Shaughnessy No.: 014503
Date Out of EAB: MAR 14 1988

To: Lois Rossi
Product Manager PM #21
Registration Division (TS-767)

From: Emil Regelman, Supervisory Chemist
Environmental Chemistry Review Section #3
Exposure Assessment Branch/HED (TS-769C)

Thru: Paul F. Schuda, Chief
Exposure Assessment Branch/HED (TS-769C)

Attached, please find the EAB review of...

Reg./File # : 31910-1

Chemical Name: Disodium ethylene bis dithiocarbamate

Type Product : Fungicide

Product Name : Nabam

Company Name : ALCO Chemical Corporation

Purpose : Review of an Aerobic Aquatic Metabolism Study and an Anaerobic Aquatic Metabolism Study.

Action Code: 660

EAB #(s): 80303

Date Received: 1/13/88

Total Reviewing Time: 3 days

Date Completed: 3/11/88

Monitoring Study Requested: _____

Monitoring Study Volunteered: _____

Deferrals to: ____ Ecological Effects Branch

____ Residue Chemistry Branch

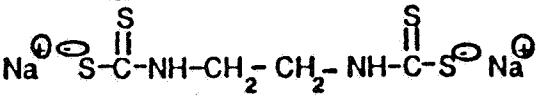
____ Toxicology Branch

1. CHEMICAL:

chemical name: disodium ethylene bis dithiocarbamate

common name: Nabam

structure:



2. TEST MATERIAL:

Disodium ethylene- ^{14}C-1,2-bisdithiocarbamate; 97% radiopure; specific activity: 5.0 mCi/mmole.

3. STUDY/ACTION TYPE:

Review of an Aerobic Aquatic Metabolism study and an Anaerobic Aquatic Metabolism study. These studies were submitted in response to the April, 1987 Nabam Registration Standard.

4. STUDY IDENTIFICATION:

A.

Obrist, John J. "Aerobic/Aquatic Metabolism of Nabam." Performed by Hazleton Laboratories, Wisconsin for ALCO Chemical Corporation, Tennessee. Study completion date- September 11, 1987. Received by EPA on January 13, 1988. Accession number: 403726-01.

B.

Obrist, John J. "Anaerobic Aquatic Metabolism of Nabam." Performed by Hazleton Laboratories, Wisconsin for ALCO Chemical Corporation, Tennessee. Study completion date- September 29, 1987. Received by EPA on January 13, 1988. Accession number 403726-02.

5. REVIEWED BY:

Dana Spatz
Chemist, ECRS #3
EAB/HED/OPP

Date: MAR 11 1988

6. APPROVED BY:

Emil Regelman
Supervisory Chemist, FCRS #3
EAB/HED/OPP

Date: MAR 14 1988

7. CONCLUSIONS:

A. Aerobic Aquatic Metabolism Study

This study does not fulfill EPA data requirements for registering pesticides for the following reasons:

a. TLC is not an acceptable technique for making a positive identification of metabolites. More sensitive analytical techniques, such as GC-Mass Spectrometry, must be utilized.

b. All metabolites at levels greater than 10% of the applied radioactivity were not identified.

c. Total recovery of applied radioactivity on Day 29, the last sampling date of the study was only 83.1%.

B. Anaerobic Aquatic Metabolism Study

This study does not fulfill EPA data requirements for registering pesticides for the following reasons:

a. TLC is not an acceptable technique for making a positive identification of metabolites. More sensitive analytical techniques, such as GC-Mass Spectrometry, must be utilized.

b. All metabolites at levels greater than 10% of the applied radioactivity were not identified.

8. RECOMMENDATIONS:

The registrant should repeat the Aerobic Aquatic Metabolism study in order that a positive identification of all metabolites may be made. If samples are still available for the Anaerobic Aquatic Metabolism study, they should be re-analyzed for positive metabolite identification. If they are no longer available, the study should be repeated.

9. BACKGROUND:

The Nabam Registration Standard was issued in April, 1987.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES:

I.

A. Study Identification:

Obrist, John J. "Aerobic/Aquatic Metabolism of Nabam." Performed by Hazleton Laboratories, Wisconsin for ALCO Chemical Corporation, Tennessee. Study completion date-September 11, 1987. Received by EPA on January 13, 1988. Accession number: 403726-01.

B. Materials and Methods:

^{14}C-Nabam (disodium ethylene-^{14}C-1,2-bisdithiocarbamate) was added to approximately 2 grams of sieved lake sediment and 20 ml of water at a rate of 12.8 ppm. The fortified samples were connected to an aerobic incubation manifold apparatus through which air was drawn. Temperature was kept at 25°C ± 1°C and the apparatus was kept in the dark. The apparatus was connected to a series of two traps: one containing ethylene glycol to trap organic volatiles and one containing 2-ethoxyethanol:ethanolamine (1:1) to trap CO_2.

Duplicate samples were removed for analysis at 0, 1, 2, 5, 8, 15, and 29 days. Traps were also sampled at each sampling period.

The amount of trapped CO_2 and organic volatiles was determined by LSC.

Non-extractable sediment residues were quantitated using a sample oxidizer.

Aliquots of all the aqueous fractions were applied to a TLC plate along with available standards and the plates were developed in $CHCl_3$:MeOH:water (6:4:1, solvent system 1). All TLC plates developed in solvent system 1 were autoradiographed.

A profile of the distribution of the radioactivity on the TLC plates was made using a linear analyzer.

For enhanced separation, samples for days 0, 1, 2, 8, and 29 were analyzed using a more polar solvent system (CH_2Cl_2:MeOH:HoAc, 70:25:1, solvent system III) to get a second profile of the radioactivity.

One sample was chosen, (sample 19, day 5, 91.5% ETU) to further purify by TLC and identify by GLC.

C. Reported Results:

The total radioactivity remaining in the aqueous fraction decreased from a mean value of 95.1% at day 0 to 56.9% at day 29.

The sediment-bound radioactivity increased as the study progressed, with a mean value of 13.0% of the applied radioactivity associated with the extracted sediment on day 29.

The total radioactivity detected as organic volatiles remained at less than 0.1% of the applied radioactivity. The total radioactivity detected as CO_2 increased from less than 0.1% on day 1 to a mean value of 13.2% on day 29.

Individual recoveries of the samples ranged from 110.9% day 5 to 81.7% at day 29. The mean recovery of all values was 99.7%. The ^{14}C-recoveries from plate scrapings ranged from 81.8% to 91.0% (Table 1).

Metabolic product identifications are given in Table 2.

D. Study Author's Conclusions:

Aerobic aquatic incubation caused Nabam, fortified at approximately 10 ppm, to degrade rapidly. The quantity of radioactivity that could be associated with parent material was only 36.8% at day 0. No half-life was calculated. The major identified products of this degradation were ETU, EBIS, and EU. Approximately 13.2% of the total applied radioactivity was observed as $^{14}CO_2$ and 13.0% as sediment-bound residues by day 29. Less than 0.1% of the applied radioactivity was observed as volatile organic compounds. Mean total recoveries ranged from 109.0% at day 5 to 83.1% at day 29 with a mean recovery of 99.7% for the study.

E. Reviewer's Discussion and Interpretation of Study Results:

The rapid breakdown of Nabam in the first day was most likely due to the instability of Nabam in the presence of moisture and oxygen and not due to aerobic aquatic metabolism.

EAB is concerned about the poor material balance for day 29. The registrant has stated that a probable cause for this was that the capacity of one trap was most likely exceeded. The

new study should clarify this point.

Due to the large difference in the distribution results of days 15 and 29, a day 22 sample might be appropriate.

II.

A. Study Identification:

Obrist, John J. "Anaerobic Aquatic Metabolism of Nabam." Performed by Hazleton Laboratories, Wisconsin for ALCO Chemical Corporation, Tennessee. Study completion date-September 29, 1987. Received by EPA on January 13, 1988. Accession number 403726-02.

B. Materials and Methods:

Sieved sediment (2g) and 20 ml of lake water were placed in an anaerobic incubation chamber and purged with a continuous flow of humidified N_2 for 32 days. After 32 days of anaerobic incubation, samples were fortified with an aqueous solution of ^{14}C-Nabam to give a final concentration of 9.4 ppm. The fortified samples were connected to an anaerobic incubation apparatus through which humidified N_2 was drawn continuously. The incubation apparatus was connected in series to two traps, one containing ethylene glycol to trap organic volatiles, and one containing 2-ethoxyethanol:ethanolamine (1:1) to trap CO_2. The incubation apparatus was kept at 25°C ± 1°C in a dark room.

Duplicate samples were removed for analysis on days 0, 8, 30, 63, 91, 121, 141, 183, 218, 240, 276, 290, 337, and 365 Trapping media were sampled and replaced on days 30, 63, 91, 121, 141, 183, 218, 240, 276, 290, 337, and 365.

Nonextractable radioactivity was quantitated by oxidation analysis of the sediment using a sample oxidizer.

The amount of trapped CO_2 and organic volatiles was determined by LSC.

Aliquots of the aqueous extracts were applied to strips on TLC plates along with available standards and the plates were developed in $CHCl_3$:MeOH:water (6:4:1), solvent system I.

A profile of the distribution of the radioactivity on the TLC plates was made using a linear analyzer. All the TLC plates that were developed in solvent system 1 were autoradiographed.

The day 91 aqueous extract sample was chosen for metabolite purification and identification by Mass Spectrometry. The sample was concentrated and purified by TLC. The purified sample was submitted for gas chromatography-mass spectrometry analysis.

C. Reported Results:

The total radioactivity remaining in the aqueous fraction ranged from a mean value of 110.0% (day 0) to 90.5% (day 365). The sediment-bound radioactivity showed a slight increase over the study period. It ranged from a mean value of 2.9% (day 0) to 5.4% (day 290).

The total radioactivity detected as organic volatiles remained below the limits of detection throughout the study. The total radioactivity detected as CO_2 gradually increased throughout the study to only 0.3% of the applied radioactivity.

Individual recoveries of the samples ranged from 113.3% at day 141 to 96.0% at day 365. The mean recovery of all values was 106.1%. The amount of radioactivity recovered by the scraping procedure varied from 74.0% for day 0 to 93.7% for day 8. The overall mean of the eight different samples was 83.8%.

Metabolic product identifications are given in table 3.

D. Study Author's Conclusions:

Nabam degraded rapidly when incubated in an anaerobic aquatic system fortified at 10 ppm. The quantity of radioactivity that could be associated with parent material was only 33.0% at day 0. No half-life was calculated. The major products of this degradation were identified as ETU, EBIS, and EU by co-chromatography. The ETU was also identified by mass spectrometry.

E. Reviewer's Discussion and Interpretation of Study Results:

The rapid breakdown of Nabam in the first day was most likely due to the instability of Nabam in the presence of moisture and oxygen and not due to anaerobic aquatic metabolism.

The results indicate that ETU is the major metabolite of the degradation of Nabam under anaerobic aquatic conditions.

Levels of ETU peaked on sampling day 218 (93.8%) and decreased to 73.9% on day 365.

11. COMPLETION OF ONE-LINER:

Not applicable.

12. CBI APPENDIX:

Not applicable.

STRUCTURES OF NABAM AND RELATED COMPOUNDS

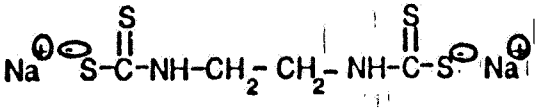
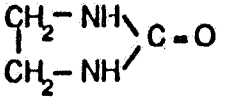
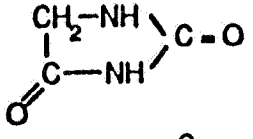
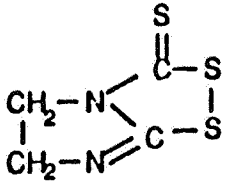
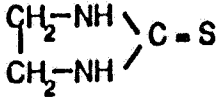
Compound	Common Name	Structure
Disodium ethylene - ^{14}C - 1,2 - bis dithiocarbamate	Nabam	
2 - Imidazolidine	Ethylene Urea or EU	
2,4 - Imidazolidinethione	Hydantoin	
Ethylene bis - isocyanate sulfide	EBIS	
2 - Imidazolidinethione	Ethylenethiourea or ETU	

Figure 1

AEROBIC AQUATIC

Table 1

Material Balance for TLC Procedure*

Sample Number	Day	Amount Applied (dpm)	Amount Recovered (dpm)	Recovery (%)
11	1	29,917	24,459	81.8
16		30,275	25,825	85.3
19	5	31,537	28,693	91.0
6		31,141	25,663	82.4
8	15	30,554	26,653	87.2
17		27,743	23,252	83.8

* Radioactivity in the aqueous fraction was applied to and recovered by scraping from representative TLC plates.

Aerobic Aquatic

Table 2

Mean Percent Distribution of the Radioactivity In the Aqueous Fraction Resulting from Aerobic Aquatic Incubation of ^{14}C-Nabam*

Compound	Interval (Days) 0	1	2	5	8	15	29
Origin**	36.8	3.9	6.7	3.1	1.9	1.6	12.7
Unresolved 1	3.7	2.5	4.6	1.7	1.6	1.6	0.7
Hydantoin	1.5	3.6	2.8	0.4	1.0	1.6	0.1
Peak 1	<0.1	5.4	<0.1	<0.1	<0.1	<0.1	<0.1
Unresolved 2	10.0	5.8	3.4	0.8	4.4	8.0	1.8
EU	4.8	8.9	4.8	3.7	16.1	14.3	4.9
Unresolved 3	5.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
ETU	32.3	27.9	44.9	89.1	65.8	66.7	36.4
Unresolved 4	<0.1	<0.1	2.1	1.9	1.7	<0.1	0.4
Peak 2	<0.1	17.2	20.9	<0.1	<0.1	<0.1	<0.1
EBIS	1.0	18.5	10.3	1.1	3.3	2.6	<0.1
Unresolved 5	<0.1	<0.1	<0.1	0.5	<0.1	<0.1	<0.1
Total	95.3	93.7	100.5	102.3	95.8	96.4	57.0

* Individual values are in Appendix F. Metabolites are listed in order of appearance on the TLC plate from origin to solvent front using $CHCl_3$:MeOH:Water (6:4:1).

** The value for Day 0 represents the maximum amount of Nabam remaining after fortification.

ANAEROBIC AQUATIC

Table 3

Percent Distribution of the Radioactivity in the Aqueous Fraction Resulting from Anaerobic Aquatic Incubation of ^{14}C-Nabam[a]

Matrix	Interval (Days) 0	0	34	63	91	121	141	183	218	240	276	290	337	365
Origin[b]	33.0	24.9	12.3	7.0	13.6	8.6	8.2	10.2	1.8	2.8	14.9	10.7	4.8	4.5
Peak Y	17.9	26.3	29.8	-	-	-	-	-	-	-	-	-	-	-
Unresolved 1	-	-	-	5.9	0.8	2.6	8.4	1.0	1.1	1.0	1.6	1.7	1.4	1.8
Hydantoin	6.8	4.9	3.5	1.2	0.3	0.8	2.9	<0.1	<0.1	<0.1	0.2	0.2	0.2	0.4
Unresolved 2	2.0	1.1	1.1	1.3	0.5	4.2	14.5	0.7	0.2	0.1	0.4	0.4	0.5	0.7
EU	4.2	7.6	8.2	6.3	15.5	15.7	9.1	9.6	4.5	5.0	14.0	7.7	9.9	6.9
Unresolved 3	1.7	0.9	0.7	-	-	-	-	-	-	-	-	-	-	-
ETU	15.2	34.3	44.0	76.5	68.2	64.8	64.7	78.6	93.8	95.8	71.3	78.7	82.2	73.9
Unresolved 4	2.6	1.1	1.1	1.0	1.2	3.1	-	0.8	0.3	0.7	0.2	-	0.2	0.4
EBIS	26.6	0.9	0.3	0.2	0.1	1.7	1.5	0.2	0.4	0.1	0.4	0.5	0.8	0.1
Unresolved 5	-	-	-	-	-	1.0	-	-	-	<0.1	-	-	<0.1	-
Total	110.0	102.5	101.0	99.4	100.2	102.5	109.3	101.1	102.1	105.5	103.0	99.9	100.0	90.7

- Not determined.

a Mean of duplicate samples; individual values are in Appendix F. The solvent system for TLC analysis was $CHCl_3$:MeOH:water (6:4:1). Metabolites are listed in order of increasing Rf.

b The value for Day 0 represents the maximum amount of Nabam remaining in samples after fortification.

AEROBIC AQUATIC

Table

Representative TLC R_f Values of Nabam-Related Compounds

Common Name	Chemical Name	Solvent System I	Solvent System II	Solvent System III
EU	2-Imidazolidine	0.48	0.30	0.65
Hydantoin	2,4-Imidazolidinethione	0.32	0.46	0.65
EBIS	Ethylene bis-isothiocyanate sulfide	0.88	0.61	0.95
ETU	2-Imidazolidinethione	0.62	0.50	0.82

Solvent System I = $CHCl_3$:MeOH:water (6:4:1).
Solvent System II = EtOAc:MeOH (4:1).
Solvent System III = CH_2Cl_2:MeOH:HOAc (70:25:1).

AEROBIC AQUATIC

Table

Mean Percent Distribution and Total Recovery of Applied Radioactivity*

Matrix	Interval (Days) 0	1	2	5	8	15	29
Sediment/Water							
Combined aqueous extract	95.1	93.4	100.3	102.3	95.7	96.2	56.9
Nonextractable	1.3	4.7	5.6	6.7	5.9	6.4	13.0
Volatiles							
CO_2 trap**	NA	<0.1	<0.1	<0.1	0.2	0.7	13.2†
Volatile organic trap††	NA	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total recovery#	96.4	98.1	105.9	109.0	101.8	103.3	83.1

NA Not applicable.

* Mean of duplicate values. Individual sample values are in Appendix E.
** CO_2 trap = ethanolamine:2-ethoxyethanol (1:1).
† The capacity of the trap was most likely exceeded since the volume of the trapping solution was substantially reduced on Day 28 (30 mL) from the usual 100 mL (apparent evaporation) and the solution had a reddish color. Approximately 70 mL of fresh trapping solution was added on Day 28.
†† Volatile organic trap = ethylene glycol.
# The mean recovery for the study was 99.7%.

ANAEROBIC AQUATIC

Table

Representative R_f Values of Nabam-Related Compounds

Common Name	Chemical Name	Solvent System I	Solvent System II
EU	2-Imidazolidine	0.48	0.32
Hydantoin	2,4-Imidazolidinethione	0.32	0.56
EBIS	Ethylene bis-isothiocyanate sulfide	0.88	0.76
ETU	2-Imidazolidinethione	0.62	0.56

Solvent System I = $CHCl_3$:MeOH:water (6:4:1).
Solvent System II = EtOAc:MeOH (4:1).

ANAEROBIC Aquatic

Table

Percent Distribution and Total Recovery of Applied Radioactivity[a]

Matrix	Interval (Days) 0	8	30	63	91	121	141	183	218	240	276	290	337	365
Sediment/Water														
Combined aqueous extract	110.0	102.5	100.6	99.2	100.0	102.2	109.1	101.1	102.1	105.4	102.9	99.7	100.0	90.5
Nonextractable	2.9	4.8	4.4	4.3	4.0	3.4	4.0	4.1	3.6	3.1	4.2	5.4	4.0	5.2[b]
Volatiles (Cumulative Parent)														
CO_2 trap[c]	NA	NA	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3
Volatile organic trap[d]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total recovery	112.9	107.3	105.1	103.6	104.1	105.8	113.3	105.4	105.9	108.7	107.3	105.3	104.3	96.0

NA Not applicable.

a Mean of duplicates. Individual sample values are in Appendix E.
b This includes half of the value recovered in the excess water and methanol from Sample No. 12.
c CO_2 trap = ethanolamine:2-ethoxyethanol (1:1).
d Volatile organic trap = ethylene glycol.

ANAEROBIC Aquatic

Table

Material Balance for TLC Procedure[a]

Sample Number	Day	Amount Applied (dpm)	Amount Recovered (dpm)	Recovery (%)
19	0	20,985	17,059	81.3
04		20,479	15,163	74.0
10	8	21,010	19,678	93.7
25		21,347	17,618	82.5
11	30	20,706	17,881	86.4
23		22,219	17,741	79.8
06	63	22,677	19,953	88.0
20		22,290	18,951	85.0

The overall mean of the TLC procedure for these samples was 83.8%.

a Radioactivity in the aqueous fraction was applied to and recovered by scraping from representative TLC plates.