Project to develop Animal Welfare Risk Assessment Guidelines on Transport[1]

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Summary

Transport of farm animals is a critical phase for the welfare of the animals because they are exposed simultaneously to a variety of stressors that may result in high levels of fearfulness and pain, inducing psychological and physical stress, thus compromising their welfare. To assess animal welfare during transport, the influential components of animal welfare need to be first established. .

The key objective of the present report is to develop the Risk Assessment (RA) Guidelines and working methodology related to the welfare aspects of transport, for food producing animals including fish. The first chapter of the technical report presents the main components for assessing animal welfare especially referred to transport.

The second chapter gives an overall bibliographic review with regards to the main species transported in Europe, namely pigs, cattle, sheep and goats, horses, poultry, rabbits and fish. For each species the main means of transport (road, sea and air) are described and when possible quantitative information about animal trade flows in Europe are given. Moreover for each species a literature review was carried out to identify the main hazards in every transport phase: preparation for transport, loading and unloading, space allowance, feeding and watering, vehicle design, journey plan, and driving quality.

The following chapter of the report describes RA methodology applied to animal welfare during transport. Different target populations have been defined in order to score the hazards within each particular population. The Consortium decided to depict some scenarios considering the following variables: the species of animals being transported, animal categories within each species, means of transport, duration of the transport and thermal environment during the transport. Among each species, the following categories were dealt with: pigs (post-weaning piglets, slaughter pigs, breeding pigs), cattle (calves, heifers, beef cattle and cows), sheep and goats (lambs, ewes, kids), horses (broken, unbroken, mares with foals and stallions), poultry (one-day-old chicks, broilers, hens, spent hens, ducks and quails, and turkey), rabbits (breeding rabbit and slaughter rabbit), fish (salmon, trout, eel, catfish, carp). For each species, the main types of transport in the EU were chosen in order to give an overview of possible scenarios. For each scenario short transport (less than 8 hours) and long transport (more than 8 hours) were considered. In addition, the thermal conditions during transport were described. Since temperatures that define the thermoneutral zone depend on the species and may vary among breeds and age of the animal, for each animal category the three scenarios - neutral, below and above the thermoneutral zone – were separately classified.

The first step of a RA is the hazard identification (HI). The hazards vary according to the categories of animals. The Consortium decided to separate the hazards accordingly to three different categories: mammals, which are loaded moving on their own feet; rabbits and poultry, which are transported in cages; and fish, which have different needs and peculiarities.

Hazards during animal transport were categorized in two groups: 1) hazards related to facilities and 2) hazards related to management and to the caused adverse effect. Hazards related to facilities were those related to the design of loading facilities (driveways, ramps, lifts, etc), design of the vehicle (vibration characteristics, insulation, ventilation, flooring,

compartment size), design of the cages (flooring, size, material), design of drinking and feeding devices, etc. Hazards related to management were those in which men's attitude towards animals may have a negative impact. One of the most important hazards affecting the welfare of animals during transport is the behaviour of stockmen during loading and unloading. Moreover drivers may contribute directly to poor animal welfare by inappropriate driving, or with poor management of the stationary vehicle (direct sunlight during hot weather, wind and low temperatures exposure during cold weather). The stocking density also represents a major hazard.

The adverse effects of each hazard have been classified according to the outputs of the Welfare Quality® project. Welfare Quality® enables overall assessment of welfare by turning the different components of welfare into 4 welfare principles: good feeding, good housing, good health, appropriate behaviour. Each welfare principle is subdivided into several criteria (absence of prolonged hunger, absence of prolonged thirst, comfort during resting, thermal comfort, ease of movement, absence of injuries, absence of disease, absence of other pain, expression of social behaviours, expression of other behaviours, good human-animal relationship and absence of general fear).

In the Hazard Characterisation (HC), which is the second step of the RA, the quantitative assessment of likelihood that an adverse effect can occur for a given exposure to a hazard was scored.

The third step of the RA is to determine the level of hazard exposure according to the principle of RA, where exposure assessment (EA) is described as the quantitative and/or qualitative evaluation of the likelihood of hazards to welfare occurring in a given animal population.

The fourth step of RA is the risk characterisation (RC) where the identified hazards are ranked in terms of level of risk estimates. The risk estimate is an indicator at the population level, considering not only the likelihood of the animals of that population being exposed to a given hazard, but also the likelihood of the animals experiencing an adverse welfare effect. In addition, in order to give the correct importance to the given hazard, risk managers should consider the magnitude of the adverse effects, which represents the potential animal welfare adverse effect at the individual level, as long as the animal is exposed to the hazard and experiences that adverse effect.

The final section of the technical report presents examples of the whole RA process applied to three chosen scenarios, which describe the most common transport practices within Europe for food producing animals: 1) slaughter pigs transported by road for less than 8 hours in above thermal neutrality conditions; 2) heifers transported by road for more than 8 hours in below thermal neutrality conditions; 3) rainbow trout at finish weight (250 g.) pump loaded transported by road for less than 8 hours in above thermal neutrality conditions. Only the highest ten scored hazard, in terms of risk estimates (median, 5th and 95th percentiles) were presented as a histogram along with their attached magnitude values.

Key words: risk assessment, animal welfare, animal transport.

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Background

A self mandate was launched in September 2007 (EFSA-Q-2007-168) to develop the Risk Assessment Guidelines for Animal Welfare, where three main animal welfare issues have been identified, namely: Stunning and Killing, Transport, and Housing and Management. A harmonised definition of Animal Welfare, including the relationship with Animal Disease, should be also addressed in the framework of this self mandate. The main animal welfare issues (Stunning and Killing, Transport, and Housing and Management) will be dealt with separately. The deliverables from the different projects will be assembled and evaluated in order to produce the final Risk Assessment Guidelines on Animal Welfare, under the EFSA self mandate framework.

As a first step on the implementation of the self mandate a grant under the framework of Article 36 was awarded in December 2007. The awarded consortium of organisations finalised a Report on the RA methodology for animal welfare in relation to the stunning and killing methods in December 2008.

The second animal welfare issue to be dealt with under the EFSA self mandate is Animal Transport. The transportation of animals is an important activity of the farming industry, involving about 360 million heads of livestock (not including poultry and fish) per year in the EU. Live animals such as cattle, sheep, pigs and horses are transported long distances across Europe and beyond, on journeys which are often several thousand kilometres long. In particular, horse transport for slaughter from Central and Eastern Europe is a particular area of concern. In 2004, approximately 150,000 equines were being imported in the Member States (including the intra-EU trade) for slaughter, mainly by road.

It has been widely reported that the transportation of animals for short or long periods, and the related handling, loading, and unloading, may cause stress on animals. A range of clinical, behavioural, physiological, and immunological changes have been documented in different animal species by, for example, increases in heart rate, increased adrenal cortical activity, decreased immunity, increased morbidity and mortality due to infectious diseases after transport.

An Article 36 Grant was awarded in September 2008 to define the Risk Assessment Guidelines and working methodology related to the welfare aspects of transport, for food producing animals including fish; whenever relevant for the risk assessment methodology to be developed, information on non food producing animals may also be included.

Terms of reference

With the scope to develop animal welfares risk assessment guidelines on transport of farm animals, the following objectives have been stated in the granted project:

1. Review of the current available knowledge on transport conditions, physiological and behavioral needs of transported animals and welfare standards for food producing

animals (cattle, pigs, poultry, horses, sheep, goats and fish), including minor commercial species like deer, rabbits and ratites.

The scientific review will be based on latest (last 20 years) available literature, peer-reviewed scientific papers, published documents scientific reports, including OIE norms and standards and other relevant International Organizations, and expert opinions. This review will look at the available information of previous AHAW and SCAHAW reports and opinions on the subject.

2. Collection of information on all critical points of animal welfare will be considered for the different means of transport (including vehicle design, environmental conditions, journey duration, handling, loading and unloading.) All major transport means including land, sea and air will be covered by the project.

3. Review of the current knowledge of potential hazards identified internationally (including 1. feeding and watering, 2. Preparation for transport, 3. Loading and unloading, 4. Vehicle design, 5. Floor & Space allowance, 6. Transport times, 7. Microclimate throughout transportation, 8. Resting time during journey and before slaughter, 9. Handling method and facilities, etc) and assess the adequacy and usefulness of current standards and procedures in compliance with minimum welfare standards.

4. Develop a comprehensive list of potential hazards based on scientific available information in relation to severity, duration and the likelihood of an individual animal being affected and specify the relevant hazards for different species qualitatively, semi-quantitatively and quantitatively.

5. Formulate the selection criteria for scientific data to be considered in the RA procedure and for the use of consultation group (expert opinion) on exposure assessment decision making.

6. Review the current international practices and standards on hazard characterization in order to evaluate the feasibility of using them as welfare monitoring points during transportation.

7. Improve the current hazard characterization technique for a comprehensive list of hazards.

8. Review current literature on the welfare consequences of exposure to different specific hazards in different species, including frequency, intensity and duration of hazard exposure.

9. Propose an improved list of monitoring points and methodology based on scientific measures and on risk analysis technique.

1. Review of the literature

1.1. Animal welfare definition

Animal welfare can be defined in various ways, but there is a growing consensus that whatever the definition, it has to include three elements: the animal's emotional state, its biological functioning and its ability to show normal patterns of behaviour (Duncan and Fraser, 1997; Mendl, 2001).

According to the animal's emotional state approach, animal welfare involves the subjective feelings of animals, so that welfare will be reduced by negative subjective states such as pain and fear, and will be improved by positive states such as comfort and play. Neurophysiology can provide insight into the similarities between human and non-human brains and therefore into the kind of emotions and perceptions that animals are likely to have (Manteca, 1998). The group of structures hypothetically responsible for the expression and sensation of emotions are often referred to as the limbic system. The brain circuits underlying the basic emotions appear to be common in their structure between humans and non-human mammals, so it is likely that man and higher vertebrates experience emotions in a comparable way (Wiepkema and Koolhaas, 1992). However, feelings being subjective states of the animal, their assessment is very difficult.

Measures based on biological functioning and on the animals' ability to cope with the environment also provide relevant information on welfare. Both failure to cope and difficulty in coping would indicate poor welfare (Broom, 1986; 2001). When the control systems regulating body state and responding to dangers are not able to prevent displacement of state outside the tolerable range, a situation of different biological importance is reached. An animal could run into three different situations regarding the capacity to face difficulties created by the environment. One possible situation could be that the environment is particularly difficult for the animal, which cannot overcome the difficulties. The animal in this situation could die or suffer multifactorial diseases. Another possibility could be that the environment is not so difficult for the animal which would eventually manage to adapt to it, although the adaptation would have been a difficult process. The difficulty of the adaptation refers to the cost of the adaptation process for the animal. This cost is the result of two parts: on one hand, the negative consequences of the stress response and on the other hand the possible negative consequences of the behaviour changes shown by the animal. This stress response is similar among different species. The stress response is mediated to a great extent by the hormone corticotrophin releasing factor (CRF) which is secreted by the hypothalamus and has an anxiogenic action. Plasma levels of glucocorticoids, catecholamines, prolactin and endorphins as well as heart rate are among the most frequently used parameters to study short-term welfare problems. Behaviour changes include a reduction of feed consumption and an inhibition of the reproductive behaviour. The third possible situation could be that the environment is appropriate for the animal so the adaptation process is not difficult for it and there is no biological cost for the animal.

In relation to the third definition, animal welfare depends on the similarity between the animal's behaviour and the species' "natural" behaviour. However, this definition is not completely

appropriate: firstly, "natural" conditions are not always good for animal welfare; secondly, the domestication process could have modified some aspects of the animals' biology and domestic animals may be able to adapt to an "artificial" environment with more ease than their wild ancestors; finally, it is difficult to define what is "natural" because animals have a great capacity of adaptation. Despite the problems of this definition, there is evidence that the performance of some of these behaviours is important for animal welfare because the achievement of their functional consequences, in the absence of the behaviours themselves, is not sufficient to reduce their motivation (Petherick and Rushen, 1997).

The three elements mentioned above are by no means contradictory; in fact they are closely interrelated. For example, when animals are prevented from performing a particular behaviour pattern, a stress response may follow (Mason et al., 2001). Also, negative emotional states often result from the animal's inability to show appropriate behavioural responses and thereby failing to cope with the situation (Broom, 2001).

1.2.   Animal welfare components

Transport of farm animals is a critical phase for the welfare of the animals because they are exposed simultaneously to a variety of stressors that may result in high levels of fearfulness and pain, inducing psychological and physical stress (Grandin, 1997) thus compromising their welfare. To assess animal welfare during transport, the influential components of animal welfare need to be established. The Five Freedoms developed by the Farm Animal Welfare Council of the UK (Farm Animal Welfare Council, 1992) provided a first framework to achieve this. These freedoms, which represent ideal states rather than actual standards for animal welfare, include: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; and freedom from fear and distress.

Welfare is multidimensional and its overall assessment requires a multi-criteria evaluation. Welfare Quality® has developed a system to enable overall assessment of welfare, where the different components of welfare to be covered are turned into 4 welfare principles that correspond to the questions:

- Are the animals properly fed and supplied with water?
- Are the animals properly housed?
- Are the animals healthy?
- Does the behaviour of the animals reflect optimized emotional states?

Each of these four principles comprises several criteria, with a total of 12 criteria (Botreau et al., 2007; Table 1). Each criterion represents a separate aspect of animal welfare. The criteria reflect what is meaningful to animals as understood by animal welfare science. The set of criteria considers the following points: 1) exhaustive (containing every important viewpoint), 2) minimal (banning redundant or irrelevant criteria), 3) independent of each other (Botreau et al., 2007).

Table 1. Principles and criteria of animal welfare as developed in the Welfare Quality® project (Botreau et al., 2007).

Principles	Criteria
Good feeding	1. Absence of prolonged hunger
	2. Absence of prolonged thirst
Good housing	3. Comfort around resting
	4. Thermal comfort
	5. Ease of movement
Good health	6. Absence of injuries
	7. Absence of disease
	8. Absence of other pain
Appropriate behaviour[1]	9. Expression of social behaviours
	10. Expression of other behaviours
	11. Good human-animal relationship
	12. Absence of general fear

Taking all these elements into account, the same 4 principles and 12 criteria could be applied when evaluating the adverse effect of hazards on animal welfare during transport in a risk assessment scheme.

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1.3. Animal welfare components during transport

1.3.1. Good feeding

Good feeding includes two elements or criteria: absence of prolonged hunger and absence of prolonged thirst.

During transport, animals are usually deprived of food and this undernutrition might result in hunger. There are several reasons why prolonged hunger may result in poor welfare. First, hunger causes stress and, if sufficiently prolonged or severe, it can lead to debilitation, loss of body condition, immunosuppression and disease. Consequently, prolonged hunger may result in inadequate biological functioning and it is likely to be an unpleasant emotional state (Webster, 1995; Kyriazakis and Savory, 1997). For example, pigs are fasted to reduce gut content during the pre-slaughter period and to prevent the release and spread of bacteriain the faeces during transport and lairage as well as the spillage of gut contents during carcass evisceration (Faucitano et al., 2008). Fasting before slaughter, within reasonable limits, is beneficial for the welfare of pigs as it prevents vomiting and hyperthermia. However a prolonged fasting period causes hunger, aggressiveness (Warriss, 1994), weakness, lethargy and sensitivity to cold (Gregory, 1998). On the other hand, cattle have a greater ability to withstand the rigours of transport and especially the disruption in their normal intake of food if they have been appropriately fed before loading.

Thirst is the sensation that accompanies dehydration. During short transports, animals might be deprived of water. Prolonged thirst causes stress and, if long-lasting or severe, may lead to debilitation, loss of body condition and disease. Thirst also reduces food intake, which in turn may cause all the welfare problems that result from prolonged hunger. During long transports, thirst can occur if animals are given water of poor quality or dirty, when access to water is difficult, either because there is an insufficient number of drinking troughs for the number of animals being transported or the supply system is not properly designed and constructed. Thirst can also occur when unsuitable drinking troughs are being used for the species or categories of animals being transported and/or when the animals are not used to the water devices. Dehydration is most common in animals that are transported long distances, during dry hot weather and when airflow through the moving truck is high. The ability to cope with dehydration varies between species and upon age (Gregory, 1998). Suckling animals are particularly susceptible to dehydration because they have not learned how to drink from a trough and therefore fail to drink the water provided. Poultry and rabbits are transported in containers and to supply water under these conditions is difficult.

1.3.2. Good housing

Good housing includes three elements: comfort during resting, thermal comfort and ease of movement. The means of transport should be designed to ensure that animals are able to satisfy their needs concerning comfort around resting and thermal comfort (neither too hot nor too cold) and to provide enough space for the animal to be able to move around freely.

Lack of comfort around resting is likely to reduce resting time. To satisfy its need of comfort around resting, each animal shall have enough space to stand up, lie down and turn around. Minimal lying area requirements can be calculated according to body weight. Animals are often strongly motivated to rest and preventing them from doing so may cause them distress. Insufficient resting space may involve increased competition and aggression. Lack of comfort around resting may be a consequence of an excessive stocking density or inadequate facilities, particularly inadequate flooring. Rest can be impaired when there is huddling due to low temperature or when there is too much noise or vibration in the vehicle. Overcrowding may increase mortality (heat stress) and injuries, particularly during hot and humid weather (Faucitano, 2000). There is no doubt that deaths occurring during transport indicate a clear compromise of animal welfare. Poor driving (sudden braking or acceleration or over-rapid cornering) can make some animals collapse and trample their companions, consequently jeopardizing their comfort during resting. An inadequate design of the cages, of the containers or of the pens in the lorry can bring the animals to use abnormal sequences of movements to lie down and get up, thereby increasing the risk of injury. The containers used for transport of poultry or rabbits are put one on top of the other inside the vehicle; this may cause urine and faeces to fall on the animals placed underneath affecting their resting. Lying behaviour is an important element of behavioural thermoregulation. In pigs, the combination of measurements of energy metabolism and animal behaviour has shown that the comfort behaviour is reached when they are lying on their side and touching each other. Lying in sternal recumbency or huddling means that it is too cold, and pigs attempt to reduce heat loss. When it is too warm, pigs lie down quickly, they maintain a relatively wide separation between individuals and they increase their respiration rate (Santos et al., 1997). Therefore, high temperature increases the space each animal needs to rest.

Thermal comfort and the relationship between animals and their thermal environment are explained using the concept of thermoneutral zone. This is defined as the range of ambient temperatures that provides a sensation of comfort and minimises stress. Temperatures which are too low or too high cause cold and heat stress respectively. The temperatures that define the thermoneutral zone depend on the species and may also vary among breeds of the same species. Even animals of the same breed may respond differently to ambient temperatures if they have been raised in different environments. Furthermore, the level of production and the amount and type of food given to the animals previous to transport can all influence their response to the thermal environment. The effects of the thermal environment are not solely dependent on air temperature but on "effective temperature", which is the end-result of the interaction between air temperature, relative humidity, ventilation and flooring. Temperatures which are too low or too high cause stress, which can lead to disease and even death if it is severe or prolonged. Heat stress also increases the amount of water required and can therefore increase the risk of prolonged thirst if water supply is limited (NRC, 1981). Pigs and poultry have great difficulty in losing heat and may therefore suffer heat stress at ambient temperatures close to the upper limit of their thermoneutral zone and at high humidity. Heat stress may result from poor ventilation, excessive duration of stops during the journey, with lack of forced ventilation in the vehicle, inadequate design of the vehicle and an overly high stocking density.

The ease of movement, the ability of animals to turn round, to groom, to get up, to lie down and to stretch their legs or wings, have long been considered basic requisites for good welfare (Brambell Committee, 1965). These movements are part of the behavioural repertoire of all species, and animals are highly motivated to perform them. They are also important to maintain the adequate functioning of the body. Difficulty of movement may be caused by a lack of space in the cage, for example hens or broilers transported to the slaughterhouse or in lorries with a low deck height for the transport of farm animals. An excessive stocking density in the lorry or in the cages may also prevent animals from moving normally. The inadequate design of cages and vehicles may prevent animals from lying down and getting up normally. The presence of dominant individuals, particularly when stocking density is high or when mixing unfamiliar animals, may curtail the movement of subordinate animals. Animals tied during vehicle movement can also impair normal movement. Slipping and falling due to an inadequate floor when loading, unloading and moving to lairage may induce fear and pain, and increase stress levels (Gregory, 1998). The slope of ramps is an important aspect when loading or unloading animals. There are important differences between species in their response to negotiating steep ramps, for example, pigs have more difficulties than sheep or cattle.

1.3.3. Good health

Good health is an important component of animal welfare and it can be defined as the absence of injuries, disease and pain.

Injuries can cause acute and/or chronic pain and may be determined by abuse or rough handling, the latter being more common when animals are loaded and unloaded during transport. Injuries can be the result of an inadequate design of the vehicle or of the cages used to transport animals (e.g. slippery floors, sharp protrusions). Fighting with other animals can also cause injuries; this is more common when animals are mixed with unacquainted individuals (particularly in pigs and to some degree cattle) and when animals have to compete for access to feed, water or resting space. The presence of bruises in the carcasses of animals at the slaughterhouse is a serious welfare problem because they are a reflection of bad welfare practices such as rough handling while loading and unloading, high stocking density, excessive fasting duration before slaughter, etc.

Absence of disease is a basic requisite for good welfare. Fit animals at the start of the journey may fall sick, or get injured during transport. Diseases can cause pain and interfere with normal behaviour. It is well documented that transportation of mammals, birds and fish can spread both animal and zoonotic diseases. Infectious diseases in transported animals can be caused by pathogens already present in the animals before transport and from pathogens directly or indirectly transmitted between the transported animals during or subsequent to the transportation. A significant source of infection can also be contaminated transport vehicles and related equipment and persons. A variety of stressors involved in transport are key factors for increasing the susceptibility to infections of transported animals and increasing the shedding of infectious agents in already infected animals.

Poor conditions during transport may cause injury, debilitation or even death, particularly in pigs and poultry. These problems can be exacerbated during longer journeys. Pigs often suffer from motion sickness due to vibration, acceleration, braking and cornering. Motion sickness has been defined as retching, chewing, foaming at the mouth, and sniffing at the air. Pigs exposed to rough journeys tend to have higher cortisol levels and are more likely to become travel sick if presented with food before travelling (Bradshaw et al., 1996a). It is therefore advisable to fast pigs before transport. However, if journeys are long this can lead to unacceptably long periods without food. Long feed withdrawal times during transport may also lead to a more favourable environment for the growth of *Escherichia coli*, *Campylobacter* and *Salmonella* species (Martin-Pelaez et al., 2008). The gastrointestinal tract of most pigs can act as a reservoir for enterohaemorrhagic *Escherichia coli*, *Campylobacter* and *Salmonella* species. These enterobacteria usually become established and multiply within the caecum during transport. Furthermore, stress can result in increased emptying of the caeca into the colon and faster evacuation of the digest. Therefore, long fasting periods and stress increase *Salmonella* shedding in infected animals and even determines excretion in silent carriers. Animals exposed to *Salmonella* can start to shed it in the faeces within 2 h.

Pain is defined as an aversive emotional experience and is therefore a welfare problem. When moving animals during loading and unloading, the combination of high speeds and poorly designed handling systems is detrimental to animal welfare because handling the animals at this rate requires considerable coercion and triggers the use of goads and sticks. Shocking animals with electric goads results in lesions and pain, and significantly raises heart rate, open mouth breathing and many other physiological indicators of distress.

1.3.4. Appropriate behaviour

The principle 'Appropriate behaviour' includes the expression of social and other behaviours, good human-animal relationship and the absence of general fear.

All farm species are social animals and as such are strongly motivated to have contact with conspecifics. Positive social interactions such as social licking have a desirable effect on welfare for at least two reasons. First, they have been shown to elicit pleasant physiological responses. Second, they reduce the negative effects of stressful events; this is known as "social buffering" of the stress response (Kikusui et al., 2006). Negative social interactions, such as aggression, impair animal welfare. Aggression may result in injuries, pain and, in extreme cases, death of the animal. Moreover, aggression leads to fear and stress within the whole group (Fraser and Rushen, 1987). Fear is an aversive emotional state and is therefore a welfare problem. Stress may compromise body functioning by impairing immune function and decreasing food intake. Also, negative social interactions may interfere with the expression of normal behaviour, particularly in low ranking animals, and reduce food intake and resting time which may lead to debilitation and health problems, such as lameness. Disruption of social groups (through mixing unacquainted animals during transport, for example) may lead to an increase in aggressive behaviour and a reduction in positive social interactions. Mixing of unacquainted animals has adverse effects on welfare and production, mainly because animals fight with each other in order to establish dominance relationships,

with the most aggressive interactions being typically shown during the first few hours after grouping (Meese and Ewank, 1972).

The quality of stockmanship has a profound effect on the animals' welfare (Boivin et al., 2003). For instance, despite centuries of domestication, exposure to human beings remains one of the most potentially alarming experiences for many farm animals. More specifically, unless they have become accustomed to human contact of either a neutral or positive nature, the predominant reaction to people is of fear (Duncan, 1990; Jones, 1997). When a person approaches closer than a certain distance, domestic animals try to escape. This critical distance, which defines the flight zone, varies among species and individuals of the same species, and depends upon previous contact with humans. Animals reared in close proximity to humans may have a smaller flight zone, whereas those kept in free range or extensive systems may have flight zones which may vary from one to many metres. The sudden penetration of the flight zone may cause a panic reaction and should therefore be avoided. The stockpersons' behaviour, which can vary from calm, gentle, frequent and "friendly" to rough, rushed or infrequent, is a major variable determining animals fear of or confidence in humans and, hence, the quality of the human-animal relationship. Positive handling can result in animals showing less fear of novel objects (Hemsworth et al., 1996) which could further reduce the stressfulness of transport. Loading and unloading are the most stressful phase of transport (Hall and Bradshaw, 1998). Loading and unloading require the proximity to humans which can cause fear in the animals. Moreover, cattle, sheep and pigs have considerable difficulty negotiating steep ramps, which can lead to rough handling and excessive and inappropriate use of electric goads during loading.

Fear and anxiety are two emotional states induced by the perception of a danger or a potential danger, respectively, that threaten the integrity of the animal (Jones 1987; Boissy, 1995). Fear causes a stress response which, if long lasting, may negatively affect body functioning by impairing the immune function, the reproductive performance, and food intake and conversion. Fear has a relatively important genetic component. Therefore, some breeds or individuals are likely to be more easily frightened than others. In front of a threatening situation, animals adopt adaptive behaviours such as escape, freeze, back off, shake or other behaviours including lying down. Social species which collaborate in defence against predators, such as pigs, vocalize a lot when caught or hurt. Species which are unlikely to be able to defend themselves, such as sheep, vocalize far less, probably because such an extreme response merely would give information to the predator that the animal attacked is severely injured and hence unlikely to be able to escape. Therefore, it is wrong to assume that an animal which is not vocalising is not injured or disturbed by what is being done to it. Slaughter red meat animals have wide-angle vision but only have limited forward binocular vision and poor depth perception. This means that they can detect objects and movements beside and behind them, but can only judge distances directly ahead. A sudden movement during loading, shadows, blinding lights or objects, discontinuities in floor texture and colour may all induce fear and its consequences. On the other hand, these animals can hear over a greater range of frequencies than humans and are more sensitive to higher frequencies. High sound levels produced during the journey, vibration of the vehicle and human vocalization represents a source of stress. Fear becomes a welfare problem particularly when animals

encounter new or unexpected stimuli, (e.g. a sudden noise, an unfamiliar smell, drafts, inappropriate handling), or situations, e.g. a new environment in the vehicle, change of cage from the farm to a new and clean one in the lorry.

2. ANIMAL TRANSPORT IN THE EUROPEAN UNION

2.1. Pigs

2.1.1. Road transport

The most common means of transport for pigs is the road vehicle. Pigs are usually transported in large trucks, equipped with a loading lift and which may hold over 200 animals. Pigs are transported either within national boundaries or internationally. There is relatively little information about the duration of journeys and the distances travelled by pigs within national territory. In the UK, the results provided by Warriss and Bevis (1986) and Riches et al. (1996) indicated that the average time pigs spent in transit was 2 to 3 hours, corresponding to distances of between 80 and 105 km. In Spain, Gispert et al. (2000) surveyed 116 commercial pig transports to the slaughterhouse comprising 15695 pigs and observed that in 47 of these transports the means transport time was <2h whereas in 69 of these transports the means transport time was >2h. In a transport survey conducted in Europe from 1992 to 1995 (AIR3-CT92-0262), the majority of pigs in all countries travelled less than 2h with average distances of 100 km or less. Most of these transports concern slaughter pigs to the abattoir.

Long distance transport (> 8 hours) of pigs is still very common. The EU database (TRACES) recorded 97252 pig transports across Europe and 35.4% of these transports were long distance transports. Long distance transports include piglets, slaughter pigs and breeding pigs. The Netherlands exports two million pigs a year to Spain, Italy and Eastern Europe. Some of them are transported for slaughter but most are piglets for fattening (Table 2). During long distance transport, any deficiencies in transport conditions are likely to have more serious consequences than in shorter national journeys (Warriss, 1998).

Table 2. Long distance pig exports from the Netherlands to southern and Eastern Europe (2007).

Importing Member State	No. of piglets exported for fattening	No. of pigs exported for slaughter
Spain	977433	6007

Italy	354188	112597
Hungary	185245	39900
Poland	269288	16472
Croatia	153087	1058
Romania	101191	11994
Slovakia	45929	2556
Greece	9114	-
Czech Republic	12640	25184
Bulgaria	9430	-
Albania	3405	-
Slovenia	0	-

Source: Long distance animal transport in Europe (2008).

Denmark and Germany also export pigs to Southern and Eastern Europe. Most of the trade consists in piglets for growing (Table3).

Table 3. Piglet exports from Denmark and Germany to Southern and Eastern Europe (2007).

Importing Member State	Exporting member: Denmark	Exporting member: Germany
Spain	188	299520
Italy	179745	46699
Hungary	6027	8301
Poland	40868	3529
Romania	5746	2921
Czech Republic	13724	41682

Source: Long distance animal transport in Europe (2008).

"The welfare of animals during transport (details for horses, pigs, sheep and cattle)" adopted on 11 March 2002 (SCAHAW, 2002) reviewed the existing scientific literature regarding the

effects on welfare of loading densities, travelling times, resting times and watering and feeding interval during transport. In this section, the most recent relevant scientific literature about the effects of transport and associated handling on the welfare of animals is reviewed.

Currently, transport of pigs by train is rare because of the difficulties of the animals being transported to a station and reloaded, increasing the adverse effects of loading and lengths the journey.

2.1.2. Sea transport

There is no information regarding sea transport of pigs in the EU but this type of transport exists in the USA where pigs are transported by sea from the USA and Canada to the Hawaiian Islands (PETA, 2008).

2.1.3. Air transport

The use of aircraft is limited to breeding animals because it is expensive. There is transport of sows/boars by air from Denmark to China (http://logisticstoday.com/logistics_services /outlog_story_6647/).

2.1.4. Literature review: identification of the main hazards in each transport phase

2.1.4.1. Preparation for transport

One of the most important requirements to achieve a good welfare during transport is that transported animals are fit. Severely lame or weak, emaciated animals are not fit for transport (Grandin, 2001). The most serious problem with a lack of fitness for transport occurs in cull breeding stock. Weak animals are more likely to fall down in a truck without being able to get back up again. Non ambulatory animals (any animal that due to age, injury, metabolic or systemic disease, etc., is unable to stand or walk without assistance) on the farm are almost impossible to load onto a truck in a low-stress manner. A major factor causing unfitness in pigs is over-selection for meat production. Modern hybrid pigs, which have been selected for rapid growth, leanness, and a large loin area, are often prone to stress that causes the pig to become non-ambulatory.

Pigs can be taken directly from the home pen to the transport vehicle or they can have an indirect transfer, where pigs are held away from the main herd for a period of time before being loaded onto the transport vehicle. Irrespective of the system used, if pigs are not kept in their original group until loading takes place, fighting amongst animals could occur and this could lead to elevated levels of plasma and salivary cortisol (Geverink et al., 1996).

Pigs are fasted for some time before collection irrespective of the length of the planned journey as they can suffer from travel sickness. Averós et al. (2008), in a multilevel logistic regression model, observed that pigs that were not fasted had doubled the risk of mortality irrespective of whether the pigs were injured or not. Moreover, there is strong evidence that fasting will decrease muscle glycogen stores and improve pork muscle quality (Leheska et al.,

2002). However, the available literature for an optimal fasting time in relation to travel is contradictory. Warriss (1994) recommended that pigs' last feed should be arranged for between 4 and 12 h before loading. However, Warriss (1998) suggested that 4 h may be too short a time to prevent pigs from vomiting during transport if the transport duration is short or the driving technique is rough. Gispert et al. (1996) measured the effects of feed withdrawal for less than 12 h, for 12-18 h and for more than 18 h on mortality in five slaughter plants and observed that the lowest overall mortality occurred in the 12-18 h group. Gispert et al. (2000) in the same study also observed that the lowest level of cortisol was measured in the group of animals fasted on farm from 12 to 18 h (7.5 μg/100 ml). This fasting time allows pigs to better cope with stress compared to pigs fasted for >18 h (8.8 μg/100 ml) or for <12 h (8.4 μg/100 ml). The elevation of the cortisol levels either during short or long periods of fasting can be due to the occurrence of travel sickness when pigs are transported on full stomach or can also be caused by the increasing demand for energy supply. In pigs, fat mobilization, as the main source of energy, starts after about 16 h of fasting. Currently, a maximal feed withdrawal of 16-24 h before slaughter is recommended. After this time, animals should be fed with moderate amounts of food (Velarde, 2008).

2.1.4.2. Loading and unloading

Most authors agree that loading and unloading are the most stressful processes during transport in pigs. Stephens and Rader (1982) compared handling to transportation and they found that handling caused more disturbances than the trip itself. Poor handling manifests itself in a number of ways leading to economic losses and welfare concerns.

One of the factors that can influence the stress level experienced by pigs during handling and transportation is the distance that pigs are moved from their home pen to the lorry. Ritter et al. (2007) observed that moving pigs for a long distance during loading (61.0 to 91.4 m), compared with a short distance (0 to 30.5 m) increased the incidence of open-mouth breathing after loading (24.9 vs. 11.0%) and tended to increase the incidence of non ambulatory and injured pigs during loading and at the slaughterhouse. The number of pigs moved per group during loading can affect stress and ease of movement. Lewis and Glone (2007) studied the effects of different group sizes of pigs ranging from 1 to 10 pigs/group moved from home pens to a transport vehicle on cardiovascular responses, time and handling measures. They observed that as the group size increased, heart rate, handling difficulty and time to complete the journey increased. Moving pigs in groups of 5 or 6 caused them less elevation of the heart rate than moving themin larger groups . The total time required to load a truck was similar when 10 pigs were moved at a time as when 5 or 6 pigs were moved at a time. The same authors conclude that considering heart rate elevation and time to load a livestock trailer, moving 5 or 6 pigs at a time is optimum for both time savings and heart rate elevation during handling.

Rough handling by untrained people during loading increases stress levels in the animals transported (Grandin, 2002). The design of the facilities also can affect the handling which animals are subject to. Handling of slaughter pigs during loading/unloading becomes more difficult when the corridor's width they are moved through is narrower than 90 cm because

two animals cannot walk down side by side (Grandin, 2002). The inadequate maintenance of the facilities (sharp protrusions on the walls, gaps and potholes on the floor) can cause injuries in the animals affecting their welfare. Moreover, Averós et al. (2008) observed that the risk of mortality during the journey increased when the loading time per pig was reduced, particularly when the levels of recorded injuries were high and the pigs had not been fasted. Rabaste et al. (2007) observed that rough handling increased the frequency of climbing, slipping and turning around during unloading from the truck in barrows. In this study, pigs handled roughly were moved as quickly as possible with an electric prod, whereas pigs handled gently were moved slowly with a plastic board during unloading, and a whip (used to tap on the back, only when necessary) on their way to the restrainer. Pig movement can be also affected by air movement, shadows and lighting. Pigs have a tendency to move from a darker area towards a brighter area, but they will not approach blinding light (Grandin, 1982).

Pigs have difficulties in negotiating steep ramps. Warris et al. (1991) observed that between 0º and 20º, slope had little effect on the time taken to ascend it. However, above 20º the time taken to ascend increased linearly. Furthermore, the pig's heart rate increases as the angle of a loading ramp increases (Van Patten and Elshof, 1978). Mayes (1978) studied a pig's stride width and found that cleats (2.5 cm x 2.5 cm) on ramps must be spaced 20 cm on the centres to fit the normal walking stride of an animal and missing cleats can cause leg injuries in the animals.

2.1.4.3. Space allowance

Optimal stocking density for pigs during transport has been a subject for debate in recent years. EU Directive 95/29/EC and Council Regulation (EC) No 1/2005 state that all pigs should as a minimum be able to stand and lie down naturally and recommended a stocking density of 0.42 m^2 per 100 kg pig. The value of 0.42 m^2 per 100 kg pig was suggested by Lambooij et al. (1985), as a suitable compromise between welfare, meat quality and transport economy for long distance transports (2 days). However, several equations have been proposed to calculate the minimum space required for pigs (Table 4).

Table 4. Equations used to predict minimum space requirements for groups of pigs under various conditions. Warriss (1998).

Equation	Space allowed (m² for a 100 kg pig)	Reference
$A = 0.021 W^{0.67}$	0.459	FAWC (1991)
$A = 0.01 W^{0.78}$	0.363	Randall (1993)
[*]$A = 0.18 W^{0.67}$	0.394	Petherick and Baxter (1981)

| [†]$A = 0.048 \ W^{0.67}$ | 1.050 | Petherick and Baxter (1981) |
| [#]$A = 0.027 \ W^{0.67}$ | 0.590 | Petherick and Baxter (1981) |

[*]Minimum space required for sternal recumbency

[†]Minimum space required for lateral recumbency

[#]Minimum space on fully-slatted floors to maintain growth and food conversion efficiency

Some authors stated that more space during transport allows pigs to lie down and rest more quickly with better welfare (Lamboij and Engel, 1991; Warris et al., 1998b). On the other hand, other authors concluded that pigs do not tend to lie down during short distance transports and a fairly high stocking density that allows pigs to support one another during transport will reduce the risk of injury (Barton-Gade and Christensen, 1998). Barton-Gade (2000) investigated the effect of stocking density during short distance transports on certain stress measures and pig behaviour. Four different stocking densities were aimed for: 0.35, 0.39, 0.42 and 0.50 m² per 100 kg pig. There was no systematic change in the percentage of carcasses with unacceptable skin damage connected to stocking density. Braking and acceleration combined with turns or roundabouts disturbed pigs irrespective of stocking density. There is a general trend towards fewer pigs standing and more pigs sitting and lying as transport progressed for both stocking densities (0.36 and 0.42 m²/100 kg pig). There were always more pigs standing and fewer pigs sitting with 0.42 than with 0.36 m². The results of this experiment showed that there is no increased tendency for pigs with more space to lie during transport. Moreover, there was little effect of stocking density on measures of stress such as blood cortisol or early rigor mortis development. Chevillon et al. (2003) also measured the influence of three loading densities (0.42, 0.50 and 0.60 m²/pig) during a 36-hour-transport on the amount of feed ingested throughout the transport period, weight loss, carcass yield, meat quality and the behaviour of the pigs in the truck. They observed that increasing the floor area per pig beyond 0.42 m²/pig did not result in an increased feed consumption. The analysis of the effect of the three loading densities on loss of live weight, carcass yield percentage, final pH and carcass appearance showed no statistically significant difference. The currently prescribed loading density of 0.42 m²/pig seems optimal because pigs can lie down or stand up in their natural position to feed and drink successively. Hence, the increase in floor area per pig in the truck of 20 to 40% did not result in an improvement in terms of observed feed consumption, loss of live weight, carcass yields and parameters of meat quality and carcass appearance. This statement is not in agreement with Ritter et al. (2007) who evaluated the effect of floor space (0.396, 0.415, 0.437, 0.462, 0.489 or 0.520 m²/pig) of the trailer during transport on the incidence of transport losses (dead and non-ambulatory pigs) on arrival at the slaughterhouse, and observed that floor space influenced the incidence of pigs dead on arrival, of total non-ambulatory pigs and of fatigued pigs. The incidence of dead animals was generally higher for the 3 lowest transport floor spaces (0.396, 0.415 and 0.437 m²/pig) respect to the 2 greatest transport floor spaces (0.489 and 0.520 m²/pig) and the authors concluded that transport floor space had a major effect on transport losses and suggested that these losses were minimized at a floor space of 0.462 m²/pig or

greater. However, Gispert et al. (2000) evaluated the effect of two different stocking densities <0.40 m^2/100 kg pig vs. >0.40 m^2/100 kg pig in five Spanish pig commercial abattoirs and observed higher lactate concentrations in the stocking density of <0.40 m^2/100 kg pig compared with >0.40 m^2/100 kg pig (121.7 vs 111.1 mg/100 ml) which indicated that pigs arrived more exhausted at the exsanguinations point when they were transported at higher stocking densities.

2.1.4.4. Feeding and watering

In the EU, after 24 h (with continuous access to water), pigs must be unloaded, allowed to rest for 24 h and provided with food before continuing the journey (1/2005/EC). Becker et al. (1989) suggested that because feed and water deprivation requires changes in energy metabolism and fluid regulation, extended transportation times could be expected to increase the demand on these physiological systems. Chevillon et al. (2003) reported that pigs eat from 2 to 5 times more when the truck stops than when the means of transport is in motion. Furthermore, feeding and watering pigs on the truck would avoid the stress of unloading and mixing in the staging point compartments (Chevillon et al., 2003).

2.1.4.5. Vehicle design

The sensitivity of pigs to extreme temperatures is well known, but experiments on how ambient temperatures interact with other transport variables appears not to have been reported. The temperatures encountered by pigs during transit can vary up to approximately 20ºC. This variation in temperature within the vehicle is related to variation in the outside temperature and to the amount of water and heat produced by pigs during transport (Kettlewell et al., 2001). Given that the thermoneutral zone for pigs is 26-31ºC, the air temperature should not exceed 30ºC (Randall, 1993).

Pig welfare during transit is highly dependent on vehicle design and driving skill as well as the quality of the road. A higher mortality rate is usually reported in the front compartment, immediately behind the driver's cabin, where the ventilation rate is poor (Barton-Gade et al., 1996). When the vehicle is in motion, ventilation is not compromised if the openings are sufficiently large and go along the length of the vehicle at the pig height. Chevillon et al. (2004) recommended openings of 40 cm in hot weather conditions to ensure good ventilation (300 m^3/h of air flow per pig) inside the truck; in cold weather conditions, openings should be partially or fully closed to reduce the air flow to 113 m^3/h per pig. The use of mechanical/forced ventilation reduces deaths in the truck (Nielsen, 1982), particularly when the vehicle is stationary. A forced ventilation with a capacity of 75m^3 per pig (120 pigs in all) during transport at temperatures of 20ºC, combined with an intermittent misting system at temperatures of 25ºC, resulted in low transport mortality (0.01%) (Christensen and Barton-Gade, 1999). Moreover, Chevillon (1998) observed that spraying pigs for 5 minutes at the end of loading reduced body temperature by 10%.

The floor type is also important for the comfort of pigs during transport. Christensen and Barton-Gade (1996) recommended lightweight rubber for its anti-skid and anti-noise

properties. Guàrdia et al. (2009) also observed that when the lorry was equipped with polyester and iron, animals had lower risks of lesions. The risk of having at least slight skin damage increased from 11.5% and 11.7% for polyester and iron, respectively, to 16.4% for aluminium. Furthermore, in previous studies, Guàrdia et al. (2004, 2005) observed that the use of polyester and aluminium reduced the risk of dark, firm and dry (DFD) meat, which is possibly due to the lower negative effects of physical stress caused by the need to keep the standing position. However, despite the consistency between these studies showing that the type of flooring of the lorry exerts an effect on skin damage and pork meat quality, results are not fully conclusive. Overall, results discard iron as the surface of choice but do not contribute to evidence that the polyester, with better anti-slip, with noise and insulator properties, is the most comfortable flooring for transportation.

Other important physical factors that may affect the welfare of the animals during transport are vibration and noise. Exposure of pigs to simulated transport (noise and vibration) led to an increase in plasma vasopressin, which is an indicator of travel sickness (Forsling et al., 1984). Randall and Bradshaw (1998) performed direct behavioural observation of individual pigs transported for commercial purposes for symptoms of travel sickness (sniffing, foaming at the mouth, chomping, and retching or vomiting) during short (100 min) and long journeys (4.5 h). They observed that on both short and long journeys pigs exhibited symptoms of travel sickness. During the long journeys 26% of pigs (13 out of 50) vomited or retched while 50% showed advanced symptoms of foaming and chomping. In conclusion, vibration is potentially an important source of stress during transport but there is little information on the frequencies and magnitudes of vibration which are important.

2.1.4.6. Journey plan

Council Regulation (EC) No. 1/2005 stipulates that pigs are to be unloaded, fed and allowed to rest for 24 hours at control posts whenever the duration of transport exceeds 24 hours. However, Bradshaw et al. (1996a) suggested that, because loading and unloading is a very stressful period, and the animals become travel sick, unloading the pigs during a long distance journey in order to rest them and allow them food and water (and subsequently re-loading them back onto the vehicles with full stomachs), may be the worst possible course of action. Moreover, Chevillon et al. (2003) performed two transport runs lasting 36 hours (20 hours transport - 9 hours rest stop - 7 hours transport) to study if it would be desirable to unload pigs at the control post and observed that the heart rate records of unloaded pigs showed peaks due to stress and (or) effort during unloading and reloading operations. In addition, whether or not the pigs were unloaded at the control post had a small effect on the feed and water consumption, on the weight loss or carcass yields. Finally, analysis of behaviour failed to show better levels of rest and feeding in unloaded pigs.

The season in which pigs are transported may have negative effects on the comfort of animals. An increase in mortality has been reported in hot humid conditions (Abbot et al., 1995). However, Gosálvez et al. (2006) assessed the effect of season on pigs transported to slaughterhouses in 496 Spanish commercial journeys involving 90366 pigs and observed that mortality was not affected by the season in which pigs were transported; this is probably due

to the fact that during the warmer seasons, drivers took precautions to protect animals from extreme conditions, such as undertaking journeys at night, reducing loading densities and showering animals. On the other hand, Werner et al. (2007) studied the impact of season on mortality rates during transport and lairage and observed that in the summer months of June, July and August, between 1999 and 2003, more pigs died (0.18%) in comparison to other seasons (winter: 0.13%; spring: 0.15%; autumn: 0.13%). However, Gosálvez et al. (2006) and Dalla Costa et al. (2007) observed higher carcass bruises in winter, which led them to conclude that cold temperatures, acting as a grouping stimulator, increased climbing and fighting.

It is generally accepted that the duration of the journey has a negative impact on pig welfare. Malena et al. (2007) studied the effect of the distance on mortality rates in slaughter pigs, young sows,sows, and boars during transport in the period from 1997 to 2006 to determine which categories of pigs appeared to be the most susceptible to transport-induced stress; they observed that the lowest mortality rates occurred at short transport distances (<50 km and 51-100 km), as compared to long transport distances (101-200 km, 201-300 km, and >300 km). However, Gosálvez et al. (2006) observed that if journey planning and handling at loading were adequately performed, distances (from <50 km to >100km) did not impair the welfare of slaughter pigs.

Pérez et al. (2002) observed that in normal Spanish commercial conditions (0.36 m^2/100 kg pig), pigs subjected to 15 min transport showed a more intense stress response (leukocytosis with lymphocytosis, greater lactate and cortisol concentrations) than pigs subjected to 3 h transport, when they were immediately slaughtered on arrival at the slaughterhouse. Transport for 3 h may have allowed the animals to adapt to the transport conditions and act as a resting period like a lairage. Probably pigs subjected to short transport would need longer lairage time as resting time.

Werner et al. (2007) analysed slaughter information from a large commercial slaughter company in Germany and observed that 1 h and 8 h journeys had both a negative impact upon welfare with regard to mortality. In particular, short journeys lead to more pathological findings during veterinary inspection at the abattoir, leading to higher incidences of poor meat quality. Authors suggested that animals transported for a short period have less time to recover from loading-dependent excitement. Regarding long transport, Brown et al. (1999) observed that a total journey time of 8 to 16 h under good conditions, even without access to water, appeared to be acceptable from an animal welfare point of view. However, Brown et al. (1999) also found that pigs appeared to become dehydrated after longer journeys, as indicated by the increases in the concentration of plasma total protein and albumin, but not in osmolarity. The most severe dehydration occurred in pigs transported for 24 h.

2.1.4.7. Driving quality

A journey can be characterized as "rough" or "smooth" by accelerometers fitted to the body of the animal (Cockram et al., 1996) or to the vehicle (Bradshaw et al. 1996). When there are many shock events as it occurs when road conditions are poor or driving is inconsiderate,

heart rate may increase (Hall and Bradshaw, 1998). Hambrecht et al. (2005) studied the effects on stress level of two transport conditions: short (50 min) and smooth, and long (3h) and rough transport and observed that long and rough transport did not result in elevated cortisol. It is possible that during longer transports, animal have more time to adapt to transport conditions after the stressful events, and actually arrive in a better condition at the slaughterhouse than after a short transport. However, in the current experiment the last third of the journey was smooth for all pigs, which might have promoted a general habituation to transport in the long transport group.

2.2. Cattle

2.2.1. Road transport

In Europe cattle transport is mainly done by road. In 2008 it was reported (Eurostat, 2009) that about 88 million cattle were kept in the European Union. Nearly all of them were transported at least once during their life time. Table 5 shows a few examples of transportation routes between Member States for special categories of cattle.

In principle there is a difference to be made between long and short journeys. The requirements for short journeys of up to 8 hours are less than those for transports which exceed this duration. Regulations for special animal transport vehicles are given in the European Council Regulation (EC) No 1/2005. Due to the fact that in the EC Regulations the specifications for short journeys are relatively few, the variation of possible transport vehicle design is much broader. Short journeys are very common for transport to slaughterhouses and to rearing farms. In 2007 about 46 million meat cattle were produced in Europe (Faostat, 2008). These cattle had to be transported at least once, as it were, to the slaughterhouse but often a second transport was incurred through the transport of calves from rearing to fattening farms.

Long journey transport vehicles, in order to be approved according to EC Regulations, have to provide adequate in-journey microclimate, and feeding and watering facilities. Also the special requirements of each cattle category - calves, heifers, lactating cows or bulls - must be recognized and taken into account. Because of the economic factors bound to provide these facilities, specialized means of transport are usually constructed for large numbers of animals. Long journey transport of cattle within Europe is done for both breeding and slaughter cattle. For these long journeys specialized semitrailers, goose neck trailers or truck-trailer combinations are used.

Table 5 Selected examples of traffic flows of different cattle categories in Europe in 2005 (Eurostat (2006) cited in Corson and Anderson, 2008)

Lightweight calves (80-160kg) for slaughter	Fattened calves (130-300kg) for slaughter	Heifers (over 300kg) for slaughter	Cows (over 300kg) for slaughter (generally

route	No animals	route	No animals	route	No animals	route	No animals
						spent dairy cows)	
Germany to Netherlands	20000	The Netherlands to Belgium	30000	France to Italy	21000	Portugal to Spain	474000
Spain to France	13000	Belgium to the Netherlands	25000	Spain to Italy	14000	Germany to the Netherlands	12000
Germany to Italy	10000	France to Italy	23000	Ireland to Spain	4000	Belgium to France	12000
France to Italy	7000	Germany to the Netherlands	12000			The Netherlands to Germany	8000
Hungary to Slovenia	7000	Germany to Spain	7000				
Poland to Spain	6000	Portugal to Spain	5000				
Poland to the Netherlands	6000	France to Spain	4000				

In 2006 about 29913 heifers were exported from the EU to Russia. The number of exported heifers increased in 2007 to 61338. The main exporters were Germany and the Netherlands (Eurostat, 2008). The transport of the heifers from the Netherlands to the Eastern part of Russia – a journey of more than 6000 km – can require up to 80 hours (Transport in Europe, 2008). Many calves are also transported for fattening, for example in 2006 Ireland exported 100 000 calves and Italy imported approximately 300 000 calves (Eurostat, 2008).

2.2.2. Sea transport

For intra-EU trade cattle are shipped by sea from ports in Ireland and in the United Kingdom or from the Azores to the European continent. Cattle are also exported from French and Spanish ports to North Africa and to the Middle East. Starting points of sea transports for slaughter cattle are Ireland (Port Waterford, Cork and Greenore), Italy (Trieste), Slovenia (Koper) and France (Marseille) (Appelt, 2001). In 2006 the UK exported about 128000 cattle to the continent of Europe (Phillips, 2008). Most sea transport is done by roll-on/roll-of Ferries.

2.2.3. Air transport

Air transportation of cattle is very rare because of its high costs. Only a few valuable breeding cattle are shipped by air. Air transport is regulated by the guidelines of Live Animal

Regulations of the International Air Transport Association, which gives detailed information regarding dimension and design of cages and the handling of transported animals.

2.2.4.   Literature review: identification of the main hazards in each transport phase

2.2.4.1. Preparation for transport

Grandin (2003) stated that loading physically fit, healthy animals on the vehicle is the single most important factor to maintain an adequate level of welfare during transport. So selection of animals for transport is a major factor in assuring animal welfare during transport. Along with the Council Regulation (EC) No 1/2005, the OIE animal welfare guideline specifies criteria for unfit animals (OIE, 2005) such as sick, injured, weak, disabled or fatigued animals or cows in an advanced state of pregnancy and newborn calves with unhealed umbilical cord.

Mixing unfamiliar cattle from different social groups before or during transport can induce a significant risk of threatening and fighting behaviour (Kenny and Tarrant, 1987, Warriss, 1990). In a survey on bulls transported to slaughter under commercial conditions, Mounier et al. (2006) studied the influence of transfer conditions and previous handling in relation to the decline of meat pH determined by an increase of stress. Their results suggested that social aspects, like the presence of bulls from the same finishing group, can limit stress and improve the pH decline. Additionally, they observed that events and management before transport also affected pH decline. Cattle are calmer if they are accustomed to handling.

Before transport cattle should be well rested and fed with sufficient good quality feed which should be withdrawn 12 hours before loading (Eldrige et al. 1989). The effect of 8 hours fasting prior to 8 hours road journeys was investigated by Earley et al. (2006). Animals that were fasting before transport lost 9,4% of live weight compared to a 7,2% live weight loss of non fasting transported animals. The non-transported but fasted control groups lost about 6% of their weight. Physiological and haematological measurements showed no significant differences, so Earley et al. (2006) concluded that 8 hours of transport, even when cattle were fasted 8 hours prior to transport, did not appear to have negative effects on animal welfare.

In an investigation on the energy metabolism of cattle during long transport, all the cattle showed a catabolic energy metabolism. In the second part of the transportation, bulls and heifers only showed a ketonic metabolism for different reasons (Marahrens et al., 2003). In bulls, mixing of social groups plays a roll due to fighting behaviour and social rank order. Thus they concluded that for long transportation of cattle, habituation of animals to feeding during transport in accordance to their home feeding regime as well as rumen physiology is important because unaccustomed feed can induce lower acceptance resulting in lower feed intake.

2.2.4.2. Loading and unloading

In order to load cattle in a lorry, the cattle must be driven from the stable to the ramp. To improve animal welfare and reduce bruises and stress driveways must be constructed and

ordered in a livestock friendly way. Facilities and structures of cattle driveways are described by Grandin (1997). Cattle are driven forward more easily if they walk in single file (Grandin, 1980) with little change of direction and without dead ends in order to prevent turns.

Von Holleben et al. (2003) recognized that cattle are calmer and show less turning around when driven in groups with more than three cattle per group, when they have uniform side protections of equal height (minimum 1,5 m) and when they are not mixed with unfamiliar animals. Due to the fact that cattle should be driven in groups, a minimum width of 2m is recommended for driveways, gates and loading ramps because at least two cattle should be able to stand side by side. Balking can be prevented if driveways are brightly illuminated, never the less without shadows and glare (Grandin, 1997).

To evaluate stress during loading and unloading Maria et al. (2004) developed a scoring system which assesses time and behavioural events of the unloading/loading process. The results indicated that loading was more stressful than unloading and that higher scores implied significantly higher levels of stress. This scoring system evaluates events that can adversely affect the welfare of the animal during loading, transport and unloading, for e.g. falls, balks, reversals, aggressive bouts, mounts, jumps, slips, eliminations, vocalisation and use of electric prods. These events are weighted according to their severity. To avoid negative effects as referred to by Maria et al. (2004) floor surface and ramp design are fundamental.

For an optimal loading of cattle on the means of transport, the ramp slope is of great importance. Different studies have led to a consensus that ramp slopes should not be steeper than 20% (11°) (Eldridge et al., 1989, Lapworth, 1990, von Holleben et al., 2003; Grandin and Gallo, 2008). Grandin et al. (2000) recommended a slope of 11°, but if other factors such as non-slip floors and cleats were optimised, maximum slopes of 20-25° could be climbed without significant problems. Inter cleat distances should be at 20-30 cm. For concrete ramps, stair steps should have a 10 cm rise and a 30 cm (Grandin, 2000) to 50 cm tread (Lapworth, 1990). Steps between loading ramp and floor should not be higher than 15 cm. Side protections of ramps should be solid and at least 150 cm high (von Holleben et al., 2003).

2.2.4.3. Space allowance

Required space allowances are stipulated in the Council Regulation (EC) No 1/2005. The required space depends not only on the animal size and weight but also on their physiological condition, on the meteorological conditions and on the likely journey time. For example for heavy cattle (550 kg) the specified space is 1.3-1.6m^2, which corresponds to the requirements for standing cattle of the FAWC (1991) recommended by Randall (1993).

Overloading increases the risk of bruising (Tarrant, 1990) and therefore the risk of pain. The recommended space should allow the animal to stand in a natural position or alternatively to lie down. According to Knowles, (1990) cattle prefer to stand in a perpendicular position during long transports although it was also shown that cattle lie down if they have enough space to do so. It is often recognized that the number of cattle which lie down increases with extended journey times because of fatigue. Warris et al. (1995) observed that during 15 hours

29

of transport no cattle laid down. Knowles et al. (1999) noted that most cattle preferred to lie down after 24 hours of transport and Marahrens et al. (2003) noted that only 20% of bulls laid down during a 29 hour journey. Contrarily Honkavaara et al. (1993) reported that stocked cattle laid down 2-3 hours after the beginning of transport, thus indicating that cattle may prefer lying down if there is enough space.

Apart from stocking density, cattle behaviour is also influenced by the height of the compartment and external factors like ventilation. To provide adequate airflows the overhead space should be at least 20 cm above the highest part of the tallest animal carried, when standing in any normal position (SCAHAW, 2004b). In order to prevent bruising due to mounting behaviour of bulls, any mounting behaviour device should not be >20cm above withers (von Holleben et al., 2003).

Table 6. Space requirements predicted for standing animals

Equation	*Space allowed* $(m^2$ *for a 300 kg cattle)*	*Space allowed* $(m^2$ *for a 400 kg cattle)*	*Space allowed* $(m^2$ *for a 550 kg cattle)*	*Reference*
A =0.021 W$^{0.67}$	0.959	1.16	1.44	FAWC (1991)
A =0.01 W$^{0.78}$	0.855	1.07	1.37	Randall (1993) Transport up to 5h

2.2.4.4. Feeding and watering

Food and water deprivation lead to energy deficits, which in turn lead to catabolic metabolism, loss of body weight and fatigue. Long transport feeding and watering processes are stipulated by Council Regulation (EC) No 1/2005.

Feed should be adapted to the given species which means checking for adequate quality, energy content and sufficient quantity (Marahrens et al., 1999, 2003). Late state pregnant and lactating cows have a higher energy demand during transport and should be fed according to that. Feeding calves during long transport is only possible if they are weaned and accustomed to roughage. During transport it is technically impossible to feed calves on board of the vehicle with milk or milk replacer.

It is often reported that cattle lose weight during transport. Deprivation during transport results in weight loss caused by excretion, exhalation and metabolism. Excretion plays a particularly important role considering the fact that gut contents can account for 12-25% of animals live weight. Knowles et al. (1999) observed with increasing transport duration an increase of live weight loss. After unloading, all groups showed initially a depressed feed and water intake, but after recovery it rose to a higher level than before transport.

The duration of feed and water supply is important for a sufficient intake particularly for ruminants. One hour rest after 14 hours of transport seems to be insufficient for adequate water intake (Knowles et al., 1999). Marahrens et al. (2003) recommended a minimum feeding interval of up to 3 hours for long journeys. Furthermore, the access to feed and watering cups is affected by the stocking density. For instance Knowles et al. (1999) observed that at a stocking density of 0,27m^2/100 kg, 42% of cattle during a one hour resting period did not drink although water was offered to them and that animals on double deck lorries often drank significantly less than those on single deck lorries. Drinking facilities for cattle should have an open water expanse with a minimum of 3 cm water depth and a minimum flow rate of 3 l per minute. To assure access to water cups for all animals at least 2 cups per pen should be present.

2.2.4.5. Vehicle design

Ventilation systems are essential because during journeys wide ranges of differing weather conditions are common. Where changes in geographical surroundings occur ventilation systems should help containing temperatures within a thermoneutral zone. Theses zones are characterised by a lower and upper critical air temperature. Although for cattle there are no absolute criteria for thermal and temperature requirements because of their ability to adjust, the sudden transfer to different environments may cause acute heat stress (Randall, 1993). Wathes et al. (1983) reviewed literature which indicated that the ideal temperature for store cattle and cows lies below 20°C and for 1-month-old calves below 25°C. Temperatures down to 0°C are acceptable. However, the thermoneutral zone depends on many factors, e.g. feeding levels, floor type, air speed and number of animals in the group (Randall, 1993). Further factors like humidity and wetness of coat can also influence the acceptable temperature range.

Ventilation systems are either free or forced systems. Free ventilation systems are common in vehicles used for short (less than 8 hours) journeys, whereas forced systems are a requirement for long journey vehicles. According to the Council Regulation (EC) No 1/2005 the minimum air flow rate of fans should not be lower than 60m^3/h per 100 kg live weight.

Wikner et al. (2003) observed for a Swedish commercial cattle transport the variation between the inside temperature of the vehicle and the outside temperature. During summer and winter the average temperature inside the vehiclewas 3-6°C higher than the outside temperature. It was also seen that during the summer there was an increase of temperature and humidity within the standing vehicle (during loading time), whereas in winter the temperature and humidity dropped. In this context the efficacy of forced ventilation systems becomes especially important with regard to transports from Northern Europe to Mediterranean regions and their hot climates (SCAHAW, 2004). For instance frequent stops due to traffic or border controls in hot climates can lead to heating up the vehicle interiors resulting in hazards for livestock (Grandin and Gallo, 2008). Ventilation is also important in containing the increase of ammonia in faeces and urine and of carbon dioxide from exhalations inside the vehicle. Wikner et al. (2003) could not report hazardous increases of these gases in their studies of Swedish commercial cattle transporters either during winter or summer.

Poor suspension can also affect animal welfare. Excessive vibrations can lead to symptoms ranging from nausea to muscular fatigue. Van de Water et al. (2003) observed emotional and physical stress in calves due to vibration. In combination with a frequency range from between 2 and 8 Hz, vibration induced a reaction of fear, and higher frequencies caused muscular fatigue (Van de Water et al., 2003).

Non-slippery floor surfaces are essential for preventing falls (Grandin and Gallo, 2008). Bedding material can be of aid although usually required only for long transport journeys. Adequate bedding material should be dry with high ability to soak up fluids. Sufficient amounts of bedding allow for more comfort and facilitate the resting of animals.

2.2.4.6. Journey plan

According to Council Regulation (EC) No 1/2005, cattle can be transported for short journeys up to 8 hours without rest and feeding and watering. Transportation time starts with the loading of the first animal and ends with the unloading of the last animal. For exceeded transport duration there are special requirements. After a journey time of 14 hours a rest of at least one hour for watering and feeding is prescribed, before they can be transported for 14 hours again. After this transportation period of 29 hours a rest of 24 hours is required.

The effects on cattle transported by road for up to 15 hours were studied by Warriss et al. (1995). Cattle transported for 5 hours lost 4.6% of their bodyweight, compared to an increasing bodyweight loss of cattle during a 15 hours journey (7,0%). Due to the fact that loading is a very stressful part of the transport, the cortisol level increased in the first part of the transport but the animal recovered when the journey continued. A progressive increase of creatine kinase was measured during longer journeys. Consequences of disrupted feeding was detected by an increase of free fatty acids, beta-hydroxybutyrate and urea concentration. Slight effects of dehydration were measured by an increase of albumin, total plasma protein and osmolality which was quickly rectified by access to water. Based on these observations Warriss et al. (1995) concluded that cattle transport up to 15 hours under good conditions is acceptable from an animal welfare viewpoint.

Knowles et al. (1999) investigated the physiological and behavioural effects of different journey periods from 14 up to 31 hours. The majority of measured variables changed during the journeys and some progressively with the length of the journey. However, the change between 15 and 31 hours was not extreme and the major effect was observed in the first 15 hours. Behaviour measurements showed an increase number of cattle lying down after 24 hours probably due to fatigue (Knowles et al. 1999). Minka et al. (2007) observed an increase of injuries in the second part of the 10-12 hours of the journey in contrast with the first 4 hours.

2.2.4.7. Driving quality

Driving quality should also be mentioned here. Most losses of balance resulting in fall are associated with bad cornering and breaking more as a result of accelerations than of

vibrations. Kenny and Tarrant (1987) reported that 95% of all losses of balance by livestock on moving vehicles were caused as a result of poor driving skills. Broom (2003) observed that the hitting of animals by the stock handlers and vehicle obstruction due to rough driving increased injuries of transported animals. Tarrant and Grandin (2000) concluded that the skill of the driver and the quality of the road appeared to be important in determining transport stress.

2.3. Sheep and goats

2.3.1. Road transport

Small ruminants are frequently transported for long distances: every year in the EU, more than 41.000 tons of meat are produced from animals that have been transported from one EU country to another or come from outside the EU (European Commission 2005) and more than half a million of small ruminants are imported to EU. Most of the sheep and goats are transported on road. Transport could be a multi factorial stressing experience in small ruminants (Augustini C. & Fisher K., 1982; Baxter S.H., Baxter M.R. et al., 1983; Carbajal S. & Orihuela A., 2001; Connell J., 1984; Dow J.K.D., 1976; Ewing S.A., Lay D.C. & von Borell E.H., 1999; Grandin T., 1998; Hails M.R., 1978; Hall SJG & Bradshaw RH, 1998; Kluczek J.P. & Mitotajczyk J., 1983; Lewis C.J., 1986; Moss R., 1982; Warriss P.D., Edwards J.E., Brown S.N. & Knowles T.G., 2002) and several elements are part of it: health status, human-animal interaction, loading and unloading procedures, transport characteristics (for example driving style, length of the journey, staging points, road characteristics), climate conditions, vehicle design (drinkers availability, space allowance and others). For example, during transport a significant reduction of the percentage of time dedicated to activities like rumination and resting has been observed (Cockram M.S. et al., 1996). Also a variety of negative behaviours have been observed during transport (Bradshaw R.H. et al., 1996; Black H. et al., 1994; Knowles T.G., 1998; Knowles T.G. et al., 1993; 1995; 1998; Schmiddunser A., 1994; Schmiddunser A., 1995) such as: restlessness, kicking, teeth grinding, rolling, anorexia, apathy, reduction in the frequency of sterno-abdominal decubitus.

Even so, welfare of small ruminants during transport has been relatively less studied when compared to other species. This could be due to the fact that economical loss caused by poor transport on the quality of the final product is less evident than in other species (Knowles T.G., 1995) or also because ovine do not show their discomfort and pain with vocalization because of a natural tendency of "silent suffering" (Buchenauer D., 1994).

One of the reasons for commercial animal transport is to take the animals to the abattoir and the meat quality could reflect signs of stress. The stress reduces the glycogen stock, increases the pH and diminishes the level of glucose. These effects could lead to changes in the structure and chemical composition of proteins that eventually alters the meat quality as in its colour, tenderness, water holding capacity and durability (Gill C.O. and Newton K.G., 1979; Hood D.E. & Tarrant P.V., 1981). Generally speaking it is believed that ovine meat is less prone to these effects when compared to other species' meat (Knowles T.G., 1998). Some studies have reported the presence of pH alteration, and consequently similar Dark, Firm and

Dry (DFD) lesions, in meat derived from lambs subject to long transport (Bray A.R., Graafhuis A.E. & Chrystal B.B., 1989). According to Morris 15% of lamb carcases produced in Australia, where the mean journey length is considerably high, could be classified as DFD.

2.3.2. Sea transport

Small ruminants are mainly transported by sea only between Australia,New Zealand and the Middle East Countries. Most of the intra EU transport from islands, such as from Sardinia to mainland Italy or from the UK to mainland Europe, foresee the use of roll on roll off truck transport.

2.3.3. Air transport

No information was found on small ruminants air transport.

2.3.4. Literature review: identification of the main hazards in each transport phase

2.3.4.1. Preparation for transport

It is very important to thoroughly evaluate the clinical conditions of the animals prior to travel (fitness to travel): the level of risk can be reduced by selecting animals that are best fit to travel (OIE, 2006); in particular animals at a late pregnancy status, or just after delivering, or very young lambs, should never be transported, whereas wounded or unfit to travel animals should be transported only under exceptional circumstances (European Commission, 2002).

Handling the animals before transport could reduce the negative effects of the transport itself. In fact Rodway et al in 1993 observed that the level of beta-endorphins is significantly lower if the animals are regularly handled for two weeks before transport compared to control animals that were not handled at all before transport.

Some genetic characteristics could have an influence on the level of stress during transport. For example autochthonous breeds are generally more stress resistant compared to highly selected breeds (Hall S.J.G., 1998; Romeyer A. & Bouissou M.F., 1992; Torres – Hernandez G. and Hohenboken W., 1979). In general extensively kept animals are more prone to stress than intensively kept ones (Markowitz T.M. et al., 1998; Neindre P.L. et al., 1996). Animals from different farms kept in the same pen or vehicle show agitation and agonistic behaviour (Pearson A.J. and Kilgour R., 1980): mixing up animals of different origin before transport should be avoided (Connell J., 1984).

2.3.4.2. Loading and unloading

The loading and unloading procedures could cause stress due to rough or inappropriate animal handling (Dwyer C.M. & Bornett H.L.L., 2004). Sheep suffer of sever stress when

they are handled in groups smaller than five individuals (Kilgour R., 1978) and the transport of a single sheep is undoubtedly unadvisable (Carbajal S. & Orihuela A., 2001).

It has been shown that sheep display a great variety of behavioural reaction in association with novel experiences (Broom D.M. et al., 1996): this could be an important factor to be considered especially during loading and unloading procedures as well as in transit. Furthermore sheep are very prone to avoid unpleasant previous experiences (Rushen J., 1990): the reluctance to use a vehicle ramp could reduce the speed and efficacy of loading (Grandin T., 1987), the aversion to walk a path, due for example to fear of an unpleasant experience, could last from 12 weeks (Rushen J., 1986) up to a year (Hutson G.D., 1985).

Generally the first animal of a group has the tendency to hesitate when entering a darker zone (Hitchcock D.K. & Hutson G.D., 1979a). Small ruminants are generally reluctant to step out on the unloading ramp (Hitchcock D.K. & Hutson G.D., 1979b): in these species the heart rate steeply increases during loading on vehicle, as in pigs (Stephens D.B. & Rader R.D., 1982), but differently to the latter, in sheep the changes occur independently from the type of used device (a conventional ramp versus hydraulic pump loading bay) (Parrot R.F. et al., 1998). Small ruminants are gregarious animal and they have a strong social instinct with a lateral vision therefore they tend to walk side by side; a wider loading ramp is preferable to a narrow one, with a slope between 15° and 20° (Fraser A.F., Broom D.M., 1990). Particular attention should be given to the unloading procedure, which is more traumatic than the loading one (Hitchcock D.K. & Hutson G.D., 1979b). In general, the destination place should be lightened and brighter than the position where they are coming from (Gonyou H.W., 2000). Especially during unloading the animals could be pushed roughly and pulled by the fleece; this behaviour could evoke fear and distress and of course bruising and pain. In order to reduce the rough handling and to strictly avoid the use of the electric prod, it could be useful to follow the principles of "visual field" and "flight zone" as indicated by Grandin: operators should stand by the limit of the visual field, which is between 191° and 306° in sheep depending on the abundance of wool, and at the limit of the flight zone (the limit distance at which the animal reacts with flight). Particularly important is the human-animal relationship in loading and unloading small ruminants. OIE guidelines on welfare of animals during transport and Council Regulation (EC) No 1/2005 foresee a Certificate of Competence for those who are willing to handle animals and to drive vehicles for animal transport. The final objective of this training is to qualify all persons involved in animal handling and transport, endorsing a culture of animal welfare friendliness (Caporale V. et al., 2005).

2.3.4.3. Space allowance

To define an optimum value for stocking density during animal transport, many variables should be considered such as the breed, the body weight, the ventilation system (Randall J.M., 1993), and the presence/absence of wool (Knowles T.G., Warriss P.D., Brown S.N. & Edwards J.E., 1998). In sheep the Farm Animal Welfare Council (FAWC, 1991; 1994) recommends a stocking density of $0,021 x W^{0,67} m^2$ (where W is the animal weight in kg.) but this value has been indicated as insufficient for some weight categories (Knowles T.G., 1998). A survey

conducted in the UK in 2002 showed that more than 30% of commercial sheep transport had a stocking density above the FAWC recommendations (Warriss P.D. et al., 2002). Considering the importance of resting, a stocking density of 0,22 m2/sheep is believed to be too much in a 12 h. journey (Hutson G.D., 1985; Knowles T.G., Warriss P.D., Brown S.N. & Edwards J.E., 1998), because most of the animals would not have enough room to lay down (Cockram M.S. et al., 1996), whereas a stocking density 0,27 m2/sheep is just sufficient for the resting needs of the animals. With an even lower density (above 0,40 m2/sheep) the time spent in resting behaviour would sensibly increase (Buchenauer D., 1997). In Europe the Council Regulation (EC) No 1/2005 accepted most of the indications above mentioned, categorizing them according to the means of transport (by road, railway, sea or air), the species (ovine and caprine) and body weight (above or less 55kg.), physiological state (pregnant or not pregnant) and the presence/absence of wool (see Table 1). A possible objection to the regulation limits regards the density ranges, which vary from an acceptable limit to a value that is not satisfactory on the light of scientific literature. Also a very low stocking density could have potential adverse effects during transport: during vehicle movements animals continuously try to find their balance in order to stand still (Hall S.J.G. et al., 1998) and the increase of density could reduce the slipping accidents and losses of balance (Cockram M.S., 1996). Abrupt accelerations and decelerations could cause further stress to the animals because they fall more frequently (Hall S.J.G. et al., 1998).

Table 7 Stocking density for sheep and goats under Council Regulation (EC) No 1/2005 (m^2/animal)

Categories	railway	road	Air (both species)	Sea (both species)
Shorn sheep (< 26 kg and > 55 kg)	0.20-0.30	0.20-0.30	25 kg: 0.2 50 kg: 0.3 75 kg: 0.4	20-30 kg: 0.24-0.265 30-40 kg: 0.265-0.29 40-50 kg: 0.29-0.315 50-60 kg: 0.315-0.34 60-70 kg: 0.34-0.39
Shorn sheep (>55 kg)	>0.30	>0.30		
Not shorn sheep (<55 kg)	0.30-0.40	0.30-0.40		
Shorn sheep (>55 kg)	>0.40	>0.40		
Late pregnant sheep (<55 kg)	0.40-0.50	0.40-0.50		
Late pregnant sheep (>55 kg)	>0.50	>0.50		
Goats (<35 kg)	0.20-0.30	0.20-0.30		
Goats (>35 kg and < 55 kg)	0.30-0.40	0.30-0.40		

Goats (>55 kg)	0.40-0.75	0.40-0.75		
Late pregnant goats (<55 kg)	0.40-0.50	0.40-0.50		
Late pregnant goats (>55 kg)	>0.50	>0.50		

2.3.4.4. Feeding and watering

One of the main concerns of commercial operators is the loss of weight during animal transport. Warris et all (Warriss P.D. et al., 1990) observed that sheep transported for 3-6 hours have a post slaughtering carcase weight 1-7% lighter than animals that have not been transported at all. These losses are directly proportional to the level of stressing factors. This effect could appear to be not that important when singularly considered, nevertheless in modern intensive breeding system it could result in serious economic loss. Others observed a loss of weight in relation to animal density and food availability during transport (Ewbank R. & Kent J.E., 1990). Some farmers have the habit of fasting animals before slaughtering them for two main reasons: to have the minimum live weight to pay and the minimum possible quantity of gut contents to dispose. It is important to remember that in general small ruminants under stressing conditions tend to cease feeding, but after 12 h (Knowles T.G., 1998) or 5 h (Cockram M.S. et al., 1999) of travelling time their priority starts to be again feeding and drinking. The fasting effect on metabolism could be also indicated by the level of plasma free fatty acid (Jackson R.E. et al., 1999) caused by the mobilization of reservoir of metabolic energy (Annison E.F., 1960; Warriss P.D. et al., 1989). When describing the consequences of deprivation of food and water during long transport in small ruminants, many researchers agreed with others who studied fasting in farming condition: in sheep after 24 h of transport, an 8 h rest is sufficient to have a complete recovery (Knowles T.G. et al., 1995); a shorter resting period would be insufficient (Parrot R.F. et all 1998). Others (Cockram M.S. et al., 1997) suggested a resting time of at least 12 h after a long journey. It has also been demonstrated that after a 24 h transport, it takes at least the same amount of hours to regain the pre-journey values in terms of body weight, plasmatic urea, total protein and albumin. For beta hydroxybutyrate (BHB), CPK and plasma osmolality the recovery time would be at least 48 h (Knowles T.G. et al., 1996). Haematocrit is another important parameter because it can be correlated with dehydration (Hall S.J.G. et all 1999). On the other side the lack of water during a 12 h journey has shown no consequences on the PT and vasopressin levels (Cockram M.S. et al., 1996). During breaks feeding and watering should be rationally planned, the correct ratio forage/fodder should be given, because even if hay is essential for a proper rumination and to prevent ruminal bloat, it increases water consumption and reduces the amount of time spent in a resting position (decubitus) (Cockram M.S. et al., 1999).

2.3.4.5. Vehicle design

The vehicle microclimate can largely influence the welfare of transported animals. Defining the thermal comfort in sheep is not very easy and is linked to the breed and the presence/absence of wool, the Lower limit of Thermal Comfort varies from –10°C to +20°C and the Upper limit of Thermal Comfort varies from +28°C to 40°C. Thermal shock is quite common in sheep, which use polypnea to reduce body temperature (Bligh J., 1963a; Bligh J., 1963b; Hales J.R. & Webster M.E. 1967). Heat stress during transport, which is also linked to stocking density and type of vehicle, could be partly avoided giving access to water to animals every two hours (Barbour D., 1999). Goats, which are less hard-coated than sheep, are more susceptible to cold temperature (Hafez E.S.E. 1968); in particular low temperatures, associated with high humidity and strong wind, could determine a rapid decrease in body temperature (Hafez E.S.E., 1968). Many environmental parameters can objectively be measured, giving further information on travelling conditions of the animals that are transported. The Council Regulation (EC) No 1/2005 prescribes the installation of devices able to monitor the temperature, but humidity, air speed, the level of noxious gases, the accelerating forces could also be monitored (Ruiz de la Torre J.L. et al., 2001). These parameters could also easily be transmitted in real time, through web/GIS systems, to a receiving station which could constantly monitor them (Fiore G., 2006; Gebresenbet G. et al., 2003). As for other species, in the ovine, some microclimate parameters could be very helpful (EFSA, 2004). When the limits of Thermo Neutral Zone (TNZ) are overstepped, the animal organism reacts with thermoregulation which inevitably brings to energy consumption and stress (Charles D.R., 1994; Richards S.A., 1973).

2.3.4.6. Journey plan

During transport small ruminants undergo several potentially stressing procedures such as: isolation, forced movements, interaction with new animals (Grandin T., 1987); these procedures could increase the frequency of heart rate, the cortisol, the beta-endorphin and prolactin haematic levels (Parrot R.F. et all 1998). Also the level of salivary cortisol has been studied as a stress indicator (Fell L.R. et al., 1985; Kilgour R. & de Langen H., 1970; Locatelli A. et al., 1985). In general the principal variations of these parameters can be observed within the first three hours, in fact even if the journey is prolonged of further 12 hours there is not a significant increase in these values (Broom D.M. et al,. 1996).

Also intra country long transport is very common (Knowles T.G., 1998). British studies have demonstrated that lambs transported for longer distances have shown greater mean levels of blood cortisol, plasma osmolality, creatine phosphokinase (CPK), bruises and ecchymosis on carcases than animal transported for short distance (Jarvis A.M. et al., 1996; Warriss P.D. et al., 1990), nevertheless traumatic events generally occur within the first five hours of transport, when the animals pass most of the time on their feet before getting down in the resting position (Cockram M.S.et al., 1996).

2.3.4.7. Driving quality

The vehicle movement, when the loading density is quite low, could cause distress to sheep and goats during transport, because animals are forced to continually balance the effects of the movement forces (Hall et al., 1998) and the frequency of balance lost diminishes with the increase of loading density (Cockram et al., 1996). Undoubtedly a rough driving style, with abrupt acceleration and deceleration, could cause further stress to the animals which more frequently lose their balance and fall.

2.4. Horses

At present, transport of horses across Europe is common for racing, breeding, horse shows, deliveries to buyers of horses, veterinary appointments, and slaughter. Ensuring the safety and the value of the horses during transportis important for both welfare and commercial reasons.

2.4.1. Road Transport

The most common way to transport horses is by road. Horses are usually transported in trucks, which may hold several animals. Horses can be transported either within national boundaries or internationally. Duration and distances of national horse transport depends on the different European Member States. International journeys tend to be longer and the transport conditions are important for the consequences on the horses' status. In 2007 about 600,000 horses were slaughtered in Europe (Eurostat, 2009). Therefore live horses are transported across Europe every year and long distance transport (> 8 hours) of horses is very common. The three largest exporting countries are Poland, Romania and Spain. Italy is the major importer.

Table 8 Export of horses to Italy for slaughter in 2006 and 2007

Exporting member state	No. of horses imported by Italy in 2006	No. of horses imported by Italy in 2007
Poland	26269	17608
Belarus	2715	1057
Lithuania	2744	818
Bulgaria	2973	1183
Romania	208	11180
Spain	10774	7519
France	5407	3207

Source: Eurostat, (2009)

Horse transport by train is not common because the animals should be transported to a station and reloaded, increasing the adverse effects of loading and length of the journey.

2.4.2. Sea transport

There is no information regarding sea transport of horses within the EU. This means of transport seems to be not important inside the EU even if it is commonly used for long trade between South America, especially Argentina, and the EU.

2.4.3. Air transport

The use of aircrafts is limited to sport horses and some breeding animals because it is very expensive. No data were found on air transport for commercial trade of horses.

2.4.4. Literature review: identification of the main hazards in each transport phase

The report of the E.U. Scientific Committee on Animal Health and Animal Welfare "The welfare of animals during transport (details for horses, pigs, sheep and cattle)" adopted on 11 March 2002 reviewed the existing scientific literature about the effects on welfare of loading densities, travelling times, resting times and watering and feeding intervals during transport. On January 2006, the Council Regulation (EC) No. 1/2005 came into force.

2.4.4.1. Preparation for transport

There are some differences between horses and other farm animals and so the transport conditions should be adapted to this specie.
Horses that travel with incomplete or no partitions can have aggressive behaviour that leads to fights; this can happen with unfamiliar groups of horses or aggressive horses, especially considering that a substantial number of the equines transported long distances for slaughter are entire males (World Horse Welfare, 2008).
Some reports say that the effort of a horse during transport can be compared with that of a walk of the horse that would last all the journey time, because they constantly have to adjust to the movements of the vehicle (Doherty et al., 1997). The increased heart rate and electro-myographic activity (Giovagnoli et al., 2002) seem to be influenced by emotional and physical stress, determined by road conditions and driving style. Some slaughter horses are tethered in stalls where they are fattened, they have no muscle development or condition and are totally unused to exercise..
For all these reasons, horses are 16.5 times more likely to be injured during transport than cattle (Stefancic and Martin, 2005).
One of the most important requirements to achieve a good welfare during transport is that transported animals are fit. Severely lame or weak, emaciated animals are not fit for transport (Grandin, 2001).

2.4.4.2. Loading and unloading

All transport vehicles should be equipped with ramps of sufficient size to ensure a safe loading/unloading (Stull, 1999).

Friends et al. (2000) established that horses transported at medium and low-density showed a slight increase in activity after 55 minutes of on-board truck rest, hinting that one-hour stops may give horses a meaningful rest. The loading/unloading activities are very stressful and resting on the truck could be a good solution but additional research is needed.

Other studies considered the immunological response and found that a 12 h rest period between two 12 h transport periods interrupted the transport-related lymphocytes decline. Furthermore, rough handling by untrained people during loading increases stress levels in the animals transported (Grandin, 2002).

2.4.4.3. Space allowance

Lots of studies have been made to standardize the space allowance in horse transport, and to determine how the density influences the welfare of transported animals.

The space allowance concerns other factors: the ability of the animals to thermo-regulate effectively, ambient conditions, particularly environmental temperatures and whether the animals should be allowed to stand up after accidental falls.

Horses are social animals but they have a flight or fight instinct. It is safer and less stressful for them to travel in individual compartments. In contrast, cattle and sheep, when scared, will herd together for safety. When stocking densities increase, horses may not be able to adopt balancing strategies because the high density does not allow them any freedom to change their behaviour. The researchers travelling in the trailer observed a high incidence of aggressive horses repeatedly biting an adjacent horse in an apparent effort to get the horse to move away (Gibbs and Friend, 2000).

Some farmers believe that fairly high stocking densities allow horses to support one another during transport but all researches demonstrated that this is completely untrue; higher densities increase the number of falls and injuries during transport and the injuries are more severe (Stull, 1999; Iacono et al., 2007; Collins et al., 2000): in a research done by Collins, (2000) the high density 1.28 horse/m^2 brought greater injuries (32) than the low density 2.28 horse/m^2 (6). In the same study falls are more frequent in the high densities than in the lower ones (40% vs 17 %).

Tarrant and Grandin (1993) stated that when animals went down at a high stocking density, they were trapped on the floor by the remaining animals 'closing over' and occupying the available standing space.

The high-density horses also have a difficult time finding a position or place for their neck and head (indispensable to maintain balance). Some horses adopted a strategy of keeping their heads below leg level while most horses kept their heads relatively high and occasionally positioned their heads on or over the backs of other horses.

For commercial practices it would appear that the best condition is to leave some space (World Horse Welfare, 2008) to allow a brace position.

2.4.4.4. Feeding and watering

Horses require 18.9-75.5 L of water per day depending on horse breed/type, activity, and weather conditions. Transport, novel surroundings, strange tasting water, or unusual routines can decrease water consumption in horses (Gibbs and Friend, 2000).

Dehydration is a problem during transport. Access to water seems to minimize the effect of transport, and many researches established that horses lose weight (affecting welfare and business aims) during transport depending on watering: 4.0% in watered, 12.8% in non-watered horses after 30 h of transport (Reece et al., 2000).

Respiration, heart rate, sodium, chloride, total protein and osmolality were significantly elevated in the non-watered horses and exceeded the normal reference ranges. These studies, conducted by Friend in 2000 established that transporting a healthy horse for more than 24 h during hot weather and with no watering caused severe dehydration.

Providing slaughter horses with access to water onboard trucks appears to be a useful method to reduce dehydration or to delay its onset. The duration of access to water depends on density and can be longer in penned horses because of dominant horses and agonistic behaviours; in some studies, highly motivated horses were blocked from drinking by more aggressive horses. So, the on-board watering system has to be constructed in a way that all the horses are able to drink, providing adequate manoeuvring space or, if mobile, placing troughs on both sides of the truck (Gibbs, 2000).

2.4.4.5. Vehicle design

Partitions must be full length to the floor and of a rigid material. No sharp edges or projections that can cause injury should be present. This should prevent the risk of falls and kicking/biting behaviours.

Several authors (Gibbs et al., 2000) have commented that when given free choice, horses will not choose to stand at a 90˚ angle to the direction of travel of the vehicle during transport, because they cannot move their weight from front to back to balance as they would naturally. Herringbone pattern or rearward facing transport has been associated with reductions of efforts and consequent injuries.

In fact, when facing the direction of travel, horses try to avoid injuries to the head and chest and then are forced to carry their head in an uncharacteristically high position. This unnatural carriage of the head tires the horse by upsetting the natural equilibrium of the ligamentum nuchae, an elastic ligament that allows the horse to maintain its head at withers height without getting tiring.

Several studies agree that horses seemed to find transportation less physically stressful when they were facing backward (Smith et al., 1994; Kusunose and Tonkai, 1996; Waran et al., 1996). It is hypothesized that rear-facing horses can better absorb the shock at impact during

decelerations using the gluteus muscles strength and exposing the rump to impact areas instead of their head and chest.

2.4.4.6. Journey plan

It is generally accepted that the duration of the journey has a negative impact on animal welfare.

The incremental rise in cortisol concentration that has been reported during transport may influence the immune system and contribute to disease susceptibility. A 12 hours rest-stop interrupted the transport-related decline in the limphocyte subpopulations (Stull, 2008).

Reece et al. (2000) stated that serum sodium, chloride and protein concentrations dramatically increased after 24 h of transport, indicating that even healthy horses suffered severe dehydration and fatigue if transported for more than 24 h.

According to the study of Friend (2000) transport for more than 28 h even with periodic access to water will likely be harmful due to increasing fatigue.

In fact, Council Regulation (EC) No 1/2005 stipulates that horses have to be unloaded, fed and allowed to rest for 24 hours at control posts whenever the duration of transport exceeds 24 hours.

2.4.4.7. Driving quality

A journey can be influenced by the driving quality and road conditions. The driver's ability influenced the levels of emotional stress (Hearth Rate) by inducing continuous horse postural adjustments finalised to balance preservation (Giovagnoli et al., 2002).

2.5. 2.5. Poultry

2.5.1. Road transport

Most poultry transports are by road (SCAHAW, 2004). The vehicles for poultry transport are very specific due to the fact that poultry have to be crated in the vehicle. These crates have different dimensions for each poultry species and category. Furthermore there are systems to secure the cages in the vehicle. Nevertheless there are also poultry species (for example Ostriches) which are herded in the vehicle (Appleby et al., 2004).

There are data available from Faostat (2009) about different poultry species slaughtered in the European Union, which also give some information about the numbers of transported poultry.

Table 9. Number of Slaughtered (1000 Head) poultry species within the EU in 2007 (may include official, semi-official or estimated data)

Species	Number (x1000 Head)

Bird meat	2000
Chicken meat	5721823
Duck meat	167616
Goose and guinea fowl meat	20557
Turkey meat	221429

Source: Faostat (2009)

However it has to be taken into account that in most cases different poultry species as well as poultry categories have to be transported several times in their life. For example, chicks of laying hens are transported from hatcheries to rearing farms and then from the rearing farms to egg producing farms. After their productive egg laying life, the so-called "spent hens" are transported to slaughterhouses or rendering plants. Broilers on the other hand are transported from hatcheries directly to fattening farms and then to processing sites.

The conditions as well as the equipment concerning the transport of chicks differ in various ways.

2.5.2. Sea transport

There is no information available about sea transport of poultry.

2.5.3. Air transport

The annually released International Air Transport Association guidelines give detailed information on the dimensions of cages and required equipment (e.g. watering place, feeder, perches) for poultry transport. Air transport occurs mostly for breeder birds and chicks (SCAHAW, 2004).

2.5.4. Literature review: identification of the main hazards in each transport phase

2.5.4.1. Preparation for transport

Broilers are normally deprived of feed 8 -12 hours before transport in order to prevent carcass contamination during the slaughtering process (Bayliss and Hinton, 1990). Water is generally withdrawn one hour before the beginning of the catching.

There are various studies on the consequences of this procedure.

Warriss et. al (1988) investigated the effect of food deprivation. They measured hepatic and muscle glycogen concentrations and pH of fed and fasted broilers (at different times up to 36

44

h) after slaughtering. Within 6 hours of food withdrawal, liver glycogen was reduced to negligible concentrations (<1mg /g) and the initial pH was elevated. No effect was apparent in the *M. pectoralis superficialis* (PS), but in the *M. biceps femoris* (BF) values were reduced (after 12 h the reduction was significant) and the ultimate pH values were elevated in the fasted group in the BF, but not in the PS (Warriss et al., 1988).

In another investigation Warriss et al. (1993) additionally found reduced plasma glucose concentrations after 10 h fasting before transport. Furthermore, the results of these investigations suggested that transported birds became dehydrated and the depletion of body glycogen stores might be associated with the perception of fatigue (Warriss et al., 1993). Animals could suffer the adverse effects of hunger and thirst due to their catabolic metabolism. In combination with high temperatures and humidity this effect would be strengthened.

2.5.4.2. Loading and unloading

Many processes of poultry transport are highly automated, especially loading and unloading. The loading and unloading of the birds are closely related to handling. For this reason poultry handling is a potent stressor due to the rare previous human contact and the increased risk of injuries (Kettlewell and Mitchell, 1994).

In order to reduce bird activity, catching takes place in dim light (Nicol and Scott, 1990). Knowles (1994) investigated different lighting intensities during husbandry and during catching of laying hens and considered that a change of lighting intensity facilitates the catching process.

Catching and crating poultry have to be considered as part of the transport process and can be performed either manually or mechanically by so-called poultry or chick harvesters.

However both approaches might result in management and facility-related hazards.

Concerning the facilities, the crates represent a risk during themanual catching. For example, the openings through which the birds are put might be too small, especially if several animals are loaded at the same time. Additionally birds might suffer different adverse effects, e.g. trapped extremities, bruises, wounds and fractures. Laying hens are particularly susceptible to that because of the fragility of their bones due to calcium demand for eggshell formation and the reduction of movements (in the case of battery cages). It was also pointed out that 30 % of animals arriving at the processing plants have been found to have one or more freshly broken bones due to animal handling (Knowles, 1994).

However the husbandry system has to be taken into account for other reasons. For example hens have to be taken out of battery cages and loaded into transport crates, which increases the risk of injuries. Hens in "alternative" systems like percheries or free range might be able to escape and the equipment in such systems aggravates manual catching whereas mechanical catching is nearly impossible (SCAHAW, 2004).

According to management it is important to employ well-skilled and trained personnel in order to avoid unnecessary suffering of the animals. The welfare of the birds depends on the attention and consciousness of catching teams (Kettlewell and Turner, 1985).

The facilities of mechanical catching and crating (the harvester, belt conveyor, crates) have to be well designed and maintained;Moreover, their employment, and therefore the management, is important.

Schneider (2000) investigated that there is a significantly higher number of dead animals on arrival after mechanical catching respect to manual catching due to heart failure and fatal injuries (fractures, liver ruptures, and haematomas of spleen or kidney). However, these are correlated with the speed of the conveyor belt. If the speed is too high the stocking density in the crates increases and the birds might die of shock and hyperthermia (Schneider, 2000). Knierim and Gocke (2003) explained that a greater amount of experience of the catching crew to use the machine significantly decreased the prevalence of injuries. However, environmental factors like season, ambient temperature and humidity have to be taken into account.

The process of unloading is also stressful because in most cases birds have to be pulled out of the crate, which is inevitably associated with handling. However there are systems like the "cage dump module" where birds are dumped out of the modules on a conveyor belt (Bayliss and Hinton, 1990), but this procedure can also lead to welfare problems.

2.5.4.3. Space allowance

There are different data available in legislation about the space allowances required for the different categories of poultry.

The Council Regulation (EC) No 1/2005 for example determined a particular area in cm^2 according to the weight (in kg) of the different categories of poultry. However, there is no requirement concerning the height of the containers in the regulation (Table 10).

Table 10. Minimum floor areas in poultry containers.

Category	Area in cm^2
Day-old chicks	21-25 per chick
Poultry other than day-old chicks: weight in kg	**Area in cm^2 per kg**
< 1,6	180-200
1,6 to < 3	160

3 to < 5	115
> 5	105

These figures may vary depending not only on the weight and size of the birds but also on their physical condition as well as on the meteorological conditions and the likely journey time.

In the report "Land Transport of Poultry" (SCRAM, 1998) the following requirements for space and height are stipulated (Table 11 and Table 12)..

Table 11. Transport container space requirements

Category	Floor space
Day-old chicks	$400 – 475$ chicks per m^2
Poultry less 1,0 to 1,6 kg	40 birds per m^2
Poultry 1,6 to 2,2 kg	36 birds per m^2
Poultry 2,2 to 3,0 kg	28 birds per m^2
Poultry 3,0 to 5,0 kg	20 birds per m^2
Poultry more than 5,0 kg	100 cm^2 per kg

Table 12 Transport container height requirements

Category	Minimum height (cm)
Day-old chicks, turkey poults, ducklings	12
Broiler chickens	23
Starter pullets, ducks, spent hens, meat and layer breeders	25
turkeys	32 or greater

The German executive order to the Council Regulation (EC) No 1/2005 stipulates minimum requirements for chicken, guinea fowl, pheasant, ducks, turkey and geese (Table 13).

Table 13. Chicken, guinea fowl, pheasant, duck, turkey and goose.

Live weight (kg per animal)	Area (cm^2/ kg live weight)	Minimum height of container (cm)
1,0	200	23
1,3	190	23
1,6	180	23
2,0	170	23
3,0	160	23
4,0	130	25
5,0	115	25
10,0	105	30
15,0	105	35
30,0	105	40

Table 14. One-day chicks

Species	Area per animal cm^2	Number of animals per container or container section	
		Minimum	Maximum
chicken, guinea fowl, pheasant, duck	25	10	105
goose, turkey	35	8	40

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These are minimum requirements and it is crucial to take the season, journey length, ambient temperatures and humidity as well as the existence or absence of forced ventilation into account. High crating densities increase the economic gain of the transporter, but birds are less able to cope with their environment with behavioural adaptation, e.g. by regulating their body temperature. Especially in hot weather conditions stocking densities have to be reduced in order to facilitate air movement and protect birds from increasing heat and humidity within the crates. In this case the over-head space and therefore the height of the crates have to be additionally taken in consideration.

Nevertheless excessively low crating densities could cause injuries if the birds are overthrown (Delezie et al., 2007). They also concluded from their studies that birds at high crating densities experienced high stress levels and therefore crating density often overruled other effects, like feed withdrawal or transportation itself.

2.5.4.4. Feeding and watering

In the case of commercial poultry transport feeding and watering during transport is not common due to the fact that poultry are transported in crates or modular systems. These systems lack in a technical and practicable possibility to install feeding and watering facilities (SCAHAW, 2004). The Council Regulation (EC) No 1/2005 requires feeding and watering of poultry if the journey lasts more than 12 hours disregarding loading and unloading time.

Furthermore, the careful control of food deprivation before transport has to be taken into account.

2.5.4.5. Vehicle and crates design

There are different types of vehicles for road transportation of poultry (semitrailers, truck-trailer-combinations, articulated lorries, etc.). These vehicles can be equipped in various manners. For example, fully closed systems with forced ventilation; adjustable, detachable/removable curtain sides (e.g. from tarpaulin, nets); with or without solid headboards; roofs can be solid or liftable and the rear can be opened or closed.

Forced ventilation systems are not commonly used in commercial poultry transporters apart during the transport of newly hatched chicks.

Webster et al. (1993) investigated the thermal comfort zones of chicken in open or closed systems without forced ventilation. The thermal comfort zone is reliant on the plumage and on the circumstances of the transport. Therefore Webster et al. (1993) compared well feathered and poorly feathered birds and windless and different wind speed (Table 15).

Table 15: Thermal comfort zones for chicken within a travelling module at different wind speeds, modified after Webster et al. (1993)

Wind speed	Thermal comfort zone for well feathered birds (°C), for example broilers	Thermal comfort zone for poorly feathered birds (°C), for example spent hens
Windless	6,5-22	24-28
0,5 m/s	15-26	28-32
3,3 m/s	24-32	33-35

From these values Webster et al. (1993) determined the lower and upper critical temperatures of birds with respect to the condition of the plumage, wind speed, and vehicle design (Table 16).

Table 16: Limits to the range of ambient (outdoor) temperatures at which birds will be thermally comfortable in closed and open vehicles, modified after Webster et al. (1993)

	Closed (°C)	Open (°C)
Well feathered birds at rest	-8 to +8	-3 to +13
Well feathered birds in motion	+7 to +18	+18 to +26
Poorly feathered birds at rest	+9 to +14	+14 to +19
Poorly feathered birds in motion	+20 to 23	+26 to +29

There are different means of systems for crating birds in the vehicle described by Bayliss and Hinton (1990).

Widely out of use are the so-called "loose crates". The crates are unloaded from the vehicle and the birds are loaded through flapped openings in the broiler house. Full crates are loaded onto the vehicle in stacks of eight crates. At the processing plant the loose crates are unloaded and placed singly on a conveyor. More automated systems allow the unloading of stacks of crates by a forklift. The loose or fixed crates are mostly used for the transport of spent hens, and the modules are mostly used for broiler chickens (Kettlewell and Mitchell, 1994)

Another system is equipped with fixed crates. In this system two banks of permanently fixed crates hold in a framework which is fixed to the vehicle's floor. Therefore the birds have to be carried to the vehicle.

Furthermore there are different modular systems which consist of a metal frame that contains between four and sixteen compartments or crates. These modules are taken from the vehicle by a forklift, brought into the broiler house and placed close to where the birds are caught. After catching and crating the birds, the modules are loaded by forklift onto the vehicle. There are about four different modular systems that vary in the number of compartments, in the openings and in the loading/unloading systems ("Multiple floor module", "Captive drawer modules", "Unrestrained drawer modules", or "cage dump modules") (Bayliss and Hinton, 1990).

Normally crates have solid bottom parts to prevent the birds from contamination with faeces (Swarbrick, 1986). However this leads to a reduced ventilation rate. All modules generally are made of perforated metal or plastic grid (Kettlewell&Mitchell, 1993).

Newly hatched chicks are usually transported in disposable cardboard boxes (SCAHAW, 2004). There might be some differences in the equipment of the vehicle, particularly in regard to the ventilation system, as they are all fully closed and temperate.

Another important stressor to welfare of poultry during transport is vibration. Randall et al. (1994) stated that the magnitudes of vibration in the containers were at a level considered fairly uncomfortable even for humans.

The experiments of Abeyesinghe et al. (2001) support older studies from Randall et al., (1994) and Warriss et al., (1997) on the aversion of broiler chickens to vibration. They conducted a "discrete-choice technique" and found that the birds significantly avoid the vibration treatment (frequency: 2 Hz; acceleration: $1m \ s^{-2}$).

2.5.4.6. Journey plan

In Council Regulation (EC) No 1/2005 it is required for poultry to be provided with suitable food and water, in adequate quantities save the case of a journey lasting less than:

(a) 12 hours disregarding loading and unloading time; or

(b) 24 hours for chicks of all species, provided that it is completed within 72 hours from the hatching. These requirements lead to reduction of transport times. By disregarding loading and unloading time, some stressful processes, that in fact extend the journey length for the animals, are not taken into account. These processes include for example catching, crating and handling, loading crates onto the vehicle and after the transit, the unloading of crates from the vehicle and lairage time. Warriss et al. (1990) found journey length from loading to unloading with a maximum of 12,8 h. Spent hens seem to be transported for the longest journeys (Knowles, 1994) due to the fact that they have a low economic value and therefore

just few plants want to process them. Like described above the transport of laying hens is a major welfare problem.

The investigations of Warriss et al. (1992) showed that mortality increased with longer journey times. The overall incidence of birds dead on arrival was 0,156 % in journeys not exceeding 4 hours in duration and 0,283 % for journeys longer than 4 hours. Thus, mortality was about 80 % higher during longer journeys (Warriss et al., 1992).

2.5.4.7. Driving quality

Poor driving quality, for example sudden accelerations, sudden breaks or vibrations reduce the welfare of animals. Bad road conditions could aggravate this effect. Animals could suffer from pain, injuries or fractures. In case of poultry, due to the fact that stocking density is high, loss of balance and therefore falls are rare and therefore barely described.

2.6. Rabbits

2.6.1. Road transport

Rabbit production is important in the Mediterranean area, especially in France, Italy and Spain. In Spain, 125 million rabbits were transported in 2001. In order to minimize costs, processors usually arrange rabbits to be picked up from intermediary collection points. The transport chain may thus consist of two distinct "parts": rabbits being transported by individual producers to the nearest collection point where they wait for an indeterminate time before being loaded; and rabbits being taken on to the processor (Jolley, 1990). In recent years the number of low throughput rabbit abattoirs in Spain has decreased substantially, increasing transport times (Buil et al., 2004).

Under commercial conditions, rabbits are conducted to the abattoir using commercial lorries which has two or three axles and a loading capacity ranging from 1,500 to 6,000 rabbits. Rabbits are usually carried in crates. The crates are placed on the vehicle in multi-floor crate stands. These may be stackable crates, of such a size that a person can lift one of them, or they may be modular units that have to be lifted with a forklift vehicle (Broom, 2008; Verga et al., 2009).

2.6.2. Sea transport

No data are available on rabbit sea transport for commercial purposes.

2.6.3. Air transport

No data are available on rabbit air transport for commercial purposes.

2.6.4. Literature review: identification of the main hazards in each transport phase

2.6.4.1. Preparation for transport

Ashby et al., (1980), Purdue (1984) and Coppings et al. (1989) carried out a number of experiments on the effects of different periods of feed or water withdrawal up to 24 hours before transport and slaughter. It was observed that rabbits lose between 3 and 4 % of their weight when fasted during 12 h, the weight loss increased from 6 to 10% after 24 h of fasting and arrived at 10 and 12% after 36 and 48 h of fasting.

Transport has an effect on intestinal content depending if the animals have been fasted prior to the transport. In this sense, rabbits fasted for 12 to 24 h before transport had higher intestinal content losses compared with rabbits that were not fasted (Jolley, 1990). Copping et al. (1989) observed that the feed in the intestinal tract could have a a protective effect as opposed to the adverse effect of transport.

2.6.4.2. Loading and unloading

The man-animal relationship plays a key role in the commercial rearing of all species, but assumes particular importance in rabbits, due to their shyness and diffidence towards man (Trocino and Xiccato, 2006). The handling and loading of rabbits is very different from that of the larger mammals because it involves manual catching that can have an important effect on welfare (Broom, 2008). Rough rabbit handling has been reported to increase pre-slaughter mortality and main carcass defects such as haemorrhages, bruises and broken bones (Verga et al., 2009).

Loading can be carried out in two ways: either in transport crates filled on the farms, or by collecting and placing, even throwing, animals into crates fixed on a truck. Rabbits on the upper truck levels are often subjected to a greater number of falls than on lower levels. In a comparative study of these two different loading methods, Fenelap (National Federation of the Rabbit Farmer Associations) showed that rabbits loaded into fixed crates had a reduction of 0.44% of carcass quality compared with those crated on the farm (Ouhayoun, 1992). Without regard to the crating method, careful rabbit handling to reduce trauma has been reported as a crucial factor to reduce pre-slaughter mortality and main carcass defects such as haemorrhages, bruises and broken bones (Verga et al., 2009).

Buil et al. (2004) in a survey on rabbit transport performed in Spanish abattoirs observed that all the farms used a cage system for transport. Pre-loading cages (different from the transport cages where the animal are located while waiting to be loaded) were not normally used. Instead, many farmers (75%) used the cages from the feedlot for transport. Almost three quarters of the farmers had multi-floor cages on a roller stand, and the mean number of tiers on each stand was six. A hydraulic lift was used to load multi-floor cages.

In one experiment carried out by Marìa et al. (2006), corticosterone showed a tendency to increase from basal levels in all transported animals, the increase being significant only during transport using the rough loading method. Neutrophilia and lymphocytopenia were

significant for all rabbits, independently of their position in the truck or of the loading method. Stress parameters were more influenced by transport and handling itself rather than by specific conditions related to different loading methods or crate position in the truck.

2.6.4.3. Space allowance

Absolute minimum space allowances are determined by the physical dimensions of animals, but this will not be sufficient to allow good welfare. Acceptable minimum allowances will be dependent on other factors as well. These include the ability of the animals to thermoregulate effectively, the ambient conditions, particularly environmental temperature and whether the animals should be allowed enough space to lie down if they so wish (Broom, 2008).

For commercial practice it would appear that the best condition was 10 rabbits/crate with a space allowance of 0.06 m2/rabbit of 2 to 3 kg. This density may have to be reduced in hot conditions (i.e. above 18-20°C) (EFSA, 2004a). Buil et al. (2004) studied the procedures for loading, transport and unloading of rabbits in Spain and observed that the mean density per cage was 353.7 ± 127.5 cm^2/animal.

2.6.4.4. Feeding and watering

During the period between catching at the farm and hanging at the abattoir line, rabbits are kept without feed and water. Due to caecotrophy, rabbits are usually considered to be very resistant to hunger. They are relatively resistant to thirst too, and can survive 4 to 8 days of water withdrawal without any irreversible damage. Furthermore, they can reasonably withstand food and water deprivation for 24 hours without significant adverse effects on bodyweight and carcass quality (Jolley, 1990; EFSA, 2004a).

Caecotrophy is nutritionally important for rabbits as they obtain large amounts of proteins and water-soluble vitamins (B and K) through the action of bacteria in the hind gut. Such recycling of faeces probably provides good protection from starvation and may explain why rabbits are considered to be resistant to such conditions (Jolley, 1990). However, crating, transportation and food withdrawal can cause the rupture of caecotrophy practice (Jolley, 1990; EFSA, 2004a).

2.6.4.5. Vehicle and crate design

Physical conditions within vehicles during transport can affect the extent of stress in animals. Similarly, the design of loading and unloading facilities is of great importance (Broom, 2008).

Buil et al (2004), in a survey on rabbit transport performed in Spanish abattoirs, observed that most of the trucks used (76.2%) were small with 2 axles (61.9%), and an average floor area of 13.80 m^2. This allows room for approximately 15 multi-floor cages roller stands. The lorry floor was made of steel in 33.3% of the cases and very rarely covered by litter. The roof and walls were made of different materials, but normally aluminium (23.8%) and steel (14.3%). Almost half of the trucks had an insulated roof. In most of the lorries (76.2%), the

environmental conditions during transport were not controlled, and they were lacking artificial light, mechanical ventilation or temperature control. Close environmental control in the crates or modules on the vehicle is difficult, mainly because on most vehicles ventilation is passive and is impeded by the close stacking of adjacent crates. When lorries are full of rabbits, the ventilation inside tends to be poor, particularly when the truck stops, either during the journey, or on arrival at the abattoir so rabbits on the inside of a load may suffer hyperthermia (Jolley, 1990; cited by Verga et al., 2009). Liste et al. (2006) found that rabbits placed in the middle and bottom of crate stacks showed higher levels of some stress indicators (blood glucose, and corticosterone) than those located at the top floor without regard to journey length. The lack of mechanical ventilation together with the use of side curtains as protection against precipitation, wind and solar radiation can lead to high ammonia levels (20 to 30 ppm) which can greatly weaken the rabbits' upper respiratory tract and open the door to bacteria.

There is a high risk of heat stress, associated with poor welfare, when rabbits are transported; perhaps a higher risk than in transported poultry or pigs. In rabbits, the sweat glands are not functional and perspiration (the evacuation of water through the skin) is not very efficient due to the fur. Heat dissipation is carried out by using three devices: general body position, breathing rate and peripheral temperature. In addition the nasal mucosa and the ear play a big role in that respect (Fayes et al., 1994; Lebas et al., 1997). These systems work between 0° and 30°C but when ambient temperatures reach (and mainly when they exceed) 35°C rabbits can no longer regulate their internal temperature and hyperthermia sets in (Lebas *et al.*, 1997). Extreme conditions, such as temperatures above 35º or low humidity (below 55%), were found to be detrimental for rabbit welfare (EFSA, 2004). The mean room temperature for rabbits is recommended to be 18 ºC with a range of 15-22 ºC. The lower critical temperature is -7 ºC and the higher critical temperature is 28.3 ºC (Lidfors, Edström and Lindberg, 2007). De la Fuente Vázquez (2003) observed that rabbits transported in summer displayed more compromised welfare than rabbits transported in winter, and they were more threatened when they were transported in summer and subjected to heat at high stocking density. In one experiment carried out by De la Fuente (2007), rabbits exposed to heat were the most affected of all three groups, although cold, noise and mixing with unfamiliar rabbits also had a detrimental effect on physiological and meat quality parameters. In relation to the temperature conditions to maintain animal welfare during transport, Luzi et al. (1994) observed that transport of rabbits to the slaughterhouse when the temperature was between 0 and 6ºC produced weight loss of 1.5%, whereas when the temperatures were between 9 and 12ºC or between 15 and 22ºC, weight loss was 3.8 and 3.9%, respectively. Leoni et al. (2000) stated that the optimum temperature for rabbit transport was between 5 and 13ºC because transport losses were lower.

Rabbits rely in part on changes in body position to adjust heat losses, curling up to save heat, stretching out to lose heat, so crate design may interfere with this ability to adapt to changes in environmental temperature (Jolley, 1990). Travel crates are generally made of plastic (60cm wide, 30cm high and about 100cm long) and they are provided with loading doors (EFSA, 2004a). There is evidence of severe problems when some breeds and sizes of rabbits are transported in crates designed for chickens (EFSA, 2004b). Rabbits need to assume

adequate postures to dissipate heat e.g. sitting up or lying flat with ears extended in both of these positions. Practical experience shows that there can be severe ear damage when ears protrude through the tops of crates, although this problem is not often reported and is probably limited to some kinds of containers (EFSA, 2004b). The procedures for loading, transport and unloading of rabbits in Spain from December 2003 to March 2004 were surveyed by Buil et al. (2004). The number of farms surveyed was 60. The size of the cages varied but most (68%) were between 3500 cm^2 to 5000 cm^2 (medium sized cage). The mean height of the cages was 30.7 ± 9.3 cm.

Rabbits panic if placed on a smooth surface, preferring to be placed on rough material where they feel more grip, for example towelling, sackcloth, or wire mesh. Flooring type can predispose to aggression. UFAW (1988) recommended the use of solid floors for rabbit transport crates, in order to prevent contamination with urine and faeces from higher to lower crates, but spillage may still occur promoting aggression between the animals (EFSA, 2004a).

Growing rabbits prefer plastic net floor to wire mesh floor. Plastic has low thermal conductivity therefore it may give a sensation of warmth so rabbits prefer staying on plastic (rabbits choose plastic floors if they can). In addition, plastic could reduce foot pad injuries. On the other hand, rabbits can chew plastic floors and the risk of diseases (coccidiosis) is high due to the rapid build up of moisture and faeces. Since the floor type did not affect their productive traits, plastic net floor is advantageous from the viewpoint of animal welfare (Princz et al., 2009)

The continually changing odours inevitably associated with transport are likely to have more influence on rabbits than in other meat species. Defecation and urination odors are important in territorial marking and social hierarchy in rabbits. A rabbit within an environment containing its own or a familiar odour feels "at home". Therefore, rabbits placed in a clean transport crate, or in one with unfamiliar odours, experience a stressful situation (Jolley, 1990).

Rabbits placed for 2 h either in or near a machine designed to simulate transport, which exposed them to vibration stress and sound, had a respiration rate of 120/min (normally 65/min). Respiration returned to normal values after 20 min to 4 h, depending upon the initial level of response (Stephens and Adams, 1982). It seemed that apparently, rabbits did not have the capacity to adapt to noise (De la Fuente Vázquez, 2003).

2.6.4.6. Journey plan

The transport duration is related both to the duration of the fasting animals may suffer and to the stress of the transport itself.

Luzi et al. (1992) carried out a survey in the North of Italy for over a year and looked at transport distances of 25, 50, 100 and 150 km in intensively farmed rabbits. Carcass yield seemed not to be influenced by distance whereas body weight losses were highly correlated with both increasing travel times (from 1.4% for 1 hour transport to 4.6% for 7 hours) and the

trucks' internal temperatures (from 1.5% at 0-6ºC to 3.8% above 9ºC) (Luzi et al., 1992; Crimella et al., 1991). Season had no effect but this may be due to the fact that transport was always carried out at night, so differences in environmental temperatures were not extreme (Luzi et al., 1992; Crimella et al., 1991). The most critical conditions seemed to stem from night temperatures above 18-20ºC, with a relative humidity of 70-75%, transport times over 4 hours, and distances above 100 km (Luzi et al., 1992).

2.6.4.7. Driving quality

There is no information available on the incidence of driving quality over rabbit welfare during transport.

2.7. Fish

The transport of live fish of aquaculture falls within the scope of Council Regulation (EC) No 1/2005, with some applicative limitations related to the peculiarities of the species being transported, but the logbook and the navigation system are not required for long journeys by road, as well as the Certificate of Competence for drivers and guardians. The certificate of homologation for the lorries required for the long journey is released on the basis of the requirements contained in Annex I, Chapter II. Finally, regarding the requirement of training, the regulation stresses the obligation of an adequate professional training in order to reduce as much as possible the lack of good welfare practice; this training should also be made available to all those who are not required to obtain a certificate of fitness. A large variety of transportation techniques are used in aquaculture, but all should aim at minimising stress, optimising water quality and oxygen levels, and minimising the build up of metabolic wastes and ammonia (Ashley, 2006).

2.7.1. Road transport

Transport of live fish is a regular practice on many fish farms, used following harvest, during grading or sorting, to take fish to short-term live storage, to stock ponds in the same or other farms for breeding or growing; or to bring live fish to market. The time of transport varies according to the distance to be covered and the methods being used; on the farm the transport time is usually very short (few minutes) or short (up to 30 minutes). Outside the farm the transport time is habitually longer, varying from a few hours to one or two days. A tank carried on the back of flat bed lorries is a frequent method of transporting live fish. The vast majority of the trout transported in the UK travel in this manner. Although lorries take much longer to cover the same distance than helicopters, they are less stressful for the fish and for that reason better for longer journeys than helicopters. Transport tanks on vehicles should have smooth walls free of chips that could harbor pathogens, and be insulated to minimize temperature changes. The tank material can also have an influence on maintaining the temperature, mainly if the water and air temperature are very different. Numerous tanks now are made of fiberglass or aluminum, with an insulating material such as polyurethane sandwiched in the middle. Most tanks are made of 3/16-inch (7.62 cm/40,64 cm) aluminum

or l/4-inch (2,54 cm/10,16) thick fiberglass. Un-insulated fiberglass or marine plywood tanks work well for short trips. Thermal conductivity (k) of classic hauling tank materials expresses the thermal properties. Thermal conductivity is the amount of heat in British Thermal Units (BTU) that is transmitted per 1h per 1 ft^2 through material 1 inch thick for each degree °F difference between the two surfaces of the materials.

Table 17. Thermal conductivity of different materials used in fish transport

MATERIAL	Thermal conductivity (k)
Plywood (Douglas Fir)	0.8
Aluminum (1100 alloy)	1532
Fiberglass	0.25
Polyurethane	0.16
Urethane	0.18
Expanded polystyrene (extruded)	0.26

Source: the American Society of Heating, Refrigerating and Air-Conditioning Engineers ASHRAE (1981).

The lower is the k value, the better are the insulating properties of the material. Metal tanks are less likely to be broken or damaged. Any hardware on the tank should be aluminum, stainless steel or another rust-resistant material.

However, during long transports, where the ambient temperature is much different than the tank temperature, a chiller or heater might be necessary to maintain the temperature of the tank within the desired range.

Insulation means that little or no ice is required to maintain temperatures on long trips. Urethane foam, plastic foam and corkboard are common insulating materials used as filler between tank walls.

The hatch should be large enough to prevent damage to the fish during loading, and lids should fit tightly so that fish and water are not lost en route. The tanks should be supplied with oxygen and compressed air. The oxygen supply should have the capacity to sustain twice the nominal biomass of fish in the tank, so oxygen levels can be increased rapidly in the event of a problem, or if the actual number of fish is greater than intended due to weighing or counting errors. The additional aeration helps circulate the water within the tank, and remove carbon dioxide. However, many transport containers have a lid and are 'closed'. These tanks can pose a secondary problem. Carbon dioxide that has been stripped from the water can cause an increase in the partial pressure of CO_2 in the airspace above the water, which might

eventually prevent CO_2 from off gassing from the water. In this case, any type of opening in the top of a transport container can provide a pathway for atmospheric air exchange.

Any compressor for the air supply must be oil free to avoid contaminating the water. The aeration rate will depend upon the type and size of fish. Tank capacities range from 75 gallons to 2,500 gallons (from l. 337.5 to l. 187.500) with the most common tanks holding 100 to 300 gallons (l.450 to l.350). Tank depth is usually 28 inches to 32 inches (cm. 71.12 to cm. 81.28). Most tanks are rectangular and may have from one to eight compartments with varying arrangements. The hauling unit should be compatible with its intended use. A light pickup can handle a 100 gallon (450 l) tank while a 3/4 ton pickup can pull a gooseneck trailer with two 350 gallon (1575 l) tanks. Water is heavy: one gallon weighs 8.3 pounds (1 pound is 453.6 g). Some units are attached to the frames or flatbed of trucks. Other units are portable and can be lifted manually or with a winch and/or pulleys. Gooseneck trailers with electric brakes are also used to carry transport tanks. The pickup truck used to pull the gooseneck is then available for other farm work. Specially designed self-contained transport vehicles are common in situations where they are used regularly to haul large quantities of fish. A producer can save money by purchasing used equipment in good condition. Recommended gross weight limits for vehicles and tonnage weight of the trailer for safety reasons must be followed.

More than one compartment is needed if several fish sizes or species require transport at the same time. Large or long tanks must contain baffles to reduce water sloshing that can injure fish and create dangerous driving conditions. Welds in single, long tanks may break if the truck bed warps. This can be avoided if smaller, standard tanks with the same carrying capacity are placed side by side. The top doors or lids of the tank should be large if fish will be loaded with a boom and fish basket.

Large doors make fish removal easier if dip netting is required. The tank drain can be located either in the rear or side of the tank. Side outlets allow easy unloading of fish to holding vats or ponds by means of chutes or extended discharge pipes. These reduce rough handling caused by dip-netting. The discharge opening should be large enough to let easily pass the largest fish hauled and should be equipped with a quick release gate or plug.

Drain openings can be rectangular or round with round drains usually 3 to 8 inches (cm 7.62 to cm 20.32) in diameter. These may be controlled with inside rubber stoppers, outside expansion plugs or threaded caps. A sliding inside gate, over the drain opening, is useful to control the rate of discharge and permit removal of outside cap or plug without releasing water or fish.

A sloped false bottom permits the complete discharge of water and fish with minimal extra handling. The bottom of the drain opening should be flush with the tank bottom. A lip on this drain prevents total emptying of water and fish. Tanks should be equipped with an overflow drain to maintain water level and allow agitators to function at the proper operating depth. Electrical outlet boxes should be available for easy hookup of agitators. A central junction box with wires leading to individual outlets works well.

Another feature is an air vent or scoop to permit air circulation in the space between the water surface and tank top. Adequate ventilation is important especially on long trips, to reduce accumulation of carbon dioxide. Tanks may have clear, vertical tubes or panels for determining fish weights by calibrated water displacement rather than by scale weighing. This method requires that water transfer with fish be minimized. It is commonly used in the trout industry where fish pumps and dewatering towers are used to move fish from production or holding units to transport tanks.

Purpose-built tanks are commonly made of fiberglass and may be insulated to minimize temperature changes; oxygen is provided via aerator or gas bottles to maintain dissolved oxygen levels and to blow off excess of carbon dioxide. The most sophisticated live fish transporters may have built-in refrigeration, an ammonia stripping system, and an in-cab console continually monitoring water parameter quality. In between these extremes lies the majority of smolt transport tanks, often modular unit which can be carried on a lorry or even by helicopter, although an increasing proportion of smolt is now transported in specialized well-boats.

2.7.2. Sea transport

Well-boats allow fish to be moved in a boat that contains a pool of seawater within its hull. The fish are kept in this pool, and the water is refreshed by continually pumping water through the hull of the boat. The seawater quality is maintained at a high level by continuously pumping in or re-circulating seawater. The water in the well-boat is therefore not static, unlike during transfer by helicopter bucket or lorry-borne tank. Seawater transport of fish in Scotland is currently dominated by Norwegian based companies that lease well-boats to Scotland. These boats probably comprise the most modern well-boat fleet in the world. Purpose built well-boats have the superstructure at the front to give good view of the well-boat. There is sophisticated remote monitoring on board with video camera observations of the wells, and dissolved oxygen sensors with alarms. Well-boats are used for transporting smolts to on-growing sites and harvest fish to slaughter.

Many salmon farms are remote from the killing station to which the harvested fish are brought live, usually by well-boats.

2.7.3. Air transport

This method is used principally to move salmon smolts over short distances. Very high numbers of fish are crowded in a small volume of highly oxygenated water (twice the volume of water to fish) and carried in a purpose-built tank or "bucket" slung underneath a helicopter. When they have reached their destination, the fish are "poured out" into sea cages or into a waiting well-boat. Helicopters represent an expensive means of transport and are mainly used for moving smolts and broodstock to cages. The duration is typically short (maximum times approximately 20 mn.) and high densities are used (300-400 kg/m³) to maximize the number of fish moved. To sustain the high density of fish, the water is supersaturated with oxygen before the fish are added and there is continuous oxygenation during transport. The "buckets"

used for transporting the fish are specifically designed: when lowered into the water at the destination, a spring-loaded float lever releases the hatch so the fish can swim out.

A current study has evaluated the risks associated with air-transport of large size fish. Strong pressure changes, during rapid changes in flight levels, have been reported to cause decompression effects such as micro-gas bubble formation in the tissues. Decompression effects have been described in detail for altitude simulations by Whitaker et al. (1945) as potentially causing gas bubble trauma (Alderdice and Jensen, 1985) and gas bubble disease (Bouck, 1980; Marking, 1987).

Small number of fish are often transported in polythene bags to which is added a little water (the volume of water should be ample in proportion to biomass of the fish, for example, to transport fish for two or three hours, a bag measuring 60 x 180 cm. is adequate for a maximum of perhaps four 200-300 g. fish). For long transport, oxygen is much more important than water, and there should be twice as much air space in the sealed bag as water space. The bag should be inflated with oxygen, the open ends closed securely; the bag should be placed in a rigid water-tight polystyrene container. Ice may be added in hot weather to lower metabolic rate during the journey; besides, the fish should be protected as much as possible from loud noise, bright light, and temperature extremes.

2.7.4. Literature review: identification of the main hazards in each transport phase

2.7.4.1. Preparation for transport

Transportation involves capture, loading, transport, unloading and stocking and it can induce large stress responses that can affect fish over a prolonged recovery period (Specker and Schreck, 1980; Davis and Parker, 1986; Schreck et al., 1989; Iversen et al., 1998) and the susceptibility of fish to disease (Winton, 2001).

Fish have a similar nervous system to mammals to communicate nociception from the body to the brain. Pain perception and suffering in fish have been debated for some years and our understanding of these questions may have large impact on the way we handle and slaughter farmed fish. It is important to separate pain (nociception) and pain perception. Some scientists argue that fish, birds and some mammals, do not perceive pain because they lack a neocortex, which is important in pain perception in most mammals (Rose, 2002). Other brain structures, however, may have the same functions as the neocortex (Braithwaite & Huntingford, 2004) and there are studies indicating that fish have numerous pain receptors and show long term behavioural indicators when exposed to pain stressors (Sneddon et al., 2003).

The health of the fish plays an important role during and after the transport. According to art. 15 of the Proposal for a Council Decision COM (2005) 297 final, fish shall be checked before transport and unfit or unhealthy fish shall not be transported, except for therapeutic reasons. Fish which die during transport shall be separated from the live fish as soon as possible, unless such operation adversely affects the welfare of those remaining. Fish shall be inspected regularly. It is essential that oxygen levels in transport tanks are maintained above the level

set as a critical value for different fish species. Carbon dioxide levels are kept low and excessive changes in water temperature and pH are avoided.

Transport equipment shall be cleaned and disinfected where appropriate to avoid a spread of disease and in a manner which is not harmful to the fish.

Internal physiological mechanisms responsible for adapting to stressor include nervous, hormonal and immunological mechanisms (Selye, 1973). Transported fish are often exposed to multiple stressors within a short duration. Stressors that affect fish can be categorized into acute (short-term) or chronic (long-term) stressors (Davis, 2006). Acute stressors include hauling densities (Piper et al., 1982), handling (Maule et al., 1988; Cech et al., 1996), confinement (David & Parker, 1986), abrupt changes in water quality (Weirich and Tomasso, 1991; Carmichael et al., 1992), improper acclimation and conditioning fish to a new environment (Carmichael et al., 1984; Brick and Cech, 2002). Chronic stressors include extended periods of poor water quality, improper stocking densities and improper diets. Severe stress might result in immediate mortality, presumably through ion loss (McDonald & Milligan, 1997), whereas chronic stress often results in a severely compromised immune function and/or a decrease in energy stores (Portz et al., 2006). Moreover, the exact impact of stress depends on its severity and duration, as well as on the health of the fish (Noga, 2000).

The primary stress responses involve release of stress hormones (e.g. adrenalin, cortisol), which lead to secondary stress responses that stimulate oxygen uptake and transfer, mobilization of energy substrates, and reallocation of energy away from growth and reproduction. This mobilizing of energy and attention to handle the stressor is named "fight or flight" response, which in nature is a process that is essential for survival. Dysfunction or epizootic diseases are induced by stress (Wedemeyer, 1970; Carmichael et al., 1984). Transport stress in fish can be diagnosed clinically by a decrease in the plasma osmolality in freshwater fish or an increase in osmolality in saltwater fish (Carmichael et al., 1984; Robetson et al., 1988). In the absence of hematologic data, failure of live fish to orient properly in the water column during or after transport is an indication of transport-induced stress. Signs of transport stress may not appear until a few days after transport (Tomasso et al., 1980). Most works on transport stress have concentrated on the prevention of the stress rather than treating the problem. So the predominant strategy is that the fish be in good health and fit for transport and that light sedation be used to lower the water temperature. The general condition is that any procedure is stressful to fish, and all the fish exhibit most, if not allthe physiological and biochemical responses to stressor which are seen in the mammals.

"To minimize stress anesthesia has been used" (EFSA, 2004). Anesthetics are widely used prior to and during transport to slow down the metabolism of the fish, thus reducing oxygen uptake and decreasing CO_2 level and ammonia production. Only a light sedation should be used if anesthetics are used during transport (Wedemeyer, 1997); however, "anesthetised" fish cannot be used for human consumption and the only anesthetic licensed for fish in the EU is not approved for such use" (EFSA, 2004). Fish anesthesia can be administered by chemical and non-chemical methods. Non-chemical methods include lowering body temperature either by snow or crushed ice. Carmichael and Tomasso (1988) and Johnson (2000) reported that ice

is commonly used to cool transport water, whereas chillers are less common. One pound of ice (0.45 kg) will lower the temperature of two gallons (9 l) of water by 5.5° C (Timmons et al., 2002). If ice is used and it is made from a chlorinated water source, sodium thiosulphate ($Na_2S_2O_3$) or sodium sulphite (Na_2SO_3) should be added to remove the residual chlorine. Sodium tiosulphate at the rate of 7.4 ppm for each ppm of any chlorine in the ice must be used.

The immediate mortality associated with transport stress is presumably caused by blood ion disturbances (McDonald & Milligan, 1997). Marine bony fish must drink large amounts of seawater to prevent dehydration because of the movement of water from their body into the surrounding seawater environment as a result of their hypotonic condition (Moyle & Cech, 1988). Conversely, freshwater fish are hypertonic, they therefore gain water and lose electrolytes. The net loss of sodium and chloride ions in freshwater fish, associated with the stress of transport, means that certain species may benefit from dilute salt solutions or a current during, before or after transportation (Carmichael, 1984; Pickering, 1992; Wedemeyer, 1997)

During excitement and in stressful circumstances (which typically occur in transport), epinephrine (adrenaline) is released into the bloodstream, thus affecting the permeability of water across the gill epithelia in fish (Moyle & Cech, 1988). This increases the water gain and blood ion loss in freshwater fish and the loss of water and ion influx in marine fish, resulting in a disturbance of the osmoregulatory homeostasis (Portz et al., 2006). Because of these conditions, a general procedure for transporting many freshwater fish is to add salts to their transport water. Many studies have documented the advantages of using salt during and after the transport of various species (Collins & Hulsey, 1963; Tomasso et al., 1980; Johnson & Metcalf, 1982; Carmichael et al., 1984; Mazic et al., 1991; Barton & Zitzow, 1995; Cech et al., 1996; Swanson et al., 1996). In freshwater, salt ($NaCl$) has the potential to alleviate or reduce osmoregulatory dysfunction by decreasing the gradient between the water and the fish blood (Mazic et al., 1991). Isotonic conditions for freshwater fish are approximately one-third the salt concentration of seawater (Moyle & Cech, 1988).

Most of the studies listed above used 5–10% salt solutions in their hauling experiments (freshwater fish). Mazeaud et al. (1977) also stated that marine fish stop drinking when stressed as a result of a gastric muscular contraction induced by catecholamine. Catecholamine is considered to be a hormone that is released under stressful situations in an attempt to adapt to or to avoid the stressor (Wedemeyer, 1996).

2.7.4.2. Loading and unloading

Care should be taken to prevent injury and stress to fish during capture, loading, transportation and unloading. Abrupt temperature changes, periods of hypoxia and any deterioration in the water quality due to excretory products should be avoided. Excessive crowding of fish prior to management procedures can be stressful, with potential decreases in oxygen levels and water quality, increased chances of abrasion, and rapid changes in light intensity (Humane Slaughter Association, HSA 2005). Stress during crowding may also affect

response to further stressors such as net capture (Schreck et al., 1989; Ruane et al., 2002). Recovery periods are therefore often beneficial, particularly during transport (Iversen et al., 1998; Jonssonn et al., 1999). Stressors associated with crowding can be kept to a minimum with appropriate management techniques. HSA guidelines (2005) suggest that good crowding management includes a slow and gentle technique, assessment of water quality, the addition of oxygen to the water if levels fall below a critical 6 mg/l, and, perhaps most importantly, close monitoring of the behaviour and activity of the fish. A simple scoring system is given to help train staff to recognize acceptable levels of activity and stress (HSA, 2005). Handling and transport are inherently stressful events (Barton et al., 1980; Davis and Schreck, 1997; Sharpe et al., 1998). It is very important to saturate or supersaturate the water with oxygen (O_2) prior to placing a heavy load of fish into a transport tank. It must also be noted that confinement and capturing prior to transport also pose a threat of deterioration of Dissolved Oxygen (DO) levels within the holding area; this is due to the initial 30–60 minutes in the transport container which are critical for the increased activity of the fish (Piper et al., 1982).

Removal from the water elicits a maximal emergency physiological response and should only be carried out when absolutely necessary. Air exposure for 3 minutes resulted in a 50-fold increase in plasma cortisol levels within 30 minutes in gilthead sea bream (Arends et al., 1999). Care must be taken at all stages to avoid abrasions and removal of scales and the fish's protective mucous coat, which serves as a physical and chemical barrier to infection besides being important in osmoregulation and locomotion. Where applicable, this should involve the use of wet hands and nets appropriate for the species, keeping the fish moist during handling. Excessive weight loading on fish at the bottom of nets and brailles should also be avoided (Conte, 2004; HSA, 2005). The use of a braille lining allows some water to be retained in the net and, therefore, provides some protection from abrasion. Moving water along with the fish should cause fewer injuries and appears to be the least stressful technique (FAWC, 1996). As such, the use of fish pumps and transfer pipes appear to be preferable for welfare. However, effective management should ensure that the design of the system provides appropriate speed and delivery rate, minimizing the potential of abrasion and the time spent in the pipe. Prolonged periods in pipes, particularly in warm conditions, should be avoided and pipes should be flushed through to ensure no fish are left inside after use (HSA, 2005). A cumulative effect on cortisol levels was seen when turbot (Scophthalmus maximus L.) were confined a second time, 4 hours after an initial net capture (Waring et al., 1997). A 24-hour recovery period between multiple handling procedures avoided this cumulative effect. The provision of a recovery period following transport is clearly important for welfare and subsequent survival (Erikson et al., 1997; Iversen et al., 1998; Tipping, 1998; Jonssonn et al., 1999; Finstad et al., 2003; Iversen et al., 2003).

If at all possible, fish should be moved without removing them from the water. McDonald and Milligan (1997) highlighted several references that reported that exposure to air after exercise for even short periods of time can have a significant impact on mortality rates. However, handling fish in nets is almost inevitable during transport procedures.

Habituation of the fish pre-transportation (Schreck et al., 1995) has shown that salmon handled before transport appeared to recover more rapidly than fish which had not been

conditioned. However this is not considered feasible for normal transport purposes. Development of a fish pump that can rapidly transport large quantities of live fish without injury has important economic and marketing implications. The majority of fish had skin bruises resulting from excessive crowding of large fish at high densities into the pump insertion port. Fin injury, frayed fins, was largely attributed to the culture holding conditions rather than a pump effect as suggested by relatively high fin abrasion of un-pumped control and quality control fish. Nearly all body injuries were non-lethal. Careful acclimation to new environmental temperatures, water chemistry and light levels (Mork and Gulbrandsen, 1994) are also important for the well-being. It might also be beneficial to use the transport time as an acclimation process between the arrival water and the pre-transport water.

The loading process is a more severe stressor than the transport itself, with plasma cortisol returning to resting levels during the time in the well-boats in four out of five transports. Only minor plasma cortisol increases are observed during unloading. It may be that the well-boats provide an important recovery function (Iversen et al., 2005). According to Specker and Schreck (1980), the greatest stress response occurs during loading and during the first few hours of transport in Coho salmon (Oncorhynchus kisutch) smolts.

Fish should be acclimated to receiving water if it is much different from the transport water. The preferred method to reduce the stress associated with the acclimation process is to mimic the water from which the fish were taken. When hauling fish, this involves the transport container water and the receiving water after transport. Abrupt changes in water parameters, such as temperature, pH, hardness and salinity, should be avoided (Noga, 2000). However, as mentioned previously, adding salts as well as reducing the hauling temperature of freshwater species can be beneficial during the hauling procedure. Timmons et al. (2002) recommended that if the temperature changeexceeded 5.5°C in 20 minutes and if the pH differed by more than one unit, 10% of the tank water should be changed every 10-20 minutes with the receiving water until it returned to normality. Most fish seem to tolerate a rapid drop in temperature better than the equivalent rise in temperature (Noga, 2000). "Delayed mortality syndrome" and "hauling loss" are terms used to reference fish mortality that is associated with transport and conditioning to a new environment. Delayed mortality might occur days or even weeks after transport depending on the underlying cause and severity.

2.7.4.3. Space allowance

The number (or weight) of fish that can be successfully transported depends on the quality and on the temperature of the water, the duration of the transport, the fish size and species.

As a general rule, as the transport time increases (particularly >8 hours) the carrying capacity should decrease. While some species adapt well to high loading densities (Barton et al., 2000; Ruane and Komen, 2003) others show prolonged elevated cortisol levels following confinement procedures (Barnett and Pankhurst, 1998; Barton et al., 2003). Barton et al. (2005) have also shown that a fish's capacity to respond to acute stressors such as crowding may be altered by the effects of long term holding conditions. Suggested hauling densities for various species of fish are reported by Piper et al. (1982).

2.7.4.4. Feeding and watering

Fish should be deprived of food prior to transport. Fasting fish for at least 24 h is recommended to reduce not only the accumulation of faeces and ammonia in the tank (Carmichael et al., 2001), but also the demand of biological oxygen because fish with empty stomach consume less oxygen. Suspended solids from fish faeces can pollute the water as well as physically damage the gills of fish. Mechanical type cartridge filters connected to a submersible pump have excellent filtration capabilities and can easily be mounted onto a transport box.

2.7.4.5. Water quality

The water that is put into the tanks is the key to a successful transport. It should be from the same source as the water in which the fish have been reared, and it should be taken from the inlet rather than the pond itself (or outlet) to ensure that it is free from contamination and suspended solids. Live fish are generally transported in water, which quality changes progressively during transport. Major changes occur in the concentration of the chemicals. A prerequisite is that the quality of raw water be good. For example, fresh water is not acidified and there is a low content of metals (like aluminum) in the water that could cause extra problems for the fish. Because fish are poikilotherms, the surrounding water is critical to their physiological reaction rates. As their body temperature increases, biochemical reaction rates increase too. Conversely, as their body temperature decreases, metabolic processes decrease. Thus, cooling the transport water has advantages. Wedemeyer (1997) found that by reducing the hauling water by 10°C, most warm water species will reduce oxygen consumption and ammonia production by 50% and, therefore, recommends lowering the hauling water temperature by 5–10°C. However, caution must be used in the cooling process to ensure that there is not too much of a gradient difference between the holding water temperatures and the hauling temperatures as an abrupt change in temperature itself could be a stressor. Temperature control involves maintaining the water temperature during transport within a desired range. At higher temperatures there is too little dissolved oxygen, and the fish are more prone to stress. According to a study on the observation of fish behaviour at Fiskeriforskning in Norway, the fish tend to spend most of their time in a narrow range of temperature, defined as their preferred temperatures (Fry, 1974; Johnson & Kelsch, 1998). This active behaviour has been termed "behavioural thermoregulation" (Reynold & Casterlin, 1979), indicating that the fish actively regulates its body temperature by selecting an adequate environment. The preference chamber allows the fish to move freely in a temperature gradient, and a temperature preference can be decided by monitoring the temperature at which the fish spends its time.

Dissolved oxygen (DO) is mainly used by fish for their respiration. Bacterial activity and oxidation process will also use oxygen in the presence of organic matter. DO consumption by fish varies with species, with water temperature, with fish activity and fish size.

The dissolved oxygen concentration will be lower in warm water.

Various methods have been used to achieve and maintain proper DO levels throughout fish transport, including compressed gaseous oxygen, agitators, aerators and liquid oxygen. Using the correct diffuser is also important for efficiency. Smaller bubbles from fine-pore diffusers have a greater air to water surface area compared with the same volume of gas with larger bubbles. This is important with regard to the amount of oxygen needed for transport. Agitators are inefficient compared with pure O_2 injection through diffusers, but are important in removing CO_2. However, agitators can cause excessive foaming in salt water (Carmichael et al., 2001). Carmichael et al. (1992) recommend a combination of agitators and pure oxygen diffusers for high-density transports.

Oxygen is the parameter that limited the carrying capacity, and subsequently, its flow rate had to be high.

However, there is no single physiological parameter with the qualities of a "universal welfare indicator" showing sensitive and linear responses to all factors which may cause a potential distress to the animal. Quantitative measurements of ventilation represent, perhaps, the most promising indicators of a wide range of important welfare factors used in physiological telemetry. We know, for example, that ventilation is significantly affected by factors such as hypoxia, hypercarbia and changes in water pH or metabolite levels (Smith & Jones, 1982; Borch et al., 1993; Wasielesky et al., 1997), toxic or sub-toxic levels of metabolites and xenobiotics in feed or water (Handy et al., 1993; McKim et al., 1999), anemia (Smith & Jones, 1982), parasite infections (Laitinen et al., 1996; Finley & Forrester, 2003), disease (Byrne et al., 1991), as well as during the general stress response such as shelter, fear and pain (Fischer, 2000; Sneddon, 2003).

Carbon dioxide is produced as a by-product of fish metabolism. It is usually not a cause of clinical problems on its own; however, high concentration of carbon dioxide can exacerbate hypoxia caused by low dissolved oxygen, both of which are common in the early morning hours (Tucker, 1985; Schwedler et al., 1985). Fresh water at equilibrium contains about 2 mg/l CO_2. Wedemeyer (1996) points out that a high concentration of CO_2 can be a greater risk than elevated ammonia levels during transport. Wedemeyer (1996) also recommends keeping CO_2 concentrations below 30–40 mg/l during transport. However, he warns that if the DO is not saturated, this level might be reduced.

Acidity is closely related to pH and alkalinity.

Ammonia is produced as a by-product from fish metabolism and is primarily excreted through the gills by diffusion (Colt & Armstrong, 1981). In the aquatic environment, ammonia exists in two forms in equilibrium: as un-ionised ammonia, NH_3, and as ionized ammonium, NH_4; un-ionised ammonia is much more toxic than the ionized form. The customary UK practice is therefore to express total ammonia concentration as just the amount of nitrogen present-i.e. Total Ammonia Nitrogen (TAN)- rather than trying to include the variable hydrogen component (Dosdat et al., 1995). The un-ionised ammonia fraction is referred to as N-NH_3 and the ionized ammonium as N-NH_4. High levels of ammonia are frequently associated with high stocking density (Tucker et al., 1979; Tucker, 1985). The accumulation of ammonia can

be minimized by fasting fish prior to transport and/or adding ammonia-reducing agents to the transport water.

Nitrite is formed from the oxidation of ammonium (NH_4) in the aquatic environment. Nitrite is toxic to fish as it diffuses from the blood plasma into the red blood cells, where it oxidises the Fe^{2+} in haemoglobin (Hb) to the Fe^{3+} oxidation state, converting haemoglobin into methaemoglobin (metHb). MetHb lacks the capacity to bind oxygen, therefore the oxygen transport system in the fish is disabled, resulting in hypoxia.

Although many freshwater transport protocols might be similar, it is worth noting differences among marine and freshwater species, including osmoregulatory differences (Moyle & Cech, 1988) and sensitivity to certain water-quality parameters. It must also be noted that acceptable water-quality parameters might even differ within a species depending on life stage, health and previous holding conditions.

2.7.4.6. Salmon

There are relatively few hatcheries compared with the number of on-growing farm. The two most common farming systems are flow-through freshwater tanks up to the smolt stage and sea cages up to the time of slaughter. Broodstock are normally transferred back to freshwater tanks prior to spawning (EFSA, 2008a).

The on-growing stage in sea cages represents normally 50-70% of life stage (EFSA, 2008a). Smoltification is the process by which salmon parr undergo physiological changes which will allow them to adapt to a saltwater environment. The smolts may be transferred in tanks by land, by air or by well-boat. In Atlantic salmon (*S. salar L.*) smolts, many of the disease outbreaks take place during the first months of transfer by sea, following transport in well boats (Iversen et al., 2005). Prior to slaughter, Atlantic salmon are often deprived of food for a few days or weeks. However, the FAWC and HSA recommend that salmon should not be totally deprived of food except during a period of up to 72 hours before slaughter or a handling procedure.

Wedemeyer (1996) found that, when transporting salmonids, a typical protocol is to fast the fish for 48–72 hours prior to transport. Water temperature between 5 and 7° C is a widely used protocol in many salmonid transports (Wedemeyer, 1996). In salmonids aquaculture, it is recommended that fish transport and handling is performed when water temperatures are low (Barton et al., 2000). The acceptable concentration of DO for Atlantic salmon is 6.0-7.0 mg/l. Levels of carbon dioxide above 12 mg/l have a negative impact. The water quality parameters are < 10mg/l of carbon dioxide. Their optimal temperature is closer to 10°-18°C. Salmon are able to survive exposure to pH in the range 6.5-8.5. An excess of 4.0 mg/l mineral acids is lethal for most salmonids. Salmonids exposed to continuous levels exceeding 0.03 mg/l have reduced growth rates and can develop environmental gill disease. The maximum level of un-ionized ammonia (NH_3) should not exceed 0.02mg/l for all stages (EFSA, 2008a). Accepted tolerance level of $N-NH_3$ for Atlantic salmon is < 0.01 mg/l. Levels exceeding

0.03mg/l ($<$ 0.03 N-NO$_3$) induce methemoglobinemia in which the iron in the hemoglobin molecules is reduced and cannot transport oxygen.

2.7.4.7. Trout

In Europe commercial trout farming is already well developed. It is used for various purposes in a wide diversity of systems, but so far there has been no systematic survey of production systems and husbandry procedures at a European level (EFSA, 2008b). The trout species of the greatest importance in aquaculture is undoubtedly the rainbow trout (*Salmo gairdneri*). A recent study, using electromyogram telemetry, indicated that rainbow trout showed vigorous swimming activity and elevated oxygen consumption during transport (Chandroo et al., 2005). While activity levels returned to baseline within 48 hours, beyond this period, swimming performance, measured as critical speed and endurance, was still affected. Fasting for trout is recommended for up to 48 hours only. FAWC guidelines also suggest that fish should not be deprived of food for any other reason such as conditioning and adjustment of body composition (FAWC, 1996). Phillips and Brockway (1954) reported that trout fasted for 63 hours produced half as much ammonia as recently fed fish. Piper et al. (1982) pointed out that with trout the maximum permissible weight is directly proportional to their size. The minimum concentration of DO needed for both rainbow and brown trout is $>$ 5.0 mg/l, but it is not allowed to fall to that level and it is maintained in a fully saturated state. A level of carbon dioxide $<$ 10 mg/l is acceptable. Temperatures around 12-17°C are considered optimal for rainbow trout. Rainbow trout and brown trout show significant mortality when exposed to a water pH $\leq$ 4. Trout can cope with acute high pH ($>$ 9.0) exposure for short-term periods. A pH level of 6.5-8.5 is generally considered to be optimum. A maximum level of 0.01 mg/l of un-ionized ammonia in the water is recommended. Levels above 0.1 mg/l NO$_2$ in the water can be toxic though the effect of other ions in the water will affect its toxicity.

2.7.4.8. Eel

Despite technological advances, it is not yet possible to complete the eel's life-cycle in captivity.

The rapid expansion of eel farming in Japan since about the middle of the 19th century, aroused considerable interest in intensive farming of this group and eel culture enterprises have developed in a number of countries in Europe, especially Italy, Germany and France.

Although there are 16 species of eels, the most important ones, from the point of view of large-scale aquaculture, are *Anguilla Anguilla* in Europe and *Anguilla Japonica* in Japan and Taiwan.

As indicated earlier, eel culture is based on seed eel collected from the wild. In Europe the collection of elvers is done either during winter and spring or in the beginning of summer, in June and July, when they ascend the rivers. Elvers need careful handling after capture and during rearing. It is a common practice to condition them after capture for a day, in special bamboo baskets or tanks. They can be transported to distant farms, packed in wooden boxes.

Intercontinental shipments of elvers have also been made in polythene bags, after conditioning at low temperatures of 4-7° C (EFSA, 2008c).

Transport of glass eels can be by road tanker or by air freight. Transport by road is a little different from transporting any other aquaculture species. The key variables are temperature, dissolved oxygen, pH and metabolic by-products. Regular monitoring and control of some of these parameters is considered as good husbandry practices and should be carried out if potential problems are to be avoided. The transport of glass eels by air freight is different from other aquaculture species. Glass eels have the ability to survive outside water for some 30 hours providing they are kept in a cool and moist environment. This unique feature allows glass eels to be shipped over large distances which would have taken days by road but can be completed in hours by air. The key variables (e.g. temperature, oxygen, density of fish) are set at the start of the transport, as they cannot be monitored or varied during transportation and require greater precision than under other transportation methods. The concentration of DO for European eel is > 4.0 mg/l. A level of carbon dioxide < 0.6 mg/l is acceptable. Their optimal temperature is between 22 and 26° C. In an insulated production unit, optimal temperatures are of 25 °C. (Tesch, 2003). Optimum pH values for the eel are reported as being between 7 and 8 (Tesch, 2003). Maximum concentrations of un-ionized ammonia should not exceed 0.05 to 0.1 mg/l with levels of $N-NH_3 < 0.01$ mg/l.

Compared with other freshwater fish species, *Anguilla Anguilla* is tolerant to nitrite, the intermediate product during nitrification. The accepted tolerance of nitrite is $N-NO_2 < 0.01$. Water nitrite concentrations should be below 30 mg/l (Kamstra et al., 1996). Water nitrate levels (as opposed to nitrite) as high as 500 mg/l are acceptable.

2.7.4.9. Channel catfish

The catfishes of the world are represented by at least 2000 species in over 25 families within the order Siluriformes. The wels catfish (Silurus *glanis*) is the only native catfish species of Europe. Catfishes are found in commercial fisheries, sport fisheries and aquaculture, as ornamental fish, and on endangered species list. Most catfishes are strictly freshwater species, although two families contain saltwater species. Most wels catfish are about 1.3-1.6 metres long. At 1.5 m they can weigh 15-20 kilograms. The minimum acceptable concentration of DO for Channel catfish is 5 mg/l (Tucker, 1985). Their optimal temperature is around 28° to 30°C. The water in the hauling tanks should be within 3°C of the sources water in which the fish are held before hauling. Piper et al. (1982) recommend that Channel catfish hauling temperatures be of 7.7–10°C in winter and 15.6–21.1° C in summer. However, Piper et al. (1982) warn that for Channel catfish fry transport, the hauling water should not be cooled down. Channel catfish are able to survive exposure to pH extremes as low as 4 and 10 (Swingle, 1961; Tucker, 1985). For optimum health, however, pH should remain in the range of 6.5 to 9.0. (Swingle, 1961; Tucker, 1985). Ammonia is the principal nitrogen waste product produced by catfish. Toxic ammonia levels will be noticed late in the afternoon when pH is highest.

2.7.4.10.Carp

Carp are a morphologically diverse family and size can range from a few centimeters to over 2.5 meters. Of all the species of finfish or shellfish used for aquaculture, carp undoubtedly have the oldest history. The common carp (*Cyprinus carpio*) is probably one of the few aquaculture species that can be considered to have been domesticated. The concentration of DO for carp is > 3.0 mg/l and the optimum level of carbon dioxide should be < 6.0 mg/l. Their optimal temperature ranges from 25° to 30°C. Carp are able to survive exposure to pH 6.0-9.0. The maximum concentration of $N-NH_3$ is < 0.1mg/l. The accepted tolerance level of $N-NO_2$ is < 0.1 mg/l.

2.7.4.11.Transport of fry

The period of rapid growth of young fish may be completed in a land-based tank unit or on floating pens in fresh water. If fry are to be grown in lochs or lakes, they need to be transported at the appropriate time. Fry are usually transported in insulated tank at optimum stocking densities, with controlled temperature and oxygen levels. Temperature shock must be avoided. Prolonged transport results in the accumulation of metabolic waste, which can be dangerous. Disinfectant used to clean the tanks must be removed completely by rinsing.

3.   Risk Assessment methodology applied to animal welfare during transport

3.1.   Risk Assessment on animal welfare background and state of the art

Risk analysis typically includes three parts: Risk Assessment (RA), Risk Management (RM) and Risk Communication (RC). In the European Union risk assessment in food safety is under the responsibility of the European Food Safety Authority (that deals with RA and RC). The European Commission mainly deals with RM and RC. RA application in the area of animal welfare is relatively new, whereas it is a widely used predicting tool in the areas of epidemiology and food safety.

The objective of risk assessment is to identify and characterize potential hazards (e.g. to human health or animal welfare or to food safety), to estimate the probability and magnitude of adverse effects resulting from exposure to those hazards and to determine the resulting risks.

RA could be also described as a systematic process to estimate the likelihood and severity of a hazard impact and it includes four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. RA should be science-based, well documented, objective, repeatable, transparent and open to review.

• Science-based: the process should be based on the best available evidence, i.e. on results that have been obtained by relying on recognized scientific methods.

• Well-documented: i.e. it should be assured that all available scientific information is considered and kept available for review.

• Objective: i.e. the problems to be addressed in RA should be clearly stated.

• Repeatable: i.e. a group of experts other than the one engaged in RA should on the basis of the information compiled reach the same conclusions.

• Transparent: The methodologies and data used for RA should be clearly documented and uncertainties should be clearly identified and taken into consideration in the final assessment.

Risks could be assessed quantitatively (when enough data are available), semi-quantitatively (when available data are rather deficient) or qualitatively (when quantitative data are not available). The outcome of a qualitative risk assessment is unavoidably less objective than the others even if sometimes it is the only possible option.

In food RA terminology, a hazard is a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. The risk is a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food (Codex Alimentarius, WHO, 1999).

In parallel to the Codex Alimentarius, RA methodology states that a hazard in animal welfare RA is a precise factor with a potential to cause a negative animal welfare effect, measured by one or more welfare indicators.

A risk in animal welfare is a function of the probability of a negative animal welfare effect and the severity of that effect, consequential to the exposure to a hazard(s).

The level of confidence in the final estimation of risk varies according to the variability, uncertainty, and assumptions recognized and integrated in the different risk assessment steps.

Uncertainty arises in the evaluation and extrapolation of information obtained from epidemiological, experimental, and laboratory animal studies. Uncertainty could be treated formally in conducting more studies or quasi-formally in using expert opinions or informally by making judgment.

Variability is a biological phenomenon (inherent dispersion) and it is not reducible. Reduction in variability is not an improvement in knowledge, but instead it would reflect a loss of information. (EFSA, 2009n)

Over the past few years, since the first Scientific Colloquium held in Parma on 1-2 December 2005 (Principles of Risk Assessment of Food Producing Animals: Current and Future Approaches), during which the peculiarity of AW compared to other branches of study was widely recognised, various studies have been conducted in order to systematize the RA approach in AW.

Below, the list of works that have been investigating the subject answering requests from the EU, through EFSA:

- the welfare of animals during transport (2004a)
- standards for the microclimate inside animal road transport vehicles (2004b)
- welfare aspects of the main systems of stunning and killing the main commercial species of animals (2004c)
- welfare aspects of the castration of piglets (2004d)
- welfare aspects of various systems of keeping laying hens (2005a)
- the impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits (2005b)
- welfare of weaners and rearing pigs: effects of different space allowances and floor (2005c)
- the aspects of the biology and welfare of animals used for experimental and other scientific purposes (2005d)
- welfare aspects of the main systems of stunning and killing applied to commercially farmed deer, goats, rabbits, ostriches, ducks, geese (2006a)
- risks of poor welfare in intensive calf farming systems (2006b)
- animal health and welfare risks associated with the import of wild birds other than poultry into the European Union (2006c)
- animal health and welfare in fattening pigs in relation to housing and husbandry (2007a)
- Animal health and welfare aspects of different housing and husbandry systems for adult breeding boars, pregnant, farrowing sows and unweaned piglets (2007b)
- Animal Welfare aspects of the killing and skinning of seals (2007c)
- The risks associated with tail biting in pigs and possible means to reduce the need for tail docking considering the different housing and husbandry systems (2007d)
- Animal welfare aspects of husbandry systems for farmed Atlantic salmon (2008a), farmed trout (2008b), European eel (2008c), European seabass and gilthead seabream(2008d) and farmed common carp (2008e)
- Species-specific welfare aspects of the main systems of stunning and killing of farmed Seabass and Seabream (2009a), farmed Atlantic Salmon (2009b), farmed Rainbow Trout (2009c), farmed Eels (Anguilla Anguilla) (2009d), farmed Carp (2009e), farmed tuna (2009f), farmed turbot (2009g)
- General approach to fish welfare and to the concept of sentience in fish (2009h)
- the overall effects of farming systems on dairy cow welfare and disease (2009i)
- welfare of dairy cows in relation to leg and locomotion problems (2009j), udder problems (2009k), behaviour, fear and pain (2009l), metabolic and reproductive problems (2009m) based on a risk assessment with special reference to the impact of housing, feeding, management and genetic selection
- Animal Welfare Risk Assessment Guidelines on Stunning and Killing (2009n)

3.2. Risk Assessment Methodology

3.2.1. Rationale of the scenarios proposed

The first step to develop a risk assessment is to define the target population in order to score the hazards within each particular population. When a RA on animal welfare during transport in Europe is to be conducted, the number of transport options to be analysed is substantial and a quantitative approach to RA will inevitably lead to extremely time-consuming exercises. Animal transportation for commercial purposes is a common practice within the EU and millions of animals are moved every year between member countries and outside them, therefore descriptions of transport processes cannot be standardized and a multitude of differences exists. For example as regards the climatic conditions, they vary a lot according to different European climatic regions, (the Mediterranean region compared to the northern part of Europe). For reasons of practicability, a list of limited scenarios that describe the most common transport practices within Europe for food producing animals were created (Annex 1).

The Consortium decided to describe the scenarios considering only the following variables: the species of animals being transported, animal categories within each species, the means of transport, the duration of the transport and the thermal environment during the transport.

- Species:
  - Swine
  - Cattle
  - Small Ruminants
  - Equine
  - Poultry
  - Rabbit
  - Fish.

- Animal categories:
  - Swine: post-weaning piglets, slaughter pigs, breeding pigs
  - Cattle: calves, heifers, beef cattle and cows
  - Small ruminants: lambs, ewes, kids and goats
  - Equine: broken, unbroken, mares with foals and stallions
  - Poultry: one-day-old chicks, broilers, hens, spent hens, ducks and quails, and turkey.
  - Rabbits: breeding rabbit and slaughter rabbit
  - Fish: salmon, trout, eel, catfish, carp

- Means of transport
  - Road
  - Sea

- Air

- <u>Transport duration</u>
  - Short (< 8 h)
  - Long (> 8 h)

- <u>Thermal environment</u>
  - Neutral: animals exposed to a thermal environment within their species-specific thermoneutral zone
  - Above: animals exposed to temperatures above their specie-specific thermoneutral zone
  - Below: animals exposed to temperatures below their specie-specific thermoneutral zone

According to the Council Regulation (EC) No 1/2005, short transport refers to transports of less than 8 hours and long transport refers to transports of more than 8 hours. Journey times shall not exceed 8 hours, except when road vehicles meet special requirements. In that case, cattle and sheep can be transported for 28 hours (with a rest of at least one hour after 14 hours), after which they must be unloaded and given food, water and at least 24 hours of rest. If the higher vehicle standards are attained, pigs and horses can be transported for 24 hours, after which they must be unloaded and given food, water and at least 24 hours of rest. Similarly, where the higher vehicle standards are attained, un-weaned animals can be transported for 18 hours (with a rest of at least one hour after nine hours), after which they must be unloaded and given food, water and at least 24 hours of rest. This pattern of travel and rest can be repeated indefinitely. When journeys exceed eight hours, the vehicles should meet certain standards.

Farm animals are adapted to a given thermal environment and during the journey, the animals might face different thermal environments. Thermal comfort and the relationship between animals and their thermal environment are defined with the term thermoneutral zone. This is defined as the range of ambient temperatures that provides a sensation of comfort and minimises stress. Too low or too high temperatures cause cold and heat stress respectively. The temperatures that define the thermoneutral zone depend on the species and may also vary among breeds and age of the animals. It has to be considered that animals could be potentially exposed to changes of thermal environment within the same journey, according, for example, to meteorological conditions or altitude variations. These changes could cause additional welfare impairments to the animals, however, thermal environment changes were not considered for RA calculation.

A list of scenarios (annex 1) was prepared by the WG taking into consideration the criteria previously explained. In the annex 1 for every species the main types of transport in the EU were chosen in order to give an overview of possible scenarios. An example related to swine is indicated in the table below.

Table 18. Example of annex 1 related to pigs.

Scenarios	Species	Animal categories	Means of transport	Transport duration	Thermal environment
1	pigs	piglets	road	short	above
2	pigs	piglets	road	short	below
3	pigs	piglets	road	short	neutral
4	pigs	piglets	road	long	above
5	pigs	piglets	road	long	below
6	pigs	piglets	road	long	neutral
7	pigs	slaughter	road	short	above
8	pigs	slaughter	road	short	below
9	pigs	slaughter	road	short	neutral
10	pigs	slaughter	road	long	above
11	pigs	slaughter	road	long	below
12	pigs	slaughter	road	long	neutral
13	pigs	breeding	road	short	above
14	pigs	breeding	road	short	below
15	pigs	breeding	road	short	neutral
16	pigs	breeding	road	long	above
17	pigs	breeding	road	long	below
18	pigs	breeding	road	long	neutral

3.2.2. Hazard identification

The second step of a risk assessment is to identify possible hazards. The hazards vary according to the categories of animals. Moreover, the effect of a certain environmental factor on the welfare of the animal, or the prevalence of a certain problem, may differ according to the type of animal within the same species. The welfare effects of a slippery or uncomfortable

floor could be more severe in dairy cows at the end of their productive life than in young bulls. The prevalence of broken wings could be much higher in spent hens than in broilers. The higher mortality rates observed in young sows, sows, and boars, as compared to growing pigs, may be related to the fact that these animals have been excluded from breeding due to decreased performance, possibly the result of poor physical condition and this may lead to impaired health and combined with transport induced-stress, to a higher mortality. Different ages of the animals within the same species, or weaned versus un-weaned animals may constitute additional variables within the same species that may significantly affect the risk of poor welfare during transport. As consequence, different types of animals may face different hazards or different overall risk characterization scores of the same hazard.

A common list of hazards identified for transported animals is provided in tables 19, 20 and 21: the first one applies to pigs, cattle, sheep and goats and equine, the second one applies to rabbit and poultry, and the third one applies to fish.

The WG decided to separate the hazards accordingly to the peculiarity of three different categories. In fact, mammals that can be loaded moving on their own feet share most of the hazards, whereas rabbits and poultry that have similar size and are transported in cages, could be considered as a standing alone category. Finally, fish that have such different needs and peculiarities, have been treated separately. In chapter 3 the main hazards have been identified according to the main transport phase for each species.

These hazards are categorized in two groups: 1) hazards related to the facilities and 2) hazards related to the management.

Hazards related to facilities are those related to the design of the loading facilities (driveways, ramps, lifts etc), design of the vehicle (vibration characteristics, insulation, ventilation, flooring, compartment size), design of the cages (flooring, size, material), design of drinking and feeding facilities, etc.

Hazards related to management are those in which the men's attitude towards animals may have a negative impact. One of the most important hazards affecting the welfare of animals during transport is the behaviour of people during loading and unloading (Lambooij et al., 1999). In fact, people who are moving the animals may cause fear or pain deliberately or accidentally. Moreover, apart from the handling hazards, this category also includes other hazards where the decision taken can have an influence in animal welfare. For instance, those who drive vehicles may contribute directly to poor welfare in the animals by driving too fast around corners, or by violent braking or acceleration. They may also subject animals to extremes of temperature by leaving stationary vehicles in direct sunlight during hot weather or by exposing animals to wind and low temperatures during cold weather. Finally, the stocking density during a transport depends on the number of animals in each compartment. The most important difference between the two categories is that hazards related to facilities cannot be easily controlled or modified in a short term whereas management hazards can be more easily controlled and modified.

The hazards are also linked to the related Welfare Quality criteria as previously described in chapter 2. This categorizing criterion allows putting a focus on the welfare of the animal by assessing the individual adverse effect type of a hazard.

In tables 18, 19 and 20 a common list of hazards during animal transport is classified according to the two categories (facilities and management) and the welfare criteria that can be affected by the adverse effect caused by the hazards (1: absence of prolonged hunger, 2: absence of prolonged thirst, 3: comfort around resting, 4: thermal comfort, 5: ease of locomotion, 6: absence of injuries, 7: absence of disease, 8: absence of other pain, 9: expression of social behaviours, 10: expression of other behaviours, 11: good human-animal relationship, 12: absence of fear).

Table 19. Common list of Hazards in Mammals

Hazard	WQ® criteria
Facilities	
Too long driveway design	5
Too narrow driveway	6,11
Inadequate driveway design (dead ends)	11
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the driveway	6
Inadequate floor condition (gaps, steps, potholes, sloping) in the driveway	5,6,11,12
Inadequate floor surface (too slippery, too rough) in the driveway	5,6,11,12
Too steep up/down ramp	5,6,12
Too narrow ramp	6,11
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the ramp	6
Inadequate floor condition (gaps, steps, potholes, sloping) in the ramp	3,5,6,11,12
Inadequate floor surface (too slippery, too rough) of the ramp	3,5,6,11,12
Inadequate inter-cleat ramp distance	5,6

Missing cleats in cleat ramps	5,6
Inadequate stairsteps	5,6
Steps between ground and ramp and loading decks	6,12
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the lift	6
Inadequate floor condition (gaps, steps, potholes, sloping) in the lift	3,5,6,11,12
Inadequate floor surface (too slippery, too rough) in the lift	3,5,6,11,12
Insufficient width of the gate	6,11
Insufficient height of the gate	6,11
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the gate	6
Inadequate lighting (blinding light, shadows, sudden change of light, glaring objects)	12
High frequency sounds	12
High intensity sounds	12
Unfamiliar smell	12
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the container	6
Inadequate container roof design (gaps, holes, protrusions, projections)	4,6
Inadequate floor condition (gaps, steps, potholes, sloping) in the vehicle	3,5,6,11,12
Inadequate floor surface (too slippery, too rough) in the vehicle	3,5,6,11,12
No partitions in the vehicle	6,9
Not solid partitions	6,9
Too big apertures in the partitions	6,9

Partitions of not enough height	6,9
Partitions closed at the top (disturbs airflow)	4
Inadequate structure of sides (sharp protrusions, sharp angles, open sides, short sides) in the partitions	6
Not reflecting bright colour of the roof	2,4
Inadequate roof insulation	2,3,4
Inadequate roof condition (gaps, holes, protrusions, projections)	3,5,6,11,12
Too low deck height	2,4,5,6,7,10
Too high deck height	6,9
Drafts blowing towards animals	3,12
Too much vibration in the vehicle	5,6,7
Inadequate inspection openings in the vehicle	4,6,7
No supply of water	2,4
Drinking facilities not adjusted to animal species/category	2,4
Inadequate number of drinking troughs/watering cups/nipples	2,4,9
Inadequate water flow rate	2,4
Inadequate position of drinking facilities	2,4
Non-isolated cups/conduits/tank (low temperature)	2,4
Inadequate refilling facilities	2,4
Insufficient tank capacity	2,4
Lack of appropriate feeding devices	1,9
Lack of feeding stuff storage	1,9
Inappropriate position of feed storage (contamination of feed)	1,9
Inadequate natural ventilation (too high air exchange/distribution)	3,4,7

Inadequate natural ventilation (too low air exchange/distribution)	2,3,4,7
Lack of forced ventilation	2,3,4,7
Too low ventilation rate	2,3,4,7
Lack of sensors of temperature and humidity in animal compartment	2,3,4,7
Lack of alert system in the driver compartment	2,3,4,7
Inadequate ventilation apertures distribution	2,3,4,7
Inadequate ventilation apertures design	2,3,4,7
Inadequate air quality (air contaminated with exhaust gases)	7
Inadequate air quality (moderate or severe dust level)	7
Inadequate air quality (not sufficient O2)	7
Lack of cooling devices	4
Inadequate cooling devices	3,4,7
__Management__	
No fasting	7
Too long fasting duration	1,4,5,6,7,9
Lack of water until loading	2,4
Inadequate feed quality in the vehicle	1
Inadequate feed quantity in the vehicle	1,4,5,6,7,9
Inadequate feeding interval	1
Inadequate duration of feeding	1
Inadequate water supply in the vehicle	2,4
Inadequate water quality in the vehicle	2,4
Lack of training to use the drinking facilities	2

Inappropriate previous positive contact with humans	6,11,12
Transport of animals not fit to travel	5,6,11
Uncleanliness of vehicle and insufficient disinfection	7
No planning of loading/unloading schedule	2,4
Stationary vehicle due to delay in traffic operation	2,4,7
No plans for emergency	6,7
Lack of preparation for the journey (road conditions)	5,6
Inappropriate handling (hitting, lack of motivation)	6,11,12
Use of unnecessary driving tools (sticks, prods)	6,8,11,12
Too short loading time	5
Inadequate number of animals in the moving group	11
Continuity of race	5
Incorrect tethering	6
Mixing unfamiliar animals	6,7,9
Separating familiar animals	9
Moving animals individually	9
Mixing horned/unhorned animals	6
Mixing of animals of different age/size	6,12
No availability of bedding	3,4
Inappropriate bedding (type and/or quality and/or quantity)–in combination with temperature	3,4
Absence of enrichment material for animals	6,7,10
Overcrowding	1,2,3,4,5,6,7,9
Multiple loading during the same trip	7,9

Weaning and transport on same day	1,2
Lack of monitoring during transport by the driver	6,7
Inadequate resting time	1,2,3
Rough driving, bad driving technique	3,5,6,7
Swaying of vehicle	3,5,6,7
Disregarding adaptation of forced ventilation to changing climatic conditions	4

Table 20. Common list of Hazards in Caged Animals

Hazard	WQ® criteria
Facilities	
Inadequate preloading installations (no protection from wind, sun)	4
Inadequate preloading installations (lack of adequate ventilation)	4
Inadequate crate design (sharp objects)	6
Crate gate too small	6
Crate's height too low	4,5,6,10
Solid crate floor	4
Non solid crate floor	7,9
Mesh size too big	6
Mesh size too small	6
Inadequate crate material (iron)	4,6
Smooth floor surface in the crate	6,9,12
Inadequate floor condition (gaps, steps, potholes, sloping) in the crate	6
Use of unclean transport crates	12

Poor maintenance of catching facilities	6,8,11,12
Use of loading tools in bad condition	6,12
Overcrowding (harvester)	4,6,9
Undercrowding (harvester)	6
Inadequate stacking of crates	4,6,9,12
Too rough handling (forklift → vibration)	6,12
Inadequate lighting (blinding light, shadows, sudden change of light, glaring objects)	12
Inadequate overall noise	12
Unfamiliar smell	12
Drafts blowing towards animals	3,12
Too much vibration in the vehicle	5,6,12
Absence of roof in the vehicle	4
Absence of side protection in the vehicle	3,4
Inadequate roof condition (gaps, holes, protrusions, projections)	3,5,6,11,12
Inadequate crate subjection in the vehicle	6
Absence of feeding facilities	1
Absence of drinking facilities	2
Inadequate inspection openings in the vehicle	4,6,7
Inadequate natural ventilation (too high air exchange/distribution)	3,4,7
Inadequate natural ventilation (too low air exchange/distribution)	2,3,4,7
Lack of forced ventilation	2,3,4,7
Inadequate air quality (air contaminated with exhaust gases)	7
Inadequate air quality (moderate or severe dust level)	7

Inadequate air quality (not sufficient O2)	7
__Management__	
Too long fasting	1
Inappropriate previous positive contact with humans	6,11,12
Transport of animals not fit to travel	5,6,11
Uncleanliness of vehicle and insufficient disinfection	7
No planning of loading/unloading schedule	2,4
Stationary vehicle due to delay in traffic operation	2,4,7
No plans for emergency	6,7
Lack of preparation for the journey (road conditions)	5,6
Inappropriate handling (hitting, lack of motivation)	6,11,12
Overcrowding	1,2,3,4,5,6,7,9
Mixing unfamiliar animals	6,7,9
Separating familiar animals	9
No availability of bedding	3,4
Inappropriate bedding (type and/or quality and/or quantity)–in combination with temperature	3,4
Absence of enrichment material for animals	10
Multiple loading during the same trip	7,9
Lack of monitoring during transport by the driver	6,7
Inadequate resting time	1,2,3
Rough driving, bad driving technique	3,5,6,7
Swaying of vehicle	3,5,6,7

Table 21.Common list of Hazards in Fish

Hazard	WQ® criteria
Facilities	
Inadequate pumping system (aspiration pressure above maximum according to different species)	6
Inadequate pumping system (aspiration pressure below minimum according to different species)	6
Inadequate pumping system (pipe section below the average size)	6
Inadequate water flow speed	6
Inadequate access hole design (sharp edges)	6
Insufficient width in the access hole	6
Inappropriate design of the container (sharp protrusions, sharp edges)	6
Inappropriate inspections facilities in the container	7
Poor suspension in the vehicle	5
Excessive pression in tyres of the vehicle	5
Vehicle in bad conditions	5
Inadequate water temperature (lack of temperature monitoring system)	4
Inadequate water temperature (lack of cooling system)	4
Inadequate water temperature (temperature above maximum according to different species)	4
Inadequate water temperature (temperature below minimum according to different species)	4
Inadequate water temperature (sudden change of temperature)	4
Oxygen level of water too high (failure in the oxygen delivery system)	7
Oxygen level of water too high (lack of an oxygen level monitoring	7

system)	
Oxygen level of water too high (super saturation)	7
CO$_2$ level of water too high (decrease in gas exchange)	7
NH$_3$ level of water too high	7
Nitrite level of water too high	7
Aluminium contamination of water (pH reduction)	7
Other metals contamination of water	7
Inadequate water exchange	7
Inadequate aeration system	7
**Management**	
Lack of previous interaction with humans	12
Untrained personnel (rough handling)	6
Crowding	6
Uncleanliness of vehicle and insufficient disinfection	7
Transport of animals not fit to travel	7
Too short fasting duration	7
Too long fasting duration	1
Inappropriate planning of the transport (Selection of an inadequate vehicle for the transport conditions)	7
Stationary vehicle due to delay in traffic operation	7
Stops for inspection/BIPs/Customs	7
No planning of loading/unloading schedule	7
No plans for emergency	7
Inadequate planning of the route (road conditions, climate,	7

duration)	
Delay for the animals to be unloaded	7
Improperly operated pumps and pipes (too high water pressure)	6
Improperly operated nets (too crowded)	6
Mixing unfamiliar animals	6
Mixing animals of different incompatible sizes and species	6
Overcrowding	6
Rough driving, bad driving technique	5
Swaying of vehicle	5
Lack of monitoring during transport by the driver	5
Lack of education/competence of the driver	7
Lack of motivation of the driver	7
Disregarding temperature measurement by the driver	7

The list of hazards was then adapted to all investigated species and scored accordingly with the applied scenario (annex 2). The species-specific hazard list was evaluated according to the identified scenarios (Tables 28, 29, 30, 31, 32, 33 and 34).

For every species-specific scenario the list of hazards was scored (whether a hazard was relevant or not) by putting a non applicable (n.a.) in case of non occurring hazard within the given scenario. In the case that a hazard was affecting more than one welfare criteria, and therefore was provoking more than one adverse effect, the hazard was repeated in as many times as many welfare criteria it affected. An example is indicated in the following table 22. Note that, for readability reasons, only a part of the table is indicated and the complete one is in the annex.

SCENARIO: Slaughter pigs short transport by road in warm conditions			
Hazard identification			
Hazard identified	**Hazard description**	**Adverse effect description**	**Adverse effect type**
FACILITIES and STRUCTURES			
LOADING/UNLOADING			
a.) Driveway			
Inadequate driveway design	Too long (> 60m)	Fatigue	Ease of movement
Inadequate driveway design	Too narrow (<90cm)	Bruising, wounds	Injuries
Inadequate driveway design	Too narrow (<90cm)	Human intervention to move animals	Human-animal relationship
Inadequate driveway design	Continuity of race (dead ends)	Difficult movement	Ease of movement
Inadequate structure of sides	Sharp protrusions, sharp angles, open sides, short sides (<1m)	Bruising, wounds	Injuries
Inadequate floor surface	Slippery	Bruising, wounds	Injuries
Inadequate floor surface	Slippery	Difficult movement	Ease of movement
Inadequate floor surface	Slippery	Reluctant to move	Fear
Inadequate floor condition	Gaps, potholes, steps	Bruising, wounds, fractures	Injuries
Inadequate floor condition	Gaps, potholes, steps	Difficult movement	Ease of movement
Inadequate floor condition	Gaps, potholes, steps	Need of human intervention to move animals	Human-animal relationship
b.) Ramp			
Inadequate ramp design	Too steep up/down (> 20°)	Bruising, wounds, fractures	Injuries
Inadequate ramp design	Too steep up/down (> 20°)	Difficult movement	Ease of movement
Inadequate ramp design	Too steep up/down (> 20°)	Reluctant to move	Fear
Inadequate ramp design	Too narrow (<90cm)	Bruising, wounds	Injuries

Table 22. Section of the annex 3 related to Hazard Identification

3.2.3. Hazard characterization

Generally speaking the objective of hazard characterization (HC) is to review and describe the consequences of the exposure to one or several hazards in terms of magnitude and likelihood of the adverse effect for the individual animal.

3.2.4. Magnitude

The magnitude of the adverse effect represents the potential animal welfare adverse effect at the individual level, given that the animal is exposed to the hazard and experiences that adverse effect, and it is expressed by multiplying the severity by the duration.

The severity of the adverse effect was subjectively scored, on a scale from 1 to 4, by the members of the working group based on the available scientific information about the level of physiological and behavioural responses of the animal to the hazard. Table 23 illustrates the principles used to score the severity.

Table 23 Severity scores of the adverse effects.

Evaluation	Score	Explanation
Mild	1	Minor changes from normality, indicative of pain, malaise, frustration, fear or anxiety (applicable to all species)
Moderate	2	Moderate changes from normality, indicative of pain, malaise, frustration, fear or anxiety. Clear change in adrenal or behavioural reactions, such as motor responses and/or vocalisations, according to each species behavioural patterns.
Severe	3	Substantial changes from normality, indicative of pain, malaise, frustration, fear or anxiety. Strong change in adrenal or behavioural reactions, such as motor responses and/or vocalisations according to each species behavioural patterns.
Very severe	4	Extreme changes from normality, indicative of pain, malaise, frustration, fear or anxiety, usually in several measures, that could be life-threatening if they persist.

The duration of the effect was expressed on a scale from 1 to 5 proportional to the time that the animal was believed or expected to be experiencing the adverse effect, once it would be exposed to the hazard (see table 24). In doing so, the duration of the adverse effect was scored independently from the actual destiny of the animal (e.g. even if the animal was transported to be slaughtered within the next few hours, the duration of the adverse effect of the hazard on the animal was considered as if the life of the animal was continuing).

Table 24. Duration scores of the adverse effect

Score	Criteria
1	The effect lasts up to 3 hours
2	The effect lasts between 3 and 8 hours
3	The effect lasts between 8 and 24 hours
4	The effect lasts between 24 and 72 hours
5	The effect lasts more than 72 hours

For the final estimation of the magnitude of the adverse effect, the severity and the duration scores were adjusted in order to give even weighting to the scores. Therefore, the magnitude of the adverse effect was calculated as follows:

$$\textbf{Magnitude} = \textbf{Severity/4 * Duration/5}$$

3.2.4.1. Quantitative assessment of percentage of likelihood that an adverse effect occurs

As a fundamental part of the HC, the quantitative assessment of likelihood that an adverse effect can occur for a given exposure to a hazard was also scored. Therefore experts on animal transport were asked to score the likelihood according to their knowledge. The expert opinion was modelled using a Beta-Pert distribution that requires three parameters: minimum, most likely and maximum. The three parameters were expressed as a percentage from 0 to 100%.

3.2.4.2. Qualitative assessment of uncertainty

The qualitative assessment of uncertainty for each assessment was also scored according to the availability of any scientific evidence, in agreement with the definition given in table 25.

Table 25. Qualitative uncertainty scores.

Low	Solid and complete data available; strong evidence provided in multiple refs; authors report similar conclusions.
Medium	Some but no complete data available; evidence provided in small number of refs; authors' conclusions vary from one to another. Solid and complete data available from other species which can be extrapolated to the species considered.
High	Scarce or no data available; rather evidence provided in unpublished reports, based on observations or personal communications; authors' conclusions vary considerably between them.

3.2.5. Exposure assessment

The following step was to assess the level of hazard exposure according to the principle of RA where exposure assessment (EA) is described as the quantitative and/or qualitative evaluation of the likelihood of hazards to welfare occurring in a given animal population.

The likelihood of each exposure to the hazard was estimated quantitatively as a Pert probability distribution function, specifying minimum, most likely and maximum values, similar to the assessment of the probability of adverse effects.

Qualitative assessment of the uncertainty related to the exposure was scored by applying again the scores as indicated in table 25.

Due to the fact that transport is by its nature a very limited process in time, the WG decided not to take into consideration the duration of the EA.

Also the intensity, which describes the intensity of the effect of the hazard, was not accounted within EA, because it refers to a qualitative evaluation that was already described under the Hazard Identification part, namely in the column of adverse effect type.

The section of the annex 3 referred to RC and EA, with an example of scored hazards, is illustrated in the following table 26.

Hazard characterization						Exposure Assessment			
Magnitude		Quantitative assessment of likelihood			Qualitative assessment of the uncertainty (1-3)	Quantitative assessment of P. of Exposure			Qualitative assessment of the uncertainty (1-3)
Severity 1-4	Duration categories	min (%)	ml (%)	max (%)		min (%)	ml (%)	max (%)	
1	1	5	20	40	high	5	15	25	high
3	5	2	10	15	high	5	15	30	high
2	2	25	45	65	high	5	15	30	high
2	1	5	15	35	high	2	5	15	high
3	5	5	15	30	high	5	15	30	high
3	5	1	3	5	high	10	20	30	high
2	1	15	20	35	high	10	20	30	high
2	1	20	40	50	high	10	20	30	high
3	5	5	15	25	high	5	15	30	high
2	1	20	40	55	high	5	15	30	high
2	1	10	30	50	high	5	15	30	high
3	5	5	10	15	high	5	15	30	high
2	1	30	50	80	high	5	15	30	high
2	1	20	35	55	high	5	15	30	high

Table 26. Section of the annex 3 related to the Hazard Characterization and Exposure Assessment

3.2.6. Risk characterisation

The main goal of risk characterisation is to rank all the identified hazards in terms of level of risk estimates. The risk estimate is an indicator at the population level, considering not only the likelihood of the animals of that population being exposed to a given hazard, but also the likelihood of the animals to experience an adverse welfare effect if they are exposed. However, in order to give the correct importance to the given hazard, the risk manager should also consider the magnitude of the adverse effect, otherwise he could be misled and come to wrong conclusions. The magnitude of the adverse effect represents the potential animal welfare adverse effect at the individual level, given that the animal is exposed to the hazard and experiences that adverse effect.

The calculations were based on the following formulas:

Magnitude = Severity/4 * Duration/5

Probability of occurrence= Distribution of Exposure * Distribution of Likelihood

Risk = Probability of Occurrence * Magnitude

Risk Estimates = described using Risk median, Risk 5th and 95th Percentile

Risk estimation was calculated by @Risk® (Palisade) software which is an „Add Inn" for Microsoft Excel® and Monte Carlo simulation with 10.000 iterations. The risk estimates distribution was described using the risk median, its 5th and 95th percentile.

The qualitative assessment of the uncertainty on the risk output was derived accordingly to a classification matrix (Table 27) used for the calculation of the product of both the uncertainty evaluations, namely the one related to the likelihood and the one related to the exposure.

Table 27. Classification matrix of the qualitative assessment of the uncertainty.

		Exposure Uncertainty		
		High	**Medium**	**Low**
Adverse Effect Uncertainty	**High**	**High**	**High**	**High**
	Medium	**High**	**Medium**	**Medium**
	Low	**High**	**Medium**	**Low**

The Consortium decided to calculate the outputs only in three defined scenarios out of the many possible ones indicated in the following tables 28, 29, 30, 31, 32, 33 and 34. The choosing criteria were based on the level of knowledge of the experts and, in the fish case, on the peculiarity of the transported species. The three chosen scenarios were:

1. Slaughter pigs transported by road for less than 8 hours in above thermal neutrality conditions.

2. Heifers transported by road for more than 8 hours in below thermal neutrality conditions.

3. Rainbow trout at finish weight (250 g.) pump loaded transported by road for less than 8 hours in above thermal neutrality conditions.

The annex 3 is divided into three sections: Hazard Characterisation (HC), Exposure Assessment (EA) and Risk Characterisation.

HC and EA sections include all values agreed by the experts and used to calculate the Risk Characterisation for each listed hazard, within the three considered scenario.

In the graphics appendix, only the highest ten scored hazards, in terms of risk estimates (median, 5th and 95th percentiles) are presented as a histogram along with their attached magnitudes values.

In the following table a section of the output are shown, only risk estimates and magnitude were used to produce the following figures (1, 2, 3, 4, 5 and 6).

Risk Characterization												
Risk estimate [CI95%]	**Qualitative uncertainty of the risk estimate**	**Dist. P. Exposure**	**Dist Likelihood**	**Probability of Occurrence**	**Median P. Of occurrence**	**P95 of P of occurrence**	**P05 of P of occurrence**	**Magnitude**	**Risk**	**Median risk**	**P95 Risk**	**P05risk**
14,848 [6,543 - 27,632]	high	15	20,8333	312,5	296,960	552,635	130,855	0,05	15,625	14,8480	27,6317	6,54275
108,344 [48,560 - 195,098]	high	15,833	9,5	150,416	144,458	260,130	64,7460	0,75	112,812	108,343	195,098	48,5595
137,691 [70,987 - 229,576]	high	15,833	45	712,5	688,454	1147,87	354,936	0,2	142,5	137,691	229,575	70,9873
9,207 [3,690 - 20,597]	high	6,1666	16,6666	102,777	92,0672	205,968	36,8953	0,1	10,2777	9,20672	20,5968	3,68953
176,677 [78,239 - 339,195]	high	15,833	15,8333	250,694	235,569	452,260	104,318	0,75	188,020	176,676	339,195	78,2387
43,876 [23,620 - 70,769]	high	20	3	60	58,5009	94,3585	31,4934	0,75	45	43,8757	70,7688	23,6201
42,294 [27,146 - 63,298]	high	20	21,6666	433,333	422,94	632,982	271,464	0,1	43,3333	42,294	63,2982	27,1464
75,598 [48,654 - 108,364]	high	20	38,3333	766,666	755,979	1083,64	486,539	0,1	76,6666	75,5979	108,364	48,6539
168,903 [78,253 - 307,346]	high	15,833	15	237,5	225,204	409,794	104,337	0,75	178,125	168,903	307,345	78,2529
59,751 [30,552 - 100,074]	high	15,833	39,1666	620,138	597,508	1000,74	305,521	0,1	62,0138	59,7508	100,074	30,5521
45,071 [20,982 - 82,544]	high	15,833	30	475	450,705	825,442	209,817	0,1	47,5	45,0705	82,5442	20,9817
113,942 [57,219 - 195,743]	high	15,833	10	158,333	151,922	260,991	76,2924	0,75	118,75	113,942	195,743	57,2193

Table 28. Section of the annex 3 related to risk characterization

	Species	Animal categories	Means of transport	Transport duration	Thermal environment
1	pigs	piglets	road	short	above
2	pigs	piglets	road	short	below
3	pigs	piglets	road	short	neutral
4	pigs	piglets	road	long	above
5	pigs	piglets	road	long	below
6	pigs	piglets	road	long	neutral
7	pigs	slaughter	road	short	above
8	pigs	slaughter	road	short	below
9	pigs	slaughter	road	short	neutral
10	pigs	slaughter	road	long	above
11	pigs	slaughter	road	long	below
12	pigs	slaughter	road	long	neutral
13	pigs	breeding	road	short	above
14	pigs	breeding	road	short	below
15	pigs	breeding	road	short	neutral
16	pigs	breeding	road	long	above
17	pigs	breeding	road	long	below
18	pigs	breeding	road	long	neutral

Table 29: List of possible scenarios during pigs transportation

Scenarios	Species	Animal categories	Means of transport	Transport duration	Thermal environment
1	cattle	heifers	road	short	above
2	cattle	heifers	road	short	below
3	cattle	heifers	road	short	neutral
4	cattle	heifers	road	long	above
5	cattle	heifers	road	long	below
6	cattle	heifers	road	long	neutral
7	cattle	heifers	sea	long	above
8	cattle	heifers	sea	long	neutral
9	cattle	heifers	air	long	neutral
10	cattle	beef cattle	road	short	above
11	cattle	beef cattle	road	short	below
12	cattle	beef cattle	road	short	neutral
13	cattle	beef cattle	road	long	above
14	cattle	beef cattle	road	long	below
15	cattle	beef cattle	road	long	neutral
16	cattle	beef cattle	sea	long	above
17	cattle	beef cattle	sea	long	below
18	cattle	beef cattle	sea	long	neutral
19	cattle	cows	road	short	above
20	cattle	cows	road	short	below
21	cattle	cows	road	short	neutral
22	cattle	cows	road	long	above
23	cattle	cows	road	long	below
24	cattle	cows	road	long	neutral
25	cattle	calves	road	short	above
26	cattle	calves	road	short	below
27	cattle	calves	road	short	neutral
28	cattle	calves	road	long	above
29	cattle	calves	road	long	below
30	cattle	calves	road	long	neutral
31	cattle	calves	air	long	neutral

Table 30: List of possible scenarios during cattle transportation

Scenarios	Species	Animal categories	Means of transport	Transport duration	Thermal environment
1	sheep and goats	adults	road	short	above
2	sheep and goats	adults	road	short	below
3	sheep and goats	adults	road	short	neutral
4	sheep and goats	adults	road	long	above
5	sheep and goats	adults	road	long	below
6	sheep and goats	adults	road	long	neutral
7	sheep and goats	adults	sea	long	above
8	sheep and goats	adults	sea	long	below
9	sheep and goats	adults	sea	long	neutral
10	sheep and goats	young	road	short	above
11	sheep and goats	young	road	short	below
12	sheep and goats	young	road	short	neutral
13	sheep and goats	young	road	long	above
14	sheep and goats	young	road	long	below
15	sheep and goats	young	road	long	neutral
16	sheep and	young	sea	long	above

	goats				
17	sheep and goats	young	sea	long	below
18	sheep and goats	young	sea	long	neutral

Table 31: List of possible scenarios during sheep and goats transportation

Scenario	Specie	Animal	Means of	Transport	Thermal
1	equine	broken	road	short	above
2	equine	broken	road	short	below
3	equine	broken	road	short	neutral
4	equine	broken	road	long	above
5	equine	broken	road	long	below
6	equine	broken	road	long	neutral
7	equine	broken	sea	long	above
8	equine	broken	sea	long	below
9	equine	broken	sea	long	neutral
10	equine	unbroken	road	short	above
11	equine	unbroken	road	short	below
12	equine	unbroken	road	short	neutral
13	equine	unbroken	road	long	above
14	equine	unbroken	road	long	below
15	equine	unbroken	road	long	neutral
16	equine	unbroken	sea	long	above
17	equine	unbroken	sea	long	below
18	equine	unbroken	sea	long	neutral
19	equine	mares with foals	road	short	above
20	equine	mares with foals	road	short	below
21	equine	mares with foals	road	short	neutral
22	equine	stallions	road	short	above
23	equine	stallions	road	short	below
24	equine	stallions	road	short	neutral

25	equine	stallions	road	long	above
26	equine	stallions	road	long	below
27	equine	stallions	road	long	neutral
28	equine	stallions	sea	long	above
29	equine	stallions	sea	long	below
30	equine	stallions	sea	long	neutral

Table 32: List of possible scenarios during horse transportation

Scenario	Specie	Animal categories	Means of	Transport	Thermal
1	poultry	broiler	road	short	above
2	poultry	broiler	road	short	below
3	poultry	broiler	road	short	neutral
4	poultry	hens	road	short	above
5	poultry	hens	road	short	below
6	poultry	hens	road	short	neutral
7	poultry	spent hens	road	short	above
8	poultry	spent hens	road	short	below
9	poultry	spent hens	road	short	neutral
10	poultry	one day old	road	short	above
11	poultry	one day old	road	short	below
12	poultry	one day old	road	short	neutral
13	poultry	one day old	road	long	above
14	poultry	one day old	road	long	below
15	poultry	one day old	road	long	neutral
16	poultry	one day old	air	long	neutral
17	poultry	ducks	road	short	above
18	poultry	ducks	road	short	below
19	poultry	ducks	road	short	neutral
20	poultry	quails	road	short	above
21	poultry	quails	road	short	below
22	poultry	quails	road	short	neutral
23	poultry	turkey	road	short	above
24	poultry	turkey	road	short	below
25	poultry	turkey	road	short	neutral

Table 33: List of possible scenarios during poultry transportation

Scenarios	Species	Animal categories	Means of transport	Transport duration	Thermal environment
1	rabbit	breeding	road	short	above
2	rabbit	breeding	road	short	below
3	rabbit	breeding	road	short	neutral
4	rabbit	breeding	road	long	above
5	rabbit	breeding	road	long	below
6	rabbit	breeding	road	long	neutral
7	rabbit	slaughter	road	short	above
8	rabbit	slaughter	road	short	below
9	rabbit	slaughter	road	short	neutral
10	rabbit	slaughter	road	long	above
11	rabbit	slaughter	road	long	below
12	rabbit	slaughter	road	long	neutral

Table 34: List of possible scenarios during rabbit transportation

Scenarios	Animal categories	Species	Means of transport	Transport duration	Thermal environment
1	fish	salmon	road	short	above
2	fish	salmon	road	short	below
3	fish	salmon	road	short	neutral
4	fish	salmon	air	short	above
5	fish	salmon	air	short	below
6	fish	salmon	air	short	neutral
7	fish	salmon	sea	short	neutral
8	fish	trout	road	short	above
9	fish	trout	road	short	below
10	fish	trout	road	short	neutral
11	fish	eel	road	short	above
12	fish	eel	road	short	below
13	fish	eel	road	short	neutral
14	fish	catfish	road	short	above
15	fish	catfish	road	short	below
16	fish	catfish	road	short	neutral
17	fish	carp	road	short	above
18	fish	carp	road	short	below
19	fish	carp	road	short	neutral

Table 35: List of possible scenarios during fish transportation

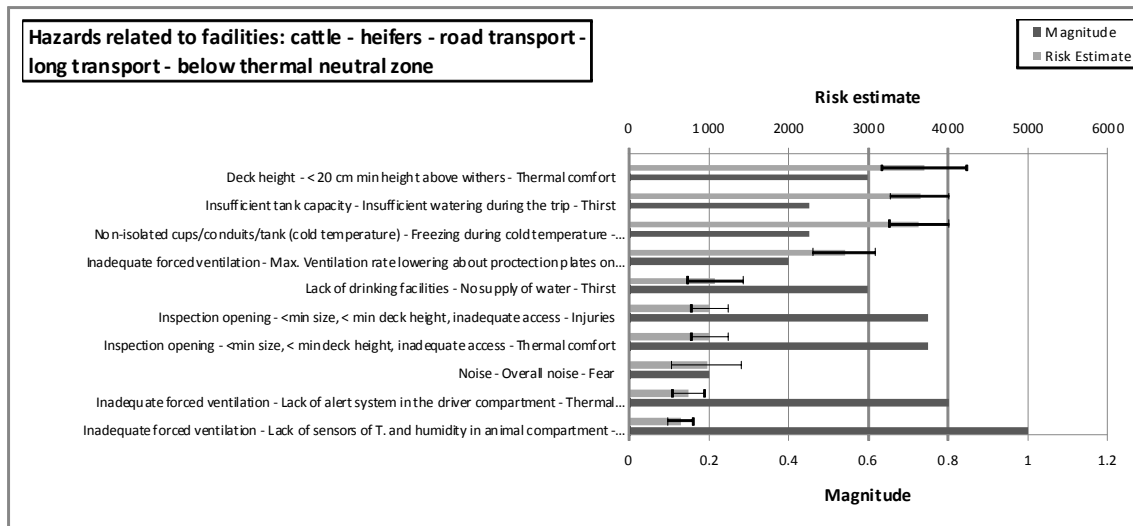**Hazards related to facilities: cattle - heifers - road transport - long transport - below thermal neutral zone**

Figure 1: Ten highest hazards related to facilities during road transport (> 8 hours) of heifers in below thermal neutral zone
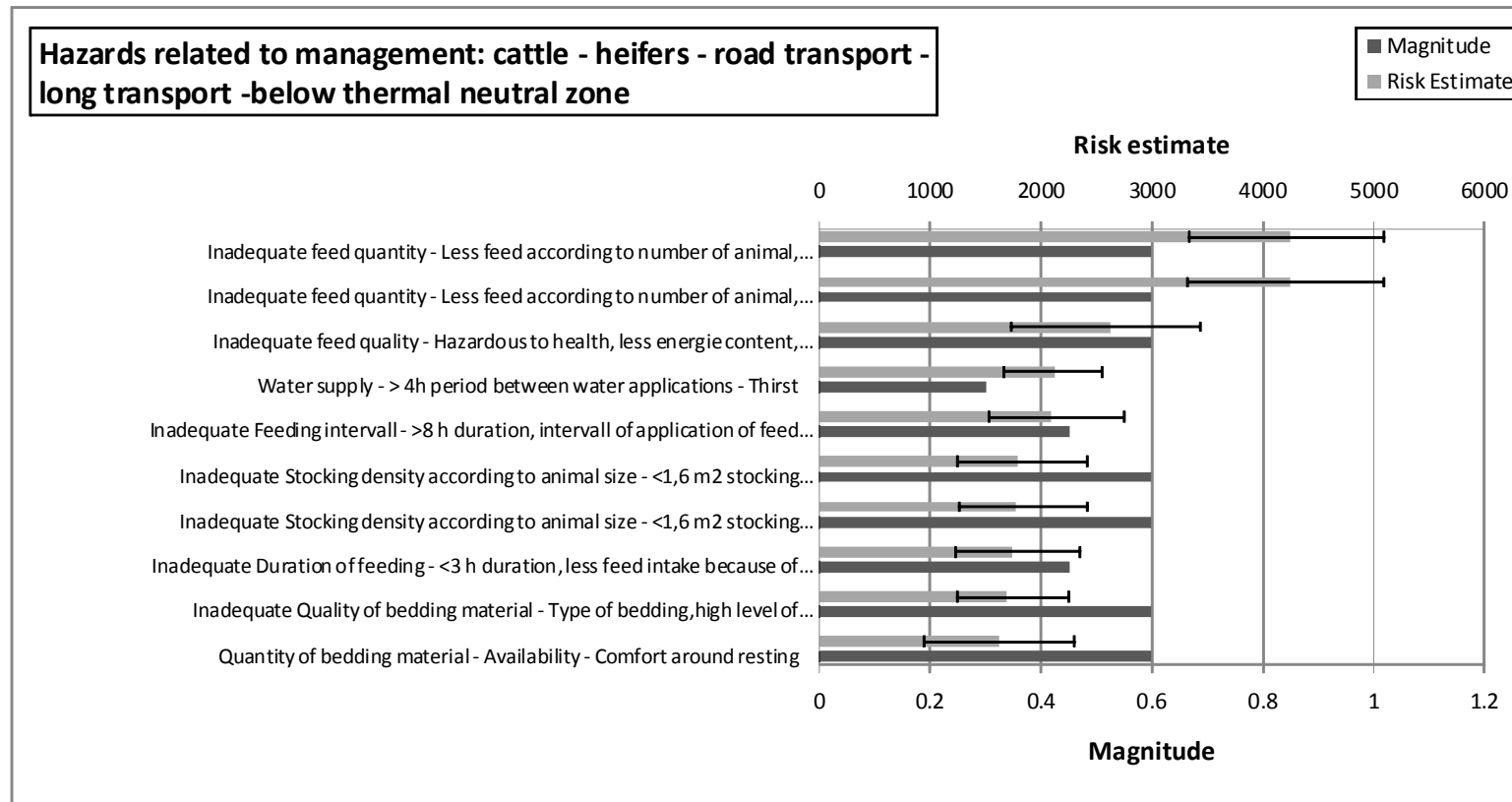
Figure 2: Ten highest hazards related to management during road (> 8 hours) transport of heifers in below thermal neutral zone

Figure 3: Ten highest hazards related to facilities during road transport (< 8 hours) of slaughter pigs in warm conditions
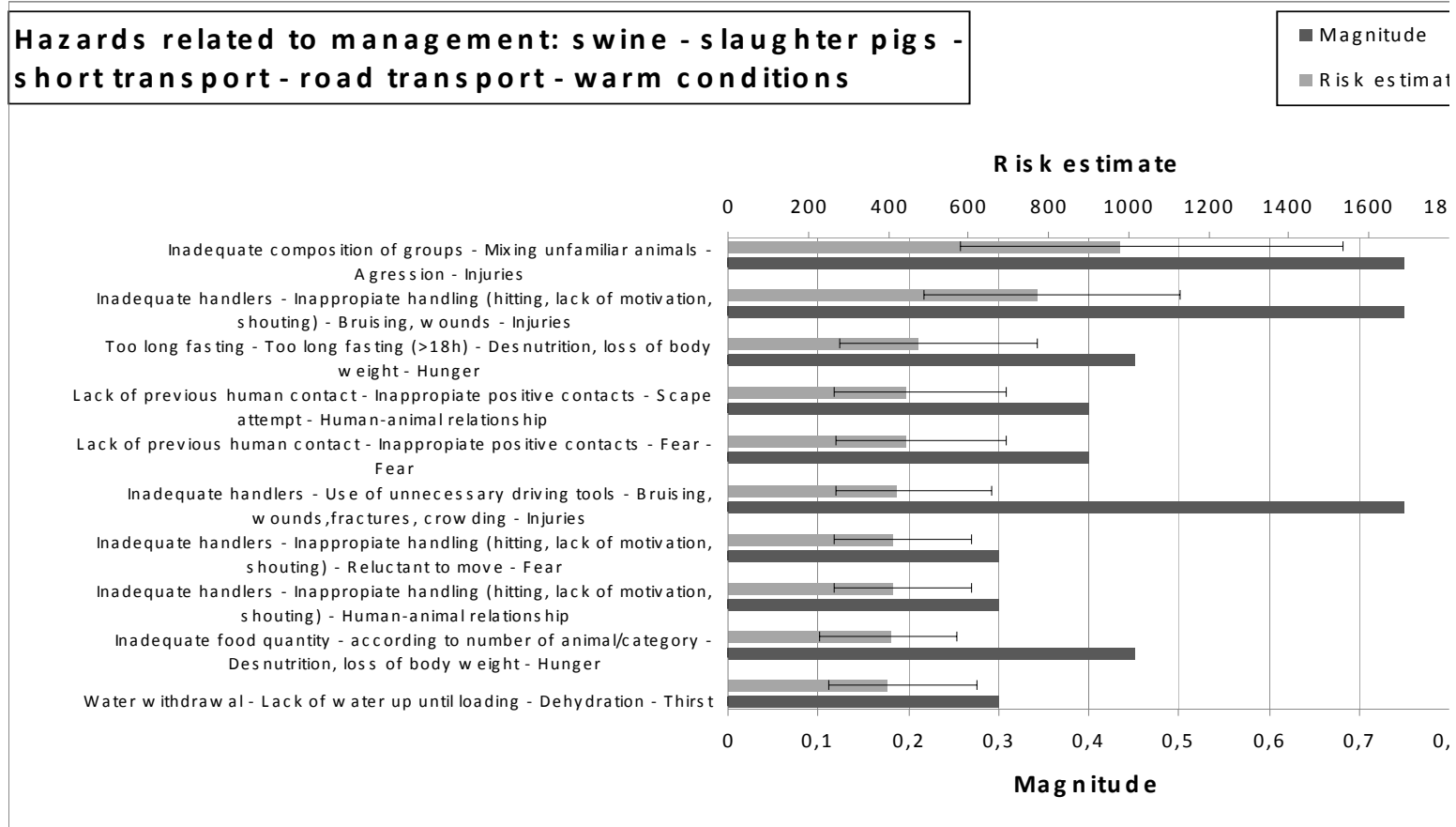
Figure 4: Ten highest hazards related to management during road transport (< 8 hours) of slaughter pigs in warm conditions

Hazards related to facilities: Fish - rainbow trouts (finished weight 250g) - road transport (pump loading) - short transport (6h) - hot weather (28°C)

Legend:
- Magnitude
- Risk estimate

Risk estimate axis: 0, 2000, 4000, 6000, 8000, 10000

Categories (top to bottom):
- Oxygen level of water too high (>5,0 mg/l) - Super saturation - Disease
- Indequate water temperature (12-17° C) - T above 24 °C - Thermal comfort
- Inadequate water exchange - Raccomanded 1 l/min/kg biomass - Disease
- Indequate water temperature (12-17° C) - Lack of a T monitoring system -…
- Indequate water temperature (12-17° C) - T above 22°C and below 24 °C -…
- Indequate water temperature (12-17° C) - Failure of cooling system -…
- Indequate water temperature (12-17° C) - Sudden change of T° - Thermal…
- NH3 level of water too high (>0,01 mg/l) - NH3 higher than 0,012 mg/l -…
- Nitrite (<0,1mg/l) - Nitrite higher than 0,1 mg/l - Disease
- Aluminium contamination of water - pH reduction - Disease

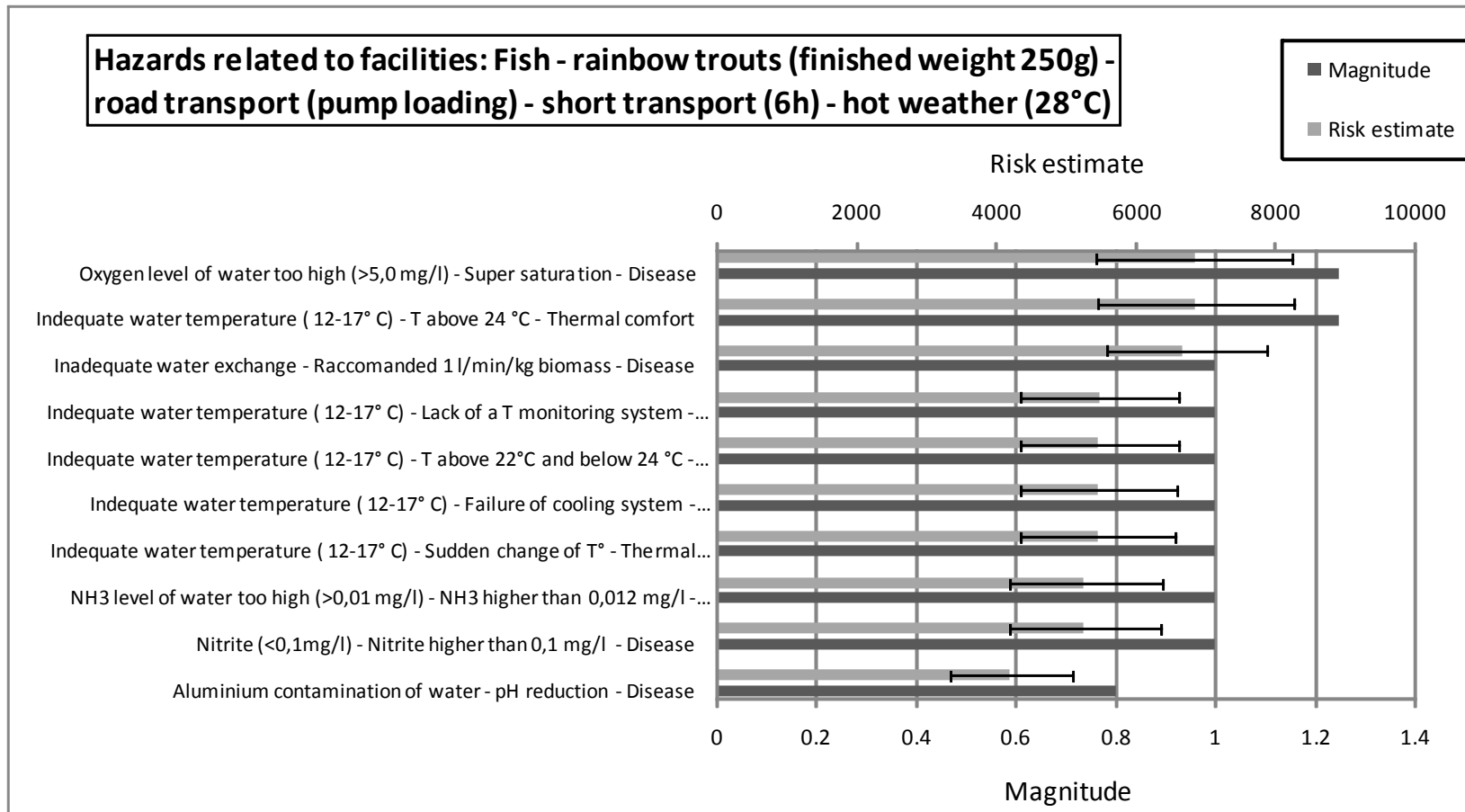Magnitude axis: 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4

Figure 5: Ten highest hazards related to facilities during road transport (< 8 hours) of rainbow trout in warm conditions

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Glossary

Animal transport

'Transport' means the movement of animals effected by one or more means of transport and the related operations, including loading, unloading, transfer and rest, until the unloading of the animals at the place of destination is completed (Council Regulation (EC) No 1/2005)

Risk Analysis

A process consisting of three components: risk assessment, risk management and risk communication.

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 Management

The process of weighing policy alternatives in the light of the results of risk assessment and, if required, selecting and implementing appropriate control options (i.e. prevention, elimination, or reduction of hazards and /or minimisation of risks) options, including regulatory measures.

Risk Communication

The interactive exchange of information and opinions concerning the risk and risk management among risk assessors, risk managers, consumers and other interested parties.

Hazard

Any factor, occurring from the pre-loading activities to the end of the unloading process, with the potentiality to cause a potential adverse effect on animal welfare.

Hazard characterization

The qualitative or quantitative evaluation of the nature of the adverse effects associated with the hazard.

Hazard Identification

The identification of any factor, occurring from the pre-loading activities to the end of the unloading process, capable of causing adverse effects on animal welfare.

Risk

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A function of the probability of an adverse effect and the severity of that effect, consequential to a hazard for animal welfare.

Risk Characterization

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 animal population based on hazard identification, hazard characterization, and exposure assessment.

Risk Estimate

Output of Risk characterization.

Exposure Assessment

The quantitative and/or qualitative evaluation of the likelihood of hazards to welfare occurring in a given animal population.

Likelihood

The probability of the individual animal suffering the adverse effect of a hazard, assuming exposure to a given scenario.

Quantitative Risk Assessment

A risk assessment that provides numerical expressions of risk and an indication of the attendant uncertainties (stated in the 1995 Expert Consultation definition on Risk Analysis).

Qualitative Risk Assessment

A risk assessment based on data which, while forming an inadequate basis for numerical risk estimations, nonetheless, when conditioned by prior expert knowledge and identification of attendant uncertainties, permits risk ranking or separation into descriptive categories of risk.

Sensitivity Analysis

A method to examine the behaviour of a model by measuring the variation in its outputs resulting from changes to its inputs.

Transparent

Characteristics of a process where the rationale, the logic of development, constraints, assumptions, value judgments, decisions, limitations and uncertainties of the expressed determination are fully and systematically stated, documented, and accessible for review.

Uncertainty Analysis

A method used to estimate the uncertainty associated with model inputs, assumptions and structure/form.

Appendices

APPENDIX A