Acts and Regulations concerning the Care and Use of Fish in Norwegian Research

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Contents

Important comments on the document

Underlined text indicates direct web-links available in the electronic version, and for the paper version the web-addresses are provided at the end of the document. Some of the links are only available in Norwegian and then the Norwegian title is used. In some cases, links to both Norwegian and English versions are available.

We have interpreted the Acts and Regulations to the best of our knowledge and apologise for any misinterpretations. This report should not be used as a legal document.

The report is written in English and will be part of the study material for FELASA category C courses for fish researchers in Norway.

This document will be continuously updated and it is therefore important to include the edition number when referring to the document. If you have any suggestions for changes/corrections please contact us and we will consider it for the next edition.

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Introduction

This report is part of a project financed by the Norwegian Food Safety Authority to establish a platform for increasing implementation of "the 3 R's" in Norwegian research.

The 3R's

- **Replace** - replace animal experiments where possible with alternatives
- **Reduce** - reduce the number of experiments, and the number of animals in each experiment, to an absolute minimum
- **Refine** - refine experiments that have to be carried out, so that the animals undergo the minimum of discomfort, and such that the scientific quality is as high as possible

It is an ethical dilemma to use live animals in research, so the number of animals and their suffering should be kept to a minimum. The main aim of the project is therefore to define a 3R-stategy that will provide additional and improved research results per animal used in research.

Since over 90% of animals used in research in Norway are fish, the project is mainly focussed on these species. We have previously issued a report entitled "An analysis of the fish used in research in Norway in 2003: Numbers, species and use".

The aim of the present report is to provide an overview of Acts and Regulations governing the care and use of fish in research in Norway. This includes Regulations that demand the use of fish in the development of medicines (including fish vaccines), testing of chemicals and toxin diagnosis. Emphasis is also placed on how this legislations affects implementation of the 3 R's.

A fourth R, Relevance, also needs to be questioned in accordance to Acts and Regulations. This is due to the fact that most fish used for research in Norway are used for testing of side-effects of fish-vaccines (batch testing). Even though the laboratory tests show little side-effects the problems with side-effects due to fish vaccines in the fish farming industry continues to be a large problem. The lack of consistency between the laboratory tests and the field results questions the relevance of the mandatory testing.

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Summary

- **There is a need for better definitions concerning fish welfare**
    - Researchers are still debating whether fish have the ability to suffer and this makes it difficult to define terms in the legislation. More knowledge in this area is needed, it should be emphasised that research will never provide a complete picture of how fish feel.
    - Acts and Regulations should be based on current assumptions on the needs of fish, including their perception of pain and suffering. Legislators must therefore be kept updated on the latest knowledge in this field.
- **Regulations for the care of fish in research**
    - The "Akvakulturdriftsforskrift" covers not only farmed fish but also fish in research facilities, and provides many details on their care. It is important to note that exemptions from this regulation can only be made by the Norwegian Food Safety Authority (Mattilsynet) and not by the Norwegian Animal Research Authority (Forsøksdyrutvalget).
    - The "Akvakuturdriftsforskrift" and a range of guidelines state that the health and welfare of the fish need to be monitored, but do not provide details on how the monitoring should be provided. A set of general guidelines is needed, and a separate paper on "Guidelines for health and welfare monitoring of fish in research" will be published by this Centre.
- **Regulations that demand the use of fish in research**
    - The European pharmacopoeia and other international regulations often state exactly how many fish that must be used, and how, for example, vaccine or chemical testing should be conducted. It is difficult to implement the 3 R's in these regulations.
    - The end-point in fish models is often death, and many regulations do not allow refinements of the method. LC_{50} tests are, for example, still used for testing chemicals on fish. Changes need to be made to allow more humane end-points, since this is an important part of the implementation of the 3 R's.
    - The report "An analysis of the fish used in research in Norway in 2003: Numbers, species and use" concludes that most fish are used for batch testing of vaccines according to the European pharmacopoeia. This illustrates the impact of international regulations on the number of fish used in Norway.
- **Changes in the legislation**
    - The Norwegian Animal Welfare Act, the EU Directive 86/609 and the Council of Europe's Convention ETS 123 are all currently being revised.
    - The Norwegian Food Safety Authority provides a website with updated Norwegian regulations. However, there is also a need for a website that is updated continuously covering the international regulations that govern the use of fish in research in Norway.

Main conclusions

- There is a need for implementation of the 3 R's in Regulations that make it mandatory to use live fish. For example, Regulations for the testing of fish vaccines and toxins do not in many cases allow any refinement of the method or reduction of the number of fish. The relevance of the mandatory tests also needs to be questioned.
- The lack of clear definitions of fish welfare has resulted in vague legislation that can be interpreted in many ways. Better definitions are necessary to ensure correct interpretation.
- A website with the latest science-based knowledge on the care and use of fish in research, together with the latest data on the ability of fish to feel pain would be of great help to fish researchers, technicians and legislators. There is also a need for an updated website on Acts, Regulations and guidelines related to research using fish.

The Animal Welfare Act

The Norwegian Animal Welfare Act No. 73 of 20th December 1974 (*Dyrevernloven*) and the Regulation relating to experiments with animals (*Forskrift om forsøk med dyr*) both include fish in their definitions of an animal. This is not the case in many countries. The Act is now being revised and is scheduled to be finalised in 2006.

The main principle and the scope of the Act according to Article 2 is as follows: "*Animals shall be treated well, and consideration shall be given to the instinctive behaviour and natural needs of animals, so that there is no risk of causing them unnecessary suffering.*" What is to be defined as "unnecessary" must be considered in each specific case according to current scientific data on, among other things, pain perception, on the purpose or aim of the study and on society's ethical standards. For example, fishing for food is considered "necessary" and is therefore allowed, while "catch and release" of fish is not considered "necessary" as the purpose is for humans to enjoy a sport and it therefore questioned in Norway.

Researchers still debate whether fish have the ability to suffer or not and this makes the term "unnecessary suffering" vague when it comes to fish welfare (Rose 2002, Sneddon 2003). Better definitions and knowledge on fish welfare are therefore of major concern. In the last decade there has been an ever increasing focus on fish welfare issues, and in connection with the on-going revision of the Animal Welfare Act it is considered essential to implement new fish welfare legislation. The first step in the revision was to establish a working group. The Group's mandate was to identify society's attitude towards animal welfare, to establish new ethical standards, to describe how the different species of production and companion animals are kept, to identify important welfare issues for each species and to suggest new means for improving animal welfare and addressing the problems that had been identified. The proposal of the working group was presented in December 2002: "Stortingsmelding nr. 12 (2002-2003) Om dyrehold og dyrevelferd". This Parliamentary Report on animal welfare was debated in the Norwegian parliament and approved by the Government in June 2003. In the Report it is explicitly stated that though there is still some doubt whether fish feel pain in a similar way as mammals, they must be treated as if they are capable of feeling pain and fear and thus are able to suffer.

Articles 21 & 22 in the current Animal Welfare Act state that animals cannot be used in research without a licence from a committee. The Committee is organised under The Norwegian Food Safety Authority and is called the Norwegian Animal Research Authority (NARA, *Forsøksdyrutvalget*). Today the NARA consists of 8 members who are appointed for a 4-year period. The secretary of the committee is the only full-time employee.

The Regulation relating to experiments with animals

1996-01-15 No. 23 The Norwegian Regulation relating to experiments in animals (*Forskrift om forsøk med dyr*) describes the work of the Norwegian Animal Research Authority (NARA) and how biological experiments shall be conducted. NARA approves laboratory animal facilities and personnel including a "responsible person" (*Ansvarshavende*) for each unit.

The “responsible person” alone approves of most experimental studies carried out at the unit and these approvals are later revised by NARA within a month. The regulation provides a list of experiments that can not be approved by the “responsible person” alone and that have be approved directly by the NARA, for instance experiments that are assumed to cause “prolonged or significant pain”. The definition of “significant pain” for fish, however, is not defined. In 2003 only 36 fish of a total of about 400.000 fish used in Norwegian research were considered to suffer "significant pain" and all these fish had radio-transmitters surgically implanted in their abdomen. Challenge trials where fish are submitted to lethal doses of infectious agents were, for example, not defined as studies causing “significant pain”. The practice of one person at each unit making most decisions has been questioned and criticised and is currently under discussion.

Field studies outside laboratory units must always be approved directly by the NARA.

The same reporting criteria are used for all laboratory animals and no differentiation is made between different fish species or ages. Adult salmon are, for example, reported in the same statistics as cod larvae. The problems with vague definitions on how to report the number of fish being used have been highlighted in the report: “An analysis of the fish used in research in Norway in 2003: Numbers, species and use” (Knudsen *et al.* 2005).

The Norwegian Regulation states that housing and environmental conditions must satisfy the guidelines in Appendix A of the European Convention (ETS 123). As mentioned below this Appendix does not cover fish at present, but fish will be included in the revised version. The new National Animal Welfare Act in Norway along with the new Appendix A will create the need for a new or revised regulation governing the care and use of fish in research.

The European Convention (ETS 123)

Fish are included in the European Convention for the “Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (1986) and Norway was one of the first countries to sign the agreement. Species-specific regulations are provided in Appendix A: “Guidelines for accommodation and care of animals”. The Appendix does not include fish today (October 2005), but a revision of the Appendix has started and the new draft includes fish. The draft provides general regulations to control parameters such as water quality, health, handling, transport and housing. Species-specific guidance on rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*), tilapiine cichlids, zebra fish (*Danio rerio*), sea bass (*Dicentrarchus labrax*), Atlantic halibut (*Hippoglossus hippoglossus*), Atlantic cod (*Gadus morhua*), turbot (*Scophthalmus maximus*) and African catfish (*Clarias gariepenus*) will be available in the background document drawn up by a group of experts, but this is not yet available.

Appendix B describes how statistics on the number of animals used for experimental and other scientific purposes should be reported. The parties have agreed on some changes in the statistics (Daniel Cangemi, Secretary of the Conventional Committees on Animal Welfare, personal communication). The statistics from different countries vary greatly, making it difficult to compare data and they are therefore published separately for each country. For some reason the Norwegian data are not shown on the Council of Europe’s web-site.

There is also a convention covering farm animals, the European Convention for the Protection of Animals kept for Farming Purposes (EST No. 87). Recommendations concerning cattle, sheep and other mammals have already been made and recommendations for farmed fish will

be discussed at a meeting in December 2005 (Daniel Cangemi, Secretary of the Conventional Committees on Animal Welfare, personal communication).

The EU Directive (86/609/EEC)

Norway is not member of EU, but EU Directives still have a major influence on our legislations through the EEA agreement. Most countries that have ratified the European Convention (ETS 123) are also members of the EU and therefore have to conform to "The Council Directive 86/609/EEC **of 24th November 1986 on the approximation of Acts, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes".** In 2000, the European Union itself adopted the ETS 123, making it compulsory to adopt the same changes into the Directive, but this process requires the approval of the European Parliament. The Directive is now being revised, but it will probably not include guidelines for accommodation and care.

The main object of the Directive is to ensure harmonisation of Acts concerning laboratory animals in the EU member states and ensure that the number of animals used and their suffering is kept to a minimum. It includes all non-human vertebrates except for foetal and embryonic forms. Fish are not mentioned in particular, but the general articles are suitable also for fish.

It is interesting to note the definition of "experiment": *"any use of an animal for experimental or other scientific purposes which may cause it pain, suffering, distress or lasting harm, including any course of action intended, or liable, to result in the birth of an animal in any such condition, but excluding the least painful methods accepted in modern practice (i.e. 'humane' methods) of killing or marking an animal"*. The definition of 'pain, suffering and distress' in fish therefore becomes a major issue and should be defined in the new version of the Directive.

All 15 EU member states in 2001 agreed upon 8 tables on "Statistics on the number of animals used for experimental and other scientific purposes" (published in 2005). Hopefully this agreement will provide data that are comparable over time and between different countries. These statistics run in parallel to the statistics mentioned in Appendix B of ETS 123.

Acts and Regulations for farmed fish in Norway

The Act of 14th June 1985 No. 68 relating to aquaculture (*Oppdrettsloven*), §2 states "The term aquaculture means any activity involving the feeding or handling of live fish and shellfish for consumption, feed production, reproduction, stocking, including sea ranching, research or educational purposes." Fish research is thereby defined as part of aquaculture. The Acts and Regulations governing aquaculture apply therefore also for fish in research.

The Act of 1st Jan. 2004 No. 124 ("The Food Act", "*Matloven*") involves all aspects of food production and animal health including fish. This new Act replaces many previous Acts including the "Fish Diseases Act" ("*Fiskesykdomsloven*"). The Food Act is the legal basis for

a number of regulations of relevance to fish research, and the most important ones are mentioned below.

An overview of most Regulations is presented on the website of The Norwegian Food Safety Authority. An English translation of all Acts and Regulations related to farmed fish was last updated in 2003 (also in English). However, many changes have been made over the last two years and these new Regulations have not yet been translated.

1995-01-01 No. 99 "Forskrift om fortegnelse over sykdommer hos fisk og andre akvatiske dyr som omfattes av matloven". This Regulation provides a list of fish diseases to which the Act is especially applicable. The list is adapted to Norwegian conditions and the diseases are divided into three groups: A, B and C.

1990-02-05 No. 144 relating to instructions for A, B and C diseases (English version in 2003)
This Regulation provides information on how detection of diseases in groups A, B and C are to be handled. Diseases in Group A are considered exotic in Norway, and thus any detection will result in stamping-out procedures (slaughter or destruction of all fish). Detection of diseases in Group B has to be reported to the authorities and different actions are then taken depending on the circumstances. The authorities also have the possibility of making general or local decisions on diseases in Group C.

1999-12-20 No. 1310 relating to vaccination of domestic animals, wildlife, fish and other aquatic animals (Vaksineforskriften) (English version in 2003) According to this Regulation, vaccination is allowed against diseases in Group C, but not against diseases in Group A and Group B, with the exceptions of furunculosis and infectious pancreatic necrosis (IPN).

2004-12-22 No. 1785 "Akvakulturdriftsforskriften" This Regulation has replaced several older ones, including no. 1397 The Hatchery Regulation (*Settefiskforskriften*), No. 1409 Operating and Disease Prevention Regulation (*Drifts- og sykdomsforskriften*), No. 278 Health Control Regulation (*Helsekontrollforskriften*) and parts of No. 509 Disease Control Regulation (*Sykdomsforskriften*). The Regulation is related to three Acts; The Food Act (No. 124), the Aquaculture Act (No. 68) and the Animal Welfare Act (No.73).

This Regulation provides guidelines both for monitoring of the health and welfare of the fish and also for water quality and other environmental factors. It states the minimum number of health inspections that are necessary and the number of fish that needs to be examined in relation to different categories of fish. Broodstock fish and young fish are, for example, to be given a minimum of 12 health examinations a year, while larger fish for food production need only 6 inspections. The monitoring of health and welfare is "risk based" (*risikobasert*) which opens for adjustments to the risk situation for the particular fish group at a given location. Monitoring and interpretation of risk factors therefore becomes a major issue.

The Norwegian Ministry of Fisheries and Coastal Affairs has issued guidelines to help people with the interpretation of the regulation (58 pages). Chapters 1, 2, 3 and 7 apply to fish in research. The guidelines state that exemptions from one or more of the provisions in the regulation may in exceptional cases be granted if there is a good reason for it. Furthermore it

is stated that it is not the intention of this Regulation to be a hindrance to research approved by the NARA but it is necessary to ensure all fish good welfare. The Norwegian Food Safety Authority is the only body that may grant an exemptions from the provisions given in the Regulation in pursuance of the Animal Welfare Act. It is also stated that certain types of research facilities will not be granted exemptions as they resemble commercial aquaculture farms.

The number of details in this Regulation is extensive, and both the Regulation and the guidelines need to be read in detail by everyone who works with farmed fish, including those involved in fish research. A table with acceptable values of some water quality parameters is provided for salmonids in freshwater and further tables for other fish species will hopefully be available in the future. Norms for expected mortality rates are provided for salmonids of different sizes and this is of major importance when an increase in mortality is to be evaluated.

As regards monitoring of fish welfare, §6 states that a sufficient number of personnel with necessary knowledge of fish welfare are needed. 'Knowledge of fish welfare' must be documented every fifth year (starting in 2010) using a plan approved by the Norwegian Food Safety Authority (not available yet).

The health monitoring must be carried out by authorised animal health personnel according to **The Act of 15th June 2001 No.75 (*"Lov om veterinærer og annet dyrehelsepersonell"*, *Dyrehelsepersonelloven*)**. As far as fish health is concerned, the Act equates veterinarians and fish health biologists (M.Sc. in Aquamedicine). Some exemptions to the types of health monitoring that can be performed by non-authorised personnel are also listed in the guidelines.

2004-03-19 No. 537 "IK-Akvakultur" states that the person responsible for an aquaculture establishment, including research facilities, is responsible for documentation that the establishment is run in accordance to all Acts and Regulations (*Internkontroll*). The one-page regulation is followed by three pages of guidelines which provide details on how this monitoring should be carried out. A **web-site** for additional help with implementation of "IK-Akvakulture" is also available in Norwegian. The Norwegian Food Safety Authority inspects the documentation mandatory by The Food Act (No. 124) and the Animal Welfare Act (No. 73), while The Directorate of Fisheries controls Regulations under The Aquaculture Act (No. 68).

Other Regulations

Regulations concerning field studies

Field studies outside approved laboratory facilities constituted 10% of all fish used in research in 2003. In these cases there are several Acts and Regulations concerning wild fish that must be followed.

Act No. 47 15th May 1992 relating to salmonids and fresh-water fish etc. protects wild species of salmonids and freshwater fish along with their habitats. §8 forbids importation of these fish species without permission from the government. The rest of the Act is mainly related to fishing regulations.

1991-07-04 No. 509 relating to prevention, control, and eradication of diseases in aquatic organisms mainly concerns wild fish since most of the clauses related to fish farming have been moved to no. 1785 "*Akvakulturdriftsforskriften*". There are, however, still clauses covering disease eradication and control. The Regulation also addresses how to avoid spread of disease from one river or water system to another.

Regulations on import, export and movement of fish

1991-07-04 No. 509 relating to prevention, control, and eradication of diseases in aquatic organisms (*Sykdomsforskriften*), §19, regulates the importation of fish for use in research and states that they have to be proven free from diseases in Group A. Certificates must be issued by the official veterinary authority or other regulatory authorities at the place of departure and a number of other requirements are stated.

2003-10-14 No. 1239 *"Forskrift om dyrehelsemessige vilkår ved omsetning og import av akvakulturdyr og akvakulturprodukter" (Omsetningsforskriften)* provides additional regulations for the importation and movement of fish. Separate paragraphs cover importation from countries outside Europe and between different zones. This Regulation also includes zebrafish and other aquarium fish. Transport documents must include a health statement that the fish satisfy the requirements laid down in **The European Council Directive of 28th January 1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products (91/67/EEC).**

2003-05-30 No. 661 describes a range of protection measures to be taken in connection with import and export of aquatic animals and products thereof (English version in 2003).

1997-12-20 No. 193 relates to the transport of aquatic organisms (English version in 2003). This Regulation states how fish can be transported and provides requirements for vehicles, tanks and other equipment used to move the fish.

1998-12-31 No. 1484: *Forskrift om tilsyn og kontroll ved innførsel og utførsel av levende dyr, annet avlsmateriale og animalsk avfall innen EØS, og ved innførsel av levende dyr fra land utenfor EØS.* This Regulation covers the supervision and control of import and export of live animals, other breeding material and animal waste within the EEA, and also covers the import of live animals from third-party countries (English version in 2003).

Regulations on disinfection

1997-02-20 No. 194 regarding the cleaning and disinfection of aquaculture sites etc. *(Desinfeksjonsforskriften)* (English version in 2003) states which disinfectants that can be used and how buildings, tanks and other equipments are to be cleaned and disinfected.

1997-02-20 No. 192 relating to disinfection of intake water and effluent water from aquaculture related operations *(Vannbehandlingsforskriften)* (English version in 2003). This Regulation states how water that is taken into or released from research stations is to be disinfected. For research stations using salmonids or freshwater fish the reduction requirements for the disinfection method on the water inlet is $3\log_{10}$ (99,9%) of *Aeromonas salmonicida subsp. salmonicida* and IPN-virus. There are no general rules for treatment of the water intake for marine fish. Research stations assigned to challenge trials in category A need to show a minimum of $5\log_{10}$ (99,999%) reduction of IPN-virus at the water outlet, while

stations only assigned to challenge trials in category B and C need $5\log_{10}$ (99,999%) reduction of *Yersinia ruckeri*.

Legislation on genetically modified fish

The Gene Technology Act 1993-04-02 No. 38 *(Genteknologiloven)* regulates the use of genetically modified animals in Norway.

2001-12-21 No. 1602 *Forskrift om innesluttet bruk av genmodifiserte dyr* provides detailed requirements for the housing and management of genetically modified organisms including zebrafish.

1998-11-13 No. 1066: *Forskrift om transport og import av genmodifiserte organismer* regulates transport and import of genetically modified organisms.

Testing of medicinal products

A large percentage of laboratory animals, including fish, are used for developing and testing of medicines and vaccines. The exact number of fish used for these purposes is not possible to obtain with today's official statistics.

1992-12-04 No. 132 *Lov om legemidler m.v. (Legemiddelloven)* and
1999-12-22 No. 1559 *Forskrift om legemidler (Legemiddelforskriften)* are, respectively, the main Act and Regulation concerning pharmaceuticals in Norway and primarily govern sale and marketing.

2004-12-16 No. 1657 The Norwegian Pharmacopoeia contains the official standards for production and quality control of medicinal products. It consists of the European Pharmacopoeia, 5th Edition, and *"Norske legemiddelstandarder 2004 med Addendum 2005"*.

The European Pharmacopoeia includes several monographs for fish vaccines:
A: 01/2002:0062 Vaccines for veterinary use
B: 01/2002:1580 Vibriosis (cold-water) vaccine (inactivated) for salmonids
C: 01/2002:1581 Vibriosis vaccine (inactivated) for salmonids
D: 01/2004:1521 Furunculosis vaccine (inactivated, oil adjuvant) for salmonids

Monograph A describes general requirements for production control of vaccines for animal use, including fish vaccines. It focuses on consistent and safe production without, among other things, contamination. Monographs B-D provide more details for safety and potency testing of these particular vaccines.

Safety tests
Safety testing is in principle carried out by administering a double dose of vaccine to the fish and then observing the animals for side-effects for 21 days. A minimum of 50 fish of each species is used and three different batches of vaccines need to be tested. A minimum of 150 fish per species for which the vaccine is intended is therefore needed.

Potency tests
Potency testing is performed by challenging a minimum of 100 vaccinated and 100 unvaccinated fish by injection of the virulent microorganism. The cumulative percentage mortality in the vaccinated group of fish is registered when 60% of the control fish are dead. The relative percentage survival (RPS) is calculated and should be not less than 90% in monograph B, 75% in C and 80% in D.

Most of the vaccines used on salmonids in Norway today are polyvalent (often 4-6 antigens per vaccine) and a potency test is necessary for each antigen (200 fish per antigen). Potency testing of a hexavalent vaccine therefore requires a minimum of 1,200 fish. The testing described is performed a limited number of times during development of the vaccine in order to document safety and potency of the product.

The challenge models required by the monographs often involve injection of the challenge-organisms. These models are often not the most suitable methods for documentation of the effect of the vaccine, thus in order to evaluate efficacy during development of products, additional bath or cohabitation models may need to be performed (Kjersti Gravningen, personal communication).

Batch tests
Batch testing, which is performed on each production batch of the vaccine, employs 10 fish for safety testing and 60 fish (30 vaccinated and 30 control fish) per antigen for potency testing. Only the most sensitive fish species for which the vaccine is intended need to be tested. A minimum of 370 fish (360 for potency and 10 for safety) is therefore needed per batch for a hexavalent vaccine.

The size of a batch depends, among other things, on the production system and success rate, and may vary from 100-500 litres. The vaccines used for salmonids normally contain 10,000 doses per litre. It is important to note that the vaccines tested in Norway may be used in other countries such as Chile, Ireland and Canada, while the vaccines used in Norway may be tested in other countries such as the Netherlands (Intervet Norbio) and Canada (Scanvacc).

The number of batches that need to be tested in a final bulk production may be reduced by performing tests to show stability of the production of at least 10 batches (***Stability testing***) according to the Position Paper "Date requirements for removing the target animal batch safety test for immunological veterinary medicinal products in the EU" (EMEA/CVMP/865/03/Final).

The challenge trials for batch potency testing can be replaced by validated tests based on antibody response in the fish. In this case, 25 fish are vaccinated and compared to 10 unvaccinated fish. The antibody level must be as high as the results from batches showing adequate potency results by challenge trials. These serological tests may therefore reduce the number of fish needed for testing of a batch of a hexavalent vaccine from 360 to 35.

The European Medicines Agency (EMEA) provides useful guidelines to the European legislations in this field like for instance:

- Guideline on adjuvants in vaccines (EMEA/CVMP/VEG/17/03/2004)
- Guideline on statistical principles for veterinary clinical trials (EMEA/CVMP/816/00-Final)

- Guidelines for production and control of immunological veterinary medical products Volume 7B pages 91-97 provides specific requirements for safety, efficacy and potency testing of live and inactivated vaccines for fish including batch testing. An appendix provides a list of infectious agents of which farms providing fish for the trials must be free.

VICH GL9 (GCP) (2000) Good Clinical Practice. This is an international guideline (EU, Japan and USA) from the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicine Products (VICH). It provides guidance on how to design and conduct clinical studies of veterinary products, and states which documents that need to be obtained before, during and after clinical testing. Inspections should be made by a relevant regulatory authority, which in Norway is the Norwegian Medicines Agency *(Statens legemiddelverk)*. This agency has provided a Norwegian guideline for clinical testing of vaccines for fish, ***"Retningslinjer vedrørende klinisk utprøving av vaksiner til fisk"***.

1996-06-28 No. 693 ***"Forskrift om forskrivning, tilvirkning og distribusjon m.v. av medisinfôr til dyr, fugler, fisk og andre akvatiske organismer"*** relating to prescription, manufacturing and distribution etc. of medicated feed to animals, birds, fish and other aquatic organisms (English version in 2003) . Involves medicated feed for all animals including fish.

Testing of chemicals and toxins

EU is working on new legislation for approval of chemicals, called the "Registration Evaluation and Authorisation of Chemicals" (REACH). Norwegian legislation will have to adapt to this new EU Directive and this may influence the number of laboratory animals required for chemical testing.

We have only found one Norwegian legal document *(Aktivitetsforskriften)* that makes it mandatory to use live fish for the testing of chemicals and toxins. International regulations that concern animal testing in Norway have not been investigated in the process of compiling this document.

In the Norwegian statistics for the use of animals in research, testing of chemicals and toxins is reported in category H (2.4) "Protection of human, animal and environment by testing of toxicity or safety, including testing for products and equipment for use in veterinary- and human medicine." The number of fish used in relation to the various legislative documents or the various tests is therefore not known.

Methods for testing chemicals and toxins may be assigned by ISO, OECD (The Organisation for Economic Cooperation and Development) or OSPAR (Convention for the Protection of the Marine Environment of the North-East Atlantic). Free copies of the OECD Guidelines for the Testing of Chemicals can be downloaded from the Internet, after application for a free 7-day password.

OECD Guidelines for testing of chemicals on fish:

No. 203 Fish, Acute Toxicity test
The aim of the test is to determine the concentration which kills 50% of the fish (LC_{50}). Six

freshwater fish species are recommended for the test, including zebrafish and rainbow trout. Preparations for replacing this test with a fish embryo test are in progress and will be submitted to OECD in late 2005 (Braunbeck *et al.*, 2004). Braunbeck *et al.* (2004) also provide a good overview (Figure 15) of the terminology used for eggs, embryos and larvae of fish.
According to Gunvor Knudsen, who examined all applications for the use of fish in Norwegian research in 2003, most fish for toxicity testing were used in accordance with OECD 203.

No. 204 Fish, Prolonged Toxicity test
This test is used instead of no. 203 when appropriate. The aim of the test is to determine threshold levels of lethal effect and levels of observed effect, which may include among other things abnormal swimming behaviour and changes in body weight. The same six fish species as in 203 are recommended.

No. 210 Fish, Early-life Stage Toxicity test
The aim in this study is to estimate the "lowest observed effect concentration" (LOEC) and "no observed effect concentration" (NOEC). The test begins with placing fertilised eggs in the test chamber and continues until the fish are feeding. Mortality, deformities, abnormal behaviour and other parameters are registered. Five fish species are recommended for this test (Appendix 3), but an additional list of 15 well-documented species is also listed as possibilities (Appendix 5). These include several species of salmonids.

No. 212 Fish, Short-term Toxicity test on Embryo and Sac-fry Stages
As in no. 210, the aim of this test is LOEC and NOEC. Cod and other fish species with high mortality rates in the control groups cannot be used in test no. 210. Test 212 is a possibility for these fish species due to the short timespan. The sensitivity is less than in no. 210.

No. 215 Fish, Juvenile Growth test
The aim of this test is to estimate the concentration that would cause an x% variation in growth rate (EC_x), *i.e.* EC_{10} or EC_{25}. Alternatively, LOEC and NOEC may be determined. The fish are simply measured before and after exposure to the substance and compared to a control group. Rainbow trout is the only recommended fish species in this test, but zebrafish and medaka can be used (Appendix 2).

No. 305 Bioconcentration: Flow-through fish test
The test consists of two phases: the exposure (uptake) period (28 days) and post-exposure (wash-out). Fish groups are exposed to the test substance in different concentrations for 28 days and then moved to clean water. Concentrations of the substance are measured in the fish and in the water at different time points. Both freshwater and marine fish have been used.

2002-07-16 no. 1139 ***Forskrift om klassifisering, merking m.v. av farlige kjemikalier*****.** This is the Regulation for classification of dangerous chemicals. Methods for animal testing are provided in Appendix V of EU-directive 67/548/EEC. Method C1 is a copy of OECD test number 203, C13 - OECD 305, C14 - OECD 215 and C15 - OECD 212. OSPAR Harmonised Offshore Chemical Notification Format (HOCNF) documentation is needed for all chemicals.

2001-09-03 no. 1157 ***Forskrift om utføring av aktiviteter i petroleumsvirksomheten (Aktivitetsforskriften)*****,** § 56, states how chemicals from the petroleum industry are to be tested on algae *(Skeletonema costatum),* copepods *(Acartia tonsa),* mud shrimp (*Corophium volutator*) and/or fish. The fish and shrimp test must be performed according to "OSPAR

Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry, 1995". This method is a modified version of the LC_{50} OECD test no. 203 "Fish Acute Toxicity test". The main modification is adaptation to two marine fish species: turbot (*Scophthalmus maximus*) and sheepshead minnow (*Cyprinodon variegatus variegates*).

Petroleum chemicals are first to be tested on copepods and algae, and only chemicals with a low toxicity to these organisms need to be further tested on fish. The "OSPAR Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry (1995) will furthermore be updated in 2005 to limit testing on fish (Ann Mari Vik, Norwegian Pollution Control Authority *(Statens forurensningstilsyn)*, personal communication).

LC_{50} testing on mammals (OECD test no. 401) is no longer allowed. "Acute Oral Toxicity tests" are used instead (no. 420 Fixed Dose Procedure (FDP), no. 423 Acute Toxic Class Method (ATC), no. 425 Up-and-Down Procedure (UDP), Botham 2002). These new tests also involves testing on laboratory animals, but the number of animals and suffering have both been drastically reduced. These tests are often refered to in the mass media, and it is therefore important for fish researchers to notice that these are all tests on mammals and do not concern fish.

Fish screening assays for the detection of endocrine substances

Substances with endocrine effects ("endocrine disruptors") may result in several changes in fish, that can be detected by a variety of different methods. The easiest test is to look for changes in the behaviour or morphology of the fish. OECD has provided a review paper on these fish assays. This review paper will most likely become the background material for future OECD guidelines. The review also includes an introduction to endocrinology in fish which can be useful for most fish researchers. Three fish species are used in these tests: the fathead minnow (*Pimephales promelas*), medaka (*Oryzias latipes*) and zebrafish (*Danio rerio*). The review also includes general information about these fish species and Table 3.1 provides pros and cons in the choice between them that is also useful in other fish models.

Guidelines

Guidelines for the care and use of fish in research, along with monitoring and reporting of health and welfare, are sparse compared to those available for mammalian laboratory animals. It is worth noting that there are more fish species than all other vertebrate species combined. Despite the fact that fish are studied in almost all biological disciplines (Powers, 1989), most guidelines for the care and use of fish in research are general documents that encompass all fish species in all types of research (DeTolla *et al.* 1995; Casebolt *et al.*, 1998; CCAC). There is a great need for more species-specific guidelines and in some cases research disciplines may require particular guidelines (http://zfin.org/zf_info/zfbook/zfbk.html).

Researchers have an ethical obligation to extract as much knowledge as possible from each animal used. Lack of standardisation of parameters such as genotype, water quality and handling procedures in fish research often lead to incomparable results and therefore unnecessary use of large numbers of fish. Harmonisation of health and welfare monitoring is one important factor in the standardisation process and a separate paper on this matter will therefore be published by this Centre.

Monitoring of fish welfare was included in a large report to the Norwegian Research Council in 2005 entitled *"Forskningsbehov innen dyrevelferd i Norge"*. The report provides a good overview of the ability of fish to feel pain and stress. Common welfare and diseases problems are discussed, and there is a chapter on indicators for monitoring of fish welfare. Today's indicators such as behaviour and health are mentioned, together with discussions on possible welfare indicators that may be available in the future, such as physiological and molecular parameters.

Another Norwegian report on monitoring of fish welfare will soon be published on www.mattilsynet.no with the title *"Dyrevelferd i akvatisk dyrehold - herunder fremtidens dyrehold"*. This report focuses especially on how fish welfare can be monitored either directly by examining the fish or indirectly by monitoring the environment. The report will focus on need for "on-farm" indicators to document the well-being of the fish.

The Fisheries Society of the British Isles (FSBI) has published a very thorough briefing paper on fish welfare that provides several definitions of welfare criteria in fish. The paper does not state what is acceptable and what is unacceptable, but provides a good overview of background knowledge. It also provides information on acute and chronic stress responses in fish and look at ways of measuring fish welfare.

The Office International des Epizooties (OIE) is an international organisation for animal health with 167 member states, including Norway. The Aquatic Animal Health Standard Commission (AAHSC) is responsible for fish health issues applied by the "Aquatic Animal Health Code" which from 2005 includes fish welfare. Two *ad hoc* groups, led by Dr. Tore Håstein from Norway, are at the moment working on guidelines for transport and slaughter of fish.

The organisation FELASA has already provided guidelines for the health monitoring of other laboratory animals (Rehbinder *et al.*, 1998; Nicklas *et al.*, 2002) and hopefully in the future guidelines will be available also for fish. An overview of guidelines and other resources on the care and use of fish in research is provided on http://oslovet.veths.no/fish.

Links and references

English translations of all Norwegian laws and regulations related to fish farming (2003): http://dyrehelsetilsynet.mattilsynet.no/dyrehelse/publikasjoner/engelskutgaveaugust2003.pdf

The Norwegian Animal Welfare Act and Regulation governing animal experimentation: http://www.fdu.no/fdu/regelverk/

ETS 123, new Appendix A: http://www.coe.int/T/E/Legal_affairs/Legal_co-operation/Biological_safety,_use_of_animals/Laboratory_animals/GT%20123%20(2004)%201%20Appendix%20A%20final%20for%20adoption%20DRAFT.pdf

ETS 123, Appendix B: http://conventions.coe.int/Treaty/EN/Treaties/html/123-B.htm

ETS 123, revised version of Appendix B: http://www.coe.int/T/E/Legal_affairs/Legal_co-operation/Biological_safety%2C_use_of_animals/Laboratory_animals/Res%20interpretation.asp#TopOfPage

EU Directive 86/609/EEC: http://europa.eu.int/eur-lex/lex/LexUriServ/LexUriServ.do?uri=CELEX:31986L0609:EN:HTML

EU Directive 86/609/EEC, Revision:
http://europa.eu.int/comm/environment/chemicals/lab_animals/revision_en.htm#Next%20Steps

Statistical data from the EU:

http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/com/2005/com2005_0007en01.pdf

Act of 14 June 1985 No. 68 relating to aquaculture *(Oppdrettsloven)*:
http://www.ub.uio.no/ujur/ulovdata/lov-19850614-068-eng.pdf

Act No 47 of 15 May 1992 relating to salmonids and fresh-water fish etc.:
http://www.ub.uio.no/ujur/ulovdata/lov-19920515-047-eng.pdf

EU directive 28 January 1991 concerning the animal health conditions governing the placing on the market of aquaculture animals and products (91/67/EEC):
http://europa.eu.int/smartapi/cgi/sga_doc?smartapi!celexapi!prod!CELEXnumdoc&lg=en&numdoc=31991L0067&model=guichett

The European Pharmacopoeia: http://online.pheur.org/entry.htm

The European Medicines Agency: http://www.emea.eu.int/htms/general/contacts/CVMP.html

The rules governing medicinal products in the European Union, Volume 7B:
http://pharmacos.eudra.org/F2/eudralex/download/volpdf/vol7/vol7ben.pdf

VICH GL9 (GCP) (2000): http://vich.eudra.org/pdf/2000/Gl09_st7.pdf

REACH: http://europa.eu.int/comm/enterprise/reach/overview.htm

OECD-library: http://miranda.sourceoecd.org/vl=2187489/cl=115/nw=1/rpsv/home.htm

Braunbeck *et al.* 2004: http://www.altex.ch/pdf/artikel/altex_2_2005_Braunbeck.pdf

67/548/EEC Annex 5: http://ecb.jrc.it/testing-methods/

OSPAR protocol for fish and shrimp testing:
http://www.ospar.org/documents/dbase/publications/p00037_Parcom%20Protocols.pdf

OECD Review on fish screening assays for the detection of endocrine substances:
http://appli1.oecd.org/olis/2004doc.nsf/linkto/env-jm-mono(2004)18

DeTolla *et al*: http://dels.nas.edu/ilar_n/ilarjournal/37_4/37_4Guidelines.shtml

CCAC guidelines:
http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Fish/Fish%20Guidelines%20English.pdf

FSBI briefing paper on fish welfare (2002): http://www.le.ac.uk/biology/fsbi/welfare.pdf

Links to Regulations available only in Norwegian

Årsrapporter fra Forsøksdyrutvalget: http://www.fdu.no/fdu/om/dbaFile3843.html

Matloven: http://www.lovdata.no/cgi-wift/ldles?doc=/all/nl-20031219-124.html

Mattilsynets liste over forskrifter: http://www.mattilsynet.no/fisk/regelverk

Forskrift om liste over fiskesjukdommer:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19950101-0099.html

Vaksineforskriften:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19991220-1310.html

Instrukser for A, B, C sykdommer:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19900205-0144.html

Akvakulturdriftsforskriften: http://www.lovdata.no/for/sf/fi/xi-20041222-1785.html

Merknader til Akvakulturdriftsforskriften:
http://www.mattilsynet.no/multimedia/archive/00012/Merknader_til_forskr_12075a.pdf

Lov om veterinærer og annet dyrehelsepersonell: http://www.lovdata.no/all/nl-20010615-075.html

IK-Akvakultur forskriften med merknader:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20040319-0537.html

Web-site for hjelp ved innføring av IK-Akvakultur:
http://62.113.154.12/ik_veileder

Forskrift om forskrivning, tilvirkning og distribusjon m.v. av medisinfôr til dyr, fugler, fisk og andre akvatiske organismer:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19960628-0693.html

Forskrift om forebygging, begrensning og utrydding av sykdommer hos akvatiske organismer:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19910704-0509.html

Omsetningsforskriften:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20031014-1239.html

Forskrift om særskilte beskyttelsestiltak ved innførsel av akvatiske dyr og produkter av disse:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20030530-0661.html

Forskrift om transport av akvatiske organismer:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19970220-0193.html

Forskrift om rengjøring og desinfeksjon av akvakulturanlegg m.v.:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19970220-0194.html

Vannbehandlingsforskriften:
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19970220-0192.html

Genteknologiloven: http://www.lovdata.no/all/hl-19930402-038.html

Forskrift om innesluttet bruk av genmodifiserte dyr (dyreforskriften):
http://www.lovdata.no/for/sf/ho/xo-20011221-1602.html

Forskrift om merking, transport, import og eksport av genmodifiserte organismer:
http://www.lovdata.no/for/sf/md/xd-20050902-1009.html

Legemiddelloven: http://www.lovdata.no/all/hl-19921204-132.html

Legemiddelforskriften: http://www.lovdata.no/for/sf/ho/xo-19991222-1559.html

Retningslinjer vedrørende klinisk utprøving av vaksiner til fisk:
http://www.legemiddelverket.no/klut/retningslinjer-fiskevaksine.htm

Forskrift om utføring av aktiviteter i petroleumsvirksomheten (aktivitetsforskriften):
http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20010903-1157.html

Rapport om Forsknings behov innen dyrevelferd i Norge:
http://www.forskningsradet.no/CSStorage/Flex_attachment/82-02156-4%20Dyrevelferd.pdf

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Powers DA (1989): Fish as model systems. Science 246:352–358

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