Environmental Impact Analysis Report

Clorsulon 8.5% Oral Suspension for Cattle

A. January 10, 1985

B. Merck Sharp & Dohme Research Laboratories
Merck & Co., Inc.

C. P.O. Box 2000
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D. Environmental Information

1. Describe the Proposed Action

(a) Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., has filed a New Animal Drug Application for Clorsulon 8.5% Oral Suspension to be administered to cattle at a dose rate of 7 mg/kg of body weight for the treatment and control of mature and immature liver fluke, Fasciola hepatica.

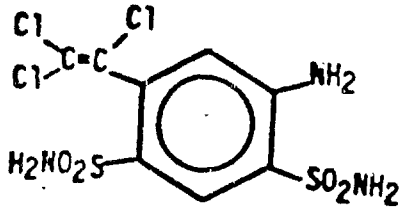
A drug withdrawal period of eight days prior to slaughter of cattle for food has been established.

(b) Physical and Chemical Properties Are as Follows:

Clorsulon, also known as MK-0401 (or MK-401), is 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide. The CAS registry number is [60200-06-8].

1. Describe the Proposed Action

The molecular structure is shown below:



The molecular formula and molecular weight are $C_8H_8Cl_3N_3O_4S_2$ and 380.6, respectively.

Clorsulon is produced by synthesis and is a white, crystalline compound with a melting point of 203-205°C when crystallized from water.

It absorbs ultraviolet light at 325, 267 and 227 nm (E% 119, 457 and 954). The absorption spectrum is essentially pH independent over the pH range of 1 to 12 and is also largely solvent independent. The NMR spectrum is consistent with the structure.

1. Describe the Proposed Action

Clorsulon is readily soluble in ethyl acetate, acetone, methanol or ethanol, and slightly soluble in ethyl ether and water. From pH 1 to 9, clorsulon has a water solubility of about 1 mg/ml. Above pH 10, the solubility increases, reaching about 10 mg/ml in 0.1 N NaOH.

The compound can be recovered from 2.5 N aqueous NaOH solution after 16 hours at room temperature essentially unchanged, but one hour reflux of this solution causes substantial decomposition. It is stable in methanesulfonic acid at 140° for 2 hours, and exchanges one of the benzene protons with CH_3SO_3D under these conditions. It is stable in the presence of p-chlorothiophenol in EtOH at room temperature for more than 1 month; no traces of secondary products can be detected by thin layer chromatography. The trichlorovinyl group in various intermediates is stable to sodium p-chlorothiophenolate in refluxing isopropanol over 16 hours, chlorosulfonic acid at 110° for 2.5 hours, fuming nitric acid, or refluxing aqueous sulfuric acid.

1. Describe the Proposed Action

Clorsulon displays only about a 0.3% weight loss when heated up to 200°C. Utilizing the Clausius-Clapeyron equation, the vapor pressure for clorsulon at 25°C may be calculated as 4.60×10^{-13}.

A sample of clorsulon stored for one week at 76% relative humidity exhibited a weight gain of only 0.14%, demonstrating the non-hygroscopic nature of this compound.

Clorsulon, like other substituted aniline-2,4-disulfonamides[1] reacts with acylating agents, aldehydes and urea to form adducts.

The pKa of the sulfonamide groups would be expected to be about 10, as compared to 10.2 for benzenesulfonamide. This is reflected in the increased water solubility above pH 10. The pKa of the 4-amino group would be less than the pKa for aniline (4.7) because of the electron-withdrawing substituents on the ring.

1. Describe the Proposed Action

Potentiometric titration with HCl indicated a pKa of 0.4 ± 0.2. Thus, clorsulon would be neutral from about pH 1 to about pH 9-10.

Based upon radioactivity measurements, the octanol distribution coefficient for clorsulon is 15.1; i.e.,

$$K_D \text{ of } \frac{\text{octanol}}{\text{pH 7 buffer}} = 15.1$$

(c) Pharmacology

Extensive chemical modification and biological testing of benzenesulfonamides resulted in the selection of clorsulon as a flukicide.[2,3] Doses of 10 to 15 mg/kg were required for greater than 90% effectiveness against immature liver flukes in calves and sheep, while a dose of 3.75 mg/kg was completely effective against mature flukes. Comparison of chromatographic and radiochemical data obtained from animals treated with radiolabeled drug demonstrated the presence of drug in blood and milk, and drug plus metabolite,

1. Describe the Proposed Action

which was hydrolyzable back to parent drug, in the urine.(4)

The pharmacokinetic basis for the efficacy of clorsulon against mature Fasciola hepatica was studied in experimentally infected rats.(5) Oral doses of 6.25 and 12.5 mg/kg showed that the drug rapidly entered the blood, attained a maximum concentration approximately 4 hours after dosing and then decreased exponentially. Approximately 75% of the circulating drug was in the plasma and the remainder was in the erythrocytes. Drug levels in F. hepatica paralleled those in the blood after a lag time. Clorsulon was shown to be a potent inhibitor of rat erythrocyte carbonic anhydrase. It was postulated that clorsulon reached liver flukes via a mechanism involving ingestion of red cells containing the drug bound to carbonic anhydrase. Further in vitro studies(6) indicated clorsulon caused a potent concentration-dependent inhibition of glucose uptake by mature flukes. The drug blocked the normal pathway of glucose metabolism by inhibiting 3-phospho glycerate

1. Describe the Proposed Action

kinase and phosphoglyceromutase. Other enzymes in the glycolytic pathway utilized by F. hepatica were not inhibited. The interactions between clorsulon and both phosphoglycerate kinase and phosphoglyceromutase were further characterized.[7-9]

Recently, the dose-dependent pharmacokinetics and efficacy of clorsulon against old, and young-mature infections of F. hepatica in the rat were studied.[10] At doses below 4 mg/kg, clorsulon was predominately bound to erythrocytes. At higher doses, it was distributed equally between the red cells and the plasma. The amount of clorsulon in the blood increased linearly up to 6 mg/kg, then began to saturate. Drug levels in F. hepatica increased in direct proportion to the blood level. Clorsulon was more effective against older fluke infections than against younger ones. It was suggested that the increased efficacy and uptake of clorsulon against older infections of F. hepatica in rats was the result of the age of the host rather than the parasite.

1. Describe the Proposed Action

Clorsulon was found to be ineffective at doses of 6 and 12 mg/kg against immature Paramphistomum microbothrium and fifth stage Haemonchus contortus and Trichostrongylus colubriformis in sheep. Clorsulon was also inactive at dosages of up to 100 mg/kg against H. contortus, Ostertagia circumcincta, T. axei and Cooperia spp. in sheep.

In an in vivo assay used to evaluate the prophylactic efficacy of compounds to control the development of avian chronic respiratory disease using Mycoplasma gallisepticum, clorsulon was ineffective at a dose of 250 mg/kg. Similarly, clorsulon, at an oral dose of 25 mg/kg, was ineffective in an infectious enteritis screen using Escherichia coli in hamsters.

Clorsulon was tested in in vitro antibacterial and antifungal screens. For the Antibacterial test, a stock solution of the sample to be tested was prepared in 25% methanol. Dilutions of this solution were

1. Describe the Proposed Action

made into Tryptone Glucose Extract Agar (Difco) and the agar was poured into sterile petri dishes. After hardening, the plates were streaked with an aqueous suspension of the test organism. The inoculated plates were incubated at 35-37°C and examined after 24 hours for the presence or absence of growth.

Clorsulon was inactive at 400 ppm against:

BACTERIAL SPECIES	STRAIN NO.
Aerobacter aerogenes	MB 1503
Pseudomonas aeruginosa	MB 418
Staphylococcus aureus morse 8	S8-Morse 8
Staphylococcus aureus	MB 2865
Salmonella pullorum	MSD 3198
Escherichia coli	MB 2884
Pseudomona aeruginosa	MB 2245
Klebsiella pneumoniae	MB 3123
Streptococcus Pyogenes	MB 2874
Pasteurella multocida	MSD 1590

1. Describe the Proposed Action

BACTERIAL SPECIES	STRAIN NO.
Bordetella bronchiseptica	MB 3551
Bordetella bronchiseptica	#74
Pasteurella multocida	PM9
Escherichia coli	#59
Salmonella typhimurium	#58

For the antifungal test, a stock solution of the sample to be tested was prepared in 25% methanol. Dilutions of this solution were made into Sabouraud Maltose Agar (Difco) and the agar was poured into sterile petri dishes. After hardening, the plates were streaked with an aqueous spore suspension of the test organism. The inoculated plates were incubated at 28-30°C and examined after 7 days for the presence or absence of growth.

Clorsulon was inactive at 400 ppm against Pullularis pullulans and Aspergillus niger.

1. Describe the Proposed Action

In an in vivo pharmacometric screening procedure, clorsulon was administered, i.p., to mice. The drug was evaluated for inhibition of dopamine β-hydroxylase, restoration of the anticonvulsant action of methazolamide (central noradrenergic agonistic activity), antiaddiction (anileridine) potential, production of postural asymmetries in mice with unilateral caudate lesions (dopamine agonistic or antagonistic activity), antagonism of amphetamine-induced hyperactivity (tyrosine hydroxylase inhibition, major tranquilizer activity), production of head twitch (central serotonin-like agents), antagonism of tetrabenazine-induced ptosis and loss of exploratory activity (antidepressant activity), antagonism of Metrazol- and electroshock-induced convulsions (anticonvulsant and minor tranquilizing activity), effects on pupil diameter, exploratory activity,

1. Describe the Proposed Action

righting reflex, and for the production of tremor, tonic convulsions, clonic convulsions, excitement, ptosis and ataxia. Finally, the 1- and 24-hour mouse mortality rate, as well as post-electroshock mortality, were determined. Clorsulon proved to be inactive in all the parameters tested; that is, the ED_{50} in each case was greater than the highest level tested of 150 mg/kg.

Clorsulon was found to be weakly active in an assay using sheep blowfly larvae (Lucilia cuprina). One ml of acetone solutions of clorsulon was added to adsorbent cotton wool in a tube. After 24 hours to allow evaporation of the acetone, 1 ml of sheep serum and 20 to 30 first instar larvae were added to the plug. The tubes were held in a culture room for 24 hours and the larvae were examined for viability and development. Clorsulon added at 63 and 125 ppm showed no or moderate activity, while application at levels of 250 to 1000 ppm showed moderate or partial activity in this assay.

1. Describe the Proposed Action

(d) Toxicity

Clorsulon, a fasciolicide for use in food animals, was negative for mutagenic activity in both the Ames test and in Chinese hamster V-79 cells. In addition, assays for the induction of either unscheduled DNA synthesis or DNA strand breaks in human fibroblast cells, with or without a rat liver microsomal activa tion system, were both negative. In vivo cytogenetic studies in mice revealed that clorsulon produced a significant increase in chromosomal breaks and micronuclei only at doses exceeding 500 mg/kg that were demonstrated to be cytotoxic to the bone marrow. Since no clastogenic activity was found at non-cytotoxic dose levels, no genotoxic hazard would be expected from the proposed use of clorsulon.

Teratogenicity studies with clorsulon were conducted in mice and rabbits at dose levels of 2, 10 and 50 mg/kg/day. Decreased fetal weight at the highest dose level was the only treatment-related finding in both species. No evidence of teratogenicity was found in

1. Describe the Proposed Action

either species administered clorsulon at dose levels up to 50 mg/kg/day.

In rat reproduction studies no effects on the mating performance, reproductive status, or post-implantation survival rates were found in females treated with up to 100 mg/kg/day, the highest dose level tested. An increase in mean gestation length was found in females treated with 100 mg/kg/day, while the average pup weight per litter on Day 1 postpartum was decreased in this group. By Day 7 of lactation this effect on pup weight was no longer evident. Dose levels of up to 30 mg/kg/day produced no adverse effects on any of the parameters examined.

In a 13-week oral toxicity study in rats following *in utero* exposure, no treatment-related effects were observed at dose levels up to 30 mg/kg/day. At a dose level of 300 mg/kg/day, slight increases in alkaline phosphatase activity were produced in male rats only.

1. Describe the Proposed Action

In a 14-week oral toxicity study in dogs, no treatment-related effects were produced at dose levels up to 8 mg/kg/day. At doses greater than or equal to 32 mg/kg/day, neutropenia and anemia were evident. These changes were accompanied by bone marrow hyperplasia and splenic hemosiderosis, indicating that the anemia was hemolytic in origin.

In a 54-week chronic oral toxicity study in rats following *in utero* exposure at doses up to 80 mg/kg/day, no drug-related effects were produced. In addition, lifetime carcinogenicity studies in rats and mice at dose levels up to 49 and 300 mg/kg/day, respectively, showed no evidence of a carcinogenic effect.

Based on the lowest no-observable-effect level of 8 mg/kg/day from the 14-week dog study and the extremely low tissue residue levels present in food animals, it is concluded that a substantial margin of safety exists for the proposed therapeutic use of clorsulon.

Table 1. Results of Acute, Subacute, Chronic, and Teratology Studies of MK-401.

Type of Study	Species	Duration	Doses (mg/kg/day)	Primary Observations Antemortem	Postmortem
Acute Oral	Mouse (M,F)	-	-	-	LD_{50} > 20 gm/kg
Acute Oral	Rat (M,F)	-	-	-	LD_{50} > 10 gm/kg
Acute i.p.	Rat (M,F)	-	-	-	LD_{50} 938-845 mg/kg
Oral Range-Finding	Rat	29-Day	100, 300, 900	All doses - salivation, retarded body weight gain (males only)	900 - inc. liver weight, liver necrosis
				300, 900 - inc. alk. phos.	300, 900 - liver cell enlargement
Oral Toxicity	Rat	13-Week (in utero exposure)	2, 20, 100	2, 20 - inc. gestation length	No treatment-related changes
					Incidental findings: All doses - inc. thyroid weight (females)
					20, 100 - urinary bladder hyperplasia
					2, 20 - salivary neoplasia
Oral Toxicity	Rat	14-Week	55	None	No treatment-related changes
					Incidental findings: Salivary-ducted hyperplasia in 2 treated and 1 control rat
					Salivary carcinoma in 1 control rat
					No increase in thyroid weight
Oral Toxicity	Rat	14-week	3, 30, 300	None	None (Focus of inc. mitotic activity in bladder of 2 high dose rats considered to be of no significance.)

Table 2. Mutagenic and Bone Marrow Toxicity Studies of MK-401.
Summary of Significant Findings.

Type of Study	Species	Duration	Doses (mg/kg/day)	Results
Ames Bacterial Mutagen Tests	-	-	2500 µg/plate	Negative
Micronucleus Test	Mouse	2 or 5 days	500, 1000, 2000	Significant increase in micronuclei (combined analysis) at 1000, 2000 mg/kg/day
Micronucleus Test	Mouse	2 days	2000 mg/kg/day	Non-significant increase in micronuclei
Bone Marrow Toxicity Study	Mouse	2 days	500, 1000, 2000	Focal vacuolation and focal necrosis of bone marrow at all dose levels
Cytogenic Study - Acute	Mouse	1 day	500, 1000, 2000	Significant increase in chromosome breaks at all dose levels Significant increase in chromosomal rearrangements 24 hours postdosing at 500 and 2000 mg/kg/day
Cytogenic Study - Subacute	Mouse	5 days	500, 1000, 2000	Significant increase in chromosomal breaks at 2000 mg/kg/day
Cytogenic Study - Acute and Subacute	Mouse	1 and 5 days, respectively	100, 250, 500	Negative
Alkaline Elution Assay (DNA strand breaks)	Human lung Fibroblast Cells	-	0.01→3 mM	No DNA strand breaks produced
Unscheduled DNA Synthesis	Human lung Fibroblast Cells	-	0.3 → 3 mM	Negative
Point Mutation Assay	V-79 Chinese Hamster Cells	-	0.3 → 3 mM	Negative

09

1. Describe the Proposed Action

In 1982, 1.4 million livers were condemned because of liver fluke damage.[11] This loss has been estimated at $5 million annually.[12] In addition, indirect losses, including reduction in average daily body weight gain and lower feed conversion ratios in feedlots, reduced milk production in dairy cattle, and reduced herd performances in cow-calf operations, may be far greater. There is evidence that the prevalence of F. hepatica is increasing in the United States, particularly in Western states where irrigated pastures are increasingly used.[12]

Clorsulon 8.5% Oral Suspension for cattle provides a convenient, ready-to-use method of control of liver flukes without leaving hazardous or potentially dangerous wastes which require careful handling, storage, transport and disposal.

The beneficial effect resulting from clorsulon use would be the reduction in the number of livers

1. Describe the Proposed Action

condemned annually, and reduction of indirect losses due to fascioliasis.

(f) Potential Market, Handling and Storage

Fascioliasis due to F. hepatica occurs primarily in Gulf Coast states and in Western states in regions where suitable soil and sufficient moisture support populations of snail intermediate hosts.(12) States having high losses from fascioliasis account for 40% of the United States' cattle population, though livers from only 3 to 4% of all cattle slaughtered are condemned because of liver fluke damage.(12)

No special handling and storage requirements are the environmental compartments affected by approval of the New Animal Drug Application for Clorsulon would be the usage sites, the animal waste disposal sites (fields fertilized with excreta from dosed animals), manufacturing sites, and the discharge and ground water at each of these sites.

1. Describe the Proposed Action

The usage sites will be large and small cattle feedlots. Since the drug will be dosed orally, the primary environment affected by the use of clorsulon in cattle will be the feedlots, due to the excretion of the drug by treated cattle via their feces and urine, and the fields fertilized by the excreta from dosed animals.

A secondary environment affected by the excretion of clorsulon would be the water run-off from cattle feedlots and the water percolating through fields fertilized with wastes from treated animals. Data relevant to drug levels and the fates of clorsulon in feces, soil and water are discussed in Section 2(a).

Another secondary environment would be the manufacturing sites and the associated discharge waters. The environmental impacts at these sites are discussed in Section 3.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers

The projected use of clorsulon in cattle involves the oral administration of the drug at a dose level of 7 mg/kg body weight. The animals may be contained in a pasture, a small independent feedlot or a large commercial feedlot. Generally, the cattle will receive 2 to 3 doses of the drug per year.

Most cattle will be dosed with clorsulon in the cattle feedlots. The following calculations based on the U.S. Environmental Protection Agency publication (Reference 13, Development Document for Effluent Limitations Guidelines and New Source Performance Standards for the FEEDLOTS - Point Source Category, U.S. Environmental Protection Agency, Washington, D.C. 20460, January, 1974) show the expected concentration of clorsulon and metabo-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

lites in the "Raw Waste" (manure) of a feedlot. These calculations show that the concentration in the manure will be only 0.66 parts per million.

Included is a flow diagram (Figure 1) from the reference (loc. cit.) showing the daily raw waste produced in a typical feedlot operation in which a 270 kg calf entered the operation and in 130-180 days reached a market animal weight of about 477 kg. During this period the animal would be treated once with clorsulon at a dose level of 7 mg/kg.

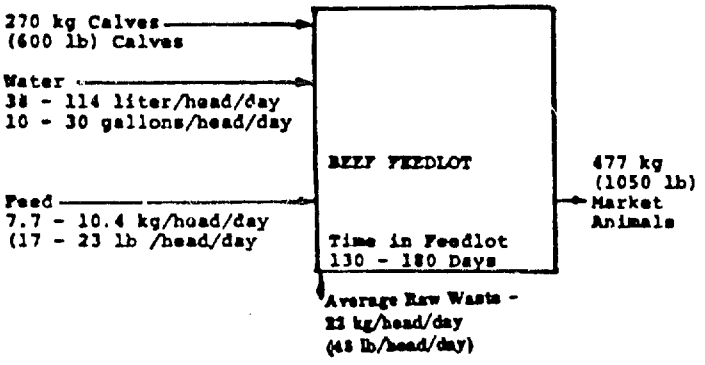


FIGURE 1
TYPICAL BEEF FEEDLOT FLOW DIAGRAM

111

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

The following calculations show the average concentration of clorsulon and its metabolites in the waste produced by a single animal. This concentration, of course, would not change regardless of the actual number of cattle treated in the feedlot.

Weight of steer		270 kg
Dose of clorsulon	x	7 mg/kg
Weight of clorsulon dosed	=	1890 mg
Waste produced per steer per day		22 kg
Total Time in feedlot		130 days
Total waste produced		2860 kg

Concentration of drug and metabolites in waste:

$$\frac{1890 \text{ mg dose}}{2860 \text{ kg waste}} = \frac{0.66 \text{ mg}}{\text{kg}} = 0.66 \text{ ppm}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

i) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

During the first seven days following drug administration, about 90% of the radiolabel in an administered dose was found in both the urine (25%) and the feces (65%).

If one considered the first-week's waste, 90% of the administered dose would be excreted. Thus, the concentration of drug-equivalent in the first-week's waste would be (0.90 x 1890 mg)/(7 days x 22 kg/waste/day) = 11.0 mg/kg (ppm). Two cattle studies have been conducted where the urine and feces were collected for the first seven days. In the first study, four calves were dosed intraruminally with radiolabeled (^{35}S) clorsulon at 6.6 mg/kg b.w. Urine and feces were collected daily from each animal during the first seven days after

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

dosing. Daily quantitative measurements of the amounts of urine and feces collected and the amounts of ^{35}S-clorsulon found in these excreta were determined and reported for three of the test animals. Peak residue levels in the urine and feces of these three animals were usually found to be about 35 ppm and to occur on the second day after dosing. An averaging of the seven days of data collected on each animals urine and feces resulted in a range of average (seven-day) values of from 14.0-17.7 ppm in urine and 15.2-16.8 ppm in feces. The residue level in the combined first week's waste (urine and feces) was 15.68 mg/kg from a 6.6 mg/kg dose.

In the second study, four steers were dosed intraruminally with ^{14}C-clorsulon at 14.9 mg/kg b.w. The urine and feces were collected daily for each

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

animal during the first seven days after dosing. Daily quantitative measurements of the urine and feces collected and the amounts of ^{14}C-clorsulon found in these excreta were determined and reported for three of the test animals. Peak residue levels in the urine and feces were usually found to be about 80-105 ppm and 60-90 ppm, respectively, and to occur on the second day after dosing. An averaging of the seven days of data collected on each animals urine and feces resulted in a range of average (seven day) values of from 32.3-37.2 ppm in urine and 30.1-37.9 ppm in feces. The residue level in the combined first week's waste (urine and feces) was 33.03 mg/kg from a 14.9 mg/kg dose.

The second report also determined that: 1) in the first seven days following dosing about 90% of the administered dose was found in the urine (25% of

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

1) Estimated Concentration of Potentially Bioactive Substances in Excreta of Steers (Cont'd)

total) and feces (65% of total); and 2) more than half of the amount excreted was found in the first three days.

The residue level in the combined first weeks waste from the first study corresponds to 16.6 mg/kg calculated for a 7 mg/kg dose, while the residue level from the second study corresponds to 15.5 mg/kg calculated for a 7 mg/kg dose. Thus, a level of about 16 ppm appears to be "the worst-case" level of residue in the excreta.

2) Estimated Concentration of Potentially Bioactive Substances Expected in Runoff from Open Air Animal Growing Facility

Based upon a 7 mg/kg dose for a 270 kg steer in an open air feedlot at 220 steers/acre (200 sq.ft./

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

2) Estimated Concentration of Potentially Bioactive Substances Expected in Runoff from Open Air Animal Growing Facility (Cont'd)

calf, Reference 14), the concentration of clorsulon drug-residue in a 2-inch runoff can be estimated to be 2.0 ppm, using 102,750 kg/acre-inch of water:

$$\frac{1890 \text{ mg}}{\text{steer}} \times \frac{220 \text{ steers}}{\text{acre}} \div 205{,}500 \text{ kg} = 2.0 \text{ mg/kg}$$

This assumes all the drug-residue will be contained in the runoff.

3) Estimated Concentration of Potentially Bioactive Substances Expected When Excreta of Target Animals Are Incorporated into Agricultural Soil as Fertilizer

Using the average estimated concentration of drug-residue in feedlot wastes, 0.66 ppm, from Section 2(a)1), an application rate of 4.5×10^3 kg/acre (about 5 tons/acre), and a weight of 909,000 kg soil per acre at 6-inch depth, the concentration of drug-residue in the soil would be only 0.0033 ppm.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(a) Introductions Into the Environment Through use of Clorsulon

3) Estimated Concentration of Potentially Bioactive Substances Expected When Excreta of Target Animals Are Incorporated into Agricultural Soil as Fertilizer (Cont'd)

$$\frac{0.66 \text{ mg MK-0401}}{\text{kg feces}} \times \frac{4.5 \times 10^3 \text{ kg feces}}{\text{acre}} \div \frac{909{,}000 \text{ kg soil}}{\text{acre}} = 0.0033 \text{ ppm}$$

Using the worst-case estimated concentration of drug-residue, Section 2(a)1), and 12 tons per acre (1.09×10^4 kg/acre), about the highest application rate recommended by the United States

Department of Agriculture (Reference 15), the concentration of drug-residue in the top 6 inches of soil from use of the feces from dosed steers as fertilizer would be:

$$\frac{16.0 \text{ mg MK-0401}}{\text{kg waste}} \times \frac{1.09 \times 10^4 \text{ kg waste}}{\text{acre}} \div \frac{909{,}000 \text{ kg soil}}{\text{acre}} = 0.13 \text{ ppm}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

1) Water Solubility

The solubility of clorsulon was essentially constant from pH 1 to pH 9 at 0.7 to 0.9 mg/ml. Above pH 10, the solubility increased, being about 10 mg/ml in 0.1 N NaOH.

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer

The distribution coefficient of clorsulon in 1-octanol and phosphate buffer (pH 7) was determined at three different equilibration times by using ^{14}C-labeled clorsulon.

Aliquots of ^{14}C-clorsulon (200 µl each) were transferred from a stock solution to 15 ml centrifuge tubes and evaporated to dryness. 1-Octanol (5 ml) was added, vortexed to mix and was followed by the addition of 5 ml 0.1 M sodium phosphate buffer

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer (Cont'd)

(pH 7). The tubes were stoppered and vigorously shaken for 5 minutes and centrifuged for 5 minutes to separate phases. The contents were agitated at room temperature in a water bath for 1 hour, 24 hours and 48 hours after centrifugation. (Controls were prepared for each time period in the same manner.)

Three aliquots of 1 ml each were removed from the octanol layer for scintillation counting. The rest of the octanol phase was removed and decanted.

The remaining phosphate layer was re-centrifuged to eliminate any residual octanol. Three aliquots of 1 ml each were withdrawn from the phosphate phase for scintillation counting.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer (Cont'd)

All aliquot samples were mixed with 15 ml Insta-Gel (Packard) and counted for 50 minutes in a Packard Tri-Carb Liquid Scintillation Spectrometer. Octanol and phosphate buffer blanks were used for background correction.

The decompositions per minute (DPM) for the 3 (1 ml) aliquots of octanol and buffer were averaged and multiplied by the total volume of 5 ml, then added together to obtain the total DPM recovered and then divided by the starting DPM in the 200 µl spike, giving the percent recovered.

5 x (Av. of octanol DPMs + Av. of buffer DPMs) = Total DPM

$$\frac{\text{Total DPM}}{\text{Starting DPM}} = \text{\% Recovered}$$

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(5) Fate of Potentially Bioactive Chemicals in the Environment

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer (Cont'd)

Then, the K_D was calculated by

$$K_D = \frac{\text{Av. octanol DPM}}{\text{Av. buffer DPM}}$$

The values of K_D at 1 hour, 24 hours and 48 hours equilibration time were 14.2, 15.8 and 15.2, respectively, for an average value of 15.1. The recovery for total radioactivity was 94%, 97% and 99.1%, respectively.

The buffer layer from the 24-hour equilibration was evaporated to dryness in the hood at room temperature under nitrogen. The corresponding octanol layer was evaporated to dryness in an oil bath at 150° over a weekend. The residues were resuspended in 100 µl acetonitrile, and 20 µl of each was chromatographed on an octadecylsilane high per-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer (Cont'd)

formance liquid chromatography (HPLC) column using 20% acetonitrile in phosphate buffer (pH 3.2 with Et_3N) for 25 minutes, followed by a gradient to 100% acetonitrile.

After evaporation of the two fractions from the 24-hour equilibration, relatively little of the radioactivity in the fractions was soluble in acetonitrile, as shown by counting aliquots of 10 µl and 20 µls, respectively, in Instagel in a scintillation counter. HPLC of the acetonitrile/ soluble material indicated that the phosphate buffer fraction had radioactivity at the clorsulon elution time and in the acetonitrile gradient area. The octanol fraction showed radioactivity only in the acetonitrile gradient area. It has been concluded that because of the low solubility

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

2) Distribution Coefficient (K_D) of Clorsulon in 1-Octanol and Phosphate Buffer (Cont'd)

of the radioactivity from each fraction and the high heat and long period for evaporation of the octanol fraction, the clorsulon possibly was degraded. However, there is no evidence of clorsulon degradation during the equilibration time periods due to the fact that the K_D's remained constant for all assay times, 1 hour, 24 hours and 48 hours.

A K_D (K_{OW}) of 15 is very low and indicates that clorsulon should not tend to bioaccumulate in organisms in the environment. This is also demonstrated in its rapid clearance in the metabolism studies.

3) Dissociation Constants

The pKa of the sulfonamide groups would be expected

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

3) Dissociation Constants (Cont'd)

to be about 10, as compared to 10.2 for benzenesulfonamide. This was confirmed by the increased solubility above pH 10.

The pKa of the 4-amino group would be expected to be less than the pKa for the amino group of aniline (4.7) because of the electron-withdrawing substituents on the ring. From the water solubility data, Section 2(b)1), and the lack of pH-dependent changes in the absorption spectrum, the amino group of clorsulon does not become protonated until below pH 1. This was confirmed by potentiometric titration with HCl, where the pKa of the amino group was determined to be 0.4 ± 0.2.

Thus, clorsulon would be neutral from about pH 1 to about pH 9-10.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

4) Vapor Pressure

Clorsulon displayed only about a 0.3% weight loss when heated up to 200°C. Utilizing the Clausius-Clapreyron equation, the vapor pressure for clorsulon at 25°C may be calculated as 4.60×10^{13}.

5) Soil Adsorption/Desorption of Clorsulon

Aqueous solutions of ^{14}C-labeled clorsulon were equilibrated with Iowa silt loam soil to determine the adsorption of the drug onto soil. Iowa silt loam soil was collected at Newton, Iowa and stored in a cold room. A portion of the soil, sifted through a #20 mesh sieve, had a clay loam soil texture, a pH of 5.0, a cation exchange capacity of 24.29 ME/100 g and was 26% sand, 46% silt, 28% clay and 4.56% organic matter. Additional batches were sifted as needed. The batch used for this study had 4.3×10^5 fungal colony forming units and 1.7×10^7 bacterial colony forming units per gram.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

A stock solution of clorsulon was prepared by mixing 6.25 mg of cold clorsulon with 1 ml ^{14}C-labeled clorsulon stock solution in 25 ml methanol. The clorsulon had about 110 DPM/ microgram. Aliquots of 10.0, 5.0, 2.0, 1.0, 0.50 and 0.10 ml of this stock solution were placed into 25 ml volumetric flasks and the methanol was evaporated under a stream of nitrogen. The flasks were filled to volume with filtered, deionized water. The solutions were 100, 50, 20, 10, 5 and 1 ppm (micrograms/ml) in clorsulon, respectively. The absorbence of the 10 ppm solution was determined directly at 267 nm. Triplicate 1.0 ml aliquots were counted from each solution, and the exact concentration of each and the specific activity of the clorsulon was determined from the scintillation and optical density data.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

Five grams of Iowa silt loam soil was placed into each of 13 fifteen-milliliter centrifuge tubes. One tube served as a blank, and 10.0 ml of the filtered, deionized water was added. Duplicate ten-milliliter aliquots from each of the clorsulon solutions were added to the other tubes. A preliminary experiment established 2 hours as sufficient for equilibration of clorsulon with Iowa silt loam soil, so the tubes were securely stoppered, wrapped in foil to exclude light, and mixed on a rotary mixer for 2 hours at room temperature. The tubes were then centrifuged, and triplicate 1.0-ml aliquots were withdrawn for scintillation counting.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

The desorption of clorsulon was studied by removing a total of 6.0 ml from one sample at each concentration and replacing it with 6.0 ml of filtered deionized water. These solutions were then mixed for 2 hours on a rotary mixer in the dark. Triplicate 1.0-ml samples were taken for scintillation counting. Only one treatment was made on each sample.

The integrity of the clorsulon following a 6.0-ml desorption was determined by Reverse Isotope Dilution Assay (RIDA). This was done to assure that unchanged clorsulon was the desorbed material.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

Scintillation counts were converted to concentration using specific activity data (111 DPM/mcg clorsulon). The amount remaining in solution following adsorption was measured directly by scintillation counting. The amount of drug bound to the soil in the adsorption experiment was calculated by the difference between the known amount added and the amount remaining in solution.

For desorption, the free concentration was also measured directly by scintillation counting. The bound clorsulon was determined by the difference between the amount remaining in solution following desorption and 40% of the amount of drug remaining in solution following adsorption, plus the amount bound following adsorption, i.e., bound (after

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

desorption) = [0.4 x free (after adsorption) + bound (after adsorption)] - free (after desorption).

The adsorption and desorption data were fitted to the Freundlich binding equation:

$$(x/m) = kc^{1/n}$$

where x is the amount of adsorbate per mass m of adsorbent, c is the solution concentration at equilibrium, and k and n are constants. This equation is easily converted to the form of a linear equation:

$$\log (x/m) = \log (k) + 1/n \times \log (c).$$

The slope of a log-log plot of solution concentration (free) vs. concentration per mass of soil (bound) is therefore equal to 1/n, and the intercept is equal to log (k).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

The values of the Freundlich constant, k, and of n for adsorption of clorsulon to Iowa silt loam soil were 1.57 and 1.06, respectively. The corresponding values for desorption were 1.97 and 1.05.

The adsorption distribution constant (Kd) can be calculated by averaging the ratio of the bound concentrations divided by the free concentrations for each absorption data point. From the calculated value, Kd=1.4, the binding to organic carbon can be calculated (Koc = Kd/% organic carbon x 100). For clorsulon with Iowa silt loam soil, this value of Koc is 1.4/4.55 x 100 = 30.8. These values indicate that clorsulon is not very tightly bound to soil and appears to be environmentally very mobile. Clorsulon should therefore readily transfer into the aquatic environment.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

5) Soil Adsorption/Desorption of Clorsulon (Cont'd)

About 15 days following the adsorption experiment and after desorption, one of the 50 mcg/ml samples was analyzed by RIDA of the ethyl acetate extract of the aqueous phase. Seventy-eight percent of the radioactivity recovered was intact clorsulon. It is not clear whether the loss of drug is soil related or due to some other process or both. A preliminary experiment had determined that aqueous clorsulon was stable in the presence of soil for at least 44 hours. HPLC analysis revealed that all of the radioactivity in aliquots from ^{14}C-labeled clorsulon with Iowa silt loam soil corresponded to clorsulon. There were no degradation peaks in the chromatograms.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer

The stability of clorsulon in soil and in the feces from a dosed steer was determined in samples exposed to an outdoor summer environment.

For clorsulon in soil, ^{14}C-labeled clorsulon was mixed into Iowa silt loam soil [See Section 2(b)5)] to a level of about 25 ppm. The clorsulon in methanol was dropped onto a small portion of soil in an amber jar. The soil was then dried under a stream of nitrogen. Additional soil was added, each addition equaling the amount already in the jar. After each addition, the soil was thoroughly tumbled and mixed by hand.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

Ten 10-g samples were weighed. Two were used as zero-time samples; the remaining eight were placed into sintered-glass funnels.

Five 10-g samples of untreated Iowa silt loam soil were used as controls. Four of the five were placed into sintered-glass funnels.

For clorsulon in feces from a dosed steer, threeday post-dose feces from steer number 3005, study RN-209 was used, as the total residue level was about 25 ppm. Two 3-g portions were used for zero-time samples while eight 3-g portions were placed into sintered-glass funnels.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

The pre-dosing feces from the same animal served as control samples. One 3-g sample was used as a zero-time sample, and four 3-g samples were placed into sintered-glass funnels.

The glass funnels, placed into Erlenmeyer flasks, were placed into a plastic tub so that they could not spill. The tub was placed outdoors, exposed to rain and sunlight. A graduated cylinder was used to collect and measure rainfall. The daily temperatures from local weather services were recorded, and additional information about cloudiness was recorded.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

The samples were placed outdoors on June 23, 1983, in an enclosure at Rahway, New Jersey. Samples were assayed at 0, 7, 19, 35 and 60 days. Percolated rain water was collected and frozen until time of assay.

The weather was generally hot and sunny between June 23, 1983 (Day 0) and August 22, 1983 (Day 60). About 1 inch of rain fell during the first 7 days, only a trace from Days 7 to 19, about 1/2 inch from Day 19 to Day 35, and then 1.7 inches from Day 35 to Day 60.

At assay, the total weights of the soil samples and feces samples were determined. A weighed amount of distilled water was added to each, along with a

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

known amount of unlabeled clorsulon. After homogenation, aliquots were taken for combustion. Each homogenate was divided in half. One portion of each was extracted with ethyl acetate. The ethyl acetate was blown dry and the residue was resuspended in 9:1 ethanol:water which was extracted with isooctane. The ethanol:water layer was dried and the sample was resuspended in methanol for chromatography. The other portion of the zero- and 7-day samples were adjusted to 0.5 N with HCl, refluxed overnight, then extracted as above.

At assay, the frozen percolated rain water collections were pooled and a known amount of unlabeled clorsulon was added. The total percolated radio-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

activity from each sample was determined by weighing and counting an aliquot. Each sample was subdivided. One portion of each was extracted as above. The other portion of two of the one-week percolates was extracted after adjusting to 0.5 N with HCl and refluxing overnight.

The level of clorsulon in the samples was determined by RIDA. The clorsulon in each sample was isolated by HPLC and its specific activity was measured.

No attempt was made to exhaustively extract the radioactivity from the outdoor samples, since the RIDA procedure required isolation of only enough

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

cold carrier to determine the specific activity. Each sample of soil, feces or water was extracted twice with ethyl acetate, the final ethyl acetate volume being less than 15 ml. The percent of radio-activity extracted was determined for the zero-time, 7- and 19-day samples only. For the zero-time soil samples, 94% and 102% of the radioactivity was extracted from duplicate samples. For the 7- and 19-day samples, the percent of radioactivity extracted was only 52 to 60% of the total in the soil. HPLC showed almost all of this extractable radioactivity as parent drug. For the water which percolated through the 7- and 19-day soil samples, only 4 to 7% of the radioactivity present extracted into ethyl acetate. HPLC

7. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

analysis of the extracts from the 7-day percolated water showed most of the extractable percolated radioactivity to be compounds more polar than the drug.

For the zero-time feces samples, only 53 to 55% of the radioactivity present extracted into ethyl acetate. For the 7- and 19-day feces samples, only 23 to 46% of the radioactivity was extractable with ethyl acetate, as compared to only 3 to 8% extraction of the percolated radioactivity. Again, HPLC analysis of the ethyl acetate extractable radioactivity from feces samples indicated it was mostly drug, and the majority of the extractable material in the percolated water samples was more polar than the drug.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

Thus, the degradation products of clorsulon in soil and in the feces of dosed steers were polar compounds which percolated from the soil, and were too polar to be effectively extracted with ethyl acetate.

Tables 3 and 4 contain the data obtained from the outdoor stability study of clorsulon in soil and in the feces of a dosed steer. Results from the duplicate samples at each time point are listed. At time zero, there was no percolated water, so all the radioactivity was in the soil or feces. The percolation of radioactivity generally followed the amount of rainfall, being greatest during Days 0 to 7 and from Days 35 to 60. By Day 60, over 70% of the radioactivity had percolated from the soil

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

samples, as compared to only about 30% from the feces samples. The RIDA % values in all the percolated water samples were less than 1%. This resulted from the very low photostability of aqueous clorsulon, as discussed in Section 2(b)8).

The RIDA % data for clorsulon in soil and in feces were fit to simple exponential decay equations. The best fit for the clorsulon in soil data points gave a t_0 value of 103.8% clorsulon and a half-life of 19.6 days (R = -0.989). The corresponding values for clorsulon in feces were 41.7% for the t_0 level and a half-life of 32.2 days (R = -0.945).

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

These half-life values represent the stabilities of the clorsulon remaining in the soil and feces only. Since the polar decomposition products would have probably leached out of the soil and feces more rapidly than clorsulon, the samples would have been "enriched" in clorsulon after each rainfall. Thus, the half-lives probably represent an upper limit for the degradation of clorsulon during the summer. Since the percolated clorsulon decayed rapidly upon percolation, tnere is no way of correcting the half-lives to account for the preferential percolation of the decomposition products.

An attempt was made to hydrolyze any clorsulon conjugates back to "free" clorsulon. Chromatograms of the ethyl acetate extractable radioactivity

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

from the zero-day samples subjected to reflux in 0.5 M HCl showed no resolved cold carrier clorsulon peak, and little radioactivity eluting where the endogenous clorsulon would be expected. Most of the extractable radioactivity corresponded to degradation material less polar than clorsulon. No further analysis of these compounds was made. The half-lives determined from the data in Tables 3 and 4, therefore, represent the degradation of "free" clorsulon. The slower degradation of clorsulon in feces as compared to clorsulon in soil, may represent a contribution to the "free" clorsulon level from hydrolysis of conjugate back to clorsulon, or a protective action towards photolysis by the feces.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

TABLE 3
CLORSULON OUTDOOR STABILITY STUDY
CLORSULON IN SOIL

DAYS	% DPMs IN SOIL	% DPMs IN PERCOLATE	RIDA% IN SOIL	RIDA% IN PERCOLATE
0	100.0	--	104.1	--
0	100.0	--	113.5	--
7	63.7	36.3	68.0	0.6
7	56.1	43.9	69.6	0.3
19	56.3	43.7	61.2	0.5
19	61.3	38.7	57.6	0.6
35	51.5	48.5	34.7	0.4
35	55.1	44.9	28.7	0.2
60	28.5	71.5	12.6	0.2
60	27.8	72.2	11.1	0.2

TABLE 4
CLORSULON OUTDOOR STABILITY STUDY
CLORSULON IN FECES

DAYS	% DPMs IN FECES	% DPMs IN PERCOLATE	RIDA% IN FECES	RIDA% IN PERCOLATE
0	100.0	--	41.3	--
0	100.0	--	50.3	--
7	90.2	9.8	34.4	0.5
7	90.6	9.4	30.1	0.6
19	86.8	13.2	38.8	0.4
19	84.9	15.1	23.4	0.7
35	88.9	16.1	16.6	0.5
35	80.3	19.7	18.0	0.6
60	70.7	29.3	11.7	0.2
60	67.4	32.6	12.6	0.3

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

6) Outdoor Stability Study of Clorsulon in Soil and of Clorsulon in Feces from a Dosed Steer (Cont'd)

Because of degradation of percolated clorsulon and the inability to hydrolyze clorsulon conjugates back to parent drug, the "stability" of clorsulon in an outdoor environment could not be determined.

However, after 60 days, the RIDA % of clorsulon in soil decreased from essentially 100% to about 12%, while the clorsulon level in feces from a dosed steer decreased from about 42% to about 12% of the total radioactivity. The calculated half-lives were 19.6 and 32.2 days, respectively, for the conversion of clorsulon to degradation products in soil and in feces in an outdoor environment. Over 70% of the radioactivity in the soil sample percolated with rain water, as compared to only 30% of the radioactivity in the feces.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

7) Aqueous Stability of Clorsulon

The stability of clorsulon in water at pH 5, 7 and 9 at 24.0 6 0.5°C was determined. ^{14}C-labeled clorsulon was equilibrated in the dark in stoppered tubes in a constant temperature bath. Samples were periodically assayed by RIDA to determine the stability of the drug.

The following buffer solutions were made with Millipore-filtered, distilled water:

1. pH 5: 0.05 M sodium acetate adjusted with acetic acid.
2. pH 7: 0.05 M potassium phosphate monobasic adjusted with sodium hydroxide.
3. pH 9: 0.05 M sodium borate adjusted with acetic acid.

All buffers were filtered through 0.2-micron filters. ^{14}C-labeled clorsulon in methanol was used to prepare the stock solution. Three hundred microliters of this solution was diluted to 3.0 ml with methanol. One-hundred microliters of this stock solution was added to each assay tube. The methanol in the assay tubes was evaporated and 5.0

7. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

7) Aqueous Stability of Clorsulon (Cont'd)

ml of appropriate filtered buffer was added to each. Each tube contained 340 ng clorsulon/ml (340 ppb).

The assay tubes were securely stoppered and shaken. The tubes were wrapped in foil to exclude light and placed into a 24 ± 0.5°C water bath until time for assay. One sample of each pH value was prepared for analysis at approximately 0, 1, 3, 7, 14 and 29 days, for a total of eighteen tubes.

At assay time, the tubes were opened and a known amount of unlabeled clorsulon was added and mixed. The clorsulon was extracted with two portions of ethyl acetate. The volume of the ethyl acetate extracts was noted and aliquots were removed for scintillation counting. The remainder of the extracts were evaporated and the residues were dissolved in methanol. The specific activity of the clorsulon isolated by HPLC was determined to allow calculation of the percent of the intact drug at assay time.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

7) Aqueous Stability of Clorsulon (Cont'd)

The specific activity was calculated by measuring the ultraviolet absorbence at 267 nm of collected HPLC fractions to determine the total concentration of clorsulon. Each fraction was aliquoted for scintillation counting to determine the radioactivity per milliliter. Knowing the total radioactivity per tube and the amount of cold clorsulon added at assay time, the theoretical specific activity of the clorsulon was calculated to be 32200 DPM/mg. Dividing the observed specific activities by this number and multiplying by 100 gave the percent of the intact drug in the samples.

The data showed that there was no significant degradation of aqueous clorsulon maintained in the dark at pHs 5, 7 and 9 for at least 29 days at 24°C.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

8) Light Stability of Clorsulon

The stability of aqueous clorsulon in direct sunlight (through glass) was determined.

All glassware used in the experiment was autoclaved to exclude microbial contamination.

Two-tenths milliliter of ^{14}C-labeled clorsulon in methanol was added to a 100-ml volumetric flask. This solution was evaporated to dryness. The sample was reconstituted with distilled water and diluted to the mark. Triplicate 1.0-ml aliquots were taken for scintillation counting. The solution was then filtered through a 0.20-micron sterilization filter unit. Triplicate aliquots of this sterile solution were taken for scintillation counting. The solution concentration decreased only about 1% upon filtration. Five milliliter

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

8) Light Stability of Clorsulon (Cont'd)

portions of this filtered solution were aseptically pipetted into sterile centrifuge tubes and stoppered. The experimental concentration of clorsulon was 331 ng/ml (ppb). As a control, duplicate samples were securely wrapped in foil to exclude light. Also one tube containing 5 ml of distilled water contained a thermometer to measure temperature at each time point. The amount of incident light was quantitated using a solar radiation integrator.

A preliminary experiment had shown that after 240 minutes, the clorsulon was completely degraded. Therefore, one sample was prepared for analysis at 5, 10, 20, 30, 45 and 60 minutes, and duplicate samples for zero time and for the dark control. The total number of samples was ten.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

8) Light Stability of Clorsulon (Cont'd)

The samples were placed outdoors at 3:33 p.m. on September 12, 1983, in Rahway, New Jersey.

At assay times, the temperature and amount of solar radiation were recorded. The tubes were opened and 100 microliters of cold-carrier clorsulon (2.70 mg/ml) was added and mixed. The samples were extracted with two portions of ethyl acetate. The volume of the extracts was recorded and 0.1-ml aliquots were removed for scintillation counting. The remainder of the extracts were evaporated and the residues dissolved in 100 microliters of methanol. The specific activity of clorsulon isolated by HPLC was determined to calculate the amount of the intact drug at assay time.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

8) Light Stability of Clorsulon (Cont'd)

Knowing the total radioactivity per tube and the amount of cold clorsulon added at assay time, the theoretical specific activity of the clorsulon was calculated to be 25522 DPM/mg. Dividing the observed specific activities by this number and multiplying by 100 gave the percent of intact drug in the samples.

During the one-hour experiment, the specific activity of the dark samples was unchanged. The specific activity of the drug in the samples exposed to light decreased with time. The logarithms of the RIDA% data versus time were fitted to the linear regression equation to obtain the best fit of the data to an exponential decay.

The half-life (t 1/2) for degradation is then,

$t\ 1/2 = \frac{\ln(2)}{k}$, where k is the rate constant for exponential decay.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

8) Light Stability of Clorsulon (Cont'd)

In the mid-afternoon with hazy sunshine with shadows, the cumulative solar radiation in 60 minutes was 37 mWh/cm^2. The rate constant (k) was 0.0363 min^{-1} and the half-life (t 1/2) was 19.08 min. The mean squared correlation coefficient (r^2) was 0.945 for the light samples.

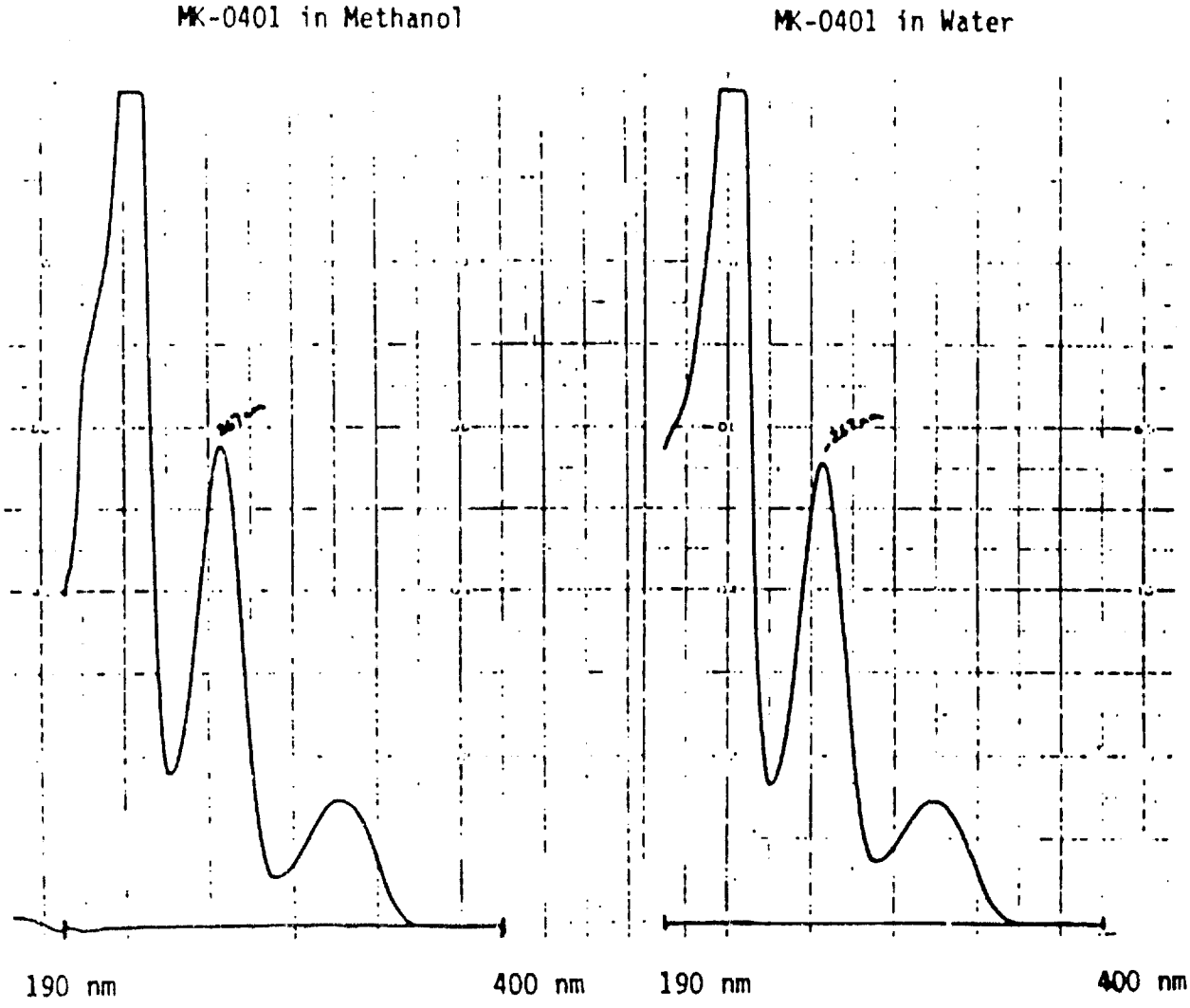
Thus, clorsulon is very unstable in solution when exposed to sunlight. However, stock solutions of clorsulon appeared to be stable for up to one month when exposed to laboratory flourescent lights.

There was little or no significant degradation of clorsulon in the dark under these experimental conditions.

Figure 2 includes the ultraviolet-visible spectra of clorsulon in methanol and in water.

FIGURE 2

ULTRAVIOLET SPECTRA OF MK-0401 IN METHANOL AND IN WATER



2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

9) Conclusions on the Biodegradation of Clorsulon

The biodegradation of clorsulon in soil was studied by trapping carbon-14 labeled carbon dioxide released from break-down of radiolabeled test compound in three soil types. One-half mg of clorsulon and 50 g of soil was added to 250 ml flasks (3 replicates) with sufficient water to remoisturize the soil to 50-70 percent of the field moisture capacity. The effluent air from each flask was passed through a series of scintillation vials which served as traps. The first vial served as a backflow trap. The second and third vials contained universal scintillation cocktail to trap any bolatiles. The fourth trap served as another backflow trap, while the fifth and sixth vials contained an amino based scintillation fluid to trap carbon dioxide. The radioactivity in each vial was determined, daily at first, then at weekly intervals. The system was flushed through the traps

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(b) Fate of Potentially Bioactive Chemicals in the Environment

9) Conclusions on the Biodegradation of Clorsulon (Cont'd)

daily. As a reference, carbon-14 labeled dextrose was similarly treated to check the viability of the soils. One series of flasks containing untreated soil provided background corrections for the scintillation data.

The biodegradation of clorsulon was studied in three soil types, sandy loam, silt loam and clay loam. At 22 days on test, the cumulative mean degradation as a percent of the dose on soil for clorsulon was less than 0.2% for all three soil types. In the same test system, glucose was degraded 24.92, 9.34 and 10.12 in sandy loam, silt loam and clay loam, respectively.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification

Communities of microbes in the soil normally convert ammonia to nitrite and nitrates, an essential part of the nitrogen cycle. Some levels of clorsulon appear to cause a significant reduction in initial conversion of ammonia to nitrites in loam soil and loamy sand soil (the only two soils tested). Triplicate samples of each soil type were treated with 0, 0.05, 0.2 2.0 or 20 mg of clorsulon/kg soil and kept under controlled standard laboratory conditions for up to five weeks. A known amount of ammonia was added to sets of these samples at either 0, 2 or 4 weeks and the respective soils were tested one week later (at either weeks 1, 3 or 5) for soil concentrations of ammonia, nitrite and nitrate. The relative amounts of ammonia, nitrite, and nitrate present one week after the ammonia addition are used to

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

estimate the potential for clorsulon to affect the sequential process of soil nitrification.

Two soil types were used in the study, a loam and a loamy sand. The soils were collected fresh from sites in Maryland and allowed to air dry for several days before sieving through a 2-mm wire mesh. The sieved soils were characterized by A & L Eastern Agricultural Laboratories, Inc. of Richmond, Virginia as follows:

Soil Analysis

Texture	Loamy Sand	Loam
Source	Burtonsville, MD	Laurel, MD
Percent Sand	85.0	29.0
Percent Silt	7.8	46.8
Percent Clay	7.2	24.2
Percent Organic Matter	1.2	3.0
pH	5.4	6.2
Field Capacity Percent	6.24	28.05

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

All calculations were performed on a dry-weight basis.

Twenty grams (dry weight) of soil were weighed into 250-ml amber screw-cap bottles. Triplicate samples were prepared for the four concentrations, untreated control, and sterile control samples of each soil type and sampling period. One representative sample was also prepared for each soil type for later microbial analysis. Next, the moisture content of the loam soil samples was adjusted by addition of 0.5 ml of deionized water to each sample. The loamy sand soil was left at its original moisture content.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

Soil samples were then incubated at 22 ± 2°C for a period of 3 days to stabilize the microbial population and to establish a uniform moisture content. After the 3-day pretreatment period, the sterile control samples were sterilized 3 times for 1 hour using 15 psig steam at 121°C.

After soil pretreatment a representative sample from each soil type was used to estimate the fungal and bacterial population by a modified plate procedure. Both soil types had between 10^6 and 10^7 colony-forming units per gram each of bacteria, fungi and actinomycetes.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

A stock solution of clorsulon was prepared in deionized water at a concentration of 0.40 mg/ml for subsequent dosing of soils. Each concentration (0.05, 0.2, 2.0 and 20.0 ppm), sterile controls and untreated controls were made from stock solution mixed with deionized water in each test vessel containing 20 g (dry weight) of preincubated soil.

The samples were capped immediately after the addition of each dose and shaken for 30 seconds by hand to distribute dose evenly before placing them back into the 22 ± 2°C incubator until analysis.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

Ammonia [100 ppm -N as $(NH_4)_2SO_4$] was applied to each set of soil samples concurrently after 0, 2 and 4 weeks of incubation with test material. Seven days after this ammonia application, 100 ml of 2 N aqueous potassium chloride solution was added to each sample followed by vigorous shaking for 30 minutes on a mechanical reciprocal shaker. After shaking, samples were allowed to settle for 15 minutes before a 10-ml sample was decanted and centrifuged at 1000 g for 20-30 minutes to remove particulate matter suspended in the aqueous solution.

The resulting particulate-free solution was then diluted by a predetermined factor so that it could be analyzed for each parameter using a Technicon

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

Auto-Analyzer II with a nitrate-nitrite manifold for NO_3^- and NO_2^- analysis and an ammonia manifold for NH_4^+ analysis.

Printouts for each sample were obtained from the Auto-Analyzer II in the form of peaks. The height of these peaks was converted to ion concentration by direct comparison to a standard curve derived from a set of standards of different known concentrations. These standards were run before and after each set of test samples to give a representative calibration curve for the Auto-Analyzer over the time of analysis.

The results showed that most, but not all, doses of clorsulon had no significant effect in either

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

1) Effect of Clorsulon on Microbial Nitrification (Cont'd)

soil upon the normal microbial conversion of ammonia to nitrite and nitrite to nitrate. The highest dose of clorsulon tested (20 ppm) caused a statistically significant reduction ($\alpha = 0.05$) in the conversion of ammonia to nitrite in both soil types tested. At 20 ppm clorsulon, the process converting ammonia to nitrite appears to have been inhibited at the beginning of the test, with less pronounced effects persisting over time.

2) Effect of Clorsulon on Microbial Respiration

Soil respiration indicates general soil microbial activity, measures decomposition rates and carbon cycling. Some levels of clorsulon appear to cause significant reduction in the respiration seen in loam soil and loamy sand soil. Triplicate samples

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

of each soil type were treated with 0.0, 0.05, 0.2, 2.0 or 20 mg of clorsulon/kg soil and kept under controlled standard laboratory conditions for 20 days. The percent of CO_2 given off by each soil sample measured soil respiration and CO_2 evolution was monitored regularly to measure clorsulon effects on soil respiration.

The same soil types were used as in the test determining the effects of clorsulon on microbial nitrification [Section 2(c)1)].

Twenty-five grams (dry weight) of soil were weighed into 250-ml amber screw-cap bottles with Teflon septum tops. Triplicate samples were prepared for the 4 concentrations, untreated control

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

and sterile control samples of each soil type. One representative sample was also prepared for each soil type for later microbial analysis. Next, the moisture content of the loamy sand samples was adjusted by addition of 0.5 ml of deionized water to each sample. The loam was left at its original moisture content.

Soil samples were then incubated at 22-25°C for a period of 3 days to stabilize the microbial population and to establish a uniform moisture content. Prior to the 3-day pretreatment period, the sterile control samples were sterilized 3 times for 1 hour each time using 15 psig steam at 121°C.

A stock solution of clorsulon was prepared in deionized water at a concentration of 0.50 mg/ml for subsequent dosing of soils. Each concentra-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

tion (0.05, 0.2, 2.0 and 20.0 ppm), sterile controls and untreated controls were made from stock solution mixed with deionized water in each test vessel containing 25 g (dry weight) or preincubated soil.

The samples were capped with Teflon-coated rubber septa and screw caps immediately after the addition of each dose and shaken for 30 seconds by hand to distribute dose evenly before flushing for 20 minutes with CO_2-free air and placing them into a 22-25°C incubator until analysis.

The percent carbon dioxide in the head gas of the respirometer vessels was determined at 24 and 48 hours after soil treatment and subsequently at 48-hour intervals thereafter with the final analysis conducted at 20 days. Carbon dioxide levels in each respirometer were determined by withdrawing 250 µl of gas from the vessel using a 500-µl

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

Pressure-Lok syringe and analyzing the sample by gas chromatography. The GC system consisted of a Hewlett-Packard 5880 GC with TCD coupled to a Hewlett-Packard 5880 GC Series GC terminal for peak recording and integration. The GC column, Porpak R (6' x 1/8" stainless steel, 100/120 mesh), utilized an oven temperature of 50°C, an injection port temperature of 250°C, and a detector temperature of 250°C with helium used as the carrier gas. This GC system supplied a good separation of CO_2 from other gases in a reasonable amount of time (approximately 2.8 minutes). Each sample was injected and allowed a run time of 3.5 minutes so the entire CO_2 peak was completely integrated.

At each sampling interval, external standards of 0.01, 0.1, 0.2 and 1.0 percent CO_2 were prepared to calibrate the gas chromatograph. These stan-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

dards were injected throughout the sampling sequence to assure that operating conditions in the GC did not change and to obtain a more representative calibration curve for the instrument on each day.

All respirometer vessels of a soil type were flushed with CO_2-free air when the controls for that soil type were determined to have a CO_2 content in the head gas of 1.0 percent or greater upon analysis. CO_2-free air was obtained by bubbling bottled breathing air through a 1 N solution of aqueous sodium hydroxide. The CO_2- free air was then dried by passing it through 2 feet of 8-mesh Drierite Anhydrous $CaSO_4$ (W.A. Hammond Drierite Co., Xenia, Ohio). Bottles were flushed

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

by inserting two syringe needles through the septum top of each bottle and hooking the CO_2-free air source up to one of the needles while the other acted as an exhaust. The respirometers were flushed for a period of 25-30 minutes in groups of 6-10 at a time.

For each day's analysis a calibration curve of average peak area versus percent CO_2 of external standards was prepared. These plots were found to be very linear with correlation coefficients of 0.976 and greater.

The percent CO_2 in each sample was determined by linear regression using the calibration curve for

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

the day the sample was analyzed and correlating the peak area with percent CO_2 in the sample. Then the mean and standard deviation for the triplicate samples in each concentration were computed to give the average percent CO_2 in each group of samples. Finally, the following formula was used to calculate the daily increase in total percent CO_2.

Total percent CO_2 evolved = (A - S) + F

where

A = Average percent CO_2 in sample concentration.

S = Average percent CO_2 in sterile control samples.

F = Sum of previous samples and flushes.

The results of the respiration of the 2 soil types were computed as the mean of replicates versus time for all treatment levels and controls.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

2) Effect of Clorsulon on Microbial Respiration (Cont'd)

From the results, the following was concluded about the effects of the test material clorsulon on the microbial respiration of the 2 soils used in this study:

Most, but not all, doses of clorsulon significantly reduced loam soil respiration as compared to untreated loam soil. Clorsulon levels as low as 0.05 mg/kg appeared to (within two weeks) significantly reduce loam soil respiration. The lowest clorsulon dose that significantly reduced loamy sand soil respiration was 0.2 mg/kg. The 2.0 mg/kg dose level also had this effect, however, the 20 mg/kg dose level did not significantly reduce loamy sand soil respiration.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon

A study was performed to determine the toxicity of clorsulon to eight higher-plant species over a 14-day exposure period as measured by shoot weight following application of clorsulon to soil. The 8 plant species used in the test were:

Oats	(Avena sativa),
Corn	(Zea mays),
Tomato	(Lycopersicon esculentum),
Lettuce	(Lactuca sativa),
Bean	(Phaseolus vulgaris),
Turnip	(Brassica rapa),
Pea	(Pisum sativum) and
Sunflower	(Helianthus annus).

The study was conducted at the Elphinstone Research Centre field station of Inveresk Research International Limited, Musselburgh, Scotland.

Seed of each plant species was purchased from Dodd Seed Merchants, Haddington, Scotland and W.K. McNair, Seed Merchants, Edinburgh, Scotland.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon (Cont'd)

Batches of seeds were placed in Petri dishes containing moistened filter paper and left to germinate in darkness at ca 20°C.

Clorsulon was initially mixed with quartz sand to provide a dilution series at the following concentrations:

10 g of test substance + 90 g quartz sand (A)

10 g of A + 90 g quartz sand (B)

10 g of B + 90 g quartz sand (C)

10 g of C + 90 g quartz sand (D)

The actual mixing was carried out by hand.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon (Cont'd)

Following preparation, each dilution mixture was thoroughly mixed with aliquots of John Innes No. 1 Compost in the proportion of 10 g clorsulon/sand mixture to 990 g of soil to provide target concentrations of 1, 10, 100 and 1000 mg

$$\frac{\text{clorsulon}}{\text{kg test soil}}.$$

The John Innes No. 1 Compost is a mixture of two parts loam, one part peat and one part sand.

Six pre-germinated seeds of each species were placed at a depth of ca 1.5 cm in each 7-cm plant pot containing the relevant soil mixture. Four replicates of each test concentration were used. After planting, each block of samples was randomized and maintained under a 16-hour photoperiod in a growth room at a temperature of 20°C for 14

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon (Cont'd)

days. The soil moisture content was adjusted daily to ca 80% of the moisture-holding capacity.

A daily record of any observable phytotoxic events was made for each group of plants. Where phytotoxic damage was recorded, the degree of damage was based on individual assessment of the plants in comparison to control plants on a scale of 0-3 as follows:

0 - no damage

1 - up to 20% damage

2 - 20-50% damage

3 - greater than 50% damage

In addition, the fresh weight of the shoots for each pot was determined after 14 days of incubation. The EC_{50} (median effective concentration)

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon (Cont'd)

was calculated by linear regression on the shoot weight vs. Arithmetic-Logarithmic concentration scaling. The EC_{50} values represent the estimated concentration of the clorsulon in the soil required to reduce shoot weight to half the observed mean control value. The results are listed below:

Plant Species	EC_{50} mg Clorsulon kg soil	95% Confidence Limits	
Oats	1031	290	6464
Corn	212	36	4095
Tomato	28	9.4	93
Lettuce	489	193	1621
Bean	898	72	626758
Turnip	11	3.0	38.3
Pea	>1000	--	--
Sunflower	117	46	356

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

3) Phytotoxicity of Clorsulon (Cont'd)

For each species, there was a progressive decrease in shoot weight with increasing concentration of clorsulon.

The confidence limits were quite wide which is to be expected in a study where logarithmic spacing of test doses is employed.

Because of the wide confidence limits, safe concentration estimation (1 percent of the lower confidence limit) affords levels for some of the plant species which are below the "worst-case" estimates for clorsulon in feces and in fields fertilized with manure from dosed animals, but above the levels estimated in plowed fields under typical conditions. It also provides an estimate above on observable effect concentration.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum

The toxicity of clorsulon to the freshwater green alga Selenastrum capricornutum was determined in a growth inhibition test.

A stock solution of the test substance was prepared by dissolving 0.800 g of clorsulon in 1 liter of micropore-filtered test medium. The pH of the stock solution was adjusted to that of the test medium (7.7). The test solutions for the growth inhibition test were prepared by diluting the stock solution with micropore-filtered medium. In order to check the added concentration of the test substance as well as the stability of the test compound, during the test, analyses were performed.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum (Cont'd)

At the start as well as the end of the growth inhibition test, samples were taken from the background-control flasks for determination of total organic carbon.

The freshwater green alga Selenastrum capricornutum, which belongs to the order of Chlorococcales (class Chlorophyceae), was used as the test organism. A preculture of algae in the exponential growth phase was prepared. The tests were carried out with suspensions of algae initially containing about 1×10^4 cells/ml. At the start and end of the growth inhibition test, the morphology of the algae was examined microscopically.

During the growth inhibition test, the pH of the medium in the highest-concentration flask fell

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum (Cont'd)

sharply (to about pH 3.7) during the incubation period. The change in pH was accompanied by development of a deep yellow color. Therefore, phosphate buffer was added to the medium in the next series. To prevent precipitation of calcium phosphate, the initial calcium chloride concentration of 35 mg/l in the algal medium was reduced to 10 mg/l. Finally, for the purpose of minimizing growth of algae on the walls of the (glass) vessels, the test was conducted in plastic 250 ml Costar Tissue Culture Flasks with screw caps.

A preculture of algae in the exponential growth phase was prepared and from this culture a suspension of algae containing about 1 x 10^6 cells/

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum (Cont'd)

ml was made. The required concentrations of test substance (0, 10, 32, 100, 320, 560 and 800 mg/l) were prepared by dilution of the stock solution of the test substance with micropore-filtered medium. The test was carried out in duplicate with algae, and complemented with a single background-control series of test substance without algae. One millilite~ of the algal suspension was transferred to each flask containing 100 ml of test substance solution. All the flasks were incubated in a thermostated room at 20 ± 2°C, on an "algal mill".

The mill rotated the culture flasks continuously past a set of fluorescent lamps, ensuring that all

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum (Cont'd)

culture flasks placed at one and the same level received the same amount of light (7-10 K lux). One sample was taken from each flask once a day on four consecutive days and the number of alga cells per ml of each sample was determined with a Coulter counter model F using an aperture of 70 µm.

In this study the EC_{50} was the concentration of test substance that reduces the growth rate of the algae by 50%, as calculated from a parametric model.

In the growth inhibition test, the "no observed effect concentration" (NOEC) was estimated from the comparison of the growth curves of the treated algal suspensions with those of the algal blanks.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Alga Selenastrum capricornutum (Cont'd)

By means of the parametric model, the EC_{50} was calculated to be 520 mg/l, with a 95% confidence interval of 468-578 mg/l. The variance of the test was 1.2. In unbuffered medium, the EC_{50} was calculated to be 570 mg/l, with a 95% confidence interval of 390-820 mg/l.

By comparison of the growth curves of algal suspensions exposed to the test substance with those of algal blanks, the NOEC of clorsulon was estimated to be 100 mg/l.

Other observations were that the added phosphate buffer prevented the pH of the algal suspensions from falling appreciably during the incubation

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

4) Effect of Clorsulon on the Al[illegible] Selenastrum capricornutum (Cont'd)

period, that microscopical examination of the cells at the end of the incubation period revealed no morphological abnormalities, that the medium with test compound quickly turned yellow, suggesting that the compound is unstable to light, and that a determination of TOC in 100 mg of clorsulon/l at the start and end of the test period revealed no changes of concentration during the test.

5) Effects of Clorsulon on Daphnia magna

Clorsulon, in various concentrations in DSW water, was tested for toxicity against Daphnia magna in a 48-hour static test. DSW water is a medium prepared by addition of several salts to a groundwater from a locality near Linschoten, The Netherlands. It is suitable for growing several species of water organisms, including Daphnia, has a pH about 8 and a hardness, expressed as $CaCO_3$, of about 210 mg/l.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

5) Effects of Clorsulon on Daphnia magna (Cont'd)

After a range-finding test, 14 concentrations of clorsulon were tested, 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56, 100, 180, 320, 560, 855 and 1000 mg/l. These concentrations were divided into two test series: 1.0-100 mg/l and 100-1000 mg/l, the 100 mg/l being tested twice. The test solutions (DSW water containing the above-mentioned concentrations of clorsulon) containing less than 855 mg/l were prepared from a stock solution containing 560 mg of clorsulon per liter of DSW water, by adding the appropriate volumes of stock solution to DSW water.

DSW water was taken as a control (blank). The stock solution and test solutions containing 560, 855 and 1000 mg/l were prepared by weighing the appropriate amounts of clorsulon, adding these to DSW water and stirring for about 20 hours.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

5) Effects of Clorsulon on Daphnia magna (Cont'd)

The tests were performed in 150-ml all-glass beakers containing 100 ml of the test solutions and covered with a watch glass. Four beakers containing five Daphnia, less than 24 hours old, were taken for each concentration. (n = 20 except for the 100 mg/l concentration where n = 40.) The test lasted 48 hours.

Only one series of untreated controls was used and the results of the two test series were pooled.

As t = 0 hours and 48 hours the pH and the oxygen concentration of the test solutions were measured. The test solutions were not renewed. The test solutions were kept in the dark at 20 ± 1°C and not aerated; the Daphnia were not fed.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

5) Effects of Clorsulon on Daphnia magna (Cont'd)

The pH of the test solutions varied between 7.8 and 8.2, while the oxygen concentrations of the test solutions were greater than 7 mg/l.

After 24 hours and 48 hours, the immobile and dead animals were counted, the dead ones were removed, and the condition of the survivors was compared with that of the control animals in the blanks.

The LC_{50} value and its confidence interval was calculated by a parametric model.

No difference was made between "living" and "mobile" animals. Thus, the EC_{50} (E = immobilization) values were equal to the LC_{50} values. The

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

5) Effects of Clorsulon on Daphnia magna (Cont'd)

results of the test, expressed as LC_{50} values, are given in Table 5.

Table 5. Results of the Test of Clorsulon with Daphnia magna

Time (hr)	LC_{50} (mg/l)	95% Confidence interval (mg/l)
24	1144	708-1848
48	356	288- 440

The condition of the Daphnia exposed for 48 hours to 56 mg and less clorsulon per liter of DSW water was the same as that of the control animals in the blanks. In other words, the NOEC in this acute test was 56 mg of clorsulon per liter of DSW water.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida

Clorsulon was tested for toxicity against the earthworm, Eisenia foetida, in artificial soil. Both range-finding and definitive tests were run.

For the range-finding test, 10.0 g of clorsulon was dispersed in 200 ml of water to form a suspension. The suspension was kept from settling by constant stirring with a magnetic stir bar and stir plate. Twenty milliliter of this 50 mg/ml suspension solution was dosed to 1 kg of dry soil for the 1000 mg/kg concentration. For the 100 mg/kg through 0.1 mg/kg concentrations, five 1 to 10 dilutions of the original stock were made in series. To obtain the proper doses, 200 ml of the second, third, fourth and fifth dilutions in this series were dosed directly to 1 kg of artificial soil.

The definitive test was conducted at concentra-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

tions of 130, 216, 360, 600 and 1000 mg/kg with 4 replicates per concentration and 4 replicate controls. Because of the insolubility of clorsulon in 200 ml of water at the 3 highest dose levels, all doses were mixed with quartz sand. For each concentration the proper amount of test material per kilogram of soil was weighed into a vial on a Mettler AE-163 balance and mixed with 10 g of finely ground quartz sand. These 10 g of dosed sand were then mixed with 1 kg of prepared artificial soil and roller mixed using a Norton roller mill. The even distribution of test material by dry mixing with sand was checked in a separate experiment with ^{14}C-labeled benzoic acid.

The test media consisted of 835 g quartz sand, 100 g peat, 50 g bentonite clay and 5 g of dried cow manure for each container. About 10 g $CaCO_3$ was

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

then added to maintain a pH of 7.0 ± 0.5. All components were weighed into a 1-gallon glass jar followed by 10 minutes of mixing using a Norton roller mill.

The earthworm species Eisenia foetida was used as the test organism. The worms were supplied by Super Stuff Incorporated, Houston, Texas. Worms were maintained in a 50:50 mixture of horse manure and peat at pH 7.0 with ionic conductivity of less than 6.0 mmho. All worms used in the test were mature with clitellum and were selected from both breeding boxes at the laboratory. Average and range of live-worm weight were taken prior to their addition to test containers and at test termination for the definitive test.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

For each dose level in the definitive test, 1 kg of the test substrate was added to a 1-gallon flint glass jar. Appropriate concentration of previously dosed 10 g quartz sand was added into the jar and the contents mixed mechanically with the Norton roller mill for 10-15 minutes. Four replicates were set up for each concentration. After mixing, the entire contents of the jar were removed and placed into the 1.5-liter glass test container. Moisture content was adjusted by the addition of about 350 ml delonized water. Final concentrations of the test chemical were based on a total of 1 kg artificial soil per test container.

Each worm was weighed individually before addition to a test container so an average and range of weight of worms in each test container could be determined. Ten worms were added to the soil

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

surface of each test container. Moisture content of 3 replicates in each concentration was determined and the test containers were covered with watch glasses to reduce soil substrate drying.

The test system was each container of soil containing 10 worms. All test systems were identified with project number, test compound concentration, and replicate number.

The test containers were maintained in a Forma Scientific environmental chamber at 20 ± 2C in continuous light. Temperatures were monitored daily and continuously by using a 7-day telethermometer and by direct reading from an NBS traceable calibrated thermometer inside the chamber.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

Mortality was assessed on Days 7, 14 and 28 by emptying the soil into a tray and sorting out the worms. Worms were classed as dead, moribund, soft and flaccid, flaccid, or normal on each mortality assessment day. When the soil substrate was returned to the test container at the 14-day mortality assessment, 5 g of cow manure was added and mixed into each to supply food to the worms.

The test was terminated at 28 days. At that time, individual live weights of remaining worms were determined along with the moisture content of the test substrate. The moisture content was determined in all 4 replicates of each concentration and reported as an average of the 4.

There was no worm mortality at any of the doses tested, however, elongated worms were seen at all doses $\geq$ 10 mg/kg.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

Since there were no mortalities in any treated sample in the range-finding test, doses for the definitive test were made between the two highest concentrations of 100 mg/kg and 1000 mg/kg. These doses were 130, 216, 360, 600 and 1000 mg/kg. All worms were fed during testing. The mean weight of the control worms decreased during the definitive test, while the mean weight of the worms in each treated soil increased.

Since mortalities were 12.5 percent or less in all concentrations an LC_{50} value for the test material could not be determined for the range of concentrations used in the study. Because of the low mortality rate in all concentrations it is concluded

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

6) Effect of Clorsulon on the Earthworm Eisenia foetida (Cont'd)

that the LC_{50} of clorsulon to Eisenia foetida is greater than 1000 mg of test chemical per kilogram of dry soil.

Sublethal effects (flaccid, soft and flaccid and moribund) were also monitored. Significant sublethal effects were evident in all treatment groups by either 7 or 14 days and became progressively more severe throughout the 28-day experiment.

A reference toxicant test using copper sulfate was conducted on Eisenia foetida. Since water was the solvent for the reference toxicant, it was added to the artificial soil with the water used to adjust the moisture content to 25 percent and worms added thereafter. The 28-day LC_{50} for Eisenia foetida exposed to the reference toxicant, copper sulfate, was calculated using Probit method to be 320 mg of $CuSO_4$ per kilogram of dry artificial soil.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

7) Effect of Clorsulon on Poecilia reticulata (Guppy) and Lepomis macrochirus (Bluegill Sunfish)

Clorsulon was tested for aquatic toxicity in short-term tests with two freshwater fish species, Poecilia reticulata (guppy) and Lepomis macrochirus (bluegill sunfish).

After a range-finding test, five concentrations of clorsulon were tested, 180, 320, 560, 855 and 1000 mg/l. The test solutions were prepared by weighing the appropriate amounts of clorsulon, adding these to DSW water and stirring for about 20 hours. DSW water is a water suitable for growing several species of water organisms and is prepared by addition of several salts to a ground-water from a locality near Linschoten, The Netherlands.

DSW water was taken as a control. The tests were performed in 1-liter all-glass beakers each containing 1 liter of test solution and covered with a watch glass for the guppy, and in 2 liters of test

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

7) Effect of Clorsulon on Poecilia reticulata (Guppy) and Lepomis macrochirus (Bluegill Sunfish) (Cont'd)

solution in all-glass tanks covered with a glass plate for the bluegill sunfish.

The guppies used in this test were about one month of age and averaged about 1.5 cm in length and 0.035 g in weight, and the bluegill sunfish averaged 2.7 cm in length and 0.2 g in weight.

The fish were observed daily for mortality and abnormal behavior for the entire 96-hour test period. The sublethal behavior checks included observations for color changes and impairment in swimming ability.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

7) Effect of Clorsulon on Poecilia reticulata (Guppy) and Lepomis macrochirus (Bluegill Sunfish) (Cont'd)

Two beakers containing 10 fish were taken for each concentration. The test lasted 96 hours; the solutions were renewed every day, and the pH and oxygen concentrations of the fresh and spent solutions measured. The test solutions were kept at 24 ± 1°C and aerated; the fish were not fed. After 3, 6, 24, 48, 72 and 96 hours, the dead fish were counted and removed, and the condition of the survivors was compared with that of the control fish in the blanks.

For Poecilia reticulata, the pH of the test solutions varied between 7.8 and 8.4, while the oxygen concentrations of the test solutions were greater than 7.6 mg/l. For Lepomis macrochirus,

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

7) Effect of Clorsulon on Poecilia reticulata (Guppy) and Lepomis macrochirus (Bluegill Sunfish) (Cont'd)

the pH of the test solutions varied between 7.9 and 8.4, while the oxygen concentrations of the test solutions were greater than 6.6 mg/l.

In agreement with the solubility data, there was some undissolved material left at 1000 mg/l. The results of this concentration were not taken into account in estimating the LC_{50}.

The 96-hour LC_{50} (and 95% confidence interval of clorsulon for Poecilia reticulata could not be estimated because insufficient mortality occurred in the test animals. The lowest concentration which caused mortality within the 96-hour test period was the 560 mg/l dose. At concentrations $\geq$ 560 mg/l, the guppies grew progressively impaired. The number of animals exhibiting these effects and when these sublethal effects were first seen were not noted.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

7) Effect of Clorsulon on Poecilia reticulata (Guppy) and Lepomis macrochirus (Bluegill Sunfish) (Cont'd)

The 96-hour LC_{50} (and 95% confidence interval) of clorsulon for Lepomis macrochirus could not be estimated because of insufficient mortality in the test animals. The lowest clorsulon concentration which caused bluegill mortality within 96 hours was the 855 mg/l dose. At concentrations $\geq$ 320 mg/l, the fish grew progressively darker in color and their swimming ability was progressively impaired. At doses $\geq$ 560 mg/l the fish remained on the bottom of the test tanks in extremely poor condition. The number of animals exhibiting these effects and when these sublethal were first seen were not noted.

8) Conclusions on the Effects of Clorsulon on the Environment

Clorsulon's octanol/water partition coefficient (Kow = 15), its biodegradation half-life (t 1/2 estimated to be $\geq$ 1000 days), its lack of volatil-

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

8) Conclusions on the Effects of Clorsulon on the Environment

ity (Vp = 5 x 10^{-13} mm Hg at 25°C), its solubility (s = 0.8 g/l) and its soil/water distribution coefficient (Kd = 1.4) can be used to estimate its ecotoxicologic effects and exposure potential (16). From these data, and other stability data presented in Sections D(2)(b), it can be determinated that clorsulon would persist in the environment, being resistant to biodegradation and to hydrolysis. Clorsulon would leach into water due to its low soil/water distribution coefficient though limited by its low solubility. Clorsulon would not bioaccumulate due to its low octanol/water partition coefficient. Photodegradation would probably be the major breakdown route for clorsulon in the environment.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(c) Effects of Potentially Bioactive Chemicals in the Environment

8) Conclusions on the Effects of Clorsulon on the Environment

The low solubility of clorsulon will limit its effects on aquatic organisms, as its solubility is below the LC_{50} values determined for Daphnia and fish. Its LC_{50} value for earthworms is higher than the "worst-case" concentration in soil or feces. Clorsulon could be phytotoxic to some plant species at its "worst-case" levels, but not at levels expected in fertilized fields under typical use conditions.

As can be discerned from the data presented in Section 1(c) and (d) clorsulon has very low toxicity for mammalian species tested.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing

A secondary environmental effect would result from the discharge of by-products from the chemical manufacturing process for clorsulon.

The following summarizes the environmental effects from the bulk manufacture of clorsulon at the Stonewall, Virginia plant:

The manufacturing process generates an aqueous waste stream which results from quenching and extraction operations. The aqueous stream will contain water, mixed salts, and small fractions of organic solvents such as chloroform and methylene chloride. The aqueous stream will be treated in the plant's wastewater treatment facility. Treatment consists of equalization, neutralization, one-stage nitrification, secondary settling, polishing bio-oxidation on two trickling filters, sludge dewatering and incineration. Effluent from the plant's treatment facility will be discharged into the south fork of the Shenandoah River. Discharge

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

to the river will be in compliance with NPDES Permit Number VA0002178 which is administered by the Virginia State Water Control Board.

A solvent-based liquid waste stream results from vacuum concentration and centrifuge operations. The solvent-based stream will contain organics such as hexane, chloroform, methylene chloride and toluene. The solvent-based stream will be shipped offsite to a registered hazardous waste management facility. The plant's disposition of the solvent waste stream will be subject to and in compliance with the Virginia State Hazardous Waste Management Regulations, Section 6.0.

In addition to the solvent waste stream, hazardous solid waste will be generated during certain filtering operations. This waste stream will contain filter aid, wet with organic solvents, and residue for disposal. The hazardous solid waste will be shipped offsite to

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

a registered hazardous waste management facility. The plant's disposition of this stream will be subject to and in compliance with the Virginia State Hazardous Waste Management Regulations, Section 6.0.

Non-hazardous solid waste (trash, paper, plastic, etc.) generated during the manufacturing process will be burned in an onsite incinerator. The trash incinerator will be subject to and in compliance with the Commonwealth of Virginia Regulations for the Control and Abatement of Air Pollution. The trash incinerator will operate under Permit Number 20524 which is administered by the Virginia Air Pollution Control Board.

Air emissions of volatile organic compounds (voc) are produced during centrifuge and vacuum drying operations. Where appropriate, voc emissions will be controlled by condensers, scrubbers, or a combination of

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

the two devices. Air emissions will be subject to and in compliance with the Virginia Regulations for the Control and Abatement of Air Pollution, Section III and Appendix M. Air emissions will be discharged subject to Permit Number 20524 which is administered by the Virginia Air Pollution Control Board.

The control and disposition of air emissions, aqueous waste, solvent-based waste, hazardous solid waste, and non-hazardous solid waste will comply with the above-mentioned environmental regulations.

The following summarizes the environmental effects resulting from bulk manufacture of clorsulon at the Flint River, Georgia plant:

The manufacturing process generates an aqueous waste stream which results from quenching and extraction operations. The aqueous stream will contain water, mixed salts, and small fractions of organic solvents

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

such as chloroform and methylene chloride. The aqueous stream will be discharged to the plant's wastewater treatment facility. Treatment consists of equalization, neutralization, primary clarification, activated sludge, secondary settling, thickening, sludge dewatering and offsite sludge disposal. Sludge disposal will be subject to and in compliance with Georgia Hazardous Waste Management Rules, Chapter 391-3-11-.08. -- Standards Applicable to Generators of Hazardous Waste. Effluent from the Plant's treatment facility will be discharged into the Flint River. Discharge to the river will be in compliance with NPDES Permit Number GA 000 1619 which is administered by the Georgia Department of Natural Resources.

A solvent-based liquid waste stream results from vacuum concentration and centrifuge operations. The solvent-based stream will contain organics such as hexane, chloroform, methylene chloride, and toluene. The solvent-based stream will be shipped offsite to a

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

registered hazardous waste management facility. The plant's disposition of this stream will be subject to and in compliance with the Georgia Hazardous Waste Management Rules, Chapter 391-3-11-.08.

In addition to the solvent waste stream, hazardous solid waste will be generated during certain filtering operations. This waste stream will contain filter aid, wet with organic solvents, and residue for disposal. The hazardous solid waste stream will be shipped off-site to a registered hazardous waste management facility. The plant's disposition of this stream will be subject to and in compliance with the Georgia Hazardous Waste Management Rules, Chapter 391-3-11-.08.

Non-hazardous solid waste (trash and paper) generated during the manufacturing process will be shipped off-site to a local landfill.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

Air emissions of voc are produced during centrifuge and vacuum drying operations. Where appropriate, voc emissions will be controlled by condensers, scrubbers, or a combination of the two devices. Air emissions will be in compliance with the Rules of the Georgia Department of Natural Resources, Environmental Protection Division, Chapter 391-3-1 -- Air Quality Control. Air emissions will be discharged subject to Air Quality Permit Number 28330478468.

The control and disposition of air emissions, aqueous waste, hazardous solid waste, and non-hazardous solid waste will comply with the above mentioned environmental regulations.

The following summarizes the environmental effects from the formulation of clorsulon at the Barceloneta, Puerto Rico plant:

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

A product-washing step generates the aqueous waste stream. The aqueous stream will contain water and some organic solvent such as ethanol. The aqueous-based stream will be pretreated before discharge to the Barceloneta Regional Wastewater Treatment Plant. Pretreatment will consist of mixing, neutralization, clarification, equalization, solvent and oil removal, and final neutralization. Discharges from the treatment plant will be subject to NPDES Permit Number PR 0021237 which is administered by the U.S. Environmental Protection Agency.

Air emissions of voc are produced during centrifuge and vacuum drying operations. Where appropriate, voc emissions will be controlled by condensers, scrubbers, or a combination of the two devices. Air permits will be subject to and in compliance with the Regulations for the Control of Atmospheric Pollution; the air regulations are administered by the Environmental Quality Board (EQB) of Puerto Rico.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

Non-hazardous solid waste (general trash, cardboard, paper and plastics) generated during the formulation process will be destroyed by onsite incineration. The incinerator will be subject to and in compliance with the Regulations for the Control of Solid Waste administered by the EQB. The incinerator will operate under permits PFE-09-0981-0780-III-0 issued by the EQB Air Program and I-82-0032 issued by the EQB Solid Waste Program.

The control and disposition of air emissions, aqueous waste, and non-hazardous solid waste will comply with the above mentioned regulations.

The following summarizes the environmental effects from the formulation of clorsulon at the Rahway, New Jersey plant:

A product-washing step generates the aqueous waste stream. The aqueous waste stream will contain water

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

and some organic solvent such as ethanol. The aqueous waste stream will be equalized and pH adjusted before being discharged to the Linden-Roselle Sewerage Authority Treatment Plant. Discharge of the aqueous stream to the treatment plant will be subject to local ordinances ("Regulations, Conditions and Restrictions with the use of Public Sewers in Linden, New Jersey"). Discharges to the treatment plant will also be in compliance with a NJPDES application submitted to the New Jersey Department of Environmental Protection.

Air emissions of voc are produced during centrifuge and vacuum drying operations. Where appropriate, voc emissions will be controlled by condensers, scrubbers, or a combination of the two devices. Air permits will be subject to and in compliance with the New Jersey Administrative Code (N.J.A.C.) 7:27 - Bureau of Air Pollution Control Regulations.

2. Discuss the Probable Impact of the Action on the Environment (including primary and secondary consequences)

(d) Impact of Manufacturing (Cont'd)

Trash and paper (non-hazardous solid waste) generated during the formulation process will be burned in an onsite incinerator. The trash incinerator will be subject to and in compliance with N.J.A.C., 7:27. The incinerator will operate under Permit Number 01451 which is administered by the New Jersey Department of Environmental Protection.

The control and disposition of air emissions, aqueous waste, and non-hazardous solid waste will comply with the above mentioned environmental regulations.

3. Describe the Probable Adverse Environmental Effects that Cannot Be Avoided

Based on the discussion in Section D(2), it is not anticipated that any substantial adverse effect on the environment will occur when the new animal drug application for clorsulon is approved. Of course, any manufacturing process must make some contribution of products to the environment. However, as indicated in Section D(2), the liquid, solid and air disposal of by-products from the manufacturing process is done under the applicable environmental requirements of various laws. Furthermore, such wastes from the clorsulon process would make a negligible contribution to the waste problem of modern industrial society.

4. Give Alternatives to the Proposed Action

Since hexachlorethane was removed from the market in 1979, losses from fascioliasis were considered important enough in 19 states to apply for emergency authorization to use the experimental drug albendazole in cattle. Albendazole currently is the only material approved for use against liver flukes in cattle. Albendazole, however, cannot be used frequently or within 180 days prior to time of slaughter.

5. Describe the Relationship Between Local Short-term Uses of the Environment with Respect to the Proposed Action and the Maintenance and Enhancement of Long-term Productivity

Short-term effects upon the environment, as discussed in Section D(2), are not expected because of the low levels of noxious compounds which will be present in the environment and because of the relatively low toxicity of the drug (e.g., LD_{50} towards fish tested was higher than the maximum solubility of the drug). Also, as discussed, there would be minimal short-term effect of the disposal of by-products from the manufacturing process upon the productivity of the environment.

These same factors also would mitigate against any long-term detrimental effects on the environment.

Short- and long-term beneficial effects from the use of clorsulon could be substantial in terms of producing healthier cattle, allowing cattle to realize their full genetic potential to utilize feed more efficiently, and eliminating losses from morbidity and liver condemnation

5. Describe the Relationship Between Local Short-term Uses of the Environment with Respect to the Proposed Action and the Maintenance and Enhancement of Long-term Productivity (Cont'd)

from fascioliasis. Taken together, this means that more food for man (beef protein) can be produced per pound of feed without increasing the need for such feed and the resulting expenditure of energy.

6. Describe any Irreversible and Irretrievable Commitment of Resources

The raw materials used to manufacture clorsulon are common organic compounds, inorganic acids and ammonia -- all of which are in ample supply. Energy commitment would be nominal.

Also, some of the raw materials used in the process are recycled or recovered for use. Though some of the raw materials are irretrievable, the proportion used in the clorsulon process compared to the total annual production of them would be minimal.

7. Discuss the Objections Raised by Other Agencies, Organizations or Individuals

We know of no agencies, organizations or individuals who have questioned the effect on the environment from the use of clorsulon for the treatment of immature and adult Fasciola hepatica in cattle.

8. If the Proposed Action Should Be Taken Prior to 90 Days from the Circulation of a Draft Environmental Impact Statement or 30 Days from the Filing of a Final Environmental Impact Statement, Explain Why

The information presented in this environmental impact analysis report amply documents the position that the approval of the new animal drug application for clorsulon by the Food and Drug Administration does not constitute a major agency action which would significantly affect the quality of the human environment. Thus, there is no reason for the Agency to prepare and circulate for comments a Draft Environmental Impact Statement.

9. Analyze Whether the Benefit to the Public of the Proposed Action Will Outweigh the Action's Potential Risk to the Environment

The benefits to be obtained from the use of clorsulon as discussed in Sections (2) and (5) outweigh any potential risk to the environment.

9. Analyze Whether the Benefit to the Public of the Proposed Action will Outweigh the Action's Potential Risk to the Environment (Cont'd)

The risk to the environment can scarcely be identified whereas the benefit in terms of savings from economic loss to the cattle producer and the consumer are substantial.

In view of the severe worldwide shortage of protein food and animal feed, the benefits from the use of an agent such as clorsulon are critically needed. Any conceivable risk to the environment would be negligible in comparison.

E. Certification

The undersigned applicant/petitioner certifies the information furnished in this Environmental Impact Analysis Report is true, accurate and complete to the best of his knowledge.

Date:

Kenneth D. Davis
(Signature)

Director, Regulatory Affairs
(Title)

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