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BASIC INFORMATION FOR THE DEVELOPMENT OF THE ANIMAL WELFARE RISK ASSESSMENT GUIDELINES

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Foreword

At present, according to our knowledge, there is no available literature about Welfare Risk Assessment and no published existent model yet. The main difficulty in welfare seems to be the clear identification of an endpoint, that is the case or adverse event, which is basilar for the definition of risk. This difficulty is mainly due to welfare nature: it's a complex multidimensional phenomenon that takes into account all the aspects of animals' condition related to the satisfaction of their basic needs. While it's certainly possible to make an effort toward a qualitative approach, even with lacking information, a quantitative approach requires as first instance to achieve a reliable sharable appraisal of welfare.

This report is meant to set the basic information, which will allow the EFSA - Animal Health and Welfare Panel - to start the development of Animal Welfare Risk Assessment Guidelines. The basic information includes the definition of Risk Assessment and a brief description of the existing models of this same, the definition of Animal Welfare and the different approaches to its evaluation (relative to Welfare Indicators). The report takes into consideration the main Animal Welfare issues for farm and laboratory animals referring to the Area of Expertise of the researchers involved in the field of Animal Welfare, the different species and topics for Animal Welfare, and the links between Animal Welfare and Food Safety. A complete list of Centres of Excellence and Experts in Animal Welfare and Risk Assessment, and a Database of References in the same fields of interest are also provided, according to the specified selection criteria.

A. Definition of Risk Analysis

Risk analysis is a complex process consisting of three components: risk assessment, risk management and risk communication (Ahl *et al.*, 1993). There are several possible fields of application of risk analysis; anyway usually the generic aim of performing a risk analysis is to make available an instrument to ensure public health protection (WHO, 1999). For example in microbiology elements, the quantitative risk analysis have been included in the wider field of the HACCP (Hazard Assessment and Critical Control Points) in order to assess the social impact of the risks of unhealthy food consumption. Other examples of application of risk analysis can be found in food security, either for chemicals, biological or microbiological hazards, in infectious diseases prevention, primarily for international trade, in environmental protection, in safety and health at work, just to remember few of them.

Risk assessment (RA), according to the WHO definition, is the scientific evaluation of known or potential adverse health effects resulting from food borne hazards. Codex Alimentarius Commission (WHO, 1999) guidelines and principles for the conduct of microbiological risk assessment are available. According to the OIE definition (2006) risk assessment "means the evaluation of the likelihood and the biological and economic consequences of entry, establishment, or spread of a pathogenic agent within the territory of an importing country". In general, the RA is a qualitative or quantitative evaluation process of the probability that an adverse event happens in a population as a consequence of the exposure to potential chemical, physical or microbiological hazards. Ahl *et al.* (1993) state that risk assessment is "the process of identifying a hazard and evaluating the risk of a specific hazard, either in absolute or relative terms. This process includes estimates of uncertainty and is objective, repeatable and scientific". According to a definition by Miller *et al.* (1993), risk assessment is an "evaluation of the likelihood of an adverse event occurring and of the magnitude of impact should it occur". In other words, risk assessment consists in the evaluation, either qualitatively or quantitatively, of the probability and the potential impact of some hazard. The risk assessment process should be scientifically-based, well-documented, flexible, consistent and open to review (Miller *et al.*, 1993).

Despite the specific field of interest, the basic step of risk assessment is to clearly define the objectives, identifying the content and the form of the output of the analysis. The objectives definition is a task of the risk manager. For example, referring to the most popular sector of application, that is food safety, and in particular to prevention of food borne infectious diseases, the risk assessment provides both a point and an interval estimate of the probability of occurrence of disease in population exposed to pathogens due to unhealthy food consumption. In other words the risk assessment produces an incidence measure of infection/clinical disease in the exposed population provided with the respective degree of uncertainty.

The risk estimate can be achieved either with a qualitative or a quantitative method. The first one is chosen in case of absence of reliable data or quite usually in case of a first evaluation aimed to verify if the risk for human health related to a certain exposure is worth examining more deeply.

According to WHO definition, qualitative risk assessment is 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 (WHO, 1999). The qualitative approach first step consists in describing available information. It implies to systematically gather all scientific edited material, to critically review it and to summarize it. The result of a qualitative approach of risk assessment is a categorical risk estimate. The risk is defined in a subjective manner, said, for example, nil, very low, low, medium, high and very high or in other similar manner. The advantage of this approach is that it can be carried on even with lacking data and that it doesn't require particular mathematical information. The main disadvantage is that its result could be easily subject to misinterpretation of the final user. Even though the risk assessment can be either qualitative or quantitative, if possible and according to data availability, it is always desirable to carry on a quantitative evaluation.

The quantitative risk assessment provides numerical expressions of risk and indication of the attendant uncertainties (WHO, 1999). The quantitative approach can be deterministic or probabilistic/stochastic. The former makes use of single values as input data, such as a mean value or a value describing the worst situation. For example, considering the risk of infection by unhealthy food consumption, we would take into account the mean daily intake of that particular food. This type of approach provides a point estimate. Usually variability measures like standard deviation and precision measures like standard error (and confidence intervals) are provided together with mean values. As an alternative the upper limit value of the confidence interval can be considered in order to obtain an estimate in case of the worst situation. The deterministic approach produces always a point estimate ignoring the variability of the phenomena and the uncertainty of the parameters included in the model (Miller *et al*., 1993). Variability consists of physiological difference existing among individuals; it is effect of chance and function of the system (Vose, 2000). Uncertainty is due to lack of scientific evidence or incompleteness, which is to poor knowledge of the parameters that characterise the physical system that is being modelled. Variability is not reducible through either study or further measurement, but may be reduced through changing the physical system (Vose, 2000). High quality information availability reduces uncertainty and increases the reliability of the risk estimate. The stochastic approach takes into account variability and uncertainty by including in the model not just single values but casual variables and their respective probability distributions. Also the output of such a model is a probability distribution (of the variable of interest). So the preliminary step of the stochastic approach is identifying the most proper distribution for each variable considered. The well known "what if" procedure allows to change the values of the parameters each time and to evaluate change in risk but has two big disadvantages: it's unthinkable to apply it in case of great number of parameters and/or large range of values and moreover it assigns to each value of a parameter the same probability to occur (Tranquillo, 2005).

Quantitative risk analysis using Monte Carlo simulation, instead, generates a number of possible scenarios. It takes account for every possible value that each variable could take and weights each possible scenario by the probability of its occurrence making use of its probability distribution. Taking into account parameters' variability, the model provides the probability distribution of the outcome (Vose, 2000). This technique involves the random sampling of each probability distribution to produce hundreds or even thousands of scenarios (Vose, 1997; Vose, 2000). Each probability distribution is sampled in a manner that reproduces the distribution's shape. The distribution of the values calculated for the model outcome therefore reflects the probability of the value that could occur (Vose, 2000). An interesting aspect of the Monte Carlo approach consists in the possibility to perform a sensitivity analysis on results, for investigating the impact of the change of each single parameter on the output (WHO, 1999). Examining existing correlations among single inputs and respective outputs makes possible to define the relative importance of each component of the model and then to orientate the control actions against most important points of the chain.

Bayesian inference should be applied in risk assessment to take into account prior estimates of parameters. A Bayesian approach would imply three main steps: first, determining the prior estimate of parameters in form of confidence distribution, second, finding an appropriate likelihood function for the observed data and, last, calculating the posterior estimate of the parameter by multiplying the prior distribution and the likelihood function, then normalising so that the result is a true distribution of confidence (Vose, 2000). In other words, the prior estimate represents the actual knowledge of the parameter before observing real data, is a measure of the uncertainty. Likelihood expresses the probability of observing the values really observed assuming a given value of the parameter. The posterior estimate summarizes these two information.

Risk assessment can be seen as a stepwise procedure composed by WHO (1999):

1. Hazard identification
2. Hazard characterisation
3. Exposure assessment
4. Risk characterisation

The hazard identification consists of the identification of biological, chemical and physical agents able of causing adverse health effects (WHO, 1999). A hazard is defined as any event which has the potential to produce harm (Miller, 1993). Hazard identification is mainly a qualitative process. Hazards can be obtained from scientific literature, from databases exploration or from opinions of experts. At this step, hazards are evaluated in order to select those that represent a real threat to animal or human health.

Hazard characterisation consists of the qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard (WHO, 1999) even in terms of severity and duration of adverse effects. In addition, according to data availability, a dose-response assessment should be performed in absence of a well-known dose-response relationship. Expert opinions are very important tools as they can enable to devise ranking systems useful to characterise severity and/or duration of disease.

The exposure assessment identifies the qualitative and/or quantitative evaluation of the exposure to biological, chemical and physical agents (WHO, 1999) from all different possible sources.

Risk characterisation consists of the process of determining the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment (WHO, 1999). For example in food borne diseases, among factors that must be considered for exposure assessment are included frequency of contamination of foods by pathogenic agent, the level of contamination in those food over time and patterns of consumption related to socio-economical state, regional differences, consumers preferences and behaviours.

Key elements in performing a risk assessment are: defining a suitable model; determining probability distributions for all uncertain variables of the model; including any dependencies between these uncertain variables; analysing the model's results and investigating its behaviour.

Risk assessment is a complex task involving the creation of a logic chain (risk pathway) among several factors important for the occurrence of the specific adverse effect in the exposed population (source of the hazard, exposure routes relevant to that hazard and their consequences), and that it requires a big level of interaction and integration among different fields. First approach to risk analysis is creating a working group composed of all different requested abilities. Such a process implies that every time we are going to plan a risk analysis, we must focus on the particular problem we are dealing with and there is no possibility to make use of an existent general model to modify and adapt to our present necessities. We must define the objectives of the analysis, that is we must define the adverse event we are interested to; we have to know the cause-effects relationship between events, and we must draw the concatenation of all possible events that intervene modulating, lowering or increasing or simply modifying, the probability and the modality of occurrence of that event. Briefly, every time the risk assessment is a unique procedure.

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B. Index of the Guidelines

This chapter provides definitions of animal welfare, which are used for the risk assessment. Welfare can be measured in a scientific way using a combination of methods, which include measurements of presence of pathology, performance, physiology, and behaviour.

The status of animals has been the object of philosophical concern for a very long time (see review by Ouedraogo and Le Neindre, 1999). Animals are now defined as “sentient creatures” in European law and no longer just as agricultural products (Treaty of Amsterdam, 1997); that change reflects ethical public concern about the quality of life of the animals.

During last years, some authors (reviewed by Fraser, 2003) have suggested a new definition of animal welfare through three different views. The first one stresses the biological functioning of the animal considering health, growth, production and reproduction activity. This view regards the possibility to “take care” of animals in a wide sense as the main expression of good welfare. The second view emphasis the “affective state” of animals, as pain, suffering, feelings and emotions, in the term of the correspondence between happy animals and high level of animal welfare. The third view is the chance for animal to live in as natural condition as possible, performing all the behaviours of its repertoire.

This three views of animal welfare and the associated value frameworks, which need not be mutually exclusive, have been included as criteria for animal welfare risk assessment. From a general point of view animal welfare is achieved when a sentient animal fits and feels good, although different authors have described animal welfare by various definitions. The report of the Scientific Veterinary Committee on the welfare of cattle kept for beef production (SCAHAW, 2001) gives a scientific summary on this subject.

The definitions of welfare, which overlap in meaning, are grouped according to their content into four categories.

1) Descriptive types of definitions (Brambell Report, 1965). Welfare is a broad term that embraces both the physical and mental well-being of the animal. Any attempt to evaluate welfare, therefore, must take into account the scientific evidence available concerning the feelings of animals that can be derived from their structure and function and also from their behaviour.

2) Definitions referring to an animal being in harmony with its environment (Hughes, 1976). Animal welfare is “a state of complete mental and physical health, where the animal is in harmony with its environment” (Hughes, 1976). The animal has not only physical but also behavioural requirements. The welfare of the animal is basically the way the animal feels about, and is affected by, its environment and not the environment per se. It can result in positive mental states (pleasure) or negative mental states (fear, pain, etc). That definition is very close to the one of human health: "a state of complete physical, mental and social well being, and not merely the absence of disease or infirmity" (WHO, 1946).

3) Definitions referring to adaptation to or control of the environment by the animal (Wiepkema, 1982; Broom, 1986). Broom (1986) defined the welfare of an animal as “its state as regards its attempts to cope with its environment”. The author proposes that welfare includes both feelings, which are part of coping systems, and health and that there is a continuum in welfare between very good in ideal conditions to very poor in a detrimental environment (Broom, 1996). The welfare is measurable using a wide range of indicators and is estimated by measuring the efforts the animal is prepared to make to reach the ideal state. When the adaptation capacities of the animal are overwhelmed the welfare of the animal is poor.

4) Definitions concerned with the subjective experience of the animal (Duncan and Petherick, 1989; Dawkins, 1990). The animal's perception of its environment cannot solely be inferred from our own human perception but needs to be evaluated from the animal's perspective. The definition of welfare that refers only to feelings is narrower than that proposed by Broom. In this report a wide range of coping systems are considered and all aspects of health are taken into account when discussing welfare. The welfare can be measured in particular by studying the physiological disorders provoked by the situation but also by the motivation of an animal to obtain some features of the environment (food, companion, bedding, etc.) or to perform some specific behaviours (feeding, social interaction, etc.) (Veissier *et al.*, 2000).

The above mentioned definitions of Animal Welfare were established during these last decades in a complex debate about Animal Welfare, following cultural developments of the societal view about the relationship between man and farm animals. They are related to the human perception of animals and to the concept of what the proper treatment for farm animals should be. In brief, each definition should fit the context of use to which it is more suitable.

Before the development of the Guidelines, a specific definition of Animal Welfare will be pointed out, giving a prominence to the Risk Assessment perspective. The new definition must be considered the links between Animal Welfare and Risk Analysis, looking at the chronic stress as a main source of risk in relation to Food Safety.

Welfare Indicators

Welfare analysis is multidimensional including health, physiology and behaviour and is based on many specific indicators originated from detailed scientific studies. Comprehensive descriptions have been reported in books (Broom and Johnson, 1993; Squires, 2003; Webster, 2005) and previous reports on welfare of the Scientific Committee on Animal Health and Animal Welfare. Scientific studies can provide evidence of the consequences for animal diseases, physiology and behaviour if their needs are not satisfied (see Report on the Welfare of Intensively Kept Pigs; SCAHAW, 1997).

Welfare indicators may be divided in four categories (Smidt, 1983).

1. *Pathological indicators*

Good welfare contributes to the definition of a good health status. Thorough clinical examination should be performed to differentiate between clinical and sub-clinical conditions, acute versus chronic diseases. Epidemiological studies on a large number of animals are necessary to obtain meaningful estimates, enabling the assessment of welfare in different situations.

2. *Physiological indicators*

Physiological measures are useful indicators of animal welfare. The activations of the hypothalamic-pituitary-adrenal cortex and the sympathetic systems are important physiological mechanisms related to the animal's efforts to cope with the environment. The opioid response can be also used as physiological indicator for measuring animal welfare. Other biochemical variables can be useful for analysing the welfare of animals: for example those related to the nutritional status of the animals, to the damage and/or to the changes of specific tissues (heart, muscle, liver, adrenal glands, etc). Any evaluation of physiological indicators should be corrected for the age of animals; there is evidence that the ability to experience distress and pain is different in young and adult animals (Fitzgerald, 1999; EFSA, 2005).
Immune functions are an important report system, since immune responses, stress and inflammation are an ancestral, overlapping set of responses, developed in the phylogenetic evolution of vertebrate animals for the neutralization of stimuli perturbing body homeostasis (Ottaviani and Franceschi, 1998).

It is worth dissecting this issue in models of acute versus chronic stress. Acute stressing events well beneath the host's threshold for coping are not dangerous and often conducive to useful learning experiences. Interestingly, transient acute stresses may be even associated to a better immune response: such events may be thus thought of as nature's adjuvant under field conditions. Surely, this is not true of severe, long-distance journeys of calves and pigs, which show in fact distinct signs of serious inflammatory reactions and immunosuppression, usually peaking at day 4-5 after transportation. The detrimental effects of chronic stress can be appreciated by means of clinical immunology tests (complement, lysozime, serum bactericidal activity), which can reveal a substantial decrease of the host's immune competence for environmental pathogens, which paves the way to opportunistic microbial infections.
Immunological parameters are also affected by iatrogenic causes, such as the administration of some illegal growth-promoting substances. The effects of corticosteroids on leukocyte formula and lymphocyte blast transformation and of beta-agonists drugs on oxidative stress could be an important evidence for quality assurance schemes at the farm level. These could be sided by a panel of *post mortem* inspection parameters on carcasses related to animal welfare (Brambilla, 1997): percent muscle within the established breed-specific values, percent drip loss, PSE and DFD conditions, pinpoint haemorrhages on carcasses and adrenal abnormalities.

3. *Behavioural indicators*

The diversity of behaviour is one important measure of the ability of animals to cope with the environment. It is a major tool for assessing not only the negative impact of constraints but also the positive effects of environmental features. The activities of the animal in situations with minimal constraints should be known in order to assess the changes in behaviour of the animals when they are under constraints. The usual environment of farm animals has to be defined carefully in order to take into account the effects of the domestication process. Since domestic animals can differ in their genetic and physiological status from the non-domestic populations, they may have problems to cope outside the farming environment.

4. *Productive indicators*

Any decrease in production and reproduction performance can be used as indicator of poor welfare, but a high level of productivity is not equal to high level of animal welfare. The correlation between welfare and production can be depicted as a bell-shaped curve (Mellor and Stafford, 2001): beyond a narrow range in which the dose response is positive, response rapidly decreases and even reverses after the plateau level. As a result, intensive production is may be related to no-specific disease.

Conclusions about welfare should always be based on all available indicators, properly weighed, including diseases, physiological status, behavioural and performance records. Whenever new parameters should be chosen, the effectiveness of experimental studies needs to be carefully verified. The validity of old and new indicators has to be proved by a satisfactory number of studies under experimental conditions, or epidemiological surveys, published on peer-reviewed scientific journals. After the test on a small number of animals, effects of a single indicator may be identified by the development of a protocol on farm, which considers the interaction with other factors.
The approach to Animal Welfare seeks to include a comprehensive connotation, in different views trough which the evaluation may be performed: the first one stresses the biological functioning, the second is based on affective states, and the last considers the natural living of animals. Every approach utilises different categories of indicators, but certainly none of these categories will be developed alone to define a good welfare status, but all of them shall be analysed to define an Hazard for Animal Welfare.

Each single category of indicators has a special significance as well as particular problems, e.g. the dependence on various internal and external factors. The combination of single category in an integrated system of indicators will be carried out for a comprehensive Animal Welfare Risk Assessment.

As a consequence of the particular relevance of Risk Assessment for Animal Welfare, and according to the need of an approach which takes into consideration a system of integrated indicators, a main task of the Guidelines will define the Risk and Control Points in relation to the criteria described below.

<u>Design Criteria (resource-based)</u>
The different housing systems need to be described for each type of production, as such information is essential to identify the sources of risks of poor welfare. Since provision of resources, management and stockmanship are the bases of good husbandry; because of that the first step is the analysis of design criteria.

The main categories should be identified as follow:

- *Housing System*: issue related to the housing, and in particular the house type and location, different type of floor and the bedding material, space allowance, pen design and fence, disease pen, the handling facilities (activity areas, passage ways, etc.), feeding and drinking facilities, all the microclimate conditions (ventilation and other cooling systems, temperature, relative humidity, air velocity and chill factor, dust, light intensity, noise, gases concentrations, etc.);
- *Management*: issue related to the management, like mutilations, genetics, feeding, grouping of animals, weaning, human-animal interaction, disease management (drug consumption, replacement rate, anticipated slaughter, etc.), warning system, cleaning procedures.

<u>Performance Criteria (animal-based)</u>
The behaviour and physiology of animals under farm condition may be described to classify the hazard and risk characterisations. Before starting the analysis of a specific risk factor involved in the definition of welfare in farm animals, the specific behaviour may be described for each species and each production categories. In relation to the welfare problem which is under discussion, the specific behaviour may be included:

- environmental perception,
- learning,
- social behaviour,
- puberty and reproduction,
- foraging behaviour,
- locomotion and resting,
- reaction to climatic conditions.

Pathological indicators
Pathological findings may be useful indicators related to a large variety of different diseases. Concerning the risk definition, different indicators may be analysed in particular:

- mortality and morbidity, which are clear evidence of a sensible reduction of animal health,
- body damage and lesions, which are often related to the human activity and management,
- respiratory diseases,
- pathological findings related to locomotion,
- gastro-enteric diseases,

- metabolic diseases.

Pathological indicators may be supported by a detailed scientific bibliography and a broad epidemiological survey, which defines connection between health status and welfare.

Physiological indicators

Studying hormones from neuro-endocrine system (e.g. adrenal hormones into the blood) are valid indicators of welfare in farm animals, as well as the measured physiological changes (e.g. heart and respiratory rate, body temperature) and some biochemical markers (e.g. acute phase proteins). The analysis of such physiological indicators, and the interpretation of related mechanisms, may be difficult to conduct, even if their effects on target tissues and different metabolic processes are studied. Physiological indicators may be supported by a detailed scientific bibliography, which defines connection between physiological changes and welfare.

The critical links among stress, inflammation and immune response suggest a preferential use of immunological parameters. These may apply to complex experimental studies regarding activation and differentiation of immune effector cells, cytokine networks and the like, as well as to field surveillance of welfare conditions. In this case, clinical immunology parameters (serum complement, lysozyme and bactericidal activity, acute phase proteins) clearly indicate the competence of the innate immune system and ongoing responses to environmental pathogens and other major stressors, as well as the likelihood of opportunistic diseases in the herd. These tests can be combined on a large scale with the assessment of T cell function (mitogen-driven blast transformation on whole blood), haematological and clinical chemistry parameters. A combined immunological approach to the evaluation of welfare in cattle has already been described (Amadori *et al.*, 1997).

Behavioural indicators

Welfare reduction can be assessed by observing behaviour, since modifications are expected when animals have difficulties or are unable to manage constraints. In particular, specific indicators should be identified in:

- comfort behaviour included resting,
- social interaction (e.g. aggressiveness),
- feeding behaviour,
- abnormal behaviour (e.g. stereotypy),
- locomotion, in respect to changes in movement patterns and rhythms, etc.,
- fear,
- signs of pain, which are different in young and old animals and may be carefully pointed out.

Behavioural indicators may be supported by a detailed scientific bibliography.

Productive indicators

Related to specific production (housing system, transport, mutilation, etc.), the target performance may be identify.

Productive indicators may be supported by a detailed scientific bibliography, which defines connection between production and reproduction changes and welfare.

Links among Animal Welfare/Animal Health/Food Safety

The link between animal welfare, animal health and food safety has been recognised internationally (OIE, 2005). The crucial issues which set the relationship between animal welfare and food safety can be grasped by outlining a correct conceptual framework about chronic stress. Chronic stress implies an overstimulation of the physiological coping mechanisms (Broom and Johnson, 1993). As such, stressing stimuli are perceived as a threat to homeostasis, which triggers complex defence circuits (Moberg, 1985). These relate to neuroendocrinological and behavioural responses, which leads to important changes and adjustments of fundamental physiological functions. If these changes are not on the wane within a reasonable time frame, pre-pathology and, eventually, overt diseases ensue: this is just the most evident negative feature of a complex adaptation process (Amadori *et al.*, 1997) in intensive farms.

The following flow chart about the consequence of poor welfare on food safety can be thus envisaged:

Chronic Stress
↓
Opportunistic Diseases
↓
Xenobiotics
↓
Food Safety

The higher demand of antibiotics and chemotherapy products in "problem" herds can be accounted for by the above reasons related to poor animal welfare; the resulting higher risk of residues in the food chain sets the fundamental link between animal welfare and food safety.

Whenever animals are forced to severe, prolonged coping reactions with a considerable energy expense, welfare is poor and a serious depression of the immune system turns out as one of the negative outcomes. In farm animals, models of chronic stress are more relevant to this issue. The consequences of stress on immune functions are in fact generally adaptive in the short term, whereas they can be damaging when stress is chronic. Chronic stress in farm animals may derive from different conditions which can be grouped as follows:

- housing condition, including microclimate conditions and feeding,
- management, including human-animal relationship,
- possibility of perform a defined behavioural repertoire,
- the possibility of catching infections or conditioned diseases
- metabolic stress and modification of the physiological status.

It can be argued that the high energy demand for coping under these conditions forces animals to re-define their metabolic priorities to the detriment of the immune response. In addition, a conflict often arises between immune response and productive performance under conditions of high infectious pressure: the *M. hyopneumoniae* model in pigs is a very convincing example of this crucial link (Pointon *et al.*, 1985). A similar conflict can be envisaged as regards energy expense for milk production and immune effectors functions in the early lactation period of high-production dairy cows (LeBlanc *et al.*, 2006).

Beside stress-induced immunosuppression, two other links between animal welfare and animal health can be defined (Broom and Johnson, 1993):

- poor housing conditions which imply changes of the usual standing and laying postures: as a result, the risk of lesions gets substantially higher;
- genetic selection for high production traits and the adoption of related feeding parameters: a metabolic stress ensues which paves the way to metabolic diseases.

For example, there is undoubtedly a high correlation between large milk output and bovine mastitis; pending a formal, direct demonstration of this assumption, convincing indirect evidence stems from the experiments carried out on a potent metabolic stressor like recombinant bovine somatotropin (SCAHAW, 1999). Such a condition of metabolic stress can be appreciated in pigs, as well. Huge genetic improvements were obtained as regards several production traits, well beyond the very needs of both farmers and consumers. As a result, fattening pigs must be even kept on a strict diet to limit daily weight gain to 650-700 grams/day, thus avoiding the animals' too early reaching of the accepted slaughter weight for ham production. Logistics and structures of several pig farms have not matched indeed these huge genetic advances. Hence, animals show reduced coping ability to environmental conditions, the main problem factor being a poor match between huge muscle growth and cardio-vascular capacity: this causes in turn chronic ipoxia in tissues and a related, dramatic increase of oxidative stress (Brambilla *et al.*, 2002). The appearance of serious infectious syndromes in the 90ies such as PRRS and PMWS is undoubtedly related to these new swine phenotypes, probably more susceptible to the detrimental effects of ubiquitous viruses like PRRSV and PCV-2 (Van Gucht *et al.*, 2003.).

In the framework of food safety, proper attention should be paid to consumers' perception of food quality. Unfortunately, established habits and misleading advertising often prompt to despicable consumer preferences such as lack of infiltrating fat in swine and bovine meat, let alone pale veal meat; substantial risks of poor animal welfare and/or illegal drug administration are thus overlooked. Correct, science-based information of consumers will be a crucial issue, liaising animal welfare and food safety in the near future.

In this section, the relation between Animal Welfare and Food Safety is outlined, as a lack of welfare results in the chronic stress for the animal. This situation leads to a need of increase in drug administration, in order to contrast the onset of various diseases as well as to settle physiological modifications. From this point of view, any residual product could be a risk for food safety.

Immune System Response to Stress Factors: Issues

The stress response is a conserved, physiological coping reaction to adverse environmental conditions, as diverse as physical and/or psychological constraints, injuries, trauma, poor microclimate and others. In this respect, immune responses, stress and inflammation are an ancestral, overlapping set of responses aimed at the neutralization of stimuli perturbing body homeostasis. The complex interaction between the immune system and the stress/inflammation complex has mainly developed in the phylogenetic evolution on the basis of a redundant, diversified system of cytokines and chemokines. Such a complexity can be accounted for by the extreme variety of tasks to be performed and of the necessary fine tuning of the relevant effector mechanisms.

"Psycho-sensitive stimuli/behavioural response" and "Antigenic stimuli/immune response" are indeed two subsystems of a unitary integrated complex aimed at providing optimal conditions for the host's survival and adaptation. This can be adequately grasped having in mind the crucial role of pro-inflammatory cytokines in the induction of sickness behaviour (lethargy, anorexia, curtail of both social and reproductive activities).

Owing to the above, it can be argued that the immune system and other homeostatic control systems share important regulatory factors, even though they formally perform diverse, apparently diverging physiological functions. As a result, the canonical boundaries between immune and neuro-endocrine control systems can be no longer recognized in a *continuum* of homeostatic circuits, in which a single recognized effector function is part of a wider strategy for better survival and adaptation. Such strategy is based upon networks of multidirectional signalling and feedback regulations effected by neuroendocrine- and immunocyte-derived mediators.

The effector mechanisms in the stress response are remarkably similar for both infectious and non–infectious stimuli, albeit differently modulated. Therefore, a cytokine response can be mounted in different forms and extent by the host after exposure to both infectious and non-infectious stimuli. In this conceptual framework, microbial infections are just one category of stressing agents, which modulate the cytokine response for a better performance of the innate and adaptive immune responses.

In this scenario, the immune system is affected by the stress response and, in turn, it affects the stress/inflammatory response, on which the cytokine network can exert important regulatory functions.

The inflammatory response is started by the host to achieve better fitness in managing adverse environmental conditions (infectious or non-infectious) and then curtailed to avoid major tissue damages. According to the danger theory, the usual limits of the inflammatory response can be only trespassed in case of major infectious threats, the priority being the very survival of the host. Thus, a strict control over start and extent of the inflammatory response is mandatory for a successful outcome of the coping reaction. Pro-inflammatory cytokines of the immune system (IL-1, IL-6, TNF-α) play a critical role in mounting and directing the inflammatory response. At the same time, the immune system displays important regulatory functions which start at very beginning of the inflammatory response.

The immune system is affected by the stress response. Whenever animals are forced to severe, prolonged coping reactions with a considerable energy expense, welfare is poor and a serious depression of the immune system turns out as one of the negative outcomes. In view of that, clinical immunology tests (complement, lysozime, serum bactericidal activity) reveal a substantial decrease of the host's immune competence for environmental pathogens, which paves the way to opportunistic microbial infections. Interestingly, the above functions relate to the innate immune system, which does not have memory and acts irrespective of antigenic specificities by recognition of conserved microbial PAMPs.

A defective innate immune response forces the host to a wider use of the adaptive immune response (antibody and cytotoxic T lymphocytes), which is by far more demanding in terms of energy expense. Consecutively, the efficiency of the adaptive immune response is also poor under conditions of chronic stress: the dramatic failure of potent Foot-and-Mouth Disease vaccines in Holstein cattle reared under hot climate conditions in Saudi Arabia is a very convincing demonstration of this tenet.

Which rationale, which explanation in terms of phylogenetic evolution can be proposed for immuno-suppression under stress conditions? It is worth dissecting this issue in models of acute versus chronic stress. Acute stressing events well beneath the host's threshold for coping are not dangerous and often conducive to useful learning experiences. Interestingly, transient acute stresses may be associated to a better immune response: such events may be even thought of as nature's adjuvant under field conditions. Surely, this is not true of long-distance journeys of calves and pigs, which show in fact distinct signs of serious inflammatory reactions and immunosuppression, usually peaking at day 4-5 after transportation. As regards farm animals, models of chronic stress are by far more relevant to this issue. It seems in fact that the consequences of stress on immune functions are generally adaptive in the short term, whereas they can be damaging when stress is chronic.

Chronic stress in farm animals may derive from several conditions which can be grouped as follows:

- climate and microclimate conditions (temperature, humidity, draught, etc.),
- microbial infectious pressure,
- pain, fear, inability to perform a defined behavioural repertoire,
- barren environment, boredom,
- inadequate diet,
- metabolic stress for both milk and meat production.

It can be argued that the high energy demand for coping under these conditions forces animals to re-define their metabolic priorities to the detriment of the immune response. This is clearly shown by the leptin model: under starvation (chronic stress) leptin is shut off by adipocytes, which leads to serious defects of immune effector functions. Also, secondary antibody responses and immunological memory may be energetically costly and therefore down-regulated during food restriction.

In addition, a conflict often arises in farm animals between immune response and productive performance under conditions of high infectious pressure: the *M. hyopneumoniae* model in pigs is a very convincing example of this crucial link. A similar conflict can be envisaged as regards energy expense for milk production and immune effector functions in the early lactation period of BLAP cows.

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C. Models of Risk Assessment

Best available evidences

As above mentioned, risk analysis is a complex procedure that allows organizing and integrating the available information on an adverse event, to estimate its probability taking into account the variability and the uncertainty of the phenomenon and to logically make decisions about its management. We also said that it should be properly documented and substantiated by the scientific literature and other sources like experts' opinion. Codex Alimentarius Commission state' scientific evidence may be limited, incomplete or conflicting. In such cases, transparent informed decisions will have to be made on how to complete the risk Assessment process. The importance of using high quality information when conducting a risk assessment is to reduce uncertainty and to increase the reliability of the risk estimate' (WHO, 1999). For this reason, it's necessary to undertake a preliminary process of research, acquisition and integration of data (facts, numbers, opinions) that should be carried on in order to select the best available information according to the scientific thinking (OIE, 2000). Moreover, criteria for the evaluation of the quality of the data and requisites to scientifically evaluate the utility of a study for the evaluation of the risk should be clearly defined and universally shared. This reasoning is the basis of the Evidence Based Medicine. The modern scientific thinking has developed standardized instruments and methods, according to Research Methodology subject, that allow selecting such best available evidences. They make use of the so called second sources (evidence based) of publishing. Primary sources of publishing are represented by current studies published on literature while second sources are systematic revisions and meta-analysis studies. Third sources (opinion based) are experts, tractates and traditional reviews. Systematic revisions provide a qualitative evaluation of the whole of the available data. Meta-analysis studies permit to summarize the information coming from the best quality data selected by systematic revisions. This type of approach guarantees a standardized and unique method of evaluation of available data. So, the first step for welfare risk assessment should consists in critically reviewing all the literature available making use of such instruments in order to select the best available information and to become conscious of the scientific level achieved, especially in the case it was poor.

Qualitative risk assessment

A qualitative assessment could be started listing all the possible hazards against the welfare of animals, which is against their needs as they are known by the five freedoms. Qualitative assessment can be practically conducted making use of tables, scenario trees (Miller *et al.*, 1993) or algorithms (Palmer *et al.*, 2005), anyway it must follow always the same pathway. After the hazard identification, the next step is making a hazard characterization by categorising the severity of the adverse event. This point is carried on in order to describe the impact of the hazards. It is possible to do it through a qualitative description like "nil", "very low", "low", "medium", "high" or like "slight adverse", "adverse", "moderately serious", "serious" and "very serious" (Vose, 2000). The important thing is to graduate the severity of the effect in an increasing (or decreasing) manner. A further step consists in describing the exposure level to each hazard. This means to quantify the exposure to the specific hazard in terms of occurrence like very rare, rare, moderately frequent, frequent and very frequent or in another clear descriptive way. The most proper number of categories must be chosen. These qualitative categories can reflect percentile distributions described by experts' opinion. Anyway, a basic issue is to make transparent the procedure for hazard identification and characterisation and for exposure assessment. It could be possible that information is not available for each hazard exposure: this lack of information must be clearly declared. Information sources can be literature or experts' opinion but in both cases must be properly substantiated.

The risk evaluation is made on both the information gathered on hazard characterization and exposure. It's necessary to preliminary state the criteria that will be used in order to decide which hazards will be considered negligible instead of minor or major: for example events very frequent and very serious should certainly be considered major risk while events with low occurrence and low damage should be regarded as negligible. Usually a table is created with columns reporting exposure categories and rows reporting hazard characterization but every practical modality is utilizable. This qualitative examination could be aimed to select factors worthwhile analysing deeper, even with a quantitative analysis, or just to provide qualitative elements for decisions making.

As alternative, there is the possibility to conduct a semi quantitative analysis based on some scores given to each category of the impact (impact score) and of the probability of occurrence of the event (probability score). It allows calculating a measure, sometimes described as "severity" of the risk, given by the impact score multiplied by the probability score. In this way risk can be graduated on the severity score (Vose, 2000).

An Example by EFSA

The only present available effort of welfare risk assessment is given by the EFSA Scientific Report on "The risk of poor welfare in intensive calf farming system" (EFSA, 2006), which adopts the definitions of the Codex Alimentarius Commission for risk analysis terminology focusing the attention to risks concerning the welfare of calves. It utilises a multidisciplinary approach making use of expertise of a working group composed of experts in different fields. The first step consists in listing and describing the needs of calves and then to list the hazards able to compromise these needs, making explicit the link between need and hazard. Then they characterized the impact of each hazard as slight adverse effect, adverse effect, moderately serious effect, serious effect and very serious effect. The exposure assessment was made taking into account the differences existing between different rearing systems. Scoring categories of exposure were very rare (1-20%), rare (21-40%), moderately frequent (41-60%), frequent (61-80%) and very frequent (81-100%). The result was a classification of the hazards in major risks and minor risks.

The quantitative approach: a possible prospective

Considering the quantitative approach, we stated that the quantitative analysis is aimed to achieve a measure of risk. None of the studies listed below treat risk assessment but we will examine them because in our opinion they represent the first necessary step to carry on a quantitative assessment of risk of welfare. These studies are an effort to achieve a measure of welfare that is an overall score of welfare. They obviously imply that it must be perfectly clear what welfare is before to measure it. Then the further step to follow in order to reach a quantitative measure of risk could be, given a validated scale to measure welfare, deciding a cut-off value on a scientific base (this could also mean to do it according to experts' opinion). This cut-off value would make possible to select a cohort of farms in 'good welfare state'. Following them for a period of time would make able to observe possible change in their welfare state that is to count the number of farms that pass from the good welfare state to the not-good welfare one. The risk measure would be given by the ratio of this number to the total number of farms of the cohort. We could then study the relationship between this risk measure and several farms features in order to define risk factors and obtain measures of relative risk. More over the value of risk would be a somewhat proxy of a factor, such as for example of a pathology. Certainly this work perspective will necessary require long time and many efforts and a cost-benefits reasoning would be useful in judging if it is worth-while to be followed.

Assessing and overall measure of welfare

There are several opinions against the possibility of making an overall welfare assessment. They are mainly based on the fact that welfare is not a constant feature but a transient state which has multiple attributes and which is different under various circumstances. Welfare varies in nature, it varies over time and it varies between individuals in a group. So a very important step would be specifying the exact circumstances of it (Bracke *et al.*, 1999). Another voice against welfare overall assessment is based on the impossibility to scientifically prove animal consciousness. On the other hand Bracke *et al*. (1999) state that every scientific evidence is just somewhat statistically significant, not a real prove and that instead of demanding proof the task of the overall welfare assessment should be to provide the best possible solution or prediction of what the welfare status is, based on the scientific data that are available at the time the assessment is made, being open to eventual change in the future. Since welfare is a complex attribute, a large number of variables (behaviour, physiology, productivity and disease), must be taken into account. At the present several efforts to assess welfare, founded on scores attribution to aspects relevant for welfare, are available in literature. They are mainly based on a 'linear model' in which the scores assigned to each aspect are summed in order to give a global welfare score. An example is given by the Freedom food, which was an initiative of the Royal society for the Prevention of Cruelty in Animals (RSPCA) in the UK (Bracke *et al.*, 2005). Whay *et al.* (2003) tried to validate the Freedom Food scheme for dairy cattle by comparing it with animal-based parameters. In USA the schemes 'Free Farmed' and 'Humane Raised' rose on the model of this UK experience.

The Animal Needs Index (ANI) of Bartussek

In Austria an Animal Needs Index (ANI) (Bartussek, 1999) has been in development for 15 years. ANI-35L-systems exist and are in use now for cows, young and beef cattle, calves, laying hens, fattening pigs (including piglets) and pregnant sows in Austria. The ANI principally considers five aspects of the animal's environment:

1. the possibility of mobility,
2. social contact,
3. condition of flooring for lying, standing and walking,
4. climatization (including ventilation, light and noise),
5. the intensity or quality of human care.

Within each field, several species-specific criteria are graded by points. The overall sum of the points gives the ANI-value. Despite the score, the respect of certain minimal conditions is requested.

The ANI was created by Bartussek belonging to the Federal Research Institute for Agriculture in Alpine Regions, in Austria. The 35 ANI-system is based on different principles:

1. assessing housing conditions at farm level according to animal welfare;
2. scaling conditions by points: better housing conditions get more points and vice versa (according to the scientific background);
3. summing up points gives ANI-value (index system);
4. compensating poor conditions by better conditions above minimum requirements;
5. minimum requirements to avoid suffering damage and unacceptable stress by overstretching ability of adaptation (legal threshold);
6. welfare categories by grouping ANI-values (index numbers);
7. covering whole range of husbandry systems in practice and all species and forms of utilization within one general system;
8. system must be result of broad negotiation between parties involved.

According to the global score, ranging from about minus 10 to plus 45 points, 6 categories of housing conditions are created: not suitable (<11 points), scarcely suitable (11-16), little suitable (16.5-21), fairly suitable (21.5-24), suitable (24.5-28), very suitable (<28) (Bartussek, 1999). Anyway, despite the reasoning below the scale, this scoring system lacks of validation.

Brake model for overall welfare assessment

Bracke *et al.* (1999) examine each different approach available in literature and distinguish between two main different types, one aimed to provide tables, the other to create schemes to assess welfare. In the first case, tables can be applied just to the specific housing system for which has been created, while schemes can be applied to every case in point. The latter in fact are more aimed to provide a general method to calculate an overall welfare score.

In both the approaches, anyway, a list of attributes relevant for welfare must be defined. For each attribute then two or more levels, representing housing systems' properties, are defined and ranked. According to the chosen level a score is given to each attribute. A basic condition to perform the overall welfare assessment is to list all the relevant attributes for welfare. Bracke *et al.* (1999), according to the literature, consider relevant attributes environment, behaviour, pathological status and physiology. They focus their attention also on other three points: on scores standardisation, suggesting the use of a range between 0 and 10 points, on the weighting of attributes, that must be explicit and on the rules, not necessarily of additive nature, for the calculation of the overall welfare score. They also point out the necessity to use a standardized vocabulary in welfare assessment.

The same authors (Bracke *et al.*, 2002a) developed a model for overall welfare assessment in pregnant sows. It takes into account a list of 11 needs, each one covering one of the animals' behavioural systems (feed intake, thermoregulation, elimination, exploration, rest and locomotion). They built a relational database with five primary tables containing the list of needs, the attributes, the weighting categories and the housing systems respectively. The model contains 37 attributes, some environmental based, others animal-based or management-related, each one with between two and nine mutually exclusive levels. Levels represent discrete classes describing the welfare relevant properties of each housing system. In two secondary tables are reported links between attributes and needs and links between attribute levels, weighting categories levels, types and scientific statements. There are 352 statements, extracted by 12 different sources, selected as being truly scientific (i.e. referring directly to empirical observations and being relevant to distinguish between housing systems on welfare grounds). The model, according to the attributes description, assigns attributes scores and weighting factors and then calculates the overall welfare score as a weighted average of the attributes scores. Each statement is linked to at least one attribute in order to provide a scientific base to the model first, secondly to weight the attributes. Ranking of different levels belonging to an attribute requires itself scientific evidence. In this model the worst level received a score of 0 while the best received a score of 1. Intermediate levels received scores in direct proportion to their ranks. For weighting of the scores 12 weighting categories were used. The more scientific evidence was available, the higher was the weighting factor. Weighting scores were positive for favourable weighting categories and negative for adverse weighting categories. Point were assigned basing on dimensions of intensity, duration and incidence and the type of weighting category, that is the quality or nature of the scientific measurement, was defined. Weighting factors are calculated as the difference between the best and the worst levels and so indicates the importance of the attribute. Despite the deep calculations for best and worst levels scores, taking into account unique types of categories and maximum scores, the rationale for this calculation is that the impact of any novel scientific statement mainly derives from its ability to identify a new weighting category (establishing a new welfare performance criterion) or from its ability to increase the weighting score of a weighting category. Attribute scores, instead, are calculated for each level of an attribute on a scale from 0 to 1 proportional to their rank. As stated before, the overall welfare score for a housing system is calculated as the weighted average of the attributes scores that is the sum of the attributes scores multiplied by the weighting factor of each attribute in the model, divided by the total sum of weighting factors. They reported that they made a 'validation' of this model comparing it to others like the ANI by Bartussek or to the Behavioural Deprivation Index by Fraser and that they found a good value of concordance (Bracke *et al*., 2002a). It's clear that the best thing would be to perform a comparison with a gold standard or to experimental data but they both are not available. In a following article they tried a validation using experts' opinion (Bracke *et al*., 2002b). Twenty-nine pig-welfare experts were asked to fill a form assigning scores from 0 to 10, as weighting scores for each item. They were also asked to rank the levels of the attributes and to give a confidence score (scale 0 to 10), for the entire set of weighting factors, to their confidence in the validity of their own set of weighting factors. In the questionnaire all the housing systems considered in the previous work were included together to others, which verify the prediction ability of the model. The Spearman rank correlation coefficient Rho was used to determine the correlation between the model and the median expert scores. They found a Kendall's coefficient of 0.73 ($P<0.001$) at the 55 percentile level. Kendall's coefficient of concordance was used to examine the degree of consensus between the experts and between the experts and the model. They obtained a degree of concordance significant but low (0.43; $P<0.001$) for concordance among the experts. The experts resulted just moderately confident that their own weighting factors represented the actual importance of the attributes (median confidence score of 7.00). Even though the absolute scores given by the experts differed considerably from the scores calculated with the model, the model correlated well with experts' opinion. For the set of 15 housing systems, Spearman's Rho was 0.92 ($P < 0.01$). For the eight novel system Rho was 0.78 ($P<0.05$). The authors found also a good ability of the model in distinguishing between housing systems and attributes (Bracke *et al*., 2002a).

<u>A different approach: the composite measurement scale methodology</u>

Another approach is based on the composite measurement scale methodology, widely used in social fields (for example for psychological-attitudinal tests and market researches). In this approach the items of the questionnaire represent different aspects of the attribute of interest. Each item can be dichotomised (1/0 or yes or no response) or ordinal. The sum of the response collected for each item gives the global score, the objective measure of the complex phenomenon under study. The underlying theories, the Measurement Theory and the Classical Test Theory (Streiner and Norman, 1999) offer a proper methodology to obtain objective measure of multidimensional attributes. These theory states that the selected items measure and are correlated with a latent variable, not directly measurable, which is the phenomenon of interest. In this approach the questionnaire, regarded as a real measurement instrument, needs a validation process in order to check that the basic assumptions of the underlying theory are not violated. As it happens for every instrument, the validity of the measures we obtain depends on validity, reliability and sensibility to changes of the scale. The validity of a scale is given by the extent in which it is able to detect exactly what it is supposed to. Usually different types of validity are identified: content validity, criterion validity and construct validity (OIE, 2000). It is worth noting that validity does not affect the measurement instrument itself but the use of it. That is the reason for which the respect of a particular type of validity does not imply the respect of another type of validity. The content validity concerns the ability of the items selected from a semantic domain, to measure the latent variable, and requires two basic applications: an exhaustive specification of the domain and the selection of the items conceptually connected to the domain. Since it is quite difficult to define the semantic boundaries of a concept of interest and that this operation is strongly affected by subjective ness, then the choice of the items more proper to represent a domain is a hard task. The content validity is not enough to guarantee that the scale we are building is valid but it is undoubtedly useful as a guide in judging the semantic relation between the items and the concept of interest. The criterion validity is defined as the correlation among a group of items, which is supposed to be connected to a latent variable and to some external variable called "criterion variable" that measures the latent variable. There are two types of criterion validity: the concurrent validity, in the case the criterion variable already exists at the evaluation time and the predictive validity, in the case the criterion variable has to be determined in the future. The criterion validity provides a synthetic measure of validity: the correlation coefficient between the scale and the criterion. This modality of validation can not always be applied because it requires the availability of instruments already able to measure the object of interest: the criterion should be the most accurate measure ("Gold standard") of the phenomenon and would act as a verifying norm. In the case of welfare, objective instruments of evaluation to be used as criterion variable are clearly not available. The construct validity refers to the theoretical relation of the variable score of a scale with a construct variable or a latent variable. The items of a scale are the mean to evaluate a construct not directly observable or measurable. Since this underlying phenomenon can assume different values, it is said "latent variable". The latent variable is regarded as the cause of the response modalities of the items, that is that the value of the latent variable forces the items to assume a predetermined value. If this is true, the value of the latent variable and the values of the items will be highly correlated. When the items are strongly correlated, the scale is said "internally consistent". A high correlation among items suggests that all the items measure the same thing, so a high correlation among items implies a high relation between items and the latent variable. The most used measure of the internal consistency (reliability) of a scale is the α of Cronbach coefficient (Streiner and Norman, 1999).

$$\alpha \text{ of Cronbach} = \frac{p}{p-1}\left(1 - \frac{\sum_j s_j^{\,2}}{s_Y^2}\right)$$

where:

p = number of the items of the scale,

$s_j^{\,2}$ = variance of each item (uncommon variance or residual variance),

$s_Y^{\,2}$ = total variance.

This coefficient represents the proportion of total variability of the score of the rating scale explained by the true score of the underling latent variable. Values of the α of Cronbach upper or equal to 0.70 are usually regarded as indicators that the rating scale is consistent within and so that it is 'reliable'. Since the α depends on the number of the items, some Authors argue that in case of scale with few items a lower cut-off value of the α, equal to 0.60, could be acceptable. A basic assumption for the estimate of α is the existence of no more than one latent variable that is that the scale is one-dimensional. This assumption can be verified by a homogeneity analysis conducted according to the HOMALS procedure (Homogeneity Analysis by Alternating Least Square). This procedure allows to test the hypothesis of the existence of more dimensions, each one orthogonal to the others, that is to postulate the existence of different independent latent variables underling the multivariate relations among the items. This procedure extracts dimensions underling the multivariate relationships of the nominal data assigning numerical values (said optimal quantifications) to both the different modalities of the singular item and the observation units. For each dimension, the quantification is calculated *a posteriori*, according to the response frequency pattern of the items. The optimal quantifications allow calculating for each item the discrimination measures that represent the variance of the recoded items. Since a discrimination measure is expression of the variability of the optimal quantification, its value indicates the contribution of the item in explaining the variability of the data.

As an alternative to the HOMALS procedure, the optimal quantifications can also be obtained by the Multiple Correspondence Analysis as indicated by Greenacre (1993).

It is possible to verify the goodness of fit of the first dimension, as explained in Greenacre (1993) by the estimate of the τ coefficient.

$$\tau = \frac{\left(\frac{p}{p-1}\right)^2 \left(\lambda - \frac{1}{p}\right)^2}{\overline{\Phi^2}} 100$$

where $\overline{\Phi^2}$ represents the mean of the contingency coefficients of Pearson (phi-square = chi-square/n) of all the possible couples of categorical variables, and is calculated, after the extraction of all the possible Eigen values, as follows:

$$\overline{\Phi^2} = \left(\frac{p}{p-1}\right)\left(\sum \lambda_r^2 - \frac{\sum m_j - p}{p^2}\right)$$

where λr = Eigen value of the dimension (r),
m_j= number of the modalities of the item j,
p= number of the items.
Values of τ upper to 0.50 are usually regarded as indicators of one-dimensionality of the scale.
The optimal quantifications of the items enable to estimate the discrimination measures of the items that represent an important indication to select the items of the definitive scale. The optimisation of the length of the scale is reached through the elimination of those items that for different reasons do not add any contribute to the measure of the latent variable (poor correlation with the other items, poor or no variance, poor correlation with the scale, negative correlation). After the individuation of the items to be included in the definitive scale, the next step is the homogeneity- one-dimensionality analysis of the scale performed by the estimate of the index τ.

There are different assignment modalities of the global score of a scale. If the items are dichotomised (1/0 as yes or no response) all the "1" selected answers can be summed; otherwise, if the items are more adapt to a Likert model of equidistance between categories, the score is given summing all the answers. After the optimal quantifications have been calculated, their values can be scaled respect to the lowest of their values in order to obtain an absolute zero. So, despite the type of item we are dealing with, the global score can be calculated as the sum of the answers values obtained after the assignment to each item of the scaled optimal quantifications. The advantage of this method is that the items, initially listed by a group of experts and included in a questionnaire as the most pertinent to welfare, are then selected, on the answers basis, with a statistical procedure which takes in account the importance of each item in explaining the variability of the latent variable. The definitive questionnaire, made of the selected items, can then be validated with experimental data or experts' opinion becoming a real measurement instrument that can be used to reveal change in time in welfare state.

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D. <u>Laboratory Animals: Main Issues for Science-based Assessment of Animal Welfare</u>

Animal experiments should only be performed when no alternative is available and when the benefit outweighs the suffering of the animal. One of the main issues is how we can decide about the requirements (flow chart for decision) and if it is possible to adopt the "Bateson's Cube of Decision" (Bateson, 1986) as effective methodological tool to decide on the feasibility of a particular experiment. This model indicates the necessity to take into account the relationships between three factors:

- therapeutic value of a particular research,
- scientific validity,
- level of sufferance inflicted to the animals.

So, additional issue would be how these three factors can be taken into account when deciding on a particular animal experiment.

Beside legal and moral obligations to safeguard welfare, discomfort and stress can lead to endocrinological and immunological changes, thus jeopardizing results; the critical links between health/welfare and reliability of experimental results shall be defined. Discomfort and stress (behavioural dimension) can lead to behavioural abnormalities; methodological and practical aspects in the evaluation and solution of behavioural problems play an important role.

The five freedoms adequately depict welfare of farm animals, but in the case of laboratory animals shall be define if their ability to cope with the environment is more important. To cope with the environment could not be enough anymore for a modern conception of animal welfare (Buchanan-Smith *et al.*, 2005). So, the following issues could be identified:

- Is there a proper definition of welfare for laboratory animals?
- Is the quality of the coping reaction an adequate tool to evaluate the level of welfare?
- Can we actively promote welfare?
- Positive and negative features of predictable versus non-predictable environments: How the level of welfare can be related to the possibility for the animal to control and intervene on its own captive environment (Sambrook and Buchanan-Smith, 1997)?

Laboratory animals have needs and show behaviour related to incentive motivation: the role of these components shall be outlined for animal welfare. Preference tests and behavioural observations are used to assess needs and wanting of the animals, but the adequacy and reliability for laboratory animals are not clear, if the tests are related to long-term priorities of the animals. The key point is: "How can we realistically relate preference test results with the welfare of animals in relation to what they want?"

Theories coming from studies of economics are now applied to the study of welfare in animals: if and how are they relevant to the understanding of the level of welfare of an animal (Mason *et al.*, 1998)? The assessment of welfare can be based on one or more of the following approaches: functional, physiological data / feelings / behaviour. Their applicability to laboratory animals and the definition of priorities among the three approaches shall be taken into consideration.

With regard to physiological data, radio-telemetry has enabled some parameters to be measured in stress-free animals, emphasize the interest about the non-invasive techniques. Experiments are often a combination of invasive versus non-invasive techniques. The relationship between non-invasive techniques and quality of the experimental data is still to be analysed, as well as the most important factors (in a rank of importance) which determine the choice of an alternative, less invasive technique in a particular experimental protocol (Pollo *et al.*, 2004).

Laboratory animals have adapted to captive life, but they still show similarities to their wildlife counterparts. The main issue should be if these similarities are important and if they should be thought of in terms of housing and handling. Another point of discussion shall be the use of the natural ethogram as a reliable method of reference to provide an adequate level of welfare in captivity and what the limits and the advantages for the use of this methodology are.

Barren, restrictive and socially deprived housing conditions interfere with the development and function of brain and behaviour. How to improve housing conditions, including if the structuring of the cage/pen environment is more beneficial and appropriate than large floor areas, shall be considered as well as the enrichment of environments, which gives to the animals the opportunities to adequately perform their behavioural repertoires. The minimum requirements to define an enriched environment and the priority among social environment / physical environment / nutritional aspects are under discussion.

General specifications of housing of laboratory animals in Europe are given in the European Convention for the protection of vertebrate animals used for experimental and scientific purposes from the Council of Europe and the Directive 86/609/EEC. The need for new, more precise rules shall be investigate; as a matter of fact, the revision is already in the works (first draft of the European Directive, perhaps in 2007): "which aspects of the current Directive are expected to be changed and how?" And also: "Are the current national directives and guidelines taking efficiently into account the recent progress in the field of the study of animal welfare? Are the contributions from disciplines outside the scientific quarters relevant? (Philosophy, Social science, etc.)? What about the importance of local ethical committees?"

The 3 Rs are not *sensu strictu* part of an index for risk analysis; it is our understanding in fact that 3 Rs efforts will anyhow continue unabated and pave the way to a lesser need for laboratory animals. However, some links should be envisaged between 3 Rs activity and investigations into welfare. Issue: how can the 3 Rs relate to critical features of welfare? Is there sort of functional connection between the two of them? What is the theoretical and functional relation between the 3Rs? How can we implement efficiently the 3Rs model? What are the limits of the model? There is also all of the questions related to the evolution of the interpretation of the 3Rs model.

The production of transgenic animals may imply suffering: e.g. overproduction of growth hormone, chronic kidney and liver dysfunction, etc. If the phenotype of transgenic animals is critical for licensing and how the general rule described above (are the requirements stricter?) is applied to such animals shall be considered.

The sanitary conditions are likely to play a major role for welfare, since high infectious pressure can lead to abnormal activation of the immune system and waste of food energy, as well as to outbreaks of disease. The links between health and welfare, relative importance of microbiological and serological disease control programmes shall be taken into consideration.

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E. Area of Expertise

The scientific production of the Experts and the Centres of Reference is grouped in relation to the species pointed out in the studies, but during the development of the Guidelines they should be divided also in relation to the specific expertise in the following areas:

- RISK ASSESSMENT with a specific expertise on Animal Welfare studies
- HEALTH AND FOOD SAFETY with a specific expertise on the connection with Animal Welfare
- ANIMAL WELFARE respect to the different species, detailed below:
  - *Cattle (calves, dairy cattle and beef cattle)*
  - *Buffalo,*
  - *Sheep,*
  - *Goats,*
  - *Swine (weaners and finishers, lactating and pregnant sows),*
  - *Horses for meat production,*
  - *Farmed domestic rabbits,*
  - *Poultry (laying hens, broilers, other poultry),*
  - *Farmed fish,*
  - *Minor farmed species,*
  - *Laboratory Animals.*

When each Expert or group of Experts will define the Critical Points and Risk for the species detailed above, the analysis has to consider the following topics, in order to cover the different approaches to Animal Welfare evaluation:

- *resource-based approach*
  - Housing:

    housing system, location, floor, bedding, space allowance, pen design, fence, disease pen, handling facilities, feeding, drinking, microclimate conditions, ventilation, cooling systems, temperature, relative humidity, air velocity, chill factor, dust, light intensity, noise, gases concentrations, etc.;
  - Management:

    genetics, feeding, grouping, handling, weaning, human-animal relationship, disease management, mutilations, warning system, cleaning procedure, etc.

- *animal-based approach*
  - Behavioural indicators:

    comfort, resting, social interactions, aggressiveness, abnormal behaviour, stereotypies, locomotion, movement patterns, rhythms, fear, pain, motivation, environmental perception, learning, feeding, social, playing and reproductive behaviour, foraging and grazing, etc.;
  - Pathological indicators:

    health, mortality, morbidity, body damage, lesions, respiratory diseases, gastro-enteric diseases, locomotion disease, reproductive disease, metabolic disease, etc;
  - Physiological indicators:

    hormones, neuro-endocrine system, adrenal hormone, physiological changes, heart rate and HRV, respiratory rate, body temperature, biochemical marks, ACTH, corticosteroids, catecholamines, acute phase proteins, etc.;
  - Productive indicators:

    reproductive performance, weight gain, body condition score, etc.

F. Definition of Selection Criteria and Ranking of Centres of Excellence and Experts

Database of References

The scientific production of the last 5 years (2002-2006) was recorded in a Database of References about the current researches on Animal Welfare issues. The database was created using an integrated web-based platform (ISI Web of Knowledge), whose sources are three bibliographic databases (Web of Science, CAB Abstract, Inspec). Only English written papers were take into consideration; for some species and some topics, when the available information was scarce, the bibliographic search was extended to the last 10 years (or more if necessary) and to languages other than English.

Articles are divided into the different species and different topics as follows:

- ✓ *Food Safety in respect to Animal Welfare;*
- ✓ *Welfare of:*
  - *Cattle (Calves, Dairy Cattle, Beef Cattle)*
  - *Buffalo,*
  - *Sheep,*
  - *Goats,*
  - *Swine,*
  - *Horses for meat production,*
  - *Farmed domestic rabbits,*
  - *Poultry (Laying hens, Broilers, Other poultry),*
  - *Farmed fish,*
  - *Minor farmed species,*
  - *Transport (General and Specific articles for each species),*
  - *Stunning and killing,*
  - *Laboratory Animals.*

In each section, articles are divided according to the type of document:
- Book,
- Scientific Journal,
- Conference Paper,
- Other type of publication (detailed in the text).

A list of the scientific opinions (to present) issued in the Animal Welfare field upon requests from the Commission was added at the end of the list of scientific papers as an inclusive folder of references useful to identify the main issues.

The EC legislation on the protection and welfare of animals was also reported.

COMPLETE LIST OF SCIENTIFIC PAPERS ON ANIMAL WELFARE IS QUOTED IN ANNEX I.

List of Centre of Excellence

The list of Centres in Animal Welfare field was a consequence of the analysis of the Database of References, following the criteria defined above.

The Centres were ranked according to the times in which they came out into the Database of References, listed in Annex I, as the address of the corresponding author. A list of additional Centres involved in Animal Welfare researches was included.

LIST OF EUROPEAN AND INTERNATIONAL CENTRES OF REFERENCE ARE QUOTED IN ANNEX II.

List of Experts

The examination of the Database of References, quoted in Annex I, formed a list of authors; the authors in this list are ranked according to the number of citations. Only the authors coming out, at least ones, as 'first author' are included. Should more than three classes of citations result, only the first two/three are reported, to avoid too long a list. At the end, a list of Experts from the board of the main scientific journals and association in the field of Applied Ethology and Animal Welfare was given.

LIST OF EXPERTS ARE QUOTED IN ANNEX III.

The lists of the Centres of Excellence and Experts in Animal Welfare and Risk Assessment, listed in Annex II and Annex III respectively, could not include all the people involved in these research fields, as they represent the result of a specific search. It is evident that the use of different criteria might result in different lists of Centres of Excellence and Experts. As the search is based on the scientific production of the last 5 years, this document will be regularly revised and the lists updated.

G. Project planning proposal for the development of Animal Welfare Risk Assessment Guidelines

1st Step

Definition of the Animal Welfare, in respect to Risk Assessment

2nd Step

The Area of Intervention of the Guidelines in Animal Welfare field is not specified at a general level, but it has been considered for each different species, detailed below:

- *Cattle (calves, dairy cattle and beef cattle)*
- *Buffalo,*
- *Sheep,*
- *Goats,*
- *Swine (weaners and finishers, lactating and pregnant sows),*
- *Horses for meat production,*
- *Farmed domestic rabbits,*
- *Poultry (laying hens, broilers, other poultry),*
- *Farmed fish,*
- *Minor farmed species,*
- *Laboratory Animals.*

The main issues for Animal Welfare, which need to be considered in the definition of the Guidelines, will be included in five categories:

- Housing,
- Management,
- Human-animal relationship,
- Transport,
- Slaughtering.

Facing the Risk Definition for each species, it will be necessary to identify specific issues for the different and most widespread farming systems, e.g. extensive, intensive, organic farming.

3rd Step

Creation of a working methodology taking into consideration the Risk, expressed as:

- Behavioural modification,
- Pathological modification,
- Physiological modification,
- Reduction in Productive and Reproductive performance,

and Critical Points for each category of Risk will be defined for:

- Housing,
- Management
- Transport,
- Slaughtering

4th Step

Development of comprehensive approach to evaluate Risk Assessment for Animal Welfare: as a consequence of the evident difficulty in developing a quantitative Welfare Risk Assessment, it would be more profitable focus on a qualitative Risk Assessment considering the Risk and Critical Points mentioned below. Just in a second phase of developing of the Guidelines, the efforts could be shifted on a quantitative approach.