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SPECIES-SPECIFIC WELFARE ASPECTS OF THE MAIN SYSTEMS OF STUNNING AND KILLING OF FARMED EELS *(Anguilla anguilla)*[1]

Scientific Opinion of the Panel on Animal Health and Welfare

(Question N° EFSA-Q-2008-440)

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PANEL MEMBERS

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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 aspects of the main systems of stunning and killing of farmed eels in the EU.

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 for eels. Areas of welfare concern were identified, as well as guidance for future research. The risk assessment was mainly based on expert opinion, due to the limited amount of quantitative and published peer reviewed data on the effects of the hazards associated with stunning and killing of eels. Pre-slaughter steps, immediately before stunning and killing, which had a direct impact on eel welfare were included in the risk assessment. Stunning and killing methods that are not commercially used in the EU were briefly described but excluded from the risk assessment.

For eels there are four methods currently practised in the EU: 1. Whole body electrical stunning in water with desliming and evisceration; 2. Salt bath, desliming and evisceration; 3. Ammonia, washing and evisceration; and 4. Immobilization by exposure to ice (and salt), washing and evisceration.

The most important hazards in the pre-slaughter stages were associated with unloading and poor water quality in the holding tank during lairage. Therefore, eel welfare is best served if they are slaughtered as soon as possible after arrival at he slaughterhouse.

Currently there are no stunning methods commercially available that immediately induce unconsciousness in all eels until death. Based on the risk assessment, electrical stunning

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immediately followed by a killing method is the preferred practically available method. Electrical stunning methods, as currently practised, however, should be improved. Evidence indicates that commercial electrical stunning systems do not guarantee an immediate loss of consciousness for a sufficiently long period for all eels. On-going research indicates that a higher voltage and current in combination with a killing method shows the potential to overcome these deficiencies.

The other three stunning/killing methods (2-4 above) have high risk scores and involve severe pain and stress, especially the salt and ice method which does not result in unconsciousness before evisceration.

Of the experimental methods, the captive needle pistol induces immediate unconsciousness and insensibility in eels and may be developed further into a suitable alternative stunning method. Methods used in other fish species other than those described in this Opinion may also be applicable to eels. The opportunity to develop new methods for slaughtering eels is considerable and should be encouraged.

To the experts' knowledge depopulation for disease control has not occurred. If a disease outbreak would require culling eels on a farm, there is no obvious method of choice. Appropriate methods for emergency killing on-farm also need to be developed.

Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and validated, robust and practically feasible welfare indicators should be developed.

Key words: Fish, European Eel (*Anguilla anguilla*), animal welfare, risk assessment, pre-slaughter, slaughter, stunning, killing.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Directive 93/119/EC[2] 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[3], 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, this EFSA report[4] 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 form 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 European Commission

In view of the above, the Commission requested 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)*

2 OJ L 340, 31.12.1993, p. 21–34

3 http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_ahaw_02_ej45_stunning_en.pdf?ssbinary=true

4 http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/opinion_ahaw_02_ej45_stunning_report_v2_en1,1.pdf?ssbinary=true

- European turbot *(Scophtalmus maximus)*
- common carp *(Cyprinus carpio)*
- farmed tuna *(Thunnus* spp.

This Scientific Opinion relates with the welfare aspects on the main systems of stunning and killing of farmed eels (*Anguilla anguilla*)

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The scientific coordination for this Scientific Opinion has been undertaken by the EFSA AHAW Panel Scientific Officers Oriol Ribó, Franck Berthe, Ana Afonsa, Tomasz Grudnik, Jordi Tarrés-Call and particularly Sofie Dhollander.

1. Scope and objectives of the scientific opinion

The scope of this report is the animal welfare aspects of stunning and killing of farmed European eel, *Anguilla anguilla.*

Welfare aspects of the farming phase of eels as well as the transport of eels were not included in this report (for the farming phase see EFSA's scientific report of the animal welfare aspects of husbandry systems for farmed European eel, (EFSA, 2008).

Pre-slaughter steps were only considered if evidence existed for a direct impact on welfare immediately before and during stunning and killing.

Product quality is not part of the assessment although some references to quality aspects are provided in the text that could be used and evaluated in further socio-economic studies on slaughtering methods for eels.

Emergency killing at production units for disease control purposes has been included in the report and assessment. Humane killing of individual fish was not included.

Food safety issues are dealt with 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. Biology of farmed eels and husbandry systems

The genus *Anguilla* comprises about 100 species. The species *Anguilla anguilla* L. (European eel) is widespread over Northern, Western and Mediterranean Europe from the elver stage onwards. This species is able to live in water with a temperature in the range of 3 to 30 ºC (Quéro, 1997)

The European eel is a catadromous fish species, long lived (on average males 8-11 years, females 12-18 years depending upon latitude) and the life cycle involves several metamorphic changes before the final adult spawning migration to the Sargasso Sea.

Unlike other fish species, the gills of *Anguilla anguilla* L. are completely covered with a skin sack with one small opening near to each pectoral fin. Once filled with water these sacks function as "physiological lungs" when an eel is outside the water. Due to this characteristic an eel is able to survive outside the water for a considerable time. The blood of an eel is not only oxygenated by the gills, but additionally by the skin and the swim bladder. An oxygen content below 2.5 mg/l O_2 at 21º C in water may be critical for eel (Hill, 1969; Tesch, 2003).

The juvenile stages, glass eel or elvers, are consumed as food or, relevant for this report, the glass eels are caught and farmed in aquaculture to commercial size. In Europe approximately 5100 tonnes of eel were produced in farms in 2008 (www.feap.info).

Intensive production systems (e.g. recirculation systems) are largely based on stock reared from glass eel whilst the extensive systems (e.g. ponds) have selected wild eel ranging in size from 5 to 150 g for production. The latter practice is changing with more of these units either establishing their own starter units for glass eels or buying juvenile eels that have been grown from glass eels. Extensive description of farming systems and practices can be found in the scientific report of the animal welfare aspects of husbandry systems for farmed European eel, (EFSA, 2008).

3. Stunning / killing process

3.1. General

EFSA has launched a questionnaire to all Member States inquiring about the methods in use for the stunning/killing of eels on a commercial scale. Salt bath and evisceration, aqueous ammonia solution and evisceration, immobilisation by the exposure to ice water (and salt) and evisceration; and whole body electrical stunning in water, desliming and evisceration were reported for the commercial stunning/killing of eels.

3.1.1. Recognition of consciousness, unconsciousness and death in eels

Movement or lack of it is not a good a indicator of consciousness/unconsciousness in eels. Directed (voluntary) movements such as avoiding a dip-net or moving backwards when grabbed by the tail are clear signs of consciousness. Undirected movements may occur even when an eel is brain dead and has lost all sensation, e.g. after decapitation.

Eels have an extraordinary capacity to breathe through the skin (a lack of breathing movements does not mean an eel is dead). Eels produce a lot of mucus due to stress, which itself might be a sign of consciousness.

The only reliable method to assess unconsciousness is on the basis of EEG recordings, including evoked responses. Death can be ascertained on the basis of combined measurements of ECG and EEG (Lambooij *et al.*, 2002a and 2002b). However this is only possible under experimental conditions and in practice other parameters validated through EEG and ECG should be used. Clinical methods for assessment of consciousness which are in use for other fish species (c.f. Kestin *et al.*, 2002) are not applicable to eels.

3.1.2. Animal welfare indicators

Validated, robust and practically feasible indicators are necessary to evaluate the welfare of eels associated with slaughter procedures. Preferably such indicators should be animal based (‘output indicators’) to most closely reflect the effect of the procedures on the welfare status of the animal. At present no reliable and practically feasible welfare indicators for eels are available. An approach in such a situation is to describe ‘best practices’ and define ‘input’ parameters (e.g. voltage, duration of stun, etc.) thereby limiting hazards and risks for impaired welfare. Such best practices and input variables can be defined under laboratory conditions where more sophisticated welfare measures such as EEG and ECG can be carried out. However, for eels, these input parameters are insufficiently defined for commercially used slaughter procedures. Thus there is a need for research to specify input parameters and animal based welfare indicators.

3.1.3. The steps from harvesting to killing of eels

Figure 1 illustrates the most common steps that occur in the process of harvesting to stunning and killing of eel. Only the pre-slaughter steps which have a direct impact on the welfare immediately before and during stunning and killing were considered to be within the terms of reference and will be described in section 3.2. The report focuses on the commercially applied stunning / killing methods which are described in section 3.3

Stunning and killing methods that are not commercially used in the EU MS are described under section 3.4. These methods were not included in the risk assessment.

Where no specific reference is given, the information is based on the opinion and practical experience of the expert of the working group. The knowledge of the expert on eels (Lambooij Bert, van de Vis Hans, and Oehlenschläger Jörg) is based on laboratory experiments and intensive observations in the field.

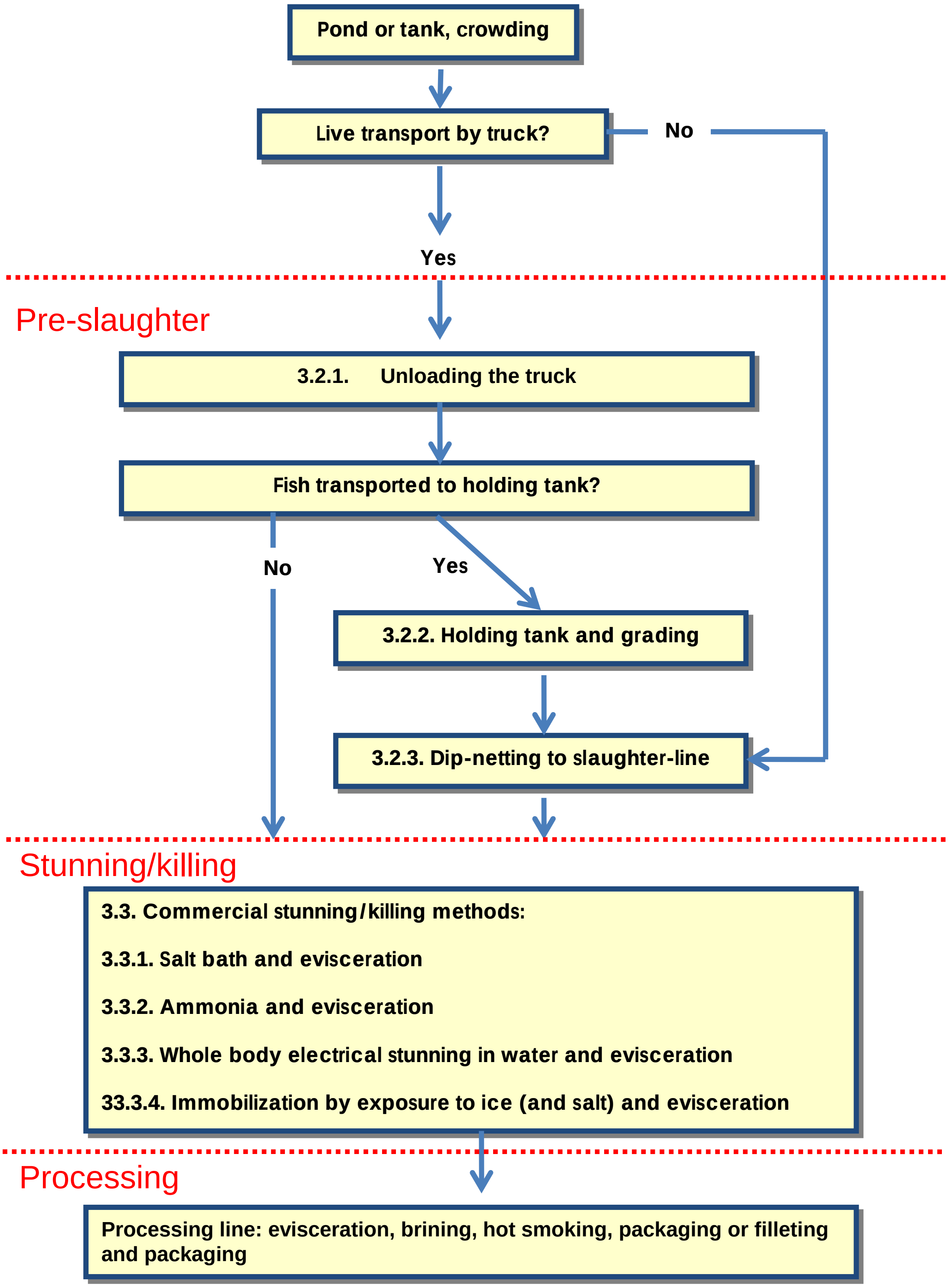

Figure 1: Steps from harvest to killing of eels

3.2. Pre-slaughter

3.2.1. Unloading the truck

Once the vehicle arrives at the slaughter facility, unloading is carried out by opening a shutter in the tank, resulting in a flow of eels and water into a container. The noise and vibrations caused by unloading may cause stress. This operation may involve a fall of around 1-2 m. On the basis of expert opinion and practical experience on production sites this can result in mechanical damage to eels such as bruises and wounds. When closing the shutter of the tank for batch-wise unloading, individual animals may be crushed. In addition, the weight of the layers of eels in the container may cause mechanical pressure for eels below. Subsequently, the container is transported to holding tanks by a forklift and eels may jump out of the transport container and fall 2 m or so, which may result in bruises and wounds, and then die from asphyxia, or be suddenly exposed to daylight and a change in temperature. A sudden change in temperature is considered to be stressful to eels. Further indicators to impaired welfare are an excess of mucus production and damage to skin and eyes.

For eels that are transported in cartons without water, unloading is carried out manually. No specific additional hazards were identified during this step which was not described earlier (Tesch, 2003).

3.2.2. Holding tank and grading

Generally, lairage prior to slaughter may last up to 2 days. Unfavourable environmental conditions and factors may affect the welfare of eels during that period. The relevant parameters were identified in EFSA's scientific report of the animal welfare aspects of husbandry systems for farmed European eel, (EFSA, 2008).

Since the period of lairage is relatively short the most important risk factor which may occur is poor water quality (pH, ammonia, nitrate, nitrite, CO_2, temperature, etc) and a high stocking density. For optimal conditions in the holding tank we refer to EFSA's scientific report of the animal welfare aspects of husbandry systems for farmed European eel, (EFSA, 2008). How often poor water quality in holding tanks occurs and the exact impact on welfare is not known.

Jumping out of the holding tank occurs and can lead to stress, mucus production, bruises, drying out and dying from asphyxia.

It has been reported that the white and black colour of the tanks are stressful for *Tilapia* and it is likely that this also applies to eels (Van Eys, 1981)

The possible negative effect of starvation or mixing established groups of eels on welfare is unknown.

Occasionally grading takes place in the slaughter house instead of on the farm. The eels are placed in the grader by dip-netting or tipping of the container. The grader consists of grids of different sized slits to sort the eels according to size. The sorted eels then fall into separate containers which proceed to the slaughter-line. This grading process can involve stress due to handling involved (e.g. with a dip-net), tissue damage (bruises) due to falling, temporary crowding on the grid, and exposure to air.

3.2.3. Dip-netting and emptying the container

Eels taken out by dip-net from the transport container, or the container emptied by turning involving a drop of 1-2m, can result in tissue damage such as bruises and wounds as the nets can be abrasive and the handling can be rough.

3.3. Commercial stunning / killing methods

3.3.1. Salt bath and evisceration

Live eels are placed in a dry tank and the weight of the layers of eels may cause some mechanical pressure on those below. Subsequently, NaCl or a combination of NaCl and aqueous Na_2CO_3 is added to the eels which results in denaturation of the mucus proteins which results in clotting. Moreover the upper layer of skin may be damaged or partly removed. During exposure to salt the eyes will become opaque.

To remove the clotted slime and salt, eels are transferred into a special mixing and rotating machine (tumbler construction or mixer principle) and are washed with water for about 10 minutes. The whole process is called ‘desliming’ and takes approximately 30 minutes. The conditions in the ‘salt bath’ (e.g. ratio of salt to eels, the combination of salts and duration) used varies between companies. The salt bath is a cheap, easy and relatively labour free way to render eels suitable for processing and to simultaneously remove the slime.

Eels make extremely vigorous attempts to escape from salt (Van de Vis *et al*., 2003) and take a long time to lose consciousness. Based on Visual Evoked Response (VER) data this may take more than 10 min (Van de Vis *et al*., 2001). Based on behavioural data and eels’ reaction to stimulation, it may even take more than 25 min. When the water is added to the tumbler, active movements of the eels can sometimes still be observed. It is probable that body movements stop due to muscular exhaustion. If the animals ultimately die as a result of the process, it is probably osmotic shock that kills them. However, it is likely that most eels treated with salt are eviscerated before they are dead (Verheijen and Flight, 1997). Killing eels in salt is now considered bad practice in Germany and has been prohibited since April 1999 (*Anon*, 1997). In The Netherlands, the Animal Welfare Council (an advisory body to the Government) proposed to ban this method (RDA report 2003).

3.3.2. Ammonia and evisceration

Eels are put into a dry container and a 25% ammonia solution is added to them (at the ratio 100 kg dry eels/100 ml ammonia solution). After approximately 4 min the eels are paralysed and the container is then filled with water. The eels are left in the container for approximately 20 min before it is emptied and the slime is washed off the eels (either by tumbler or replacing the water) which are then processed.

The ammonia solution causes denaturation of the mucus proteins which results in loosening of the mucus layer on the skin. Moreover the upper layer of skin may be damaged and during the exposure the eyes become opaque or white.

As with the salt bath, ammonia is a cheap, easy and relatively labour free procedure to render eels suitable for evisceration and to simultaneously remove the slime. During exposure to ammonia eels make extremely vigorous attempts to escape (Kuhlmann and Münkner, 1996). Immediately after exposure to ammonia eels start to bleed from the gill openings and they take up to 15 min to die, based on behavioural observations (Kuhlmann and Münkner, 1996). It is likely that body movements stop due to exhaustion. The animals ultimately die as a result of the exposure to ammonia in combination with the occurring osmotic shock. All eels are dead before evisceration. Exposing eels to ammonia is now considered bad practice in Germany and has been prohibited since 1999 (Anon, 1997). In The Netherlands the Animal Welfare Council (an advisory body to the Government) proposed to ban this method (RDA report 2003).

3.3.3. Whole body electrical stunning in water with de-sliming and evisceration

For the whole body electrical stunning method eels are placed in a fresh water tank of about 50cm to 100cm in diameter and an electrical current is passed. Two plate electrodes are placed on opposite sides of the tank and cover the whole area. The required stunning parameters as given in the German and Austrian legislation (Anon, 1997 and 2004) are shown in Table 1. The duration of the stun required in this legislation is at least 5 minutes.

Appendix 3, Part II 3.10 of the German Directive for the protection on animals in context with slaughtering and killing (Anon, 1997) states that “For the stunning of fish in water bath stunning devices the electrodes must be of such a size and arrangement that in all parts of the stunning device a uniform electrical stream through the fish is guaranteed. Fishes and electrodes must be completely covered by water.”

Appendix 3, Part II 3.11 of the same directive states that “For the electrical stunning of eels potable water with an electrical conductivity of below 1000 (µS/cm) has to be used. Prior to stunning the electrical conductivity of the water in the stunning device has to be measured und the current density necessary for stunning has to be adjusted. For this purpose the voltage has to be adjusted so that between the electrodes an alternating current in ampere (A) per square decimetre (dm2) of the current supplying electrode surface is flowing” as shown in Table 1. In the 2004 Austrian directive, Annex G (Anon., 2004) (Instructions for the storage and killing of food fishes, frogs, crustacean and molluscan shellfish) the text and the table are identical (except for some linguistic differences) with those in the German directive.

Table 1: Parameters given in German and Austrian legislation (Anon. 1997 and Anon 2004)

Electrical conductivity of the water (µS/cm)	**Current density (A/dm2)**
< 250	0.10
> 250 < 500	0.13
> 500 < 750	0.16
> 750 < 1000	0.19

The calculated corresponding voltages for electrodes 50 cm apart for a given conductivity are shown in Table 2. Measurements of EEG end ECG have shown that the method laid down in the German and Austrian legislation does not stun all eels (Kuhlmann *et al.*, 2000).

For de-sliming, eels are transferred into a tumbler and a saturated chalk milk solution ($Ca(OH)_2$), 10% sodium carbonate (Na_2CO_3) or 3% ammonia solution (ratio of alkaline solutions: eels = 3:97) is added. The use of the de-sliming agent chalk milk has ecological and economic advantages. The de-sliming loss is between 5 and 6% (Kuhlmann *et al.*, 2000). De-sliming takes 10 min ad 10 r.p.m., during which any living eel will die.

The eels are then washed with water for about 10 min to remove the clotted slime.

Table 2: Calculated voltages for 50 cm electrode distances at varying conductivity based on data in Table 1

Voltage (V)	Conductivity (µS/cm)
250	200
160	500
135	600
120	800

In the experiments of Lambooij *et al*. (2002e) the behaviour of eels was observed and they were able to move freely in the water (with a conductivity of 500 µS/cm) before and after stunning at 50 V with electrodes at 16 cm apart (equivalent to 160 V at 50 cm electrode distance) and 0.17 A/dm2. After a 3 sec electrical stun, some eels turned upside down (suggesting unconsciousness) but changed back again to a normal position after 10 sec. All the other eels showed very active swimming behaviour immediately after the electrical stun and stopped swimming after 75 sec and remained in the normal rest position. After electrical stunning with a voltage of 50 V at a water conductivity of 500 µS for more than 10 sec, some eels turned upside down and stopped breathing for 1 minute, followed by sluggish activity. In a parallel experiment (Lambooij *et al.*, 2002b) three out of 7 eels fitted with EEG electrodes did not show any general epileptiform fit after a one second stun. These results reveal that under these electrical parameters stunning is not instantaneous; rather a number of eels may just show no movement as a result of immobilisation and exhaustion.

Clearly additional research is necessary to define best practices and clear input parameters for whole body electrical stunning. Potential monitoring points for this method could refer to conductivity of the water, the applied voltage, amperage and behaviour and time until evisceration (in order to guarantee that eels are unconscious during de-sliming and until death).

Sensory and instrumental assessments of flesh quality did not show any downgrading caused by electrical stunning (Morzel and Van de Vis, 2003).

3.3.4. Immobilisation by exposure to ice (and salt)

Eels are placed in a dry tank and 2 % of NaCl (weight/weight) is added. Subsequently 25% (weight/weight) ice water is added (the process is not monitored with respect to temperature when it is performed). The weight of the layers of eels in the container may cause mechanical pressure and tissue injury to the eels below. The aim is to immobilise the eels whilst keeping them alive. Eels attempt to escape during this procedure when they are exposed to salt and temperature shock The eels are left overnight and are killed the next day by evisceration. During evisceration the slime is washed off by or replacing the water.

A variant of this method is used in Denmark where no salt is added to the ice water. After arrival at the slaughterhouse, eels are kept in well-water at 5 to 10 C, depending on the time of the year. Before slaughter they are put into ice water at 0 to 2 C until they are immobilised and they are then eviscerated.

Evisceration alive is clearly a welfare issue since it is expected that all eels are conscious. This expectation of the experts is based on the lack of rigor mortis that is indicative of death (one of the signs of death is a settling of the blood in the lower portion of the body, causing a purplish red discoloration of the skin).

3.4. Other stunning / killing methods

Apart from the above stunning/killing methods that are applied in commercial settings there are some additional methods that are either only used on small scale or are still under development. These are briefly described below but are not included in the Risk Assessment.

3.4.1. Decapitation with evisceration,

The head is separated from the body by a sharp cut resulting in bleeding although there may be some recoil in the arteries that will stem blood flow. Decapitation causes death through anoxia due to blood loss.

Verheijen and Flight (1997) report loss of reactions in severed eels heads 30 min after decapitation and Van de Vis *et al*. (2003) reported that it took 13 min before brain function, determined using EEGs, was lost after decapitation. Thus, decapitation would appear to expose eels to considerable periods of suffering.

3.4.2. Neck-cut with evisceration

A neck-cut is applied to immobilise the animal to facilitate evisceration. The neck-cut consists of cutting the spinal cord just behind the head, without disruption of the soft tissue and flow of blood to the brain. It has been reported by Flight and Verheijen (1993) that eels may survive a neck-cut under laboratory conditions.

3.4.3. Experimental electrical stunning

3.4.3.1. Head-only stunning

In head only stunning electricity is applied to the head of an eel by a pair of stunning tongs in air. A general epileptiform seizure was observed with 255 V, 545 mA for 1 sec. The general epileptiform seizure, as measured on the EEG, was characterised by a tonic/clonic phase and an exhaustion phase that lasted for at least half a minute (this period can be prolonged by a longer exposure time to the current). During the general epileptiform insult eels showed tonic contractions alternating with clonic ones.

When eels were placed in water after head only stunning, two behavioural phases were distinguished. Limited tonic and clonic contractions combined with backward swimming were followed by heavy clonic contractions combined with uncoordinated movements such as jumping out of the water. A distinct exhaustion phase was not observed in all eels (Lambooij *et al.*, 2002b) and to prevent recovery, a suitable killing method should be applied immediately after the electrical stunning.

3.4.3.2. Improved whole body stunning

For the whole body electrical stunning method eels are placed in a fresh water tank and an electrical current is passed between two plate electrodes placed on the opposite sides of the tank. These electrodes cover the whole area of both sides. In the experiments of Lambooij *et al.*, (2002b) the electrical stunning parameters to induce unconsciousness were on average 194 V (across electrodes at 16 cm distance) and 0.64 A/dm2 for 1.6 sec. The eels are rendered unconscious and insensible instantaneously as demonstrated by the occurrence of a general epileptiform seizure on the EEG which is characterized by a tonic/clonic contraction and an exhaustion phase.

In another experiment with a similar voltage (200 V, electrodes at 16 cm distance) it was shown that unconsciousness can be prolonged if eels were stunned in fresh water with a conductivity of 500µS/cm for approximately 1 sec, followed by 50 V for 5 min. Although eels showed heart fibrillation after stunning, they nevertheless recovered. To kill eels during the 5 min exposure to the electrical current, the water was de-oxygenated by flushing with nitrogen as soon as the electricity was switched on. The oxygen saturation of the water decreased from 74 to 23 % at 22 C. This results in hypoxia during the period of unconsciousness and insensibility. After this combined treatment there was no brain activity (measured by EEG), no responses to pain stimuli and the eels did not recover.

3.4.4. Captive needle method

A nail gun was modified to drive a hollow needle into the brain to inject pressurised air in the brain and the spinal cord (Hillebrand *et al*., 1996). This 'captive needle pistol' (developed for poultry) was adapted to eels regarding its length and shape of the needle (Lambooij *et al.*, 2002d). A cone shaped needle of 16 mm in length with a diameter of 2 mm was used, which forced the air through 3 holes positioned at the end of the needle. The holes were positioned in 3 directions radially 120^{o}, with one pointing towards the spinal cord.

Eels were restrained using tyribs and then mechanically stunned using a captive needle pistol with a shooting pressure of 8 bar and an air injection of 3 bar during 1.5 sec. Indices for the immediate induction of unconsciousness and insensibility were the appearance of theta and delta waves tending to an iso-electric line i.e. no brain activity on the EEG. To overcome severe clonic seizures some eels had to be stunned twice to facilitate evisceration. A few eels showed some slow muscle contractions after stunning for more than one hour. No responses to pain stimuli on the EEG or with respect to behaviour were observed. The captive needle pistol using air pressure is an easy and suitable alternative stunning method for individual eels. However, more research is needed to make this method more feasible for application in practice

3.4.5. Chilling and freezing

The basic effect of cooling down is lowering eels metabolism. Lambooij *et al.* (2002c) exposed eels to iced water until their body temperature was 5 C and then transferred them to brine at -18 C for approximately 5 minutes. EEG indices for the induction of unconsciousness and insensibility were the appearance of theta and delta waves, and no response to pain stimuli. Unconsciousness was induced in most eels at a body temperature of on average 8 C after 12 min in the iced water. However, it was observed that 5 % of eels were not stunned at a body temperature of 5 C. The heart rate decreased from 24 to 7 beats/minute and became irregular during cooling. When placed in brine the EEG showed rapid and extreme depolarisation of the membranes, which started after 27 sec. The ECG showed heart fibrillation in all eels and none recovered.

In another experiment, groups of eels and single eels were placed in ice water at 0 C. The observation of unrestrained eels revealed four phases. Animals were: 1) swimming around in the water; 2) attempting to escape from the ice water; 3) pressing their nose to the wall or corner while showing clonic muscle contractions; and 4) breathing only, while all other muscle activity was totally suppressed. Afterwards they were transferred to cold brine at -18 C, and none of the eels recovered (Lambooij *et al*., 2002c).

Hypothermia is not considered acceptable for stunning/killing of eels, because it does not induce an immediate loss of consciousness and does not reduce the ability to feel pain. Also the exposure to ice water and the brine may be painful (Lambooij *et al*, 2002c).

3.5. Emergency killing for disease control

To the experts' knowledge emergency killing for disease control has not occurred. Eels are not a susceptible species for any of the listed diseases in Annex II CD 2006/88 (EC, 2006).

If a disease outbreak would require culling eels on a farm, there is no obvious method of choice. Development of an appropriate method for on farm stunning/killing therefore requires additional research (e.g. mobile electrical stunning devices).

If the product is not destined for human consumption, an overdose of anaesthetic could be considered suitable for killing moribund or diseased eels under humane conditions. A solution of the chosen anaesthetic is added to a container of a size suitable to contain the number and size of eels to be killed. Any licensed anaesthetic may be used, at a dose appropriate to the amount, size and the development of the eels. The implementation of anaesthesia for mass killing of eels, however, can become problematic since the exact dosing in function of the size can be very complicated (Kuhlmann *et al.*, 2000).

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 D. The risk assessment applied to the stunning and killing of farmed eels is described in chapter 4.1 and 4.2.

4.1. Application of the risk assessment approach to stunning and killing eels

Eels are either i) taken directly to slaughter on arrival at an abattoir, or ii) kept at the abattoir for up to two days. The hazards associated with both approaches were assessed. The method of stunning and killing was not associated with the probability of being held at the slaughterhouse.

The assumption that exposure to the hazard resulted in all the fish suffering the adverse effect held for all hazards except two (unloading at slaughterhouse (Table 6, hazard 1) and unloading container into tank (Table 7, hazard 8). All eels are unloaded, hence exposed to the hazard, however, only a proportion of the population suffer the adverse effect (mainly physical trauma). For this hazard, an estimate of the proportion of the population that experienced the adverse effect was made (Appendix A).

Different definitions of intensity for hazards that occurred pre-slaughter and those arising during stunning and killing were needed (Tables 3 and 4 below).

Table 3: Intensity categories for adverse effects arising from hazards associated with pre-slaughter

EVALUATION	SCORE	IN WATER
MILD	1	The animal is minimally affected as evidenced by minor changes in behaviour such as somewhat increased swimming activity. Tissue damage such minor skin lesions Normal active responsiveness (e.g. easily escape capture by netting)
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	Signs might include energetic and purposeful escape behaviour (head or tail out of water), rapid movement Excess mucus production, the eels become pale. Severe tissue damage such as damaged skin, broken bones, bruises and/or eye injury. Extreme lethargy, cannot escape capture by net. Convulsions when conscious. Out of water drying out causing death (Tesch, 2003) Colour changes of flesh (DFD and PSE flesh be observed in the eviscerated eels)

Table 4: Intensity categories for adverse effects arising from hazards associated with stunning /killing

EVALUATION	SCORE	DESCRIPTION
MILD	1	Minor trauma, Normal active responsiveness (e.g. easily escape capture by netting)
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	Signs might include energetic and purposeful escape behaviour (head or tail out of water), rapid movement Excess mucus production, the eels become pale. Extreme lethargy, cannot escape capture by net. Gill bleeding, extreme trauma to skin

The same categorisation for duration of the adverse effect was used for pre-slaughter and stunning/killing hazards (Table 5 below).

Table 5: Duration categories for adverse effects arising from hazards associated with pre-slaughter and stunning /killing

Duration (minutes)	Score
< 5[1]	1
5 – 15	2
>15 – 60	3
> 60	4

[1]adverse effects with a duration of less than one second are not scored

4.2. Pre-slaughter hazards

4.2.1. Eels slaughtered directly on arrival at the abattoir

Seven hazards were identified (Table 6) (details in Appendix A) that occur pre-slaughter to eels which are slaughtered directly on arrival at an abattoir. The risk score ranged from 0.08 to 33. The highest ranking risks were unloading (Table 6, hazard 2), exposure to sudden change in temperature and to daylight, scores of 33, 25 and 25, respectively. These hazards have high scores because all the eels are affected and duration of the adverse effect may last up to 30 minutes. Other hazards had the highest magnitude scores, indicating a more severe impact on the fish which were affected. Unloading at slaughter (Table 6, hazard 1) and jumping out of the transport container results in adverse effects which have high (Table 6, hazard 3) intensity scores, and had the highest magnitude scores, but affect only a small proportion of the population.

The sum of the risk scores of all the hazards was 92.

Table 6: Risk and magnitude scores for pre-slaughter hazards

	Hazard	Adverse effects	Risk score	Magnitude
1	unloading at slaughterhouse (with a drop)	Fall (about 2m) causing stress observed, pale, mucus produced, skin and muscle damage, broken bones	0.08	75
2	unloading at slaughterhouse (with a drop)	Stress from unloading exposure to noise, vibrations	33.33	33
3	jumping out of transport container	Stress observed, pale, mucus produced, skin and muscle damage, broken bones, drying out	0.08	75
4	piling up in internal transport	Mechanical pressure on eels (upper layer not affected) causing bruises, difficulty to breath	8.33	8
5	trapped in shutter of tank	Crush trauma leading to death	0.08	75
6	sudden change in temperature	Temperature shock, stress from rapid change in temperature	25.00	25
7	sudden exposure to daylight (from dark)	Stress	25.00	25

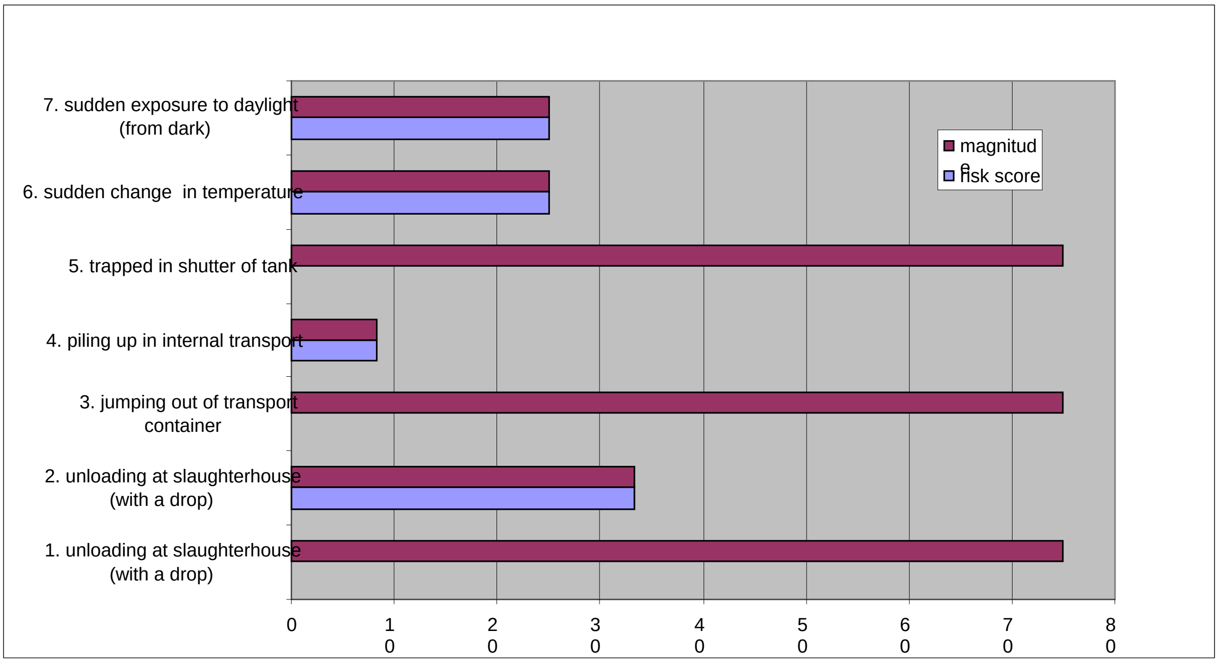

Figure 2: Pre-slaughter hazards, no lairage – risk and magnitude scores

4.2.1.1. Variability and uncertainty

Variability is captured by estimates of the minimum and maximum values of the probability of exposure to the hazard. For all but two hazards (Table 6, hazards 3 and 5) the entire population was considered to be exposed to these hazards and the minimum and maximum values were the same as the most likely value (equal to one), which resulted in a variability of 0 of these risk score estimates (Appendix A). The estimate for the probability that unloading leads to physical trauma (Table 6, hazard 1) was thought to be very low but the estimate was not known with any precision (range between 0 and 2%, most likely value 0.1%). Similarly, the proportion of eels jumping out of the container (Table 6, hazard 3) or trapped by the shutter door of the transport (Table 6, hazard 5) was also known to be very low but the estimates carried a wide, skewed distribution (Appendix A).

Due to the scale necessary to visualise the magnitude of the risk scores and the lack of variability of a number of the hazards the error bars were not included in Figure 2.

For nearly all the hazards the uncertainty score was two. There was good consensus amongst the working group experts about the choice of estimates, however, there is no or very little supporting published data (hence an uncertainty estimate of two). The exception was exposure to temperature change for which published data exists (and was given an uncertainty score 1, Appendix A).

4.2.2. Eels held for up to two days at the abattoir

The pre-slaughter hazards discussed in the previous section were rescored for eels which were kept in lairage at the slaughterhouse (Table 7, details in Appendix B). The period of lairage was assumed to be two days. The duration score for two hazards: unloading at a slaughter house leading to physical trauma and jumping out of transport container increase from 30 minutes to 2 days. This resulted in the score for both hazards increasing from 0.08 to 0.10, and the magnitude increasing from 75 to 100 (i.e. the maximum score).

Eels held in lairage at the slaughterhouse experienced an additional 11 hazards associated with lairage for up to two days (details in Appendix B). The risk scores ranged from 0.10 to 33.3. Nine of the 11 hazards had the highest duration score (Table 7, hazard 11) because the adverse effects were judged to last up to two days, i.e. the duration of their time in lairage. Only the adverse effects for dip netting and grading were judged to be short lived, 10 and 3 minutes, respectively. Unloading from transport container into holding tank (with a drop) (Table 7, hazard 9) resulting in mild stress had the highest score because whilst the intensity was low (score 1) the entire population experienced the hazard (and its effect). The second highest scoring hazard was a sudden change in temperature (score 11). Very poor water quality and dip netting prior to slaughter were the third and fourth highest ranked hazard (scoring 5 and 4.17, respectively). Four hazards had the maximum magnitude score (100) - unloading from transport container into holding tank (with a drop) (Table 7, hazard 8) leading to physical trauma, very poor water quality, jumping out of the container.

The sum of the risk scores for the additional 11 hazards associated with holding eels in lairage for 2 days was 63.

Table 7: Risk and magnitude scores for hazards associated with lairage

	Lairage hazards	Adverse effects	Risk score	Magnitude
8	unloading from transport container into holding tank (with a drop)	fall (about 2m) causing stress observed, pale, mucus produced, skin and muscle damage, broken bones	0.10	100
9	unloading from transport container into holding tank (with a drop)	mild stress	33.33	33
10	sudden change in temperature	temperature shock, stress from rapid change in temperature	11.00	33
11	poor water quality (pH, DO, water temp)	physiological stress	3.33	33
12	very poor water quality (pH, DO, water temp)	severe physiological stress	5.00	100
13	very high density (>250 kg/ m3)	physiological stress	1.67	33
14	white or black tanks	stress	3.33	33
15	grading mechanical before stunning	stress due to exposure to air, physical damage from fall, abrasion by bars of the selection grid	0.67	33
16	jumping out of holding tank (1)	stress observed, pale, mucus produced, skin and muscle damage	0.10	100
17	jumping out of holding tank (2)	death from asphyxia after drying out	0.10	100
18	dip-netting (immediately before slaughter)	abrasion from net and rubbing between fish	4.17	8

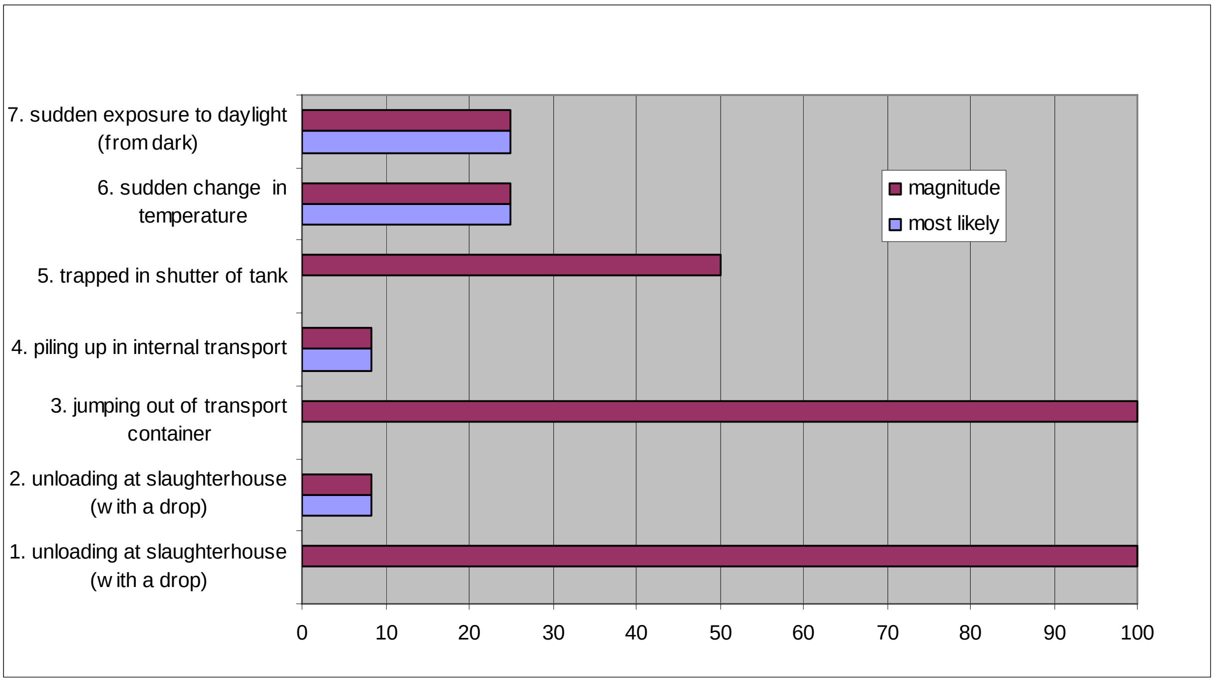

Figure 3: Risk and magnitude scores for hazards associated with lairage

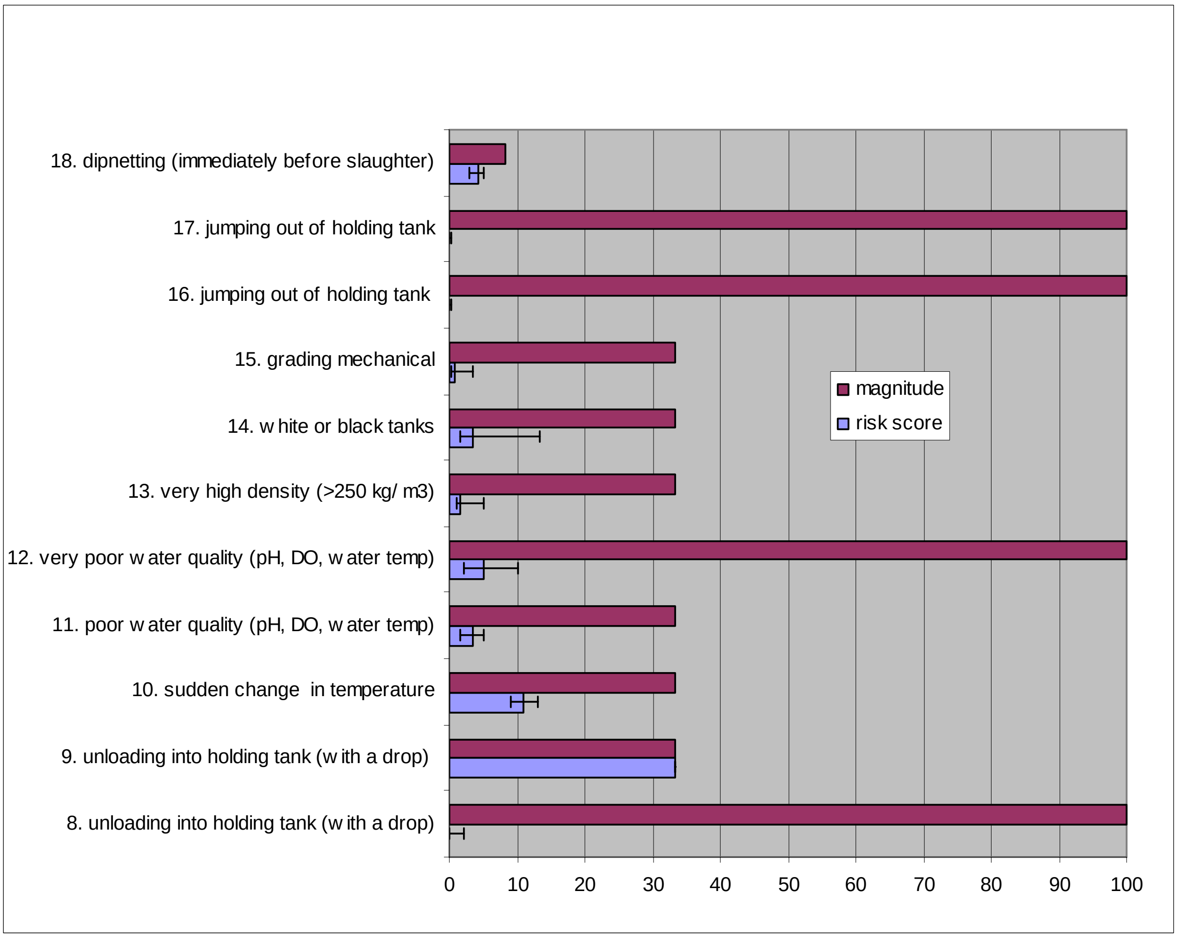

Figure 4: Additional risk and magnitude scores for hazards associated with lairage (error bars represent the variability of the risk score estimates)

4.2.2.1. Variability and uncertainty

There was considerable variability around the probability of exposure for a number of the hazards. Of the lower ranking scores, unloading into the holding tank (Table 7, hazard 8) and stocking at a high density had the highest variability (Appendix B) (wide range from minimum to maximum estimate of the probability of exposure to the hazard).

Of the high ranking hazards the scores for white or black tanks and very poor water quality had the highest variability.

The uncertainty score was 2 for all hazards, except change in water temperature and water quality, for which there is consistent published data, and thus a low uncertainty score (table 7, hazard 12). Due to the scale necessary to visualise the magnitude of the risk scores and the lack of variability of a number of the hazards the error bars were not included in figure 3.

4.3. Stunning and killing hazards

Seven methods of stunning and killing were assessed (Table 8, details in Appendix C). One or two hazards were identified for each method. For each method of stunning / killing the risk scores for the hazards were summed and the magnitude scores averaged (Table 8). It should be noted that different definitions for intensity of the adverse effect were used for killing and stunning hazards compared with the pre-slaughter hazards.

The risk scores range from 13 for electricity to 117 for the ice and salt treatment. All methods of stunning and killing included evisceration. Evisceration was a hazard in three of the four methods because some, or all in the case of salt and ice, of the eels were considered to be conscious when eviscerated (Appendix C).

Ice and salt had the highest risk score (117) because the eels have prolonged exposure (16 hours) to a solution of ice and salt which was considered to result in a moderate adverse effect (intensity score 2), but did not result in unconsciousness when eviscerated (intensity score 3). The variation of the method without salt was considered to have the same risk score (same moderate intensity and long duration and probability to exposure to hazard and adverse effect of 1)

The use of salt and ammonia had very similar scores: 76 and 75, respectively. All eels exposed to ammonia were judged to be unconscious or dead after 15 minutes, thus evisceration was not a hazard. Exposure to ammonia was judged to be a severe hazard resulting in death. Similarly, salt treatment causes a severe insult resulting in death or unconscious in nearly all eels, thus evisceration had a low risk score (but a magnitude score of 50) (Appendix C).

The magnitude score (50) for evisceration whilst conscious may be considered low as other slaughter hazards had higher scores, e.g. 75 for salt treatment. Six of the seven hazards arising from the stunning and killing methods received the highest intensity score of 3. Of these some will have more severe adverse effects than the others, but they all met the established criteria for the highest score. This is a limitation of the scoring system. It was estimated that eels only suffer the adverse effect of evisceration for 5 minutes before they expire. The system of scoring used gives the same score for severe hazards of short duration as mild hazards of long duration.

The risk score for electricity (13) is considerably lower than the other methods. The hazards arise from an estimated low percentage of the eels electrically stunned that experience too low an electrical current or are eviscerated when conscious (Appendix C).

Table 8: Risk and magnitude scores for the hazards associated with each stunning /killing method

Stunning/killing method	Description of adverse effect of killing hazard	Risk score			Magnitude score
		most likely	min	max	
Salt treatment		**76[a]**			**63[b]**
Salt treatment	severe pain, osmotic shock, exhaustion, stripping off layer of skin (change of skin colour)	75	75	75	75
Evisceration	severe pain, trauma, tissue damage	1	0.5	2.5	50
Ammonia treatment		**75 [a]**			**75[b]**
Ammonia treatment	extensive bleeding, severe pain, exhaustion, dehydration, intoxication with ammonia	75	75	75	75
Ice and salt		**117 [a]**			**58[b]**
ice and salt (2% salt and 25% ice water) for 16 hours	temperature shock, exhaustion, skin irritation, exhaustion, dehydration	67	67	67	67
Evisceration	severe pain, trauma,	50	50	50	50
Electricity		**13 [a]**			**38[b]**
Insufficient current/voltage	escape behaviour, pain, stress	10	2.5	11.25	25
Evisceration	severe pain, trauma, if conscious	3	0.05	3.5	50

[a] sum of risk scores for the hazards of that stunning/killing method; [b] average score for the hazard of that method

4.3.1.1. Variability and uncertainty

There was variability around only three hazards. For the majority of hazards the entire population was exposed hence most likely, minimum and maximum values for the probability of exposure to the hazard were equal to one. However, the proportion of the population which was eviscerated when conscious following salt treatment and electricity has not been systematically investigated. Thus whilst the percentage was considered low, it was not known with precision (hence a wide estimate range).

The uncertainty score for most of the hazards was two (Appendix D). Field observations are consistent and clear however, there are no published data supporting the choice of parameters.

4.4. Overall comparison of methods of stunning and killing eels

The total scores for the stunning and killing methods (i.e. summed pre-slaughter and slaughter hazards) are given in Table 9. The scores for the pre-slaughter hazards do not vary with the slaughter method.

Eels are exposed to 19 or 20 hazards (Table 9). Most of these hazards occur in the pre-slaughter period, but the hazards causing the most extreme adverse effects occur directly from the method used to kill the eels (through exposure to salt, ammonia and evisceration).

Table 9: The overall ranking of methods for eels stunning and killing

Category of hazard		Slaughter method			
		salt treatment	ammonia treatment	ice and salt	electrical
Pre-slaughter hazards (no lairage)	*risk score*	92	92	92	92
	number of hazards	7	7	7	7
Pre-slaughter hazards (2 days lairage)	*risk score*	63	63	63	63
	number of hazards	11	11	11	11
slaughter hazards	*risk score*	76	75	117	13
	number of hazards	2	1	2	2
total	***risk score***	**231**	**230**	**272**	**168**
	number of hazards	**20**	**19**	**20**	**20**

5. Conclusions

- EEG recordings are the only reliable method to assess unconsciousness in eels and death can only be ascertained on the basis of combined measurements of ECG and EEG.
- At present there are no validated and robust indicators available to evaluate in practice the welfare of eels associated with slaughter procedures
- The most important hazards in the pre-slaughter phase are associated with unloading and poor water quality in the holding tank.
- Improved management at the slaughterhouse could reduce the exposure of eels to sudden changes in temperature or light and suboptimal water quality.
- Eels are exposed to additional hazards if kept in lairage at the slaughterhouse and, therefore, their welfare is best served if they are slaughtered as soon as possible after arrival.
- Currently there is no stunning method commercially available that immediately induces unconsciousness in all eels until death.
- Based on current knowledge and practical experience, and from the risk assessment, electrical stunning immediately followed by a killing method is the preferred practically available method for slaughter of eels.
- The currently practiced electrical stunning methods should be improved. Evidence indicates that commercial electrical stunning systems do not guarantee to induce immediate loss of consciousness and for a sufficiently long period. On-going research indicates that a higher voltage and current in combination with a different killing method shows potential to overcome these deficiencies.
- With the exception of electrical methods, all the other methods of stunning/killing (salt bath and evisceration; ammonia and evisceration and immobilization by exposure to ice (and salt) and evisceration) had high risk scores and involve severe pain and distress,

especially the salt and ice method which does not result in unconsciousness before evisceration

- The captive needle pistol injecting air into the brain induces immediate unconsciousness and insensibility in eels and may be developed further into a suitable alternative stunning method for individual eels under good welfare conditions

6. Recommendations

- The hazards associated with unloading, moving and keeping eels at the abattoir should be mitigated through improved devices, practices and management and better training of personnel in all steps of the procedure.
- Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced and relevant practical welfare indicators developed.
- Eels should be kept at slaughterhouses for as short a time as possible before slaughter.
- Electrical stunning and simulations of a killing method followed should be further developed to improve welfare of eels at slaughter
- At present we cannot recommend any method other than electrical stunning since it is the best available method for stunning eels prior to evisceration.

7. Recommendations for further research

- Valid, robust and practically feasible indicators to evaluate the welfare of eels during slaughter procedures need to be developed.
- The existing electrical stunning methods currently practised need to be improved. Variations in voltages, currents, frequencies and combinations of them need to be tested and monitoring points defined.
- The captive needle method need to be further developed with respect to equipment for application in practice.
- Appropriate methods for emergency killing on farm need to be developed
- Clinical indicators for loss of consciousness in eels need to be established and validated by registration of EEG and ECG.

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APPENDICES

APPENDIX A: PRE-SLAUGHTER HAZARDS – EELS TAKEN DIRECTLY TO SLAUGHTER ON ARRIVAL AT THE SLAUGHTERHOUSE

	Hazard	Adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability (exposure to the hazard)			Risk score[3]			Magnitude
			score[1]	minutes	score[2]	most likely	min	max	most likely	min	max	
1	unloading at slaughterhouse (with a drop)	fall (about 2m) causing stress observed, pale, mucus produced, skin and muscle damage, broken bones	3	30	3	0.001 [a]	0 [a]	0.02 [a]	0.08	0	1.5	75
2	unloading at slaughterhouse exposure to noise and vibrations (with a drop)	stress from unloading exposure to noise, vibrations	2	5	2	1	1	1	33.33	33.33	33.33	33
3	jumping out of transport container	stress observed, pale, mucus produced, skin and muscle damage, broken bones, drying out	3	30	3	0.001	0.0005	0.0015	0.08	0.04	0.11	75
4	piling up in internal transport	mechanical pressure on eel (upper layer not affected)	1	2	1	1	1	1	8.33	8.33	8.33	8
5	trapped in shutter of tank	crush trauma leading to death	3	30	3	0.001	0.0001	0.002	0.08	0.008	0.15	75
6	sudden change in temperature	temperature shock, stress from rapid change in temperature	1	30	3	1	1	1	25	25	25	25
7	sudden exposure to daylight (from dark)	stress	1	10	3	1	1	1	25	25	25	25

[1]1=mild, 2=moderate, 3=severe; [2] 1=<5min, 2=5-15min, 3=15-60 min, 4 =>60min; [a] probability that exposure lead to the adverse effect (100% of population exposed to this hazard); [3]The risk scores allow a ranking but the absolute figures are not on a linear scale

APPENDIX B: PRE-SLAUGHTER HAZARDS – WHEN EELS TAKEN ARE HELD FOR TWO DAYS PRIOR TO SLAUGHTER

	Hazard	Adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability (exposure to the hazard)			Risk score[3]			Magnitude
			score[1]	Minutes	score[2]	most likely	min	max	most likely	min	max	
1	unloading at slaughterhouse (with a drop)	fall (about 2m) causing stress observed, pale, mucus produced, skin and muscle damage, broken bones	3	2880	4	0.001 [a]	0 [a]	0.02 [a]	0.1	0	2	100
2	unloading at slaughterhouse exposure to noise and vibrations (with a drop)	stress from unloading exposure to noise, vibrations	1	2	1	1	1	1	8.33	8.33	8.33	8
3	jumping out of transport container	stress observed, pale, mucus produced, skin and muscle damage, broken bones, drying out	3	2880	4	0.001	0.0005	0.0015	0.10	0.05	0.15	100
4	piling up in internal transport	mechanical pressure on eels (upper layer not affected)	1	2	1	1	1	1	8.33	8.33	8.33	8
5	trapped in shutter of tank	crush trauma leading to death	3	5	2	0.001	0.0001	0.002	0.05	0.005	0.1	50
6	sudden change in temperature	temperature shock, stress from rapid change in temperature	1	2880	3	1	1	1	25	25	25	25
7	sudden exposure to daylight (from dark)	stress	1	10	3	1	1	1	25	25	25	25

[1]1=mild, 2=moderate, 3=severe; [2] 1=<5min, 2=5-15min, 3=15-60 min, 4 =>60min; [a] probability that exposure lead to the adverse effect (100% of population exposed to this hazard);
[3]The risk scores allow a ranking but the absolute figures are not on a linear scale

ADDITIONAL PRE-SLAUGHTER HAZARDS – WHEN EELS TAKEN ARE HELD FOR TWO DAYS PRIOR TO SLAUGHTER

	Hazard	Adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability (exposure to the hazard)			Risk score[3]			Magnitude
			score[1]	Minutes	score[2]	most likely	min	max	most likely	min	max	
8	unloading from transport container into holding tank (with a drop)	fall (about 2m) causing stress observed, pale, mucus produced, skin and muscle damage, broken bones	3	2880	4	0.001 [a]	0 [a]	0.02 [a]	0.10	0.00	2.00	100
9	unloading from transport container into holding tank (with a drop)	mild stress	1	2880	4	1	1	1	33.33	33.33	33.33	33
10	sudden change in temperature	temperature shock, stress from rapid temp change	1	2880	4	0.33	0.27	0.39	11.00	9.00	13.00	33
11	poor water quality (pH, DO, water temp)	physiological stress	1	2880	4	0.1	0.05	0.15	3.33	1.67	5.00	33
12	very poor water quality (pH, DO, water temp)	severe physiological stress	3	2880	4	0.05	0.02	0.1	5.00	2.00	10.00	100
13	very high density (>250 kg/ m3)	physiological stress	1	2880	4	0.05	0.03	0.15	1.67	1.00	5.00	33
14	white or black tanks	physiological stress	1	2880	4	0.1	0.05	0.4	3.33	1.67	13.33	33
15	grading mechanical before stunning	S tress due to exposure to air, physical damage from fall, abrasion in bars	2	10	2	0.02	0.01	0.1	0.67	0.33	3.33	33
16	jumping out of holding tank	stress observed, pale, mucus produced, skin and muscle damage	3	2880	4	0.001	0.0005	0.0015	0.10	0.05	0.15	100
17	jumping out of holding tank	die from asphyxia after drying out	3	2880	4	0.001	0.0005	0.0015	0.10	0.05	0.15	100
18	dip-netting (immediately before slaughter)	abrasion from net and rubbing between fish	1	3	1	0.5	0.35	0.6	4.17	2.92	5.00	8

[1]1=mild, 2=moderate, 3=severe; [2] 1=<5min, 2=5-15min, 3=15-60 min, 4 =>60min; [a] probability that exposure lead to the adverse effect (100% of population exposed to this hazard);
[3]The risk scores allow a ranking but the absolute figures are not on a linear scale

APPENDIX C: SLAUGHTER HAZARDS

	Hazard	Adverse effect	Intensity of the adverse effect	Duration of the adverse effect		Probability (exposure to the hazard)			Risk score[3]			Magnitude
			score[1]	Minutes	score[2]	most likely	min	max	most likely	min	max	
1	**salt treatment**											
	salt treatment	extreme pain, osmotic shock, exhaustion, stripping off layer of skin (change of skin colour)	3	25	3	1	1	1	75	75	75	75
	evisceration	extreme pain, trauma	3	5	2	0.02	0.01	0.05	1	0.5	2.5	50
2	**ammonia treatment**											
	ammonia treatment	extensive bleeding, extreme pain, exhaustion, dehydration, intoxication with ammonia	3	15	3	1	1	1	75	75	75	75
3	**ice and salt**											
	ice and salt (2% concentration) for 16 hours	temperature shock, exhaustion, skin irritation, exhaustion, dehydration	2	960	4	1	1	1	66.7	66.7	66.7	67
	evisceration	extreme pain, trauma,	3	5	2	1	1	1	50	50	50	50
4	**electrical**											
	insufficient current/voltage	escape behaviour, pain, stress	3	2	1	0.4	0.1	0.45	10	2.5	11.3	25
	evisceration	extreme pain, trauma, if conscious	3	5	2	0.05	0.001	0.07	2.5	0.05	3.5	50

[1]1=mild, 2=moderate, 3=severe; [2] 1 =<5min, 2=5-15min, 3=15-60 min, 4 = >60min; [3]The risk scores allow a ranking but the absolute figures are not on a linear scale

APPENDIX D: 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[5].

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.

[5] 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.

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, 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 B 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.

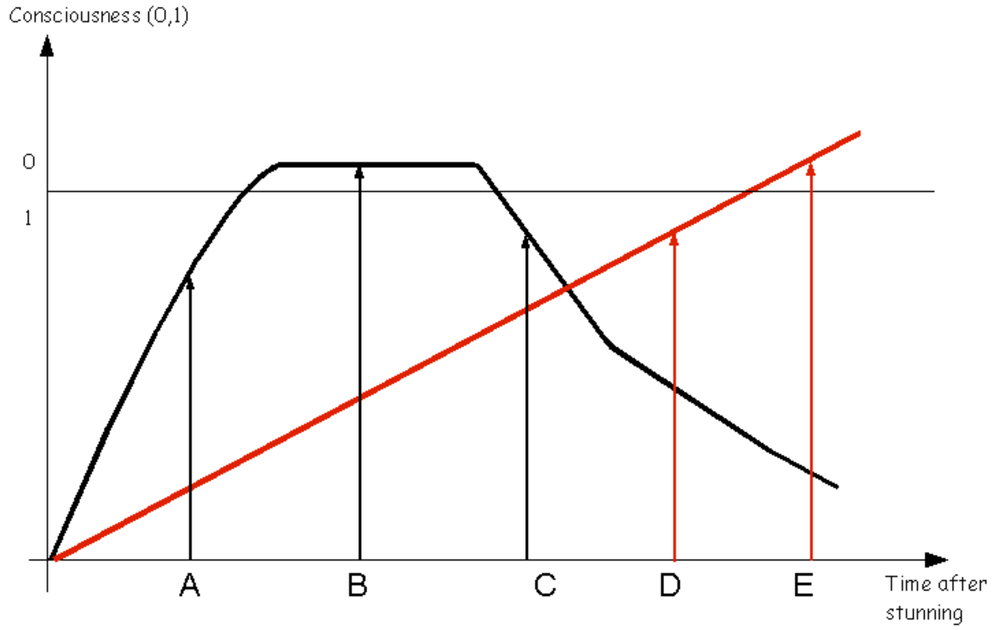

Figure 5. Time to unconsciousness (insensibility) following stunning / killing (horizontal grey line indicates consciousness threshold above which killing takes place without an adverse effect).

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

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 10. 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 11. 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.

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.
Catadromous	catadromous fish live in fresh water, breed in the sea
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.
DFD meat	Dark Firm Dry: animal stressed for a long time before 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.
Glass eels	All stages before entering the farming

	process
Harvest	The killing, slaughtering and processing of fish.
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
Juvenile	Early life stage of fish from 4 to 16 weeks approximately, beginning from glass-eels to on-grower.
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.
PSE meat	Pale Soft Exudative: animal stress just before slaughter
Risk	A function of the probability of an adverse effect and the intensity 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 but more accurately as an alternative term for magnitude.
Size-grading	Sorting the eels according to size
Slaughter	The killing of animals for human consumption.
Slaughterhouse	Any establishment used for slaughtering fish.
Starvation	A period of food deprivation such that the animal metabolises tissues that are not food reserves but are functional tissues.
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.
Tyribs	Self-closing plastic strip (here used to restrain eels)
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
Well-water	Water out of a well and therefore with different temperatures depending on time of year.

Abbreviations

A	ampere
AHAW	Animal Health and welfare
D_adverse	effect the duration of the adverse effect
DFD meat	Dark Firm Dry
EFSA	European Food Safety Authority

EEG	electroencephalogram
EC	European Commission
ECG	electrocardiogram
EU	European Union
mar	milli-ampere
mV	milli-volts
MS	Member States
µS	micro-Siemens
P_hazard	L the probability that the hazard occurs
PSE meat	Pale Soft Exudative
SS_adverse	effect the intensity of the adverse effect
V	volts