CEFUROXIME SODIUM

First draft prepared by

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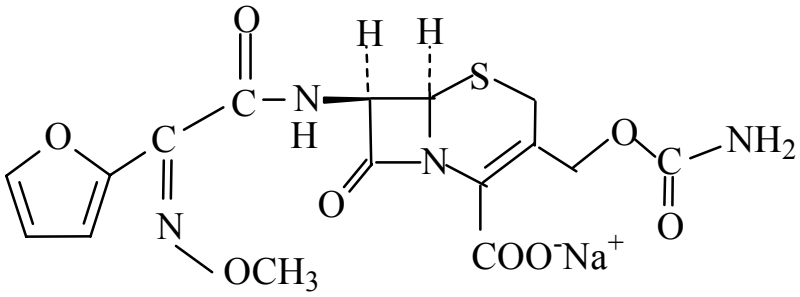
IDENTITY

Chemical name: [6R-[6α, 7β(Z)]]-3-[[aminocarbonyl)methyl]-7-[[2-furanyl(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt
{ Chemical Abstracts Service (CAS) name; CAS number 556238-63-2}

Sodium (Z)-(6R,7R)-3-(carbamoyloxymethyl)-7-[2-(2-furyl)-2-(methoxyimino)acetamide]-8—oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate {International Union of Pure and Applied Chemistry (IUPAC) name}

International Non-Proprietary Name: none allocated (BAN and rINN is cefuroxime sodium)



Structural formula:

Molecular formula: $C_{16}H_{15}N_4NaO_8S$

Molecular weight: 446.4

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Cefuroxime sodium

Appearance: White or faintly yellow powder

Melting point: Decomposes on heating

Solubility: Freely soluble in water and buffered solutions (500 mg/2.5 mL water, pKa in water 2.5); soluble in methanol; very slightly soluble in ethyl acetate, diethyl ether, octanol, benzene and chloroform (Merck)

Stability: Solutions are stable at room temperature for about 13 h, approximately 10% decomposition over 48 h at 25 °C

Ultraviolet maximum: 274 nm (water)

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

General

Cefuroxime is a second-generation cephalosporin antibacterial with activity against a range of gram-positive bacteria, as well as many gram-negative organisms. It is used in veterinary medicine for the treatment of mastitis. It is also used in human medicine as either the sodium salt or as the 1-acetoxyethyl ester (known as cefuroxime axetil).

Dosage

Cefuroxime sodium is available in two formulations intended for veterinary use, Spectrazol ™ Dry Cow (12.5% cefuroxime sodium) and Spectrazol ™ Wet Cow (9.22% cefuroxime sodium). Both preparations are in a base which is largely composed of liquid paraffin BP. Spectrazol ™ Dry Cow is a long-acting preparation used to treat sub-clinical mastitis in dry cows and to prevent new infections during the dry period. It is applied using a single dose syringe to deliver 375 mg of cefuroxime into the test canal, for a total dose of 1.5 g per animal. Spectrazol ™ Wet Cow is an intra-mammary product used in the treatment of clinical mastitis which is supplied as a single syringe that is injected into one quarter of the udder. Treatment of all four quarters results in a dose of 1 g of cefuroxime per animal.

PHARMACOKINETICS AND METABOLISM

Laboratory animals

Rats

In a series of experiments to measure pharmacokinetic parameters and distribution, ^{14}C-cefuroxime was administered to rats by intravenous or intra-muscular injection, or by intravenous infusion (Nanbo *et al*, 1979). Intravenous administration to male rats at a dose of 25 mg/kg bw ^{14}C-cefuroxime resulted in concentrations in whole blood of 45 mg/L at 5 minutes after treatment. The elimination followed a bi-phasic profile, with half-lives of 0.44 h and 17.25 h. Following intravenous infusion of male rats with 50mg/mL/kg ^{14}C-cefuroxime by intravenous infusion in the hind leg at a rate of 0.69 mL/h for 40 minutes, the peak concentration in whole blood was about 60 mg/L was at the end of the infusion, diminishing rapidly within 3 h with a half-life of 0.98 h. Within 24 h, only about 2% of the concentration seen at the end of infusion remained. The half-life following intra-muscular injection with a dose of 25 mg/kg bw was 0.78 h, with the highest concentration appearing within 15 to 30 min of administration.

Following intravenous administration at a dose of 25 mg/kg bw, elimination of ^{14}C-cefuroxime was rapid in rats. In males, 71.2% of the dose was excreted in the urine within 6 h, and 79.3% within 72 h. In females, 74.4% of the dose was excreted in urine in the first 6 h, with 81.3% eliminated in urine over 72 h. In males. 16.6% of the dose was excreted in faeces in 24hand 18.6% in 72 h, while in females, faeces contained 14.8% of the dose in the first 24 h and 16.9% after 72 h. In male rats, 13.5% of the dose was excreted in the bile within 1 h of treatment, reaching 17.8% after 48 h. The peak excretion in both urine and bile was within 15 to 30 min of treatment. In a rat which had bile from a treated rat introduced into the duodenum, the excretion rate relative to the radioactivity of the bile in the duodenum was 5.1% in the bile and 21.9% in the urine over 48 h, indicating that about 27% of the dose was reabsorbed from the intestinal tract. Thin layer chromatographic analysis indicated that >95% of the residue in urine and plasma was parent compound, while about 72% of the residue excreted in the bile of rats within 24 h was parent compound. A small amount of an unidentified metabolite was observed in the rat bile samples.

Continuous administration experiments were also conducted in which three male rats received 25 mg/kg ^{14}C-cefuroxime by intra-muscular injection once daily for 14 days, with naturally excreted urine and faeces collected for analysis prior to each administration. Additional male rats received a similar treatment regimen, but were killed after either 1 or 7 doses. Rats which received each treatment regimen were killed at 1 and 3 days after final administration and tissues were analyzed for radioactive residues.

The excretion rates were unchanged in urine and faeces following the repeated doses. At 72 h following final administration, 82.4% of the dose was excreted in urine and 16.3% in faeces.

Whole body autoradiography measurements were conducted on rats which received a dose of ^{14}C-cefuroxime by intravenous injection into the caudal vein. A high level of radioactivity was observed in the bladder urine within 5 min of intravenous administration, with a similar activity on the kidneys. Other areas showing high activity included the contents of the small intestine, artery walls, liver, trachea, blood and skin, as well as the lung, salivary gland, eyeball wall, adrenal gland, testes and seminal vesicle. Activity was low in the central nervous system. Weak activity was observed in the gastric contents at 30 min. At 24 h, low activity was observed in the contents of the large intestine and kidneys, with a high level of activity in the kidneys from the medulla to the cortex. Slight activity was observed in the liver, but not in other internal organs or tissues. At 72 h, there was still a low level of activity in the kidney medulla and slight activity in the contents of the large intestine, but no observed activity in other internal organs or tissue.

Analysis of tissues collected following intravenous administration to male rats showed highest concentrations in kidneys, declining from 443 ±99 mg equiv/kg at 5 min, to 54 ±5 mg equiv/kg at 30 min and 11.7 ±0.6 mg equiv/kg at

72 h. Liver samples showed a similar pattern, declining from 117 ±8 mg equiv/kg at 5 min, to 47 ±3 mg equiv/kg at 30 min and 0.94 ±0.09 mg equiv/kg at 72 h. Concentrations in fat and muscle were 7.9 and 7.1 mg equiv/kg at 5 min, respectively, and were not detected at 72 h. Analysis of tissues and fluids from pregnant female rats indicated little transfer to the foetus or amniotic fluid. Samples of milk contained the highest concentrations of ^{14}C-cefuroxime at 1 h following intravenous administration (approximately 18% of the whole blood level) and at 24 h. concentrations were similar in milk and in whole blood. Following repeated intramuscular injections at a dose of 25 mg/kg bw, highest residues were found in kidney (58.1 ±24.7 mg equiv/kg), followed by liver (3.2 ±0.9 mg equiv/kg) 72hafter completion of 14 successive days of treatment. No residues were detected in fat or muscle samples.

Dogs

Experiments to determine distribution, elimination and pharmacokinetics were also conducted with male beagle dogs (Nanbo *et al*, 1979). Three dogs each received 25 mg/kg bw of ^{14}C-cefuroxime by intravenous injection, with blood samples being collected at 5, 15 and 30 minutes and at 1, 2, 3, 4, 6, 8 and 24 hours following administration. Concentrations observed in whole blood were 33 mg equiv/L 5 min post-administration, following a bi-phasic elimination as observed in rats, with half-lives of 0.66 and 34.8 h. At 24 h, concentrations had declined to 2% of those observed in the 5 min sample. Analysis of urine and faecal samples indicated that 84.8% of the dose was excreted in urine within 12 hours, with 90.2% eliminated over 72 h. Faecal elimination accounted for 8.1% of the dose at 12hand 8.3% at 72 h. As observed in the experiments with rats, >95% of the residue in plasma and urine samples from dogs was unchanged parent compound.

Food producing animals

Cattle

Eight lactating dairy cows (402–599 kg bw at start of study) were administered ^{14}C-cefuroxime sodium by intra-mammary infusion following three successive milkings at a nominal dose of 250 mg cefuroxime per quarter, for a total dose of 3000 mg (Ferguson and Batten, 1996). Urine and faeces were collected at 24-hr intervals from two of the treated cows which were held in metabolism cages. Samples were collected from one day prior to initial administration of drug to seven days after the final administration, at which time all animals in the study were killed. Blood samples were collected from all 8 cows at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168 and 192 h. following the initial administration. Milk was collected twice daily from all 8 cows for analysis. Tissue samples (muscle, fat, kidney, liver, udder) were collected at slaughter for metabolic profiling. Details of residue testing on milk and tissue samples are provided under the discussion of "Tissue Residue Depletion" which follows in the next section. The study was conducted in compliance with GLP.

Approximately 95% of the total dose of ^{14}C-cefuroxime administered to the two cows held in metabolism cages was accounted for, as shown in Table 1. The majority of the drug was excreted in the milk, followed by urine as the principle route of elimination for drug absorbed from the udder. Most elimination of absorbed drug through urine and faeces occurred within 72 h of administration.

Table 1. Elimination of ^{14}C-cefuroxime from lactating cows administered by intra-mammary infusion at a total dose of 3000 mg per animal (1000 mg at each of 3 successive milkings)

Sample tested	Administered dose eliminated (%)	
	Animal 1	Animal 2
Milk	81.6	75.0
Urine	6.0	13.9
Faeces	3.0	6.2
Cage wash	3.7	1.0
Total	94.3	96.1

Peak concentrations of ^{14}C-cefuroxime appeared in blood (0.10 mg equiv/kg) and plasma (0.126 mg equiv/kg) at 24 h following final administration, declining steadily to concentrations near 0.01 mg equiv/kg at 192 h post-administration. At 96 h post-treatment, no ^{14}C-cefuroxime was detected (limit of detection 0.018 mg equiv/kg) in blood samples from 3 of the 8 animals. However, radiolabelled residues were detectable in plasma samples from two of these animals to 120 and 144 h, respectively (limit of detection 0.012 mg equiv/kg). The apparently bi-phasic elimination observed suggested that there was distribution in a compartment other than blood/plasma within the body.

Following overnight digestion with protease enzyme (*Subtilisin carlsberg,* ca. 80 mg), kidney homogenates (20 g) were centrifuged and the supernate was applied to a C-18 solid phase extraction cartridge. The cartridge eluent fraction containing cefuroxime was analyzed by liquid chromatography using two different columns (C8, -NH_2) and mobile phases. Urine samples were analyzed by liquid chromatography without extraction, using the same liquid chromatographic columns and conditions. Milk samples were also analyzed by liquid chromatography-mass

spectrometry to assess residue depletion, as described under "Tissue Residue Depletion", as well as under the chromatographic conditions described in this section to identify the extent of metabolic breakdown.

Liquid chromatographic analysis of urine samples indicated that approximately 20.4% of the radiolabelled material was parent compound, while three other polar eluents accounted for 29.3%, 19.6% and 29.1% of the total radiolabelled residues. Only 59.7–66.8% of the total radiolabelled residue was extractable from kidney, of which very little co-eluted with parent cefuroxime. On the amino LC column, most radioactivity was observed to elute in two diffuse regions, one of which was more polar than the parent compound and accounted for 14.8–32.3% of the applied radioactivity. The second region, also more polar than the parent, was 18.5 - 67.6% of the applied radioactivity. Using the C-8 LC column, from 2.3 - 27.2% of the radioactivity co-eluted with parent compound, while three other polar regions accounted for 17.9 -39.8%, 4.0 - 65.9% and 0 - 57.6% of the applied radioactivity. A similar pattern of small and inconsistent peaks was observed for milk extracts analyzed on this latter LC column. None of these compounds was identified, with parent compound appearing from these studies to be the appropriate marker residue.

In a separate study, ten dry cows (450 - 650 kg bw) were administered an intramuscular injection into the right gluteal muscle of either 30% cefuroxime aqueous suspension or 30% cefuroxime suspension in glycerol formol at a dose of 14 mg/kg bw (Silley and Rudd, 1986). This study did not include a statement of GLP compliance. The animals were divided into two groups, with Group 1 receiving the glycerol formol suspension and Group 2 receiving the aqueous suspension. The treatment was repeated 30 days later, as a crossover, with the treatments being reversed for the two groups. Following each treatment, blood samples were collected from each animal at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8 and 12 hours. A pre-treatment sample was also collected from each animal. Serum was analyzed for cefuroxime using an agar diffusion method, with the procedure adjusted to the expected concentration range. The limit of detection using the more sensitive assay was 0.02 mg/L. Data were analyzed using a computer program which assumed first-order elimination kinetics during the terminal phase (plot of log cefuroxime concentration versus time). The approach proved valid in all cases, with the program determining peak antibiotic concentration, the time to peak concentration, Area Under the Curve (AUC), Ultimate Half Life (UHL) and the time above selected antibiotic concentrations. As shown by the results in Table 2, the aqueous formulation was more readily bioavailable.

Table 2. Pharmacokinetic parameters for cefuroxime in cattle following intramuscular administration as either a 30% aqueous suspension or a 30% glycerol formol suspension at a dose of 14 mg/kg bw

Parameter	Aqueous suspension	Glycerol formol suspension
Peak concentration (mg/L)	18.5 ±6.0	5.0 ±3.3
Time to peak (hr)	0.3 ±0.1	2.1 ±1.7
AUC (mg/L x hr)	28.0 ±5.5	20.5 ±6.1
UHL (hrs.)	1.1 ±0.2	2.2 ±0.6
Time above 1 mg/L (hr)	4.5 ±0.3	6.9 ±2.8
Time above 2 mg/L (hr)	3.2 ±0.4	4.3 ±1.7

A study was also conducted in which a single calf was treated by intravenous injection with a 30% cefuroxime axetil solution in glycerol formal at a dose rate of 14 mg/kg bw (Silley and Rudd, 1987). Blood samples were collected before treatment and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours. Serum samples were assayed by a microbiological agar diffusion method, as in the study described above. It was noted that in this study, the rate of conversion of cefuroxime axetil to the microbiologically active form of cefuroxime was a factor in the results. The observed half-life for cefuroxime was 0.8 h, with a peak concentration of 11.7 mg/L. This study did not include a statement of GLP compliance.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled residue depletion studies

Cattle

Eight lactating dairy cows (402 - 599 kg bw at start of study) were administered ^{14}C-cefuroxime sodium in a GLP study by intra-mammary infusion following three successive milkings at a nominal dose of 250 mg cefuroxime per quarter, for a total dose of 3000 mg (Ferguson and Batten, 1996). Milk was collected twice daily for seven days following the last administration. Following the morning milking on day 7, the cows were killed and liver, kidney, muscle, fat and udder were sampled for analysis. Analysis for radiolabelled residues was conducted by combustion of each test portion, followed by determination of radioactivity by scintillation counting. All radioassays were performed using a minimum of three replicates. Limits of detection were as follows: milk, 0.009 mg equiv./kg; muscle, 0.020 mg equiv./kg; fat, 0.060 mg equiv./kg; liver and kidney, 0.015 mg equiv./kg; udder, 0.016 mg equiv./kg. In addition, milk was tested for antimicrobial residues using an agar diffusion assay with a limit of detection of 0.06 mg/kg. Kidneys from four of the animals were also tested for antimicrobial residues by the agar diffusion method, with an LOD of 0.06 mg/L of kidney

extract. Individual samples from the fifth milking after final administration of the drug were also tested for antimicrobial residues using the Delvotest P100.

Milk extracts were also analyzed for cefuroxime residues by liquid chromatography-mass spectrometry using the methodology described in the section "Methods of Analysis for Residues in Tissues and Milk" (Todd and McCormack, 1996). Pools prepared for the 1st, 2nd, 3rd, 5th, 7th, 9th, 11th and 13th milkings were extracted twice with chilled 1M HCl, centrifuging after each extraction. The supernates from the two extractions of each pooled sample were combined, washed with dichloromethane, centrifuged and the aqueous layer taken. The dichloromethane layer was then washed with 1 M HCl and, after centrifugation, the aqueous layer was removed, the two aqueous supernates were combined and applied to a C-18 solid phase extraction cartridge. The eluent fraction containing cefuroxime residues was analyzed by liquid chromatography, using C-8 and -NH_2 columns, as described earlier in the metabolic profiling discussion.

Residue concentrations in tissue samples, measured as ^{14}C-cefuroxime equivalents, had declined to near or below the limits of detection seven days after the final administration of the drug. No residues were detected in fat or muscle samples, while residues in liver and kidney were 0.035 ±0.011 mg equiv./kg and 0.101 ±0.046 mg equiv./kg, respectively. Highest residues (0.294 ±0.210 mg equiv./kg) were found in the udder, the site of drug administration. No microbiologically active residues were found in the kidney extracts, using the agar diffusion assay. In milk, residues depleted rapidly over a period of several days following treatment, as shown in Table 3. Highest concentrations were observed in the period immediately following the three successive treatments (milkings designated as -2, -1 and 1, taken after treatments 1, 2 and 3, respectively). Milk samples taken at the 5th milking were positive when measured undiluted using the Delvotest P100.

Table 3. Depletion of ^{14}C-cefuroxime residues in milk following three intra-mammary infusion treatments at a nominal total dose level of 3000 mg/cow. Residue results are not corrected for recoveries, which were reported as 69 - 88%)

Milking #	Mean concentration ^{14}C-cefuroxime equivalents (mg/kg)	Cefuroxime residue concentration in pool sample by LC-MS (mg/kg)	Antimicrobial residue concentration in pool sample by disc assay (mg/kg)
-2	178.6 ±71.0	NA[1]	NA[1]
-1	112.2 ±24.6	NA[1]	NA[1]
1	268.9 ±88.0	56.4	147.1 (182.9)*
2	37.8 ±14.6	7.57	9.13 (11.4)*
3	15.7 ±6.69	1.99	6.45 (<6.20)*
4	2.67 ±1.64	NA[1]	NA[1]
5	1.49 ±0.83	<0.01	<0.06
6	0.41 ±0.23	NA[1]	NA[1]
7	0.36 ±0.25	ND[2]	<0.06
8	0.17 ±0.13	NA[1]	NA[1]
9	0.11 ±0.06	ND[2]	<0.06
10	0.06 ±0.04	NA[1]	NA[1]
11	0.07 ±0.06	ND[2]	<0.06
12	0.04 ±0.02	NA[1]	NA[1]
13	0.05 ±0.04	ND[2]	<0.06
14	0.03 ±0.02	NA[1]	NA[1]

[1] NA = Not analyzed; [2] ND = Not detected (limit of detection, 0.01 mg/kg); * Results calculated for zones measured with ten- and hundredfold dilutions of the pooled sample from the treated cows with commercial milk containing no inhibitory substances.

Residue depletion studies with unlabelled drug

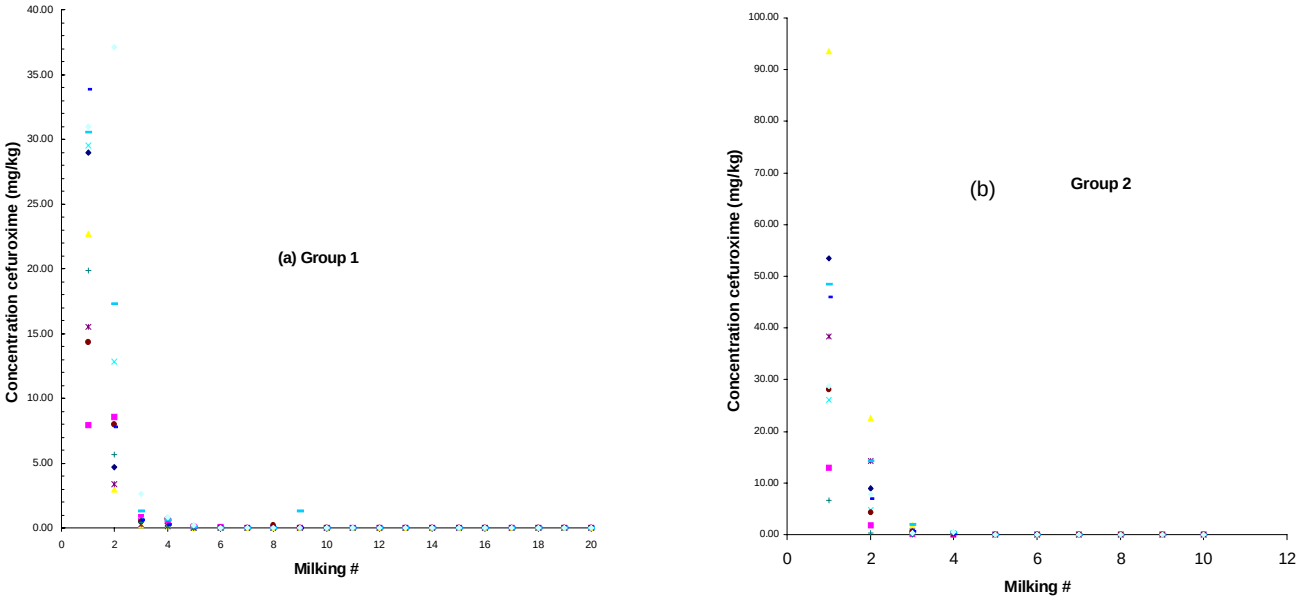
Cattle

In a non-GLP study, four lactating dairy cattle were each treated by intra-mammary injection of 250 mg cefuroxime sodium, or a dose of 1 g per animal per treatment (Kilpatrick and Rudd, 1985). Each cow received a total of three treatments at approximately 12-hour intervals, after each milking. Two cows were slaughtered at 12 hours following treatment, while the other two cows were slaughtered after 24 hours withdrawal. Samples of liver, kidney, heart and skeletal muscle were collected and analyzed by a microbiological agar diffusion assay with a limit of detection of 0.01 mg/kg for cefuroxime. At 12 hours withdrawal, residues of cefuroxime detected in kidneys from the two animals were 0.12 and 0.06 mg/kg, respectively, while residues in livers were 0.09 and 0.04 mg/kg. No residues were detected in the

heart or muscle samples from these animals, nor in any of the tissue samples collected from the animals slaughtered at 24 hours withdrawal.

In a subsequent study, 20 lactating cows, divided into two groups of ten, were infused in all four quarters at three successive milkings with cefuroxime sodium (250 mg per quarter per treatment) and milk from each cow was tested for 15 days following the last infusion (Davies and Kilpatrick, 1986). Samples were analyzed by agar diffusion techniques, with the assay parameters adjusted to suit the concentration range of the residues. Some samples which contained unusually high concentrations of antibiotic were also analyzed by paper chromatography, with bioautographic detection to confirm that the response in the agar diffusion assay was due only to cefuroxime residues.. The "Group 1" cows received their third infusion with antibiotic three days prior to commencement of the first treatment of "Group 2". Some anomalous results, particularly in one "9th milking" sample from Group 1 (1.34 mg/kg) suggested that cross-contamination of some samples may have occurred, as the treatments and initial milking of the "Group 2" cows coincided with this sample collection from "Group 1". The results from both groups, however, show substantial consistency. There is an initial high concentration of residues in the first milking (6.65 - 93.60 mg/kg), declining to <0.01-2.65 mg/kg by the third milking, <0.01-0.21 mg/kg by the fifth milking and 0.01 mg/kg in subsequent milkings (not including the two anomalous results from Group 1).

Figure 1. Depletion of cefuroxime residues in milk from cows in (a) Group 1 and (b) Group 2.



In a preliminary study to determine residues in tissues of cattle treated at the end of the lactating period, two dairy cows each were treated at drying off with cefuroxime sodium by infusion of 375 mg cefuroxime into each quarter for a total dose 1.5 g cefuroxime per animal (Tait, 1986a). The animals were slaughtered at seven days after infusion and samples of skeletal muscle, liver, kidney, subcutaneous fat, omental fat and renal fat were collected and analyzed by a microbiological agar diffusion assay with a limit of detection of 0.01 mg/kg for cefuroxime. No residues of cefuroxime were detected in any tissues in this study, which was carried out to GLP standards.

In a second study, three dairy cows were treated at drying off with cefuroxime sodium by infusion of 375 mg cefuroxime into each quarter for a total dose 1.5 g cefuroxime per animal (Tait, 1986b). As in the previous study, the animals were slaughtered at seven days after infusion. Samples of liver, kidney, heart and skeletal muscle were collected and analyzed by a microbiological agar diffusion assay with a limit of detection of 0.01 mg/kg for cefuroxime. No residues of cefuroxime were detected in tissues in this study, which was carried out to GLP standards.

Inhibition of starter cultures

A series of studies were conducted to assess the potential effect of cefuroxime residues on dairy starter cultures. In an initial investigation, the effect of 0.01 and 0.1 mg/kg cefuroxime on acid production by 40 dairy starter cultures was examined. An inhibition of acid production greater than 20% was found in 18 cultures, while 2 only *Streptococcus* strains and a thermophilic lactic culture were susceptible at the lower concentration. Only two strains showed >50% inhibition of acid production at 0.01 mg/kg cefuroxime, the guideline suggested by the International Dairy Federation as a measure of significant inhibition (International Dairy Federation, 1991).

A subsequent study was conducted, both in the presence and absence of milk, with ten pure starter cultures currently used in the dairy industry for production of cheese, fermented milk products and yoghurt (Watson, P, 1998a). While all test strains showed some sensitivity to cefuroxime, none was inhibited at concentrations >0.016 mg/kg and results were

similar in both the presence and absence of milk. Further work was conducted using mixed starter cultures, in the presence and absence of milk (Watson, 1998b). This work was considered more representative of industry practice, but used lower levels of inoculant than are typical for industrial application to enable measurement of microbial inhibition. Again, the results were not affected by the presence of milk and higher minimum inhibitory concentrations (0.0125 to 4 mg/kg) were observed than in studies with single cultures. An additional study was then conducted to determine the effect of six mixed commercial dairy starter cultures on milk acidification, using continuous pH measurement (Watson, 1999a). This study demonstrated that the effects of cefuroxime on milk acidification were dependent on both concentration and culture. Two cultures were unaffected at the three concentrations tested (0.05, 0.1, 0.2 mg/kg), one was affected only at the two higher concentrations and three were affected at all concentrations.

One further study was conducted with the organism that demonstrated concentration dependence and one of the organisms which was affected at all concentrations, using 10 repeated experiments at a concentration of 0.05 mg/kg cefuroxime in milk (Watson, 1999b). It was observed that the organism (DWC1913) which showed no effect at this cefuroxime concentration in the previous study was not affected, while with the susceptible organism (DWC1912) the time taken for the pH to decrease to 5.00 increased significantly and that there was a significant increase in pH four hours after the start of incubation. A final study was conducted using the same two organisms at concentrations of 0.05, 0.075 and 0.01 mg/kg cefuroxime in milk, repeating each experiment 5 times (Watson, 1999c). At 0.01 mg/kg, acid production by the more sensitive organism (DWC1912) was reduced by 49.6% compared to a positive control, a result considered to be significant with respect to International Dairy Federation guidelines. This study demonstrated that a concentration of 0.05 mg/kg cefuroxime in milk would not be significant in terms of the International Dairy Federation guidelines for the two cultures tested, which may be considered representative of those tested in the preceding studies.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

Two screening tests routinely used for the detection of antimicrobial residues in milk, the Delvotest P and the Accusphere.01, were evaluated for the detection of residues of cefuroxime in fresh whole milk (Crosse and Davies, 1984). A dilution series was prepared by diluting a stock solution of the antibiotic in phosphate buffer (100 mg free acid/L) with fresh whole milk. The two test kits were used according to manufacturer's instructions, on three separate occasions. Using the Accusphere.01, the minimum concentration of cefuroxime detected was 0.04 mg/kg, while the minimum concentration detected with the Delvotest P was 0.03 - 0.04 mg/kg. Comparative tests were also conducted using cephalonium, cephazole and penicillin G. Both tests were considered equally sensitive for cefuroxime, based on the results. The numbers of replicates tested to determine test performance were inadequate by current standards, but still give an indication of the expected detection capabilities of these tests for cefuroxime residues in milk.

A liquid chromatographic assay was reported for the analysis of cefuroxime residues in milk at concentrations from 0.005 - 0.100 mg/kg (Bakes and Tait, 1987). After addition of 1 mL of chloroform to a 10 mL test portion of fresh milk to precipitate proteins, the test portion was centrifuged for 5 min at 2000g and the supernate was diluted to 100 mL with water and loaded onto a Sep Pak C-18 solid phase extraction cartridge. Before loading with sample, the cartridge was first washed with 15 mL of acetonitrile. The cartridge should not be allowed to dry before the supernate is loaded. Following loading, the cartridge was washed with 10 mL of water, dried with 180 mL of air, followed by washing with 2 mL of butan-1-ol:diethyl ether (1:1, v:v), then another 180 mL of air. Cefuroxime was eluted with 1 mL of 5% (v/v) acetonitrile in water. The test portions were analyzed by injecting 100 µL of the final extract into a liquid chromatograph equipped with a Spherisorb S5 ODS2 (fully endcapped C-18) column, 25 cm x 0.45 cm and a UV-detector set at 274 nm. The analysis uses a gradient (flow rate 1 mL/min) starting with an initial mobile phase of 20% methanol in water containing 8 mM octane sulphonic acid (mobile phase A), changing over 10 min to 80% mobile phase B (75% methanol in water containing 8 mM octane sulphonic acid). This composition is held for 2 minutes, then the composition is returned to mobile phase A. Under these conditions, cefuroxime elutes with a retention time of 4.5 to 5.5 min. Using fortified samples, analytical recoveries were estimated to be 62% and relative standard deviations (n = 3) determined at concentrations within the linear range 0.005 - 0.100 mg/kg was <5%. Chromatograms of extracts of milk known to be free of cefuroxime residues contained no interfering peaks.

A method developed under GLP has also been reported which uses LC-MS for the analysis of cefuroxime residues in milk from cattle (Todd and McCormack, 1996). A 5 mL test portion of milk is shaken and then sonicated for 10 min with 15 mL cold 1N hydrochloric acid in a 50 mL polyethylene centrifuge tube. The phases are separated by centrifugation at 2000 rpm, then the acidic supernate is decanted into a fresh tube. The solid residue remaining in the first tube is re-extracted, as above, with 10 mL of hydrochloric acid, the supernates are combined and 10 mL of dichloromethane are added. After shaking, the phases are allowed to separate, using centrifugation if required. The aqueous layer is transferred to a 100 mL polypropylene bottle and the dichloromethane layer is washed with 10 mL 1N HCl. The aqueous layer is removed and combined with the aqueous layer from the first wash in the 100 mL bottle. The combined aqueous phases are sparged with nitrogen for 5 min to remove all dichloromethane, then loaded on a 500 mg C-18 solid phase extraction cartridge which has been pre-conditioned with 10 mL methanol, followed by 10 mL of water. After sample loading, the cartridge is washed with 2 mL 80:20 water/ methanol (v:v) and the cefuroxime is then eluted into a 5 mL volumetric flask with 4.5 mL of elution solution, 50:50 water/methanol (v:v), adding elution solution to the flask to bring the contents to the mark (5 mL).

Analysis is then conducted by injecting 100 µL of the final extract onto a Spherisorb-NH_2 column, 5 µm particle size, 25 cm x 4.6 mm i.d. The column is held at room temperature, with a flow rate of 0.1 mL/min of mobile phase, using a 20:1 split ratio into the mass spectrometer. Cefuroxime elutes at approximately 9 min using an elution system comprised of two mobile phases (Mobile Phase A: 50:50 v:v water/acetonitrile with 0.01M ammonium acetate; Mobile Phase B: 75:25 v:v water/acetonitrile with 0.1M ammonium formate). The gradient is 100% Mobile Phase A from 0 to 11 min, switching to 100% Mobile Phase B from 12 to 18 min, then back to 100% Mobile Phase A from 18 to 20 min. The mass spectrometer is operated in electrospray, positive ion mode, monitoring m/z 364, using the match of retention time with a standard and the presence of the same ion in the standard and the sample as confirmatory criteria. Mean recoveries were 90-97%, with precision of 4.4 to 11.2% within the linear range tested (0.010 - 0.100 mg/kg). The limit of quantitation calculated was 0.010 mg/kg, with a limit of detection of 0.005 mg/kg. No interference was observed from known blank milk samples and the related compounds cephalonium and cephalexin did not interfere in the analysis.

A method developed under GLP was also reported for the determination of cefuroxime residues in edible tissues by liquid chromatography - mass spectrometry, or LC-MS (Croucher, 1998). The method was applied to muscle, liver, kidney and fat samples, using fortification of known blank control samples with cefuroxime to establish method performance. To a 1 g test portion of tissue in a polypropylene centrifuge tube, add 15 mL of acetonitrile/water (50:50, v:v), macerate for 1 min, then centrifuge. Filter the supernate through glass wool into a fresh centrifuge tube, add 15 mL hexane and shake vigorously. Allow the phases to separate, centrifuge if required, then aspirate the hexane layer to waste and transfer the remaining aqueous phase to a 250 mL round bottom flask. Rotary evaporate at <45 °C to remove all residual acetonitrile (this is a critical step), then transfer the aqueous extract to a polypropylene tube, using water washes to make up to 40 mL. Remove a 20 mL aliquot, adjust volume to 40 mL with water and load this on a 100 mg ENV+ SPE cartridge which has been pre-conditioned with 1 mL of methanol, followed by 1 mL of water. Use a vacuum setting of 5 psi for sample; loading. Elute the cefuroxime from the cartridge with 1.5 mL of 1:1 (v:v) water/methanol into a 2 mL volumetric flask and dilute to the mark with elution solution.

The extract is then analyzed LC-MS (electrospray, positive ion) using a Hypersil SAX column, 15 cm x 3.2 mm, and a mobile phase consisting of methanol:water:formic acid, 50:50:0.1% (v:v:v) + 0.025M ammonium formate at a flow rate of 0.5 mL/min the injection volume is 100 µL. Under these conditions, cefuroxime elutes in about 8 min and is detected in single ion mode by monitoring m/z 364.

Table 4. Validation of analytical methods for residues of cefuroxime in edible tissues and milk using LC or LC-MS.

Parameter	Method		
	LC (milk)	LC-MS (milk)	LC-MS (tissues)
Recovery	62%	>90%	85-110%
Calibration curve	5-100 mg/kg	0.005-0.100 mg/kg	0.005-0.100 mg/kg
Linearity	r ≥ 0.99	Linear; r not reported, but sample curve provided.	r ≥ 0.99
Limit of Detection	Not reported	0.005 mg/kg[a]; (0.003 mg/kg)[b]	0 -0.003 mg/kg[c]; 0.003 -0.017 mg/kg[b]
Limit of Quantitation	Not reported	0.010 mg/kg[d]	0.050 mg/kg for all tissues[d]
Repeatability	≤10% (n = 3)	n=5 at 3 levels - <11.2%	n=5 at 3 levels - <15% for all tissues

[a] Determined as 3xS/N (signal-to-noise of background). [b] Statistically determined by least squares regression of results from fortified controls for n=5, 3 concentrations. [c]20 controls per matrix, using 5 sources - LOD determined as mean S/N for controls + 3 standard deviations. [d] Lowest concentration at which acceptable accuracy and precision were demonstrated on fortified samples.

The validation was carried out at 0.050, 0.100 and 0.200 mg/kg for all tissues (fat, liver, kidney, muscle), with a limit of quantitation established in all cases as 0.050 mg/kg. Recoveries were generally >80%, with precision (relative standard deviation) <15% for all tissues and concentrations. The experimental limit of detection was <20 ng/g for cefuroxime in all tissues. No interference was encountered from controls or from tissues fortified with amoxicillin, ampicillin, benzylpenicillin, cephalonium or cephalexin. Concentrations remained stable in tissues stored at -20 °C for one month. Extracts from liver were stable under refrigerated storage for 7 days, but there was about a 15% loss of residue from kidney extracts over this time period.

The performance characteristics of the LC and LC-MS methods with respect to method-specific validation requirements are summarized in Table 4. No data were provided on analyte stability in milk, either during sample storage or analysis, although samples in at least one study (Davies and Kilpatrick, 1986) were stored at -20 °C prior to analysis. Cefuroxime is stable in standard solutions for at least 1 month at 4 °C, while cefuroxime residues in liver extracts are stable up to 7 days. Kidney extracts lose 15% of analyte at 4 °C. over a 7-day period. Cefuroxime residues remain stable in tissues for

one month when samples are stored at -20 °C. No intra-laboratory (precision) or inter-laboratory (reproducibility) trials of the methods were reported, nor were results of ruggedness testing reported. No interference was reported from co-extractives or, for the LC-MS methods, from other similar compounds tested. Accuracy of the methods is reported as Recovery.

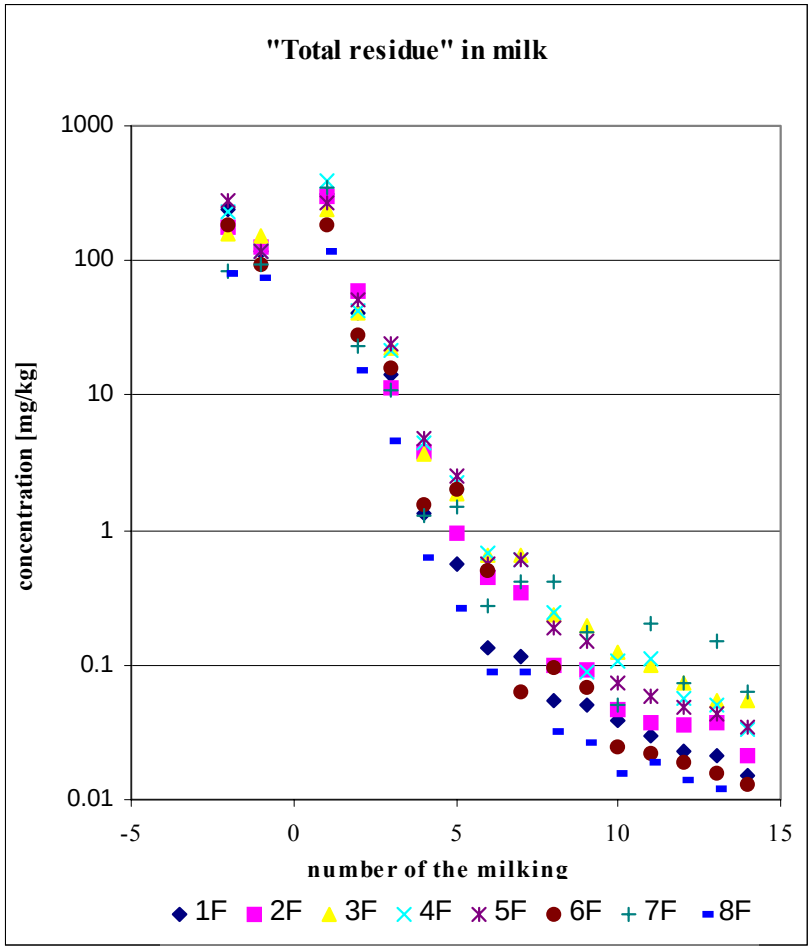
APPRAISAL

Cefuroxime is a second-generation cephalosporin antibacterial used in veterinary medicine for the treatment of mastitis. It has activity against a range of gram-positive bacteria, as well as many gram-negative organisms and is also used in human medicine as either the sodium salt or as the 1-acetoxyethyl ester (known as cefuroxime axetil).

Information was provided on metabolism and distribution in both laboratory (rats, dogs) and food (cattle) animals, with generally consistent results across species. Cefuroxime is excreted via urine and faeces, with more than 95% of the total dose eliminated in urine and faeces within 72 h. The highest residues of parent compound are found in kidney, with lower concentrations in liver. Residues are much lower in muscle and fat. Residues in all tissues and in milk are primarily parent compound. Metabolism is limited, with no significant metabolites identified in any of the studies in laboratory or food animals.

Residue depletion studies were conducted to GLP standards using both ^{14}C-cefuroxime and unlabelled compound in dairy cattle. While total radiolabelled residues remain at 1.49 mg equiv/kg at the fifth milking following treatment, analysis of these samples by liquid chromatography (LOD, <0.01 mg/kg) and by disc assay (LOD, <0.06 mg/kg) indicated cefuroxime residues were below the limits of detection of these methods. Total residues measured as ^{14}C-cefuroxime equivalents were <0.1 mg equiv/kg at the tenth and subsequent milkings.

Figure 2. Depletion of "total residue" of cefuroxime in milk (mg equiv cefuroxime/kg)



Plotting the data provided for the individual milkings in Table 3 on a semi-log scale clearly demonstrates the bi-phasic kinetics of the depletion of the total residues (see Figure 2). The cow-to-cow variability is greatest during the terminal depletion phase.

From the depletion data, “tolerance limits” may be calculated for every time point (milking number) on the basis of the geometric mean plus 3.732 standard deviations (for n=8, according to the “Documenta Geigy” Statistical Tables). These limits - as a function of the number of the milking are given in Table 5.

Table 5. "Tolerance limits" of the residue concentration in individual milkings as a function of the number of milkings after the last administration of the drug

Milking number:	1	2	3	4	5	6	7
	Tolerance limit [mg of parent drug equivalents/Kg]						
a)"total residue"	1083.323	186.788	107.145	34.589	23.518	5.806	9.206
b) parent drug	220.584	52.496	193.168	27.822	12.814	3.121	1.432

Milking number:	8	9	10	11	12	13	14
	Tolerance limit [mg of parent drug equivalents/Kg]						
a) "total residue"	3.135	1.102	0.676	1.273	0.386	0.712	0.278
b) parent drug	0.183	0.020					

Using the data from the studies on metabolites which have been performed with bulk milk samples (Ferguson and Batten, 1996), the ratio between radioactive parent drug and radioactive "total residue" may be calculated (see table 6). From these ratios, the fraction of the above tolerance limits which corresponds to parent drug may be calculated. These data are given in table 5 under "b) parent drug".

Table 6. Ratio of the concentrations of radiolabelled parent drug and radiolabelled "total residue" in bulk milk obtained from individual milkings

Milking number	"Total residue"	Fraction co-eluting with the parent compound			% of total residue	Ratio: parent drug/"total residue"	
		Solvent1	Solvent 2	Mean		found	predicted *
	mg of parent drug equivalents/kg						
1	238.8	76.150	61.920	69.035	28.9	0.289	0.237
2	36.09	6.378	7.399	6.889	19.1	0.191	0.208
3	13.8	2.530	2.064	2.297	16.6	0.166	0.178
4							0.149
5	1.308	0.091	0.073	0.082	6.27	0.063	0.120
6							0.090
7	0.296	0.014	0.022	0.018	6.08	0.061	0.061
8							0.032
9	0.112	0.006	0.002	0.004	3.57	0.036	0.002
11	0.057		0.002				
13	0.052		0.001				

* Predicted values were obtained through linear regression analysis with the number of the milking as independent variable and ratio found as the dependent variable.

A second approach which may be applied is based on the observation that the initial phase (milking 1-6) and the final phase (milking 7-14) of depletion may be separately described either by a single exponential term on a linear scale or by a single linear term on a semi-log scale. In Table 7, the results obtained when these calculations are performed either separately for the milk samples of every individual cow or for all samples of all animals together are given. These calculations use the following formula:

$$\log_{10} C_n = \log_{10} a + b \times n$$

where C_n is the concentration at a given milking number n, a is the extrapolated concentration immediately after the last dosing ("zero time") and b is a rate constant. The variability of the rate constants is surprisingly low.

Table 7. Description of the depletion of "total residue" from milk using linear regression

Cow:	1F	2F	3F	4F	5F	6F	7F	8F	All cows
	Results obtained with the data from milking 1-6								
n	6	6	6	6	6	6	6	6	48
a	6.0545	5.9169	5.7645	5.8910	5.8572	5.5785	5.7855	5.5198	5.7960
b	-0.6697	-0.5711	-0.5047	-0.5228	-0.5135	-0.4937	-0.5736	-0.6206	-0.5587
r	-0.9934	-0.9935	-0.9905	-0.9865	-0.9941	-0.9683	-0.9662	-0.9914	-0.9563
	Results obtained with the data from milking 7-14								
n	8	8	8	8	8	8	8	7	63
a	2.7377	3.2593	3.6390	3.6212	3.6605	2.8016	3.2172	2.6079	3.1474
b	-0.1126	-0.1413	-0.1451	-0.1525	-0.1610	-0.1245	-0.1003	-0.1238	-0.1276
r	-0.9757	-0.9151	-0.9589	-0.9272	-0.9390	-0.9269	-0.6951	-0.9062	-0.6614

The parameters of the right column (all cows) may then be used to calculate tolerance limits on the basis of linear regression and to calculate the fraction corresponding to parent drug (see Table 8). In the same way, the data for all 20 cows from the study with unlabelled drug (Davies and Kilpatrick, 1986) in which the residues were determined based on antimicrobial activity may be evaluated. These results are given in the last column of Table 8.

Table 8. Tolerance limits of "total residue" and parent drug concentrations as a function of the number of milkings - calculations based on linear regression

Parameter set used for the calculations	Number of the milking	"Total residue"	F	Parent drug	Antibiotic activity
		mg equivalents per kg of milk		mg equivalents per kg of milk	mg equivalents per kg of milk
n: 48 a: 5.7960 b: -0.5587	1	787.539	0.2370	186.670	150.782
	2	205.388	0.2077	42.653	29.045
	3	54.871	0.1783	9.784	5.843
	4	15.158	0.1490	2.258	1.237
	5	4.330	0.1196	0.518	0.272
	6	1.267	0.0902	0.114	0.061
n: 63 a: 3.1474 b: -0.1276	7	0.927	0.0609	0.056	0.014
	8	0.660	0.0315	0.021	
	9	0.476	0.0022	0.001	
	10	0.348			
	11	0.260			
	12	0.198			
	13	0.153			

There is good agreement between the concentrations of parent drug extrapolated from the results of the study with radiolabelled cefuroxime (Ferguson and Batten, 1996), the antimicrobial activity found in this study for samples of pooled milk and the values obtained with microbiological methods measuring residues of unlabelled drug (Davies and Kilpatrick, 1986). It may therefore be concluded that in the second phase of the elimination of residues in milk (7th milking and subsequent), parent compound appears to be the major relevant residue with antimicrobial activity.

Cattle which received cefuroxime by intra-mammary administration were free of detectable residues at slaughter seven days following treatment in studies with the unlabelled drug. Residues were detectable in kidneys and livers of cattle at 12hfollowing treatment, but these were ≤0.12 mg/kg. No residues were detectable in muscle at 12 h, or in liver, kidney and muscle samples of animals slaughtered at 24hpost-treatment. In a study with ^{14}C-cefuroxime, no residues were detected in fat or muscle samples at seven days following treatment, while residues in liver and kidney were 0.035 ±0.011 mg equiv./kg and 0.101 ±0.046 mg equiv/kg, respectively. These studies indicate that there is some persistence of residues in milk following treatment, but that there is a low risk of residues in edible tissues following intra-mammary administration of the drug.

Analytical methods were described which included screening tests for bulk milk, a microbiological disc assay, a liquid chromatographic (LC) assay and liquid chromatography-mass spectrometry (LC-MS). The LC-MS methods were validated according to existing EU criteria for drug registration, but have not been subjected to interlaboratory trials. However, sufficient detail and validation experiments were conducted to demonstrate "fitness for purpose" in the hands of the developing laboratories. Other laboratories wishing to use these methods should generate performance data and should consider ruggedness testing of the methods prior to implementation. The liquid chromatographic assay has also been validated using a "criteria" approach and most of the required elements have been addressed, although no data on ruggedness testing, or on multi-analyst or inter-laboratory trials were provided. The methods have adequate sensitivity for regulatory purposes.

MAXIMUM RESIDUE LIMITS

In recommending MRLs, the Committee took into account the following factors:

- An ADI of 0-30 μg per kg of body weight was established by the Committee, which results in a maximum daily intake of 1800 μg for a 60 kg person.
- The primary residue with microbiological activity in milk is parent compound. The parent compound, cefuroxime, is therefore the appropriate marker residue in milk.
- Available data were not sufficient to permit the establishment of MRLs for edible tissues.
- A suitable analytical method is available for analysis of cefuroxime parent compound residues in cows milk.

On the basis of the above considerations, the Committee recommended a temporary MRL of 50 μg/kg for cefuroxime parent compound in milk from cattle. No MRLs in tissues are recommended.

The MRL recommended above would result in a daily maximum intake of 75 μg, based on a daily food intake of 1.5kg of milk.

The Committee requested the following information to be provided by 2004:

1. Data to clarify whether the residues other than parent compound found in milk are primarily due to metabolism or to a non-metabolic decomposition of parent cefuroxime in milk.
2. Identification of the non-parent residues found in milk, including an assessment of their potential toxicity.

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