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IN VIVO MUTAGENICITY STUDIES WITH TRICHLOROETHYLENE AND OTHER SOLVENTS (PRELIMINARY RESULTS)

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1. INTRODUCTION

During 1976 a report was published by the National Cancer Institute (1), referring on the results obtained on a long-term car cinogenicity study developed on TCE. In these experiments TCE was administered by gavage, 5 days a week, for a period of 78 weeks to B6C3F1 mice and to Nendel Osborne rats (both females and males were treated for the two species). The treatments were at the maximum to lerated dose (MTD): this was for rats of 1300 mg/kg and for mice of 2000 mg/kg (males) and 1400 mg/kg (females); a second dose was also given (the half of the MTD). During treatments, the doses for both species were reduced.

The final data of these experiments have concluded that TCE did not produce tumours in rats at the MTD (1300 mg/kg); but it produced hepatocellular carcinoma in both male and female mice with a statistical significant difference from the control; the in cidence of tumours was higher in males compared to females.

In 1977, HENSCHLER et al. (2) questioned the results obta ined by the NCI experiment, on the basis that the carcinogenic effect reported by the NCI could have been due, largely, to the types and amounts of epoxides present in TCE sample used for long-term study, namely epichlorohydrin and 1,2-epoxybutane. Moreover, these Authors analyzed a sample of TCE similar to that employed by the NCI and

they observed the presence of 0.42% of epoxides employed as stabilizers. Mutagenic analyses developed by the same Authors, by using TA1CO strain of Salmonella in the Ames test, demonstrated that epichlorohydrine and 1,2-epoxybutane were strong mutagens for Salmonella, whereas trichloroethylene of pure grade was not mutagenic.

On the basis of HEINSCHER'S criticism (2) and of other Authors (3) we have investigated the mutagenicity of different types of TCE (technical and pure grade) containing different types of stabilizers, (including epichlorohydrine): the mutagenic experiments have been performed in vivo, in the host mediated assay, on NMRI mice, by treating the animals by gavage (similarly to the procedure adopted by NCI on the same strains of mice); other solvents, such as epichlorohydrine, trichloroethane and cyclohexanone have also evaluated in the same study.

The aim of the present study was the possibility to verify by a direct methodology the criticism developed by HEINSCHER (1), in order to promote further studies on trichloroethylene for a more accurate evaluation of its potential mutagenic as well carcinogenic risk.

2. BIOLOGICAL MATERIAL AND GENETIC END POINT

Yeast cell gene-mutation test in *Schyzosaccharomyces pombe* is based on the treatment of a haploid double mutant strain - P_1 strain - of the genotype *Prad6-60/rad 10-109/h⁻*: it allows the evaluation of the frequency of forward gene-mutations induced in five different genetic loci located on the three chromosomes of this yeast.

The strain employed consists of purple colonies, and the induced mutants are represented by white colonies: they are of the genotype *ade6-60/ade X*. The second "ade" mutation may have occurred in one of the following 5 genetic loci, namely *ade1*, *ade2*, *ade4*, *ade5*, *ade6*.

Forward mutations induced by chemicals in each one of these 5 loci have been shown to consist of base-pair substitution as well of insertion/deletion base-pairs; seventy chemicals of different molecular nature have been so far tested in this mutational assay. (see 7).

3. HOST MEDIATED ASSAY

Intraperitoneal assay. The animals (mice or rats) are starved overnight before the experiment. The yeast cell suspension is inoculated into the peritoneal cavity of mice (25-30 gr of body wheight) by injection (1.5 ml of suspension containing 2.10^9 cells/ml). The chemical is administered by gavage (0.5 ml per animal per treatment). After different incubation times, the animals are killed by cervical dislocation, the peritoneal cavity is open and cells are recovered by a pipette and by washing the peritoneal cavity with 1 ml of sterile distilled water. The cell suspension is counted on the microscope and appropriately diluted according to the assay: a dilution to 1.5×10^3 cells/ml is prepared and 0.2 ml of this suspension is spread on a yeast extract agar plate.

ANALYSIS OF THE RESULTS

The induction of genetic effect is evaluated by regression analysis, according to equation

$$y = a + bx$$

where (y) is the induced frequency of genetic effect to be evaluated, and (x) is the dose of the mutagen.

The regression coefficient (b) is the slope of the linear relationship observed and represent the amount by which the induced frequency (y) increases for a unit increase of the dose (x); the coefficient (a) represents the frequency of genetic effect produced at a dose x = 0.

In the present cases, the correlation factor (r) has been calculated for an evaluation of the statistical significance of the data.

5. TREATMENT WITH A CHEMICAL IN VITRO

5.1. Yeast cell suspension

One fresh deep-red yeast colony grown on YEA is resuspended in a 50 ml YEL broth and allowed to grow for 36 hours at 30°C on a shaker. After this incubation time yeast cells are washed and collected by centrifugation (3 min. at 2500 x g). Cell titer is adjusted at 4×10^8 cell/ml in YELD and 1 ml of such a suspension is inoculated in a sterile test tube (approx 30 ml volume).

1 ml of S-9 mix, if metabolic activation is required, is added to each test tube, otherwise 1 ml of phosphate buffer is added.

5.2. Treatment procedure

The test substance is added in a final volume of 0.1 ml to the cell suspension.

Routinely the compound is tested with the following doses: 100, 30, 10, 1, 0.3, 0.1, 0.03 mMolar.

The cells suspensions are allowed to grow for 16 hours in a shaking water bath at 32°C. At the end of the treatment the cell are washed twice, counted at the microscope and are plated for evaluation of the presence of eventually induced mutants.

Only a sample of treated suspensions, according to the following criterin, are plated: 1. the control, 2. the highest dose treated suspension which is grown up the control, 3. a suspension which is weakly inhibited, 4. a suspension which is intermediately inhibited (see the example).

5.3. Plating procedure

0.1 ml of cells suspension containing approximatively 2-3 x 10^3 cell are inoculated in 3 ml soft agar (45°C) and poured onto a YEA petri dish (100 mm diameter). Usually 10 petri dishes per dose are plated.

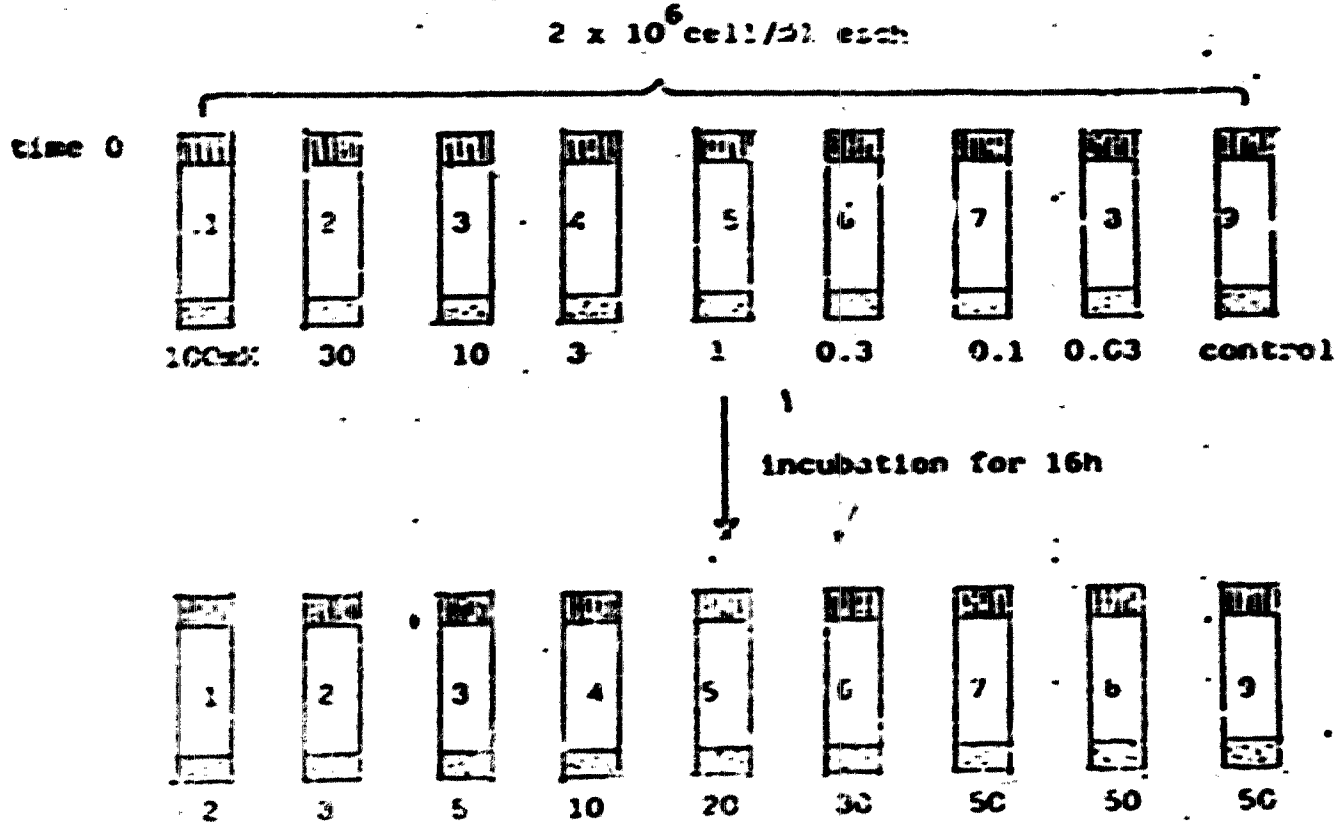
3 x 0.1 ml of the same cell suspension diluted 1 x 10^{-1} are plated with the same procedure for the population evaluation. For this the colony are allowed to grow for 5 days at 32°C; then 1-2 days at +4°C are requested to increase the pod pigmentation of the colony.

5.4. Scoring of the Mutants

All the plated petri dishes are scored for white complete ore sectored mutant colony among the red ones.

Survival and mutants induction are scored on the same plates for each treatment series: in this case all colonies which have survived the chemical treatments are scored.

5.5. Example


2 x 10^6 cell/ml each
time 0
1
2
3
4
5
6
7
8
9
100
30
10
3
1
0.3
0.1
0.03
control
incubation for 16h
1
2
3
4
5
6
7
8
9
2
3
5
10
20
30
50
50

3.6. Materials

a. YEA (per liter)= glucose 30 gr, yeast extract 5 gr, Agar 15 g

b. YEL (per liter)= glucose 20 gr, yeast extract 5 gr,
adenine 75 mgr

c. YELP (per liter)= glucose 40 gr, yeast extract 10 g.
adenine 150 mgr

d. S-9 mix (per 10 ml)= 3 ml S-9, NADP 30 mgr, Glucose-6-P 60 mgr
Glucose 6-P-Dehidrogenase 200 µl, $MgCl_2$ 400 µl
from a 500 mM solution. Phosphate buffer pH 7.4 0.1 M
0.04 ml.

e. Soft agar (per liter Agar 8 gr)

f. Phosphate buffer = 0.1 M pH 7.4

METABOLIC ACTIVATION SYSTEM FOR IN VITRO EXPERIMENTS

A post-mitochondrial fraction of the liver homogenate (S-9) is used as an activation system. The procedure for its preparation is the following.

A liver is obtained from 3 rats, which hawbeen treated five days before with i.p. injection of Aroclor 1254 at a dose of 500 mg/kg. The livers are wheighed, minced, suspended in a buffer, and homogenized on ice. The homogenate is centrifuged twice (at 900 x g for 10 min. at 4°C.; at 9,000 x g for 20 min. at 4°C.). The supernatant (S-9) is decanted and it represents the post-mitochondrial fraction to be employed during the treatment.

To the S-9 preparation (0.1 ml) the following cofactors are added:

NADP	mg	3.0/ml
Glucose-6-P	"	6.0/ml
Glucose-6-P-Dehydh.	µl	20/ml
$MgCl_2$	"	40/ml

7. CHEMICAL COMPOUNDS TASTED ON Schizosaccharomyces pombe WITH DIFFERENT METHODOLOGIES "in vitro" AND "in vivo".

With the forward mutation system of Schizosaccharomyces pombe (wild type and a more sensitive derivative strain, P_1) a certain number of chemical compounds of different nature (solid, liquid, gas) have been tested for the evaluation of their mutagenic potential. A schematic summary of the results so far obtained are reported in the following list. The quantitative data have been reported in several scientific paper since 1964; compounds 43-50 have been assayed in the Laboratoire de Génétique, University of Liege (Belgium), under the leadership of prof. J. HOUTSCHEN.

Several different methodologies have been applied in the mutation studies, including in vitro assays with and without metabolic activation, and in vivo assays. In the host mediated assay with this latter procedure yeast cells have been inoculated into the animal via intraperitoneal, via intrasanguineous and via intragastric.

Besides the listed compounds, 20 other chemicals whose chemical formula is not yet known to us have been tested: their analysis is part of the "International Programme for the evaluation of short-term tests for Carcinogenicity" sponsored by MRC of England, ICI, and NIEHS of USA (Mutation Res. 54, 203-205, 1978).

CHEMICAL COMPOUNDS TESTED ON THE FORWARD MUTATION SYSTEM OF THE YEAST Schizosaccharomyces pombe.

1. N-METHYL-N-NITROSO URETHANE	M		
2. N-ETHYL-N-NITROSO URETHANE	M		
3. URETHANE	inactive		
4. N-METHYL URETHANE	inactive		
5. N-ETHYL URETHANE	inactive		
6. METHYLMETHANE SULFONATE	M		
7. ISOPROPYL METHANE SULFONATE	M		
8. ETHYLMETHANE SULFONATE	M		
9. ETHYLENEIMINE	M		
10. DIMETHYL SULFATE	M		
11. DI-EPOXYBUTANE	M		
12. N-NITROSO-N-METHYL-N-NITROGUANIDINE	M		
13. HYDROXYLAMINE	M		
14. NITROUS ACID	M		
15. CAFFEINE	inactive		
16. VINYL CHLORIDE	M	ma	hma
17. CHLOROETHYLENE OXIDE	M		
18. CHLORO ACETALDEHYDE	inactive	ma	hma
19. CHLORO ETHANOL	inactive	ma	
20. STYRENE	(M)		hma
21. STYRENE OXIDE	M		hma
22. CHROMATE	M		
23. MERCURY CHLORIDE	M		

34. DIMETHYL NITROSO AMINE	N	ma	hma
35. TRICHLORO ETHYLENE	inactive	ma	hma
36. EPOXY-1,1,2-TRICLOROETHANE	N		
37. 1,4-DICHLORO BUTENE-2	N		
38. 1,4-DICHLORO BUTENE-2,3-EPOXIDE	N		
39. 2,4-DIAMINO ANISOLE	N	ma	
40. CARMINIC ACID	inactive	ma	hma
41. CYCLOPHOSPHAMIDE	N		hma
42. NIYONYCIN C	N		
43. DIMETHYLSULFOXIDE	inactive	ma	
44. METHANOL	inactive	ma	
45. ETHYLENE GLYCOL	inactive	ma	
46. 2-METHOXY ETHANOL	inactive	ma	
47. 2-PROPANONE	inactive	ma	
48. MYCAMYCIN	N		
49. PRAZIQUANTEL	inactive	ma	hma
50. DIFLUOROCHLORO METHANE	inactive	ma	hma
51. TRIIODIO-ISO FTALDIAMIDE	inactive	ma	hma
52. ICR-170	N		
53. PARAXON	N	ma	(-)
54. DICHLORVOS	N	ma	(-)
55. TRICHLORFON	N	ma	(-)
56. FENITROTHION	N		
57. ETHYLAZINPHOS	N		

48. METHYLAZINPHOS	M		
49. METHYL PARATHION	M		
50. ETHYL PARATHION	inactive		
51. DIMETHOATE	M		
52. METHYL BROMOPHOS	M		
53. ETHYL BROMOPHOS	inactive		
54. MALATHION	inactive		
55. TRIMETHYL-PHOSPHATE	M		
56. TRIETHYL-PHOSPHATE	M		
57. ATRAZINE	M	ma	hma
58. EPICHLOROHYDRINE	M		hma
59. METHYLUREA	inactive		hma
60. NITROUS ACID + METHYLUREA	M		hma
61. AMINOPYRINE	inactive		hma
62. NITROUS ACID + AMINOPYRINE	M		hma
63. DIMETHYLAMINE	inactive		hma
64. NITROUS ACID + DIMETHYLAMINE	M		hma
65. 1,1,1 TRICHLOROETHANE	inactive		hma
66. 2-[p-(1-OXO-2-ISOINDOLINYL)-PHENYL] BUTYRIC ACID			
67. 5-METHYL-PIRAZIN-2-CARBOXILIC-4-OXIDE ACID			
68. UV LIGHT	M		
69. X RAYS	M		
70. SPONTANEOUS	M	ma	hma
71. CYCLOHEXANONE	inactive		hma
+ 20 other compounds			

M = mutagenic ma = in vitro: metabolic activation

hma = in vivo: host mediated assay

8. RESULTS

In the following tables and figs. the results so far obtained in the several performed experiments are reported; the composition of chemical's samples employed in the mutagenic analyis is also encluded.

TABLE 1

CONTAMINANTS OF A TECHNICAL GRADE SAMPLE OF TRICHLOROETHYLENE NONTEDISON EMPLOYED IN MUTAGENICITY TEST

Compound	% w/w
TRICHLOROETHYLENE	99
1,2-EPOXYBUTANE	0.10
ETHYLACETATE	0.04
EPICHLORHYDRIN	0.09
N-METHYL PYRROLE	0.02
DI-ISOBUTYLENE	0.03

TABLE 2

MUTAGENICITY OF TCE TECHNICAL GRADE ON *S.pombe* BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE (16 HOURS OF INCUBATION)

Treatment	Dose mg/kg	Animal n°	Forward n°	Mutants M.F.x10^{-4}	M ± S.E.
NONE	-	1	5/56400	0.88	
"	-	2	6/72100	0.83	0.94±0.08
"	-	3	8/72540	1.10	
TCE	500	1	12/6660	1.80	
"	"	2	12/108240	1.11	1.33±0.33
"	"	3	7/39380	1.77	
"	"	4	6/90900	0.66	
TCE	1,000	1	5/58680	0.85	
"	"	2	5/56210	0.89	1.32±0.19
"	"	3	16/76230	2.09	
"	"	4	6/41200	1.46	
TCE	2,000	1	1/20200	0.50	
"	"	2	8/39700	2.69	1.61±0.45
"	"	3	12/83330	1.44	
"	"	4	3/18700	1.80	
MMS	50	1	15/28700	5.22	4.16±0.[illegible]
"	"	2	21/54300	3.86	
"	"	3	18/52700	3.41	

Regression analysis (x=mg/kg; y=F.M.x10^{-4})

$y = 1.0379 + 0.000299x \quad r = 0.86399^{**}$

TABLE 9

MUTAGENICITY OF TCE TECHNICAL GRADE ON S.typhe BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE (16 HOURS OF INCUBATION)

Treatment	Dose mg/kg	Animal n°	Forward n°	Mutants M.F.x10^{-4}	M ± S.E.
NONE	-	1	5/56400	0.88	
"	-	2	6/73100	0.83	0.84±0.08
"	-	3	8/72040	1.10	
TCE	500	1	12/6660	1.80	
"	"	2	12/108240	1.11	1.33±0.15
"	"	3	7/39380	1.77	
"	"	4	6/90900	0.66	
TCE	1,000	1	5/58680	0.85	
"	"	2	5/56210	0.89	1.32±0.19
"	"	3	10/76230	1.09	
"	"	4	8/41200	1.46	
TCE	2,000	1	1/20200	0.50	
"	"	2	8/29700	1.69	1.61±0.41
"	"	3	12/83330	1.44	
"	"	4	3/18700	1.80	
NMS	50	1	15/28700	5.22	4.16±0.[illegible]
"	"	2	21/54300	3.86	
"	"	3	18/52700	3.41	

Regression analysis (x mg/kg; y = F.M.x10^{-4})

$y = 1.0370 + 0.000.99x$ $r = 0.86399^{**}$

TABLE 3

CONTANINANTS OF A PURE GRADE SAMPLE OF TRICHLOROETHYLENE ACHIEVISON EMPLOYED IN MUTAGENICITY TESTS

Compound	% w/w
TRICHLOROETHYLENE	99.66
CLOROFORM	10 ppm
CARBON TETRACHLORIDE	17 "
1,1,2-TRICHLOROETHANE	21 "
1,1,2,2-TETRACHLOROETHANE	14 "
OTHER CONTANINANTS	16 "

p-Tert. BUTHYLCATECHOL used as a stabiliser.

TABLE 4

MUTAGENICITY OF A PURE GRADE SAMPLE OF TCE ON S.pombe BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE

Treatment	Dose mg/kg	Incubation hours	Animal n°	Forward mutants n°	M.F.x10-4	M±S.E
NONE (*)	-	3	1	3/40530	0.60	
"	-	"	2	1/39716	0.25	0.60±0.39
"	-	"	3	5/32140	1.55	
NONE (*)	-	6	1	3/39318	0.76	
"	-	"	2	2/37606	0.53	0.74±0.13
"	-	"	3	3/30435	0.93	
NONE (*)	-	16	1	8/18450	2.10	
"	-	"	2	6/29017	2.00	1.46±0.60
"	-	"	3	1/39716	0.25	
TCE	1,000	16	1	6/55200	1.03	
"	"	"	2	7/36655	1.90	1.59±0.22
"	"	"	3	3/14900	2.00	
"	"	"	4	3/22045	1.36	
TCE	2,000	3	1	4/57600	0.69	
"	"	"	2	2/34020	0.59	0.84±0.20
"	"	"	3	3/24500	1.23	
TCE	3,000	6	1	1/22750	0.29	
"	"	"	2	1/34506	0.26	0.44±0.10
"	"	"	3	3/33960	0.75	
TCE	2,000	16	1	4/27200	1.47	
"	"	"	2	3/31610	0.95	1.36±0.31
"	"	"	3	5/29930	1.67	

continua

DMNA	100	16	1	102/40182	20.70	
"	"	"	2	45/23344	19.20	16.73±3.24
"	"	"	3	47/45291	10.30	

(*) average CONTROL 1.[illegible] ± 0.22

<u>Regression analysis</u> (x = mg/kg y = F.M. x 10^{-4})

y = 1.5177 - 0.0[illegible]x r = 0.[illegible] (NS)

TCE PURE GRADE SAMPLE

TRICHLOETHYLENE	99.98	%
CHLOROFORM	10	ppm
CARBON TETRACHLORIDE	17	"
1.1.2-TRICHLOROETHANE	21	"
1.1.2.2-TETRACHLOROETHANE	14	"
OTHER CONTAMINANTS	16	"

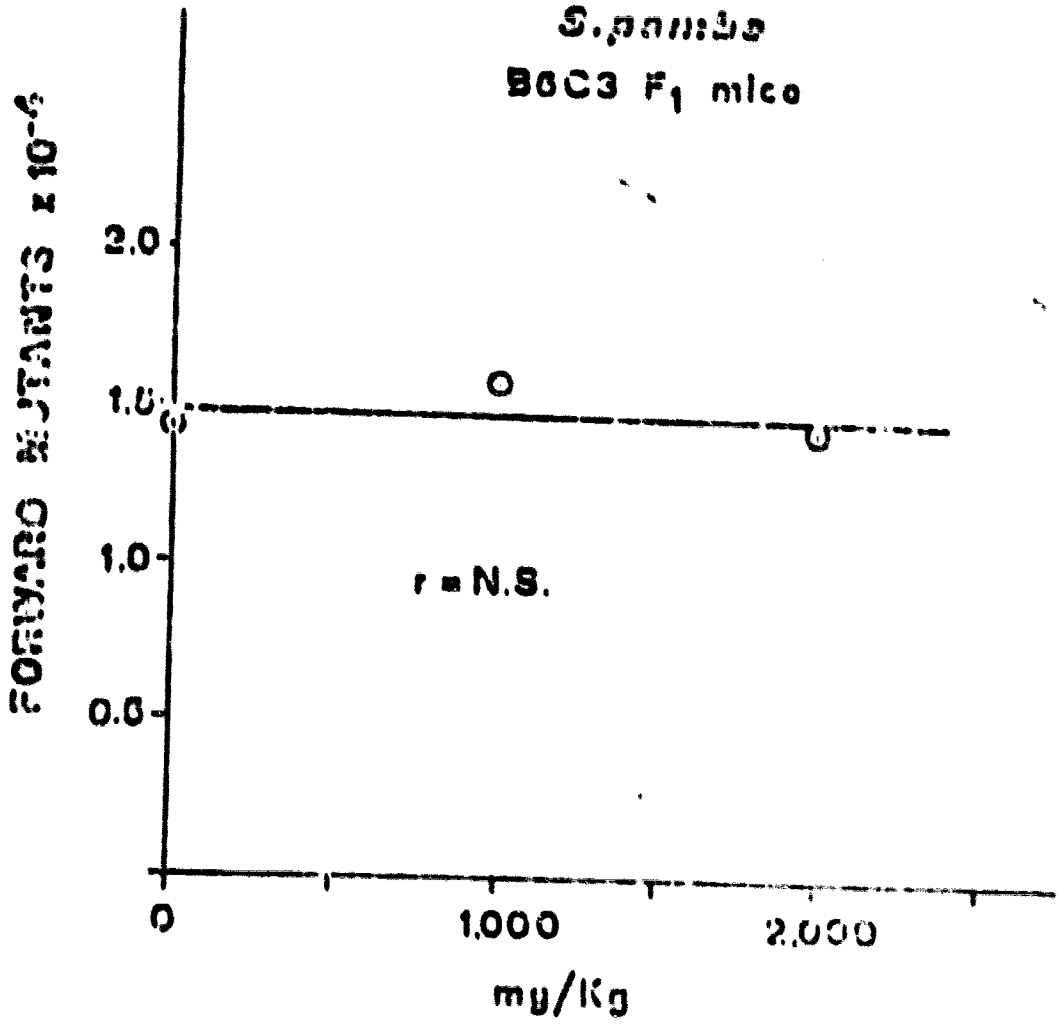


FIG. 2

TCE PURE GRADE SAMPLE

TRICHLOROETHYLENE	99.98 %
CHLOROFORM	10 ppm
CARBON TETRACHLORIDE	17 "
1.1.2.- TRICHLOROETHANE	21 "
1.1.2.2 - TETRACHLOROETHANE	14 "
OTHER CONTAMINANTS	16 "

S. pomba

B6C3 F_1 mice

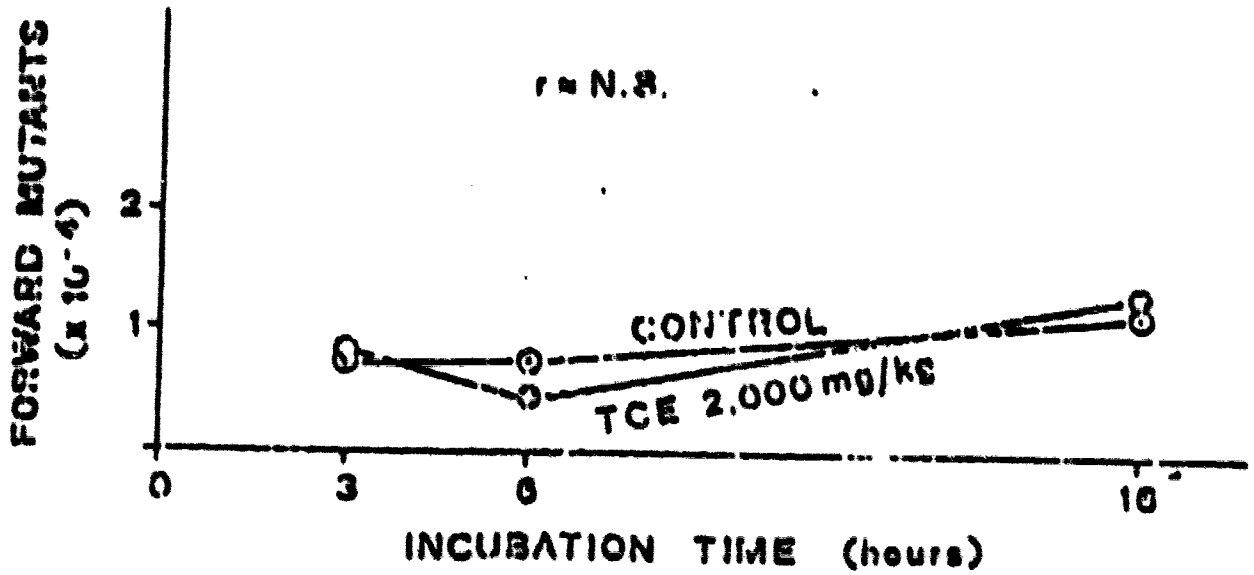


FIG. 3

TABLE 5

MUTAGENICITY OF EPICHLOROHYDRIN ON *S.typhi* BY MEANS OF AN *in vitro* ASSAY (10 HOURS OF INCUBATION).

Treatment	Dose mM	Survival %	Forward mutants n°	M.F.x10^{-4}
NONE	-	100	3/16461	1.82
EPC	0.25	98.9	8/17328	4.62
"	0.50	119.0	14/15156	9.24
"	1.00	58.4	17/7168	23.72
"	2.00	12.9	5/1584	31.56

Regression analysis (x = mM; y = M.F. x 10^{-4})

$y = 2.37 + 15.76x$ $r = 0.938^{***}$

EPICHLOROHYDRIN

S.pemba

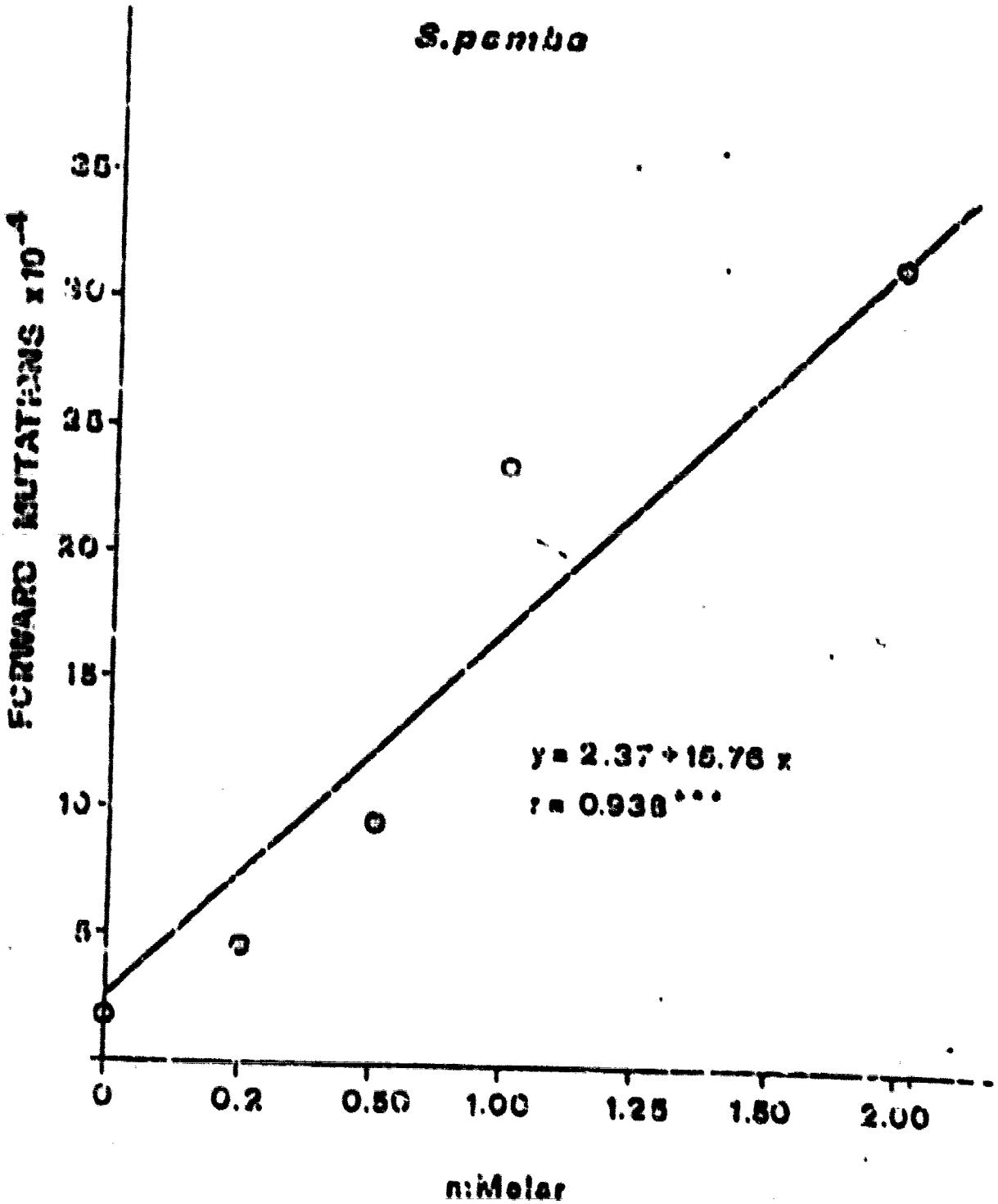


FIG. 4

TABLE C

MUTAGENICITY OF EPICHLOROHYDRIN ON S.pombe BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE
(16 HOURS OF INCUBATION)

Treatment	Dose mg/kg	Animal n°	Forward Mutants n°	M.F.×10^{-4}	M±S.E.
NONE	-	1	26/135680	1.91	
"	-	2	34/109256	1.79	1.68±0.30
"	-	3	42/311520	1.34	
EPC	2	1	24/767350	0.31	
"	"	2	96/620000	1.54	1.50±1.20
"	"	3	50/480000	1.00	
"	"	4	53/168600	3.14	
EPC	10	1	47/217910	2.15	
"	"	2	40/269656	1.48	2.05±0.51
"	"	3	39/155232	2.51	
EPC	50	1	31/114576	2.70	
"	"	2	29/79772	3.63	3.71±1.05
"	"	3	35/72896	4.86	

Regression analysis (x = mg/kg; y = M.D. × 10^{-4})

$y = 1.95 + 0.0747x$ $r = 0.99^{***}$

EPICHLOROHYDRIN

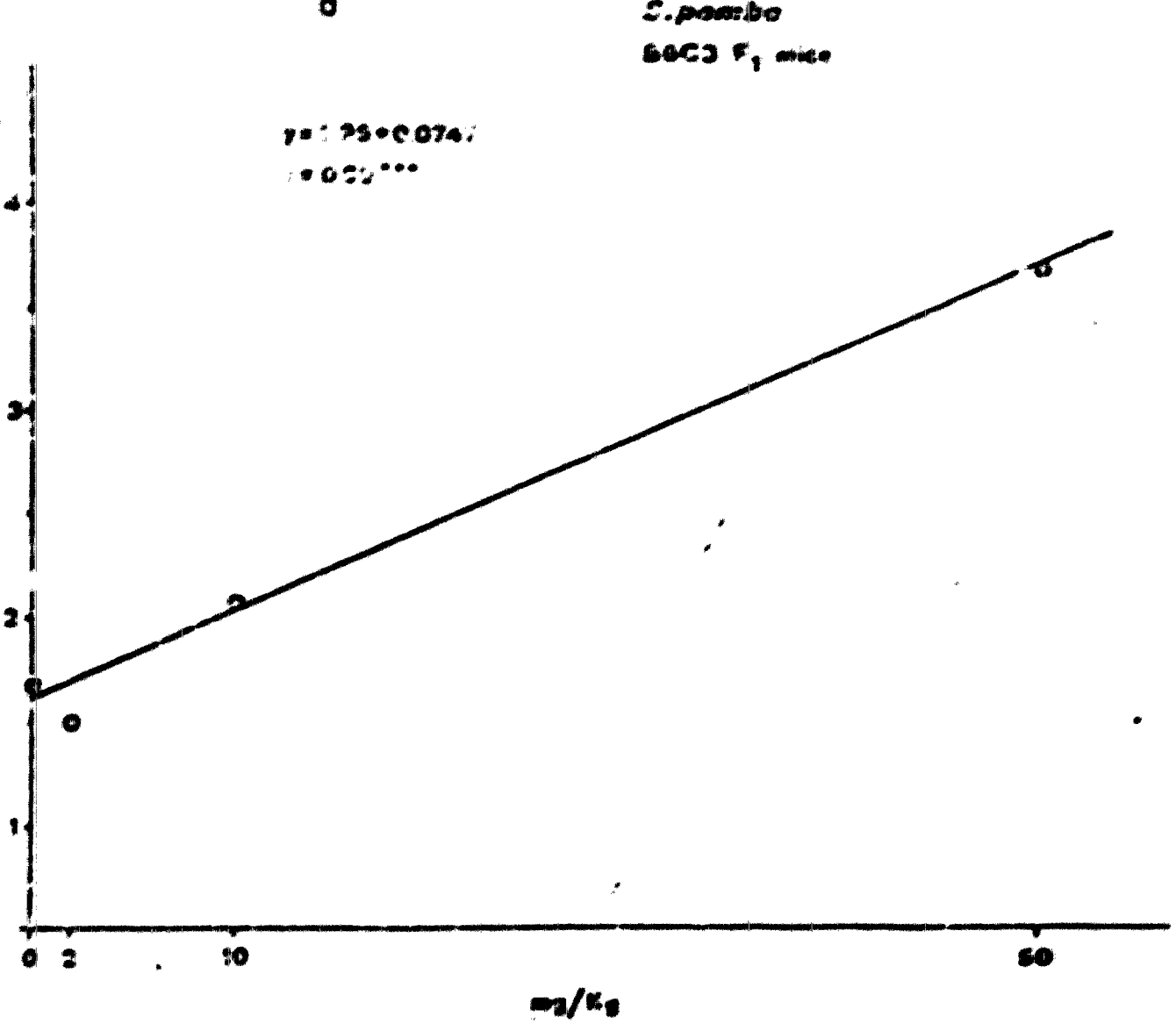


FIG. 5

TABLE 7

SUMMARY OF MUTAGENICITY DATA OF TCE SAMPLES EVALUATED FOR THE EPICHLORHYDRINE CONTENT

Treatment	Regression analysis	Specific mutag. activity mg/kg
TCE (distilled sample)	y = 1.51 - 0.000065x	N.S.
TCE (technical sample)		
TCE	y = 1.037 + 0.00299x	0.00299
EPC	y = 1.030 + 0.0337x	0.0377
EPICHLORHYDRIN	y = 1.95 + 0.0747x	0.0747

Test: FORWARD MUTATIONS

YEAST (*S. pombe*) + H.I.A. (18 h)

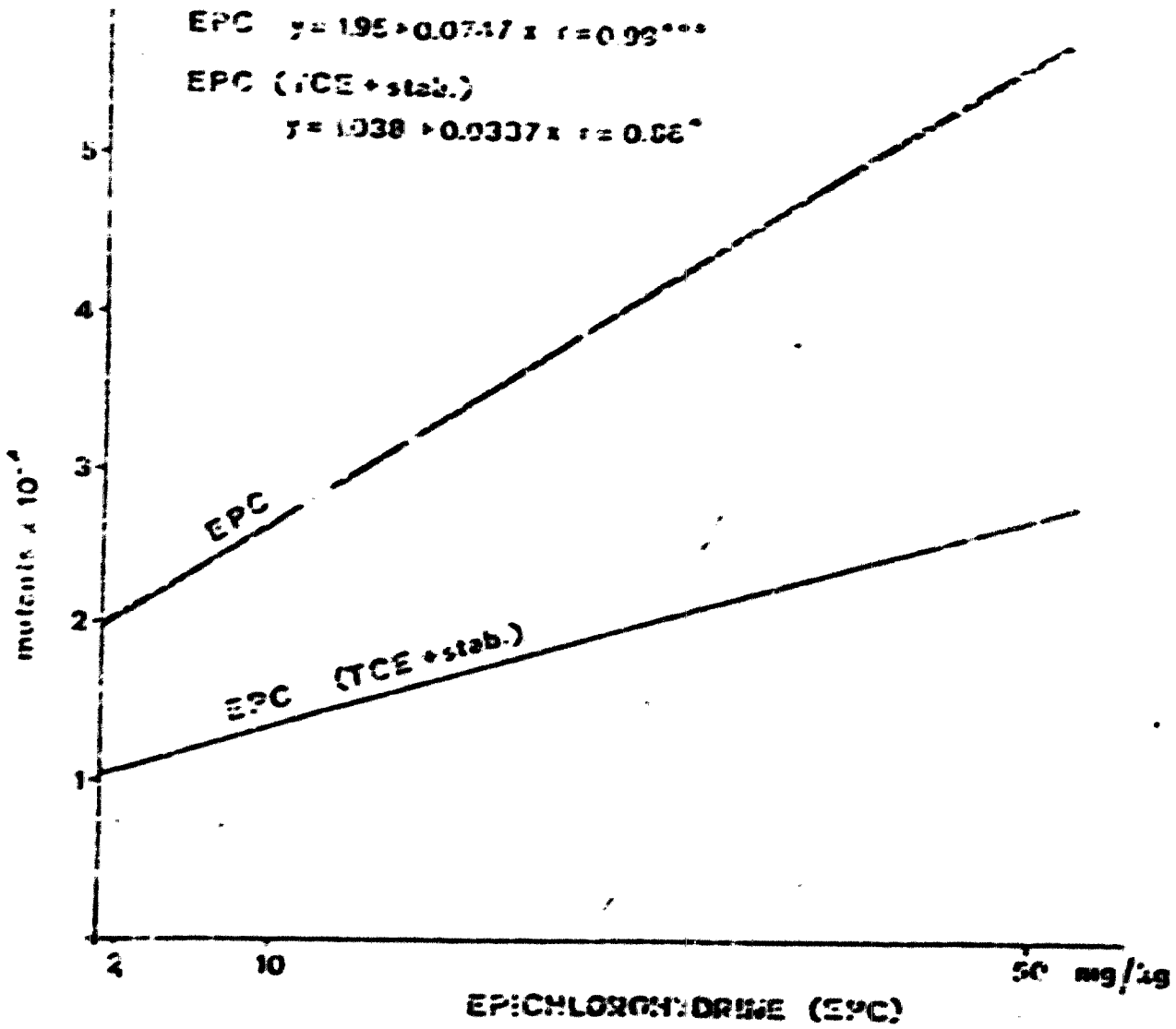


Fig. 6

TABLE 8

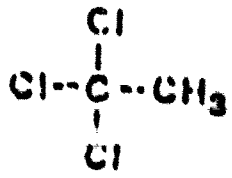
MUTAGENICITY OF 1,1,1-TRICHLOROETHANE ON S.bombe BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE

TREATMENT mg/kg	Inc.time (hours)	DOSE mg/kg	ANIMAL n°	FORWARD MUTANTS n°	M.F.x10^{-4}	M±S.E.
NONE	3h	-	1	3/[illegible]6520	0.[illegible]	
"		-	2	1/39[illegible]16	0.25	0.80±0.39
"		-	3	5/32140	1.55	
NONE	6h	-	1	3/39312	0.76	
"		-	2	2/37806	0.53	0.76±0.13
"		-	3	3/30[illegible]5	0.9[illegible]	
NONE	16h	-	1	4/18450	2.10	
"		-	2	6/29017	2.00	1.45±0.60
"		-	3	1/39716	0.25	
1,1,1-TCE	3h	5,000	1	1/20913	0.47	
"		"	2	1/29422	0.33	0.27±0.14
"		"	3	0/3[illegible]179	-	
1,1,1-TCE	6h	5,000	1	6/[illegible]253	1.2[illegible]	
"		"	2	2/[illegible]743	0.[illegible]	0.[illegible]±0.28
"		"	3	1/[illegible]	0.[illegible]	
1,1,1-TCE	16h	5,000	1	4/[illegible]853	1.0[illegible]	
"		"	2	7/[illegible]	1.[illegible]	0.97±0.06
"		"	3	5/[illegible]320	0.85	

Regression analysis (x = h, y = F.M. x10^{-4})

y = 0.642 + 0.0149 x = 0.35 (NS)

1,1,1-TRICHLOROETHANE



S. pomona

B6C3 F_1 mice

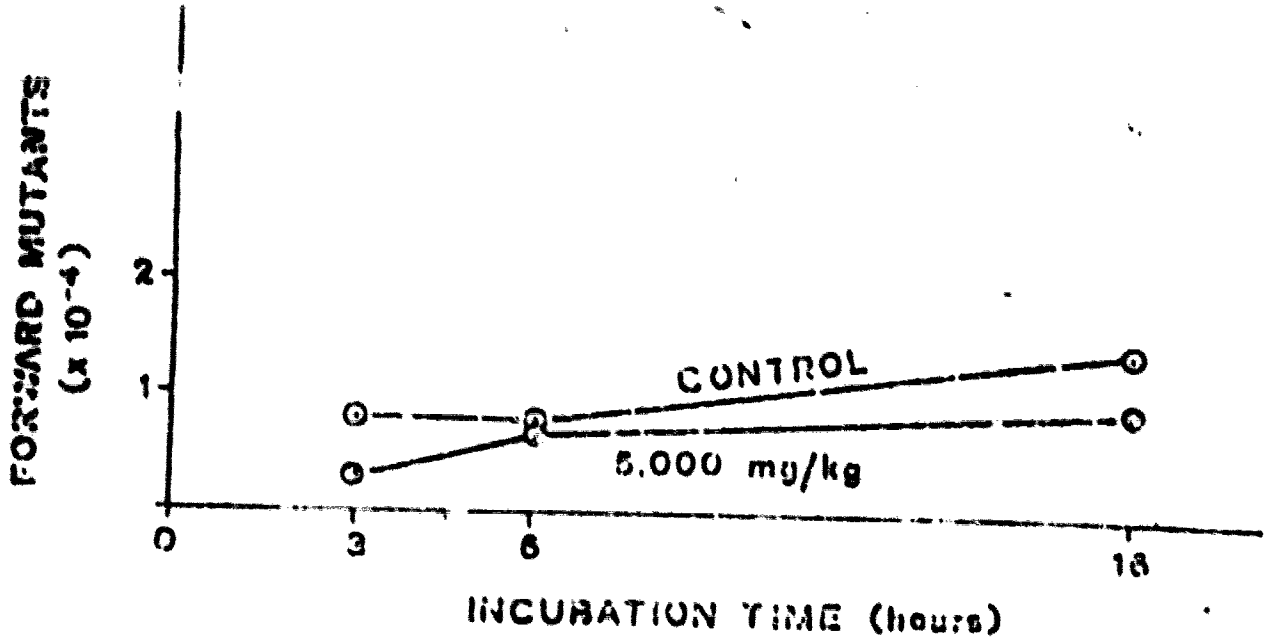


Fig. 7

TABLE V

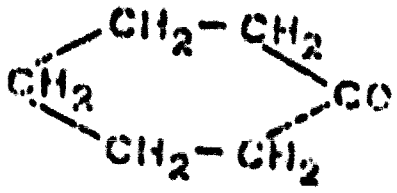
MUTAGENICITY OF CYCLOHEXANONE ON S.pombe BY MEANS OF THE HOST MEDIATED ASSAY ON B6C3F1 HYBRID MICE (INCUBATION TIME: 16 HOURS).

TREATMENT	DOSE mg/kg	ANIMAL n°	FORWARD MUTANTS n°	N.F.x10⁻⁴	M±S.E.
NONE	-	1	2/65000	0.30	
"	-	2	3/66[illegible]80	0.43	0.47±0.19
"	-	3	4/58500	0.60	
CYCL.	300	1	3/58400	0.51	
"	"	2	3/64000	0.46	0.46±0.05
"	"	3	2/48000	0.41	
CYCL.	600	1	2/50720	0.39	
"	"	2	3/71040	0.42	0.42±0.03
"	"	3	2/42880	0.46	
CYCL.	1,200	1	3/55360	0.54	
"	"	2	2/55500	0.36	0.42±0.10
"	"	3	2/54400	0.36	

Regression analysis (x = mg/kg y = F.M.x10⁻⁴)

y = 0.408 + 0.0000[illegible]4x r = 0.87 (ns)

CYLOHEXANONE



S. pombe

B6C3 F_1 mice

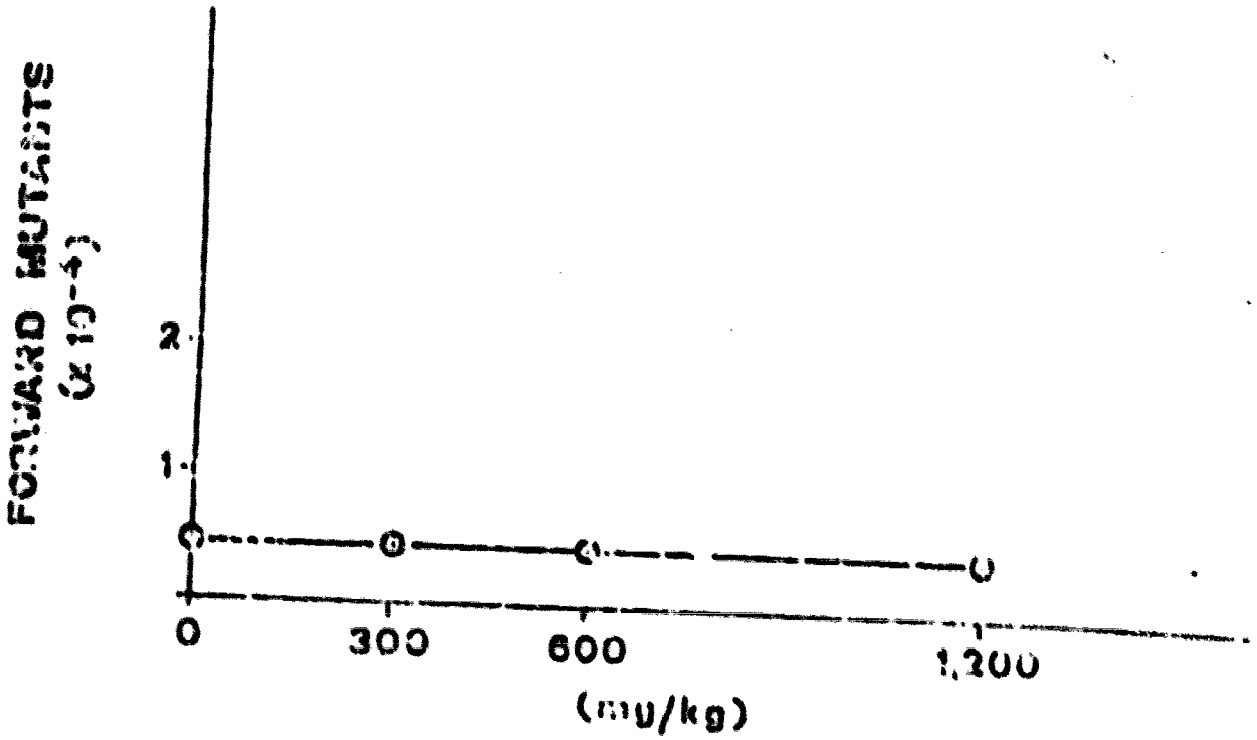


FIG. 8

8. DISCUSSION

On the basis of our studies, it seems reasonable to conclude that the carcinogenic effect described in the ICI's experiments might be due to the presence of epichlorohydrins, on the basis of mutagenic experiments presented in this paper.

In Table 10 the results of many mutagenic studies so far published for TCE are reported: in the majority of these studies no indications were included to the actual composition of samples employed in the analysis: at the present it is not possible to draw some conclusion on the possible mutagen activity of trichloroethylene (3).

In the experimental conditions employed in the present study other solvents, such as 1,1,1-trichloroethane and cyclohexanone have resulted negative.

It is therefore of extreme urgency to develop more appropriate methodological studies with trichloroethylene of known chemical composition in different mutational assays.

TABLE 10

RESULTS OF MUTAGENICITY SHORT-TERM STUDIES DEVELOPED ON UNSTERILIZED TCE SAMPLES (a)

Organism	Technique	Results
1. *Salmonella typhimurium*	in vitro	+
2. *Escherichia coli*	in vitro	+
3. *Schizosaccharomyces pombe*	in vitro	-
	h.m.a. (mice)	+
	h.m.a. (rats)	-
4. *Saccharomyces cerevisiae*	in vitro	+
	h.m.a. (mice)	+
	h.m.a. (rats)	+
5. Chinese hamster	in vitro	-
6. *Drosophila melanogaster*	in vivo	+
7. Mouse (bone marrow)	in vivo	-
8. Hamster (bone marrow)	in vivo	-
9. Mouse (Somatic mutation)	in vivo	+
10. *Tradescantia* (Somatic mutation)	in vivo	+

9702/1394

10. REFERENCES

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