MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES

3 4456 0262438 2

ORNL/TM-10472

OAK RIDGE NATIONAL LABORATORY

MARTIN MARIETTA

Test of Carcinogenicity in Mouse Skin: Methylenedianiline, γ Glycidyloxytrimethyloxysilane, γ Aminopropyltriethoxysilane and a Mixture of *m*-Phenylenediamine, Methylenedianiline, and Diglycidylether of Bisphenol-A

J. M. Holland
L. H. Smith
E. Frome
M. J. Whitaker
L. C. Gipson

OAK RIDGE NATIONAL LABORATORY
CENTRAL RESEARCH LIBRARY
CIRCULATION SECTION
4500N ROOM 175
LIBRARY LOAN COPY
DO NOT TRANSFER TO ANOTHER PERSON
If you wish someone else to see this report, send in name with report and the library will arrange a loan.

OPERATED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road, Springfield, Virginia 22161
NTIS price codes—Printed Copy: A03; Microfiche A01

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

ORNL/TM-10472

TEST OF CARCINOGENICITY IN MOUSE SKIN: METHYLENEDIANILINE, γGLYCIDYLOXYTRIMETHYLOXYSILANE, γAMINOPROPYLTRIETHOXYSILANE AND A MIXTURE OF m-PHENYLENEDIAMINE, METHYLENEDIANILINE, AND DIGLYCIDYLETHER OF BISPHENOL-A

J. M. Holland*
L. H. Smith
E. Frome
M. J. Whitaker
L. C. Gipson

Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

Date Published - June 1987

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37831
operated by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
Under Contract No. DE-AC05-84OR21400

*J. M. Holland left the Laboratory in 1982 and this document was prepared by R. J. M. Fry.

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES

3 4456 0262438 2

TABLE OF CONTENTS

	Page
List of Figures	iii
List of Tables	iv
Abstract	1
Introduction	2
Materials, Methods, and Experimental Design	2
Results and Analysis	5
Acute Toxicity	5
Carcinogenicity	5
Mortality	7
Discussion	8
Acknowledgments	9
References	10
Tables (1-12)	11-22
Figures (1-12)	23-34

LIST OF FIGURES

Figure		Page
1	Cyclohexane, females. Dose: 116.7 mg/week.	23
2	γGlycidyloxytrimethyloxysilane, females. Dose groups (DGRP), mg/week: A-78, B-62.4, C-46.8 in cyclohexane. . . .	24
3	γAminopropyltriethoxysilane, females. Dose groups (DGRP), mg/week: A-43.8, B-29.2, C-14.6 in cyclohexane. . .	25
4	Cyclohexane, males. Dose: 116.7 mg/week.	26
5	γGlycidyloxytrimethyloxysilane, males. Dose groups (DGRP), mg/week: A-78, B-62.4, C-46.8 in cyclohexane. . . .	27
6	γAminopropyltriethoxysilane, males. Dose groups (DGRP), mg/week: A-43.8, B-29.2, C-14.6 in cyclohexane. . .	28
7	Methanol, females. Dose: 116.7 mg/week	29
8	m-Phenylenediamine/Methylenedianiline/Diglycidylether of Bisphenol-A, females. Dose groups (DGRP), mg/week: A-10.8, B-5.4, C-2.7 in methanol.	30
9	Methylenedianiline, females. Dose groups (DGRP), mg/week: A-4.5, B-2.25, C-1.12 in methanol	31
10	Methanol, males. Dose: 116.7 mg/week	32
11	m-Phenylenediamine/Methylenedianiline/Diglycidylether of Bisphenol-A, males. Dose groups (DGRP), mg/week: A-10.8, B-5.4, C-2.7 in methanol.	33
12	Methylenedianiline, males. Dose groups (DGRP), mg/week: A-4.5, B-2.25, C-1.12 in methanol	34

LIST OF TABLES

Table		Page
1	Material tested in phase 1	11
2	Experimental design	12
3	Acute toxicity following daily dermal application of test materials	13
4	Organ weights in females following continuous dermal application of test materials	14
5	Organ weights in males following continuous application of test materials	15
6	Treatment related organ weight changes in animals exposed to the test materials	16
7	Cumulative skin tumor frequency	17
8	Frequency of grossly observed neoplasms for materials applied in methanol	18
9	Frequency of grossly observed neoplasms for materials applied in cyclohexane	19
10	Mortality analysis for materials applied in methanol	20
11	Mortality analysis for materials applied in cyclohexane	21
12	Body weight trends	22

ABSTRACT

Application of graded concentrations of four test substances in acetone to the skin of C3H mice five times a week for two weeks resulted in the following: γaminopropyltriethoxysilane at concentration of 100 and 50 wt/vol % was a severe and mild skin irritant in female C3Hf/Bd respectively and in males a mild skin irritant. Methylenedianiline at a concentration of 10 wt/vol % in methanol killed 4/9 females and 1/9 males. When acetone was the solvent 3/10 females and 3/10 males died within 2 weeks. No mortality or skin damage occurred with γglycidyloxytrimethyloxysilane or the mixture of m-phenylenediamine, methylenedianiline and diglycidylether of bisphenol A (MDA). A study of the effects of a two-year, three times a week, topical application of the four test materials revealed no significant increase in skin tumors. The incidence of liver tumors appeared to be increased by exposure to methylenedianiline and with the mixture (MDA). Significant and dose-dependent increases in mortality were found in male mice with MDA and increased mortality at the highest dose (10.8 mg/week) in females. In the case of methylenedianiline excess mortality was found in both sexes. The precise cause of excess mortality from dermal absorption of the materials applied over a two-year period was not established.

INTRODUCTION

The purpose of the study was to determine whether or not γ aminopropyltriethyoxysilane methylenedianiline, γ glycidyloxytrimethyloxysilane, and a mixture of m-phenylenediamine, methylenedianiline and diglycidylether of bisphenol A (MDA) are carcinogenic to the skin of mice. The mixture was tested in order to detect interactions. Given the possibility of carcinogenicity of the test materials a positive control was included. Benzo(a)pyrene [B(a)P] was applied at three dose levels so that relative potency estimates could be made if any of the test materials proved to be carcinogenic. Since many experiments with B(a)P have been carried out by us using the C3Hf/Bd mouse current results could be checked with previous results (1, 2).

In order to detect whether protracted exposure of the skin to the materials results in sufficient absorption to cause systemic effects, mortality was assessed and major organ systems were examined in mice dying and in those killed at the end of two years.

Materials, Methods and Experimental Design

The sources of the four test agents were as follows: the mixture composed of m-phenylenediamine, methylenedianiline, and diglycidylether of bisphenol-A from Maja, Ltd., methylenedianiline from Allied Chemical; and γglycidyloxytrimethyloxysilane and γaminopropyltriethoxysilane from Union Carbide Corporation (Table 1). The positive control agent benzo(a)pyrene was purchased from Sigma Chemical Company. The vehicles were acetone, methanol and cyclohexane obtained from Fisher Scientific Company. All agents and vehicles were kept in the dark at room temperature in well-stoppered amber

glass containers. Dilutions of each agent were made biweekly and kept in glass-stoppered Erlenmeyer flasks within an unlighted fume hood.

Male and Female C3Hf/Bd mice were produced by the animal facility of this laboratory and supplied free of enzootic murine viruses and endo- and ectoparasites. Mice were randomly entered into the experiment when 8 to 10 weeks old. They were housed five per cage which contained ground corn cob bedding and were given Purina 5010-C chow and hyperchlorinated water ad libitum. Mice were maintained for the duration of the study in a closed-colony limited access conventional animal facility on 12 hour light - 12 hour dark cycle with gold lights.

Prior to commencing the chronic test, acute toxicity testing of the four agents was carried out to determine the maximum tolerated dose. The agents were applied to the clipped skin of young adult mice in a volume of 50 µl and at various concentrations. The tests consisted of exposing 10 animals of both sexes 5 days a week for 2 weeks to various doses of each agent dissolved in vehicle. Clinical and pathological responses that were assessed measured included body weight change, final organ weights, mortality, and skin irritation. From these data, three doses of each agent were selected that were predicted to produce minimal systemic toxicity from chronic dermal exposure.

For the definitive chronic study, each of the four agents was applied in a volume of 50 µl to the clipped skin of 9 to 12 week-old mice on Monday, Wednesday and Friday of each week for a period of 24 months. A 24-month cut-off was chosen because the spontaneous mortality rate increases rapidly after this time. The hair of the mice was clipped one week prior to the first application and reclipped as required. Three doses of each agent were

tested on 40 male and 40 female mice. The doses and solvents used as vehicles are shown in Table 2.

The selection of a solvent is often determined by the capability to solubilize rather than its benign nature. It was necessary to use cyclohexane as a solvent for γ glycidyloxytrimethyloxysilane and γ amino-propyltriethoxysilane. Cyclohexane may not be without effects, however it is a good solvent and in C3Hf/Bd mice has not produced malignant tumors, in particular, skin tumors, or increased significantly the mortality rate of aging mice (2). As indicated in Table 2, Methanol was used as the solvent for the mixture and methylenedianiline and cyclohexane for the other materials under test.

Control groups of 40 males and 40 females were as follows: the positive control substance benzo(a)pyrene in methanol or cyclohexane (0.01%, 15 μg per week) and methanol or cyclohexane as vehicle control substances. Thirty mice of each sex were also included as untreated room controls. Vital statistics recorded for each mouse included birth date, date exposure began, date the first skin tumor was observed, and death date. Two weeks, after the appearance of the first skin tumor, mice were sacrificed. Interim body weights were determined at 6, 12 and 18 months of exposure. At the time of death, mice were weighed and necropsied. All major organs were examined carefully for tumors. Abnormalities were recorded on necropsy cards and tissues taken to confirm the gross diagnosis. Skin tumors and suspect skin lesions were routinely obtained for histopathologic evaluation.

RESULTS AND ANALYSIS

Acute Toxicity

Information about acute toxicity is required both for the assay of potential toxic effects as a result of dermal exposure and also in order to select exposure levels for the assessment of carcinogenicity. Lastly, the initial studies are used to determine the appropriate solvent.

The results of the acute toxicity study are shown in Tables 3-6. In Table 3 it can be seen that the two higher exposure levels of γaminopropyltriethoxysilane in acetone caused skin damage in female C3Hf/Bd mice. In the case of the highest concentration the damage was sufficient to cause ulceration. The effects were less marked in the male mice. The highest doses of methylenedianiline in acetone caused the death of 3 of 10 mice of each sex. When methanol was the solvent 4 of 10 females and 1 of 10 males died within the two week test period.

The changes in body weight over the relatively short period of 2 weeks did not show trends that are of a help in assessing the toxicity of the materials. Some information indicative of systemic toxicity is obtained from changes in organ weights. The changes in liver, kidney and spleen in male and female mice are shown in Tables 4 and 5, respectively. Changes in spleen weight were the most marked. In Table 6 are given the dose-dependent changes in organ weight relative to methanol controls.

Carcinogenicity

None of the materials under test which were applied to the skin over a 24-month period caused a significant increase in skin tumors. The study involved 3 doses with about 2-4 fold range. In fact, the only tumors noted

(and they were benign papillomas) were noted in cyclohexane solvent control groups. These results may be compared with the dose-dependent and marked increase in skin cancer with B(a)P in either solvent which were used as positive controls. These positive controls make it possible to establish relative potency.

The experiment was designed to test skin carcinogenicity and the result of this test indicates no carcinogenicity at relatively high doses per week and at high total doses (Table 7).

The frequency of all other neoplasms were recorded at autopsy and the observed incidences of tumors in the major organs are shown in Tables 8 and 9. The results raise the question of carcinogenicity of the mixture and methylenedianiline, in only one organ, namely the liver. Liver tumors in C3H mice occur at a very high natural incidence. Increased incidences can occur in this strain, especially in males with agents or conditions not usually considered carcinogenic. For example, the protein content of the diet and the number of mice per cage can alter the incidence of the hepatic tumors (2). Furthermore, some agents, whether or not recognized carcinogens, appear to advance the time of appearance of tumors rather than increase the cumulative lifetime incidence. This observation raises question about the significance of any positive finding. Nevertheless, the crude incidences that have not been age-adjusted suggest, especially in the case of females, that methylenedianiline increases the incidence of liver tumors. We do not consider C3Hf mice the most appropriate test animal for liver carcinogenicity but the observation that a systemic effect may have occurred and its significance may require further study. However, the increase in the incidence of liver tumors in the female mice indicates that there may be a

carcinogenic effect.

Mortality

Mortality data is precise and the endpoint is incontrovertable. On the other hand the amount of information that may be obtained depends on the analysis. The differences in mortality between the groups especially those exposed to the test materials compared to solvent controls are shown in Figs. 1-12 and the analyses are shown in Tables 10 and 11. In the case of γ glycidyloxytrimethoxysilane one dose group in females and with γ aminopropyltriethoxysilane a dose group in males showed significant increase in mortality from controls. However, the significance of these findings is not clear because a dose-response relationship has not been established and the affected mice are in neither the high-dose or the low-dose groups (Table 11). In the case of methylenedianiline and the mixture (MDA) there were significant and dose-dependent increases in mortality in both sexes (Tables 8 and 9). The assay indicated that both methylenedianiline and the mixture (MDA) are toxic when absorbed through the skin in sufficient quantity. The weight changes over the 2-year period of application are shown in Table 12 and are consistent with the mortality data.

DISCUSSION

The main objective of this study was to determine whether or not the test materials could cause skin cancer. The answer is that the materials are not skin carcinogens under the conditions of the test. However, the materials are toxic especially methylenedianiline and the mixture of m-phenylenediamine, methylenedianiline and diglycidylether of bisphenol-A. The question of carcinogenicity for organs other than the skin was not resolved but there is no evidence of a carcinogenic effect on the lung, at least in this strain. The results indicate that methylenedianiline increases the crude incidence of liver tumors but it has been noted the C3H mouse is unusually susceptible to liver tumor and the significance of the finding requires further study.

ACKNOWLEDGMENTS

The authors are grateful to the animal care specialists, and to Mrs. Faye Young for secretarial help.

REFERENCES

1. J. M. Holland, D. G. Gosslee, L. C. Gipson and M. J. Whitaker. ORNL-5375, (1978).
2. J. M. Holland, D. A. Wolf and B. R. Clark. Environ. Health Perspect. 38, 149 (1981).
3. C. Peraino, R. J. M. Fry and E. Staffeldt. J. Natl. Cancer Inst. 51, 1349, (1973).
4. D. R. Cox. J. Royal Statistical Society B 34, 187-220 (1972).
5. F. E. Harrel. SUGI Supplemental Libary User's Guide, Version 5 Edition, Cary, NC: SAS Institute, Inc. (1986).

Table 1. Material tested in phase 1.

ID No.	Principal component(s)	Wt %	Source	CAS No.	Batch No.
2	m-Phenylenediamine	56.7	Maja, Ltd.		
	Methylenedianiline	30.3		Eutectic mix	9000032JP
	Diglycidylether of Bisphenol-A	13.0			
3	Methylenedianiline	*	Allied Chemical	101-77-9	170-C-16
4	γ Glycidyloxy-trimethoxysilane	*	Union Carbide Corporation	2530-83-8	1117MI021580
5	γ Aminopropyl-triethoxysilane	*	Union Carbide Corporation	919-30-2	1131T50825 80

*The information available about these materials was limited.

Table 2. Experimental Design

Compound	CAS No.	Test ID	Solvent	Concentration w/v%	Dosage* mg/wk
m-Phenylenediamine					
Methylenedianiline	Mixture	2	Methanol	6,3,1,5	10.8,5.4.2.7
Diglycidylether of bisphenol-A					
Methylenedianiline	101-77-9	3	Methanol	3,1.5,0.75	4.5,2.25,1.12
γ Glycidyloxy-trimethyloxysilane	2530-83	4	Cyclohexane	50,40,30	78,62.4,46.8
γ Aminopropyl-trimethyloxysilane	919-30	5	Cyclohexane	30,20,10	43.8,29.2,14.6

*Corrected for density, based upon three applications per week Monday, Wednesday, and Friday in 50 μl of the solvent.

Table 3. Acute toxicity following daily dermal application of test materials.

ID No.	Solvent	Concentration (wt/vol %)	No. of mice Female	No. of mice Male	No. of Deaths Female	No. of Deaths Male	Body Weight Change (%) Female	Body Weight Change (%) Male	Skin Irritation Female	Skin Irritation Male
2	Acetone	10	10	10	0	0	+4.1	-2.5	0	0
		5	10	10	0	0	+4.5	+7.7	0	0
		2.5	10	10	0	0	+3.3	+2.9	0	0
2	Methanol	10	10	10	0	0	+0.8	+2.6	0	0
		5	9	10	0	0	-0.4	+4.0	0	0
		2.5	9	10	0	0	-1.4	+4.0	0	0
3	Acetone	10	10	10	3	3	+6.7	+5.0	0	0
		5	10	10	0	0	-0.4	+3.6	0	0
		2.5	10	10	0	0	+4.6	-1.3	0	0
3	Methanol	10	9	9	4	1	-1.7	+0.7	0	0
		5	10	9	0	1	+4.8	+2.0	0	0
		2.5	9	10	0	0	-3.1	+4.5	0	0
4	Acetone	100	10	9	0	0	+6.6	+6.4	0	0
		50	10	10	0	0	+7.3	+3.1	0	0
		25	10	10	0	0	+7.1	+5.6	0	0
5	Acetone	100	10	10	0	0	+1.2	+4.7	Severe	Mild
		50	10	10	0	0	+5.6	+5.3	Mild	0
		25	10	9	0	0	+9.1	+6.8	0	0
Methanol solvent control			10	10	0	0	+4.4	+4.6	0	0

Table 4. Organ weights in females following continuous dermal application of test materials.

ID No.	Solvent	Concentration (wt/vol. %)	No. of Animals	Average tissue weight, gm (±se) Liver	Kidney	Spleen
2	Acetone	10	10	1.68(.030)	0.443(.005)	0.169(.008)
		5	10	1.39(.045)	0.360(.016)	0.135(.004)
		2.5	10	1.50(.034)	0.369(.008)	0.124(.003)
2	Methanol	10	10	1.64(.051)	0.417(.014)	0.139(.007)
		5	9	1.60(.042)	0.417(.009)	0.132(.005)
		2.5	9	1.84(.072)	0.438(.011)	0.125(.010)
3	Acetone	10	7	1.73(.058)	0.395(.014)	0.198(.013)
		5	10	1.52(.049)	0.367(.016)	0.169(.009)
		2.5	10	1.63(.058)	0.363(.013)	0.152(.008)
3	Methanol	10	5	2.03(.055)	0.455(.013)	0.204(.010)
		5	10	2.00(.045)	0.436(.008)	0.186(.008)
		2.5	9	1.59(.037)	0.431(.010)	0.131(.005)
4	Acetone	100	10	1.62(.038)	0.432(.009)	0.130(.005)
		50	10	1.53(.034)	0.424(.012)	0.125(.003)
		25	10	1.51(.036)	0.420(.007)	0.115(.003)
5	Acetone	100	9	1.50(.053)	0.429(.009)	0.158(.014)
		50	10	1.52(.041)	0.444(.008)	0.138(.010)
		25	10	1.45(.047)	0.415(.015)	0.111(.002)
Methanol control		-	10	1.63(.062)	0.427(.015)	0.110(.003)

Table 5. Organ weights in males following continuous application of test materials.

ID No.	Solvent	Concentration (wt/vol. %)	No. of Animals	Average tissue weight, gm (±se) Liver	Kidney	Spleen
2	Acetone	10	10	1.94(.058)	0.679(.020)	0.166(.004)
		5	10	1.77(.065)	0.632(.025)	0.121(.003)
		2.5	10	1.58(.022)	0.523(.010)	0.090(.002)
2	Methanol	10	10	1.63(.042)	0.565(.014)	0.127(.004)
		5	10	1.75(.056)	0.631(.020)	0.115(.003)
		2.5	10	1.80(.037)	0.628(.009)	0.103(.003)
3	Acetone	10	4	2.21(.132)	n.t.	0.292(.035)
		5	10	2.15(.073)	0.541(.022)	0.227(.013)
		2.5	8	1.93(.077)	n.t.	0.128(.010)
3	Methanol	10	9	2.20(.044)	0.608(.012)	0.235(.009)
		5	9	1.81(.083)	0.569(.018)	0.168(.005)
		2.5	10	1.61(.020)	0.600(.018)	0.120(.003)
4	Acetone	100	9	1.73(.057)	0.648(.024)	0.098(.003)
		50	10	1.82(.040)	0.636(.016)	0.098(.003)
		25	10	1.77(.043)	0.628(.016)	0.097(.003)
5	Acetone	100	10	2.90(.047)	0.693(.010)	0.125(.008)
		50	10	1.96(.069)	0.649(.025)	0.109(.005)
		25	9	1.75(.033)	0.609(.018)	0.106(.004)
Methanol solvent control		-	10	1.71(.023)	0.605(.011)	0.009(.021)

Table 6. Treatment related organ weight changes in animals exposed to the test materials.

		Dose-dependent changes in organ weight relative to methanol control		
Material	Sex	Liver	Kidney	Spleen
2	Female	nc[1]	nc	↑
	Male	↑[3]	↑[3]	↑
3	Female	↑[2]	↑[2]	↑
	Male	↑	nc	↑
4	Female	nc	nc	↑
	Male	nc	↑	nc
5	Female	nc	nc	↑
	Male	↑	↑	nc

[1] nc, no change.

[2] Methanol only.

[3] Acetone only.

Spleen weight proved to be the most sensitive indicator, followed by liver and kidney. Given the known tendency for materials 2 and 3 to form Schiff bases with acetone as a solvent the differences in apparent toxicity as a function of solvent could reflect responses to the Schiff base reaction products and not the starting material.

Table 7. Cumulative Skin Tumor Frequency

Material (solvent)	Exposure rate (mg/wk)	No. of Histologically Confirmed Skin Tumors (sexes combined)			
		Papilloma	Squamous Carcinoma	Fibroma	Other
2[a] (Methanol)	10.8	0	0	0	0
	5.4	0	0	0	0
	2.7	0	0	0	0
3[a] (Methanol)	4.5	0	0	0	0
	2.25	0	0	0	0
	1.12	0	0	0	0
4[a] (Cyclohexane)	78.0	0	0	0	0
	62.4	0	0	0	0
	46.8	0	0	0	0
5[a] (Cyclohexane)	43.8	2	0	0	0
	29.2	0	0	0	0
	14.6	0	0	0	0
B(a)P (Cyclohexane)	0.015	11	60	0	0
	0.0075	14	43	1	1
	0.00375	28	19	1	2
B(a)P (Methanol)	0.015	3	75	0	0
	0.0075	11	62	0	0
	0.00375	10	23	4	0
Cyclohexane[b]	116.7	1	0	0	0
Methanol[b]	119	0	0	0	0

[a]40 animals per sex, per dose.

[b]100 animals per sex, per dose.

Table 8. Frequency of Grossly Observed Neoplasms for Materials Applied in Methanol.

Material ID No.	Sex	Dose (mg/wk)	Observed Tumor Incidence (%)				
			Liver	Spleen	Kidney	Ovary/Testis	Lung
2*	F	10.8	25	0	4	40	0
		5.4	25	0	0	55	8
		2.7	20	0	0	68	10
	M	10.8	55	0	0	0	0
		5.4	68	0	0	0	2
		2.7	60	0	0	0	5
3*	F	4.5	85	2	4	52	10
		2.25	25	0	0	40	10
		1.12	22	0	2	55	8
	M	4.5	95	0	2	0	12
		2.25	75	0	0	0	2
		1.12	55	0	0	0	8
Methanol**	F	119	11	2	2	49	16
	M	119	53	0	0	0	9

*40 animals per sex, per dose.

**100 animals per sex.

Table 9. Frequency of Grossly Observed Neoplasms for Materials Applied in Cyclohexane.

Material ID No.	Sex	Dose (mg/wk)	Observed Tumor Incidence (%)				
			Liver	Spleen	Kidney	Ovary/Testis	Lung
4*	F	78	12	0	2	60	15
		62.4	18	0	0	52	2
		46.8	12	0	0	52	15
	M	78	62	0	2	0	8
		62.4	60	0	2	2	12
		46.8	42	0	2	0	2
5*	F	43.8	12	0	0	45	8
		29.2	12	0	0	50	12
		14.6	18	0	4	50	12
	M	43.8	48	0	0	2	15
		29.2	58	0	2	0	15
		14.6	58	0	0	0	18
Cyclo-hexane**	F	117	18	0	3	44	10
	M	117	52	0	1	0	13

*40 animals per sex, per dose.

**100 animals per sex, per dose.

Table 10. Mortality Analysis for Materials Applied in Methanol.

Material ID No. (or solvent)	Sex	Dose (mg/wk)	24 Month survival (%)	Risk Coefficients* $\hat{\beta}$	sd	$\frac{\hat{\beta}}{sd}$**
Methanol	F	119	60.7	-	-	-
	M	119	62.9	-	-	-
2	F	10.8	20.5	1.14	0.24	4.7
		5.4	53.8	0.27	0.27	
		2.7	61.5	0.02	0.28	
	M	10.8	32.5	0.78	0.24	3.2
		5.4	35.0	0.74	0.24	3.1
		2.7	37.5	0.53	0.25	2.1
3	F	4.5	15.4	1.39	0.24	5.8
		2.25	25.7	0.97	0.25	3.9
		1.12	35.9	0.82	0.25	3.3
	M	4.5	35.0	0.83	0.24	3.5
		2.25	35.9	0.66	0.25	2.6
		1.12	62.5	-0.12	0.29	

*From the proportional hazards regression model $\lambda_j(t) = \lambda_o(t)e^{\hat{\beta}_j}$ where $\lambda_o(t)$ denotes the "force of mortality" at age t for the vehicle control group so that $e^{\hat{\beta}_j}$ (j = dose group A, B, C) is the "relative risk" for dose group j. The natural log of the relative risk, $\hat{\beta}$, is positive if the risk in treated animals is increased over that in the control groups.

**For $\frac{\hat{\beta}}{sd} > 1.95$ $P \leq 0.05$. Values are shown only if $P \leq .05$.

See reference 4 for details of proportional hazards regression model of Cox (1972) and reference 5 for the computational procedure.

Table 11. Mortality Analysis for Materials Applied in Cyclohexane.

Material ID No.	Sex	Dose (mg/wk)	24 Month survival (%)	Risk Coefficients* $\hat{\beta}$	sd	$\frac{\hat{\beta}}{sd}$**
	F	78	48.7	0.30	0.24	
		62.4	32.5	0.55	0.23	2.4
4		46.8	52.6	0.01	0.26	
	M	78	51.3	0.14	0.26	
		62.4	51.3	0.08	0.27	
		46.8	47.5	0.16	0.26	
	F	43.8	50.0	0.12	0.25	
		29.2	48.6	0.03	0.26	
5		14.6	61.5	-0.06	0.26	
	M	43.8	48.6	0.30	0.26	
		29.2	40.5	0.52	0.25	2.1
		14.6	50.0	0.11	0.26	

*See Table 10.

**See Table 10.

Table 12. Body Weight Trends

Material (solvent)	Sex	Dose (w/v%)	Average Weight (±se), g			
			6 Months	12 Months	18 Months	24 Months
2	F	6%	26.7(0.4)	28.8(0.5)	29.7(0.6)	25.4(0.7)
(Methanol)	M		33.8(0.3)	34.4(0.4)	34.1(0.5)	28.1(0.6)
3	F	3%	29.5(0.3)	30.8(0.4)	32.6(0.4)	28.2(1.0)
(Methanol)	M		35.2(0.4)	36.6(0.5)	35.0(0.6)	26.7(0.7)
4	F	50%	27.9(0.3)	28.5(0.4)	30.4(0.5)	27.2(0.7)
(Cyclohexane)	M		33.4(0.3)	34.4(0.4)	34.6(0.5)	28.9(0.8)
5	F	30%	28.5(0.3)	29.9(0.3)	31.0(0.4)	27.9(0.6)
(Cyclohexane)	M		34.3(0.6)	35.9(0.7)	36.1(0.6)	28.2(0.9)
B(a)P	F	0.01%	29.4(0.3)	31.1(0.4)	N.D.	29.5(0.3)
(Cyclohexane)	M		34.4(0.3)	36.5(0.4)	N.D.	34.4(0.3)
(Cyclohexane)	F	100%	28.9(0.3)	30.2(0.4)	31.0(0.5)	26.9(0.5)
(Cyclohexane)	M		33.5(0.5)	35.9(0.5)	34.6(0.6)	27.6(0.4)
B(a)P	F	0.01%	29.7(0.4)	31.0(0.4)	N.D.	29.1(0.4)
(Methanol)	M		35.3(0.4)	35.5(0.4)	N.D.	34.0(0.5)
(Methanol)	F	100%	28.8(0.4)	29.9(0.5)	29.6(0.7)	27.4(0.4)
(Methanol)	M		36.3(0.5)	37.5(0.6)	37.1(0.6)	29.3(0.5)
Untreated	F	-	N.D.	N.D.	33.1(0.8)	30.1(0.9)
Untreated	M		N.D.	N.D.	44.3(0.6)	34.4(1.8)

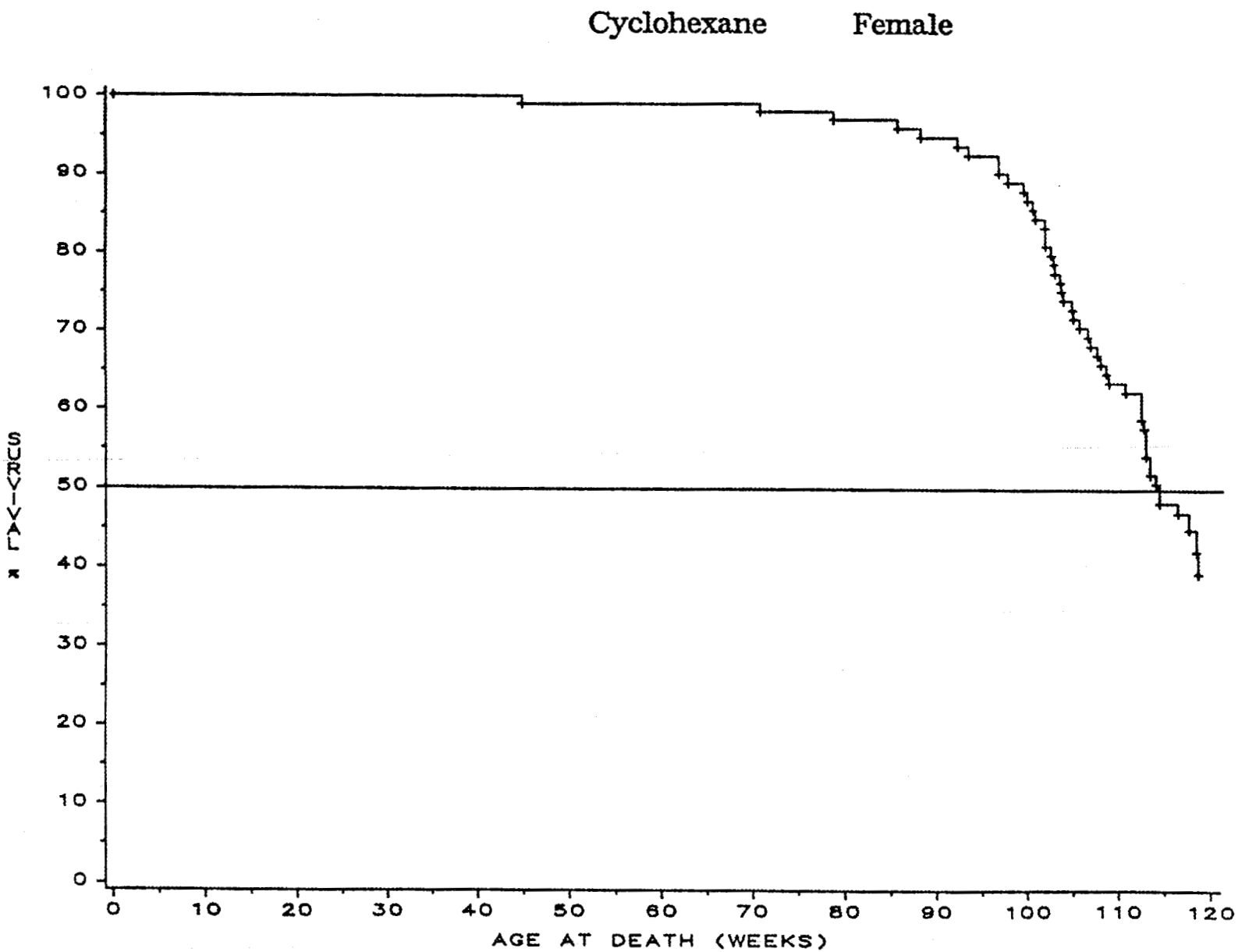

Fig. 1. Survival of female C3Hf/Bd mice treated with cyclohexane (solvent) 3 treatment/wk for 24 months, total dose/wk: 116.7 mg.

γ Glycidyloxytrimethyloxysilane Female

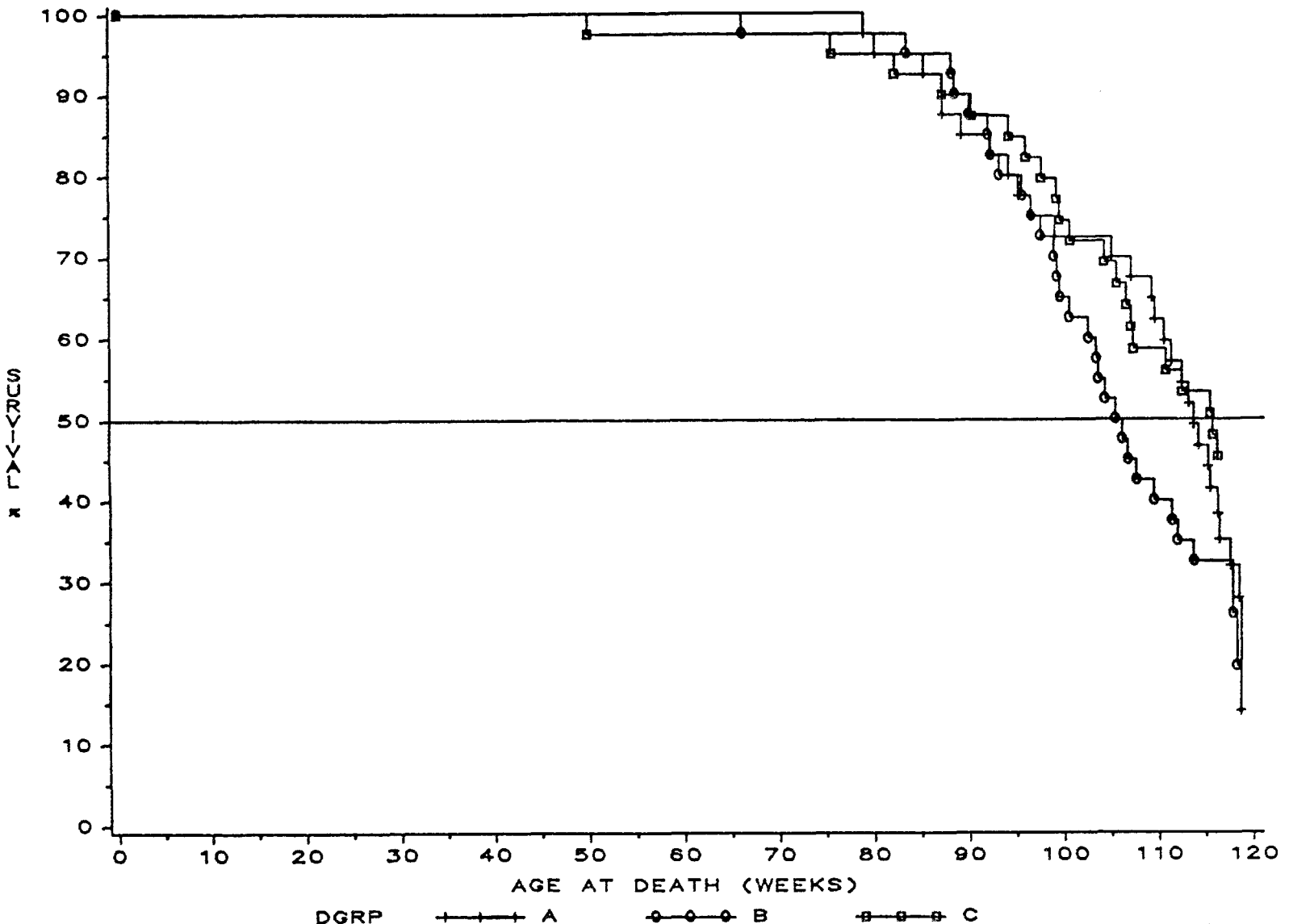

Fig. 2. DGRP. A: 78, B: 62.4, C: 46.8 mg/week in cyclohexane, 3 treatments/wk for 24 months.

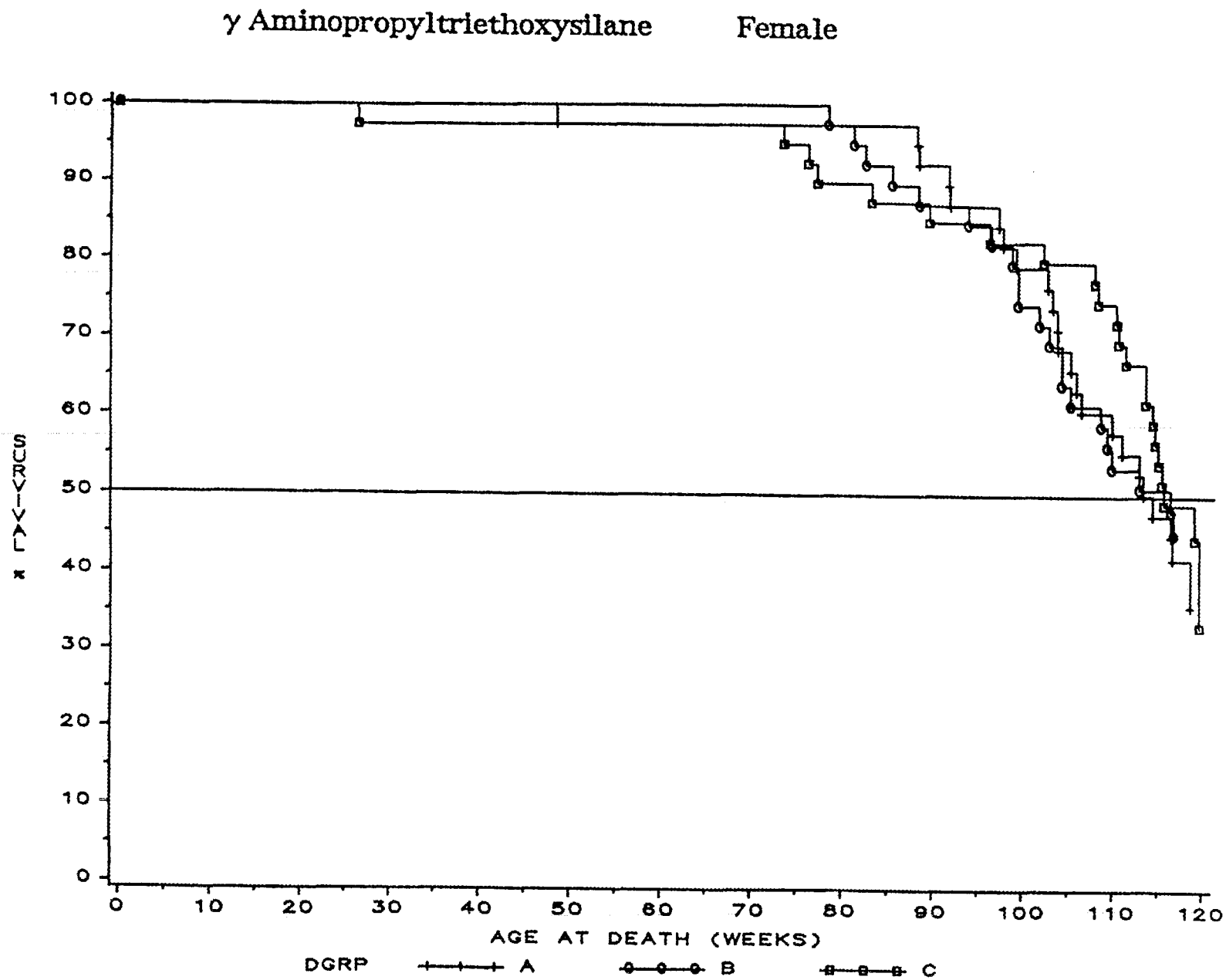

Fig. 3. DGRP. A: 43.8, B: 29.2, C: 14.6 mg/wk in cyclohexane, 3 treatments/wk for 24 months.

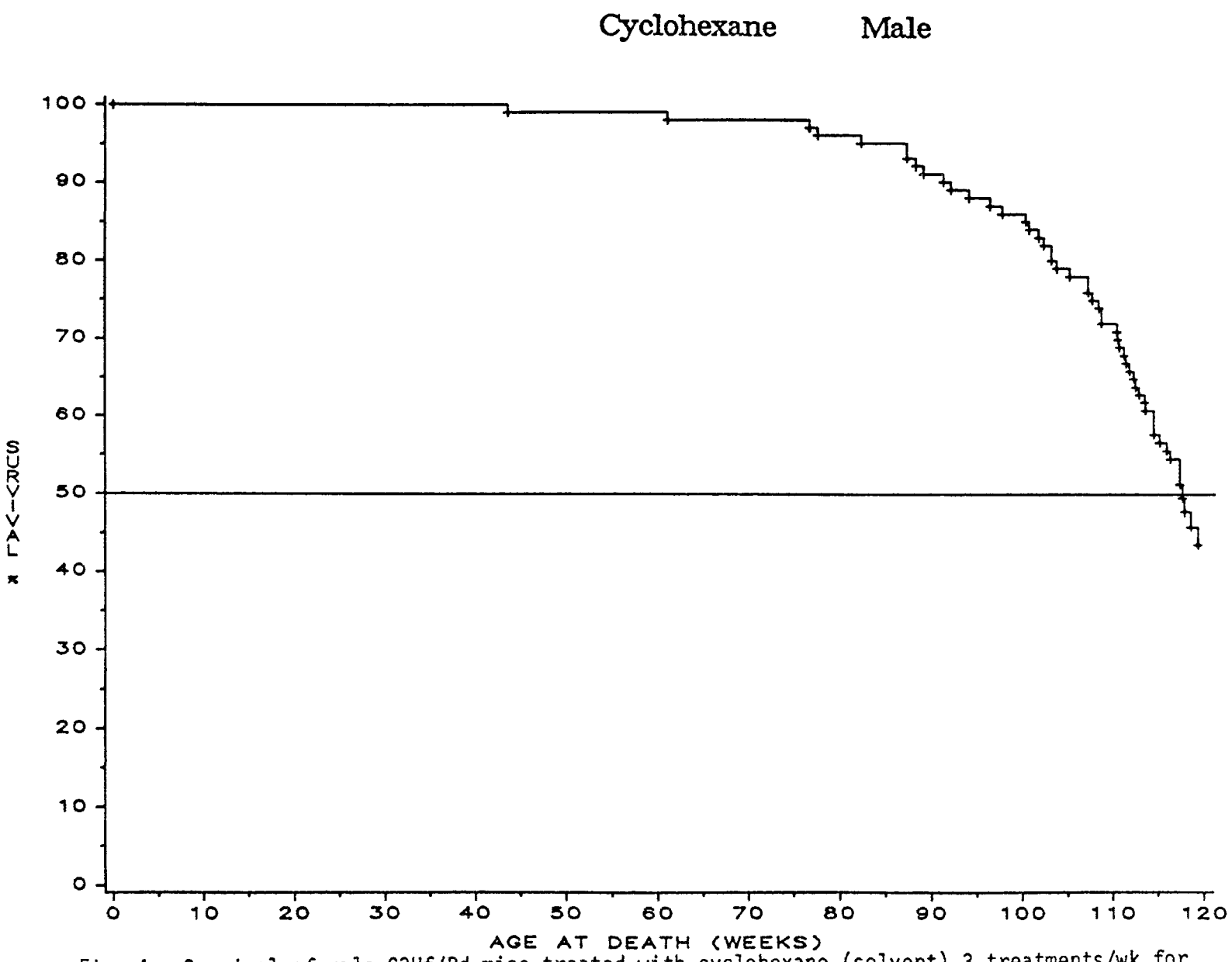

Fig. 4. Survival of male C3Hf/Bd mice treated with cyclohexane (solvent) 3 treatments/wk for 24 months, total dose/wk: 116.7 mg.

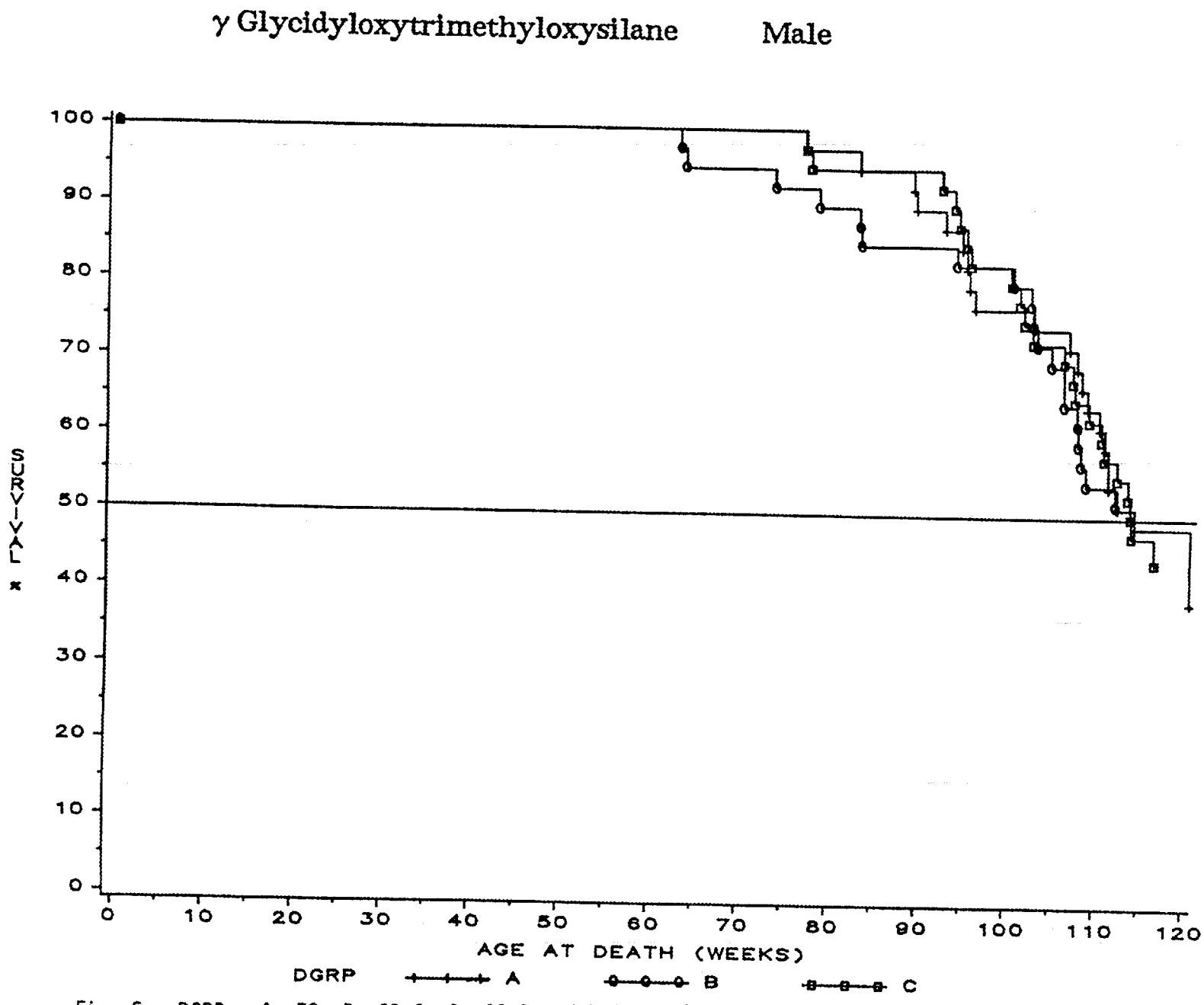

Fig. 5. DGRP. A: 78, B: 62.6, C: 46.8 mg/wk in cyclohexane, 3 treatments/wk for 24 months.

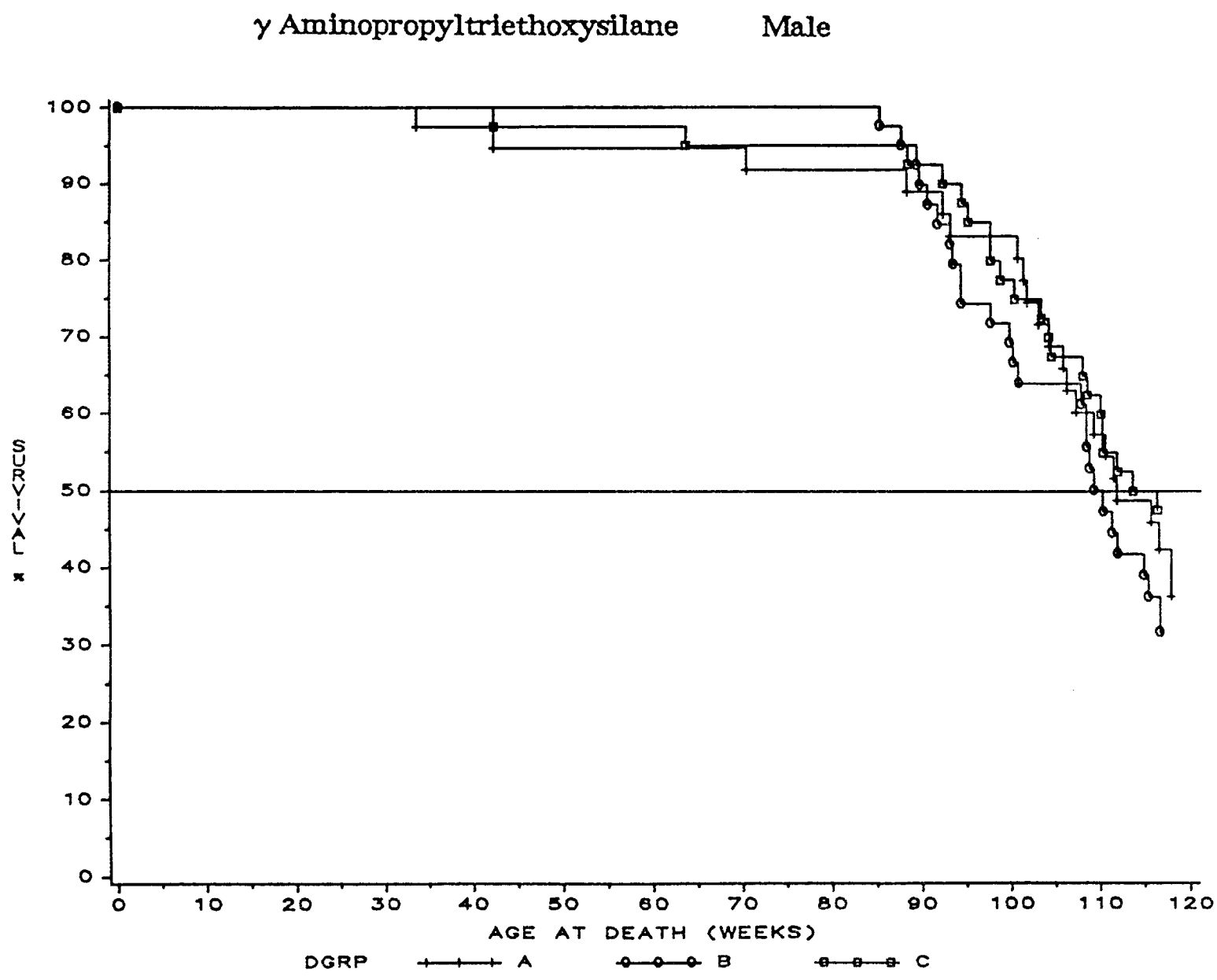

Fig. 6. DGRP. A: 43.8, B: 29.2, C: 14.6 mg/wk in cyclohexane, 3 treatments/wk for 24 months.

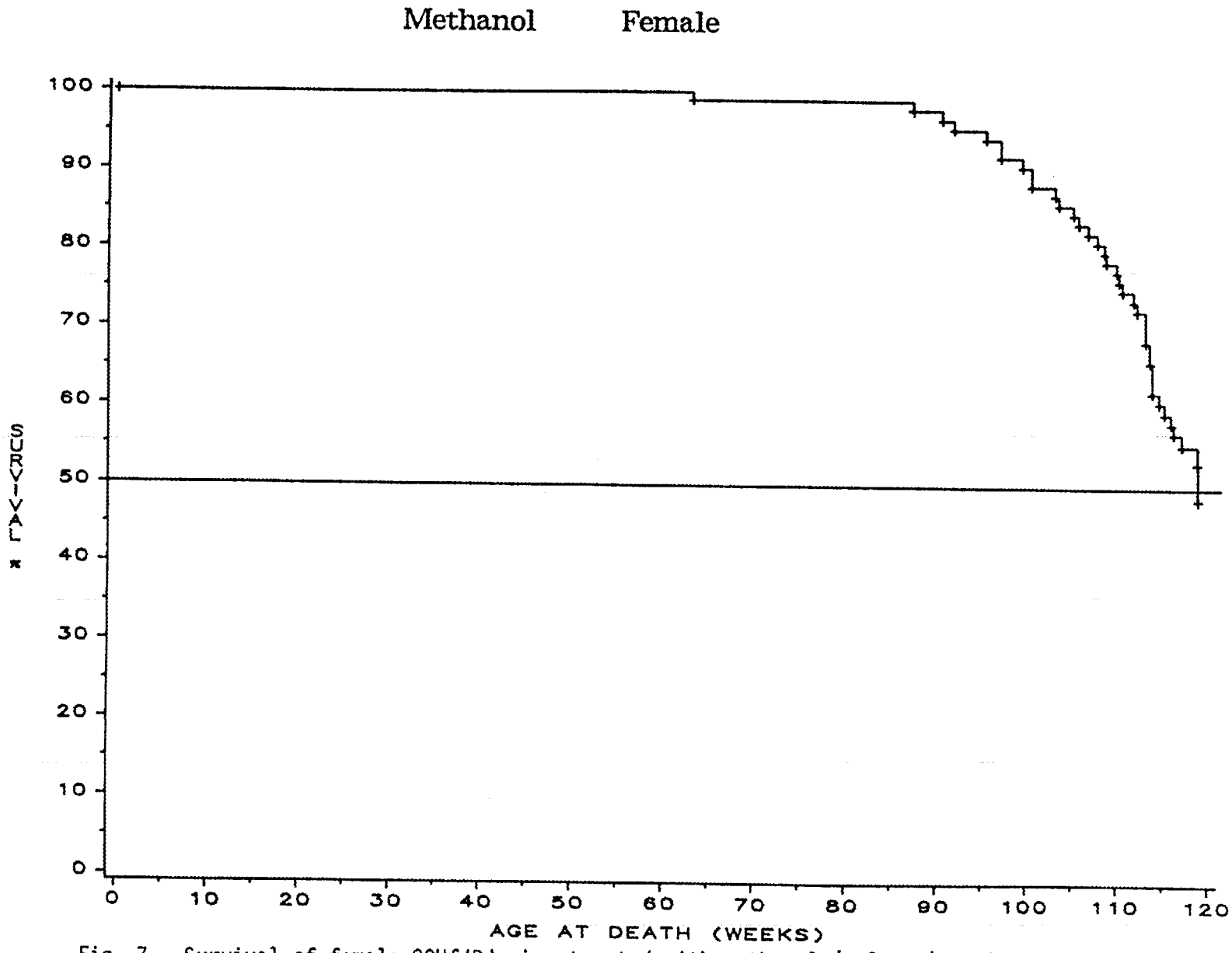

Fig. 7. Survival of female C3Hf/Bd mice treated with methanol (solvent) 3/wk for 24 months total dose/wk: 116.7 mg.

m-Phenylenediamine/Methylenedianiline/Diglycidylether of bisphenol-A Female

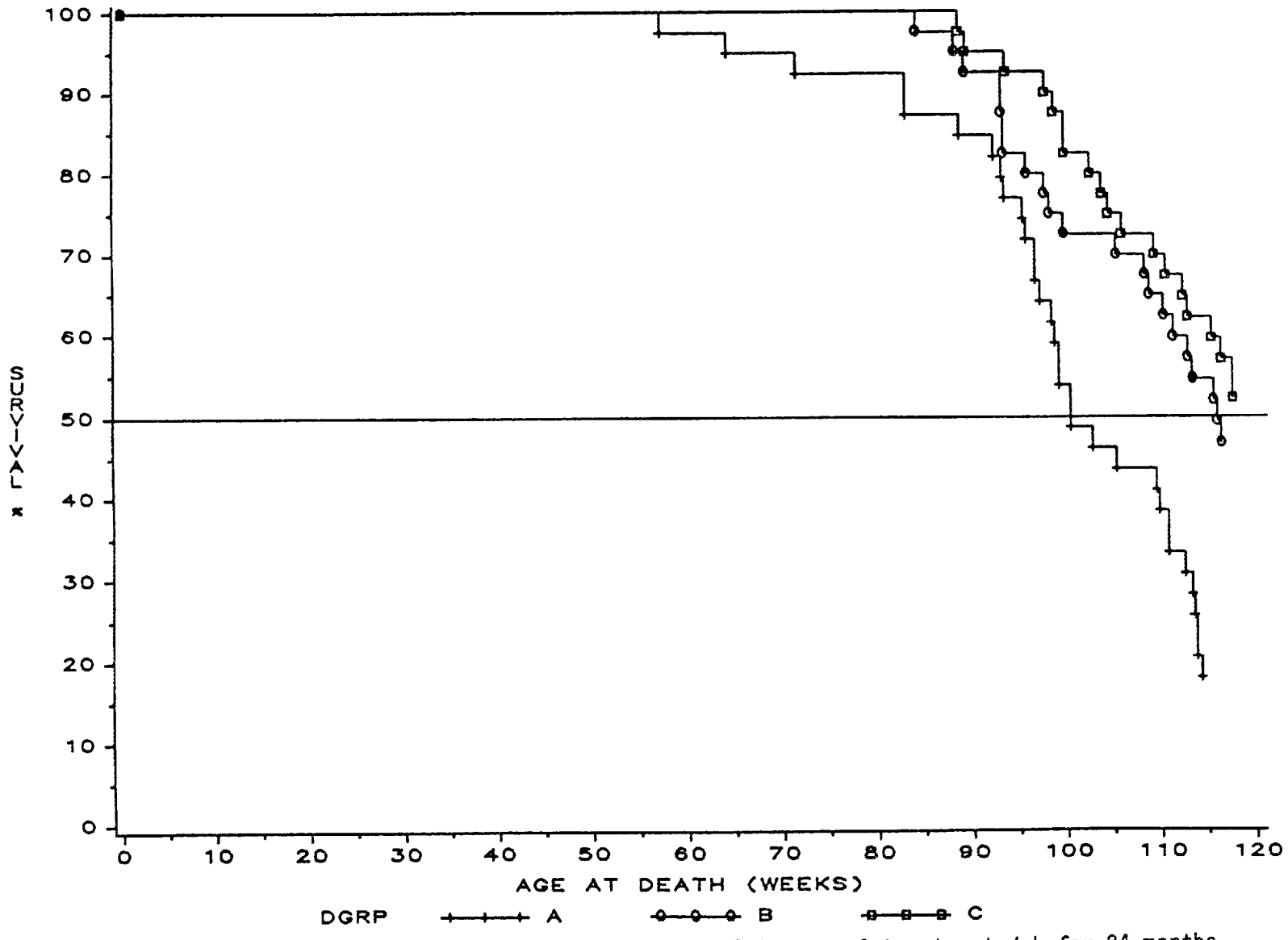

Fig. 8. DGRP. A: 10.8, B: 5.4, C: 2.7 mg/wk in cyclohexane, 3 treatments/wk for 24 months.

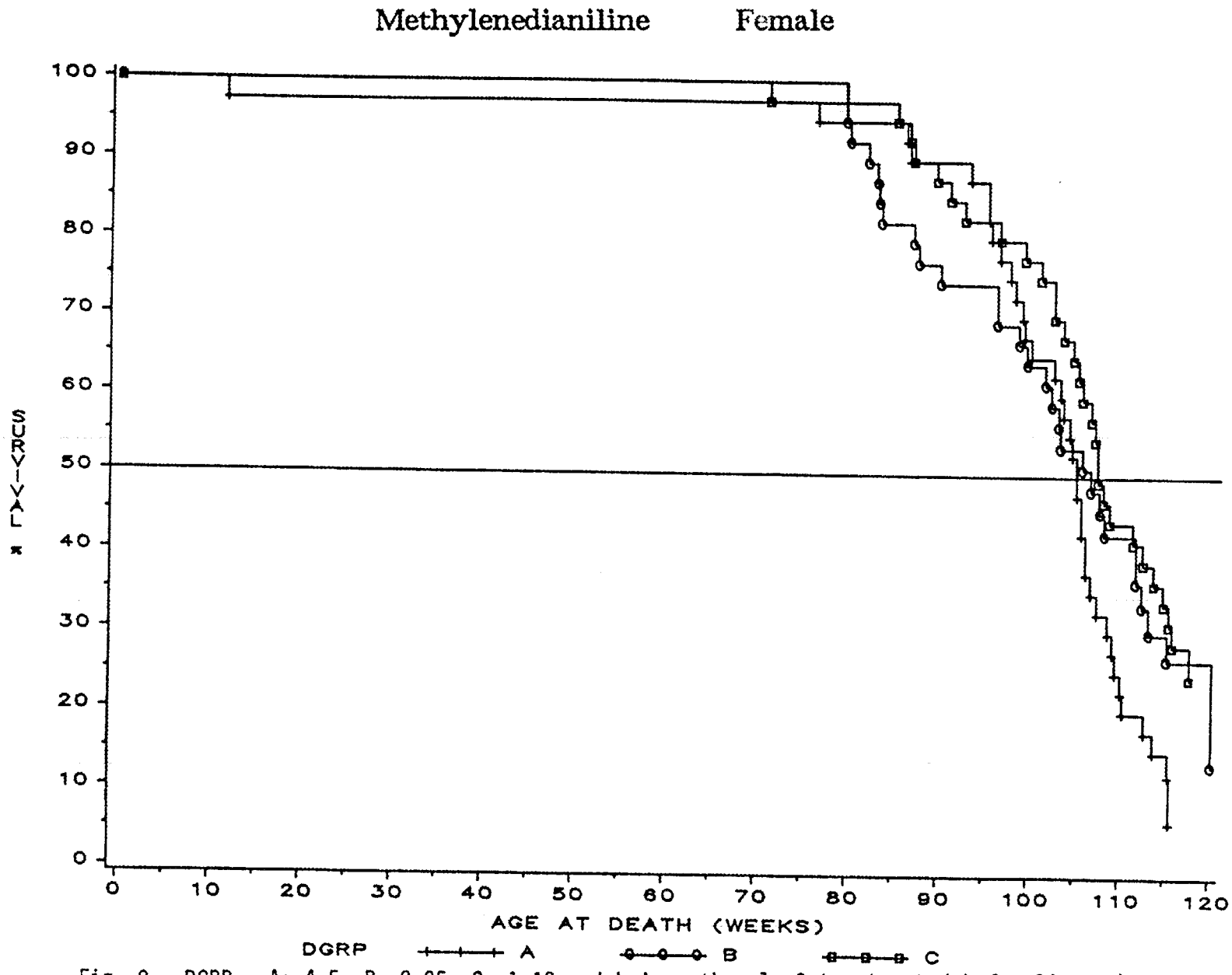

Fig. 9. DGRP. A: 4.5, B: 2.25, C: 1.12 mg/wk in methanol, 3 treatments/wk for 24 months.

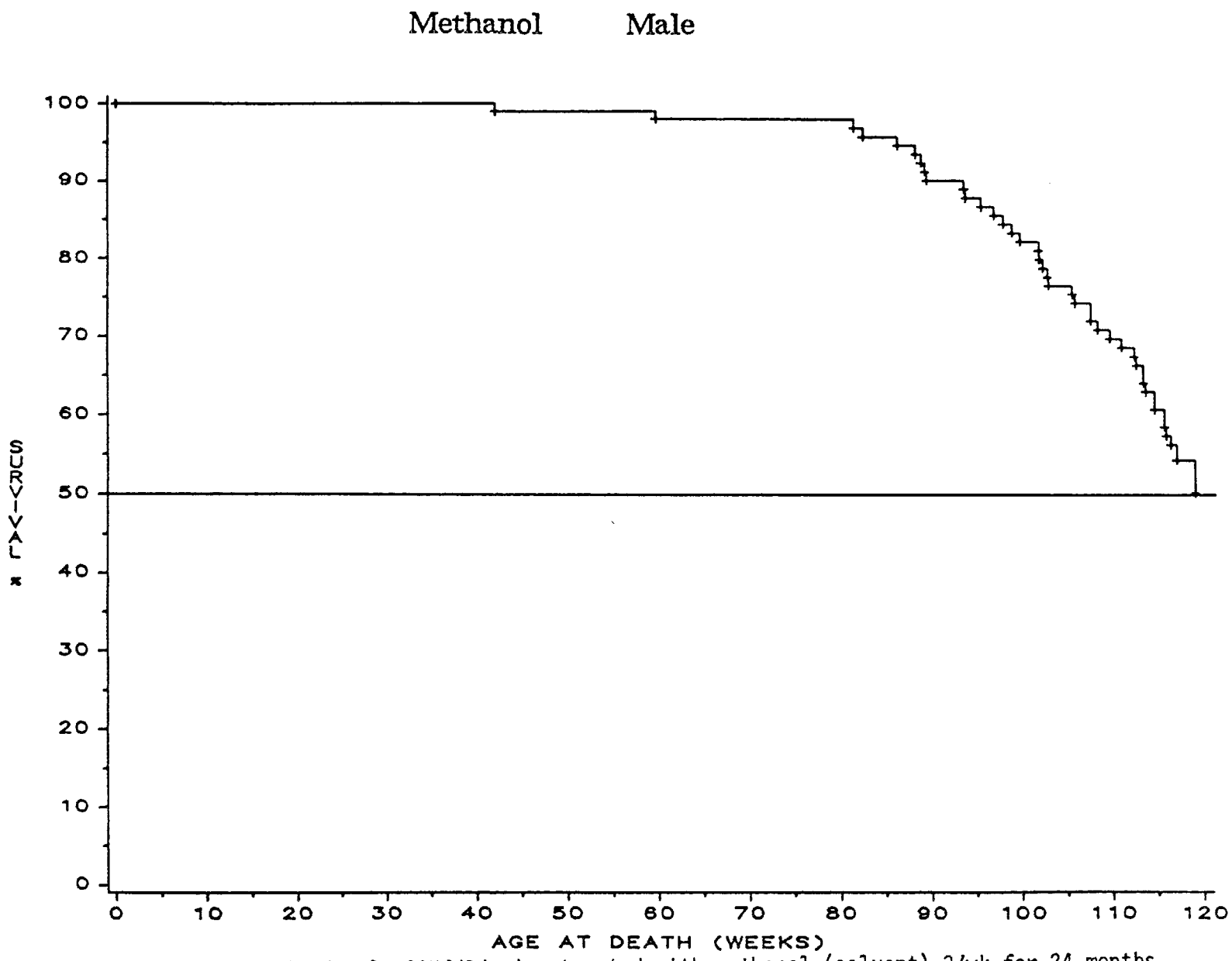

Fig. 10. Survival of male C3Hf/Bd mice treated with methanol (solvent) 3/wk for 24 months, total dose/wk: 116.7 mg.

m-Phenylenediamine/Methylenedianiline/Diglycidylether of bisphenol-A Male

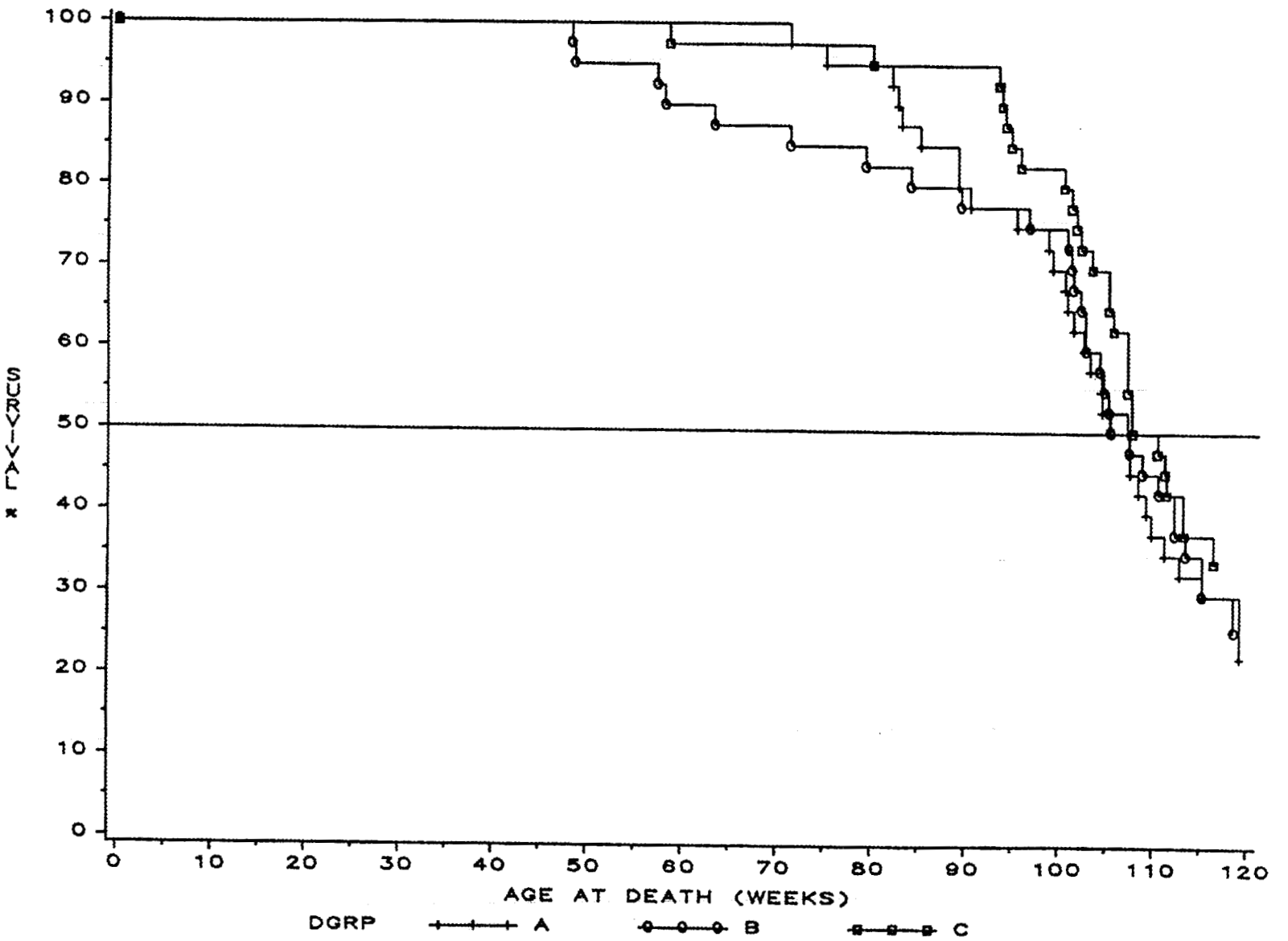

Fig. 11. DGRP. A: 10.8, B: 5.4, C: 2.7 mg/wk in methanol, 3 treatments/wk for 24 months.

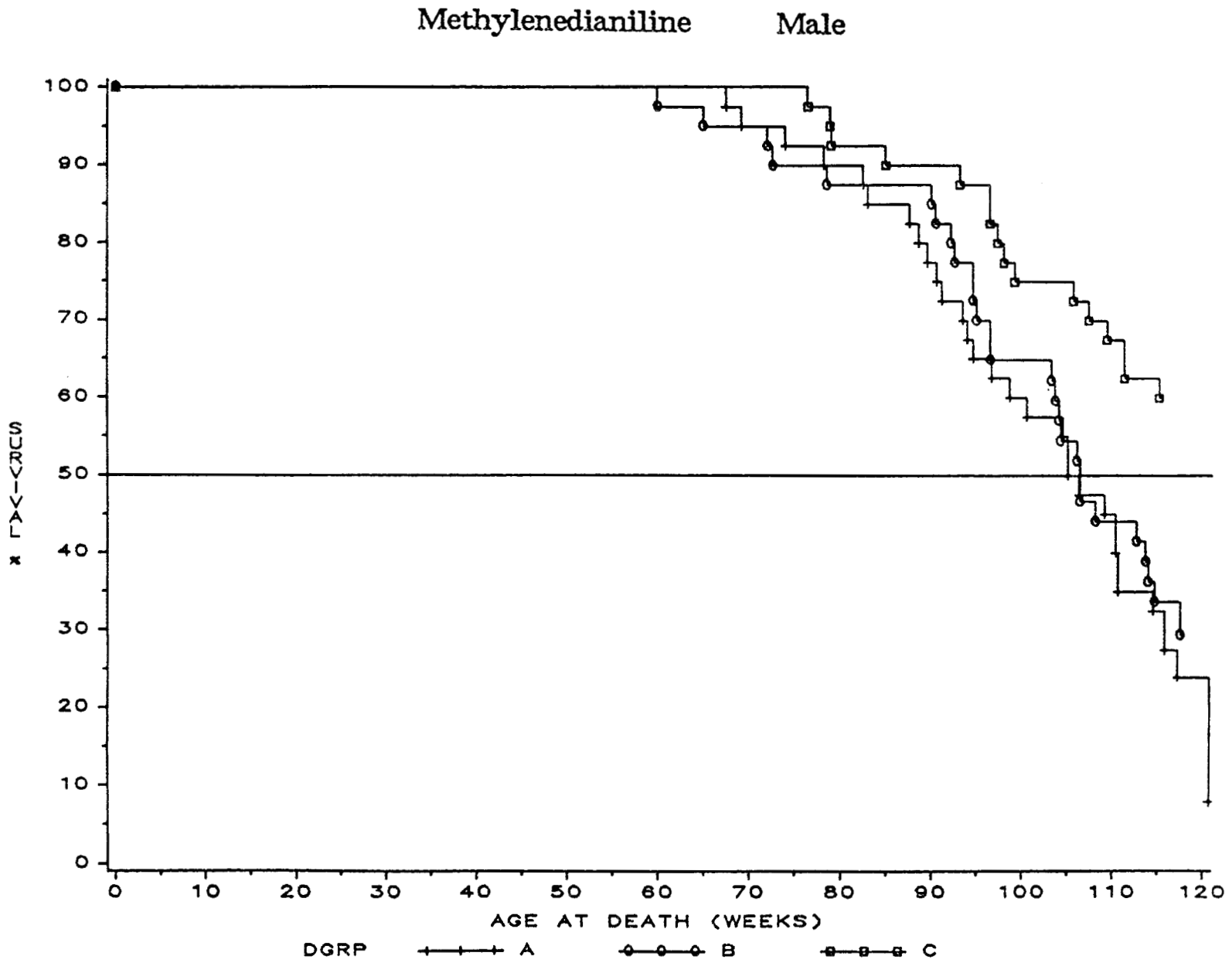

Fig. 12. DGRP. A: 4.5, B: 2.25, C: 1.12 mg/wk in methanol, 3 treatments/wk for 24 months.

INTERNAL DISTRIBUTION

1. E. Frome
2. R. J. M. Fry
3. L. C. Gipson
4. W. Harper
5. S. V. Kaye
6-21. G. Love
22. C. J. Oen
23. C. R. Richmond
24. L. H. Smith
25. W. E. Smith
26. M. J. Whitaker
27. H. R. Witschi
28. Central Research Library
29. Document Reference Section
30. Y-12 Technical Library
31. Laboratory Records
32. Patent Office

EXTERNAL DISTRIBUTION

33-34. Technical Information Center

Upjohn Company, Kalamazoo, Michigan 49001

35. J. M. Holland

DOE - Oak Ridge Operations

36. Assistant Manager, Energy Research and Development