ENVIRONMENTAL ASSESSMENT FOR CLINACOX™

1. DATE

May 20, 1996

2. NAME OF APPLICANT

Mallinckrodt Veterinary, Inc.

3. ADDRESS

421 East Hawley Street
Mundelein, Illinois 60060

4. DESCRIPTION OF THE PROPOSED ACTION

A. Requested Approval

The Applicant has requested approval of NADA 140-951 providing for the marketing of CLINACOX™ (diclazuril) Type A Medicated Article (premix) containing 0.2 % diclazuril. The premix will be incorporated into the rations of broiler chickens to attain a level of 1 ppm diclazuril. The medicated rations will be fed to chickens for the prevention or control of coccidiosis, a debilitating protozoal disease of chickens.

B. Need for the Action (Intended Use)

Coccidiosis is a ubiquitous, severe, and costly disease affecting broiler chickens which is caused by a protozoan parasite of the genus *Eimeria*. The parasite multiplies in the intestinal tract of the host and causes damage to intestinal tissues resulting in malabsorption, diarrhea, and sometimes death. Approximately 10 new animal drugs are currently approved for use in prevention and/or control of coccidiosis. Problems have been recognized with each of these products, such as: marginal effectiveness against some species of coccidia; low margins of safety; or high required feeding levels. Diclazuril is effective against all species of *Eimeria* at the lowest feeding level of any approved product, and possesses a very low order of toxicity, resulting in a wide margin of safety. As a result, it offers a needed alternative to the available armamentarium of coccidiosis prevention and control measures.

C. Production Locations

1. Drug Substance (Diclazuril)

Diclazuril will be produced at a foreign facility owned by Janssen Pharmaceutica, Beerse, Belgium. The facility has two manufacturing plants which are currently in operation and are producing diclazuril for non-US markets. The plants, located in Beerse and Geel, Belgium, have similar equipment, are centrally managed (including safety and environmental management), and share common services such as maintenance, QA/QC, warehouses, and supplies. Production of diclazuril at these sites was approved by the Permanent Deputation of the Provincial Council of Antwerp on April 23, 1987 (Beerse) and November 26, 1987 (Geel). The permit numbers and certain other environmental and manufacturing information described below are considered confidential by Janssen Pharmaceutica. A complete file of the confidential information has been provided in support of this application (Janssen Pharmaceutica, 1996).

The Janssen Pharmaceutica Beerse plant is located on a parcel of 148 acres. The site is surrounded with residential type housing and primary access is from state road N 14 connecting Antwerp to Turnhout.

The Janssen Pharmaceutica Geel plant is located on a parcel of 99 acres in the Geel industrial area. The industrial area is bounded to the north by the Albert Channel and to the south by highway E 313 connecting Antwerp to Achen.

2. Drug Product (CLINACOX Premix)

Diclazuril will be transported in sealed containers to the Mallinckrodt Veterinary, Inc. facility in Terre Haute, IN. The diclazuril will be blended into the Type A Medicated Article (premix) at the premixing facility. The CLINACOX premix plant is located on a 42 acre site zoned for industrial manufacturing at 1331 South First Street, Terre Haute, IN. The site is bounded on the east by South First Street, on the north and south by other industrial properties, and on the west by a cinder access road which runs along the Wabash River.

3. Finished Poultry Feeds

CLINACOX will be distributed to feed mills throughout the United States, including its territories and possessions, for the production of medicated feed for the poultry industry.

D. Locations of Use

Poultry production occurs throughout the United States, but primarily in rural areas in the South Central and South Atlantic regions of the country.

E. Disposal Locations

1. Diclazuril Manufacturing and Distribution

Solid waste resulting from manufacturing, testing, and packaging, or from rejected or outdated drug substance, will be transported to the licensed waste processor Indaver N.V., Antwerp, Belgium.

Waste solvents will be hauled for recycling and reuse in relevant industries by either of two licensed waste processors, De Neef Chemical Recycling, Heist op den Berg, Belgium, or Chemical Manufacture and Recycling Limited, Rye Harbour, England.

Packaging material waste, not contaminated with pharmaceutical substances, will be collected selectively and recycled by a specialized waste contractor, Watco Waste Center, Beerse, Belgium.

2. Premix Manufacturing and Distribution

Rejected, returned or expired drug products will be disposed of by landfilling at a facility which is permitted by the US Environmental Protection Agency (EPA), or by a state environmental agency which has received delegated authority from EPA. The facility currently used in Terre Haute, Indiana is Victory Environmental Services, Inc. which is permitted by the Indiana Department of Environmental Management (IDEM) to accept municipal and special wastes, under permit number OPP 84-2.

3. Poultry Feed Mixing and Distribution

Waste CLINACOX (containing 0.2 % diclazuril) and poultry feed (containing 1 mg/kg diclazuril) from feed mills that prepare and distribute poultry feeds will be handled according to industry standards to minimize release into the environment.

Any accidental or indirect introductions of diclazuril via animal feed at points of use (e.g., poultry farms) would be at the 1 mg diclazuril per kg feed concentration which is comparable to the 2.2 mg/kg (dry weight) or 0.55 mg/kg (wet weight) poultry litter concentration (see Item 6.C.2. below).

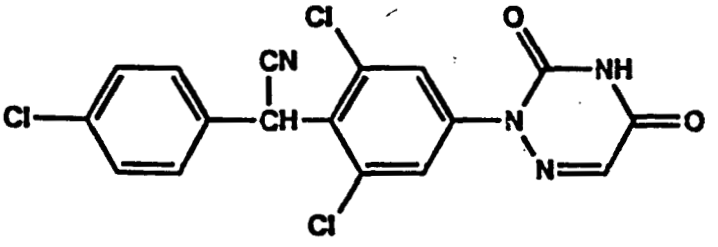
5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

The active ingredient (drug substance) in CLINACOX is diclazuril. Information on the identity of diclazuril is summarized in Table 1.

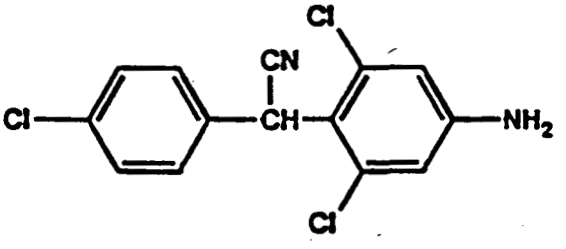
Table 1. Identification of Drug Substance in CLINACOX

Parameter	Description
Established Name (USAN)	diclazuril
Brand/Proprietary Name	CLINACOX
Chemical Name	(±)-2,6-dichloro-α-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzeneacetonitrile
CAS Registration Number	101831-37-2
Other Identification Number	R64433 (by Janssen Pharmaceutica)
Molecular formula	$C_{17}H_9C_{13}N_4O_2$
Molecular weight	407.64
Structural formula	See Figure 1
Physical description	white to light beige powder
Known metabolite	DM5 (see Figure 1)

Figure 1. Structures of Diclazuril and Metabolite DM5



Diclazuril



Metabolite DM5

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. Manufacture of Diclazuril: Janssen Pharmaceutica (Beerse & Geel, Belgium)

1. Substances Expected to be Emitted

The materials used in the manufacture of diclazuril, and thus possibly emitted into the environment, are considered confidential information by Janssen Pharmaceutica. A complete listing of those substances has been provided in support of this application (Janssen Pharmaceutica, 1996).

2. Controls Exercised

All wastewaters are discharged to central two-stage biological wastewater treatment systems (WWTS). Air emissions are discharged to the atmosphere through two-stage scrubber systems. No treatment of solid waste is done at the manufacturing plants. Solid wastes are temporarily stored in appropriate containers and at regular times hauled by a licensed transporter to a licensed high temperature waste incinerator.

3. Citation of, and Statement of Compliance with, Applicable Emissions Requirements

a. Wastewater

The effluents from the WWTS are discharged in compliance with the requirements set forth in the Janssen Pharmaceutica wastewater discharge permits. The permits were obtained from the Flemish regional authorities (Permanent Deputation of the Provincial Council of Antwerp) dated March 10, 1994 (Beerse) and September 3, 1992 (Geel).

b. Air Emissions

Air emissions are in compliance with the requirements set forth in the Janssen Pharmaceutica operation permits. The permits were obtained from the Flemish regional authorities (Permanent Deputation of the Provincial Council of Antwerp) dated April 23, 1987 (Beerse) and November 26, 1987 (Geel).

c. Solid Waste

Solid waste handling is done in compliance with the Flemish regional waste legislation. Because no treatment takes place at the site, no special permit for treatment is required. However, monthly and yearly reports to the authorities are required.

d. Occupational Standards

The facility will make available to employees the detailed Material Safety Data Sheet (MSDS) for diclazuril, included in Appendix 1. The MSDS for each of the raw material substances is also available to employees.

The design and use of facilities, buildings, equipment, and procedures for manufacturing, packaging, distribution, and disposal of the drug substance meet current standards of operation (building and energy regulations), pharmaceutical production (Good Manufacturing Practices), environmental protection, and occupational exposure.

Occupational standards in Belgium are prescribed in the national General Regulation for Labor Patronage (Algemeen Reglement voor Arbeidsbescherming). The Janssen facilities in Belgium comply with these standards.

e. Compliance Statement

Janssen Pharmaceutica is currently in compliance with all applicable local and national environmental laws and emission requirements. A compliance statement for this facility, signed by the responsible company official, is contained in Appendix 2.

4. Effect of Approval on Compliance with Current Emission Requirements

Diclazuril is currently produced at the Beerse and Geel plants. Approval of the proposed action will result in an increase in the existing production of diclazuril but will have no impact on compliance with current emission requirements.

B. CLINACOX Premixing Facility: Mallinckrodt Veterinary, Inc. (Terre Haute, IN)

1. Substances Expected to be Emitted

Individual substances expected to be emitted during the premix operation are listed in Table 2.

Table 2. Substances Expected to be Emitted: Mallinckrodt Veterinary, Inc. Facility in Terre Haute, IN		
Substance	**CAS No.**	**Wastestream(s) or Media Expected to be Emitted Into**
Diclazuril	101831-37-2	Air emissions, wastewater, solid waste
Soybean oil	8001-22-7	Wastewater, solid waste
Calcium carbonate	1317-65-3	Air emissions, wastewater, solid waste
Wheat middlings	N/A	Air emissions, wastewater, solid waste
Colloidal silicon dioxide	60676-86-0	Air emissions, wastewater, solid waste

2. Controls Exercised

The premixing facility was constructed in 1991 solely for CLINACOX premixing. The facility is entirely enclosed, with controlled heating and ventilation. Air emissions of particulates are abated by dust collection systems. Floor drains within the process areas discharge to the Terre Haute Wastewater Treatment Plant. In order to minimize potential discharges of wastewater, initial cleaning of the equipment will be conducted using dry methods. Disposal of solid waste will be minimized by the use of highly-controlled procedures and properly designed, maintained, and operated equipment for the production of CLINACOX premix. Persons entering the processing and packaging areas must have arms and legs covered (long sleeves, pants) and wear gloves, safety glasses, and other equipment which may be specified by the department. For example, due to possible exposure to dust and airborne particulates, employees must wear a dust respirator and the use of contact lenses is prohibited.

3. Citation of, and Statement of Compliance with, Applicable Emissions Requirements

a. Wastewater Requirements

Wastewater discharges and permitting are regulated under the Clean Water Act,

Indiana Regulation 327 IAC 5, and the Terre Haute Pretreatment Ordinance. Process wastewater discharges from the facility are made under provisions of the Terre Haute Wastewater Treatment Plant Pretreatment Permit 1099B [expiration date June, 1997]. Discharges of non-contact cooling water and stormwater runoff to the Wabash River are regulated under National Pollutant Discharge Elimination System (NPDES) permit IN 0003328. [The Terre Haute site's NPDES permit expired in October, 1995. A renewal application was submitted to the IDEM in April, 1995. Because the renewal application was submitted in a timely manner, the existing permit remains in effect until a new permit is issued.]

b. Air Emissions Requirements

Air emissions and permitting are regulated under the Clean Air Act, Indiana Regulation 326 IAC 2, and administered by the Vigo County Air Pollution Control Office. The operating permit for the CLINACOX premix facility is 04-2834-01-95 [expiration date January, 1998].

c. Solid Waste Requirements

Disposal of solid wastes is regulated by requirements mandated under the Federal Resource Conservation and Recovery Act (RCRA) and Indiana Regulations 329 IAC 2.21 and 329 IAC 3.1. All solid wastes are disposed of at appropriately permitted solid waste disposal facilities. Currently, solid wastes from the Terre Haute facility are disposed of at the Victory Environmental Services, Inc. landfill, which is permitted by the IDEM for landfill disposal of municipal and special wastes. Special waste disposal approvals have been granted by the IDEM and can be renewed in less than 60 days.

d. Occupational Standards

The facility will make available to employees the detailed Material Safety Data Sheet (MSDS) for diclazuril, included in Appendix 1.

The facility maintains a program for continued monitoring and documentation of compliance with the applicable occupational standards. Employee exposure monitoring will be conducted for the following dusts:

Wheat dust with a Threshold Limit Value (TLV) of 4 mg/m^3 and an Occupational Safety and Health Administration Permissible Exposure Limit of 10 mg/m^3.

Calcium carbonate with a TLV of 10 mg/m^3.

Silicon dioxide with a TLV of 10 mg/m^3.

e. Compliance Statement

The Mallinckrodt Veterinary, Inc. facility (Terre Haute, IN) is currently in compliance with all applicable local and national environmental laws and emission requirements. A compliance statement for this facility, signed by the responsible company official, is contained in Appendix 3.

4. Effect of Approval on Compliance with Current Emission Requirements

Approval of the use of CLINACOX will not adversely affect compliance with current emissions requirements and occupational standards.

C. Expected Introduction Amount and Concentrations

1. Metabolism and Excretion of Diclazuril by Broiler Chickens

Diclazuril metabolism and excretion has been characterized in single and repeated-dose poultry studies using ^{14}C-diclazuril labeled in the cyano [nitrile] group (Janssen Pharmaceutica, 1987b, 1988b, 1989d). In the repeat dosing study, male and female broiler chickens were dosed orally at 0.045 mg/kg body weight per day (equivalent to 1 ppm in the feed) from day 28 to day 41 of age. At steady state after repeated oral dosing (approximately one week), about 90 % of the administered daily dose was excreted each day. Mass balance data indicated that about 82 to 84 % of the total administered dose of diclazuril was recovered in excreta as a radioactive fraction within 10 days of administration of the final dose. Parent (unchanged) diclazuril in the excreta accounted for 50.3 % (females) or 56.0 % (males) of the total administered dose.

The primary metabolite in the excreta, DM5 (Figure 1; formed as result of hydrolytic cleavage of the triazine ring), accounted for 5.6 to 8.3 % of the total administered dose and 11 to 18 % of sample radioactivity during the post-dosing time interval. The remaining fraction of radioactivity in the excreta, about 23 %, was made up of other diclazuril metabolites. At steady state, three metabolites (DM3, DM7, and DM8) were detected in addition to DM5, each accounting for 3 to 5 % of the excreta radioactivity. At various time periods after dosing was completed, several additional metabolites (DM1, DM2, DM4, DM6) were detected in excreta, one metabolite (DM6) accounting for about 7 % of the sample radioactivity and the others in the range of 2 to 4 %.

In summary, study data indicate that about 30 % of administered diclazuril was excreted as metabolites. About 53 % of dosed diclazuril was excreted unchanged. The remaining radioactivity (17 %) remained *in vivo* or was eliminated by other routes [additional studies with chickens and rats show that up to 3 % of the administered radioactivity is excreted as

$^{14}CO_2$, $S^{14}CN^-$, $^{14}CN^-$, or $H^{14}CN$ (Janssen Pharmaceutica, 1987a)]. Based on the lack of metabolites in the bile, it was concluded that DM5 and the other metabolites in excreta originated from degradation of unabsorbed diclazuril by the intestinal microflora.

2. Amount of Diclazuril Present in Poultry Litter

The expected introduction concentration for parent diclazuril, in this case, the concentration in poultry litter, serves as the basis for all subsequent calculations of concentrations of diclazuril in relevant environmental compartments (i.e., soil, surface water, sediment). Utilizing available metabolism and excretion data, the litter concentration may be determined based on the method of Harrass et al. (1991):

$$\{1\} \quad \textit{Litter concentration (mg/kg)} = \frac{\textit{(Individual daily dose; mg/day)}\ (1 - f)}{\textit{(Mass of litter generated per animal; kg/day)}}$$

where

f = fraction of drug that is metabolized, or absorbed and not excreted

The total individual daily dose of diclazuril (mg/day) is calculated by multiplying the feed consumption (kg/day) by the concentration in feed (1 mg diclazuril/kg). This dose may also be expressed on a body weight basis (i.e., mg/kg/day). Using historical data for feed consumption and body weights for broiler chickens during the latter part of the growout period[1], calculated daily doses of diclazuril are presented in Table 3.

Daily doses corrected for body weight peak on day 31 and slowly decline thereafter; however, total doses expressed in mg/day tend to rise over time as feed consumption increases and peak on day 40. Using equation *{1}* and the day 40 peak dose (0.133 mg/day) to calculate an upper bound estimate, the maximum expected concentration of parent diclazuril in poultry litter is 2.20 mg/kg on a dry weight basis (0.55 mg/kg on a wet weight basis). This upper-bound estimate is based on the following assumptions:

Approximately 53 % of the daily dose of diclazuril is excreted as parent compound in poultry excreta (f = 0.47). Study data discussed previously indicate that the remaining 47 % of administered diclazuril is metabolized (most likely by gut microflora) and excreted as DM5 and several other metabolites, remains *in vivo* as unchanged parent compound or metabolites, or is eliminated as volatile metabolic products (e.g., CO_2, CN).

[1] The growout period ranges from 6 to 7.5 weeks for broiler chickens (Mitchell et al., undated)

Table 3. Historical Data of Body Weight and Feed Consumption in Broiler Chickens and Daily Doses of Diclazuril When Present in Feed at 1 ppm[1]

Chicken Age (day)	Body Weight (kg)	Feed Consumption (kg/day)	Daily Dose of Diclazuril	
			mg/day	mg/kg/day
28	0.954	0.094	0.094	0.098
29	1.010	0.103	0.103	0.102
30	1.070	0.111	0.111	0.104
31	1.129	0.119	0.119	0.121
32	1.185	0.111	0.111	0.094
33	1.243	0.120	0.120	0.096
34	1.295	0.119	0.119	0.092
35	1.354	0.121	0.121	0.089
36	1.413	0.124	0.124	0.088
37	1.472	0.124	0.124	0.084
38	1.526	0.122	0.122	0.080
39	1.584	0.123	0.123	0.078
40	1.647	0.133	0.133	0.081
41	1.709	0.128	0.128	0.075
Mean	1.328	0.118	0.118	0.090

[1] Data taken from Janssen Pharmaceutica, 1987b.
Original source of data: Holsheimer, J. P., Spelderholt Institute for Poultry Research, Beekbergen, The Netherlands; I.P.S. Communication No. 385.

According to the USDA Fact Sheet on Animal Manure Production (USDA, undated), broilers excrete 0.28 pounds (0.127 kg) per day of fresh manure with a moisture content of 75 %. Converting to dry weight, this equates to 0.032 kg per day. The latter figure is used because it produces a higher litter concentration when used in equation 1, and because soil amendment rates for poultry litter are typically expressed on a dry weight basis.

A typical broiler production house will be full for 6 cycles per year (6-7.5 weeks growout plus 1-2 weeks between cycles). Total manure production from a group of houses with a combined capacity of 100,000 birds (0.032 kg/bird/day X 45 days X 100,000 birds X 6 cycles) would be approximately 864,000 kg (950 tons) per year. This would be spread on 190 acres of cropland at the rate of 5 tons/acre.

Diclazuril concentrations in poultry manure and in litter as amended to soil are assumed to be equal. In reality, poultry litter as amended to soil would typically include diluents (e.g., sawdust, wood shavings, peanut hulls) used in poultry operations which would decrease the concentration of diclazuril in the mass of litter amended to soil.

3. Concentration of Diclazuril in Soil After One Application of Manure

Harrass et al. (1991) also presented a model for predicting soil concentrations of chemicals in manure that incorporates the following parameters:

1) the typical application rate for poultry litter applied as fertilizer (1.1 kg/m^2 or 5 tons/acre; applied as dry weight);
2) the depth to which litter is incorporated into soil (top 15 cm of soil); and
3) the average soil density (1,350 kg/m^3).

The model yields the following dilution rate for diclazuril in soil:

$$\{2\} \quad Dilution\ Rate = \frac{(Application\ Rate)\ (100\ \%)}{(Soil\ Depth)\ (Soil\ Density)} = 0.54\ \%$$

The soil concentration of parent diclazuril immediately following an application of poultry litter is calculated as the product of the concentration of the unchanged drug in the poultry litter (2.2 mg/kg expressed on a dry weight basis as described in Item 6.C.2., above) and the dilution rate as per the following equation:

$$\{3\} \quad C_{(soil)} = \frac{(Concentration_{litter})\ (Dilution\ Rate)}{100\ \%} = 0.012\ mg/kg\ (ppm) = 12\ ppb$$

This estimate is conservative in that it does not take into account any biotic or abiotic degradation of diclazuril that has occurred in litter in the time interval between when the manure was excreted and when it was actually amended to soil. In addition, the litter concentration has been calculated assuming that there are no diluents (e.g., saw dust, wood chips) present in the litter, which is rarely the case.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

A. Identification of Substance of Interest

The majority (~ 53 %) of diclazuril given in repeated dose studies is excreted unchanged, and no metabolites or structurally related substances comprise greater than 10 % of the excreted dose. Therefore, the active drug substance of CLINACOX, diclazuril, is the only substance quantitatively evaluated in this environmental assessment.

B. Physical/Chemical Characterization

1. Melting Point

The melting point of diclazuril was determined by the capillary method to be approximately 295 °C (Janssen Pharmaceutica, 1985a).

2. Density

As determined by the pycnometer method, the mean density of diclazuril is 1.614×10^3 kg/m^3 at 20 °C (Janssen Pharmaceutica, 1989c).

3. Dissociation Constant (pKa)

The dissociation constant was determined using OECD Guideline 112 and found to be 5.92, extrapolated to pure water (Janssen Pharmaceutica, 1989a). This study indicates that diclazuril will dissociate in low pH environments.

4. Water Solubility

Using the flask method (OECD Guideline No. 105), the water solubility of diclazuril was determined to be less than 10^{-6} g/L (< 1 ppb) (Janssen Pharmaceutica, 1986a). However, because this method may not be accurate for substances with very low solubility, diclazuril solubility is reported as less than 10 ppb.

5. Vapor Pressure

The vapor pressure of diclazuril was determined in two separate studies using different methodologies. Using the spinning rotor method, the vapor pressure at 10 °C (282 °K) was 5.85 x 10^{-7} torr (State University of Utrecht, 1986). To confirm this value, the vapor pressure was determined by a capillary gas chromatography method and calculated to be 4.91 x 10^{-9} torr at 25 °C (298 °K), corrected for being a solid at room temperature, and 2.3 x 10^{-6} torr, uncorrected (Mallinckrodt Veterinary, 1988). Taken together, these data indicate that the vapor pressure of diclazuril is very low and that diclazuril will not volatilize under environmental conditions.

6. Octanol/Water Partition Coefficient

The n-octanol/water partition coefficient was initially determined to be 3.5 X 10^4 (log K_{ow} = 4.54) at pH 8 (Janssen Pharmaceutica, 1989b). Subsequently, the partition coefficient was characterized over the pH range of about 5 to 8 as follows:

1.0 X 10^4 (log K_{ow} = 4.0) at pH 4.98

3.0 X 10^4 (log K_{ow} = 4.5) at pH 7.03

2.5 X 10^4 (log K_{ow} = 4.4) at pH 8.00

(Mallinckrodt Veterinary, 1989). These data indicate that over the relevant environmental pH range, the octanol/water partition coefficient is greatest at about pH 7, and that diclazuril is lipophilic. The results suggest that there is some potential for diclazuril to bioconcentrate in biota.

7. Ultraviolet-Visible Absorption Spectrum

The ultraviolet-visible (UV-Vis) absorption spectrum of diclazuril was originally determined over the pH range of 1.5 to 11.0 in buffer-methanol and water-methanol solutions (Janssen Pharmaceutica, 1986b). This study was subsequently repeated in accordance with FDA TAD 3.05 at 25 °C over the pH range from 1.1 to 13.0 (Janssen Pharmaceutica, 1991a). Results of the latter study are summarized in Table 4.

Results of both studies indicated that some absorption may be expected in the tail range above 290 nm. As a result, there is the potential for photodegradation of diclazuril.

Table 4. Ultraviolet-Visible Spectra of Diclazuril: Peak Maxima and Band Widths[1]

pH	Maximum 1 (nm)	Band width[2] (nm)	Maximum 2 (nm)	Band Width[2] (nm)	Maximum 3 (nm)	Band Width[2] (nm)
1.1	218 - 219	26 - 28			276 - 277	50 - 52
5.6					274 - 276	50
6.2	219	26			274 - 276	50 - 52
9.0	221	24	249	*	269	84
13.0					269 - 271	52 - 58

[1] Three determinations were made at each pH. Ranges are given when values were not the same for all three determinations.

[2] Band width at half of peak maximum.

* Cannot be determined due to overlap.

C. Environmental Fate Studies

The environmental fate, distribution, and depletion of diclazuril was evaluated using test protocols generally following those outlined in the FDA Technical Assistance Handbook. Studies were conducted on adsorption/desorption to soils, hydrolysis, aqueous photolysis, and aerobic biodegradation in soils.

1. Adsorption/Desorption to Soils

Soil adsorption and desorption coefficients for diclazuril were determined using ^{14}C-diclazuril and three different soil types (Springborn Life Sciences, 1989a). All tests were conducted in triplicate. A nominal solution concentration of 0.50 mg diclazuril/L in 0.01 M $CaCl_2$ or deionized water was used in the preliminary and screening tests to determine the time required to reach equilibrium and an approximation of the sorption coefficients for a silty clay loam, sandy loam, and silt loam soil. Estimated adsorption K_d values were 204 for silty clay loam, 1824 for sandy loam, and 1009 for silt loam. Estimated desorption K_d values were 84, 301 and 902, respectively.

Because of mass balance recovery variations, one leg of this definitive study was repeated using Mississippi silty clay loam, diclazuril concentrations of 0.50, 0.25, 0.10, and 0.05 mg/L, and the same experimental protocol. The resulting adsorption K_d of 720 and desorption K_d of 93 for silty clay loam validated the results of the larger study.

While the use of test concentrations above the water solubility of diclazuril (< 10 g/L) precludes the establishment of definitive adsorption/desorption coefficients, the study indicates that diclazuril strongly adsorbs to soil and would not be mobile in soil environments. These expectations are consistent with the physicochemical properties of diclazuril discussed earlier (i.e., very low water solubility; moderately high octanol/water partition coefficient).

2. Hydrolysis

The hydrolysis potential of diclazuril was studied in aqueous buffers over the pH range of 5 to 9 (Springborn Life Sciences, 1987a). Study data indicate that diclazuril is stable in pH 5, 6, 7, and 8 buffers and is rapidly hydrolyzed at pH 9. Because pH values above 8 do not occur in the majority of natural waters, hydrolysis is not expected to be a significant depletion mechanism for diclazuril in surface waters or in the interstitial water of most soils.

3. Aqueous Photolysis

The photolysis of diclazuril in the aquatic environment under conditions of natural sunlight was studied using ^{14}C-radiolabeled compound (Janssen Pharmaceutica, 1993c). The method used was similar to that described in TAD 3.10 except that the study was conducted at a single pH. Diclazuril at a concentration of 0.5 ppm in an acetonitrile solution, and the reference actinometer (p-nitroacetophenone-pyridine), were exposed aseptically to sunlight for 14 days in sealed borosilicate glass vials at an average pH of 6.6. For each sampling period, four test vials and three reference vials were taken for analysis. Three foil wrapped vials were also sampled as dark controls. Sampling was conducted at 0, 1, 2, 4, 10, 20, 24, 28 hours and 2, 3, 4, 6, 10, and 14 days of exposure.

Actinometer samples were analyzed by HPLC as per published protocol. The actinometer concentration steadily decreased with exposure time and was reduced to approximately half of the initial concentration by day 14 of exposure to sunlight. Diclazuril test samples were analyzed by radioassay and radio-HPLC for determinations of total activity and parent / transformation compounds, respectively. Radioactivity recoveries ranged from 88.9 to 108 % of the input dose. The volatile phase of the exposed test solutions never exceeded 1 % of the initial dose. Radio-HPLC analysis showed a decrease in diclazuril concentration over time with the parent compound accounting for 76.2 % of initial radioactivity after 14 days of light exposure. Dark controls showed little or no decrease in

diclazuril concentration over time. Five apparent degradation products appeared in the test solutions. None of these fractions reached 10 % of the initial dose at any time during the study. One fraction co-eluted with the reference compound 2,6-dichloro-α-(4-chlorophenyl) benzeneacetonitrile indicating a degradation product resulting from the breakdown and / or removal of the parent compound's heterocyclic ring. The opening of the heterocyclic ring may provide chemical sites open to microbial degradation.

The photoreaction quantum yield of diclazuril was determined by combining the concentration data of diclazuril with the HPLC results for p-nitroacetophenone in the simultaneously exposed actinometer solutions. Depending on the calculation method used ("forced fit" or "free fit" regression analysis), the value of the reaction quantum yield (Φ_{dc}) was determined to be 4.40×10^{-4} ("forced fit") or 2.22×10^{-4} ("free fit"). Based on these two quantum yield values, diclazuril photodegradation rate constants and half-lives were calculated as a function of latitude and season (Table 5). Calculated half-lives for the summer ranged from about 10 to 25 days over a latitude of 30° to 50°. Photodegradation rates were smallest during the winter with half-lives ranging from about 29 to 308 days. Taken together with the UV-Vis absorption spectrum data presented earlier, it is concluded that diclazuril is photodegradable in water and that a photodegradation half-life would be in the range of several weeks to several months depending upon the season.

Table 5. Photodegradation Rate Constants and Half-Lives for Aqueous Diclazuril as a Function of Latitude and Season[1]

Parameter	Latitude	Spring	Summer	Fall	Winter
Quantum yield (Φ_{dc}) = 4.40 x 10^{-4} ("forced fit" regression)					
Rate Constant	50°	3.54 x 10^{-2}	5.49 x 10^{-2}	1.41 x 10^{-2}	4.46 x 10^{-3}
(K_{dE}) in day^{-1}	40°	4.67 x 10^{-2}	6.25 x 10^{-2}	2.55 x 10^{-2}	1.22 x 10^{-2}
	30°	5.70 x 10^{-2}	6.79 x 10^{-2}	3.80 x 10^{-2}	2.41 x 10^{-2}
Half-life	50°	19.6	12.6	49.3	155
(days)	40°	14.8	11.1	27.2	56.8
	30°	12.2	10.2	18.2	28.8
Quantum yield (Φ_{dc}) = 2.22 x 10^{-4} ("free fit" regression)					
Rate Constant	50°	1.79 x 10^{-2}	2.77 x 10^{-2}	7.09 x 10^{-3}	2.25 x 10^{-3}
(K_{dE}) in day^{-1}	40°	2.36 x 10^{-2}	3.15 x 10^{-2}	1.29 x 10^{-2}	6.15 x 10^{-3}
	30°	2.87 x 10^{-2}	3.42 x 10^{-2}	1.92 x 10^{-2}	1.21 x 10^{-2}
Half-life	50°	38.8	25.0	97.8	308
(days)	40°	29.4	22.0	53.9	113
	30°	24.1	20.3	36.2	57.1

[1] Midseason dates and clear sky conditions.

4. Aerobic Biodegradation in Soils

The aerobic biodegradation of diclazuril in soil was evaluated in several studies. In an initial soil biodegradation study, evolution of carbon dioxide was used to characterize complete biodegradation (mineralization) of radiolabeled diclazuril in three soils (Springborn Life Sciences, 1987b). Results indicated that minimal mineralization occurred; less than 5 % of the initial ^{14}C-diclazuril dose added to the soil was recovered as $^{14}CO_2$ or as ^{14}C-volatile products over the 74-day test period. The evolution of CO_2 from the glucose controls was lower than would be expected; it was only 30.6 - 45.0 %. This suggests that either the microbial inoculum was weak, a significant portion of the CO_2 evolved was not captured, or significant carbon was incorporated into microbial biomass. If there was in fact greater biodegradation in the glucose control than was accounted for by ^{14}C-volatile products, then the same could apply to diclazuril, meaning that the study results underestimated degradation.

In a supportive study of the aerobic biodegradation of diclazuril in soil, unlabeled test compound was added at one dose level (nominal 10 ppb) to neat sandy loam soil and to sandy loam soil fortified with dextrose (ABC Laboratories, 1991). Dextrose added to sandy loam soil served as the positive control. Sterile controls using 10 ppb diclazuril added to autoclaved soils were included in the study. The aerobic metabolism of the test material was monitored for 64 days. Soils were extracted with acetonitrile and the extract was analyzed by HPLC for determinations of parent compound and possible transformation products. Soil amended with diclazuril plus dextrose was observed to produce CO_2 at a rate similar to that produced by the positive control (dextrose only). This suggests no suppression or toxicity of diclazuril to the microflora at 10 ppb. HPLC analysis of test soil extracts showed a reduction of parent diclazuril to approximately 10 % of the initial dose at day 64, suggesting that it is readily degradable. Extracts of the autoclaved controls at day 64 contained approximately 78.4 % of the applied dose of diclazuril, suggesting that the parent compound is readily extractable and that physical factors were not responsible for the reduction of parent diclazuril in the unsterilized soil. Because of the limitations of the HPLC analysis with unlabeled material, no characterization of potential transformation products was made and results should be considered only qualitative; however, these study data indicate that some portion of the parent diclazuril molecule was biodegraded.

In order to overcome the limitations of the previous two studies, a definitive study of diclazuril biodegradation in soils was conducted using ^{14}C-diclazuril (ABC Laboratories, 1992). The study was conducted, with some modifications, according to methods recommended in TAD 3.12. Radiolabeled-diclazuril (99.8 % purity; labeled at the cyano carbon) was added to three soil types (sandy loam, loam, and silt loam) at 10 and 100 ppb. Radiolabeled ^{14}C-glucose was tested concurrently as the positive control. The aerobic soil

metabolism of the material was monitored for 64 days. Soils samples were extracted with acetonitrile for determination of degradation products.

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Evolution of ^{14}C-volatiles was observed in all three soils at both dose levels (Table 6), indicating that some mineralization of diclazuril did occur. By day 64, the cumulative percentages of the day 0 initial measured dose (IMD) recovered as volatiles ($^{14}CO_2$) from the three soils ranged from 7.25 to 13.36 % and from 5.34 to 15.32 % for the 10 ppb and 100 ppb treatments, respectively (using 90 - 110 % Recovery Data). Biodegradation was greatest in silt loam and least in sandy loam; degradation was slightly more rapid at the lower dosage level (10 ppb).

Table 6. Evolution of Volatiles ($^{14}CO_2$) after Dosing of ^{14}C-Diclazuril to Soils (90 - 110 % Recovery Data)

Soil Level	Study Day	Cumulative Volatiles ($^{14}CO_2$) Evolved (% IMD)[1]		
		Soil A sandy loam	Soil B loam	Soil C silt loam
10 ppb	0	0.00	0.00	0.00
	32	5.32	(6.39)[2]	13.36
	64	7.25	10.36	(54.62)[2]
100 ppb	0	0.00	0.00	0.00
	14	1.04	0.85	2.20
	32	2.68	3.48	5.99
	48	3.81	5.51	11.12
	64	5.34	(9.75)[2]	15.32

[1] Initial measured dose (IMD) calculated as the sum of extractable + bound residues at day 0.

[2] Not within 90 - 110 % recovery range; values in parentheses are those determined using all data.

Extractable residues comprised a decreasing proportion of the applied dose throughout the duration of the study in all soil types and at both dose levels. Estimates of the proportion of initial dose activities attributable to the parent compound by HPLC analysis (90 - 110 % Recovery Data) at test termination were about 46 % and 33 % for loam and silt loam, respectively, at both dose levels. [Note: The HPLC method was not suitable for analysis of sandy loam samples]. Similar estimates using TLC analysis were slightly lower (28.5 - 43.8 %); values for sandy loam were 40.6 % (10 ppb) and 48.9 % (100 ppb).

No metabolites were found at greater than 10 % of the applied dose by either HPLC or TLC analysis. Consequently, the metabolites were not chemically characterized. However, an attempt was made to identify additional ^{14}C-bound residues in the post-extracted soil samples (ABC Laboratories, 1994). Retained soil samples were intensively extracted using a new solvent mixture of methanol:acetic acid (50:0.5; v:v). Samples were analyzed using radio-analysis (LSC) and compound-specific analysis (TLC). Approximately 45 % of the ^{14}C-activity was recovered from the original bound residues of day 64 soil samples; 41 - 52 % for 10 ppb samples; 38 - 47 % for 100 ppb samples. TLC results indicated that 32 % (silt loam) to 81 % (sandy loam) of the newly extracted ^{14}C-activity was parent compound for 100 ppb, day 64 samples. [Note: 10 ppb samples were not analyzed for parent compound or metabolites due to insufficient ^{14}C-activity in the extracts].

Taking into account the additional extractable ^{14}C-residues identified, the original mass balance results (ABC Laboratories, 1992) were modified (ABC Laboratories, 1994). Average ^{14}C-mass balance was at or above 100 % for all three soils at both dose levels. In most treatments, there was a rapid increase in soil-bound ^{14}C-residues followed by a plateau or small decrease on day 64. This suggests that the (uncharacterized) bound residues were still available for further biodegradation. The amount of total extractable residues decreased steadily over time. Initially, 81 to 98 % of IMD of soil residues were extractable, decreasing to 41 to 76 % of IMD on day 64. There was a parallel decrease in the extractable residues identified as parent compound. [Note: Residue data are only available for the 100 ppb dose]. On day 0, the amount of parent compound in extractable residues was approximately 77 to 94 % of IMD (86 - 96 % of total extractable residues). On day 64, these residues had decreased to about 38 to 67 % of IMD (67 - 88 % of total extractable residues). In parallel to the decreases in total extractable residues and extractable parent compound, there was a steady increase in the evolution of ^{14}C-volatiles (Table 6), suggesting that some of this fraction was mineralized to $^{14}CO_2$ or degraded to other ^{14}C-volatiles. Support for mineralization to CO_2 is provided by the fact that there was no apparent buildup of identified metabolites, despite the finding that extractable parent compound residues steadily decreased over time.

The ^{14}C mass balance data for 10 and 100 ppb soils on day 64 is presented in Table 7. Bound residues ranged from about 13 to 29 % of IMD. Total extractable residues ranged

from about 56 to 78 % of IMD, the majority of which was parent compound. Total extractable degradation products (determined by difference) ranged from about 9 to 19 % of IMD. Diclazuril biodegradation was greatest in silt loam and least in sandy loam when measured by both evolution of volatiles and formation of extractable degradates.

Table 7. Average ^{14}C-Mass Balance of ^{14}C-Diclazuril in Soils Dosed at 10 and 100 ppb (90 - 110 % Recovery Data; TLC Analysis)

Soil Level	Soil Type	Study Day	Volatiles ($^{14}CO_2$) (% IMD)[1]	Bound Residues (% IMD)[1]	Extractable Residues (% IMD)[1]		
					Total	As Parent Compound[2]	Degradation Products (by difference)
10 ppb	Sandy loam	64	7.25	19.34	69.34	ND[3]	---
	Loam	64	10.36	28.45	71.22	ND[3]	---
	Silt loam	32*	13.36	29.37	57.96	ND[3]	---
100 ppb	Sandy loam	64	5.34	21.27	76.03	66.70 [87.73]	9.33
	Loam	48†	5.51	13.17	78.05	65.43 [83.83]	12.62
	Silt loam	64	15.32	27.51	56.45	37.71 [66.80]	18.74

[1] Initial measured dose (IMD) was calculated as the sum of extractable and bound residues at day 0.

[2] Values in brackets are the amounts of parent compound as a percentage of the total extractable residues.

[3] Not determined due to insufficient ^{14}C-activity in extracts.

* Data are shown for day 32 because data for day 64 were not within the 90 - 110 % recovery range; day 64 values based on all data were 54.62, 38.41, 41.15, and ND[3], respectively.

† Data are shown for day 48 because data for day 64 were not within the 90 - 110 % recovery range; day 64 values based on all data were 9.75, 33.93, 73.30, 61.65 [84.11], and 11.65, respectively.

Further characterization of extractable ^{14}C-residues is presented in Table 8. Two extractable diclazuril metabolites were identified by TLC analysis. Metabolite 1 comprised about 3 to 6 % of IMD; metabolite 2 about 1.5 % of IMD. Uncharacterized extractable degradation products made up about 5 to 12 % of IMD.

Table 8. Characterization of Extractable ^{14}C-Residues in Soils Dosed with ^{14}C-Diclazuril at 100 ppb (90 - 110 % Recovery Data; TLC Analysis)

Soil Type	Study Day	Extractable ^{14}C-Residues (% IMD)[1]			
		Parent Compound	Metabolite 1 (Rf = 0.00)	Metabolite 2 (Rf = 0.86)	Total Other[2]
Sandy loam	64	66.70	2.85	1.43	5.05
Loam	48*	65.43	4.52	1.59	6.51
Silt loam	64	37.71	5.70	1.43	11.61

[1] Initial measured dose (IMD) was calculated as the sum of extractable and bound residues at day 0.

[2] Determined by difference by subtracting the combined extractable residues of parent and metabolites 1 and 2 from the total extractable residues (Table 7).

* Data are shown for day 48 because data for day 64 were not within the 90 - 110 % recovery range; day 64 values based on all data were 61.65, 2.47, 1.57, and 7.61, respectively

D. Predicted Ultimate Degradability of Diclazuril by Analogy

The soil degradation studies described in the previous subsection confirm some degradation of parent diclazuril over a 64-day period and the formation of metabolites (each less than 10% of the initial dose) along with some CO_2 or other volatiles (most likely the cyano-moiety which held the ^{14}C label). Available biodegradation data for several structural analogs of diclazuril support the ultimate degradation (mineralization) of diclazuril. That is, there are microbial degradation pathways for the diclazuril metabolites and therefore complete degradation is

expected in the environment with sufficient time. The basis of ultimate degradation is provided below; further details and literature references were provided in support of this application (Environ, 1996).

The cleavage of the asymmetrical triazine ring portion of diclazuril has been documented in the formation of the DM5 metabolite (Janssen Pharmaceutica, 1989d); see Figure 1. While symmetrical triazine rings are often quite stable (e.g., the herbicide triazines), the presence of the keto-groups on the diclazuril triazine ring will make it vulnerable to hydroxylation at those points. The triazine portion of diclazuril is therefore expected to undergo complete biodegradation.

The chlorinated diphenyl ethane moiety (metabolite DM5) is structurally analogous to several chlorinated aromatic hydrocarbons that have been extensively tested for biodegradability. A close analogy is to p,p'-dichlorodiphenylmethane (DDM). This ring compound has been shown to be aerobically degraded (i.e., ring cleavage) by microorganisms. Degradation occurs in days, but is likely to be slower for diclazuril and DM5 because of an additional chlorine substitution on one of the phenyl rings and the di-*ortho* substitution pattern of these chlorines. Dechlorination and ring cleavage have been demonstrated for similar compounds with one to three chlorine substitutions on aromatic rings over a period of a few days to several years. Cleavage products are usually chlorobenzoic acids and chloroacetophenones which are susceptible to further microbial degradation and complete mineralization. Because the microorganisms responsible for this degradation are known to occur commonly in the environment, all evidence indicates that diclazuril and its degradation products will ultimately and completely biodegrade in the environment.

E. Half-life Estimates for Diclazuril

1. For Parent Diclazuril Based on Formation of Total Degradation Products

Because of the location of the ^{14}C-radiolabel on the cyano group, evolution of CO_2 or other volatiles does not demonstrate complete mineralization of diclazuril; however, it does confirm that some degradation of parent diclazuril occurred. The same can be said based on detection of actual degradation products (e.g., metabolites 1 and 2) in extracted residues. The degradation half-life of parent diclazuril in soils may be estimated from the analytical data for total degradation products (i.e., volatiles and non-parent extractables). From this data (Tables 6 and 7) and conservatively assuming that all non-extractable soil-bound residues are parent compound, an estimate can be made of the total amount of diclazuril degradation products. This in turn forms the basis for estimating degradation rates and soil half-lives for parent diclazuril. Data for these parameters are presented in Table 9. [Note: Only data for the 100 ppb soil level are shown. The amount of parent compound in soil extracts at the 10 ppb dose could not always be determined because of

insufficient ^{14}C-activity; however, apparent degradation rates based on decreases in total extractable residues were higher for 10 ppb soils than for 100 ppb soils, therefore, data in Table 9 for 100 ppb soils represent conservative estimates of degradation rates and half-lives for diclazuril.] Degradation rates for parent diclazuril ranged from 0.00229 to 0.00532 day^{-1}; estimated soil half-lives range from 130 to 303 days. Half-lives were shortest in silt loam and longest in sandy loam soils.

Table 9. Degradation Rates and Half-Lives of ^{14}C-Diclazuril in Soils at 100 ppb (90 - 110 % recovery data; TLC Analysis; Rates based on formation of degradates)

Soil Type	Study Day	^{14}C-Activity (% IMD)			Degradation Rate[4] (day^{-1})	Soil Half-life (days)
		Volatiles[1] ($^{14}CO_2$)	Total Parent Compound[2]	Extractable Degradation Products[3]		
Sandy loam	64	5.34	87.97	9.33	0.00229	303
Loam	48*	5.51	78.60	12.62	0.00378	183
Silt loam	64	15.32	65.22	18.74	0.00532	130

[1] From Table 6.

[2] From Table 7; sum of "bound residues" plus "extractable residues as parent". [Note: In this analysis it was conservatively assumed that all bound residues were parent compound].

[3] From Table 7.

[4] Degradation rate = formation rate of total degradation products ("volatiles" plus "extractable degradation products").

* Data are shown for day 48 because data for day 64 were not within the 90 - 110 % recovery range; day 64 values based on all data were 9.75 % IMD, 61.65 % IMD, 11.65 % IMD, 0.00334 day^{-1}, and 207 days, respectively.

2. For Ultimate Degradation Based on Rates for Analogous Structures

As discussed above, there is considerable published information for analogous structures indicating that both parent diclazuril and its metabolite DM5, including the chlorinated diphenyl ring moiety, will ultimately and completely degrade in the environment. Estimated degradation half-lives for analogous chlorinated aromatic hydrocarbons range from as little as several days, to as much as 10 years or more. Generally, the more highly chlorinated, the greater the hindrance of degradation and the longer half-life. Because diclazuril and DM5 are only lightly chlorinated compared to many analogous structures and because these chlorines are found only on the phenyl rings, the ultimate degradation half-lives of both diclazuril and DM5 are expected to be at the middle or lower end of this spectrum, most likely on the order of several years.

F. Expected Environmental Concentrations (EEC's)

1. Predicted Concentration of Diclazuril in Soil after Fertilization with Poultry Litter

As described in Items 6.C.*2.* and 6.C.*3.* above, Harrass et al. (1991) presented a model for predicting concentrations of chemicals in manure, and in soil to which the manure is applied as fertilizer. For diclazuril, those predictions are 2.2 mg/kg in poultry manure and 12 ppb in soil. These estimates are conservative in that they do not take into account any biotic or abiotic degradation of diclazuril that occur in litter in the time interval between when the manure was excreted and when it was actually amended to soil. In addition, the litter concentration has been calculated assuming that there are no diluents (e.g., saw dust, wood chips) present in the litter, which is rarely the case.

The soil concentration of diclazuril at steady state following repeated annual applications of poultry litter as fertilizer may be estimated by the method of Larson and Cowan (1995) and Cowan et al. (1995). The maximum steady state soil concentration (Equation 3) is determined by multiplying the single-addition soil concentration (C_{soil}) by the appropriate steady state biodegradation factor (BF_{ssb}).

$$C_{soil}\ (\text{maximum steady state}) = C_{soil}\ (\text{single application}) \times BF_{ssb}$$

where

$BF_{ssb} = 1/(1 - BF)$

BF = fraction remaining at the end of one residence time

$= e^{-0.693(1/(BHL/RT))}$

[where BHL = biodegradation half-life; RT = residence time = 1 year if applications are made once per year]

Based on the data for volatile and non-volatile degradation products, an estimated biodegradation half-life of parent diclazuril in soil is on the order of 1 year (Table 9).

C_{soil} (single application) = 12 ppb

BF = 0.50 (where BHL = biodegradation half-life = 1 year; RT = 1 year)

BF_{ssb} = 2.00

C_{soil} (maximum steady state) = 12 ppb x 2.00 = 24 ppb

The parent diclazuril concentration in soil after repeated applications of poultry litter, accounting for the degradation observed in the laboratory tests, is estimated to stabilize at about 24 ppb. Photodegradation could further reduce the level of parent diclazuril if exposed to sunlight at the soil surface.

While there are no data from the diclazuril soil degradation studies to predict a half-life for total mineralization, the information on analogous structures supports a conclusion of their ultimate degradation. Data for analogous structures suggests that half-lives of DM5 and similar diclazuril degradation products should be on the order of a few months to several years. If, for example, the half-life for complete mineralization of diclazuril was 2 years (i.e., BF = 0.707; BF_{ssb} = 3.41), then the concentration of diclazuril and degradation products would stabilize in about 7 years at a concentration of about 41 ppb after repeated annual applications. If the mineralization half-life were 5 years (i.e., BF = 0.87; BF_{ssb} = 7.69), then the concentration of diclazuril and degradation products would stabilize after about 40 years of annual applications at a concentration of about 92 ppb. At a 10-year mineralization half-life (i.e., BF = 0.93; BF_{ssb} = 14.94), the steady state soil concentration would be about 179 ppb (diclazuril plus degradation products).

2. Predicted Surface Water Concentrations of Diclazuril as a Result of Soil Runoff

An approximation for the maximum release of diclazuril from soil runoff can be used to predict a maximum surface water concentration. Such a scenario is based on a 5 cm (2 inch) rainfall occurring one day after application of poultry litter containing diclazuril; all precipitation becomes runoff; and 1 % of the diclazuril in the soil migrates to the surface water via the runoff. The 5 cm rainfall represents a moderately heavy storm event and is consistent with modeling results presented below. The 1 % runoff value is derived from a series of simulations using the Simulator for Water Resources in Rural Basins (SWRRB)

computer model to determine worst-case runoff loads for pesticides[2] applied to agricultural soils (Aquatic Effects Dialogue Group, 1992). In SWRRB, runoff simulations were run for an 8-year period and included a 5 cm rainfall one day after pesticide application for each year. In these simulations, the percentage of pesticide application contained in runoff was related to water solubility (Table 10). Based on the solubility of diclazuril in water (< 10 ppb; see additional discussion below), no more than 1 % of diclazuril is expected in runoff and perhaps as little as 0.1 %. This is consistent with soil adsorption/desorption data for diclazuril which indicate that it is strongly bound to soil.

Table 10. Relationship of Runoff Percentage to Water Solubility for Pesticides (Aquatic Effects Dialogue Group, 1992)

	Solubility of Pesticide in Water			
	100 ppm	< 100 ppm & > 1 ppm	1 ppm	1 ppb
Percentage of pesticide application contained in runoff	5 %	2 %	1 %	0.1 %

Based on the expected maximum concentration of diclazuril in poultry litter (2.2 ppm or 2.2 mg/kg) and the expected application rate for poultry litter as fertilizer (1.1 kg/m^2):

- The application rate of diclazuril to soil is 2.42 mg/m^2 (2.2 mg/kg x 1.1 kg/m^2);
- The amount of diclazuril contained in runoff, assuming 1 % migration from litter to water, would be 0.024 mg/m^2 (0.01 X 2.42 mg/m^2);
- A 5 cm runoff is equivalent to a water volume of 50.0 L/m^2 (5.0 cm x 10,000 cm^2/m^2 x 0.001 L/cm^3); and
- The maximum concentration of dissolved diclazuril in surface runoff would therefore be 0.48 ppb (0.024 $mg/m^2 \div 50.0$ $L/m^2 = 0.00048$ mg/L). Dilution into a receiving water body would reduce the surface water concentration.

[2] Diclazuril runoff is expected to be less than for pesticides because poultry litter is mixed into soil as a fertilizer, whereas pesticides are typically sprayed onto plants and/or soils and not directly incorporated into soil, making them more susceptible to loss in runoff.

In reality, the dissolved concentration of diclazuril in surface water cannot exceed the water solubility. The dissolved or bioavailable form is the appropriate form to be used when making comparisons to aquatic toxicity data. The water solubility of diclazuril was measured at < 1 ppb (reported as < 10 ppb because of potential methodological limitations). The estimated maximum concentration in surface runoff (0.48 ppb) is not much different from the measured solubility limit.

Diclazuril is not expected to persist in surface water. Photodegradation data for the compound in water (summarized in Item 7) show average diclazuril photolysis half-lives ranging from about 10 to 25 days in summer, and from about 29 to 308 days in winter, depending upon the geographical latitude. The observed photolysis of the heterocyclic ring observed in this study suggests that sites for microbial degradation may be liberated following exposure of the compound to sunlight. The biodegradation of the compound observed in the soil studies suggests that the same process may continue for diclazuril complexed with sediments or dissolved in water.

3. Predicted Concentration of Diclazuril in Sediments as a Result of Soil Runoff

As a simple approximation, the maximum concentration of diclazuril in sediments can be estimated to be comparable to the maximum steady state concentration in soil that has received poultry litter (24 ppb). This is based on a scenario in which litter-amended soil erodes during a heavy rainfall event and deposits in a nearby waterway to form sediment. The scenario is very conservative in that it does not consider "dilution" or mixing of diclazuril-bound sediment with sediment that is already in place or that is subsequently transported in from upstream or other overland sources, nor does it consider subsequent degradation.

G. Summary of Environmental Fate

In summary, the physicochemical properties of diclazuril indicate that it has a very low water solubility and is not volatile. It would be expected to adsorb strongly to soils and, based on its octanol/water partition coefficient, might bioconcentrate in biota. Soil studies confirmed that diclazuril adsorbs strongly to soil and therefore would not be expected to be mobile in a soil environment. Additional environmental fate studies demonstrated that diclazuril will photodegrade in water and that biodegradation of parent diclazuril occurs in a variety of soils. The estimated soil half-life for biodegradation of parent diclazuril, based on the soil studies, was on the order of 1 year. The experimental data confirm multiple biodegradation products after 64 days, including: ^{14}C-volatiles, at least two ^{14}C-metabolites (nonvolatile), and non-labeled degradation products. Available information on analogous structures indicates that diclazuril and its degradation products will ultimately and completely degrade in the

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environment. Based on data for these analogous structures, half-lives for diclazuril degradation products are expected to be on the order of several months to several years.

The predicted maximum concentration of parent diclazuril in soils after a single application of poultry litter as fertilizer is 12 ppb. Accounting for biodegradation observed during the laboratory studies, the predicted upper-bound concentration of parent diclazuril in soil at steady state after repeated applications is in the range of 24 ppb. Assuming soil erodes and is deposited as sediment, an upper-bound estimate of the sediment concentration is the concentration occurring in soil. Based on its low water solubility and strong adsorption to soils, the maximum runoff concentration (and maximum surface water concentration) of diclazuril is estimated at about 0.5 ppb or less. In any case, the concentration of dissolved diclazuril in surface water cannot exceed its solubility in water (measured at < 1 ppb, and reported at < 10 ppb).

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

The potential environmental effects of diclazuril and the dose-response relationships for these effects were characterized in studies with a variety of terrestrial and aquatic organisms, including birds, earthworms, plants, fish, aquatic invertebrates, and microorganisms. The uptake and bioaccumulation potential of diclazuril were also characterized in many of these same species. In addition, numerous studies with laboratory animals were conducted as part of the human safety evaluation of diclazuril; data from these studies provide insight into potential environmental effects on wildlife. Results of all the environmental effects studies are summarized below and used to evaluate and predict the likelihood of adverse effects occurring as a result of CLINACOX (diclazuril) use in poultry feed.

A. Toxicity and Bioaccumulation of Diclazuril to Terrestrial Plants and Invertebrates

Ecotoxicology studies conducted with diclazuril on terrestrial plants and invertebrates include: seed germination and root elongation, plant seedling growth, plant bioaccumulation, subacute earthworm toxicity, earthworm bioaccumulation, acute oral and reproductive toxicity in mallard ducks, and reproductive toxicity in Japanese quail. When available, studies were conducted according to TAD protocols given in the FDA Technical Assistance Handbook.

1. Effects of Diclazuril on Seed Germination and Root Elongation in Six Plant Species

The effects of diclazuril on the seed germination and root elongation of six plant species (corn, cucumber, pinto bean, rye, soybean, and wheat) were studied using the TAD 4.06 protocol (Springborn Life Sciences, 1991b). Preliminary tests were carried out on each species to establish exposure concentrations for the definitive tests. The preliminary test

concentrations were 1, 10, 100, and 830 ppm (nominal) active ingredient. No adverse effects were observed in the preliminary tests[3]. In the definitive studies, corn, cucumber, and ryegrass were tested at a nominal concentration of 830 ppm; pinto beans, soybean, and wheat were tested at a nominal concentration of 700 ppm. Measured concentrations for the nominal 830 ppm and 700 ppm treatments were 580 ppm and 660 ppm, respectively. Seeds of the six plant species were incubated in petri dishes containing the treatment concentration of diclazuril in a solvent, solventless control water, or solvent control. Seeds were observed daily for germination and morphological abnormalities. At the end of the exposure period, fifteen seedlings were selected from each treatment replicate and measured for radicle length.

Seed germination was not affected by exposure to diclazuril and no diclazuril effects on seedling radicle length were observed in any species when compared to the nonsolvent control. A retrospective review of study data indicated that both the preliminary and definitive studies lacked sufficient statistical power to determine No Observed Effect Concentrations (NOECs) for soil because of the variability in the root elongation endpoint in both the control and treatment groups.

2. Effects of Diclazuril on Seedling Growth in Six Plant Species

In conjunction with the seed germination and root elongation study (above), the effects of diclazuril on the growth of seedlings of the same six plant species were determined using TAD protocol 4.07 (Springborn Life Sciences, 1991c). Through use of radiolabled diclazuril, this study also attempted to quantify plant tissue residues.

A preliminary test was performed with nominal diclazuril concentrations of 1.0, 10, 100, and 1000 ppm in silica sand. No adverse effects were observed in the preliminary test, although stimulatory effects on growth were noted in soybeans at 10 and 100 ppm. These were not considered to be adverse effects nor necessarily compound related, therefore, a nominal concentration of 1000 ppm was chosen for the definitive test. The average measured concentration of diclazuril in the definitive test was 720 ppm.

In the definitive test, germinated seedlings were transplanted into pots containing neat silica sand support medium, or silica sand containing 720 ppm ^{14}C-diclazuril. Five replicates of five seedlings were prepared for the control and test concentration for each species. Plants were provided with nutrient medium and grown in a growth chamber for 21 days. At the end of the growth period, the seedlings were evaluated for shoot length, shoot weight, and root weight. No adverse effects were observed in any of the plants

[3] Stimulation of growth was observed in several species, but this effect did not appear to be concentration dependent. Because diclazuril will be incorporated into soil via poultry litter, and because the intended use of this litter is as a fertilizer on agricultural crops, stimulation of growth is not considered an adverse effect.

exposed to diclazuril. As was the case for the seed germination and root elongation study, retrospective analysis indicated that because of study limitations there was insufficient statistical power to establish a precise NOEC. At study termination, plants were also analyzed for ^{14}C-activity to give an indication of diclazuril uptake. Whole plant ^{14}C-residues were found but were not characterized as parent compound and / or metabolites. The study design did not allow for a determination of whether these residues were actually incorporated into tissues or whether they were adsorbed to external plant surfaces.

3. Effects of Diclazuril on Seedling Emergence and Plant Growth in Three Species (Supplemental Study)

A supplemental study, conducted according to OECD Guideline No. 208, investigated the effect of diclazuril in soil on seedling germination (emergence) and growth of wheat, radishes, and lettuce (Janssen Pharmaceutica, 1993a). Tests were carried out in soil treated with diclazuril at nominal concentrations of 0.1, 1, 10, and 100 ppm. Lettuce, radish, and wheat seeds were grown for 25, 18, and 25 days, respectively. Diclazuril did not adversely affect the emergence and growth of radishes or wheat at soil concentrations up to 100 ppm. For lettuce, a dose-related effect on emergence was observed at the highest soil concentration (100 ppm). At test termination, emergence of lettuce at 100 ppm was reduced about 15 % compared to controls. Lettuce growth was not adversely affected by diclazuril at any soil level, but statistically significant stimulation of growth was observed in the 0.1 and 10 ppm exposures.

4. Effects of Diclazuril on Seedling Growth in Five Species and Plant Bioconcentration in Two Species (Definitive Study)

In order to address concerns over the lack of statistical power in the previously described plant studies, as well as other study design limitations, a definitive seedling growth and plant bioconcentration study was conducted (Plant Research Technologies, 1996). The seedling growth portion of this study followed the procedures of TAD 4.07 and utilized the findings of a preliminary test (Plant Research Technologies, 1995) to select plant species and treatment concentrations. Plant bioconcentration was evaluated using modified procedures of the plant uptake and translocation test (EPA TSCA Environmental Effects Testing Guidelines; CFR 797.2850). Both studies were conducted over a 21-day period with diclazuril dosed directly to sand in order to best approximate the exposure route that would occur after soil incorporation of poultry litter containing diclazuril.

Five plant species, three monocots (corn, wheat, and ryegrass) and two dicots (tomato and cucumber), were chosen based on the results of the preliminary test. Nominal soil concentrations used in the definitive study were 0.5, 5, 10, 20, and 1000 ppm; actual measured concentrations were 0.5, 5, 10, 23, and 914 ppm, respectively. Endpoints quantified on day 21 were plant morphology / survival, shoot height, shoot weight, and

root weight. No morphological abnormalities or statistically significant adverse effects were found for any of the plant species at any of the exposure concentrations. A statistically significant increase in root weight was seen in corn at the 23 and 914 ppm levels but this is not considered an adverse effect. Based on these results, it is concluded that the No Observed Adverse Effect Level (NOAEL) in soil is 914 ppm for all five species.

The evaluation of plant uptake and bioconcentration included two plant species, a dicot (cucumber) and a monocot (corn), and three nominal concentrations of ^{14}C-diclazuril (0.5, 5, and 20 ppm), all of which were below the NOAEL. All of these concentrations were well above the predicted maximum soil concentration for diclazuril (24 ppb). During this test, sand and seedlings from different treatments were removed at scheduled intervals (7, 14, and 21 days after dosing), separated, and combusted to determine: 1) the recovery of radioactivity from plants, rinsates, and sand; 2) the distribution of radioactivity in shoots and roots of the plants; and 3) the bioconcentration factors for ^{14}C-residues in the plants. On day 21, from 67 to 77 % of the initially applied radioactivity was recovered in tests systems; recovery was similar to that in unplanted reference controls. Study results indicated that after 21 days of exposure, very little of the radioactivity was taken up and translocated by the plants. In both species, the root was the primary site of accumulation; less than 5 % of the total radioactivity taken up occurred in the shoot tissues. Bioconcentration factors (BCF) for roots, shoots, and whole plants, expressed on a tissue fresh weight basis, are presented in Table 11. The BCF was typically slightly higher for corn than for cucumber, and the BCF for roots was two orders of magnitude larger than for shoots. Whole plant BCF values rarely exceeded one. The maximum root BCF was 3.7 and the maximum shoot BCF was 0.025. Using the predicted upper-bound concentration of 24 ppb of parent diclazuril in soil at steady state after repeated applications, the maximum diclazuril content of roots grown in such soil would be 3.7 X 24 = 88.8 ppb (0.0888 ppm). As noted below, this is far less than the NOEL's observed for avian or mammalian species in toxicity tests (20 ppm or higher), and thus would not be expected to pose a risk of adverse effects on organisms which consume plant roots.

Day 21 root samples were further analyzed to determine the nature of the bioconcentrated ^{14}C-residues. [Note: BCF values in Table 11 are expressed as total ^{14}C-residues]. Root tissues of both corn and cucumber at all three exposure levels were extracted and analyzed. Extracts of cucumber root tissues contained greater than 80 % of their original radioactivity; those of corn roots contained approximately 50 % of their original activity. Profiles of the extracts were performed on samples from the 5 ppm level using HPLC-β-Ram analysis. One major component was found in each of the extracts. The identity of this extract was confirmed to be parent diclazuril by coinjection and coelution with ^{14}C-labeled and unlabeled diclazuril.

Table 11. Plant Bioconcentration Factors[1] for Uptake of ^{14}C-Diclazuril from Soil

Soil Level	Cucumber					Corn				
	Root	Shoot	Whole Plant			Root	Shoot	Whole Plant		
	Day 21	Day 21	Day 7	Day 14	Day 21	Day 21	Day 21	Day 7	Day 14	Day 21
0.5 ppm	1.50	0.020	1.16	0.86	0.58	3.73	0.025	1.54	1.66	1.30
5 ppm	0.64	0.008	0.50	0.25	0.24	1.40	0.009	0.41	0.40	0.48
20 ppm	0.31	0.005	0.24	0.18	0.13	0.54	0.004	0.19	0.21	0.19

[1] Bioconcentration factor = concentration of diclazuril in fresh tissue / diclazuril soil concentration.

5. Toxicity and Bioconcentration of Diclazuril in Earthworms

The subacute toxicity of diclazuril to the earthworm *(Lumbricus terrestris)* was studied in a 28-day test generally following the procedures of TAD 4.12 (Springborn Life Sciences, 1991a). This study also included a measurement of diclazuril bioconcentration in the earthworms. Two preliminary range-finding studies were performed; the first using nominal soil concentrations of 0.1, 1, 10, 100, and 1000 ppm; the second using a nominal concentration of 990 ppm. Survival was not affected by diclazuril treatment in either test. Based on the results of the preliminary tests, a definitive test was performed using ^{14}C-diclazuril (1100 ppm; measured concentration) in artificial soil. In the definitive test, there were no significant differences in weight change or survival between controls and treatment groups indicating that the NOEL for diclazuril in soil is > 1100 ppm. Analysis of ^{14}C-activity in earthworms collected on day 28 and soil samples collected on days 0 and 14 provided data for determination of a bioconcentration factor. The bioconcentration factor, calculated by dividing the mean tissue concentration by the mean soil concentration, was 0.55 indicating that diclazuril (and/or its metabolites) did not accumulate in the earthworm to a concentration greater than in the surrounding media.

An additional supplemental study of the toxicity of diclazuril to a second species of earthworm, *Eisenia foetida*, was conducted according to OECD Guideline No. 207

(Janssen Pharmaceutica, 1988a). A range-finding test was conducted with nominal diclazuril concentrations of 0.1, 1, 10, 100, and 1000 ppm in soil. No mortality was observed at the end of the 14-day test period. A definitive study was then carried out at nominal concentrations of 80, 150, 280, 500, and 900 ppm (four replicates of 10 worms per concentration and control). Tests with reference substances (copper sulfate and chloroacetamide) were run concurrently. Mortality of worms exposed to reference compounds was similar to that reported in the scientific literature. No mortality occurred in worms exposed to diclazuril and no significant or dose-related changes in weight were found. In summary, results confirm the findings of the study conducted with *Lumbricus terrestris* and indicate that diclazuril does not affect earthworms at 900 - 1100 ppm in soil.

6. Oral Toxicity of Diclazuril in Mallard Ducks

The acute oral toxicity of diclazuril was determined in mallard ducks using the EPA TSCA Guideline published in 40 CFR 797.2175 (Bio-Life Associates, 1995a). In this study, 18-week old ducks (five males and five females per group) were administered diclazuril by gelatin capsule at doses of 0, 464, 681, 1000, 1470, and 2150 mg/kg and observed for a period of 14 days. All birds appeared to be normal and active throughout the test. There were no differences in survival, body weight, feed consumption, or gross pathology between treated birds and controls. It is concluded that the LD_{50} and NOEL for diclazuril in mallards is greater than 2150 mg/kg, therefore, diclazuril would be classified as practically non-toxic according to EPA guidelines (Environmental Protection Agency, 1985).

7. Reproductive Toxicity of Diclazuril to Mallard Ducks

The effect of diclazuril on parental and reproductive parameters of mallard ducks was investigated in a pilot dietary toxicity study performed according to the EPA TSCA Guideline presented in 40 CFR 797.2150 (Bio-Life Associates, 1995b). Diclazuril was administered in the diet to six groups of mallards (5 females and 5 males per group) at 0, 10, 30, 100, 300, and 1000 ppm for four consecutive weeks. All birds were approaching their first breeding season at study initiation. A wide variety of parental (F_0 generation) and reproductive parameters were quantified during the study including: adult body weights and body weight changes, feed consumption, mortality and morbidity, signs of toxicity, egg production, eggshell thickness, egg quality, hatchability, stage of embryonic development, number of hatchlings, body weights of hatchlings on days 1 and 14, hatchling survival, and gross pathology of ducklings. No deaths or clinical signs of toxicity were observed in the F_0 generation during the study. There were no adverse effects on body weights (or weight changes), feed consumption, or reproductive success of the parental generation or in the body weights or survival of the F_1 generation. In addition, mean eggshell thickness for treated groups (0.394 - 0.405 mm) was similar to that of controls (0.401 mm) and showed no dose-related response. It is concluded that

the NOEL in this study was 1000 ppm based on the lack of reproductive effects and toxicity for both the F_0 and F_1 generations.

8. Reproductive Toxicity of Diclazuril to Japanese Quail

The findings of non-GLP dissertation research conducted with the Japanese quail, *Coturnix coturnix japonica* (Huter, 1989) are supportive of the avian reproductive study results (above). This study determined the effect of diclazuril at various dietary concentrations on egg yield, egg quality, fertility, and hatchability. Two feeding trials were conducted; one with diclazuril feed concentrations of 1, 5, and 10 ppm; one with concentrations of 10, 20 and 50 ppm. Each 42-day trial included a 14-day acclimation period, 14 days of medication, and 14 days of follow-up with no diclazuril. Egg parameters were determined daily. After collection, eggs were incubated for 18 days and reproductive outcome was followed. In both trials, no differences were observed between control birds and those treated with diclazuril. Diclazuril produced no adverse effects on egg production, fertility, or hatchability at up to 50 ppm in the feed.

9. Toxicity of Diclazuril in Studies Conducted to Determine Human Food Safety

The toxicity of diclazuril has been extensively characterized in numerous *in vitro* and *in vivo* laboratory studies conducted to demonstrate the safety of this compound to humans. Studies have included testing of reproductive toxicity, embryotoxicity and teratogenicity, and carcinogenicity. Results of these toxicity studies are briefly summarized in Table 12; for a full description see the Human Food Safety section of the Freedom of Information summary for CLINACOX. Genotoxic potential was also characterized in a battery of studies. There was no evidence of genotoxicity, teratogenicity, or carcinogenicity in any of the studies, although diclazuril appeared to be slightly fetotoxic (decreased mean body weight) in Wistar rats at doses of 200 ppm and above in feed. The FDA established a safe concentration for diclazuril based on the NOEL of 50 ppm in feed (equivalent to 2.5 mg/kg body weight/day) for fetotoxicity in the rat teratology and reproduction studies.

Results of a repeated oral dose study with broiler chickens indicate that diclazuril will not accumulate in plasma or tissues to a higher level than in their diet (Janssen Pharmaceutica, 1987b). Chickens dosed with ^{14}C-diclazuril at 0.045 mg/kg body weight (equivalent to 1 ppm in the feed) exhibited steady state levels of total radioactivity within six hours. Residual levels of radioactivity at steady state were 589, 386, 324, 193, 58, and 87 ppb for plasma, liver, kidney, skin/fat, pectoral muscle, and femoral muscle, respectively. Total radioactivity depleted at a similar rate for plasma and tissues with half-lives of about 2.5 days and within 10 days after the last administered dose, 95% of administered radioactivity was recovered in excreta. These data indicate that significant bioaccumulation of diclazuril in birds or wildlife through ingestion of environmental residues is extremely unlikely and that any accumulated compound will be quickly excreted.

Table 12. Results of Animal Toxicity Tests with Diclazuril			
Species	**Type of Test**	**Exposures**	**NOEL**
Rat	Oral toxicity (1 year)	0, 16, 63, 250, and 1000 ppm in feed	63 ppm
Dog	Oral toxicity (1 year)	0, 5, 20, 80 mg/kg via gelatin ampules	20 mg/kg
Rabbit	Embryotoxicity and teratogenicity	0, 40, 80, 160 mg/kg by gavage	160 mg/kg
Rat	Embryotoxicity and teratogenicity	0, 200, 400, 800, 1600 ppm in feed (1st test)	None
		0, 12.5, 50, 200 ppm in feed (2nd test)	50 ppm
Rat	2-Generation reproduction	0, 50, 200, and 800 ppm in feed	50 ppm

B. Toxicity and Bioaccumulation of Diclazuril to Aquatic Organisms

The acute and chronic toxicity and the possibility for bioaccumulation of diclazuril were variously evaluated in studies with fish, aquatic invertebrates, and algae. Most of these studies were conducted according to TAD guidelines.

1. Acute Toxicity of Diclazuril to Bluegill Sunfish

An acute toxicity test was performed with diclazuril on the bluegill sunfish, *Lepomis macrochirus*, using the procedures of TAD 4.11 (Janssen Pharmaceutica, 1993b). Fish were exposed to nominal concentrations of diclazuril (0.16, 0.51, 1.6, and 16 mg/L) using dimethylsulfoxide as a carrier solvent under static conditions. Measured concentrations of diclazuril were significantly lower than nominal at dose levels above 1.6 ppm. The 96-hour LC_{50} expressed as a measured concentration was 0.58 ppm. The lowest measured concentration tested (0.15 mg/L) produced 10 % mortality in 96 hours. No mortality was observed in the water or solvent controls.

A second acute toxicity test was performed using a flow-through test system (Janssen Pharmaceutica, 1995b). Fish were exposed to nominal concentrations of diclazuril (0.01,

0.1, 1.0, and 10 mg/L) using dimethylsulfoxide as a carrier solvent. Mean measured concentrations of diclazuril were significantly lower than nominal at all dose levels (highest concentration achieved was 0.26 mg/L). No mortality was observed in the water controls or in the 0.01, 1.0, or 10 mg/L diclazuril groups. In the solvent control one fish died after 72 hours. In the 0.1 mg/L concentration one fish died after 72 hours and a second fish died after 96 hours. The three deaths were regarded as natural mortality.

The LC_{50} value (0.58 mg/L) and the concentration causing 10% mortality (0.15 mg/L) in the static test are orders of magnitude above the solubility limit of diclazuril in water (< 0.010 mg/L). Based on the limited mortality exhibited at the lowest concentration tested in the static system (0.15 mg/L), and the lack of mortality in the flow-through system (at up to 0.26 mg/L), it is reasonable that no mortality would be observed at the saturation limit of diclazuril in natural waters.

2. *Bioconcentration of Diclazuril in Bluegill Sunfish*

Bioconcentration of ^{14}C-diclazuril in bluegill sunfish, *Lepomis macrochirus*, was investigated in a flow-through system according to the EPA TSCA Guideline presented in 40 CFR 797.1560 (Huntingdon Life Sciences, 1996). Bioconcentration was evaluated at two nominal concentrations (0.1 and 1.0 µg/L) that were well below the 96-hour bluegill LC_{50}, using dimethylsulfoxide (DMSO) as a carrier solvent. Measured concentrations throughout testing were relatively consistent; mean measured concentrations were 108 % and 102 % of nominal (0.108 and 1.02 µg/L) . The fish were exposed to diclazuril in water for 28 days (uptake phase), followed by a 28-day depuration period with no exposure. Fish and exposure media were sampled seven times during the uptake phase (days 1, 3, 7, 10, 14, 21, and 28) and five times during depuration (on days 29, 31, 35, 42, and 56 of the test) and analyzed for ^{14}C-diclazuril by liquid scintillation counting. No mortality or sub-lethal effects were observed during the test. Steady state concentrations of diclazuril in fish were achieved after approximately 10 days of exposure at both concentrations. The steady state bioconcentration factor (BCF) was 160X for both doses. The BCF is considerably below the predicted BCF of 1191 to 7211 based on the octanol/ water partition coefficient (log K_{ow} 4.5) of diclazuril. Diclazuril was eliminated rapidly from fish, with elimination half-lives of 3.3 or 4.4 days for the 0.108 and 1.02 µg/L doses, respectively.

3. *Acute Toxicity of Diclazuril to* Daphnia magna

Acute toxicity of diclazuril to the aquatic invertebrate, *Daphnia magna*, was evaluated according to the protocol outlined in TAD 4.08 (Janssen Pharmaceutica, 1992b). Daphnids were exposed to nominal concentrations of diclazuril (0.58, 1.8, 5.8, 18, and 24 mg/L) using dimethylsulfoxide as a carrier solvent under static conditions. Measured concentrations of diclazuril were significantly lower than nominal at levels above 1.8 ppm.

No daphnid immobilization was observed after 48 hours at the nominal concentration 0.58 mg/L (measured concentration of 0.63 mg/L), while 100 % immobilization was observed at 48 hours at nominal concentrations of 1.8 and 5.8 mg/L (measured concentrations of 1.99 and 1.34 mg/L, respectively). The percent of immobilization was intermediate (20 - 30 %) at higher nominal test concentrations (measured concentrations were 0.38 - 0.43 mg/L). Because of this variability in immobilization at higher concentrations, no LC_{50} values were calculated. It is reasonable to expect that the LC_{50} for daphnids under the conditions of this experiment is between 0.63 and 1.99 mg/L. These concentrations are orders of magnitude greater than the solubility of diclazuril in water in the absence of a carrier solvent (< 0.010 mg/L). Based on the low levels (0 - 30 %) of immobilization at the lower measured concentrations tested in this experiment (0.38 - 0.63 mg/L), it is expected that diclazuril would produce no immobilization of daphnids at the saturation limit of the compound in natural waters.

4. *Chronic Toxicity of Diclazuril to* Daphnia magna

The chronic toxicity of diclazuril was investigated in a 21-day reproduction study with *Daphnia magna* using the procedures of TAD 4.09 (Janssen Pharmaceutica, 1995d). Daphnids were exposed to nominal concentrations of diclazuril of 0.01, 0.02, 0.04, 0.08, and 0.16 mg/L using dimethylsulfoxide as a carrier solvent. The test was conducted under static-renewal conditions with test media renewal three times per week. Diclazuril concentrations were measured by HPLC and confirmed to be greater than 80 % of nominal concentrations for all treatments. The behavior and physical appearance of daphnids was not affected by exposure to diclazuril at any concentration. Similarly, there were no significant differences (Dunnett's T test; $\alpha = 0.05$) in mortality or reproduction between controls and daphnids exposed to diclazuril (Table 13). The NOEL is 0.16 mg/L, the highest concentration tested. These results confirm the expectation from the acute study reported above that no immobilization will occur at levels at or near the water solubility of diclazuril.

Table 13. Effect of Diclazuril on Mortality and Reproduction of *Daphnia magna*		
Nominal Concentration	**% Mortality (Day 21)**	**Reproduction (Day 21, cumulative)**
Control	0	1661
Solvent control	20.0	2121
0.01 mg/L	0	2249
0.02 mg/L	5.3	2262
0.04 mg/L	20.0	2258
0.08 mg/L	5.0	2340
0.16 mg/L	5.0	2414

5. The Toxicity of Diclazuril to Unicellular Algae

Effects of diclazuril on algal growth were studied using the unicellular green alga, *Selenastrum capricornutum*, and OECD Guideline 201 "Alga Growth Inhibition Test" (Janssen Pharmaceutica, 1992a). Algal cultures were exposed for 72 hours to nominal concentrations of diclazuril (0.0048, 0.025, 0.150, 0.850, and 4.8 mg/L) dissolved in growth media using dimethylsulfoxide as a carrier solvent. Measured concentrations of diclazuril were highly variable. At the highest dose tested, the measured concentration was significantly lower than the nominal. *Selenastrum* growth rates were reduced 17 % and 27 % at measured concentrations of 0.76 and 1.07 mg/L, respectively, but the EC_{50} for growth inhibition was above the maximum measured concentration tested (1.1 mg/L) and could not be quantified. Because 1.1 mg/L is significantly above the saturation limit of diclazuril in water (< 0.01 mg/L), effects on algae are not expected at the saturation limit of the compound in natural waters. In support of this conclusion, microbial growth inhibition tests with two blue-green algae, *Anabaena cylindrica* and *Nostoc muscorum*, indicated no effects at diclazuril concentrations of 100 and 1000 ppm, respectively (see discussion in Item 8.D.*1*).

C. Toxicity and Bioaccumulation of Diclazuril in Sediment-Dwelling Organisms

The toxicity and bioaccumulation of diclazuril in a sediment-dwelling organism, the midge *Chironomus tentans*, was investigated in a definitive 28-day study (ABC Laboratories, 1995). Second instar (age 10 days) midge larvae were exposed to ^{14}C-diclazuril in sediment with an organic carbon content of about 1.1 % at nominal concentrations of 0, 6.25, 12.5, 25.0, 50.0, and 100 mg/kg (equivalent oven-dried weight). Measured sediment concentrations were 0, 7.3, 14.2, 26.2, 57.4, and 112 mg/kg, respectively. Parameters monitored included mortality, growth of larvae, and emergence of adult midges (% emergence; time to first emergence). Midge larvae were collected and combusted on study days 7 and 14 for determination of diclazuril bioaccumulation. Bioaccumulation was also determined for adult midges collected from emergence through study day 28 at the NOEL exposure concentration. The NOEL for diclazuril toxicity to larvae (based on survival and weight) was 7.3 mg/kg on day 14. The NOEL for toxicity to adults (based on survival and emergence) was also 7.3 mg/kg. The bioaccumulation factor (BAF), based on total recovered radioactivity for day 14 larvae was 0.97. [Note: All BAF calculations were based on dry weight tissue and sediment concentrations]. The BAF for day 7 larvae exposed at this level was similar (1.04); however, there was an apparent enhancement of diclazuril bioaccumulation in emergent adults (BAF = 6.0) likely due to loss of the exoskeleton by midges undergoing metamorphosis from the larval to the adult state.

D. Effects of Diclazuril on Ecologically Important Microorganisms

The potential effects of diclazuril on microorganisms (bacteria, fungi, algae) found in water and soil were investigated in several studies.

1. Microbial Inhibition of Diclazuril

The *in vitro* activity of diclazuril was investigated in 11 pathogenic and saprogenic fungi species and 11 pathogenic bacterial species (Janssen Pharmaceutica, 1985b). The development of *Trichophyton mentagrophytes* was significantly inhibited at 100 ppm and there was no growth of *Candida albicans* at 100 ppm. All other species tested were unaffected by diclazuril.

In order to address the question of whether diclazuril would inhibit microbes that carry out ecosystem functions (e.g., nutrient recycling, degradation of natural and anthropogenic wastes), a second microbial inhibition study with diclazuril was conducted using species selected from the list provided in the FDA Technical Assistance Document 4.02 (Janssen Pharmaceutica, 1991b). Microorganisms used in the study were *Mycobacterium butyricum, Bacillus megaterium, Clostridium multifermentans, Rhosopseudomonas acidophilia, Anabaena cylindrica, Thiobacillus perometabolis, Desulfovibrio vulgaris,*

Bacillus firmus, Pseudomonas stutzeri, Arthrobacter globiformis, Trichoderma viride, Schizophyllum commune and *Streptomyces murinus*. Bacteria and fungi were cultured in duplicate on neat growth media (controls), media with 0.25 % and 2.5 % ethanol (solvent controls), and media containing 0.25 % or 2.5 % ethanol plus 10 or 100 ppm diclazuril, respectively. Blue-green algae were cultured in neat Bold's mineral medium (control) and mineral medium containing 10 and 100 ppm diclazuril. Growth of the organisms was expressed as mean colony diameter or as percent of control growth.

There was no difference in mean diameters of microbial colonies or colony growth between the 0.25% ethanol controls and the 10 ppm test concentration or between the 2.5 % ethanol controls and colonies cultured on 100 ppm diclazuril with the exception of a growth stimulation in the lignin-degrading organism *Schizophyllum commune* at 100 ppm. The blue-green alga *Anabaena cylindrica* exhibited no inhibition of growth at 10 and 100 ppm diclazuril when compared to the media control.

In order to better characterize the potential ecotoxic effects of diclazuril, two additional definitive studies of microbial growth inhibition were conducted according to the procedures of TAD 4.02 (Janssen Pharmaceutica, 1995a, 1995b)[4]. Pure cultures of bacteria (*Azotobacter beijerinckii, Arthrobacter globiformis*), fungi (*Penicillium digitatum, Trichoderma viride*), and a blue-green algae (*Nostoc muscorum*) were tested for their sensitivity to diclazuril using the agar plate dilution technique. Testing was conducted over the nominal concentration range of 1 to 1000 ppm using dimethylsulfoxide (DMSO) as a carrier solvent. Measured concentrations ranged from about 0.98 ppm up to 1080 ppm. Test results were expressed in terms of the minimum inhibitory concentration (MIC), the lowest concentration causing complete inhibition of growth.

Testing indicated that the MIC's for the fungi, *Penicillium digitatum* and *Trichoderma viride*, the blue-green alga, *Nostoc muscorum*, and the bacterium, *Azotobacter beijerinckii*, were above 1000 ppm; no growth inhibition was observed at the highest concentrations tested for these organisms. For the bacterium *Arthrobacter globiformis*, complete inhibition of growth was found at 10 ppm (the MIC); however, normal growth was observed in each of the triplicate inoculations at 5 ppm. Overall, results are consistent with those of earlier studies, indicating that diclazuril will inhibit microbial growth only when present at concentrations of 10 ppm and greater.

2. *Effect of Diclazuril on Soil Respiration*

Additional information relating to the potential effects of diclazuril on microorganisms is provided in a soil respiration study (Janssen Pharmaceutica, 1990). Two soils, a silt loam

[4] The two studies were identical except that one (Janssen Pharmaceutica, 1995a) used UV absorption spectrometry to measure diclazuril concentrations, while the other (Janssen Pharmaceutica, 1995b) used an HPLC assay.

and a sand, were used in this study. Test concentrations were 0, 0.1 and 1.0 mg diclazuril/kg. Testing was carried out in biometers which were sampled periodically for CO_2 production. Treatments included addition of diclazuril to:

1) fresh soil to which $(NH_2)_2SO_4$-glucose had been added;
2) fresh soil to which lucerne meal had been added;
3) fresh soil to which nothing had been added; and
4) aged soil that had been preincubated for four weeks.

Tests results were mixed: no significant differences in respiration were found for 9 of 24 combinations (mostly in fresh soils); stimulation was found in 6 of 24 combinations (again mostly in fresh soils); and inhibition was found in 9 of 24 combinations (mostly in aged soils). Evidence suggests that inhibition of respiration in aged soils was the result of carbon starvation that led to a reduction in the quantity of soil microflora. Considering the lack of effects found in the microbial inhibition studies at levels much higher than those in this study, it did not appear that diclazuril had any significant detrimental effect on soil respiration.

E. Predicted Environmental Effects Associated with Diclazuril Use in Poultry

1. Comparison of the Terrestrial Toxicology Data to the Estimated Soil Concentration

No adverse environmental impacts from the use of diclazuril in poultry feed are predicted for terrestrial organisms. This conclusion is based on a comparison between diclazuril toxicity to terrestrial species (Table 14) and estimated soil concentrations resulting from the use of poultry litter. The estimated maximum concentration of parent diclazuril in soil immediately following amendment with poultry litter (12 ppb) is more than five orders of magnitude below the no effect levels in plants and earthworms and over 800 times below concentrations showing no effects in ecologically important microorganisms. Calculations of the parent diclazuril concentration after repeated soil applications of poultry litter, using available biodegradation data for diclazuril, yield a steady-state concentration estimate of 24 ppb, which is still over 400 times below adverse effect concentrations for microbial species.

The above estimated soil concentrations are for parent diclazuril. If it is assumed that the poultry metabolites and soil degradation products of diclazuril are as toxic to biota as is parent diclazuril. [Note: It is possible that organisms were exposed to some degradation products during the toxicity tests and therefore the observed effects reflect such exposure]. If a 10-year mineralization half-life is used to predict a steady-state concentration of total diclazuril (i.e., parent, degradation products, and metabolites initially present in poultry excreta) in soil, the resulting soil concentrations of up to 179 ppb are still well below (over 50 times) the no observed adverse effect levels (NOAEC) in the terrestrial plant, animal, and microbial species tested.

Table 14. Summary of Key Toxicity Data for Diclazuril		
Organism	**Effect Parameter**	**Concentration[1] (media)**
Microbes	MIC (Minimum Inhibitory Concentration)	10 ppm (culture media)
Terrestrial Plants	NOAEL (No Observed Adverse Effect Level)	≥ 914 ppm (soil)
Earthworm	subacute NOAEL	≥ 1100 ppm (soil)
Bird	reproduction NOAEL	≥ 1000 ppm (diet)
Mammal	reproduction NOAEL	50 ppm (diet)
Fish	acute LC_{50}	0.58 ppm (water)
Daphnid	chronic NOAEL	≥ 0.16 ppm (water)
Midge	chronic NOAEL	7.3 ppm (sediment)
Algae	chronic EC_{50}	≥ 1 ppm (water)

[1] Use of ≥ indicates that no adverse effects were observed at the highest concentration tested.

2. Comparison of the Aquatic Toxicology Data to the Estimated Runoff Concentration

No adverse environmental impacts from the use of diclazuril in poultry feed are predicted for aquatic organisms. This conclusion is based on a comparison between the toxicity of diclazuril to aquatic species (Table 14) and the estimated maximum runoff concentrations of diclazuril from agricultural soil treated with poultry litter. The maximum estimated dissolved concentration of diclazuril in surface runoff (0.48 ppb which is also near the approximate water solubility limit) is 333 times lower than the lowest chronic NOAEC value for aquatic organisms (daphnid; 160 ppb). [Note: Bioavailability and aquatic toxicity was enhanced by the use of carrier solvents]. Additionally, dilution of surface runoff into receiving waters and subsequent degradation through the demonstrated

processes of aerobic biodegradation and photolysis can be expected to further reduce exposure to diclazuril in surface water.

The above analysis is based upon single application diclazuril levels in soil of 12 ppb and in runoff water of 0.48 ppb. Changing the soil levels to maximum steady state (24 ppb diclazuril) or maximum total degradation products using a 10 year half-life (179 ppb diclazuril plus metabolites and degradants) would increase maximum runoff concentrations to 0.96 and 7.16 ppb, respectively. [Note: It is extremely unlikely that the concentration would be this high due to solubility limits]. These concentrations are still 166 or 22 fold less than the daphnid chronic NOAEC, respectively.

3. Comparison of the Sediment Toxicology Data to the Estimated Sediment Concentration

No adverse environmental impacts from the use of diclazuril in poultry feed are predicted for sediment-dwelling organisms. This conclusion is based on a comparison between the toxicity of diclazuril to midge exposed in sediment (7.3 ppm) to the maximum estimated sediment concentration of diclazuril. The chronic NOAEC for midge was nearly three orders of magnitude above the estimate of diclazuril in sediment, which was based on soil (12 ppb diclazuril after a single application) eroding during a storm event and being deposited as sediment.

If the analysis is again based upon steady state (24 ppb diclazuril in sediment) or total degradation products using a 10 year half-life (179 ppb diclazuril plus metabolites and degradants in sediment), it is still concluded that sediment-dwelling organisms would not be adversely affected. These sediment levels are still 300 or 40 fold below the chronic NOAEC for the midge, respectively.

F. Summary of Environmental Effects

The extensive evaluation of the environmental fate and effects of diclazuril predicts no adverse environmental effects will occur if this material is released into the environment under normal poultry feed practices and conservative estimates (more likely to overpredict than underpredict) of diclazuril concentrations in soil, water and sediment. The established toxicity thresholds for a wide range of terrestrial, aquatic, and sediment-dwelling plant and animal species are well above the estimated maximum exposure concentrations of parent diclazuril, as well as those for parent diclazuril plus metabolites and degradants.

The evaluation of diclazuril uptake and bioconcentration potential in terrestrial, aquatic and sediment-dwelling organisms indicates that diclazuril does not accumulate to levels significantly above the surrounding media. Because of the low uptake and accumulation of

diclazuril in plants, earthworms, or midge larvae, dietary exposures would be considerably less for wildlife consuming plants, worms, or insect larvae which live in soils amended with poultry litter or nearby sediments. Although bioaccumulation was somewhat higher in fish, the fish bioconcentration study showed that diclazuril residues decreased rapidly following exposure. Based on the studies that could be used to predict toxicity to wildlife, no effects would be expected for species consuming diclazuril through their diet. The fish BCF value of 160 would equate to a fish tissue residue of 160 ppb at a water concentration of 1 ppb diclazuril (at or near the approximate water solubility limit). This fish concentration is thus still orders of magnitude below adverse effect levels for birds and mammals exposed to diclazuril in their diets.

Finally, there is considerable evidence from both diclazuril studies and from studies of analogous chemical structures that diclazuril is not a persistent chemical; it will degrade in the environment and will not build up to toxic levels following repeated applications of poultry litter to agricultural soil.

9. USE OF ENERGY AND RESOURCES

The energy requirement for diclazuril manufacture for the US is approximately 0.1% of the total energy used for drug production at the two Janssen Pharmaceutica production plants (Janssen Pharmaceutica, 1996). The manufacture of CLINACOX (diclazuril) does not require use or consumption of any special raw materials or resources. All raw materials are chemicals which are commercially available. No impact from waste disposal on threatened or endangered species or properties in the National Register of Historic Places is expected as a result of manufacturing or disposal of the drug product.

10. MITIGATION MEASURES

As discussed in Item 6, extensive mitigation measures and/or emissions controls have been implemented at the foreign facilities and the Mallinckrodt Veterinary Terre Haute facility involved in production of diclazuril and CLINACOX. Internal and external inspections help assure that mitigation practices are maintained. Other practices in place to mitigate potential environmental impacts include:

a] Spill-control procedures, as required by federal, state, and local regulation or by company policy, will be used wherever there is potential for adverse effect;

b] Personnel hygiene and health, safety, and GMP/GLP training are monitored regularly;

c] Waste minimization is practiced in accordance with regulations or when there is an opportunity for resource conservation. Examples include recovering and redistillation of synthesis solvents, and recycling of cardboard, paper, glass, and metals.

The use and/or disposal of CLINACOX is not expected to have an adverse impact on the environment and therefore additional mitigation measures are not required.

11. ALTERNATIVES TO THE PROPOSED ACTION

In accordance with Council on Environmental Quality regulations, the only alternative to the proposed action would be non-approval. No adverse environmental impacts have been identified for the manufacturing, packaging, labeling, quality control testing, distribution, or use of the product. Because of the strict environmental controls and mitigation practices exercised, no environmental impact can be associated with the proposed approval, and thus adoption of the alternative of non-approval is not warranted.

12. LIST OF PREPARERS

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13. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate, and complete to the best of the knowledge of Mallinckrodt Veterinary, Inc.

May 31, 1996
Date

Donald G Campbell
Signature

Senior Research Scientist
Title

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15. APPENDICES

1. MSDS sheet for diclazuril

2. Janssen Pharmaceutica (Beerse, Belgium) drug manufacturing facility environmental compliance statement

3. Mallinckrodt Veterinary, Inc. (Terre Haute, IN) premix formulation facility environmental compliance statement

SAFETY INFORMATION	VIF 087	Page 1
Product name: **Diclazuril**	R 64433	Nov. 1995
	Replaces : Aug. 1993	

I PRODUCT INFORMATION

Molecular formula: $C_{17}H_9Cl_3N_4O_2$

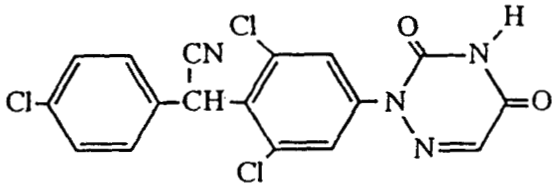
Molecular weight: 407.64

CAS No: 101831-37-2

EINECS No: not known

Janssen code: 036593

Structural formula:



II SUMMARY

White to light-beige powder. The substance is a coccidiostatic (a drug for coccidiosis, a parasitic disease). Avoid dust formation during handling. Ensure proper earthing and use anti-static materials. Render the reactor inert with nitrogen before charging with the substance.

The substance can be absorbed in the body by inhalation and ingestion. Upon intoxication notify the medical service or a physician.

When handling, wear proper gloves (plastic), closed working clothes, a pair of safety glasses and a half mask with a P2 dust filter whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P2), an Airstream anti-dust helmet or a full face mask with a P2 or P3 dust filter, whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P3), a breathe-easy with a P3 filter or a mask of an equivalent standard.

In the event of a spillage or breakage, the substance should be carefully recovered, scooped up and taken away in a closed receptacle to have it destroyed as chemical waste.
Rinse containers and adjuvants used with a suitable solvent before transfer to the washing room.
Protection code: A

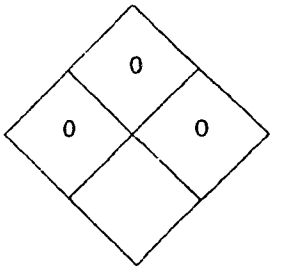
III STORAGE AND TRANSPORTATION INSTRUCTIONS

Hazard symbols: 	*class:* no regulations *ADR-RID margin No:* *symbol:*	*UN:*

Nature of special hazards: Very toxic to aquatic organisms.

Safety recommendations: Do not breathe dust.
Use appropriate containment to avoid environmental contamination.

Hazard rating



Packaging materials

Internal: plastic bag in fibre drum or pallet box.

ADR-RID: no regulations

IATA: no regulations

IV CLEARANCE OF SPILLED PRODUCT

Procedure:
Upon rupture or spillage, carefully collect and scoop up spilled product and store in a closed container for destruction as chemical waste.
Wear proper gloves (plastic), closed working clothes, a pair of safety glasses and a half mask with a P2 dust filter whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P2), an Airstream anti-dust helmet or a full face mask with a P2 or P3 dust filter, whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P3), a breathe-easy with a P3 filter or a mask of an equivalent standard when cleaning up.

V PHYSICAL-CHEMICAL CHARACTERISTICS

- *Appearance (20°C) :* white to light-beige powder
- *Melting point:* inapplicable — *Melting range:* 290.8 -292.6 °C
- *Boiling point:* inapplicable — *Boiling range:* inapplicable
- *Azeotrope:* inapplicable
- *Relative density (d20/4) :* 1.628 kg/dm3
- *Vapour pressure:* 0.00000185 mbar/20 °C
- *Vapour density (air=1):* irrelevant
- *Surface tension:* irrelevant
- *Specific conductance:* not known
- *Solubility in water:* <1 µg/l 20 °C (pH = 6.5)
- *Solubility in fats:* 55 g/l in Fettsimulans HB 307
- *Distribution coefficient octanol/water:* log P = 4.54
- *Solubility in solvents (g/100ml)*

methanol :	0.0053	*methylene chloride :*	0.0077
ethanol :	0.0026	*N,N-dimethylformamide :*	2.4
2-propanol :	0.0009	*acetone :*	0.055

VI REACTIVITY

- *Stability*

  Light : 7 days at 17000 lux: stable

  Water : not hygroscopic

  Temperature: qualitatively stable at 175°C-8h (TLC-control).

  pH: - 1N HCl (5 days - 100 °C): stable
  - H2O (5 days - 100 °C, pH = 6.3): stable
  - 0.01 N NaOH: not stable

- *Avoid contact with :* oxidants

- *Decomposition products :* not known

- *Combustion products :* nitrous oxides, HCl, Cl2, HCN, CO, CO2, water

- *Risk of polymerization :* not known

- *Risk of statical accumulation ;* ground

VII FIRE AND EXPLOSION HAZARD

- *Flash point :* inapplicable
- *Explosion range vol. % in air :* inapplicable
- *Auto-ignition temperature:* inapplicable
- *Fire class* *20 °C :* 1
  100 °C : 1
- *Auto-ignition temperature (BAM-furnace dispersed dust) :* > 570 °C
- *Ignition class deposited dust :* not known
- *Dust explosion class* *fine :* 1
  micronised : not known
- *Pmax :* 8.4 bar
- *Lower explosion limit :* <15 g/m3
- *Minimum ignition energy :* 1000 g/m3 <250 J
- *KSt value :* 129 bar.m/sec
- *Hazard class* *drying :* 0
  grinding : 1

- *Exothermicity DSC :* endothermic signal as of 291°C with max. at 297°C. Slight exothermic signal as of 307°C with max. at 326°C; heat content: 198 J/g

- *Exothermicity open*
  dynamic : first exothermic signal at 315°C
  isothermic : not known
- *Exothermicity under air supply*
  dynamic : melting endoth. as of 285°C , no exoth. signal up to 300° C
  isothermic : not known
- *Exothermicity under inert gas*
  dynamic : not known
- *Exothermicity mini-autoclave:* not known

- *Explosion hazard*
  Shock sensitivity class : 1 (not sensitive to shock)
  Friction sensivity : not sensitive to friction
  Thermic sensitivity : not sensitive to flame

VIII FIRE CONTROL

[X] water [X] powder [ ] halones [X] CO_2 [X] sand

[X] foam [ ] others

Personal means of protection : gloves, protective clothing and respiratory protection for the fire brigade

Remarks : Cool containers threatened by the fire with atomized water.

IX HEALTH

IX-1 PHARMACOLOGIC ACTIVITY

coccidiostatic agent

IX-2 LIMIT VALUES

TLV-TWA : not known

STEL : not known

AAC : 0.0175 mg/m3

Threshold of smell : inapplicable

RSI : inapplicable

IX-3 PERSONAL PROTECTION

- *Eyes :* a pair of safety glasses

- *Skin:* suitable gloves (plastic), closed working clothes

- *Respiration :* a half mask with a P2 filter whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P2), an Airstream anti-dust helmet or a full face mask with a P2 or P3 filter, whether or not in combination with a multi-purpose carbon filter (e.g., an ABEK-P3), a breathe-easy with a P3 filter.

- *Protection code :* A

IX-4 SYMPTOMS

- *Oral route :* atoxic

- *Eyes :* rabbit: non irritant

- *Skin :* not known

- *Inhalation :* not known

IX-5 FIRST AID

- *Oral route :* Allow the victim to rinse his mouth with water. Notify the medical service or a physician.

- *Eyes :* rinse the eyes thoroughly with running water for 10-15 minutes. Keep the eyelids open and turn the eyes in all directions during rinsing.

- *Skin :* Immediately remove soiled clothing and wash the skin with water and soap.

- *Inhalation :* immediately remove the victim from the hazard zone and take him into the fresh air. Apply artificial respiration upon breathing difficulties or respiratory arrest. Keep the victim warm and quiet. Immediately notify the medical service or a physician.

IX-6 MEDICATION

To be applied exclusively by a physician :

symptomatic treatment.

X TOXICITY DATA

X-1 ACUTE TOXICITY

- *Oral route :* rat LD50: >5000 mg/kg muis LD50: >5000 mg/kg

- *Eyes :* rabbit: no irritation

- *Skin :* rabbit LD50: >4000 mg/kg

- *Inhalation :* rat LC50: >2.24 mg/l (max. concentration)

- *Explanation :* inapplicable

X-2 SUBACUTE TOXICITY

Oral administration of doses of 5, 20 and 80 mg/kg to rats and dogs for 3 months brought about mildly toxic effects at 20 mg/kg (liver changes in rats) (m) and moderately toxic effects at 80 mg/kg (liver changes in rats and dogs).

X-3 GENOTOXICITY

- *Structural analysis :* not suspected of genotoxicity

- *Mutagenesis tests :* Non-mutagenic in:
  1. DNA-repair test with rat hepatocytes and SOS-chromotest with Escherichia coli.
  2. Ames test with Salmonella typhimurium.
  3. Sex-linked recessive lethal test with Drosophila melanogaster.
  4. Chromosome aberration test with human lymphocytes.
  5. Micronucleus test with mce and the dominant lethal test with male mice.
  6. Mouse lymphoma test (mammalian cell gene mutation test).

X-4 ECOTOXICOLOGIC PROPERTIES

- *Acute toxicity fish :*	species : bluegill	LC50: 0,58 mg/l
- *Acute toxicity daphnia :*	LC_{50} : not known	NEL : not known
- *Acute toxicity algae :*	EC_{50} : >4,8 mg/l	NEL : not known
- *Decomposability :*	BOD : not known	
	COD : 1.03	
	BOD/COD :	

XI REFERENCES

- List of active Janssen R-compounds R 64433; 08.11.93
- Safety test lab no. 1987/009A and 1992/022A.
- PC-CHAR 85-71, 85-137, 86-34, 94-1, 89-29, 86-42, 92-32, 92-37.
- PC-Results 87-7.
- PC-Results 89-32.
- EEC guideline L110 A/79 dated 04.05.93.
- Histology Dept. report nos. V5597, V5868, V5598, V5834, V5964, V6081, V6139 and V5909.
- Toxicology Dept. report nos. V6078, V6079 and exp. nrs. 1920 and 1921.
- Report no V88.198/280469 (TNO); V6684 (Janssen).
- Environmental dept.: rep. no. AASc/003, AFLm/003 and ADK6/0016.

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JANSSEN

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PHARMACEUTICA

naamloze vennootschap

Beerse, April 22, 1996

JANSSEN PHARMACEUTICA N.V., Turnhoutseweg 30, 2340 Beerse, Belgium

herewith certifies that the manufacturing facilities in Beerse and Geel are:

1) in compliance with all regional and national environmental laws;

2) in compliance with, or are on an enforceable schedule to be in compliance with, all emission requirements set forth in all permits;

and 3) that approval and the subsequent increase in production of the drug substance diclazuril at the facilities is not expected to affect compliance with current emission requirements or compliance with environmental laws.

Sincerely,

for JANSSEN PHARMACEUTICA N.V.

Wils Roger,
Vice President Environmental Affairs

Turnhoutseweg 30
B-2340 Beerse, België
Tel.: 014/60.21.11
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P.R.: 000-0003331-33
Telegramadres: JANSSENPHARMA
BTW: BE 403.834.160
H.R.: Turnhout 4203

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MALLINCKRODT VETERINARY

Rick Chiesa
Site Manager - Terre Haute

Mallinckrodt Veterinary, Inc.
1331 South First Street
Terre Haute, Indiana 47802 USA

Telephone (812) 232-0121

May 8, 1996

TO WHOM IT MAY CONCERN:

This is to certify that, to the best of our knowledge, Mallinckrodt Veterinary's manufacturing plant at Terre Haute, Indiana, is in compliance with all applicable federal, state, and local emissions requirements, and is expected to remain in compliance when CLINACOX (diclazuril 0.2% premix) is produced at the site.

J. Richard Chiesa
Site Manager - Terre Haute