The Director General

Maisons-Alfort, 15 February 2013

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the use of zinc oxide in the diet of piglets at weaning to reduce the use of antibiotics

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).
Its opinions are made public.

On 12 March 2012, ANSES received a formal request from the General Directorate for Food (DGAL) to conduct a risk/benefit assessment of the use of zinc oxide in the diet of pigs at weaning, as an alternative to antibiotics for the prevention and treatment of diarrhoea.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Measure 19 of the national plan to reduce the risk of antibiotic resistance in veterinary medicine (which contains 40 measures to be implemented by 2017) considers the development of alternatives to antibiotics (AB) that would lead to a decrease in their use. In particular, this measure provides for an assessment of the risks and benefits of potential alternative products. In this context, therefore, ANSES was requested to investigate zinc oxide (ZnO), which can be used to reduce diarrhoea in post-weaning piglets.

At weaning, piglets often suffer from digestive disorders and growth retardation caused by changes to their diet and stress related to batching. To limit the losses caused by these problems, a large proportion of animals systematically receive a preventive antibiotic treatment in the form of medicated feed (containing colistin, or colistin sometimes in combination with another antibiotic).

As an alternative to these antibiotic treatments, some European countries already authorise the use of high doses of ZnO in the diet of piglets, in the form of medicated premix, during this weaning period. There is currently no such authorisation in France.

In this context, ANSES was asked to:

- ✓ conduct a risk-benefit assessment of the use of zinc oxide in the diet of piglets (its efficacy for animals, and risk to humans, animals and the environment);

✓ issue an opinion on the value of using this treatment to reduce the risk of antimicrobial resistance.

In addition, the expert group expanded the scope of investigation of this assessment, by compiling an initial inventory of other alternatives to antibiotic therapy to combat post-weaning digestive disorders in piglets.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in compliance with Standard NF X 50-110 "Quality in expertise activities - General requirements of competence for an expertise activity (May 2003)".

ANSES set up a multidisciplinary expert working group (the Zinc Oxide WG) to respond to this formal request. The WG reported to the Expert Committees (CES) on "Feed" (leader) and "Animal Health" and to the French Commission for Veterinary Medicinal Products (CNMV). The efficacy of zinc oxide and the risks for humans, animals and the environment associated with its use as a medicinal product were analysed on the basis of an internal expert appraisal by the French Agency for Veterinary Medicinal Products (ANMV).

The methodological and scientific aspects of the expert appraisal were regularly submitted by the Working Group to the CESs. The report produced by the Working Group takes account of observations and additional information supplied by the members of the CESs.

This expert appraisal was therefore conducted by a group of experts with complementary skills.

The CES on "Animal Health", the French Commission for Veterinary Medicinal Products (CNMV) and the CES on "Feed" (leader) adopted the collective expert appraisal work and its findings and recommendations at their meetings on 9 January, 15 January and 22 January 2013 respectively and informed ANSES's General Directorate accordingly.

3. ANALYSIS AND CONCLUSIONS OF THE CESS AND THE CNMV

3.1. Risk/benefit analysis of the use of zinc oxide

- **Weaning and digestive disorders**

Weaning, especially early weaning, is a real cause of distress in piglets: separation from the mother, move to a different building, mixing of piglets from different litters, change of feeding and watering system, and of course change of diet. Aged 3 to 4 weeks, the piglets are still immature in many ways (digestive tract, enzyme system, immune system).

All these factors lead to changes in the intestinal flora that are conducive to the uncontrolled multiplication of pathogenic bacteria. Even with optimised food formulas and comfort and hygiene conditions, digestive disorders subsequent to weaning are common. Colonisation of the gut by a significant quantity of pathogenic strains of *Escherichia coli* leads to various clinical manifestations depending on the bacteria's virulence factors (diarrhoea, oedema disease, haemorrhagic gastroenteritis or septicaemia) (Fairbrother and Gyles, 2012).

Even when these risk factors are mitigated, the use of preventive or metaphylactic antibiotic treatments after weaning is often the only way to control colibacillosis. Oral administration of colistin is common because colibacillus are still mostly susceptible to this antibiotic (aminoglycosides can also be used). However, the existence on some farms of persistent colibacillosis related to multi-drug resistant bacteria should be noted.

During this risk period for piglet rearing, the use of zinc oxide could be considered as an alternative to antibiotic treatments, due to its identified effects on the intestinal microbiota and on the animal's immune function.

In Europe, the situation varies regarding the use of zinc oxide as a veterinary medicinal product: some countries have not authorised it (this is currently the case in France), while others allow it in high doses (3100 ppm of ZnO) as a medicated premix in the diet of piglets, for the "prevention" or "prevention and treatment" of post-weaning diarrhoea.

It should be noted that the existence of these marketing authorisations in certain European Union Member States would allow France to seek a referral to EMA's Committee for Medicinal Products for Veterinary Use (CVMP) under Article 35 of Directive 2001/82/EC. The opinion issued on the benefit-risk ratio could lead to a Community Decision that would apply to all countries of the EU.

- **Effect of zinc oxide on colibacillosis**

ZnO has been identified as having an effect on the microbial ecosystem and digestive tract. In the intestinal microbiota, it has a stabilising effect, by acting on certain species of lactic acid bacteria and anaerobic bacteria, and by promoting stability and increased diversity of Enterobacteriaceae. The intestinal epithelial barrier is itself strengthened at the intercellular junctions, while the possibility of strains *of E. coli* adhering to intestinal cells is reduced.

In addition, different literature sources indicate that supplementation with ZnO also has a beneficial effect on the local and systemic immune function in piglets during the immediate post-weaning period, although not all the mechanisms have been identified and described with accuracy (Li *et al.*, 2006; Martinez-Montemayor *et al.*, 2008; Ou *et al.*, 2006; Sargeant *et al.*, 2010).

In the available studies seeking to demonstrate the efficacy of ZnO in combating coliform diarrhoea, the medicated feed was always distributed before the onset of diarrhoea. It therefore acts by 'preventing' diarrhoea in piglets in early post-weaning, and these studies cannot be used to claim a 'treatment' indication for established diarrhoea. Overall, they show a reduction in the number of piglets with diarrhoea and an improvement in the consistency of faeces, compared to the untreated control group.

Nor is it possible, based on the currently available literature, to extrapolate the efficacy of the ZnO premix to severe forms of coliform diarrhoea, to oedema disease or to haemorrhagic forms, because the symptoms presented in the literature are always those of moderate disease without mortality (Katouli *et al.*, 1999; Molist *et al.*, 2011; Ou *et al.*, 2006; Poulsen and Larsen, 1995; Slade *et al.*, 2011).

All the tests studied were based on the administration of ZnO for seven days or more. Efficacy has only been demonstrated for 14 days but it is not currently possible to validate this as the optimal duration of treatment, as none of the studies tested administration over periods between seven and 14 days. Similarly, the dose used in field studies is always 3100 ppm of ZnO. According to the literature, a lower dose (2500 ppm of ZnO) may be sufficient. It is therefore not possible to determine unambiguously the optimal dose and duration of treatment.

Finally, it should be emphasised that while the different efficacy studies available can be used to qualify the intrinsic efficiency of ZnO, they cannot be used to compare it to medicinal products that already have a marketing authorisation in pigs, whether these are anti-diarrhoeals (aluminal, kaopectate) or anti-infectives (colistin). It is therefore impossible to say how the efficacy of ZnO compares to that of colistin.

In the absence of clinical trials comparing ZnO and colistin in particular, it is impossible to say whether ZnO could replace colistin or if it could be used in addition to or in combination with colistin.

- **Chemical characteristics of ZnO and risk to humans and animals**

The premixes based on zinc oxide already used in Europe are mainly 100% zinc oxide, without any added excipient.

Because zinc oxide is described in the European Pharmacopoeia currently in effect and zinc salts are listed in the Annex to Regulation (EU) No 37/2010, its use in a medicinal product is possible. Its purity and inorganic impurity content are subject to regulation.

It is therefore possible to have a pharmaceutical grade product, compared to certain illegal practices such as overdosing with additives, or even hazardous ones such as using drying bedding containing zinc oxide in combination with undesirable substances. These practices make it impossible to quantify the doses used, which may have an effect on both the animal's health and the environment.

In addition, levels up to three times the dose used in medicated premix are well tolerated by piglets.

Furthermore, the low level of zinc accumulation in animal foodstuffs, combined with the low consumption of pork from post-weaning piglets, suggest that the risk to consumers is negligible. The risk for handlers is also negligible when they comply with risk management measures associated with handling of the medicated premix.

- **Use of zinc oxide and risk to the environment**

The environmental risk of using zinc oxide, administered at 3100 ppm for 14 days post weaning, was assessed according to the applicable guidelines for veterinary medicinal products. Zinc has a high affinity for soil, and because of its non-degradable nature it accumulates there. The assessment indicates a toxic risk for the different environmental compartments (sediment, water and soil).

The calculation of the expected zinc concentrations in soil thus shows that the continuous input of zinc oxide from the medicated premix leads to soil gradually being enriched in zinc, and this is in addition to the amounts produced at present from the waste of animals receiving feed containing Zn (raw materials and additives).

In addition, the metal's accumulation in soil also leads to contamination of ground water and surface water, resulting from leaching, drainage and runoff: this presents a risk for the 'Algae' and 'Crustacea' taxa that live in surface water, as well as for sediment-dwelling organisms.

The environmental risk assessment was also extended to the various zinc salts due to zinc's speciation in soil (transformation of ZnO into other forms of Zn, once released into the environment).

Regarding the water compartment, a risk of toxicity for sediment-dwelling organisms appears in the first year of slurry application, and after two to five years of consecutive application on the same plot for freshwater organisms, depending on the water hardness. Concerning the soil compartment, a risk of toxicity appears after 12 years of consecutive slurry application on the same plot.

However, it should be noted that this environmental risk assessment, carried out according to the applicable guidelines for veterinary medicinal products, took into account the zinc content only due to slurry from post-weaning piglets concerned by the treatment under study. It did not include the effects of diluting the zinc in this slurry by mixing it with less concentrated slurry from animals in the fattening stage on the same farms.

When this dilution effect is included, it shows that if fattening pigs are present on the farm (post-weaning/fattening farms or breeding-fattening farms that account for 94% of French pig farms), the environmental impact of the zinc oxide is reduced.

Moreover, in these farms with fattening units, the environmental risk of zinc oxide can also be reduced by husbandry measures relating to diet: reducing zinc intake from additives during the fattening phase (the animals' zinc requirements are lower than the maximum regulatory limits) and enhancing the zinc in feed through the addition of exogenous phytases would offset the environmental impact of using zinc oxide during the post-weaning period.

Treating slurry by removing nitrogen is a possible management strategy under the Nitrates Directive (Directive No. 91/676/EEC), which authorises a maximum annual application of 170 kg N/ha. This practice leads to an increase in the zinc/nitrogen ratio and therefore in the amount of zinc spread. The environmental impact is increased in this case.

- **Bacterial resistance, antibiotics and zinc oxide**

Colistin is widely used in pig farms in Europe, for the prevention or therapeutic treatment of gastrointestinal diseases in post-weaning piglets.

A study conducted in 2008 among 83 French breeding-fattening farms assessed the relative share of polypeptide antibiotics (including colistin) compared to other antibiotics acquired, and their destination. Almost all farms (93%) had acquired polypeptides and 90% of these antibiotics were colistin for post-weaning administration (Chauvin *et al.*, 2002; Chauvin, 2010).

Resistance to colistin in *E. coli* strains of animal or human origin has undergone relatively little study, probably because of the unreliability of agar diffusion tests, related to the poor dissemination of this antibiotic in this medium. Methods based on determination of minimum inhibitory concentrations (MICs) should be used. Studies conducted in France show that rates of resistance in digestive *E. coli* from healthy animals (commensal flora) remain very low (below 1%), while in sick pigs these rates exceed 5%.

The emergence of resistance to colistin in pig farms should therefore be noted, even though the level is currently low. This is why the recommendations state that antibiotics, including colistin in piglets, should be used with caution, and such use must be accompanied by monitoring of the level of resistance to colistin and, more generally, to all antibiotics.

Although the use of zinc oxide is being investigated as an alternative to antibiotics, it should be emphasised that the possibility of selection of bacterial resistance to zinc cannot be ruled out. Resistant isolates of different bacteria (including *E. coli)* have been found in environments with high concentrations of this element.

The mechanism of resistance to Zn is based on the expression of bacterial efflux pumps. These are either specific to Zn, or likely to respond to other compounds such as antibiotics, leading to a slight reduction in bacterial susceptibility with regard to these inhibitors.

In addition, Zn resistance genes are sometimes located on the same mobile genetic elements as antibiotic resistance genes, thus inducing co-selection of antibiotic resistance. This is particularly true with *Staphylococcus aureus:* administration of ZnO leads to an increase in the occurrence of methicillin-resistant *S. aureus* (MRSA) in the nasal cavities of treated pigs (Agerso *et al.*, 2012).

- **Initial inventory of alternatives to antibiotics in post-weaning piglets**

Some potential alternatives to the use of antibiotics in post-weaning piglets were studied. They are largely based on nutritional alternatives that aim to provide nutrients for the

commensal bacteria, probiotics or yeast cell walls, enzymes, plant extracts or organic acids.

Organic acids and probiotics appear to present the most promising results, although the specific conditions under which their efficacy was demonstrated remain to be determined, especially in severe forms of gastrointestinal infections.

Oral vaccine strategies are being developed against the main serotypes of pathogenic *E. coli* or against oedema disease, but they are confronted with both the difficulty of immunising very young animals, and the diversity of pathogenic strains in the field.

In any case, in the present state of knowledge, it is impossible to say whether these measures would be sufficient in the event of infection by highly pathogenic strains, especially in cases of oedema disease, due to its rapid evolution.

3.2. Conclusions

Following the benefit/risk analysis of the use of ZnO in post-weaning piglets, the Expert Committees (CES) on "Feed" and "Animal Health", and the CNMV validated the findings of the Working Group in the following terms:

- ✓ The regulatory status of ZnO as a medicated premix for piglets has not been harmonised within the European Union.
- ✓ Because ZnO is described in the European Pharmacopoeia currently in effect and Zn salts are listed in the Annex to Regulation (EU) 37/2010, its use in a medicinal product poses no particular problem. Its purity and inorganic impurity content are subject to regulation.
- ✓ The efficacy of ZnO in preventing moderate post-weaning diarrhoea in piglets has been demonstrated at a dose of 3100 ppm and for a maximum period of 14 days, but this dose still needs to be optimised. ZnO can therefore be regarded as a means of preventing these digestive disorders. However, neither its efficacy in preventing serious forms or oedema disease, nor its curative efficacy have to date been documented.
- ✓ The assessment of the environmental risk of ZnO as a medicated premix (spreading slurry from post-weaning piglets) indicates a toxic risk for the different environmental compartments (sediment, water and soil). The level of impact depends on the context of use, specifically the type of farm and the Zn content in the feed throughout the rearing period.
- ✓ Phenomena of bacterial resistance to Zn and cross-resistance or co-selection of resistance to certain antibiotics have been described in the literature, but it is impossible to anticipate how they will develop if ZnO is used.
- ✓ The use of antibiotics such as colistin is still an effective practice with regard to established bacterial digestive infections. However, it should be noted that resistance to colistin is an emerging phenomenon in pig farming, even though the level is currently low.
- ✓ No comparison of the efficacy of ZnO with that of colistin in controlling post-weaning diarrhoea has yet been documented. It was therefore not possible to assess the comparative benefits of colistin and ZnO.
- ✓ In terms of quantitative risk assessment, comparing the risks associated with the use of colistin and those of zinc oxide does not seem appropriate based on the currently available data, due among other things to the major scientific uncertainties concerning the development of resistance to colistin and changing phenomena of bacterial resistance to Zn or co-selection of antibiotic resistance in the short and medium term.

- ✓ Many other alternatives to antibiotics for controlling colibacillosis at weaning are being studied, and although some are promising, their use presupposes a scientific validation of their efficacy. However, it is likely that these alternatives will not be sufficient in the event of infection with highly pathogenic strains.

3.3. Recommendations

As a result of its findings, the CESs and the CNMV validated the recommendations of the Working Group:

- ✓ With regard to the environmental risk associated with the use of zinc oxide as a medicated premix in post-weaning piglets, management measures to offset this risk must be considered. The first is to only use zinc oxide in farms with fattening units, and which do not treat the slurry before application. In addition, in these farms with fattening units, other husbandry measures should also be proposed, such as reducing levels of zinc as a feed additive during the fattening phase. As the maximum regulatory limits for zinc are higher than the pig's needs during this phase, it can be reduced and the use of phytases in feed can enhance this intake, both nutritionally and environmentally.
- ✓ Because the demonstrated efficacy of zinc oxide in the case of severe digestive disorders and oedema disease or established diarrhoea has not been documented, the use of antibiotics in these situations, and colistin in particular, cannot be excluded. Colistin should, however, be used prudently and accompanied by monitoring for bacterial resistance.
- ✓ Any authorisation of zinc oxide as a medicated premix for post-weaning piglets should be accompanied by the establishment of a system for monitoring resistance to zinc, cross-resistance or co-selection of resistance to certain antibiotics, as well as annual monitoring of amounts used at national level.
- ✓ Marketing authorisation applications for medicated premixes based on zinc oxide should include evidence substantiating:
    - o the precise indication, in terms of the targeted conditions and alleged mode of action (prevention/treatment);
    - o the dosage;
    - o the environmental risk.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions and recommendations of the Working Group, and of the CESs and the CNMV to which it reported.

ANSES emphasises that although it was able to conduct an intrinsic risk-benefit assessment of the zinc oxide product for its use in the context of digestive diseases in post-weaning piglets, it was not able to compare this usage to that of antibiotics (including colistin):

- the comparative benefit could not be established: comparing the efficacy of the two product types requires field trials using both compounds, which have thus far not been carried out.
- the comparative risk was not studied: this approach was confronted with firstly the difficulty of comparing a predominantly environmental risk with a risk of antimicrobial resistance, and secondly the fact that such a comparative approach requires non-scientific criteria to be taken into account, whether they be economic, political or sociological.

It seems that this overall comparative approach is actually the responsibility of the risk manager, when considering the options and making the final decision.

The Director General

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