EXHIBIT 13

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FROM (NAME—LOCATION—PHONE) Dept. of Medicine & Environmental Health — T.J. Long, G2WD 4-8851

DATE : December 26, 1984

SUBJECT : CP 76100: Lifetime Carcinogenicity Study in Mice

REFERENCE : IR-77-223

TO : *R.W. Street C2SC

CC. E.E. Debus, C2SC
V.C. Espenschied
T.W. Fuhremann
R.L. Harness, C2NA
L.A. Suba, C2SC
Toxdata

Ct25AM

file Pendulf Metabolite

The accompanying report has been reviewed and accepted. A quality assurance review was performed by International Research and Development Corporation. A summary of the methods and results and an evaluation of the conclusions presented in this report are summarized below.

METHODS

CP 76100 was administered by gavage as an aqueous solution of the sodium salt to Charles River CD®-1 mice daily for 104 weeks. Dosing was at a constant volume of 10 ml/kg at dosage levels of 50, 150, and 500 mg/kg/day. Seventy male and 70 female mice were dosed at each level. A control group of 70 mice of each sex received a solution of NaCl (5.0 mg Na+/ml) at the same dosing volume. The concentration of sodium in the control dosing solution was selected to equal that received by the high dose group.

The mice were observed daily for mortality and overt signs of toxicity. A detailed physical examination of each animal was performed weekly. Individual body weights and food consumption measurements were recorded weekly for the first 14 weeks and biweekly thereafter. The following hematological parameters were measured for 10 mice/sex/group at 12 and 24 months: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leukocyte counts, platelet count, and reticulocyte count.

Complete postmortem examinations were performed on all animals dying spontaneously, sacrificed in extremis, or sacrificed at the twelve month interim and 24-month terminal sacrifice periods. The following tissues were examined microscopically: adrenals, brain, eyes and Harderian glands, gall bladder, heart, esophagus, stomach, duodenum, jejunum, ileum, large intestine, kidneys, urinary

*received report

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bladder, prostate, testes with epididymides, ovaries, cervix uteri, liver, lung and mainstem bronchi, lymph nodes, mammary gland, salivary glands, sciatic nerve, pancreas, pituitary, skin, spinal cord, spleen, thymus, trachea, thyroid/parathyroid, sternum (bone marrow), and other tissues with lesions. In addition, 10 animals/sex/group were examined microscopically as follows: 3 coronal sections through the head which included the nasal cavity, paranasal sinuses, tongue, oral cavity, nasopharynx, and middle ear.

Tumor incidences were statistically analyzed by the testing laboratory employing life table methods and Chi-square analysis to assess differences between control and treated groups. Analysis for the presence of a linear trend was performed both with and without adjustment for time of death (life table method). In addition, Monsanto analyzed the data for differences between group incidences by the Fisher Exact test and for the presence of a linear trend by the Cochran-Armitage test.

RESULTS

During the first twelve months of the study, mortality was higher in treated male mice as compared to controls (See Table 1). Percent mortality was 4.3, 7.1, 14.3, and 11.4% for the control, low, mid, and high dose level males respectively. For the remainder of the study, mortality was similar for control and treated males. At study termination, survival for mid- and high-dose males was 6 and 10 percent less than control, respectively. For female mice, survival was similar for all groups throughout the study. At study termination, survival in high dose females was 5% lower than controls. Body weight and food consumption were similar for control and treated mice throughout the study. Although occasional differences were statistically significant, no consistent differences were observed. No treatment-related changes were observed in appearance or behavior.

There were no test material related effects observed in the hematological data for either sex at either of the sampling periods. Occasional differences were observed between control and treated groups. However, due to large variability, lack of dose-response, and the absence of appropriate similar findings in both sexes, none of these differences were considered to be treatment related. There were no compound-related macroscopic or microscopic changes observed during necropsy or during

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microscopic examination. All changes observed were considered to be spontaneous or incidental in nature and commonly encountered lesions for mice of this age, sex, and strain.

There were several statistically significant differences for adjusted trend of life table data for some tumors among males. This included percent animals with tumors, harderian gland adenoma, liver hemangioma and malignant lymphocytic lymphoma. These differences were considered to have resulted from and reflected the pattern of earlier deaths in the high dose animals. This resulted in earlier discovery of clinically silent tumors or the recording of non life-threatening tumors when death occurred early for other reasons. The only statistically significant differences in unadjusted trend in any group of tumors or individual tumors among males were for malignant lymphocytic lymphoma and liver hemagioma. When analyzed by the Cochran-Armitage test (Table 2), no linear trend was observed for lymphocytic lymphoma. Also, the combined incidence for histiocytic plus lymphocytic lymphomas observed for high dose males in this study (7%) falls within the historical control range of this laboratory (0-15%) for malignant lymphomas. In addition, the incidence of lymphocytic lymphomas in treated female mice was significantly less than in control females (Table 2). There was a statistically significant trend (Cochran-Armitage) for liver hemangioma. However, since both benign and malignant tumors of blood vessels are not unusual tumors in mice, the low incidence observed in this study (2/70 males) was not considered to be indicative of a treatment-related effect. The testing laboratory's historical control range for this tumor in male CD-1 mice is 0-2.0%.

Similarly, for female mice there were several significant differences for adjusted trend of life table data. These included alveolar bronchiolar carcinoma, malignant lymphocytic lymphoma, malignant histiocytic lymphoma and ovarian adenoma. For alveolar bronchiolar, carcinoma there was no statistically significant trend for unadjusted data or when analyzed by the Cochran-Armitage test (Table 2). In addition, the high dose incidence (6%) was lower than the mean historical control incidence (7.2%) for the testing laboratory. The unadjusted trend was statistically significant for histiocytic and for lymphocytic lymphomas. However, the trend for lymphocytic lymphomas was negative and was, therefore, not an adverse treatment effect. The trend for histiocytic lymphoma was not statistically

significant however for male mice when analyzed by the Cochran-Armitage test (Table 2). Also, in high-dose males the incidence of histiocytic lymphomas was less than in the concurrent control group. The combined incidences of these two tumors in female mice at the high dose level (17%) was less than that for concurrent controls (23%) and was within the testing laboratory's historical control range (3.3-27.0%) for malignant lymphomas. The incidence of ovarian adenomas in the high dose group (4%) was well within the laboratory's historical control range (0-18%). Additionally, no statistically significant trend was observed for unadjusted data or when analyzed by the Cochran-Armitage test (Table 2). Finally, none of the tumor incidences observed in female mice were elevated when compared to control incidences by the Fisher Exact test.

In summary, none of the tumors observed in this study were considered to be the result of treatment with CP 76100.

CONCLUSIONS

Treatment of male and female mice with CP 76100 by gavage at dosages of 50, 150, and 500 mg/kg/day elicited no treatment-related changes in appearance, behavior, body weight, food consumption, hematological parameters, or macroscopic and microscopic pathology. Mortality was increased in treated male mice during the first twelve months of treatment. For the remainder of the study, mortality was similar for control and treated males. Mortality for control and treated female mice was similar throughout the study.

Under the conditions of this study, CP 76100 was not considered to be carcinogenic in mice at dosages up to and including 500 mg/kg/day.

Timothy J. Long

Timothy J. Long, PhD
Senior Product Toxicologist
Monsanto Company
Department of Medicine and
Environmental Health

/jb

Table 1. Mortality

Dosage-(mg/kg/day)		Cumulative Mortality (%)* Months: 12	18	24
0	male	4.3	14.3	32.8
	female	5.8	13.0	39.1
50	male	7.1	12.8	32.8
	female	8.7	13.0	42.0
150	male	14.3	21.4	38.6
	female	7.1	14.3	42.8
500	male	11.4	21.4	42.8
	female	7.1	15.7	44.3

*Figures do not include animals sacrificed at the 12-month interim and 24-month terminal sacrifices.

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Table 2. Incidence of Neoplastic Lesions

Dosage (mg/kg/day)	% Incidence 0	50	150	500	Linear[1] Trend
Animals with Tumors (%)					
Male	40	50	47	51	No
Female	52	55	48	50	No
Total Animals with Benign Tumors					
Male	21	36*	33	29	No
Female	26	23	22	28	No
Total Animals with Malignant Tumors					
Male	23	26	20	29	No
Female	36	39	34	26	No
Harderian Gland Adenoma					
Male	6	9	7	13	No
Female	4	6	7	4	No
Liver Hemangioma					
Male	0	0	0	3	Yes
Female	0	0	0	0	No
Liver Adenoma					
Male	3	9	6	7	No
Female	3	2	0	1	No
Liver Total Tumors					
Male	21	26	16	29	No
Female	17	20	17	13	No
Alveolar Bronchiolar Carcinoma					
Male	1	3	3	1	No
Female	1	1	4	6	No
Lung Total Tumors					
Male	21	26	27	18	No
Female	20	28	26	24	No

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Dosage (mg/kg/day)	0	50	150	500	Linear[1] Trend
Lymphoreticular lymphoma-lymphocytic					
Male	0	6	3	7*	No
Female	20	16	7*	7*	Yes
Lyphoreticular lymphoma-histiocytic					
Male	3	4	4	0	No
Female	3	1	7	10	Yes
Ovarian Adenoma					
Female	0	1	4	4	No

*Statistically different from controls ($p \leq 0.05$) by Fisher Exact Test

[1]Cochran-Armitage test for linear trend ($p \leq 0.05$).

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DEPARTMENT OF MEDICINE & ENVIRONMENTAL HEALTH

Monsanto Company
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
Phone: (314) 694-1000

November 9, 1984

Dale E. Johnson, PhD
International Research and
Development Corporation
500 Main Street
Mattawan, Michigan 49071

RE: Lifetime Carcinogenicity Study with CP 76100 in Mice (IR-77-223)

Dear Dale,

As a follow-up to our telephone conversation several weeks ago, I want to reemphasize the need to finalize this report before the end of 1984. Back as early as June, 1984 this report was supposed to have been in final form, but several issues still remain unresolved. In particular, the following items were pointed out to you in our last conversation:

1) Pages 15 and 16 (Vol. 1) - In the mortality tables, the reported mortality for high dose males and mid dose females for the 12-24 month period do not agree with the numbers reported for this period in Table 1, pgs. 25-30.

2) Several discrepancies between statements in sections 3a and 3b (Vol. 1, pg. 19) and data in Appendix I (trend and homogeneity analyses).

3) Page 20 (Vol. 1) - The entire first paragraph, beginning with line 3 (..."This falls within the range...") has numerous transpositions and does not make sense as written.

4) Table 13 (Vol. 1, page 142) - The total number of female control mice with neoplasia (reported as 35) does not agree with the number reported in Appendix G (36).

Please address these issues so that we can get a final report issued within the month. If you still have further questions, please call me. As this report has been long, long overdue for completion, I'm sure we would all like to see it completed immediately.

Sincerely,

Timothy J. Long

Timothy J. Long, PhD
Senior Product Toxicologist

MCE 0329819

bcc: T.W. Fuhremann
F.R. Johannsen

dli

Monsanto DEPT. OF MEDICINE AND ENVIRONMENTAL HEALTH

TRIP REPORT No. _____

BY: T.J. Long SECTION: Toxicology

TRIP DATE: 5/24-25/84 SHEET 1 OF 1

LOCATION VISITED

International Research & Development Corporation (IRDC)
Mattawan, Michigan

IN ATTENDANCE

Dale Johnson
Barry Benson - (IRDC)
Ward Ritter
QA Staff

T. Long
C. Russell
M. Chatel - (Monsanto)
S. Haag
A. Uelner

PURPOSE

To finalize the long overdue report on the lifetime carcinogenicity study in mice with CP 76100, tour the laboratory facilities and familiarize myself with IRDC data packages.

REPORT SUMMARY & CONCLUSIONS

Following an extensive audit of the lifetime carcinogenicity study with CP 76100 in mice (IR-77-223) by Monsanto's quality assurance unit, numerous errors were detected in the pathology data package. This meeting was intended to resolve these issues and finalize the report. Although most of our concerns had adequately been addressed, several minor issues will require resolution by the pathologist. In the next several weeks a final draft report will be submitted to staff toxicology for final approval. It appeared that a good deal of effort had been extended by IRDC staff to resolve all issues of concern.

Discussions with Dale Johnson and the manager of acute studies were held to assess IRDC's ability to run acute toxicity and irritation screens (ODES), DOT skin corrosivity tests and skin sensitization studies with guinea pigs. The acute toxicity testing facilities were also toured. IRDC appears to be well equipped and staffed to give us reliable data and quite rapid report turn around time. Copies of generic protocols for our review will be forthcoming.

REPORT DISTRIBUTION

T.W. Fuhremann
F.R. Johannsen
G.J. Levinskas
A.F. Uelner
R.W. Street

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T.J.L. 6/4/84
PREPARED BY DATE
T.J. Long

Monsanto DEPT. OF MEDICINE AND ENVIRONMENTAL HEALTH

TRIP REPORT No. _____

BY: F.R. Johannsen SECTION: Toxicology

TRIP DATE: April 14-15, 1982 PAGE 1 OF _____

LOCATION VISITED

International Research & Development Corp.
Mattawan, Michigan

IN ATTENDANCE

James L. Schardein (IRDC)
Fred R. Johannsen

PURPOSE

See Report Summary & Conclusions

REPORT SUMMARY & CONCLUSIONS

1. A draft report of study IR-81-229, rat teratology study with DMAC, was reviewed as was some raw data on fetal malformations. The final report will be issued in the next 2 weeks following final Q.A. review.

2. An incomplete draft of the 2-generation rat reproduction study (IR-79-358) with Maleic Anhydride was reviewed. Histopathology compilation was, as yet, incomplete. A final draft report will be issued about July 30, 1982 for this study. In lieu of what appears to have been more histopathology done on this study than indicated by protocol or protocol amendment, it is suggested that R.D. Short follow up on final cost projections with IRDC upon receipt of the final draft report. Plans should be made to review raw data from this study at the next scheduled visit at IRDC.

3. The following are scheduled dates of issuance of final drafts for each of the following studies:

   a. Lifetime chronic mouse study with CP 76100 (IR-77-223)

      Histopath finished - Oct. 1982
      Draft report - Feb. 1983

REPORT DISTRIBUTION

R.C. Dirks
T.W. Fuhremann
P.H. Hobson
G.J. Levinskas
D.P. McFadden
R.B. Oleson
J.H. Senger
R.D. Short
A.F. Uelner

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F.R.J. PREPARED BY

4/21/82 DATE

b. 21-day rabbit dermal with MON 2139 (IR-80-009)

Final report - April 30, 1982

c. 21-day rabbit dermal with AVADEX® BW (IR-81-316)

Draft report - June 15, 1982

d. Rat teratology with MON 4606 (IR-81-344)

Draft report - May 28, 1982

e. Rat reproduction study with MON 097 (IR-80-053)

F_o interim report - May 14, 1982
F_o audited report - Aug. 3, 1982

f. Rat teratology with Propachlor (IR-81-264)

Draft report - June 15, 1982

/dlj

Monsanto

MAR 18 1982

FROM (NAME—LOCATION—PHONE) Dept. of Medicine & Environmental Health S.M. Haag-G2WC

DATE : March 16, 1982

SUBJECT : Dosing Solution Analysis for IRD 77-223 - Lifetime Carcinogenicity Study in Mice with CP 76100

REFERENCE :

TO : S. Dubelman - U2C

cc: T. W. Fuhremann - G2WD
F. B. Oleson - G2WD
D. B. Sharp - U2E

File

Findings from the DMEH Quality Assurance review of the analytical data package for IRD 77-223 have been previously summarized in memos dated November 5, 1980 and February 10, 1982.

The deficiencies noted are not of sufficient magnitude to preclude confirmation that animals were dosed as specified in the protocol and the technical aspects of the analysis appear to be correct, with ample QC samples to validate methodology. We do not know, however, what impact the deficiencies cited would have on the conclusions reached in a regulatory agency audit.

S. M. Haag

cac

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IN - 10 (REV. 8/77)

Monsanto

FROM (NAME LOCATION PHONE) Dept. of Medicine & Environmental Health R. C. Dirks - G2WD, 4-8818

DATE: March 15, 1982

SUBJECT: Diet Analysis--Lifetime Toxicity Study in Mice with CP 76100

REFERENCE: IR-77-223; MAPC Job/Project #7163, Report #MSL-1893

TO *R. W. Street - C2SL

cc: E. E. Debus - C2SC
T. W. Fuhremann - G2WD
E. C. Spurrier - C2NA
Toxdata

The accompanying report contains the results of the stability and dosing solution analyses of the sodium salt of N-Nitrosoglyphosate (CP 76100) for the referenced study. Analyses were performed by the Research Division of Monsanto Agricultural Products Company (MAPC). A review of the data and an evaluation of the conclusions presented in this report are summarized below.

Methods

The test material, CP 76100, was supplied to International Research and Development Corporation by MAPC in the appropriate concentrations (5, 15, and 50 mg/ml) for dosing the test groups. Before submission, accuracy of the test concentrations were verified by MAPC-Research. To ensure that correct dosage levels were maintained, samples of the CP 76100 test solutions were assayed periodically by MAPC-Research throughout the study.

Results

Results of the test solution analyses showed that N-Nitrosoglyphosate (CP 76100) remained stable in all three test concentrations for the duration of the study. The average amount of CP 76100 found in all test solutions was 98.8, 93.7, and 116.9% of target concentrations for weeks 1-3, 52-54, and 103-105, respectively.

Conclusion

On the basis of these studies, it is concluded that the target concentrations of CP 76100 were accurately prepared and the test solutions were stable for the duration of the study. Therefore, assuming adequate dosing procedures, each animal received a dose of test material within ±10% of the target dose throughout the study.

Richard C. Dirks

Richard C. Dirks

/cld

MCE 0329824

*Receives report

IN-10M (REV. 2/78)

Monsanto

TR-77-223 (Dist. Analysis)

MAPC Research - St. Louis
(CO./DIV./DEPT./LOCATION)

Final REPORT
(TYPE OF REPORT)

REPORT NO.: MSL-1893

JOB/PROJECT NO.: 7163

DATE: October 20, 1981

TITLE: ANALYSIS OF DIET SOLUTIONS IN THE CP 76100 LIFE-TIME TOXICITY STUDY IN MICE CONDUCTED BY INTERNATIONAL RESEARCH AND DEVELOPMENT CORP., MATTAWAN, MICHIGAN

AUTHORS: C. M. Lottman

WORK DONE BY: C. M. Lottman

GROUP LEADER: S. Dubelman

ABSTRACT: A CP76100, life-time toxicity study in mice was conducted by International Research and Development Corp., Mattawan, Michigan (401-075). Analysis of a representative number of samples of diet solutions shows that CP76100 was stable throughout the duration of the study and that the concentrations of diet solutions actually received by the test animals corresponded closely to protocol. Control diet solutions were found to contain less than <0.01 mg/ml of CP76100 the sensitivity of our method. All samples were analyzed by high pressure liquid chromatography with a post-column Griess reaction detection system.

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REPT. NO.: MSL-1893 COPY NO.: 15

AUTHORS: C. M. Lottman

TITLE: ANALYSIS OF DIET SOLUTIONS IN THE CP76100 LIFE-TIME TOXICITY STUDY IN MICE CONDUCTED BY INTERNATIONAL RESEARCH AND DEVELOPMENT CORP., MATTAWAN, MICHIGAN

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I. INTRODUCTION

Chemical assays of diet preparations used in animal toxicity studies are required to confirm that pesticide levels being administered to test animals actually correspond to proposed concentrations throughout the term of the study.

A life-time study in mice with CP76100, the sodium salt of N-nitrosoglyphosate (N-nitroso-N-phosphonomethylglycine), was conducted by International Research and Development Corp. Test solutions of CP76100 were supplied by MAPC in appropriate concentrations for dosing the test groups. The administration of test solutions was carried out by IRDC. Samples of the CP76100 test solutions were assayed periodically throughout the study by MAPC-Research.

II. CONCLUSIONS

HPLC analysis of the diet solutions demonstrate that CP76100, the sodium salt of N-nitrosoglyphosate, was stable throughout the duration of the study and that test solutions were on an average of 102.4% of proposed levels.

III. MATERIALS AND METHODS

A. Sampling

Once a week, IRDC removed aliquots from CP76100 control, 5.0 mg/ml, 15.0 mg/ml, and 50.0 mg/ml test solutions and sent them to MAPC for analysis.

B. Analysis

The test solutions were analyzed by diluting samples to give a concentration between 3-4 micrograms per milliliter and injecting 40 microliters into a high pressure liquid chromatograph fitted with a post-column Griess reactor and a UV detector (546 nm).

Peak heights of samples were measured by electronic integration and compared via computer to a calibration curve made from appropriate standards of CP76976. (N-Nitrosoglyphosate, free acid)

HPLC data were corrected to give CP76100 equivalents and the concentration of original solutions were

calculated using the appropriate dilution factors. Controls were injected without dilution. Results for controls were < 0.01 milligrams per milliliter, the sensitivity of our method.

Details of the method are presented in Appendix A.

IV. RESULTS AND DISCUSSION

Assay results for the CP76100 diet solutions are presented in Table I. Samples from the control level (NaCl 5.0 mg/ml) were free of CP76100 (<0.01 mg/ml).

Samples from the 50 mg/kg/day level averaged 102.6% of expected, 150 mg/kg/day level averaged 102.7% of expected, and 500 mg/kg/day level averaged 101.8% of expected.

Results for stock solutions are summarized in Table II. Stock solutions averaged 99.1% of expected.

These results indicate that the CP76100 diet solutions were stable throughout the term of the toxicity study and that correct dose levels were maintained.

Table I

CP76100 DIET MONITORING PROGRAM

LIFE-TIME TOXICITY STUDY IN MICE

IRDC 401-075

Week No.	Date	Test Level Analyzed mg/kg/day	Dose Level Analyzed mg/ml	Found mg/ml Control	Found mg/ml Dosing Sample	% of Planned Concentration Found
1	8/2/79	50.0	5.0	<0.01	4.8	96.0
4	8/23/79	"	"	"	5.2	104.0
7	9/13/79	"	"	"	4.5	90.0
8	9/20/79	"	"	"	4.7	94.0
11	10/11/79	"	"	"	5.1	102.0
14	11/1/79	"	"	"	5.0	100.0
17	11/22/79	"	"	"	4.97	99.4
20	12/13/79	"	"	"	5.0	100.0
23	1/3/80	"	"	"	4.9	98.0
26	1/24/80	"	"	"	5.1	102.0
29	2/14/80	"	"	"	5.1	102.0
32	3/6/80	"	"	"	5.0	100.0
36	4/3/80	"	"	"	4.7	94.0
39	4/24/80	"	"	"	5.2	104.0
42	5/15/80	"	"	"	5.2	104.0
48	6/26/80	"	"	"	5.2	104.0
51	7/17/80	"	"	"	5.3	106.0
54	8/7/80	"	"	"	4.75	95.0
57	8/28/80	"	"	"	4.75	95.0
60	9/18/80	"	"	"	4.82	96.4
63	10/9/80	"	"	"	5.4	108.0
66	10/30/80	"	"	"	5.2	104.0
69	11/20/80	"	"	"	5.1	102.0
72	12/11/80	"	"	"	5.2	104.0
75	1/1/81	"	"	"	5.3	106.0
78	1/22/81	"	"	"	4.9	98.0
81	2/12/81	"	"	"	5.1	102.0
84	3/5/81	"	"	"	4.9	98.0
87	3/26/81	"	"	"	5.2	104.0
90	4/16/81	"	"	"	5.0	100.0
93	5/7/81	"	"	"	5.3	106.0
96	5/28/81	"	"	"	5.8	116.0
99	6/18/81	"	"	"	5.8	116.0
102	7/9/81	"	"	"	6.0	120.0
105	7/30/81	"	"	"	6.0	120.0
				Average	5.13	102.6%

Table I (continued)

CP76100 DIET MONITORING PROGRAM

LIFE-TIME TOXICITY STUDY IN MICE

IRDC 401-075

Week No.	Date	Test Level Analyzed mg/kg/day	Dose Level Analyzed mg/ml	Found mg/ml Control	Found mg/ml Dosing Sample	% of Planned Concentration Found
2	8/9/79	150.0	15.0	<0.01	14.95	99.7
5	8/30/79	"	"	"	15.10	100.7
9	9/27/79	"	"	"	14.5	96.7
12	10/18/79	"	"	"	15.1	100.7
15	11/8/79	"	"	"	15.3	102.0
18	11/29/79	"	"	"	14.95	99.7
21	12/20/79	"	"	"	14.0	93.3
24	1/10/80	"	"	"	15.0	100.0
27	1/31/80	"	"	"	15.3	102.0
30	2/21/80	"	"	"	15.8	105.3
33	3/13/80	"	"	"	14.9	99.3
34	3/20/80	"	"	"	14.5	96.7
37	4/10/80	"	"	"	16.8	112.0
40	5/1/80	"	"	"	15.1	100.7
43	5/22/80	"	"	"	14.8	98.7
46	6/12/80	"	"	"	16.1	107.3
49	7/3/80	"	"	"	14.9	99.3
52	7/24/80	"	"	"	14.4	96.0
55	8/14/80	"	"	"	16.0	107.0
61	9/25/80	"	"	"	15.5	103.3
64	10/16/80	"	"	"	15.8	105.3
67	11/6/80	"	"	"	15.8	105.3
70	11/27/80	"	"	"	15.4	102.7
73	12/18/80	"	"	"	15.4	102.7
76	1/8/81	"	"	"	14.5	96.7
79	1/29/81	"	"	"	15.2	101.3
82	2/19/81	"	"	"	15.1	100.7
85	3/12/81	"	"	"	14.8	98.7
88	4/2/81	"	"	"	15.1	100.7
91	4/23/81	"	"	"	15.1	100.7
94	5/14/81	"	"	"	16.4	109.3
97	6/4/81	"	"	"	17.6	117.3
100	6/25/81	"	"	"	18.0	120.0
103	7/16/81	"	"	"	17.7	118.0
				Average	15.4	102.7%

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Table I (continued)

CP76100 DIET MONITORING PROGRAM

LIFE-TIME TOXICITY STUDY IN MICE

IRDC 401-075

Week No.	Date	Test Level Analyzed mg/kg/day	Dose Level Analyzed mg/ml	Found mg/ml Control	Found mg/ml Dosing Sample	% of Planned Concentration Found
3	8/16/79	500.0	50.0	<0.01	50.4	100.8
6	9/6/79	"	"	"	46.7	93.4
10	10/4/79	"	"	"	50.1	100.2
13	10/25/79	"	"	"	48.3	96.6
16	11/15/79	"	"	"	49.5	99.0
19	12/6/79	"	"	"	48.9	97.8
22	12/27/79	"	"	"	49.2	98.4
25	1/17/80	"	"	"	50.3	100.6
28	2/7/80	"	"	"	51.0	102.0
31	2/28/80	"	"	"	49.4	98.8
35	3/27/80	"	"	"	49.1	98.2
38	4/17/80	"	"	"	50.3	100.6
41	5/8/80	"	"	"	51.5	103.0
44	5/29/80	"	"	"	53.0	106.0
47	6/19/80	"	"	"	54.3	108.6
50	7/10/80	"	"	"	52.8	105.6
53	7/31/80	"	"	"	45.0	90.0
55	8/14/80	"	"	"	52.7	105.0
56	8/21/80	"	"	"	46.7	93.4
59	9/11/80	"	"	"	48.3	96.7
62	10/2/80	"	"	"	51.2	102.4
65	10/23/80	"	"	"	53.1	106.2
68	11/13/80	"	"	"	50.6	101.2
71	12/4/80	"	"	"	50.9	101.8
74	12/25/80	"	"	"	53.0	106.0
77	1/15/81	"	"	"	46.9	93.8
80	2/5/81	"	"	"	52.4	104.8
83	2/26/81	"	"	"	51.0	102.0
86	3/19/81	"	"	"	48.5	97.0
89	4/9/81	"	"	"	51.0	102.0
92	4/30/81	"	"	"	52.2	104.4
95	5/21/81	"	"	"	53.4	106.8
98	6/4/81	"	"	"	56.3	112.6
101	7/2/81	"	"	"	57.6	115.2
104	7/23/81	"	"	"	56.4	112.8
				Average	50.9	101.8%

Table II

SUMMARY OF ANALYSES OF CP76100 STOCK SOLUTIONS

LIFE-TIME TOXICITY STUDY IN MICE

IRDC 401-075

Date	Lot No.	CP76100 mg/ml	Assay mg/ml	% Expected
7/24/79	1496821	0	0	
9/25/79	"	0	0	
3/20/79	"	0	0	
5/27/80	"	0	0	
11/10/80	"	0	0	
3/18/81	1949010			
7/24/79	1496821	5.0	4.7	94.0
9/25/79	"	"	4.6	92.0
3/20/79	"	"	4.95	99.0
5/27/80	"	"	5.1	102.0
11/10/80	"	"	5.1	102.0
3/18/81	1949010	"	5.2	104.0
		Average	4.94	98.8%
7/24/79	1496821	15.0	13.8	92.0
9/25/79	"	"	14.1	94.0
3/30/79	"	"	15.1	101.0
5/27/80	"	"	15.3	102.0
11/10/80	"	"	15.4	102.7
3/18/81	1949010	"	15.2	101.0
		Average	14.8	98.7%
7/24/79	1496821	50.0	47.9	95.8
9/25/79	"	"	49.4	98.8
3/30/79	"	"	47.8	95.6
5/27/80	"	"	51.9	103.8
11/10/80	"	"	51.4	102.8
3/18/81	1949010	"	51.4	102.8
		Average	49.96	99.9%

V. APPENDIX A

Analytical Method With Typical Chromatograms

ANALYTICAL METHOD FOR CP76100 IN TOXICOLOGY DIET SOLUTIONS

SCOPE

The analytical procedure given determines levels of CP76100 (sodium salt of N-nitrosoglyphosate) in aqueous solutions used for dosing animals in toxicology studies.

SUMMARY

The analytical method described is for the chemical assay of solutions of CP76100 used in toxicology studies. The procedure consists of diluting a sample of diet solution to give an appropriate concentration for assay by high pressure liquid chromatography with post-column Griess Reaction and detection by UV absorption.

SENSITIVITY

0.25 microgram per ml.

APPARATUS AND EQUIPMENT

Volumetric flasks and pipettes in the usual range of sizes.

Gelman Acrodisc disposable filter assembly 0.45 µm pore size.

Filter paper, 47 mm diameter 0.22 µm, Millipore Cat. No. GSWP04700.

REAGENTS

A. Analytical Standards

Weigh and dissolve 0.1000 g of N-nitrosoglyphosate (CP76976) in 1000 ml of filtered deionized water. This concentrate contains 100 micrograms of CP76976 per milliliter. Subsequent dilutions of this concentrate are made as follows:

Milliliters Concentrate	Standard Dilution	Concentration Micrograms per ml
1.0	100.0	1.0
2.0	100.0	2.0
3.0	100.0	3.0
5.0	100.0	5.0

HPLC standard solutions and dilutions are made with deionized water filtered through a 0.22 µ filter.

CP76976 solutions will decompose when exposed to UV light; therefore, precautions should be taken to avoid exposure to light such as storing in amber bottles under refrigeration.

PROCEDURE

Aliquots of CP76100 diet solutions are diluted appropriately to produce an analytical sample of 3 to 4 µg/ml concentration. A portion of this sample is filtered through a Gelman Acrodisc disposable filter assembly (0.45 µm pore size).

HPLC GRIESS POST COLUMN REACTOR SYSTEM

N-nitrosoglyphosate (CP76976) may be analyzed by using a high pressure liquid chromatograph interfaced with a detector specific for those compounds which hydrolyze in dilute acid to give nitrite. The detector is based on the use of the Griess reagent and the components needed for the construction of this detection system are outlined below. A general schematic and flow diagram is presented in Figure 1 while subsequent Figures 2-4 present detailed assembly diagrams for the areas labeled A, B and C in Figure 1. Several general comments concerned with the assembly and maintenance of this detection system are also presented.

A. Equipment and Supplies

Waters 6000A pump

Waters U6K injector (for manual injection) or Varian 8500 autosampler.

Waters Model 440 absorption detector fitted with a 546 nm filter.

Spectrum 1021 Filter Amplifier

MCE 0329836

Technicon Proportioning pump I.

Technicon heating bath (105-A-101-01, 37° C) modified by placing heating element under control of a Therm-O-Watch model L7-600.

Pump tube (Technicon 116-0549P03) 0.05 ml/min - Orange/Blue - one required.

Pump tube (Technicon 116-0549P06) 0.23 ml/min - Orange/White - three required.

Pump tube (Technicon 116-0549P08) 0.42 ml/min - Orange/Orange - two required.

Pump tube (Technicon 116-0549P11) 1.00 ml/min - Grey/Grey - one required - several extra pieces are useful for sleeving 1/16" teflon and stainless steel tubing.

Cactus "HS" Connector (Technicon 116-0207-05) - one required.

"A10" Connector (Technicon 116-B034-01) - two required.

"PT4" Connector (Technicon 116-B038-01) - one required.

Mixing Coils (Technicon 116-0127-04) - two required.

Heating Bath Coil [Technicon 105-1128-02 (inner) or 105-1123-02 (outer)] - one required.

C3 Debubbler (Technicon 116-0202P03) - one required.

Pulse Suppressor (Technicon 116-B044P02) - two required.

N5 Nipples (Technicon 116-0002P01) - seven required.

N8 Nipples (Technicon 116-0003P01) - thirteen required.

N13 Nipples (Technicon 116-0061P01) - two required.

Tubing, Acid Flex (Technicon 116-0529P02) - two feet- used for sleeving all bath exit connections.

Tubing, Polyethylene (Technicon 116-0454-01) - two feet - used for sleeving all glass/glass and all glass/N5 connections.

Tubing weights (Technicon 116-0454-01).

Tubing, Tygon 1/6" i.d. x 1/8" o.d. - enough for reagent lines from bottles to pump tube connections.

Tubing, Teflon - 3 mm i.d. x 1/16" o.d. - six feet - for cooling bath.

Beaker (2 liter) filled with water to serve as a cooling bath.

Glass tubing.

B. Reagents

Brij 35, 6% Solution (Fisher CS-285-2 diluted 1-5).

Hydrobromic Acid, 24% Solution (Mallinckrodt 0410 diluted 1-2).

Methanol LC grade.

Potassium dihydrogen phosphate. HPLC grade.

Phosphoric Acid, concentrated. HPLC grade.

N-1-napthylethylene diamine dihydrochloride (NED) (Fisher Scientific N-30), 0.1% solution in distilled water.

Sulfanilamide (Aldrich S652-5), 1% solution in 10% HCl.

Technicon Wetting Agent (Technicon T21-0332) - 1 ml/liter.

C. Buffer Solution Preparation

Prepare 0.07 M potassium dihydrogen phosphate (KH_2PO_4) by dissolving 38.1 g in four liters of 17.5% (v/v) methanol/deionized water. This solution is allowed to cool to room temperature and then is adjusted to pH 2.2 with concentrated phosphoric acid. Normal HPLC degassing procedures are followed as the solution is filtered through an 0.22 µm millipore filter.

D. HPLC Conditions

Column:	Partisil SAX, 25 cm x 4.6 mm i.d.
Column Temperature:	Ambient
Buffer Flow Rate:	1.5 ml/min
Pressure:	∿1500 psi

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E. General Comments

During the construction of all sleeved glass/glass and glass/N5 nipple connections the glass should be moistened with 2 drops of cyclohexanone to insure a good seal. Every effort should be made to have the pieces which are being connected to be butted together.

After the system has been constructed all lines should be conditioned by pumping an 0.01% Technicon wetting solution through them for 4-6 hours followed by a distilled water rinse for an equal period of time.

When starting turn all pumps and the detector on for 30 minutes prior to use. If an air bubble becomes trapped in the detector cell it can be removed by disconnecting the line from the cell to the AAI pump and alternately drawing and forcing liquid through the cell with a syringe containing water until the bubble is removed. Distilled water or a dilute Brij solution should be pumped through the detector system for 30 minutes prior to turning the autoanalyzer pump off. It is advised that all pump tubes be replaced at one week intervals.

F. Quantitation

Sample quantitation is based on the relative peak height or peak area of the sample to standard peak heights or areas across the range of expected sample concentrations.

G. Reference

Singer, G.M.; Singer, S.S. and Schmidt, D.G.; J. Chromatogr., 133 (1977) 59-66.

CALCULATIONS

Quantitation of analytical samples is done by interpolation from a standard calibration curve of peak area or peak height versus CP76976 concentration in micrograms per milliliter.

Because the HPLC standards are made of CP76976 (M.W. 198.08) and the diet solutions contain CP76100 (M.W. 220.07), the sodium salt of CP76976, HPLC data must be corrected to give the equivalent CP76100 concentrations. Thus,

CP76976 conc. x 1.111 = CP76100 conc.

When HPLC data are converted to CP76100 equivalents, the concentration of the original CP76100 diet solution is calculated by multiplication by the appropriate dilution factor.

Figure 1

GENERAL N-NITROSO DETECTOR SCHEMATIC

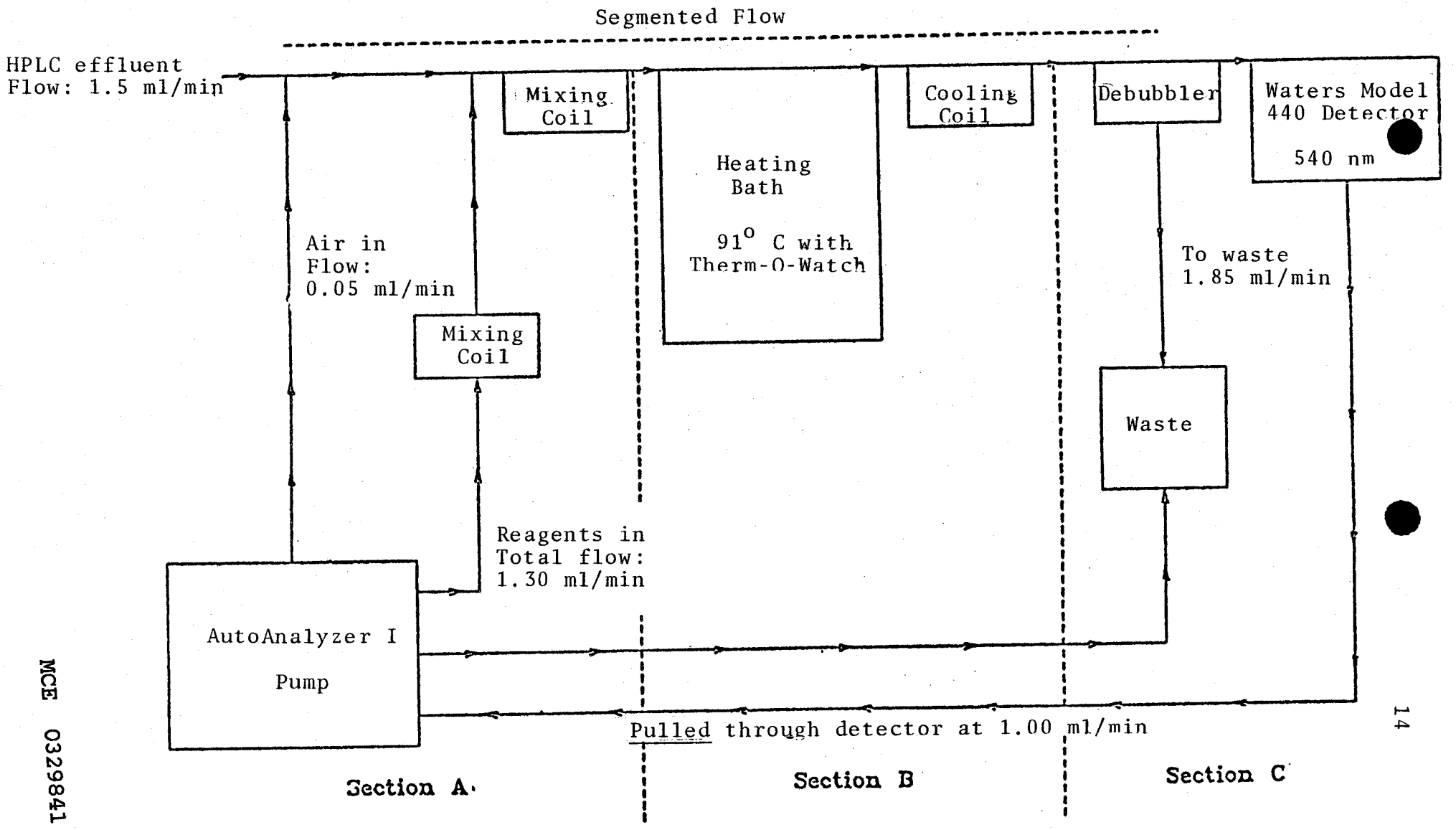

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Figure 2

Detailed Section A of General Schematic (Manifold to Heater)

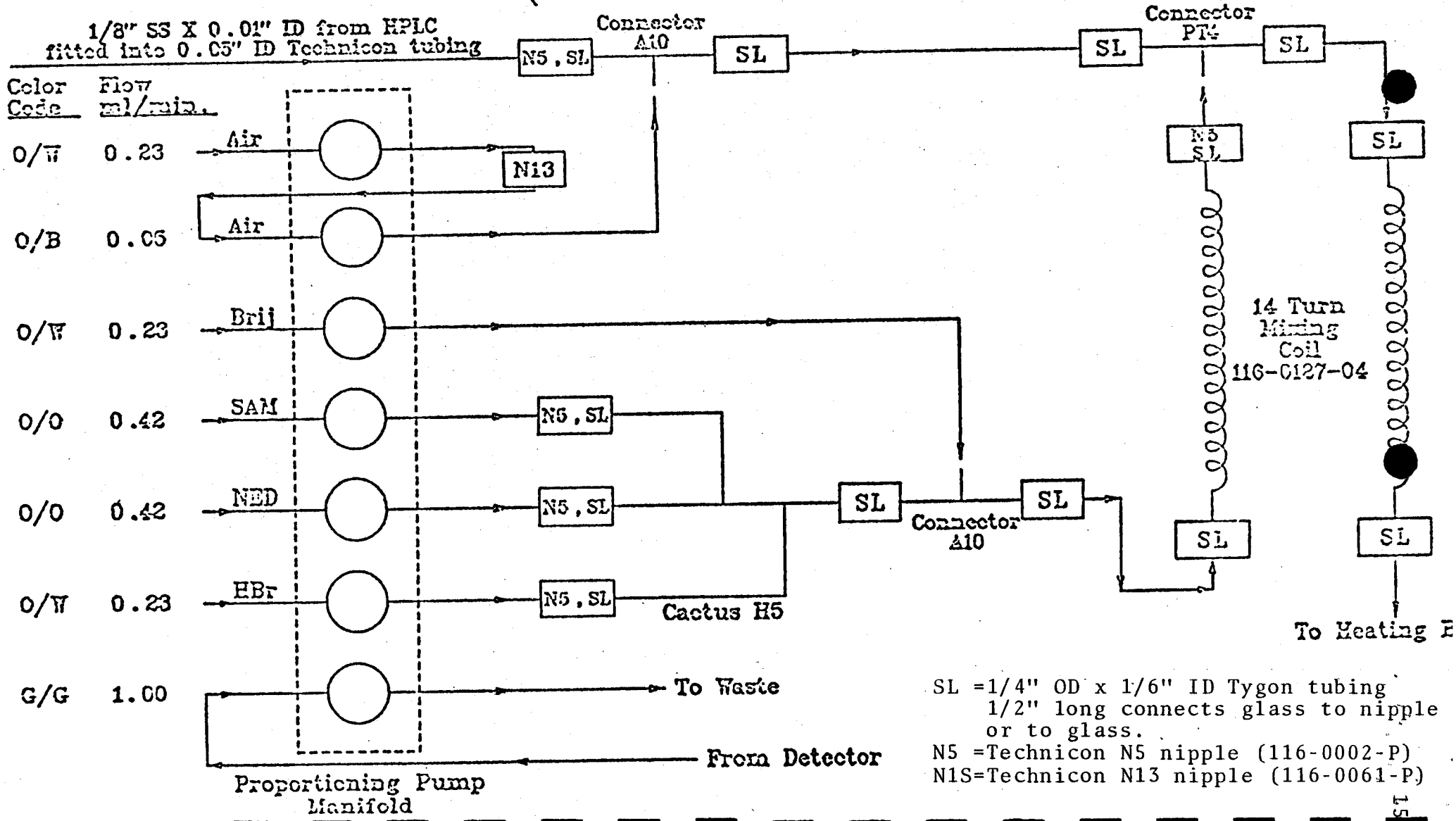
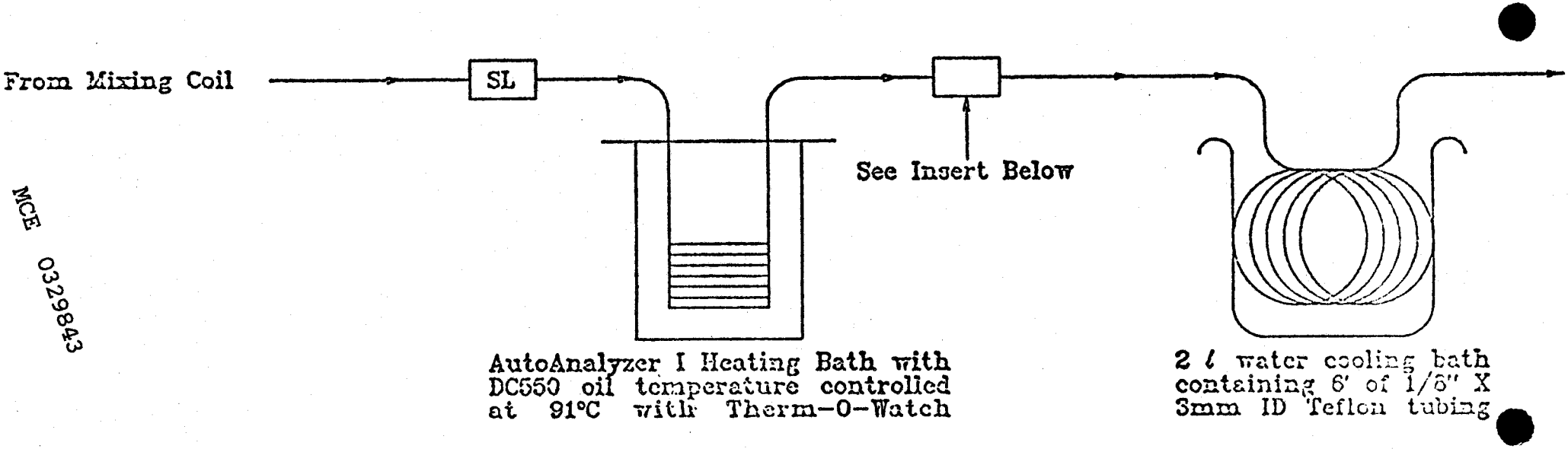


Figure 3

Detailed Section B of General Schematic (Heating and Cooling Area)



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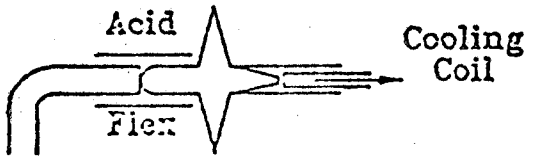


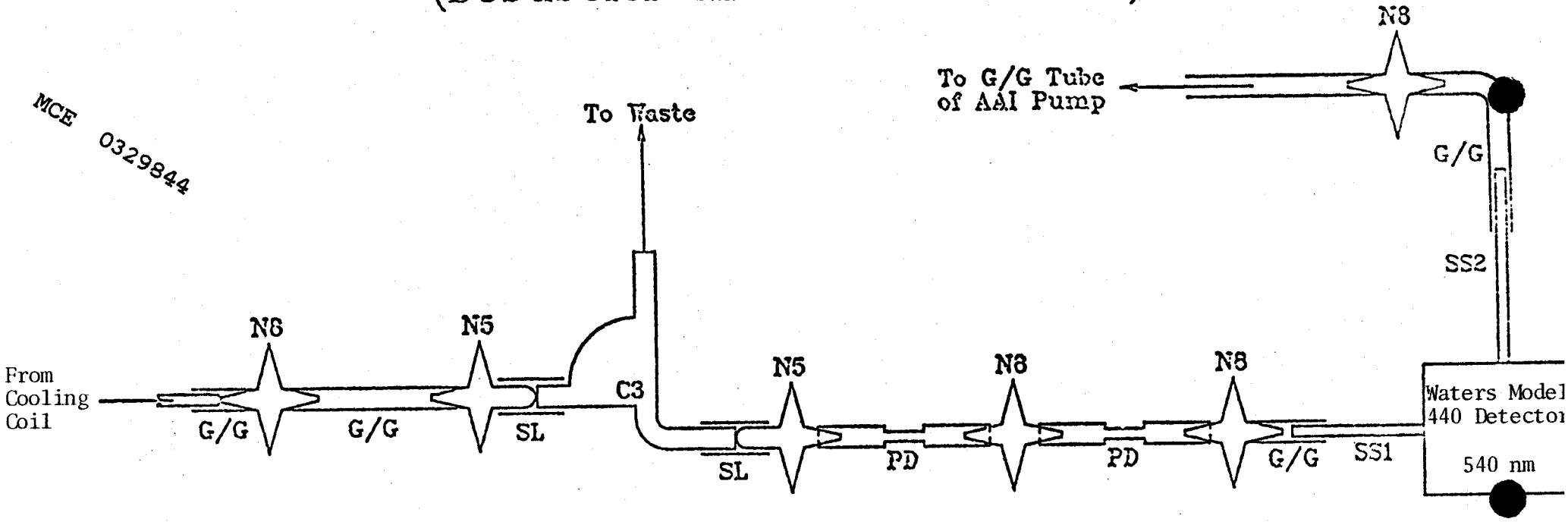
3/4" sleeve of acid flex tubing with N5 nipple to 2" of 0.051 ID (G/G) Technicon pump tube sleeved to 1/8" X 3 mm ID Teflon tubing.

SL=1/4" OD X 1/8" ID Tygon tubing 1/2" long connects glass to nipple or to glass.

16.

Figure 4

Detailed Section C of General Schematic (Debubbler and Detector Areas)

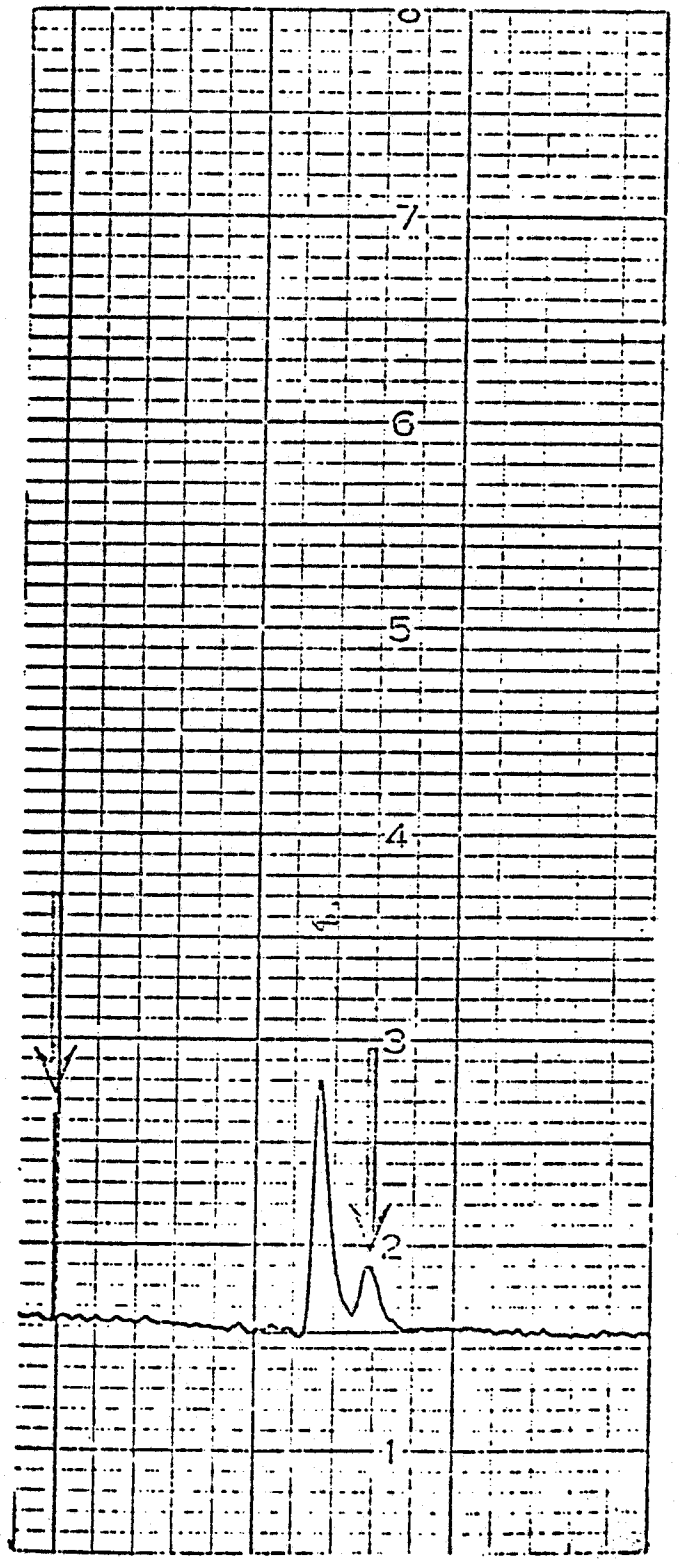


C3 = Technicon C3 debubbler (116-0202P03)
G/G = Technicon 0.05" id tubing
N5 = Technicon N5 nipple (116-0002-P01)
N8 = Technicon N8 nipple (116-0003-P01)
PD = Technicon pulse suppressor (116-B044-P02)
SL = 1/4" OD x 1/8" ID Tygon tubing
1/2" long connects glass to glass
SS1 = 1/8" x 0.01" ID x 2" long SS
tubing for detector cell entry line
SS2 = 1/8" x 0.02" ID x 6" long SS
tubing for detector cell exit line

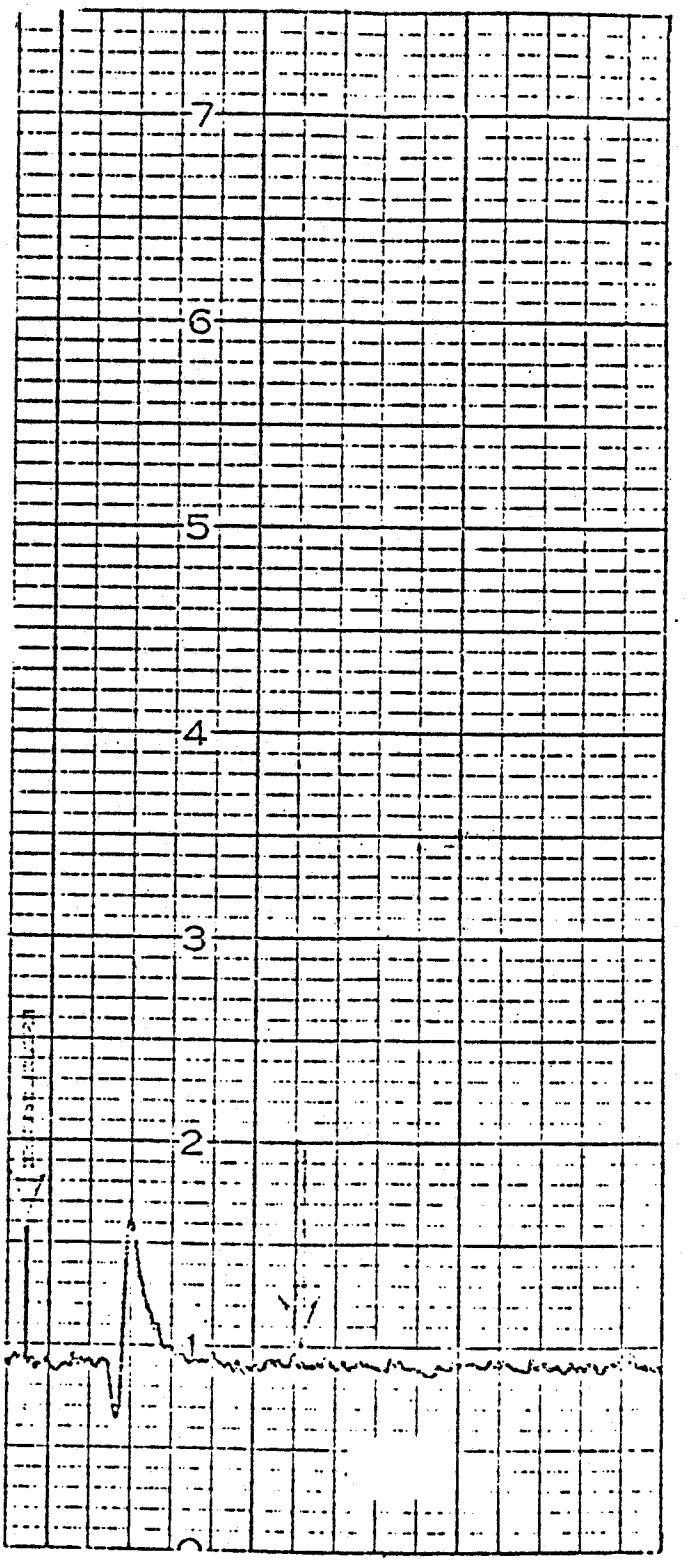
17.

CP76100 DIET MONITOR

0.25 µg/ml Std.



Control
Diet Solution



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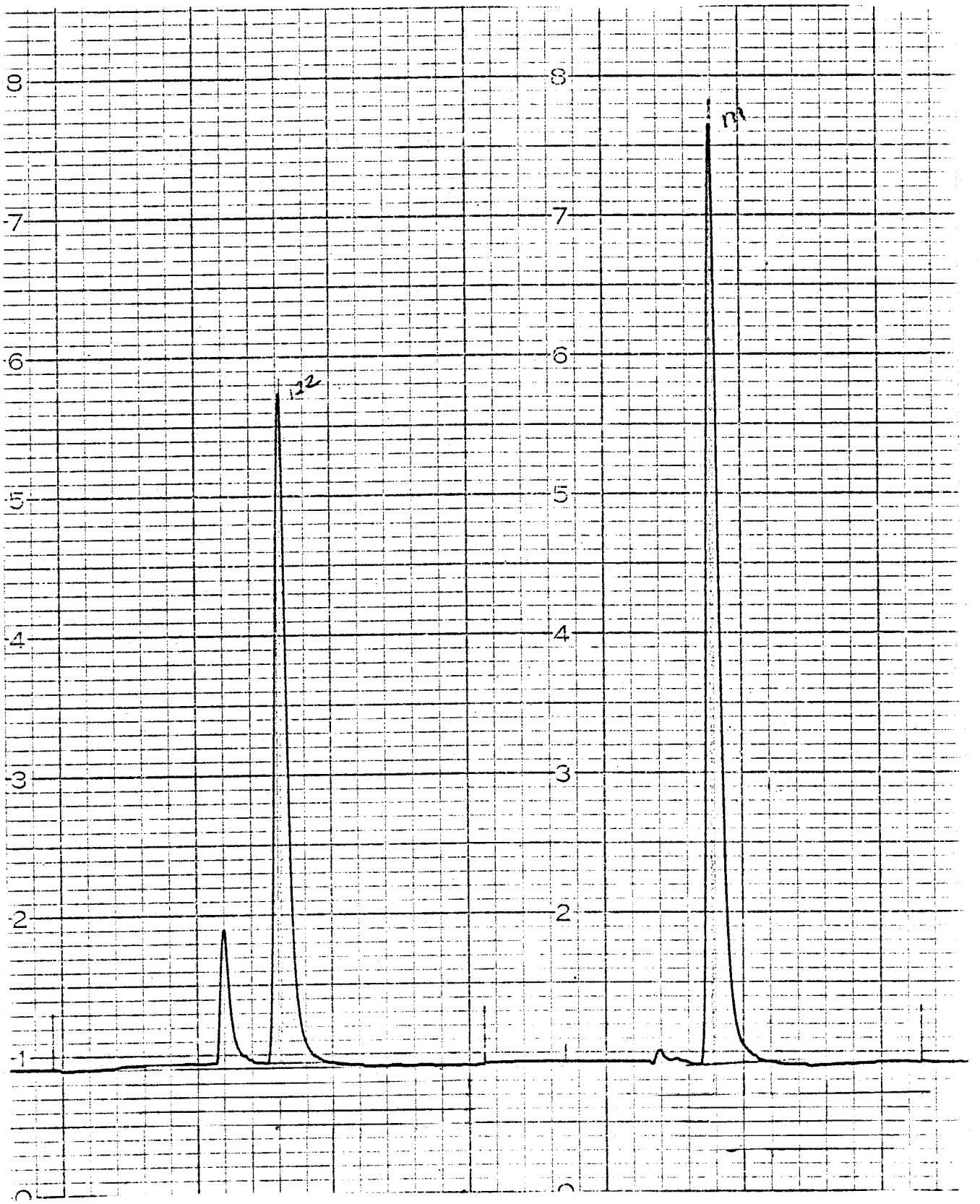
CP76100 DIET MONITOR



15.0 mg/ml

5.0 standard

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VI. APPENDIX B

All analytical data on CP76100 can be found in Monsanto notebooks 1503401 and 1889301.

VII. APPENDIX C

Project Cost Estimate

CML 3 man-months

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