

confine

OK
K-003034-028

T35.1-2-(50)
K3034



THE DOW CHEMICAL COMPANY

TEXAS DIVISION
FREEPORT TEXAS

PV

Texas Medical Department

Report No: TMD 64-3
Date: Oct. 15, 1965

PROGRESS REPORT: CHROMOSOME EVALUATION OF ANIMALS EXPOSED TO CHRONIC LEVELS OF ETHYLENIMINE AFTER 7 MONTHS EXPOSURE.

D.J. Kilian, M.D.
R.L. Schwebel, D.V.M.
Marjorie S. Bengel, B.S.

RESTRICTED

FOR USE WITHIN

THE DOW CHEMICAL CO.

CONCLUSIONS:

On March 13-26, seven months post-exposure, the animals used in the chronic exposure study on ethylenimine, conducted at the Biochemical Research Laboratory in Midland, were sacrificed. Bone marrow preparations following in vivo colchicine were taken on all species (except canines) as opposed to the conventional tissue culture methods used at the pre-exposure and two month post-exposure phases. (See reports No. TMD 64-1 and TMD 64-2).

Considerable time has been devoted to this project with 279 chromosome preparations made, 2790 slides stained and filed, 309 photomicrographs taken and six karyotypes prepared. All the slides have been so prepared as to last indefinitely for future reference if the need arises. Thorough investigation of each slide would take two people approximately five years to complete. Such time expenditure is impossible and unwise.

From the information presently available there is no evidence, thus far developed, that ethylenimine has produced any significant chromosome aberrations in the test animals. However, it must be pointed out that a thorough and careful statistical evaluation of all the material has not been possible because of the tremendous number of man hours involved in such a project.

Ethylenimine: a study, from chromosome eval
Chromosomes: effect of ethylenimine on
Distribution:

Texas Division: Dr. E.B. Barnes, T.E. Brown, R. Buell, C.A. Christian, J.M. Leathers, H. McClure, M. Pruitt, K.M. Tolleson, Medical Department.

Midland Division: B.B. Holder, M.D.

Corporate Division: H.L. Gordon, M.D., R.H. Boundy, V.K. Rowe, D.D. Irish, R. Stewart, M.D., J. Johnson.

Texas Bioproducts: V. Johnston, D.V.M.

Midland Bioproducts: W.R. Dickson

Pitman-Moore Division: B. Slomka, M.D., J. Robertsen, D.V.M., T.L. Kerley, Ph.D.

CRI: Texas Division

CRI: Midland Division

RECEIVED
OCT 18 1965

DO 142616
CONFIDENTIAL

CENTRAL RESEARCH INDEX

During our course of chromosome investigation the following experts outside the company have been consulted at various times for their help and guidance on the technical aspects of chromosome preparation and regarding the need and interpretation of the work.

Mary L. Alexander, Ph.D., Genetics Foundation, Department of Zoology, University of Texas, Austin.

Marvin Fuchs, M. D., F. A. C. P., Internist and Allergist, Washington, D. C., and consultant to Chemirad Corporation, producer of ethylenimine for many years.

A. Clark Griffin, Ph.D., American Cancer Society, Professor of Biochemistry, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston.

Charles H. Kine, M. D., Professor of Occupational Medicine, University of California Medical Center.

T. C. Hsu, Ph.D., Section of Cytology, Department of Biology, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston.

Don Micks, M. D., Professor, Department of Preventive Medicine and Public Health, University of Texas Medical Branch, Galveston.

Harvey Rosenberg, M. D., Pathologist, Director of Laboratories, Texas Children's Hospital, Houston.

Anil K. Sinha, Ph.D., Geneticist, Department of Pathology, Texas Children's Hospital, Houston.

Wilson S. Stone, Ph.D., Professor, Head of Genetics Foundation, Department of Zoology, University of Texas, Austin.

Doctor of Medicine Theiss, Medical Director, B. A. S. F., Ludwigshafen, Germany, producer of ethylenimine for the past 30 years.

Wayne Truax, M. D., Assistant Professor, Hematologist and Cancer Chemotherapist, Department of Internal Medicine, University of Texas Medical Branch and former member of the Atomic Bomb Casualty Team at Hiroshima, Japan.

Shung-Jun Yang, Ph.D., Geneticist, formerly of the Department of Pathology, Texas Children's Hospital, now with Stanford University in California.

Results:

The attached charts are summaries of the results of the blood culture samples of the pre-exposure and two month exposure studies (Report Nos. TMD 64-1 and TMD 64-2) and the blood culture and/or in vivo colchicine bone marrow preparations of the seven month exposure group of each of the animals tested. These charts also include the progress to date on numbers of slide preparations made, cells photographed, and karyotypes done on each animal tested.

Discussion:

At the terminal phase (seven month post-exposure) bone marrow preparations were done on rats, guinea pigs and rabbits by administration of in vivo colchicine in dosages of 2.5 mgm/kg three hours prior to sacrificing. In view of some questionable findings in the blood chemistries, which were possibly due to the in vivo colchicine, conventional tissue cultures (as done on the previous controls) and bone marrow preparations were done on the dogs. Fibrinogen increased in the tissue cultures to the extent that most of the preparations were not satisfactory for chromosome investigation.

The bone marrow preparations were processed by placing marrow from the femur in Difco tissue culture media, adding three mcg colchicine to each culture to arrest mitosis, incubating for two hours, then swelling the cells in hypotonic citrate for 30 minutes at 37.5°C, and "fixing" in the usual manner with Carnoy's.

Control dog #905 (pre-exposure to air) and dog #908 (two-month exposure to 3.5 ppm ethylenimine) have been thoroughly investigated. The remaining 560 slide preparations on canines have received only scattered attention. From the photographs made at this time no significant abnormalities have been seen.

Three rabbits (#237, 252, 177) of the seven month exposure to 3.5 ppm and one rabbit (#179) of the unexposed controls have been thoroughly investigated. These four revealed an extremely high incidence of aneuploidy. Because of these findings a geneticist, Dr. Amil K. Sinha, Texas Children's Hospital, was consulted. It was his opinion that these findings were probably due to technique.

None of the 830 slides prepared on the guinea pigs have been closely investigated. From the few photographs made at this time, no abnormalities have been seen.

From the rats a total of 910 slides have been prepared for investigation. These are from the two month and seven month exposure groups only. The pre-exposure samples were unsatisfactory due to technical problems. From the photographs made at this time no gross chromosome changes have been seen.

After being on recovery for three months bone marrow preparations were done on nine rats which had been exposed to 5.0 ppm ethylenimine. Ten slides per animal were prepared but have not been investigated.

Also available on file are two bone marrow smears per each of 49 rats that had short term exposure to ethylenimine. No chromosome investigations have been made on these.

DO 142619
CONFIDENTIAL

CANINES

	SEX	ANIMAL NO.	PRE-EXPOSURE B.L.D. CULTURE # Mitosis	Slides Prepared	Cells Photomicrographed	Karyotypes	2 MONTH EXPOSURE B.L.D. CULTURE # Mitosis	Slides Prepared	Cells Photomicrographed	Karyotypes	- TERMINAL - 7 MONTH EXPOSURE		Slides Prepared	Cells Photomicrographed	Karyotypes	BONE MARROW SMEARS
											BLOOD CULTURE # Mitosis	BONE TISSUE PREPARATIONS # Mitosis				
UNEXPOSED CONTROLS	F	905	No Samples				No Samples				Some - poor due to fibrin.	Satisfactory	20	1		2
	M	910	"				"				"	"	20	1		2
AIR EXPOSED CONTROLS	F	905	Many	20	16	1	Some	20	6		Some - contaminated	"	30	1		2
	M	911	Many	20	3		Some	20	6		Some - poor due to fibrin.	"	30	1		2
3.5 PPM EXPOSURE	M	914	Few	20	2		EXPIRED									
	F	908	Many	20	2		Many	20	16	SACRIFICED						
	M	900	No Samples				No Samples				Some - poor due to fibrin	"	30	1		2
	M	908	"				"				"	"	30	1		2
0.45 PPM EXPOSURE	F	907	Many	20	1		Some	20	5		Some - poor due to fibrin.	"	30	1		2
	M	913	Many	20	3		Some	20	5		Some	"	30	1		2
0.15 PPM EXPOSURE	F	906	Many	20	2		Some	20	6		Some - poor due to fibrin	"	30	1		2
	M	912	Many	20	1		Some	20	6		"	"	30	1		2
TOTALS				160	30	1		140	50				300	10		20

DO 14262C
CONFIDENTIAL

RABBITS

	SEX	ANIMAL NO.	PRE-EXPOSURE BLD. CULTURE # Mitosis	Slides Prepared	Cells Photographed	Necropsies	2 MONTH EXPOSURE BLD. CULTURE # Mitosis	Slides Prepared	Cells Photographed	Necropsies	- TERMINAL - 7 MONTH EXPOSURE		Slides Prepared	Cells Photographed	Necropsies	BONE MARROW SLIDES
											BLOOD CULTURE # Mitosis	BONE MARROW PREPARATIONS # Mitosis				
UNEXPOSED CONTROLS	M	258, 219,	254, 201, 202										50	5		10
	F	179, 181,	263, 264, 255									Many Many	50	5		10
AIR EXPOSED CONTROLS	M	256	Fungus cont.	10			None--fibrin clot					Contaminated				
	M	187	Many	10	2		Some	10	3			Many Many	10 30			2 6
	M-267, F-260		F-272											3		
2.5 PPM EXPOSURE	M	229	Many	10	9	4	EXPIRED									
	M	232	Many	10	1		EXPIRED									
	M	228					Some-- poor separation	10	3			Many	10	6		2
	F	177										Many	10	20		2
	F	237										Many	10	20		2
	F	252										Many	10	22		2
0.45 PPM EXPOSURE	M	173	Many	10	2		None-- fibrin clot					Many	10	1		2
	M	199	Many	10	1		EXPIRED									
	M-266, F-236		F-246									Many	30	3		6
0.15 PPM EXPOSURE	M	299	Many	10			EXPIRED									
	M	230	Many	10			None-- fibrin clot		1			Some	10	1		2
	M-257, F-224		F-247, F-262									Many	40	4		8
TOTALS				80	12	4		20	7				270	90		57

DO NOT WRITE IN THESE SPACES

GUINEA PIGS

	SEX	ANIMAL NO.	PRE-EXPOSURE BLD. CULTURE # Mitosis	Slices Prepared	Cells Photographed	Karyotypes	2 MONTH EXPOSURE BLD. CULTURE # Mitosis	Slices Prepared	Cells Photographed	Karyotypes	-TERMINAL- 7 MONTH EXPOSURE		Slices Prepared	Cells Photographed	Karyotypes	BONE MARROW SLICES
											BLOOD CULTURE # Mitosis	BONE MARROW PREPARATIONS # Mitosis				
UNEXPOSED CONTROL	M	5936					NONE - Contaminated									
AIR EXPOSED CONTROLS	M	5956-00	Many	10	9	1						Many	10			2
	N	5963-01	Many	10	2							Many	10			2
	M	5967-01	NO SAMPLE				NO SAMPLE					NO SAMPLE				
	M	5957-01,	5959-01, 5961-01,		5962-00,		5964-00, 5966-00					Many	60			12
	F	6034-00,	6035-01, 6037-00,		6037-01,		6039-01, 6040-00, 6041-01,					Many	110			22
5.0 PPM EXPOSURE	M	5971	Many	10	10		EXPIRED									
	M	5968-00	Many	10	10							Many	10			2
	M	5972-01,	5973-01, 5975-01,		5976-00,		5977-01, 5978-00					Many	60			12
	F	6048-00,	6049-01, 6050-00,		6051-01,		6053-01, 6055-01, 6056-00,				6057-01	Many	80			16
1.2 PPM EXPOSURE	M	5980-00	Many	10	6							Many	10			2
	M	5984-00	CLOTTED BLOOD - No growth									Many	10			2
	M	5981-01,	5982-01, 5983-01,		5985-01,		5987-01, 5988-00, 5989-01,				5990-00, 5991-01	Many	90			18
	F	6058-00,	6059-00, 6060-00,		6061-00,		6062-00, 6064-00, 6065-01,				6067-01, 6068-00	Many	90			18
0.2 PPM EXPOSURE	M	5992-00,	Many	10	1							Many	10			2
	M	5995-01	Many	10	1							Many	10			2
	M	5993-01,	5994-00, 5996-00,		5997-01,		5999-01, 6000-00, 6001-01,				6002-00	Many	80			16
	F	6070-00,	6071-01, 6072-00,		6073-01,		6074-00, 6076-00, 6077-01,					Many	110			22
TOTALS				70	39	1							750			150

CONFIDENTIAL
DO NOT REPRODUCE

GUINEA PIGS

	SEX	ANIMAL NO.	PRE-EXPOSURE BLD. CULTUR. # Mitosis	Slides Prepared	Cells Photographed	Karyotypes	2 MONTH EXPOSURE BLD. CULTUR. # Mitosis	Slides Prepared	Cells Photographed	Karyotypes	- TERMINAL - 7 MONTH EXPOSURE		Slides Prepared	Cells Photographed	Karyotypes	BONE MARROW SMEARS
											BLOOD CULTURE # Mitosis	BONE MARROW PREPARATIONS # Mitosis				
LINEXPOSED CONTROL	M	5936					NONE... Contaminated									
AIR EXPOSED CONTROLS	M	5956-00	Many	10	9	1						Many	10			2
	M	5963-01	Many	10	2							Many	10			2
	M	5967-01	NO SAMPLE				NO SAMPLE					NO SAMPLE				
	M	5957-01,	5959-01, 5961-01,		5962-00,		5964-00, 5965-00,					Many	60			12
	F	6034-00,	6035-01, 6036-00,		6037-01,		6039-01, 6040-00, 6041-01,					Many	110			22
5.0 PPM EXPOSURE	M	5971	Many	10	10		EXPIRED									
	M	5968-00	Many	10	10							Many	10			2
	M	5972-01,	5973-01, 5975-01,		5976-00,		5977-01, 5978-00					Many	60			12
	F	6048-00,	6049-01, 6050-00,		6051-01,		6053-01, 6055-01, 6056-00,				6057-01	Many	80			16
1.2 PPM EXPOSURE	M	5980-00	Many	10	6							Many	10			2
	M	5984-00	CLOTTED BLOOD - No growth									Many	10			2
	M	5981-01,	5982-01, 5983-01, 5985-01,				5987-01, 5988-00, 5989-01,				5990-00, 5991-01	Many	90			18
	F	6058-00,	6059-00, 6060-00, 6061-00,				6062-00, 6064-00, 6065-01,				6067-01, 6068-00	Many	90			18
0.2 PPM EXPOSURE	M	5992-00,	Many	10	1							Many	10			2
	M	5995-01	Many	10	1							Many	10			2
	M	5993-01,	5994-00, 5996-00,		5997-01,		5999-01, 6000-00, 6001-01,				6002-00	Many	80			16
	F	6070-00,	6071-01, 6072-00, 6073-01,				6074-00, 6076-00, 6077-01,					Many	110			22
TOTALS				70	39	1							750			150

DO 142623
CONFIDENTIAL

RATS

	SEX	ANIMAL NO.	PRE-EXPOSURE BLD. CULTUR. # Mitosis	Slides Prepared	Cells Photographed	Karyotypes	2 MONTH EXPOSURE IN VIVO COCH. BONE MARROW # Mitosis		- TERMINAL - 7 MONTH EXPOSURE		Slides Prepared	Cells Photographed	Karyotypes	BONE MARROW SMEARS
							Slides Prepared	Cells Photographed	BLOOD CULTURE # Mitosis	BONE MARROW PREPARATIONS # Mitosis				
UNEXPOSED CONTROLS	M	123-01,	124-II, 125-02,	126-03,	127-12,		128-21, 130-01,	132-11, 133-02,	134-20	Many	100			20
AIR EXPOSED CONTROLS	M	157					Many	10	8	SACRIFICED				
	M	158					Many	10	6	SACRIFICED				
	M	464												
	M	159, 160,	164, 165, 166,	167,	169,	70				NONE-- tube broken				
	F	459, 462,	463, 465, 466,	467,	469,	468,	470			Many	80			16
										Many	90			18
3.5 PPM EXPOSURE	M	191-00					Many	10	12	SACRIFICED				
	M	192-01					Many	10	12	SACRIFICED				
	M	195-00,	196-01, 198-II,	199-02,	200-21,		202-22, 203-II,	204-02, 205-12,	206-21	Many	100			20
	F	495-00,	496-01, 497-10,	500-21, 501-00,			503-10, 504-12, 505-12, 506-21			Many	90			18
0.45 PPM EXPOSURE	M	229					Many	10	6	SACRIFICED				
	M	230					Many	10	10	SACRIFICED				
	M	233-10,	235-02, 237-00, 238-01, 239-10,				240-II, 241-02, 242-12			Many	80			16
	F	543-12,	544-01, 545-10, 546-II, 547-02,				548-20, 549-00, 551-10, 553-02, 554-21			Many	100			20
0.15 PPM EXPOSURE	M	265					Many	10	7	SACRIFICED				
	M	266					Many	10	7	SACRIFICED				
	M	267-00,	268-01, 270-II, 272-20, 273-01,				274-10, 275-II, 276-02, 277-12,	278, 21		Many	100			20
	F	567-00,	568-01, 569-10, 570-II, 571-02,				572-12, 573-00, 574-01, 578-20			Many	90			18
TOTALS								80	68		830			136

DO 142624
CONFIDENTIAL

DO 142624
CONFIDENTIAL

RATS - Ethyleneimine-Recovery
3 Mos. Post-Terminal Exposure

5.0 PPM
EXPOSURE

SEX	ANIMAL NO.	IN VIVO COLCHICINE BONE MARROW <i>% Mitosis</i>	Slides Prepared	Cells Photographed	REMARKS
M	208-01	Poor	10		Bone marrow preparations from both femurs were made in the same manner as described for the dogs in the "Discussion" section of this report. (Paragraph 2)
M	209-01	"	10		
M	210-11	"	10		
M	216-12	"	10		
F	512-10	Poor	10		Over an hour elapsed before these femurs were put into the media. As a result bone marrow smears were not attempted, however, Dr. Sadek, Pathologist, Midland Division, has some smears on the animals.
F	513-00	"	10		
F	514-01	"	10		
F	516-11	"	10		
F	517-02	"	10		

DO 142625
CONFIDENTIAL