AGENCE FRANÇAISE
DE SÉCURITÉ SANITAIRE
DES ALIMENTS

AFSSA–Request no. 2009-SA 0288
Related request no. 2001-SA-0075

Maisons-Alfort, 19 May 2010

OPINION*

THE DIRECTOR-GENERAL

of the French Food Safety Agency on the risk assessment of effluents from processing plants of Category 1, 2 and 3 animal by-products intended to be reused for the irrigation of food and feed crops

REVIEW OF THE REQUEST

The French Food Safety Agency (AFSSA) received a request for an Opinion on the risk assessment of effluents from processing plants of Category 1, 2 and 3 animal by-products intended to be reused for the irrigation of food and feed crops.

CONTEXT

On 18 November 2009, the Directorate General for Food (DGAL) requested that AFSSA conduct a 'risk assessment accompanied by recommendations for managing the use of liquid effluents from plants that collect, store, handle and process Category C1, C2 and C3 by-products of animal origin as per Regulation (EC) no. 1774/2002, as well as from slaughterhouses'.
In agreement with the DGAL, AFSSA restricted the scope of the assessment to the reuse of liquid effluents for the irrigation of food and feed crops (since green spaces come under AFSSET's field of expertise).

For the record, a draft interministerial Order from March 2001 "determining from a health perspective the technical requirements, implementation and monitoring procedures applicable to the use of water originating from urban wastewater treatment for the watering or irrigation of crops and green spaces" prepared by the French Ministries of Health, the Environment and Agriculture was submitted by the Directorate General for Health (DGS) to AFSSA for an opinion. AFSSA produced a report entitled "*Réutilisation des eaux usées traitées pour l'arrosage ou l'irrigation*" [Reuse of treated wastewater for watering or irrigation] in November 2008. AFSSET, which received a request regarding a later version of the Order, issued an Opinion on 8 October 2009[1].

EXPERT ASSESSMENT METHOD

The collective expert assessment was conducted by experts from the 'Water', 'Transmissible spongiform encephalopathies (TSE)', 'Animal Health' and 'Microbiology' Scientific Panels. The Opinion was presented and its conclusions validated during meetings of the TSE Scientific Panel on 16 April, the Animal Health Scientific Panel on 7 April, the Microbiology Scientific Panel on 14 April and the Water Scientific Panel on 4 May 2010.

27-31, avenue
du Général Leclerc
94701
Maisons-Alfort cedex
Tel 01 49 77 13 50
Fax 01 49 77 26 13
www.afssa.fr

FRENCH
REPUBLIC

* This opinion takes into account the erratum dated 22 March 2011, modified on page 15, concerning the publication by Brown et Gajdusek, 1991.

[1] AFSSET Opinion of 8 October 2009 regarding the draft interministerial Order on the use of water originating from urban wastewater treatment for the irrigation of crops and green spaces.

To estimate the risk with regard to the possible reclamation of effluents for the irrigation of food and feed crops (intended for humans or animals), the experts reviewed the different regulations in effect, as well as actual practices. The assessment then looked at identifying (and characterising) hazards in raw effluents from the various plants, as well as the efficacy of the different treatments used in industrial processes with regard to these hazards.

The assessment focused on biological risks by examining the hazards related to unconventional transmissible agents (pathogens responsible for prion diseases) and conventional transmissible agents (bacteria, viruses, parasites, etc.).

In addition, a one-day symposium was organised with the industry professionals and ministerial services concerned, prior to the assessment, as well as a tour of a Category 1 and 2 animal by-product processing plant.

GLOSSARY

Animal by-product (ABP) processing plants

Slaughterhouse:
Facility used to slaughter and trim animals whose meat is intended for human consumption. In 2010, there were 285 slaughterhouses for domestic ungulates in France, of which 215 were multi-species slaughterhouses and 70 specialised slaughterhouses (including 40 for small ruminants) (source: DGAL). Mixed slaughterhouses may have species-dedicated slaughter lines (ergonomic set-up);

Animal by-product processing plant:
Facility that treats Category 1 material prior to final disposal (Category 1 processing plant or, according to the former term, 'rendering plant'), prior to its final disposal or new processing or use (Category 2 processing plant), or that transforms Category 3 material into processed animal proteins and other processed products;

Intermediate plant:
Facility that handles or temporarily stores unprocessed Category 1, 2 or 3 material for its transportation to a final destination. Depending on the category of by-product, a distinction is made between Category 1 or 2 intermediate plants and Category 3 intermediate plants. Certain activities may take place, such as the removal of hides or skins or *post mortem* inspections (Category 1 and 2), sorting and/or cutting and/or refrigeration or block freezing and/or temporary storage (Category 3);

Storage plant:
Facility other than an intermediate plant that temporarily stores processed products for final use or disposal;

Sorting of material and pre-treatment of wastewater

Screenings:
Visible and solid material retained by the screen-rake (filter located at the intake of conduits conveying wastewater from slaughterhouses or animal by-product processing plants when pre-treatment is required). The mesh size of the screens is regulated at 6 mm. As a result, the screen-rake separates solid by-products (Categories 1 or 2 depending on the species) from effluents. The solid material from ruminants (cattle and small ruminants) is classified as Category 1;

Grit:

Animal matter or visible, solid sediment retained by degritting systems that serve as a pre-treatment;

Treatments and uses for effluents

Wastewater treatment plant (**STEP):**
System designed for the purification of wastewater (effluents) and comprised of structures and service equipment. The term '*STEP urbaine'* (urban treatment plant) is used for plants under municipal or inter-municipal operation, and *'STEP privée'* (private treatment plant) for others.

Solid sludge (Order of 8 January 1998[2]):
Dehydrated primary or secondary sludge, which, when stored at a height of 1 metre, forms a slope of at least 30°;

Stabilised sludge (Order of 8 January 1998):
Primary or secondary sludge that has undergone a stabilisation treatment consisting in inhibiting or completing fermentation (the addition of quicklime for example);

Hygienised sludge (Order of 8 January 1998):
Sludge that has been treated to reduce any pathogens it contains to an undetectable level.

Clarification:
Stage that separates biological sludge from treated water.

Naturally aerated lagoon system:
Biological treatment process comprising a series of wastewater lagoons that are not artificially aerated.

Ultrafiltration:
Pressure-driven membrane filtration operation, comprising membranes with an average pore size of between 2 and 100 nanometres (nm). The corresponding cut-offs vary between 2000 and 500,000 Da. The cut-off is the diameter of particles retained with a reduction of 1 log.

Sterilisation/heat treatments:
For the purpose of this report, the sterilisation of effluents refers to the application of a heat treatment at 133°C at a pressure of at least 3 bars for 20 minutes as provided for by Regulation (EC) 1774/2002.

Spraying:
Supply of water from above, so that it falls as artificial rain on plants.

Irrigation:
Supply of water to compensate for a lack of water in the soil with regard to the needs of plants[3].

Infiltration:
Transfer of water through the unsaturated zone of the soil to the infiltrating water body or table.

---

[2] Order of 8 January 1998 laying down the technical requirements applicable to landfarming on agricultural soil.

[3] **According to the draft Order as it was submitted on the reuse of treated wastewater, there are several types of irrigation:**

1. gravity irrigation: water is supplied to plants by filling small basins, by borderstrips or canals, by irrigation trenches, channels or furrows;
2. localised irrigation:
   a. subsurface: water is supplied via perforated pipes, micro-irrigation emitters or buried lines;
   b. surface: water is distributed using emitters or spraylines near the plant;
3. spray irrigation: pressurised water is supplied to plants in the form of artificial rain using spray heads or sprinklers.

Sludge/landfarming:
Addition of a mix of water and solid matter to crops for soil improvement purposes. The application rate is determined according to the agronomic value of what is being spread.

Hazards:

Unconventional transmissible agents (UTAs):
UTAs or prions are unconventional infectious agents as they are mainly of proteinaceous origin. They are formed of aggregates rich in abnormally folded prion protein (PrP) and are responsible for transmissible spongiform encephalopathies (TSEs).

Conventional transmissible agents (CTAs):
Viral pathogens, prokaryotes (bacteria) or eukaryotes (parasites).

Other terms used:

Pastureland:
Land covered with grass or other herbage grazed by or used as feedingstuffs for farmed animals.

Specified risk material (SRM):
Tissues or organs liable to contain TSE infectivity and listed in EC Regulation no. 999/2001 and the Order of 17 March 1992[4]. They are systematically removed from the food chain (human and animal) and destroyed.

DISCUSSION

AFSSA's line of reasoning is based on the expert assessment of the Water, TSE, Animal Health and Microbiology Scientific Panels, and is broken down into three sections: status report on regulations, status report on actual practices, and the identification and characterisation of biological hazards.

[4] Order regarding the conditions that slaughterhouses must meet for the production and marketing of fresh meat and that lays down the health inspection conditions for these plants.

I. Status report on regulations

1. European Community Regulation on animal by-products (EC 1774/2002[5])

Regulation (EC) No. 1774/2002 lays down the health rules concerning Category 1, 2 and 3 (C1, C2, C3) animal by-products (ABPs) not intended for human consumption (and originating from collection, storage, handling and processing plants).

This Regulation provides for requirements on:

- adequate facilities for cleaning and disinfecting receptacles receiving animal by-products, as well as vehicles (particularly wheels);
- the cleaning of containers, receptacles and vehicles used to transport unprocessed material to incineration plants;
- the wastewater disposal system;
- the treatment of wastewater in contact with Category C1, C2, C3 ABPs:
  - in Category C1 and C2 processing and intermediate plants,
  - from incineration and co-incineration plants coming from the cleaning of containers used to transport unprocessed material,

so as to ensure that no pathogens remain.

The Regulation also provides for a pre-treatment stage (referred to as screening), which involves sieves with openings that do not exceed 6 mm to retain solid particles from the wastewater.

This wastewater pre-treatment stage is provided for:

- Category 1 processing plants,
- premises from which specified risk material (SRM) is removed,
- slaughterhouses and Category 2 processing plants.

It should be noted that the Regulation's scope of application concerns only the fate of material retained by the 6-mm filter.

After pre-treatment, the wastewater from Category 1 or 2 processing plants is treated in the same capacity as the wastewater from plants that receive only Category 3 material in accordance with relevant national or community legislation. The provisions are outlined below.

The following are defined as Category 1 by-products (*cf.* description in Annex 1):

- Cadavers (or parts of cadavers) of animals that died from or were suspected of being infected by a transmissible spongiform encephalopathy (TSE) or slaughtered as an eradication measure;
- Bodies of wild animals suspected of being infected with a communicable disease;
- SRM and cadavers of animals containing SRM (ruminants);
- Material containing prohibited or controlled substances, or environmental contaminants;
- Material of animal origin collected during the treatment of wastewater from C1 processing plants and other premises from where specified risk material is removed, including screenings, grit, grease and oil mixtures, sludge and material removed from drains from those premises, unless such material contains no SRM;
- Bodies of domestic, zoo or circus animals;

[5] REGULATION (EC) No. 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption, repealed by REGULATION (EC) No. 1069/2009 of the European Parliament and of the Council of 21 October 2009, applicable as from 4 March 2011.

- Bodies of research animals;
- Catering waste from means of transport operating internationally;
- Mixtures of material as soon as they contain Category 1 by-products as defined below.

C1 by-products are:

- either directly incinerated in an approved plant;
- or treated in an approved processing plant, by applying a heat treatment (formalised Methods 1 to 4). The product obtained is then incinerated or co-incinerated or, in some cases, buried.

The Regulation defines Category 1 material processing methods according to size, as shown in Table 1:

Table 1: ***Definition of Category 1 material processing methods***

Method	Size	Treatment
1	50 mm max	133°C / 20 min / 3 bars
2	150 mm max	T°C > 100°C / 125 min; T°C > 110°C / 120 min and T°C > 120°C / 50 min
3	30 mm max	T°C > 100°C / 95 min; T°C > 110°C / 55 min and T°C > 120°C / 13 min
4	30 mm max presence of added grease	T°C > 100°C / 16 min; T°C > 110°C / 13 min; T°C > 120°C / 8 min and T°C > 130°C / 3 min

Note that for processed animal proteins, only Method 1 has an inactivating effect on TSE agents (with a reduction of 2 to 7 log depending on the strain) (Giles K *et al.*, 2008; Taylor, 2000). However, they can be treated by any of the methods from 1 to 4 since they are then incinerated. In this case, the proteins must be permanently marked (with glutaraldehyde for example).

The following are defined as C2 by-products (*cf.* description in Annex 1):

- Manure and digestive tract content;
- Materials collected when treating wastewater from slaughterhouses or from Category 2 processing plants;
- Material containing residues of veterinary drugs;
- Imported products that fail to comply with health requirements;
- Cadavers of animals other than those in Category 1;
- Mixtures of Category 2 and 3 by-products.

Depending on its nature, Category 2 material can:

- Either be treated as Category 1 material and thus incinerated;

- Or, for manure, digestive tract content, milk and colostrum, be shipped to an approved biogas production or composting plant, landfarmed or converted into compost or biogas;
- Or, after processing in a Category 2 processing plant, be incinerated or co-incinerated, buried, converted into compost, biogas, organic fertiliser or shipped to an oleochemical plant.

The following are defined as C3 by-products (*cf.* description in Annex 1):

- Co-products from slaughtered animals that are fit for human consumption but not used for commercial reasons;
- Parts of slaughtered animals that are free of communicable disease;
- By-products from agri-food production;
- Former foodstuffs;
- Blood, skin and hides, appendages, not salvaged at the slaughterhouse;
- Catering waste.

Category 3 by-products are:

- Either treated as Category 1 material and thus incinerated;
- Or processed in Category 3 processing plants, technical plants, petfood plants, biogas production or composting plants.

2. Reuse of effluents

Few French regulatory texts make explicit reference to the effluents treated in wastewater plants. For this reason, the experts felt it was also relevant to study the regulations on landfarming, a practice that has similar impacts on human and animal health as the reuse of treated effluents. The following sections thus outline both the regulations governing the reuse of treated effluents and those governing landfarming, bearing in mind that the latter does not apply to the subject matter of this report.

a. General context

- **Order of 22 June 2007**[6] on the collection, transport, and treatment of wastewater in agglomerations lays down the minimal technical requirements as well as the monitoring thereof, in particular:
  - Article 6 on the connection of non-domestic effluents to the collection system;
  - Article 10 on the discharge of effluents treated in wastewater plants (STEP) specifies in particular that "in the event that treated effluents cannot be discharged into the surface water, the treated effluents can either be disposed of by infiltration into the soil, if the soil is suitable for this mode of disposal, or reused for watering green spaces or irrigating crops, in accordance with the provisions laid down by Order of the Minister of Health and the Minister of the Environment.";
  - Annexes I to IV determining the minimal performances of the two categories of treatment plant in agglomerations in terms of maximum concentration and/or minimum yield to be reached for parameters characterising the organic pollution load; nitrogen and phosphorus requirements apply to zones sensitive to eutrophication;

[6] Order of 22 June 2007 on the collection, transport, and treatment of wastewater in agglomerations, as well as the monitoring of their operations and efficacy, and on individual wastewater systems that receive a gross load of organic pollution greater than 1.2 kg/day of BOD.

- Annex V listing the substances that non-domestic effluents must not contain in concentrations likely to lead to the production of sludge or non-compliant discharge into the receiving water body.

Note that no requirements for the microbiological quality of wastewater or treated wastewater are provided for in this ministerial Order.

- **Order of 8 January 1998**[7] laying down the technical requirements applicable to landfarming on agricultural soil outlines :
  - the concept and management of landfarming ;
  - the quality of sludge and safe practice ;
  - monitoring procedures including sludge analyses for trace metal compounds and selenium, trace organic compounds, and characterisations of their agronomic value for which the threshold values are listed in the annexes. Provision is also made to search for any substance or microorganism that could be found in significant quantities in sludge due to the nature of the treated effluents. Soil is also monitored.

In certain specific landfarming provisions, the hygienised sludge produced must comply with the count limits set for *Salmonella spp*, enteroviruses, viable pathogenic helminth eggs, and this as soon as the treatment unit is under operation. Heat-resistant coliforms are counted during the characterisation phase of the process, as well as during monitoring.

Minimum isolation distances and landfarming timeframes vary according to the nature of the activities to protect and the type of sludge being spread, i.e.:

- for pastureland or forage crops:
  - three weeks before livestock is turned out to pasture or the harvest of forage crops for hygienised sludge.
  - six weeks before livestock is turned out to pasture or the harvest of forage crops for other types of sludge.
- for land allocated for growing fruits and vegetables, with the exception of fruit tree farming: no landfarming during the growing period, regardless of the type of sludge;
- for land intended or allocated for growing fruits and vegetables in direct contact with the soil, or liable to be consumed raw:
  - ten months before the harvest, and during the harvest itself for hygienised sludge.
  - eighteen months before the harvest, and during the harvest itself for other types of sludge.

b. <u>Regulatory framework specific to certain classified facilities for the protection of the environment (plants treating Category 1, 2 and 3 animal by-products)</u>

- **Amended Orders of 12 February 2003** regarding:
  - requirements applicable to classified facilities subject to authorisation under Section 2730 (treatment of by-products of animal origin, including scraps, inedible offal and cadavers, excluding activities referred to by other sections of the nomenclature, and diagnosis, research and teaching facilities) ;
  - requirements applicable to classified facilities subject to authorisation under Section 2731 (disposal sites for by-products of animal origin, including scraps, inedible offal and cadavers, excluding skin/hide disposal sites, and diagnosis, research and teaching facilities).

These two Orders govern the treatment of effluents following 6 mm pre-filtration, as described earlier, and their discharge into the natural environment.

The collected effluents are divided into 3 categories:

- uncontaminated rainwater ;

[7] Order of 8 January 1998 laying down the technical requirements applicable to landfarming on agricultural soil.

- contaminated water and water that has been in contact with raw materials or surfaces contaminated by raw materials ;
- other types of water (wash water, including gas wash water, blow-down water, black water, etc.).

The destination of effluents and the type of treatment to be applied depend upon the category of the effluent. Moreover, the number of discharge outlets into the natural environment must be reduced to a minimum.
Provisions regarding the discharge of effluents into the natural environment (Annex I of these Orders) were drawn from the Order of 22 June 2007. Neither of the two Orders contains requirements for the microbiological quality of treated wastewater.
Provisions regarding landfarming made only in the interest of the soil or for crop nutrition (Annex II) are modelled on the provisions of the Order of 8 January 1998.
In certain specific cases (e.g. landfarming on pastureland or forage crops or land intended or allocated for growing fruits and vegetables, in direct contact with the soil, or liable to be consumed raw), landfarming is only permitted if there is "no risk associated with the presence of pathogens for certain activities".

Moreover, the Orders of 25 April 2008[8] amended the Orders of 12 February 2003 by abolishing the obligation to autoclave effluents at 133°C, for 20 minutes, at 3 bars, as per Articles 36 and 27 (Articles on contaminated water and water that has been in contact with Category 1 or 2 raw materials or with surfaces contaminated by Category 1 or 2 raw materials). Provision is now made for effluents to be treated so as to comply with the discharge threshold values (like the physico-chemical parameters) specified in Annex I. In addition, the plants must be equipped with effluent pre-treatment systems to retain and collect material of animal origin. This involves a screening pre-treatment system as described in Regulation (EC) no. 1774/2002.

According to this same Order, sludge from the treatment of Category 1 and 2 raw materials (unless exemption is granted by the Prefect) is retreated in the circuit for Category 1 and 2 material depending on the origin of the sludge (or, in the case of mixed categories, in the treatment circuit for Category 1 material). Sludge originating from plants treating Category 3 by-products may be reclaimed/converted.

c. <u>Regulatory framework specific to slaughterhouses</u>

- **Amended Orders of 30 April 2004** regarding:
  - the general requirements applicable to classified facilities for the protection of the environment subject to authorisation under Section 2010 'Slaughter of animals' which sets the requirements applicable to slaughterhouses for meat animals, poultry, rabbits and farmed game animals;
  - the general requirements applicable to classified facilities for the protection of the environment subject to declaration under Section 2010 'Slaughter of animals' which sets the requirements applicable to classified facilities included in an establishment that has at least one facility subject to authorisation when these facilities are not governed by the prefectoral order of authorisation.

These two Orders govern in particular the treatment and discharge of effluents, i.e.:
- water resulting from the activity (processing, washing);
- black water (toilet waste).

They stipulate:
- connection to a collective wastewater system under certain conditions,
- discharge into the natural environment subject to compliance with threshold values, which may require, in addition to screening (mesh: 6 mm), prior treatment of

[8] Order of 25 April 2008 amending the Order of 12 February 2003 regarding requirements applicable to classified facilities subject to authorisation under Section 2730 (treatment of by-products of animal origin) and the Order of 25 April 2008 amending the Order of 12 February regarding requirements applicable to classified facilities subject to authorisation under Section 2731 (disposal sites for flesh, cadavers, scraps and inedible offals of animal origina, excluding skin/hide disposal sites).

wastewater (consistency of parameters and threshold values by reference to the abovementioned Orders). The number of discharge outlets into the natural environment must be reduced to a minimum. The direct or indirect discharge into a groundwater body, even after wastewater treatment, is prohibited.

- a separated collection system[9].
- that 6 mm screenings (by-products) and unprocessed slaughterhouse by-products, including blood, cannot be landfarmed. In the case of ruminant slaughterhouses, they are incinerated.
- that without prejudice to restrictions laid out by the regulation for health reasons, effluents, with the exclusion of black water, that have undergone the pre-treatment specified in these Orders[10], can be landfarmed when the farmer does not possess a treatment plant. The requirements regarding the irrigation of farmland are outlined in less detail.

Note that there are no requirements as to the microbiological quality of treated wastewater in these ministerial Orders and that there is no specific regulation for the discharge of wastewater with respect to the risk associated with unconventional transmissible agents (UTAs).

This analysis of Community and national regulations highlights that wastewater from plants with activities related to the collection and processing of animal by-products and liable to contain UTAs are no longer compulsorily heat treated. This specific issue will be examined in the follow-up to the Opinion.

II. Status report on actual practices

Field data were obtained on the basis of information received from industry professionals during their hearing, or during the tour of the Category 1 and 2 by-product processing plant. Annex 2 shows the flowchart for effluent treatment and conversion at the site toured.
Another major source of information is the results of the survey conducted by the French Ministry of Ecology, Energy, Sustainable Development and the Sea on the treatment of effluents from Category 1 and/or 2 processing plants (April 2010).

- According to the trade union of French animal by-product industries (SIFCO):

"*Different types of wastewater treatment are implemented, depending on the installations.*

Of eleven C1 and C2 by-product processing plants (the first ten sites representing 99.9% of the tonnage of processed by-products):
- 4 sites have only a private wastewater treatment plant (STEP),
- 3 sites have a private treatment plant and thermal oxidizer(s),
- 3 sites have only thermal oxidizer(s),
- 1 site recycles all of its wastewater in the processing circuit for Category 1 and 2 animal by-products.

Of 44 intermediate plants processing Category 1 and 2 by-products:

[9] A stormwater collection system that is separate from the wastewater collection system.
[10] Pre-treatment that comprises *a minima* screening and, if necessary, macrostraining, degritting and grease removal.

- 3 sites have a private treatment plant (including one that sends its treated water to a C1-C2 processing plant),
- 41 sites send their wastewater to a C1-C2 processing plant.

Of 27 Category 3 by-product processing plants:
- 10 sites have only a private treatment plant,
- 5 sites send their wastewater to an urban treatment plant,
- 2 sites send from a private treatment plant to an urban treatment plant,
- 2 sites have a private treatment plant and thermal oxidizer(s),
- 1 site sends its wastewater to an urban treatment plant and has thermal oxidizer(s),
- 2 sites have only thermal oxidizer(s),
- 4 sites send their wastewater to a C3 processing plant,
- 1 site sends its wastewater to a C1-C2 processing plant.

Of 21 intermediate plants processing Category 3 by-products:
- 1 site has a private treatment plant,
- 13 sites send their wastewater to an urban treatment plant,
- 3 sites send directly for irrigation, infiltration or drainage,
- 4 sites send their wastewater to a C1 or C3 processing plant."

- According to the trade association Célene[11], "a survey was conducted in 2009 on 48 slaughter businesses (38 meat-animal slaughterhouses and 10 poultry slaughterhouses). Three-quarters of the businesses were connected to the wastewater system, while the others had their own treatment plant. The businesses with their own treatment plant were those that treated the most tonnage and more than half of the tonnage treated (54%) was in facilities equipped with an independent treatment plant. This figure was as high as 80% in the poultry sector".

1. Category 1 and 2 animal by-product processing plants and intermediate plants

According to the professionals interviewed at the hearing, the treated water corresponds to contaminated rainwater, water from the treatment of gaseous discharge from plants (particularly the vapour produced by cookers), water from the cleaning of containers, floors, buildings and vehicle wash stations, fluids from raw materials (pressed or not), skinning lines and flotation grease. This water is macrostrained, pre-filtered and then treated in private treatment plants when they exist. Facilities equipped with a thermal oxidizer (initially designed for the treatment of condensation) can dispose of part of their effluents using this process.
When the wastewater treatment is complete, the sludge from private plants is recycled in the treatment circuit for Category 1 material (at the head of the process). Landfarming on cropland and pastureland is subject to authorisation by the Prefect. Where there is no risk associated with the presence of pathogens, regulations provide for a 21-day withholding period between landfarming and turning livestock out to pasture. This period increases to six weeks if no report has been made on the absence of risk associated with the presence of pathogens.

Effluents can be discharged into the natural environment or used to irrigate crops (maize, wheat and rapeseed) as well as plantations (trees) or for infiltration. If they are used to water feed crops or pastureland, there is a 21-day withholding period between the last application and grazing or harvest. Note that while regulations (particularly the aforementioned Orders of 12 February 2003) make no provision for the irrigation of crops or plantations, this has already been done.

During their hearing, industry professionals indicated that the sectors treating effluents from Category 1 and 2 animal by-product processing plants and certain Category 1 and 2 intermediate plants can

[11] Association with members from the following groups of related trades: CNADEV (Comité national des abattoirs et ateliers de découpe de volailles, lapins et chevreau) / Coop de France Bétail et Viande / FIA (Fédération des Industries Avicoles) / FNEAP (Fédération Nationale des Exploitants d'abattoirs prestataires de service) / FNICGV (Fédération Nationale de l'Industrie et des Commerces en Gros des Viandes) / SNIV-SNCP (Syndicat national de l'industrie de la viande and the Syndicat national du commerce du porc).

use membrane filtration processes. The example of a site using ultrafiltration was given, claiming the retention of particles greater than 40 nm.

According to the results of a survey conducted by the French Ministry of Ecology, Energy, Sustainable Development and the Sea, of the 13 French plants that treat Category 1 and 2 by-products:
- 7 continue to use heat treatments for their effluents (133°C/20 min/3 bars);
- 6 have stopped sterilising their effluents, that are sent (except in one case) after pre-treatment to a treatment plant specific to the installation. Note that 3 installations declared that they had implemented an ultrafiltration system.

With regard to the aqueous discharge of the 6 plants that stopped using heat treatment:
- 4 plants discharge into rivers;
- 1 plant reclaims water for irrigation or disposes of it by infiltration;
- no information is available for the last plant.

Lastly, with regard to the fate of the sludge from these 6 plants:
- 4 plants reintroduce sludge into the by-product treatment circuit.
- 2 plants have authorisation from the Prefect for landfarming.

The Order of 25 April 2008 makes it possible to discharge into the environment effluents from the treatment of Category 1 animal by-products that have not undergone prior heat treatment (133°C/20 minutes/3 bars).

The experts visited a Category 1 and 2 processing plant in which effluents still undergo Method 1 heat treatment (133°C/20 min/3 bars) before being discharged into the natural environment or used for irrigation (willow trees and meadows belonging to the facility).

2. Slaughterhouses:

According to the professionals interviewed at the hearing, wastewater is screened with a mesh less than or equal to 6 mm, sometimes macrostrained with a mesh between 0.6 and 0.75 mm, then passes through a grease flotation unit, before being collected and sent to a treatment plant (private or urban).
According to ICPE[12] regulations, material recovered during water treatment after the 6-mm screen is considered waste: it can be used for landfarming, composting or methanisation (subject to compliance with regulations). The sludge originating from wastewater treatment (whether the plant is private or urban) is landfarmed (subject to compliance with regulations).
Note that depending on the species of animal treated and the different workshops, slaughterhouses may have to handle material from the three categories.

3. Category 3 by-product intermediate plants and Category 3 animal by-product processing plants:

According to the professionals interviewed at the hearing, the wastewater from these 48 plants, which represent 90% of the member plants of SIFCO, is degritted, macrostrained through a screen of 6 mm at most, de-greased and stored in sealed buffer tanks that make it possible to smooth production out evenly over a week. The water is then sent to urban treatment plants in 19 facilities (40% of cases) or treated in private facilities (treatment plant, thermal oxidizer). It is then 'burned' in 6-7% of cases in thermal oxidizers or treated by biological processes, clarified or ultrafiltered before being stored in naturally aerated lagoons and discharged into the environment (direct discharge, irrigation and infiltration) in 53% of cases.

[12] *Installation Classée pour la Protection de l'Environnement*, or Classified installation for the protection of the environment.

Water from treatment plants designated for the treatment of effluents from Category 3 intermediate plants and Category 3 by-product processing plants can be used to irrigate crops, but when it is used to water feed crops or pastureland, there is a 21-day withholding period between the last application and grazing or harvest. The sludge from these installations is spread on cropland and pastureland or converted into energy. There is a 21-day withholding period between landfarming and turning livestock out to pasture.

III. Identification and characterisation of biological hazards

1. Characterisation of potential biological hazards in effluents originating from different facilities

A number of pathogens can contaminate the liquid effluents from slaughterhouses and collection, storage, handling and processing plants for Category C1, C2 and C3 by-products of animal origin. These agents can, particularly when their initial concentration in these effluents is sufficiently high, be found in the sludge and/or water originating from wastewater treatment plants.
They have two possible origins: the animal itself (living animal, carcass, internal organs, tissue fragments, etc.) or the plant's surfaces that can be contaminated by agents likely to persist in the environment (*Listeria monocytogenes*, *Salmonella enterica*, etc.). The list of pathogens hosted by the animal depends on its species, its geographic origin and its health condition. These agents will also differ depending on the type of plant concerned (slaughterhouse or collection, storage, handling and processing plant for Category C1 to C3 by-products of animal origin).
The tables below only take into account those pathogens whose resistance or capacity to sporulate enable them to persist significantly in effluents, with particular focus on food-borne agents.

a. Analysis of hazards associated with unconventional transmissible agents

Unconventional transmissible agents (UTAs) are pathogens responsible for transmissible spongiform encephalopathies (TSEs). TSEs are a group of fatal neurodegenerative disorders that affect humans and animals. These consist mainly of BSE (classical and atypical) in cattle and scrapie (classical and atypical) in small ruminants. The most common TSEs in humans are Creutzfeldt-Jakob diseases (sporadic, familial, iatrogenic and new variant CJD), familial fatal insomnia, kuru and Gerstmann-Staüssler-Scheinker syndrome. Other wild or domestic species are susceptible to developing TSEs (cats, deer, etc.). To date, these diseases have not been found naturally in pigs or poultry.

UTAs replicate predominantly in the central nervous system of the infected host. Infectivity titres at the terminal stage of the disease vary according to the strain of the agent and the infected species, but can commonly reach up to $10^{10}LD_{50}$[13]/g of brain tissue. The lymphoid organs are also frequently infected, particularly in the case of classical scrapie, but to a lesser degree. Some of these tissues (central nervous system and lymphoid tissue) are contained in SRM.

Only the UTA responsible for classical BSE is currently classified as a zoonotic pathogen[14], since it is responsible for the variant form of CJD. The BSE-L agent, responsible for cases of atypical BSE discovered recently, has a high zoonotic potential (Beringue *et al.*, 2008; Comoy *et al.,* 2008; Kong *et al.*, 2008). While classical scrapie has always been considered non-zoonotic, this affirmation nevertheless warrants some qualification. In a recent Opinion (dated 7 April 2010), AFSSA considered that while the transmission of scrapie to humans had not been established, it could not be excluded either. The discovery of atypical scrapie is too recent to benefit from sufficient testing to assess its zoonotic potential.

i. Resistance to inactivation procedures

UTAs are among the infectious agents most resistant to conventional decontamination procedures (dry heat, irradiation, etc.). Only certain chemical (sodium hypochlorite at 11 ppm, 1M NaOH, 1 hour) or heat (autoclaving at 134-138°C, 20 min, 3 bars) treatments can achieve a significant reduction in infectivity titres. Worthy of note, these procedures generally involve UTA inactivation and not sterilisation, and significant variations in resistance can be observed depending on the strain of UTA (Giles *et al.,* 2008). Highly infectious tissue or material has to be incinerated at a temperature greater than 800°C with combustion or pyrolysis for destruction (Circular DGS/5 C/DHOS/E 2 no. 2001-138 of 14 March 2001).

[13] Lethal dose for 50% of the inoculated population.

[14] Disease that can spread from animals to humans or from humans to animals.

ii. Persistence in the environment

UTAs are capable of persisting in the environment for years without losing their infectivity. This notion, which dates back many years, came from the observation that sheep could develop scrapie by grazing on meadows previously occupied by sick animals (Greig *et al.*, 1940). An Icelandic epidemiological study that followed up on attempts to eradicate scrapie in the country in the early 1980s showed that UTAs were capable of persisting in the environment for a minimum of 16 years (up to 21 years), and still be highly infectious (Georgsson *et al.* 2007). The same observations were reproduced in experiments in the USA with chronic wasting disease in deer and elk (Miller *et al.*, 2004), which is physiopathologically very similar to sheep scrapie.

Studies based on scrapie models adapted to laboratory rodents (263K hamster strain) showed that burying infected brain tissue for 3 years only reduced its infectious load by approximately sixty. (Brown, Gajdusek, 1991). This same study also showed that the bulk of the infectivity remained at the burial site. Runoff water did not seem to have a major 'percolator' effect. This study was reproduced more recently by Seidel *et al.* (2007). The 263K hamster strain was still capable of orally infecting recipient animals 21 months after burial in the ground. An extract of contaminated soil and soil from the neighbouring area mixed with water to which detergent had been added (sodium dodecyl sulphate (SDS)) also proved to be infectious, but to a lesser degree. The molecular mechanisms at the origin of this persistence are highly probably tied to the fact that the pathological prion protein (PrPSc), the basic component of UTAs, is adsorbed strongly and highly effectively to different types of soil (chalk, sandy soils), as well as to their mineral components (quartz, montmorillonite and, to a lesser degree, kaolinite) (Johnson *et al.*, 2006; Genovesi *et al.*, 2007; Ma *et al.,* 2007). The desorption of the PrPSc requires very drastic treatments (e.g. SDS, 100°C). Thus changes in ionic strength, non-denaturing detergents and chaotropic agents are not very effective (Johnson *et al.*, 2006; Rigou *et al.*, 2006). Johnson *et al.* also conducted experiments to study the consequences of adsorption of UTAs to montmorillonite on their oral infectivity, in order to mimic the natural route of infection of the host. A first study by their group (Johnson *et al.,* 2006) showed the initial infectivity titres holding steady (Johnson *et al.,* 2007). A more in-depth study by this group (Johnson 2007) showed that when they are in low quantities in the soil, the UTA-particle complexes in the soil have a detectable infectivity that is markedly higher than that of the non-complexed UTAs, the increase in titre nearing 3 log of infectivity (perhaps due to better bioavailability or lower degradation).

Available studies unequivocally conclude that the adsorption of UTAs in the soil is persistent, reduces their migration and can lead to a potentiating effect. It is an acknowledged fact that this environmental reservoir of infectivity contributes significantly to the horizontal transmission of TSEs, particularly scrapie in small ruminants, and chronic wasting disease in deer and elk. This horizontal transmission is a key factor in the persistence and propagation of these two TSEs, and contributes to their quasi-enzootic character in certain geographic areas.

iii. Quantitative aspects of unconventional transmissible agents

The risk of UTA, which increases with the age of animals, concerns scrapie (classical and atypical) in small ruminants and BSE (epizootic and atypical cases) in cattle.
BSE is a disease for which an active monitoring process, based on the detection of PrPSc in the brain of animals, was implemented in the early 2000s. Each year, approximately 300,000 cattle of more than 24 months of age are treated in Category 1 processing plants (data: DGAL) and tested for the presence of prion protein. In slaughterhouses, all animals over 48 months of age are tested (1.8 million tests per year) and SRM is removed. 2009 data reported 11 cases of BSE (6 classical, 2 type-H, 3 type-L)[15].

Only random samples are taken for scrapie (classical and atypical) in small ruminants. There is thus less certainty about the number of cases in slaughterhouses and rendering plants. The estimated cumulative prevalence of classical and atypical scrapie is around 1 per 1000 in France. Of the

[15] Data: AFSSA Lyon

400,000 adult sheep treated in Category 1 plants each year (2009 data), 400 are thus probably affected by scrapie. This estimate is consistent with exhaustive samples taken from 2005 to 2007 (over 300 cases/year), to which is added the SRM that is removed at the slaughterhouse and disposed of as Category 1 by-products.

To date, therefore, a significant number of cases of animal BSEs transit through slaughterhouses and collection, storage, handling or processing plants of Category C1 or C2 by-products of animal origin. With regard to the organisation of the sectors and the epidemiological development of diseases, the risk associated with UTAs is significantly higher for C1 processing plants and concerns small ruminants more particularly.

b. Analysis of hazards associated with conventional transmissible agents

i. Identification of conventional transmissible agents

The hazards associated with conventional transmissible agents (CTAs) that could be emitted into plant wastewater can be classified into three categories: usual, occasional or exceptional hazards.

Usual pathogens (or potential pathogens) (Table 2) are those likely to be hosted in animals regardless of their status, including when they are reputed to be healthy or when their condition, controlled during the *ante mortem* clinical examination, provides no grounds to believe that there is any risk to consumers. These animals can in fact be carriers (occasional or permanent) of a variety of pathogens (or potential pathogens), found on their skin and skin appendages, their mucous membranes, in their digestive tract or in their tissues.
The intestine in particular can host, without having any clinical effect on the animals, bacteria (enterobacteria such as *Escherichia coli*, *Salmonella enterica* and *Yersinia sp.*, *Listeria sp.*, *Erysipelothrix rhusiopathiae*, spore-forming bacteria such as *Clostridium perfringens* and *Clostridium botulinum*, mycobacteria like *Mycobacterium avium* subsp. *paratuberculosis*, etc.), enterotropic viruses (*Enterovirus*, *Rotavirus*, *Norovirus*, etc.), protozoans (cryptosporidia and other coccidia), similar organisms (microsporidia, *Giardia*, *Blastocystis*) and helminths. Futhermore, some environmental bacteria are naturally found in soil (*Clostridium sp., Listeria sp.,* etc.) and can be absorbed during grazing.
This list also includes pathogens that are widespread in certain livestock farms and more or less controlled through sanitary practices and vaccination. Examples from pig farming include *Parvovirus* and the *Circovirus* associated with piglet wasting disease (PWD).

Table 2: ***List of usual pathogens (CTAs) with a potential impact on animal and/or human health in France:***

Bacteria	Viruses	Parasites
Brachyspira pilosicoli (pg, plt) *Brachyspira hyodysenteriae* (pg) ***Heat-tolerant Campylobacter*** (pg, plt) ***Chlamydophila psittaci*** (plt) ***Clostridium perfringens*** (all species) ***Clostridium tetani*** (mam) ***Clostridium botulinum*** **(**plt) Other *Clostridium sp.* (mam) ***Erysipelothrix rhusiopathiae*** (pg, sp, plt) ***Escherichia coli*** **enteropathogens** ***Listeria monocytogenes*** (all species)	*Birnavirus* (plt: Gumboro disease) *Circovirus* (pg: piglet wasting disease) *Enterovirus* (pg) *Herpesvirus* (plt: duck plague) *Herpesvirus* (plt: Marek's disease) ***Hepevirus*** **for Hepatitis E** (pg) *Norovirus* (pg) *Parvovirus* (pg: parvovirus) *Parvovirus* (plt: Derszy's disease) *Pestivirus* (cw: mucosal disease) *Reovirus* (plt) *Rotavirus* (cw: Rotaviral enteritis) *Rotavirus* (pg: Rotaviral enteritis) *Sapovirus* (pg)	*Ascaris sp.* ***Balantidium coli*** (pg) *Capillaria spp.* (rum, plt) ***Cryptosporidium sp.*** (rum, hr, pg) Coccidia (rum, plt) ***Giardia intestinalis*** **Microsporidia** (mam, plt) *Oxyurus sp.* (gt) *Parascaris equorum* (hr) Strongyles (all species) *Strongyloides sp.* (all species) ***Toxocara spp.*** *Toxocara vitulorum* (cw) *Trichuris sp.* (rum)

Mycobacterium avium (plt, pg) *Mycobacterium avium subsp. paratuberculosis* (rum) ***Rhodococcus equi*** (hr) ***Salmonella spp.*** (all species) ***Staphylococcus sp.*** (all species) ***Streptococcus sp.*** (all species) ***Yersinia enterocolitica*** (all species) ***Yersinia pseudotuberculosis*** (plt, rab)		***Trichuris suis*** (pg)

Comment 1: Pathogens with a potential impact on animal health and human health appear in bold in the table.

Comment 2: The main emitting species are indicated in parentheses, using the following abbreviations:

cw: cows	gt: goats	hr: horses	rab: rabbits
mam: mammals	pg: pigs	plt: poultry	sp: sheep
rum: ruminants			

Occasional pathogens (Table 3) are observed in two situations:

- During the slaughterhouse health inspection, certain infections, such as metritis, mastitis, arthritis and abscesses, are discovered in animals and justify a full or partial seizure.
- Animals disposed of as part of the plan of action against certain animal diseases still found in France can be taken to the slaughterhouse, when the risk of spread is limited and controllable: this is the case of ruminants from TB-infected herds, pigs from brucellosis-infected herds, chickens and turkeys from flocks recognised as infected by *Salmonella* Typhimurium or Enteritidis.

Table 3: List of occasional pathogens (CTAs) with a potential impact on animal and/or human health in France:

Bacteria	Viruses	Parasites
Arcanobacterium pyogenes (pg, plt) ***Bacillus anthracis*** (mam) ***Brucella suis* biovar 2** (pg) *Brucella ovis* (sp) ***Burkholderia pseudomallei*** (hr) ***Chlamydophila ovis*** (rum) ***Chlamydophila abortus*** (rum) *Corynebacterium ovis* (sp, gt) *Coxiella burnetii* (rum) ***Francisella tularensis subsp. holarctica*** ***Leptospira interrogans*** (mam) ***Mycobacterium bovis*** (mam) *Salmonella Abortus ovis* (sp)	*Coronavirus* (pg: transmissible gastroenteritis) *Coronavirus* (cw: enteritis) *Lagovirus* (rab: hemorrhagic viral disease) *Paramyxovirus* (plt: Newcastle disease) *Pestivirus* (sp: border disease)	*Dicrocoelium sp.* (sp) ***Fasciola hepatica*** (rum, pg, hr) *Paramphistomum sp.* (rum)

Comment 1: Pathogens with a potential impact on animal health and human health appear in bold in the table.

Comment 2: The main emitting species are indicated in parentheses, using the following abbreviations:

cw: cows	gt: goats	hr: horses	rab: rabbits
mam: mammals	pg: pigs	plt: poultry	sp: sheep
rum: ruminants			

Exceptional pathogens (Table 4) are associated with the introduction or reintroduction of a disease that has been absent from Metropolitan France, and that is epizootic (such as foot and mouth disease, pig plague or influenza from a highly pathogenic viral strain) or non-epizootic (e.g. *Brucella abortus* or *Brucella melitensis* brucellosis) in nature.

Animals from herds that are suspected of having or are affected by an epizootic disease are not normally sent to the slaughterhouse. There is a risk, nevertheless, if animals arrive at the slaughterhouse before the disease has been diagnosed and the health warning has been given (the disease is discovered during *ante-* or *post-mortem* examinations at the slaughterhouse), if the epizootic disease is not under control, or if the slaughterhouse is located in a restricted area.
In the case of a non-epizootic disease, some animals can be sent to the slaughterhouse, under exceptional circumstances, or within the regulatory framework (ruminant brucellosis in particular).

Table 4: ***List of exceptional pathogens (CTAs) with a potential impact on animal and/or human health in France:***

Bacteria	Viruses	Parasites
Brucella abortus (cw) ***Brucella melitensis*** (sp, gt, cw) ***Brucella suis* biovar 1 and 3** (pg) ***Burkholderia mallei*** (hr)	*Aphtovirus* (rum, pg: foot and mouth disease) *Asfivirus* (pg: African pig plague) *Enterovirus* (pg: swine vesicular disease) *Herpesvirus* (pg: Aujeszky's disease) *Orthomyxovirus* (plt: highly pathogenic avian influenza) *Pestivirus* (pg: classical pig plague) *Teschovirus* (pg: Teschen disease)	***Linguatula serrata*** (rum, rab, hr, pg)

Comment 1: Pathogens with a potential impact on animal health and human health appear in bold in the table.

Comment 2: The main emitting species are indicated in parentheses, using the following abbreviations:
cw: cows gt: goats hr: horses rab: rabbits
mam: mammals pg: pigs plt: poultry sp: sheep
rum: ruminants

ii. Case of slaughterhouses

Before entering the slaughterhouse, animals are placed in holding rooms where their faeces and urine is collected in the form of liquid or solid manure, and does not, *a priori*, enter the normal circuit of slaughterhouse wastewater. However, these areas are scrubbed down and rinsed, and this wash water does enter the collection system.

During the different stages of carcass preparation and collection of offal and by-products, the pathogens carried by wastewater are primarily those found in or passing through the digestive tract (emitted particularly when washing casings). They correspond to what were described above as usual pathogens. The pathogen load can be high due to the large number of animals slaughtered.
Pathogens can also come from tissue fragments and various liquids spilled on the floor or equipment (during bleeding, skinning and splitting) and carried away with the wash water. They correspond mainly to what were defined above as occasional pathogens. The reinforcement of Food Chain Information (FCI) accompanying production animals to the slaughterhouse enables a more effective identification of some of these occasional pathogens. It is nevertheless important to note that certain pathologies will not be discovered until inspection at the slaughterhouse.

In conclusion, barring any exceptional incidents, any CTAs that may be found in slaughterhouse wastewater correspond mainly to pathogens of the digestive tract flora and the skin, and pathogens already present in a majority of herds. They are thus related to the CTAs found in the effluents from livestock farms and slaughterhouse holding rooms. However, the slaughterhouse could have a

greater diversity of pathogens than livestock farms, since the slaughtered animals are of multiple origins and come from herds of varying health profiles.

iii. Case of collection, storage, handling and processing plants of Category C3 by-products of animal origin

Category 3 by-products from slaughterhouses and processing plants can be considered free from pathogens, with the exception of possible surface contaminants, mainly bacteria originating from the digestive tract or storage and processing premises (*Salmonella enterica*, *Listeria monocytogenes*, *Campylobacter sp.*, *Clostridium perfringens*, etc.), identical to those discharged into urban wastewater as a result of meat processing operations and during home processing or the disposal of kitchen waste.

In conclusion, any CTAs that may be found in the effluents of these plants (C3) are mainly surface contaminants. Very occasionally, and particularly in periods of epizootic disease, they might consist of specific pathogens in inapparent carrier animals.

iv. Case of collection, storage, handling and processing plants of Category C1-C2 by-products of animal origin

Pathogens liable to contaminate liquid effluents from these plants mainly come from discharge (faeces, exudates, blood, etc.) accumulated in collection vehicle bins, tanks and storage areas, unloading and processing areas, as well as tissue debris (blood, placenta, etc.) carried by vehicle, surface and equipment wash water. They vary depending on the species of animal, geographic area and disease causing the animals' death or seizure from slaughterhouses.
However, these plants handle a much wider range of animal species that can include carnivores and omnivores, wild and domestic. Moreover, *Echinococcus* cestoda eggs and roundworm eggs could contaminate the wastewater from these plants.

All of the conventional pathogens listed in Tables 2 to 4 may be found in effluents from collection, storage, handling and processing plants of Category C1-C2 by-products of animal origin. Moreover, these plants receive cadavers and animals slaughtered for health policy measures, which, in addition to the aforementioned hazards, involve a wide variety of specific, unforeseeable hazards responsible for the death or euthanasia of animals.

c. Ranking of conventional transmissible agents by risk of transmission to animals and humans in raw effluents

The risk of contamination for humans and animals depends in particular on the likelihood of emission of pathogens into raw effluents, their concentration and pathogenicity, and lastly the likelihood of causing a food-borne infection at the concentrations encountered.
In the case of parasites, their impact will also depend on their direct infecting power (example of *Cryptosporidium spp*. oocysts), the period of maturation in the soil (roundworm eggs) or the possibility of reaching an environment that facilitates the encounter with an intermediary host (e.g. gastropods as intermediary hosts of trematodes). The main pathogens included are thus those emitted frequently, at sufficiently high initial concentrations and with a resistance or capacity to produce spores that enables them to persist in effluents.
Pathogens carried frequently by wild or domestic animals (dogs, cats) were not included, and most likely contribute to a dissemination that is significant but not caused by effluents.
Wastewater treatment, whether or not combined with withholding periods before turning animals out to pasture or heat treating plants, should then help control these hazards.

i. Regarding animal health

The agents belonging to the group of pathogens described earlier as 'usual hazards', emitted regularly and in greater quantity due to the high number of animals that host them, are no doubt the most likely to contaminate treated water from slaughterhouses. These pathogens generally do not differ from those emitted by animals into effluents from livestock production. One can consider, however, that using this water for irrigation and watering can contribute to the introduction of pathogens into livestock that were until then free of them.

The risk of spreading pathogens belonging to the group of 'occasional hazards' is more difficult to pinpoint, due to the quantitatively lower and probably irregular emission in the case of slaughterhouses. The risk is, however, higher for rendering plants.

The hazard associated with the emergence of cases of anthrax (*Bacillus anthracis*) or epizootic diseases like foot and mouth disease (*Aphtovirus*) or classical pig plague (*Pestivirus*), is managed earlier in infected livestock, and taken into account through special effluent management measures for the rendering plants concerned.
The domestic species put out to graze on irrigated pastureland are primarily ruminants and horses (this is usually not the case for pigs, poultry and rabbits).

Regardless of the facility of origin, other than UTAs, the main pathogens that are carried specifically by raw effluents to irrigated pastureland and present a risk of transmission to ruminants and horses are thus the usual pathogens liable to be transmitted through the digestive system, in particular:
- bacteria: *Erysipelothrix rhusiopathiae*, *Escherichia coli*, *Listeria monocytogenes*, *Mycobacterium sp.*, *Rhodococcus equi*, *Salmonella spp.*, *Yersinia sp.*;
- viruses: *Pestivirus* (mucosal disease), *Rotavirus*;
- parasites *Cryptosporidium spp*, coccidia, strongyles and roundworms.

ii. Regarding human health

In terms of human health, among the pathogens listed in Tables 1, 2 and 3, only those with the likelihood of carrying an infection at the given concentrations were included.

• Bacteria

Heat-resistant *Campylobacter*
The high prevalence of healthy carriage of *Campylobacter* by livestock is most likely the result of frequent contamination of effluents. Since human contamination is possible with very low quantities of *Campylobacter*, raw effluents are thus a potential source of human contamination.

Shiga toxin-producing *Escherichia coli* (STEC)
Cattle and sheep are the main reservoirs of STEC. This asymptomatic intestinal carriage is responsible for the contamination of the environment and raw effluents at concentrations that are most likely low, but that present a potential risk of human contamination by the ingestion of raw plants irrigated by these effluents.

Salmonella
In the majority of cases, the main salmonella serotypes are found in the digestive tract of animals, with no clinical signs.
Effluents contain salmonella at concentrations that are low (less than 100 bacteria/mL), but that present a potential risk of human contamination by the ingestion of raw vegetables irrigated by these effluents.

Yersinia enterocolitica and *Y. pseudotuberculosis*
The main animal reservoir of *Y. enterocolitica* is the pig, and less frequently other species. *Y. pseudotuberculosis* is above all found in birds, as well as in wild and domestic mammals (especially lagomorphs and rodents).
While moderately resistant in the environment, *Yersinia enterocolitica* can contaminate raw effluents from pig slaughterhouses and be found at potentially hazardous concentrations for consumers of raw vegetables irrigated by these effluents.

• Parasites:

In Metropolitan France, *Cryptosporidium* oocysts, microsporidia spores, certain fungi with, apparently, high zoonotic potential, are major problems. These agents are, as a rule, not highly pathogenic in immunocompetent hosts and reach low concentrations in countries with a high level of hygiene, which makes them more difficult to detect. However, in the wastewater from slaughterhouses and Category 1 and 2 by-product processing plants, these infecting forms could be concentrated.

Cryptosporidium
The species of this genus are monoxenous (= a single host) and infect the gastrointestinal tract of a number of vertebrate species including humans. Ubiquitous, resistant to chemical disinfection, these parasites constitute a non-negligible environmental infectious risk that can cause severe infections in the immunodepressed (AFSSA, 2002).

Giardia spp

There are several species of these flagellated protozoans, depending on the host. *G. intestinalis* is the most common protozoan in human intestinal infections. This parasite infects the small intestine of humans and occasionally of a number of mammals (cattle, sheep, horses, pigs, dogs, etc.). It is extremely widespread worldwide and responsible for a high morbidity rate (diarrhoea can become chronic in children). The most frequent route of contamination is the ingestion of water contaminated by cysts.

Microsporidia
Over the past few years, these microsporidia, which are pathogenic to humans, have been found in a wide range of seawater and freshwater vertebrates and invertebrates (Mathis *et al,* 2005). For patients who are immunodepressed or being treated with immunosuppressive drugs, the microsporidia can cause severe digestive, visceral and systemic symptoms.

*Blastocystis spp (*Straminopiles*)*
Noel *et al*., 2006 demonstrated that the same species are encountered in humans and a number of animal species, which suggests a high zoonotic potential (Souppart *et al.,* 2009, 2010). *Blastocystis spp.* is implicated in irritable bowel syndrome and other inflammatory bowel disorders.

The conventional transmissible agents that can be found in effluents and that present a potential risk of human contamination are thus heat-resistant *Campylobacter*, shiga-toxin producing *Escherichia coli*, *Salmonella spp*. and *Yersinia enterocolitica* for bacteria, and *Cryptosporidium spp*., Microsporidia, *Blastocystis* and *Giardia spp.* for parasites.

2. Persistence of biological hazards in treated effluents

a. Case of unconventional transmissible agents

To date, there has been no direct proof of contamination associated with the presence of UTAs in treated effluents. It is important, however, to point out that there are currently no suitable technical means for detecting the presence of these agents or prion protein in treated effluents and/or sludge. What is more, few studies have experimentally modelled the specific fate of the infectivity of UTAs and/or pathological prion protein in the water leaving treatment plants and/or in an aquatic environment. However:

- Hinckley *et al.* (2008) experimentally contaminated untreated wastewater with an experimental hamster UTA (strain 263K). They showed that this water, once treated, was not infectious and that the bulk of the pathological PrP and infectivity settled with the sludge. The 'quantitative' significance of this result is nonetheless limited by the fact that the measure of infectivity was performed by bioassay on recipient animals, infected orally, a route known for its poor efficacy in this model.
- Maluquer de Motes *et al*. (*2008*) intentionally contaminated the wastewater of an urban treatment plant with a scrapie-infected sheep brain homogenate and a BSE-infected cattle brain homogenate. The protein was detected up to 15 days post inoculation for BSE and up to 45 days post inoculation for scrapie. The reduction in the amount of protein gives reason to believe that the initial infectivity titre would not drop more than 2 log of infectivity.

While the effect of activated sludge wastewater treatment on UTA infectivity has not been specifically studied, a number of factors resulting in particular from experiments on the degradation of these agents by microbial flora and proteases give reason to believe that the reduction in infectivity would be relatively modest. While certain enzymes are capable of significantly reducing the infectivity of UTAs (Yoshioka *et al.* 2007: Dickinson *et al.* 2009), the necessary experimental conditions (temperature, pH conditions, presence of detergents), as well as the bacteria that produce these enzymes, are not those found in the sludge. These effects are, once again, dependent upon the strain. BSE infectivity thus seems to be particularly resistant to this type of treatment (Langeveld *et al*., 2007). Also worthy of note, naturally infected animals can excrete infectivity in their faeces (Tamguney *et al*., 2009), which is a sign of resistance to intestinal microbiota.

In order to provide quantitative data on the persistence of UTAs in effluents that are treated and then landfarmed, the fate of the infectivity of a scrapie-infected brain during the different stages of

inactivation/treatment carried out in C1-type plants was estimated using a textbook example[16]. The initial data used for the simulation stemmed from the preliminary results of experiments underway in the INRA/ENVT laboratory[17] (French national institute for agricultural research/Toulouse national veterinary school), which indicate that a dose the equivalent of 2 mg of brain in the terminal phase of classical scrapie kills 70% of animals infected orally (po). This dose corresponds approximately to the LD_{50} (lethal dose killing 50% of infected animals). The central nervous system of a sheep (which weighs 200 to 300 g) thus contains approximately 100,000 to 150,000 LD_{50} po.

Based on this approximation, two different scenarios can be examined by simulation (*cf.* Table 5):

Case 1: the central nervous system (125,000 LD_{50} po) enters the head of the C1 process.
The brain will be heat treated using Method 1, 2, 3 or 4. Method 1 will enable a reduction of infectivity averaging 3 log (but variable by strain). The other methods are not considered capable of achieving a significant reduction. The infectious particles then pass through the 6-mm filter. Assuming a particle with a 6-mm diameter is spherical, it will have a mass of approximately 0.1-0.2 g. A 6-mm filter retains an estimated 99% of the infected brain. It has also been estimated that the bulk of infectivity (99%) is found in the sludge, based on the publication by Hinckley *et al.,* 2008. At this stage in the process, Method 1 water treatment achieves at best a 7-log reduction in infectivity, while the other treatments only enable a 4-log reduction (12.5 LD_{50} po). The lack of sterilisation of effluents incident to the provisions of the Orders of 25 April 2008 does not allow for any further reduction. Landfarming does not significantly reduce prion infectivity, due to the persistence and adsorption of these pathogens in the soil and the lack of diluting effect in the landfarmed surface. The landfarming of effluents from C1-type processing plants that have treated a scrapie-infected brain could, without heat treatment of effluents, theoretically lead to the oral contamination of 12 animals at a dose equivalent to the LD_{50}. When the potentiating effect of adsorption in the soil (Johnson *et al.,* 2007) is included in the simulation, the risk is multiplied by 1000 and thus 1250 animals can, in theory, be contaminated at a dose equivalent to the LD_{50}.

Case 2: the central nervous system (125,000 LD_{50} po) contaminates the wash water of vehicles or intermediate plants. This water, in contact with infected central nervous systems, could contain 0.1 to 1% of contaminated tissue (125-1250 LD_{50} po). As in the first scenario, two treatments enable a 4-log reduction in infectivity; 1) the 6-mm filter, 2) sludge treatment. The landfarming of treated water does not, in this case, present a theoretical risk, barring a potentiating effect. In the event such an effect exists, 12.5 animals could be infected at a dose equivalent to the LD_{50}.

Note that the same type of analysis can be made for BSE. It must be borne in mind that the minimal infectious dose is in the order of 1 mg (Wells *et al.,* 2007), that the central nervous system weighs 1500g and that heat treatments will be less effective.

[16] Note that this exercise is restrictive since, generally speaking, the entire sheep enters with a significant contribution of lympoid tissue into the calculation of the animal's overall infectivity.
[17] Personal communication from O. Andreoletti.

Table 5: Estimate of risk based on a calculation to determine orders of magnitude.

Working hypothesis: 1 brain infected by scrapie passes through the circuit

	Best case*	Worst case**	Comments
sheep Oral LD_{50} (g)	0.002	0.002	Andreoletti *et al.*, unpublished data (> LD_{50})
sheep Oral LD_{50} (brain)	125000	125000	1 sheep brain = 200 to 300 g
Head of C1 process	-	-	
Heat treatment with Method 1* to 4**	125	125000	Estimated 3-log reduction for Treatment 1 and nothing for the others
6-mm filter	1.25	1250	Estimated 99% retention by the filter
Separation Sludge/Effluents	0.0125	12.5	Estimated 99% of infectivity goes into sludge, 1% into effluents (Hinckley *et al.,* 2008)
Method 1 Sterilisation	0.0000125	NA	Estimated 3-log reduction for scrapie
Adsorption in the soil/potentialisation	**0.0000125**	**12500**	Estimated absence of effect to a potentiating effect of 3 log (Johnson *et al.,* 2007)
If no sterilisation of effluents	**0.0125**	**12500**	
	-	-	
Wash water from vehicles and intermediate plants	-	-	
sheep oral LD_{50}	125	1250	Estimated that this water can contain the equivalent of 0.1 to 1% of tissue
6-mm filter	1.25	12.5	Estimated 99% retention by the filter
Separation Sludge/Effluents	0.0125	0.125	Estimated 99% of infectivity goes into sludge, 1% into effluents (Hinckley *et al., 2008*)
Method 1 Sterilisation	0.0000125	NA	Estimated 3-log reduction for scrapie
Adsorption in the soil/potentialisation	**0.0000125**	**125**	Estimated absence of effect to a potentiating effect of 3 log (Johnson *et al.*, 2007)
If no sterilisation of effluents	**0.0125**	**125**	

NA: not applied

In conclusion, this simulation suggests that in the absence of heat treatment specifically for the prion (133°C/20 min/3 bars), the wastewater treatment process cannot guarantee the sterility of UTAs responsible for scrapie in particular.

b. Case of conventional transmissible agents

The recommended proposals in AFSSA's report of November 2008 were based on the "identification of pathogenic microorganisms likely to be found in water and easily measurable" and their prevalence, on one hand; and the WHO's quantitative risk assessment process on the other. This process is carried out on the basis of an acceptable risk set at "10^{-6} DALY[18] lost per person per year (pppy) for all pathogens" and makes it possible to determine that a total reduction of 7 log is required for this health objective to be met, taking into account the most sensitive uses of treated wastewater (irrigation of root vegetables consumed raw). The WHO breaks down this 7-log reduction for pathogens as follows:

- a 4-log reduction needs to be obtained by the treatment process,
- a 2-log reduction due to the decrease in pathogens on irrigated plants[19],
- a one-log reduction due to the cleaning of vegetables in the home.

Different proposals for bacterial, viral and parasitic indicators have been studied, with in the end a pertinent choice of three indicators representative of the efficacy of treatments implemented: intestinal enterococci, F-specific RNA bacteriophages and spores of sulphite-reducing anaerobes. The main pathogens in animal and human health retained following the hazard analysis do not demonstrate greater resistance than the proposed efficacy indicators. Their reduction in treated water will thus be at least equal to that of the indicators.
E. coli monitoring helps ensure over time the efficacy of the treatment process described earlier.

In conclusion, with regard to UTAs, AFSSA reiterates the recommendations made in its 2008 report *Réutilisation des eaux usées traitées pour l'arrosage ou l'irrigation* (particularly in terms of reduction values and threshold values), and stresses the importance of determining the efficacy of different types of physical, chemical and/or biological treatments for effluents, combined with a global monitoring strategy for the smooth operation of the treatment process.

c. Efficacy of ultrafiltration

The professionals indicated that a certain number of processing plants perform ultrafiltration on their treatment plant effluents before discharge into the environment (which was confirmed by the French Ministry of Ecology, Energy, Sustainable Development and the Sea survey). It is important to stress that ultrafiltration is not a recognised method for eliminating prion UTAs (Circular DGS/5 C/DHOS/E 2 no. 2001-138 of 14 March 2001). The size of infectious particles associated with UTAs is not accurately known and can vary according to the infecting strain (Tixador *et al.*, 2010).
In the past, the size of the smallest infectious particle reported during inactivation experiments by ionising radiation corresponded to a PrPSc dimer, or approximately 60 kDa (Bellinger-Kawahara *et al.,* 1988, Gibbs *et al.,* 1978). It now appears that soluble forms of PrPSc are nevertheless highly infectious (Berardi *et al.*, 2006), which probably explains why ultrafiltration, even if it is potentially capable of reducing the infectious load of a sample infected by several log, is not totally inactivating. Thus a significant amount of infectivity can persist after the sample has passed through very small filters (15 nm), such as those traditionally used for the preparation of pharmaceutical products derived from plasma (Yunoki *et al.* 2008; 2010). This method is considered to achieve a 4-log reduction in infectivity at best (Yunoki *et al.*, 2010; You *et al.,* 2010).
Moreover, the membranes implemented in treatment processes are used for periods exceeding 5 years and flaws in porosity affecting their performance can emerge over time. And yet, to date, it is not possible to reveal flaws under 200 nm.

[18] Disability Adjusted Life Years: The DALY is used as a unit of risk measurement, taking into account the number of years lost following a premature death and the number of years with a disability.
[19] The WHO report does not specify the timeframe in which these reductions are reached.

IV. Conclusions: Estimation of risk regarding the possible reclamation of effluents for crop irrigation and recommendations

In conclusion, AFSSA recommends:

a) that the reuse of all effluents originating from animal by-product processing plants be conditioned by the prescriptions of the 2008 AFSSA report entitled *Réutilisation des eaux usées traitées pour l'arrosage ou l'irrigation*. This report recommends in particular:

- Regarding animal health:

-- that Category A[20] water may be used on pastureland and fields producing fresh fodder with no additional constraints;

-- that Category B[21] water may be used on pastureland and fields producing fresh fodder when respecting a 3-week withholding period (21 days) before turning animals out to pasture or harvest [22];

-- that Category C[23] water may be used on cereal or fodder crops but only for gravity or localised irrigation (all spray irrigation is prohibited).

- Regarding human health:

-- that Category A water may be used on market garden, fruit and vegetable crops with no additional constraints;

-- that Category B water may be used on market garden, fruit and vegetable crops on condition that these foodstuffs undergo suitable industrial heat treatment;

-- that Category C water may be used for fruit trees but only for gravity or localised irrigation (all spray irrigation is prohibited).

b) for effluents originating from collection, storage, handling or processing plants of C1/C2 by-products of animal origin that have been in direct or indirect contact with risk material[24], to use for the irrigation of feed and food crops only those effluents purified in treatment plants and heat treated in accordance with European Regulation 1774/2002 (Method 1: 133°C/20 min/3 bars).

Lastly, given the persistence of prion infectivity in the soil, AFSSA stresses the risks associated with reallocating the use of land that has been irrigated with effluents originating from plants reserved for C1-C2 by-products. It thus recommends only reallocating these fields for food and feed crops on the basis of a case-by-case study depending on the nature of the reallocation.

[20] Category A: suspended solids (SS) less than 15 mg/L and chemical oxygen demand (COD) less than 60 mg/L O_2, reduction of at least 4 log shown on three indicators, with an *E. coli* count of less than 250 CFU/100 mL (count corresponding to the swimming water quality limits for *E. coli*).

[21] Category B: SS and COD compliant with regulations on the discharge of treated wastewater for treatment plant outfall outside of the irrigation period, with a reduction of at least 3 log shown on three indicators, with an *E. coli* count of less than 10,000 CFU/100 mL.

[22] AFSSA proposes to modify the REUT report on this matter, by requiring a 3-week period (21 jours) in keeping with the Order of 8 January 1998 (for sludge) and the amended ICPE Orders of 12 February 2003.

[23] Category C: SS and COD compliant with regulations on the discharge of treated wastewater for treatment plant outfall outside of the irrigation period, with a reduction of at least 2 log shown on a combination of three indicators, with an *E. coli* count of less than 10,000 CFU/100 mL.

[24] Included in these effluents: all water in contact with Category 1 or 2 material or surfaces contaminated by this material.

The Director-General

Marc Mortureux

Annex 1: Description of animal by-product categories stipulated by Regulation (EC) 1774/2002

Category 1 by-product

Category 1 material shall comprise animal by-products of the following description, or any material containing such by-products:

a) all body parts, including hides and skins, of the following animals:

i) animals suspected of being infected by a TSE in accordance with Regulation (EC) No. 999/2001 or in which the presence of a TSE has been officially confirmed,

ii) animals killed in the context of TSE eradication measures,

iii) animals other than farm animals and wild animals, including in particular pet animals, zoo animals and circus animals,

iv) experimental animals as defined by Article 2 of Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes,

v) wild animals, when suspected of being infected with diseases communicable to humans or animals;

b) i) specified risk material, and ii) where, at the time of disposal, specified risk material has not been removed, entire bodies of dead animals containing specified risk material;

c) products derived from animals to which substances prohibited under Directive 96/22/EC have been administered and products of animal origin containing residues of environmental contaminants and other substances listed in Group B (Point 3), of Annex I to Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC, if such residues exceed the permitted level laid down by Community legislation or, in the absence thereof, by national legislation;

d) all animal material collected when treating wastewater from Category 1 processing plants and other premises in which specified risk material is removed, including screenings, materials from desanding, grease and oil mixtures, sludge and materials removed from drains from those premises, unless such material contains no specified risk material or parts of such material;

e) catering waste from means of transport operating internationally; and

f) mixtures of Category 1 material with either Category 2 material or Category 3 material or both, including any material destined for processing in a Category 1 processing plant.

Category 2 by-product

Category 2 material shall comprise animal by-products of the following description, or any material containing such by-products:

a) manure and digestive tract content;

b) all animal materials collected when treating wastewater from slaughterhouses other than slaughterhouses covered by Article 4, Section 1 (Point d), or from Category 2 processing plants,

including screenings, materials from desanding, grease and oil mixtures, sludge, and material removed from drains from those premises;

c) products of animal origin containing residues of veterinary drugs and contaminants listed in Group B (Points 1 and 2) of Annex I to Directive 96/23/EC, if such residues exceed the permitted level laid down by Community legislation;

d) products of animal origin, other than Category 1 material, which are imported from non-Member countries and, in the course of the inspections provided for in Community legislation, fail to comply with the veterinary requirements for their importation into the Community, unless they are returned or their importation is accepted under restrictions laid down under Community legislation;

e) animals and parts of animals, other than those referred to in Article 4, that die other than by being slaughtered for human consumption, including animals killed to eradicate an epizootic disease;

f) mixtures of Category 2 with Category 3 material, including any material destined for processing in a Category 2 processing plant, and

g) animal by-products, other than Category 1 or Category 3 material.

Category 3 by-product

Category 3 material shall comprise animal by-products of the following description, or any material containing such by-products:

a) parts of slaughtered animals, which are fit for human consumption in accordance with Community legislation, but are not intended for human consumption for commercial reasons;

b) parts of slaughtered animals, which are rejected as unfit for human consumption but are not affected by any signs of diseases communicable to humans or animals, and derive from carcasses that are fit for human consumption in accordance with Community legislation;

c) hides and skins, hooves and horns, pig bristles, and feathers originating from animals that are slaughtered in a slaughterhouse after undergoing ante-mortem *inspection, and were fit, as a result of such inspection, for slaughter for human consumption in accordance with Community legislation;*

d) blood obtained from animals, other than ruminants that are slaughtered in a slaughterhouse, after undergoing ante-mortem *inspection, and were fit, as a result of such inspection, for slaughter for human consumption in accordance with Community legislation;*

e) animal by-products derived from the production of products intended for human consumption, including degreased bones and greaves;

f) former foodstuffs of animal origin or former foodstuffs containing products of animal origin, other than catering waste, which are no longer intended for human consumption for commercial reasons or due to problems of manufacturing or packaging defects or other defects which do not present any risk to humans or animals;

g) raw milk originating from animals that do not show clinical signs of any disease communicable through that product to humans or animals;

h) fish or other sea animals, except sea mammals, caught in the open sea for the purposes of fish meal production;

i) fresh by-products from fish from plants manufacturing fish products for human consumption;

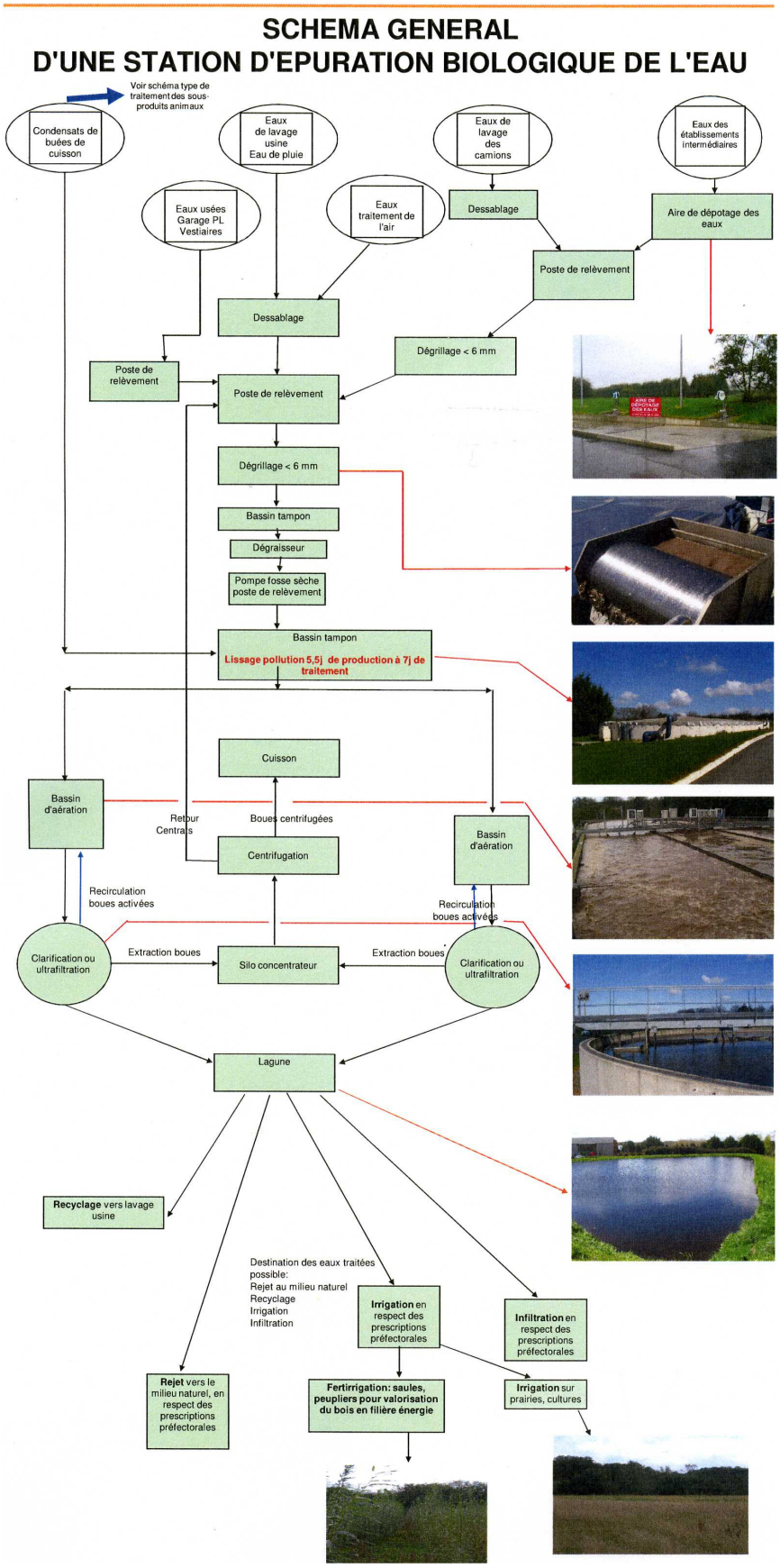
j) shells, hatchery by-products, and cracked egg by-products originating from animals which did not show clinical signs of any disease communicable through that product to humans or animals;

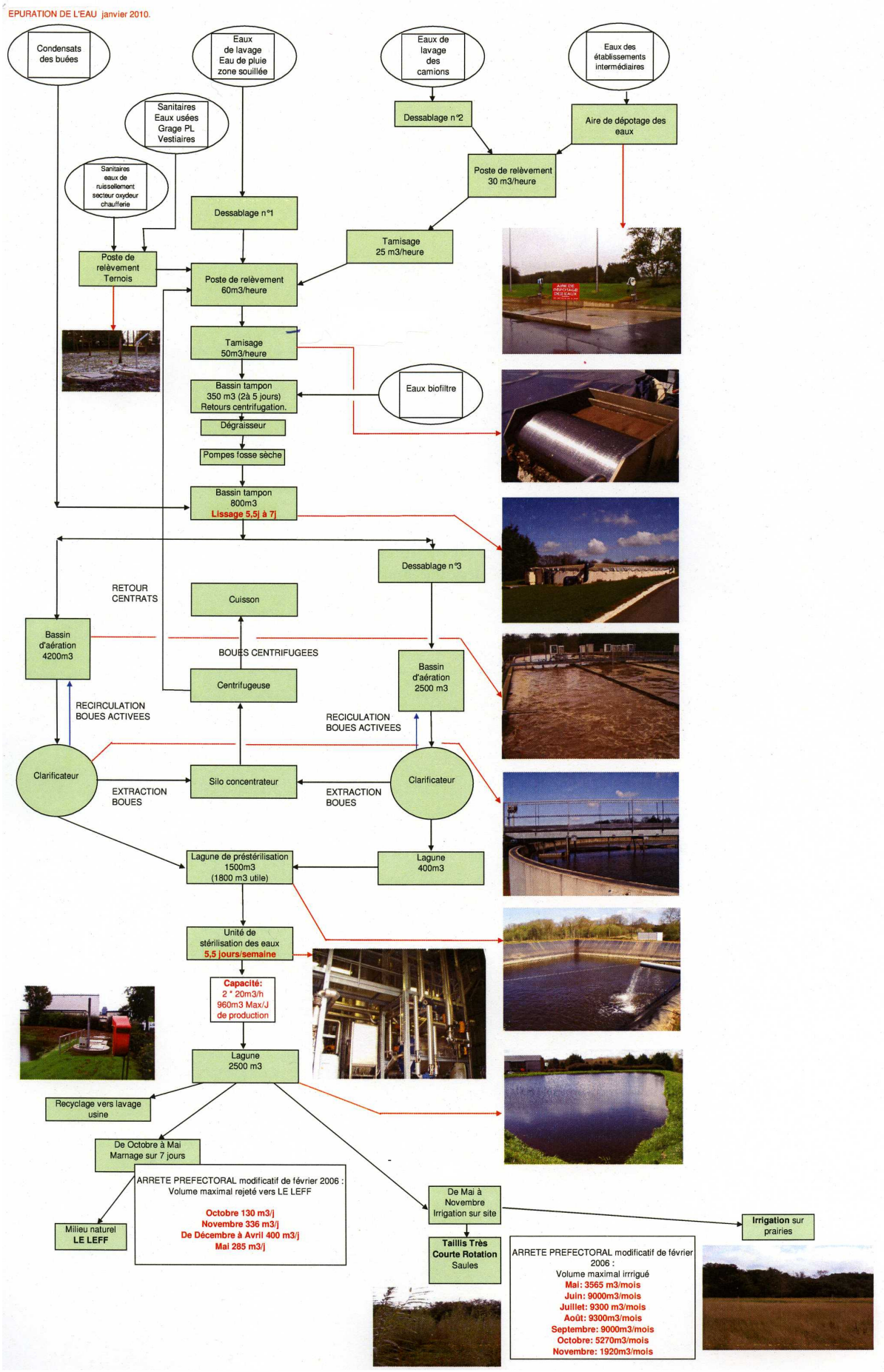
k) blood, hides and skins, hooves, feathers, wool, horns, hair, and fur originating from animals that did not show clinical signs of any disease communicable through that product to humans or animals, and

l) catering waste, other than as referred to in Article 4, Section 1 (Point e).

Annex 2: Examples of water treatment in animal by-product processing plants (source: SIFCO)

Réunion AFSSA-SIFCO du 15-01-10 – Document préparatoire du 7 janvier 2010




EPURATION DE L'EAU janvier 2010.
Condensats des buées
Eaux de lavage Eau de pluie zone souillée
Eaux de lavage des camions
Eaux des établissements intermédiaires
Sanitaires Eaux usées Grage PL Vestiaires
Sanitaires eaux de ruissellement secteur oxydeur chaufferie
Dessablage n°2
Aire de dépotage des eaux
Poste de relèvement 30 m3/heure
Dessablage n°1
Tamisage 25 m3/heure
Poste de relèvement Ternois
Poste de relèvement 60m3/heure
Tamisage 50m3/heure
Eaux biofiltre
Bassin tampon 350 m3 (2à 5 jours) Retours centrifugation.
Dégraisseur
Pompes fosse sèche
Bassin tampon 800m3 Lissage 5,5j à 7j
Dessablage n°3
RETOUR CENTRATS
Cuisson
Bassin d'aération 4200m3
BOUES CENTRIFUGEES
Centrifugeuse
Bassin d'aération 2500 m3
RECIRCULATION BOUES ACTIVEES
RECICULATION BOUES ACTIVEES
Clarificateur
EXTRACTION BOUES
Silo concentrateur
EXTRACTION BOUES
Clarificateur
Lagune de préstérilisation 1500m3 (1800 m3 utile)
Lagune 400m3
Unité de stérilisation des eaux 5,5 jours/semaine
Capacité: 2 * 20m3/h 960m3 Max/J de production
Lagune 2500 m3
Recyclage vers lavage usine
De Octobre à Mai Marnage sur 7 jours
ARRETE PREFECTORAL modificatif de février 2006 : Volume maximal rejeté vers LE LEFF
Octobre 130 m3/j
Novembre 336 m3/j
De Décembre à Avril 400 m3/j
Mai 285 m3/j
Milieu naturel LE LEFF
De Mai à Novembre Irrigation sur site
Irrigation sur prairies
Taillis Très Courte Rotation Saules
ARRETE PREFECTORAL modificatif de février 2006 :
Volume maximal irrrigué
Mai: 3565 m3/mois
Juin: 9000m3/mois
Juillet: 9300 m3/mois
Août: 9300m3/mois
Septembre: 9000m3/mois
Octobre: 5270m3/mois
Novembre: 1920m3/mois

KEY WORDS

Animal by-products, reuse, liquid effluents, irrigation.

BIBLIOGRAPHIC REFERENCES

AFSSA. 2002 Rapport sur les « Infections à protozoaires liées aux aliments et à l'eau »: « Evaluation scientifique des risques associés à *Cryptosporidium sp.* » [Report of 2002 on "Protozoan infections related to foodstuffs and water": "Scientific assessment of the risks associated with *Cryptosporidium sp.*"]

AFSSA *Rapport de novembre 2008 concernant la réutilisation des eaux usées traitées pour l'arrosage ou l'irrigation.* [Report of November 2008 on the Reuse of treated wastewater for watering or irrigation]

AFSSA. Sheet on *Campylobacter* spp.

AFSSA.2008. Sheet on enterohaemorragic *Escherichia coli* (EHEC).

AFSSA.2009. Sheet on *Salmonella* spp.

AFSSA (7 April 2010) *avis relatif au risque zoonotique des différentes souches connues d'EST chez les petits ruminants*. [Opinion on the zoonotic risk of the various known strains of TSE in small ruminants]

AFSSA.2006b. Sheet on *Yersinia enterocolitica, Yersinia pseudotuberculosis*

AFSSA.2009. Sheet on *Salmonella* spp.

Bellinger-Kawahara CG, Kempner E, Groth D, Gabizon R, Prusiner SB. Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. Virology. 1988 Jun;164(2):537-41

Berardi VA, Cardone F, Valanzano A, Lu M, Pocchiari M. Preparation of soluble infectious samples from scrapie-infected brain: a new tool to study the clearance of transmissible spongiform encephalopathy agents during plasma fractionation. Transfusion. 2006 Apr;46(4):652-8.

Beringue, V., L. Herzog, et al. (2008). "Transmission of atypical bovine prions to mice transgenic for human prion protein." Emerg Infect Dis **14**(12): 1898-901.

Brown, P. and D. C. Gajdusek (1991). "Survival of scrapie virus after 3 years' interment." Lancet **337**(8736): 269-70.

Comoy, E. E., C. Casalone, et al. (2008). "Atypical BSE (BASE) transmitted from asymptomatic aging cattle to a primate." PLoS One **3**(8): e3017.

Dickinson J, Murdoch H, Dennis MJ, Hall GA, Bott R, Crabb WD, Penet C, Sutton JM, Raven ND Decontamination of prion protein (BSE301V) using a genetically engineered protease. J Hosp Infect. 2009 May;72(1):65-70.

Genovesi, S., L. Leita, et al. (2007). "Direct detection of soil-bound prions." PLoS One **2**(10): e1069.

Georgsson, G., S. Sigurdarson, et al. (2006). "Infectious agent of sheep scrapie may persist in the environment for at least 16 years." J Gen Virol **87**(Pt 12): 3737-40.

Gibbs C J, Gajdusek D C, and Latarjet R: Unusual resistance to ionizing radiation of the viruses of Kuru, Creutzfeldt-Jakob disease, and scrapie**.** Proc Natl Acad Sci U S A. 1978 December; 75(12)

Giles, K., D. V. Glidden, et al. (2008). "Resistance of bovine spongiform encephalopathy (BSE) prions to inactivation." PLoS Pathog **4**(11): e1000206.

Greig JR (1940) Scrapie: Observation on the transmission of the disease by mediate contact. Vet J 96: 203–206

Hinckley, G. T., C. J. Johnson, et al. (2008). "Persistence of pathogenic prion protein during simulated wastewater treatment processes." Environ Sci Technol **42**(14): 5254-9.

Humphrey, T., O'Brien, S., Madsen, M. 2007. Campylobacters as zoonotic pathogens: A food production perspective. Int. J. Food MIcrobiol. 117, 237-257.

Johnson, C. J., J. A. Pedersen, et al. (2007). "Oral transmissibility of prion disease is enhanced by binding to soil particles." PLoS Pathog **3**(7): e93.

Johnson, C. J., K. E. Phillips, et al. (2006). "Prions adhere to soil minerals and remain infectious." PLoS Pathog **2**(4): e32.

Kong, Q., M. Zheng, et al. (2008). "Evaluation of the human transmission risk of an atypical bovine spongiform encephalopathy prion strain." J Virol **82**(7): 3697-701.

Langeveld, J; Groschup, MH; Buschmann, A; Becher, D; Wang, JJ; Shih, JCH, Enzymatic Degradation of PrPSc Fails to Dis-Infect Bovine BSE Brain Homogenates. Oral communication Neuroprion 2007 Edinburgh, Scotland, UK 26-28 Sept. 2007

Lejeune J.T., Besser T.E., Hancock, D.D. 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. Appl. Environ. Microbiol. 67, 3053-3057.

Loukiadis, E., Kérourédan, M., Beutin, L., Oswald, E., Brugère, H. 2006. Characterization of Shiga toxin gene (stx)-positive and intimin gene (eae)-positive *Escherichia coli* isolates from wastewater of slaughterhouses in France. Appl. Environ. Microbiol. 72, 3245-3251.

Ma X, Benson CH, McKenzie D, Aiken JM, Pedersen JA (2007) Adsorption of pathogenic prion protein to quartz sand. Environ Sci Technol 41: 2324–2330

Maluquer de Motes, C., M. J. Cano, et al. (2008). "Detection and survival of prion agents in aquatic environments." Water Res **42**(10-11): 2465-72.

Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. Clin Microbiol Rev. 2005 Jul;18(3):423-45. Review

Miller, M. W., E. S. Williams, et al. (2004). "Environmental sources of prion transmission in mule deer." Emerg Infect Dis **10**(6): 1003-6.

Mommeja, F. Contamination des effluents d'abattoir par des *Escherichia coli* producteurs de shiga toxines. Dissémination environnementale et conséquences en santé publique. Thèse de Doctorat Vétérinaire, Ecole Nationale Vétérinaire de Toulouse, 2004.

Munch, B., Larsen, E.H., Aalbaek, B., 1987. Experimental studies on the survival of pathogenic and indicator bacteria in aerated and nonaerated cattle and pig slurry. Biol. Wastes, 22, 49-65.

Rigou, P., H. Rezaei, et al. (2006). "Fate of prions in soil: adsorption and extraction by electroelution of recombinant ovine prion protein from montmorillonite and natural soils." Environ Sci Technol **40**(5): 1497-503.

Seidel, B., A. Thomzig, et al. (2007). "Scrapie Agent (Strain 263K) can transmit disease via the oral route after persistence in soil over years." PLoS One **2**(5): e435.

Souppart L, Sanciu G, Cian A, Wawrzyniak I, Delbac F, Capron M, Dei-Cas E, Boorom K, Delhaes L, Viscogliosi E. Molecular epidemiology of human Blastocystis isolates in France. Parasitol Res. 2009 Aug;105(2):413-21.

Tamgüney G, Miller MW, Wolfe LL, Sirochman TM, Glidden DV, Palmer C, Lemus A, DeArmond SJ, Prusiner SB. Asymptomatic deer excrete infectious prions in faeces. Nature. 2009 Sep 24;461(7263):529-32

Taylor, D. M. (2000). "Inactivation of transmissible degenerative encephalopathy agents: A review." Vet J **159**(1): 10-7.

Tixador P, Herzog L, Reine F, Jaumain E, Chapuis J, Le Dur A, Laude H, Béringue V. The physical relationship between infectivity and prion protein aggregates is strain-dependent.PLoS Pathog. 2010 Apr 15;6(4)

Wells, G. A., T. Konold, et al. (2007). "Bovine spongiform encephalopathy: the effect of oral exposure dose on attack rate and incubation period in cattle." J Gen Virol **88**(Pt 4): 1363-73.

Yoshioka, M., Y. Murayama, et al. (2007). "Assessment of prion inactivation by combined use of Bacillus-derived protease and SDS." Biosci Biotechnol Biochem **71**(10): 2565-8.

You B, Aubin JT, Le-Hir G, Arzel A, Laude H, Flan B. In vitro infectivity assay for prion titration for application to the evaluation of the prion removal capacity of biological products manufacturing processes. J Virol Methods. 2010 Mar;164(1-2):1-6.

Yunoki M, Tanaka H, Urayama T, Hattori S, Ohtani M, Ohkubo Y, Kawabata Y, Miyatake Y, Nanjo A, Iwao E, Morita M, Wilson E, MacLean C, Ikuta K Prion removal by nanofiltration under different experimental conditions. Biologicals. 2008 Jan;36(1):27-36. Epub 2007 Sep 24.

Yunoki M, Tanaka H, Urayama T, Kanai Y, Nishida A, Yoshikawa M, Ohkubo Y, Kawabata Y, Hagiwara K, Ikuta K. Infectious prion protein in the filtrate even after 15 nm filtration. Biologicals. 2010 Mar;38(2):311-3.