Study Title

Environmental Assessment for Enrofloxacin BAYTRIL® 3.23% Concentrate Antimicrobial Solution

Guideline

21 CFR Part 25

Authors

G. G. Gagliano

F. T. McNamara

Sponsor

Bayer Corporation

Agriculture Division

Animal Health

P.O. Box 390

Shawnee Mission, Kansas 66201-0390

Table of Contents

Table of Contents

List of Appendices

1.0 Date
February, 1996

2.0 Name of Applicant
Bayer Corporation, Agriculture Division, Animal Health

3.0 Address
Bayer Corporation
Agriculture Division
Animal Health
P.O. Box 390
Shawnee Mission, Kansas 66201-0390

Inquiries regarding this assessment should be directed to:
Greg Gagliano
Phone: 913-268-2751

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, (enrofloxacin), for use in poultry, specifically chickens and turkeys. Enrofloxacin is a fluoroquinolone antibiotic that will provide the poultry industry an efficacious product for the treatment of *Escherichia coli* infections in chickens and *E. coli* and *Pasteurella multocida* (fowl cholera) in turkeys. An estimated population of 6.7 billion birds is anticipated to benefit from this product during the first year of introduction.

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 in chicken houses and turkey raising operations. Finished products will be stored in distribution centers throughout the United States prior to transportation to veterinary clinics. BAYTRIL® 3.23% Concentrate Antimicrobial 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

Proposed Bulk Drug Specifications

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® 3.23% Concentrate Antimicrobial Solution formulation.

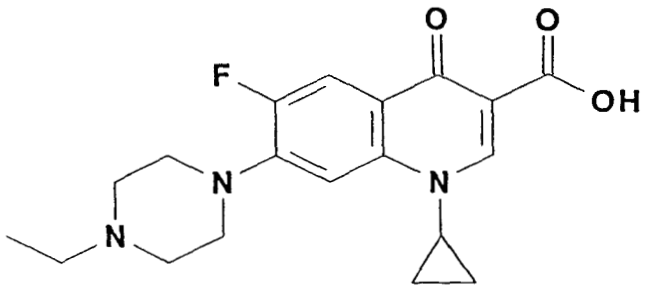
5.2.1 Enrofloxacin

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 poultry for treatment of *E. coli* infections in chickens and turkeys and fowl cholera in turkeys .

5.2.2 Propylene Glycol
CAS Registry No.: 000057-55-6
Molecular Formula: $C_3H_8O_2$
Molecular Weight: 76.1
Description: Viscous, hygroscopic liquid

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 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® 3.23% Concentrate Antimicrobial Solution 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® 3.23% Concentrate Antimicrobial 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® 3.23% Concentrate Antimicrobial 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 BAYTRIL® 3.23% Concentrate Antimicrobial Solution is for the treatment of *E. coli* infections in chickens and *E. coli* and *P. multocida* (fowl cholera) infections in turkeys. The label dose is 25 to 50 ppm in drinking water for 3 to 7 days. The drug will be packaged in one-quart and one-gallon containers.

To identify what the significant excretion products are from chickens and turkeys treated with BAYTRIL® 3.23% Concentrate Antimicrobial Solution, excreta were analyzed.

Excreta from male and female turkeys previously treated with ^{14}C-enrofloxacin equivalent to the recommended treatment rate (50 ppm in the drinking water for 7 days; Bayer Report No. 106544-1) were analyzed to determine the metabolites excreted from turkeys. Of the radioactivity in the excreta, 69% was comprised of enrofloxacin and enrofloxacin conjugates, 23% as ciprofloxacin and a ciprofloxacin conjugate, 1% as oxociprofloxacin, and 7% as bound residues. Two unknown metabolites each represented $\leq$0.4% of the total radioactivity in the excreta. Enrofloxacin and ciprofloxacin are the primary metabolites excreted by turkeys and represent 92% of the excreted radioactivity. The remaining residues each represent <10% of the administered dose (Bayer Report No. 106632).

Excreta from male and female chickens previously treated with ^{14}C-enrofloxacin equivalent to the recommended treatment rate (50 ppm in the drinking water for 7 days;

Bayer Report No. 106543-1) were analyzed to determine the metabolites excreted from chickens. Of the radioactivity in the excreta, 87% was comprised of enrofloxacin, 1% was a mixture of hydroxylated enrofloxacin and a conjugate of enrofloxacin, 2% was ciprofloxacin, 7% as bound residues, and five minor metabolites each represented <1% of the excreted radioactivity. The primary residues excreted by chickens are enrofloxacin and ciprofloxacin; the remaining residues each represent <10% of the administered dose (Bayer Report No. 106937).

Based on these excretion profiles, enrofloxacin and ciprofloxacin are the only significant substances introduced into the environment from the use of enrofloxacin with chickens and/or turkeys.

Statistics from the United States Department of Agriculture indicate that there were approximately 6.4 billion chickens and approximately 288 million turkeys raised in the United States in 1992 (USDA, 1993). Most of the production was centered in the states of (in descending order of production): Arkansas, Georgia, Alabama, North Carolina, Mississippi, Texas, Maryland, Delaware, California, and Virginia (USDA, 1993). Most of the turkey production was centered in the states of (in descending order) North Carolina, Minnesota, California, Arkansas, Missouri, Virginia, Indiana, Pennsylvania, Iowa, and South Carolina (USDA, 1993).

Manure Handling and Disposal Practices

The major use of BAYTRIL® 3.23% Concentrate Antimicrobial Solution will be in broiler houses and turkey raising operations. A general description of broiler and turkey management practice is provided to support an understanding of terrestrial and aquatic exposures resulting from the use of BAYTRIL® 3.23% Concentrate Antimicrobial Solution.

Turkeys

Large turkey houses contain about 25,000 birds and are set twice per year. In a typical turkey house operation the excreta is mixed with sawdust and wood shavings (in a 3 to 1 ratio). The manure is removed from the houses once per year (after the second setting) and typically is spread immediately over agricultural fields. On occasion, the litter may be stockpiled prior to being spread.

Dry litter 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 3 to 5 dry tons per acre (4 dry tons average) based on the nitrogen content of the soil, the nitrogen content of the litter, and the nitrogen requirements of the crop being planted in the field.

Chickens

Large broiler houses contain about 23,000 birds and are usually set six times per year. In a typical broiler house operation the excreta is mixed with rice hulls/wood shavings (in a 5 to 1 ratio). The manure is removed from the houses once per year (after the sixth setting) and typically is spread immediately over agricultural fields. On occasion, the litter may be stockpiled prior to being spread.

Dry litter 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 3 to 5 dry tons per acre (4 dry tons average) based on the nitrogen content of the soil, the nitrogen content of the litter, and the nitrogen requirements of the crop being planted in the field.

Currently, biodegradation of broiler and turkey litter is limited to composting of wet cakes (clumps of litter) removed from the houses between sets. Carcasses of dead birds are also added to this compost pile. The final composted litter and carcasses are spread over agricultural fields.

To determine the concentration of drug residues which could be applied to an agricultural field, the following scenario and assumptions were used:

Turkeys

A typical turkey house will have 25,000 birds per set. Morbidity ranges from 2 to 25 percent for *E. coli* and up to 100 percent for fowl cholera (Bayer Veterinary technical services estimates). The USDA does not compile information on turkey morbidity (Donna Carver, poultry specialist - personal communication, 1995), but these estimates appear to be reasonable. Birds contracting E. *coli* infections are usually 3 to 4 weeks old and birds contracting fowl cholera are usually 10 to 12 weeks old. It is assumed that all birds with these two diseases will be treated with enrofloxacin. In actual practice, an entire house is treated for disease not just individual birds. Therefore, it is assumed that one setting out of 4 (25 percent) will be treated with enrofloxacin. This equates to one house being treated once every other year.

Although young birds are those typically treated, for this assessment a conservative estimate is used, i.e. adult birds will be treated (15 lbs). This is a very conservative estimate and represents a worst-case scenario since large birds ingest more drug than smaller birds.

An adult 15-lb turkey produces approximately 0.71 lbs of wet manure per day with a solids content of 25.5 percent (Ohio State University, 1992). This equates to 0.18 lbs of dry manure per day (0.71 lbs x 0.255 solids = 0.18 lbs dry manure solids).

At a rate of 0.18 lbs dry manure/day, for one setting (180 days), a turkey house with 25,000 birds will produce:

(25,000 birds) x (0.18 lbs dry manure/day/bird) x (180 days/setting) =

8.1×10^5 lbs dry manure per setting or 3.67×10^5 kg dry manure per setting.

Essentially, the amount of drug ingested by a bird is nearly 100 percent excreted by the bird. The concentration of drug residues in the manure of turkeys treated with enrofloxacin can be estimated from the recommended dosage and the average amount of manure produced by commercially raised turkeys. The treatment dosage can be calculated based on the label rate of 50 mg/L (ppm) in drinking water for 7 days and water consumption per bird (0.64 L/bird/day) as follows:

$$\frac{(50 \text{ mg enrofloxacin/L}) \times (0.64 \text{ L/bird/day}) \times (7 \text{ days}) \times (25{,}000 \text{ birds/setting})}{(3.67 \times 10^5 \text{ kg dry manure/setting})} =$$

15.3 mg drug residues/kg dry manure

This represents the maximum concentration of residue in the excreta from turkeys. In actual practice the drug residues in the litter will be less since the excreta from treated birds will be mixed with excreta from untreated birds.

Chickens

A typical broiler house will have 23,000 bird per set. Morbidity ranges from 2 to 3 percent (Bayer Veterinary technical services estimates) for *E. coli* infections. The USDA does not compile information on chicken morbidity (Donna Carver, personal communication, 1995), but these estimates appear to be reasonable. Birds contracting *E. coli* infections are usually young (1 to 4 weeks old). It is assumed that all infected birds will be treated with enrofloxacin. In actual practice, an entire house is treated for disease not just individual birds. Therefore, it is assumed that one setting out of 6 (17 percent) will be treated with enrofloxacin. This equates to one house being treated once every year and is a very conservative estimate since it assumes a morbidity rate nearly 6 times higher than what occurs in actual broiler operations.

Although young birds (1 to 4 weeks old) are those typically treated, for this assessment a conservative estimate is used, i.e. adult birds will be treated (4 lbs). This is a very conservative estimate and represents a worst-case scenario since large birds ingest more drug than smaller birds. As with turkeys, the amount of drug ingested by a chicken is nearly 100 percent excreted by the bird. A 4-lb broiler chicken produces approximately 0.28 lbs of wet manure per day with a solids content of 25.2 percent (Ohio State University, 1992). This equates to 0.071 lbs of dry manure per day (0.28 lbs x 0.252 solids = 0.071 lbs dry manure solids).

At a rate of 0.071 lbs dry manure/day, for one setting (42 days), a broiler house with 23,000 birds will produce:

(23,000 birds) x (0.071 lbs dry manure/day/bird) x (42 days/setting) =

68,586 lbs dry manure per setting or 31,110 kg dry manure per setting.

The concentration of drug residues in the manure of chickens treated with enrofloxacin can be estimated from the recommended dosage and the average amount of manure produced by commercially raised broiler chickens. The treatment dosage can be calculated based on the label rate of 50 mg/L (ppm) in drinking water for 7 days and water consumption per bird (0.12 L/bird/day) as follows:

$$\frac{(50 \text{ mg enrofloxacin/L}) \times (0.12 \text{ L/bird/day}) \times (7 \text{ days}) \times (23{,}000 \text{ birds/setting})}{(31{,}110 \text{ kg dry manure/setting})} =$$

31.1 mg drug residues/kg dry manure

This represents the maximum concentration of residue in the excreta from chickens. In actual practice the drug residues in the litter will be less since the excreta from treated birds will be mixed with excreta from untreated birds.

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 which may influence its fate in the environment. Report summaries for the environmental fate studies for enrofloxacin and ciprofloxacin are presented in Appendix 3. The following data relate to the fate of enrofloxacin and ciprofloxacin in the environments where the drug will be used.

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 poultry excreta, 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 chicken excreta and turkey excreta was also evaluated. The results are presented in the following table.

Manure/Excreta	Chicken	Turkey
pH	7.3	6.0
Organic Carbon (%)	35.3	32.7
Organic Matter (%)	60.7	56.2
Cation Exchange Capacity (meq/100g)	19.3	38.7
Adsorption (%)	44 - 63	29 - 41
Desorption (%)	33 - 54	51 - 72
K_d	139	64.6
K_{oc}	395	198

As demonstrated by the K_d and K_{oc} values, enrofloxacin also binds to poultry excreta, although not nearly to the extent as with soil.

The sorption of enrofloxacin was up to 63% to chicken excreta, and up to 41% to turkey excreta. The percent desorption of the adsorbed enrofloxacin was up to 54% from chicken excreta and up to 72% for turkey excreta (Bayer Report No. 106557). This study demonstrates that enrofloxacin binds relatively tightly to chicken and turkey feces. However, whatever residues desorb from poultry excreta, will bind tightly and nearly irreversibly to soil upon contact with litter and 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 (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

Although the sorption of ciprofloxacin to poultry excreta was not directly studied, based on the similarity of the enrofloxacin and ciprofloxacin sorption behavior on the same soils and on cattle manure (Bayer Report No. 106557), ciprofloxacin should also bind to poultry excreta to approximately the same extent as enrofloxacin did (discussed on previous page).

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 broiler or turkey houses (only if very windy) or during application of litter 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.

Runoff from Commercial Houses

Runoff from turkey and broiler houses is not expected to contribute drug residues from litter to aquatic environments based on current management practices. Precipitation cannot infiltrate the litter since the houses are roofed. Overland runoff cannot infiltrate the litter since the houses typically have a concrete foundation that extends 18 to 24 inches above the soil line. Therefore, runoff from commercial houses could occur only during significant floods.

Composting Facilities Runoff

Runoff from composting bins will also be negligible since the bins are relatively small (4 feet high by 3 feet deep by 5 feet wide) and are covered with a roof to reduce the chance for infiltration from precipitation.

Amended Cropland Runoff

There is a somewhat greater possibility of runoff from agricultural fields amended with litter 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, 1995). 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 poultry 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:

Litter application rate = 5 tons dry litter per acre
Chicken excreta to litter ratio = 5:1 (83% excreta)
Turkey excreta to litter ration = 3:1 (75% excreta)

Chicken excreta application rate = 0.83 x 5 tons = 4.15 tons per acre (3,765 kg/acre)
Turkey excreta application rate = 0.75 x 5 tons = 3.75 tons per acre (3,402 kg/acre)

Total residues in chicken excreta = 31.1 mg ai/kg excreta
Total residues in turkey excreta = 15.3 mg ai/kg excreta

Soil concentration from applied chicken litter:

(31.1 mg/kg) x (3,765 kg/acre) = 117,092 mg total residues/acre
= 0.258 lbs total residues/acre

Estimated soil concentration from applied turkey litter:

(15.3 mg/kg) x (3,402 kg/acre) = 52,051 mg total residues/acre
= 0.115 lbs total residues/acre

The range of enrofloxacin and ciprofloxacin residues applied per acre can be calculated based on the percentages excreted (87% enrofloxacin, 2% ciprofloxacin for chickens, and 69% enrofloxacin and 23% ciprofloxacin for turkeys):

Chickens:

(0.258 lbs total residues/acre) x (0.87 enrofloxacin) = 0.224 lbs enrofloxacin/acre

(0.258 lbs total residues/acre) x (0.02 ciprofloxacin) = 0.005 lbs ciprofloxacin/acre

Turkeys:

(0.115 lbs total residues/acre) x (0.69 enrofloxacin) = 0.079 lbs enrofloxacin/acre

(0.115 lbs total residues/acre) x (0.23 ciprofloxacin) = 0.026 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 37,893 to 61,006 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:

	Enrofloxacin		Ciprofloxacin	
Parameter	Broilers	Turkeys	Broilers	Turkeys
Application Rate (lbs/acre)	0.224	0.079	0.005	0.026
Soil K_{oc}	74635	74635	37893	37893
Solubility at pH 7 (ppm)	250	250	59.1	59.1
Soil incorporation (inches)	6	6	6	6
Soil half-life (days)	696	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 EECs were calculated to be:

EEC_{water} for enrofloxacin = 0.000016 ppm to 0.000046 ppm

EEC_{water} for ciprofloxacin = 0.0000012 ppm to 0.0000062 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 = 0.00000419 ppm to 0.00000552 ppm

4-day EEC_{water} for ciprofloxacin = 0.00000012 ppm to 0.00000148 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

Chicken and Turkey House Operations

Chicken and turkey houses are non-natural settings and are, therefore, depauperate in flora and fauna. However, some inquiline species, such as mice and voles may be found in and around these operations. Because there is no habitat for wildlife nor any way for larger animals (i.e. birds) to enter a broiler or turkey house, no terrestrial exposures are expected within these structures.

Amended Croplands

The greatest exposure of enrofloxacin drug residues will be through application of litter from treated birds 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 litter inadvertently.

Turkeys

Litter 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, an upper bound of the range of application rates (5 tons dry litter/acre) is used to estimate soil concentrations of drug residues. Litter is composed of 75 percent turkey excreta and 25 percent absorbent material (wood shavings, sawdust, rice hulls, etc.). Therefore, the mass of manure in the litter is 3.75 tons manure/acre (0.75 x 5 tons litter/acre) or 3,402 kg manure/acre.

The litter 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 total drug residues in the top 6 inches of soil are calculated as follows:

$$EEC_{soil} = \frac{(15.3 \text{ mg ai/kg manure}) \times (3{,}402 \text{ kg manure/acre})}{925{,}121 \text{ kg soil/acre}} = 0.056 \text{ mg residues/kg soil}$$

The two main residues of interest that are excreted in turkey excreta are enrofloxacin and ciprofloxacin. These have been shown to be excreted as 69 percent enrofloxacin and 23 percent ciprofloxacin (Bayer Report No. 106632). Therefore, the total drug residues in soil can be converted into enrofloxacin and ciprofloxacin concentrations based on these percentages.

(0.056 mg total residues) x (0.69 enrofloxacin) = 0.039 mg enrofloxacin/kg soil

(0.056 mg total residues) x (0.23 ciprofloxacin) = 0.013 mg ciprofloxacin/kg soil

Chickens

Litter 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, an upper bound of the range of application rates (5 tons dry litter/acre) can be used to estimate soil concentrations of drug residues. Litter is composed of 83 percent chicken excreta and 17 percent absorbent material (wood shavings, sawdust, rice hulls, etc.). Therefore, the mass of manure in the litter is 4.15 tons manure/acre (0.83 x 5 tons litter/acre) or 3,765 kg manure/acre.

The litter is typically incorporated into the top 6 inches of soil by plowing. The mass of the top 6 inches of soil (previously determined) is 925,121 kg/acre-6 inches.

Therefore, the expected environmental concentration (EEC) of total drug residues in the top 6 inches of soil are calculated as follows:

$$EEC_{soil} = \frac{(31.1 \text{ mg ai/kg manure}) \times (3{,}765 \text{ kg manure/acre})}{925{,}121 \text{ kg soil/acre}} = 0.127 \text{ mg residues/kg soil}$$

The two main compounds of interest that are in chicken excreta are enrofloxacin and ciprofloxacin. These have been shown to be excreted as 87 percent enrofloxacin and 2 percent ciprofloxacin (Bayer Report No. 106937). Therefore, the total drug residues in soil can be converted into enrofloxacin and ciprofloxacin concentrations based on these percentages.

(0.127 mg total residues) x (0.87 enrofloxacin) = 0.110 mg enrofloxacin/kg soil

(0.127 mg total residues) x (0.02 ciprofloxacin) = 0.003 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; Cowan *et al.*, 1995). 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 23.0 ppm in the enrofloxacin acute *Daphnia* study (Appendix 3). Using EEC estimates, the range of the Risk Quotients was calculated to be 500,000 to 1,437,500 (see table on following page). This means that the estimated concentration of enrofloxacin in water is half a million to nearly 1.5 million times lower than the lowest concentration of enrofloxacin that affects *Daphnia*. Thus, there is little likelihood that enrofloxacin used to treat poultry entering the aquatic environment would adversely affect *Daphnia*.

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	0.000016 - 0.000046	404,348 - 1,162,500
Trout	Enro	>196	33.5	74501	0.000016 - 0.000046	728,261 - 2,093,750
Daphnia	Enro	79.9	23.0	106595	0.000016 - 0.000046	500,000 - 1,437,500
Daphnia	Enro (chronic)	N/A	9.80	106790	0.000016 - 0.000046	213,043 - 612,500
Hyalella	Enro	> 206	< 12.7	106788	0.000016 - 0.000046	< 276,087

Species	Compound	LC_{50} (ppm)	NOEC (ppm)	Bayer Report No.	EEC_{water} Range (ppm)	Risk Quotient Range
Bluegill	Cipro	> 9.85	≥ 9.85	106791	0.0000012 - 0.0000062	≥ 1,588,710
Trout	Cipro	> 9.4	≥ 9.4	106775	0.0000012 - 0.0000062	≥ 1,516,129
Daphnia	Cipro	> 9.90	≥ 9.90	106596	0.0000012 - 0.0000062	≥ 1,596,774
Hyalella	Cipro	> 10.2	2.24	106783	0.0000012 - 0.0000062	361,290 - 1,866,667

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 *Mycrocystis* 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. This 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, 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, muscle, and skin with adhering fat tissues of broiler chickens and turkeys treated with ^{14}C-enrofloxacin was very rapid (Bayer Report Nos. 106543-1 and 106544-1).

Based on the exposure estimates and toxicity data, enrofloxacin and ciprofloxacin entering an aquatic environment from runoff from agricultural fields amended with excreta 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 litter. The main route of entry for drug residues in terrestrial vertebrate and invertebrate species is through ingestion of excreta 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 to 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 are presented in the tables below.

Species	Compound	Oral LD_{50} (mg/kg)	Bayer Report No.	EEC_{soil} Range (mg/kg)	LD_{50} /EEC_{soil} Ratio Range
Mouse (male)	Enro	> 5,000	73075	0.039 - 0.110	> 128,205
Mouse (female)	Enro	4,336	73075	0.039 - 0.110	39,418 - 111,179
Rat (male)	Enro	> 5,000	73075	0.039 - 0.110	> 128,205
Rat (female)	Enro	> 5,000	73075	0.039 - 0.110	> 128,205
Rabbit (male/female)	Enro	500 - 800	73075	0.039 - 0.110	4,545 - 12,821

Species	Compound	Study Type	NOEL (mg/kg)	Bayer Report No.	EEC_{soil} Range (mg/kg)	NOEL /EEC_{soil} Ratio Range
Dog	Enro	Subchronic	3	73775	0.039 - 0.110	27 - 77
Mouse	Enro	Chronic	150	74229	0.039 - 0.110	1,364 - 3,846
Rats	Enro	Chronic	5.3	74387	0.039 - 0.110	48 - 136
Rats	Enro	Reproduction	6.25	73892	0.039 - 0.110	57 - 160
Rabbit	Enro	Embryotoxicity	25	73705	0.039 - 0.110	227 - 641

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 poultry litter containing 0.110 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/0.110 mg enro) = 273 kg soil (602 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 highest EEC_{soil} values (0.110 ppm enrofloxacin and 0.013 ppm ciprofloxacin) the resulting Risk Quotients would be $\geq$ 9,000 for earthworms exposed to enrofloxacin and 76,923 for earthworms exposed to ciprofloxacin. Therefore, no adverse effects on earthworms are expected in and around agricultural fields amended with excreta 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).

However, the experimental design in the guideline for seed germination and root elongation (TAD 4.06) is an unrealistic one. The use of blotter paper soaked in a solution containing enrofloxacin 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 0.039 to 0.110 ppm for enrofloxacin in soil, the resulting Risk Quotient Range would be 83 to 233.

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.

$$(0.54 \text{ ppm} \times 34)/0.013 = 1{,}412$$

$$(0.54 \text{ ppm} \times 34)/0.003 = 6{,}120$$

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)	$NOEC/EEC_{soil}$ Ratio Range
Cucumber	Enro	Seed germination	≥ 882	106661	0.039 - 0.110	> 22,615
Cucumber	Enro	Root elongation	0.27	106661	0.039 - 0.110	2.5 - 6.9
Cucumber	Enro	Seed germ (soil)	9.1	74576	0.039 - 0.110	83 - 233
Cucumber	Enro	Root elongation (soil)	9.1	74576	0.039 - 0.110	83 - 233
Wheat	Enro	Seedling growth	< 0.13	74583	0.039 - 0.110	< 1.2
Tomato	Enro	Seedling growth (soil)	9.5	74511	0.039 - 0.110	86 - 244
Wheat	Enro	Seedling growth (soil)	4.7	74511	0.039 - 0.110	43 - 121
Lettuce	Cipro	Seed germination	≥ 900	106911	0.003 - 0.013	> 300,000
Lettuce	Cipro	Root elongation	0.54	106911	0.003 - 0.013	41 - 180
Tomato	Cipro	Seedling growth (soil)	≥ 49	74511	0.003 - 0.013	> 16,333
Wheat	Cipro	Seedling growth (soil)	≥ 49	74511	0.003 - 0.013	> 16,333

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 excreta 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)	$NOEC/EEC_{soil}$ Ratio Range
Pseudomonas	Enro	12.5	1.3	106599	0.039 - 0.110	12 - 33
Pseudomonas	Cipro	10	1	106750	0.003 - 0.013	77 - 333
Arthrobacter	Enro	12.5	1.3	106599	0.039 - 0.110	12 - 33
Arthrobacter	Cipro	10	1	106750	0.003 - 0.013	77 - 333
Azotobacter	Enro	1.3	< 1.3	106599	0.039 - 0.110	< 12
Azotobacter	Cipro	1	< 1	106750	0.003 - 0.013	< 77
Anabaena	Enro	12.5	1.3	106599	0.039 - 0.110	12 - 33
Anabaena	Cipro	10	1	106750	0.003 - 0.013	77 - 333
Aspergillus	Enro	> 250	≥ 250	106599	0.039 - 0.110	≥ 6,410
Aspergillus	Cipro	> 60	≥ 60	106750	0.003 - 0.013	≥ 20,000
Penicillium	Enro	> 250	≥ 250	106599	0.039 - 0.110	≥ 6,410
Penicillium	Cipro	> 60	≥ 60	106750	0.003 - 0.013	≥ 20,000
Trichoderma	Enro	> 250	≥ 250	106599	0.039 - 0.110	≥ 6,410
Trichoderma	Cipro	> 60	≥ 60	106750	0.003 - 0.013	≥ 20,000

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 ≥ 4,545.

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 tables.

Species	Study Type	LD_{50} (mg/kg)	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 oral	165 mg/kg	73314
Mouse	Chronic oral	323 mg/kg	74229
Rat (male)	Chronic oral	5.3 mg/kg	74387
Rat (female)	Chronic oral	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® 3.23% Concentrate Antimicrobial Solution will instruct users that this product is for use in animals only and should be kept out of the reach of children. The label will further instruct users to avoid contact with eyes and in case of

contact, immediately flush eyes with copious amounts of water for 15 minutes. Spillage of the product onto the skin is not an acute human 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. Nevertheless, the label will state "Wash hands with soap and water after handling" and will include recommendations concerning human handling for individuals with a history of hypersensitivity to quinolones. Based on the magnitude of the toxicity values, and users following the label warnings, 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 flock will not be treated with enrofloxacin; we estimate that only one flock in a given house will need treatment every two years. 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 poultry 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® 3.23% Concentrate Antimicrobial 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 human health or 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 quality product with no expected adverse effects on human health or the environment. A no-action alternative would result in the deprivation of a beneficial drug to the poultry 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: 8/12/96

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

Date: 8/12/96

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.

Donna L. Lawrence for J. I. H. Phillip Date: 8-12-96

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

14.0 References

American Society of Agricultural Engineers (ASAE). 1992. Proceedings of the National Livestock, Poultry and Aquaculture Waste Management Workshop. 29-31 July 1991, Kansas City, Missouri. ASAE Publication 03-92.

Barnthouse, L. W., G. W. Suter, S. M. Bartell, J. J. Beauchamp, R. H. Garnder, E. Lindner, R. V. O'Neill, and A. E. Rosen. 1986. User's Manual for Ecological Risk Assessment. ORNL-6257. Oak Ridge National Laboratory, Oak Ridge, TN.

Carver, Donna. 1995. Personal communication. Poultry Specialist, USDA-APHIS, Centers for Epidemiology and Animal Health, Fort Collins, CO.

Clark, K. E., A. P. C. Goras, and D. MacKay. 1990. Model of organic chemical uptake and clearance by fish from food and water. **Environmental Science and Technology**. 206: 831-832.

Cowan, C. E., D. J. Versteeg, R. J. Larson, and P. J. Kloepper-Sams. 1995. Integrated Approach for Environmental Assessment of New and Existing Substances. **Regulatory Toxicology and Pharmacology** 21: 3-31.

Ohio State University. 1992. Ohio Livestock Manure & Wastewater Management Guide. Bulletin 604.

USDA. 1993. **Agricultural Statistics 1993**. USDA, National Agricultural Statistics Service, Washington, D.C.

USEPA. 1988. Superfund Exposure Assessment Manual. EPA/540/1-88/001. Office of Remedial Response, Washington, D.C.

USEPA. 1995. GENEEC version 1.2. Office of Pesticide Programs, Washington, D.C.

USFDA. 1987. Environmental Assessment Technical Assistance Handbook. Food and Drug Administration, Washington, D.C.

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 H2O)
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

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.

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.

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

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

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

The Dow Chemical Company
Midland, Michigan 48674
Emergency 517 - 636-4400

Product Code: 70531 Page: 1

Product Name: PROPYLENE GLYCOL USP

Effective Date: 05/20/92 Date Printed: 06/14/93 MSDS:000248

1. INGREDIENTS: (% w/w, unless otherwise noted)

Propylene glycol CAS# 000057-55-6 99%

This document is prepared pursuant to the OSHA Hazard Communication Standard (29 CFR 1910.1200). In addition, other substances not 'Hazardous' per this OSHA Standard may be listed. Where proprietary ingredient shows, the identity may be made available as provided in this standard.

2. PHYSICAL DATA:

BOILING POINT: 370F, 188C
VAP PRESS: 0.08 mm Hg @ 20C, 68F
VAP DENSITY: 2.62
SOL. IN WATER: Complete
SP. GRAVITY: 1.038 20/20C, 68F
APPEARANCE: Colorless liquid.
ODOR: Odorless.

3. FIRE AND EXPLOSION HAZARD DATA:

FLASH POINT: 218F, 103C
METHOD USED: PMCC

FLAMMABLE LIMITS
LFL: 2.6%
UFL: 12.5%

EXTINGUISHING MEDIA: Water fog, alcohol foam, CO2, dry chemical.

FIRE & EXPLOSION HAZARDS: Not available.

FIRE-FIGHTING EQUIPMENT: Wear positive-pressure, self-contained breathing apparatus.

(Continued on page 2)

4. REACTIVITY DATA:

STABILITY: (CONDITIONS TO AVOID) Stable under normal storage conditions.

INCOMPATIBILITY: (SPECIFIC MATERIALS TO AVOID) Oxidizing material.

HAZARDOUS DECOMPOSITION PRODUCTS: Propionaldehyde, carbon monoxide in the presence of limited oxygen in a fire situation.

HAZARDOUS POLYMERIZATION: Will not occur.

5. ENVIRONMENTAL AND DISPOSAL INFORMATION:

ACTION TO TAKE FOR SPILLS/LEAKS: Small spills: Cover with absorbent material, soak up and sweep into a drum. Large spills: Dike around spill and pump into suitable containers.

DISPOSAL METHOD: Reprocess or burn in an approved incinerator in accordance with all federal, state, and local requirements.

6. HEALTH HAZARD DATA:

EYE: May cause slight transient (temporary) eye irritation. Corneal injury is unlikely.

SKIN CONTACT: Prolonged contact is essentially nonirritating to skin. Repeated exposure may cause slight flaking, tenderness, and softening of skin.

SKIN ABSORPTION: A single prolonged exposure is not likely to result in the material being absorbed through skin in harmful amounts. The LD50 for skin absorption in rabbits is >10 g/kg.

INGESTION: Single dose oral toxicity is extremely low. The

(Continued on page 3)

6. HEALTH HAZARD DATA: (CONTINUED)

oral LD50 for rats is 21-33.7 g/kg. No hazards anticipated from ingestion incidental to industrial exposure.

INHALATION: A single prolonged (hours) inhalation exposure is not likely to cause adverse effects. Mists are not likely to be hazardous.

SYSTEMIC (OTHER TARGET ORGAN) EFFECTS: Repeated excessive ingestion may cause central nervous system effects.

CANCER INFORMATION: Did not cause cancer in long-term animal studies.

TERATOLOGY (BIRTH DEFECTS): Birth defects are unlikely. Exposures having no adverse effects on the mother should have no effect on the fetus.

REPRODUCTIVE EFFECTS: In animal studies, has been shown not to interfere with reproduction.

MUTAGENICITY (EFFECTS ON GENETIC MATERIAL): Results of in vitro ('test tube') mutagenicity tests have been negative. Results of mutagenaicity tests in animals have been negative.

7. FIRST AID:

EYES: Irrigate immediately with water for at least 5 minutes.

SKIN: Wash off in flowing water or shower.

INGESTION: No adverse effects anticipated by this route of exposure.

INHALATION: No adverse effects anticipated by this route of exposure incidental to proper industrial handling.

(Continued on page 4)

7. FIRST AID: (CONTINUED)

NOTE TO PHYSICIAN: No specific antidote. Supportive care. Treatment based on judgment of the physician in response to reactions of the patient.

8. HANDLING PRECAUTIONS:

EXPOSURE GUIDELINE(S): AIHA WEEL is 50 ppm total; 10 mg/m3 aerosol only. There is no OSHA PEL or ACGIH TLV for propylene glycol.

VENTILATION: Good general ventilation should be sufficient.

RESPIRATORY PROTECTION: When airborne exposure guidelines and/or comfort levels may be exceeded, use an approved air-purifying respirator.

SKIN PROTECTION: Use impervious gloves when prolonged or frequently repeated contact could occur.

EYE PROTECTION: Use safety glasses. Where contact with liquids is likely, chemical goggles are recommended because eye contact with this material may cause pain, even though it is unlikely to cause injury.

9. ADDITIONAL INFORMATION:

SPECIAL PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE: Exercise reasonable care and caution.

MSDS STATUS: Revised section 6.

REGULATORY INFORMATION: (Not meant to be all-inclusive--selected regulations represented.)

NOTICE: The information herein is presented in good faith and believed to be accurate as the effective date shown above. However, no warranty, express or implied, is given. Regulatory requirements are subject to change and may differ from one location to another; it is the buyer's responsibility to ensure that its activities comply with federal, state or provincial, and local laws. The following specific information is made for the purpose of complying with numberous federal, state or provincial, and local laws and regulations. See MSD Sheet for health and safety information.

U.S. REGULATIONS

SARA HAZARD CATEGORY: This product has been reviewed according to the EPA "Hazard Categories" promulgated under Sections 311 and 312 of the Superfund Amendment and Reauthorization Act of 1986 (SARA Title III) and is considered, under applicable definitions, to meet the following categories:

Not to have met any hazard category

CANADIAN REGULATOINS

WHMIS INFORMATION: The Canadian Workplace Hazardous Materials Information System (WHMIS) Classification for this product is:

This product is not a "Controlled Product" under WHMIS.

CANADIAN TDG INFORMATION: For guidance, the Transportation of Dangerous Goods Classification for this product is:

(Continued on page R-2)

REGULATORY INFORMATION (CONTINUED)

Not regulated.

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 Aldriche
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

MATERIAL SAFETY DATA SHEET

SECTION 1. - - - - - - - - - - CHEMICAL IDENTIFICATION- - - - - - - - - -

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

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

CAS #:100-51-6
MF: C7H8O

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

SECTION 3. - - - - - - - - - - HAZARDS IDENTIFICATION - - - - - - - - - -

LABEL 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

SECTION 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.

CONTINUED ON NEXT PAGE

olland
rich Chemie
lephone: 3238991301
x: 3238991311

Spain
Aldrich Quimica
Apt. de Correos, 161
28100 Alcobendas, Madrid
Telephone: 3416619977
Fax: 3416619642

Italy
Aldrich Chimica
Via Gallarate, 154
20151 Milano
Telephone: 39233417340
Fax: 39238010737

Switzerland
Aldrich Chemie
Industriestrasse 25
CH-9470 Buchs
Telephone: 41817552723
Fax: 41817567420

Belgium
Aldrich Chemie
K. Cardenplein 6
B-2880 Bornem
Telephone: 3238991301
Fax: 3238991311

Czech Republic
Aldrich, s.r.o.
Krizikova, 27
180 00 Prague-8
Telephone: 42224225285
Fax: 42224224031

India
Aldrich-India
84/158 Saidarjung Enclave
New Delhi 110 029
Telephone: 91116886872
Fax: 91116886873

France
Aldrich-Chimie S.a.r.l.
L'Isle D'Abeau Chesnes
B.P. 701
38297 St. Quentin Fallavier
Cedex

Japan
Aldrich Japan
Kyodo Bldg. Shinkanda
10 Kanda-Mikuracho
Chiyoda-Ku, Tokyo
Telephone: 81332580155

Australia
Aldrich Chemicals
Unit 2
10 Anella Ave.
Castle Hill, NSW 2154
Telephone: 6128999977

United Kingdom
Aldrich Chemical Co. Ltd.
The Old Brickyard, New Road
Gillingham, Dorset SP8 4JL
Telephone: 4474822211

Brazil
Aldrich-Brazil
Rua Sabara, 566-conj.53
01239-010 Sao Paulo, SP
Telephone: 55112311666

Germany
Aldrich-Chemie GmbH & Co.KG
Riedstrasse 2, D-89555 Steinheim
Telephone: 4973329970
Fax: 4973291939

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 Aldrich
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

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 AND 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.

CONTINUED ON NEXT PAGE

olland
Sigma Chemie
Telephone: 3238991301
ax: 3238991311

Spain
Aldrich Química
Apt. de Correos, 161
28100 Alcobendas, Madrid
Telephone: 3416619977
Fax: 3416619642

Italy
Aldrich Chimica
Via Gallarate, 154
20151 Milano
Telephone: 39233417340
Fax: 39238010737

Switzerland
Aldrich Chemie
Industriestrasse 25
CH-9470 Buchs
Telephone: 41817552723
Fax: 41817567420

Belgium
Aldrich Chemie
K. Cardijnplein 8
B-2880 Bornem
Telephone: 3238991301
Fax: 3238991311

Czech Republic
Aldrich, s.r.o.
Krakovská, 27
180 00 Prague-8
Telephone: 42224225285
Fax: 42224224031

India
Aldrich-India
84/158 Saidanjung Enclave
New Delhi 110 029
Telephone: 91116886872
Fax: 91116886873

France
Aldrich-Chimie S.a.r.l.
L'Isle D'Abeau Chesnes
B.P. 701
38297 St. Quentin Fallavier
Cedex

Japan
Aldrich Japan
Kyodo Bldg. Shinkanda
10 Kanda-Mikuracho
Chiyoda-Ku, Tokyo
Telephone: 81332580155

Australia
Aldrich Chemicals
Unit 2
10 Aneta Ave.
Castle Hill, NSW 2154
Telephone: 61296899777

United Kingdom
Aldrich Chemical Co. Ltd.
The Old Brickyard, New Road
Gillingham, Dorset SP8 4JL
Telephone: 44747822211

Brazil
Aldrich-Brazil
Rua Sabara, 566-conj.53
01239-010 Sao Paulo, SP
Telephone: 55112311866

Germany
Aldrich-Chemie GmbH & Co.KG
Riedstrasse 2, D-89555 Steinheim
Telephone: 497329970
Fax: 4973291939

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
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

CUST#: 130680
PO#: G54P10117

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

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

PPEARANCE 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

ECTION 10. - - - - - - - - - -STABILITY AND REACTIVITY - - - - - - - - -

NCOMPATIBILITIES
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.
AZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS
TOXIC FUMES OF:
CARBON MONOXIDE, CARBON DIOXIDE

ECTION 11. - - - - - - - - - - TOXICOLOGICAL INFORMATION - - - - - - - -

CUTE 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.

CONTINUED ON NEXT PAGE

olland
Inch Chemie
elephone: 3238991301
x: 3238991311

Spain
Aldrich Quimica
Apt. de Correos, 161
28100 Alcobendas, Madrid
Telephone: 3416619977
Fax: 3416619642

Italy
Aldrich Chimica
Via Gallarate, 154
20151 Milano
Telephone: 39233417340
Fax: 39238010737

Switzerland
Aldrich Chemie
Industriestrasse 25
CH-9470 Buchs
Telephone: 41817552723
Fax: 41817567420

Belgium
Aldrich Chemie
K. Cardinplein 6
B-2880 Bornem
Telephone: 3238991301
Fax: 3238991311

Czech Republic
Aldrich, s.r.o.
Krizkovo. 27
180 00 Prague-8
Telephone: 42224225285
Fax: 42224224031

India
Aldrich-India
84/158 Safdarjung Enclave
New Delhi 110 029
Telephone: 9111686872
Fax: 9111686873

France
Aldrich-Chimie S.a.r.l.
L'Isle D'Abeau Chesnes
B.P. 701
38297 St. Quentin Fallavier
Cedex

Japan
Aldrich Japan
Kyodo Bldg. Shinkanda
10 Kanda-Mikuracho
Chiyoda-Ku, Tokyo

Australia
Aldrich Chemicals
Unit 2
10 Anella Ave.
Castle Hill, NSW 2154

United Kingdom
Aldrich Chemical Co. Ltd.
The Old Brickyard, New Road
Gillingham, Dorset SP8 4JL
Telephone: 44747822211

Brazil
Aldrich-Brazil
Rua Sabara, 566-conj.53
01239-010 Sao Paulo, SP
Telephone: 55112311856

Germany
Aldrich-Chemie GmbH & Co.KG
Riedstrasse 2, D-89555 Steinheim
Telephone: 4973329970
Fax: 4973291939

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 Aldricher
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

CUST#: 130680
PO#: G54P10117

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

TARGET ORGAN(S):
CENTRAL NERVOUS SYSTEM
TECS NO: DN3150000
BENZYL ALCOHOL
RRITATION 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

OXICITY 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
IVN-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

ARGET 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.

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

CONTINUED ON NEXT PAGE

olland nch Chemie elephone: 3238991301 : 3238991311

Spain Aldrich Química Apt. de Correos, 161 28100 Alcobendas, Madrid Telephone: 3416619977 Fax: 3416619642

Italy Aldrich Chimica Via Gallarate, 154 20151 Milano Telephone: 39233417340 Fax: 39238010737

Switzerland Aldrich Chemie Industriestrasse 25 CH-9470 Buchs Telephone: 41817552723 Fax: 41817567420

Belgium Aldrich Chemie K. Cardijnplein 8 B-2880 Bornem Telephone: 3238991301 Fax: 3238991311

Czech Republic Aldrich, s.r.o. Krizkova, 27 180 00 Prague-8 Telephone: 42224225285 Fax: 42224224031

India Aldrich-India B4/156 Safdarjung Enclave New Delhi 110 029 Telephone: 91116886872 Fax: 91116886873

France Aldrich-Chimie S.a.r.l. L'Isle D'Abeau Chesnes B.P. 701 38297 St. Quentin Fallavier Cedex

Japan Aldrich Japan Kyodo Bldg. Shinkanda 10 Kanda-Mikuracho Chiyoda-Ku, Tokyo Telephone: 81332580155

Australia Aldrich Chemicals Unit 2 10 Anella Ave. Castle Hill, NSW 2154 Telephone: 6128999977

United Kingdom Aldrich Chemical Co. Ltd. The Old Brickyard, New Road Gillingham, Dorset SP8 4JL Telephone: 44747822211

Brazil Aldrich-Brazil Rua Sabara, 566-conj.53 01239-010 Sao Paulo, SP Telephone: 55112311866

Germany Aldrich-Chemie GmbH & Co.KG Riedstrasse 2, D-89555 Steinheim Telephone: 497329970 Fax: 49732991939

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
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

CUST#: 130680
PO#: G54P10117

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

SECTION 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.

SECTION 14. - - - - - - - - - - TRANSPORT INFORMATION - - - - - - - - - -

CONTACT ALDRICH CHEMICAL COMPANY FOR TRANSPORTATION INFORMATION.

SECTION 15. - - - - - - - - - - REGULATORY INFORMATION - - - - - - - - - -

REVIEWS, STANDARDS, AND REGULATIONS
EPA 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

SECTION 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.

CONTINUED ON NEXT PAGE

Holland
Aldrich Chemie
Telephone: 3238991301
Fax: 3238991311

Spain
Aldrich Quimica
Apt. de Correos, 161
28100 Alcobendas, Madrid
Telephone: 3416619977
Fax: 3416619642

Italy
Aldrich Chimica
Via Gallarate, 154
20151 Milano
Telephone: 39233417340
Fax: 39238010737

Switzerland
Aldrich Chemie
Industriestrasse 25
CH-9470 Buchs
Telephone: 41817552723
Fax: 41817567420

Belgium
Aldrich Chemie
K. Cardijnplein 8
B-2880 Bornem
Telephone: 3238991301
Fax: 3238991311

Czech Republic
Aldrich, s.r.o.
Krizikova, 27
180 00 Prague-8
Telephone: 42224225285
Fax: 42224224031

India
Aldrich-India
B4/158 Safdarjung Enclave
New Delhi 110 029
Telephone: 91116886872
Fax: 91116886873

France
Aldrich-Chimie S.a.r.l.
L'Isle D'Abeau Chesnes
B.P. 701
38297 St. Quentin Fallavier
Cedex

Japan
Aldrich Japan
Kyodo Bldg. Shinkanda
10 Kanda-Mikuracho
Chiyoda-Ku, Tokyo
Telephone: 81332580155

Australia
Aldrich Chemicals
Unit 2
10 Anella Ave.
Castle Hill, NSW 2154
Telephone: 6128999977

United Kingdom
Aldrich Chemical Co. Ltd.
The Old Brickyard, New Road
Gillingham, Dorset SP8 4JL
Telephone: 44747822211
Fax: 44747823779

Brazil
Aldrich-Brazil
Rua Sabara, 566-conj.53
01239-010 Sao Paulo, SP
Telephone: 55112311666
Fax: 55112579079

Germany
Aldrich-Chemie GmbH & Co.KG
Riedstrasse 2, D-89555 Steinheim
Telephone: 4973329970
Fax: 4973291939

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 Aldrich
Telex: 26 843 Aldrich MI
FAX: (414) 273-4979

CUST#: 130680
PO#: G54P10117

PRODUCT #: 305208 NAME: BENZYL ALCOHOL, 99+%
MF: C7H8O

Holland
Aldrich Chemie
Telephone: 3238991301
Fax: 3238991311

Spain
Aldrich Química
Apt. de Correos, 161
28100 Alcobendas, Madrid
Telephone: 3416619977
Fax: 3416619642

Italy
Aldrich Chimica
Via Gallarate, 154
20151 Milano
Telephone: 39233417340
Fax: 39238010737

Switzerland
Aldrich Chemie
Industriestrasse 25
CH-9470 Buchs
Telephone: 41817552723
Fax: 41817567420

Belgium
Aldrich Chemie
K. Cardijnplein 8
B-2880 Bornem
Telephone: 3238991301
Fax: 3238991311

Czech Republic
Aldrich, s.r.o.
Krizikova, 27
180 00 Prague-8
Telephone: 42224225285
Fax: 42224224031

India
Aldrich-India
84/158 Siddarjung Enclave
New Delhi 110 029
Telephone: 91116886872
Fax: 91116886873

France
Aldrich-Chimie S.a.r.l.
L'Isle D'Abeau Chesnes
B.P. 701
38297 St. Quentin Fallavier
Cedex

Japan
Aldrich Japan
Kyodo Bldg, Shinkanda
10 Kanda-Mikurecho
Chiyoda-Ku, Tokyo
Telephone: 81332580155

Australia
Aldrich Chemicals
Unit 2
10 Anella Ave
Castle Hill, NSW 2154
Telephone: 6128999977

United Kingdom
Aldrich Chemical Co. Ltd.
The Old Brickyard, New Road
Gillingham, Dorset SP8 4JL
Telephone: 44747822211
Fax: 44747823779

Brazil
Aldrich-Brazil
Rua Sabara, 566-conj.53
01239-010 Sao Paulo, SP
Telephone: 55112311866
Fax: 55112579079

Germany
Aldrich-Chemie GmbH & Co.KG
Riedstrasse 2, D-89555 Steinheim
Telephone: 497329970
Fax: 4973291939

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	Düsseldorf, 12.09.95
	2211 - Bi -	

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 Be...

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.: 106544-1

Title: Addendum 1: Total Radioactive Residue Depletion and Metabolism of [4-^{14}C] Enrofloxacin in Turkeys: A Total Residue Depletion Study in Turkeys Following Oral Administration of [4-^{14}C] Enrofloxacin

Authors: C. E. Heird

Reference: Southwest Bio-Labs, Inc.
SBL Study No. 9332t

Summary: Turkeys (10 to 11 weeks old at first dose) were administered enrofloxacin for 7 consecutive days by oral gavage. Each bird was given a total daily dose of [^{14}C]-enrofloxacin equivalent to the maximum recommended treatment rate (50 ppm drinking water) based on water consumption of 800 mL per day. Each bird was dosed with one-third of the total daily dose three times per day. The birds were sacrificed at 6, 10, 15, and 24 hours after the final dose was administered. Excreta, liver, muscle, and skin with adhering fat were collected for analysis. The depletion of the total ^{14}C residue from the tissues was monitored by combustion and radioassay of an aliquot of each tissue from each bird. Briefly, the highest residue levels were in the liver at each withdrawal interval. At 6 hours, liver contained approximately 8.6 ppm, and this level rapidly declined to approximately 3.0 ppm by 24 hours. Muscle contained 2.1 ppm at 6 hours, and this level rapidly declined to approximately 0.43 ppm by 24 hours. Skin with adhering fat contained 0.9 ppm at 6 hours, and this level rapidly declined to approximately 0.27 ppm by 24 hours.

Bayer Report No.: 106632

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

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

Reference: Bayer Research Park
Bayer Study No. EN191402

Summary: Excreta from male and female turkeys previously treated with ^{14}C-enrofloxacin equivalent to the maximum recommended treatment rate (50 ppm in the drinking water for 7 days; Bayer Report No. 106544) were analyzed to determine the metabolites excreted from turkeys. The excreta collected 24 hours after the treatment initiation and 6 or 15 hours after the cessation of treatment were analyzed. No substantial difference in the distribution of the metabolites was noted for the excreta collected from the male and female birds nor for the excreta at either collection interval. Therefore, the average distribution of the metabolites was determined for the turkeys. Of the radioactivity in the excreta, 69% was comprised of enrofloxacin and enrofloxacin conjugates, 23% as ciprofloxacin and a ciprofloxacin conjugate, 1% as oxociprofloxacin, and 7% was bound residues. Two unknown metabolites each represented $\leq$0.4% of the total radioactivity in the excreta. Enrofloxacin and ciprofloxacin are the primary metabolites excreted by turkeys and represent 92% of the excreted radioactivity. The remaining residues each represent <10% of the administered dose.

Bayer Report No.: 106543-1

Title: Addendum 1: Total Radioactive Residue Depletion and Metabolism of [4-^{14}C] Enrofloxacin in Broiler Chickens: A Total Residue Depletion Study in Broiler Chickens Following Oral Administration of [4-^{14}C] Enrofloxacin

Author: C. E. Heird

Reference: Southwest Bio-Labs, Inc.
SBL Study No. 9324c

Summary: Broiler chickens (22 days old at first dose) were administered [^{14}C]-enrofloxacin for 7 consecutive days by oral gavage. Each bird was given a total daily dose of enrofloxacin equivalent to the maximum recommended treatment rate (50 ppm drinking water) based on water consumption of 200 mL per day. Each bird was dosed with one-third of the total daily dose three times per day. The birds were sacrificed at 6, 10, 15, and 24 hours after the final dose was administered. Excreta, liver, muscle, and skin with adhering fat were collected for analysis. The depletion of the total ^{14}C residue from the tissues was monitored by combustion and radioassay of an aliquot of each tissue from each bird. Briefly, the highest residue levels were in the liver at each withdrawal interval. At 6 hours, liver contained approximately 4.9 ppm, and this level rapidly declined to approximately 0.09 ppm by 24 hours. Muscle contained 0.9 ppm at 6 hours, and this level rapidly declined to approximately 0.01 ppm by 24 hours. Skin with adhering fat contained 0.4 ppm at 6 hours, and this rapidly level declined to approximately 0.01 ppm by 24 hours.

Bayer Report No.: 106937

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

Authors: A. M. Kasper, D. W. Irwin, and B. A. Shadrick

Reference: Bayer Research Park
Bayer Study No. EN191402

Summary: Excreta from male and female chickens previously treated with ^{14}C-enrofloxacin equivalent to the recommended treatment rate (50 ppm in the drinking water for 7 days; Bayer Report No. 106543) were analyzed to determine the metabolites excreted from chickens. The excreta collected 24 hours after the treatment initiation and 15 hours after the cessation of treatment were analyzed. No substantial difference in the distribution of metabolites was noted for the excreta collected from the male and female birds nor for the excreta at either collection interval. Therefore, the average distribution of the metabolites was determined for the chickens. Of the radioactivity in the excreta, 87% was comprised of enrofloxacin, 1% was a mixture of hydroxylated enrofloxacin and a conjugate of enrofloxacin, 2% was ciprofloxacin, 7% was bound residues, and five minor metabolites each represented <1% of the excreted radioactivity. The primary residues excreted by chickens are enrofloxacin and ciprofloxacin; the remaining residues each represent <10% of the administered dose.

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 and 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.

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. 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.

Soil Type Soil Series and Source	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.: 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*)

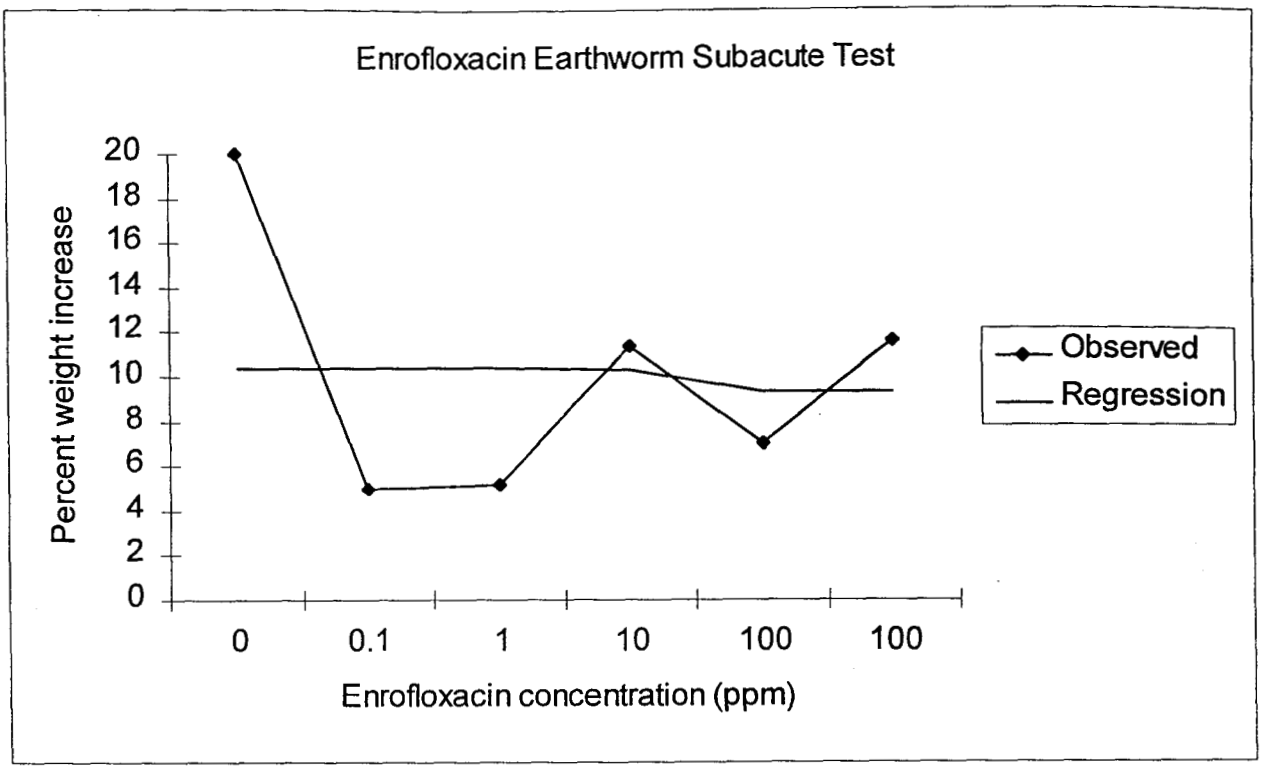
Author: T. B. Waggoner

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 $\geq$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.