UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES

01/31/11

MEMORANDUM

SUBJECT; Review and Assess the Hydrolysis Studies Data of CONTRAM ST-1

PC Code: 054702	DP Barcode No.: DP 385718
Decision No.:400806	Registration No.: 5485-G
Petition No.: N/A	Regulatory Action: Registration Review/Environmental Fate and Transport/Hydrolysis/PRIA
Risk Assessment Type: Single Chemical	Case No.: N/A
TRX No.: N/A	CAS No.: 5625-90-1
MRID No(s).: 475713-01, 475558-21C, 475558-21, 480648-02, 480648-03, 480648-04	40 CFR 158.340, 180.1001

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Conclusions: The results of the four hydrolytic studies discussed below indicate the active, namely: N,N-Methylenebismorpholine (active in Contram ST-1) is NOT stable under different experimental conditions (pure aqueous medium, and in simulated body fluids) and degrades fast and at a similar half life under the varied conditions of the experiments. These measured half life varied from 6 seconds to less than a day. In simulated body fluids (Gamble's solution), however, the half life of hydrolytic stability was estimated to be between five to 8 months (higher 95% confidence limit to lower 95% confidence limit respectively)

Background: The Lubrizol Corporation has submitted a set of hydrolysis data for their product CONTRAM SR-1, for registration as a new use (non-food, indoor) under FIFRA section 2(mm). This product will be used as a metalworking fluid (MWF).
The active in the product is: N,N-Methylenebismorpholine. It is shown to degrade into morpholine and formaldehyde in aqueous medium.
The rationale for these studies was two-fold: a) to determine the hydrolysis half life of the active in pure aqueous medium and b) to determine the half life of the active in non-aqueous medium which is similar to the actual use conditions, that is, the metalworking fluid. The importance of these studies is to show the release rate of formaldehyde at the actual use condition half life of degradation process which results in the formation of formaldehyde when the active is used as the MWF.

Methodology I: **GC Method**

Abiotic degradation (hydrolysis as a function of pH) was conducted at pH 1.2, 4, 5, 7, and 9 using different buffering agents. Prepared samples (sample size: 0.5 g/l and in duplicate) were kept in the dark. Samples for pHs 4, 7, and 9 were kept at 50° C ± 0.5 ° C for 2. 4 hours before testing for hydrolysis; while the samples for pH 1.2 were maintained at 37 ± 0.5 ° C for 24 hour duration. All

running samples were made in acetonitrile. Standard samples were prepared @ 25 mg/L level in acetonitrile.
All samples were run and analyzed as a function of time (maximum sample running duration: 18 minutes) using Gas chromatography (GC) with running conditions noted below.

Running Conditions:

GC System: HP 5890, incorporating auto sampler and workstation
Column: RTX-5 Amine (30m x 0.32 id x 1.0 μm film)
Oven temp Program: initial 50 ° C for 1 min; rate 15 ° C/min; final 290 ° C for 3 minutes;
On-column injection temp: oven track mode;
FID temp: 300 ° C
Inj. Volume: 1 μL;
Retention time: 12 minutes

Calculations:

At any given time, the concentration of sample solution was calculated by sing the equation:

$C_s = P_{sp}/P_{spa} \times C_{st} \times D \times 1/1000$ ---------- (1)

Where: C_s = sample concentration (g/L); P_{sp} = mean peak area of sample solution; P_{spa} = mean peak area of standard solution; C_{st} = nominal standard concentration (25 mg/L); D= sample dilution factor (20)

Results:

Using equation 1, it was estimated that all for pHs used in the study, that is, 1.2, 4, 5, 7, and 9, the half life for hydrolysis of the active is less than one day.

Methodology II **HPLC Method**

The HPLC method was used in combination with some known chemical reactions in a two step process: a) HPLC was used to separate the active and its degradation product (morpholine and formaldehyde); b) derivativize formaldehyde with a color agent to characterize it. HPLC is equipped with a Post Column Reactor (PCR) which captures the derivativized formaldehyde and characterized with UV spectra measured at 410 nm.
Degradation happens very fast in the HPLC column when the aqueous solution is injected into the HPLC column.
Standards of Bismethylenemorpholine, and formaldehyde were run ahead of the samples, and the recovery of the standards was about 103%

The HPLC runs of the active were made at concentration between 1000-2000pm levels, which is the recommended use level of the product.

Running Conditions:

Instrument: Agilent Series 1100; Column: (Waters): YMC ODS-AQ: 5 μm, 120 A, 2.0 x 25 mm; temperature controller: waters; Static mixture: ASI binary, 25 μL cartridge; Post Column Reactor (PCR): ASI, 1.00 ml, stainless steel, 3000 p.s.i, 150 $^{\circ}$C; Reagent Pump: Waters
HPLC injection of size 10 μL was injected into the HPLC column at 30 $^{\circ}$C and signal detected through UV at 140 nm; the PCR column conditions were: 0.300 ml/min sample was pumped through the reagent pump and the PCR temperature was maintained at 95 $^{\circ}$C.

Calculations:

Percent recovery of Contram ST-1 with formaldehyde is calculated from the chromatogram of the standard solution:

Recovered Contram ST-1 = ((Area-Intercept)/Slope x (FW_{active})/FW_{HOCO})

Where: Area = chromatogram Band area, mAUx sec
Intercept = Formaldehyde working standards Regression= 290 mAU x sec
Slope= Formaldehyde working standards regression 168 mg/Kg/mAU x sec
FW_{active} = formula wt of Bis-morpholine = 186.25 g/mole
FW_{HCHO} = 30.03 g/mole
The half life of the active in the column was estimated to be about six seconds.

Methodology III:

This study was conducted on the active when it is present in aqueous soluble Metal Working Fluid (MWF). The study was conducted on HPLC attached to Post Column Reactor (PCR). A number of chromatograms were developed over a period of time: three data sets were generated based on these chromatograms: a) HPLC chromatograms developed for 64 days; b) chromatograms that were developed for 45 days and third one for 30 days. The first data set was obtained from a solution which consisted of 50% water, and 50% semi-synthetic base which itself consists of emulsifiers, mineral oil and performance components. This is called semi-synthetic metal working fluid. Data set 3 was generated with treated soluble oil based Lubrizol 5375 base

package. Soluble oil base comprises of emulsifiers, performance components and mineral oil which make a soluble oil metalworking fluid; it is diluted with 3-10% water.
The HPLC Methodology used for this part was similar to Methodology II. In this method the residual active is extracted in chloroform, which will not dissolve formaldehyde. This extract can be analyzed through normal phase HPLC; chromatograms of various times are taken, and with the help of the band areas (of various times), amount of the active is estimated in metal working fluid. This response vs. time plot can be sued to monitor (and determine) the amounts of ST-1

Calculations:

The data form all three sets are plotted as response (mAUx s/g) vs. time (days); the plotted data was regressed in excel to extract a regression equation:
Response (mAU x s/g) = 313- 1.08 x time (days)..... (1)
From this equation, it is assumed that the intercept value represent the zero time (days), and ½ of the intercept is the half life of the degradation of Contram ST-1 in metalworking fluid. With 95% confidence limit, the half life of the Contram ST-1 was estimated between 5 months to 8 months (upper 95% confidence limit = 5 months; lower 95% confidence limit = 8 months.)

Running Condition:

HPLC: Same as for Methodology II; column: Restek, Ultra Cyano 2.1 x 150 mm; Temperature controller: waters, TCM II; Static Mixer: ASI, Binary, 25 µL cartridge; PCR: ASI; 1.00 ml, stainless steel, 3000 p.s.is; 150 ° C; reagent pump: dionex, GP-40; Regulator: Upchurch backpressure pump; HPLC injection: 50 µL; flow: 300 µl/min; detection: 410 nm; column temperature: 30 ° C;PCR: flow from reagent pump: 300 µL/min; reactor temperature: 92 ° C

Methodology IV:

The HPLC methodology used for the hydrolysis of Contram ST-1 in experiments II and II discussed above was also for this part of the study. This study was conducted in modified **Gamble's solution**[a]
Rest of the methodology in running the hydrolysis was the same and this experiment also estimated the hydrolytic half life in gamble's solution was about six seconds as determined in the previous method. The samples were analyzed in duplicate.

The running conditions of the HPLC column, PCR were the same as in methodology II.

Conclusions:

The results in all possible combinations in methodologies II, III and IV are the same: The hydrolytic half life i.e. estimated to be the same, that is, about six seconds in HPLC column when the concentrations of active used for the experiments were the same as the actual level in commercial settings (1000-2000 ppm).
This experiments attempts to show that under simulated biological conditions (Gamble's solution) the hydrolytic stability of the active did not change, and that within six seconds the product degrades. Thus the likelihood of finding the active in vivo conditions (through inhalation route) is minimum.

Note a: Gamble's solution is a synthetic body fluid, and was originally made as a complex mixture of inorganic salts (chlorides, carbonates, and phosphates of alkali and alkaline earth metals), and designed to mimic the salt balance of extra cellular fluid (present at the rate of : ng/cm^2/hour). The original Gamble solution is modified) to include organic ingredients and proteins. Some modified Gamble solution does not include proteins, as these may adversely the reproducibility of the experiments such as the present study.

RASSB Conclusions and Recommendations:

RASSB has noted that the studies were not conducted according to the GLP to meet the FIFRA requirements (40 CFR, Part 160). It is also not clear to RASSB if these studies were conducted under EU or OECD Good Laboratory Practices (GLP). RASSB, therefore recommends: 1) these studies are acceptable as supplemental.
2) The conclusions of the stability of the active in metalworking fluid are based on one type of metalworking fluid, and appears to be on the high side (5 or 8 months).In the absence of confirmatory data from other sources (open literature studies etc.), RASSB is inclined to introduce an uncertainty factor of two from the high 95% confidence limit of 5 months and recommend a half life of about 2.5 months (75 days), unless shown otherwise by additional studies.

Bibliography

MRID#	Subject
475713-01/05:	Determination of Hydrolysis Time for Contram ST-1
480648-03:	Determination of Hydrolysis Time for Contram ST-1 by HPLC
CA 475558-21:	Half Life of Contram ST-1 in Aqueous-Soluble Oil Metalworking Fluid
480648-02:	Supplemental Information for MRID 475558-21: Half life of Contram ST-1 in Aqueous Soluble Oil Metalworking Fluid
480648-04:	Determination of Hydrolysis Time For Contram ST-1 by HPLC in Water and Modified Gamble's Solution, a Pseudo-Alveolar Fluid
475558-21C:	Half Life of Contram ST-1 in Aqueous Soluble Oil Metalworking Fluid