Study Title

Environmental Assessment for Enrofloxacin BAYTRIL® 100 Injectable Solution

Guideline

21 CFR Part 25

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1.0 Date

February, 1996

2.0 Name of Applicant

Bayer Corporation, Agriculture Division, Animal Health

3.0 Address

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4.0 Description of the Proposed Action

4.1 Request Approval and Need for Action

This environmental assessment is necessary for the approval of the new animal drug, BAYTRIL® 100 Injectable Solution (enrofloxacin), for use in cattle. Enrofloxacin is a fluoroquinolone antimicrobial product for the treatment of bovine respiratory disease (BRD).

4.2 Location Where the Product Will Be Produced

The drug substance will be produced by Bayer AG in Wuppertal, Germany. The formulation and packaging will be done at Bayer Corporation's Animal Health manufacturing facility in Shawnee Mission, Kansas, USA.

4.3 Location Where the Product Will Be Used

The ultimate use of the finished product will be on cattle ranches and feedlots. Finished products will be stored in distribution centers throughout the United States prior to transportation to veterinary clinics. BAYTRIL® 100 Injectable Solution is a prescription drug. Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

4.4 Locations Where Product Will Be Disposed

Disposal of unused drug product may result during manufacturing activities, from the discarding of returned goods, or from end-user disposal of individual units of empty or partly empty finished product containers. The present infrastructure at the proposed manufacturing sites provides for the ultimate disposal of product through landfilling and incineration.

4.5 Type of Environment Present At and Adjacent to Manufacturing Locations

The areas around the manufacturing facility in Wuppertal, Germany and Shawnee Mission, Kansas, USA are characterized by mixed use land patterns consisting of residential, commercial, and industrial areas.

5.0 Identification of Chemical Substances of the Proposed Action

5.1 Chemical Process

The materials listed below are used in the chemical process by the manufacturing facility in Wuppertal, Germany for the synthesis of enrofloxacin, the final active drug:

fluoroquinolone carboxylic acid
N-ethyl piperazine
butyl glycol
acetic acid
anhydrous ethyl alcohol with toluene
activated carbon
diatomaceous earth
ammonium hydroxide (25%)
purified water

<u>Proposed Bulk Drug Specifications</u>
Greater than 99 percent enrofloxacin and less than 1 percent each of desfluoro compound and ciprofloxacin

5.2 Pharmaceutical Formulation

Copies of the Material Safety Data Sheets for the final drug product and for the ingredients used in the formulation of the drug product (except water, which is non-hazardous) are presented in Appendix 1. The following describes the main properties of the components used in the BAYTRIL® 100 Injectable Solution.

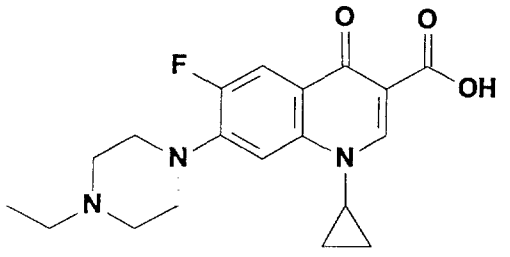
5.2.1 Enrofloxacin

Synonyms and Abbreviations
enrofloxacin
Baytril®
Bay Vp 2674
CAS Registry No. 93106-60-6

Chemical Name
1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid

Molecular Formula
$C_{19}H_{22}FN_3O_3$

Structural Formula



Molecular Weight
359.4

Melting Point
222 - 226°C

Solubilities in Water
1100 mg/L at pH 5.0; 25°C (Bayer Report No. 73353)
250 mg/L at pH 7.0; 25°C (Bayer Report No. 73353)
600 mg/L at pH 9.0; 25°C (Bayer Report No. 73353)

Vapor Pressure
$< 1 \times 10^{-7}$ mm Hg at 25°C (Bayer Report No. 73409)

n-Octanol/Water Partition Coefficients
K_{ow} = 0.4 at pH 5.0 (Bayer Report No. 73390)
K_{ow} = 3.1 at pH 7.0 (Bayer Report No. 73390)
K_{ow} = 0.7 at pH 9.0 (Bayer Report No. 73390)

Classification
Therapeutic: synthetic antibiotic.
Pharmacological: in cattle for treatment of bovine respiratory disease (BRD).

5.2.2 L-Arginine
CAS Registry No.: 74-79-3
Molecular Formula: $C_6H_{14}N_4O_2$
Molecular Weight: 174.20
Description: white crystals or crystalline powder, faint odor

5.2.3 Benzyl Alcohol
CAS Registry No.: 100-51-6
Molecular Formula: C_7H_8O
Molecular Weight: 108.13
Description: colorless liquid

5.2.4 *n*-Butyl Alcohol
CAS Registry No.: 71-36-3
Molecular Formula: $C_4H_{10}O$
Molecular Weight: 74.12
Description: colorless liquid

5.2.5 Water
CAS Registry No.: 7732-18-5
Molecular Formula: H_2O
Molecular Weight: 18.1
Description: colorless liquid

6.0 Introduction of Substances into the Environment
Portions of the materials listed in Section 5 will be released into the environment as a result of the proposed action. These will be generated from the manufacturing site in the form of air emissions, liquid waste streams, and solid waste.

6.1 Production Site
Enrofloxacin has and will continue to be manufactured for world wide use at the Bayer AG facilities in Wuppertal, Germany. The substances emitted from the facilities during manufacturing are ethanol, ammonia, butyl glycol, and general waste water from process operations. Emissions are controlled by Bayer AG's department of environmental

protection (WV-Umweltschutz) in compliance with the applicable German laws and pertinent regulations as described in Appendix 2.

6.2 Formulation Site

The BAYTRIL® 100 Injectable Solution formulation will be manufactured at the Bayer Corporation's Animal Health facility in Shawnee Mission, Kansas, USA. The manufacture of the formulation will consist of mixing the bulk enrofloxacin (from Germany) with other formulation components to form a homogeneous solution. The formulation site is located in a suburban area. The 53-acre facility is surrounded by mixed (residential/commercial) land use patterns.

Air Emissions

Air emissions from the formulation process for the BAYTRIL® 100 Injectable Solution will consist of both minor particulate and volatile organic compound (VOC) emissions. Particulate emissions are controlled by a dust collector with an efficiency of 99.998 %. VOC emissions are vented through the building's HVAC system which contains no special control devices. There are no Hazardous Air Pollutant (HAPS) emissions from the process.

Liquid

Liquid waste streams resulting from the pharmaceutical formulation and packaging operations will consist of residue waste waters from sanitary use and washing operations which will be discarded to the Public Owned Treatment Works (POTW) operated by the Johnson County Unified Wastewater District #1.

Bayer Corporation has been issued Industrial Wastewater Discharge Permit #92TC101 for its pharmaceutical operations and is regulated by the Johnson County Wastewater District, controlling authority as described in 40 CFR Part 403 under the authority of the State of Kansas, KSA 19-27, 168. All discharges associated with the formulation of the BAYTRIL® 100 Injectable Solution are regulated by the effluent limitations and conditions established in this permit. Production of this product should not cause any violations of existing parameters as set forth by the Johnson County Environmental Department.

Liquid wastes arising from rejected raw materials, rejected batches, or samples will be incinerated at an EPA-licensed Treatment Storage and Disposal Facility (TSDF) in accordance with Resource Conservation and Recovery Act (RCRA) regulations. The Bayer facility is currently registered as a hazardous waste generator and holds EPA I.D. #KSD007162407. Production of this product will not change our regulated status as a Large Quantity Generator.

Solid
Solid waste will consist of cardboard, paper, and plastics. Cardboard material will be baled for recycling. All other material will be disposed of in a sanitary landfill.

Statement of Compliance
The site selected is already a manufacturing facility for animal health drugs. It is currently in full compliance with all applicable environmental laws and regulations. The proposed action will not affect the overall status of the facility.

Approval of the proposed action will result in minor increases of air emissions exhausted to the atmosphere, liquid wastewater discharged to the POTW, and solid wastes destined for landfill disposal or recycling. In the long term, the approval will result in the use of resources confined to raw materials and utilities in the manufacturing area. Operations will be conducted in compliance with all applicable regulations enforced by the local, state, and federal levels as appropriate. The following environmental regulations or standards are cited as applicable to the proposed action:

Clean Air Act, as amended
Kansas Air Quality Act, as amended
Solid Waste Disposal Act, as amended
Clean Water Act, as amended
Kansas Hazardous Waste Management Act, as amended
Johnson County Code of Regulations for Sanitary Sewer Use, as amended
Resource Conservation and Recovery Act, as amended

6.3 Use Sites

The intended use of this product is the treatment of bovine respiratory disease in cattle, excluding cattle intended for dairy production or in calves to be processed for veal. The drug will be packaged in 100-ml amber glass bottles. The label dose is 2.5 to 5.0 mg/kg of body weight for 3 to 5 days. The dosage will depend on the severity of the disease. The maximum use of active ingredient (enrofloxacin) results from the multiple injection regime (5.0 mg/kg/day x 5 days = 25 mg). Therefore, this environmental assessment addresses the exposures resulting from the maximum dose of the product since it represents the worst case exposure scenario.

To identify what the significant excretion products are from cattle treated with BAYTRIL® 100 Injectable Solution, excreta were analyzed.

Urine and feces from cattle were collected after five days of treatment by daily subcutaneous injection with ^{14}C-BAYTRIL injectable formulation at the maximum recommended dose of 5 mg a.i. per kg of body weight per day (Bayer Report No. 106564). As representative samples, the urine and feces collected 24 hours after the first dose and 24 hours after the last dose in the male and female animals were analyzed. Approximately 46% of the excreted radioactivity was contained in the urine. Of the radioactivity in the urine, 42% was comprised of ciprofloxacin or ciprofloxacin conjugates and 4% was comprised of enrofloxacin. Approximately 54% of the excreted radioactivity was contained in the feces from the cattle. Of the radioactivity in the feces, 24% was comprised of enrofloxacin and 10% was ciprofloxacin. Therefore, the primary residues excreted by cattle are enrofloxacin and ciprofloxacin; the remaining residues each represent <10% of the administered dose. Based on this excretion profile, environmental fate and effects studies were conducted using enrofloxacin and ciprofloxacin.

Approximately 70 percent of the drug will be used in feedlots with the remaining 30 percent distributed between cow-calf stocker operations, pasture, and farm feeders. The majority of use will be in feedlots. The remaining usage will be on individual free-ranging animals in pastures.

The environmental exposure is considered negligible for non-feedlot operations since the occurrence of feces and urine containing enrofloxacin and its metabolites will be so infrequent in the overall scheme of a large pasture setting. The maximum exposure will result from feedlot use, and therefore, the remainder of this environmental assessment will focus on exposures originating from feedlot use of the drug.

Statistics from the United States Department of Agriculture indicate that there were approximately 40 million beef cattle on feed in the United States in 1994 (David Dargatz, beef specialist - personal communication, 1995). Most of the production was centered in the states of (in descending order of production): Texas, Nebraska, Kansas, Colorado, Iowa, Oklahoma, California, Minnesota, Illinois, South Dakota, Idaho, Arizona, and Washington (USDA, 1995). Up to 9 percent of the cattle raised in feedlots in Kansas and Nebraska from 1979 to 1984 required treatment for disease, and up to 70 percent of those cattle treated each year had symptoms of respiratory disease (Edwards, 1985).

Manure Handling and Disposal Practices

The major use of the antibiotic, enrofloxacin, will be in cattle feedlot operations. A general description of feedlot management practice is provided to support an understanding of terrestrial and aquatic exposures resulting from the use of BAYTRIL® 100 Injectable Solution.

Animals undergoing therapeutic drug treatment are housed in a separate hospital pen area for 3 to 7 days. After the treatment and observation period is complete, the animals are returned to their pens of origin. The manure is removed from the hospital pens and placed in a stockpile with manure from untreated cattle. Currently, biodegradation of manure through composting and/or biogasification are not generally used in the feedlot industry due to the high costs associated with these practices. However, it is common for manure stockpiles to be turned often to facilitate drying and volume reduction. Dry manure is applied to fields once or twice a year (fall or spring), but only one application per field is made within a 12-month period. Application rates range from 6 to 30 dry tons per acre (20 dry tons average) based on the nitrogen content of the soil, the nitrogen content of the manure, and the nitrogen requirements of the crop being planted in the field.

Under the U.S. Environmental Protection Agency feedlot effluent guidelines (1995a), all feedlots with a greater than 1,000 head capacity must handle runoff through the use of drains leading to stormwater detention basins or lagoons. Runoff water does not enter natural aquatic ecosystems. The lagoons must be capable of handling a 1-in-25 year storm event. In the event of heavy rainfall or if the year is particularly dry, the lagoon water will be pumped for crop irrigation. These crops, in turn, are fed back to cattle. Otherwise, the water level in the lagoon is reduced due to evaporation. The bottom sediments are removed from the lagoon on an as-needed basis, usually once every 7 to 10 years. The sediments are mixed with manure for land application.

To determine the concentration of drug residues being applied to an agricultural field, the following scenario and assumptions were used. In a state such as Colorado, 94 percent of the feedlots range from < 1,000 to 8,000 head of cattle (Colorado Cattle Feeders, 1995). For the purposes of this environmental assessment, a weighted average of feedlot size was used (2,000 head) with a morbidity rate range of 6 percent (Edwards, 1995) to 12 percent (Bayer veterinary estimates) for bovine respiratory disease. The USDA does not compile information on feedlot morbidity (David Dargatz, personal communication, 1995), but these estimates appear to be reasonable. It is assumed that all cattle with BRD will be treated with enrofloxacin. Most herds of cattle move through a feedlot in 120-day cycles. For the purposes of this environmental assessment, it is assumed that three herds of cattle (2,000 head per herd) move through this feedlot in a year. In actual practice, small herds of 50 to 60 head move in and out of the feedlot at different times.

Therefore, in a typical 2,000-head feedlot the number of cattle treated for bovine respiratory disease would be:

At 6% morbidity: (2,000 head/herd) x (3 herds/year) x 0.06 = 360 head treated

At 12% morbidity: (2,000 head/herd) x (3 herds/year) x 0.12 = 720 head treated

It is assumed that all cattle with BRD will be treated with enrofloxacin. Cattle that generally contract BRD are those animals that recently arrive at the feedlot since the stress of travel and transfer make them more susceptible to disease. The cattle arriving at a feedlot (and therefore, likely to be treated) range in weight from 500 - 750 lbs (mean 600 lbs or 270 kg). A 1000 lb animal produces approximately 60 lbs of wet manure per day with a solids content of 11 percent (Ohio State University, 1983). This equates to 6.6 lbs of dry manure per day (60 lbs x 0.11 solids = 6.6 lbs dry manure solids). For purposes of this environmental assessment, it is assumed that all the treated cattle are 600 lbs and produce 6.6 lbs of dry manure per day.

Manure is usually stockpiled for up to 12 months before land application. At a rate of 6.6 lbs dry manure/day, for 365 days (12 months), a feedlot with three 2,000 head herds moving through will produce:

(3 herds) x (2,000 head/herd) x (6.6 lbs dry manure/day/head) x (365 days/year) =

14.45×10^6 lbs dry manure per year or 6.56×10^6 kg dry manure per year.

Since enrofloxacin and its metabolites are nearly completely excreted via urine and feces, the manure excreted during an individual animal's treatment period may contain up to 6750 mg of drug residue [(270 kg/animal) x (5 mg ai/kg/day) x (5 days)]. Assuming that all drug residues in urine bind to the manure, the concentration of drug residues in the manure stockpile would be approximately:

At 6% morbidity: $$\frac{(6000 \text{ head/year}) \times (0.06) \times (6750 \text{ mg drug excreted/head})}{6.56 \times 10^6 \text{ kg manure/year}}$$

$$= 0.37 \text{ mg drug residues/kg manure}$$

At 12% morbidity: $$\frac{(6000 \text{ head/year}) \times (0.12) \times (6750 \text{ mg drug excreted/head})}{6.56 \times 10^6 \text{ kg manure/year}}$$

$$= 0.74 \text{ mg drug residues/kg manure}$$

7.0 Fate of Emitted Substances in the Environment

The fate and transport of enrofloxacin and its major metabolite, ciprofloxacin, in the environment are dependent on the chemical and physical properties of these compounds. Several studies have been conducted to evaluate the characteristics of enrofloxacin and ciprofloxacin which may influence their fate in the environment. Report summaries for the environmental fate studies for enrofloxacin and ciprofloxacin are in Appendix 3. The following data relate to the fate of enrofloxacin and ciprofloxacin in the environments where the drug will be used.

Generally, these data show that enrofloxacin's and ciprofloxacin's properties; namely their tight binding to soil, relatively high water solubility, rapid photolysis in water, and non-volatile nature, predict a negligible exposure in air, minimal exposure and residence in water and biota, but persistence and immobility in soil and sediment.

Enrofloxacin Physical Chemical Data

The aqueous solubility of enrofloxacin was determined to be 1100 ppm at pH 5, 250 ppm at pH 7, and 600 ppm at pH 9 (Bayer Report No. 73353). The *n*-octanol/water partition coefficient (K_{ow}) of enrofloxacin was determined to be 0.4 at pH 5, 3.1 at pH 7, and 0.7 at pH 9 (Bayer Report No. 73390). The vapor pressure of enrofloxacin is $< 10^{-7}$ mm Hg (Bayer Report No. 73409).

The dissociation constants (pK_a) were experimentally determined to be 6.27 and 7.73 for enrofloxacin using potentiometric titration methods (Bayer Report No. 73955). As a consequence, enrofloxacin will be present in the environment in an ionic form. The ionic species include a cation, an anion and a dipolar zwitterion.

Under moderate conditions (pH 5, 7, and 9 at 50°C) enrofloxacin does not readily undergo hydrolysis (Bayer Report No. 106423). However, enrofloxacin is rapidly photolyzed in water with half-lives of 20.6 minutes at pH 5, 3.4 minutes at pH 7, and 14.3 minutes at pH 9 (Bayer Report No. 106562). The electromagnetic absorption spectrum of enrofloxacin exhibits maximum absorbance at 271 to 276 nm and a broad shoulder peaking between 322 and 344 nm at pH 5, 7, and 9 (Bayer Report No. 73954).

Enrofloxacin Environmental Fate Data

The adsorption and desorption of compounds in soil and manure can significantly influence transport processes in the environment. The adsorption of a compound to soil is described by the adsorption coefficient, K_d. A large K_d value indicates that the compound is strongly associated with soil or manure. The adsorption/desorption potential of enrofloxacin was determined for cattle manure, and four types of soil.

K_d values for enrofloxacin were determined in four soil types which represent a range in naturally occurring soil types, including a silt loam, clay loam, sandy loam, and loam. The results are presented in the table below. Nearly complete (> 99.7 %) sorption of enrofloxacin to the soils occurs rapidly when exposing soils to ^{14}C-enrofloxacin. Desorption of the bound enrofloxacin from the soils was insignificant (< 0.26%) under the conditions specified by the FDA guideline (5 ml of 0.01 *M* $CaCl_2$/g soil). As demonstrated by the very large K_d and K_{oc} values, enrofloxacin tightly binds to soils and is immobile (Bayer Report No. 106555).

Parameters	Soil Type; Soil Series; and Source			
	Silt Loam; Drummer; Champaign, IL	Clay Loam; Bearden; Casselton, ND	Sandy Loam; Tifton; Meigs, GA	Loam; Morley; Allen Co., IN
pH	6.2	7.7	5.5	5.5
Organic Carbon (%)	1.9	1.7	1.3	1.1
Organic Matter (%)	3.3	3.0	2.2	2.0
Cation Exchange Capacity (meq/100g)	24.5	29.6	4.5	8.0
Sand/Silt/Clay (%)	23/26/51	27/31/42	70/12/18	36/24/40
Adsorption (%)	99.9	99.8	99.7	99.9
Desorption (%)	0.06	0.09	0.18	0.07
K_d	5502	3466	970	3915
K_{oc}	289568	203906	74635	355941

In addition, the adsorption/desorption behavior of enrofloxacin on cattle feces was also evaluated. The results are presented in the following table.

Cattle Manure	
Parameter	Value
pH	8.7
Organic Carbon (%)	37.6
Organic Matter (%)	64.7
Cation Exchange Capacity (meq/100g)	40.9
Adsorption (%)	67 - 85
Desorption (%)	11 - 30
K_d	367
K_{oc}	976

The sorption of enrofloxacin to cattle manure was up to 85%. The percent desorption from the manure of the adsorbed enrofloxacin was up to 30% (Bayer Report No. 106557). This study demonstrates that enrofloxacin binds relatively tightly to cattle feces. However, whatever residues desorb from cattle feces, or residues in the urine, will bind tightly and nearly irreversibly to soil upon contact with soil.

Aerobic biodegradation of enrofloxacin in soil was tested in three soils types. The results indicated that degradation in soil was slow. The half-life of enrofloxacin in three soil types ranged from 359 to 696 days. In addition, biodegradation of enrofloxacin in cattle manure was tested. The half-life of enrofloxacin in the feces from cattle not previously

treated with enrofloxacin was 468 days, and the half-life of enrofloxacin in the feces of cattle previously treated with ^{14}C-enrofloxacin was 142 days (Bayer Report No. 106560). However, enrofloxacin was shown to be degraded by fungi *in vitro* (Bayer Report No. 106772). This suggests that whenever enrofloxacin residues desorb from manure and/or soil, the unbound enrofloxacin can be degraded in the natural environment since this group of fungi are commonly found in soils. The process will occur more slowly in the natural environment since the desorption of enrofloxacin from soil is a very slow process.

Ciprofloxacin Physical Chemical Data

The aqueous solubility of ciprofloxacin was determined to be 292 ppm at pH 5, 59.1 ppm at pH 7, and 200 ppm at pH 9. The *n*-octanol/water partition coefficient (K_{ow}) of ciprofloxacin was determined to be 0.0852 at pH 5, 0.165 at pH 7, and 0.0360 at pH 9. The vapor pressure of ciprofloxacin is $< 10^{-7}$ mm Hg (Bayer Report No. 106436).

Under moderate conditions (pH 5, 7, and 9 at 50°C), ciprofloxacin does not readily undergo hydrolysis (Bayer Report No. 106430). However, ciprofloxacin is rapidly photolyzed in water with half-lives of 46.4 minutes at pH 5, 9.0 minutes at pH 7, and 23.1 minutes at pH 9 (Bayer Report No. 106563).

The dissociation constants (pK_a) were experimentally determined to be 5.71 and 9.59 for ciprofloxacin using potentiometric titration methods (Bayer Report No. 106436). As a consequence, ciprofloxacin will be present in the environment in an ionic form. The ionic species include a cation, an anion and a dipolar zwitterion.

These data indicate that ciprofloxacin is very similar to enrofloxacin with respect to physical chemical properties.

Ciprofloxacin Environmental Fate Data

K_d values for ciprofloxacin were determined in four soil types which represent a range in naturally occurring soil types, including a silt loam, clay loam, sandy loam, and loam. The results are presented in the table below. Nearly complete (> 98.7 %) sorption of ciprofloxacin to the soils occurs rapidly when exposing soils to ^{14}C-ciprofloxacin. Desorption of the bound ciprofloxacin from the soils was insignificant (< 0.69%) under the conditions specified by the FDA guideline (5 ml of 0.01 *M* $CaCl_2$/g soil). Based on the high K_d and K_{oc} values, ciprofloxacin tightly binds to soils and is immobile (Bayer Report No. 106556).

Parameter	Soil Type; Soil Series; and Source			
	Silt Loam; Drummer; Champaign, IL	Clay Loam; Bearden; Casselton, ND	Sandy Loam; Tifton; Meigs, GA	Loam; Morley; Allen Co., IN
pH	6.2	7.7	5.5	5.5
Organic Carbon (%)	1.9	1.7	1.3	1.1
Organic Matter (%)	3.3	3.0	2.2	2.0
Cation Exchange Capacity (meq/100g)	24.5	29.6	4.5	8.0
Sand/Silt/Clay (%)	23/51/26	27/31/42	70/12/18	36/24/40
Adsorption (%)	99.5	99.3	99.4	98.7
Desorption (%)	0.27	0.36	0.33	0.62
K_d	918	601	544	1479
K_{oc}	48341	35342	41841	134465

The adsorption/desorption behavior of ciprofloxacin on cattle feces was also evaluated. The results are presented in the following table.

Cattle Manure	
Parameter	Value
pH	8.7
Organic Carbon (%)	37.6
Organic Matter (%)	64.7
Cation Exchange Capacity (meq/100g)	40.9
Adsorption (%)	71 - 84
Desorption (%)	10 - 24
K_d	399
K_{oc}	1062

The sorption of ciprofloxacin to cattle manure was up to 84%. The percent desorption from the manure of the adsorbed ciprofloxacin was up to 24% (Bayer Report No. 106557). This study demonstrates that ciprofloxacin also binds relatively tightly to cattle feces. However, whatever residues desorb from feces, or residues in the urine, will bind tightly and nearly irreversibly to soil upon contact with soil.

Aerobic biodegradation of ciprofloxacin in soil was tested in three soils types. The results indicated that degradation in soil was slow. As minimal degradation occurred over the 65-day study period, half-lives of ciprofloxacin in the three soil types were not calculated (Bayer Report No. 106561).

The physical and chemical properties of enrofloxacin and ciprofloxacin are very similar. Moreover, the environmental fate of enrofloxacin and ciprofloxacin in and on soil, manure, and water are also similar. Although there are differences in actual values, the practical differences are not significant. For example, the K_{oc} values for enrofloxacin range from 74,635 to 355,941 for enrofloxacin compared to K_{oc} values of 35,342 to 134,465 for ciprofloxacin on the same soils. Although the enrofloxacin K_{oc} values are higher by a factor of 2 to 3, this difference isn't of practical significance when any compound with a K_{oc} value greater than 1000 is considered immobile, and the K_{oc} values for both compounds exceed the K_{oc} classification of 1000 by a minimum of 35 times.

These data indicate that ciprofloxacin will behave very similar to enrofloxacin in the environment.

7.1 Atmospheric Environment

Enrofloxacin is not expected to enter the atmospheric environment. The drug and its major metabolite, ciprofloxacin, tightly bind to excreta and soil. In addition, both enrofloxacin and ciprofloxacin have very low vapor pressures ($< 10^{-7}$ mm Hg). A possible route of entry into the atmospheric environment is via fugitive dusts from manure stock piles (only if very dry) or during application of manure to agricultural fields (once a year). However, only a small fraction of airborne particulates are respirable. The inhalable fraction of suspended particulates are those particles ≤ 10 μm in diameter (USEPA, 1988). Also, as discussed later in this assessment, the relative toxicity of enrofloxacin is very low to animal life. The very small quantities of enrofloxacin from fugitive dusts will not pose a toxic hazard. Therefore, respirable fugitive dusts containing enrofloxacin and ciprofloxacin are not likely to be generated in sufficient quantities to be of concern.

7.2 Aquatic Environment

Theoretically, movement of enrofloxacin and ciprofloxacin into aquatic systems could occur from runoff.

<u>Pasture Runoff</u>

Runoff from pastures is not expected to contribute significant amounts of drug residues from manure to aquatic environments based on current practices of treating animals in pasture settings. Sick animals are taken from the pasture and placed in a hospital pen for a 3- to 5-day treatment period and then released. In a typical 50-head cow-calf operation with a BRD morbidity rate of 5 percent (Perino, 1992), approximately two to three

animals would be treated. The amount of manure excreted by three animals in a 100- to 300-acre pasture is not significant as determined by:

(3 head) x (6.6 lbs dry manure/day/head) x (5 days) = 99 lbs manure (45 kg)

This manure would then be spread out over the pasture (100- to 300-acres in this example).

Feedlot Runoff

Because enrofloxacin and ciprofloxacin bind so tightly to manure and particularly to soil, runoff under any conditions is unlikely. Moreover, with feedlots runoff catchment lagoons are mandatory as required by USEPA National Pollution Discharge Elimination System. These retention systems are designed to prevent a discharge to aquatic systems from feedlot runoff. Therefore, feedlots present little potential for exposure to aquatic systems.

Amended Cropland Runoff

There is a somewhat greater possibility of runoff from agricultural fields amended with manure from treated animals. Runoff from agricultural fields is affected by many factors which are quantitatively difficult to evaluate. These factors include location of receiving bodies of water, slope steepness and complexity, soil and weather conditions, soil type, and buffer strips.

For the purposes of this environmental assessment, a worst-case scenario was used to determine the expected environmental concentration (EEC) of drug residues in water from cropland runoff (USEPA, 1995b). In the scenario, the EEC_{water} is calculated using the Generic Expected Environmental Concentration model (GENEEC). This computer-based model utilizes basic chemical parameters (solubility, photolysis, hydrolysis, K_{oc}, and soil degradation) combined with application rate and method of soil incorporation to determine the EEC. The model considers the reduction in dissolved drug residues due to adsorption to soil or sediment, incorporation depth, degradation in soil before washoff to a water body, and degradation of the residues within the water body. The model assumes that the runoff from a 10 hectare (24.7 acre) field is entering a 1 hectare (2.47 acre) by 2 meter (6.56 feet) deep pond.

Prior to running the model using the input parameters and assumptions for cattle feedlot operations, a sensitivity analysis was conducted. A set of "standard" parameters was used, and one parameter was varied at a time to see the effect that parameter had on the final maximum EEC value. The parameters that were varied were soil K_{oc} , photolysis, application rate, soil half-life, and aqueous solubility. Without question, the application rate has the greatest effect on the maximum EEC. The parameter with the second greatest effect was soil K_{oc}. The other parameters have more of an influence on the "die-away" of a chemical in the pond rather than the instantaneous maximum concentration.

The range of expected concentrations of drug residues in an acre of amended cropland soils can be calculated as follows:

Low application rate = 9,072 kg manure/acre (10 tons/acre)
High application rate = 27,216 kg manure/acre (30 tons/acre)
Residues in manure at 6% morbidity rate = 0.37 mg ai/kg manure
Residues in manure at 12% morbidity rate = 0.74 mg ai/kg manure

Lower bound:
(0.37 mg ai/kg manure) x (9,072 kg manure/acre) = 3,357 mg total residues/acre
= 0.007 lbs total residues/acre

Upper Bound:
(0.74 mg ai/kg manure) x (27,216 kg manure/acre) = 20,140 mg total residues/acre
= 0.044 lbs total residues/acre

The range of enrofloxacin and ciprofloxacin residues applied per acre can be calculated based on the percentages excreted (28% for enrofloxacin, 52% for ciprofloxacin):

(0.007 lbs total residues/acre) x (0.28 enrofloxacin) = 0.002 lbs enrofloxacin/acre

(0.044 lbs total residues/acre) x (0.28 enrofloxacin) = 0.012 lbs enrofloxacin/acre

(0.007 lbs total residues/acre) x (0.52 ciprofloxacin) = 0.004 lbs ciprofloxacin/acre

(0.044 lbs total residues) x (0.52 ciprofloxacin) = 0.023 lbs ciprofloxacin/acre

Based on actual land amendment practices, only one application of manure was assumed for a given field per year. The soil K_{oc} value for enrofloxacin ranges from 74,635 to 355,941 depending on soil type (Bayer Report No. 106555). The soil K_{oc} value for ciprofloxacin ranges from 35,342 to 134,465 depending on soil type (Bayer Report No. 106556). The aerobic soil/manure half-life for enrofloxacin ranges from 468 to 696 days (Bayer Report No. 106560). A half-life in soil for ciprofloxacin could not be calculated (Bayer Report No. 106561), but could be graphically estimated at 727 days for the purposes of using the GENEEC model. Neither enrofloxacin nor ciprofloxacin readily undergo hydrolytic degradation (Bayer Report Nos. 106423 and 106430). Since neither solubility nor photolysis affect the instantaneous maximum EEC (based on sensitivity analysis), the solubility and photolysis values at pH 7 were used for both enrofloxacin and ciprofloxacin. The model assumes that a rain event occurs immediately after application (0 days) which represents worst case conditions.

To summarize, the range of input parameters for the GENEEC model are:

Parameter	Enrofloxacin		Ciprofloxacin	
	Low Value	High Value	Low Value	High Value
Application Rate (lbs/acre)	0.002	0.012	0.004	0.023
Soil K_{oc}	355941	74635	134465	35342
Solubility at pH 7 (ppm)	250	250	59.1	59.1
Soil incorporation (inches)	6	6	6	6
Soil half-life (days)	359	696	727	727
Days until rainfall	0	0	0	0
Hydrolysis	0	0	0	0
Photolysis at pH 7 (days)	0.00236	0.00236	0.00062	0.00062
Aquatic metabolism	0	0	0	0

Based on these assumptions the range of instantaneous maximum EEC's were calculated to be:

EEC_{water} for enrofloxacin = 0.00000025 ppm to 0.0000025 ppm

EEC_{water} for ciprofloxacin = 0.00000070 ppm to 0.0000056 ppm

Environmental Processes

The GENEEC model utilizes environmental fate data to determine die-away of the compounds in water in addition to the instantaneous maximum concentration (time = 0) previously described. Based on its rapid photodegradation and tendency to partition into sediments, enrofloxacin and ciprofloxacin concentrations in water are predicted by the model to be reduced by 70 to 75 percent 4 days after a runoff event. For example, the ranges for 4-day EEC_{water} are predicted to be:

4-day EEC_{water} for enrofloxacin = 0.000000006 ppm to 0.000000064 ppm

4-day EEC_{water} for ciprofloxacin = 0.00000017 ppm to 0.00000139 ppm

This would reduce the already low exposure potential to aquatic life to even lower levels in a relatively short time period.

7.3 Terrestrial Environment

Pasture Operations

The terrestrial environment is not expected to receive enrofloxacin drug residues in any significant quantities from cow-calf pasture operations. The animals treated will be

scattered over wide areas of grazing land, and the manure from these cattle will be so widespread that drug residues will be localized to the immediate location of the manure.

Feedlot Operations

Feedlots are non-natural settings and are, therefore, depauperate in flora and fauna. However, some inquiline species, such as mice, voles, starlings, cowbirds, house sparrows, magpies, and blackbirds may be found in and around feedlots. Because there is no suitable habitat for wildlife in a feedlot, no terrestrial exposures are expected on feedlot grounds.

The feedlot profile usually contains a compacted interfacial layer of manure and soil that provides a biological seal that reduces water infiltration rate (ASAE, 1992). This zone of low infiltration restricts the leaching of drug residues into groundwater. Feedlot managers maintain this impermeable layer by carefully removing manure during cleaning operations. In addition, research has shown that runoff lagoons are partially self-sealing due to clogging of soil pores by bacterial cells and organic matter, thereby reducing the opportunity for drug residues leaching into groundwater (ASAE, 1992). In addition to these general precautions for any chemical, as described previously, enrofloxacin and ciprofloxacin residues is reduced even further by very strong sorption to soil and manure and are classified as immobile.

Amended Croplands

The greatest exposure of enrofloxacin drug residues will be through application of manure from treated cattle to cropland. The exposure will be limited to those species being planted in the amended fields and to flora and fauna in fringe areas (e.g., hedge rows, buffer strips) that may receive runoff or direct application of manure inadvertently.

Manure application rates depend on many factors including manure analysis, physical and chemical soil characteristics, type of crop, yield goal, soil drainage, climate, groundwater depth and geology (ASAE, 1992). For the purposes of this environmental assessment, the lower and upper bounds of a range of application rates (10 tons dry manure/acre and 30 tons dry manure/acre) are used to estimate soil concentrations of drug residues.

Manure is typically incorporated into the top 6 inches of soil by plowing. The mass of the top 6 inches of soil is determined by bulk density. The bulk density of a typical agricultural silt loam is 1.5 g/cm^3 . From this, the mass can be determined by:

(4,046.9 m^2 /acre) x (0.1524 m/6 inches) x ($1x10^6$ cm^3/m^3) x (1.5 g/cm^3) x (1 kg/1000g) = 925,121 kg/acre-6 inches.

Therefore, the expected environmental concentration (EEC) of totals drug residues in soil are calculated as follows:

At 6% morbidity and 10 tons/acre (9,072 kg/acre) application rate:

$$EEC_{soil} = \frac{(0.37 \text{ mg ai/kg manure}) \times (9{,}072 \text{ kg manure/acre})}{925{,}121 \text{ kg soil/acre}} = 0.004 \text{ mg residues/kg soil}$$

At 12% morbidity and 30 tons/acre (27,216 kg/acre) application rate:

$$EEC_{soil} = \frac{(0.74 \text{ mg ai/kg manure}) \times (27{,}216 \text{ kg manure/acre})}{925{,}121 \text{ kg soil/acre}} = 0.022 \text{ mg residues/kg soil}$$

The two main compounds of interest that are excreted in cattle feces and urine are enrofloxacin and ciprofloxacin. These have been shown to be excreted as 28 percent enrofloxacin and 52 percent ciprofloxacin (Bayer Report No. 106564). Therefore, the total drug residues in soil can be converted into enrofloxacin and ciprofloxacin concentrations based on these percentages.

At 6% morbidity and 10 tons/acre:

(0.004 mg total residues) x (0.28 enrofloxacin) = 0.001 mg enrofloxacin/kg soil

(0.004 mg total residues) x (0.52 ciprofloxacin) = 0.002 mg ciprofloxacin/kg soil

At 12% morbidity and 30 tons/acre:

(0.022 mg total residues) x (0.28 enrofloxacin) = 0.006 mg enrofloxacin/kg soil

(0.022 mg total residues) x (0.52 ciprofloxacin) = 0.011 mg ciprofloxacin/kg soil

Environmental Processes

As discussed previously, based on their extremely high K_{oc} values, enrofloxacin and ciprofloxacin are strongly sorbed to particulates which decreases bioavailability and mobility. USFDA (1987) has stated that compounds having a log K_{oc} greater than 3, such as enrofloxacin and ciprofloxacin, are considered tightly bound to organic matter in soil and are considered immobile. These compounds do not readily degrade in soil; however, there is evidence that several groups of fungi can significantly degrade enrofloxacin (Bayer Report No. 106772).

8.0 Environmental Effects of Released Substances

The effects of enrofloxacin and ciprofloxacin on many organisms have been evaluated in many TAD and TAD derived studies, and the results of these studies are summarized below in each of the appropriate sections. In addition, the results of the effects studies are compared with the estimated environmental concentrations to characterize possible risk.

Risk characterization is the process of estimating the nature and likelihood of effects by combining exposure estimates with the effects observed from toxicity studies. A well accepted method for describing potential risk to flora and fauna from environmental exposure to a compound is the Toxicity Exposure Ratio or Risk Quotient (Barnthouse *et al.*, 1986). This is simply the division of some toxicological benchmark by the estimated environmental concentration. If the quotient is less than one (NOEC/EEC, LC_{50}/EEC, etc.) then a toxic effect is expected to occur.

In the case of enrofloxacin and ciprofloxacin, the Risk Quotient describes how many times lower the EEC is compared to the no observed effect level for a given study and test species. The no observed effect concentration (NOEC) was chosen as the toxicological benchmark as it is a much more conservative value compared to the median lethal concentration (LC_{50}). For example, there were no observable sublethal effects at concentrations at or below 18.6 ppm in the enrofloxacin acute bluegill study. The range of the Risk Quotients was calculated to be 7,440,000 to 74,000,000. This means that the estimated concentration of enrofloxacin in water is 7.4 to 74 million times lower than the lowest concentration of enrofloxacin that affects bluegill. Thus, there is little likelihood that enrofloxacin used to treat cattle entering the aquatic environment would adversely affect bluegill.

8.1 Aquatic Organisms

Although it is not likely that enrofloxacin residues will reach water bodies due to its manufacture and/or end use, several aquatic toxicity studies were conducted according to guidelines set forth in CVM's Technical Assistance Documents. Studies were conducted with enrofloxacin and ciprofloxacin for the fish, bluegill and rainbow trout; the waterflea, *Daphnia*; the amphipod, *Hyalella*; the green alga, *Selenastrum*; and the blue-green alga, *Microcystis*. Summaries of these studies are presented in Appendix 3. The results (LC_{50} and NOEC values) are presented in the table below. Using the EEC_{water} estimates previously discussed in Section 7.2 of this document, risk quotients for enrofloxacin and ciprofloxacin are reported below.

Species	Compound	LC_{50} (ppm)	NOEC (ppm)	Bayer Report No.	EEC_{water} Range (ppm)	Risk Quotient Range
Bluegill	Enro	79.5	18.6	74507	0.00000025 - 0.0000025	7,440,000 - 74,000,000
Trout	Enro	>196	33.5	74501	0.00000025 - 0.0000025	13,400,000 - 134,000,000
Daphnia	Enro	79.9	23.0	106595	0.00000025 - 0.0000025	9,200,000 - 92,000,000
Daphnia	Enro (chronic)	N/A	9.80	106790	0.00000025 - 0.0000025	3,920,000 - 39,200,000
Hyalella	Enro	> 206	< 12.7	106788	0.00000025 - 0.0000025	< 5,080,000

Species	Compound	LC_{50} (ppm)	NOEC (ppm)	Bayer Report No.	EEC_{water} Range (ppm)	Risk Quotient Range
Bluegill	Cipro	> 9.85	≥ 9.85	106791	0.00000070 - 0.0000056	≥ 1,758,928
Trout	Cipro	> 9.4	≥ 9.4	106775	0.00000070 - 0.0000056	≥ 1,678,571
Daphnia	Cipro	> 9.90	≥ 9.90	106596	0.00000070 - 0.0000056	≥ 1,767,857
Hyalella	Cipro	> 10.2	2.24	106783	0.00000070 - 0.0000056	400,000 - 3,200,000

Clearly, these data and Risk Quotient values indicate that there will be insignificant risk to the two of the trophic levels tested (fish and invertebrates). The effects of enrofloxacin and ciprofloxacin on the growth of *Selenastrum* and *Microcystis* were also evaluated, (Bayer Report Nos. 106657, 106940, 10633, and 106627). Risk Quotients could not be determined for *Selenastrum* or *Microcystis* since there was substantial degradation of the test compound over the course of the testing period and a definitive NOEC could not be determined. The degradation observed was very likely due to aqueous photolysis, however, photodegradation is an unavoidable artifact of the experimental design for conducting algae toxicity tests. Algae testing requires a strong and almost constant light source in order for the algae to grow and it is not possible to conduct these tests under low light conditions in order to avoid photodegradation. Although Risk Quotients could not be calculated, the studies did show that these two species are more sensitive to enrofloxacin and ciprofloxacin than other aquatic life. This is not unexpected for *Microcystis* since this species is a prokaryote and very bacteria-like which would make it more sensitive to an antibiotic.

In the natural environment, aqueous photolysis and compartmental partitioning will reduce the concentration in the water will be reduced, by up to 75% in four days, as estimated by the GENEEC model. Populations of blue-green algae, such as *Microcystis*, would not be exposed to the predicted maximum concentration for very long which would allow for recovery. From an ecological perspective, the trophic level in which blue-green algae reside contain many, many more groups of algae including green algae, such as *Selenastrum*, which have been shown to be very insensitive to enrofloxacin and ciprofloxacin. Even if a slight, temporary decline in blue-green algal populations were to occur, the function of the ecosystem at the algal trophic level would not change as the insensitive species would still be present in a primary producer role.

With regard to possible bioaccumulation in aquatic organisms, the *n*-octanol/water partition coefficient (K_{ow}) is an indication of the lipid solubility and membrane permeability of a chemical, and therefore can be used to predict the likelihood of the chemical to bioaccumulate in biota. According to USFDA (1987), chemicals with K_{ow} values less than 10, such as enrofloxacin and ciprofloxacin, are not expected to undergo significant bioconcentration. The bioconcentration potential for enrofloxacin and

ciprofloxacin in aquatic organisms can be further estimated from the K_{ow} using the following regression equation (Clark *et al.*, 1990):

$$\text{Log BCF (bioconcentration factor)} = 0.76(\log K_{ow}) - 0.23$$

Using this equation and the highest log K_{ow} for enrofloxacin (0.49 at pH 7) and ciprofloxacin (-0.78 at pH 7), the predicted BCFs are approximately 1.38 and 0.150, respectively. This indicates that both enrofloxacin and ciprofloxacin have a low propensity to bioconcentrate since bioconcentration is not observed unless BCFs are several orders of magnitude higher (Clark *et al.*, 1990). This is also empirically supported by tissue residue studies which showed that the depletion of [^{14}C] residues from the liver, kidneys, muscle, fat, injection sites, and other tissues of cattle treated with ^{14}C-enrofloxacin was biphasic and very rapid (Bayer Report No. 106579).

Based on the exposure estimates and toxicity data, enrofloxacin and ciprofloxacin entering an aquatic environment from runoff from agricultural fields amended with manure containing these drug residues are not expected to have any effects on aquatic organisms.

8.2 Terrestrial Organisms

The exposure of terrestrial organisms to drug residues is only expected to occur from contact with soil/manure containing enrofloxacin in cropland soils amended with manure. The main route of entry for drug residues in terrestrial vertebrate and invertebrate species is through ingestion of manure and/or amended soils containing drug residues. Dermal exposure is considered a negligible route of entry since enrofloxacin and its metabolites do not exhibit strong lipophilicity that would allow significant dermal absorption of drug residues into an organism. For example, the dermal LD_{50} and NOEC for enrofloxacin were greater than 2,000 ppm for albino rabbits (Bayer Report No. 73606). Likewise, inhalation is considered a minor route of entry since enrofloxacin and ciprofloxacin are not volatile, and fugitive dusts containing drug residues are not expected to occur in sufficient quantities and for sufficient duration as represent a significant exposure. The inhalation LC_{50} for white rats exposed to enrofloxacin was greater than 3547 mg/m^3 (Bayer Report No. 73466).

For estimation purposes, enrofloxacin mammalian toxicity data can be used to approximate possible risk from ciprofloxacin exposure. As enrofloxacin is generally more toxic than ciprofloxacin, the use of enrofloxacin toxicity data is more conservative and protective when determining risk to terrestrial mammals.

Several toxicological studies were conducted on terrestrial species including mice, rats, rabbits, earthworms, six species of crops, fungi, and bacteria. Summaries of these studies are presented in Appendix 3.

8.2.1 Mammals

The results from mammalian toxicity tests and calculated Risk Quotient Ranges using soil EEC values are presented in the tables below.

Species	Compound	Oral LD_{50} (mg/kg)	Bayer Report No.	EEC_{soil} Range (mg/kg)	Risk Quotient Range
Mouse (male)	Enro	> 5,000	73075	0.001 - 0.006	> 806,450
Mouse (female)	Enro	4,336	73075	0.001 - 0.006	699,350 - 4,336,000
Rat (male)	Enro	> 5,000	73075	0.001 - 0.006	> 806,450
Rat (female)	Enro	> 5,000	73075	0.001 - 0.006	> 806,450
Rabbit (male/female)	Enro	500 - 800	73075	0.001 - 0.006	80,640 - 800,000

Species	Study Type	Compound	NOEL (mg/kg)	Bayer Report No.	EEC_{soil} Range (mg/kg)	Risk Quotient Range
Dog	Subchronic	Enro	3	73775	0.001 - 0.006	484 - 3,000
Mouse	Chronic	Enro	323	74229	0.001 - 0.006	52,100 - 323,000
Rats	Chronic	Enro	5.3	74387	0.001 - 0.006	855 - 5,300
Rats	Reproduction	Enro	6.25	73892	0.001 - 0.006	1,000 - 6,250
Rabbit	Embryotoxicity	Enro	25	73705	0.001 - 0.006	4,030 - 25,000

The EEC_{soil} values are those derived in Section 7.3 of this document. Based on these soil concentrations, the amount of soil that an mammal would need to ingest on a daily basis to reach the chronic NOEC is a physical impossibility. For example, the subchronic oral NOEL for dogs was based on a continuous 90-day exposure to enrofloxacin. For a 10-kg dog to be exposed to a daily 3 mg/kg dose via a field amended with cattle manure containing 0.062 mg enrofloxacin/kg soil , it would have to consume daily for 90-consecutive days:

(3 mg/kg body wt) x (10-kg body wt) x (1 kg soil/0.062 mg enro) = 484 kg soil (1,067 lbs)

Therefore, based on the exposure estimates and toxicity data, enrofloxacin entering a terrestrial environment via manure containing this drug residue is not expected to have adverse effects on mammals.

8.2.2 Earthworms

Two 28-day studies (one for enrofloxacin and one for ciprofloxacin) were conducted to determine the effects of soil-incorporated compound on the earthworm (*Lumbricus terrestris*). Summaries of these studies (Bayer Report Nos. 74123 and 106793) are presented in Appendix 3. The results of the studies indicated that the NOEC for enrofloxacin, based on growth and survival, was greater than or equal to 1000 ppm (nominal) and that the NOEC for ciprofloxacin was greater than or equal to 1000 ppm (nominal, 885 ppm measured).

Based the EEC_{soil} range of 0.001 to 0.006 ppm for enrofloxacin, the resulting Risk Quotients would be 161,290 to 1,000,000 for earthworms exposed to enrofloxacin. Based on the 0.002 to 0.011 ppm EEC_{soil} range for ciprofloxacin, the Risk Quotients for earthworms exposed to ciprofloxacin are 77,630 to 465,790. Therefore, no adverse effects on earthworms are expected in and around agricultural fields amended with manure containing enrofloxacin and ciprofloxacin residues.

8.2.3 Plants

Seed Germination and Root Elongation

Seed germination and root elongation were monitored for seeds of soybean, lettuce, ryegrass, wheat, tomato, and cucumber exposed to solutions of enrofloxacin in a study conducted according to TAD Guideline 4.06 (Bayer Report No. 106661). The study showed that enrofloxacin concentrations ranging from 1 to 882 ppm (1 to 1000 ppm nominal) had no effect on the germination of the seeds of the six species, thus the no effect concentration for enrofloxacin on seed germination was at least 882 ppm. The most sensitive species to enrofloxacin for root growth was cucumber with a NOEC of 0.27 ppm (0.25 ppm nominal).

The experimental design in the guideline for seed germination and root elongation (TAD 4.06) requires the use of blotter paper soaked in a solution containing enrofloxacin. This design does not represent real world conditions and it cannot account for the strongly sorptive nature of enrofloxacin in soil. Therefore, another seed germination and root elongation study was conducted using the same basic techniques and procedures as in the previously described study, but substituting soil for blotter paper (Bayer Report No. 74576). The least sorptive soil, as determined in the soil adsorption/desorption study (Bayer Report 106555) was used. Cucumber was chosen as the test species since it was the most sensitive species for root elongation effects. The NOEC was determined to be 9.1 ppm for both seed germination and root elongation. Thus, soil greatly reduced the effect of enrofloxacin on cucumber root elongation by at least 34-fold:

TAD 4.06 root elongation NOEC = 0.27 ppm
Soil based root elongation NOEC = 9.1 ppm
(9.1 ppm)/(0.27 ppm) = 34

Using the NOEC, 9.1 ppm, for the most sensitive plant species grown in the presence of soil, and the range of EEC_{soil} of 0.001 to 0.006 ppm for enrofloxacin in soil, the resulting Risk Quotient Range would be 1,470 to 9,100.

Likewise, a seed germination and root elongation study was conducted for the metabolite, ciprofloxacin, using the same six plant species (Bayer Report No. 106911). The study showed that ciprofloxacin concentrations ranging from

2.2 to 900 ppm (2 to 1000 ppm nominal) had no effect on the germination of the seeds of the six species, thus the no effect concentration for ciprofloxacin on seed germination was at least 900 ppm. The species most sensitive to ciprofloxacin effects on root elongation was lettuce with a NOEC of 0.54 ppm (0.50 ppm nominal).

A seed germination and root elongation study for ciprofloxacin in the presence of soil was not conducted since ciprofloxacin, like enrofloxacin, binds so tightly to soil that it was not likely to be bioavailable under these soil conditions. Assuming that the phytotoxicity on root elongation is reduced by 34-fold as occurred with enrofloxacin (discussed above) since ciprofloxacin, like enrofloxacin, binds so tightly to soil, the Risk Quotient Range can be calculated as.

$$(0.54 \text{ ppm} \times 34)/0.011 = 1{,}669$$

$$(0.54 \text{ ppm} \times 34)/0.002 = 9{,}180$$

Seedling Growth

The effect of enrofloxacin on seedling growth was evaluated according to TAD Guideline 4.07 using the same species tested under the seed germination and root elongation guideline studies (Bayer Report No. 74583). The most sensitive species tested was wheat with a NOEC < 0.13 ppm. Ciprofloxacin was not tested for effects on seedling growth since it has historically demonstrated less toxicity than enrofloxacin for many other species, and consequently, the enrofloxacin values can be used to conservatively estimate ciprofloxacin toxicity.

To provide more realistic data, a seed germination and root elongation study in soil was conducted to more accurately assess the toxicity that might occur under field conditions (Bayer Report No. 74511). This study clearly showed a substantial decrease in toxicity of enrofloxacin in the presence of soil. Since the seed germination and root elongation study showed such a dramatic decrease in toxicity, a seedling growth study in soil was also conducted. The species tested were wheat, the most sensitive species identified in the seedling growth study conducted in sand, and tomato, a representative dicot. Both enrofloxacin and ciprofloxacin were tested. The more sensitive species tested to enrofloxacin under soil testing conditions was wheat with a NOEC of 4.7 ppm. Tomato and wheat exposed to soil treated with ciprofloxacin were equally sensitive with a NOEC of greater than or equal to 49 ppm.

The summaries for these studies are presented in Appendix 3. The results for the most sensitive species are shown in the table below along with the EEC ranges and, for risk characterization purposes, the calculated Risk Quotient Range.

Species	Compound	Study Type	NOEC (ppm)	Bayer Report No.	EEC_{soil} Range (ppm)	Risk Quotient Range
Cucumber	Enro	Seed germination	≥ 882	106661	0.001 - 0.006	> 142,250
Cucumber	Enro	Root elongation	0.27	106661	0.001 - 0.006	44 - 270
Cucumber	Enro	Seed germ (soil)	9.1	74576	0.001 - 0.006	1,470 - 9,100
Cucumber	Enro	Root elongation (soil)	9.1	74576	0.001 - 0.006	1,470 - 9,100
Wheat	Enro	Seedling growth	< 0.13	74583	0.001 - 0.006	< 21
Tomato	Enro	Seedling growth (soil)	9.5	74511	0.001 - 0.006	1,530 - 9,500
Wheat	Enro	Seedling growth (soil)	4.7	74511	0.001 - 0.006	760 - 4,700
Lettuce	Cipro	Seed germination	≥ 900	106911	0.002 - 0.011	≥ 78,950
Lettuce	Cipro	Root elongation	0.54	106911	0.002 - 0.011	47 - 280
Tomato	Cipro	Seedling growth (soil)	≥ 49	74511	0.002 - 0.011	> 25,780
Wheat	Cipro	Seedling growth (soil)	≥ 49	74511	0.002 - 0.011	> 25,780

Soil had a dramatic effect on decreasing the NOECs of terrestrial plants. Therefore, based on the exposure estimates and toxicity data (particularly that data generated in the presence of soil), enrofloxacin and ciprofloxacin entering a terrestrial environment via manure containing these drug residues are not expected to have adverse effects on plants.

8.2.4 Microorganisms

Microbial growth inhibition studies were conducted for enrofloxacin (Bayer Report No. 106599), and its major metabolite, ciprofloxacin (Bayer Report No. 106750), using seven representative soil species. The organisms tested included three bacterial species (*Pseudomonas aeruginosa, Arthrobacter picolinophilus, Azotobacter vinelandii*), a blue-green alga (*Anabaena flos-aquae)*, and three fungal species (*Aspergillus clavatus, Penicillium canescens, Trichoderma hamatum*). The summaries for these studies are presented in Appendix 3. The results are shown in the table below along with the EEC ranges (calculated in Section 7.3 of this document) and, for risk characterization purposes, the calculated Risk Quotient Range.

Species	Compound	MIC (ppm)	NOEC (ppm)	Bayer Report No.	EEC_{soil} Range (ppm)	Risk Quotient Range
Pseudomonas	Enro	12.5	1.3	106599	0.001 - 0.006	209 - 1,300
Pseudomonas	Cipro	10	1	106750	0.002 - 0.011	88 - 530
Arthrobacter	Enro	12.5	1.3	106599	0.001 - 0.006	209 - 1,300
Arthrobacter	Cipro	10	1	106750	0.002 - 0.011	88 - 530
Azotobacter	Enro	1.3	< 1.3	106599	0.001 - 0.006	< 210
Azotobacter	Cipro	1	< 1	106750	0.002 - 0.011	< 90
Anabaena	Enro	12.5	1.3	106599	0.001 - 0.006	209 - 1,300
Anabaena	Cipro	10	1	106750	0.002 - 0.011	88 - 530
Aspergillus	Enro	> 250	≥ 250	106599	0.001 - 0.006	≥ 250,000
Aspergillus	Cipro	> 60	≥ 60	106750	0.002 - 0.011	≥ 31,580
Penicillium	Enro	> 250	≥ 250	106599	0.001 - 0.006	≥ 250,000
Penicillium	Cipro	> 60	≥ 60	106750	0.002 - 0.011	≥ 31,580
Trichoderma	Enro	> 250	≥ 250	106599	0.001 - 0.006	≥ 250,000
Trichoderma	Cipro	> 60	≥ 60	106750	0.002 - 0.011	≥ 31,580

An additional, non-guideline study was conducted using the two most sensitive species (*Arthrobacter, Azotobacter*) to determine the bioavailability of soil-bound enrofloxacin residues to microorganisms (Bayer Report No. 107124). The results of this study showed that the strong adsorption of enrofloxacin to a soil/manure matrix made it unavailable to microorganisms. No inhibitory effects were observed up to the highest concentration tested (500 mg a.i./kg soil). Therefore, under conditions which microorganisms will be exposed in the environment, the NOEC to EEC_{soil} ratio would be ≥ 83,333.

Based on the exposure estimates and toxicity data, enrofloxacin and ciprofloxacin entering a terrestrial environment via manure containing these drug residues are not expected to have adverse effects on microorganisms.

Bacterial Resistance Development

Development of antibiotic resistance by bacteria has relevance to human and animal health. Although enrofloxacin and ciprofloxacin bind tightly to soil and that soil-bound enrofloxacin was clearly shown to be almost completely non-bioavailable, the possibility exists for residues in soil to select for resistant organisms. The Center for Veterinary Medicine has determined that resistance development is not considered to have potential for significant impacts on ecological process, and accordingly, it is more appropriate to consider this issue under the FD&C Act.

8.3 Human Exposure

Several toxicity studies were conducted to assess the acute, subchronic, and chronic effects in mammals. The summaries for these studies are presented in Appendix 3. The results are presented in the following table.

Species	Study Type	LD_{50}	Bayer Report No.
Rat (male & female)	Acute oral	> 5,000	73075
Mouse (male)	Acute oral	> 5,000	73075
Mouse (female)	Acute oral	4,336	73075
Rabbit (male & female)	Acute oral	500 to 800	73075

Species	Study Type	NOEL	Bayer Report No.
Rat	Subchronic oral	40 mg/kg	73194
Dog	Subchronic oral	3 mg/kg	73775
Rat	Teratology oral	50 mg/kg	73159
Rabbit	Teratology oral	25 mg/kg	73705
Rat	Reproduction	165 mg/kg	73314
Mouse	Chronic	323 mg/kg	74229
Rat (male)	Chronic	5.3 mg/kg	74387
Rat (female)	Chronic	7.2 mg/kg	74387

Species	Study Type	Results	Bayer Report No.
Mouse	Carcinogenicity	No carcinogenic effect up to 10000 ppm	74229
Rat	Carcinogenicity	No carcinogenic effect up to 6000 ppm	74230

During manufacturing, engineering controls and industrial hygiene precautions will be utilized to effectively minimize exposure to workers. The controls will be in compliance with regulations set forth by the Occupational Safety and Health Commission (OSHA). Based on these protective measures and the mammalian toxicity data, workers producing and formulating enrofloxacin will not be adversely affected by the proposed action.

The label for the BAYTRIL® 100 Injectable Solution will instruct users that this product is not for human use and should be kept out of the reach of children. The label will further instruct users to avoid letting the formulation contact their eyes. Spillage of the product onto the skin is not a practical health concern since it is neither dermally toxic

(LD_{50} > 2000 mg/kg , the highest level tested) nor is it a dermal irritant or sensitizer. The potential for parenteral toxicity from inadvertent self-injection is minimal as the oral LD_{50} is in the range of 5000 mg/kg and the intravenous LD_{50} is approximately 220 mg/kg. To simulate the intravenous LD_{50}, a 60 kg human would require an injection greater than 120 ml. Based on the magnitude of the toxicity values and the fact that the drug is in a liquid formulation, users of the product will not be adversely affected by the proposed action.

8.4 Uncertainty Analysis

There is uncertainty associated with any estimate of environmental exposure and the effects this exposure may have on environmental receptors. Uncertainty analysis identifies the uncertainty from each phase of the risk assessment process and provides an evaluation of the impact of the uncertainties on the overall assessment.

The two phases in this risk assessment are toxicity evaluation and risk characterization. The uncertainty associated with toxicity evaluation primarily lies with the inability to test all species and/or all life-stages of an organism under natural conditions. However, the species chosen for toxicity evaluation represent broad classes of organisms that are of economic, recreational, or ecological importance. In addition, the test methodologies generally represent worst case conditions. For example, fish were exposed to a constant concentration of the test article for 96-hours in clean laboratory water. In the environment, fish would be exposed to a pulsed dose that may only last for a few hours and the compound would be subjected to many processes which would reduce exposure (photolysis, adsorption, etc.). This adds conservatism to the toxicity estimates.

The uncertainty associated with risk characterization is the estimation of exposure levels. It is not possible to obtain field estimates of exposure to pre-approved drugs, so estimates are made using basic algorithms and validated mathematical models. However, to reduce the uncertainty associated with the estimated soil and water concentrations, the "worst case" scenario is addressed so that the estimates are very conservative. For example, every herd will not be treated with enrofloxacin. Also, the Risk Quotients were calculated using no observed effect concentrations (NOECs) even for acute studies which are much lower than the median effect concentration (LC_{50} or EC_{50}). This produces a more conservative estimate of risk.

The increased conservatism of estimates and assumptions reduce the uncertainty associated with toxicity evaluation and risk characterization. Therefore, the use of enrofloxacin to treat cattle is not expected to result in any adverse environmental impact.

9.0 Use of Resources and Energy

Production and formulation of enrofloxacin will occur at facilities already producing and formulating enrofloxacin for use in cats and dogs. The operations for the production and

formulation of the BAYTRIL® 100 Injectable Solution are not expected to use unusual amounts of energy and resources.

10.0 Mitigation Measures

The proposed action is not expected to have any adverse impact on the environment. Adherence to state and federal guidelines, process engineering controls, personal safety equipment, and standard industrial hygiene practices will be effective in minimizing exposure to enrofloxacin in production and formulation facilities.

11.0 Alternatives to the Proposed Action

The only alternative to the proposed action is one of no action. This alternative is not being considered since resources and facilities are being used efficiently to produce a product with no expected adverse effects on or the environment. A no-action alternative would result in the deprivation of a beneficial drug to the cattle industry.

12.0 List of Preparers

The following personnel of Bayer Corporation, Agriculture Division, Animal Health were responsible for the preparation of this environmental assessment:

Date: 12-20-96

Gregory G. Gagliano, M.S.
Scientific and Regulatory Specialist
Research and Development

Date: 12/20/96

F. T. McNamara, M.S.
Manager, Biochemistry and
Pesticide Registrations
Research and Development

13.0 Certification of Authenticity

The undersigned official certifies that the information presented in this environmental assessment is true, accurate, and complete to the best of his knowledge.

Date: 12/20/96

J. I. H. Phillip
Vice-President,
Research and Development

14.0 References

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APPENDIX 1
Material Safety Data Sheets

Bayer

MATERIAL SAFETY DATA SHEET

BAYER CORPORATION
AGRICULTURE DIVISION
12707 West 63rd
Shawnee Mission, KS 66216-1846

TRANSPORTATION EMERGENCY
CALL CHEMTREC: 800-424-9300
DISTRICT OF COLUMBIA: 202-483-7616

NON-TRANSPORTATION
BAYER EMERGENCY PHONE...: (800) 422-9874
BAYER INFORMATION PHONE.: (913) 631-4800

I. PRODUCT IDENTIFICATION:

PRODUCT NAME.....: Enrofloxacin
PRODUCT CODE.....: 68-0490-00
CHEMICAL FAMILY.....: Antimicrobial - Quinoline Compound
CHEMICAL NAME.....: 1-cyclopropyl-7-(4-ethyl-1-piperazinyl) -6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid
SYNONYMS.....: Baytril, Bay VP 2674
FORMULA.....: C19 H22 F N3 O3

II. HAZARDOUS INGREDIENTS:

INGREDIENT NAME /CAS NUMBER	EXPOSURE LIMITS	CONCENTRATION (%)
Enrofloxacin 93106-60-6	OSHA : Not Established ACGIH: Not Established	% Not Noted

III. PHYSICAL PROPERTIES:

PHYSICAL FORM.....: Solid
APPEARANCE.....: Crystalline
COLOR.....: Pale yellow to yellow
ODOR.....: Not established
ODOR THRESHOLD.....: Not established
MOLECULAR WEIGHT.....: 359.4
pH .....: 6.5 - 7.5 (1 g in 100 mL H20)
BOILING POINT.....: Not applicable
MELTING/FREEZING POINT....: 222-226 C / Not applicable
SOLUBILITY IN WATER .....: < 0.01 g/100 mL @ 20 C
SPECIFIC GRAVITY .....: Not applicable
BULK DENSITY.....: Not established
VAPOR PRESSURE .....: Not applicable

Continued on next page

IV. FIRE AND EXPLOSION DATA:

FLASH POINT.....: Not established
EXTINGUISHING MEDIA.....: Water; Carbon Dioxide; Foam
SPECIAL FIRE FIGHTING PROCEDURES: May release toxic gases. Fight fire from upwind position. Wear self-contained breathing apparatus and full protective clothing. Contain runoff to prevent entry into sewers or waterways.

V. HUMAN HEALTH DATA:

ROUTE(S) OF ENTRY.....: Inhalation; Skin Contact; Eye Contact

HUMAN EFFECTS AND SYMPTOMS OF OVEREXPOSURE:
ACUTE EFFECTS OF EXPOSURE.....: None known; however, animal studies have shown that it can be irritating to the eyes.
CHRONIC EFFECTS OF EXPOSURE...: None known

CARCINOGENICITY.....: This product is not listed by NTP, IARC or regulated as a carcinogen by OSHA.

MEDICAL CONDITIONS
AGGRAVATED BY EXPOSURE.....: None known

VI. EMERGENCY AND FIRST AID PROCEDURES:

FIRST AID FOR EYES.....: Hold eyelids open and flush with copious amounts of water for 15 minutes. Call a physician if irritation develops or persists after flushing.
FIRST AID FOR SKIN.....: In case of skin contact, remove contaminated clothing and wash affected areas with plenty of soap and water. Get medical attention if irritation develops or persists. If signs of intoxication (poisoning) occur, get medical attention immediately.
FIRST AID FOR INHALATION: Remove to fresh air or uncontaminated area. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention as soon as possible.
FIRST AID FOR INGESTION.: If ingestion is suspected, call a physician or poison control center. Drink one or two glasses of water and induce vomiting by touching back of throat with finger, or, if available, by administering syrup of ipecac. If syrup of ipecac is available, administer 1 tablespoonful (15 mL) of syrup of ipecac followed by 1 to 2 glasses of water. If vomiting does not occur within 20 minutes, repeat the dose once. Do not induce vomiting or give anything by mouth to an unconscious person.

Continued on next page

VI. FIRST AID PROCEDURES (Continued)

NOTE TO PHYSICIAN.....: Treat victim symptomatically. In case of poisoning, it is also requested that Miles Inc., Agriculture Division, Shawnee, KS, be notified. Telephone: 800-422-9874 (working hours) or 913-268-2700 (non-working hours)

VII. EMPLOYEE PROTECTION RECOMMENDATIONS:

EYE PROTECTION REQUIREMENTS.....: Goggles
SKIN PROTECTION REQUIREMENTS.....: Avoid skin contact. Wear long sleeves and trousers to prevent dermal exposure.
HAND PROTECTION REQUIREMENTS.....: Chemical-resistant gloves
RESPIRATOR REQUIREMENTS.....: When necessary under the conditions of use, wear a NIOSH-approved dust/mist respirator.
VENTILATION REQUIREMENTS.....: Control exposures through the use of general and local exhaust ventilation.
ADDITIONAL PROTECTIVE MEASURES.....: Clean water should be available for washing in case of eye or skin contamination. Educate and train employees in safe use of the product. Follow all label instructions. Launder clothing after use. Wash thoroughly after handling.

VIII. REACTIVITY DATA:

STABILITY.....: This is a stable material.
HAZARDOUS POLYMERIZATION...: Will not occur.
INCOMPATIBILITIES.....: Not established
INSTABILITY CONDITIONS.....: Not established
DECOMPOSITION TEMPERATURE..: Not established
DECOMPOSITION PRODUCTS.....: May release toxic gases if heated to decomposition

IX. SPILL AND LEAK PROCEDURES:

SPILL OR LEAK PROCEDURES....: Isolate area and keep unauthorized people away. Do not walk through spilled material. Avoid breathing dusts and skin contact. Avoid generating dust (a fine water spray mist, plastic film cover, or floor sweeping compound may be used if necessary). Use recommended protective equipment while carefully sweeping up spilled material. Place in covered container for reuse or disposal. Scrub contaminated area with soap and water. Rinse with water. Use dry absorbent material such as clay granules to absorb and collect wash solution for proper disposal. Contaminated soil may have to be removed and disposed. Do not allow material to enter streams, sewers, or other waterways.

Continued on next page

IX. SPILL AND LEAK PROCEDURES (Continued)

WASTE DISPOSAL METHOD.....: Follow container label instructions for disposal of wastes generated during use in compliance with the product label. In other situations, bury in an EPA-approved landfill or burn in an EPA-approved incinerator. Do not reuse container.

X. SPECIAL PRECAUTIONS & STORAGE DATA:

STORAGE TEMPERATURE(MIN/MAX): Not established
SHELF LIFE.....: Not established
SPECIAL SENSITIVITY.....: Protect from light
HANDLING/STORAGE PRECAUTIONS: Store in a cool, dry place. Do not store near materials intended for the use or consumption by humans.

XI. SHIPPING INFORMATION:

TECHNICAL SHIPPING NAME.....: Not Applicable
FREIGHT CLASS BULK.....: Not Applicable
FREIGHT CLASS PACKAGE.....: Drugs and Medicines*
PRODUCT LABEL.....: Not Noted

DOT (HM-181) (DOMESTIC SURFACE)

PROPER SHIPPING NAME.....: Drugs and Medicines
HAZARD CLASS OR DIVISION .....: Non-Regulated

* released to value as described in NMFC 6000

IMO / IMDG CODE (OCEAN)

PROPER SHIPPING NAME.....: Not Applicable
HAZARD CLASS DIVISION NUMBER...: Non-Regulated

ICAO / IATA (AIR)

PROPER SHIPPING NAME.....: Not Applicable
HAZARD CLASS DIVISION NUMBER...: Non-Regulated

XII. ANIMAL TOXICITY DATA:

TOXICITY DATA FOR: Enrofloxacin Active Ingredient (Technical Drug Substance)
ACUTE TOXICITY

Continued on next page

XII. ANIMAL TOXICITY DATA (Continued)

ORAL LD50.....: Rat: >5000 mg/kg
DERMAL LD50.....: Rabbit: >2000 mg/kg
INHALATION LC50....: Rat: >3547 mg/m3
EYE EFFECTS.....: Rabbit: Irritant, reversible in 7 days
SKIN EFFECTS.....: Rabbit: Not a primary irritant
SENSITIZATION.....: Guinea pig: Not a sensitizer
OTHER ACUTE EFFECTS: None
SUBCHRONIC TOXICITY...: Dog: NOEL: 3 mg/kg
CHRONIC TOXICITY.....: Rat: NOEL: 100 ppm
CARCINOGENICITY.....: Non-carcinogen
MUTAGENICITY.....: Suspect
DEVELOPMENTAL TOXICITY: Rabbit: NOEL: 25 mg/kg
REPRODUCTION.....: Rat: NOEL: 10 mg/kg
NEUROTOXICITY .....: No data available

XIII. FEDERAL REGULATORY INFORMATION:

OSHA STATUS.....: This product is hazardous under the criteria of the Federal OSHA Hazard Communication Standard 29 CFR 1910.1200.
TSCA STATUS.....: This product is exempt from TSCA Regulation under Section 3 (2)(B)(vi) when used for pharmaceutical application.
CERCLA REPORTABLE QUANTITY..: None
SARA TITLE III:
SECTION 302 EXTREMELY HAZARDOUS SUBSTANCES..: None
SECTION 311/312 HAZARD CATEGORIES.....: Immediate Health Hazard
SECTION 313 TOXIC CHEMICALS.....: None
RCRA STATUS.....: If discarded in its purchased form, this product would not be a hazardous waste either by listing or by characteristic. However, under RCRA, it is the responsibility of the product user to determine at the time of disposal, whether a material containing the product or derived from the product should be classified as a hazardous waste. (40 CFR 261.20-24)

XIV. OTHER REGULATORY INFORMATION:

NFPA 704M RATINGS: Health Flammability Reactivity Other

Continued on next page

XIV. OTHER REGULATORY INFORMATION (Continued)

1	0	0	0
0=Insignificant	1=Slight	2=Moderate	3=High 4=Extreme

Bayer's method of hazard communication is comprised of Product Labels and Material Safety Data Sheets. NFPA ratings are provided by Bayer Corproation as a customer service.

XV. APPROVALS:

REASON FOR ISSUE.....: Revise to new format; change company name; make general revisions
PREPARED BY.....: V. C. Standart
APPROVED BY.....: D. C. Eberhart
TITLE.....: Product Safety Manager
APPROVAL DATE.....: 10/12/94
SUPERSEDES DATE.....: 12/09/88
MSDS NUMBER.....: 20184

This information is furnished without warranty, expressed or implied, except that it is accurate to the best knowledge of Bayer Corporation. The data on this sheet relates only to the specific material designated herein. Bayer Corporation assumes no legal responsibility for use or reliance upon these data.

L-Arginine
Common Name
Cat # 04250
Unit package size 200 mg

MATERIAL SAFETY DATA SHEET
UNITED STATES PHARMACOPEIAL CONVENTION, INC.

address:
12601 Twinbrook Parkway
Rockville, MD 20852 USA

emergency and information
telephone calls:
(301) 881-0666

Jerome A. Halperin
Responsible Party

07-03-89
date prepared

WARNING STATEMENT

WARNING! REFERENCE STANDARD; NOT FOR HUMAN CONSUMPTION; AVOID INGESTION, INHALATION, SKIN CONTACT. FOR CHEMICAL TEST AND ASSAY USE ONLY.

SECTION 1 - IDENTITY

COMMON NAME	L-Arginine
SYNONYMS	n/a
CAS NUMBER	74-79-3 (L-arginine); 1119-34-2 (hydrochloride)
RTECS NUMBER	CF1934200 (L-arginine); CF1995500 (hydrochloride)
CHEMICAL NAME	L-Arginine
CHEMICAL FAMILY	Amino acid
THERAPEUTIC CATEGORY	Ammonium detoxicant; diagnostic aid
FORMULA	$C_6H_{14}N_4O_2$

SECTION 2 - HAZARDOUS INGREDIENTS

	NAME	PERCENT	THRESHOLD LIMIT VALUE (UNITS)
PRINCIPAL HAZARDOUS COMPONENT(S)/[Chemical & Common name(s)]	L-Arginine	Pure Material	Not Established

SECTION 3 - PHYSICAL AND CHEMICAL CHARACTERISTICS (Fire & Explosion Data)

BOILING POINT	n/a
SPECIFIC GRAVITY (H_2O = 1)	1.30
VAPOR PRESSURE (mm Hg)	n/a
PERCENT VOLATILE BY VOLUME (%)	n/a
VAPOR DENSITY (AIR = 1)	6.0
EVAPORATION RATE	n/a
SOLUBILITY IN WATER	Freely soluble
REACTIVITY IN WATER	n/a
APPEARANCE AND ODOR	White crystals or crystalline powder, faint odor

n = not applicable

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L-Arginine
Common Name
Cat # 04250

MATERIAL SAFETY DATA SHEET
UNITED STATES PHARMACOPEIAL CONVENTION, INC.

FLASH POINT	n/a
FLAMMABLE LIMITS IN AIR % BY VOLUME	LOWER Non-flammable UPPER Non-flammable
EXTINGUISHER MEDIA	Water spray, dry chemical, carbon dioxide or foam as appropriate for surrounding fire and materials.
AUTO-IGNITION TEMPERATURE	n/a
SPECIAL FIRE FIGHTING PROCEDURES	As with all fires, evacuate personnel to safe area. Firefighters should use self-contained breathing equipment and protective clothing.
UNUSUAL FIRE AND EXPLOSION HAZARDS	This material is assumed to be combustible. As with all dry powders it is advisable to ground mechanical equipment in contact with dry material to dissipate the potential buildup of static electricity. When heated to decomposition material emits toxic fumes. Emits toxic fumes under fire conditions.

SECTION 4 - PHYSICAL HAZARDS

'ABILITY	( ) Unstable (X) Stable
CONDITIONS TO AVOID	Material is stable from a safety point of view. Absorbs CO_2 from the air.
INCOMPATIBILITY (MATERIALS TO AVOID)	Strong oxidizers.
HAZARDOUS DECOMPOSITION PRODUCTS	When heated to decomposition material emits toxic fumes. Emits toxic fumes under fire conditions.
HAZARDOUS POLYMERIZATION	( ) May Occur (X) Will Not Occur

SECTION 5 - HEALTH HAZARDS

THRESHOLD LIMIT VALUE	None established
SIGNS AND SYMPTOMS OF OVEREXPOSURE	[L-Arginine CAS RN: 74-79-3 Mutation Data [RRRRRRTECS]] [Arginine Hydrochloride CAS RN: 1119-34-2 LD_{50}: 12 mg/Kg oral-rat; LD_{50}: 3793 mg/Kg mg/Kg intraperitoneal-rat; Reproductive Effect [RTECS]] Possible allergic reaction to dust if inhaled, ingested or in contact with skin.

/a = not applicable

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ACUTE	Eye, skin and/or respiratory tract irritation
CHRONIC	Possible hypersensitization
PRECAUTIONS TO CONSIDER	Persons developing hypersensitivity (anaphylactic) reactions must receive immediate medical attention.Material may be irritating to mucous membranes and respiratory tract. As a general rule, when handling USP Reference Standards avoid all contact and inhalation of dust, fumes, mist, and/or vapors associated with the material. Keep container tightly closed and use with adequate ventilation; wash thoroughly after handling. Individuals working with chemicals should consider all chemicals to be potentially hazardous even if their individual hazards may be uncharacterized or unknown.
MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE	Hypersensitivity to material
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN	NATIONAL TOXICOLOGY PROGRAM ( ) Yes (X) No I.A.R.C. Monographs ( ) Yes (X) No OSHA ( ) Yes (X) No OTHER n/a
	ACGIH TLV: n/a — OTHER EXPOSURE LIMIT(S) USED: n/a
OSHA PERMISSIBLE EXPOSURE LIMIT:	Not established
OTHER EXPOSURE LIMIT USED	Not established
EMERGENCY AND FIRST AID PROCEDURES	Remove from exposure. Remove contaminated clothing. Persons developing serious hypersensitivity reactions must receive immediate medical attention. Upon eye or skin contact, flush affected area with copious quantities of water. Obtain medical attention. If not breathing give artificial respiration. If breathing is difficult give oxygen.
1. INHALATION	May cause irritation of respiratory tract. Remove to fresh air.
2. EYES	May cause irritation. Flush with copious quantities of water.
3. SKIN	May cause irritation. Flush with copious quantities of water.
4. INGESTION	May cause irritation. Flush out mouth with water.

'a = not applicable

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L-Arginine
Common Name
Cat # 04250

MATERIAL SAFETY DATA SHEET
UNITED STATES PHARMACOPEIAL CONVENTION, INC.

SECTION 6 - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (SPECIFY TYPE)	Approved dust mask
VENTILATION	Adequate
LOCAL EXHAUST	Recommended
MECHANICAL (GENERAL)	Recommended
OTHER	n/a
PROTECTIVE GLOVES	Rubber
EYE PROTECTION	Safety goggles
OTHER PROTECTIVE CLOTHING OR EQUIPMENT	Appropriate laboratory apparel, protect exposed skin

SECTION 7 - SPECIAL PRECAUTIONS AND SPILL/LEAK PROCEDURES

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE	Store in tight container as defined in the United States Pharmacopeia. This material should be handled and stored per label and other instructions to ensure product integrity.
THER PRECAUTIONS	Avoid contact with eyes, skin or clothing. Avoid breathing dust or mist. Use with adequate dust control. Wash thoroughly after handling. Wear fresh clothing daily. Wash contaminated clothing before reuse. Do not permit eating, drinking or smoking near material.
STEPS TO BE TAKEN IN CASE MATERIAL IS SPILLED OR RELEASED	Wear approved respirator and chemically compatible gloves. Vacuum or sweep up spillage. Avoid dust. Place spillage in appropriate container for waste disposal. Wash contaminated clothing before reuse. Ventilate area and wash spill site.
WASTE DISPOSAL METHODS	Dispose of waste in accordance with all applicable Federal, State and local laws.

'a = not applicable

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L-Arginine
Common Name
Cat # 04250

MATERIAL SAFETY DATA SHEET
UNITED STATES PHARMACOPEIAL CONVENTION, INC.

NOTICE: The information contained herein is applicable solely to the chemical substance when used as a USP Reference Standard and does not relate to any other use of the substance described. Its use is intended by persons having technical skill and at their own discretion and risk. The information has been developed by USP staff from sources considered reliable but has not been independently verified by the USP. Therefore, the USP Convention cannot guarantee the accuracy of the information in these sources nor should the statements contained herein be considered an official expression. NO REPRESENTATION OR WARRANTY, EXPRESS OR IMPLIED, INCLUDING THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE is made with respect to the information contained herein.

ATTENTION:

This Product is Sold as a Reference Standard for Use In Chemical Analysis Not For Human Consumption.

= not applicable

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chemists helping chemists in research & industry
aldrich chemical co., inc.
P.O. Box 355, Milwaukee, Wisconsin 53201 USA

Telephone: (414) 273-3850
TWX: (910) 262-3052 Aldrichem Mil
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

ATTN: SAFETY DIRECTOR
LARRY THOMAS
MILES INC
ANIMAL HEALTH DIVISION
BOX 4913
KANSAS CITY MO 64120-0013

DATE: 04/13/95
CUST#: 130680
PO#: G54P10117

M A T E R I A L S A F E T Y D A T A S H E E T

CTION 1. - - - - - - - - - - CHEMICAL IDENTIFICATION- - - - - - - - - -

PRODUCT #: 01620-8 NAME: BENZYL ALCOHOL, 99+%

CTION 2. - - - - - - COMPOSITION/INFORMATION ON INGREDIENTS - - - - - -

CAS #:100-51-6
MF: C7H8O

NONYMS
BENZAL ALCOHOL * BENZENECARBINOL * BENZENEMETHANOL * BENZOYL ALCOHOL * HYDROXYTOLUENE * ALPHA-HYDROXYTOLUENE * METHANOL, PHENYL- * NCI-C06111 * PHENOLCARBINOL * PHENYLCARBINOL * PHENYLMETHANOL * PHENYLMETHYL ALCOHOL * ALPHA-TOLUENOL *

CTION 3. - - - - - - - - - - - HAZARDS IDENTIFICATION - - - - - - - - -

BEL PRECAUTIONARY STATEMENTS
HARMFUL
HARMFUL IF SWALLOWED.
IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.
RISK OF SERIOUS DAMAGE TO EYES.
TARGET ORGAN(S):
CENTRAL NERVOUS SYSTEM
IN CASE OF CONTACT WITH EYES, RINSE IMMEDIATELY WITH PLENTY OF WATER AND SEEK MEDICAL ADVICE.
WEAR SUITABLE PROTECTIVE CLOTHING.
HYGROSCOPIC

CTION 4. - - - - - - - - - - - FIRST-AID MEASURES- - - - - - - - - - -

IN CASE OF CONTACT, IMMEDIATELY FLUSH EYES WITH COPIOUS AMOUNTS OF WATER FOR AT LEAST 15 MINUTES.
IN CASE OF CONTACT, IMMEDIATELY WASH SKIN WITH SOAP AND COPIOUS AMOUNTS OF WATER.
IF INHALED, REMOVE TO FRESH AIR. IF NOT BREATHING GIVE ARTIFICIAL RESPIRATION. IF BREATHING IS DIFFICULT, GIVE OXYGEN.
IF SWALLOWED, WASH OUT MOUTH WITH WATER PROVIDED PERSON IS CONSCIOUS. CALL A PHYSICIAN.
WASH CONTAMINATED CLOTHING BEFORE REUSE.

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CUST#: 130680
PO#: G54P10117

PRODUCT #: 3016208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

ECTION 5. - - - - - - - - - - FIRE FIGHTING MEASURES - - - - - - - - - -

XTINGUISHING MEDIA
WATER SPRAY.
CARBON DIOXIDE, DRY CHEMICAL POWDER OR APPROPRIATE FOAM.
PECIAL FIREFIGHTING PROCEDURES
WEAR SELF-CONTAINED BREATHING APPARATUS AND PROTECTIVE CLOTHING TO PREVENT CONTACT WITH SKIN AND EYES.
NUSUAL FIRE AND EXPLOSIONS HAZARDS
EMITS TOXIC FUMES UNDER FIRE CONDITIONS.

ECTION 6. - - - - - - - - - ACCIDENTAL RELEASE MEASURES- - - - - - - - -

WEAR SELF-CONTAINED BREATHING APPARATUS, RUBBER BOOTS AND HEAVY RUBBER GLOVES.
COVER WITH DRY LIME OR SODA ASH, PICK UP, KEEP IN A CLOSED CONTAINER TO HOLD FOR WASTE DISPOSAL.
VENTILATE AREA AND WASH SPILL SITE AFTER MATERIAL PICKUP IS COMPLETE.

ECTION 7. - - - - - - - - - - HANDLING AND STORAGE- - - - - - - - - - -
REFER TO SECTION 8.

ECTION 8. - - - - - - EXPOSURE CONTROLS/PERSONAL PROTECTION- - - - - -

CHEMICAL SAFETY GOGGLES.
RUBBER GLOVES.
NIOSH/MSHA-APPROVED RESPIRATOR.
SAFETY SHOWER AND EYE BATH.
MECHANICAL EXHAUST REQUIRED.
DO NOT BREATHE VAPOR.
AVOID CONTACT WITH EYES, SKIN AND CLOTHING.
WASH THOROUGHLY AFTER HANDLING.
SEVERE EYE IRRITANT.
HARMFUL LIQUID.
KEEP TIGHTLY CLOSED.
STORE IN A COOL DRY PLACE.

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CUST#: 130680
PO#: G54P10117

PRODUCT #: 3016208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

ECTION 9. - - - - - - - PHYSICAL AND CHEMICAL PROPERTIES - - - - - - -

PEARANCE AND ODOR
COLORLESS LIQUID
BOILING POINT: 205 C
MELTING POINT: -15 C
FLASHPOINT 201 F
93C
AUTOIGNITION TEMPERATURE: 817 F 435C
VAPOR PRESSURE: 3.75MM 77 C 13.3MM 100 C
VAPOR DENSITY: 3.7
SPECIFIC GRAVITY: 1.045

CTION 10. - - - - - - - - - -STABILITY AND REACTIVITY - - - - - - - - -

CO. ATIBILITIES
STRONG OXIDIZING AGENTS
A MIXTURE OF BENZYL ALCOHOL AND 58% SULFURIC ACID DECOMPOSED VIOLENTLY WHEN HEATED TO 180 C. BENZYL ALCOHOL CONTAINING 1.4% HYDROGEN BROMIDE AND 1.1% OF AN IRON(2) SALT POLYMERIZED EXOTHERMALLY WHEN HEATED ABOVE 100 C.
MAY DECOMPOSE ON EXPOSURE TO MOIST AIR OR WATER.
ZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS
TOXIC FUMES OF:
CARBON MONOXIDE, CARBON DIOXIDE

CTION 11. - - - - - - - - - - TOXICOLOGICAL INFORMATION - - - - - - - -

UTE EFFECTS
HARMFUL IF SWALLOWED.
MAY BE HARMFUL IF INHALED.
MAY BE HARMFUL IF ABSORBED THROUGH THE SKIN.
CAUSES SEVERE EYE IRRITATION.
CAUSES SKIN IRRITATION.
MATERIAL IS IRRITATING TO MUCOUS MEMBRANES AND UPPER RESPIRATORY TRACT.
CAN CAUSE CNS DEPRESSION.

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CUST#: 130680
PO#: G54P10117

PRODUCT #: B016208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

TARGET ORGAN(S):
CENTRAL NERVOUS SYSTEM
'ECS NO: DN3150000
BENZYL ALCOHOL
:RITATION DATA

SKN-MAN 16 MG/48H MLD	CTOIDG 94(8),41,79
SKN-RBT 10 MG/24H OPEN MLD	AMIHBC 4,119,51
SKN-RBT 100 MG/24H MOD	CTOIDG 94(8),41,79
EYE-RBT 750 UG OPEN SEV	AMIHBC 4,119,51
SKN-PIG 100% MOD	FCTXAV 11,1011,73

)XICITY DATA

ORL-RAT LD50:1230 MG/KG	FCTXAV 2,327,64
IPR-RAT LD50:400 MG/KG	NPIRI* 1,6,74
IVN-RAT LD50:53 MG/KG	TXAPA9 18,60,71
IAT-RAT LD50:441 MG/KG	TXAPA9 18,60,71
ORL-MUS LD50:1360 MG/KG	GISAAA 50(7),81,85
IPR-MUS LD50:650 MG/KG	JPMSAE 75,702,86
IN-MUS LD50:324 MG/KG	AIPTAK 135,330,62
ORL-RBT LD50:1040 MG/KG	JPETAB 84,358,45
SKN-RBT LD50:2 GM/KG	NPIRI* 1,6,74
ORL-GPG LD50:2500 MG/KG	GISAAA 50(7),81,85
ORL-BWD LD50:100 MG/KG	TXAPA9 21,315,72

RGET ORGAN DATA
SENSE ORGANS AND SPECIAL SENSES (MIOSIS)
BEHAVIORAL (ALTERED SLEEP TIME)
BEHAVIORAL (SOMNOLENCE)
BEHAVIORAL (TREMOR)
BEHAVIORAL (EXCITEMENT)
BEHAVIORAL (ATAXIA)
BEHAVIORAL (COMA)
LUNGS, THORAX OR RESPIRATION (CHRONIC PULMONARY EDEMA OR CONGESTION)
LUNGS, THORAX OR RESPIRATION (DYSPNAE)
LUNGS, THORAX OR RESPIRATION (OTHER CHANGES)
GASTROINTESTINAL (HYPERMOTILITY, DIARRHEA)
KIDNEY, URETER, BLADDER (OTHER CHANGES)
ONLY SELECTED REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) DATA IS PRESENTED HERE. SEE ACTUAL ENTRY IN RTECS FOR COMPLETE INFORMATION.

CTION 12. - - - - - - - - - - ECOLOGICAL INFORMATION - - - - - - - - - - -
DATA NOT YET AVAILABLE.

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PO#: G54P10117

PRODUCT #: B016208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

ECTION 13. - - - - - - - - - - DISPOSAL CONSIDERATIONS - - - - - - - - - -

DISSOLVE OR MIX THE MATERIAL WITH A COMBUSTIBLE SOLVENT AND BURN IN A CHEMICAL INCINERATOR EQUIPPED WITH AN AFTERBURNER AND SCRUBBER. OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.

ECTION 14. - - - - - - - - - - TRANSPORT INFORMATION - - - - - - - - - -

CONTACT ALDRICH CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION.

ECTION 15. - - - - - - - - - - REGULATORY INFORMATION - - - - - - - - - -

EVIEWS, STANDARDS, AND REGULATIONS
PA FIFRA 1988 PESTICIDE SUBJECT TO REGISTRATION OR RE-REGISTRATION
FEREAC 54,7740,89
OEL-RUSSIA:STEL 5 MG/M3;SKIN JAN93
OEL IN BULGARIA, COLOMBIA, JORDAN, KOREA CHECK ACGIH TLV
OEL IN NEW ZEALAND, SINGAPORE, VIETNAM CHECK ACGIH TLV
NOHS 1974: HZD 11360; NIS 85; TNF 7284; NOS 68; TNE 138757
NOES 1983: HZD 11360; NIS 102; TNF 14657; NOS 102; TNE 334686; TFE 195361
EPA GENETOX PROGRAM 1988, NEGATIVE: E COLI POLA WITHOUT S9
EPA TSCA CHEMICAL INVENTORY, JUNE 1993
EPA TSCA TEST SUBMISSION (TSCATS) DATA BASE, JULY 1994
NTP CARCINOGENESIS STUDIES (GAVAGE):NO EVIDENCE:RAT,MOUSE
NTPTR* NTP-TR-343,89

ECTION 16. - - - - - - - - - - OTHER INFORMATION- - - - - - - - - - -

THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT DOES NOT PURPORT TO BE ALL INCLUSIVE AND SHALL BE USED ONLY AS A GUIDE. ALDRICH SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR FROM CONTACT WITH THE ABOVE PRODUCT. SEE REVERSE SIDE OF INVOICE OR PACKING SLIP FOR ADDITIONAL TERMS AND CONDITIONS OF SALE.

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MATERIAL SAFETY DATA SHEET

CUST#: 130680
PO#: G54P10117

PRODUCT #: 3016208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

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1-BUTANOL
1-BUTANOL
1-BUTANOL

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SUBSTANCE IDENTIFICATION

SUBSTANCE: **1-BUTANOL** CAS-NUMBER 71-36-3

TRADE NAMES/SYNONYMS:
BUTYL ALCOHOL; N-BUTYL ALCOHOL; N BUTANOL; BUTANOL; 1-BUTYL ALCOHOL; BUTYL HYDROXIDE; CCS 203; HEMOSTYP; METHYLOLPROPANE; PROPYL CARBINOL; RCRA U031; STCC 4909117; UN 1120; NA 1120; A384; A383; A383SK; A400; A399; A399S; A398; C4H10O; ACC15400

CHEMICAL FAMILY:
Hydroxyl, aliphatic

MOLECULAR FORMULA: C-H3 C-H2-C-H2-C-H2-O-H

MOLECULAR WEIGHT: 74.12

CERCLA RATINGS (SCALE 0-3): HEALTH=3 FIRE=3 REACTIVITY=0 PERSISTENCE=0
NFPA RATINGS (SCALE 0-4): HEALTH=1 FIRE=3 REACTIVITY=0

COMPONENTS AND CONTAMINANTS

COMPONENT: 1-BUTANOL PERCENT: 100
CAS# 71-36-3

OTHER CONTAMINANTS: NONE

EXPOSURE LIMITS:
BUTYL ALCOHOL:
50 ppm (150 mg/m3) OSHA ceiling (skin)
50 ppm (150 mg/m3) ACGIH ceiling (skin)
50 ppm (150 mg/m3) NIOSH recommended ceiling (skin)
100 ppm (300 mg/m3) DFG MAK TWA;
200 ppm (600 mg/m3) DFG MAK 5 minute peak, momentary value, 8 times/shift

Measurement method: Charcoal tube; 2-propanol/carbon disulfide; gas chromatography with flame ionization detection; (NIOSH Vol. III # 1401, Alcohols II).

5000 pounds CERCLA Section 103 Reportable Quantity
Subject to SARA Section 313 Annual Toxic Chemical Release Reporting

OSHA revoked the final rule limits of January 19, 1989 in response to the 11th Circuit Court of Appeals decision (AFL-CIO v. OSHA) effective June 30, 1993. See 29 CFR 1910.1000 (58 FR 35338)

PHYSICAL DATA

DESCRIPTION: A clear, mobile, colorless liquid, with a strong, pungent characteristic odor similar to fusel oil. BOILING POINT: 243 F (117 C)

MELTING POINT: -128 F (-89 C) SPECIFIC GRAVITY: 0.8098 VOLATILITY: 100%

VAPOR PRESSURE: 6.5 mmHg @ 25 C EVAPORATION RATE: (butyl acetate=1) 0.5

SOLUBILITY IN WATER: 7.7% ODOR THRESHOLD: 10 ppm VAPOR DENSITY: 2.55

SOLVENT SOLUBILITY: Soluble in ethanol, ether, acetone, benzene, most organic solvents

VISCOSITY: 2.940 cP @ 70 F

FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARD:

Dangerous fire hazard when exposed to heat or flame.

Vapors are heavier than air and may travel a considerable distance to a source of ignition and flash back.

Vapor-air mixtures are explosive above flash point

FLASH POINT: 99 F (37 C) (CC) UPPER EXPLOSIVE LIMIT: 11.2%

LOWER EXPLOSIVE LIMIT: 1.4% AUTOIGNITION TEMP.: 650 F (343 C)

FLAMMABILITY CLASS(OSHA): IC

FIREFIGHTING MEDIA:
Dry chemical, carbon dioxide, water spray or alcohol-resistant foam (1993 Emergency Response Guidebook, RSPA P 5800.6).

For larger fires, use water spray, fog or alcohol-resistant foam (1993 Emergency Response Guidebook, RSPA P 5800.6).

Alcohol foam
(NFPA 325M, Fire Hazard Properties of Flammable Liquids, Gases, and Volatile Solids, 1991)

FIREFIGHTING
Move container from fire area if you can do it without risk. Apply cooling water to sides of containers that are exposed to flames until well after fire is out. Stay away from ends of tanks. For massive fire in cargo area, use unmanned hose holder or monitor nozzles; if this is impossible, withdraw from area and let fire burn. Withdraw immediately in case of rising sound from venting safety device or any discoloration of tank due to fire. Isolate for 1/2 mile in all directions if tank, rail car or tank truck is involved in fire (1993 Emergency Response Guidebook, RSPA P 5800.6, Guide Page 26)

Extinguish only if flow can be stopped. Use water in flooding amounts as a fog, solid streams may not be effective. Cool containers with flooding amounts of water, apply from as far a distance as possible. Avoid breathing toxic vapors, keep upwind.

Water may be ineffective (NFPA 325M, Fire Hazard Properties of Flammable Liquids, Gases, and Volatile Solids, 1991)

TRANSPORTATION DATA

U.S. DEPARTMENT OF TRANSPORTATION SHIPPING NAME-ID NUMBER, 49 CFR 172.101:
Butanols-UN 1120

U.S. DEPARTMENT OF TRANSPORTATION HAZARD CLASS OR DIVISION, 49 CFR 172.101:
3 - Flammable liquid

U.S. DEPARTMENT OF TRANSPORTATION PACKING GROUP, 49 CFR 172.101:
PG II

U.S. DEPARTMENT OF TRANSPORTATION LABELING REQUIREMENTS, 49 CFR 172.101 AND SUBPART E:
Flammable liquid

U.S. DEPARTMENT OF TRANSPORTATION PACKAGING AUTHORIZATIONS:
EXCEPTIONS: 49 CFR 173.150
NON-BULK PACKAGING: 49 CFR 173.202
BULK PACKAGING: 49 CFR 173.242

U.S. DEPARTMENT OF TRANSPORTATION QUANTITY LIMITATIONS 49 CFR 172.101:
PASSENGER AIRCRAFT OR RAILCAR: 5 L
CARGO AIRCRAFT ONLY: 60 L

TOXICITY

N-BUTYL ALCOHOL (1-BUTANOL):
IRRITATION DATA: 20 mg/24 hours skin-rabbit moderate; 405 mg/24 hours skin-rabbit moderate; 50 ppm eye-human; 2 mg eye-rabbit severe; 2 mg/24 hours eye-rabbit severe.
TOXICITY DATA: 25 ppm inhalation-human TCLo; 8000 ppm/4 hours inhalation-rat LC50; 28400 mg/m3 inhalation-mammal LC50; 3400 mg/kg skin-rabbit LD50; 790 mg/kg oral-rat LD50; 3484 mg/kg oral-rabbit LD50; 1760 mg/kg oral-dog LDLo; 2 gm/kg subcutaneous-dog LDLo; 2200 mg/kg subcutaneous-mouse LD50; 310 mg/kg intravenous-rat LD50; 377 mg/kg intravenous-mouse LD50; 243 mg/kg intravenous-cat LDLo; 1122 mg/kg intraperitoneal-rat LD50; 603 mg/kg intraperitoneal-mouse LD50; 3500 mg/kg unreported-rabbit LDLo; mutagenic data (RTECS); reproductive effects data (RTECS).
CARCINOGEN STATUS: None.
LOCAL EFFECTS: Irritant- inhalation, skin, eye.
ACUTE TOXICITY LEVEL: Moderately toxic by ingestion; slightly toxic by inhalation and dermal absorption.
TARGET EFFECTS: Central nervous system depressant. Poisoning may also affect the liver and kidneys.
AT INCREASED RISK FROM EXPOSURE: Persons with chronic respiratory, skin or eye diseases.

ADDITIONAL DATA: Alcohol may enhance the toxic effects.

HEALTH EFFECTS AND FIRST AID

INHALATION:
N-BUTYL ALCOHOL (1-BUTANOL):
IRRITANT/NARCOTIC. 8000 ppm Immediately Dangerous to Life or Health.
ACUTE EXPOSURE- The substance is quite odorous, which usually provides adequate warning, but olfactory senses readily become fatigued. 25 ppm may cause mild irritation of the respiratory tract. Exposure to 50 ppm may cause headache and vertigo; higher concentrations may cause marked irritation, sore throat, coughing, nausea, shortness of breath, pulmonary injury, and central nervous system depression with headache, dizziness, dullness, and drowsiness. Exposure to 8600 ppm produced giddiness, prostration, narcosis, ataxia, and death in some animals.
CHRONIC EXPOSURE- Inhalation of vapor concentrations as low as 0.03 ppm may affect light-sensitivity of dark-adapted eyes and the electrical activity of the brain. Prolonged inhalation caused auditory nerve and vestibular injury resulting in severe vertigo and hearing loss in workers exposed to N-butyl and isobutyl alcohols. Lung hemorrhage, reduced erythrocyte count, lymphocytosis, albuminuria, early degenerative changes of the liver, and cortical and tubular degeneration in the kidneys were reported in animals following prolonged exposure to 100 ppm. Reproductive effects have been reported in animals.

FIRST AID- Remove from exposure area to fresh air immediately. Perform artificial respiration if necessary. Keep person warm and at rest. Treat symptomatically and supportively. Get medical attention immediately.

SKIN CONTACT:
N-BUTYL ALCOHOL (1-BUTANOL)
IRRITANT.
ACUTE EXPOSURE- Liquid contact with the skin may cause irritation with redness and dryness. The substance may be absorbed through intact skin. Single applications to the skin of rabbits resulted in slight to moderate irritation.
CHRONIC EXPOSURE- Repeated or prolonged contact has been reported to cause drying, cracking, and eczematoid dermatitis of the fingers and hands due to the defatting action of the liquid. Application of 42 to 55 ml/kg/day for 1 to 4 consecutive days to rabbits resulted in 100% mortality.

FIRST AID- Remove contaminated clothing and shoes immediately. Wash with soap or mild detergent and large amounts of water until no evidence of chemical remains (at least 15-20 minutes). Get medical attention immediately.

EYE CONTACT:
N-BUTYL ALCOHOL (1-BUTANOL):
IRRITANT.
ACUTE EXPOSURE- Application of 1 drop of the liquid to rabbit eyes caused moderate temporary injury which was rated 7 on a scale of 10.
CHRONIC EXPOSURE- Repeated or prolonged exposure to vapors may result in conjunctivitis, vacuolar keratitis, characterized by very fine transparent vacuoles resembling tiny bubbles of gas in the epithelial layers of the cornea has been reported in workers exposed to butyl alcohol and other solvents. Exposure to 200 ppm for 10 years caused conjunctival and corneal edema resulting in lacrimation, blurring of vision, corneal inflammation, photophobia, and a burning sensation, with inflammation clearing within a few days with no residual damage.

FIRST AID- Wash eyes immediately with large amounts of water or normal saline, occasionally lifting upper and lower lids, until no evidence of chemical remains (at least 15-20 minutes). Get medical attention immediately.

INGESTION:
N-BUTYL ALCOHOL (1-BUTANOL):
NARCOTIC.
ACUTE EXPOSURE- Ingestion may cause abdominal pain, nausea, vomiting, and diarrhea. Ingestion of high doses caused complete paralysis, constriction of pupils, reduction in corneal, pupillary, and ciliary reflexes, nystagmus, salivation, reduction of body temperature and respiration, deep narcosis, marked hepatic hyperemia, degeneration of liver and kidneys, and death in animals.
CHRONIC EXPOSURE- Prolonged ingestion in drinking water resulted in liver damage in rats.

FIRST AID- If the person is conscious and not convulsing, induce emesis by giving syrup of ipecac (keeping the head below the hips to prevent aspiration), followed by water. Repeat in 20 minutes if not effective initially. In patients with depressed respiration or if emesis is not produced, perform gastric lavage cautiously (Dreisbach, Handbook of Poisoning, 12th Ed.). Treat symptomatically and supportively. Gastric lavage should be performed by qualified medical personnel. Get medical attention immediately.

ANTIDOTE:
No specific antidote. Treat symptomatically and supportively.

REACTIVITY

REACTIVITY
Stable under normal temperatures and pressures.

INCOMPATIBILITIES:
N-BUTYL ALCOHOL (1-BUTANOL):
ALKALI METALS: Liberates flammable hydrogen gas; sharply increases pressure.
ALUMINUM: Liberates flammable hydrogen gas; sharply increases pressure.
T-BUTANOL: Possible explosion hazard.
CHROMIC ANHYDRIDE: Ignition reaction.
OXIDIZERS (STRONG): Fire and explosion hazard.
PLASTICS, RUBBER, COATINGS: May be attacked.
SEE ALSO: Alcohols.

ALCOHOLS
ACETALDEHYDE: Violent condensation reaction.
BARIUM PERCHLORATE: Formation of highly explosive perchloric ester on refluxing.
CHLORINE: Formation of highly explosive alkyl hypochlorites.
DIETHYL ALUMINUM BROMIDE: Spontaneous ignition.
ETHYLENE OXIDE: Possible explosion.
HEXAMETHYLENE DIISOCYANATE: Possible explosion in absence of solvent.
HYDROGEN PEROXIDE + SULFURIC ACID: Possible explosion.
HYPOCHLOROUS ACID: Formation of highly explosive alkyl hypochlorites.
ISOCYANATES: Possible explosion in absence of solvent.
LITHIUM ALUMINUM HYDRIDE: Vigorous reaction.
NITROGEN TETROXIDE: Possible explosion.
PERCHLORIC ACID (HOT): Dangerous interaction.
PERMONOSULFURIC ACID: Possible explosion on contact with primary or secondary alcohols.
TRI-ISO-BUTYL ALUMINUM: Violent reaction.

DECOMPOSITION
Thermal decomposition products may include toxic oxides of carbon.

POLYMERIZATION
Hazardous polymerization has not been reported to occur under normal temperatures and pressures.

STORAGE AND DISPOSAL

Observe all federal, state and local regulations when storing or disposing of this substance.

Storage

Store in accordance with 29 CFR 1910.106.

Bonding and grounding: Substances with low electroconductivity, which may be ignited by electrostatic sparks, should be stored in containers which meet the bonding and grounding guidelines specified in NFPA 77-1983, Recommended Practice on Static Electricity.

Store away from incompatible substances.

Keep container tightly closed. Protect from exposure to air or light.

Disposal

Disposal must be in accordance with standards applicable to generators of hazardous waste, 40CFR 262. EPA Hazardous Waste Number U031.

****

CONDITIONS TO AVOID

Avoid contact with heat, sparks, flames, or other sources of ignition. Vapors may be explosive and poisonous; do not allow unnecessary personnel in area. Do not overheat containers; containers may violently rupture and travel a considerable distance in heat of fire.

****

SPILL AND LEAK PROCEDURES

OCCUPATIONAL SPILL:
Shut off ignition sources; no flares, smoking or flames in hazard area. Stop leak if you can do it without risk. Water spray may reduce vapor; but it may not prevent ignition in closed spaces. For small spills, take up with sand or other noncombustible absorbent material and place into containers for later disposal. For larger spills, dike far ahead of spill for later disposal. Keep unnecessary people away; isolate hazard area and deny entry. Stay upwind; keep out of low areas.

Reportable Quantity (RQ): 5000 pounds
The Superfund Amendments and Reauthorization Act (SARA) Section 304 requires that a release equal to or greater than the reportable quantity for this

DATE: 06/24/95 ACCT: 548625012
INDEX: M51745692 CAT NO: A39920 PO NBR: 54G32603

substance be immediately reported to the local emergency planning committee and the state emergency response commission (40 CFR 355.40). If the release of this substance is reportable under CERCLA Section 103, the National Response Center must be notified immediately at (800) 424-8802 or (202) 426-2675 in the metropolitan Washington, D C. area (40 CFR 302.6).

PROTECTIVE EQUIPMENT

VENTILATION.
Provide local exhaust ventilation to meet published exposure limits.
Ventilation equipment should be explosion-proof if explosive concentrations of dust, vapor or fume are present.

RESPIRATOR:
The following respirators and maximum use concentrations are recommendations by the U.S. Department of Health and Human Services, NIOSH Pocket Guide to Chemical Hazards; NIOSH criteria documents or by the U.S. Department of Labor, 29 CFR 1910 Subpart Z.
The specific respirator selected must be based on contamination levels found in the work place, must not exceed the working limits of the respirator and be jointly approved by the National Institute for Occupational Safety and Health and the Mine Safety and Health Administration (NIOSH-MSHA)

N-BUTYL ALCOHOL

1000 ppm- Any powered air purifying respirator with organic vapor cartridge(s).
Any chemical cartridge respirator with a full facepiece and organic vapor cartridge(s).

1250 ppm- Any supplied-air respirator operated in a continuous flow mode

2500 ppm- Any air-purifying full facepiece respirator (gas mask) with a chin-style or front- or back-mounted organic vapor canister
Any self-contained breathing apparatus with a full facepiece.
Any supplied-air respirator with a full facepiece.

8000 ppm- Any supplied-air respirator with a full facepiece and operated in a pressure-demand or other positive pressure mode.

Escape- Any air-purifying full facepiece respirator (gas mask) with a chin-style or front- or back-mounted organic vapor canister.
Any appropriate escape-type self-contained breathing apparatus

FOR FIREFIGHTING AND OTHER IMMEDIATELY DANGEROUS TO LIFE OR HEALTH CONDITIONS:

Any self-contained breathing apparatus that has a full facepiece and is operated in a pressure-demand or other positive-pressure mode.

Any supplied-air respirator that has a full facepiece and is operated in a pressure-demand or other positive pressure mode in combination with an auxiliary self-contained breathing apparatus operated in pressure-demand or other positive-pressure mode.

CLOTHING:
Employee must wear appropriate protective (impervious) clothing and equipment to prevent repeated or prolonged skin contact with this substance.

GLOVES:
Employee must wear appropriate protective gloves to prevent contact with this substance.

EYE PROTECTION:
Employee must wear splash-proof or dust-resistant safety goggles to prevent eye contact with this substance.

Emergency eye wash: Where there is any possibility that an employee's eyes may be exposed to this substance, the employer should provide an eye wash fountain within the immediate work area for emergency use.

AUTHORIZED - FISHER SCIENTIFIC, INC.
CREATION DATE: 10/01/84 REVISION DATE: 11/18/94

-ADDITIONAL INFORMATION-

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APPENDIX 2
Environmental Compliance Statement

State Office of the Environment Düsseldorf
(Original has the Coat of Arms of the Administrative District of Düsseldorf)

State Office of the Environment, Schanzenstreet 90, 40549 Düsseldorf

Information will be given by
H. Biermann

Tel. No.: 0211/5778-348

To
The Company
Bayer AG
Business Group Animal Health
attn. Dr. Neukam

51368 Monheim

Your Reference, Your communication of	My Reference 2211-Bi-	Düsseldorf, 07. 09. 95

Concerning: Environmental Assessment

Dear Dr. Neukam,

The manufacture of Enrofloxacin at your premises in Wuppertal-Elberfeld has been approved in accordance with the Federal Imission Control Act (Bundes-Immisionsschutzgesetz). This occurred till lately by the approval of the President of the Administrative District of Düsseldorf issued on 28. 04. 1994, Certificate: 55.8851. 1/3859.

Such approval is only given if it has been ensured that humans, animals and plants, the soil, the water and the atmosphere as well as cultural and other properties are protected from harmful environmental effects and from risks, substantial detriment and substantial inconvenience and that measures commensurate with the state of technology have been taken to protect against harmful effects upon environment.

In addition other public regulations, in particular for the protection of nature, the landscape and water must not bar the intended action.

Prior to approval being issued, all the relevant environmental aspects have therefore been checked with the participation or the responsible authorities. This guarantees that at the time of approval all valid regulations are adhered to.

The facilitiy is also subject of the specific supervision by the state environmental authorities. To my knowledge no complaints worthy of note are pending.

Yours faithfully

By authority
(Biermann)

(Original signed)

Staatliches Umweltamt Düsseldorf

Staatliches Umweltamt, Schanzenstr. 90, 40549 Düsseldorf

Firma
Bayer AG
Geschäftsbereich Tiergesundheit
z. Hd. Herrn Dr. Neukam

51368 Monheim

Auskunft erteilt:
H. Biermann

Durchwahl:
0211 / 5778-348

Ihr Zeichen und Tag

Mein Zeichen
2211 - Bi -

Düsseldorf, 12.09.95

Betr.: FDA - Environmental assessment;

Sehr geehrter Herr Dr. Neukam,

die Herstellung von Enrofloxacin in Ihrem Werk in Wuppertal-Elberfeld ist gemäß den Vorschriften des Bundes-Immissionsschutzgesetzes konzessioniert. Dies geschah zuletzt mit dem Genehmigungsbescheid des Regierungspräsidenten Düsseldorf vom 28.04.1994, AZ: 55.8851.4.1/3859.

Eine derartige Genehmigung wird nur erteilt, wenn sichergestellt ist, daß Menschen, Tiere und Pflanzen, der Boden, das Wasser, die Atmosphäre sowie Kulturgüter und sonstige Sachgüter vor schädlichen Umwelteinwirkungen und vor Gefahren, erheblichen Nachteilen und erheblichen Belästigungen geschützt werden und nach dem Stand der Technik Vorsorge gegen schädliche Umwelteinwirkungen getroffen wird.

Außerdem dürfen andere öffentlich-rechtliche Vorschriften - insbesondere auch aus dem Natur-, Landschafts- und Gewässerschutz - dem Vorhaben nicht entgegenstehen.

Vor Erteilung einer solchen Genehmigung sind daher unter Beteiligung der zuständigen Behörden alle relevanten Umweltbelange überprüft worden. Damit ist sichergestellt, daß zum Zeitpunkt der Genehmigung die gültigen Vorschriften eingehalten werden.

Die Anlage unterliegt darüber hinaus der besonderen Überwachung durch die staatlichen Umweltbehörden. Erwähnenswerte Beanstandungen liegen nach hiesiger Erkenntnis nicht vor.

Mit freundlichen Grüßen

Im Auftrag

(Biermann)

Fernsprecher: Schanzenstr. 90, (0211) 5778-0, Telefax 5778-134
nach Dienstschluß in dringenden Fällen: (0201) 714488 (Nachrichten und Bereitschaftszentrale)

(7.)

APPENDIX 3
Study Report Summaries

Bayer Report No.: 73353

Title: Water solubility of BAY Vp 2674

Authors: T. B. Waggoner

Reference: ABC Laboratories, Columbia, Missouri
ABC Study No. 34529

Summary: Following TAD Guideline 3.01, the aqueous solubility of ^{14}C-labeled BAY Vp 2674 was determined at pH 5, 7, and 9. Triplicate test systems were prepared in phosphate buffers at each level, and duplicate analyses of each test system were made on each analysis day. Measurements of radioactivity were made with a liquid scintillation counter. Values reported were the means of all values obtained from three or more analysis days. The solubility at pH 5 was 1100 mg/L, the solubility at pH 7 was 250 mg/L, and the solubility at pH 9 was 600 mg/L.

Bayer Report No.: 73409

Title: Vapor Pressure of BAY Vp 2674

Authors: T. B. Waggoner

Reference: ABC Labs, Columbia, Missouri
ABC Study No. 73409

Summary: Vapor pressure of BAY Vp 2674 was determined according to TAD Guideline 3.03 utilizing a gas-saturation technique and analysis by HPLC and fluorescence detection. Three separate determinations were made. At 25°C, the vapor pressure was less than 1×10^{-7} mm Hg (torr).

Bayer Report No.: 73390

Title: *n*-Octanol/Water Partition Coefficient for Bay Vp 2674

Authors: T. B. Waggoner

Reference: M. C. Bowman and Associates, Pine Bluff, Arkansas
Bowman Study No. 2674RPTH

Summary: Following TAD Guideline 3.02, the partition coefficient was determined at pH 5, 7, and 9 for two concentrations, 10 and 100 ppm, corresponding to 4% and 40% of the solubility at pH 7 (250 ppm, 25°C). The *n*-Octanol/water partition coefficients at 25°C were 0.4 (pH 5), 3.1 (pH 7), and 0.7 (pH 9).

Bayer Report No.: 106564

Title: Analysis of [2,3-Piperazinyl $^{13}C_2$]/[4-^{14}C]Enrofloxacin Residues in Cattle Urine and Feces

Authors: A. M. Kasper and B. A. Shadrick

Reference: Bayer Research Park
Bayer Study Number EN191401

Summary: Urine and feces from male and female cattle previously treated with ^{14}C-enrofloxacin at the maximum recommended treatment rate (5 mg/kg/day administered subcutaneously for 5 consecutive days; Miles Report No. 106579) were analyzed to determine the metabolites excreted from cattle. The urine and feces collected 24 hours after the first dose and 24 hours after the last dose were analyzed. Approximately 46% of the excreted radioactivity was contained in the urine. Of the radioactivity in the urine, 42% was comprised of ciprofloxacin or ciprofloxacin conjugates and 4% was comprised of enrofloxacin (2%), ring-opened oxociprofloxacin (1%), desethylene enrofloxacin (0.1%), and three unknown metabolites (1%). Approximately 54% of the excreted radioactivity was contained in the feces from the cattle. Of the radioactivity in the feces, 24% was comprised of enrofloxacin, 10% was ciprofloxacin, 7% was 7-aminofluoroquinolone, 4% was oxociprofloxacin, 3% was ring-opened oxociprofloxacin, 1% was 7-acetoaminofluoro-quinolone, 1% was desethylene enrofloxacin, and 5% was bound residues. The primary residues excreted by cattle are enrofloxacin and ciprofloxacin; the remaining residues each represent <10% of the administered dose. The distribution of the metabolites was approximately the same in the male and female cattle.

Bayer Report No.: 73955

Title: Dissociation Constant for Enrofloxacin (Bay Vp 2674)

Author: T. B. Waggoner

Reference: M. C. Bowman and Associates
Mount Ida, Arkansas

Summary: The study was conducted according to TAD Guideline 3.04. The dissociation constant (pK_a) was determined using a titration/potentiostatic method whereby solutions of enrofloxacin are titrated with standardized acid and base solutions. The pK_a values were determined to be 6.27 an 7.73.

Bayer Report No.: 106423

Title: Hydrolysis of ^{14}C-Enrofloxacin in Buffered Aqueous Solutions

Authors: R. Fernando, S. M. Schwietzer, R. A. Kok, and P. Y. Yen

Reference: Battelle Columbus Operations, Columbus, Ohio
Battelle Study No. SC930295
(Bayer Study No. EN072401)

Summary: The stability of enrofloxacin in aqueous buffers was determined according to methods and procedures set forth by TAD 3.09. The stability was determined at pH 5, 7, and 9 for 5 days at 50°C. The method of identification and quantification of ^{14}C-enrofloxacin was by HPLC with a radiochemical flow detector. No hydrolysis of enrofloxacin occurred under the test conditions.

Bayer Report No.: 106562

Title: Photodegradation of [4-^{14}C]Enrofloxacin in Sterile Buffer

Authors: A. M. Kasper, B. A. Shadrick, and T. E. Dement

Reference: Bayer Study No. EN082401

Summary: The photodegradation of enrofloxacin was evaluated in sterile buffers at pH 5, 7, and 9 in accordance with the FDA TAD 3.10. An actinometer reference chemical was employed as an approximate measure of sunlight intensity as specified in the TAD. The photolysis experiments were conducted in sterile buffer solutions at an initial concentration of 5 ppm enrofloxacin. The first-order photodegradation rate constants for enrofloxacin at pH 5, 7, and 9 were 0.0337, 0.2029, and 0.0486 days^{-1}, respectively. The degradation half-lives for enrofloxacin were calculated to be 20.6 min at pH 5, 3.4 min at pH 7, and 14.3 min at pH 9. Enrofloxacin rapidly degraded into various photoproducts which in turn were further transformed. Attempts to identify the transient photoproducts were unsuccessful. After 41 hours of irradiation, only numerous minor components remained; no individual photoproduct represented more than 10% of the applied radioactivity.

Bayer Report No.: 73954

Title: Absorption Spectra for Enrofloxacin (Bay Vp 2674)

Author: T. B. Waggoner

Reference: M. C. Bowman and Associates
Mount Ida, Arkansas

Summary: The study was conducted according to TAD Guideline 3.05. Absorption spectra for enrofloxacin were determined in the ultraviolet (350 - 190 nm) and visible (800 - 350 nm) regions of the electromagnetic spectrum. Analyses of triplicate solutions of 10 μg enrofloxacin/ml 0.05 M phosphate buffer at three pHs indicated the major absorption maximum at 271 nm, a secondary doublet at 322 and 344 nm, and a minor, poorly defined maximum, at 225 nm.

Bayer Report No.: 73742

Title: Soil Adsorption Constant for Enrofloxacin

Authors: T. B. Waggoner

Reference: M. C. Bowman and Associates
Mount Ida, Arkansas

Summary: A sorption/desorption study was conducted according to TAD 3.08. Soil adsorption constants were determined for three different types of soil (silty clay loam, silty loam, silt loam) ranging in pH from 6.0 to 7.3 and organic matter content from 1.8 to 2.6 percent. Greater than 99% adsorption of enrofloxacin was observed for all three soil types. The adsorption constants (K_d) determined ranged from 861 to 3,900. The K_{oc} values ranged from 81,300 to 185,670.

Bayer Report No.: 106555

Title: Sorption/Desorption of ^{14}C-Enrofloxacin on Soils by the Batch Equilibrium Method

Authors: T. R. Fernando, L. A. Burrows, D. S. First, and P. Y. Yen

Reference: Battelle Columbus Operations, Columbus, Ohio
Battelle Study No. SC930281
Bayer Study No. EN182101

Summary: A sorption/desorption study was conducted according to TAD 3.08. ^{14}C-Enrofloxacin in 0.01 *M* $CaCl_2$ was applied to four soils (loam, silt loam, clay loam, and sandy loam) in order to determine the maximum sorption of enrofloxacin to the soils, the minimum amount of time required to reach maximum sorption, and the relative desorption of enrofloxacin from the soils. The 0.01 *M* $CaCl_2$ was used to approximate the ionic conditions present in the natural environment, and the $CaCl_2$ did not inhibit the sorption of the enrofloxacin to the soils. The results of the study are summarized in the following table.

Parameters	Soil Type; Soil Series; and Source			
	Silt Loam; Drummer; Champaign, IL	Clay Loam; Bearden; Casselton, ND	Sandy Loam; Tifton; Meigs, GA	Loam; Morley; Allen Co., IN
pH	6.2	7.7	5.5	5.5
Organic Carbon (%)	1.9	1.7	1.3	1.1
Organic Matter (%)	3.3	3.0	2.2	2.0
Cation Exchange Capacity (meq/100g)	24.5	29.6	4.5	8.0
Sand/Silt/Clay (%)	23/26/51	27/31/42	70/12/18	36/24/40
Adsorption (%)	99.9	99.8	99.7	99.9
Desorption (%)	0.06	0.09	0.18	0.07
K_d	5502	3466	970	3915
K_{oc}	289568	203906	74635	355941

Nearly complete (> 99.5%) sorption of enrofloxacin to the soils occurred within 2 hours of exposing the soils to the enrofloxacin solution. Desorption of the bound enrofloxacin from the soils was slow (< 0.26%) under the conditions specified by the FDA (5 ml of 0.01 *M* $CaCl_2$/g soil). Enrofloxacin is tightly bound to soils.

Bayer Report No.: 106557

Title: Sorption/Desorption of ^{14}C-Enrofloxacin in Cattle Manure and Poultry Excreta and ^{14}C-Ciprofloxacin in Cattle Manure

Authors: M. D. Williams, L. G. Heim, and P. Y. Yen

Reference: ABC Laboratories, Columbia, Missouri
ABC Study No. 41452
Bayer Study No. EC182401

Summary: The study was conducted following methods and procedures set forth in TAD 3.08, except that manure was substituted for soil. ^{14}C-Enrofloxacin solution was applied to cattle manure, chicken excreta, and turkey excreta in order to determine the maximum sorption of enrofloxacin to the manure/excreta, the minimum amount of time required to reach maximum sorption, and the relative desorption of enrofloxacin from the manure/excreta.

Parameters	Manure/Excreta		
	Cattle	Chicken	Turkey
pH	8.7	7.3	6.0
Organic Carbon (%)	37.6	35.3	32.7
Organic Matter (%)	64.7	60.7	56.2
Cation Exchange Capacity (meq/100g)	40.9	19.3	38.7
Adsorption (%)	67 - 85	44 - 63	29 - 41
Desorption (%)	11 - 30	33 - 54	51 - 72
K_d	367	139	64.6
K_{oc}	976	395	198

The sorption of enrofloxacin was up to 85% to cattle manure, 63% to chicken excreta, and 41% to turkey excreta. The percent desorption of the adsorbed enrofloxacin was up to 30% from cattle manure, 54% from chicken excreta, and 72% from turkey excreta. Maximum sorption occurred within approximately 2 hours.

Similarly, ^{14}C-ciprofloxacin was applied to cattle manure.

Parameters	Cattle Manure
pH	8.7
Organic Carbon (%)	37.6
Organic Matter (%)	64.7
Cation Exchange Capacity (meq/100g)	40.9
Adsorption (%)	71 - 84
Desorption (%)	10 - 24
K_d	399
K_{oc}	1062

The sorption of ciprofloxacin was up to 84% to the manure. The percent desorption of the adsorbed ciprofloxacin was up to 24% from the manure. Maximum sorption occurred within approximately 2 hours. The sorption/desorption of ciprofloxacin to poultry excreta is expected to be similar to that of enrofloxacin.

Bayer Report No.: 106560

Title: Aerobic Biodegradation of [4-^{14}C]Enrofloxacin in Soil and Feces

Authors: A. M. Kasper, B. A. Shadrick, and V. A. Marlow

Reference: Bayer Study No. EN042101

Summary: The aerobic biodegradation of [4-^{14}C]enrofloxacin in 3 soils, in feces of cattle not previously treated with enrofloxacin, and in feces of cattle previously administered a series of subcutaneous injections of [^{14}C]enrofloxacin was investigated in accordance with TAD 3.12. The test systems were maintained for 64 days, and over the course of the 64 days only trace amounts of $^{14}CO_2$ were measured indicating that enrofloxacin mineralized very slowly. At the end of the 64-day study, 13 to 15% of the enrofloxacin in the 3 soil test systems had degraded to a combination of desethylene ciprofloxacin, ciprofloxacin, and hydroxylated enrofloxacin. At the end of the 64-day study, the degradation of enrofloxacin added to the feces from untreated cattle was similar to that determined for the 3 soil systems. At the end of the 64-day study, the amount of enrofloxacin in the feces from cattle treated with ^{14}C-enrofloxacin had decreased from 78% (at the start of the study) to 54%. The half-life of enrofloxacin in the 3 soils ranged from 359 to 696 days. The half-life of enrofloxacin in the feces from cattle not previously treated with enrofloxacin was 468 days, and the half-life of enrofloxacin in the feces of cattle previously treated with ^{14}C-enrofloxacin was 142 days.

Bayer Report No.: 106772

Title: Biodegradation of ^{14}C-Enrofloxacin and ^{14}C-Ciprofloxacin by *Aspergillus clavatus, Cunninghamella elegans, Trichoderma hamatum,* and *Phanerochaete chrysosporium*

Authors: Z. Yan, D. Harris, and I. Kelley

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41591
Bayer Study No. EC041401

Summary: This study was conducted in support of biodegradation studies in manure and soil (TAD 3.12). ^{14}C-Enrofloxacin and ^{14}C-Ciprofloxacin solutions were applied to cultures of four fungi, *Aspergillus clavatus, Cunninghamella elegans, Trichoderma hamatum,* and *Phanerochaete chrysosporium*, in order to determine their potential to biotransform and mineralize the test compounds. During the 35-day test period, 100% biotransformation of enrofloxacin to five degradates occurred in the *Phanerochaete* culture, 22% in the *Cunninghamella* culture, 19% in the *Aspergillus* culture, and 15% in the *Trichoderma* culture. Ciprofloxacin degradation was slower than that of enrofloxacin, and 36% biotransformation of ciprofloxacin to four degradates occurred in the *Phanerochaete* culture, 23% in the *Cunninghamella* culture, 40% in the *Aspergillus* culture, and 25% in the *Trichoderma* culture. Approximately 2% of the ^{14}C-enrofloxacin was recovered from the *Phanerochaete* culture as $^{14}CO_2$, 0.02% from the *Cunninghamella* and *Trichoderma* cultures, and 0% from the *Aspergillus* culture. Less than 2% of the ^{14}C-ciprofloxacin was recovered from the *Phanerochaete* culture as $^{14}CO_2$ and approximately 0.1% from the *Cunninghamella, Trichoderma*, and *Aspergillus* cultures.

Bayer Report No.: 106436

Title: Physical Chemical Properties of Ciprofloxacin

Authors: J. Blasberg, P. Y. Yen, and V. A. Marlow

Reference: ABC Laboratories, Inc.
ABC Labs Study No. 41483
Bayer Study No. CN201301

Summary: The studies were conducted according to TAD 3.01 (Water Solubility), TAD 3.02 (*n*-Octanol/Water Partition Coefficient), TAD 3.03 (Vapor Pressure), TAD 3.04 (Dissociation Constant), and TAD 3.05 (UV-Visible Absorption Spectrum).

The aqueous solubility of ciprofloxacin was determined to be 292 ppm at pH 5, 59.1 ppm at pH 7, and 200 ppm at pH 9. The *n*-octanol/water partition coefficient of ciprofloxacin was determined using 3 buffered aqueous systems: pH 5 K_{OW} = 0.0852, pH 7 K_{OW} = 0.165, and pH 9 K_{OW} = 0.0360. The vapor pressure of ciprofloxacin is < 10^{-7} mm Hg (torr). The dissociation constants of ciprofloxacin are pK_a = 5.71 and pK_b = 9.59. The UV-visible absorption spectrum was obtained for ciprofloxacin, and absorption maxima were observed at 315 and 328 nm in pH 5 buffer, 322 and 333 nm in pH 7 buffer, and 321 and 334 nm in pH 9 buffer.

Bayer Report No.: 106430

Title: Hydrolysis of ^{14}C-Ciprofloxacin in Buffered Aqueous Solutions

Authors: T. R. Fernando, S. M. Schwietzer, R. A. Kok, and P. Y. Yen

Reference: Battelle Columbus Operations, Columbus, Ohio
Battelle Study No. SC930297
Bayer Study No. CN027401

Summary: The stability of ciprofloxacin in aqueous buffers was determined according to methods and procedures set forth by TAD 3.09. The stability was determined at pH 5, 7, and 9 for 5 days at 50°C. The method of identification and quantification of ^{14}C-ciprofloxacin was by HPLC with a radiochemical flow detector. No hydrolysis of ciprofloxacin occurred under the test conditions.

Bayer Report No.: 106563

Title: Photodegradation of [4-^{14}C]Ciprofloxacin in Sterile Buffer

Authors: A. M. Kasper, B. A. Shadrick, and T. E. Dement

Reference: Bayer Study No. CN082401

Summary: The photodegradation of ciprofloxacin was evaluated in sterile buffer at pH 5, 7, and 9 in accordance with the FDA TAD 3.10. An actinometer reference chemical was employed as an approximate measure of sunlight intensity as specified in the TAD. The photolysis experiments were conducted in sterile buffer solutions at an initial concentration of 5 ppm ciprofloxacin. The first-order photodegradation rate constants for ciprofloxacin at pH 5, 7, and 9 were 0.0149, 0.769, and 0.0300 days^{-1}, respectively. The degradation half-lives for ciprofloxacin were calculated to be 46.4 min at pH 5, 9.0 min at pH 7, and 23.1 min at pH 9. Ciprofloxacin rapidly degraded into various photoproducts which in turn were further transformed. Attempts to identify the transient photoproducts were unsuccessful. After 41 hours of irradiation only numerous minor components remained; no individual photoproducts represented more than 10% of the applied radioactivity.

Bayer Report No.: 106556

Title: Sorption/Desorption of ^{14}C-Ciprofloxacin on Soils by the Batch Equilibrium Method

Authors: T. R. Fernando, L. A. Burrows, D. S. First, and P. Y. Yen

Reference: Battelle Columbus Operations, Columbus, Ohio
Battelle Study No. SC930282
Bayer Study No. CN182101

Summary: The study was conducting according to TAD 3.08. ^{14}C-Ciprofloxacin in 0.01 *M* $CaCl_2$ was applied to four soils (loam, silt loam, clay loam, and sandy loam) in order to determine the maximum sorption of ciprofloxacin to the soils, the minimum amount of time required to reach maximum sorption, and the relative desorption of enrofloxacin from the soils. The 0.01 *M* $CaCl_2$ was used to approximate the ionic conditions present in the natural environment, and the $CaCl_2$ did not inhibit the sorption of the ciprofloxacin to the soils. The results of the study are presented in the following table.

Parameter	Soil Type; Soil Series; and Source			
	Silt Loam; Drummer; Champaign, IL	Clay Loam; Bearden; Casselton, ND	Sandy Loam; Tifton; Meigs, GA	Loam; Morley; Allen Co., IN
pH	6.2	7.7	5.5	5.5
Organic Carbon (%)	1.9	1.7	1.3	1.1
Organic Matter (%)	3.3	3.0	2.2	2.0
Cation Exchange Capacity (meq/100g)	24.5	29.6	4.5	8.0
Sand/Silt/Clay (%)	23/51/26	27/31/42	70/12/18	36/24/40
Adsorption (%)	99.5	99.3	99.4	98.7
Desorption (%)	0.27	0.36	0.33	0.62
K_d	918	601	544	1479
K_{oc}	48341	35342	41841	134465

Nearly complete (> 99.1%) sorption of ciprofloxacin to the soils occurred within 2 hours of exposing the soils to the ciprofloxacin solution. Desorption of the bound ciprofloxacin from the soils was slow (< 0.69%) under the conditions specified by the FDA guideline (5 ml of 0.01 *M* $CaCl_2$/g soil). Ciprofloxacin is tightly bound to soils.

Bayer Report No.: 106561

Title: Aerobic Biodegradation of [4-^{14}C]Ciprofloxacin in Soil

Authors: A. M. Kasper, B. A. Shadrick, and V. A. Marlow

Reference: Bayer Study No. CN042101

Summary: The aerobic biodegradation of [4-^{14}C]ciprofloxacin in 3 soils was investigated following TAD Guideline 3.12. The test systems were maintained for 65 days, and over the course of the 65 days only trace amounts of $^{14}CO_2$ were measured indicating that ciprofloxacin mineralized very slowly. At the end of the 65-day study, the extracted residues accounted for 82 to 97% of the applied radioactivity and were comprised of ciprofloxacin (75 to 93% of the extracted residues) and 3 components (each less than 5% of the extracted residues).

Bayer Report No.: 73694

Title: Biological Effects of Enrofloxacin on Rainbow Trout, Bluegill Sunfish and Daphnia

Author: T. B. Waggoner

Reference: ABC Laboratories
Columbia, MO

Summary: Three acute toxicity tests were conducted to assess the toxicity of enrofloxacin to trout, bluegill and *Daphnia magna*. The fish studies were conducted under flow-through conditions for a 96-hour period. The *Daphnia* study was conducted under flow-through conditions for a 48-hour period. Fish were exposed to nominal concentrations of 0.62, 1.2, 2.5, 5.0, and 10 mg enrofloxacin/L. No mortality or sublethal effects were observed in any test level for either bluegill or rainbow trout after 96-hours of exposure. No LC_{50} could be determined for either species. The NOEC for bluegill and trout was $\geq$ 10 mg/L.

Daphnia were exposed to nominal concentrations of 0.72, 1.2, 2.5, 4.3, and 10 mg enrofloxacin/L. No mortality or sublethal effects were observed in any test level after 48-hours of exposure. No EC_{50} could be determined. The NOEC was $\geq$10 mg/L.

Bayer Report No.: 74507

Title: Acute Toxicity of Enrofloxacin to the Bluegill (*Lepomis macrochirus*) Under Static Renewal Conditions

Author: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Study No. EN810301

Summary: A 96-hour acute fish toxicity test was conducted according to TAD Guideline 4.11. An initial range-finding test was conducted as a limit test using one concentration (200 mg/L nominal) to see if any mortality would occur at the limit of solubility. Since mortality was observed in this test, a second range-finding test was conducted as per the TAD using a range of concentrations (100, 10, 1 mg/L) to determine the test levels for the definitive study. The results from both range-find tests, as presented in the report, were combined. The results of the second range-finding test were used to establish the concentrations for the definitive test. Bluegill were exposed for 96 hours under static renewal conditions to mean measured concentrations of 18.6 - 198 mg enrofloxacin/L of water (ppm). The test solutions were renewed after the initial 48 hours of exposure had elapsed (Day 2). Test solutions were analyzed for actual concentration of test compound on Day 0 in the freshly made test solutions, on Day 2 in the freshly made test solutions and on Day 4 in the old test solutions. The comparison of the Day 2 and Day 4 results demonstrated that after 48 hours in the test system, the test compound was stable. These solutions did not show significant degradation and had recoveries ranging from 81 to 103 percent of nominal.

In addition, the stability information is supported by a stability check performed during method validation for enrofloxacin. The stability check showed that after 96 hours (without renewal), there was still 88 percent enrofloxacin parent still present in the test system.

Singly, or combined, these data demonstrated that enrofloxacin was stable in the test solutions over the course of the study.

The 96-hour LC_{50} was 216 ppm (the concentration lethal to 50% of the bluegill), the low effect concentration was 33.5 ppm, and the no effect concentration was 18.6 ppm.

Bayer Report No.: 74501

Title: Acute Toxicity of Enrofloxacin to the Rainbow Trout (*Oncorhynchus mykiss*) Under Static Renewal Conditions

Author: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Study No. EN812201

Summary: A 96-hour acute fish toxicity test was conducted according to TAD Guideline 4.11. An initial range-finding test was conducted as a limit test using one concentration (200 mg/L nominal) to see if any mortality would occur at the limit of solubility. Since mortality was observed in this test, a second range-finding test was conducted as per the TAD using a range of concentrations (100, 10, 1 mg/L) to determine the test levels for the definitive study. The results from both range-find tests, as presented in the report, were combined. The results of the second range-finding test were used to establish the concentrations for the definitive test. Rainbow trout were exposed for 96 hours under static renewal conditions to mean measured concentrations of 18.5 to 196 mg enrofloxacin/L of water (ppm). The test solutions were renewed after the initial 48 hours of exposure had elapsed (Day 2). Analysis of the 48-hour old test solutions demonstrated no significant degradation (92 to 100 percent recovery). The test solutions at test termination (also 48 hours old) were also analyzed. These solutions did not show significant degradation and had recoveries ranging from 100 to 106 percent.

In addition, the stability information is supported by a stability check performed during method validation for enrofloxacin. The stability check showed that after 96 hours (without renewal), there was still 88 percent enrofloxacin parent still present in the test system. Photodegradation did not occur to any significant effect during the toxicity test as demonstrated by the analytical values. The lack of photodegradation in the study was probably due to the fact that the light energy was two orders of magnitude lower than used for the photolysis study and was of a different quality.

The 96-hour LC_{50} was >196 ppm, the low effect concentration was 61.9 ppm, and the no effect concentration was 33.5 ppm.

Bayer Report No.: 106595

Title: Acute Toxicity of Enrofloxacin to *Daphnia magna*

Authors: S. L. Hicks, M. Muckerman, H. Murrell

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41281

Summary: A static acute toxicity test was conducted according to TAD Guideline 4.08. *Daphnia magna* were exposed for 48 hours to mean measured concentrations of enrofloxacin ranging from 5.60 to 96.8 ppm. Test solutions were analyzed at 0 hours, 24 hours and 48 hours during the study. The test compound was stable during the conduct of the study and the analytical results showed that the measured concentrations ranged from 87 to 97% of nominal at 0 hours, 86 to 97% of nominal at 24 hours, and 86 to 97% of nominal at 48 hours. The 48-hour EC_{50} (the concentration of enrofloxacin which has an effect on 50% of the daphnids) was 79.9 ppm, and the NOEC (no observed effect concentration) was 23.0 ppm. Observed effects included staying on the bottom of the test flask, erratic movement, and trailing extraneous material.

Bayer Report No.: 106790

Title: Chronic Toxicity of Enrofloxacin to *Daphnia magna*

Authors: S. L. Hicks, J. Veltri, and A. D. Forbis

Reference: Analytical Bio-Chemistry Labs, Inc.
ABC Study No. 41300

Summary: A chronic toxicity test was conducted according to TAD Guideline 4.09. *Daphnia magna* were exposed under static renewal conditions to mean measured concentrations of enrofloxacin ranging from 0.61 to 20 ppm for 21 days. Freshly prepared test solutions were analyzed on Days 0, 4, 11 and 18, and old test solutions were analyzed on Days 7, 14 and 21. The comparison of the data between Day 4 and Day 7, Day 11 and Day 14, and Day 18 and Day 21 demonstrated that the test compound was stable in the test system during the study. The solutions did not show significant degradation and had recoveries ranging from 77 to 101 percent of nominal. The 21-day No Observed Effect Concentration (NOEC) was determined to be 9.8 ppm, and the Lowest Observed Effect Concentration (LOEC) was determined to be 20 ppm. The Maximum Acceptable Toxicant Concentration (MATC) was determined to be 14 ppm.

Bayer Report No.: 106788

Title: Acute Toxicity of Enrofloxacin to *Hyalella azteca* Under Static Conditions

Authors: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Miles Study No. CN821401

Summary: An acute toxicity test was conducted according to TAD Guideline 4.10. *Hyalella azteca* were exposed for 96 hours under static renewal conditions to mean measured concentrations of enrofloxacin ranging from 12.5 to 206 mg /L (ppm). The test solutions were renewed after the initial 48 hour exposure period. Test solutions were analyzed for actual concentration of test compound on Day 0 in the freshly prepared test solutions, on Day 2 in the freshly prepared test solutions and on Day 4 in the old test solutions. The comparison of the Day 2 and Day 4 results demonstrated, after 48 hours in the test system, the test compound was stable during the study. These solutions did not show significant degradation and had recoveries ranging from 101 to 107 percent of nominal. The 96-hour LC_{50} (the concentration of enrofloxacin lethal to 50% of the *Hyalella*) was greater than 206 ppm (the maximum solubility of enrofloxacin in the test system).

Bayer Report No.: 106657

Title: Effect of Enrofloxacin Technical on Growth of the Green Alga (*Selenastrum capricornutum*)

Authors: G. G. Gagliano and L. M. Bowers

Reference: Bayer Research Park
Bayer Study Number EN881601

Summary: An algae growth study was conducted according to TAD Guideline 4.01. Green algae (*Selenastrum capricornutum*) were exposed to initial concentrations of enrofloxacin ranging from 0.016 to 1.04 ppm for 14 days. The test compound appeared to degrade (probably due to photolysis) during the course of the study.

The photolysis study for enrofloxacin (Bayer Report No. 106562 submitted August 30, 1995 under INAD 4586) demonstrated that the half-life of the compound under the conditions of the photolysis study (significantly more intense irradiation) is a matter of minutes. Adsorption of the compound to cell walls is possible, but this would be a worst case exposure situation since the compound is in direct contact with the algae cells. With enrofloxacin, photolysis and adsorption are artifacts of the standard experimental design for algae growth tests under TAD 4.01

The Day 0 concentrations were used for calculating the no observed effect levels and MICs. The data show that inhibition in the two highest test levels for enrofloxacin (0.50 and 0.99 ppm) occurred early in the study, and that the algae in these test levels showed significant recovery by the end of the study. The recovery most likely correlated with the degradation of the test compounds, and the inhibition coincided with the concentrations of test compound near or at the Day 0 measured concentrations.

Based on the initial measured concentrations, the No Observed Effect Concentration (NOEC) was 0.25 ppm for maximum standing crop (a measurement of the biomass of the culture), and the minimum inhibitory concentration was 0.50 ppm. Based on initial measured concentrations, the NOEC for the maximum growth rate was 0.13 ppm, and the minimum inhibitory concentration was 0.25 ppm.

Bayer Report No.: 106940

Title: Growth Rate Effect of ^{14}C-Enrofloxacin to *Microcystis aeruginosa*

Authors: D. W. Gledhill, A. D. Forbis, and T. Leak

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41576

Summary: An algae growth study was conducted according to TAD Guideline 4.01. *Microcystis aeruginosa* were exposed to initial concentrations of enrofloxacin ranging from 0.143 to 4.50 ppm for 18 days.

The photolysis study for enrofloxacin (Bayer Report No. 106562 submitted August 30, 1995 under INAD 4586) demonstrated that the half-life of the compound under the conditions of the photolysis study (significantly more intense irradiation) is a matter of minutes. Adsorption of the compound to cell walls is possible, but this would be a worst case exposure situation since the compound is in direct contact with the algae cells. With enrofloxacin, photolysis and adsorption are artifacts of the standard experimental design for algae growth tests under TAD 4.01

Based on the initial concentrations, the no-observed effect level was 0.143 ppm for the maximum standing crop (a measurement of the biomass of the culture), and the minimum inhibitory concentration was 0.283 ppm. For significant effects on the maximum growth rate, the no-observed effect level was 2.22 ppm, and the minimum inhibitory concentration was 4.50 ppm.

Bayer Report No.: 106791

Title: Acute Toxicity of Ciprofloxacin to the Bluegill (*Lepomis macrochirus*) Under Static Renewal Conditions

Authors: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Bayer Study No. CN810302

Summary: A fish acute toxicity test was conducted according to TAD Guideline 4.11. A range-finding test was conducted at the limit of solubility (10 mg/L nominal) to determine if toxicity occurred at this concentration. Since none of the fish in the range-finding test died or showed sublethal toxic effects, Bayer chose to conduct the definitive study as a limit test. The initial definitive test was conducted as a limit test using one test concentration (10 mg/L nominal). There were 3 deaths (15% mortality) at the end of the 96-hour period observed in the limit test. However, this mortality was not statistically different from the control group mortality (0% mortality). The mortality of the bluegill in the definitive limit test (measured concentration = 10.6 mg/L) was deemed a possible concern. In order to be conservative, Bayer chose to conduct a second definitive test using five test concentrations in order to ensure accurate characterization of the toxicity of ciprofloxacin to bluegill. There were no mortalities observed in the second definitive test.

A possible explanation in the difference between the first and second studies was that each test used a different lot of bluegill obtained from different suppliers. Nevertheless, statistically, there is no difference between the results of the range-find and two definitive test results. Therefore, the toxicity was adequately characterized.

Bluegill were exposed for 96 hours to nominal concentrations of 1.19 to 9.85 ppm ciprofloxacin. The 96-hour LC_{50} (the concentration of ciprofloxacin lethal to 50% of the fish) was greater than 9.85 ppm (measured), the maximum solubility of ciprofloxacin in this test system. No abnormal physical or behavioral effects were observed on the fish at or below the 9.85 ppm test concentration.

The occurrence of turbid test solutions at the highest concentration in the trout study, but not in the bluegill study, appears to be a case of solubility differential at varying temperatures. The trout study was conducted at 12°C whereas the bluegill study was conducted at 22°C. Solubility generally increases as temperature increases.

Bayer Report No.: 106775

Title: Acute Toxicity of Ciprofloxacin to the Rainbow Trout (*Oncorhynchus mykiss*) Under Static Renewal Conditions

Authors: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Bayer Study No. CN812201

Summary: A fish acute toxicity test was conducted according to TAD Guideline 4.11. Rainbow trout were exposed for 96 hours to a nominal concentration of 10 ppm ciprofloxacin (10 mg ciprofloxacin/L dilution water). No abnormal physical or behavioral effects were observed on the fish. The 96-hour LC_{50} (the concentration of ciprofloxacin lethal to 50% of the fish) was greater than 9.4 ppm (measured), the maximum solubility of ciprofloxacin in this test system.

The Technical Assistance Document 4.11 for freshwater fish acute toxicity specifies that a graph of the concentration versus mortality line be included in the report. Accordingly, a representative graph was included as required. This dose-response curve was accurate and was depicted by a line curve defined by the two points.

Bayer Report No.: 106596

Title: Acute Toxicity of Ciprofloxacin to *Daphnia magna*

Authors: S. L. Hicks and M. Muckerman

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41282

Summary: An acute toxicity test was conducted according to TAD Guideline 4.08. *Daphnia magna* were exposed for 48 hours to a mean measured concentration of 9.90 ppm ciprofloxacin. The 48-hour EC_{50} (the concentration of enrofloxacin which has an effect on 50% of the daphnids) was greater than 9.90 ppm (the maximum solubility of ciprofloxacin in the test system). No effects on the daphnids were observed at the 9.90 ppm test level.

Bayer Report No.: 106783

Title: Acute Toxicity of Ciprofloxacin to *Hyalella azteca* Under Static Conditions

Authors: L. M. Bowers

Reference: Bayer Research Park
Stilwell, Kansas
Bayer Study No. CN821401

Summary: An acute toxicity test was conducting according to TAD Guideline 4.10. *Hyalella azteca* were exposed for 96 hours to mean measured concentrations of ciprofloxacin ranging from 1.25 to 10.2 mg /L (ppm). The 96-hour LC_{50} (the concentration of ciprofloxacin lethal to 50% of the *Hyalella*) was greater than 10.2 ppm (the maximum solubility of ciprofloxacin in the test system).

Bayer Report No.: 106633

Title: Effect of Ciprofloxacin Technical on Growth of the Green Alga (*Selenastrum capricornutum*)

Authors: G. G. Gagliano and L. M. Bowers

Reference: Bayer Research Park
Bayer Study Number CN881402

Summary: An algae growth study was conducted according to TAD Guideline 4.01. Green algae (*Selenastrum capricornutum*) were exposed for 14 days to measured initial concentrations of ciprofloxacin ranging from 0.81 to 12.8 mg ciprofloxacin/L of test water (ppm). The test compound appeared to degrade (probably due to photolysis) during the course of the study, and all endpoints were based on initial concentrations of ciprofloxacin.

The photolysis study for ciprofloxacin (Bayer Report No. 106563 submitted August 30, 1995 under INAD 4586) demonstrated that the half-life of the compound under the conditions of the photolysis studies (significantly more intense irradiation) is a matter of minutes. With ciprofloxacin, photolysis and adsorption are artifacts of the standard experimental design for algae growth tests under TAD 4.01.

The Day 0 concentrations were used for calculating the no observed effect levels and MICs. The data show that inhibition in the highest level for ciprofloxacin (12.8 ppm) occurred early in the study, and that the algae in these test levels showed significant recovery by the end of the study. The recovery most likely correlated with the degradation of the test compounds, and the inhibition coincided with the concentrations of test compound near or at the Day 0 measured concentrations.

The No Observed Effect Concentration (NOEC) for the maximum standing crop was >12.8 ppm (the maximum solubility of ciprofloxacin in this test system). For the maximum growth rate, the NOEC was 6.43 ppm, and the Minimum Inhibitory Concentration (MIC) was 12.8 ppm.

Bayer Report No.: 106627

Title: Growth Rate Effect of ^{14}C-Ciprofloxacin to *Microcystis aeruginosa*

Authors: D. W. Gledhill, A. D. Forbis, and T. Leak

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41577

Summary: An algae growth study was conducted according to TAD Guideline 4.01. *Microcystis aeruginosa* were exposed to initial concentrations of ciprofloxacin ranging from 0.0691 to 4.24 ppm for 14 days.

The photolysis study for ciprofloxacin (Bayer Report No. 106563 submitted August 30, 1995 under INAD 4586) demonstrated that the half-life of the compound under the conditions of the photolysis studies (significantly more intense irradiation) is a matter of minutes. With ciprofloxacin, photolysis and adsorption are artifacts of the standard experimental design for algae growth tests under TAD 4.01.

Based on the initial measured concentrations, the no-observed effect level was 0.0691 ppm for the maximum standing crop (a measurement of the biomass of the culture), and the minimum inhibitory concentration was 0.137 ppm. For significant effects on the maximum growth rate, the no-observed effect level was 0.0691 ppm, and the minimum inhibitory concentration was 0.137 ppm.

Bayer Report No.: 106579

Title: I. Depletion of [^{14}C] Enrofloxacin Residues in Cattle and Determination of Target Tissue. II. Determination of Desethylene Ciprofloxacin as the Marker Substance. III. Determination of the R_M for Desethylene Ciprofloxacin in Cattle Liver.

Authors: L. R. Hall and A. L. Hartz

Reference: Bayer Study Nos. EN040402 and EN040403

Summary: Male and female cattle (6 months old at first dose) were treated with [^{14}C]-enrofloxacin 10% injectable formulation under simulated field conditions. Cattle were dosed by subcutaneous injection at a rate of 5 mg enrofloxacin/kg body weight/day once per day for 5 consecutive days. At 8 hours, and 3, 7, and 14 days after the last dose was administered, the cattle were sacrificed, and the excreta, liver, kidneys, muscle, fat, injection sites, and other tissues were collected. The depletion of [^{14}C] residues from the tissues was biphasic and very rapid. The rate of depletion of enrofloxacin-related residues from the liver was the slowest compared to the other tissues, and on this basis liver was selected as the target tissue. While the major metabolites identified in the liver at the 8-hour interval were enrofloxacin and ciprofloxacin (Bayer Report No. 106580), the major radioactive residue in the liver at the later withdrawal intervals was conjugated and unconjugated desethylene ciprofloxacin. Desethylene ciprofloxacin was present in the liver at all of the withdrawal intervals and was selected as the marker residue.

Bayer Report No.: 73606

Title: Acute Dermal Toxicity of Bay Vp 2674 in Albino Rabbits

Authors: D. Eigenberg

Reference: Bayer Research Park
Stilwell, Kansas

Summary: The acute dermal toxicity of Bay Vp 2674 was tested in young adult New Zealand White rabbits. Groups of five males and five females weighing 2.81 to 3.67 kg had 2000 mg/kg of Bay Vp 2674 applied to a shaved area of the back. The dosing area was covered with an occlusive patch for 24 hours, after which the patch was removed and the animals were observed for 14 days for mortality and clinical signs. Body weights were recorded on the day of treatment and on days 7 and 14 after treatment. At the end of the study, the animals were sacrificed, and a gross necropsy was performed. No clinical signs or deaths were observed during the study, and no treatment-related gross lesions were observed at gross necropsy. Body weights increased during the study in all animals with mean body weight gains for males and females on day 14 of 0.20 kg and 0.29 kg, respectively. The amount of test material applied per unit area of skin ranged from 23 to 31 mg/cm^2. The dermal LD_{50} and the no-observable-effect level were > 2,000 mg/kg for males and females.

Bayer Report No.: 73466

Title: Acute Inhalation Toxicity Study with Bay Vp 2674 in Sprague-Dawley Rats

Author: R. Shiotsuka

Reference: Bayer Research Park
Stilwell, Kansas

Summary: The acute inhalation toxicity of Bay Vp 2674, tested as a dust, was evaluated in Sprague-Dawley rats. A group of ten male and ten female rats was exposed for four hours, by head only, to Bay Vp 2674 at a gravimetric concentration of 3547 mg/m^3 (8127 mg/m^3 nominal). This concentration was the highest attainable by the generation and exposure system used. A comparable group of rats was sham-exposed (conditioned air) to serve as controls. The deaths of two males and one female were attributed to the test substance. The compound-related clinical signs of toxicity included rales (4/17), gasping (1/17), and decreased activity (1/17). There were no toxicologically significant effects on weight gain. The compound-related gross lesions (only observed in rats found dead) included: red turbinates; red lungs; and nasal, ocular, and ventral neck stains. There were no sex-related differences in toxicity. The LC_{50} for male and female rats was greater than a gravimetric concentration of 3547 mg Bay Vp 2674/m^3. The NOEL was less than a gravimetric concentration of 3547 mg Bay Vp 2674/m^3.

Bayer Report No.: 73075

Title: Acute Oral Single Dose Studies in Rats, Mice, Rabbits and Dogs

Author: M. Schmidt

Reference: Bayer Institute for Toxicology
Wuppertal, Germany

Summary: The test substance (enrofloxacin technical) was administered as an oral suspension to Rats (Wistar), Mice (BOR:CFW1), Rabbits (Chinchilla), and Dogs (Beagle). The number of animals per sex per treatment group were: rats - 5, mice - 5, rabbits - 2, dogs - 2. The drug levels tested and duration of dosing were: rats - 630, 1000, and 5000 mg/kg (single day); mice - 1000, 2000, 4000, and 5000 mg/kg (single day); rabbits - 320, 500, 800, 1200, and 2000 mg/kg (single day); dogs - 1000 and 5000 mg/kg (single day). Observations were made for 14 days. Symptoms observed were such as reduced mobility, trembling, tonic convulsions, labored breathing, and staggering gait. Signs appeared as early as 15 minutes after exposure with some persisting for 10 days. Pulmonary congestion and hemorrhage were noted during gross necropsy. Oral LD_{50}'s; male and female rats > 5000 mg/kg; male mice > 5000 mg/kg and female mice = 4336 mg/kg; male and female rabbits ≈ 500 to 800 mg/kg. Endpoints for male and female dogs were not established due to vomition.

Bayer Report No.: 73775

Title: Subchronic Oral Toxicity in Dogs

Author: M. C. Porter

Reference: Miles Laboratories
Elkhart, Indiana

Summary: Beagles (4 animals per sex per treatment group) were administered enrofloxacin technical drug substance incorporated into feed for 91 days. The drug levels tested were 0, 100, 320, and 2,500 ppm. No deaths occurred, but abnormal gait and/or posture was observed in the high-dose animals by the second week of the study. Daily observations were conducted. Radiographic examination revealed that neither bone growth nor density were significantly affected. Physical examinations including direct ophthalmoscopy were conducted pretreatment and at termination with the only finding described in the clinical signs section. Body weights and body weight gains were not significantly different for treated and control animals which were recorded at weekly intervals. Hematology samples were collected at 2 and 6 weeks as well as at termination with no abnormal findings. Clinical chemistries were evaluated at 2 and 6 weeks, and at termination and again with no abnormalities. Urine was collected after weeks 2 and 6, and at termination with crystal formation in the urine of dogs receiving the high-dose treatment. Absolute and relative organ weights for treated animals did not differ significantly from those of controls, but testicular weights were higher for the treated animals. Superficial erosions of articular cartilage surfaces were seen in all high-dose dogs and one mid-dose male with no joint lesions in the remaining mid-dose or low-dose animals. Microscopically, the joint lesions were characterized by splitting of the articular cartilage surface and disorganization of chondrocytes. Necrosis and disintegration of hyaline cartilage was seen in some cases. A marked variation occurred in the appearance of testes including stage of maturity, diameter of lumen of seminiferous tubules, and vacuolar changes in the lining cells of the tubules. One dog from the control group and 3 dogs from the mid-dose group had signs of testicular maturity as evidenced by the production of tail-containing spermatozoa. None of the low-dose or high-dose animals had any evidence of tailed spermatozoa. Three dogs from the control group and 1 each from the mid- and high-dose groups had immature testes that were characterized by small seminiferous tubules with little or no lumen and containing a single layer of spermatogonial cells. Testes from 4 dogs, one each from the low- and high-dose groups and 2 from the mid-dose group, contained seminiferous tubules with dilated lumen and often contained more than a single layer of lining cells. Similar, but less advanced, tubules were observed for one dog from the control group. Vacuolar change in the apical parts of the spermatogonial cells occurred in 2 dogs of the low-dose group, 3 dogs of the high-dose group, and 1 dog of the

control group. The tubular morphology observed for the 2 low-dose and 3 high-dose animals appeared to be beyond the normal limits. The Agency concluded a NOEL of 100 ppm or equivalent of 3 mg/kg for this study following conduction of additional subchronic dog studies.

Bayer Report No.: 74229

Title: Chronic Toxicity and Carcinogenicity in Mice

Author: E. Bomhard

Reference: Bayer Institute for Toxicology
Wuppertal, Germany

Summary: Mice (B6C3F1) were dosed with enrofloxacin technical drug substance incorporated into feed. Fifty animals per sex per treatment group were exposed to 0, 1000, 3300 and 10,000 ppm for 24 months. The body surfaces, orifices, eyes, general behavior, posture, breathing, and excretion products of the treated animals did not show any unusual features compared to the controls when inspected twice daily. There was no evidence of treatment-induced damage to the eyes in the groups receiving up to and including 3300 ppm at the end of the study. The females in the 10,000 ppm group showed a clearly elevated incidence of focal lenticular opacity. No histological correlation could be assigned to the effect. A slightly higher increase in mortality of the males in the 3300 ppm group and females in the 10,000 dose was observed. Body weights were determined once a week with animals in the 2 lower dosed groups being significantly higher than the controls with the 10,000 ppm group occasionally higher than the controls, and the 20,000 ppm group comparable to the controls. The total white blood cell counts were decreased in the 3300 and 10,000 ppm groups for the males and in the 3300 ppm group for the females. Significantly lower hemoglobin, hematocrit, MCV, and MCH values were seen at 10,000 ppm level. Samples obtained after 12 and 24 months showed lower alkaline phosphatase levels at the 3300 and 10,000 levels. A dose-dependent trend was observed in total protein values with the reduction in total plasma protein attributed to a decrease in the globulin fraction. No specific gross pathology findings were observed at the 12-month sacrifice. After 24 months, an incidence of cecal dilation was observed which increased with increasing dose for males treated at 1000 ppm (4.0%), 3300 ppm (42.0%) and 10,000 ppm (82.0%) and for females at 3300 ppm (26%) and at 10,000 ppm (64%). No changes occurred in absolute or relative organ weights except for increases in kidney weights of females receiving 20,000 ppm. On histopathological examination, there was neither an increase in the incidence nor a decrease in the time of appearance of tumors in the dosed groups compared to the controls. Intrahepatic bile-duct changes were detected in the 3300 and 10,000 ppm dose groups. Focal papillary mucosal hyperplasia in the gall bladder was observed in the 3300 ppm males and the 10,000 ppm females. There was no evidence of carcinogenic effect in mice dosed with 1000 - 10,000 ppm enrofloxacin for two years. The Agency concluded a NOEL of 1000 ppm (323 mg/kg) for all effects.

Bayer Report No.: 74387

Title: Chronic Toxicity Study in Rats After Administration in Feed Over a Period of 2 Years.

Author: K. Leser

Reference: Bayer Institute for Toxicology
Wuppertal, Germany

Summary: Wistar rats (50 animals per sex per treatment group) were exposed to 0, 100 and 500 ppm enrofloxacin technical via feed for a 24 month period. Histological evaluation was limited only to the heart and liver. No statistically significant differences were observed compared to the controls. A NOEL for enrofloxacin had not been demonstrated by this study based on significant increases in the bile-duct hyperplasia in all the exposure groups of male rats. The Agency set the NOEL at 100 ppm after considering the spectrum of data from all the chronic rat studies and opinions submitted to CVM. The 100 ppm NOEL corresponded with doses of 5.3 and 7.2 mg enrofloxacin/kg bw for the male and female rats, respectively.

Bayer Report No.: 73892

Title: Additional Two-Generation Reproduction Study in Rats

Author: R. L. Kowalski

Reference: Miles Laboratories
Elkhart, Indiana

Summary: Sprague-Dawley rats (120 F_0 animals per sex per treatment group) were exposed to 0, 125, 300, and 2,000 ppm enrofloxacin technical drug via feed over a duration of two generations. The only definite test article-related change was seen in the testes and epididymides of mid- and high-dose F_0 and F_1 males. The change was characterized as abnormal spermatozoa. Epididymal weights of the high-dose group were decreased in both the F_0 and F_1 generation with the variation in the F_1 being statistically different. No effects on fertility or reproductive performance were noted. The Agency concluded a NOEL of 125 ppm.

Bayer Report No.: 73705

Title: Embryotoxicity/Teratogenicity Study with the Rabbit

Author: H. Becker

Reference: Research and Consulting Co.
Itingen, Switzerland

Summary: Chinchilla rabbits (16 females per treatment group) were orally dosed from day 6 through day 18 post-breeding. The drug levels tested were 0, 1, 5, 25, and 75 mg enrofloxacin/kg. No treated dams expired with one in the high-dose group aborting on day 19. A statistically significant decrease in food consumption was noted for the high-dose females. This group also had a slightly decreased body weight gain. Treatment at the higher dose caused increased post-implantation loss. No differences occurred in mean body weights of fetuses in the treated groups. Malformations observed were considered to be incidental and within the normal spontaneous range. No test article or dose-related differences were evident, and the stage of development in all groups was similar. The Agency concluded a NOEL of 25 mg/kg.

Bayer Report No.: 73584

Title: Subacute Toxicity of Enrofloxacin (Bay Vp 2674) to Earthworms (*Lumbricus terrestris*)

Author: T. B. Waggoner

Reference: M. C. Bowman and Associates
Mount Ida, Arkansas

Summary: The study was conducted according to Environmental Assessment Technical Guideline 11.13. Earthworms were exposed to 0.1, 1.0, 10, 100, and 1,000 ppm enrofloxacin for 28-days in a test soil medium. A single mortality (10%) was observed in the 0.1 and 10 ppm treatment levels. There was 20% mortality in the solvent control treatment (0.1% methanol) and no mortality in the negative control. No adverse effects were observed of the control soil or any treatment level. The no observed effect level was determined to be $\geq$ 1000 ppm enrofloxacin.

Bayer Report No.: 74123

Title: Toxicity of Enrofloxacin (Bay Vp 2674) to Earthworms (*Lumbricus terrestris*)

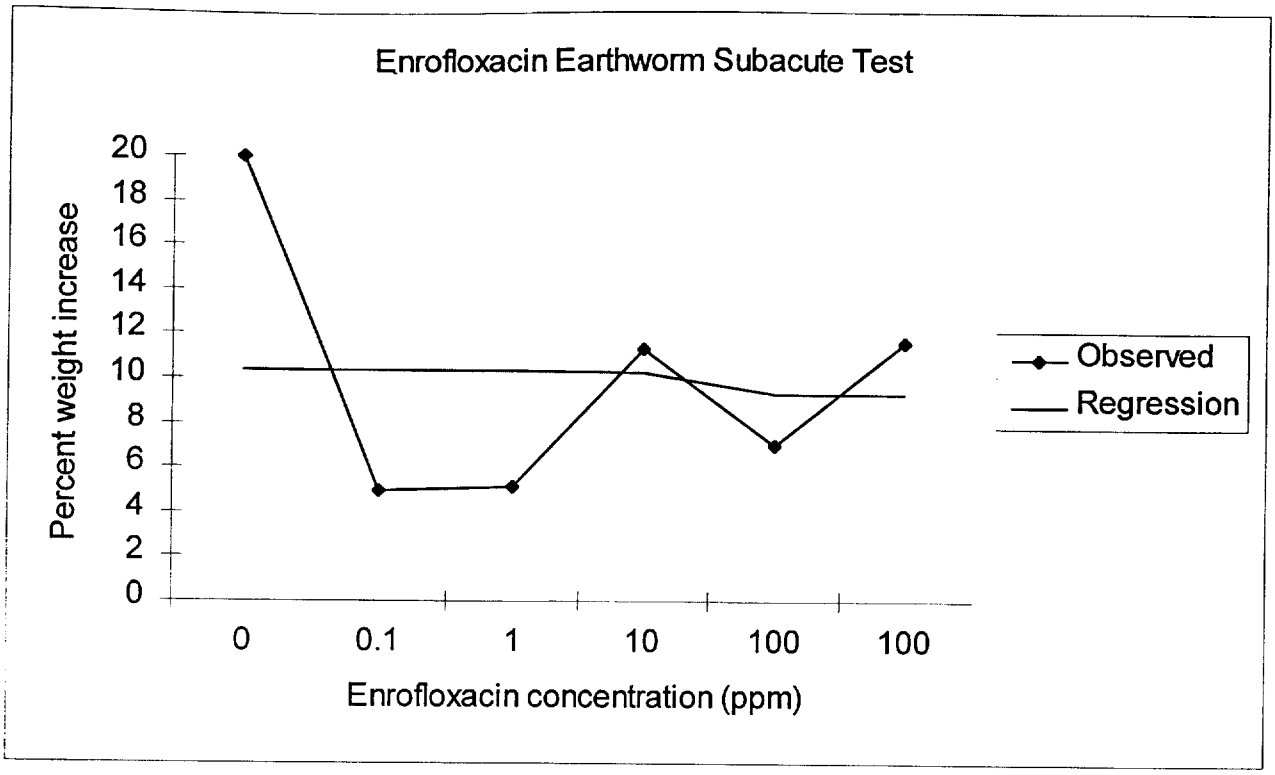
Author: T. B. Waggoner

Reference: M. C. Bowman and Associates
Mount Ida, Arkansas

Summary: The study was conducted according to TAD Guideline 4.12. Earthworms were exposed to 0.1, 1.0, 10, 100, and 1,000 ppm enrofloxacin for 28-days in a test soil medium. No mortality was observed in the control or any treatment level. No adverse effects were observed of the control soil or any treatment level. The no observed effect level was determined to be ≥ 1000 ppm enrofloxacin.

TAD 4.12 states that in experiments where substrates containing the highest levels of the test chemical fail to yield 50% mortality, a definitive test is not indicated. The enrofloxacin earthworm study was conducted as a range-find or preliminary study using one replicate per test level, as allowed by the TAD. Due to the lack of mortality in this preliminary study, a definitive study was not conducted as per TAD 4.12. Moreover, the TAD does not specify that individual worm weights are required for either a range-find or definitive test, therefore, only mean worm weights were calculated for the enrofloxacin worm study by measuring the total mass of the surviving worms and dividing that value by the number of worms weighed.

The TAD recommends regression analysis to determine if there was an observable adverse effect over the range of concentrations tested. Essentially, if the slope of the regression line for the weight data is zero or very close to zero, then there are no adverse effects with respect to growth. The regression analysis is presented below.



The regression line is defined by the equation:

Percent weight gain = [-0.010 x (enrofloxacin concentration)] + 10.37

where -0.010 is the slope of the regression line. Since the slope of the line is very close to zero, this demonstrates that there are no adverse effects on worm growth due to enrofloxacin.

Bayer Report No.: 106793

Title: Subacute Toxicity of Ciprofloxacin to the Earthworm, *Lumbricus terrestris*

Authors: D. C. England and J. Veltri

Reference: ABC Laboratories
ABC Study No. 41638

Summary: An earthworm subacute toxicity study was conducted in accordance with TAD Guideline 4.12. Earthworms, *Lumbricus terrestris*, were exposed for 28 days to concentrations of ciprofloxacin ranging from 10 to 1000 µg ciprofloxacin/g soil (ppm). Over the course of the 28-day exposure, no adverse effects, including mortality, discoloration, and decreased worm weight and mobility were noted at any of the concentrations of ciprofloxacin. The no observed effect level was determined to be ≥1000 ppm.

Bayer Report No.: 106661

Title: Seed Germination and Root Elongation Study Using Enrofloxacin

Authors: R. S. Chetram and C. Cone

Reference: ABC Laboratories, Inc. - Pan-Ag Div.
Pan-Ag Study No. 93329

Summary: The effect of enrofloxacin on the seed germination and root elongation of six species of plants was determined in accordance with FDA Technical Assistance Document Guideline 4.06. The six species represent monocots and dicots: ryegrass, wheat, soybean, tomato, lettuce, and cucumber. The seeds were incubated on paper in small petri plates or pans, and the paper was treated with the dosing solutions of enrofloxacin.

A preliminary root elongation test indicated significant reduction ($p < 0.05$) in root elongation at 1.0, 10, 100, and 1000 mg enrofloxacin/L in ryegrass and wheat. A root elongation definitive test was conducted on these two crops using the following concentrations: 1.25, 2.5, 5.0, and 10 mg/L plus positive and negative controls.

Root elongation was measured three days after treatment for wheat and six days after treatment for ryegrass. Results of statistical analyses of root elongation showed significant reductions ($p < 0.05$) for ryegrass and wheat at 1.25 mg/L. A second definitive test was conducted for ryegrass and wheat using the following concentrations: 0.031, 0.63, 1.25, and 2.50 mg/L plus positive and negative controls.

The data were statistically analyzed by ANOVA and Dunnett's test. The proportion of seeds germinated were transformed prior to analysis using an arc sine transformation. The sensitivity analysis used to check the power of the statistics was that specified by FDA TAD Guideline 5.02.

Enrofloxacin concentrations ranging from 1 to 1000 µg/mL water (ppm) had no effect on the germination of the seeds of the six species, and therefore, the no effect concentration for enrofloxacin on seed germination was 1000 ppm. Enrofloxacin did reduce the growth of the radicle (young root) which emerged from the seeds. The NOEC for soybean was 12.5 ppm, for lettuce was 2.5 ppm, for ryegrass and wheat was 0.63 ppm, for tomato was 0.50 ppm, and for cucumber was 0.25 ppm. Cucumber was the most sensitive species.

Bayer Report No.: 74576

Title: Seed Germination and Root Elongation Phytotoxicity Study With Enrofloxacin in Soil

Authors: E. Feutz and C. Lochhaas

Reference: ABC Laboratories, Inc.
ABC Study No. 42185

Summary: The study was conducted following methodologies similar to TAD Guideline 4.06. In a previous TAD 4.06 study (Bayer Report No. 106661), the species most sensitive to enrofloxacin was cucumber, and the no effect concentration (NOEC) was 0.25 pm. To determine the effect of enrofloxacin on cucumbers planted in soil, a new study was conducted. The effect of enrofloxacin on the seed germination and root elongation of cucumber was determined in accordance with FDA Technical Assistance Document 4.06, with the exception that sandy loam was used in place of the filter paper specified in the guideline. The cucumber seeds were planted in glass dishes containing soil previously amended with enrofloxacin at the nominal concentrations of 1, 10, 100, and 1000 µg/g soil (ppm). Slight effects of enrofloxacin on seed germination and root elongation were noted at the 100 ppm level.

Replicate 1 from the 1 ppm treatment and replicate 3 from the 100 ppm treatment were deleted from statistical analysis of the percent germination and radicle length data. These replicates had aberrantly low germination in comparison to all other test dishes. The cause for this, in the opinion of the study director, did not indicate a treatment response to the test chemical. The rationale for omission from statistical analysis was based on the biological observation that these replicates were outliers.

The power of the ANOVA and Dunnett's test appeared to be adequate since significant differences were detected at the levels that were visually observed to have chemical related effects.

No test concentrations between 10 and 100 ppm were tested, and the NOEC was determined to be 9.1 ppm for both seed germination and root elongation. Thus, soil greatly reduced (at least 36-fold) the effect of enrofloxacin on cucumber.

Bayer Report No.: 106911

Title: Seed Germination and Root Elongation Study Using Ciprofloxacin

Authors: R. S. Chetram and C. Cone

Reference: ABC Laboratories, Inc. - Pan-Ag Div.
Pan-Ag Study No. 93328

Summary: The effect of ciprofloxacin on the seed germination and root elongation of six species of plants was determined in accordance with FDA Technical Assistance Document 4.06. The six species (ryegrass, wheat, soybean, tomato, lettuce, and cucumber) represent monocots and dicots. The seeds were incubated on paper in small petri plates or pans, and the paper was treated with the dosing solutions of ciprofloxacin.

The data were statistically analyzed by ANOVA and Dunnett's test. The proportion of seeds germinated were transformed prior to analysis using an arc sine transformation. The sensitivity analysis used to check the power of the statistics was that specified by FDA TAD Guideline 5.02.

Ciprofloxacin concentrations ranging from 1 to 1000 µg/mL water (ppm) had no effect on the germination of the seeds of the six species, and the no effect concentration for ciprofloxacin on seed germination was 1000 ppm. Ciprofloxacin did reduce the growth of the radicle (young root) which emerged from the seeds. The NOEC for ryegrass was 3.13 ppm, for wheat was 2.50 ppm, for soybean and tomato was 1.25 ppm, for cucumber was 1.0 ppm, and for lettuce was 0.25 ppm. Lettuce was the species most sensitive to the effects of ciprofloxacin on root elongation.

Bayer Report No.: 74583

Title: Seedling Growth Phytotoxicity Test With Enrofloxacin

Authors: D. Judy and C. Lochhaas

Reference: Analytical-Biochemistry Laboratories, Inc.
ABC Study No. 42183

Summary: The effect of enrofloxacin on the seedling growth of six species of plants was determined in accordance with TAD Guideline 4.07. The six species represent monocots and dicots: ryegrass, wheat, soybean, tomato, lettuce, and cucumber. The seedlings were germinated in vermiculite prior to test initiation. On the day of test initiation, the seedlings were transplanted to sand, and the sand was treated with the dosing solutions of enrofloxacin. The seedlings were exposed to concentrations of enrofloxacin ranging from 0.12 to 8.20 μg/mL nutrient solution (ppm) for three weeks. Visual toxicological observations were made of the plants at weekly intervals. At the end of the three weeks, the NOEC for visual effects were noted for ryegrass at 0.13 ppm, soybean at 0.50 ppm, tomato at 2 ppm, lettuce at 2 ppm, and cucumber at 0.25 ppm. For wheat, visual toxicological effects were noted by the third week for the lowest concentration (0.13 ppm). Shoot length measurements were obtained at weekly intervals during the test, and shoot dry weight and root dry weight were obtained at the termination of the study. The overall NOEC for ryegrass was 0.13 ppm, for wheat was <0.13 ppm, for soybean was 1 ppm, for tomato was <0.250 ppm, for lettuce was 0.5 ppm, and for cucumber was 0.25 ppm.

Bayer Report No.: 74511

Title: FDA Seedling Growth Phytotoxicity Test With Enrofloxacin and Ciprofloxacin

Authors: D. Judy and C. Lochhaas

Reference: ABC Laboratories, Inc.
ABC Study No. 42587

Summary: In a previous study with enrofloxacin (Bayer Report No. 74583), wheat and tomato demonstrated a sensitivity to enrofloxacin. To determine the effect of enrofloxacin and ciprofloxacin on wheat and tomato seedlings grown in soil (as opposed to sand in the previous TAD 4.07 study), a new study was conducted in accordance with FDA Technical Assistance Document 4.07, with the exception that sandy loam was used in place of the sand specified in the guideline. Wheat and tomato seedlings were transplanted to soil previously amended with enrofloxacin at the nominal concentrations of 0.50, 5.0, 10, 50, and 100 µg/mL of nutrient media (ppm) or ciprofloxacin at the nominal concentrations of 0.10, 1.0, 5, 10, and 50 µg/mL of nutrient media (ppm). The seedlings were maintained in this system for 3 weeks and periodically watered with a nutrient solution containing the requisite concentration of enrofloxacin or ciprofloxacin. Shoot length, shoot dry weight, and root dry weight of the treated seedlings were measured and compared to these parameters for the untreated seedlings. The no effect level for wheat exposed to enrofloxacin was 4.7 ppm and for ciprofloxacin was >49 ppm. The no effect level for tomato exposed to enrofloxacin was 9.5 ppm and for ciprofloxacin was >49 ppm.

Bayer Report No.: 106599

Title: Microbial Growth Inhibition with Enrofloxacin

Authors: J. Wood, T. Bielefeld, and I. Kelley

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41571

Summary: The study was conducted in accordance with TAD Guideline 4.02. *Pseudomonas aeruginosa, Arthrobacter picolinophilus, Azotobacter vinelandii, Anabaena flos-aquae, Aspergillus clavatus, Penicillium canescens,* and *Trichoderma hamatum* were maintained for 4 days on growth media amended with enrofloxacin at nominal concentrations ranging from 1.3 to 250 ppm (the maximum solubility of enrofloxacin). The minimum inhibitory concentration (MIC) was 12.5 ppm for *P. aeruginosa, A. picolinophilus,* and *A. flos-aquae*. The MIC was 1.3 ppm for *A. vinelandii*. No inhibition of growth was observed for *A. clavatus, P. canescens*, or *T. hamatum*.

Bayer Report No.: 106750

Title: Microbial Growth Inhibition with Ciprofloxacin

Authors: J. Wood, T. Bielefeld, and I. Kelley

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 41572

Summary: The study was conducted in accordance with TAD Guideline 4.02. *Pseudomonas aeruginosa, Arthrobacter picolinophilus, Azotobacter vinelandii, Anabaena flos-aquae, Aspergillus clavatus, Penicillium canescens,* and *Trichoderma hamatum* were maintained for 4 days on growth media amended with ciprofloxacin at nominal concentrations ranging from 1 to 60 ppm (the maximum solubility of ciprofloxacin). The minimum inhibitory concentration (MIC) was 10 ppm for *P. aeruginosa, A. picolinophilus,* and *A. flos-aquae*. The MIC was 1 ppm for *A. vinelandii*. No inhibition of growth was observed for *A. clavatus, P. canescens*, or *T. hamatum*.

Bayer Report No.: 107124

Title: Bioavailability of Enrofloxacin in Soil and Manure and its Effect on Microbial Growth Inhibition

Authors: Z. Yan and I. Kelley

Reference: ABC Laboratories, Columbia, MO
ABC Study No. 42632

Summary: The bioavailability of enrofloxacin in soil and manure and the potential effect of this compound on the growth inhibition of two soil microbes were investigated. The test species selected, *Arthrobacter picolinophilus* and *Azotobacter vinelandii*, were the most sensitive in the FDA TAD 4.02 guideline study (Bayer Report No. 106599), with MICs of 12.5 and 1.3 mg/L on agar plates. The two species were exposed to concentrations of enrofloxacin in a soil/manure matrix ranging from 1.3 to 500 mg enrofloxacin per kg soil/manure matrix. The MICs of these bacteria in soil and manure were > 500 mg/kg. Thus, enrofloxacin was not bioavailable to the test species at concentrations as high as 500 mg/kg soil/manure and had no inhibitory effects on the growth of the test organisms.

Bayer Report No.: 73194

Title: 90-Day Oral Toxicity Study in Rats

Author: R. L. Kowalski

Reference: Miles Laboratories
Elkhart, Indiana

Summary: Sprague-Dawley rats (15 per sex per treatment group) were exposed to 0, 500, 2000, and 7500 ppm enrofloxacin for a minimum of 91 days via oral exposure through feed. No test article-related signs of toxicosis nor death occurred. A statistically significant reduction in mean body weight for males and females from the high-dose group was observed. No treatment-related trends were observed in hematological parameters. Total protein levels were significantly decreased for both sexes in the high-dose group after weeks 6 and 13. Aspartate aminotransferase was decreased for the high-dose males after 6 and 13 weeks. A decrease in total bilirubin occurred in the high-dose males. A dose-dependent increase in inorganic phosphorous was reported in the males and females after 13 weeks. A dose-dependent trend toward decreasing urine sodium output was reported for males in the mid- and high-dose groups at 6 weeks and for females in the mid- and high-dose groups after 13 weeks. Heart weights were reduced for mid-dose and high-dose animals. Mean prostate weights were significantly lower for mid- and high-dose males. Liver weights were reduced at the high-dose levels. Swollen external ears and distention of the cecum were primarily in the high-dose animals. Auricular chondropathy was observed in all groups including the controls, but appeared to be dose-related (1 control, 1 low-dose, 6 mid-dose, and 10 high-dose). Microscopic changes of questionable relationship to the test article were observed in the knee joints of 3 of 30 rats in the high-dose group. Microscopic change occurred in the epididymides and testes of male rats from the high-dose group. This change appeared to represent a degenerating spermatid. The Agency concluded a NOEL of 500 ppm (40 mg/kg).

Bayer Report No.: 73159

Title: Teratology Study in Rats

Author: G. R. Clemens

Reference: Miles Laboratories
Elkhart, Indiana

Summary: Charles River Rats (28 females) were exposed to enrofloxacin technical drug substance via oral suspension. The test levels were 0, 50, 210, and 875 mg/kg for 10 consecutive days from the sixth through the fifteenth day of gestation. All dams survived, and there were no remarkable observations attributable to the test article. Body weight gain was significantly reduced in the highest test level group. There was no statistically significant differences in any reproductive parameter for any test article group when compared with the control. Fetal weights for both males and females were significantly reduced for the high-dose group. Male and combined fetal weights were significantly reduced also for the mid-dose group. No gross morphological external and visceral changes attributable to the test article were observed in the fetuses, but they were generally smaller in the high-dose group compared to the controls. There was no increase in incidence of common skeletal variations, but a statistically significant delay in ossification was observed in the mid- and high-dose groups which accompanied the overall reduction in body weights for these groups. The Agency concluded a NOEL of 50 mg/kg/day for the study.

Bayer Report No.: 73314

Title: Two-Generation Reproduction Study in Rats

Author: G. R. Clemens

Reference: Miles Laboratories
Elkhart, Indiana

Summary: Sprague-Dawley rats (120 F_0 animals per sex per treatment group) were exposed to 0, 500, 2000, and 7500 ppm technical enrofloxacin via feed over a duration of two generations. No overt toxicity signs at any dietary concentration. General behavior and appearance was essentially normal for the F_0 and F_1 generations. Swollen pinnae were reported in both the F_0 and F_1 generations. Body weights for the high-dose males and females from the F_0 and F_1 generations were significantly reduced during most of the study. A significant reduction in food intake for the F_1 generation was seen in high-dose males. Libido was unaffected and breeding performance was considered favorable for males from both generations. No meaningful alteration occurred in any reproductive parameter for dams exposed to 500 ppm or 2000 ppm of test article in the diet. There was a marked reduction in reproductive performance in the dams receiving the 7500 ppm level. Pregnancy rates, total number of pups born, litter size, number of implantations, and birth index which were significantly reduced for the high-dose group; the length of gestation was significantly increased in the high-dose group. There was no increase in the number of stillbirths for any dose group from either generation when compared to the controls. The high-dose level significantly reduced neonatal survival and neonatal weight gain during lactation. Unilaterally, small testes were reported. No changes occurred in the female reproductive tissues. Spermatic morphological alterations were seen in the high-dose males from both generations. Cecal dilatation, unilateral testicular atrophy and reduced prostatitis were observed in the various treatment groups. The Agency concluded a NOEL of 2000 ppm (165 mg/kg) for the study.

Bayer Report No.: 74230

Title: Chronic Toxicity and Carcinogenicity in Rats

Author: E. Bomhard

Reference: Bayer Institute for Toxicology
Wuppertal, Germany

Summary: Wistar rats (60 animals per sex per treatment group) were dosed via feed with 0, 770, 2000, and 6000 ppm enrofloxacin technical drug substance for 105 weeks. Mortality increased for both males and females at the 6000 ppm level. The body weight development of males was slightly retarded at 6000 ppm. Hematology investigations were carried out after 6, 12, and 18 months and at the end of the study. Leukopenia was seen in the 770 to 6000 ppm groups for both the males and females. Temporarily decreased erythrocyte, hemoglobin, hematocrit, and MCV values were observed at the higher doses. Total protein was significantly decreased in males at all doses and at all sampling intervals. Total protein decreases were less for females. Urinalysis performed at 6-month intervals indicated the test substance did not lead to toxicologically relevant kidney damage. There was no evidence of treatment-induced changes in the refractile media, the ocular fundus, or the pupillary reflex after one year and at the end of the study. A statistically significant increase in the incidence of hepatic cysts was found in both sexes after 2000 and 6000 ppm treatments, and a biologically significant increase was found in males at 770 ppm. Both sexes showed a significant increase in the incidence of dilated caecum after 6000 ppm. Size of testes was reduced and consistency altered in male treatments at 2000 - 6000 ppm. Absolute and relative liver weights were decreased in males at 2000 ppm and above. The same decreases were seen in the females at the interim, but not at the study termination. In the 6000 ppm males, absolute weights of the brain, testes, and adrenals were significantly decreased, but the relative weights were no different than the controls. The incidence of bile-duct hyperplasia in the liver increased in a dose-related response in all treatment groups and both sexes. Sclerotic changes were statistically significant from 770 ppm in the males and from 2000 ppm in females. Cystic bile-duct hyperplasia increased with drug exposure from 770 ppm in the males and 2000 ppm in females. A marked increase in the incidence of cardiomyopathy was found in males at the 6000 ppm dose and in females dosed at 770 ppm and above. There was also a significant increase in the number of subendocardial proliferative lesions in males and females at the highest dose as well as an elevated number of subendocardial mesenchymal tumors. There was a marked increase in the number of sarcolemmal nuclei in skeletal muscles of animals in the 6000 ppm treatments. This lesion is generally associated with degenerative changes in the muscle fibers. Marked degenerative changes were also noted in the sciatic nerve. Additional

histopathological lesions included fewer females in the treated groups showed mammary alveolar development and milk secretion than the control animals. The Agency concluded a NOEL was not established for the study due to incidence of bilary duct hyperplasia and cardiomyopathy. There was no evidence of carcinogenic effect in rats dosed with 770 to 6000 ppm enrofloxacin for two years.