The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BENZOCAINE
(extension to *Salmonidae*)

SUMMARY REPORT (2)

1. Benzocaine (4-aminobenzoic acid ethyl ester (synonym: ethyl p-aminobenzoate), is a local anaesthetic of the ester type with a poor solubility in water. It is used in cattle, sheep, swine and horses for local and prolonged low epidural anaesthesia. Standard therapeutic doses of benzocaine ranged between 150 and 750 mg per animal. Benzocaine is also currently used as surface anaesthetic as ointments (0.5% benzocaine) for wounds and ulcerated surfaces in horses, cattle and sheep applied twice a day until healing.

   Currently, benzocaine is included in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Benzocaine	All food-producing species	For use as local anaesthetic only

   An application has now been submitted for an extension of the MRL classification to *Salmonidae*. Benzocaine is intended for use as a central anaesthetic in several species of fish. The exposure concentrations would range between 10 and 50 mg/l in the water bath depending on the type of fish and the sedation level required for the water borne procedure. The main use of the substance would be during vaccination of salmon parrs and smolts, 6 – 12 weeks before their transfer from fresh water into sea water. The temperature of the water would be between 4 and 10°C. The fish would be removed from the anaesthetic solution when the required level of anaesthesia had been achieved. For this use, the proposed maximum exposure time would be 15 minutes. In addition, the substance would also be used to induce light sedation during transport, for automatic counting or sorting for monitoring of sea lice numbers, etc., or to induce light anaesthesia for manual counting or sorting or manual spawning. The substance is not intended for use as an anaesthetic and tranqillizer before slaughter for human consumption.

2. In fish, anaesthetics like benzocaine administered in water (inhalants) passes over the gills during respiration and then the substance crosses the respiratory membranes to enter the circulatory system. The main metabolites identified in fish are p-aminobenzoic acid acetyl p-aminobenzoic acid and acetyl benzocaine. The less polar residues (benzocaine, acetyl benzocaine) are predominantly eliminated in effluent water, presumably through the gills. The more polar residues (p-aminobenzoic acid, acetyl p-aminobenzoic acid) are retained in the animal and excreted more slowly in the urine. Acetylation is the primary route of metabolism.

3. Published pharmacokinetic data indicate that benzocaine in fish is rapidly distributed and eliminated from plasma between the first 20 minutes after dosing in water bath. In rainbow trout kept at 12°C pharmacokinetic parameters were assessed after single intra-arterial administration of 6 (8 fish) or 9 mg/kg bw (10 fish). Blood samples from each fish were taken 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 90, 120 and 150 minutes after treatment and analysed for benzocaine with HPLC with UV-detection. Mean plasma concentrations after 2 minutes were 51.832 µg/ml in the 6 mg/kg bw dose group and 15.935 µg/ml in the 9 mg/kg bw dose group. In both groups the values declined nearly 100-fold within the next 8 minutes. After 90 minutes plasma values were

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close to or below the limit of quantification (0.025 µg/ml) of the method. The data best fitted a 3-compartment model. The steady-state volumes of distribution (V_{ss}) were calculated as 112 and 156 ml/kg for the 6 and 9 mg/kg bw groups, respectively. The corresponding values for clearance (Cl_b) were 12.5 and 18 ml/min/kg and for terminal half-life ($t_{½ß}$) 89.4 and 108.5 minutes. Mean residence time (MRT) was 8.9 and 8.7 minutes in the 6 and 9 mg/kg bw dose group, respectively. Plasma concentrations of benzocaine were highly variable within the same dose group and the higher average concentrations in the lower dose group were difficult to explain, though dose-dependent differences in distribution and elimination were suspected.

4. In rainbow trout the influence of water temperature on the pharmacokinetics of benzocaine after bath exposure (1 mg/l for 4 hours) was studied at 6°C, 12°C and 18°C. The uptake and metabolic clearance increased with higher temperatures (uptake clearance: 581 ± 170 and 1154 ± 447 ml/min/kg at 6 and 18°C, metabolic clearance: 15.2 ± 4.1 and 22.3 ± 4.2 ml/min/kg at 6 and 18°C). The apparent volume of distribution showed a (non-significant) trend towards increase with higher temperatures (2369 ± 678 and 3260 ± 1182 ml/kg at 6 and 18°C). The elimination half-life was variable but not significantly different with temperature (60.8 ± 30.3, 35.9 ± 13 and 42.4± 21 minutes at 6, 12 and 18°C). Mean plasma concentrations of benzocaine at 4 hours of exposure were 1.538 ± 0.14 µg/ml at 6°C, 1.463 ± 0.341 µg/ml at 12°C and 1.224 ± 0.127 µg/ml at 18°C. After 2.5 minutes in anaesthetic-free water the values declined to 0.242 ± 0.132 µg/ml at 6°C, 0.098 ± 0.064 µg/ml at 12°C and 0.076 ± 0.016 µg/ml at 18°C.

5. Plasma kinetics in catfish also show a rapid initial decline of concentrations of benzocaine residues, however, values in bile increased at later time points. Catfish were exposed to bath concentrations of ^{14}C-labelled benzocaine of 70 mg/l until loss of righting reflex (about 5 minutes) followed by 35 mg/l for 30 minutes at 25°C. Average concentrations in plasma and bile determined at 0, 4, 25, 144, 240 and 480 hours after treatment were 47.1 and 72 µg/ml, 2.92 and 0.556 µg/ml, 0.309 and 387 µg/ml, 0.135 and 132 µg/ml, 0.0792 and 43.8 µg/ml, and 0.0458 and 6.6 µg/ml, respectively. Half-lives were determined to be about 8 days for plasma and 3.2 days for bile. At 0 and 4 hours, benzocaine was the dominant substance detected in all tissues and fluids, with para amino benzoic acid and acetyl benzocaine also being present.

6. Metabolism and elimination of benzocaine was studied in rainbow trout (5 fish per time point) which received a single aortic dose of 20.8 µg/kg bw of ^{14}C-labelled benzocaine hydrochloride (uniformly ring-labelled). Branchial elimination was rapid and accounted for 59.2% of the dose during the first 3 hours after treatment. Renal elimination was considerably slower, amounting to 2.7% of the dose within 3 hours and to 9% of the dose within 24 hours. At 24 hours after treatment bile in the gall bladder contained 2% of the dose. In the surrounding water 3 minutes after injection of the ^{14}C-benzocaine 87.3% of the detected radioactivity was due to benzocaine and 12.7% to acetyl benzocaine. The proportions changed to 32.7% benzocaine and 67.3% acetyl benzocaine 1 hour after treatment. Urinary radiocativity at 1 hour after treatment was accounted for to 7.6% by p-aminobenzoic acid, 59.7% by acetyl para amino benzoic acid, 19.5% benzocaine and 8% acetyl benzocaine. The proportions changed over time and 20 hours after treatment para amino benzoic acid amounted to 1% and acetyl p-aminobenzoic acid to 96.6%. The main route of excretion for benzocaine and its less polar residue acetyl benzocaine was through the gills, with renal and biliary pathways being less significant. Analysis of the samples was performed by HPLC and liquid scintillation counting.

7. Limited toxicological data and on effects in humans were provided for benzocaine during the original assessment of the substance. There were insufficient data to identify a pharmacological or toxicological NOEL and consequently no ADI could be retained for benzocaine. In view of the limited use as a local anaesthetic no further data were considered necessary at that point in time.

   No further toxicological data were presented for the establishment of MRLs in fin fish. Some information concerning the use in humans was provided. Benzocaine had been used for over 50 years as a local anaesthetic for the temporary local relief of pain associated with dental conditions, teething in babies, oropharyngeal disorders and ear pain. The available preparations include sprays, gels, pastes and solutions containing up to 20% benzocaine for the surface anaesthesia of the throat and mouth. Lozenges containing up to 10 mg benzocaine may be administered for the relief of sore throats. The most commonly reported adverse reaction in humans was hypersensitivity. Positive reactions in the cutaneous sensitisation test were reported in 3.3 to 5.9% of human patients.

8. Residue depletion studies mainly found in published literature were provided for rainbow trout after water-borne treatment. Rainbow trout (3 fish per time point) exposed to a bath concentration of 50 mg benzocaine/l for 15 minutes at 12°C were killed at 0, 1, 2, 4, 8 and 24 hours after exposure. For withdrawal fish were kept in flowing, anaesthetic free water. Muscle tissue was analysed by a colorimetric method which detects benzocaine by reaction of its primary aromatic amine group. The method does not distinguish between benzocaine and its metabolites and is impeded by background levels (440 µg/kg) of primary aromatic amines in fish tissues, precluding measurement of low levels of benzocaine. Maximum concentrations of 14 010 µg/kg were detected immediately after treatment, declining to 5460 µg/kg a 1 hour, 740 µg/kg at 2 hours, 550 µg/kg at 4 hours, 670 µg/kg at 8 hours and 710 µg/kg at 24 hours after treatment.

9. A further study was performed in rainbow trout (8 to 10 fish per time point) exposed in the bath at temperature of 14 to 15°C to benzocaine concentrations of 20 mg/l for 30 minutes, 50 mg/l for 5 minutes (residues quantified after 4 hours only) and 40 mg/l for 10 minutes (residues quantified after 0, 2, 4, 6, 8 and 12 hours (9°C)). After treatment, fish were rinsed, kept in a 40 l fresh water bath until full recovery and then put into a flow-through basin for the remainder of the withdrawal period. Concentration of benzocaine and its metabolites acetyl benzocaine, para amino benzoic acid and acetyl para amino benzoic acid were assayed in muscle and skin in natural proportions by HPLC with UV-detection. The method was not validated in accordance with the requirements of Volume VI of The Rules Governing Medicinal Products in the European Community and had high variations in reproducibility (more than 20%). The reported values can therefore only be regarded as indications of likely residue levels. The limits of quantification in this study were 139, 452, 61 and 242 µg/kg for para amino benzoic acid, acetyl para amino benzoic acid, benzocaine and acetyl benzocaine, respectively. Some of the following tissue concentrations are below the respective limits of quantification but above the limit of detection and represent estimates of the actual residues. Total residues (sum of all metabolites) amounted to 28 600, 1530, 570, 530, 270 and 180 µg/kg at 0, 2, 4, 6, 8 and 12 hours After treatment with 40 mg/l for 10 minutes the concentrations for para amino benzoic acid amounted to 150 µg/kg at 0 hours and to an estimated 20 µg/kg at 4 hours after treatment, with no para amino benzoic acid being detected at later time points. Acetyl para amino benzoic acid was present at 0, 2, 4, 6, 8 and 12 hours in concentrations of 40, 170, 280, 210, 160, and 50 µg/kg. For benzocaine the corresponding values are 27 600, 1000, 160, 180, 60 and 40 µg/kg, while for acetyl benzocaine the concentrations amounted to 750, 270, 120, 120, 0 and 20 µg/kg. After treatment with 20 mg/l for 30 minutes total residues (sum of all metabolites) amounted to 1140 µg/kg at 4 hours. The values for the individual metabolites were not calculated. The concentrations of the total residues or the individual metabolites 4 hours after treatment with 50 mg/l for 5 minutes were also not calculated, however the overall relative ratio of metabolites was comparable to that observed at 40 mg/l for 10 minutes (acetyl para amino benzoic acid being somewhat more abundant than acetyl benzocaine and benzocaine).

10. The effect of temperature on elimination of benzocaine and acetyl benzocaine from edible fillet with skin of rainbow trout (10 fish/temperature and time point) was studied at 7°C and 16°C after bath exposure to 30 mg/l for 5 minutes followed by 15 mg/l for 30 minutes. Immediately after treatment fillets at both temperatures contained about 27 000 µg/kg benzocaine. At 24 hours after treatment benzocaine at both temperatures was below the limit of quantification (22 µg/kg for benzocaine) of the HPLC-method with UV-detection. At 7°C the concentration of acetyl benzocaine increased initially, reached a plateau at 1 to 4 hours after treatment, before decreasing. At 16°C the concentration of acetyl benzocaine was constant at 0 to 1 hour after treatment before decreasing. Concentrations at both temperatures were below the limit of quantification (23 µg/kg for acetyl benzocaine) 24 hours after treatment. Maximum concentrations of acetyl benzocaine at earlier time point were not reported in detail but were in the range between 500 and 1000 µg/kg.

11. Published residue depletion data after water-borne treatment were available for other fish species.

    In largemouth bass (3 fish per time point) exposed to bath concentrations of 50 mg benzocaine/l for 15 minutes at 12°C, then kept in flowing, anaesthetic free water until slaughter at 0, 1, 2, 4, 8 and 24 hours after exposure, the following concentrations were observed in muscle tissues analysed with the colorimetric method described above: 10 650 µg/kg at 0 hours, 3780 µg/kg at 1 hour, 2170 µg/kg at 2 hours, 1110 µg/kg at 4 hours, and below the control value of 470 µg/kg at 8 and 24 hours after treatment.

12. In striped bass, bluegills and largemouth bass exposed to a bath concentration of 63.2 mg/l benzocaine at 18°C, residues in tissue homogenate (whole fish without head, scales, fins and viscera) were determined by a colorimetric assay. Para amino benzoic acid was separated from benzocaine by liquid-liquid partitioning. Immediately after exposure mean concentrations in the homogenate were 25 800 µg/kg benzocaine and 900 µg/kg para amino benzoic acid, declining to 3300 µg/kg benzocaine and 200 µg/kg para amino benzoic acid at 1 hour, and 600 µg/kg benzocaine and 100 µg/kg para amino benzoic acid at 4 hours after exposure. Tissues of bluegill and largemouth bass at 1 hours after treatment contained 1500 and 2100 µg/kg benzocaine and 0 and 60 µg/kg para amino benzoic acid, respectively.

13. In catfish exposed to a bath concentration of ^{14}C-labelled benzocaine of 70 mg/l until loss of righting reflex (about 5 minutes) and then to 35 mg/l for 30 minutes at 25°C, red muscle tissue, white muscle tissue and filet (without skin) were analysed. Total radioactivity was determined by combustion analysis and liquid scintillation counting (LSC), for the quantification of benzocaine, para amino benzoic acid , acetyl benzocaine and acetyl para amino benzoic acid HPLC with reverse isotope dilution and liquid scintillation counting was used. Samples were taken at 0, 4, 25, 144, 240 and 480 hours after treatment. Immediately after exposure average concentrations of total residues were 19400 µg/kg in white muscle, 107900 µg/kg in red muscle, 32500 µg/kg in skin, 143000 µg/kg in liver, 61700 µg/kg in head kidney and 66600 µg/kg in trunk kidney, changing to 1470 µg/kg in white muscle, 6300 µg/kg in red muscle, 4260 µg/kg in skin, 29900 µg/kg in liver, 4790 and 9490 µg/kg in head and trunk kidney. Concentrations 25 hours after exposure were 99 µg/kg in white muscle, 557 µg/kg in red muscle, 413 µg/kg in skin, 3810 µg/kg in liver, 467 and 1470 µg/kg in head and trunk kidney, in most tissues reaching about 1% of the initial values. Radioactivity were still detectable after 20 days averaging 19.1 µg/kg in white muscle, 52.3 µg/kg in red muscle, 90.9 µg/kg in skin, 65.3 and 99.4 µg/kg in head and trunk kidney, 230 µg/kg in liver. The mean residue concentrations in filet (red and white muscle in natural proportions) were 36700, 2200, 192.5, 50.8, 47 and 17.6 µg/kg at 0, 4, 25, 144, 240 and 480 hours after exposure.

14. Based on the above limited residue information in rainbow trout and other fish species, the maximum amount of benzocaine-related residues from 300 g fish, 24 hours after treatment, amounts to 0.21mg (range 0.05 to 0.21 mg). However, the main use of the substance would be during the vaccination of salmon parrs and smolts, 6 to 12 weeks before their transfer from fresh water into sea water, and consequently there would be a long period between treatment and slaughter for human consumption (one year). In addition, the substance could also be used in adult fish, for example to induce light sedation for automatic counting or sorting or for light anaesthesia for manual counting or sorting or manual spawning. Taking into account the evidence of fast elimination in *Salmonidae,* it was considered unnecessary to establish MRLs for *Salmonidae*. However elimination was slower in other fish species such as catfish. The CVMP noted that significant residues could be present in *Salmonidae* immediately after treatment. Consequently benzocaine should not be used to tranquillize fish prior to slaughter.

16. Several analytical methods have been developed to determine benzocaine and its metabolites in the fish tissues: HPLC with UV detection, HPLC with electrochemical detection and colorimetric quantification. None of the quantification methods available in the literature have been validated in accordance with Volume VI of the Rules Governing Medicinal Products in the European Community.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for Veterinary Medicial Products for the inclusion of substances in Annex II of Council Regulation (EEC) No. 2377/90 and in particular that:

- animals are unlikely to be sent for slaughter during or immediately after treatment,
- in *Salmonidae*, benzocaine was rapidly eliminated;

The Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for benzocaine and recommends its inclusion in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Benzocaine	*Salmonidae*	

In addition, the CVMP recommended that during the consideration of Applications for Marketing Authorisations, Member States may consider the application of a withdrawal period.