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SCIENTIFIC OPINION

Scientific Opinion on On-site treatment of pig carcasses¹

EFSA Panel on Biological Hazards (BIOHAZ)^{2, 3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

EFSA's Scientific Panel on Biological Hazards (BIOHAZ) was asked for a scientific opinion on an alternative method for processing Category (Cat) 2 Animal By-Products (ABP)⁴. The materials to be treated are placentas and fallen pigs; this implies that the animals died due to a disease, which in most cases was not properly diagnosed. The target parameters are: i) particle size less than 150 mm and ii) heating for 10 - 12 hours at 100°C. The end-product obtained is mixed with pig slurry and used as an organic fertiliser. According to the legislation in force, before being used as an organic fertiliser, Cat. 2 material should be treated with a sterilisation process (i.e. $133^{\circ}C/20 \min/3$ bars / 50 mm particle size). The most resistant hazards identified by the applicant as target to demonstrate the risk reduction are spores of pathogenic clostridia. Due to uncertainty on the cause of the animals' death, the presence of more resistant hazards cannot be considered negligible. The sterilisation process defined in the current legislation is able to minimise the risks due to unidentified agents, such as Bacillus anthracis and TSE agents. The BIOHAZ Panel concluded that the process proposed was not properly validated experimentally under real scale conditions. In theory, it should permit a high degree of reduction of spores of pathogenic clostridia but because of several uncertainties (i.e. water evaporation, fat protective effect and particle size) it is not certain that the values of the parameters used in the theoretical calculations would apply in practice. Moreover, the proposed alternative method cannot be considered equivalent to the sterilisation process defined in the current legislation. This would be particularly relevant in the case of extremely heat resistant spores being present in the material to be treated.

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KEY WORDS

Animal By-Products, alternative methods, equivalence, on-site treatment, pig carcasses

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¹ On request from the Italian Competent Authority, Question No EFSA-Q-2008-028, adopted on 19 October 2011.

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³ Acknowledgement: The Panel wishes to thank the members of the Working Group on Assessment of Animal By-Products: Avelino Álvarez-Ordóñez, Reinhard Böhm, John Griffin and Christophe Nguyen-The for the preparatory work on this scientific opinion.

⁴ Cat. 2 ABP is defined in Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009).



SUMMARY

Following a request from the Italian Competent Authority, the Panel on Biological Hazards was asked to deliver a scientific opinion on On-site treatment of pig carcasses.

The application received concerns a new alternative method for processing Category (Cat) 2 Animal By-Products (ABP) as defined in Reg. (EC) 1069/2009⁵. The material to be treated consists of placentas and fallen pigs; this implies that the animals died due to a disease, which in most cases was not properly diagnosed.

The proposed process is a batch process to be used on-farm. It consists of the following steps: i) preheating of the material in a boiling tank, ii) mincing the preheated material, iii) heating of the minced material in a boiling tank and iv) pumping out of the heated material after cooling to 80° C. The target parameters defined for the process are: i) particle size of less that 150 mm after mincing and ii) heating of the minced carcass for 10 - 12 hours at 100° C.

The end-product obtained is intended to be mixed with pig slurry and applied as a fertiliser on land directly or after biogas production.

According to article 13 (d) of Regulation (EC) 1069/2009, before being used as an organic fertiliser, Cat. 2 material should be treated with method 1 as defined in Annex IV to Regulation (EU) 142/2011 (i.e. 133° C / 20 min / 3 bars / 50 mm particle size). Method 1 is a sterilisation process deemed to inactivate heat resistant hazards including bacterial spores with a sufficient safety margin. This method is intended to cover also risks which are not known until now taking the experience of the BSE crisis into account. Indeed, method 1 has been shown to reduce the titres of TSE agents between 2 to 3 log₁₀.

Microbiological investigation of the treated material was performed both in laboratory experiments and in full scale trials (200 to 300 kg of carcass material) done in four different farms. The most resistant hazards identified by the applicant as a target to demonstrate the risk reduction achieved by the process were spores of pathogenic clostridia. However, due to uncertainty on the cause of the animals' death, the presence of more resistant hazards cannot be considered negligible. Method 1 as defined in the current legislation, is able to minimise the risks due to unidentified agents, such as *Bacillus anthracis* and TSE agents.

The Scientific Panel on Biological Hazards (BIOHAZ) concluded that the process proposed by the applicant has not been properly validated experimentally under real scale conditions. In theory, it should permit a high degree of reduction of spores of pathogenic clostridia but because of uncertainties relating to water evaporation, the protective effect of fat and particle size, it is not certain that the values of the parameters used in the theoretical calculations would apply in practice. Moreover, the proposed alternative method cannot be considered equivalent to processing method 1. This would be particularly relevant in the case of more resistant hazards (e.g. extremely heat resistant spores) than pathogenic clostridia being present in the material to be treated.

To assess alternative methods, the Panel recommended that the relevant hazards and their level of inactivation to be targeted by the processing methods for Cat. 2 animal by-products should be specified in a more precise and detailed way. Moreover, to facilitate the assessment of the alternative methods for the treatment and the specific use of the Cat. 2 material under consideration it was recommended that i) test organisms with defined resistance patterns should be specified; and ii) the required level of quantitative risk reduction of such organisms should also be provided.

⁵ Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009).



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BACKGROUND AS PROVIDED BY THE ITALIAN COMPETENT AUTHORITY

This is to inform that this Department received the authorization requirement from Emilia Romagna Region regarding two innovative procedures for disposal animal by products under Reg. EC 1774/2002.

Indeed the mentioned Region supported and performed a research project on On-site treatment of pig carcasses.

In the reports attached we will show the results obtained, detailing every particular case as requested in the Guidelines expressly written by the European Commission for the application of alternative methods for the use or the disposal of animal by-products according to the Regulation EC 1774/2002 SANCO / 10060/ 2006.

The work group that ran the project included CRPA SpA, the Foundation CRPA Studi Ricerche and the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER).

The IZSLER contributed to the project by guaranteeing the scientific supervision and carrying out the relative infection and health analyses.

The work group formulated a system of "plant squandering/dissipation" of carcasses coming from pigs died within the farm; today these carcasses have to be transported into specialized rendering plants. The progressive structure is in compliance with reg. CE 1774/2002, most of all for what concerns the sanitary hazards.

The equipment consists in a cooking boiler where the temperature of 100°C is achieved through an oil interspace. The dissipation procedure is carried out with atmospheric pressure into 24-48 hours and completed by a proper mincing pump.

The final product can be used as zootechnical fluids for manure or for biogas production.

The application of this method into practice could drop the cost of disposal of carcasses coming from animals died within the farm, reduce the hazard of transporting potentially infected carcasses and also take advantage of a product that would be destructed.

TERMS OF REFERENCE AS PROVIDED BY THE ITALIAN COMPETENT AUTHORITY

We ask to the Unit to evaluate this project and estimate if it can be approved as alternative method for disposal of animal by products.



ASSESSMENT

1. Introduction

After the submission of this application, Reg. (EC) $1774/2002^6$, laying down rules concerning Animal By-Products (ABP), was repealed by Reg. (EC) $1069/2009^7$. However, the standard method for the production of organic fertilisers from Cat. 2 material (except manure and digestive tract content), as reported in Art. 13, (d) of Reg. 1069/2009 remains the so called "Method 1" that is currently defined in Annex IV to Regulation (EC) $142/2011^8$ (i.e. $133^{\circ}C / 20 \text{ min} / 3 \text{ bars} / 50 \text{ mm}$ particle size). Considering that, the current assessment makes reference to the legislation currently in force as regard to ABP i.e. Reg. (EC) 1069/2009 and Reg. (EU) 142/2011. In particular the assessment was performed taking into account the criteria laid down in Art. 20, point 5 of Reg. 1069/2009.

The application concerns a new processing method Category 2 material, namely placentas and fallen pigs, on pig farms.

The terminology used in this assessment conforms to the "Guidelines for applications for new alternative methods of disposal or use of animal by-products" prepared jointly by the Health and Consumer Protection Directorate-General (DG-SANCO) and the European Food Safety Authority (EFSA) (EC, 2008). The assessment only considered biological hazards.

1.1. The method as described by the applicant

The proposed process consists of the following steps:

- preheating of the material in a boiling tank;
- mincing of the preheated material;
- heating of the minced material in a boiling tank;
- pumping out of the heated material after cooling to 80°C.

The targets defined for the process are:

- particle size of less than 150 mm after mincing;
- heating of the minced carcass for 10-12 hours at 100°C.

No parameters (*e.g.* time, temperature) are defined for the preheating steps before mincing. The dossier only defined preheating as sufficient to permit mincing of the carcasses to the target particle size.

⁶ 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 (OJ L 273, 10.10.2002).

⁷ Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation) (OJ L 300, 14.11.2009).

⁸ Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive (OJ L 54, 26.2.2011).

Heating is done as a batch process, which take place in "dissipaters" (boilers heated by circulation of hot oil inside the wall of the equipment), at atmospheric pressure. Illustrations of such equipment are provided in the dossier. During experimental trials in real conditions in farms, the "dissipaters" were loaded with approximately 200 to 300 kg of minced pig carcasses and various amounts of water (from 500 1 to 1400 1). However, it is not specified if the heated material is regularly stirred and if evaporation occurs at the surface of the liquid phase of the heated material. In some trials water was added in the course of the treatment, presumably to compensate for evaporation.

No information on the mincing and preheating equipment is given.

The carcasses are stored in refrigerators before processing. The end-product is intended to be mixed with pig slurry, to be applied on lands as fertilizers or used for biogas production.

A flow diagram of the process is provided with indications of the critical control points and steps where the process parameters are monitored (particle size, time and temperature).

2. Risk categories

The application concerns animal by-products of Category 2 material as defined in the Regulation (EC) 1069/2009.

3. Identification and characterisation of risk material

The material to be treated consists of placentas and pigs dead on the farm; this implies that the animals died due to a disease, which in most cases was not properly diagnosed. The applicant proposes that the process will not be used to treat carcasses of animals killed during emergency sanitary situation.

The applicant considered several pathogens (Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Mycoplasma spp., viruses and Clostridia spp.).

Among these hazards, spores from pathogenic bacteria are the most difficult to inactivate by the process considered in this application. In particular, spores of the pathogenic clostridia, proteolytic *Clostridium botulinum* and *Clostridium perfringens*, can be present in pig or piglet intestine (Baker et al., 2010; Dahlenborg et al., 2001; Myllykoski et al., 2006; Waters et al., 2003) and carcasses could provide site for their multiplication (Dodds, 1993).

However, due to uncertainty on the cause of the death of the animals in this situation, the presence of more resistant hazards cannot be considered negligible. In particular method 1 as defined in the current legislation, is able to minimise the risks due to unidentified agents, such as *Bacillus anthracis* and TSE agents.

4. Agent risk reduction

Cat. 2 material, which includes the carcasses of animals that die on-farm, should be treated according to method 1 as defined in Annex IV to Regulation (EU) 142/2011 (i.e. $133^{\circ}C / 20 \text{ min} / 3 \text{ bars} / 50 \text{ mm}$ particle size) before being used as an organic fertiliser (Article 13 (d) of Regulation (EC) 1069/2009). Method 1 is a sterilisation process deemed to inactivate heat resistant hazards including bacterial spores with a sufficient safety margin. This method is intended to cover also risks which are not known until now taking the experience of the BSE crisis into account. Indeed, method 1 has been shown to reduce the titres of TSE agents between 2 to 3 \log_{10} (Schreuder et al., 1998).



4.1. Assessment of the experimental validation

Microbiological investigation of the treated material was performed both in laboratory experiments and in full scale trials (200 to 300 kg of carcass material) done in four different farms.

The laboratory experiments were done at 100°C, 121°C and 137°C with a sterilizer, in a broth containing pig materials inoculated with a PRRS virus suspension, a Salmonella suspension and spores of *Cl. perfringens*. Only the experiments at 100°C are representative of the proposed process conditions. The information presented in the dossier does not permit the level of reduction of the infective viruses to be calculated. The results provided in the dossier show a reduction of at least 6 log₁₀ for Salmonella and at least 5 log₁₀ for *Cl. perfringens* spores, after 12, 24, 48 hours at 100°C. This was expected, considering the D_{100°C} around 20 min for spores of *Cl. perfringens*. However, the applicant did not verify the quality of the spore suspension used (heat resistance properties and percentage of dormant spores).

The dossier gives times needed at 100°C for 90% reduction of spores ($D_{100°C}$) from *Cl. perfringens* and *Cl. botulinum* species, cited from published scientific literature, ranging from 15 to 33 min in foods or buffers around neutral pH (ICMSF, 1996; Juneja et al., 2003). These values are consistent with those reported by Kim and Foegeding (1993) for *Cl. botulinum* proteolytic, and in Labbé (1989; 2000) for the most heat resistant strains of *Cl. perfringens*. However, spores in lipids are more heat resistant (Kim and Foegeding, 1993).

In the full scale experiments, two temperature probes were placed in the dissipaters for each experiment. The 2 probes always recorded very similar temperature profiles. Total treatment times ranged from 24 to 71 h, and the time to reach the target temperature of 100° C was between 4 and 14 hours. The shortest time of exposure at 100° C recorded over all the experiments was 19 h, longer than the proposed target times of 10-12 h. However, the dossier does not indicate the location of the 2 temperature probes in the dissipaters. No microorganisms were inoculated in the treated material. The endogenous microflora of the pig carcasses used contained between 10^{6} and 10^{7} anaerobic clostridia before the treatment. After treatments, the material did not contain any anaerobic clostridia. However, the dossier does not indicate whether the clostridia enumerated on the pig carcasses before treatment were spores or vegetative cells. It is therefore not possible to conclude on the reduction of Clostridium spores experimentally obtained by the proposed process under real scale conditions.

Hence, a proper experimental validation with representative organisms was not performed.

4.2. Comparison of the theoretical risk reduction of method 1 and the proposed alternative method

Although the knowledge on heat resistance of spores of *Cl. perfringens* is scarce and the values found in the literature may not represent the true diversity of the different natural isolates, on the basis of purely theoretical calculations and assuming that the heat resistance of spores in the material to be processed is the same as in laboratory broths:

- the treatment with method 1 would achieve an order of magnitude of 1000 log₁₀ reduction of spores of pathogenic clostridia; while
- the treatment of 10 hours at 100°C proposed by the applicant should achieve between 20 to 30 log₁₀ reduction of spores of pathogenic clostridia.

These theoretical reductions calculated for *Cl. perfringens* are so high compared to the expected prevalence of these hazards in ABPs of category 2 (few \log_{10} spores of *Cl. perfringens* per g at most), that they have no practical value. They show however that the proposed method would not permit an



equivalent reduction of the risk to that achieved by processing method 1 from Regulation (EU)142/2011.

Method 1 is designed to inactivate hazards more resistant than spores of pathogenic clostridium and it is technically not possible to demonstrate equivalence with method 1 by using pathogenic clostridia spores as a test organism. Standardised more resistant test organisms are necessary, for instance as for medical equipment described in ISO 14161 (ISO, 2009) for thermal sterilisation.

In the proposed process there are other uncertainties concerning the possibility that water evaporation in the "dissipater" could reduce the heat treatment at the air/liquid interface and the possible protective effect of lipids on spore inactivation.

Moreover, the difference between the proposed process and method 1 could be even higher when taking into account the particle size of 150 mm considered in the application dossier compared to the 50 mm prescribed for method 1.

5. Risk Containment

No formal HACCP scheme is presented in the dossier. The flow diagram indicates putative critical control points for:

- pre-heating (before mincing) without indicating critical values;
- mincing, without indicating how the correct particle size of 150 mm is checked;
- heating (10-12 hours at 100°C), using temperature probes to check that the targets are obtained. The number and place of the probes are not specified.

In addition, the applicant specified that any bone remaining at the end of the treatment would be removed and stored before disposal according the Regulation (EC) 1774/2002.

Hygienic precautions as required in the treatment of Cat. 2 materials according to Reg. (EC) 1069/2009 are missing in the description of the process (e.g. separation between the clean and unclean sides, uses of protective clothing, dedicated staff).

6. Identification of interdependent processes

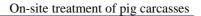
The process implies collection and storage of the pig carcasses on the farm before processing. The dossier indicates that the pig carcasses will be stored refrigerated.

Potential wastes generated by the process (e.g. waste water from cleaning, steam) and their treatments are not described by the applicant.

The end product is mixed with slurry and the mixture stored in the farm. The origin of the slurry and the potential transport of the mixture of slurry with the end product is not described by the applicant.

7. Intended end-use of the products

The end product is intended to be mixed with pig slurry and applied as a fertiliser on land directly or after biogas production.





8. Documentary evidence

See documentation provided to EFSA listed below.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- The application concerns treatment of Animal By-Products of Category 2, as defined in the Regulation (CE) 1069/2009, for organic fertilisers and biogas production. The standard processing method to be used for this purpose, called method 1, is specified under Regulation (EU) 142/2011.
- Some deficiencies were noted by the Panel in relation to the risk containment.
- The process proposed by the applicant has not been properly validated experimentally under real scale conditions.
- On the basis of theoretical calculations the process proposed by the applicant, 10-12 hours at 100°C in batches, should permit a high degree of reduction of spores of pathogenic clostridia.
- Because there are uncertainties relating to water evaporation, protective effect of fat and particle size, it is not certain that the values of the parameters used in the theoretical calculations would apply in practice.
- In any case the proposed alternative method cannot be considered equivalent in terms of log reduction to processing method 1 described under Regulation (EU) 142/2011. This would be particularly relevant in the case of more resistant hazards (e.g. extremely heat resistant spores) than pathogenic clostridia being present in the material to be treated.

RECOMMENDATIONS

- To assess alternative methods, the relevant hazards and their level of inactivation to be targeted by the processing methods for Cat. 2 animal by-products should be specified in a more precise and detailed way.
- To facilitate the assessment of the alternative methods for the treatment and the specific use of the Cat. 2 material under consideration i) test organisms with defined resistance patterns should be specified; and ii) the required level of quantitative risk reduction of such organisms should also be provided.





DOCUMENTATION PROVIDED TO EFSA

- 1. Letter "Submission of application of new alternative method of disposal animal by products under regulation (EC) n° 1774/2002". November 2007. Submitted by the Italian *Ministero della Salute, Dipartimento per la sanità pubblica veterinaria, la nutrizione e la sicurezza degli alimenti, Direzione generale della sicurezza degli alimenti e della nitrizione, Ufficio III.*
- 2. Report "Applications for new alternative methods of disposal or use of animal by-products under regulation (EC) 1774/2002 The on-site treatment of pig carcasses". November 2007. November 2007. Submitted by the Italian *Ministero della Salute, Dipartimento per la sanità pubblica veterinaria, la nutrizione e la sicurezza degli alimenti, Direzione generale della sicurezza degli alimenti e della nitrizione, Ufficio III.*
- 3. Dossier "The on-site treatment of pig carcasses". November 2007. Submitted by the Italian Ministero della Salute, Dipartimento per la sanità pubblica veterinaria, la nutrizione e la sicurezza degli alimenti, Direzione generale della sicurezza degli alimenti e della nitrizione, Ufficio III.
- 4. Letter "Additional information about our letter ref. 001608-P-05/112007DGSAN-3 with reference to Your letters ref. MH/FB/lm out 5481212 and ref. MH/FB/lm out 5481200". March 2011. Submitted by the Italian *Ministero della Salute, Dipartimento per la sanità pubblica veterinaria, la nutrizione e la sicurezza degli alimenti, Direzione generale della sicurezza degli alimenti e della nitrizione, Ufficio III.*



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