SCIENTIFIC OPINION

Scientific Opinion on safety and efficacy of CRINA® Poultry Plus (preparation of benzoic acid and essential oil compounds) as feed additive for chickens for fattening

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

CRINA® Poultry Plus (CPP) is a feed additive consisting on seven active substances: benzoic acid (83%), thymol (1.9%), eugenol (1%), benzylsalicylate (0.3%), piperine (0.1%), isoamylsalicylate (0.1%) and trans-anethole (0.1%). It is intended to be used as a zootechnical additive in complete feed for chickens for fattening (300-450 mg CPP/kg feed) to favourably affect their performance. Given the inconsistency of the data and the suggestion of adverse effects, albeit not dose-related, the FEEDAP Panel is unable to conclude on the safety of the additive at the dose range proposed. None of the components of CPP are genotoxic and in vitro assays made with the additive gave no indication of a genotoxic potential. No metabolism/residue data are available for eugenol and piperine in chickens for fattening. Consequently, the FEEDAP Panel cannot draw any final conclusion on the safety for the consumer of CPP used as feed additive in chickens for fattening. CPP should be considered as irritant for the skin and eyes, as well as skin sensitiser; no adverse effects upon inhalation are to be expected. In the absence of data on the environmental fate of piperine, isoamylsalicylate and benzylsalicylate, or the additive itself, the safety for the environment cannot be assessed. A significant effect relative to performance was shown in only one of the eight studies taken for the demonstration of efficacy. There is insufficient evidence that CPP at the recommended dose can improve the zootechnical performance of chickens. The sensory properties of the meat from chickens fed CPP are not affected.

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KEY WORDS

Zootechnical additive, benzoic acid, thymol, eugenol, piperine, safety, efficacy

1 On request from the European Commission, Question No EFSA-Q-2010-01130, adopted on 7 March 2012.
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3 Acknowledgement: The Panel wishes to thank the members of the Working Group on CRINA Poultry Plus, including Guido Rychen, for the preparatory work on this scientific opinion.


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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety for the target animal(s), consumer, user and the environment and the efficacy of the product CRINA® Poultry Plus as feed additive for chickens for fattening.

CRINA® Poultry Plus (CPP) is a feed additive consisting on seven active substances: benzoic acid (83%), thymol (1.9%), eugenol (1%), benzylsalicylate (0.3%), piperine (0.1%), isoamylsalicylate (0.1%) and trans-anethole (0.1%). It is intended to be used as a zootechnical additive (functional group “other zootechnical additives”) in complete feed for chickens for fattening at the dose of 300-450 mg/kg complete feed.

Given the inconsistency of the data and the suggestion of adverse effects, albeit not dose-related, the FEEDAP Panel is unable to conclude on the safety of the additive at the dose range proposed.

None of the components of CPP are genotoxic and in vitro assays made with the additive gave no indication of a genotoxic potential. No metabolism/residue data are available for eugenol and piperine in chickens for fattening. Consequently, the FEEDAP Panel cannot draw any final conclusion on the safety for the consumer of CPP used as feed additive in chickens for fattening.

The FEEDAP Panel considers that the product should be treated as irritant for the skin and eyes, as well as skin sensitiser. Given the particle size distribution and low dusting potential of CPP, no adverse effects upon inhalation are to be expected.

In the absence of data on the environmental fate of piperine, isoamylsalicylate and benzylsalicylate, or the additive itself, the safety for the environment cannot be assessed.

There is insufficient evidence that CPP at the recommended dose can improve the zootechnical performance of chickens. The sensory properties of the meat from chickens fed CPP are not affected.
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BACKGROUND

Regulation (EC) No 1831/2003\(^4\) establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company DSM Nutritional Products AG represented in the European Union (EU) by DSM Nutritional Products Sp. z o.o. Poland\(^5\) for authorisation of the product CRINA\(^\circledast\) Poultry Plus (a preparation of benzoic acid and essential oil compounds), when used as a feed additive for chickens for fattening (category: zootechnical additives; functional group: other zootechnical additives) under the conditions proposed in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive), EFSA received directly from the applicant the technical dossier in support of this application.\(^6\) According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 16 November 2010.

The additive is not authorised in the EU.

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the product CRINA\(^\circledast\) Poultry Plus (a preparation of benzoic acid and essential oil compounds), when used under the conditions described in Table 1.

\(^5\) DSM Nutritional Products Sp. z o.o. Poland. Tarczynska 113, 96-320- Mszczonow. Poland.
\(^6\) EFSA Dossier reference: FAD-2010-0093.
Table 1: Description and conditions of use of the additive as proposed by the applicant

<table>
<thead>
<tr>
<th>Additive</th>
<th>CRINA® Poultry Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Registration number/EC No/No (if appropriate)</td>
<td></td>
</tr>
<tr>
<td>Category(-ies) of additive</td>
<td>Zootechnical</td>
</tr>
<tr>
<td>Functional group(s) of additive</td>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Chemical formula</th>
<th>Purity criteria (if appropriate)</th>
<th>Method of analysis (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of benzoic acid and essential oil compounds having a minimum of benzoic acid 80% thymol 1% eugenol 0.5% piperine 0.05% and sum of other essential oil compounds below 0.6%</td>
<td>benzoic acid: C7H6O2 (CAS 65-85-0) thymol: C10H14O (CAS 89-83-8) eugenol: C10H12O2 (CAS 97-53-0) piperine: C17H19O3N (CAS 94-62-2)</td>
<td></td>
<td>Benzoic acid: Reversed Phase HPLC-UV Thymol, eugenol, piperine: Gas chromatography</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trade name (if appropriate)</th>
<th>CRINA® Poultry Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of the holder of authorisation (if appropriate)</td>
<td>DSM Nutritional Products</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions of use</th>
<th>Species or category of animal</th>
<th>Maximum Age</th>
<th>Minimum content</th>
<th>Maximum content</th>
<th>Withdrawal period (if appropriate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicken for fattening</td>
<td>Maximum</td>
<td>Minimum content</td>
<td>Maximum content</td>
<td>Withdrawal period (if appropriate)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>mg/kg of complete feedingstuffs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>450</td>
<td></td>
<td>none</td>
</tr>
</tbody>
</table>

Other provisions and additional requirements for the labelling

| Specific conditions or restrictions for use (if appropriate) | - |
| Specific conditions or restrictions for handling (if appropriate) | May cause sensitization by skin contact. Irritating to eyes. |
| Post-market monitoring (if appropriate) | No additional requirements further to the need for traceability and recall procedures established by Regulation No 178/2002 |
| Specific conditions for use in complementary feedingstuffs (if appropriate) | - |

Maximum Residue Limit (MRL) (if appropriate)
<table>
<thead>
<tr>
<th>Marker residue</th>
<th>Species or category of animal</th>
<th>Target tissue(s) or food products</th>
<th>Maximum content in tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>not applicable</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ASSESSMENT

During the assessment of the dossier submitted by the applicant, the FEEDAP Panel noted that star anise oil, a component of the product, could raise safety concerns because of its content of estragole, a recognized genotoxic and carcinogenic substance (EC, 2001). Following a request for supplementary information from EFSA, the applicant chose to reformulate the product by replacing the star anise oil with pure trans-anethole, thus avoiding estragole in the product.

The assessment of the product is based on the new re-formulated additive. It has been specifically indicated in the relevant sections of the opinion if this limited change in composition of the product may impact on the safety and efficacy of the additive.

1. Introduction

CRINA® Poultry Plus (later abbreviated CPP) is a feed additive consisting on seven active substances: benzoic acid, thymol, eugenol, piperine, isoamyl salicylate, benzyl salicylate and trans-anethole. It is intended to be used as a zootechnical additive (functional group “other zootechnical additives”) in complete feed for chickens for fattening with the aim to favourably affect their performance.

No other specific authorisation exists for this product as a whole in the EU. However, all components are authorised as food (Commission Decision 1999/217/EC)7 and feed (currently subject of re-evaluation as feed additives) flavourings (category: Sensory additives). Moreover, some of the components of the CPP are also authorised under other feed additive categories:

- benzoic acid (E 210) as technological (Regulation (EC) No 757/2007)8 and zootechnical (Regulation (EC) No 1730/20069 and Regulation (EC) 1138/2007);10
- thymol is a component of a zootechnical feed additive (Regulation (EU) No 1117/2010);11

2. Characterisation

2.1. Identity of the additive

The additive is a light brown, free flowing powder with a characteristic odour. Its composition is given on Table 2.

The analysis of six batches of CPP showed that the concentrations of benzoic acid (82.6 % ± 0.6), thymol (2.0 % ± 0.0), eugenol (1.0 % ± 0.1) and piperine (0.11 % ± 0.01) are consistent with the specifications given by the applicant in Table 1.12 The analysis of thymol, eugenol, isoamyl salicylate, enzyl salicylate and trans-anethole, performed on a CPP pre-blend, indicated compliance with the specifications with very low coefficients of variation (CV).

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12 Supplementary Information, Annexes 18 and 19.
The analysis of the particle size distribution of three batches of CPP showed an average diameter of 1.2 mm, with no particles below 100 μm. The dusting potential measured by the Stauber-Heubach method was 0.55 g/m³, which is rated as low.

<table>
<thead>
<tr>
<th>Table 2: CRINA® Poultry Plus typical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active substances</td>
</tr>
<tr>
<td>Benzoic acid</td>
</tr>
<tr>
<td>Thymol</td>
</tr>
<tr>
<td>Eugenol</td>
</tr>
<tr>
<td>Piperine</td>
</tr>
<tr>
<td>Isoamyl salicylate</td>
</tr>
<tr>
<td>Benzyl salicylate</td>
</tr>
<tr>
<td>Trans-anethole</td>
</tr>
<tr>
<td>Diatomaceous earth</td>
</tr>
<tr>
<td>Silicic acid</td>
</tr>
<tr>
<td>Monopropylene glycol</td>
</tr>
<tr>
<td>Soyoil</td>
</tr>
<tr>
<td>Turmeric (Rhizome)</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
</tr>
</tbody>
</table>

Three batches of the product were analysed for pesticides and undesirable substances (heavy metals, arsenic, fluorine, dioxins and sum of dioxins plus dioxins like PCBs); the values obtained did not raise concerns. The analysis for microbial contamination (Escherichia coli and Salmonella) as well as for mycotoxins, including aflatoxin B1, complied with the legal limits. The absence of residual solvents (Class I, EMEA, 2000) is ensured by the suppliers from whom the food-grade active substances are purchased.

The soy-oil used in the manufacturing process is GMO free, as stated by the applicant.

2.2. Characterisation of the active substances

The physico-chemical characteristics of the active substances of the additive are summarised in Table 3.

<table>
<thead>
<tr>
<th>Table 3: Chemically defined active substances in CRINA® Poultry Plus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active substances</td>
</tr>
<tr>
<td>Benzoic acid</td>
</tr>
<tr>
<td>Thymol</td>
</tr>
<tr>
<td>Eugenol</td>
</tr>
<tr>
<td>Piperine</td>
</tr>
<tr>
<td>Isoamyl salicylate</td>
</tr>
<tr>
<td>Benzyl salicylate</td>
</tr>
<tr>
<td>Trans-anethole</td>
</tr>
</tbody>
</table>

Benzoic acid complies with the specifications set in Commission Directive 96/77/EC (data from the analysis of three batches).

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14 Technical Dossier, Section II, Annex 2-5; Supplementary Information, Annex 22.
17 Supplementary Information, Annex 27.
Purity criteria of the other compounds are set in accordance with JECFA specifications. Analytical data obtained from three batches of thymol and eugenol and 11 batches of piperine indicate compliance with those specifications.

2.3. Manufacturing process

The manufacturing process is fully described in the technical dossier. Essentially, the individual components are blended and an anticaking agent added.

2.4. Stability and homogeneity

2.4.1. Shelf life

The applicant provided two studies. In the first study, three CPP batches were stored at 25°C for 18 months and three at 35°C for 12 months. Benzoic acid contents were measured at 0, 3, 6, 12 and 18 months. The results indicated that the benzoic content is unchanged after the 12 and 18 months periods tested.

In the second study, not completed yet, three CPP batches were stored at 25°C and three at 35°C. Benzoic acid, thymol, eugenol and piperine contents were measured at 0, 3 and 6 months. The results indicated that the four active substances are stable up to six months under both storage conditions. Data concerning longer periods of storage are not available.

2.4.2. Stability in premixtures and feedingstuffs

Stability of CPP in premixtures (containing vitamins and trace elements) was assessed during a six month period in three batches containing 30 g CPP/kg, corresponding to 25 g benzoic acid/kg. Benzoic content was measured at 0, 3 and 6 months storage. The results indicated that benzoic acid from CPP is stable during processing and storage of six months in premixtures.

Stability of CPP in complete feed was assessed during a three month period by measuring the benzoic acid content of three batches of mash feed in which CPP was introduced at the level of 360 mg CPP/kg feed, corresponding to 300 mg benzoic acid/kg. The stability of CPP during pelleting (80°C) of the same mash feed and then stored for three months was tested in three batches. The results indicated that the benzoic acid content after the three month period was 99% of the initial dose in the mash feed and 92% in the pelleted feed.

There is no evidence that benzoic acid is an appropriate marker for the stability of all CPP components.

2.4.3. Homogeneity

Homogeneity of distribution of CPP in premixtures was evaluated by measuring benzoic acid. The test was done in two premixtures with two CPP batches (four trials), six sub-samples being analysed for each trial. The results indicated an average CV of 7.2% (2.7 to 11.1%).

Homogeneity of distribution of CPP in complete feed has been evaluated by measuring benzoic acid in three batches of the same feed prepared using three distinct CPP batches and eight replicates each. The results indicate an average CV of 9.8% (9.3 to 10.9%).

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20 Technical Dossier, Section II; Supplementary Information.
23 Technical Dossier, Section II, Annex 2-1.
24 Technical Dossier, Section II, Annex 2-1.
2.5. **Conditions of use**

CPP is intended to be used in chickens for fattening in the range of 300-450 mg/kg complete feed.

2.6. **Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)**

EFSA has verified the EURL report as it relates to the methods used for the control of four active substances (benzoic acid, thymol, eugenol and piperine). The Executive Summary of the EURL report can be found in Appendix A.

3. **Safety**

3.1. **Safety for the target species**

3.1.1. **Tolerance studies**

Four trials (the three first described trials done with the previous CPP formulation (star anise oil 0.1% of the product) and the fourth one with the new formulation (0.1% trans-anethole instead)) were conducted to collect information on the consequences of high intake of CPP on chickens for fattening.

3.1.1.1. **Trial 1**

A total of 960 one-day-old “Ross M3” chickens were divided into 48 groups and randomly allocated to six treatments (doses of CPP: 0, 300, 600, 900, 1500 and 3000 mg/kg; doses analytically confirmed for benzoic acid).\(^{25}\) The chickens were given a pellet diet based on wheat, maize and soybean meal. The trial lasted 35 days. The animals from all groups were checked for health status, weight gain, feed intake, blood chemistry/haematology and necropsy with pathological examination; mortality was recorded. The data were statistically evaluated by analysis of variance followed by Newman Keuls test.

No significant differences were observed between groups as regards feed to gain ratio, blood parameters and mortality (3.1 to 4.4%). Weight gain was significantly lower in the 3000 mg/kg CPP group compared to the 900 mg/kg dose group, but not different from control. At necropsy, treated groups presented high levels of hydropericardium, proventricular hypertrophy and/or liver alterations (hypertrophy, friable liver) when compared to controls. However, a relationship with treatment could not be firmly established, due to the lack of a dose-related response (Table 4).

**Table 4:** Pathological examination of animals treated with increasing levels of CRINA® Poultry Plus (CPP).

<table>
<thead>
<tr>
<th>Treatment (mg/kg CPP)</th>
<th>Number of animals</th>
<th>Hydropericardium</th>
<th>Proventricular hypertrophy</th>
<th>Hepatic hypertrophy</th>
<th>Friable liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>16</td>
<td>13</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>600</td>
<td>16</td>
<td>13</td>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>900</td>
<td>16</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1500</td>
<td>16</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>3000</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

3.1.1.2. **Trial 2**

A total of 960 one-day-old chickens were divided into 48 groups.\(^{26}\) Two different basal diets were prepared using a commercial coccidiostat (two different coccidiostats were used). Six treatment diets were prepared by supplementing each basal diet with CPP at the following doses: 0, 300 and 3000 mg/kg.

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\(^{25}\) Technical Dossier, Section II, Annex 3-2.

\(^{26}\) Technical Dossier, Section II, Annex 3-3.
mg/kg (doses analytically confirmed for benzoic acid). Each treatment was replicated with four groups of “Ross M3” chickens and four groups of “Ross 308” chickens. The trial lasted 35 days. The animals from all groups were checked for health status, weight gain, feed intake, blood chemistry/haematology and necropsy with pathological examination; mortality was recorded. The data were evaluated by analysis of variance followed by Newman Keuls test.

No significant differences were observed between groups for weight gain, feed conversion ratio or blood parameters. Mortality was not statistically different between groups, despite a numerical increase observed with increasing CPP doses: 4.4 % (control), 5.6 % (300 mg/kg), 7.5 % (3000 mg/kg). The incidence of pathological alterations markedly increased in CPP treated groups, albeit without a dose-related response:

- hydropericardium: dose 0: 26/128 (20 %), dose 300: 66/128 (52 %), dose 3000: 54/128 (42 %)
- friable liver: dose 0: 35/128 (27 %), dose 300: 52/128 (41 %), dose 3000: 46/128 (36 %).

3.1.1.3. Trial 3

A total of 312 one-day-old chickens male “Ross 308” were randomly distributed to 24 pens and six treatments (doses of CPP: 0, 300, 450, 1500, 3000 and 4500 mg/kg (doses analytically confirmed for benzoic acid)) and fed a diet based on wheat, maize and soybean meal, and supplemented with a coccidiostat. The trial lasted 35 days. The animals from the different groups were checked for health status, weight gain, feed intake, blood chemistry/haematology and necropsy and histopathological examination of liver (including lesion scores) in the 0, 300, 3000 and 4500 mg/kg; mortality was recorded. The data were evaluated by analysis of variance. In order to assess the association between treatment doses and severity of histopathological changes, the frequency and severity of lesions were analysed by the Chi-square test.

No significant differences were observed between groups for weight gain, feed to gain ratio and mortality. The histopathological evaluation revealed a 33 % incidence of hepatic haemorrhages in the two high dose groups whereas, the incidence was only 4 % in the groups 0 and 300 mg/kg CPP. Hydropic degeneration of hepatocytes also increased at the 4500 mg/kg dose.

Further histopathological examinations were performed in liver samples (three animals from treatment 0, 300, 3000 and 4500 mg/kg CPP). Hepatocellular hypertrophy, bile duct proliferation and fatty change were seen without a clear relationship to the dose level.

3.1.1.4. Trial 4

This experiment was carried out as a randomised complete block design with a 2x4 factorial arrangement of treatments. A total of 320 Ross 308 broilers (both sexes) were distributed into four dietary treatments, corresponding to four CPP dose levels (0, 450, 3000 and 4500 mg CPP/kg). Each treatment had four replicates (a pen containing 20 birds). The birds were fed a starter diet from 0 to 21 days of age and a grower diet from 21 to 35 days of age. Birds and feed were weighed in weekly intervals. On day 0, ten birds per pen were pre-selected and marked. They were followed-up once a week by a veterinarian for general health, respiratory system, movements, faeces quality, and homogeneity. On day 35, five marked birds per pen were sacrificed for post-mortem examination, histopathology and blood chemistry. Two other marked birds were used to determine slaughter yield. The experimental data were analyzed by ANOVA. Pen was the experimental unit for production parameters, whereas bird was the experimental unit for blood chemistry, gross pathology and histopathology.

No significant treatment-related differences were found for production parameters, mortality, post-mortem examination, chemistry or haematological blood parameters.

28 Supplementary Information, Annex 16.
29 Supplementary Information, Annex 13.
Histopathological examination was performed on the liver of 16 animals per treatment. A number of degenerative and inflammatory processes were observed in all treatment groups, but no overall dose-response trend was present. At the recommended dose level (450 mg/kg) only hepatic inflammation was increased compared to controls; this change was also increased at the high dose levels, but without a dose-related pattern. Some lesions (fatty change and granuloma) were somewhat more prevalent at top dose, but others (necrosis and bile duct proliferation) were more prevalent in controls (Table 5). The severity of the lesions were not related to treatments.

Table 5: Summarised data on liver lesions in tolerance trial 4. Number of observations. (Results of 16 samples).

<table>
<thead>
<tr>
<th>Lesions</th>
<th>CRINA® Poultry Plus (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Fatty change</td>
<td>0</td>
</tr>
<tr>
<td>Inflammatory cell foci</td>
<td>6</td>
</tr>
<tr>
<td>Peri-/biliar inflammation</td>
<td>7</td>
</tr>
<tr>
<td>Necrosis</td>
<td>2</td>
</tr>
<tr>
<td>Granuloma</td>
<td>0</td>
</tr>
<tr>
<td>Large areas of inflammatory</td>
<td>2</td>
</tr>
<tr>
<td>cells</td>
<td></td>
</tr>
<tr>
<td>Bile duct proliferation</td>
<td>3</td>
</tr>
</tbody>
</table>

3.1.1.5. Microbial studies

The in vitro antimicrobial activity of CPP was tested against the following strains: E. coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212 and Bacillus subtilis ATCC 6633. The minimum inhibitory concentration values obtained for all the tested strains were equal or higher than 2400 mg/kg, suggesting that the feed concentrations of CPP would not affect these strains in rearing situations.

3.1.2. Conclusions on the safety for target species

Given the inconsistency of the data and the suggestion of adverse effects, albeit not dose-related, the FEEDAP Panel is unable to conclude on the safety of the additive at the dose range proposed.

3.2. Safety for the consumer

3.2.1. In vitro genotoxicity of CPP

According to the relevant literature and the previous assessments by JECFA of the individual components of CPP (see section 3.2.2), none of them are genotoxic.

CPP was examined in two GLP in vitro genotoxicity assays, an Ames assay and an in vitro micronucleus assay in V79 cells.

An Ames assay (OECD 471) was performed in Salmonella Typhimurium strains TA98, TA100, TA102, TA1535 and TA1537, using eight CPP concentrations (from 3 to 5000 μg/plate). No significant increase in revertant colony numbers of any of the five tested strains was observed, neither in the presence nor absence of metabolic activation (S9 mix). Inhibition of bacterial growth was observed at 2500 μg/plate and higher. A range of recognized mutagens were used as positive controls (sodium azide, 4-nitro-o-phenylene-diamine, methyl methane sulfonate, 2-aminoanthracene), all increasing the revertant colonies rate.

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30 Technical Dossier, Section III, Annex 3-6.
31 Technical Dossier, Section III, Annex 3-40.
In an in vitro micronucleous assay in V79 cells (OECD 487) five CPP concentrations were tested without S9 mix (range 15.6-250 μg/mL) and three concentrations in the presence of S9 mix (range 15.6-62.5 μg/mL). Higher concentrations were not tested due to precipitation problems. Both in the absence and presence of S9 mix, no increase in the percentage of micronucleated cells was observed. Griseofulvin, mitomycin C and cyclophosphamide, used as positive controls, all elicited significant effects.

3.2.2. Safety and carry over of CPP components

The safety for the consumer of the seven components of CPP has been already assessed by JECFA and published in World Health Organisation reports (WHO, 2000 (trans-anethole); WHO 2001 (thymol); WHO 2002a (benzyl salicylate, isoamyl salicylate); WHO 2002b (benzoic acid); WHO 2006 (eugenol, piperine)), and all but trans-anethole by EFSA in the framework of the assessment of food flavourings (EFSA 2008, 2009). No safety concerns were identified for the use in human food, with the exception of piperine for which the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) concluded that the No Observed Adverse Effect Level (NOAEL) of 20 mg piperine/kg bw/day retained by JECFA was based on an inadequate study (short duration, lack of histopathology, low group size) and that further studies were needed to identify a NOAEL (EFSA, 2008).

The safety of the residues of the active substances and derived metabolites resulting from the consumption of tissues from chickens for fattening exposed to CPP at the maximum recommended dose has been considered for each of these substances.

3.2.2.1. Benzoic acid

The safety for the consumer of sodium benzoate used as feed additive in chickens for fattening at the dose of 22 mg/kg complete feed has been assessed by FEEDAP Panel (EFSA, 2011a). It was calculated that consumer exposure would amount to 0.22 mg/person/day on the conservative basis of the standard food basket according to Regulation (EC) No 429/2008. Considering the extended capacity of animals to metabolise benzoic acid even at high dose levels (up to 500 mg/kg bw), and the absence of bioaccumulation, the proportionality of residue levels vs dose administered can be reasonably assumed. Considering a use level of 373 mg/kg complete feed for chickens, which is 17 times higher than in the former application referred to, the corresponding consumer exposure to benzoic acid from CPP would be 3.74 mg/person/day. This amount would contribute to a very limited extent to the Acceptable Daily Intake (ADI) of 300 mg/person/day retained for benzoic acid (EC, 2002a).

3.2.2.2. Eugenol and trans-anethole

Eugenol and trans-anethole have been assessed recently as feed flavourings (EFSA 2011b, 2012). The FEEDAP Panel concluded that “The lack of data on metabolism and residues in poultry precludes an assessment of consumer exposure from this source”. As no data have been supplied by the applicant for this submission, no firm conclusion can be taken on consumer safety of eugenol and trans-anethole in CPP. However, the FEEDAP Panel notes that the assessment of these compounds as feed flavourings considered as safe the maximum dose proposed for use of 25 mg/kg complete feed. The dose related to CPP use would be 5 and 55 folds lower for eugenol and trans-anethole, respectively, than for the applications as feed flavourings. Consequently, consumer exposure related to parent compound and metabolites would be considerably lower and likely without safety concern for trans-anethole. However, in the absence of metabolism/residue data for eugenol in chickens, no definite conclusion can be drawn.

3.2.2.3. Thymol
In its assessment of thymol used as feed flavouring, the FEEDAP Panel concluded that up to the maximum concentration of 6 mg/kg complete feed for chickens, no safety concern would arise for the consumer (EFSA, 2012). The maximum dose of CPP, would deliver 8.6 mg thymol/kg complete feed, which is in the same order of magnitude as the figure derived for flavouring use.

3.2.2.4. Piperine
A study on the comparative in vitro metabolism of piperine in liver subcellular fractions from the rat and hen allowed the isolation and separation of 10-13 metabolites having a similar chromatographic behaviour in both species. This qualitative observation supports the conclusion of the likely commonality of metabolic fate of piperine in the target species and laboratory rodents.\textsuperscript{34}
In the absence of residue data in tissues of chickens for fattening administered CPP at the maximum dose proposed for use, the FEEDAP Panel cannot evaluate the consumer exposure to piperine.

3.2.2.5. Benzylsalicylate and isoamylsalicylate
In a review of the toxicological data of seventeen salicylate esters, including benzylsalicylate, the authors reported that in vitro mutagenicity studies (bacterial tests) gave negative results (Belsito et al., 2007). Methylsalicylate was the only compound for which a complete toxicological package was available; the lowest NOAEL (50 mg/kg body weight/day) was identified from subchronic and chronic oral toxicity data in both rats and dogs. As a subchronic oral toxicity study of isoamylsalicylate in the rat gave a comparable NOAEL (47 mg/kg body weight/day), the authors concluded that the toxicity of the other salicylate esters (including benzylsalicylate) would likely be similar.

In mammals, benzylsalicylate and isoamyl-salicylate are hydrolysed in the gastro-intestinal tract and the liver, yielding the corresponding alcohols (benzyl alcohol and isoamyl alcohol) and salicylic acid. Benzoyl alcohol is oxidised to benzaldehyde and benzoic acid, whereas salicylic acid is further metabolised in the liver via conjugation with glycine and subsequent formation of salicyluric acid, but also glucuronide conjugation. Given the rapid and near complete excretion of salicylates and related compounds in the urine, one can conclude that absorbed salicylates and their metabolites are widely distributed via blood, with little retention in tissues.\textsuperscript{35} Data from internal and published studies have shown that carboxylic acids including benzoic and salicylic acid follow a similar fate in chickens, conjugation occurring with ornithine instead of glycine.\textsuperscript{36} Consequently, no metabolite of safety concern would appear as residues in chicken tissues. Considering the very low exposure of chickens to these two compounds and their very limited retention in tissues, the FEEDAP Panel does not identify any safety concern for the consumer.

3.2.3. Conclusion on consumer safety
None of the components of CPP are genotoxic. Furthermore, the in vitro assays made with the additive gave no indication of a genotoxic potential.

No metabolism/residue data are available for eugenol and piperine in chickens for fattening. Consequently, the FEEDAP Panel cannot draw any final conclusion on the safety for the consumer of CPP used as feed additive in chickens for fattening.

3.3. Safety for the user
No studies have been supplied concerning eye irritation and skin irritation and sensitization. The Material Safety Data Sheet (MSDS) proposed for CPP indicates that the additive is a severe eye irritation agent and a skin sensitizer, whereas eugenol is a skin irritant and a skin sensitizer. Following dermal exposure, eugenol and piperine are extensively metabolised in the gastro-intestinal tract and the liver, yielding the corresponding alcohols and their metabolites are widely distributed via blood, with little retention in tissues.

\textsuperscript{34} Technical Dossier, Section III. Annex 3-33.
\textsuperscript{35} Technical Dossier, Section III. Annexes 3-7 and 3-8.
\textsuperscript{36} Technical Dossier, Section III. Annexes 3-13 and 3-14.
irritant and may cause sensitization by skin contact. Due to the lack of data, the FEEDAP Panel considers that the product should be treated as irritant for the skin and eyes, as well as skin sensitiser.

Given the particle size distribution and low dusting potential of CPP, no adverse effects upon inhalation are to be expected.

3.4. Safety for the environment

Benzoic acid is the by far the most abundant component of CPP. In a former assessment of benzoic acid the FEEDAP Panel referenced an opinion from the Scientific Committee on Animal Nutrition (SCAN) stating that most benzoic acid administered to farm animals will be excreted as hippuric acid, an endogenous metabolic by-product. Such compounds have low potential for bioaccumulation and low toxicity for aquatic or terrestrial organisms (EC, 2002b). Overall, the Panel concluded that there was no safety concern for the environment derived from the use of benzoic acid as feed additive.

In its assessment of eugenol and trans-anethole as feed flavourings, the FEEDAP Panel concluded that “Though in view of the metabolism in the target species and the fast biodegradation, the impact on the environment from the use of eugenol, eugenyl acetate, trans-anethole and 4-allyl-2,6-dimethoxyphenol in animal feed is expected to be low.” (EFSA, 2011b). In its assessment of phenol derivatives (including thymol proposed for use in the same order of magnitude as in CPP) as feed flavourings, the FEEDAP Panel concluded that “These compounds are not expected to pose a risk for the environment when used at a dose considered safe for the target species” (EFSA, 2012). In the absence of data on the environmental fate of piperine, isoamylsalicylate and benzylsalicylate, or the additive itself, the safety for the environment cannot be assessed.

4. Efficacy

4.1. Efficacy trials

The applicant provided four efficacy trials (protocols shown in Table 6) described below. Moreover, the results of the tolerance studies, including in all cases a treatment group with the recommended dose, were also used to evaluate the efficacy of the additive. Overall results of these controlled studies (efficacy trials and tolerance studies) are given in Table 7. Two additional field experiments were also reported: they were performed in partially controlled conditions (CPP tested with unknown other feed additives, experiment carried out within a large commercial herd). The full description of the trials used to assess the efficacy of CPP can be found in Appendix B.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Doses tested (mg/kg)</th>
<th>Breed Chickens</th>
<th>Treatments</th>
<th>Genders</th>
<th>Pens per gender and treatment</th>
<th>Birds per pen</th>
<th>Birds in trial</th>
<th>Stocking density birds/m²</th>
<th>Coccidiostats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 vs 300</td>
<td>Cobb 500</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>80</td>
<td>960</td>
<td>11</td>
<td>Coccidiostat A d1-d35</td>
</tr>
<tr>
<td>2</td>
<td>0 vs 300 vs 450/150</td>
<td>Ross 308</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>34</td>
<td>1224</td>
<td>17</td>
<td>Coccidiostat B d1-d22, Coccidiostat A d23-d35</td>
</tr>
<tr>
<td>3</td>
<td>0 vs 150 vs 300 vs 450</td>
<td>Ross 308</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>62</td>
<td>2976</td>
<td>16</td>
<td>Coccidiostat B d1-d21</td>
</tr>
<tr>
<td>4</td>
<td>0 vs 200 vs 300 vs 400</td>
<td>Ross PM3</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>45.5</td>
<td>2184</td>
<td>na*</td>
<td>Coccidiostat B d1-d22</td>
</tr>
</tbody>
</table>

(*) na: not available

37 Technical Dossier, Section IV.
38 Supplementary Information, Annexes 33 and 34.
Table 7: Effects of CRINA® Poultry Plus (CPP) on zootechnical parameters of chickens for fattening.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Doses tested (mg/kg)</th>
<th>Duration (d)</th>
<th>Number of animals (replicates per treatment)</th>
<th>Body weight (g)</th>
<th>Feed intake (kg/bird)</th>
<th>Feed to gain ratio</th>
<th>Mortality (%)</th>
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<td>0</td>
<td>42</td>
<td>960</td>
<td>2711</td>
<td>4.71</td>
<td>1.78</td>
<td>3.96</td>
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<td>2727</td>
<td>4.73</td>
<td>1.76</td>
<td>4.17</td>
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<tr>
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<td>0</td>
<td>42</td>
<td>1224</td>
<td>2603</td>
<td>3.60</td>
<td>1.39</td>
<td>1.50</td>
</tr>
<tr>
<td>trial 2</td>
<td>300</td>
<td></td>
<td>(12x34)</td>
<td>2637</td>
<td>3.67</td>
<td>1.39</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>450/150</td>
<td></td>
<td></td>
<td>2648</td>
<td>3.66</td>
<td>1.38</td>
<td>2.20</td>
</tr>
<tr>
<td>Efficacy</td>
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<td>42</td>
<td>2976</td>
<td>2681</td>
<td>4.71</td>
<td>1.76 a</td>
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<tr>
<td>trial 3</td>
<td>150</td>
<td></td>
<td>(12x62)</td>
<td>2674</td>
<td>4.65</td>
<td>1.74 ab</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td></td>
<td></td>
<td>2706</td>
<td>4.68</td>
<td>1.73 b</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td></td>
<td></td>
<td>2696</td>
<td>4.66</td>
<td>1.73 b</td>
<td>6.10</td>
</tr>
<tr>
<td>Efficacy</td>
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<td>42</td>
<td>2184</td>
<td>2557</td>
<td>4.19</td>
<td>1.64</td>
<td>5.78</td>
</tr>
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<td>trial 4</td>
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<td></td>
<td>(12x45)</td>
<td>2557</td>
<td>4.19</td>
<td>1.64</td>
<td>6.05</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td></td>
<td></td>
<td>2642</td>
<td>4.34</td>
<td>1.65</td>
<td>6.28</td>
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<td>2554</td>
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<td>4.65</td>
</tr>
<tr>
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<td>960</td>
<td>2186</td>
<td>3.56</td>
<td>1.63</td>
<td>3.80</td>
</tr>
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<td>study 1</td>
<td>300</td>
<td></td>
<td>(20x8)</td>
<td>2112</td>
<td>3.42</td>
<td>1.63</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td></td>
<td></td>
<td>2186</td>
<td>3.52</td>
<td>1.61</td>
<td>3.80</td>
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<tr>
<td>Tolerance</td>
<td>0</td>
<td>35</td>
<td>960</td>
<td>2653</td>
<td>4.19</td>
<td>1.59</td>
<td>3.80</td>
</tr>
<tr>
<td>study 2</td>
<td>(+ Cocc. A)</td>
<td></td>
<td>(20x16)</td>
<td>2627</td>
<td>4.13</td>
<td>1.58</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>(+ Cocc. B)</td>
<td>2638</td>
<td>4.17</td>
<td>1.59</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td></td>
<td>(+ Cocc. B)</td>
<td>2563</td>
<td>4.12</td>
<td>1.61</td>
<td>2.50</td>
</tr>
<tr>
<td>Tolerance</td>
<td>0</td>
<td>35</td>
<td>312</td>
<td>2184</td>
<td>3.36</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>study 3</td>
<td>300</td>
<td></td>
<td>(13x4)</td>
<td>2136</td>
<td>3.32</td>
<td>1.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>450</td>
<td></td>
<td></td>
<td>2172</td>
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</tr>
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<td>Tolerance</td>
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<td>2372</td>
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</tr>
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<td>study 4</td>
<td>450</td>
<td></td>
<td>(20x4)</td>
<td>2423</td>
<td>3.59</td>
<td>1.51</td>
<td>5.62</td>
</tr>
</tbody>
</table>

* Cocc.: Coccidiostat
(a,b) in a given study, means with a different letter differ significantly (P<0.05)

Overall, there were eight feeding studies (four describe as efficacy studies and four as tolerance trials) in which the minimum and/or maximum recommended dose was compared to a control group. In only one study was a significant effect on feed to gain ratio recorded. A meta-analysis considered only four of the available eight studies and was not further considered.

In addition, two field trials were described which showed significant improvement of performance with CPP at 300 mg/kg. However, only limited conclusions can be taken from these field studies as they were not conducted in controlled conditions (CPP compared to unknown products in one study, and treated CPP animals placed in commercial herds in the other study).

4.2. Conclusions on efficacy

A significant effect relative to performance was shown in only one of the eight studies taken for the demonstration of efficacy. In the view of the FEEDAP Panel, this is insufficient to establish efficacy.

4.3. Studies on the quality of meat

A sensory evaluation was carried out to evaluate whether the taste of cooked chicken meat was affected following the use of the maximum recommended dose of CPP (450 mg/kg feed). Leg meat and breast filets from control and treated animals were submitted to a trained panel. No significant taste difference was noted.

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39 This study was done in connection with the tolerance test submitted as Supplementary information, Annex 13.
5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation\(^{40}\) and Good Manufacturing Practice.

**CONCLUSIONS**

The limited change in product formulation proposed by the applicant during the assessment of the additive (replacement of star anise oil by trans-anethole), is unlikely to significantly affect the tolerance for chickens for fattening. Given the inconsistency of the data and the suggestion of adverse effects, albeit not dose-related, the FEEDAP Panel is unable to conclude on the safety of the additive at the dose range proposed.

None of the components of CPP are genotoxic and *in vitro* assays made with the additive gave no indication of a genotoxic potential. No metabolism/residue data are available for eugenol and piperine in chickens for fattening. Consequently, the FEEDAP Panel cannot draw any final conclusion on the safety for the consumer of CPP used as feed additive in chickens for fattening.

The FEEDAP Panel considers that the product should be treated as irritant for the skin and eyes, as well as skin sensitiser. Given the particle size distribution and low dusting potential of CPP, no adverse effects upon inhalation are to be expected.

In the absence of data on the environmental fate of piperine, isoamylsalicylate and benzylsalicylate, or the additive itself, the safety for the environment cannot be assessed.

There is insufficient evidence that CPP at the recommended dose can improve the zootechnical performance of chickens. The sensory properties of the meat from chickens fed CPP are not affected.

**DOCUMENTATION PROVIDED TO EFSA**


4. Comments from Member States received through the ScienceNet.

**REFERENCES**


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EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011b. Scientific Opinion on the safety and efficacy of allylhydroxybenzenes (chemical group 18) when used as flavourings for all animal species. EFSA Journal, 9(12):2440.

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012. Scientific Opinion on the safety and efficacy of phenol derivatives containing ring-alkyl, ring-alkoxy and side-chains with an oxygenated functional group (chemical group 25) when used as flavourings for all species. EFSA Journal 2012;10(02):2573


APPENDIX A

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Crina® Poultry Plus

In the current application authorisation is sought for Crina® Poultry Plus under Articles 4(1), category of 'zootechnical additives' functional group 4(d) 'other zootechnical additives' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of Crina® Poultry Plus for chicken for fattening. The active substances in the feed additive are a preparation of benzoic acid and three essential oil compounds (thymol, eugenol and piperine). The feed additive, as proposed by the Applicant, is intended to be mixed to complete feedingstuffs at a dose ranging from 300 to 450 mg, which is equivalent to 250 to 375 mg benzoic acid per kg feedingstuffs.

For the determination of the benzoic acid in the feed additive and in the premixtures the Applicant proposed a single laboratory validated and further verified method, based on Reverse Phase High-Performance Liquid Chromatography coupled to an ultraviolet detector (RP-HPLC-UV). The following performance characteristics were reported:

- For the feed additive:
  - a standard deviation for repeatability (RSD_r) ranging from 0.5 to 1%;
  - a standard deviation for intermediate precision (RSD_ip) ranging from 0.7 to 1.3%, and
  - a recovery rate (R_{rec}) ranging from 99.7 to 101%.

- For premixtures:
  - RSD_r ranging from 0.5 to 4.5%;
  - RSD_ip ranging from 1.8 to 5.4%, and
  - R_{rec} ranging from 96 to 105%.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified RP-HPLC-UV method, submitted by the Applicant, to determine benzoic acid in feed additive and premixtures.

For the determination of the benzoic acid in feedingstuff the Applicant proposed a single laboratory validated and further verified method, based on Gas Chromatography Isotope Dilution Mass Spectrometry (GC-IDMS). The following performance characteristics were reported:

- RSD_r ranging from 1.6 to 3.3%;
- RSD_ip ranging from 1.9 to 3.9%, and
- R_{rec} ranging from 94.5 to 101%.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified GC-IDMS method, submitted by the Applicant, to determine benzoic acid in feedingstuffs.

For the determination of thymol, eugenol and piperine in the feed additive the Applicant proposed a single laboratory validated and further verified method based on Gas Chromatography coupled to a Flame Ionization Detector (GC-FID). The following performance characteristics were reported:

- RSD_r ranging from 1.5 to 4.6%;

- \( \text{RSD}_q \) ranging from 1.7 to 5.6%, and
- \( \text{R}_{\text{Re}} \) ranging from 94 to 105%.

Based on the performance characteristics presented the EURL recommends for official control the single laboratory validated and further verified GC-FID method, submitted by the Applicant, to determine thymol, eugenol and piperine in the feed additive.

For the determination of Crina® Poultry Plus in premixtures and feedingstuffs the Applicant proposed to identify only benzoic acid as appropriate marker substance for the recognition of the whole feed additive. No experimental data were provided by the Applicant for the identification of the thymol, eugenol and piperine in premixtures and feedingstuffs. Therefore, the EURL is not able to evaluate nor recommend a method for the official control to identify thymol, eugenol and piperine in premixtures and feedingstuffs.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.
Appendix B

Full description of the trials used to assess the efficacy of Crina® Poultry Plus (CPP)

Trial 1
A total of 960 one-day-old COB 500 chickens were randomly allocated to two treatments and six repetitions per treatment (three groups of males and three groups of females) (doses of CPP: 0, 300 mg/kg (doses analytically confirmed for benzoic acid)). The birds were fed a pelleted feed based on wheat, maize and soybean meal and supplemented with a coccidiostat (d1-d35). The trial lasted 42 days. The following parameters were checked: mortality, weight gain and feed intake. The data were submitted to analysis of variance.

Results indicated a similar mortality rate between groups (3.96 to 4.17 %) and overall no significant effects on weight gain, feed intake and feed to gain ratio (FCR) were found between groups.

Trial 2
A total of 1224 one-day-old Ross 308 chickens were randomly allocated to three treatments and six repetitions (three groups of males and three groups of females) per treatment (doses of CPP: 0, 300 and 450 mg/kg (for the latter dose, from 22-42 d, 150 mg/kg) (doses analytically confirmed for benzoic acid)). The birds were fed a pelleted feed based on wheat, maize and soybean meal, and supplemented by two successive coccidiostats (d1-d22, one coccidiostat, d23-d35, another coccidiostat). The trial lasted 42 days. The following parameters were checked: mortality, weight gain, feed intake and caecal microflora. Data were submitted to analysis of variance.

Results indicated a similar mortality rate between groups (less than 4 %) and overall no significant effects on weight gain, feed intake, FCR and caecal microflora were found between groups.

Trial 3
A total of 2976 one-day-old chickens were randomly allocated to six treatments and six repetitions (three groups of males and three groups of females) per treatment (doses of CPP: 0, 150, 300 and 450 mg/kg) (doses analytically confirmed for benzoic acid). The birds were given a pelleted feed based on wheat, maize and soybean meal and a coccidiostat (d1-d21). The trial lasted 42 d. The following parameters were checked: mortality, weight gain, feed intake and microbial profile. Data were submitted to a variance analysis.

Results indicated a significant improvement of FCR for both doses 300 and 450 mg/kg when compared to control animals (P< 0.05). Mortality was not significantly affected by treatments. Regarding modulation of the microflora, no clear dose-response relationships could be found.

Trial 4
A total of 2184 one-day-old Ross PM 3 chickens were randomly allocated to four treatments (doses of CPP: 0, 200, 300 and 400 mg/kg), and 12 repetitions (six male groups (40 birds per pen) and six female groups (51 birds per pen)) per treatment (doses analytically confirmed for benzoic acid). Birds were fed a pelleted feed based on wheat, maize and soybean meal, supplemented with a coccidiostat (d1-d22). The trial lasted 42 d. The following parameters were checked: mortality, weight gain, feed intake, digesta analyses and carcass evaluation. Data were submitted to analysis of variance followed by the Tukey t test.

Overall (whole period, both sexes) no significant effects were observed on either weight gain, feed intake, FCR, caecal microflora, or mortality. No differences between the various treatments were observed for carcass yield and for the microbiological counts.

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1 Technical Dossier, Section IV, Annex 4-18.
2 Technical Dossier, Section IV, Annex 4-19.
3 Technical Dossier, Section IV, Annex 4-20.
4 Technical Dossier, Section IV, Annex 4-21.
Additional field studies

Two trials were performed in Europe in controlled conditions, in research stations related to commercial feed compounders. Both trials were conducted on chickens for fattening and satisfied the minimum length of observation time specified in the EFSA guidance for tolerance and efficacy studies in target species (35 days). In both trials the treatment (recommended dose 300 mg/kg) was compared to a control. The aim of the first trial was the comparison of various treatments, including a control, a CPP, and other groups of non-defined products from the market. In the second trial control and treated chickens (n= 300) were held in separated pens (ten chickens per pen) within a flock of about 8500 chickens with the aim to simulate sanitary conditions of large commercial farms.

These two additional trials showed a similar improvement of performance (2-3 % on weight gain and 1-4 % on FCR, P<0.05) when CPP was included in the feed (at 300 mg/kg). However, only limited conclusions can be taken from these field studies as they were not conducted in controlled conditions (CPP compared to unknown products in one study, and treated CPP animals placed in commercial herds in the other study).

Efficacy findings from tolerance studies

In each tolerance study, one treatment group corresponded to the recommended CPP dose. Thus, these tolerance studies adequately designed for efficacy evaluation, could contribute to demonstrate the potential of the additive to improve efficacy. In all four tolerance studies provided, no significant differences were found concerning zootechnical parameters between control birds and birds treated with CPP at the recommended dose.

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6 Supplementary Information, Annex 33.
7 Supplementary Information, Annex 34.