# ENVIRONMENTAL ASSESSMENT

## For Use of Corid® (Amprolium) Premix, Oral Solution, and Soluble Powder in Cattle for the Prevention and Treatment of Coccidiosis

**NAME OF APPLICANT/PETITIONER:** **Huvepharma EOOD**

**ADDRESS:** **5th Floor**
**3A Nikolay Haytov Str.**
**Sofia, Bulgaria 1113**

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## LIST OF ACRONYMS

| | |
|---|---|
| AF | assessment factor |
| BW | body weight |
| CVM | Center for Veterinary Medicine |
| $DT_{50}$ | time needed for half the mass of a compound to degrade |
| $DT_{90}$ | time needed for 90% of the mass of a compound to degrade |
| dwt | dry weight |
| EA | environmental assessment |
| $EC_{50}$ | concentration resulting in 50% effect |
| EMA | European Medicines Agency |
| GLP | good laboratory practice |
| $K_{oc}$ | organic carbon-water partition coefficient |
| LOEC | lowest-observed-effect concentration |
| LogP | logarithmic n-octanol/water partition coefficient |
| NADA | new animal drug application |
| NOAEL | no-observed-adverse-effect level |
| NOEC | no-observed-effect concentration |
| OC | organic carbon |
| OECD | Organisation for Economic Co-operation and Development |
| PEC | predicted environmental concentration |
| $PEC_{soil\text{-}initial}$ | predicted environmental concentration in soil, initial |
| $PEC_{surfacewater\text{-}initial}$ | predicted environmental concentration in surface water, initial |
| pKa | negative base-ten logarithm of acid dissociation constant, $K_a$ |
| PNEC | predicted no-effect concentration |
| RQ | risk quotient |
| wwt | wet weight |

# 1. NAME AND ADDRESS OF APPLICANT

Huvepharma EOOD
5th Floor
3A Nikolay Haytov Str.
Sofia, Bulgaria 1113

# 2. PURPOSE AND NEED

## 2.1 Proposed Use and Indications

Corid® refers to any of three different formulations of the active substance amprolium, an anticoccidial used as an aid in the prevention and treatment of coccidiosis caused by *Eimeria bovis* and *E. zurnii* in calves, growing beef steers and heifers fed in confinement for slaughter, growing beef steers and heifers on pasture (stocker, feeder, and slaughter), and replacement beef and dairy heifers under one year of age; not for use in veal calves. Throughout this document, amprolium refers to the active ingredient, and Corid® refers to the finished products.

The assessment of environmental impacts for Corid® is performed according to the following regulatory guidance for preparation of an environmental assessment (EA): Center for Veterinary Medicine (CVM) Guidance for Industry #89 (CVM 2001), CVM Guidance for Industry #166 (CVM 2006), and guidance from the European Medicines Agency (EMA 2016).

## 2.2 Dosage and Route of Administration

Corid® is available in three different product formulations: Type A Medicated Article 25% (premix in feed), a 9.6% oral solution, and a 20% soluble powder. There are two dosing regimens: preventive (5 mg/kg body weight [bw]/d for 21 d) and treatment (10 mg/kg bw/d for 5 d).

# 3. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

## 3.1 Description of Active Ingredient

Amprolium, the active ingredient in Corid®, is an organic chloride salt with pyrimidine, pyridinium, and amine functionality. It is present in Corid® as a hydrochloride salt but is also available as the base molecule, which has a different CAS number. In addition to the active ingredient, the Type A Medicated Article 25% (premix in feed) also contains corn gluten and soybean oil.[1] The 20% soluble powder also contains sucrose and a small amount (1-5%) of fumaric acid (Huvepharma 2015). None of these excipients are anticipated to have an adverse impact on the environment at the levels likely to occur as a result of the proposed use of Corid®.

## 3.2 Description of the Mode of Action of the Active Ingredient

Coccidiosis is a parasitic intestinal infection common in cattle. It is caused by protozoa of the genus *Eimeria*. It can have adverse effects on feed turnover rate and weight gain (Çiçek et al.

[1] https://www.drugs.com/vet/corid-25-type-a-medicated-article.html; accessed June 16, 2023.

2007). Amprolium is an anticoccidial agent. It is a structural analog of thiamine, also known as vitamin B1, and acts by competitive inhibition of cellular thiamine transport (Park et al. 2000).

### 3.3 Physicochemical Properties

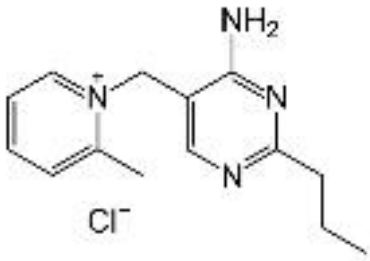
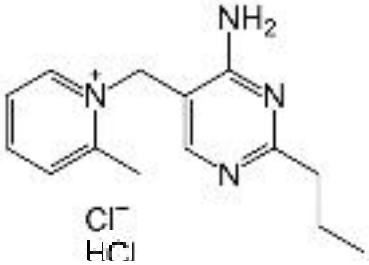
The solubility of amprolium in water was determined in a good laboratory practice (GLP) study following Organisation for Economic Co-operation and Development (OECD) Guideline 105, Water Solubility (Foster and Thomas 2014). A preliminary test indicated that the compound is highly soluble, so the definitive test used the flask method. The method involves equilibration of an aqueous solution with an excess of the solid test substance. Water solubility was determined in pH 5, 7, and 9 buffers, but high amprolium concentrations in these tests caused the actual, measured pHs to be in the range of 2–3. This is because saturation with amprolium lowers the pH of buffer solutions. The results are summarized in Table 3-1. While solubility measurements at pH of 2-3 are not environmentally relevant, the results of the aquatic toxicity tests conducted using amprolium hydrochloride indicate that water solubility was not an issue in these tests. These tests were performed at approximately neutral pH (initial pH of 6.7-8.0) and demonstrated acceptable analytical recovery of the test substance at nominal test concentrations up to 200 mg/L (see Section 7.1.1).

Amprolium's acid dissociation constant (pKa) was measured by the titration method outlined in OECD Guideline 112 (Foster and Thomas 2014). In two definitive tests, the mean measured pKa was 4.65. The compound's vapor pressure was measured using the Knudsen cell effusion model, according to OECD Guideline 104. The results were extrapolated to 20 and 25°C and can be found in Table 3-1.

The octanol-water partition coefficients of amprolium were determined at three different pH levels (Foster and Thomas 2014). The study conformed to OECD Guideline 107, Partition Coefficient (n-octanol/water): Shake Flask Method. Amprolium was added to mixtures of n-octanol and water, the mixtures were shaken and centrifuged, and each layer was analyzed separately. The test was performed in duplicate at n-octanol/water ratios of 1:1, 1:2, and 2:1 and at pH values of 5, 7, and 9. However, the pH buffers did not adequately maintain the pH at the targeted levels. pH during the study was approximately 4 in the pH 5 buffer and approximately 6.4-6.8 in the other buffers. For each pH value, the mean of six trials was computed. The results are included in Table 3-1. Despite the fact that the pH values were not maintained at the targeted levels, the results clearly demonstrate that amprolium is not anticipated to partition to lipids and will not bioconcentrate.

With the exceptions of UV-visible absorption spectrum and melting point, data are available for all physicochemical properties required for a Phase II assessment per CVM Guidance (CVM 2006). Data for these parameters are not considered crucial for the EA. These parameters are often used to assist in estimating other physicochemical and environmental fate properties if data are absent. However, data are available for key properties. Summaries of the studies used to support this EA are found in Appendix A. Note that for studies in which the substance tested was amprolium hydrochloride, if the results were not reported as amprolium, a conversion factor based on the relative molecular weights was used to express the results as amprolium.

**Table 3-1. Physical and chemical properties of amprolium**

| Standard Name | amprolium | |
|---|---|---|
| CAS No.[1] | 121-25-5 (137-88-2 for the hydrochloride) | |
| Synonyms | IUPAC name: 5-[(2-methylpyridin-1-ium-1-yl)methyl]-2-propylpyrimidin-4-amine; chloride | |
| Molecular weight[1] | 278.784 (315.242 for the hydrochloride) | |
| Molecular formula[1] | $C_{14}H_{19}ClN_4$ ($C_{14}H_{20}Cl_2N_4$ for the hydrochloride) | |
| Melting point | No data available | |
| UV/vis data | No data available | |
| Molecular structure[2] | Amprolium  | Amprolium Hydrochloride  |
| Solubility in water (mg/L)[3] | 526,730 in pH 5 buffer at 20°C[a]<br>540,320 in pH 7 buffer at 20°C[b]<br>525,820 in pH 9 buffer at 20°C[c] | |
| Octanol-water partition coefficient (LogP)[3] | -3.133 in pH 5 buffer[d]<br>-2.529 in pH 7 buffer[e]<br>-2.239 in pH 9 buffer[f]<br>(Amprolium is a charged ion at environmentally-relevant pH values) | |
| Dissociation constant (pKa)[3] | 4.65 | |
| Vapor pressure at (Pa)[3] | $4.928\times10^{-15}$ at 20°C<br>$1.769\times10^{-14}$ at 25°C | |

[1] https://pubchem.ncbi.nlm.nih.gov/compound/amprolium; accessed 11/26/18.
[2] https://en.wikipedia.org/wiki/Amprolium; accessed 11/26/18.
[3] Foster and Thomas 2014. Note: temperature was not recorded during the study.
[a] Measured pH range 2.17–2.23
[b] Measured pH range 2.60–2.67
[c] Measured pH range 2.07–2.27
[d] pH of aqueous layers 4.24–4.28, pre-dosing 5.25
[e] pH of aqueous layers 6.75–6.81, pre-dosing 7.10
[f] pH of aqueous layers 6.43–6.76, pre-dosing 8.98
Note: Numbered footnotes are citations while lettered footnotes provide further details of the information.

# 4. DESCRIPTION OF THE ECOSYSTEM AT THE SITE OF INTRODUCTION

## 4.1 Description of Proposed Product Use

Corid® is proposed for use in preventing and treating bovine coccidiosis. It is offered in three different forms: Type A Medicated Article 25% (premix in feed), 9.6% oral solution, and 20% soluble powder. Two dosing regimens are available: preventive (5 mg/kg bw/d for 21 d) and treatment (10 mg/kg bw/d for 5 d).

Introduction of amprolium to the environment would occur through the excretion of manure from treated cattle. Exposure of soil microorganisms, earthworms, and plants could occur through the transfer of amprolium residues from dung pats or from feedlot manure spread on agricultural land. Leaching from soil to groundwater and ultimately to surface water, runoff from pastures and manure applied to cropland, and direct excretion by pasture-reared cattle into streams all represent possible routes of introduction of amprolium to the water column and sediment, potentially exposing aquatic and benthic organisms. The risks, if any, to ecological receptors via these exposure pathways are assessed in this EA.

# 5. METABOLISM, DISPOSITION, AND ENVIRONMENTAL FATE

## 5.1 Metabolism and Disposition

No data are available on the metabolism of amprolium by cattle. Therefore, to be conservative, a total residue approach (i.e., the assumption that 100% of the dosed compound is excreted intact) will be adopted for exposure calculations (CVM 2001).

## 5.2 Environmental Fate

Several studies have been conducted on the environmental fate properties of amprolium. These studies are described in Appendix A, with brief summaries presented in this section.

### 5.2.1 *Photolysis*

The ability of amprolium to undergo photolysis has not been evaluated in an OECD Guideline 316-compliant study. Therefore, to be conservative, exposure calculations will assume that the compound is not subject to photolytic degradation.

### 5.2.2 *Hydrolysis*

Hydrolysis of amprolium has not been evaluated in an OECD Guideline 111-compliant study. Therefore, to be conservative, exposure calculations will assume that the compound is not subject to hydrolytic degradation.

### 5.2.3 *Adsorption and Desorption in Soil*

The adsorption and desorption kinetics of [$^{14}$C]amprolium were measured in a GLP-compliant study following OECD Guideline 106 (Dean 2024). Five U.S. soils were tested: a sandy loam (3.2% organic carbon [OC]), a loamy sand (2.5% OC), a loam (0.8% OC), a different loam (7.9% OC), and a silty loam (0.9% OC). For each test soil, Freundlich coefficients and exponents were determined for adsorption and desorption. An organic carbon-water partition coefficient ($K_{oc}$) was calculated for each soil as the ratio of the adsorption coefficient to OC

content. The results are summarized in Table 5-1. The average $K_{oc}$ for the five soils was 11,900 mL/g.

### 5.2.4 *Aerobic Degradation in Soil*

The aerobic transformation of [$^{14}$C]amprolium was measured in four U.S. soils in a GLP-compliant study following OECD Guideline 307 (Dean 2023). The soils included a sand (0.46% OC), a sandy loam (0.75% OC), a second sandy loam (2.5% OC) and a silt loam (2.5% OC). Material balance in the soil test systems (soil extractables, volatiles, and non-extractable residues) as well as [$^{14}$C]amprolium and transformation products in the soil-extractable fractions were determined for days 0, 7, 14, 30, 59, and 120.

One major transformation product that averaged above 10% of the dose was further analyzed. It was more polar than the parent compound and it was identified by LC/MS as 4-amino-2-propylpyrimidine-5-carboxylic acid. Non-extractable residues (NERs) increased over the course of the incubation period and ranged from 13.1% AR to 70.7% AR. Exhaustive extraction procedures were employed, indicating the NERs did not need to be considered as parent compound. However, the major transformation product was considered as parent compound. The dissipation half-lives ($DT_{50}$) and 90% dissipation times ($DT_{90}$) of amprolium were calculated by fitting the data to a single first-order (SFO) kinetics model. As reported by the study author, the $DT_{50}$ values for the five soils ranged from 32.4 to 135 days, and the $DT_{90}$ values ranged from 108 to 450 days. When the major transformation product is included, the $DT_{50}$ values ranged from 37.4 to 134.0 days, and the $DT_{90}$ values ranged from 124.0 to 445.0 days (see Appendix B). The average of these values (including the transformation product; see Table 5-1) were used in this EA.

**Table 5-1. Environmental fate parameters for amprolium**

| Property | Value or Finding | Reference |
|---|---|---|
| Soil organic carbon-water partition coefficients for adsorption ($K_{oc}$, L/kg) | Sandy clay loam: 9,430<br>Loamy sand: 9,960<br>Loam: 6,860<br>Loam: 3,200<br>Silty Loam: 29,800<br>Average: 11,900 | Dean 2024 |
| Photolytic degradation half-life, $DT_{50}$ (d) | n/a | n/a |
| Hydrolytic degradation half-life, $DT_{50}$ (d) | n/a | n/a |
| Aerobic dissipation half-lives, $DT_{50}$, in soil at 20°C (d) | Sand: 38.9<br>Sandy loam: 36.9<br>Silt loam: 32.4<br>Sandy Loam: 135<br>Average: 60.8 | Dean 2023 |
| 90% aerobic dissipation times, $DT_{90}$, in soil at 20°C (d) | Sand: 129<br>Sandy loam: 123<br>Silt loam: 108<br>Sandy loam: 450<br>Average: 202.5 | Dean 2023 |
| Aerobic dissipation half-lives, $DT_{50}$, in soil at 20°C (d), including major transformation products | Sand: 50.1<br>Sandy loam: 52.5<br>Silt loam: 37.4<br>Sandy loam: 134.0<br>Average: 69 | Appendix B |
| 90% aerobic dissipation times, $DT_{90}$, in soil at 20°C (d), including major transformation products | Sand: 166.0<br>Sandy loam: 174.0<br>Silt loam: 124.0<br>Sandy loam: 445.0<br>Average: 227 | Appendix B |

Data are available for all environmental fate parameters required for a Phase II assessment of a drug used in terrestrial systems. No data are available for photolysis and hydrolysis, which are optional. It will be assumed that these processes are negligible for amprolium.

## 6. EXPOSURE ASSESSMENT

### 6.1 Phase I

Predicted environmental concentrations (PECs) were calculated for a number of cattle classes. For pasture-reared cattle, these were growing beef cattle (including stocker, feeder, slaughter, replacement heifers and steers), replacement dairy heifers (2–12 months old), and beef calves. For intensively-reared cattle, these were beef cattle (including cattle raised in confinement for slaughter, replacement beef heifers, beef heifers, and steers), beef cattle in a dry lot (including replacement beef heifers, beef heifers, and steers), replacement dairy heifers (2–12 months

old), dairy calves, and veal calves. The initial PECs were calculated using conservative assumptions and inputs.

The proposed use of amprolium will result in excretion of the compound in the feces of treated cattle. As a result, the compound could enter the soil and water compartments of the environment, potentially resulting in exposure to terrestrial and aquatic plants and animals. These potential exposures are evaluated using conservative assumptions to calculate the initial PEC in soil ($PEC_{soil\text{-}initial}$). The approach follows EMA guidance (EMA 2016), adjusted to reflect conditions appropriate to the U.S.

The $PEC_{soil\text{-}initial}$, calculated using conservative assumptions, is compared to the Phase I trigger limit of 100 µg/kg. If it is less than the Phase I trigger limit, a Phase II EA is normally not required, and the assessment stops.

### 6.1.1 *$PEC_{soil\text{-}initial}$*

#### 6.1.1.1 Pasture-Reared Cattle

The approach used to calculate $PEC_{soil\text{-}initial}$ for pasture-reared cattle is described in EMEA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016) and shown in Equation 6-1 below. For this initial PEC calculation, it is assumed that the entire applied dose is excreted as the parent compound, distributed evenly over the pasture, and incorporated into the soil at a depth of 5 cm. There are two different Corid® dosing regimens available: preventive (5 mg/kg bw/d for 21 d) and treatment (10 mg/kg bw/d for 5 d). Because the preventive regimen results in a larger mass of amprolium entering the environment (105 mg/kg bw versus 50 mg/kg bw), it was used in the calculations as the more conservative option.

$$\mathrm{PEC_{soil-initial}} = \left(\frac{\mathrm{D \times Ad \times BW \times SD \times Fh}}{\rho \times \mathrm{AH \times Ds}}\right) \times \mathrm{CF} \qquad \textbf{Equation 6-1}$$

where

| | |
|---|---|
| $PEC_{soil\text{-}initial}$ | = Predicted environmental concentration in soil, initial [µg/kg] |
| D | = Daily dose of the active ingredient [mg/kg bw/d] |
| Ad | = Number of days of treatment [d] |
| BW | = Animal body weight [kg bw] |
| SD | = Stocking density [animal/ha] |
| Fh | = Fraction of herd treated [value between 0 and 1] |
| ρ | = Bulk density of dry soil [1,500 kg/$m^3$] |
| AH | = Area of 1 hectare [10,000 $m^2$/ha] |
| Ds | = Depth of penetration into soil [0.05 m] |
| CF | = Conversion factor [1,000 µg/mg] |

Rather than the default values for body weight and stocking density given in the EMA guidance (2016), values for these parameters for the specific classes of pasture-reared cattle were chosen to reflect U.S. practices.

The parameters and numerical values used in Equation 6-1 are listed in Table 6-1 along with the resulting $PEC_{soil\text{-}initial}$ for pasture-reared cattle.

**Table 6-1. $PEC_{soil\text{-}initial}$ parameters for pasture-reared cattle**

| Symbol (Units) | Value | | | Reference |
|---|---|---|---|---|
| | Growing Beef Cattle | Replacement Dairy Heifers (2–12 months old) | Beef calves | |
| Input | | | | |
| D (mg/kg bw/d) | 5 | | | Product data for preventive program (most conservative) |
| Ad (d) | 21 | | | Product data for preventive program (most conservative) |
| BW (kg bw) | 258[1] | 150[3] | 133[5] | See footnotes below table |
| SD (animal/ha) | 3.74[2] | 3.74[4] | 1.49[2] | See footnotes below table |
| Fh | 1 | | | Default value, EMA 2016 |
| ρ (kg/m$^3$) | 1,500 | | | Default value, EMA 2016 |
| AH (m$^2$/ha) | 10,000 | | | Conversion |
| Ds (m) | 0.05 | | | Default value, EMA 2016 |
| CF (µg/mg) | 1,000 | | | Conversion |
| Output | | | | |
| $PEC_{soil\text{-}initial}$ (µg/kg dwt) | 135.1 | 78.54 | 27.74 | Calculated |

[1] Recommended by animal scientists and veterinarians at CVM.
[2] Iowa, I. B. C. P. F. o. (2007). *Custom Grazing Survey 2007: Stocking Rates, Fees and Services*. Available at: https://lib.dr.iastate.edu/leopold_pubspapers/166/
[3] American Society of Agricultural Engineers (ASAE) (2005). The value of 150 kg is from Table 1.b. in ASAE (2005)
[4] Based on CVM Animal Scientists' professional judgment and an approach described in: UMASS Extension Crops, Dairy, Livestock and Equine Program. Dairy Cow Stocking Rages. University of Massachusetts Amherst, CDLW Pub. 11-4. Available at: https://ag.umass.edu/crops-dairy-livestock-equine/fact-sheets/dairy-cow-stocking-rates
[5] Based on CVM Animal Scientists' professional judgment in consideration of data provided in the following three references: Comerford, J.W., et al., Reproductive rates, birth weight, calving ease and 24-h calf survival in a four-breed diallel among Simmental, Limousin, Polled Hereford and Brahman beef cattle. J Anim Sci, 1987. 64(1): p. 65-76. Available at: https://pubmed.ncbi.nlm.nih.gov/3818492/; Dunn, B., E. Hamilton, and D. Pruitt. Characterization of the Beef Cow-Calf Enterprise of the Northern Great Plains, in South Dakota Beef Report. 2003, South Dakota State University. Available at: https://openprairie.sdstate.edu/sd_beefreport_2003/10/; Hudson, M.D., et al., Effect of weaning date (normal vs. late) on performance of young and mature beef cows and their progeny in a fall calving system in the Southern Great Plains. J Anim Sci, 2010. 88(4): p. 1577-87. Available at: https://academic.oup.com/jas/article/88/4/1577/4745741.

Solving Equation 6-1 results in an amprolium concentration in soil of 135.1 µg/kg dwt from use in pasture-reared growing beef cattle, 78.54 µg/kg dwt from use in pasture-reared replacement dairy heifers (2–12 months old), and 27.74 µg/kg dwt from use in pasture-reared beef calves. (In this document, intermediate values are reported to multiple insignificant digits to permit subsequent calculations to be checked.) These values are considered conservative because they assume that all amprolium administered to cattle is excreted as the parent compound, and they do not account for metabolism. The $PEC_{soil\text{-}initial}$ resulting from use of amprolium in pasture-reared cattle, based on growing beef cattle, is above the threshold value of 100 µg/kg presented in the CVM Phase I guidance (CVM 2001), necessitating a Phase II assessment.

#### 6.1.1.2 Intensively-Reared Cattle

The approach used to calculate $PEC_{soil\text{-}initial}$ for intensively-reared cattle is a slightly modified version of the approach described in EMEA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016). The approach was modified for U.S. agricultural practices to be based on the phosphorus content of manure, instead of nitrogen, and is shown in Equation 6-2:

$$PEC_{soil-initial} = \left(\frac{D \text{ x } Ad \text{ x } BW \text{ x } P \text{ x } Ps \text{ x } Fh}{\rho \text{ x } AH \text{ x } Ds \text{ x } Py \text{ x } H}\right) \text{ x } CF \qquad \textbf{Equation 6-2}$$

where

$PEC_{soil\text{-}initial}$ = Predicted environmental concentration in soil, initial [µg/kg]
D = Daily dose of the active ingredient [mg/kg bw/d]
Ad = Number of days of treatment [d]
BW = Animal body weight [kg bw]
P = Animal turnover rate per place per year [animal/place/yr]
Ps = Annual phosphorus spreading limit [kg $P_2O_5$/ha/yr]
Fh = Fraction of herd treated [value between 0 and 1]
Py = Phosphorus produced per place per year [kg $P_2O_5$/place/yr]
H = Housing factor [1 for animals housed throughout the year or 0.5 for animals housed for only 6 months]
ρ = Bulk density of dry soil [1,500 kg/$m^3$]
AH = Area of 1 hectare [10,000 $m^2$/ha]
Ds = Depth of penetration into soil [0.05 m]
CF = Conversion factor [1,000 µg/mg]

For this initial PEC calculation, it was assumed that the entire applied dose is eliminated as the parent compound and distributed in manure spread evenly over the field, with a conservative soil incorporation depth of 5 cm to account for no-till agriculture practices. As in the case for pasture-reared cattle (Section 6.1.1.1), the preventive dosing regimen was chosen as the more conservative scenario. The calculations were performed so that manure application was based on phosphorus, rather than nitrogen, content in manure. Manure production values were based upon data from ASAE (2005) for the "as-removed" mass of manure from earthen lots for beef cattle classes and scraped concrete lots for dairy cattle classes (representing a worst case). The value for phosphorus produced per place per year (Py) was determined based on the phosphorus content of manure (either 0.002 or 0.0035 kg $P_2O_5$/kg manure, depending on the class of cattle) multiplied by the amount of manure produced per year for that animal class (see Table 6-2). The calculations were performed for beef cattle (including cattle raised in confinement for slaughter, replacement beef heifers, beef heifers, and steers), beef cattle in a dry lot (including replacement beef heifers, beef heifers, and steers), replacement dairy heifers 2–12 months old, dairy calves, and veal calves.

**Table 6-2. Calculation of phosphorus in manure of intensively-reared cattle**

| Cattle class | kg $P_2O_5$/kg manure | Manure produced (kg/hd/d)[1] | Manure produced (kg/hd/y) | Py (kg/$P_2O_5$/place/y) |
|---|---|---|---|---|
| Beef cattle | 0.0035 | 7.5 | 2,738 | 9.58 |
| Beef cattle in dry lot | 0.0035 | 7.5 | 2,738 | 9.58 |

| | | | | |
|---|---|---|---|---|
| Replacement dairy heifers (2–12 months old) | 0.002 | 40 | 14,600 | 29.20 |
| Veal calves | 0.002 | 40 | 14,600 | 29.20 |
| Dairy calves | 0.002 | 40 | 14,600 | 29.20 |

[1] ASAE (2005).

The values used in the calculation for Equation 6-2 are provided in Table 6-3.

**Table 6-3. $PEC_{soil\text{-}initial}$ parameters for intensively-reared cattle**

| Symbol (Units) | Value | | | | | Reference |
|---|---|---|---|---|---|---|
| | Beef cattle | Beef cattle in dry lot | Replacement dairy heifers (2–12 months old) | Veal calves | Dairy calves | |
| Input | | | | | | |
| D (mg/kg bw/d) | 5 | | | | | Product data for preventive program (most conservative) |
| Ad (d) | 21 | | | | | Product data for preventive program (most conservative) |
| BW (kg bw) | 446[1] | 268[2] | 150[1] | 118[1] | 57.1[1] | See footnotes below table |
| P (animals/place/yr) | 1.0 | 1.0 | 1.0 | 1.8 | 1.8 | Default value, EMA 2016 |
| Ps (kg $P_2O_5$/ha/yr) | 280 | | | | | OSU 2006 |
| Fh | 1 | | | | | Default value, EMA 2016 |
| ρ (kg/$m^3$) | 1,500 | | | | | Default value, EMA 2016 |
| AH ($m^2$/ha) | 10,000 | | | | | Conversion |
| Ds (m) | 0.05 | | | | | Default value, EMA 2016 |
| Py (kg $P_2O_5$/place/yr) | 9.58 | 9.58 | 29.20 | 29.20 | 29.20 | See Table 6-2 |
| H | 0.5 | 0.5 | 0.5 | 1 | 1 | Default value, EMA 2016 |
| CF (1,000 µg/mg) | 1,000 | | | | | Conversion |
| Output | | | | | | |
| $PEC_{soil\text{-}initial}$ (µg/kg dwt) | 3,650 | 2,193 | 403 | 285 | 138 | Calculated |

[1] ASAE (2005). The value of 446 for beef cattle is the average of the weight of cattle entering the feedlot and cattle exiting the feedlot from Table 3.b. in ASAE (2005). The values for veal calves and dairy calves are from Table 1.b. in ASAE (2005)
[2] Recommended by animal scientists and veterinarians at CVM.

Solving Equation 6-2 results in amprolium concentrations in soil of 3,650, 2,193, 403, 285, and 138 µg/kg dwt associated with treatment of intensively-reared beef cattle, beef cattle in dry lot, replacement heifers 2–12 months, veal calves, and dairy calves, respectively. These values for $PEC_{soil\text{-}initial}$ are considered conservative because they assume that all amprolium administered to cattle is excreted as the parent compound, and they do not account for metabolism. The values are also conservative because they do not consider degradation in soil after the manure is spread on land. These conservative $PEC_{soil\text{-}initial}$ values resulting from use of amprolium in intensively-reared cattle are above the threshold value of 100 µg/kg presented in the CVM Phase I guidance (CVM 2001), necessitating a Phase II assessment.

## 6.2 Phase II

The application of manure containing amprolium could result in exposure to soil microorganisms, earthworms, and plants. Runoff from crop fields could transport the compound into nearby water bodies, potentially leading to exposure for pelagic organisms. Therefore, in the Phase II assessment, PEC values in surface water are determined. Because the average $DT_{90}$ for aerobic dissipation in the tested soils was less than 1 yr (Section 5.2.4, Table 5-1), amprolium is not considered persistent and $PEC_{soil}$ need not be recalculated to account for accumulation (CVM 2006).

### 6.2.1 *$PEC_{surfacewater\text{-}initial}$*

6.2.1.1 Direct Excretion

Treated cattle on pasture may introduce residues directly into an aquatic environment by defecation into a stream. The approach used to calculate $PEC_{surfacewater}$ from direct defecation by pastured animals is as described in EMA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016) and shown in Equation 6-3. Values used in the calculation are provided in Table 6-4. The direct excretion pathway is not applicable for intensively-reared cattle.

$$\mathrm{PEC}_{\mathrm{surfacewater-initial-dir}} = \left(\frac{\mathrm{D\ x\ Ad\ x\ BW\ x\ SD\ x\ Fe}}{\mathrm{L\ x\ W\ x\ Dw\ x\ CF}}\right) \mathrm{x\ CF} \qquad \textbf{Equation 6-3}$$

where

$PEC_{surfacewater\text{-}initial\text{-}dir}$ = Predicted environmental concentration in stream via direct excretion, initial [µg/L]
D = Daily dose of the active ingredient [mg/kg bw/d]
Ad = Number of days of treatment [d]
BW = Animal body weight [kg bw/animal]
SD = Stocking density [animal/ha]
Fe = Fraction of the total absorbed dose excreted into the stream [0.01]
L = Length of stream per hectare [m/ha]
W = Width of stream [m]
Dw = Depth of water in the stream [m]
CF = Conversion factor [1,000 µg/mg or 1,000 L/$m^3$]

**Table 6-4. $PEC_{surfacewater-initial-dir}$ parameters: direct excretion of pasture-reared cattle**

| Symbol (Units) | Value | | | Reference |
|---|---|---|---|---|
| | Growing beef cattle | Replacement dairy heifers (2–12 months old) | Beef calves | |
| Input | | | | |
| D (mg/kg bw/d) | 5 | | | Product data for preventive program (most conservative) |
| Ad (day) | 21 | | | Product data for preventive program (most conservative) |
| BW (kg) | 258 | 150 | 133 | See footnotes below Table 6-1 |
| SD (animal/ha) | 3.74 | 3.74 | 1.49 | See footnotes below Table 6-1 |
| Fe | 0.01 | | | Default value, EMA 2016 |
| L (m) | 100 | | | Default value, EMA 2016 |
| W (m) | 1 | | | Default value, EMA 2016 |
| Dw (m) | 0.3 | | | Default value, EMA 2016 |
| CF (µg/mg or $L/m^3$) | 1,000 | | | Conversion |
| Output | | | | |
| $PEC_{surfacewater-initial-dir}$ (µg/L) | 33.77 | 19.64 | 6.94 | Calculated |

Solving Equation 6-3 results in amprolium concentrations in surface water for pasture-reared cattle via direct excretion of 33.77, 19.64, and 6.94 µg/L for growing beef cattle, replacement dairy heifers (2–12 months old), and beef calves, respectively.

#### 6.2.1.2 Indirect via Runoff

The $PEC_{surfacewater}$ from runoff is calculated from the $PEC_{soil}$ using the $K_{OC}$. The average $K_{OC}$ for amprolium is 11,900 L/kg in various soils (see Section 5.2.4). The approach used to calculate $PEC_{groundwater}$ for intensively-reared and pasture-reared cattle is provided in the equations below, as described in EMA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016).

$$\mathrm{CONV_{soil}} = \frac{\rho_{soil}}{F_{solid-soil} \times \rho_{solid}} \qquad \textbf{Equation 6-4}$$

$$\mathrm{PEC_{soil-initial\_pw\_dw}} = \frac{\mathrm{PEC_{soil-initial}}}{4} \qquad \textbf{Equation 6-5}$$

$$\mathrm{PEC_{soil-initial\_pw\_ww}} = \frac{\mathrm{PEC_{soil-initial\_pw\_dw}}}{\mathrm{CONV_{soil}}} \qquad \textbf{Equation 6-6}$$

$$PEC_{groundwater} = \frac{PEC_{soil-initial\_pw\_ww} \text{ x } \rho_{soil}}{K_{soil-water} \text{ x } 1{,}000} \quad \textbf{Equation 6-7}$$

$$K_{soil-water} = (F_{air-soil} \text{ x } K_{air-water}) + F_{water-soil} + \left(F_{solid-soil} \text{ x } \frac{K_{p-soil}}{1{,}000} \text{ x } \rho_{solid}\right) \quad \textbf{Equation 6-8}$$

$$K_{air-water} = \frac{VP \text{ x } MW}{SOL \text{ x } R \text{ x } TEMP} \quad \textbf{Equation 6-9}$$

$$K_{p-soil} = F_{oc-soil} \text{ x } K_{oc} \quad \textbf{Equation 6-10}$$

where

$PEC_{groundwater}$ = Predicted environmental concentration in groundwater [µg/L]
$\rho_{soil}$ = Bulk density of fresh soil [1,700 kg/m$^3$]
$\rho_{solid}$ = Density of soil solids [2,500 kg/m$^3$]
$F_{air-soil}$ = Fraction air in soil [0.2 m$^3$/m$^3$]
$F_{water-soil}$ = Fraction water in soil [0.2 m$^3$/m$^3$]
$F_{solid-soil}$ = Fraction solids in soil [0.6 m$^3$/m$^3$]
$F_{oc-soil}$ = Weight fraction organic carbon in soil [0.02 kg/kg]
TEMP = Temperature at air-water interface [285 K]
R = Gas constant [8.314 Pa·m$^3$/mol·K]
VP = Vapor pressure [Pa]
MW = Molar mass [g/mol]
SOL = Water solubility [mg/L]
$K_{soil-water}$ = Partition coefficient, solids and water in soil (v/v) [m$^3$/m$^3$]
$K_{p-soil}$ = Partition coefficient, solids and water in soil (v/w) [L/kg]
$K_{air-water}$ = Partition coefficient, air and water in soil [m$^3$/m$^3$]
$K_{oc}$ = Water-organic carbon distribution coefficient [L/kg]
$CONV_{soil}$ = Conversion factor from dry weight to wet weight soil [1.13 kg wwt/kg dwt]
$PEC_{soil-initial\_pw\_dw}$ = Initial predicted environmental concentration in dry weight soil, corrected for a soil depth of 20 cm (i.e., $PEC_{soil-initial}$/4) [µg/kg]
$PEC_{soil-initial\_pw\_ww}$ = Initial predicted environmental concentration in wet weight soil, corrected for a soil depth of 20 cm (i.e., $PEC_{soil-initial}$/4) [µg/kg]

The predicted environmental concentration from indirect transport of the compound to surface water ($PEC_{surfacewater-initial-indir}$) is provided in Equations 6-11 and 6-12 below and is as described in EMEA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016). $PEC_{surfacewater-initial-indir}$ is equal to $PEC_{groundwater}$ divided by 3. This value represents the initial $PEC_{surfacewater}$ for indirect exposure from runoff.

$$PEC_{surfacewater-initial-indir} = \frac{PEC_{porewater}}{DF} \quad \textbf{Equation 6-11}$$

$$PEC_{groundwater} = PEC_{porewater} \quad \textbf{Equation 6-12}$$

where

$PEC_{surfacewater-initial-indir}$ = Predicted environmental concentration in surface water via runoff, initial [µg/L]
DF = Dilution factor [3]

The values used in the calculations are provided in Table 6-5. Here, $PEC_{groundwater}$ is derived from the $PEC_{soil-initial}$ values calculated in Section 6.1.1.

**Table 6-5. $PEC_{surfacewater-initial-indir}$ parameters: indirect via runoff**

| Symbol (Units) | Value | | Reference |
|---|---|---|---|
| Input | | | |
| $\rho_{soil}$ (kg/m$^3$) | 1,700 | | Default value, EMA 2016 |
| $\rho_{solid}$ (kg/m$^3$) | 2,500 | | Default value, EMA 2016 |
| $F_{air-soil}$ (m$^3$/m$^3$) | 0.2 | | Default value, EMA 2016 |
| $F_{water-soil}$ (m$^3$/m$^3$) | 0.2 | | Default value, EMA 2016 |
| $F_{solid-soil}$ (m$^3$/m$^3$) | 0.6 | | Default value, EMA 2016 |
| $F_{oc-soil}$ (kg/kg) | 0.02 | | Default value, EMA 2016 |
| TEMP (K) | 285 | | Default value, EMA 2016 |
| R (Pa·m$^3$/mol·K) | 8.314 | | Constant |
| VP (Pa) | $4.928\times10^{-15}$ | | Foster and Thomas 2014* |
| MW (g/mol) | 278.784 | | see Table 3-1 |
| SOL (mg/L) | 540,320 | | Foster and Thomas 2014* |
| $K_{soil-water}$ (m$^3$/m$^3$) | 357.2 | | Calculated |
| $K_{p-soil}$ (L/kg) | 238.0 | | Calculated |
| $K_{air-water}$ (m$^3$/m$^3$) | $1.073\times10^{-21}$ | | Calculated |
| $K_{oc}$ (L/kg) | 11,900 | | Dean 2024 |
| $CONV_{soil}$ (kg wwt/kg dwt) | 1.133 | | Calculated |
| $PEC_{soil-initial\_pw\_dw}$ (µg/kg dwt) | **Pasture-reared cattle:**<br>Growing beef cattle: 33.77<br>Replacement dairy heifers: 19.64<br>Beef calves: 6.94 | **Intensively-reared cattle:**<br>Beef cattle: 912.5<br>Beef cattle in dry lot: 548.3<br>Replacement dairy heifers: 100.7<br>Veal calves: 71.3<br>Dairy calves: 34.5 | Calculated |

| Symbol (Units) | Value | | Reference |
|---|---|---|---|
| $PEC_{soil\text{-}initial\_pw\_ww}$ (µg/kg wwt) | **Pasture-reared cattle:**<br>Growing beef cattle: 29.80<br>Replacement dairy heifers: 17.33<br>Beef calves: 6.12 | **Intensively-reared cattle:**<br>Beef cattle: 805.2<br>Beef cattle in dry lot: 483.8<br>Replacement dairy heifers: 88.9<br>Veal calves: 62.9<br>Dairy calves: 30.4 | Calculated |
| Output | | | |
| $PEC_{surfacewater\text{-}initial\text{-}indir}$ (µg/L) | **Pasture-reared cattle:**<br>Growing beef cattle: 0.047<br>Replacement dairy heifers: 0.027<br>Beef calves: 0.010 | **Intensively-reared cattle:**<br>Beef cattle: 1.28<br>Beef cattle in dry lot: 0.768<br>Replacement dairy heifers: 0.141<br>Veal calves: 0.100<br>Dairy calves: 0.048 | Calculated |

* Values derived at 20°C and in pH buffer 7 were chosen.

### 6.2.2 *Summary of PEC Values*

Table 6-6 and Table 6-7 summarize the PEC values calculated in Sections 6.1 and 6.2. As described above, these values were all derived using conservative assumptions and are considered initial PECs.

Because aquatic organisms may be exposed to amprolium via direct excretion and runoff from pasture simultaneously, the highest $PEC_{surfacewater\text{-}initial\text{-}dir}$ was added to the highest $PEC_{surfacewater\text{-}initial\text{-}indir}$ for pasture-reared cattle. This would simulate an unrealistic scenario whereby more than 100% of amprolium from pasture-reared cattle enters the aquatic system through the combination of direct excretion and runoff simultaneously.

**Table 6-6. Summary of calculated PECs for pasture-reared cattle**

| PEC | Cattle class | | | Units |
|---|---|---|---|---|
| | Growing beef cattle | Replacement dairy heifers | Beef calves | |
| $PEC_{soil\text{-}initial}$ | 135.1 | 78.54 | 27.74 | µg/kg dwt |
| $PEC_{surfacewater\text{-}initial\text{-}dir}$ | 33.77 | 19.64 | 6.94 | µg/L |
| $PEC_{surfacewater\text{-}initial\text{-}indir}$ | 0.047 | 0.027 | 0.010 | µg/L |
| Total $PEC_{surfacewater}$ | 33.82 | 19.67 | 6.95 | µg/L |

**Table 6-7. Summary of calculated PECs for intensively-reared cattle**

| PEC | Cattle class | | | | | Units |
|---|---|---|---|---|---|---|
| | Beef cattle | Beef cattle in dry lot | Replacement dairy heifers | Veal calves | Dairy calves | |
| $PEC_{soil\text{-}initial}$ | 3,650 | 2,193 | 403 | 285 | 138 | µg/kg dwt |
| $PEC_{surfacewater\text{-}initial\text{-}indir}$ | 1.28 | 0.768 | 0.141 | 0.100 | 0.048 | µg/L |

## 7. EFFECTS ASSESSMENT

This section summarizes the available data on the effects of amprolium on aquatic organisms (algae, cyanobacteria, invertebrates, and fish) and terrestrial organisms (nitrogen-transforming bacteria, plants, and earthworms). Summaries of studies submitted in the prior submission are found in Appendix A.

### 7.1 Effects on Aquatic Species

#### 7.1.1 *Algae and Cyanobacteria*

A GLP-compliant study (Benstead 2017) was conducted with amprolium hydrochloride using the freshwater green alga *Raphidocelis subcapitata* (known at the time as *Pseudokirchneriella subcapitata*) according to OECD Guideline 201. Organisms were exposed to control medium, a solvent control, a reference chemical (3,5-dichlorophenol at 2 mg/L), or amprolium hydrochloride at nominal concentrations of 0.2, 0.6, 1.9, 6.1, 19.5, 62.5, and 200 mg/L. Amprolium hydrochloride was dissolved directly into the test medium; the solvent control was included only for comparison to the reference chemical, which was not soluble in medium. Each amprolium concentration was tested in triplicate, and each of the other treatments was tested in quadruplicate. The endpoints evaluated were growth rate and yield (as measured via fluorescence) at 24, 48, and 72 h of exposure. All criteria for the validity of the study were met. pH (initially 7.80 in the negative control) changed less than 1.5 units as specified by the guideline in all solutions excluding the highest test item concentration, which had a pH at the beginning of the test of 6.03 and 7.71 at the end of the test. Analytical verification of the exposure concentrations was conducted at test initiation and termination, with recoveries ranging from 94.8 to 144% at test initiation and 67.8 to 144% at test termination. Results were expressed based on geometric mean measured concentrations, which were 0.220, 0.730, 2.557, 9.996, 27.561, 83.249, and 258.419 mg/L amprolium hydrochloride (equivalent to 0.194, 0.646, 2.261, 8.840, 24.374, 73.621, and 228.532 mg/L amprolium). The more sensitive of the two endpoints (growth rate and yield) was yield. Based on a statistical re-evaluation of the results, the $EC_{50}$ (concentration resulting in 50% effect) for yield was 41.265 mg/L amprolium. The no-observed-effect concentration (NOEC) for both endpoints was 0.220 mg/L (0.194 mg/L amprolium), and the lowest-observed-effect concentration (LOEC) for both endpoints was 0.730 mg/L (0.646 mg/L amprolium).

Gilberg et al. (2014) conducted a similar growth inhibition and yield study for amprolium hydrochloride using the freshwater cyanobacterium *Anabaena flos-aquae*. For assessment of effects of antimicrobial substances, this species is considered the most appropriate. This study also followed OECD Guideline 201 and was conducted under GLP. Cultures were exposed to control medium or to nominal amprolium hydrochloride concentrations of 9.5, 17.1, 30.9, 55.6, or 100.0 mg/L (equivalent to 8.4, 15.1, 27.3, 49.2, or 88.43 mg/L amprolium) for 72 h. Both the

initial and final pH in the test solutions was 6.7–6.8. Analytical verification of the exposure concentrations was conducted at test initiation and termination, indicating that the measured concentrations remained within 20% of the nominal concentrations; thus, the results were calculated based on the nominal concentrations. Cyanobacterial cell densities were measured at 24, 48, and 72 h of exposure. Both growth rate and yield were used to calculate study endpoints. A weak relationship between amprolium hydrochloride concentration and yield was observed that allowed extrapolation to calculate an $EC_{50}$ for yield, but because it is not advisable to report a value outside the range of the tested concentrations, the $EC_{50}$ for yield could be considered as > 88.5 mg/L as amprolium. However, upon re-evaluation, the $EC_{50}$ for yield was determined to be 100 mg/L as amprolium hydrochloride (88.5 mg/L as amprolium). The $EC_{50}$ for growth rate could not be calculated. For growth rate, upon re-evaluation the NOEC was 55.6 mg/L (equivalent to 49.2 mg/L amprolium), and the LOEC was 100.0 mg/L (equivalent to 88.5 mg/L amprolium). For yield, the, the NOEC was 55.6 mg/L (49.2 mg/L amprolium), and the LOEC was 100.0 mg/L (88.5 mg/L amprolium).

#### 7.1.2 *Aquatic Invertebrates*

Egeler et al. (2014) tested the acute toxicity of amprolium hydrochloride to *Daphnia magna* (Straus) in a GLP-compliant study conducted according to OECD 202. Organisms were exposed to test media (Elendt Medium M4 at an initial pH of 8.0) containing 0, 10, or 100 mg/L amprolium hydrochloride (equivalent to 0, 8.8, or 88.4 mg/L amprolium) for 48 h and evaluated for immobilization. Chemical analysis of exposure concentrations was conducted at the beginning and end of the test. Measured concentrations were within ±20% of nominal concentrations, so the results were reported in terms of nominal concentrations. Each treatment was replicated six times. Both criteria for test validity were met, dissolved oxygen was maintained in an acceptable range, and no daphnids were immobilized in the control treatment. After 48 h, no immobilization occurred in the 10 mg/L treatment, and only 16.7% of organisms were immobilized in the 100 mg/L treatment. Therefore, the $EC_{50}$ for immobilization is >100 mg/L (>88.4 mg/L amprolium).

#### 7.1.3 *Fish*

Gilberg and Caine (2014) conducted a study on the acute toxicity of amprolium hydrochloride to zebrafish (*Danio rerio*). The study was GLP-compliant and followed OECD Guideline 203. The exposure was conducted in reconstituted water (pH 7.3, total hardness 167.9 mg/L as $CaCO_3$). The fish were exposed for 96 h under static conditions to control water or 100 mg/L amprolium hydrochloride. Measured concentrations (at 0 and 96 h) closely approximated the nominal concentration so the nominal concentration was used as the basis for the results. No mortality occurred among control or amprolium hydrochloride-exposed fish. Based on this finding, the 96-h median lethal concentration ($LC_{50}$) for zebrafish is >100 mg/L amprolium hydrochloride, or >88.4 mg/L amprolium.

### 7.2 Effects on Terrestrial Species

#### 7.2.1 *Soil Microbes*

Jarratt (2014a) tested the effect of amprolium hydrochloride on the microbial transformation of nitrogen in soil in a GLP study following OECD Guideline 216. The study soil was a sandy loam with an OC content of 0.67% (w/w), a microbial biomass concentration of 4.8% of total OC before study initiation, and a pH of 6.0 ± 0.9. It was amended with organic matter in the form of ground lucerne, then treated with amprolium hydrochloride at concentrations of either 745.5 or

7,454.8 µg/kg dwt (equivalent to 652.0 or 6,520.1 µg/kg dwt amprolium). Each treatment group was run in triplicate, as were the negative controls and positive controls. Nitrate was extracted at d 0, 7, 14, and 28. After 7, 14, and 28 d, the overall nitrate production rates in amprolium-treated soils diverged from control by -2.76, -9.84, and -4.52%, respectively, at the lower treatment and -3.76, +5.78, and -3.36%, respectively, at the higher treatment. Based on the criterion established by CVM (2006) of a final treatment effect of ≤25% relative to the control after 28 d, the study concluded that amprolium has no significant, long-term impact on soil nitrogen transformation at concentrations ≤6,520.1 µg amprolium/kg dwt.

### 7.2.2 *Terrestrial Plants*

The effects of amprolium on seedling emergence and early growth of six terrestrial plants—wheat, onion, French bean, tomato, cucumber, and radish—were investigated in a GLP-compliant study (Jarratt 2014b) conforming to OECD Guideline 208. The test endpoints were emergence, shoot length, shoot fresh weight, shoot dry weight, and any visually apparent detrimental effects. A sandy loam soil was used. After range-finding bioassays, the definitive tests were performed either in dose-response format (for radish and French bean) at amprolium concentrations of 4.0, 8.0, 15.9, 31.8, 63.5, 127.0, and 254.0 mg/kg dwt, or in limit format (for wheat, onion, tomato, and cucumber) at a concentration of 493.9 mg/kg dwt. Control treatments were also included. Calculations were based on nominal concentrations. Plants were observed for 14 d following 50% emergence in the control. No effects were seen on emergence or survival of plants exposed to amprolium. No dose-response behavior was observed, so $EC_{50}$ could not be calculated for any of the plant species. Amprolium had a positive effect on some of the growth endpoints, so no-observed-adverse-effect levels (NOAELs) were calculated instead of NOECs.

The most sensitive species was radish, with statistically significant effects of 27% inhibition for shoot length and 22% inhibition for seedling fresh weight at the highest test level (254.0 mg/kg). Therefore, the NOAEL was 127.0 mg/kg dwt. The $EC_{50}$ can be concluded to be > 254.0 mg/kg. All endpoints demonstrated significant effects for French bean at the highest test level, resulting in a NOAEL of 254.0 mg/kg dwt. However, the degree of effect was less than 50%, so the $EC_{50}$ can be concluded to be > 254.0 mg/kg for French bean. The initially calculated effects data were subsequently re-evaluated, finding that all endpoints evaluated for cucumber and tomato were found to be statistically significantly different from the control resulting in a LOAEL of 493.9 mg/kg dwt for these species.

Table 7-1 summarizes the most sensitive (i.e., lowest, or most conservative) NOAEL or LOAEL values for each species as well as the estimated $EC_{50}$ values from the study by Jarratt (2014b).

**Table 7-1. Most sensitive toxicity values for amprolium for terrestrial plants determined by Jarratt (2014b) in Tier A study**

| Species | Endpoint | NOAEL or LOAEL (mg/kg dwt) | $EC_{50}$ (mg/kg dwt) |
|---|---|---|---|
| Cucumber | All | 493.9 | >493.9 |
| French bean | All | 254.0 | >254.0 |
| Onion | All | 493.9 | >493.9 |
| Radish | Shoot length, fresh weight | 127.0 | >254.0 |
| Tomato | All | 493.9 | >493.9 |
| Wheat | All | 493.9 | >493.9 |

A Tier B seedling emergence and growth study (Kirkwood 2022) was conducted to provide further information on terrestrial plant toxicity at higher concentrations than previously tested. This GLP-compliant study was conducted following OECD Guidance 208 and examined the effects on six species: cabbage, garden bean, oilseed rape, pea, radish, and soybean. Stock solutions of amprolium hydrochloride was mixed into the test soil, which was a loamy sand with organic matter ≤ 3%, to yield nominal test concentrations of 15, 50, 150, 450, and 1000 mg a.i./kg soil dry weight, plus control. Stock solutions were analyzed at test initiation and resulted in 98 to 100% of nominal concentrations; therefore, the study results were based on the nominal concentrations as amprolium hydrochloride, corrected for percent purity of the test substance. Percent emergence, percent survival, shoot length, and shoot dry weight were recorded on days 0, 7, and 14 after 50% of the control seeds had emerged for each species. Of these response variables, the NOEC and $EC_{50}$ values for the most sensitive response are presented in Table 7-2. (Values are converted from amprolium hydrochloride to amprolium based on the relative molecular weights; see Table 3-1).

Amprolium hydrochloride had no effect on emergence or survival for any of the six test species up to and including the highest test concentration, 1400 mg/kg amprolium hydrochloride (1238 mg/kg amprolium). However, effects were seen upon shoot length and shoot dry weight for all species except soybean. The most sensitive species were cabbage and radish, with $EC_{50}$ values of 221 and 265 mg/kg amprolium, respectively, and NOEC values of 44.2 mg/kg amprolium for both species.

**Table 7-2. Most sensitive toxicity values for amprolium for terrestrial plants determined by Kirkwood (2022) in Tier B study**

| Species | Endpoint | NOEC (mg/kg dwt) | $EC_{50}$ |
|---|---|---|---|
| Cabbage | Shoot dry weight | 44.2 | 221 |
| Garden bean | Shoot dry weight | 398 | 1,026 |
| Oilseed rape | Shoot dry weight | 133 | 672 |
| Pea | Shoot length | 133 | >1,238 |
| Radish | Shoot dry weight | 44.2 | 265 |
| Soybean | All | >1,238 | >1,238 |

Reported values converted from amprolium hydrochloride to amprolium using a factor of 0.88435

### 7.2.3 *Earthworms*

A GLP-compliant study (Scheffczyk and Lorenz 2014) following OECD Guideline 222 and ISO 11268-2 Part 2 was performed to measure the reproductive toxicity of amprolium hydrochloride to the earthworm *Eisenia fetida*. Earthworms were exposed to artificial soil (70% sand, 20% clay, 10% peat, and calcium carbonate) containing zero (control) or 100 mg/kg dwt amprolium hydrochloride (equivalent to 88.4 mg/kg dwt amprolium) and evaluated for mortality, biomass after 28 d, and reproduction after 56 d. All test validity criteria were fulfilled. No mortality occurred in either the control or amprolium hydrochloride-treated soil, and there were no statistically significant differences in biomass, or the number of juveniles produced. Thus, a NOEC of >100 mg/kg dwt amprolium hydrochloride (>88.4 mg/kg dwt amprolium) can be established for all endpoints from this limit test.

### 7.3 Tier A Effects and PNEC Calculations

Acute effects are considered at Tier A. Table 7-3 presents the Tier A toxicity data, assessment factors (AFs; from CVM guidance [CVM 2006]) and predicted no-effect concentration (PNECs) for aquatic organisms for amprolium.

**Table 7-3. Tier A aquatic effects and PNECs for amprolium**

| Test and Reference | Species | Duration and Endpoint | Toxicity Value (µg/L) | Assessment Factor | PNEC (µg/L) |
|---|---|---|---|---|---|
| Algae yield (Benstead 2017) | *Pseudokirchneriella subcapitata* | 72-h $EC_{50}$ | 41,265 | 100 | 412.65 |
| Cyanobacteria yield (Gilberg et al. 2014) | *Anabaena flos-aquae* | 72-h $EC_{50}$ | 88,500 | 100 | 885 |
| Daphnid acute toxicity (Egeler et al. 2014) | *Daphnia magna* | 48-h $EC_{50}$ | >88,400 | 1,000 | 88.4 |
| Fish acute toxicity (Gilberg and Caine 2014) | *Danio rerio* | 96-h $LC_{50}$ | >88,400 | 1,000 | 88.4 |

Table 7-4 presents the Tier A toxicity data, AFs, and PNECs for terrestrial organisms based on tests conducted with amprolium.

**Table 7-4. Tier A terrestrial effects and PNECs for amprolium**

| Test and Reference | Species | Duration and Endpoint | Toxicity Value (mg/kg dwt) | Assessment Factor | PNEC (mg/kg dwt) |
|---|---|---|---|---|---|
| Nitrogen transformation by soil bacteria (Jarratt 2014a) | -- | ≤25% effect relative to control | n/a; no significant, long-term impact predicted | n/a | n/a |
| Terrestrial plants, seedling emergence and growth (Jarratt 2014b) | Cucumber | 14-day $EC_{50}$ | >493.9 | 100 | 4.94 |
| | French bean | | >254.0 | 100 | 2.54 |
| | Onion | | >493.9 | 100 | 4.94 |
| | Radish | | >254.0 | 100 | 2.54 |
| | Tomato | | >493.9 | 100 | 4.94 |
| | Wheat | | >493.9 | 100 | 4.94 |
| Earthworm mortality, biomass, and reproduction (Scheffczyk and Lorenz 2014) | *Eisenia fetida* | 28-d NOEC for biomass, 56-d NOEC for reproduction | >88.4 | 10 | 8.84 |

**Table 7-5. Tier B Terrestrial Effects and PNECs for Amprolium**

| Test and Reference | Species | Duration and Endpoint | Toxicity Value (mg/kg dwt) | Assessment Factor | PNEC (mg/kg dwt) |
|---|---|---|---|---|---|
| Terrestrial plants, seedling emergence and growth (Kirkwood 2022) | Cabbage | 14-d NOEC | 44.2 | 10 | 4.42 |
| | Garden bean | | 398 | 10 | 39.8 |
| | Oilseed rape | | 133 | 10 | 13.3 |
| | Pea | | 133 | 10 | 13.3 |
| | Radish | | 44.2 | 10 | 4.42 |
| | Soybean | | >1,238 | 10 | 123.8 |

Toxicity values ($LC_{50}$s, $EC_{50}$s) were also evaluated from relevant published literature (Yoshimura and Endoh 2005, Hu et al 2010, Yoshimura et al 2005, Kuwabara et al 1980, and Canton et al 1976). These toxicity values were all above the toxicity values listed in the Table 7-3, Table 7-4, and Table 7-5. Therefore, the data evaluated herein is the most conservative in terms of indicating potential for environmental impacts.

## 8. RISK CHARACTERIZATION

Risk is characterized by relating exposure to effects. Risk quotients (RQs, unitless) were generated by dividing the PEC values calculated in Section 6 by the appropriate PNECs summarized in Section 7. If all RQs are less than 1, the risk assessment concludes at Tier A. RQs greater than or equal to 1 may indicate an unacceptable level of risk, necessitating a Tier B risk characterization. Table 8-1 and Table 8-2 present the Tier A RQs for amprolium in the aquatic compartment, and Table 8-3 presents the Tier A RQs for the terrestrial compartment. Note that, for simplicity, each table presents only the highest PEC value of its type. (See Table 6-6 and Table 6-7 for the complete list.) The RQs for use of amprolium in other types of cattle are lower than those shown in the tables because the PECs are lower.

**Table 8-1. Tier A RQs for aquatic organisms (for pasture-reared cattle, from both direct excretion and indirect runoff)**

| Species | PNEC (µg/L) | *$PEC_{surfacewater\text{-}initial\text{-}dir}$ (µg/L) | RQ |
|---|---|---|---|
| Algae (*Pseudokirchneriella subcapitata*) | 412.65 | 33.82 | 0.082 |
| Cyanobacteria (*Anabaena flos-aquae*) | 885 | | 0.038 |
| Daphnid (*Daphnia magna*) | 88.4 | | 0.383 |
| Zebrafish (*Danio rerio*) | 88.4 | | 0.383 |

*For pasture-reared growing beef cattle (highest of all values calculated; see Table 6-6).

**Table 8-2. Tier A RQs for aquatic organisms (for intensively-reared cattle, indirect via runoff)**

| Species | PNEC (µg/L) | *$PEC_{surfacewater\text{-}initial\text{-}indir}$ (µg/L) | RQ |
|---|---|---|---|
| Algae (*Pseudokirchneriella subcapitata*) | 412.65 | 1.28 | 0.0031 |
| Cyanobacteria (*Anabaena flos-aquae*) | 885 | | 0.00015 |
| Daphnid (*Daphnia magna*) | 88.4 | | 0.0145 |

| | | | |
|---|---|---|---|
| Zebrafish (*Danio rerio*) | 88.4 | | 0.0145 |

*For intensively-reared beef cattle (highest of all values calculated; see Table 6-7).

All RQs for aquatic organisms are below 1, as shown in Table 8-1 and Table 8-2, so it can be concluded that there is no risk to these organisms.

The risk characterization for terrestrial organisms is based on the highest $PEC_{soil\text{-}initial}$ value, which was 3,650 µg/kg dwt (equal to 3.650 mg/kg dwt).

**Table 8-3. Tier A RQs for terrestrial organisms**

| Species | PNEC (mg/kg dwt) | *$PEC_{soil\text{-}initial}$ (mg/kg dwt) | RQ |
|---|---|---|---|
| Wheat | 4.94 | 3.650 | 0.739 |
| Onion | 4.94 | | 0.739 |
| French bean | 2.54 | | 1.44 |
| Tomato | 4.94 | | 0.739 |
| Cucumber | 4.94 | | 0.739 |
| Radish | 2.54 | | 1.44 |
| Earthworm (*Eisenia fetida*) | 8.84 | | 0.413 |

*For intensively-reared beef cattle (highest of all values calculated; see Table 6-7).

The RQs for earthworms and most of the terrestrial plants are below 1, as shown in Table 8-3. RQ values for French bean and radish are reported as 1.44, because the highest concentration used in testing these species was 254 mg/kg dwt, resulting in a calculated PNEC of 2.54 mg/kg dwt.

Because the Tier A RQ values are not definitive for two plant species, the risk characterization proceeds to Tier B for terrestrial plants. Here, the PNEC values are based on the NOECs determined in the study by Kirkwood (2022).

**Table 8-4. Tier B RQs for terrestrial plants**

| Species | PNEC (mg/kg dwt) | *$PEC_{soil\text{-}initial}$ (mg/kg dwt) | RQ |
|---|---|---|---|
| Cabbage | 4.42 | 3.650 | 0.83 |
| Garden bean | 39.8 | | 0.09 |
| Oilseed rape | 13.3 | | 0.274 |
| Pea | 13.3 | | 0.274 |
| Radish | 4.42 | | 0.83 |
| Soybean | 123.8 | | 0.03 |

*For intensively-reared cattle (highest of all values calculated; see Table 6-7).

The results of the Tier B risk characterization indicate that there are no risks to terrestrial plants, based on the highest $PEC_{soil}$ value calculated for any of the eight cattle classes.

In addition to the terrestrial organisms listed in Table 8-3, toxicity data are also available for nitrogen-transforming soil bacteria exposed to amprolium (Section 7.2.1). These data indicate that the compound has no significant, long-term impact on soil nitrogen transformation at a

concentration as high as 6,520.1 µg/kg, which is well above the highest $PEC_{soil\text{-}initial}$, so it is not considered a risk to these organisms according to CVM criteria (CVM 2006).

Using conservative assumptions, all the aquatic and terrestrial RQs are less than 1; thus, no further assessment is necessary per CVM guidance (CVM 2006), and no significant impacts are expected from the proposed use of the drug.

Amprolium is also the active pharmaceutical ingredient in a number of other products currently approved for use in the U.S., as listed in Appendix C. Approved amprolium products include those used for cattle (calves only) and for poultry, including chickens (broilers, laying hens), turkeys, and pheasants. To evaluate aggregate impacts for the use of Corid® (amprolium) in cattle and all other approved animal drugs containing amprolium, the following scenarios are considered: (1) use of Corid® or other drugs containing amprolium for multiple indications in the same species on the same farm (i.e., preventative vs. treatment); (2) use of Corid® or other drugs containing amprolium in different species on the same farm (e.g., cattle, chickens); and (3) use of Corid® or other drugs containing amprolium in the same or different species on different farms in the same watershed.

To aid in evaluating these three scenarios, the $PEC_{soil}$ values were first derived for approved uses of amprolium in the various animal classes. The current EA has calculated PEC values for Corid® for multiple cattle classes, including pasture-reared beef calves and intensively-reared veal and dairy calves (see Section 6). In addition, the $PEC_{soil}$ values for broiler chickens, turkeys and laying hens were also calculated below to compare to the PEC values estimated for cattle (including calves) to ensure that the use in cattle will not substantially change the current environmental exposure.

The approach used to calculate $PEC_{soil\text{-}initial}$ for the poultry classes is described in EMEA/CVMP/ERA/418282/2005-Rev.1-Corr (EMA 2016). However, U.S.-specific practices and values were used where appropriate. A modified version of Equation 6-1 was used, adjusted to consider a daily dosage not dependent on animal body weight. The highest dose of amprolium used in poultry is 227 g/ton of feed, as used for prevention of coccidiosis in broilers and turkeys (see data for New Animal Drug Application [NADA] 012-350 in Appendix C). Therefore, the worst-case assumption will be used, that all three groups of poultry would be fed continuously during their production period at the highest dose of 227 g amprolium per ton of feed. Pheasants are not addressed since this population is small compared to the other groups.

In order to determine the daily dosage for poultry, the concentration of amprolium in feed (227 g/ton) was first converted to 250.22 mg/kg (227 g/ton × 1,000 mg/g × ton/907.184kg) and then converted to daily doses by multiplying by the daily feed intakes. The daily feed intakes were converted from annual feed intakes for broiler chickens (0.136 kg/d; see Table 8-6 below), laying hens (0.095 kg/d; see Table 8-6 below), and turkeys (0.362 kg/d; see Table 8-6 below) according to the following equations:

$$PEC_{soil-initial} = \left(\frac{DD \text{ x } Ad \text{ x } P \text{ x } Ps \text{ x } Fh}{\rho \text{ x } AH \text{ x } Ds \text{ x } Py \text{ x } H}\right) \text{ x } CF \qquad \textbf{Equation 8-1}$$

$$DD = F \times I \qquad \textbf{Equation 8-2}$$

where

$PEC_{soil\text{-}initial}$ = Predicted environmental concentration in soil, initial [µg/kg]

DD = Daily dose of the active ingredient [mg/\d]
Ad = Number of days of treatment [d]
P = Animal turnover rate per place per year [animal/place/yr]
Ps = Annual phosphorus spreading limit [kg $P_2O_5$/ha/yr]
Fh = Fraction of herd treated [value between 0 and 1]
Py = Phosphorus produced per place per year [kg $P_2O_5$/place/yr]
H = Housing factor [1 for animals housed throughout the year or 0.5 for animals housed for only 6 months]
ρ = Bulk density of dry soil [1,500 kg/$m^3$]
AH = Area of 1 hectare [10,000 $m^2$/ha]
Ds = Depth of penetration into soil [0.05 m]
CF = Conversion factor [1,000 µg/mg]
F = Concentration of the active ingredient in feed (mg/kg feed)
I = Daily feed intake (kg feed/d)
DD = Daily dose of the active ingredient (mg/d)

Manure production values were based upon data from ASAE (2005) for the "as-removed" mass of manure. The value for phosphorus produced per place per year (Py) was determined based on the phosphorus content of manure multiplied by the amount of manure produced per day for each type of poultry. We assumed that animals received medicated feed every day they were present. Therefore, the number of days of treatment is equal to the number of days in a year divided by the animal turnover rate per year provided by EMA guidance (EMA 2016). For example, the turnover rate for broiler chickens is 9 animals/year. Therefore, each individual animal is on the farm for about 41 days (365 days/9 animals) and we assume it is treated each day. The phosphorus content for broiler chicken, caged laying hens (17-70 weeks of age), and turkey litter reported in ASAE (2005) of 27.5 lb/ton (0.0137 kg $P_2O_5$/kg manure), 55.4 lb/ton (0.0277 kg $P_2O_5$/kg manure), and 15.2 lb/ton (0.0076 kg $P_2O$ kg/manure), respectively, was used.

**Table 8-5. Calculation of phosphorus in manure of poultry**

| Poultry type | kg $P_2O_5$/kg manure[1] | Manure produced (kg/hd/d)[1] | Py (kg $P_2O_5$/place/y) |
|---|---|---|---|
| Broiler | 0.0137 | 0.02 | 0.1000 |
| Laying hen | 0.0277 | 0.03 | 0.3033 |
| Turkey | 0.0076 | 0.11 | 0.3051 |

[1] ASAE (2005)

The parameters and numerical values used in Equations 8-1 and 8-2 are listed in Table 8-6 along with the resulting $PEC_{soil\text{-}initial}$ for broiler chickens, laying hens, and turkeys.

**Table 8-6. $PEC_{soil\text{-}initial}$ parameters for poultry**

| Symbol (Units) | Value | | | Reference |
|---|---|---|---|---|
| | Broilers | Laying Hens | Turkeys[2] | |
| Input | | | | |

[2] Different feed intake rates were reported for male and female turkeys. The feed intake rate for female and male turkeys are 0.219 and 0.362 kg/d. The value for male turkey is used for subsequent calculation for $PEC_{soil}$ to be conservative because higher feed intake rate would result in a higher $PEC_{soil}$.

| Symbol (Units) | Value | | | Reference |
|---|---|---|---|---|
| | Broilers | Laying Hens | Turkeys[2] | |
| F (mg/kg feed) | 250.22 | | | Product data for preventative program (most conservative) |
| I (kg feed/d) | 0.136[3,4] | 0.095[5,6] | 0.362[7,8] | See the footnotes 3-8 |
| DD (mg/d) | 34.03 | 23.77 | 54.80 | Calculation (See Equation 9-2) |
| Ad (d) | 40.56 | 365.0 | 135.2 | Product data for preventive program (most conservative) and based on turnover rate (P) |
| P (animal/yr) | 9 | 1 | 2.7 | Default value, EMA 2016 |
| Ps (kg $P_2O_5$/ha/yr) | 280 | | | OSU 2006 |
| Fh | 1 | | | Default value, EMA 2016 |
| ρ (kg/$m^3$) | 1,500 | | | Default value, EMA 2016 |
| AH ($m^2$/ha) | 10,000 | | | Conversion |
| Ds (m) | 0.05 | | | Default value, EMA 2016 |
| Py (kg P2O5/place/yr) | 0.1000 | 0.3033 | 0.3051 | See Table 9-1 |
| H | 1 | 1 | 1 | Default value, EMA 2016 |
| CF (µg/mg) | 1,000 | | | Conversion |
| Output | | | | |
| $PEC_{soil\text{-}initial}$ (µg/kg dwt) | 46,377 | 10,679 | 40,455 | Calculated |

Solving Equation 8-1 results in $PEC_{soil}$ values of 46.4 mg/kg for broiler chickens, 10.7 mg/kg for laying hens and 40.5 mg/kg for turkeys.

With the $PEC_{soil}$ values calculated, the three scenarios for aggregate assessment mentioned above were evaluated. For the first scenario of the aggregate assessment, the use of amprolium-containing drugs for other indications in cattle was evaluated. Other approved drugs for use in cattle are only approved for use in calves (including AMPROMED™ For Cattle, AMPROMED™ For Calves, Boviprol™ 9.6% Oral Solution, Amprol® 25% Type A Medicated Article, Corid® Type A Medicated Article, CORID® 9.6% Oral Solution, Amprovine 9.6% Oral Solution, Corid 20% Soluble Powder, and Amprolium 9.6% Oral Solution). However, the indications are the same as for the approval sought in this EA, namely "for use as an aid in the treatment and prevention of coccidiosis caused by *Eimeria bovis* and *E. zurnii.*" It is highly unlikely that more than one product containing amprolium would be used in the same animals

[3] Ross. (2021). *Ross 708 Parent Stock Performance Objectives*. Retrieved from https://en.aviagen.com/assets/Tech_Center/Ross_PS/Ross708-ParentStock-PerformanceObjectives-2021-EN.pdf
[4] Ross. (2021). *Ross 308 Parent Stock Performance Objectives*. Retrieved from https://en.aviagen.com/assets/Tech_Center/Ross_PS/Ross308-ParentStock-PerformanceObjectives-2021-EN.pdf
[5] HyLine. (2018). *Hy-Line Brown Commercial Layers Management Guide*. Retrieved from https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/Brown/BRN%20COM%20ENG.pdf
[6] HyLine. (2020). *Hy-Line W-36 Commercial Layers Management Guide*. Retrieved from https://www.hyline.com/filesimages/Hy-Line-Products/Hy-Line-Product-PDFs/W-36/36%20COM%20ENG.pdf
[7] Hybrid. (2011). *Female Turkey Grower Performance Guide*
[8] Hybrid. (2011). *Male Turkey Grower Performance Guide*

on the same farm for the same treatment. Thus, there is little or no potential for aggregate exposures to amprolium that are greater than the exposure from Corid® alone. In addition, the proposed dose for Corid® is the same as that for currently approved products. In other words, use of the currently approved products would not result in higher $PEC_{soil}$ values than those shown in Table 6-6 and Table 6-7, which include the use of Corid® in calves.

For the second and third scenarios, the use of multiple amprolium drug products in different species on the same farm and on different farms in the same watershed was considered. Besides cattle, the use of amprolium drug products has also been approved in poultry (see Appendix C). For evaluating the worst scenario of the environmental impact of approved amprolium drug products in poultry, the highest $PEC_{soil}$ value was calculated to be 46.4 mg/kg for broiler chickens (see Section 8.2). This $PEC_{soil}$ was based on a farm scenario, which is the most conservative evaluation. If the evaluation is expanded to look at the mixed use in a watershed, the environmental exposure from amprolium would be the same or lower than the worst scenario due to dilution (i.e., diluted by soil and runoff that contain less or no amprolium). In addition, amprolium has been approved for over 60 years in poultry and used for many years in broiler chickens, and no significant environmental impacts (e.g., population-level impacts to terrestrial or aquatic organisms) have been reported from this use. Further, the current action will not increase the use or environmental exposure in poultry.

Overall, based on the information above, the proposed use of amprolium in cattle is not expected significantly impact the environment.

## 9. SUMMARY AND CONCLUSIONS

An EA was conducted to support the use of Corid® (amprolium) in cattle to prevent and treat coccidiosis. Two dosing regimens are available, with the preventive regimen (5 mg/kg bw/d) resulting in a higher environmental exposure. This dosing regimen was assumed to provide a worst-case estimate of PECs in soil and surface water. All required data were available to estimate ecological effects for aquatic and terrestrial organisms and were used to derive PNEC values. RQs were determined by comparing the PEC values to PNEC values at Tier A. For aquatic organisms, all RQs were less than 1, indicating no risks for the aquatic compartment. For terrestrial organisms, all RQ values were less than 1 at Tier A with the exception of two species of plants where the Tier A RQ values were not definitive. At Tier B, all RQs were less than 1 for terrestrial plants. These findings are based on the highest $PEC_{soil}$ among all cattle classes. Conservative assumptions were used in the risk assessment, and it can thus be concluded that unacceptable risks to non-target aquatic or terrestrial receptors are unlikely from the use of Corid®.

## 10. MITIGATION MEASURES

Because the proposed action would not have a significant effect on the environment, no mitigation measures will be required.

## 11. ALTERNATIVES TO THE PROPOSED ACTION

The only alternative to the proposed action is the “no action” alternative, which would be the failure to approve the NADA for Corid®. However, based on our analysis in this EA, we do not

believe that significant environmental impacts will occur from this action; therefore, the “no action” alternative was eliminated from consideration.

## 12. AGENCIES AND PERSONS CONSULTED

This EA was prepared with input and assistance from members of the Environmental Branch in the Office of New Product Evaluation in FDA’s Center for Veterinary Medicine.

## 13. LIST OF PREPARERS

This EA was prepared by Exponent, Inc. under the direction of Jane Staveley and Margaret Fleming.

## 14. CERTIFICATION

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

_____

Andrea Brake, PhD
Senior Project Scientist, Regulatory Affairs
Huvepharma, Inc.

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# Appendix A. Study Summaries in Support of the Environmental Assessment of Corid® (Amprolium) Premix, Oral Solution, and Soluble Powder in Cattle for the Prevention and Treatment of Coccidiosis

**Reference:**

Benstead, R. (2017). Amprolium Hydrochloride: *Pseudokirchneriella subcapitata* Growth Inhibition Test. Fera Science, Ltd., Centre for Chemical Safety & Stewardship. Fera Study Number FR/000682.

**Title:**

Amprolium Hydrochloride: *Pseudokirchneriella subcapitata* Growth Inhibition Test

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: FR/000682 | 14 February 2017 |

**Study type:**

Freshwater alga and cyanobacteria growth inhibition (OECD Guideline 201)

**Name of test material:**

Amprolium hydrochloride, 99.1% purity

**Test design:**

This study examined the effects of amprolium hydrochloride on the growth rate and yield of the alga *Raphidocelis subcapitata* (then known as *Pseudokirchneriella subcapitata*). Algae were exposed for 72 hours to nominal amprolium hydrochloride concentrations of 0.2, 0.6, 1.9, 6.1, 19.5, 62.5, and 200 mg/L, as well as a control, reference item (3,5-dichlorophenol at 2 mg/L) and solvent control pursuant to the reference item test. The test substance was dissolved directly in test media and used to prepare the test concentrations by serial dilution. Exposures were performed in 6 200-µL wells in a well plate, for a total exposure volume of 1.2 mL. Standard OECD test medium was used. Each amprolium hydrochloride test concentration was run in triplicate, and the control, solvent control, and reference item were run in quadruplicate. The nominal initial cell concentration at the start of the test was $10^4$ cells/mL. Measurements of cell count were made via fluorescence at 24, 48, and 72 ± 4 hours of exposure. Light levels were measured at 0 and 72 hours and were found to be 83 (72 hours) to 86 (0 hours) µE $m^{-2}$ $s^{-1}$ PAR. Exposures were performed in an incubator with shaking at 150 rpm. Temperature was maintained at 23 ± 2°C. The inhibition of daily average specific growth rate and yield relative to the control after 72 hours were determined.

The concentration of amprolium hydrochloride in the test solutions was measured in duplicate samples from the bulk preparations at the beginning of the test and in samples from pooled replicates at the end of the test. Samples were filtered before analysis by liquid-chromatography/mass spectroscopy. Concentrations were measured as amprolium but are reported as amprolium hydrochloride.

**Statistical analysis:**

A step-down analysis of variance and a Bruce-Versteeg regression analysis were applied using R (v.3.2.2). Means and corresponding 95% confidence intervals were calculated. It was only possible to calculate $EC_{50}$ and $EC_{20}$ values for average specific growth rate with large extrapolation, so these were reported as greater than the highest test concentration of amprolium.

**Summary of findings:**

Recoveries ranged from 94.8 to 144% of nominal at test initiation and from 67.8 to 144% of nominal at test termination. Because the measured concentrations were not uniformly ±20% of nominal or initial, the geometric

means of measured values on days 0 and 3 were calculated and used to express the study results. These values were: 0, 0.220, 0.730, 2.557, 9.996, 27.561, 83.249, and 258.419 mg/L amprolium hydrochloride.

All validity criteria were met. The cell number increased by a factor of 163 within the 72-hour test period, greater than the criterion of 16. The coefficient of variation among specific growth rates in control cultures was 1.76%, below the maximum value of 7%. The mean coefficient of variation for section-by-section growth rates for the test period was 20.4%, below the maximum value of 35%. Light levels were maintained within the specified range. pH (initially 7.80 in the negative control) changed less than 1.5 units as specified by OECD Guideline 201 in all solutions excluding the highest test item concentration (target 200 mg/L), which had a pH at the beginning of the test of 6.03 and 7.71 at the end of the test.

The results are expressed as concentrations of amprolium hydrochloride and are summarized below.

| | $EC_{50}$ (mg/L) | $EC_{20}$ (mg/L) | $EC_{10}$ (mg/L) | NOEC (mg/L) | LOEC (mg/L) |
|---|---|---|---|---|---|
| Average Specific Growth Rate | >258.419 | >258.419 | 10.2547 (3.1602–33.2758) | 0.220 (0.1102) | 0.730 (0.0058) |
| Yield | 43.2589 (17.6379–106.0972) | 0.2802 (0.0513–1.5297) | 0.0201 (0.0019–0.2178) | 0.220 (0.1038) | 0.730 (0.0058) |

95% confidence intervals are given in parentheses for the EC values.
p values are given in parentheses for the NOEC and LOEC.

Initially calculated endpoints were subsequently re-evaluated and these results are as follows. The most sensitive endpoint was yield, with an $EC_{10}$ of 0.0216 mg/L, an $EC_{20}$ of 0.2885 mg/L, and an $EC_{50}$ of 41.2645 mg/L, as amprolium.

| | |
|---|---|
| **Study conducted by:** Fera Science, Ltd.<br><br>**Author:** Rachel Benstead<br><br>**Address:** Sand Hutton, York, Y041 1LZ, UK | **Compliance with OECD GLP:** yes, with minor exceptions<br><br>**If no, justification:** |

**Reference:**

Dean, A. (2023). $^{14}$C-Amprolium - Aerobic Transformation in Four U.S. Soils Following OECD Guideline 307. Smithers Study No. 14329.6102

**Title:**

$^{14}$C-Amprolium - Aerobic Transformation in Four U.S. Soils Following OECD Guideline 307

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma, Inc. | Smithers Study No. 14329.6102 | May 30, 2023 |

**Study type:**

Aerobic transformation in soil (OECD Guideline 307)

**Name of test material:**

[pyridine-2,6-$^{14}$C]Amprolium, Batch No. CFQ44933, purity of 99.6%;

[pyrimidine-2-$^{14}$C]Amprolium, Batch No. CFQ45107, purity of 99.6%

Non-radiolabeled reference: amprolium hydrochloride, Lot. No. R101X0, purity 99.39%

**Test design:**

The aerobic degradation of $^{14}$C[Amprolium] was assessed in four U.S. soils:

- a sand (CA Shebelut Farm) with 0.46% organic carbon and starting microbial biomass of 12.9 mg C/100 g soil;
- a sandy loam (Dodson) with 0.75% organic carbon and starting microbial biomass of 12.0 mg C/100 g soil;
- a silt loam (Iowa-Fayette) with 2.5% organic carbon and starting microbial biomass of 61.0 mg C/100 g soil;
- a sandy loam (MSL-PF) with 2.5% organic carbon and starting microbial biomass of 40.0 mg C/100 g soil.

The soils had a pH range of 6.5 to 7.9 in water and a pH range of 6.0 to 7.7 in 0.01 M $CaCl_2$. Samples were incubated in the dark and maintained at a moisture level of pF 2.0 to 2.5 in 250-mL glass screw-cap test vessels with Teflon®-lined silicone septa. Labeled [pyridine-2,6-$^{14}$C]Amprolium and [pyrimidine-2-$^{14}$C]Amprolium were applied separately to 50.0-g soil aliquots at an application rate of approximately 1.0 mg/kg. Aerobic test vessels were connected in a chain with tubing and aerated continuously using a vacuum pump through a hydration vessel (containing purified reagent water) to moisten the air in the system. Test vessels were incubated in a dark environmental chamber at 20 ± 2 ºC. Material balance in the soil test systems (soil extractables, volatiles, and non-extractable residues) as well as [$^{14}$C]Amprolium and transformation products in the soil-extractable fractions were determined for days 0, 7, 14, 30, 59 and 120 after dosing.

A method for extraction and analysis of amprolium from soil was developed during a preliminary study. At each sampling interval, duplicate samples of each soil type were removed from the environmental chamber. The replicates were extracted with approximately 150 mL of 1/1 methanol/0.5 M ammonium acetate in purified reagent water (v/v) and transferred to a 250-mL Nalgene bottle. Samples were allowed to soak in the first extraction solvent for approximately 45 minutes, sonicated for 15 minutes, shaken at 1,200 rpm by Geno grinder for 10 minutes and then centrifuged at 3,800 rpm for 10 minutes. The extract was then transferred to a glass graduated cylinder. The volume was recorded, and the sample was analyzed by Liquid Scintillation Chromatography (LSC) (duplicate aliquots of 0.100 mL) for quantification of radioactivity. Additional extraction procedures were performed up to three times with 1/1 methanol/0.5 M ammonium acetate in purified reagent water (v/v). If necessary, additional extractions were performed up to three more times with 1/1 methanol/1.0 M ammonium acetate in purified reagent water (v/v) using the procedure described above. The number of extractions were consistent between soils for each label. A 10 mL portion of the combined extracts for each soil

sample was concentrated to approximately 3 to 5 mL by nitrogen gas purging. Concentrated extracts were analyzed by LSC for radioactivity and a portion was mixed with an aliquot of non-radiolabeled test article prior to HPLC/RAM analysis for confirmation of retention time.

Volatile trapping solutions were analyzed at least at each planned time interval and confirmed by barium carbonate precipitation at select intervals. Aliquots of the post-extracted soil were analyzed by combustion to determine the content of non-extractable residues (NER). Since NER were significant (>10% applied radioactivity, AR), additional extractions using a polar solvent (tetrahydrofuran) and a non-polar solvent (hexane) were performed. Following this, radioactive residues remained >10% AR, so organic matter fractionation (OMF) was also employed. An unknown transformation product that was detected above 10% of the dose in two of the soils was investigated using LC-MS/MS

**Statistical analysis:**

The times to 50% ($DT_{50}$) and 90% ($DT_{90}$) dissipation of amprolium were calculated using Computer Assisted Kinetic Evaluation (CAKE) version 3.4 software. The Single First Order (SFO) kinetics model provided an acceptable $r^2$ value (> 0.95) and an acceptable $X^2$ error (<15). Material balance was calculated by summing the radioactivity measure in the soil extracts, volatile trapping solutions, and the NER.

**Summary of findings:**

The study demonstrated that [$^{14}$C]Amprolium degraded over time in sand, silt loam, and two sandy loam soils under aerobic conditions with half-lives ($DT_{50}$) ranging from 32.4 to 135 days, as presented below. Recalculation of the $DT_{50}$ and $DT_{90}$ was performed, to include the major transformation product (see Appendix B) and these values are also presented below.

| Soil | $DT_{50}$ (days) | $DT_{90}$ (days) | Material Balance of Applied Radioactivity (%) | Recalculated Values | |
|---|---|---|---|---|---|
| | | | | $DT_{50}$ (days) | $DT_{90}$ (days) |
| CA Shebelut Farm (sand) | 38.9 | 129 | 97.3 – 106.2 | 50.1 | 166.0 |
| Dodson (sandy loam) | 36.9 | 123 | 93.4 – 104.8 | 52.5 | 174.0 |
| Iowa-Fayette (slit loam) | 32.4 | 108 | 95.0 – 104.3 | 37.4 | 124.0 |
| MSL-PF (sandy loam) | 135 | 450 | 99.6 – 106.0 | 134.0 | 445.0 |

One major transformation product (TP 1) that averaged above 10% of the dose in two soils of [pyrimidine-2-$^{14}$C]-labeled samples was further analyzed by LC-MS/MS and identified as 4-amino-2-propylpyrimidine-5-carboxylic acid. TP 1 was more polar than the parent compound which generally implies that it is no more toxic than the parent compound. Significant $CO_2$ was formed during the incubation period indicating that ultimate biodegradation occurs for amprolium. At the end of the study (120 DAT), the average maximum $CO_2$ levels ranged from 9.6% AR (observed in MSL-PF soil samples treated with [pyrimidine-2-$^{14}$C]Amprolium) to 76.2% AR (observed in Iowa-Fayette soil samples treated with [pyridine-2,6-$^{14}$C]Amprolium). Non-extractable residues (NER) increased over the course of the incubation period. At the end of the study, the average maximum NER levels ranged from 13.1% AR (observed in Dodson soil samples treated with [pyridine-2,6-$^{14}$C]Amprolium) to 70.7% AR (observed in Iowa-Fayette soil samples treated with [pyrimidine-2-$^{14}$C]Amprolium). The NER were further characterized by extracting representative NER samples with solvents of different polarities and different dielectric constants, however, no significant radioactivity was extracted with these solvents. The NER were also characterized by organic matter fractionation into fulvic acids, humic acids, and humin fractions. The majority of the radiocarbon for both pyridine and pyrimidine labeled samples remained bound to the insoluble humins, representing permanently bound residues indistinguishable from natural products. For the pyrimidine labeled samples, the fulvic acid

| | |
|---|---|
| fraction, representing covalently bound residues that extracted under harsh base conditions also recovered significant radiocarbon. For both labels, radiocarbon associated with the humic acids represented <2% AR. | |
| **Study conducted by:** Smithers<br><br>**Authors:** Aleksandra Dean<br><br>**Address:** 790 Main Street, Wareham MA 02571 | **Compliance with OECD GLP:** yes, except that the software program used to determine the model for the degradation rate (CAKE v 3.4) is not a validated program.<br><br>**If no, justification:** |

**Reference:**

Dean, A. (2024). [$^{14}$C]Amprolium – Determining the Adsorption Coefficient ($K_{oc}$) following OECD Guideline 106. Smithers Study No. 14329.6103.

**Title:**

[$^{14}$C]Amprolium – Determining the Adsorption Coefficient ($K_{oc}$) following OECD Guideline 106.

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma, Inc. | Smithers Study No. 14329.6103 | 27 March 2024 |

**Study type:**

Adsorption and desorption in batch equilibrium (OECD Guideline 106)

**Name of test material:**

[pyridine-2,6-$^{14}$C]Amprolium, Batch No. CFQ44933, 99.6% purity

Non-radiolabeled reference: amprolium hydrochloride, Lot. No. R101X0, purity 99.39%

**Test design:**

The adsorption and desorption kinetics of amprolium were measured in batch equilibrium studies following the serial method in OECD 106. Five different U.S. soils were used:

- a sandy loam (MSL-PF) with 3.20% organic carbon
- a loamy sand (RMN-SL-PF) with 2.50% organic carbon
- a loam (CA-Hanford) with 0.81% organic carbon
- a loam (DU-L-PF) with 7.90% organic carbon
- a silty loam (Arkansas) with 0.90% organic carbon

Four tests were performed as part of this study. An initial investigation determined the potential for adsorption to various test vessels (polypropylene, Teflon, and glass) and indicated that polypropylene Falcon tubes would be appropriate, with <1% of radioactivity sorbed to the container. A preliminary test (Tier I) was performed in five soils to determine the optimum soil:solution ratio, equilibration time, and stability of the test article over 24 hours. Soil:solution ratios of 1:50 for the CA-Hanford soil and 1:100 for the other soils were indicated to be optimal. The stability of [$^{14}$C]Amprolium in the five soils at 20 to 25 ºC over 24 hours was confirmed.

In the Tier II test, adsorption and desorption kinetics were determined in all five soils using 0.01 M $CaCl_2$ solution at a nominal concentration of 1.00 mg/L. This study was performed with soil-to-solution ratios resulting from Tier I. Duplicate samples for each soil type, duplicate blanks, and duplicate soil-less controls were prepared. Test vessels were shaken by hand for approximately 30 seconds, then agitated at approximately 150 rpm on an orbital shaker in the dark at 21 ºC. Duplicate samples were removed at 2, 4, 24, and 48 hours, centrifuged, and analyzed by LSC. The criteria for adsorption and desorption equilibrium time point were determined as the earliest time point where the increase (or decrease) in the % adsorption (or % desorption) of [$^{14}$C]Amprolium is less than 5% to the following time point. After the adsorption equilibrium times were determined, the desorption kinetics experiment was performed by decanting the liquid phase, adding fresh $CaCl_2$ solution, and shaking the samples for a period equivalent to the equilibration time in the adsorption phase. Duplicate samples were analyzed by LSC. The sampling intervals for the desorption phase were 4 hours for the MSL and Arkansas soils and 24 hours for the RMN-SL-PF, CA Hanford, and DU-L-PF soils.

In the Tier III test, the Freundlich adsorption isotherms were performed in all five soils using 0.01 M $CaCl_2$ solution and the soil-to-solution ratios and equilibration times resulting from Tier I and Tier II. Testing was performed using duplicates at five nominal concentrations: 0.100, 0.300, 1.00, 3.00. and 10.0 mg/L. Two additional replicates at each concentration served as soil-less controls and duplicate blank controls were also prepared for each test system. Samples were shaken and incubated as described previously, removed at the appropriate equilibration time (4 or 24 hours) and centrifuged. Duplicate aliquots of the supernatants were analyzed for quantification of radioactivity by LSC. Select soil residues in the Tier I, Tier II, and Tier III tests were extracted and analyzed to determine the stability of the test article by HPLC-RAM. Combustion of soils was conducted to determine the total residual radioactivity after extraction.

**Statistical analysis:**

Equations were used to calculate percent absorption, $K_d$ (absorption coefficient), and $K_{oc}$ (organic carbon normalized absorption coefficient) as well as the coefficients for desorption. Data from the isotherm test were fitted to the Freundlich isotherm equation.

**Summary of findings:**

The average mass balance for the five soils ranged from 98.9 to 100% after the adsorption, desorption, extraction, and combustion analyses. Following adsorption/desorption testing, $K_d$ and $K_{OC}$ values for [$^{14}$C]Amprolium were determined at an optimal soil-to-solution ratio (i.e., of 1/100 for MSL-PF, RMN-SL-PF, DU-L-PF, and Arkansas soils and 1/50 for CA-Hanford soil). The adsorption/desorption kinetics data provided the adsorption equilibrium time of 4 hours (MSL-PF and Arkansas soils) and 24 hours (RMN-SL-PF, CA-Hanford, and DU-L-PF soils). The adsorption $K_{oc}$ in the soil samples ranged from 2,970 to 24,100 mL/g. The desorption $K_{oc}$ in the soil samples ranged from 3,820 to 24,200 mL/g. No correlations between soil properties (pH, soil clay content, and soil organic matter) and the adsorption and desorption coefficients of [$^{14}$C]Amprolium were noted.

| Soil | Mean $K_{OC}$ (mg/L) | Freundlich Adsorption Coefficient ($\mu g^{1-1/n}$ $mL^{1/n}$ $g^{-1}$) | Freundlich Adsorption Exponent | Adsorption $K_{F\ oc}$ (mL/g) | Freundlich Desorption Coefficient ($\mu g^{1-1/n}$ $mL^{1/n}$ $g^{-1}$) | Desorption $K_{F\ oc}$ (mL/g) | Freundlich Desorption Exponent |
|---|---|---|---|---|---|---|---|
| MSL-PF (sandy clay loam) | 9,430 | 310 | 0.883 | 9,700 | 405 | 12,700 | 0.890 |
| RMN-SL-PF (loamy sand) | 9,960 | 214 | 0.829 | 8,550 | 269 | 10,800 | 0.820 |
| CA-Hanford (loam) | 6,860 | 59.3 | 0.876 | 7,330 | 85.8 | 10,600 | 0.887 |
| DU-L-PF (loam) | 3,200 | 235 | 0.833 | 2,970 | 302 | 3,820 | 0.817 |
| Arkansas (silty loam) | 29,800 | 217 | 0.844 | 24,100 | 218 | 24,200 | 0.871 |
| Arithmetic mean | 11,900 | 207 | 0.853 | 10,500 | 256 | 12,400 | 0.857 |

[$^{14}$C]Amprolium can be classified as having slight to no mobility in the soils tested.

**Study conducted by:** Smithers

**Authors:** Aleksandra Dean

**Address:** 790 Main Street, Wareham MA, 02571

**Compliance with OECD GLP:** yes

**If no, justification:**

**Reference:**

Egeler, P., M. Goth, and J. Caine. (2014). Amprolium Hydrochloride: A Study on the Acute Toxicity to *Daphnia magna*. ECT Oekotoxikologie GmbH and Battelle UK Ltd. ECT Study Number 13BT4DA; BUKL Phase ID: KS/13/002

**Title:**

Amprolium Hydrochloride: A Study on the Acute Toxicity to *Daphnia magna*

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: ECT 13BT4DA<br>BUKL: KS/13/002 | 19 March 2014 |

**Study type:**

*Daphnia* sp. acute immobilization (OECD Guideline 202)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study assessed the acute toxicity of amprolium hydrochloride to *Daphnia magna* in an extended limit test format. Daphnids were cultured in Elendt Medium M4 that was renewed twice per week and were fed four times weekly with a combination of algae (*Desmodesmus subspicatus*), instant baker's yeast, and TetraMin®. The exposure was performed under static conditions in 25-mL plastic beakers covered with plastic lids and kept at 20 ± 2°C with a photoperiod of 16 hours light:8 hours dark. Light intensity was 50–1,000 lux. Neonate daphnids were exposed to nominal concentrations of 0, 10, or 100 mg/L amprolium hydrochloride for 48 hours under static conditions in Elendt Medium M4 (pH 8.0). Each concentration was tested with six replicate beakers, and each beaker contained five daphnids. Temperature was controlled at 20.5–21.6°C, and light intensity was 435–518 lux. During exposure, test solutions were not aerated or renewed, and daphnids were not fed. Samples for amprolium hydrochloride analysis were taken at the beginning and end of the exposure and measured by high-performance liquid chromatography with ultraviolet detection. The measured endpoint was immobilization of daphnids.

**Statistical analysis:**

Fisher's exact binomial test was used to assess significance of differences between control and test concentrations. The statistical software package ToxRat 2.10 Professional was used for calculations.

**Summary of findings:**

The measured concentration of amprolium hydrochloride were within ±20% of nominal concentrations, so nominal concentrations were used in the calculations. pH in the control and test solutions was 7.6 at 0 hours and 7.8 at 48 hours.

All validity criteria were met. No immobilized daphnids were observed in the control (the maximum allowable is 10%), and the dissolved oxygen concentration at the end of exposure was ≥8.2 mg/L (the minimum is 3 mg/L). Signs of disease or distress were observed in fewer than 10% of daphnids in the control. The results are summarized below.

| Nominal Amprolium Concentration (mg/L) | Total Number of Daphnids Introduced | Number of Daphnids Immobilized After 24 Hours | Number of Daphnids Immobilized After 48 Hours |
|---|---|---|---|
| 0 (control) | 30 | 0 | 0 |
| 10 | 30 | 0 | 0 |
| 100 | 30 | 5 | 5 |

Immobilization occurred in 16.7% of daphnids at 100 mg/L amprolium hydrochloride after 48 hours, indicating that the 48-hour $EC_{50}$ for immobilization is >100 mg/L. Other behavioral endpoints were observed. At the highest concentration level, reduced swimming activity was observed compared to the control.

| | |
|---|---|
| **Study conducted by:** ECT Oekotoxikologie GmbH<br><br>**Authors:** Phillip Egeler, Marika Goth, Jane Caine<br><br>**Address:** Böttgerstr. 2-14, 65439 Flörsheim am Main, Germany | **Compliance with OECD GLP:** yes<br><br><br><br>**If no, justification:** |

**Reference:**

Foster, B., and D. Thomas. (2014). Amprolium: Evaluation of Selected Physical Chemical Properties. Smithers Viscient (ESG), Ltd. Smithers Viscient Study Number 3200401.

**Title:**

Amprolium: Evaluation of Selected Physical Chemical Properties

[*Note: this study report evaluated several physical chemical properties but summaries of each have been prepared separately for clarity*]

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: 3200401 | 2 May 2014 |

**Study type:**

Vapor pressure (OECD Guideline 104)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

The vapor pressure of amprolium was established using the Knudsen cell diffusion method described in OECD Guideline 104. Approximately 6–7 mg amprolium was placed in each of two weighed aluminum crucibles with 1.0-mm-diameter orifices cold-welded on. A control consisting of 5.281 mg amprolium in a crucible with an unperforated lid was also tested. The crucibles were weighed to a precision of 1 µg and placed in an oven that was subjected to ultra-high vacuum. At predetermined intervals, the oven was brought back to atmospheric pressure and the crucibles were weighed. Vapor pressure, ρ, was calculated according to the Hertz-Knudsen relationship, as follows:

$$\rho = \frac{m}{K \times A \times t}\sqrt{\frac{2\pi \times R \times T}{M}}$$

where

m = mass loss of amprolium by effusion between measurements (kg)
A = orifice area ($m^2$)
t = time (s)
R = universal gas constant (8.314 J/mol/K)
T = temperature (K)
M = molar mass (kg/mol)
K = Clausing probability factor = $\frac{1}{1+\left(\frac{3L}{8r}\right)}$, where L = orifice depth (m) and r = mean orifice radius (m)

The tests were conducted in the range of 111–201°C, and the calculated vapor pressure in this temperature range was extrapolated to 20 and 25°C using the Clausius-Clapeyron equation.

The stability of amprolium under the test conditions was assessed by measuring tested samples, untested samples, and control samples using infrared spectroscopy.

**Statistical analysis:**

Mean extrapolated vapor pressures at 20 and 25°C were calculated.

**Summary of findings:**

The infrared spectra of the control and tested samples were the same, indicating that amprolium had not undergone chemical change during testing and supporting the validity of the results. While tests were conducted in the range of 111–201°C, measurements were only taken in the range of 151–201°C due to the difficulty of measuring weight losses at extremely low vapor pressure. The results, extrapolated to environmentally relevant temperatures, are summarized below.

| Replicate | Vapor Pressure at 20°C (Pa) | Vapor Pressure at 25°C (Pa) |
|---|---|---|
| 1 | $9.820\times10^{-15}$ | $3.522\times10^{-14}$ |
| 2 | $3.654\times10^{-17}$ | $1.704\times10^{-16}$ |
| Mean | $4.928\times10^{-15}$ | $1.769\times10^{-14}$ |

Because these results were extrapolated from a highly nonlinear data set, they are accompanied by substantial relative uncertainty. However, it can nonetheless be concluded that the vapor pressure of amprolium in the environment is insignificantly small.

**Study conducted by:** Smithers Viscient (ESG), Ltd.

**Authors:** Ben Foster, David Thomas

**Address:** Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK

**Compliance with OECD GLP:** yes

**If no, justification:**

**Reference:**

Foster, B., and D. Thomas. (2014). Amprolium: Evaluation of Selected Physical Chemical Properties. Smithers Viscient (ESG), Ltd. Smithers Viscient Study Number 3200401.

**Title:**

Amprolium: Evaluation of Selected Physical Chemical Properties

[*Note: this study report evaluated several physical chemical properties but summaries of each have been prepared separately for clarity*]

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: 3200401 | 7 May 2014 |

**Study type:**

Water solubility (OECD Guideline 105)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

The water solubility of amprolium was determined in two stages. In a preliminary test, approximately 800-mg aliquots of amprolium hydrochloride were weighed into 10-mL glass vials, and 1 mL of pH 5, 7, or 9 buffer solution was added to each. pH 5 and 7 buffers were prepared with monobasic and dibasic phosphate, and pH 9 buffer was prepared with boric acid, potassium chloride, and sodium hydroxide. The buffered amprolium test solutions were diluted by a factor of 10,000 with a 2:3 mixture of double-distilled water and high-performance liquid chromatography (HPLC)-grade acetonitrile, then analyzed via HPLC. The results indicated that the solubility of amprolium at these pHs is greater than 0.01 g/L, so the shake flask method was used for definitive testing.

Approximately 2–3 g amprolium hydrochloride was added to a flask containing a known volume (2 mL) of pH 5, 7, or 9 buffer. Six replicates were prepared at each pH. The flasks were stirred in an incubator at 30°C for 24, 48, or 72 hours (duplicate flasks at each time point), then transferred to another incubator at 20°C and equilibrated for another 24 hours. At each sampling time point, a 0.5-mL aliquot of each solution was withdrawn and centrifuged at 14,000 rpm for 5 minutes to remove particles, then diluted by a factor of 10,000 with a 2:3 mixture of double-distilled water and HPLC-grade acetonitrile before analysis via HPLC. Solution pH was also measured at each sampling time.

**Statistical analysis:**

Means and standard deviations were calculated.

**Summary of findings:**

Amprolium was highly soluble in all three buffer solutions. In the definitive test, amprolium concentrations were sufficiently high to depress the pH of test solutions to 2–3 irrespective of the presence of buffer. As such, the results reflect amprolium in the presence of the buffers, not at the target pHs. The resulting solubility values were:

526.73 g/L (pH 5 buffer, pH range 2.17–2.23); 540.32 g/L (pH 7 buffer, pH range 2.60–2.67), and 525.82 g/L (pH 9 buffer, pH range 2.07–2.27).

| | |
|---|---|
| **Study conducted by:** Smithers Viscient (ESG), Ltd.<br><br>**Authors:** Ben Foster, David Thomas<br><br>**Address:** Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK | **Compliance with OECD GLP:** yes<br><br><br><br>**If no, justification:** |

**Reference:**

Foster, B., and D. Thomas. (2014). Amprolium: Evaluation of Selected Physical Chemical Properties. Smithers Viscient (ESG), Ltd. Smithers Viscient Study Number 3200401.

**Title:**

Amprolium: Evaluation of Selected Physical Chemical Properties

[*Note: this study report evaluated several physical chemical properties but summaries of each have been prepared separately for clarity*]

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: 3200401 | 7 May 2014 |

**Study type:**

n-Octanol/water partition coefficient via shake flask method (OECD Guideline 107)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

The n-octanol/water partition coefficient of amprolium was measured using the shake flask method, which was selected based on preliminary solubility testing. Double-distilled water and n-octanol were mutually saturated by shaking a 1:1 mixture for 24 hours. Duplicate test systems at n-octanol:water ratios of 1:1, 1:2, and 2:1 were prepared and dosed with amprolium to give aqueous-layer concentrations of 0.01 M. Tests were conducted in pH 5, 7, and 9 buffers. pH 5 and 7 buffers were prepared with monobasic and dibasic phosphate, and pH 9 buffer was prepared with boric acid, potassium chloride, and sodium hydroxide. The solutions were mixed by inverting 100 times over 5 minutes, equilibrated at 25°C for 30 minutes, centrifuged, and equilibrated at 25°C for a further 90 minutes. Both aqueous and organic layers were sampled and analyzed via high-performance liquid chromatography with ultraviolet detection.

**Statistical analysis:**

Means and standard deviations of LogP were calculated.

**Summary of findings:**

The average mass balance across all 6 test systems was 101 ± 1%. Logarithmic n-octanol/water partition coefficients (LogP) were consistently low in all three solution ratios and the mean log Pow at each pH was within ± 0.3 units. Results are summarized below.

pH 5: -3.133; pH of aqueous layers was 4.24 to 4.28, pre-dosing 5.25

pH 7: -2.529; pH of aqueous layers was 6.75 to 6.81, pre-dosing 7.10

pH 9: -2.239; pH of aqueous layers was 6.43 to 6.76, pre-dosing 8.98

| | |
|---|---|
| **Study conducted by:** Smithers Viscient (ESG), Ltd.<br><br>**Authors:** Ben Foster, David Thomas<br><br>**Address:** Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK | **Compliance with OECD GLP:** yes<br><br><br><br>**If no, justification:** |

**Reference:**

Foster, B., and D. Thomas. (2014). Amprolium: Evaluation of Selected Physical Chemical Properties. Smithers Viscient (ESG), Ltd. Smithers Viscient Study Number 3200401.

**Title:**

Amprolium: Evaluation of Selected Physical Chemical Properties

[*Note: this study report evaluated several physical chemical properties but summaries of each have been prepared separately for clarity*]

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: 3200401 | 7 May 2014 |

**Study type:**

Dissociation constants in water (OECD Guideline 112)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

The acid dissociation constant of amprolium was measured using a modification of OECD Guideline 112. Early measurements had suggested that the compound is highly water soluble (>201.1 mg/mL), which called for the use of the titration method. In one preliminary and two definitive titration tests, 0.01 M amprolium solutions were titrated with 0.01 M sodium hydroxide. The preliminary titration was performed coarsely to estimate the pKa, and the definitive titrations were performed with much smaller additions of sodium hydroxide on either side of the equivalence point for greater precision. pKa was estimated in two different ways. First, it was determined geometrically from plots of pH versus volume added. Second, equivalence points were determined from plots of the first derivative of pH versus volume added.

**Statistical analysis:**

A mean pKa was calculated across both tests and both methods of estimation.

**Summary of findings:**

The estimated pKa values agreed well between duplicate tests and between the two methods of estimation. The results are summarized below.

| Test | pKa from Midpoint | pKa from Equivalence Point |
|---|---|---|
| Definitive 1 | 4.60 | 4.65 |
| Definitive 2 | 4.64 | 4.69 |

The mean of all four measured values was 4.65.

| | |
|---|---|
| **Study conducted by:** Smithers Viscient (ESG), Ltd.<br><br>**Authors:** Ben Foster, David Thomas<br><br>**Address:** Otley Road, Harrogate, North Yorkshire, HG3 1PY, UK | **Compliance with OECD GLP:** yes<br><br><br><br>**If no, justification:** |

**Reference:**

Gilberg, D., and J. Caine. (2014). Amprolium Hydrochloride: A Study on the Acute Toxicity to Zebrafish (*Danio rerio)*. ECT Oekotoxikologie GmbH and Battelle UK, Ltd. ECT Study Number 13BT4FY. BULK Phase ID: KS/13/001

**Title:**

Amprolium Hydrochloride: A Study on the Acute Toxicity to Zebrafish (*Danio rerio)*

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: ECT 13BT4FY<br>BULK: KS/13/001 | 13 March 2014 |

**Study type:**

Fish acute toxicity (OECD Guideline 203)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

The objective of this study was to determine the acute toxicity (lethality) of amprolium hydrochloride to zebrafish (*Danio rerio*) after 96 hours under static conditions. It was designed as a limit study, wherein zebrafish were exposed to one amprolium hydrochloride concentration of 100 mg/L (nominal) or control. Amprolium hydrochloride concentrations were measured in samples collected at the beginning and end of the test via high-performance liquid chromatography with ultraviolet detection. Reconstituted tap water (prepared from deionized water and specified salts) was diluted with deionized water (1:1, v/v) and supplemented with 1% artificial sea water for use in culturing and testing. This water had a pH of 7.3 and total hardness of 167.9 mg/L as $CaCO_3$). Exposures were performed in 18-L stainless steel vessels containing 7 zebrafish; there was one replicate for the treatment concentration and one for the control. Loading was 0.2–0.4 g of fish/L. Water was aerated. Temperature was maintained at 23 ± 1°C, and a 12 hours light:12 hours dark cycle was enforced. Light intensity was 100–1,000 lux. Temperature, pH, and dissolved oxygen were monitored after test solutions were brought to the target temperature and again on days 1, 2, 3, and 4 of testing. Fish were not fed during tests or in the 24 hours before test initiation. During exposure, fish were observed after 3 hours, 6 hours, 1, 2, 3, and 4 days for mortality and changes to appearance, size, or behavior relative to control fish. Mortality was established by lack of gill movement and lack of response to touching of the caudal peduncle.

**Statistical analysis:**

No statistical analyses were performed.

**Summary of findings:**

Measured amprolium hydrochloride concentrations were 104.98 mg/L at test initiation and 104.58 at test termination. Both were within ±20% of nominal concentrations, so results were reported in terms of nominal concentrations.

Both test validity criteria were met: no mortalities occurred in the control (required: ≤10% or 1 out of 7 fish), and dissolved oxygen was maintained at 7.9–8.2 mg/L, or ≥94.5% air saturation value (required: ≥60% of air saturation value). The pH during the test was between 6.25 and 7.5. No behavioral effects in the fish were seen.

| | |
|---|---|
| No mortality was observed during the test, so the 96-hour $LC_{50}$ for amprolium hydrochloride was determined to be >100 mg/L. | |
| **Study conducted by:** ECT Oekotoxikologie GmbH<br><br>**Authors:** Daniel Gilberg, Jane Caine<br><br>**Address:** Böttgerstr. 2-14, 65439 Flörsheim am Main, Germany | **Compliance with OECD GLP:** yes<br><br>**If no, justification:** |

**Reference:**

Gilberg, D., I. Löffler, and J. Caine. (2014). Amprolium Hydrochloride: A Study on the Toxicity to Blue-Green Algae (*Anabaena flos-aquae*). ECT Oekotoxikologie GmbH and Battelle UK, Ltd. ECT Study Number 13BT4AB. BULK Phase ID KS/13/002.

**Title:**

Amprolium Hydrochloride: A Study on the Toxicity to Blue-Green Algae (*Anabaena flos-aquae*)

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: ECT 13BT4AB<br>BULK: KS/13/002 | 13 March 2014 |

**Study type:**

Freshwater alga and cyanobacteria growth inhibition (OECD Guideline 201)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study was designed to measure the effects, if any, of amprolium hydrochloride on the growth rate and yield of the freshwater cyanobacterium *Anabaena flos-aquae*. After culture and incubation under test conditions, cyanobacterial inocula were introduced to test flasks at an initial cell concentration of approximately $1\times10^4$/mL. Flasks were vortexed to ensure homogeneous distributions, and cell numbers and morphological deviations were recorded microscopically. Cyanobacteria were exposed for 72 hours to nominal amprolium hydrochloride concentrations of 0, 9.5, 17.1, 30.9, 55.6, or 100.0 mg/L in an MES-Volvox medium. Exposures were performed in 300-mL Erlenmeyer flasks at controlled temperatures in the range of 22.6–23.2°C, with permanent lighting at an intensity of 48.5–57.0 µE $m^{-2}$ $s^{-1}$. Cell counts were measured via fluorometer after 24, 48, and 72 hours on a sub-sample that was sonicated for about 20 seconds. Amprolium hydrochloride concentrations were measured at 0 and 72 hours via high-performance liquid chromatography with ultraviolet detection. Each amprolium hydrochloride test concentration was run in triplicate, and six controls were run. Two additional test vessels containing 100.0 mg/L amprolium hydrochloride were maintained without cyanobacteria to track the stability of the compound during the study. Growth inhibition was expressed based upon growth rate and yield.

**Statistical analysis:**

The normality of the data was evaluated in terms of the range-to-standard-deviation ratio, and the homogeneity of variances was evaluated using Bartlett's Test. The Williams Multiple Sequential t-Test and Welch t-Test were used to determine the significance of differences between algal growth in controls and in amprolium test systems. Probit analysis with linear maximum likelihood regression was applied to develop the concentration-response function. ToxRat 2.10 Professional software was used for all calculations.

**Summary of findings:**

Measured amprolium hydrochloride concentrations were within ±20% of nominal concentrations, so nominal concentrations were used in the calculations.

All validity criteria were met. Mean biomass increased by a factor of 185.0 during the 72-hour study (greater than the minimum of 16), the mean coefficient of variation in specific growth rates on a section-by-section basis was

24.9% (less than the maximum of 35%), and the coefficient of variation in mean specific growth rates between replicate control cultures was 1.1% (less than the maximum of 10%). The pH in the control and all test solutions was in the range of 6.7-6.8 at both the initiation and termination of the test.

No statistically significant differences in growth rate were detected between controls and the highest amprolium hydrochloride test concentration, 100 mg/L, so the LOEC and NOEC for this endpoint were set at >100.0 and ≥100.0 mg/L, respectively. For yield, a statistically significant response was detected at the highest test concentration, so the LOEC was set at 100.0 mg/L and the NOEC was set at the next highest concentration, 55.6 mg/L. A weak relationship was found between yield and concentration, enabling extrapolation to an $EC_{50}$ value of 178.5 mg/L. No $EC_{50}$ for growth rate could be computed. These results are summarized below.

| Endpoint | Response Variable | Amprolium Hydrochloride Concentration (mg/L) |
|---|---|---|
| Growth rate | LOEC | >100.0 |
| | NOEC | ≥100.0 |
| | $EC_{50}$ | Not determined |
| Yield | LOEC | 100.0 |
| | NOEC | 55.6 |
| | $EC_{50}$ | 178.5* |

*Concentration extrapolated from tested range. Confidence limits could not be determined.

The initially calculated endpoints were subsequently re-evaluated. For growth rate, the NOEC was determined to be 55.6 mg/L and the LOEC 100 mg/L, as amprolium hydrochloride. For yield, the NOEC and LOEC were unchanged, but the $EC_{50}$ was determined at 100 mg/L, as amprolium hydrochloride. When expressed as amprolium, the NOEC is 49.2 mg/L, the LOEC is 89.5 mg/L, and the $EC_{50}$ is 88.5 mg/L.

| | |
|---|---|
| **Study conducted by:** ECT Oekotoxikologie GmbH<br><br>**Authors:** Daniel Gilberg, Ivonne Löffler, Jane Caine<br><br>**Address:** Böttgerstr. 2-14, 65439 Flörsheim am Main, Germany | **Compliance with OECD GLP:** yes<br><br>**If no, justification:** |

**Reference:**

Jarratt, N. (2014a). Amprolium: Nitrogen Transformation Test. The Food and Environment Research Agency, Centre for Chemical Safety & Stewardship. Fera Study Number A2FL1020.

**Title:**

Amprolium: Nitrogen Transformation Test

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: A2FL1020 | 28 April 2014 |

**Study type:**

Nitrogen transformation by soil microorganisms (OECD Guideline 216)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study measured the effects of amprolium hydrochloride on nitrogen transformation by soil microorganisms. The test soil was LUFA 2.3, a sandy loam with an organic carbon content of 0.67 ± 0.05%, a pH in 0.01 M $CaCl_2$ of 6.0 ± 0.9, and a nitrogen content of 0.08 ± 0.01%. The microbial biomass concentration was 4.8% of total organic carbon before study initiation. Soil was collected to a depth of 20 cm from a field that had not been treated with fertilizer or plant protection products for the preceding 4 years. Soil was amended with lucerne meal at a concentration of 5 g/kg dwt. 300 ± 0.1 g dwt soil was added to each of 16 1.2-L polypropylene tubs with sealable lids and 12-mm holes in the sides. A further 1.5-g powdered lucerne meal was mixed into each tub. Soils were pre-incubated in the dark for 18 days at a temperature of 20 ± 2°C. There was sufficient headspace to permit gas exchange and maintain aerobic conditions.

Amprolium hydrochloride was added to soil samples by dissolving in methanol, applying to sand, allowing the methanol to evaporate, and mixing the sand into the soil to give 745.5 and 7454.8 µg amprolium hydrochloride per kg dwt soil, or amprolium concentrations of 652.0 and 6,520.1 µg/kg dwt. Each of these concentrations was run in triplicate. Triplicate negative controls containing the corresponding amount of test item carrier (sand) and triplicate positive controls containing sodium chloride at a nominal concentration of 16.0 g/kg dwt were also run. Another 4 tubs with no amprolium treatment were run to track microbial biomass at test initiation, during the test, at test termination, and at 100 days after initiation. The latter was not analyzed because the test did not extend to 100 days. Temperature was maintained at 20 ± 2°C throughout the study. Every week, soil moisture content was measured gravimetrically and maintained in the range of 40–60% of moisture holding capacity by adding demineralized water as necessary. Soil subsamples were collected on days 0, 7, 14, and 28.

Soil subsamples were placed into centrifuge tubes, extracted by shaking for at least 60 minutes with 0.1 M potassium chloride, centrifuged for 10 minutes at 2,000 rpm, and filtered via syringe. Biomass was determined with a fumigation-extraction method, amprolium hydrochloride (as amprolium ions) was determined via liquid chromatography with tandem mass spectrometric detection, and soil nitrate was determined via high-performance liquid chromatography with ultraviolet detection. Nitrate formation was calculated from the change in concentrations over time.

**Statistical analysis:**

The nitrate data were evaluated using analysis of variance tests at a 5% significance level. Normality of the residuals was evaluated using normality tests (Anderson-Darling, Cramer-von Mises and Watson), homogeneity

of variances was evaluated using Bartlett's Test at a 5% significance level, and treatment means were compared to the control at each time point using Dunnett's Test (simultaneous two-sided 95% confidence interval around control). Genstat Version 16.1 (VSN International) was used for the statistical analyses.

**Summary of findings:**

The validity criterion of <15% mean variation in nitrate concentration among replicate control samples throughout the study was fulfilled (9.96%). The microbial biomass in the soil was 321.9 µg C/g before the study, 189.5 µg C/g during the study, and 187.4 µg C/g at study termination. Nitrate concentrations in the positive control soils deviated from the control by >25% after day 0. At day 28, overall nitrate formation rates in amprolium-treated soils were 4.52% lower (652.0 µg/kg dwt treatment) and 3.36% lower (6,520.1 µg/kg dwt treatment), respectively, than the formation rates in control soils. As reported by the study author, these differences were not statistically significant. The results are summarized below. In a subsequent re-evaluation of the data, it was observed that the differences in nitrate formation rates between the control and 6520.1 µg/kg treatment group were statistically significant for 0 - 7 days (p-value = 0.0022), 0 – 14 days (p-value = 0.0005), and 0 – 28 days (p-value <0.0001).

Overall nitrate formation rates

| | Control | 652.0 µg amprolium/kg treatment | | 6520.1 µg amprolium/kg treatment | |
|---|---|---|---|---|---|
| Days After Treatment | Mean Soil Nitrate Formation Rate (mg/kg dwt/day) | Mean Soil Nitrate Formation Rate (mg/kg dwt/day) | % Deviation from Control | Mean Soil Nitrate Formation Rate (mg/kg dwt/day) | % Deviation from Control |
| 0–7 | 5.67 ± 1.35 | 5.52 ± 0.63 | -2.76 | 5.46 ± 1.31 | -3.76 |
| 0–14 | 5.12 ± 0.44 | 4.62 ± 0.22 | -9.84 | 5.42 ± 1.32 | +5.78 |
| 0–28 | 4.29 ± 0.28 | 4.10 ± 0.05 | -4.52 | 4.15 ± 0.32 | -3.36 |

Nitrate formation rates in the treatments deviated by less than 25% from the controls. These results indicate that amprolium is unlikely to have a long-term effect on soil nitrogen transformation.

**Study conducted by:** The Food and Environment Research Agency

**Authors:** Nicholas Jarratt

**Address:** Sand Hutton, York, Y041 1LZ, UK

**Compliance with OECD GLP:** yes

**If no, justification:**

**Reference:**

Jarratt, N. (2014b). Amprolium: Terrestrial Plant Seedling Emergence Test. The Food and Environment Research Agency, Centre for Chemical Safety & Stewardship. Fera Study Number A2FL1000.

**Title:**

Amprolium: Terrestrial Plant Seedling Emergence Test

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: A2FL1000 | 17 March 2014<br>Amendment 1: 30 October 2018<br>Amendment 2: 12 May 2020 |

**Study type:**

Terrestrial plant seedling emergence and growth (OECD Guideline 208)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study examined the effects of amprolium on the emergence and growth of six types of terrestrial plant. Seedlings of cucumber (*Cucumis sativus*), French bean (*Phaseolus vulgaris*), onion (*Allium cepa*), radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), and wheat (*Triticum aestivum*) were sown into LUFA 2.3, a sandy loam test soil. The soil was collected from a field that had not been treated with any organic fertilizer or plant protection products in the preceding four years. It was received air-dried and sieved to 2 mm. Different batches of the soil were used in the preliminary and definitive tests. The one used in the definitive test had an organic carbon content of 0.67 ± 0.05%, a pH in 0.01 M $CaCl_2$ of 6.0 ± 0.9, and a water content of 6.78% of dry weight. Amprolium, a reference item (linuron), or a control (water) were mixed with soil for approximately 30 minutes in a cement mixer before apportionment to test systems. Tests were performed in porous plastic pots containing 500 g dwt soil. Pots were placed in 8-oz plastic containers that served as water reservoirs. Seeds were sown at a soil depth of approximately 5–10 mm and in a consistent pattern across test species, with sufficient spacing between each other and away from pot walls. Pots were placed in glasshouse cubicles after sowing and were arranged and periodically rearranged in a random fashion to minimize localized environmental effects. Pots were kept in glasshouse cubicles at 25 ± 10°C and given at least 16 hours of light at 300 ± 100 µmol/$m^2$/s. Temperature was monitored continuously, and light intensity was measured at least once per week.

The concentration in the application solution for the highest treatment rate was quantified by chemical analysis via liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) and test item application rates are based on the gravimetric (calculated) concentration. The preliminary test was performed in dose-response format, with amprolium applied at nominal concentrations of 0.8, 4.0, 20.0, 100.0, and 500.0 mg/kg dwt for all six plant species. In the definitive study, a dose-response test was performed for French bean and radish at nominal amprolium concentrations of 4.0, 8.0, 15.9, 31.8, 63.5, 127.0, and 254.0 mg/kg dwt, and a limit test was performed for cucumber, onion, tomato, and wheat at a nominal amprolium concentration of 493.9 mg/kg dwt. Depending on plant type, either two or three seeds were included in each pot, with 10 or 15 replicate pots, for a total of 30 replicate plants per plant type and treatment. The test was run for 14 days after 50% emergence of the control plants. Endpoints measured included seedling emergence, shoot length, fresh weight, dry weight, and any visually apparent injury as assessed on a 100-point scale. Shoot length was measured from the base of the stem to the longest leaf for onions, radish, and wheat and from the base of the stem to the apical bud for cucumbers, French beans, and tomatoes.

**Statistical analysis:**

Emergence and survival data were evaluated by analysis of variance. Percentage data were arcsine-transformed before analysis. Survival rates of 0 or 100% were not analyzed for statistical significance. NOAEL values for shoot length and fresh and dry weights were calculated by analysis of variance. Evaluations were performed using Microsoft Excel's 1-way analysis of variance function.

**Summary of findings:**

All validity criteria were met. In the control treatment, seedling emergence was ≥70% for all species, mean survival of emerged seedlings was ≥90% during the study, and seedlings exhibited no visually apparent signs of phytotoxicity apart from normal variation in growth and morphology.

There was no significant effect of amprolium on emergence or seedling survival. A significant effect (decrease) on shoot length was only reported by the study author for radish, at the highest test concentration (254.0 mg/kg). Significant effects (increase) on shoot fresh and dry weights were observed for radish and wheat. Based on visual inspection, no phytotoxicity was reported.

No dose-response behavior was observed in any of the endpoints, so $EC_{50}$ could not be calculated. Furthermore, because some of the effects of amprolium on plants were positive, it was decided to report NOAELs rather than NOECs. These are summarized below. The initially calculated effects data were subsequently re-evaluated. The NOAEL for all endpoints evaluated for cucumber, reported as 493.9 mg/kg, were found to be statistically significantly different from the control (p-value = 0.0370), and therefore the NOAEL is actually a LOAEL. The NOAEL for all endpoints evaluated for tomato, reported as 493.9 mg/kg, were found to be statistically significantly different from the control (p-value = 0.0137), and therefore the NOAEL is actually a LOAEL.

For radish, the maximum degree of inhibition seen at the highest test level (254.0 mg/kg dwt) was 27% (for shoot length) and 22% (for seedling fresh weight). Since these values are well below 50% inhibition, it can be concluded that the $EC_{50}$ > 254.0 mg/kg dwt for radish. For French bean, the $EC_{50}$ is also > 254.0 mg/kg dwt. For all other species, it can be concluded that the $EC_{50}$ > 493.9 mg/kg dwt.

| Species | NOAEL[1], Shoot Length (mg/kg dwt) | NOAEL[1], Fresh Weight (mg/kg dwt) | NOAEL[1], Dry Weight (mg/kg dwt) |
|---|---|---|---|
| Cucumber | 493.9 | 493.9 | 493.9 |
| French bean | 254.0 | 254.0 | 254.0 |
| Onion | 493.9 | 493.9 | 493.9 |
| Radish | 127.0 | 127.0 | 254.0 |
| Tomato | 493.9 | 493.9 | 493.9 |
| Wheat | 493.9 | 493.9 | 493.9 |
| [1] NOAEL values for cucumber and tomato are actually LOAEL values | | | |

**Study conducted by:** The Food and Environment Research Agency.

**Authors:** Nicholas Jarratt

**Address:** Sand Hutton, York, Y041 1LZ, UK

**Compliance with OECD GLP:** yes

**If no, justification:**

**Reference:**

Jarratt, N. (2014b). Amprolium: Terrestrial Plant Seedling Emergence Test. The Food and Environment Research Agency, Centre for Chemical Safety & Stewardship. Fera Study Number A2FL1000.

**Title:**

Amprolium: Terrestrial Plant Seedling Emergence Test

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: A2FL1000 | 17 March 2014<br>Amendment 1: 30 October 2018<br>Amendment 2: 12 May 2020 |

**Study type:**

Terrestrial plant seedling emergence and growth (OECD Guideline 208)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study examined the effects of amprolium on the emergence and growth of six types of terrestrial plant. Seedlings of cucumber (*Cucumis sativus*), French bean (*Phaseolus vulgaris*), onion (*Allium cepa*), radish (*Raphanus sativus*), tomato (*Solanum lycopersicum*), and wheat (*Triticum aestivum*) were sown into LUFA 2.3, a sandy loam test soil. The soil was collected from a field that had not been treated with any organic fertilizer or plant protection products in the preceding four years. It was received air-dried and sieved to 2 mm. Different batches of the soil were used in the preliminary and definitive tests. The one used in the definitive test had an organic carbon content of 0.67 ± 0.05%, a pH in 0.01 M $CaCl_2$ of 6.0 ± 0.9, and a water content of 6.78% of dry weight. Amprolium, a reference item (linuron), or a control (water) were mixed with soil for approximately 30 minutes in a cement mixer before apportionment to test systems. Tests were performed in porous plastic pots containing 500 g dwt soil. Pots were placed in 8-oz plastic containers that served as water reservoirs. Seeds were sown at a soil depth of approximately 5–10 mm and in a consistent pattern across test species, with sufficient spacing between each other and away from pot walls. Pots were placed in glasshouse cubicles after sowing and were arranged and periodically rearranged in a random fashion to minimize localized environmental effects. Pots were kept in glasshouse cubicles at 25 ± 10°C and given at least 16 hours of light at 300 ± 100 µmol/$m^2$/s. Temperature was monitored continuously, and light intensity was measured at least once per week.

The concentration in the application solution for the highest treatment rate was quantified by chemical analysis via liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) and test item application rates are based on the gravimetric (calculated) concentration. The preliminary test was performed in dose-response format, with amprolium applied at nominal concentrations of 0.8, 4.0, 20.0, 100.0, and 500.0 mg/kg dwt for all six plant species. In the definitive study, a dose-response test was performed for French bean and radish at nominal amprolium concentrations of 4.0, 8.0, 15.9, 31.8, 63.5, 127.0, and 254.0 mg/kg dwt, and a limit test was performed for cucumber, onion, tomato, and wheat at a nominal amprolium concentration of 493.9 mg/kg dwt. Depending on plant type, either two or three seeds were included in each pot, with 10 or 15 replicate pots, for a total of 30 replicate plants per plant type and treatment. The test was run for 14 days after 50% emergence of the control plants. Endpoints measured included seedling emergence, shoot length, fresh weight, dry weight, and any visually apparent injury as assessed on a 100-point scale. Shoot length was measured from the base of the stem to the longest leaf for onions, radish, and wheat and from the base of the stem to the apical bud for cucumbers, French beans, and tomatoes.

**Statistical analysis:**

Emergence and survival data were evaluated by analysis of variance. Percentage data were arcsine-transformed before analysis. Survival rates of 0 or 100% were not analyzed for statistical significance. NOAEL values for shoot length and fresh and dry weights were calculated by analysis of variance. Evaluations were performed using Microsoft Excel's 1-way analysis of variance function.

**Summary of findings:**

All validity criteria were met. In the control treatment, seedling emergence was ≥70% for all species, mean survival of emerged seedlings was ≥90% during the study, and seedlings exhibited no visually apparent signs of phytotoxicity apart from normal variation in growth and morphology.

There was no significant effect of amprolium on emergence or seedling survival. A significant effect (decrease) on shoot length was only observed for radish, at the highest test concentration (254.0 mg/kg). Significant effects (increase) on shoot fresh and dry weights were observed for radish and wheat. Based on visual inspection, no phytotoxicity was reported.

No dose-response behavior was observed in any of the endpoints, so $EC_{50}$ could not be calculated. Furthermore, because some of the effects of amprolium on plants were positive, it was decided to report NOAELs rather than NOECs. These are summarized below.

For the one species in which statistically significant effects were observed (e.g., radish), the maximum degree of inhibition seen at the highest test level (254.0 mg/kg dwt) was 27% (for shoot length) and 22% (for seedling fresh weight). Since these values are well below 50% inhibition, it can be concluded that the $EC_{50}$ > 254.0 mg/kg dwt for radish, the most sensitive species. For French bean, the $EC_{50}$ is also > 254.0 mg/kg dwt. For all other species, it can be concluded that the $EC_{50}$ > 493.9 mg/kg dwt.

| Species | NOAEL, Shoot Length (mg/kg dwt) | NOAEL, Fresh Weight (mg/kg dwt) | NOAEL, Dry Weight (mg/kg dwt) |
|---|---|---|---|
| Cucumber | 493.9 | 493.9 | 493.9 |
| French bean | 254.0 | 254.0 | 254.0 |
| Onion | 493.9 | 493.9 | 493.9 |
| Radish | 127.0 | 127.0 | 254.0 |
| Tomato | 493.9 | 493.9 | 493.9 |
| Wheat | 493.9 | 493.9 | 493.9 |

**Study conducted by:** The Food and Environment Research Agency.

**Authors:** Nicholas Jarratt

**Address:** Sand Hutton, York, Y041 1LZ, UK

**Compliance with OECD GLP:** yes

**If no, justification:**

**Reference:**

Kirkwood, A. (2022). Amprolium Hydrochloride – Seedling Emergence Test. Smithers Study No. 14329.6105.

**Title:**

Amprolium: Terrestrial Plant Seedling Emergence Test

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma, Inc. | Smithers No.: 14329.6105 | 27 May 2022 |

**Study type:**

Terrestrial plant seedling emergence and growth (OECD Guideline 208)

**Name of test material:**

Amprolium hydrochloride, Lot No. 7005211, 98.45% purity

**Test design:**

This study examined the effects of amprolium hydrochloride on the emergence and growth of six species of terrestrial plants. These included three members of the Brassicaceae family: radish (*Raphanus sativus*), cabbage (*Brassica oleracea*), and oilseed rape (*Brassica napus*); and three members of the Fabaceae family: garden bean or French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), and soybean (*Glycine max*). Seeds were sown into a soil comprised of a loamy sand (30%) mixed with commercially washed silica sand (70%), with a resulting organic matter content of 1.2%. Approximately 1.0 kg of soil was added to each replicate polypropylene pot. Prior to sowing, the soil was treated by adding aliquots of stock solutions of amprolium hydrochloride prepared in deionized water to obtain nominal soil concentrations of 17, 50, 150, 450, and 1400 mg a.i./kg. The control contained deionized water only. There were 10 replicate pots per treatment per species, and 4 seeds planted in each replicate pot. Seeds were impartially selected and planted at a depth of approximately 1 cm below the surface. After planting, the pots were watered on the surface; all subsequent waterings with via sub-irrigation. Three times per week, nutrient solution was added; other watering was conducted as needed. Pots were placed in a greenhouse with supplemental lighting to maintain ≥ 50 µmol/$m^2$/s, with a photoperiod of 16 hours light: 8 hours darkness and temperature of approximately 17 to 35 ºC. Air temperature and humidity were monitored continuously, and light intensity was measured daily.

The concentration of amprolium hydrochloride in each stock solution used to treat the soils was was quantified by chemical analysis via liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). Control pots were observed daily for emergence, and all pots were obserced on days 0, 7, and 14 after 50% of the control seeds had emerged per species. Endpoints measured included seedling emergence, seedling survival, individual shoot length, replicate dry weight, and any morphological abnormalities.

**Statistical analysis:**

The NOEC was determined for emergence, survival, shoot length, and shoot dry weight. For emergence and survival, the Step-Down Cochran-Armitage test or Fisher's Exact test with Bonferroni-Holm adjustment was used. Length and weight data were first checked for normality (Shapiro-Wilks Test) and homogeneity of variance (Bartlett's test). For data that were normal and homoscedastic, Williams' or Dunnett's Multiple Comparison test were used. If data were normal and heteroscedastic, Step-Down Jonckheere-Terpstra or Dunnett's T3 Multiple Comparison test were used. If data were not normal, Step-Down Jonckheere-Terpstra or Wilcoxon's Test with Bonferroni-Holm adjustment were used. The $EC_{25}$ and $EC_{50}$ values for emergence, survival, shoot length, and

shoot dry weight were determined using linear interpolation. CETIS Version 1.9 was used to perform all statistical analyses.

**Summary of findings:**

All validity criteria were met. In the control treatment, seedling emergence was ≥ 70% for all species (attaining ≥ 85%), mean survival of emerged control seedlings was ≥ 90% during the study (attaining ≥ 100%), and control seedlings exhibited no visually apparent signs of phytotoxicity apart from normal variation in growth and morphology.

Measured concentrations of amprolium hydrochloride in the stock solutions were 98 to 110% of nominal; therefore, nominal soil concentrations are the basis for presentation of results. There was no significant effect of amprolium hydrochloride on emergence or seedling survival for any species; however, shoot length and shoot dry weight were affected for all species except soybean.

**Results Based on Nominal Soil Concentrations (mg a.i./kg as Amprolium hydrochloride)**

| Species | Parameter | $EC_{25}$[a] | $EC_{50}$[a] | NOEC | LOEC |
|---|---|---|---|---|---|
| Cabbage (*Brassica oleracea*) | Percent Emergence | >1,400 (NA[b]) | >1,400 (NA) | 1,400 | >1,400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | 200 (190 - 220) | 430 (400 - 490) | 50 | 150 |
| | Shoot Dry Weight | 120 (110 - 140) | 250 (240 - 270) | 50 | 150 |
| Garden Bean (*Phaseolus vulgaris*) | Percent Emergence | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | 720 (440 - 890) | >1,400 (NA) | 450 | 1,400 |
| | Shoot Dry Weight | 590 (290 - 750) | 1,100 (870 - ND[c]) | 450 | 1,400 |
| Oilseed Rape (*Brassica napus*) | Percent Emergence | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | 460 (370 - 530) | 1,100 (1000 - 1200) | 150 | 450 |
| | Shoot Dry Weight | 320 (220 - 450) | 760 (660 - 840) | 150 | 450 |
| Pea (*Pisum sativum*) | Percent Emergence | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | >1,400 (NA) | >1,400 (NA) | 150 | 450 |
| | Shoot Dry Weight | >1,400 (NA) | >1,400 (NA) | 450 | 1,400 |
| Radish (*Raphanus sativus*) | Percent Emergence | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | 120 (80 - 170) | 610 (370 - 1100) | 50 | 150 |
| | Shoot Dry Weight | 82 (52 - 120) | 300 (150 - 440) | 50 | 150 |
| Soybean (*Glycine max*) | Percent Emergence | >1,400 (NA) | >1,400 (NA) | 1,400 | >1400 |
| | Percent Survival | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Length | >1400 (NA) | >1,400 (NA) | 1,400 | >1,400 |
| | Shoot Dry Weight | >1,400 (NA) | >1,400 (NA) | 1,400 | >1,400 |

[a] 95% confidence interval is presented in parentheses.
[b] NA = Not Applicable. EC value was empirically estimated; therefore, the corresponding 95% confidence interval could not be determined.
[c] ND = Not Determined; corresponding 95% confidence limit could not be determined. Since this confidence limit could not be determined, caution should be applied when using this value in any risk assessments.

| | |
|---|---|
| **Study conducted by:** Smithers | **Compliance with OECD GLP:** yes |
| **Authors:** Ashlee Kirkwood | |
| **Address:** 790 Main Street, Wareham, MA 02571 | **If no, justification:** |

**Reference:**

Scheffczyk, A., and P. Lorenz (2014). Amprolium Hydrochloride: Reproduction Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil. ECT Oekotoxikologie GmbH. ECT Study Number 14BT5RR.

**Title:**

Amprolium Hydrochloride: Reproduction Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil

| **Name of Sponsor Company:** | **Study number:** | **Report date:** |
|---|---|---|
| Huvepharma N.V. | Lab Study No.: 14BT5RR | 11 June 2014 |

**Study type:**

Earthworm reproduction (OECD Guideline 222, ISO 11268-2 Part 2)

**Name of test material:**

Amprolium hydrochloride, 98.9% purity

**Test design:**

This study was designed to determine the effects, if any, of amprolium hydrochloride on reproduction by the earthworm *Eisenia fetida* in soil. It was performed as a limit test, with one test treatment of 100 mg/kg dwt (nominal) and a control applied to an artificial soil assembled according to OECD Guideline 222 as follows:

- 10% sphagnum peat, finely ground and air-dried with no visible plant remains
- 20% kaolin-clay with a kaolin content >30%
- approximately 70% quartz sand with >50% fine particles in the range 50–200 µm
- 0.3–1% calcium carbonate

The peat was shredded, and all components were homogenized. The pH was adjusted to 6 ± 0.5 with calcium carbonate, and moisture content was adjusted to 40–60% of the maximum water holding capacity with deionized water. Water content was checked weekly and adjusted as necessary. Approximately 500-g dwt aliquots of soil were contained in 1-L plastic containers covered with transparent and perforated lids. Temperature was kept at 20 ± 2°C and a 16 hours light:8 hours dark regime was enforced, with a light intensity at the soil surface of 400–800 lux.

Adult worms at least 2 months old (no more than 4 weeks difference in age among worms) and weighing 250–600 mg were acclimated to the soil test conditions for 24 hours before addition of amprolium hydrochloride. The test lasted 56 days, but the adult worms were removed after 28 days and thereafter juvenile production was monitored. Ten worms were added to each container. A total of 16 containers were run: 8 with amprolium hydrochloride and 8 controls. Amprolium hydrochloride was dissolved in deionized water and an appropriate amount of stock solution thoroughly mixed in with the soil. Deionized water was added to control containers instead of amprolium hydrochloride solution.

Worms were fed with finely ground cow manure that was free of growth promoters, nematicides, and similar veterinary pharmaceuticals. Food was introduced by moistening 5 g with 10 mL deionized water and spreading on the soil surface. Feeding occurred one day after application of amprolium hydrochloride and introduction of the worms, then weekly during the first four weeks of the test and when the adults were removed on day 28. Morphological and behavioral changes and mortality were noted. The combined weight of the surviving worms in each container was recorded on day 28. Juvenile worms were sampled 56 days after test initiation by extracting in a water bath set to 55°C. Extraction efficiency was tracked by hand-sorting one replicate per treatment. One container of each treatment was sampled for pH and moisture content.

A separate GLP study was conducted using the reference material carbendazim to demonstrate the sensitivity of the test system.

**Statistical analysis:**

Biomass and reproduction data were checked for normality with the R/s test procedure and for homogeneity with Cochran's test. Treatment means were compared by analysis of variance followed by Student's t-test ($p \leq 0.05$, 2-sided for biomass, 1-sided for reproduction) and tested for statistically significant differences compared to the control. Calculations were performed with the ToxRat Professional 2.10 software package.

**Summary of findings:**

All study validity criteria were fulfilled: there was no adult mortality in the control (maximum 10%), the mean number of juveniles in the control was 364.6 (minimum 30), and the coefficient of variance for the number of juveniles in the control was 9.2% (maximum 30%). Almost all food was consumed by the worms in the test vessels at days 7, 14, 21, and 28. No effects on the behavior or morphology of adult worms were observed. In the reference test using carbendazim, the NOEC for reproduction was determined to be 0.32 mg/kg dwt, and the LOEC was 1.0 mg/kg dwt. These were within the range of 1–5 mg/kg dwt specified by the guideline, indicating acceptable sensitivity of the test system.

No mortality occurred in either the control or amprolium hydrochloride treated soil. There was no significant difference in the biomass or percent change in biomass of surviving adult worms on day 28 between control and treated soil. The NOEC for both mortality and biomass was therefore set at ≥100 mg/kg dwt, and the LOEC was set at >100 mg/kg dwt.

The mean numbers of juveniles per container were 364.6 ± 33.6 in control soil and 378.1 ± 55.2 in the treatment soil. The 3.07% increase in number of juveniles in the treatment soil relative to control was not statistically significant. The NOEC for reproduction was therefore set at ≥100 mg/kg dwt, and the LOEC was set at >100 mg/kg dwt.

| | |
|---|---|
| **Study conducted by:** ECT Oekotoxikologie GmbH<br><br>**Authors:** Adam Scheffczyk, Pascale Lorenz<br><br>**Address:** Böttgerstr. 2-14, 65439 Flörsheim am Main, Germany | **Compliance with OECD GLP:** yes<br><br><br><br>**If no, justification:** |

## Appendix B. Discussion of determination of $DT_{50}$ and $DT_{90}$ values for aerobic degradation of amprolium in soils

In the aerobic soil metabolism study conducted with [pyrimidine-$^{14}$C]amprolium and [pyridine-$^{14}$C]amprolium (Dean, 2023), the degradation rates were calculated using only the amount of amprolium recovered in the soil extracts, using the software program CAKE to perform the determinations.

For risk assessment, the degradation rate of the test substance is calculated using the worst-case scenario where metabolites and bound residues that may be available for biodegradation are added to the concentration of the test substance for rate determination. The major metabolites in the study included $^{14}CO_2$, the metabolite TP1, and bound residues. $CO_2$ is the terminal product in the biodegradation in soil and not included in the worst-case calculation since it does not contribute to further accumulation/degradation. The non-extractable residues in this study were characterized as irreversibly bound to the soil and indistinguishable from natural products (Dean 2023), therefore the bound residues were not included in the worst-case scenario calculations.

The determination of worst-case scenario degradation rates for the aerobic degradation of amprolium in soils were determined using the sum of the percent amprolium and the metabolite TP1. The input values used in the calculations are presented in Tables B-1 through B4. These values were calculated with Excel software and may include small differences in the sums due to rounding. These values were used as input in the CAKE software to determine the degradation rates with the first-order (SFO) kinetics model.

**Table B-1. Input data for $DT_{50}$ Determination in Dodson Soil**

| Days of Incubation | Amprolium | TP1 | Amprolium +TP1 |
|---|---|---|---|
| 0 | 94.2 | 0.0 | 94.2 |
| 0 | 96.0 | 0.0 | 96.0 |
| 7 | 87.0 | 0.0 | 87.0 |
| 7 | 87.1 | 0.0 | 87.1 |
| 14 | 77.5 | 1.4 | 78.9 |
| 14 | 77.9 | 2.1 | 80.0 |
| 30 | 58.6 | 4.0 | 62.6 |
| 30 | 64.4 | 4.8 | 69.2 |
| 59 | 30.1 | 12.8 | 42.9 |
| 59 | 31.6 | 16.0 | 47.6 |
| 120 | 7.5 | 8.9 | 16.4 |
| 120 | 8.9 | 9.0 | 17.8 |

**Table B-2. Input data for $DT_{50}$ Determination in Shebelut Soil**

| Days of Incubation | Amprolium | TP1 | Amprolium +TP1 |
|---|---|---|---|
| 0 | 104.1 | 0.0 | 104.1 |
| 0 | 102.7 | 0.0 | 102.7 |
| 7 | 97.1 | 0.0 | 97.1 |
| 7 | 94.7 | 0.0 | 94.7 |
| 14 | 88.0 | 0.0 | 88.0 |
| 14 | 86.7 | 1.1 | 87.8 |
| 30 | 64.0 | 5.5 | 69.5 |
| 30 | 65.5 | 5.4 | 70.9 |
| 59 | 35.7 | 12.3 | 48.0 |
| 59 | 35.1 | 12.0 | 47.0 |
| 120 | 6.4 | 9.7 | 16.1 |
| 120 | 7.7 | 11.2 | 18.9 |

**Table B-3. Input data for $DT_{50}$ Determination in Iowa Soil**

| Days of Incubation | Amprolium | TP1 | Amprolium +TP1 |
|---|---|---|---|
| 0 | 96.9 | 0.0 | 96.9 |
| 0 | 95.7 | 0.0 | 95.7 |
| 7 | 84.9 | 0.0 | 84.9 |
| 7 | 86.4 | 0.0 | 86.4 |
| 14 | 81.6 | 0.0 | 81.6 |
| 14 | 81.1 | 1.6 | 82.7 |
| 30 | 52.9 | 6.8 | 59.7 |
| 30 | 57.6 | 2.9 | 60.5 |
| 59 | 26.9 | 8.9 | 35.8 |
| 59 | 24.6 | 6.8 | 31.4 |
| 120 | 2.0 | 0.5 | 2.4 |
| 120 | 2.2 | 1.3 | 3.4 |

**Table B-4. Input data for $DT_{50}$ Determination in MSL Soil**

| Days of Incubation | Amprolium | TP1 | Amprolium +TP1 |
|---|---|---|---|
| 0 | 93.0 | 0.0 | 93.0 |
| 0 | 91.2 | 0.0 | 91.2 |
| 7 | 91.2 | 0.0 | 91.2 |
| 7 | 90.9 | 0.0 | 90.9 |
| 14 | 85.5 | 0.0 | 85.5 |
| 14 | 86.6 | 0.0 | 86.6 |
| 30 | 77.1 | 2.4 | 79.6 |
| 30 | 78.4 | 1.3 | 79.7 |
| 59 | 70.5 | 3.0 | 73.5 |
| 59 | 72.1 | 2.6 | 74.7 |
| 120 | 47.4 | 0.0 | 47.4 |
| 120 | 46.9 | 0.0 | 46.9 |

**Table B-5. Summary of Worst-Case Scenario Degradation Rates of Amprolium in Soil Under Aerobic Conditions.**

| | **SFO Model results[1]** | | | | |
|---|---|---|---|---|---|
| | **$DT_{50}$** | **$DT_{90}$** | **$R^2$** | **ChiSq(%)** | **Model** |
| **Dodson** | | | | | |
| Pyrimidine amprolium + TP1 | 52.5 | 174.0 | 0.9935 | 1.6 | SFO |
| **Shebelut** | | | | | |
| Pyrimidine amprolium + TP1 | 50.1 | 166.0 | 0.9970 | 1.6 | SFO |
| **Iowa** | | | | | |
| Pyrimidine amprolium + TP1 | 37.4 | 124.0 | 0.9840 | 5.8 | SFO |
| **MSL** | | | | | |
| Pyrimidine amprolium + TP1 | 134.0 | 445.0 | 0.9703 | 2.6 | SFO |

[1] NAFTA EPA recommended for modeling

## Appendix C. Use information for approved products containing amprolium

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 013-663 | Cocciprol™ | Amprolium (9.6% oral solution) | N | Growing chickens, laying hens and turkeys | N/A | N/A | Give amprolium at the 0.012% level (8 fluid ounces per 50 gallons) for coccidiosis outbreaks as soon as coccidiosis is diagnosed and continue for 3 to 5 days. (In severe outbreaks, give amprolium at the 0.024% level). Continue with 0.006% amprolium medicated water for an additional 1 to 2 weeks. Use as the sole source of amprolium. | As soon as coccidiosis is diagnosed and continue for three to five days at 0.012%; and treat at a lower dose for an additional 1-2 weeks. |
| 036-361 | Amprol Plus® and Aureomycin® | Amprolium Ethopabate, Chlor-tetracycline Type C medicated feed | Y | Broiler Chickens<br><br>Do not feed to egg producing chickens for human consumption | Amprolium: 227 grams per ton of feed | Feed continuously as sole ration for not more than the first 21 days of life | May be used as treatment of chronic respiratory disease caused by strains of *Mycoplasma* susceptible to chlortetracycline | Not provided |
| 114-794 | Baciferm® / Amprol HI-E® Premix | Amprolium, Bacitracin Zinc, and Ethopabate Type A medicated article | Y | Broiler Chickens up to 16 weeks.<br><br>Do not feed to laying chickens. | Amprolium 113.5 grams per ton of feed | Feed as the sole ration from the time chicks are placed on litter to market weight | Do not use as treatment for outbreaks of coccidiosis | Not provided |
| 141-156 | Amprol® / BMD® | Amprolium Bacitracin methylene disalicylate Type A medicated article | Y | Replacement chickens. | (1) Feed continuously as sole ration 36.3 to 113.5 g amprolium per ton feed depending on age | | N/A | N/A |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 141-469 | Stafac® and AMPROL® | Amprolium Virginia-mycin Type A medicated article | Y | Broiler chickens.<br><br>Not for use in laying chickens. Do not use in feeds containing bentonite. | (1) 72.6 to 113.5 g (0.008-0.0125%) amprolium per ton of Type C medicated feed<br><br>(2) Feed 113.5 to 227 g (0.0125-0.025%) amprolium per ton of Type C medicated feed | Feed continuously as the sole source of amprolium. | N/A | N/A |
| 200-205 | Albac® / Amprol Hi-E® | Amprolium Bacitracin Zinc Ethopabate Type A medicated article | Y | Broiler chickens<br><br>Not for chickens over 16 weeks of age. Do not feed to laying chickens. Not for use in feeds for breeder chickens. | Amprolium+Ethopabate: 113.5 g/ton of amprolium | Feed as the sole ration from the time chicks are placed on litter until past time when coccidiosis is ordinarily a hazard. | N/A | N/A |
| 200-464 | AMPROMED™ For Cattle | Amprolium 20% soluble powder | N | Calves[1] | 5 mg/kg | 21-Day | 10 mg/kg | 5-Day |
| 200-482 | AmproMed™ for Calves | Amprolium 9.6% oral solution | N | Calves[1].<br>Do not use in calves to be processed for veal | 5 mg/kg | 21-Day | 10 mg/kg | 5-Day |
| 200-488 | Ampromed™ P for Poultry | Amprolium 20% soluble powder (200 mg/g) | N | Growing chickens, turkeys, and laying hens | N/A | N/A | 0.024% - 20 oz in 1 gallon<br>0.012% - 10 oz in 1 gallon<br>0.006% - 5 oz in 1 gallon | Not stated |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 200-496 | AmproMed™ P for Poultry | Amprolium 9.6% oral solution (96 mg/mL) | N | Growing chickens, turkeys, and laying hens | N/A | N/A | Give amprolium at the 0.012% level (8 fl oz AMPROMED P for Poultry per 50 gallons) as soon as coccidiosis is diagnosed and continue for three to five days at 0.012%; and treat at a lower dose for an additional 1-2 weeks. (In severe outbreaks, give amprolium at the 0.024% level). Continue with 0.006% amprolium medicated water for an additional 1 to 2 weeks. Use as the sole source of amprolium. | As soon as coccidiosis is diagnosed and continue for three to five days at 0.012%; and treat at a lower dose for an additional 1-2 weeks. |
| 200-514 | Boviprol™ 9.6% Oral Solution | Amprolium (96 mg per mL of solution) | N | Calves[1].<br>Use on a herd basis only; when one or more calves show signs of coccidiosis, it is likely that the rest of the group has been exposed, and all calves in the group should be treated. | 5 mg amprolium/kg bw | Offer this solution as the only source of water for 21 days. May also be used as a drench (same dose) | 10 mg amprolium/kg bw | Offer this solution as the only source of water for 5 days. Use on a herd basis only. May also be used as a drench (same dose) |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 200-630 | CocciAid® | Amprolium 9.6% oral solution (96 mg/mL) | N | Growing chickens, turkeys, and laying hens | N/A | N/A | Give amprolium at the 0.012% level (8 fl oz per 50 gallons) as soon as coccidiosis is diagnosed and continue for 3 to 5 days. (In severe outbreaks, give amprolium at the 0.024% level.) Continue with 0.006% amprolium medicated water for an additional 1–2 weeks. Use as sole source of amprolium. | As soon as coccidiosis is diagnosed and continue for three to five days at 0.012%; and treat at a lower dose for an additional 1–2 weeks. |
| 012-350 | Amprol® 25% Type A Medicated Article | | N | Turkeys | 0.0125–0.025% in feed | Use as sole source of amprolium | N/A | N/A |
| | | | | Pheasants | 0.0175% in feed | | N/A | N/A |
| | | | | Chickens (replacement) | 0.004–0.025% in feed | | N/A | N/A |
| | | | | Chickens (laying) | 113.5 g/ton feed (0.0125%) | | 227 g/ton feed (0.025%) | Use as sole source of amprolium |
| | | | | Chickens (broilers) | 72.6–227 g/ton feed (0.008–0.025%) | | N/A | N/A |
| | Corid® Type A Medicated Article | | N | Calves[1] | 5 mg/kg bw | Use as sole source of amprolium | 10 mg/kg bw | Use as sole source of amprolium |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 013-149 | CORID® 9.6% Oral Solution, | Amprolium 9.6% oral solution (96 mg/mL) | N | Calves[1] | 5 mg amprolium/kg bw | Offer this solution as the only source of water for 21 days | 10 mg amprolium/kg bw | Offer this solution as the only source of water for 5 days. |
| | Amprovine 9.6% Solution | | N | Chickens, turkeys | N/A | N/A | 0.006% - 0.012% in drinking water | As soon as coccidiosis is diagnosed and continue for 3 - 5 days at 0.012%; and treat at a lower dose for an additional 1-2 weeks. |
| 013-461 | Amprol Hi-E,<br><br>Amprol Plus | Amprolium (25%) and ethopabate | Y | Broiler and replacement chickens less than 16 weeks of age | 113.5 g/ton in feed (0.0125%) | Use as sole source of amprolium | N/A | N/A |
| 033-165 | AMPROL® 128 | Amprolium soluble powder (20%) | N | Chickens, turkeys, laying hens | N/A | N/A | 0.012 up to 0.024 percent in drinking water (initially) then 0.006 percent | Higher dose for 3–5 days then continue with lower dose for 1–2 weeks. Offer as only source of water. |
| | Corid 20% Soluble Powder | Amprolium (20%) | N | Calves[1] | N/A | N/A | 5–10 mg amprolium/kg bw | Low dose daily for 21 days. High dose daily for 5 days. |
| 036-304 | Amprol HI-E® Plus | Amprolium (25%) with bacitracin methylene disalicylate and ethopabate | Y | Broiler chickens and replacements<br><br>Not for chickens over 16 weeks of age. Do not feed to laying chickens. Not for use as a treatment for outbreaks of coccidiosis. | 113.5 g/ton in feed | No information provided | N/A | N/A |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 095-543 | Amprol Hi-E® / Flavomycin®,<br><br>Amprol HI-E® & Bambermycins | Amprolium, bambermycins and ethopabate powder | Y | Broiler chickens | 113.5 g/ton of feed | Use as the sole source of amprolium | NA | NA |
| 130-185 | Flavomycin® and Amprol®,<br><br>Amprol 25%® and Flavomycin® | Amprolium 25% and bambermycins | Y | Broiler chickens, growing turkeys | (1) Feed 72.6 to 113.5 g amprolium per ton (0.008 to 0.0125%)<br><br>(2) Feed 113.5 to 227 g amprolium per ton (0.0125 to 0.025%) for chickens; 113.5 g/ton for turkeys | (1) feed continuously as the sole ration for prevention of coccidiosis caused by *Eimeria tenella* only, and for increased rate of weight gain and improved feed efficiency in broiler chickens.<br><br>(2) feed continuously as the sole ration for prevention of coccidiosis where immunity to coccidiosis is not desired and for increased rate of weight gain and improved feed efficiency. Use 0.0125% amprolium for most field conditions as they exist under modern management practices. Where severe coccidiosis conditions exist and where immunity is not required, use 0.025% amprolium. | NA | NA |

| NADA | Proprietary Name | Form of Amprolium | Combination Drug (Y/N) | Target Animals | Use for Prevention | | Use for Treatment | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Dose (mg/kg body weight) | Frequency of Dose / efficacy information | Dose (mg/kg body weight) | Frequency of Dose / efficacy information |
| 200-389 | Amprolium 9.6% Oral Solution | Amprolium 9.6% | N | Calves[1] | 5 mg amprolium/kg bw | Offer as the only source of drinking water for 21 days.<br><br>Can be given in drinking water or with a drench. | 10 mg amprolium/kg bw | Offer as the only source of drinking water for 5 days.<br><br>Can be given in drinking water or with a drench. |
| 200-463 | Amprolium-P 9.6% Oral Solution | Amprolium 9.6% | N | Growing chickens, turkeys and laying hens | NA | NA | Give amprolium at the 0.012% level (8 fl oz per 50 gallons). In severe outbreaks, give amprolium at the 0.024% level. | …as soon as coccidiosis is diagnosed and continue for 3 to 5 days. Continue with 0.006% amprolium medicated water for an additional 1 to 2 weeks. No other source of drinking water should be available to the birds during this time. Use as the sole source of amprolium. |

[1] For use as an aid in the treatment and prevention of coccidiosis caused by *Eimeria bovis* and *E.zurnii* in calves.