5 November 2020
EMA/CVMP/405186/2010-*corr.*[1]
Veterinary Medicines and Product Data Management

Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Isoeugenol (fin fish)

On 13 April 2011 the European Commission adopted a Regulation[2] establishing maximum residue limits for isoeugenol in fin fish, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Isoeugenol is intended for use in fin fish for sedation/anaesthesia in routine husbandry by waterborne use.

Scan Aqua AS submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 7 July 2009.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use concluded that isoeugenol should be included in Table 1 of Commission Regulation (EU) 37/2010.

Subsequently the Commission recommended on 23 December 2010 that maximum residue limits in fin fish are established. This recommendation was confirmed on 26 January 2011 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 13 April 2011.

[1] *The EPMAR was updated in November 2020 to correct in paragraph 3.5 the ADI, which was misreported in the conclusion of the original EPMAR.*
[2] Commission Regulation (EU) No 363/2011,O.J. L 100, of 14.04.2011

Summary of the scientific discussion for the establishment of MRLs

Substance name:	Isoeugenol
Therapeutic class:	Anaesthetic
Procedure number:	EU/09/169/SNA
Applicant:	ScanAqua AS
Target species:	Atlantic Salmon and rainbow trout
Intended therapeutic indication:	Sedation/anaesthesia in routine husbandry
Route (s) of administration:	Waterborne use

1. Introduction

Isoeugenol (CAS No 97-54-1) is manufactured from eugenol (obtained from clove oil) by a process of isomerisation to yield a mixture of cis- (<12%) and trans- (>87%) isoeugenol containing less than 1% residual eugenol. Clove oil is a natural product, derived from the *Eugenia caryophyllata* tree.

In veterinary medicine isoeugenol is intended to be used for sedation/anaesthesia of salmon and Rainbow trout in routine husbandry operations such as grading, vaccination and brood stock transportation. Isoeugenol is also intended to reduce the deleterious effects of harvesting on muscle quality by eliminating muscle fatigue resulting from stress when fish are captured and handled. For anaesthesia, the intended dose is 7.5 to 13.4 mg isoeugenol/l. Lower doses in the range 2.5 to 5 mg/l of isoeugenol are intended to be used for light sedation/stress reduction.

Isoeugenol is used as a flavouring agent and may be present in beer, roasted coffee, nutmeg and smoked fish. The substance is not used in human medicine.

2. Scientific risk assessment

2.1. Safety assessment

2.1.1. Overview of pharmacological properties

Literature data indicate that mature salmon (*Salmo salar* and *Oncorhynchus tshawytscha*) can take between 5 and 8 minutes of exposure at 8.5 mg isoeugenol/l before showing signs of loss of equilibrium, and most husbandry practices can be carried out after 12 to 15 minutes exposure. However, much shorter response times of approximately 2 to 5 minutes to produce handleable fish can be obtained by working with higher concentrations. In addition to the concentration used, other factors that may influence response to the substance include duration of exposure, animal factors (species, age), level of fatigue, health status and environmental factors, in particular water temperature. Lower water temperatures increase the time taken for onset of anaesthesia.

Following exposure, increasing sedation is characterised by an increase in swimming speed, ventilation and a progressive deterioration of distance perception. Thereafter there is a progressive loss of equilibrium, swimming motions become ineffective, ventilation becomes erratic and fish demonstrate insensitivity to loud noises and restraint. Exposure to isoeugenol suppresses respiratory activity. At higher concentrations of isoeugenol, respiratory depression, characterised by undetectable opercular movement, may occur with the effect that drug clearance is inhibited. In such cases, a resuscitation technique, ensuring irrigation of the gills, may aid recovery.

The mechanism of action of isoeugenol in fish has not been determined. It is hypothesised that its effects are mediated via receptors controlling cellular ion channels in a similar way to that described for local anaesthetics. Direct effects on the brain may occur due to the absence of a blood brain barrier in fish. In the rat, it has been shown that isoeugenol may exert competitive effects with acetylcholine at the neuromuscular junction.

It has been suggested that the apparent reduction in stress response observed in fish at sub-anaesthetic doses may be due to a reduction in the cortisol response mediated by hypothalamic-pituitary-interrenal interactions. However, while an apparent isoeugenol-related reduction in cortisol release was detected in some studies in which fish were exposed to stressors such as confinement and acute oxygen depletion, the findings of other studies did not detect a positive effect of isoeugenol on cortisol response.

Limited data were provided in respect of secondary pharmacodynamic effects. Short, non-GLP research reports are provided that characterise effects on cardiac and respiratory function. Anaesthesia with isoeugenol resulted in a reduction in heart rate and cardiac output. Post anaesthesia, cardiac output increased while heart rate remained fairly stable for the first hour. Thereafter, heart rate increased before plateauing and then decreasing steadily back to pre-anaesthesia values by 48 hours. Respiratory function returned to normal by 4 hours post anaesthesia.

No data were provided on secondary pharmacology in mammalian species. However, it was accepted that isoeugenol is not likely to have a pharmacological effect relevant for humans other than those that would be detected in the standard battery of toxicological tests.

Pharmacokinetic properties (in laboratory animals)

A literature study (GLP status unstated) used radiolabelled isoeugenol to investigate the disposition and metabolism of the compound in the male Fischer 344 rat. Following a single oral dose of [^{14}C]isoeugenol (156 mg/kg bw, 50 microCi/kg bw), greater than 85% of the administered dose was excreted in the urine predominantly as sulfate or glucuronide metabolites by 72 hours. Approximately 10% was recovered in the faeces, and less than 0.1% was recovered as CO_2 or expired organics. No parent isoeugenol was detected in the blood at any of the time points analysed (0.25 to 72 hours). Following intravenous administration (15.6 mg/kg bw, 100 microCi/kg bw), isoeugenol disappeared rapidly from the blood. The half-life was 12 minutes, the volume of distribution was 13.96 l/kg, mean residence time (MRT) was 11.6 minutes and the systemic clearance was 1.9 l/min/kg. Excretion characteristics were similar to those seen following oral administration.

The total amount of radioactivity remaining in selected tissues (heart, kidneys, liver, muscle, subcutaneous adipose tissue and testicular adipose tissue) by 72 hours was less than 0.25% of the dose following both oral and intravenous administration. Based on the findings of this study, it can be concluded that isoeugenol is rapidly metabolised in the rat and is excreted predominantly in the urine as phase II conjugates of the parent compound.

With respect to metabolism of isoeugenol in fish, a literature report that extrapolates from data in mammals taking into consideration the relevant enzymatic systems in fish suggests that the main elimination route is glucuronidation, and to a lesser extent sulphate conjugation. Both conjugates are more hydrophilic than the parent compound and will be readily excreted through bile/faeces or via urine.

2.1.2. Calculation of pharmacological ADI, if relevant

It was concluded that isoeugenol does not exert pharmacological effects relevant to consumer safety. Consequently it was not considered necessary to establish a pharmacological ADI.

2.1.3. Overview of toxicology

Single-dose toxicity

Only limited literature data have been provided in respect of acute toxicity. In the rat, the toxic signs observed were coma soon after treatment and scrawny appearance, with time to death ranging from 1 hour to 7 days. In the guinea pig, the toxic signs observed were depression, coma, rough fur, scrawny appearance, with time to death ranging from 3 to 6 days. Isoeugenol administered by the intraperitoneal route to mice produced distinct hypothermia.

Tolerance in target species

When isoeugenol is used in the target species at recommended doses and exposure times, it is well tolerated and exposed fish can be expected to recover uneventfully. It is clear however that mortality is a consequence of overdosage. In a literature report of a GLP compliant study, following exposure of salmon to isoeugenol at higher than the recommended concentrations (1, 2 and 3 times the high effective concentration for 3 times the exposure time required to obtain anaesthesia), there was no evidence of gross or histopathological changes to brain, gill, heart, kidney, liver or integument.

Repeated dose toxicity

Repeated dose toxicity data in mice and rats are available from GLP compliant US National Institute for Environmental Health Sciences/National Toxicology Program (NIEHS/NTP) published reports. In mice oral doses of 0, 37.5, 75, 150, 300 and 600 mg isoeugenol/kg bw were administered by gavage to groups of male and female mice five times per week for up to 14 weeks. Body weight and clinical observations were recorded weekly. At study termination, mice were necropsied and selected organs were weighed. Tissue collected at necropsy was sent for microscopic examination. No effects of isoeugenol were noted on survival, clinical observations, clinical pathology endpoints and gross necropsy findings. However, a treatment related effect on bodyweight was noted in males in the high dose group. Some changes in liver to bodyweight ratios were observed, but these changes were not considered treatment related because the magnitude of the changes was small and there was no clear dose response relationship. Histopathological changes to the olfactory epithelium and olfactory nerve fibres were detected in the high dose groups (both male and female), but not in lower dose groups.

It is suggested that the lesions in the olfactory epithelium were a consequence of local irritation due to contact with the test material. The observed atrophy of olfactory nerves is considered to have been secondary to the effects on the overlying epithelium. The NOAEL for this study was 300 mg/kg bw/day based on body weight effects seen at 600 mg/kg bw/day.

In a study in rats oral doses of 0, 37.5, 75, 150, 300 and 600 mg isoeugenol/kg bw were administered by gavage to groups of males and females five times per week for up to 14 weeks. Bodyweight and clinical observations were recorded weekly. At study termination, rats were necropsied and selected organs were weighed. Tissue collected at necropsy was sent for microscopic examination. There was an absence of any isoeugenol related changes on survival, clinical observations, clinical pathology endpoints and gross necropsy findings. A treatment related effect on bodyweight was noted for the high dose male group. No effect on weight was detected for females. At necropsy, some changes in liver to bodyweight ratios were observed: increased absolute and relative hepatic weights in the 300 and 600 mg/kg bw/day groups. The changes in liver weight were attributed to centrilobular hyperplasia. Histopathological changes to the olfactory epithelium and olfactory nerve fibres were detected in all isoeugenol dose groups, with the exception of the female 37.5 mg/kg bw/day dose group. Again, it is suggested that the effects on the nose were a result of direct contact rather than systemic exposure. The NOAEL for this study was 150 mg/kg bw based on increased hyperplasia in liver.

Reproductive toxicity, including developmental toxicity

Reproductive toxicity was investigated in two US NIEHS/NTP GLP compliant studies. Isoeugenol was administered daily by oral gavage at doses of 0, 70, 230 and 700 mg/kg bw to Sprague-Dawley rats (20/sex/dose group) in a 2-generation (3 litters/generation) reproductive toxicity study. A dose-related decrease in mean bodyweights was observed in mid- and high-dose males, and high-dose females. The aggregate number of live male pups born in all litters to F_0 parents was significantly decreased at 700 mg/kg bw/day. Combined pup weights were significantly reduced in high-dose F_2 progeny. All other reproductive parameters were comparable between groups. Sperm morphology, motility and numbers were unaffected by treatment, as was vaginal cytology and oestrus cycle activity.

In a separate out breeding programme conducted as part of this study, a significant increase in the percentage of live female pups/litter and an increase in the number of implantation sites was noted when naïve females were mated with high-dose males. Reductions in terminal bodyweights resulted in increases in some organ-to-bodyweight ratios in high-dose males and females. A significant increase in hyperkeratosis and hyperplasia of the non-glandular stomach was evident in all treated groups. Although no NOEL could be retained for systemic toxicity, the NOEL for reproductive toxicity was 230 mg/kg bw/day under the conditions of this study.

In the second reproductive toxicity study female Sprague-Dawley rats (25/dose group) were administered isoeugenol by gavage at doses of 0, 250, 500 and 1000 mg/kg bw/day from days 6 to 19 of gestation. No treatment-related maternal deaths occurred in this study. Maternal body weight and body weight gain were significantly reduced at all doses tested. Gravid uterine weights were reduced at isoeugenol doses equal to or higher than 500 mg/kg bw/day, while relative liver weights were significantly increased in all treated groups. Although the mean foetal body weight per litter was significantly reduced at 1000 mg/kg bw/day, prenatal viability was unaffected by treatment. A significantly increased incidence of unossified sternebrae was noted in high-dose pups. However, the incidence of all other morphological anomalies was comparable between treated and control groups. Although no NOEL could be retained for maternal toxicity, the NOEL for developmental toxicity was 500 mg/kg bw/day under the conditions of this study.

Genotoxicity

The genotoxicity of isoeugenol has been extensively studied. A majority of the studies are published references but three crucial *in vivo* assays have been performed by the applicant.

Isoeugenol, in the presence and absence of S9, was negative in two Ames tests in *Salmonella* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 and in the *Escherichia coli* strain WP2 uvrA using test concentrations up to 600 μg/plate. In the *Bacillus subtilis* DNA repair assay isoeugenol dissolved in ethanol produced a preferential killing of Rec- cells compared to Rec+ cells in the absence of S9. However, the outcome of this study has to be considered as equivocal since there are uncertainties due the differences in growth between the two strains. In addition, a second study on *Bacillus subtilis* DNA-repair was also reported, using isoeugenol dissolved in dimethylsulfoxide (DMSO). The results of this study were negative. Overall, isoeugenol is considered to have shown no genotoxic potential with respect to gene mutations in bacteria.

Isoeugenol induced a significant increase in sister chromatid exchange (SCE) at test concentrations of 0.25 to 0.5 mM (41 to 82 mg/l) in cultured human lymphocytes. However, as no concurrent measurement of cytotoxicity was included in this study it is not possible to draw conclusions from these results since the observed effect could be due to cytotoxicity. Moreover, isoeugenol did not induce chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9 activation. The genotoxicity of isoeugenol was investigated *in vitro* in cultured primary hepatocytes using the *in vitro* unscheduled DNA synthesis (UDS) test. There was no evidence of unscheduled DNA synthesis

(UDS) in primary cultured hepatocytes from male Fischer 344 rats and female B6C3F1 mice at isoeugenol test concentrations of up to 1 mM. Overall, there is no clear evidence of genotoxic potential of isoeugenol in the *in vitro* mammalian cell tests.

In a cell-free *in vitro* system, DNA binding of isoeugenol/metabolites was seen in the presence of a metabolic activation system (Aroclor induced rat liver S9). No apparent DNA binding activity was seen in the absence of a metabolic activation system. However, since it is known to be difficult to purify DNA samples from protein contamination, it should be emphasized that a major part of the observed binding activity may have been due to protein binding of the compound/metabolites rather than to DNA binding.

Isoeugenol was non-genotoxic at test concentrations of 164 to 2460 mg/l in the wing spot test in *Drosophila melanogaster*.

A total of three *in vivo* micronucleus tests and one *in vivo* UDS test were submitted. Two of the tests have been newly performed and were initiated following the previous CVMP evaluation of the substance, which concluded that there was equivocal evidence of genotoxic potential and that a new micronucleus test, including both male and females, could possibly provide assurance and shed light on the results of the NTP sponsored study (EMEA/CVMP/693156/2009).

The first study, an *in vivo* mammalian bone marrow erythrocyte micronucleus study in male mice with doses up to 2000 mg/kg isoeugenol, did not show genotoxic activity. However, a second study, which was a peripheral blood micronucleus test in mice, conducted on behalf of the (National Toxicology Program) NTP, showed a 3.2-fold increase in the frequencies of micronucleated erythrocytes in female mice in the highest dose-group of 600 mg/kg bw/day. In the third, newly performed study, which was an *in vivo* micronucleus test in male and female mice, there was no indication of genotoxic potential of isoeugenol when dosed up to 2000 mg/kg/day and 1500 mg/kg/day in males and females, respectively. In addition, no evidence of genotoxic potential could be observed in the rat *in vivo* UDS test in male and female rats administered doses of up to 2000 mg/kg (males) and 1250 mg/kg (females). Overall, isoeugenol is considered to be devoid of genotoxic potential.

In conclusion, the genotoxicity of isoeugenol has been extensively studied in *in vitro* and *in vivo* genotoxicity assays. Overall, the weight of evidence is sufficient to conclude that isoeugenol is not genotoxic.

Carcinogenicity

Chronic toxicity/carcinogenicity studies were conducted with isoeugenol in Fischer 344 rats and B6C3F1 mice as part of the US government-sponsored NTP. Draft reports on these studies are publicly available and have been subject to review by the NTP. Although presented in draft form the reports included sufficient information to allow for a full assessment of the studies.

In the rat carcinogenicity study males and females were exposed to isoeugenol in corn oil by gavage at doses of 0, 75, 150, or 300 mg/kg bw, 5 days per week, except holidays, for 105 weeks. A slight increase in the incidences of thymoma in thymus and carcinoma in mammary gland were seen in male rats at the highest tested dose, 300 mg/kg/day. These findings were, however, within the historical control range. Moreover, it was noted that the conduct of the study had significant weaknesses related to the route of administration (oral gavage) and the dosing regime (week days only). In conclusion, isoeugenol is not considered to be carcinogenic in rats.

In the mouse carcinogenicity study males and females were exposed to isoeugenol in corn oil by gavage at doses of 0, 75, 150, or 300 mg/kg bw, 5 days per week, except holidays, for 104 (females) or 105 (males) weeks. Hepatocellular adenoma, hepatocellular carcinoma and combined hepatocellular adenoma or carcinoma were seen in all dose groups, 75 to 300 mg/kg bw/day. In

female mice, histiocytic sarcoma (all organs) was noted in the high dose group, 300 mg/kg bw/day. These findings were close to the historical control range and could represent a random effect. Furthermore, no dose response was identified in hepatic tumour incidence in male mice. It was noted that the conduct of the study had significant weaknesses related to the route of administration (oral gavage), the dosing regime (week days only) and a number of dead females (9) observed in the high dose group over a period of 8 days. These deficiencies could have affected the final results. It was also noted that the mouse strain used (B6C3F1) is known to have a high incidence of spontaneous liver tumours and lymphomas. The CVMP concluded that although equivocal positive findings were identified in the carcinogenicity study in mice the deficiencies identified in the study and the comparison with historical data did not allow a firm conclusion to be drawn. Overall, the findings of the mouse carcinogenicity study were considered equivocal and their relevance for the human consumer remains unclear. No NOAEL could be determined from this study as effects were seen at all doses. A LOAEL of 75 mg/kg bw was determined.

Studies of other effects including immunotoxicity and neurotoxicity

Isoeugenol is well recognised as a contact sensitizing agent, particularly following application by the dermal route. The purported mode of sensitization may involve the benzylic route or the hapten ("poison ivy") route. Isoeugenol (25 μl of 0.15 to 0.61 M solution) produced positive results for increased lymph node cell proliferation following topical administration to mice. However, isoeugenol did not induce any increase in plasma IgE concentrations in the mouse IgE model, and was thus considered not to be a respiratory tract sensitizing agent.

Reports of adverse reactions involving the use of isoeugenol in humans are primarily confined to incidents of contact sensitization or allergy following dermal exposure.

No specific neurotoxicity studies were conducted. Reference was made to the pharmacodynamic studies presented previously in relation to the molecule's known sedative and anaesthetic properties. Isoeugenol was found to possess myorelaxant and anti-convulsive properties in mice at a dose of 200 mg/kg bw.

Effects on the olfactory epithelium/olfactory nerve fibres were observed in repeated–dose studies. The observed atrophy of olfactory nerves is believed to have occurred as a local effect secondarily to the loss of overlying epithelium. It is accepted that the possibility of a systemic effect can be excluded based on the findings detailed in the NTP draft report on the chronic toxicity/carcinogenicity studies. No clinical signs of neurotoxicity were observed in the 13-week or 2 year studies. Furthermore, histopathological evidence of neuronal and nerve fibre degeneration was confined to a single location (the olfactory epithelium). Based on the information available, isoeugenol is not considered to be a neurotoxin.

2.1.4. Calculation of the toxicological ADI or alternative limit

The toxicological endpoints are considered the most relevant for the safety assessment of isoeugenol. The induction of liver cancer in male mice (strain: B6C3F1) observed in a 2-year carcinogenicity study is considered as the most sensitive adverse effect of isoeugenol. Both liver adenomas and carcinomas were observed at all doses (no NOAEL identified; LOAEL: 75 mg/kg bw/day). Since the liver carcinogenesis did not arise through a genotoxic mechanism, a threshold can be assumed.

The relevance of the liver cancer seen in male mice remains unclear. Isoeugenol did not increase rates of cancer in Fischer 344 rats or female mice and no dose response was observed in male mice. In addition the B6C3F1 mouse strain is known to be highly susceptible to liver tumours as a result of genetic predisposition. Nevertheless, the possible significance of the effects seen cannot be ruled out,

particularly in light of the fact that liver hyperplasia was also noted in the 14-week repeat dose rat study.

On this basis, the mouse carcinogenicity bioassay was selected as the key study for the derivation of a health based guidance value for isoeugenol, i.e. the ADI.

Using the LOAEL of 75 mg/kg bw/day as the point of departure and using an overall uncertainty factor of 1000 (100 for inter/intra-species extrapolation, 2 for use of a LOAEL instead of a NOAEL, and 5 to account for the potential seriousness and irreversibility of the effect combined with the deficiencies of the study) a toxicological ADI of 0.075 mg/kg bw (i.e. 4.5 mg/person) can be established.

2.1.5. Overview of microbiological properties of residues

Disruption of the colonisation barrier

The growth of *Bacillus subtilis*, *Escherichia coli* and two strains of *Staphylococcus aureus* were not inhibited by isoeugenol at a dilution of 1:500, but dilutions of 1:1000 to 1:6400 were bactericidal to pure and mixed cultures of staphylococci and various intestinal bacteria. Isoeugenol concentrations of 0.03 to 0.06% inhibited the hyphal growth and sporulation of *Choanephora frispora*. Isoeugenol (1 to 2% dissolved in ethanol) did produce moderate growth inhibition *in vitro* against several bacterial species isolated from the skin, gills and intestines of common carp.

2.1.6. Calculation of microbiological ADI

While isoeugenol was seen to possess some antimicrobial activity, the effect was weak and it was therefore concluded that isoeugenol residues resulting from the ingestion of treated fish would be unlikely to exert a significant effect on the human gut flora. Based on the magnitude of the antimicrobial effects it was concluded that there is no need to establish a microbiological ADI for isoeugenol as the value of this ADI would certainly be higher than that of the toxicological ADI.

2.1.7. Observations in humans

Isoeugenol has been used as a food flavouring agent and as a fragrance agent in numerous cosmetic products for a long period of time. In the USA, the molecule has been granted "GRAS" status when employed at sufficiently low doses and used for the above purposes. Reports of adverse reactions involving the use of isoeugenol in man are primarily confined to incidents of contact sensitization or allergy. This is particularly common via the dermal route of exposure. Two adverse reaction reports in humans have been provided following the use of the Aqui-S product as a fish anaesthetic. Both reactions appear to involve vapour mist and included uneventful recoveries.

2.1.8. Findings of EU or international scientific bodies

It was noted that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated isoeugenol as a flavouring agent in 2003. No ADI was established but JECFA considered that at an exposure level of 120 µg/person/day there would be no safety concern on the use of the substance. In the USA, isoeugenol was granted "GRAS" status as a flavouring agent in 1965.

Isoeugenol is included in the EU Register[3] for flavouring agents and was evaluated by the scientific panel of food contact materials, enzymes, flavourings and processing aids (CEF) of the European Food Safety Authority (EFSA). At their meeting of 26-28 January 2010 the EFSA scientific panel adopted an

[3] Commission Decision N° 1999/217/EC

opinion on the use of isoeugenol concluding that the substance was not genotoxic and that there was no safety concern at the estimated levels of intake arising from use as a flavouring agent.

2.1.9. Overall conclusions on the ADI

The establishment of a pharmacological and a microbiological ADI was not considered necessary for the safety assessment of isoeugenol and the toxicological endpoints were considered the most relevant for the safety assessment of isoeugenol. Therefore the toxicological ADI of 75 µg/kg bw (i.e. 4500 µg/person) was established based on the application of a 1000-fold safety factor applied to the LOEL (75 mg/kg/day) derived from the assessment of tumorigenic potential in mice.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

Pharmacokinetic data were provided in Atlantic salmon only.

A non-GLP study in Atlantic Salmon showed that following initial exposure, isoeugenol was rapidly absorbed in fish and rapidly distributed to skin + muscle. Concentrations in skin/muscle increased steadily during the exposure period and in the initial stages of exposure residues in muscle were concentration dependent. After the end of the exposure period isoeugenol was rapidly eliminated. An elimination half-life of 1.44 hours in plasma, and a depletion half-life of 1.89 hours in muscle+skin was determined following exposure to 1.8 mg isoeugenol/l for 60 minutes. Isoeugenol remained quantifiable in plasma (limit of detection: 25 ng/l) for up to 12 hours after the end of exposure but was no longer detectable in plasma at 24 hours. While the dosage used in this study was lower than that recommended for treatment, it was considered adequate for characterisation of the pharmacokinetic profile of isoeugenol in the target species.

In a GLP compliant study radioactivity derived from ^{14}C-isoeugenol was detected in plasma, muscle + skin, liver, bile and kidney of salmon following immersion in 8.16 mg isoeugenol/l for one hour. Isoeugenol was the major radioactive component in extracts of muscle + skin and accounted for a mean of 85.2% (range: 78.6 to 92.2%) of total radioactive residue. Approximately 8 other extractable residues were present in muscle, none of which were quantitatively significant. Isoeugenol was present to varying extents in kidney (3.8% of total radioactive residues), liver (76.8% of total radioactive residues), bile (8.5% of total radioactive residues) and plasma (62.4% of total radioactive residues). Other residues in kidney, liver and plasma included three polar components. These other residues were not characterised further. The dosage used was lower than that recommended for treatment.

2.2.2. Residue depletion studies

Residue depletion data were provided in Atlantic salmon only.

In a GLP-compliant study immersion of Atlantic salmon for 1 hour in 8.16 mg/l isoeugenol resulted in highest total residue concentrations at the end of the treatment period being found in liver (104 mg/kg), with kidney and muscle concentrations of 55 mg/kg and 25.8 mg/kg respectively. Isoeugenol accounted for 85%, 77% and 4% of total residues in muscle, liver and kidney respectively.

In a non-GLP compliant study where Chinook salmon were immersed in isoeugenol either at 17 or 34 mg/l for 1 hour, residue concentrations declined quickly (values reduced by 92% and 86% of peak within 5 hours and were less than 0.27 mg/kg and 0.03 mg/kg respectively in muscle+skin at 11 hours post treatment (limit of detection 50 µg/kg)).

In a GLP-compliant study, residue concentrations of isoeugenol in muscle + skin fillets taken from Atlantic salmon immersed in isoeugenol at 9.9 mg/l, 14.6 mg/l or 19.9 mg/l for between 30 and 120 minutes were between 18.8 and 28.4 mg/kg. There was no difference in the residues observed with increased time of immersion.

Similarly, in another GLP-compliant study, mean residue concentrations in Atlantic salmon that had been immersed in 4.6, 6.9 and 9.2 mg/l isoeugenol for between 20 and 60 minutes were between 11.9 and 31.3 mg/kg in muscle fillets (including skin in natural proportions) and did not vary with increased immersion time.

Selection of marker residue and target tissues

The relevant target tissue for fin fish is muscle and skin in natural proportions. Immersion of Atlantic salmon for 1 hour in 8.16mg/l isoeugenol resulted in highest total residue concentrations at the end of the treatment period being found in liver (104 mg/kg), with kidney and muscle concentrations of 55 mg/kg and 25.8 mg/kg respectively. Isoeugenol accounted for more than 80% of total residues in muscle and can be considered the marker residue.

Considering that the marker to total residues was not defined but was known to be higher than 0.8, a figure of 1 was considered to be appropriate for use in the intake calculation.

2.2.3. Monitoring or exposure data

Not applicable

2.2.4. Analytical method for monitoring of residues

A HPLC method with fluorescence detection was developed and validated in a GLP approved laboratory for the determination of residues of isoeugenol in muscle/skin of Atlantic salmon. The limit of quantification of the method was 0.25 mg/kg. The method was described in an internationally recognised format and was demonstrated to be suitable for the purposes of quantifying residues. The method has been sufficiently validated according to the requirements of Volume 8 of the Rules Governing Veterinary Medicinal Products in the European Union and has been verified by the relevant European Reference Laboratory, which confirmed the suitability of the method.

2.2.5. Findings of EU or international scientific bodies

Information on MRLs established by other International bodies was not available.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

Not applicable.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

Not applicable.

3.3. Elaboration of MRLs

As exposure to isoeugenol residues may result from its use in other areas (e.g, as a flavouring agent) as well as in veterinary medicine it was considered appropriate to allocate a maximum of 40% of the ADI for veterinary uses. Taking into account the established ADI of 0.075 mg/kg, an estimated daily per capita consumption of 300 g of fish and an average human body weight of 60 kg, an MRL of 6 mg/kg could be recommended for salmon.

Calculation of theoretical daily intake of residues

The theoretical maximum daily intake arising from exposure to residues of isoeugenol in fin fish was calculated as follows:

Edible tissue or products	Daily consumption (kg)	MRL proposal (µg/kg)	Ratio of the marker/total residue	Amount per edible tissue or product (µg)
Muscle+skin	0.30	6000	1	1800

The calculated figure of 1800 µg represents a theoretical consumer daily intake of residues in fish of 40% of the ADI.

Based on available data it is estimated that the level of intake of flavouring agents in this group of hydroxypropenylbenzenes is 120 µg/capita/day. When exposure to isoeugenol as a result of its use as a flavouring agent was also considered, total exposure to residues was calculated according as follows:

ADI (µg per person)	4500 µg/person
Total used for veterinary products	1800
Total from other sources	120
Total [veterinary + other use]	1920

Taking into account also the use of the substance as a flavouring agent the total consumer intake of residues represents approximately 43% of the ADI.

3.4. Considerations on possible extrapolation of MRLs

In line with the CVMP Note for Guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMEA/CVMP/187/00-Final) the MRL proposed for salmon may be extrapolated to fin fish.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- the toxicological ADI of 0.075 mg/kg bw (i.e. 4.5 mg/person) was established as the overall ADI for isoeugenol;
- isoeugenol was retained as the marker residue;
- the ratio of marker to total residues was set at 1;
- an analytical method for the determination of residues of isoeugenol in edible tissues of Atlantic salmon (muscle and skin in natural proportions) is available and has been validated in accordance

with the requirements of Volume 8 of the Rules governing medicinal products in the European Union;

- in line with existing CVMP guidance MRLs proposed for salmon can be extrapolated to fin fish;

the Committee for Medicinal Products for Veterinary Use (CVMP) recommends the establishment of maximum residue limits for isoeugenol in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Isoeugenol	Isoeugenol	Fin fish	6000 µg/kg	Muscle and skin in natural proportions	Not applicable	Agents acting on the nervous system/Agents acting on the central nervous system

4. Glossary

ADI – Acceptable daily intake

DNA – Deoxyribonucleic acid

GLP – Good laboratory practice

HPLC – High performance liquid chromatography

LOAEL – Lowest observed adverse effect level

MRL – Maximum residue limit

NOAEL – No observed adverse effect level

NOEL – No observed effect level

NTP – National Toxicology Program

UDS – Unscheduled DNA synthesis

5. Background information on the procedure

Submission of the dossier	7 July 2009
Steps taken for assessment of the substance	
Application validated:	17 July 2009
Clock started:	18 July 2009
List of questions adopted:	11 November 2009
Consolidated response to list of questions submitted:	16 June 2010
Clock re-started:	17 June 2010
Adoption of opinion	15 September 2010