FINDING OF NO SIGNIFICANT IMPACT

for

Clorsulon (Curatrem^{TM}) Oral Suspension for Cattle

NADA 136-742
Merck Sharp & Dohme Research Laboratories

The Center for Veterinary Medicine has carefully considered the potential environmental impact of this action and has concluded that this action is not expected to have a significant impact on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

Merck Sharp & Dohme Research Laboratories of Rahway, New Jersey, has filed a new animal drug application (NADA 136-742) for the over-the-counter use of clorsulon (Curatrem 8.5% oral suspension) for the treatment of mature and immature liver flukes (Fasciola hepatica) in cattle. Cattle are to be dosed at a level of 7 milligrams (mg) of drug per kilogram (kg) of body weight (b.w.). This is equal to 7.5 milliters (mL) of the 8.5% oral suspension of clorsulon/91 kg cattle b.w. (or 1/4 fluid ounce/200 lb. b.w.). Retreatment of cattle with clorsulon may occur 2-3 times/year. The timing of retreatment for cattle is based upon geographic, climatic, and husbandry considerations. Cattle cannot be slaughtered within 8 days of dosing with clorsulon. This medication cannot be used in female dairy cattle of breeding age because a withdrawal time in milk has not been established.

Clorsulon 8.5% liver fluke drench for cattle contains 85 mg of drug active ingredient/mL of suspension. The drug active ingredient is 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide (CAS No. 60200-06-8). Clorsulon has also been known as MK-0401 or MK-401.

Merck Sharp & Dohme Research Laboratories (Division of Merck and Company) has filed the attached Environmental Impact Analysis Report (EIAR) dated January 10, 1985, in support of the proposed use of clorsulon in cattle. The EIAR asserts that the proposed use of Curatrem in cattle should result in levels of introductions of clorsulon into the environment that are not expected to result in effects upon organisms living in the environment. These assertions are based upon the firm's data and calculations. This FONSI provides the Center's interpretation of the environmental information submitted by Merck, describes mitigations of possible adverse environmental effects and defines the need for additional environmental data.

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A. Introductions of Clorsulon into the Environment

The active drug ingredient may be introduced into the environment, both through the manufacture and the use of the product. The drug manufacturing process results in chemical waste products which also will be introduced into the environment. The first step in an assessment of environmental impacts is to estimate the types and quantities of materials that could potentially enter the environment.

1. Environmental introductions of chemicals due to the manufacture of clorsulon:

The attached EIAR (pp. 118-128) identifies four locations where clorsulon will be manufactured. The attached EIAR, however, does not include quantitative estimates of the environmental introductions of chemicals that would result from the manufacture of clorsulon. The manufacture of clorsulon in bulk will take place at facilities in Stonewall, Virginia and Flint River, Georgia. The formulation of bulk clorsulon into the finished oral suspension will take place at facilities in Rahway, New Jersey and Barceloneta, Puerto Rico.

a. Bulk manufacture of clorsulon

The bulk manufacturing process will generate an aqueous waste stream, a solvent-based liquid waste stream, hazardous and non-hazardous solid wastes, and air emissions.

The aqueous waste stream from bulk manufacture will contain water, salts and small fractions of organic solvents, such as chloroform and methylene chloride. The aqueous waste streams will be treated at the wastewater treatment plant at each facility. Effluent from the treatment plants will be discharged into the Shenandoah and Flint River, respectively. Discharge to these two rivers will be in compliance with the National Pollutant Discharge Elimination System (NPDES) permits administered by the states of Virginia and Georgia, respectively.

The solvent waste streams will contain organic solvents, such as hexane, chloroform, methylene chloride and toluene. These solvents will be shipped offsite to registered hazardous waste management facilities. This disposition of solvents will be subject to the Hazardous Waste Management rules of Virginia and Georgia, respectively.

The hazardous solid wastes will consist of filter aids and residues that are wet with organic solvents. These wastes will also be shipped to registered hazardous waste management facilities (as above). The non-hazardous solid wastes (trash, paper, etc.) at Stonewall, Virginia, will be burned in an onsite incinerator subject to Virginia's air pollution control regulations and operated under a permit from that state. The non-hazardous solid wastes from Flint River, Georgia, will be disposed of by shipping them to an offsite local landfill.

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Air emissions of volatile organic compounds produced at each facility will be controlled by appropriate condensers and scrubbers subject to each state's air pollution rules and regulations. The air emissions allowed to be discharged are subject to the air quality permits administered by the respective states of Virginia and Georgia.

b. Formulation of clorsulon oral suspension

The formulation process will generate an aqueous waste stream, non-hazardous solid waste and air emissions.

The aqueous waste stream will contain water and some organic solvents, such as ethanol. The aqueous waste streams generated at the two formulation facilities will be pre-treated prior to discharge to publicly-owned wastewater treatment plants in New Jersey and Puerto Rico. Discharges to and from these wastewater treatment plants will be subject to the respective discharge elimination system permits administered by the state of New Jersey and the EPA.

The non-hazardous solid wastes (trash, paper, etc.) will be burned onsite, using incinerators subject to (and in compliance with) the respective air pollution control regulations and operated under air pollution permits from the state and territory involved.

The air emissions of volatile organic compounds produced during formulation will be controlled by appropriate condensers and scrubbers subject to the respective air pollution regulations of this state and territory. The air emissions allowed to be discharged are subject to the air quality permits administered by New Jersey and Puerto Rico, respectively.

2. Environmental introductions of chemicals due to the use of clorsulon:

Clorsulon is to be used for the treatment of liver flukes, due to Fasciola hepatica, in cattle. Figure 1 (from Foreyt and Todd, 1976) illustrates the areas of the United States where liver flukes were responsible for the condemnation of over 1 million cattle livers in 1973. The EIAR (p. 18) states that "in 1982, 1.4 million livers were condemned because of liver fluke damage." Therefore, the prevalence of this disease does not appear to have decreased. In fact, the American Association of Veterinary Pathologists (1983) state that the prevalence of F. hepatica is increasing, especially in the Western states where irrigated pastures are being increasingly used. The EIAR (p. 19) also states that "fascioliosis due to F. hepatica occurs primarily in the Gulf Coast states and Western states."

Figure 1. Summary of bovine liver condemnations for liver flukes in the United States dur-
ing 1973. Total bovine examined: 30,747,614, total condemned for flukes: 1,147,721 (3.73%)
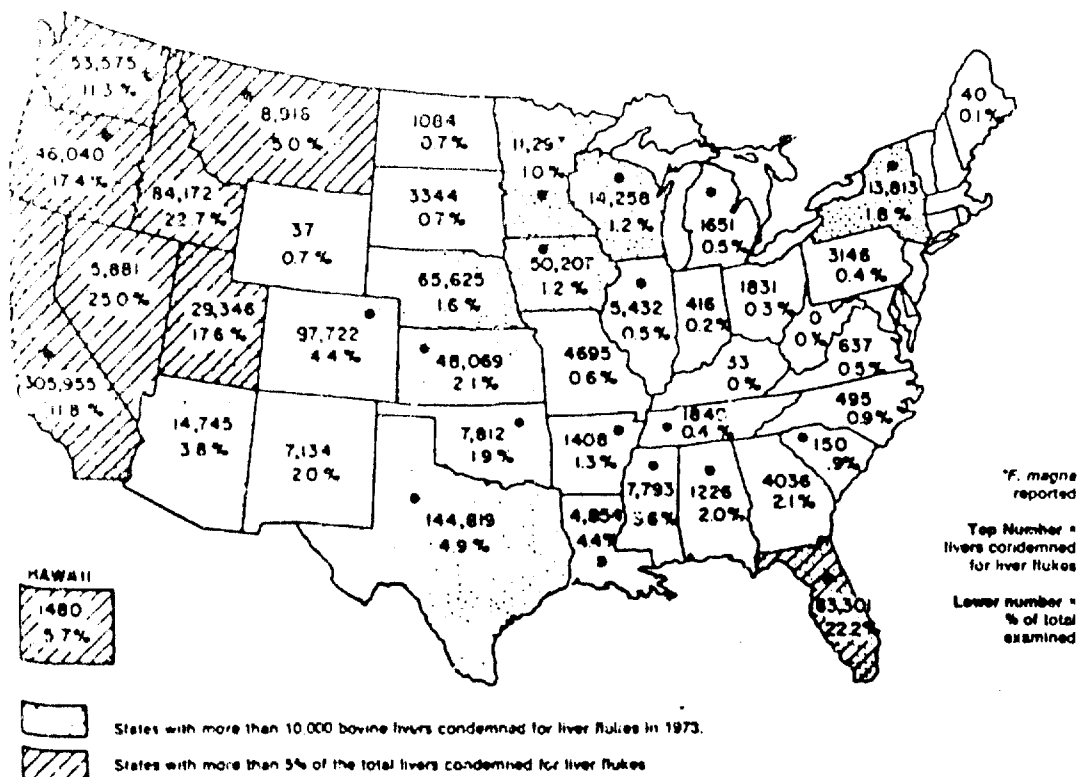
The use of clorsulon will therefore probably approximate the
distribution of bovine liver condemnations seen in Figure 1. The
heaviest use of clorsulon should therefore probably be in the
feedlot and pasture cattle grown in the Southeastern states. This
will be due to the large number of cattle present there and the
prevalence of heavy fluke infestations in the low-lying Gulf coast
areas of these states. Next in order of use would probably be the
fluke endemic areas of the Far West, the Pacific Northwest, and
the Southwest with their abundant feedlot and range populations of
cattle. This will be followed by use in the Midwest because of its
great concentration of cattle. Finally, the Northeast with its
low cattle numbers probably represents the area of smallest
utilization of clorsulon.

The EIAR (p. 19) says that states having high losses from
fascioliasis account for about 40% of the United States cattle
population. The total United States cattle population is about
100 million animals. Therefore somewhat less than 40 million
animals may be exposed to this parasitic disease. Based upon the
numbers of bovine livers condemned/year, the estimated uses of
prior flukicides, and that up to 2-3 treatments/year are possible,

a reasonable estimate of the amount of clorsulon that may be sold is probably somewhere between 5 and 10 million treatment doses/ year. Assuming an average animal treatment weight of about 600 lbs (273 kg), an average treatment dose would be about 1900 mg of drug active ingredient (7 mg/kg x 273 kg = 1911 mg). Therefore, a total of between about 9.5 and 19 metric tons of drug may be introduced into the United States environment every year.

   a. Specific environments potentially impacted by the use of
       clorsulon

The specific environments that may be impacted due to the use of clorsulon to treat cattle for liver flukes would consist primarily of 1) the clorsulon usage sites (cattle feedlots and pastures where cattle are kept), 2) the sites of cattle waste disposal (fields fertilized with the excreta from dosed animals), and 3) the environments receiving runoff from these feedlots, pastures and fields. Estimations of the amounts of clorsulon expected to be introduced into these environments first requires a brief description of how clorsulon is metabolized and how much intact clorsulon can be expected to be excreted by the cattle treated with this drug.

Clorsulon metabolism in cattle

The major route of clorsulon introduction into the environment will be through the excretion of this drug (and its metabolites) into the wastes of treated cattle. The administration of radiolabeled clorsulon to cattle demonstrated that about 90% of the drug dose was excreted in the first seven days in the feces and urine of the treated animals.

Approximately 65% of the total radiolabeled material administered was found in the cattle feces collected in the first week after dosing. About 40% of this radiolabeled material was determined to be parent drug (clorsulon), with another 35-40% being a drug conjugate that was readily converted (via hydrolysis) back to parent clorsulon. Thus, about 80% of the radioactivity excreted in cattle feces was accounted for as parent clorsulon or compounds easily converted back to clorsulon.

About 25% of the total administered dose of radiolabeled clorsulon was found in the urine collected during the first week. Approximately 70-80% of these drug residues were found to be parent clorsulon, with another 8% being a drug conjugate that also was readily converted back to parent clorsulon. Therefore, about 80-90% of the radiolabeled materials found in cattle urine appear to be clorsulon or drug conjugates readily converted to clorsulon.

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It therefore appears reasonable to conclude that the compound(s) excreted into the environment through the use of this product would consist primarily of clorsulon and metabolites that may be converted back to clorsulon. For the sake of simplicity, all subsequent calculations and evaluations will assume that all of the clorsulon given to cattle will end up in animal wastes as the bioactive parent drug.

Clorsulon levels in cattle wastes

Two studies were conducted where cattle feces and urine were collected for the first seven days following the dosing of these animals with radiolabeled clorsulon. Peak clorsulon residue levels were found in both the cattle feces and in the cattle urine collected two days after dosing. More than half of the administered dose of clorsulon was excreted in these wastes within the first three days, and about 90% of the clorsulon was excreted within the first seven days.

These two studies used cattle dosage rates of 6.6 mg drug/kg b.w. and 14.9 mg/kg. The results of both of these studies were adjusted to be equivalent to the recommended drug dose of 7 mg/kg. The (adjusted) levels of clorsulon found in the feces and urine from these two studies were very comparable and quite consistent with each other. In both studies, the day two peak levels of clorsulon found in either feces or urine were about 35-40 mg drug/kg waste. Similarly, the clorsulon levels in the first week's feces and urine ranged between 14-18 mg/kg and averaged close to 16 mg/kg. The above-measured ranges of clorsulon levels in cattle wastes should be about the same for both feedlot and pasture animals.

1) <u>Clorsulon levels at usage sites</u> - cattle feedlots and pastures

Large concentrations of cattle are frequently grown and fattened in relatively discrete locations called cattle feedlots (Thompson and O'Mary, 1983). In the United States, a large proportion of the cattle raised for meat are fattened in feedlots for about five months prior to slaughter. The dense concentrations of cattle in feedlots probably represents the situation of maximum animal wastes (and therefore maximum drug quantities introduced)/unit area.

Estimates of reasonable maximum and minimum concentrations of clorsulon that could be expected in feedlot cattle wastes were made by either assuming that the clorsulon in the first week's waste would be disposed of (approximate maximum level = 16 mg/kg), or assuming that the clorsulon would eventually become evenly distributed throughout the wastes from an entire 130-day grow-out period and then would be disposed of (approximate minimum level = 0.67 mg/kg, see calculation in 2) below).

Estimates of concentrations of clorsulon expected in the waste of pasture cattle would depend primarily upon the time after treatment. Concentrations up to about 40 mg drug/kg waste (wet weight) would appear to be the maximum expected. Such high concentrations should be restricted to the small areas where wastes are deposited by recently treated cattle.

2) Clorsulon levels at disposal sites - introductions into the terrestrial environment

Feedlot cattle wastes (and the associated drug residues) are frequently disposed of via incorporation into soil as fertilizer. The attached EIAR (pp. 21-29) estimates some of the potential environmental introductions of clorsulon from such cattle wastes. The calculations in the EIAR are based upon certain assumptions. The estimates in the EIAR attempt to define the probable range of drug concentrations expected to occur in the terrestrial and aquatic environments. Following are similar estimates made by CVM which are based upon assumptions which appear more appropriate to us.

Assumptions

    a. 220 steers/acre of feedlot (Ensminger, 1978)
    b. Average steer weight = 273 kg (USEPA, 1974)
    c. Clorsulon dose/steer = 7 mg/kg x 273 kg = 1,911 mg
    d. Time steer in feedlot = 130 days (USEPA, 1974)
    e. Waste produced/steer = 22 kg/day (USEPA, 1974) x 130 days
                                 = 2,860 kg. waste
    f. Average clorsulon level in 130 days waste
                         = 1911 mg drug/2860 kg waste
                         = 0.67 mg drug/kg waste
    g. Average clorsulon level in first week's waste
                         = 16.0 mg drug/kg waste
    h. One metric ton = 1,000 kg (2,200 lbs.)
    i. Waste incorporation rate into soil = 10.9 metric tons
                                           waste (wet wt.)/acre soil
    j. Waste is mixed into the top six inches of soil
    k. Weight of top six inches soil/acre = 909,000 kg
                                        (Jackson, 1958)
    l. Weight of rainfall/acre = 102,750 kg/acre-inch of water
    m. Two inches of rainfall runs off from the feedlot

Calculations

    a. Using the feedlot cattle wastes from one entire 130-day grow-out period:

        0.67 mg clorsulon/kg waste x 10,900 kg waste/acre ÷ 909,000 kg soil/acre = 0.008 mg clorsulon/kg soil.

b. Using the first week's feedlot cattle waste:

16 mg clorsulon/kg waste x 10,900 kg waste/acre ÷ 909,000 kg soil/acre = 0.192 mg clorsulon/kg soil.

This range of minimum and maximum expected clorsulon soil concentrations (0.008 to 0.192 mg/kg) will subsequently be contrasted and compared with the levels of clorsulon in soil demonstrated to cause lethal (and sub-lethal) effects upon organisms that live in the soil (soil microbes, plants and earthworms). The above estimated soil clorsulon levels should encompass the entire range expected from soil incorporation of feedlot cattle wastes.

In comparison to feedlot cattle, cattle on pasture are stocked at a much reduced density, therefore, the manure levels/unit area of pasture can be expected to be about 1/200 - 1/400 that found in a feedlot. Manure is seldom directly incorporated into pasture soils. These factors should prevent significant quantities of clorsulon from being introduced into pasture soils. Nevertheless, a limited distribution of high levels of clorsulon in pastures can be expected in and around the excreta of recently treated pasture cattle.

3) **Clorsulon levels in runoff** - introductions into the aquatic environment

If about four inches of rain were to fall in the period of time shortly after feedlot cattle had excreted all their clorsulon dose, and two inches of this rain escaped as runoff from the feedlot, and this runoff carried with it the entire drug burden, then the maximum drug concentration in this feedlot runoff would be about 2 mg drug/kg runoff (2 ppm).

1911 mg/steer x 220 steers/acre ÷ 205,500 kg runoff/acre = 2.05 ppm [a part per million (ppm) = 1 mg/Liter(kg) water].

The runoff from a feedlot is often mixed with the runoff from other nearby areas and this (combined) runoff is subsequently deposited into a receiving water (ditch, stream, pond, lake, etc.) where aquatic organisms could be impacted by substances in the runoff.

The clorsulon concentration in runoff is subsequently contrasted and compared with the levels of clorsulon in water demonstrated to cause lethal (and sub-lethal) effects upon aquatic organisms, such as aquatic algae, crustaceans and fish.

B. The Fate of Clorsulon Introduced into the Environment

Chemicals introduced into one of the four different environmental
compartments (air, water, soil, and biota) may stay in that medium,
however, they frequently tend to migrate into (distribute between) the
other environmental compartments. This distribution of chemicals
between these four environments will, in large part, determine whether
or not organisms present in an environment will be exposed to levels
of chemical sufficient to cause adverse effects in these organisms.

Certain simple physical/chemical measurements have been found to be
useful in approximating a chemicals potential for distribution between
air, water, soil, and biota. The pertinent physical/chemical para-
meters for clorsulon are listed below   This list is followed by
calculations and evaluations that assist in determining where
clorsulon introduced into the environment would tend to end up (its
environmental fate).

    1. Physical/chemical parameters of clorsulon
        (EIAR, pp. 1-3 and 30-69)

        a. Vapor pressure $= 4.6 \times 10^{-13}$ mm Hg (at 25°C)
        b. Water solubility $=$ 700-900 ppm (from pH 1-9)
        c. Octanol/water partition coefficient (Kow) = 15.1
        d. Soil sorption/desorption:
            Freundlich sorption coefficient (K) = 1.57
            Soil sorption distribution coefficient (Kd) = 1.4
            Soil organic carbon coefficient (Koc) = 30.7
        e. Stability in water (in dark) = does not degrade in 30 days
                                  (at pH 5, 7, or 9)
        f. Half-life in water (in sunlight) = 19 minutes
        g. U.V. absorption maxima = 227, 267, and 325 nm
        h. Soil biodegradation half-life $\geq$ 1000 days

    2. Fate evaluation

A vapor pressure of the magnitude listed above clearly indicates
that clorsulon is not a very volatile chemical and therefore is
very unlikely to distribute into the atmosphere. This
environmental compartment will not be considered further.

The water solubility measure gives an indication of the maximum
concentration of clorsulon that can reasonably be expected in the
aquatic environment. For environmental purposes, a water
solubility of about 1,000 ppm is considered a moderate level.
Therefore clorsulon is considered moderately soluble in water and
it could tend to distribute into the aquatic environment.

The octanol/water partition coefficient (Kow) gives an
approximation of a chemical's ability to distribute into
lipid-like material and thereby roughly correlates (Kenaga and

coring, 1980) with a chemicals ability to be concentrated into the fats and lipids of living things (bioconcentration). The Kow for clorsulon is 15.1 and this is a low value---by comparison the Kow for DDT is about 1,000,000. The low Kow for clorsulon indicates that it should not tend to bioaccumulate in organisms in the environment. This assessment is corroborated by the fact that clorsulon residues rapidly deplete from the tissues of cattle dosed with this chemical with most of the drug dose being excreted within the first week. Therefore clorsulon should not tend to distribute into living things and accumulate in this compartment of the environment.

The soils of the earth are an extremely heterogeneous environment. The above noted soil sorption/desorption test consisted of mixing a clorsulon solution with Iowa silt loam soil and measuring the tendency of clorsulon to adhere to the Iowa silt loam (EIAR, pp. 37-44). This result approximates the way clorsulon may distribute between water and other types of soils. From the sorption/desorption experiment the following parameters were estimated: 1) a measure of the strength of sorption of clorsulon to soil $(K = 1.59)$, 2) a measure of the relative distribution of clorsulon between soil and water $(Kd = 1.4)$, and 3) an estimate of clorsulon binding to the organic matter in this soil $(Koc = 30.7)$. From the value for $K = 1.59$, it is estimated that clorsulon should be very mobile in soil with much of it able to leach through the soil and into subsoil (USEPA, 1979). From a $Kd = 1.4$, it can be estimated that about 42% of the clorsulon in an aqueous solution would remain in the water, with only about 58% of the drug adhering to this Iowa silt loam. Finally, the high level of organic matter in this soil (4.6%) did not appear to significantly influence the quantitites of clorsulon sorbed. These values indicate that clorsulon is not very tightly bound to this soil and clorsulon would therefore appear to be environmentally very mobile. Clorsulon should therefore fairly rapidly transfer from the soil environment into the aquatic environment.

The potential stability of clorsulon in the soil and aquatic environments has been estimated in various manners. The experimental evidence demonstrating the degradation of clorsulon in soil(s) appears to be subject to differences in interpretation. Therefore a definitive soil biodegradation study was undertaken. Procedural difficulties, however, resulted in a flawed test that only roughly estimated clorsulon's half-life in soil as $\geq$ 1000 days. A more definitive test of biodegradation of clorsulon in soils has been undertaken by the drug sponsor.

The results of the tests of clorsulon's stability in water depended upon whether or not sunlight was present. When light was not present, aqueous solutions of clorsulon were very stable with no degradation seen in 30 days. The same result was seen in

clorsulon solutions exposed to fluorescent light for one month.
However, exposure of a clorsulon aqueous solution to sunlight
resulted in the rapid degradation of clorsulon to more polar
compounds. One-half of the clorsulon was estimated to have been
degraded within 19 minutes of exposure to sunlight, with a
complete degradation of clorsulon seen in about 4 hours.
Ultraviolet (U.V.) wavelengths appear to most often be responsible
for sunlight caused degradation of a chemical.

To recapitulate the fate of clorsulon in the environment --

1. Significant amounts of clorsulon should not be found in
the atmospheric environment.

2. Although clorsulon will be administered to one part of the
biota (cattle), this drug should tend to rapidly deplete from
the cattle and not tend to become redistributed to
(accumulated) in other organisms in the environment.

3. Clorsulon residues will tend to be applied directly onto
or into soils. Clorsulon does not appear to degrade much in
soils. Clorsulon will probably readily leach from these
soils into water via percolation or runoff from rainfall (or
irrigation) of these soils.

4. Clorsulon residues would therefore tend to selectively
distribute into the aquatic environment. Clorsulon in water
exposed to sunlight does appear to degrade rapidly to more
polar compounds. Photodegradation in water may be the major
breakdown route for clorsulon in the environment.

C. Potential Environmental Effects of Clorsulon

Toxic environmental effects are frequently measured by estimating an
LC50 or EC50. An LC50 (or EC50) is an estimated concentration that
would, on the average, cause mortality (or some other significant
response, e.g. inhibition , immobilization, etc.) in 50% of a
population of organisms. The 95% confidence interval (C.I.) is the
range of values within which it is 95% certain that the true LC50 (or
EC50) resides.

A key value not reported in any of the clorsulon toxicity studies is
the slope of the dose-response line. From the slope of the line,
scientists determine the rapidity of the onset of the adverse effect
as dose increases. The steeper the slope of the line, the more
suddenly mortalities occur with increasing increments of exposure. It
is from this information that the maximum concentration where the
effect would not be observed is estimated. Depending on the effect
being measured in the test and the slope of the dose-response line, a
safety factor is applied to the study results and a safe concentration
is estimated where the test organism (and the class of organism it
represents) can be expected to grow, reproduce, and behave normally.

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On average, the safety factor is one-one hundredth of the 50% effect concentration when the effect is mortality or another significant response (LC50 or EC50). This safety factor is frequently used when adequate data are not available to determine a more accurate value. The value derived from the use of this safety factor may still be inadequate to determine a "safe dose" (see phytotoxicity results below).

A variety of organisms present in either the soil, water or biotic components of the environment have been exposed to doses of clorsulon. Following is a general classification of those organisms, the levels of clorsulon they were exposed to, and the effects that these doses of clorsulon had upon either these organisms, and/or the ecological processes that these organisms are involved in.

1. Microorganisms (EIAR, pp. 8-10)

Clorsulon put in nutrient media at a dose of 400 mg/L (ppm) was found to be inactive in inhibiting the growth of a variety of pathogenic bacterial and fungal species.

2. Soil organisms (EIAR, pp. 70-91 and 103-110)

   a. Microbes

   Soil respiration and nitrification are ecological processes which are under the control of microbes normally found in soil. Soil respiration can indicate the cycling of carbon in soil by measuring general soil microbial activity and the normal decomposition of organic carbon compounds to carbon dioxide. Likewise, communities of microbes in the soil normally convert ammonia to nitrite and nitrate, an essential part of the earths nitrogen cycle.

   Samples of normal loam and loamy sand soils were exposed to doses of 0, 0.05, 0.2, 2, or 20 mg of clorsulon/kg of soil (ppm) for up to five weeks. Measurements of soil respiration and soil nitrification were taken periodically. Clorsulon did not generally have any significant effect upon the soil nitrification process in either soil. However, the highest dose of clorsulon (20 ppm), did cause a transitory decline in the conversion of ammonia to nitrate.

   Clorsulon appeared to have had more effect upon the process of soil respiration than upon soil nitrification. However, a clear dose-related response was not always evident in the soil respiration experiment. In the loam soil, the two highest doses (2 and 20 ppm) caused about a 10% and 20% decline, respectively, in the evolution of carbon dioxide from the soil. In the loamy sand soil, the two intermediate doses (0.2 and 2 ppm) caused a similar level of depressed soil respiration, however, the highest dose of 20 ppm caused no such effect.

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It appears that the range of levels of clorsulon residues that can be expected to be introduced into the terrestrial environment (from 0.008 to 0.192 ppm) will not begin to approach the dose determined to inhibit the microbes involved in the process of soil nitrification. In contrast, limited and transitory reductions in soil respiration rates may result from clorsulon introduced into soil. However, the drug level at which such an effect will be demonstrated appears to be unclear. The level of inhibition may also be related to the type of soil examined.

b. Plants

A range-finding screening of clorsulon toxicity to eight plant species [oat (Avena sativa), corn (Zea mays), tomato (Lycopersicon esculentum), lettuce (Lactuca sativa), bean (Phaseolus vulgaris), turnip (Brassica rapa), pea (Pisum sativum), and sunflower (Helianthus annus)] measured decreases in average plant shoot weight over a 14-day period. The doses of clorsulon used were 0, 1, 10, 100, or 1000 mg drug/kg of potting soil. An EC50 (dose reducing shoot weight 50%) and 95% confidence interval (C.I.) were calculated for seven of the eight species. A linear trends test was then used to determine the lowest drug dose that caused a statistically significant decrease in plant shoot weight. For five of the eight species this was 1 ppm, the lowest dose tested. Therefore, in order to estimate a potentially safe dose for these eight plant species, a safety factor of 1/100 was applied to the EC50. The data from this experiment are summarized in the following table.

Plant Species	EC50[1] (mg/kg)	95% C.I.[2] (mg/kg)	Lowest Dose[3] Decreasing Plant Shoot Weight (mg/kg)	Estimated[4] "Safe Dose" (mg/kg)
Oat	1,031	290–6,494	1	10
Corn	212	36–4,095	1	2
Tomato	28	9–93	1	0.3
Lettuce	489	193–1,621	10	5
Bean	898	72–627,000	10	9
Turnip	11	3–38	1	0.1
Pea	*	*	1,000	–
Sunflower	117	46–356	1	1

[1] concentration estimated to reduce shoot weight 50%

[2] dose interval in which the true EC50 may reside

[3] statistically significant ($\alpha = 0.05$) decrease seen at this dose

[4] 1/100 of the EC50

* unable to calculate, higher test dose(s) needed

The fact that the lowest dose tested significantly decreased
plant weights for five of the eight species examined is an
indication that damage to some plants in the environment could
occur. The lowest concentrations of clorsulon that could
cause damage to plants are uncertain. The best estimate
available is either the "safe dose" or 1 ppm, whichever
concentration is lower. Note that the "safe dose" would have
been inadequate to protect several of these plant species, as
the lowest dose significantly reducing shoot weight was found
to be near (and even below) the supposed "safe dose".

For this reason, a phytotoxicity caution statement will be
included on the product label until more definitive
phytotoxicity studies are undertaken and evaluated. These
studies will include four additional species of pasture plants
(fescue, perennial rye, clover and bermuda grass). The test
will examine (in all species) the effects of clorsulon on seed
germination and also determine the soil clorsulon doses that
do not cause decreased seedling growth.

c. Earthworms

Durgworms (Eisenia foetida) were kept under controlled
laboratory conditions and four replicate dose groups of 10
worms each (40 total) were exposed to nominal soil
concentrations of clorsulon of 0, 130, 216, 360, 600 or 1,000
mg drug/kg soil for a 28-day period. Mortality at 28 days was
seen in all treatments and ranged from 0% in controls to 12.5%
in two of the highest treatment groups. An LC50 could not be
determined from this experiment, but the LC50 appeared to be
>1,000 mg drug/kg 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 range-finding test exposed groups of 10 worms each to
clorsulon doses of 0.1, 1, 10, 100 or 1,000 mg/kg for a 28-day
period. In this test some of the worms were noted at days 14
and 28 to be "elongated." At 28 days some of the worms in the
10 and 100 ppm dose groups and all of the worms in the 1,000
ppm dose group were elongated.

Therefore, the lowest soil concentration of clorsulon that
resulted in some sublethal effects in the dungworm was 10 ppm.
The maximum level of clorsulon that could be expected in soil
under normal agricultural practice would be 0.192 ppm and this
is a >50-fold safety factor for sublethal effects. Death
(within 28 days) would not be expected until soil clorsulon
levels reached 100 ppm, a most unlikely occurrence.

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The highest concentration of clorsulon expected in cattle manure is 40 ppm (wet wt.). This peak concentration is only expected to occur on the second day after dosing. In the unlikely event that dungworms were to be continuously exposed directly to this waste, then the levels of clorsulon in the manure could conceivably cause some sublethal effects in E. foetida.

Clorsulon should therefore not be expected to impact this species of worm significantly. Note, however, that other species of earthworms are normally found in agricultural soils and there is some research indicating that dungworms may be a species of worms which is more resistant to chemical challenge (Dean-Ross, 1983; Roberts and Dorough, 1984).

3. Aquatic organisms (EIAR, pp. 86-98 and 111-115)

Some of the toxic effects of clorsulon have been determined for four species of freshwater organisms: a green alga (Selenastrum capricornutum), a crustacean (Daphnia magna), and two species of fish, the guppy (Lebistes reticulatus) and the bluegill (Lepomis macrochirus). These organisms are representatives of three important levels in the aquatic food chain - the green algae are photosynthetic producers, daphnia are herbivorous grazers on such algae, and both fish species are carnivorous and will eat small crustaceans, such as daphnia.

a. Algae

The toxicity of clorsulon to the green alga was determined by measuring the algal growth inhibition seen over an 87-hour exposure period. Algae populations were kept under constant light and exposed to nominal clorsulon concentrations of either 0, 10, 31, 100, 320, 560, or 800 mg of drug/L of growth medium. The number of algal cells/ml of medium was determined once a day for four consecutive days and the effect of clorsulon on the algal growth rate was determined by calculating the concentration of clorsulon that reduced the growth rate of the algae by 50% (EC50). Algal growth rate data were used and the EC50 (and 95% C.I.) was then calculated to be 520 mg/L (470-580 mg/L). The concentration reported to demonstrate no observed effect on the rate of growth of the algae was reported as 100 mg/L. This value does not appear to have been calculated, but was apparently estimated by a simple visual observation of the respective growth curves.

b. Crustaceans

Groups of 20 daphnia were exposed to a wide range of doses of clorsulon. The nominal concentrations tested were 0, 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56, 100, 180, 320, 560, 855, or 1,000 mg drug/L water. Two series of tests were run: 1-100 mg/L and 100-1,000 mg/L, the 100 mg/L dose being tested twice.

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only one series of untreated controls appears to have been used and the results of the two test series appear to have been pooled. The daphnids were observed for mortality daily for the duration of the 48-hour test period. The 48-hour LC50 (and 95% C.I.) was calculated to be 356 mg/L (288-440 mg/L). The highest clorsulon concentration that did not cause any daphnid mortality in 48 hours was 56 mg/L.

c. Fish

Groups of 20 of both species of fish (guppies and bluegills) were tested to determine their sensitivity to clorsulon doses of either 0, 180, 320, 560, 855, or 1,000 mg drug/L water. The guppies were about one month of age and averaged about 1.5 cm in length and 0.035 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. The 96-hour LC50 (and 95% C.I.) for guppies 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 >560 mg/L, the guppies grew progressively darker in color and their swimming ability was progressively impaired. The number of fish exhibiting these effects and when these sublethal effects were first seen could not be determined from the test information provided.

The bluegills 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 also included observations for color changes and impairment in swimming ability. A 96-hour LC50 (and 95% C.I.) likewise could not be estimated for bluegill because of insufficient mortality in the test animals. The lowest concentration which caused bluegill mortality within 96 hours was the 855 mg/L dose. At concentrations >320 mg/L, the fish grew progressively darker in color and their swimming ability was progressively impaired. At doses >560 mg/L the fish remained on the bottom of the test tanks in extremely poor condition. The number of fish exhibiting these effects and when these sublethal effects were first seen could not be determined from the test information provided.

The results of these aquatic toxicity tests are summarized in the following table, as are the estimated "safe doses" calculated by applying a 1/100 safety factor to the EC50 or LC50.

Test Species	Test Duration (Hrs.)	Test Temperature ($^{\circ}$C)	EC50[1] or LC50[2] (mg/L)	95% C.I.[3] (confidence interval) (mg/L)	Estimated "Safe Dose" (mg/L)
Green alga	87	20	520	470-580	5.2
Daphnia	48	20	356	288-440	3.6
Guppy	96	24	>855	-	-
Bluegill	96	24	>855	-	-

[1] concentration estimated to reduce algal growth by 50%
[2] concentration(s) estimated to result in the death of 50% of the daphnia or fish
[3] dose interval in which the true EC50 (or LC50) may reside
[4] 1/100 the EC50 or LC50

The estimated safe doses for the algae and daphnia are about 5 ppm and 4 ppm respectively. These concentrations are quite close to the 2 ppm estimate of the maximum concentration possible in runoff coming directly from a cattle feedlot. The volume of the receiving waters (stream, pond, etc.) for this runoff (and the additional runoff from the unexposed regions) should substantially dilute (at least 10x) the maximum clorsulon concentration possible in the aquatic environment.

The maximum clorsulon concentrations which seemed not to cause obvious acute effects in algae, daphnia, guppy and bluegill were (respectively) 100 ppm, 56 ppm, 320 ppm and 180 ppm. All of these clorsulon concentrations are considerably above the maximum clorsulon concentrations that could reasonably be expected to be introduced into the aquatic environment. Therefore it appears unlikely that the use of clorsulon in cattle would cause direct toxic effects (or even sublethal effects) upon these four freshwater species that represent organisms important in aquatic food chains.

.4. Mammals (and mammalian parasites)

The screening of clorsulon against various nematode, fluke and insect parasites of cattle and sheep demonstrated that low doses (about 10 ppm) of this drug were effective only in controlling liver flukes in calves and sheep. Much higher doses of clorsulon were found to be ineffective against several nematode species, another fluke species, and sheep blowfly larvae.

Clorsulon was tested for toxic effects in several mammalian species, including cattle. Cattle tested at levels up to 25 times the recommended dose showed no signs of toxicity over the 14-day observation period. The acute oral LD50 in rodents is >10,000 mg clorsulon/kg b.w. The results of these studies and other acute and chronic toxicity tests in several mammalian species can be seen in the attached EIAR (pp. 13-17) and in the Freedom of Information Summary. These tests appear to demonstrate that in mammals, clorsulon is not very toxic, is not teratogenic, and is not carcinogenic.

D. Conclusion

The potential for adverse environmental effects due to the use of clorsulon for liver flukes in cattle appears to center on whether 1) clorsulon introductions into the terrestrial environment could adversely affect the germination and growth of plants exposed to residues of this drug, and 2) clorsulon residues would tend to persist in the terrestrial environment.

Mitigation measures have been implemented to minimize the possibility of clorsulon causing phytotoxicity. The following actions have been taken: 1) a phytotoxicity caution statement is required on the product label, and 2) a definitive toxicity test of the effect of clorsulon upon the germination and growth of several relevant plant species has been undertaken by Merck Sharp and Dohme. A definitive test of clorsulon degradation in the terrestrial environment is also being undertaken by the drug sponsor.

E. Summary

The requested action to approve the over-the-counter use of Curatrem[TM] (clorsulon) 8.5% oral suspension at 7 mg drug/kg b.w. for the treatment of liver flukes in cattle does not appear to result in a potential for the manufacture and use of the drug to result in significiant adverse environmental impacts. Clorsulon environmental introductions should be restricted primarily to the terrestrial and aquatic environments. Transfer of clorsulon from the terrestrial to the aquatic environment (via runoff, etc.) is likely to occur. Clorsulon should not tend to bioaccumulate in organisms in the environment. Except when exposed to sunlight, clorsulon appears to be

...luble in both soil and water. The aquatic organisms tested do not appear to be very sensitive to acute clorsulon toxicity. Some organisms in the terrestrial environment may be affected by the maximum levels of clorsulon expected in soils. Mitigation measures have been taken to minimize such potential environmental impacts.

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