk scores*	
		Off site	On site
crowding	5	26.1	25.8
transfer			
Pumping	3	67.5	50.6
free flowing	2	0.8	0.6
dip-netting	1	1.7	1.7
holding unit	2	4.7	3.2
post-transport condition	1	0.5	n/a

Five of the 12 hazards arise when trout are crowded at the start of the process.

Uncertainty and variability

The exposure was not known with any precision for any of the hazards, except pumping (all the population exposed) and all had wide ranges (see Appendices B and C).

Most of the adverse effects were given an uncertainty of score of three, reflecting the lack of published data available on which to base the estimates of intensity or duration. Because of this uniformity, this has not been analysed.

4.2.2. Stunning and killing hazards

Six stunning and killing method were analysed. The number of hazards associated with each method ranged from one to five (Table 8 and Appendix D).

Table 8. Summary of risk scores for slaughter methods

	Method of killing	Sum of hazard scores (number of hazards)		
		All trout	Large trout	Portion-sized trout
A	Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration		77 (5)	
A’	Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration. Mitigation by reducing duration of air exposure on dry table, or using water flow on the table		32 (5)	
B	Manual percussive stunning -, manual cut and/or evisceration (portion size trout mainly but would also apply to larger trout)	15 (4)	15 (4)	
C	Electrical stunning - in-water (batch) system	22 (4)	23 (4)	
D	Electrical stunning - pipe line system			1 (2)
E	Ice slurry (asphyxia)	133 (2)		
F	Asphyxia	75 (1)		
G	Carbon dioxide	170 (3)	193 (3)	

The details of hazards are provided in the table below and Appendix D. Carbon dioxide has clearly the highest risk score (3 hazards with a total risk score of 170 or 193, for all trout and large trout, respectively). The other methods with high scores are ice slurry (death by asphyxia) (risk score 133), asphyxia (not in ice slurry) (risk score 75) and percussive stunning, semi-automatic (non mitigated 5 hazards with a total risk score of 77). Carbon dioxide has the highest score because not only was it judged that exposure to the gas causes a strong adverse reaction but it does not reliably result in unconsciousness, thus fish may be eviscerated or bled when conscious. Killing by asphyxia is judged to be a severe hazard. Asphyxia in ice slurry had a higher score since the temperature shock was an additional hazard (asphyxia may be less intense if the fish are chilled however it was still given the highest severity score of three). There is no potential to mitigate the risk score, the hazards are inherent to the method. The high score for method A, semi-automatic percussive stunning is mainly attributable to the fish being handled in air on a table (hazards 1 and 2). Percussive methods (A and B) and electrical stunning (C and D) were judged to reliably cause unconsciousness in the vast majority of trout. The electrical stunning methods (C and D) had the lowest scores. The pipeline system (D) had the lowest score (~1) because if does not have the hazards such as crowding and poor water quality which occur in the batch stunning system (D).

Table 9. Details of stunning and killing hazards

		Risk score most likely	min	max	Magnitude	Uncertainty
A	*Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration (large trout only)*					
1	Laying on table	67.50	60.00	71.25	75	2
2	Being handled manually	8.33	8.33	8.33	8	2
3	Mis-stun	1.25	0.75	1.75	25	2
4	Cut when conscious (large trout)	0.38	0.38	0.38	75	2
5	Evisceration when conscious (large trout)	0.03	0.03	0.03	25	2
B	*Manual percussive stunning -, manual cut and/or evisceration (portion size trout mainly but would also apply to larger trout)*					
1	Laying on table	5.00	3.00	7.00	25	2
2	Being handled manually	8.33	8.33	8.33	8	2
3	Mis-stun	1.25	0.75	1.75	25	2
4	Evisceration; if conscious	0.25	0.25	0.25	50	2
5	Cut when conscious (large trout)	0.38	0.38	0.38	75	2
C	*Electrical stunning - in-water (batch) system*					
1	Crowding in the stunning tank	16.67	16.67	16.67	17	2
2	Poor water quality	1.67	0.833	2.50	17	2
3	Mis-stun (insufficient current or voltage)	1.25	0.75	2.00	50	2
4	Mis-cut when conscious (large trout)	3.75	1.50	5.25	75	2
5	Evisceration when conscious (all sizes)	2.50	1.00	3.50	50	2
D	*Electrical stunning - pipe line system (for portion sized trout only)*					
1	Mis –stun 1(insufficient current or voltage)	0.25	0.05	0.38	25	2
2	Evisceration when conscious	0.75	0.10	0.75	50	2
E	*Ice slurry (asphyxia)*					

1	temperature shock (decrease of >10 degrees in water temperature)	33.33	26.67	40.00	67	2
2	Asphyxia	100.00	100.00	100.00	100	1
F	*Asphyxia (no cooling)*					
1	Asphyxia	75.00	75.00	75.00	75	1
G	*Carbon dioxide*	0.00	0.00	0.00	0	
1	exposure to high levels of CO_2	75.00	75.00	75.00	75	1
2	Very low water quality	50.00	50.00	50.00	50	1
3	evisceration if conscious	45.00	42.50	47.50	50	2
4	cut when conscious (large trout)	67.50	63.75	71.25	75	2

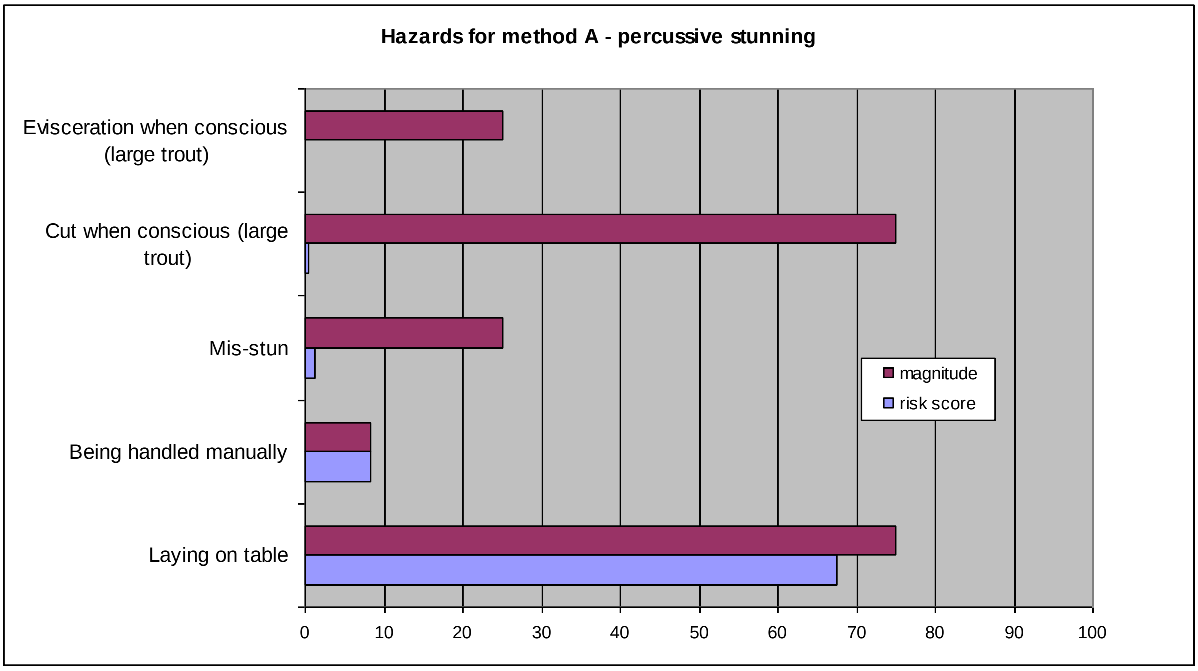

Figure 4. Scores of hazards associated with Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration

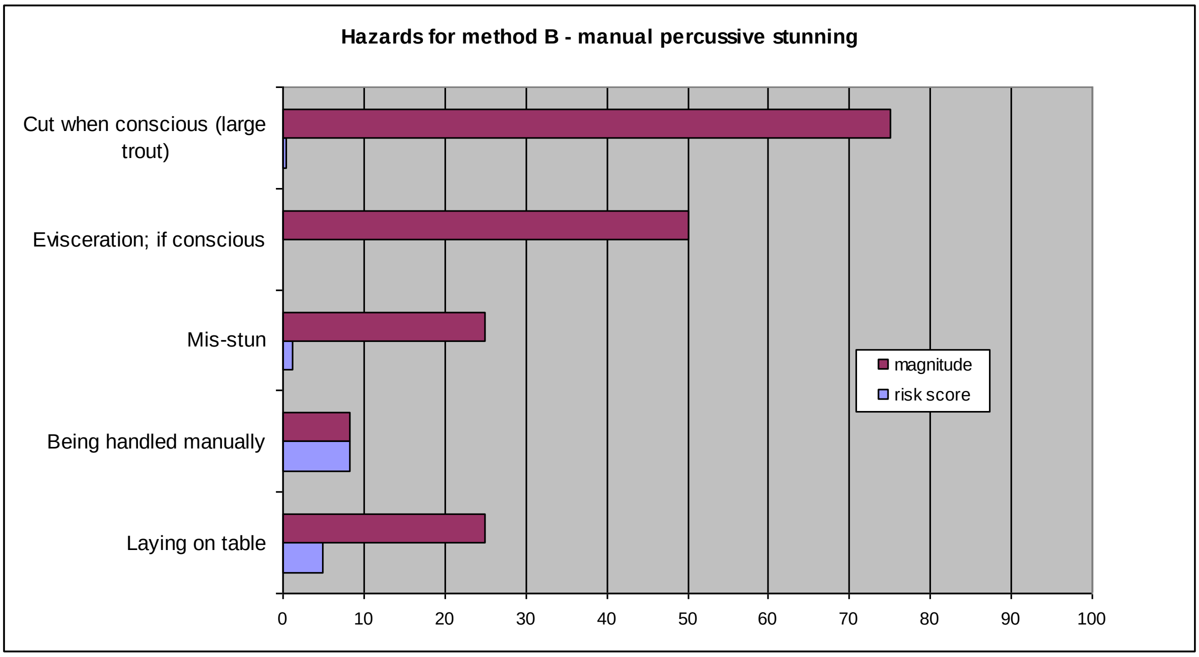

Figure 5. Scores of hazards associated with Manual percussive stunning -, manual cut and/or evisceration (portion size trout mainly but would also apply to larger trout)

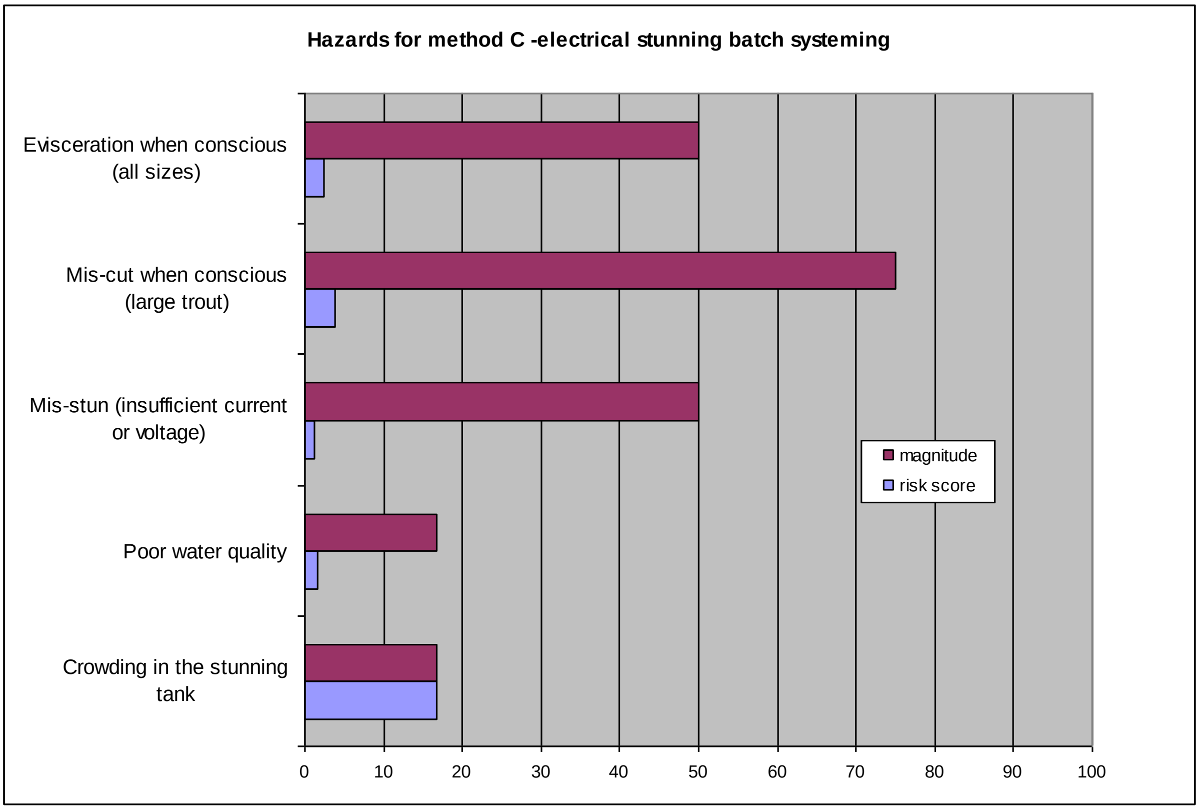

Figure 6. Scores of hazards associated with Electrical stunning - in-water (batch) system

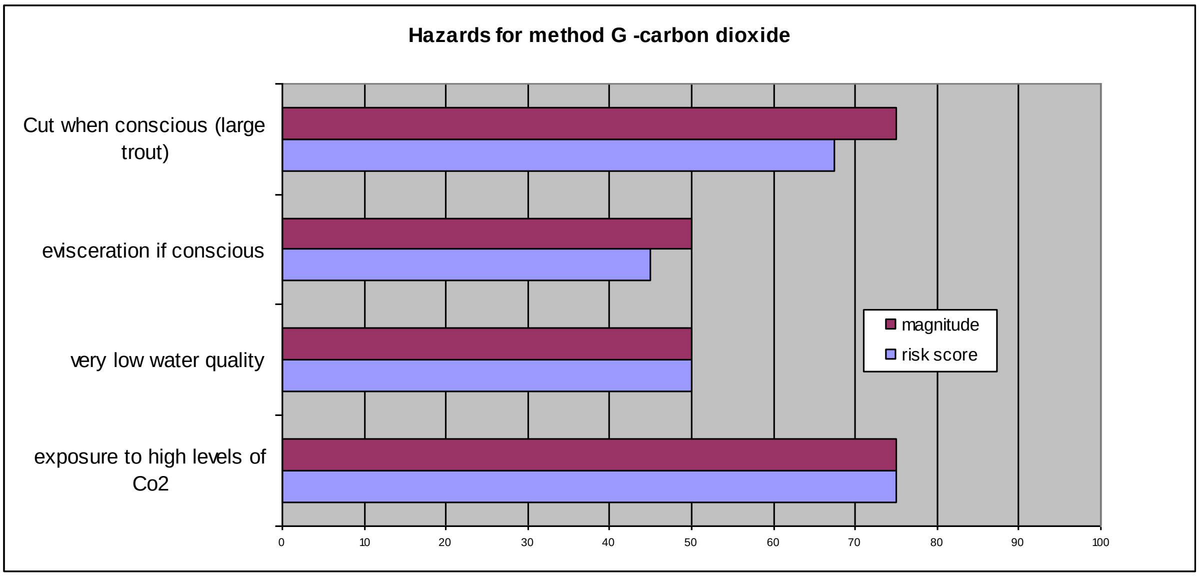

Figure 7. Scores of hazards associated with carbon dioxide slaughter

Uncertainty and variability

The uncertainty score for most hazards was two because the estimates were based on reliable and consistent field observations but few published data are available.

There was little variation around the probability of exposure for the hazards for which all the population is exposed (see Appendix D), e.g. asphyxia and exposure to carbon dioxide.

4.2.3. Pathway analysis

Table 10. Total scores for pre-slaughter pathways (on site)

	On farm slaughter Pre-slaughter						
Pathway	step 1		step 2		step 3		total pre-killing score
	description	score	description	score	description	score	
1	crowding	25.8	pumping	50.6	holding unit	3.2	79.6
2	crowding	25.8	pumping	50.6	no holding unit	0.0	76.4
3	crowding	25.8	netting	1.7	holding unit	3.2	30.7
4	crowding	25.8	netting	1.7	no holding unit	0.0	27.5

Table 11. Total scores for pre-slaughter pathways (off site)

	Off site slaughter Pre-slaughter										
Pathway	step 1		step 2		step 3		step 4		step 5		total pre-killing score
	description	score	description	score	description	score	description	score	description	score	
5	crowding	26.1	pumping	67.5	transport	0.5	pumping	67.5	holding unit	4.7	166.3
6	crowding	26.1	pumping	67.5	transport	0.5	pumping	67.5	no holding unit		161.6
7	crowding	26.1	pumping	67.5	transport	0.5	netting	1.7	holding unit	4.7	100.4
8	crowding	26.1	pumping	67.5	transport	0.5	netting	1.7	no holding unit		95.8
9	crowding	26.1	netting	1.7	transport	0.5	pumping	67.5	holding unit	4.7	100.4
10	crowding	26.1	netting	1.7	transport	0.5	pumping	67.5	no holding unit		95.8

11	crowding	26.1	netting	1.7	transport	0.5	netting	1.7	holding unit	4.7	34.7
12	crowding	26.1	netting	1.7	transport	0.5	netting	1.7	no holding unit		30.0

Table 12. Total scores for emergency killing pathways (including pre-slaughter phases)

	Emergecy killing Preslaughter										
Pathway	step 1		step 2		step 3		step 4		step 5		Total score
	description	score	description	score	description	score	description	score	description	score	
1	crowding	26.1	pumping	67.5	transport	0.5	pumping	67.5	chemical killing	50.0	211.6
2	crowding	26.1	pumping	67.5	transport	0.5	netting	1.7	chemical killing	50.0	145.8
3	crowding	26.1	netting	1.7	transport	0.5	pumping	67.5	chemical killing	50.0	145.8
4	crowding	26.1	netting	1.7	transport	0.5	netting	1.7	chemical killing	50.0	79.9
5	crowding	26.1	pumping	67.5	transport	0.5	pumping	67.5	asphyxia	75.0	236.6
6	crowding	26.1	pumping	67.5	transport	0.5	netting	1.7	asphyxia	75.0	170.8
7	crowding	26.1	netting	1.7	transport	0.5	pumping	67.5	asphyxia	75.0	170.8
8	crowding	26.1	netting	1.7	transport	0.5	netting	1.7	asphyxia	75.0	104.9
9	crowding	25.8	no transference	0	no transport	0	no transference	0	chemical killing	50.0	75.8
10	crowding	25.8	no transference	0	no transport	0	no transference	0	asphyxia	75.0	100.8

4.3. Emergency slaughter

Emergency slaughter by the methods discussed below will be preceded by one of the pre-slaughter pathways.

Two methods of emergency slaughter were considered: exposure to metacaine (or similar chemical) and asphyxia. Use of metacaine has three hazards associated with it (total risk score ~50) and asyphyxia just one (risk score 75) (see Appendix E).

4.4. Comparison of stunning and killing methods

The pre-slaughter pathways are independent of the slaughter methods, i.e. any of the slaughter methods could be preceded by any of the pre-slaughter pathways (described above). The range of total scores for each method (depending on the pre-slaughter pathway) is given in the table below.

Table 13. Total score ranges for pre-slaughter and slaughter pathways (on site)

On farm					
Pre slaughter total scores of pathways 1 to 4		killing method	Sum of risk scores of each killing method	Total score range	
Min	Max			Min	Max
27.5	79.6	A	77	104.5	156.6
27.5	79.6	B	15	42.5	94.6
27.5	79.6	C	22 (23*)	49.5 (50.5*)	101.6 (102.6*)
27.5	79.6	D	1	28.5	80.6
27.5	79.6	E	133	160.5	212.6
27.5	79.6	F	75	102.5	154.6
27.5	79.6	G	170 (193*)	197.5 (220.5*)	249.6 (272.6*)

A: Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration (large trout)
B: Manual percussive stunning – manual cut and /or evisceration (portion size trout mainly but would also apply to larger trout)
C: Electrical stunning – in water (batch) system (*: values for large trout)
D: Electrical stunning – pipe line system (for portion sized trout only)
E: Ice slurry
F: Asphyxia (no cooling)
G: Carbon dioxide (*: values for large trout)

Table 14. Total score ranges for pre-slaughter and slaughter pathways (off site)

Off site slaughter					
Pre slaughter total scores of pathways 5 to 12		Killing method	Sum of risk scores for each killing method	Total score range	
Min	Max			Min	Max
30	166.3	A	77	107	243.3
30	166.3	B	15	45	181.3
30	166.3	C	22 (23*)	52 (53*)	188.3 (189.3*)
30	166.3	D	1	31	167.3
30	166.3	E	133	163	299.3
30	166.3	F	75	105	241.3
30	166.3	G	170 (193*)	200 (223*)	336.3 (359.3*)

A: Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration (large trout)
B: Manual percussive stunning – manual cut and /or evisceration (portion size trout mainly but would also apply to larger trout)
C: Electrical stunning – in water (batch) system (*: values for large trout)
D: Electrical stunning – pipe line system (for portion sized trout only) (*: values for large trout)
E: Ice slurry
F: Asphyxia (no cooling)
G: Carbon dioxide (*: values for large trout)

5. Reference to welfare indicators and to new scientific development

Welfare indicators have not been satisfactorily assessed and validated so far. Nevertheless, observation of fish response was taken into account in this approach.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. This Scientific Opinion on stunning and killing of freshwater rainbow trout evaluated the methods currently used in farmed trout in Europe. Methods used in other fish species other than those described in this Opinion may also be applicable to trout.
2. During pre-slaughter, crowding and transport appear to significantly increase the risk of poor welfare of trout. Crowding appears as a major welfare issue.
3. During transfer of trout, pumping is likely to result in poor welfare of trout more than with free-flow or small scale dip-netting.
4. For pre-slaughter, transport increases risk of poor welfare.
5. Proper application of percussive and electric stunning ensures effective stunning of trout prior to their killing.
6. For semi-automatic percussive method, fish potentially stay exposed to air for some seconds or minutes on a table; this method may also result in poor welfare of the fish.
7. CO2, asphyxia on ice and asphyxia are the methods resulting in the poorest welfare.
8. Properly applied percussive and electric stunning induce immediate loss of consciousness and therefore result in better welfare.
9. Prolonged feed deprivation of more than 50 degree days can result in the utilisation of body fat reserves and then functional tissue; the latter is associated with poor welfare.
10. Currently it is not possible to identify welfare indicators that could be used to monitor slaughter procedures.
11. During stunning and killing usually water temperature ranges between 2 and 20 C, which has a great influence on the physiological responses of trout. At higher temperatures trout are more easily stressed.
12. If trout is out of the water for longer than 10 seconds, pathological changes may be observed within gill tissues.
13. At present there are no validated and robust indicators available to evaluate in practice the welfare of trout associated with slaughter procedures.

RECOMMENDATIONS

1. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and relevant practical welfare indicators developed.
2. Since the welfare of all farmed fish species studied has been found to be poor when they are killed by being left in air (asphyxia) or when they are exposed to carbon dioxide in water, these methods should generally not be used for any species as alternative methods are available.
3. A surveillance (monitoring) programme should be initiated so that data is available in the future for an improved risk assessment and for determining improvements over time and also for benchmarking for those involved in the slaughter of fish.

4. The opportunity to develop new methods for slaughtering trout is considerable and should be encouraged.
5. Valid, robust and practically feasible indicators to evaluate the welfare of trout during slaughter procedures need to be developed
6. Persons involved in killing fish should be trained and hence skilled in handling and welfare.
7. Handling fish intended for slaughter can cause a number of hazards. Exposing trout to air or shallow water which restricts the movements of the fish should be avoided. Crowding is inevitable but efforts should be made to minimise the stress caused.
8. During slaughter procedures, trout should not be exposed to air for longer than 10 seconds before stunning.
9. Pre-slaughter transport should be avoided when possible.
10. During the pre-slaughter period, transfer should preferably be by free-flowing water or small scale dip-netting. Pumping and large scale netting of fish should be avoided, other methods are preferable.
11. Crowding appears as a major welfare issue during pre-slaughter. This is a necessary step but should be performed with utmost care to ensure best welfare of trout.
12. Percussive and electric stunning must be properly performed to ensure best practices and lowest adverse effect on welfare of trout.
13. For semi-automatic percussive method, the risk of poor welfare could be significantly reduced by avoiding exposure to air on the stunning table. For example, reducing the duration of exposure to air or maintaining water flow over the fish would improve welfare.
14. Persons involved in killing fish should be trained and hence skilled in handling and welfare.
15. All slaughter procedures should take into account the water temperature which affects the physiology of the fish. Trout should preferably be slaughtered at temperature between 2 and 20 C.

Recommendations for further research

1. The following areas for further research were identified:
2. Welfare indicators are needed to be developed and validated. Such indicators would need to be compatible with practical use on farm.
3. Research should provide evidence based information on loss of consciousness for the different stunning methods applied to trout, under a range of temperature conditions.
4. Research should be conducted to assess welfare aspects of new stun/kill methods (e.g. CO, NO).
5. Emergency killing methods that are compatible with humane killing should be clearly established.
6. There is a need to improve methods for transfer of fish (pumping). Further research is needed to address this issue from welfare point of view.

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APPENDICES

APPENDIX A. RISK ASSESSMENT APPROACH

Introduction

Overall the risk assessment was constrained due to limited scientific data and consequently a semi-quantitative assessment was carried out often based on expert opinion. Because of this lack of data, the Panel on Animal Health and Welfare recommends that a surveillance / monitoring programme should be initiated for all the fish species so that in the future it may be possible to carry out a quantitative risk assessment.

In this section, the risk assessment method used to assess the risk to welfare of farmed fish at the time of killing is described.

Risk assessment is a systematic, scientifically based process to estimate the probability of exposure to a hazard, and the magnitude of the effects (consequences) of that exposure. A hazard in animal welfare risk assessment may be defined as a factor with the potential to cause a negative animal welfare effect (adverse effect). Risk is a function of both the probability that the hazard and the consequences (characterised by the adverse effect) occur.

Three parameters were scored to assess the importance of a hazard; the intensity of the adverse effect that the hazard causes, the duration of the adverse effect and the probability of exposure to the hazard. The population in question is the fish killed in the EU by the selected method of stunning and slaughter.

The probability of exposure to the hazard corresponds to the percentage of all fish exposed to the hazard. Thus if 4% of the all the fish killed by a particular method are exposed to a hazard there is a probability of 0.04 that any randomly selected fish within that population is exposed. The consequence of exposure can be assessed by scoring the intensity and the duration of the adverse effect in the individual. The risk assessment was based on two assumptions;

1. all fish exposed to the hazard experienced the same intensity and duration of the adverse effect.
2. in the absence of any evidence to the contrary, it is assumed that all fish exposed to the hazard experience the adverse effect[8].

Factors which adversely affect fish welfare are considered in the risk assessment. In absence of reliable data, the volume of fish slaughtered by each method is not taken into account. Thus the results are not weighted by the volume of fish slaughtered by each method.

The definitions of intensity and the categories for duration of the adverse effect used for the fish species considered in this scientific opinion are in the relevant section in each Scientific Opinion.

In the following paragraphs the risk assessment process for hazard identification and characterization and the probability of exposure to the hazard are described as well as the way they were scored. Finally the risk scoring process is described.

The general risk assessment is in line with the approach previously used in the EFSA welfare reports (EFSA, 2007a; EFSA, 2007b; EFSA 2007c; EFSA, 2008a; EFSA, 2008b; EFSA,

[8] if this assumption was not found to be sound for a particular hazard an additional parameter (probability that exposure resulted in the adverse effect) was used.

2008c; EFSA, 2008d; EFSA, 2008e) with some modifications according to the risk question posed.

Hazard identification

The objective of the hazard identification is to identify potential welfare hazards associated with each stunning and killing method. The identification was based on a review of the literature and field observations. The scope of the risk assessment included the period leading up to killing (which may be the time spent in lairage for fish killed in a slaughterhouse). The adverse effect caused by each hazard is described. In order to consistently identify hazards associated with stunning and killing, the relationship between the time from applying a stun method, unconsciousness and the point at which the killing method was applied are illustrated graphically (Figure 5).Various scenarios (A to E) in which hazards may arise were identified as follows:

'A' where a fish is killed in some potentially painful way (asphyxia, bleeding out) while it is conscious i.e. before it has been made unconscious; and

'B' represents a fish that has been stunned and is killed or it dies after it is unconscious;

'C' where a fish has been stunned but it recovers consciousness and is killed in some potentially painful way (asphyxia, bleeding out).

'D' represents a fish that, like A is killed in some potentially painful way (asphyxia, bleeding out) while it is conscious but has also suffered from the aversive nature of the stunning method; and

'E' represents a fish that has been stunned and is killed or it dies after it is unconscious but has also suffered from the aversive nature of the stunning method.

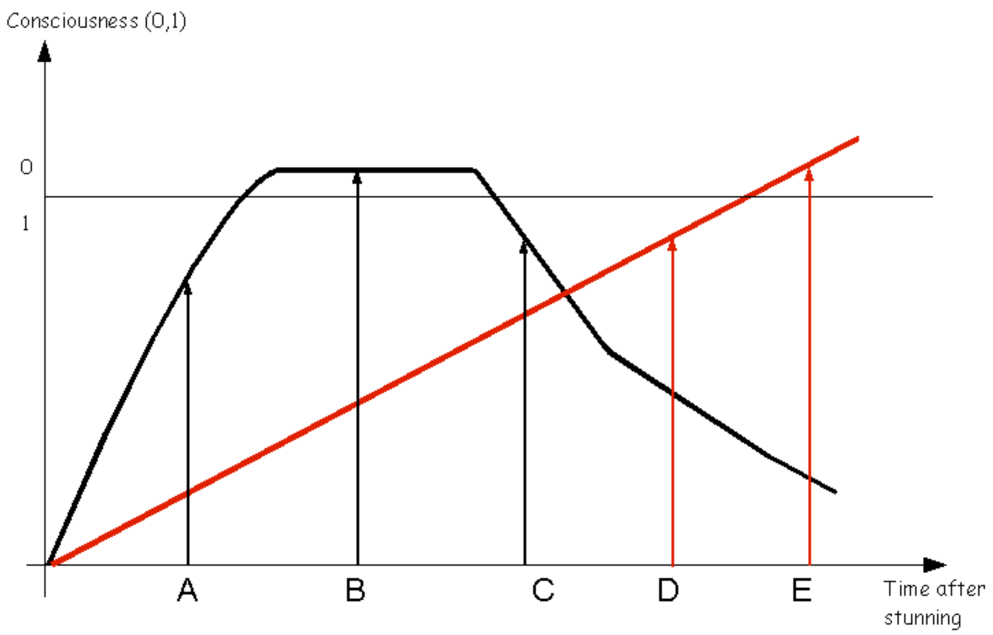

Figure 8. Time to unconsciousness (insensibility) following stunning / killing (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect).

The scenarios above do not take into account hazards arising from gathering animals during pre-slaughter or killing without stunning.

Table 15. Identification of hazards associated to pre-slaughter phases for trout

Hazard number	Identification of hazard	Description of the hazard
	Crowding	
1	Increased density	Stressed fish
2	Fish exposed to shallow water and	Primary stress reaction because of loss of water column.
	Fish exposed to air	Extreme stress, suffocation, physical collapse of gill structure
3	Poor water quality (O_2, TOC, ammonia, suspended solids, temperature)	Hypoxia, leading to acidosis, ammonia self intoxication. panic and respiratory distress. In this process may make the fish more susceptible to secondary infections if not being killed immediately after. Biological hazards (fungi, bacteria) may cause disease outbreaks.
4	Contact with the physical structures	Abrasion
5	Netting and lifting	Abrasion, exhaustion, may be exposure to air. Loading of the net and crushing the trout on the net.
	Transfer by pumping, netting or free flow	
6	Poor equipment design (adjusted to the size of the fish)	The width of the pipe should be appropriate, the inner surface should be smooth, not to cause injuries, sharp angles, junctions between pipes may damage the body surface, avoid high drops.
7	Delay in pipe or screw	Stops between pumping sessions (may last from 5 to 30 minutes, e.g. lunch break, shifts etc.): fish gets stuck. Lack of oxygen, hypoxia and stress.
8	Fish remaining in the pipe	Stop at the end of the pumping (may last for longer, even can cause death): fish gets stuck. Lack of oxygen, hypoxia and stress.
9	Getting stuck in vacuum pressure valve	Fish will get heavily injured or killed. This does not apply with the screw
10	Netting	Abrasion, exhaustion, may be exposure to air. Loading of the net and crushing the trout on the net.
	Grading	
11	Handling, exposure to air	Stress, potential physical damage.
	Holding unit	
12	Poor water quality (pH, TOC, DO, water temp, CO2, ammonia)	Hypoxia, leading to acidosis, ammonia self intoxication. Panic and respiratory distress. In this process may make the fish more susceptible to secondary infections if not being killed immediately after. Biological hazards (fungi, bacteria) may cause disease outbreaks.
13	Netting	Abrasion, exhaustion, may be exposure to air. Loading of the net and crushing the trout on the net.
	Transport	
14	Fish is in metabolic stress (e.g. after a not-well performed closed transport)	Osmo-regulatory imbalance, acidosis; Always fish dying from this step (below 1%)
15	Fish is injured	Scales off is the major injury due to crowding and pumping; minor injuries to the skin; major injuries to the skin and muscle and bones (haemorrhages, oedema, broken backs). Always fish dying from this step (below 1%)
16	Cage towing	Fresh water lake

Table 16. Identification of hazards associated to methods for stunning and killing of trout

Hazard number	Identification of hazard	Description of the hazard
	Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration	
1	Being handled manually	Distress because of being held in air and handled
2	Asphyxia	Being in air
3	Mis-stun	Too low pressure in the pressure chamber (sudden drop below 7-8 bars), hammer missed the correct location on skull, wrong orientation of the fish.
4	Not stunned (=cut if conscious)	Some fish may pass by the operator supposed to stun
5	Mis-cut; if conscious (this includes recovery of consciousness)	Failure to cut the gill arch (unsharpened knife, partial cut).
6	Exsanguination; if conscious	Fish loose gradually consciousness (10 minutes) as it bleeds out in the exsanguination tank. This also includes asphyxia in the bleeding tank.
7	Evisceration; if conscious	Failure to kill by percussive blow, or exsanguinate, or asphyxia, prior to evisceration.
	Manual percussive stunning -, manual cut and/or evisceration (portion size trout mainly but would also apply to larger trout)	
8	Being handled manually	Distress because of being held in air and handled, bigger the fish more difficult handling is
9	Asphyxia	Being in air
10	Mis-stun	Fatiguing work and exhausted staff, too high pace
11	Mis-cut; if conscious	Failure to cut the gill arch (unsharpened knife, partial cut).
12	Not stunned (=cut if conscious)	Some fish may pass by the operator supposed to stun
13	Evisceration; if conscious	Failure to kill by percussion prior to evisceration.
14	Exsanguination if conscious	Failure to kill by percussion prior to exsanguination
	Electrical stunning - in-water (batch) system	
15	Crowding in the stunning tank	High density of fish, short term stress from crowding.
16	Poor water quality	Insufficient water renewal
17	Mis-stun (insufficient current or voltage)	Electrical stimulation leading to some degree of exhaustion (low current or voltage). Current is too low to stun the fish in less that 1 second. The animal can consciously feel the electricity. Escape behaviour, pain, distress, exhaustion.
18	Exsanguination; if conscious	Fish loose gradually consciousness (10 minutes) as it bleeds out in the exsanguination tank. This also includes asphyxia in the bleeding tank.- Fish may recover from the stunning (a number of fish may recover after 2 minutes if duration of stunning was too short).
19	Mis-cut; if conscious	Failure to cut the gill arch (unsharpened knife, partial cut). - Fish may recover from electrical stunning.

32	Evisceration; if conscious	Failure to kill by percussive blow, or exsanguinate, or asphyxia, prior to evisceration..
33	Asphyxia; if conscious	If mis-cut happens, animals may die from asphyxia due to poor water quality in the exsanguination tank.
	Electrical stunning - pipe line system	
34	No stun (insufficient current or voltage)	Complete failure of the system - no stunning
35	Mis –stun 1(insufficient current or voltage)	Delayed stunning because of low level of field strength (stunning after 1 second)
36	Mis-stun 2	Insufficient stunning.because of a heterogenic electric field. Smaller fish may more likely escape the field and not get properly stunned.
37	Poor pipe design	The inner surface of the pipe must not be abrasive
38	Exsanguination; if conscious	Fish loose gradually consciousness (10 minutes) as it bleeds out in the exsanguination tank. This also includes asphyxia in the bleeding tank.
41	Evisceration; if conscious	Failure to kill by percussive blow, or exsanguinate, or asphyxia, prior to evisceration.
42	Asphyxia; if conscious	If mis-cut happens, animals may die from asphyxia due to poor water quality in the exsanguination tank..

Table 17. Identification of hazards associated to methods for emergency killing of trout, degraded fish (not for human consumption).

	Asphyxia	
1	Asphyxia, exposure to air	Only for rejected fish. Same as 30. Fish is exposed to air. By definition asphyxia and being taken out of water always result in adverse effects (collapse of the gills, hypoxia, etc…).
	Pharmaceutical methods	
2	Exposure to the chemical	Aversive effect due to the presence of the chemical substance.
3	Netting	Abrasion, exhaustion, may be exposure to air. Loading of the net and crushing the trout on the net.
4	Crowding including too low water levels	Stressed fish potentially physically damaged between fish
5	Poor water quality	Hypoxia, leading to acidosis, ammonia self intoxication. panic and respiratory distress.
6	Mis stun (Insufficient concentration of chemical and or insufficient exposure to the chemical)	They would die from asphyxia. Over excitement phase leading to stress..

Hazard characterisation

Intensity

If a fish is unconscious, by definition there is no adverse welfare effect at that time. Therefore, before assessing the intensity of any adverse effects, consideration must be given as to whether the fish is conscious or not; this is a binary judgement (i.e. degrees of un/consciousness are not assessed). There is evidence that signs associated with consciousness and unconsciousness at the time of killing apply to all fish species as they do for general anaesthesia (Kestin et al., 2002). If it is conscious, the appropriate score for the degree of intensity of the adverse effect must be selected: mild, moderate or severe. If unconsciousness is achieved or induced with no suffering, or any pain or distress is for less than one second, then it is assumed that there was no welfare hazard. The issue of consciousness is mainly relevant to hazards associated with the killing method. If unconsciousness was achieved immediately (less than one second) then it is assumed that there was no hazard associated with the proper and effective application of that method and so this was not included in the risk assessment.

Generic guidelines for defining intensity categories for pre-slaughter hazards and slaughter hazards are given in Table 10. The approach taken has been to define only the mild and severe categories; the moderate is defined as being neither mild nor severe. Thus, by default hazards which are considered to have welfare consequences which are not in the severe or mild category fall into the moderate category. This approach was taken as scientists are reasonably confident in recognising the extreme states of intensity but as these states are on a continuum, allocating a distinct moderate banding is more difficult and contentious. Appropriate descriptions for the categories of intensity will vary between species and are given for each species in the Scientific Opinion.

Additionally, different definitions of intensity for the same species may be required for hazards that occur before killing, compared with at the time of killing. The descriptions of intensity for these pre-slaughter adverse effects are given for each species in the Scientific Opinion.

Table 18. Observable signs considered by experts when scoring the intensity of an adverse effect in farmed fish arising from hazards associated with the pre-slaughter or slaughter period

Evaluation	Score	Description
Mild	1	The animal is minimally affected as evidenced by minor changes in behaviour (e.g. rapid swimming away from stimulus and then slowing down, eye position normal).
Moderate	2	The animal is affected as evidenced by behaviour changes which can be considered moderate (more pronounced than minor but not severe).
Severe	3	The animal is affected greatly, as evidenced by marked changes from normal behaviour (e.g. energetic and purposeful escape behaviour, eyes rolling, rapid and erratic swimming, swimming upside down or tilted, colliding with the net, stopping swimming for more than 5 secs, crowding of fish)

Finally, each hazard was assessed and ranked by magnitude and occurrence independently of other hazards. For some hazards there may be more than one adverse effect. For example, all fish netted will be exposed to air, but in addition they may be injured e.g. skin lesions due to contact with the net or other fish.

The duration of the adverse effect

The time during which an animal will on average experience the adverse effect was estimated in minutes. The duration of an adverse effect can be longer than the duration of the hazard, for example a miss-stun takes a fraction of a second but the adverse effect lasts until the animal is unconscious or dies. Thus the duration of the hazard is included in the duration of the adverse effect.

Different time periods may be used for the adverse effects arising from pre-slaughter hazards compared with the hazards associated with slaughter. The definitions of duration used are given in the relevant section of the Scientific Opinion (Table 5).

Exposure assessment

The exposure assessment is performed by assessing the proportion of the population of interest (i.e all fish in the EU being killed by the method in question) that is likely to experience the hazard. This proportion is equal to the probability of exposure to the hazard (P_hazard). It is recognised that the proportion of the population exposed to a selected hazard will vary depending on the farm of origin and slaughterhouse. Estimates of the most likely, maximum and minimum values for this proportion are required. The range of values provides an indication of the uncertainty of the estimate (see next section).

Uncertainty and variability

The degree of confidence in the final estimation of risk depends on the uncertainty and variability (Vose, 2000). Uncertainty arises from incomplete knowledge and/or when results are extrapolated from one situation to another (e.g. from experimental to field situations) (Vose, 2000). Uncertainty can be reduced by carrying out further studies to obtain the necessary data, however this may not always be a practical possibility. It can also be appraised by using expert opinion or by simply making a judgment.

Variability is a statistical and biological phenomenon and is not reducible by gathering further information. The frequency and severity of welfare hazards will inevitably vary between farms and countries and over time, and fish will vary individually in their responses. However, it is not always easy to separate variability from uncertainty. Uncertainty combined with variability is generally referred to as total uncertainty (Vose, 2000).

Total uncertainty associated exposure to the hazard was captured by estimates of the maximum and minimum estimates of the most likely value of the proportion of the population exposed to the hazard. For the other parameters (intensity and duration of the adverse effect) total uncertainty was scored on a scale of 1-3 (table 11).

Table 19. Scoring system for total uncertainty in intensity and duration of effect

Evaluation	Score	Description
low	1	Solid and complete data available; strong evidence in multiple references with most authors coming to the same conclusions, or Considerable and consistent experience from field observations.
medium	2	Some or only incomplete data available; evidence provided in small number of references; authors' or experts' conclusions vary, or Limited evidence from field observations, or Solid and complete data available from other species which can be extrapolated to the species being considered
high	3	Scarce or no data available; evidence provided in unpublished reports, or Few observations and personal communications, and/or Authors' or experts' conclusions vary considerably

Risk Characterisation

The scoring process

The scoring was undertaken by the working group in plenary. The estimates were based on current scientific knowledge, published data, field observation and experience (as summarised in this report).

Calculation of the risk score

All three factors (probability of exposure to the hazard; intensity of adverse effect; duration of adverse effect), were included in calculating the final risk score of a hazard. The score for each parameter was standardised by dividing the score by the maximum possible score for that parameter. Thus all parameters have a maximum value of one. The risk score is the product of the standardised scores multiplied by 100 (for ease of comparison) and thus has a maximum value of 100.

Risk score = [*(I_adverse_effect /3) * (D_adverse_effect / 4)* (P_hazard)*] * 100

Where the following are defined:

the intensity of the adverse effect *(I_adverse_effect)*

the duration of the adverse effect *(D_adverse_effect)*

the probability of exposure to the hazard *(P_hazard)*

The minimum, most likely and maximum values for *P_hazard* were used to generate minimum, most likely and maximum estimates of the risk score. If only one risk score is given it refers to the most likely. It is also assumed that hazards usually occur independently of each other.

Calculation of magnitude of adverse effect

The magnitude of the adverse effect is the product of the scores for intensity and duration according to the following formula:

Magnitude score = [(*I_adverse_effect* /3) * (*D_adverse_effect* / 4)] * 100

It has a maximum score of 100. The magnitude provides an indication of the impact of the hazard on the fish which are exposed to the hazard and experience the adverse effect. Thus a hazard that causes a prolonged and severe adverse effect but which affects only a small proportion of the population will have a low risk score but a high magnitude of severity score**.**

Worked example – mis-stun

Mis-stun may result when a concussive stunning method is used. This will give rise to an adverse effect. It was estimated that the adverse effect had a intensity score equal to 3. The duration (time from mis-stun to death or re-stun) was judged to last between one and two minutes, hence a score of 3. It was estimated that the probability that the hazard occurs was 0.04 (i.e. 4% of fish suffer a mis-stun), with minimum and maximum estimates of 0.01 and 0.10, respectively. In summary:

- score for the intensity of the adverse effect (*I_adverse_effect*) = 3
- score for the duration of the adverse effect (*D_adverse_effect*) = 3 (between one and two minutes)
- the probability that the hazard occurs (*P_hazard*) = 0.04

(ranging from a minimum estimate of 0.01 to a maximum estimate of 0.10)

Thus the risk score for this example mis-stun is:

(3/3 * 3/4 *0.04) * 100 = (1 * 0.75 * 0.04) * 100 = 3

This score has a range that is determined by the minimum and maximum estimates of the probability that the hazard occurs (*P_hazard*), 0.01 and 0.10 respectively.

Minimum score = (3/3 * 3/4 *0.01) * 100 = 0.75

Maximum score = (3/3 * 3/4 *0.1) * 100 = 7.50

The magnitude equals intensity score/3 * duration score/4 * 100; and in this example is 75:

(3/3 * 3/4) *100 = 75

Interpretation of the risk score

Due to the limited amount of quantitative data on many effects of hazards on fish stunning and killing, the risk assessment was mainly based on expert opinion. The methodology used does not give a precise numerical estimate of the risk attributed to certain hazards; however the output can be used to rank the problems and designate areas of concern, as well as, guidance for future research. The methodology does not take into account interactions between factors and assumes linearity in the scores. These assumptions cannot be tested. Secondly, the risk scoring is semi-quantitative. Thus the scores allow a ranking but the absolute figures are not on a linear scale (e.g. a risk score of 12 should not interpreted as being twice as important as a risk score of 6).

One key objective of this work is to compare different methods of stunning and slaughter within each species. This will be achieved by summing the risk scores for all the hazards arising for each method of stunning and slaughter. This figure will be used to rank and compare the methods. Risk scores are given for the commonly used methods (see Table 9). However, it should be noted that insufficient data were available to calculate the overall exposure to the hazard within the European population, i.e. how commonly are those methods actually used within the member states of the EU. For comparison purposes, this calculation is important as it quantifies more precisely the number of fish at risk for that particular method of slaughter. Moreover, a hazard with a small risk score but a high magnitude may still have serious welfare effects for a large number of fish. The converse is also true.

APPENDIX B: RISK, MAGNITUDE AND UNCERTAINTY SCORES FOR PRE-SLAUGHTER HAZARDS (OFF-SITE)

Table 20. Risk, magnitude and uncertainty scores for pre-slaughter hazards (off-sites)

	Hazard	Description of adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability of the exposure to the hazard			Risk score			Magnitude	Uncertainty
				min	score								
			1 mild, 2 moderate, 3 severe		1 = <5min, 2 = 5-10min, 3 = 10-30 min, 4 = >30 min	most likely	min	max	most likely	min	max		
	CROWDING												
1	Increased density	Stressed fish	1	30	3	0.6	0.5	0.75	15.00	12.50	18.75	25	3
2	Fish exposed to shallow water	Primary stress reaction because of loss of water column.	1	20	3	0.3	0.1	0.4	7.50	2.50	10.00	25	3
3	Fish exposed to air (>15 seconds)	Extreme stress, suffocation, physical collapse of gill structure	3	7	2	0.03	0.01	0.05	1.50	0.50	2.50	50	1

4	Poor water quality (O_2, TOC, ammonia, suspended solids, temperature)	Hypoxia, leading to acidosis or ammonia self intoxication, panic and respiratory distress.	3	2	1	0.03	0.01	0.1	0.75	0.25	2.50	25	2
5	Contact with the physical structures	Abrasion	2	120	4	0.02	0.001	0.05	1.33	0.07	3.33	67	2
	TRANSFER												
6	Vacuum pumping	stress and / or physical damage	2	120	4	1	1	1	66.67	66.67	66.67	67	2
7	Poor equipment design (adjusted to the size of the fish)	High drops and/or sharp angles and/or junctions between pipes causing damage to the body surface.	2	120	4	0.005	0.0001	0.01	0.33	0.01	0.67	67	3
8	Delay in pipe or screw	Stops between pumping sessions (may last from 5 to 30 minutes, e.g. lunch break, shifts etc.): fish gets stuck. Lack of oxygen, hypoxia and stress.	3	120	4	0.005	0.0005	0.009	0.50	0.05	0.90	100	3

9	Dip-Netting	Abrasion, exhaustion, exposure to air. Loading of the net and crushing the trout on the net.	2	1	1	0.1	0.05	0.2	1.67	0.83	3.33	17	3
	HOLDING UNIT												
10	Poor water quality (pH, TOC, DO, water temp, CO2, ammonia)	Hypoxia, leading to acidosis, ammonia self intoxication, panic and respiratory distress.	3	120	4	0.03	0.01	0.05	3.00	1.00	5.00	100	3
11	Dip-Netting in holding unit	Abrasion, exhaustion, exposure to air. Loading of the net and crushing the trout on the net.	2	1	1	0.1	0.05	0.2	1.67	0.83	3.33	17	3
	POST-TRANSPORT CONDITION												
12	Poorly performed transport	Osmoregulatory imbalance, acidosis; some fish dying from this step (below 1%)	3	120	4	0.005	0.0001	0.01	0.50	0.01	1.00	100	3

APPENDIX C: RISK, MAGNITUDE AND UNCERTAINTY SCORES FOR PRE-SLAUGHTER HAZARDS (ON-SITE)

Table 21. Risk, magnitude and uncertainty scores for pre-slaughter hazards (on-sites)

	Hazard	Description of adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability of the exposure to the hazard			Risk score			Magnitude	Uncertainty
				minutes	score								
			1 mild, 2 moderate, 3 severe		1 = <5min, 2 = 5-10min, 3 = 10-30 min, 4 = >30 min	most likely	Min	max	most likely	min	max		
	CROWDING												
1	Increased density	Stressed fish	1	30	3	0.6	0.5	0.75	15.0	12.5	18.75	25.00	3
2	Fish exposed to shallow water	Primary stress reaction because of loss of water column.	1	20	3	0.3	0.1	0.4	7.5	2.5	10.00	25.00	3
3	Fish exposed to air (>15 seconds)	Extreme stress, suffocation, physical collapse of gill structure	3	7	2	0.03	0.01	0.05	1.5	0.5	2.50	50.00	1
4	Poor water quality (O_2, TOC, ammonia, suspended solids, temperature)	Hypoxia, leading to acidosis or ammonia self intoxication, panic and respiratory distress.	3	2	1	0.03	0.01	0.1	0.75	0.25	2.50	25.00	2

5	Contact with the physical structures	Abrasion	2	20	3	0.02	0.001	0.05	1.0	0.05	2.50	50.00	2
	TRANSFER												
6	Vacuum pumping	stress and / or physical damage	2	20	3	1	1	1	50.0	50.0	50.00	50.00	2
7	Poor equipment design (adjusted to the size of the fish)	High drops and/or sharp angles and/or junctions between pipes causing damage to the body surface.	2	20	3	0.005	0.0001	0.01	0.25	0.01	0.50	50.00	3
8	Delay in pipe or screw	Stops between pumping sessions (may last from 5 to 30 minutes, e.g. lunch break, shifts etc.): fish gets stuck. Lack of oxygen, hypoxia and stress.	3	20	3	0.005	0.0005	0.009	0.38	0.04	0.68	75.00	3
9	Dip-Netting	Abrasion, exhaustion, exposure to air. Loading of the net and crushing the trout on the net.	2	1	1	0.1	0.05	0.2	1.67	0.83	3.33	16.67	3
	HOLDING UNIT												

10	Poor water quality (pH, TOC, DO, water temp, CO2, ammonia)	Hypoxia, leading to acidosis, ammonia self intoxication, panic and respiratory distress.	3	10	2	0.03	0.01	0.05	1.50	0.50	2.50	50.00	3
11	Dip-Netting in holding unit	Abrasion, exhaustion, exposure to air. Loading of the net and crushing the trout on the net.	2	1	1	0.1	0.05	0.2	1.67	0.83	3.33	16.67	3

APPENDIX D: RISK, MAGNITUDE AND UNCERTAINTY SCORES FOR STUNNING AND KILLING HAZARDS

Table 22. Risk, magnitude and uncertainty scores for stunning and killing hazards

			Intensity of the adverse effect	Duration		Probability of the exposure to the hazard			Risk score			magnitude	uncertainty
ID	Hazard	Description of adverse effect	score [a]	min	score [b]	most likely	min	max	most likely	min	max		
A	Percussive stunning – semi automatic: hand-fed system, manual cut and/or evisceration (large trout only)												
1	Laying on table	Being in air, asphyxia	3.00	2.50	3.00	0.90	0.80	0.95	67.50	60.00	71.25	75	2
2	Being handled manually	Stress response because of being handled in air	1.00	0.50	1.00	1.00	1.00	1.00	8.33	8.33	8.33	8	2
3	Mis-stun	Severe physical trauma, injury	3.00	0.50	1.00	0.05	0.03	0.07	1.25	0.75	1.75	25	2
4	Cut when conscious (large trout)	Severe physical trauma, injury	3.00	6.00	3.00	0.01	0.01	0.01	0.38	0.38	0.38	75	2
5	Evisceration when conscious (large trout)	Severe physical trauma, injury	3.00	0.50	1.00	0.00	0.00	0.00	0.03	0.03	0.03	25	2
B	Manual percussive stunning -, manual cut and/or evisceration (portion size trout mainly but would also apply to larger trout)												
1	Laying on table	Being in air, asphyxia	3.00	0.50	1.00	0.20	0.12	0.28	5.00	3.00	7.00	25	2
2	Being handled manually	Stress response because of being handled in air	1.00	0.50	1.00	1.00	1.00	1.00	8.33	8.33	8.33	8	2
3	Mis-stun	Severe physical trauma, injury	3.00	0.50	1.00	0.05	0.03	0.07	1.25	0.75	1.75	25	2
4	Evisceration; if conscious	Severe physical trauma, injury	3.00	1.50	2.00	0.01	0.01	0.01	0.25	0.25	0.25	50	2
5	Cut when conscious (large trout)	Severe physical trauma, injury	3.00	6.00	3.00	0.01	0.01	0.01	0.38	0.38	0.38	75	2
C	Electrical stunning - in-water (batch) system												
1	Crowding in the stunning tank	High density of fish, short term stress from crowding.	1.00	1.50	2.00	1.00	1.00	1.00	16.67	16.67	16.67	17	2
2	Poor water quality	Hypoxia, and respiratory distress, gill and skin irritation.	1.00	1.50	2.00	0.10	0.05	0.15	1.67	8.33	2.50	17	2
3	Mis-stun (insufficient current or voltage)	The animal experiences the electrial current causing pain, distress, exhaustion.	3.00	1.50	2.00	0.03	0.02	0.04	1.25	0.75	2.00	50	2
4	Mis-cut when conscious (large trout)	Severe physical trauma, injury	3.00	6.00	3.00	0.05	0.02	0.07	3.75	1.50	5.25	75	2

5	Evisceration when conscious (all sizes)	Severe physical trauma, injury	3.00	1.50	2.00	0.05	0.02	0.07	2.50	1.00	3.50	50	2
D	Electrical stunning - pipe line system (for portion sized trout only)												
1	Mis –stun 1(insufficient current or voltage)	Electrical stimulation leading to pain, distress, exhaustion.	3.00	0.50	1.00	0.01	0.00	0.02	0.25	0.05	0.38	25	2
2	Evisceration when conscious	Severe physical trauma, injury	3.00	1.50	2.00	0.02	0.00	0.02	0.75	0.10	0.75	50	2
E	Ice slurry (asphyxia)								0.00	0.00	0.00	0	
1	Temperature shock (decrease of >10 degrees in water temperature)	Primary stress reaction, circulatory collapse	2.00	10.00	4.00	0.50	0.40	0.60	33.33	26.67	40.00	67	2
2	Asphyxia	Stress response, escape response	3.00	10.00	4.00	1.00	1.00	1.00	100.00	100.00	100.00	100	2
F	Asphyxia (no cooling)												
1	Asphyxia	Stress response, escape response	3.00	4.00	3.00	1.00	1.00	1.00	75.00	75.00	75.00	75	2
G	Carbon dioxide								0.00	0.00	0.00	0	
1	exposure to high levels of Co2	low Ph, strong adversive reaction, ecape behaviour	3.00	3.00	3.00	1.00	1.00	1.00	75.00	75.00	75.00	75	2
2	very low water quality	Hypoxia, and respiratory distress, gill and skin irritation.	2.00	3.00	3.00	1.00	1.00	1.00	50.00	50.00	50.00	50	2
3	evisceration if conscious	Severe physical trauma, injury	3.00	1.50	2.00	0.90	0.85	0.95	45.00	42.50	47.50	50	2
4	Cut when conscious (large trout)	Severe physical trauma, injury	3.00	6.00	3.00	0.90	0.85	0.95	67.50	63.75	71.25	75	2

[a] 1 mild, 2 moderate, 3 severe , [b] 1 = <1min, 2 = 1-2 min, 3 = 2-6min, 4 = >6min

APPENDIX E: RISK, MAGNITUDE AND UNCERTAINTY SCORES FOR EMERGENCY KILLING

Table 23. Risk, magnitude and uncertainty scores for emergency killing

	Hazard	Description of adverse effect	Intensity of the adverse effect [a]	Duration of the adverse effect		Probability of the exposure to the hazard			Risk score			Magnitude	Uncertainty
				min	score[b]	most likely	min	max	most likely	min	max		
	Exposure to chemical								0.00	0.00	0.00	0	
1	Exposure to sufficient concentration of chemical	stress due ionic imbalance, release of stress hormones	2	4	3	0.999	0.999	0.999	49.95	49.95	49.95	50	2
2	Exposure to insufficient concentration of chemical	stress due ionic imbalance, release of stress hormones	2	10	4	0.001	0.001	0.001	0.07	0.07	0.07	67	2
3	Asyphyxia	Stress	3	10	4	0.0001	0.0001	0.0001	0.01	0.01	0.01	100	2
	Asphyxia												
1	Asphyxia, exposure to air	Stress response, escape response	3	4	3	1	1	1	75.00	75.00	75.00	75	2

[a] 1 mild, 2 moderate, 3 severe ; [b] 1 = <1min, 2 = 1-2 min, 3 = 2-6min, 4 = >6min

GLOSSARY / ABBREVIATIONS

Glossary

Adverse effect	The welfare consequences for an animal in terms of pain and distress when exposed to a hazard.
Asphyxia	A process where fish die from hypoxia. This may happen in some species by: taking them out of water; by partially bleeding animals out; by preventing gill movements e.g. crushing; and by reducing oxygen content of the water.
Crowding	Keeping animals at stocking densities that are high or that reduce swimming volume e.g. by hoisting a net.
Depopulation (Emergency killing for disease control)	A process of killing animals for public health, animal health, animal welfare or environmental reasons, sometimes under the supervision of the competent authority.
Dip-net	A net used to dip into a tank or cage to catch fish for the purpose of transfer of fish to another pond or facility or to market or for slaughter.
Duration	Specifically used with ‘intensity’ in the context of evaluating the magnitude of the adverse effect.
Emergency killing	The killing of animals that are injured or have a disease associated with severe pain or suffering and where there is no other practical possibility to alleviate this pain or suffering.
Exposure Assessment	The quantitative and qualitative evaluation of the likelihood of hazards to welfare occurring in a given fish population.
Hazard	Any factor with the potential to cause an adverse welfare effect on fish.
Hazard characterisation	The qualitative and quantitative evaluation of the nature of the adverse effects associated

	with the hazard.
Hazard Identification	The identification of any factor capable of causing adverse effects on fish welfare.
Hypoxia	A condition with low oxygen saturation in the water or a condition with low oxygen saturation in the water (blood).
Intensity	The quality of pain or distress per unit time
Killing	Any intentionally induced process that causes the death of an animal.
Lairage	Short-term storage of fish in a tank or other facility before slaughter. Fish may be subjected to high stocking densities or materials for short periods.
Magnitude of the adverse effects	A function of intensity and duration of welfare impairment for fish.
Pre-slaughter	Anything happening just before stunning, killing or slaughter.
Risk	A function of the probability of an adverse effect and the magnitude of that effect, consequent to a hazard for fish.
Risk Assessment	A scientifically based process consisting of the following steps: i) hazard identification, ii) hazard characterisation, iii) exposure assessment and iv) risk characterisation.
Risk Characterisation	The process of determining the qualitative or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse effects on welfare in a given fish population based on hazard identification, hazard characterisation, and exposure assessment.
Severity	Sometimes used to denote intensity.
Size-grading	Sorting the fish according to size
Slaughter	The killing of animals for human consumption.
Slaughterhouse	Any establishment used for slaughtering fish.
Stocking density:	Number of fish in a defined volume of water.
Stunning	Any intentionally induced process that causes loss of consciousness and sensibility without pain, including any process resulting in instantaneous death.
Uncertainty Analysis	Uncertainty refers to the extent to which data are supported by published evidence. A

	method used to estimate the uncertainty associated with model inputs, assumptions and structure/form. This includes also uncertainty, due to the lack of reliable publications, uncertainty in the scientific results etc.
Variability	The natural biological variation that occurs in a population of animals. Not to be confused with uncertainty as it cannot be reduced by simply decreasing uncertainty.
Visual evoked reflexes (VER)	Evoked EEG activity in the brain with a visual stimulus.

Abbreviations

A	Ampere
AHAW	Animal Health and Welfare
D_adverse	effect the duration of the adverse effect
EFSA	European Food Safety Authority
EEG	Electro-encephalogram
EC	European Commission
ECG	Electro-cardiogram
EU	European Union
mA	milli-Ampere
mV	milli-Volts
MS	Member States
µS	micro-Siemens
P_hazard	L the probability that the hazard occurs
SER	Somato-sensory evoked reflex
SS_adverse	effect the intensity of the adverse effect
TOC	Total organic carbon
V	Volts
VER	Visual evoked reflexes
VOR	Vestibulo-ocular reflex