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INTERNATIONAL ISOCYANATE INSTITUTE, INC.
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Attn: 8(d) HEALTH 7 SAFETY STUDY REPORTING RULE
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Dear Sir or Madam:

We herewith submit a copy of the following recently completed health and safety study.

"DIPHENYLMETHANE 4,4'-DIISOCYANATE (MDI-POLYMER):Evaluation of respiratory sensitization in guinea pigs following single high-level and repeated low-level inhalation induction exposure." A second report, "Guinea pig model for MDI asthma" is also enclosed-this details the determination of antibody levels for project 11322, and is part of the overall study.

Name of Chemical Substance:	benzene 1,1'-methylenebis[iscoyanato-
Common name:	generic MDI
Chemical Abstracts Service Number:	26447-40-5
Abbreviation:	MDI

Contains No CBI

Authors:

J. Pauluhn
Bayer AG
Department of Toxicology
Friedrich-Ebert-Str. 217-333
D-42096 Wuppertal
Germany

R. Dearman
Zeneca CTL
Macclesfield, Cheshire, UK

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A 04

The International Isocyanate Institute (III) project identification number (11332) has been marked on the title page of the report. The supplemental report has been assigned (11323). Please refer to the III identification number(s) in any communication regarding this study. The enclosed report does not contain any Confidential Business Information.

This study was sponsored by the International Isocyanate Institute on behalf of the following:

The Dow Chemical Company
Bayer Corporation
BASF Corporation
ICI Americas, Inc.
Lyondell Chemical Company

Very truly yours,

M.J. Blankenship
Managing Director

Enclosure: Study

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III Project 153

III Ref. 11323

Guinea pig model for MDI asthma

R Dearman

Zeneca Central Toxicology Laboratory
Macclesfield, Cheshire, UK

This report details the determination of antibody levels for Project 153. The full study protocol, methods used, other investigations are described in III Ref. 11322 In addition III Ref. 11322 describes the results of this report in context of the whole study.

Number of pages: 33

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III Project 1[illegible]

11323

CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK

REPORT NO : CTL/L/8246
153-EU-MTX : GUINEA PIG
MODEL FOR MDI ASTHMA

STUDY DETAILS

Sponsor: M A Collins
on behalf of
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The III Scientific Office
Bridgewater House
Floor 9, Whitworth Street
Manchester
M1 6LT
UK

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REPORT TYPE

Summary

AUTHORS

R J Dearman

APPROVED FOR ISSUE

DATE OF ISSUE

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STATEMENT OF COMPLIANCE

This report is based on carefully conducted research studies which, however, are not fully compliant with GLP standards. The data described in this summary report have not been audited by the CTL Quality Assurance Unit.

R J Dearman .....

Study Director

P M Hext .....

Product Manager, Zeneca Central Toxicology Laboratory

A 08

I, the undersigned, declare that this report constitutes a true record of the actions taken and the results obtained in the above study.

R J Dearman .....

Study Director

Date 15/10/98

A 09

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1. SUMMARY

Guinea pig serum samples from animals exposed by inhalation to increasing concentrations (1; 3; 10; 30mg/m^3) of polymeric MDI (pMDI) for various periods of time (15 minutes; one hour; six hours; fifteen periods of six hours) or air controls were analyzed by enzyme-linked immunosorbent assay (ELISA) for MDI-specific IgG1 antibody.

Serum samples from air exposed control guinea pigs (10 per group exposed to air for 15 minutes; one hour; six hours; fifteen periods of six hours) were all negative for anti-MDI IgG1 antibody (titres were less than 1 in 10).

Inhalation exposure to all concentrations of pMDI resulted in the production of detectable IgG1 antibody in at least a proportion of animals. There was considerable within group variation in antibody titre which reflects presumably the outbred nature of the guinea pig population. Despite this variation, it is clear that the number of responders per group and the antibody titre of serum samples showed a marked dose response relationship with respect to the total amount of pMDI received (length of exposure and concentration). Thus, for example, exposure to 3mg/m^3 pMDI for 15 minutes resulted in detectable but low titre antibody (1 in 10 to 1 in 160) in only 5 out of 10 animals. Exposure to the same concentration of pMDI for 6 hours and fifteen periods of 6 hours resulted in 9 out of 10 and 10 out of 10 antibody positive responders, respectively, with titres ranging from 1 in 10 to 1 in 1280 and 1 in 320 to 1 in 10240, respectively.

No-observable-effect-levels for pMDI inhalation were demonstrated with respect to concentrations of pMDI and exposure times which did not generate significant antibody production. Thus, compared with concurrent air-treated controls, exposure to 3mg/m^3 or 10mg/m^3 pMDI for 15mins or 1mg/m^3 pMDI for 6 hours failed to elicit significant levels of anti-MDI IgG1 antibody (not statistically significantly different from control groups at the 1% level).

2. INTRODUCTION

Guinea pig serum samples from animals exposed to polymeric MDI (pMDI) by inhalation for various periods of time or air controls in study 153-EU-MTX were received from the Department of Toxicology, Bayer AG, Wuppertal in March 1998.

The agreement with the International Isocyanates Institute was that an MDI guinea pig serum albumin (MDI-GSA) conjugate prepared using monomeric MDI for analysis of serum samples for study 114-EU-MTX (CTL/R/1211) would be utilized in the enzyme-linked immunosorbent assay (ELISA) developed for the detection of MDI-specific IgG1 antibodies in the current experiments. This MDI-GSA conjugate was prepared using monomeric MDI; such is appropriate for the determination of anti-MDI IgG1 antibody responses following exposure to polymeric MDI since not only is monomeric MDI a major constituent of polymeric MDI but also the antigenic determinants appear to be largely cross-reactive, as evidenced by the ability of conjugates prepared from monomeric MDI to detect anti-MDI antibody production in serum derived from animals exposed to monomeric or polymeric (CTL/R/2926 and CTL/L/8246).

The report on this study will be stored in the Central Toxicology Laboratory (CTL) Report Centre as stipulated in the contract.

3. MATERIALS AND METHODS

3.1 Preparation of MDI-GSA conjugate

MDI-GSA conjugate was prepared as described previously (Rattray *et al.*, 1994; CTL/R/1211). Approximately 200mg of GSA was dissolved in 20ml 0.05M sodium borate buffer (pH9.4). Approximately 60mg of monomeric MDI was added and the solution stirred at 4°C for 30 minutes. Glass vessels were used throughout as MDI reacts with plastic. The solution was dialyzed successively against phosphate buffered saline (PBS; pH7.2) and distilled water for a period of approximately 48 hours at 4°C. The conjugate was lyophilized and stored at -20°C until use.

3.2 Analysis of conjugate

The degree of substitution of the MDI conjugate was assessed using a method based upon the determination of free amino groups by reaction with 2,4,6-trinitrobenzene sulphonic acid (TNBS) as described previously (Rattray *et al.*, 1994). Approximately 1mg of MDI-GSA conjugate and 1mg of GSA were each dissolved in approximately 1 ml of 0.1M sodium borate buffer (pH9.3). 25µl of a stock solution of 1.2M TNBS in 0.1M sodium borate buffer (pH9.3) was added to each sample and the samples incubated for approximately 20 minutes at room temperature. The optical density at 420nm (OD) was measured using a Philips spectrophotometer (PU 8880uv/vis). GSA has approximately 30 readily available hapten-binding sites per molecule. The degree of substitution (moles hapten/moles protein) is calculated according to the formula:-

Substitution ratio = $\frac{(1\text{-OD sample})}{(\text{OD GSA})} \times 30$

OD sample = 0.522

OD GSA = 1.024

Substitution ratio = $\frac{(1-0.522) \times 30}{(1.024)}$

The conjugate substitution ratio was therefore approximately 15:1 moles hapten:moles protein.

3.3 Storage of serum samples

Serum samples were shipped frozen from Bayer AG, Wuppertal and arrived in good condition. On arrival, the samples were stored at -20°C until analysis which was completed by 24th April 1998.

3.4 Enzyme-linked immunosorbent assay

Plastic microtitre plates (NUNC Immunoplate type II, Nunc, Copenhagen, Denmark) were coated with 50µl per well of 5µg/ml MDI-GSA conjugate in 0.5M sodium carbonate/bicarbonate buffer (pH9.6) by overnight incubation at 4°C. This was administered on to the plate using a 12 channel adjustable repeat dispenser set to take up and dispense 50µl of fluid. The plates were flicked out and washed three times for approximately 3 minutes each wash with 100µl PBS containing 0.05% Tween 20 (PBS-Tween). Guinea pig serum samples were serially diluted in PBS-Tween. The serially diluted guinea pig serum samples were added to duplicate wells (100µl per well). Each plate also had a row of wells to which diluent (PBS-Tween) only was added (reagent blank wells). The plates were incubated for 30 minutes at 37°C. The plates were washed as before and 100µl rabbit anti-guinea pig IgG1 (Miles Scientific, Slough, UK), diluted in 1 in 2500 in PBS-Tween, was added to each well. After a further 30 minutes incubation at 37°C, plates were washed as before and 100µl peroxidase-labelled goat anti-rabbit IgG (Miles Scientific) diluted 1 in 5000 in PBS-Tween was added to each well. Following 30 minutes incubation at 37°C, the plates were washed and 100µl substrate added (1.6mg/ml 0-phenylene diamine and 0.4mg/ml urea hydrogen peroxide in 0.05M citrate phosphate buffer [pH5.0] per well). The reaction was terminated after approximately 10 minutes by the addition of 50µl of 0.5M citric acid per well.

Absorbance was measured at 450nm using an automated plate reader (Multiskan, Flow Laboratories, Irvine, Ayrshire, UK).

Serial dilutions of serum samples (1 in 10, 1 in 20, 1 in 40, 1 in 80, 1 in 160 and 1 in 320) in PBS-Tween were analyzed in preliminary ELISAs. In subsequent assays, serum samples with titres of 1 in 320 or higher were reanalyzed by ELISA using serial doubling dilutions of 1 in 10 to 1 in 20480.

3.5 Criterion for positivity for individual serum samples

The mean optical density for the reagent blank wells for each plate was calculated, as were the means of each duplicate serum sample. A reading is regarded as positive if it is higher than twice the reagent blank for the particular plate. The titre of each serum is the highest dilution which gives a positive reading.

As an additional control for the integrity of the MDI-GSA conjugate (synthesized in December 1993), selected high titre serum samples from study 114-EU-MTX were reanalyzed alongside the samples from study 153-EU-MTX and compared with the results of the previous analyses (February 1995 and May 1996; described in reports CTL/R/1239 and CTL/T/2926).

3.6 Statistical analysis

For each ELISA dilution curve for each individual serum sample, the area under the curve was calculated. The areas were considered using analysis of variance following a log transformation to stabilize the variance. Least-squares means for each group were calculated. Unbiased estimates of differences from concurrent control groups were provided by the difference between each treatment group least-squares mean with the appropriate control groups least-squares mean. Differences from controls were tested statistically by comparison of each treatment group least-squares mean with the control group least-squares mean using a two-sided student's t-test based on the error mean square in the analysis. Differences were considered to be statistically significant at the 1% level.

4. RESULTS

Repeat analyses of serum samples 26, 27 and 28 (high titre samples from study 114-EU-MTX) confirmed the integrity of the MDI-GSA conjugate and the MDI ELISA, with antibody profiles comparable to previous analyses (performed in February 1995 and May 1996) being achieved (Figure 1).

Guinea pig serum samples from study 153-EU-MTX were therefore analyzed for anti-MDI IgG1 antibody using the same ELISA protocol. Serum derived from air control animals (animal numbers 1-10, 41-50, 81-90 and 121-130 inclusive) were uniformly negative for anti-MDI IgG1 antibody (titres were less than the top dilution of serum; 1 in 10). Exposure to increasing concentrations of pMDI by inhalation resulted in a clear dose response with respect to the number of animals exhibiting detectable IgG1 antibody and the IgG1 titre observed. Profiles of anti-MDI IgG1 antibody responses for animals exhibiting titres of above 1 in 10 are displayed in Figure 2a-l. Animals exposed to 3 or 10mg/m^3 of pMDI for 15 minutes (Figure 2a, b) exhibited low titre antibody or no detectable antibody. Exposure to 30mg/m^3 of pMDI for 15 minutes resulted in low titre antibody for the majority of animals, with one animal (number 23) displaying high titre antibody (Figure 2c). Treatment with 3mg/m^3 of pMDI for one hour also resulted in low titre antibody expression (Figure 2d). Somewhat higher titres were observed following one hour exposure to 10 or 30mg/m^3 of material (Figure 2e, f), although there was considerable interanimal variation. Six hours exposure to 1mg/m^3 pMDI provoked relatively low titre antibody (Figure 2g), with increased titres and increased numbers of responders observed following exposure to 3mg/m^3 pMDI for the same period of time (Figure 2h). Inhalation exposure to 10mg/m^3 for 6 hours and to 1, 3 and 10mg/m^3 pMDI for fifteen periods of six hours resulted in high titre antibody production with less interanimal variation than that observed for lower concentrations of material (Figure 2i, j, k, l).

The anti-MDI IgG1 antibody titres derived for each individual animal are displayed in Table 1.

The distribution of anti-MDI IgG1 antibody titres within each experimental group is shown, together with the group mean and median titres, in Table 2. These data are displayed

graphically in Figure 3a-p. These data show clearly the marked relationship between the amount of pMDI exposure (concentration and time of exposure) and the antibody titre. Thus exposure to 3mg/m^3 pMDI for 15 minutes or one hour or to 1mg/m^3 pMDI for 6 hours fails to provoke detectable antibody in the majority of animals examined with median titres of <1 in 10 or 1 in 10 observed. Exposure to increasing amounts of pMDI or increased exposure periods resulted in measurable antibody production by the majority of animals. Thus, for example, mean and median titres of 1 in 3406 and 1 in 2560 were provoked by exposure to 3mg/m^3 pMDI for 15 periods of 6 hours. There was clearly some within group animal variation in titre, for example, following exposure to 30mg/m^3 pMDI for 15 minutes, the majority of animals displayed titres of 1 in 320 or less; however one animal displayed a titre of 1 in 10240. These outlying results were confirmed by repeat ELISA analyses (data not shown).

The analyses performed with the current serum samples were compared with those derived from study 135-EU-MTX (described in report CTL/T/2926). Animals were exposed for 15 minutes to 10, 100 or 900mg/m^3 pMDI. Antibody profiles are displayed in Figure 4a-c. A comparison of antibody responses observed following treatment with 10mg/m^3 pMDI for 15 minutes in the current study (Figure 2b) with the previous study (Figure 4a) reveals that consistent low titre responses have been induced in both experiments. Exposure to higher concentrations of pMDI (100 or 900mg/m^3) resulted in higher titre antibody, with treatment with 900mg/m^3 pMDI giving a similar distribution of antibody titres to those observed following exposure to 3 or 10mg/m^3 pMDI for 15 periods of 6 hours.

Finally, the statistical significance of differences between antibody responses induced by exposure to pMDI and concurrent air treated controls was analyzed (Table 3). These analyses revealed that treatment with 3 or 10mg/m^3 pMDI for 15 minutes or with 1mg/m^3 pMDI for 6 hours failed to elicit statistically significant anti-MDI IgG1 antibody responses (not significantly different from control groups at the 1% level). All other exposure regimes resulted in significant anti-MDI IgG1 antibody production ($p<0.01$).

5. CONCLUSIONS

Exposure of guinea pigs to polymeric MDI by inhalation elicited a clear dose response with respect to antibody production and the total MDI dose (concentration and time of exposure). All air control groups were negative for anti-MDI IgG1 antibody (titres of less than 1 in 10). A no-observable-effect level was demonstrated with respect to concentrations of pMDI and exposure times which did not generate significant antibody production. Thus exposure to $3mg/m^3$ or $10mg/m^3$ pMDI for 15 minutes or to $1mg/m^3$ for 6 hours failed to provoke significant anti-MDI IgG1 antibody responses compared with concurrent air treated control groups. Treatment with higher concentrations of pMDI or for longer exposure periods resulted in the production of significant IgG1 antibody in each case.

6. REFERENCES

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CTL/R/1211. Evaluation of antibody responses in guinea pigs following intradermal induction and MDI challenge.

CTL/R/1239. Evaluation of antibody responses in guinea pigs following inhalation induction and MDI challenge.

CTL/R/2926. Antibody production in the guinea pig.

7. TABLES

TABLE 1 - ANTIBODY RESPONSES FOLLOWING INHALATION EXPOSURE OF GUINEA PIGS TO pMDI

A. 15 minutes exposure

Group 1 Animal Number	Air Control titre	Group 2 Animal Number	3mg/m^3 titre	Group 3 Animal Number	10mg/m^3 titre	Group 4 Animal Number	30mg/m^3 titre
1	<1 in 10	11	1 in 20	21	1 in 160	31	1 in 20
2	<1 in 10	12	1 in 10	22	1 in 80	32	1 in 10
3	<1 in 10	13	< 1 in 10	23	1 in 80	33	1 in 10240
4	<1 in 10	14	1 in 160	24	< 1 in 10	34	1 in 160
5	<1 in 10	15	1 in 160	25	1 in 10	35	1 in 20
6	<1 in 10	16	< 1 in 10	26	1 in 320	36	1 in 20
7	<1 in 10	17	< 1 in 10	27	1 in 160	37	1 in 10
8	<1 in 10	18	1 in 10	28	1 in 40	38	< 1 in 10
9	<1 in 10	19	< 1 in 10	29	1 in 80	39	1 in 320
10	<1 in 10	20	< 1 in 10	30	1 in 10	40	1 in 160

B. One hour exposure

Group 5 Animal Number	Air Control titre	Group 6 Animal Number	3mg/m^3 titre	Group 7 Animal Number	10mg/m^3 titre	Group 8 Animal Number	30mg/m^3 titre
41	<1 in 10	51	1 in 80	61	1 in 1280	71	1 in 1280
42	<1 in 10	52	1 in 160	62	1 in 1280	72	< 1 in 10
43	<1 in 10	53	< 1 in 10	63	1 in 160	73	1 in 160
44	<1 in 10	54	< 1 in 10	64	1 in 160	74	1 in 160
45	<1 in 10	55	< 1 in 10	65	1 in 80	75	1 in 2560
46	<1 in 10	56	1 in 320	66	1 in 640	76	1 in 10
47	<1 in 10	57	< 1 in 10	67	1 in 160	77	1 in 80
48	<1 in 10	58	< 1 in 10	68	1 in 160	78	1 in 1280
49	<1 in 10	59	< 1 in 10	69	1 in 20	79	1 in 10
50	<1 in 10	60	1 in 160	70	1 in 80	80	1 in 80

C. Six hour exposure

Group 9 Animal Number	Air Control titre	Group 10 Animal Number	1mg/m^3 titre	Group 11 Animal Number	3mg/m^3 titre	Group 12 Animal Number	10mg/m^3 titre
81	<1 in 10	91	<1 in 10	101	1 in 160	111	1 in 5120
82	<1 in 10	92	1 in 160	102	1 in 160	112	1 in 640
83	<1 in 10	93	<1 in 10	103	<1 in 10	113	1 in 1280
84	<1 in 10	94	<1 in 10	104	1 in 160	114	1 in 5120
85	<1 in 10	95	<1 in 10	105	1 in 10	115	1 in 1280
86	<1 in 10	96	<1 in 10	106	1 in 20	116	1 in 1280
87	<1 in 10	97	1 in 40	107	1 in 160	117	1 in 160
88	<1 in 10	98	1 in 1280	108	1 in 1280	118	1 in 2560
89	<1 in 10	99	1 in 1280	109	1 in 160	119	1 in 2560
90	<1 in 10	100	<1 in 10	110	1 in 160	120	1 in 320

D. Fifteen times 6 hour exposures

Group 13 Animal Number	Air Control titre	Group 14 Animal Number	1mg/m^3 titre	Group 15 Animal Number	3mg/m^3 titre	Group 16 Animal Number	10mg/m^3 titre
121	<1 in 10	131	1 in 1280	141	1 in 2560	151	1 in 2560
122	<1 in 10	132	1 in 10240	142	1 in 320	152	1 in 2560
123	<1 in 10	133	1 in 640	143	1 in 2560	153	1 in 2560
124	<1 in 10	134	1 in 1280	144	1 in 10240	154	1 in 5120
125	<1 in 10	135	1 in 160	145	1 in 2560	155	1 in 20480
126	<1 in 10	136	1 in 20480	146	1 in 2560	156	1 in 5120
127	<1 in 10	137	1 in 10240	147	1 in 5120	157	1 in 1280
128	<1 in 10	138	1 in 160	148	1 in 1280	158	1 in 5120
129	<1 in 10	139	1 in 160	149	1 in 2560	159	1 in 5120
130	<1 in 10	140	1 in 10240	150	1 in 5120	160	1 in 5120

TABLE 2 - DISTRIBUTION OF ANTI-MDI IgG1 TITRES FOLLOWING INHALATION EXPOSURE OF GUINEA PIGS TO pMDI

A. 15 minutes exposure

Titre	Number of Animals			
	Air Controls Group 1	3mg/m^3 Group 2	10mg/m^3 Group 3	30mg/m^3 Group 4
< 1 in 10	10	5	1	1
1 in 10		2	2	2
1 in 20		1		3
1 in 40			1	
1 in 80			3	
1 in 160		2	2	2
1 in 320			1	1
1 in 640				
1 in 1280				
1 in 2560				
1 in 5020				
1 in 10240				1
1 in 20480				
MEAN	< 1 in 10	1 in 36	1 in 94	1 in 1096
MEDIAN	< 1 in 10	1 in 10	1 in 80	1 in 20

B. One hour exposure

Titre	Number of Animals			
	Air Controls Group 5	3mg/m^3 Group 6	10mg/m^3 Group 7	30mg/m^3 Group 8
< 1 in 10	10	6		1
1 in 10				2
1 in 20				
1 in 40		1		
1 in 80		2	2	2
1 in 160		1	4	2
1 in 320				
1 in 640			1	
1 in 1280			2	1
1 in 2560				1
1 in 5020				
1 in 10240				1
1 in 20480				
MEAN	< 1 in 10	1 in 72	1 in 402	1 in 1458
MEDIAN	<1 in 10	< 1 in 10	1 in 160	1 in 160

C. Six hour exposure

Titre	Number of Animals			
	Air Controls Group 9	1mg/m³ Group 10	3mg/m³ Group 11	10mg/m³ Group 12
< 1 in 10	10	6	1	
1 in 10			1	
1 in 20			1	
1 in 40		1		
1 in 80				
1 in 160		1	6	1
1 in 320				1
1 in 640				1
1 in 1280		2	1	3
1 in 2560				2
1 in 5020				2
1 in 10240				
1 in 20480				
MEAN	< 1 in 10	1 in 276	1 in 227	1 in 2032
MEDIAN	< 1 in 10	< 1 in 10	1 in 160	1 in 2560

B 11

D. Fifteen times 6 hour exposures

Titre	Number of Animals			
	Air Controls Group 13	1mg/m³ Group 14	3mg/m³ Group 15	10mg/m³ Group 16
< 1 in 10	10			
1 in 10				
1 in 20				
1 in 40				
1 in 80				
1 in 160		3		
1 in 320			1	
1 in 640		1		
1 in 1280		2	1	1
1 in 2560			5	3
1 in 5020			2	5
1 in 10240		3	1	
1 in 20480		1		1
MEAN	< 1 in 10	1 in 5488	1 in 3406	1 in 5504
MEDIAN	< 1 in 10	1 in 1280	1 in 2560	1 in 5120

TABLE 3 - STATISTICAL ANALYSIS OF ANTI-MDI IgG1 ANTIBODY RESPONSES

A. 15 minutes exposure

Group	MDI dose mg/m^3	Mean area under curve	S.D.	P value	Significance
1	0	7.46	1.83	-	-
2	3	6.89	4.04	0.614	NS
3	10	17.01	9.97	0.033	*
4	30	43.78	76.23	0.001	**

B. One hour exposure

Group	MDI dose mg/m^3	Mean area under curve	S.D.	P value	Significance
5	0	4.32	0.68	-	-
6	3	13.82	11.46	0.0095	**
7	10	28.77	19.50	<0.0001	**
8	30	58.04	77.50	<0.0001	**

C. Six hour exposure

Group	MDI dose mg/m^3	Mean area under curve	S.D.	P value	Significance
9	0	12.50	4.28	-	-
10	1	23.04	20.19	0.261	NS
11	3	46.76	33.97	0.0006	**
12	10	110.18	65.42	<0.0004	**

B 13

D. Fifteen times 6 hour exposures

Group	MDI dose mg/m^3	Mean area under curve	S.D.	P value	Significance
13	0	13.53	3.83	-	-
14	1	158.95	106.57	<0.0001	**
15	3	187.05	75.98	<0.0001	**
16	10	221.27	90.88	<0.0001	**

NS Not significant at 5% level

* statistically significant difference from the control group mean at the 5% level

** statistically significant difference from the control group mean at the 1% level

8. FIGURE LEGENDS AND FIGURES

Figure 1 Profiles of antibody responses in serum samples from study 114-EU-MTX

Profiles of antibody responses following intradermal sensitization of guinea pigs to pMDI in study 114-EU-MTX. Serum samples from animals number 26 (a), 27 (b) and 28 (c) are shown. Data are from analyses performed in February 1995 (; displayed in report CTL/R/1239), May 1996 (Δ; displayed in report CTL/T/2926) and March 1998 (◊).

Figure 2 Profiles of antibody responses in serum samples from study 153-EU-MTX.

Profiles of antibody responses following inhalation exposure of guinea pigs to pMDI in study 153-EU-MTX. Serum samples from group 2 (animals 11, 12, 14, 15, 18; 3mg/m^3 pMDI for 15 minutes) are shown in (a); serum samples from group 3 (animals 21-23, 25-30; 10mg/m^3 pMDI for 15 minutes) are shown in (b); serum samples from group 4 (animals 31-37, 39, 40; 30mg/m^3 pMDI for 15 minutes) are shown in (c); serum samples from group 6 (animals 51, 52, 56, 60; 3mg/m^3 pMDI for 1 hour) are shown in (d); serum samples from group 7 (animals 61-70 inclusive; 10mg/m^3 pMDI for 1 hour) are shown in (e); serum samples from group 8 (animals 71, 73-80; 30mg/m^3 pMDI for 1 hour) are shown in (f); serum samples from group 10 (animals 92, 97-99; 1 mg/m^3 pMDI for 6 hours) are shown in (g); serum samples from group 11 (animals 101, 102,. 104-110; 3mg/m^3 pMDI for 6 hours) are shown in (h); serum samples from group 12 (animals 111-120 inclusive; 10mg/m^3 pMDI for 6 hours) are shown in (i); serum samples from group 14 (animals 131-140 inclusive; 1mg/m^3 pMDIfor 15 times 6 hours) are shown in (j); serum samples from group 15 (animals 141-150 inclusive; 3mg/m^3 pMDI for 15 times 6 hours) are shown in (k); serum samples from group 16 (animals 151-160 inclusive; 10mg/m^3 pMDI for 15 times 6 hours) are shown in (l). Each line represents an individual serum sample.

C 01

Figure 3 Distribution of anti-MDI IgG1 titres following inhalation exposure of guinea pigs to pMDI.

Distribution of antibody titres following inhalation exposure of guinea pigs to pMDI in study 153-EU-MTX. Antibody titres are shown for group 1 (a), 2 (b), 3 (c), 4(d), 5(e), 6(f), 7(g), 8(h), 9 (i), 10(j), 11 (k), 12 (l), 13 (m), 14 (n), 15 (o) and 16 (p).

Figure 4 Profiles of antibody responses in serum samples from study 134-EU-MTX.

Profiles of antibody responses following inhalation exposure of guinea pigs to pMDI in study 134-EU-MTX. Serum samples from group 2 (animals 8-16 inclusive; 10mg/m^3 pMDI for 15 minutes) are shown in (a); serum samples from group 3 (animals 17-24 inclusive; 100mg/m^3 pMDI for 15 minutes) are shown in (b) and serum samples from group 4 (animals 25-28, 30-31 inclusive, 900mg/m^3 pMDI for 15 minutes) are shown in (c). Each line represents an individual serum sample.

Figure 1

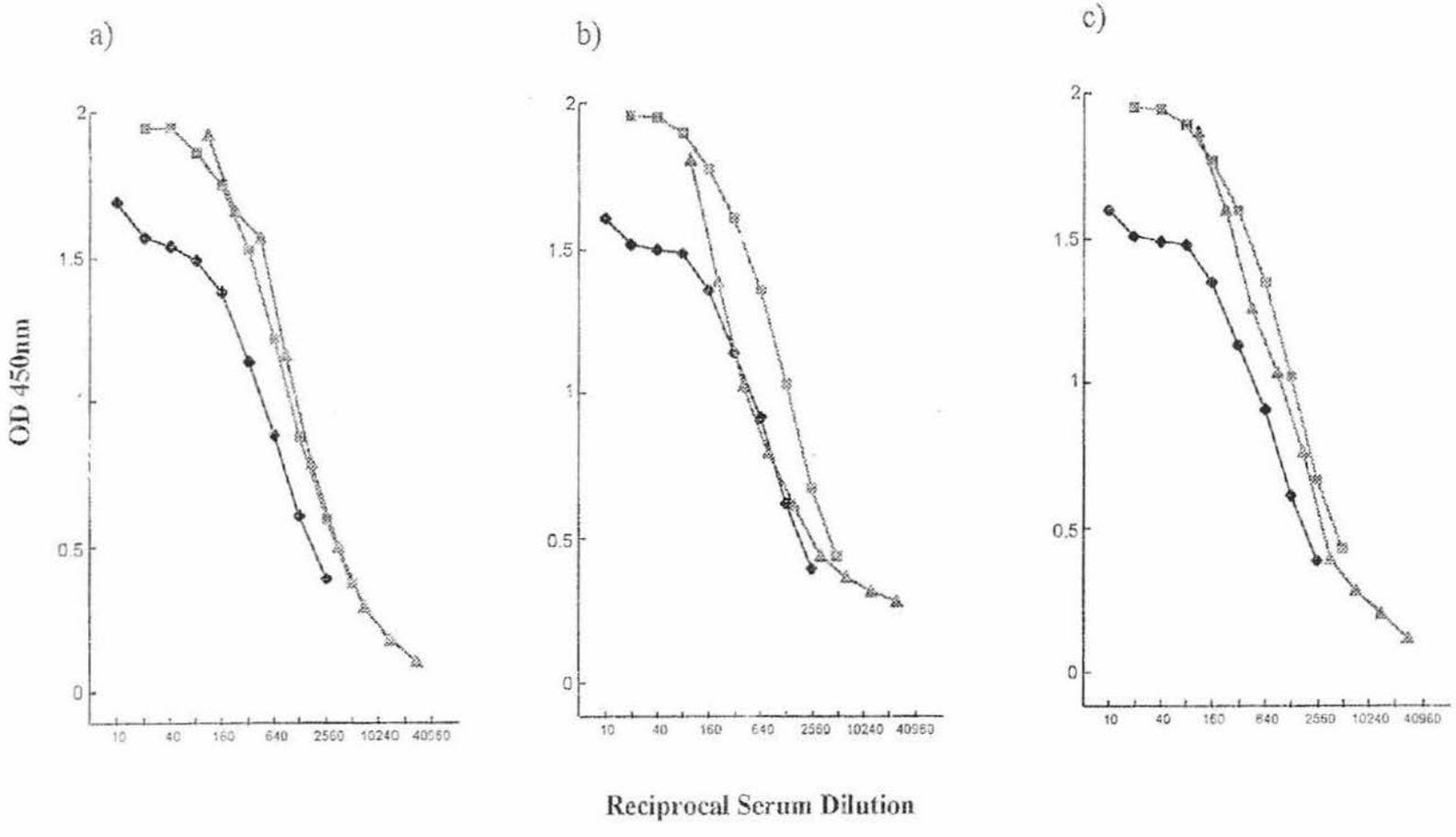

a)
b)
c)
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

Figure 2

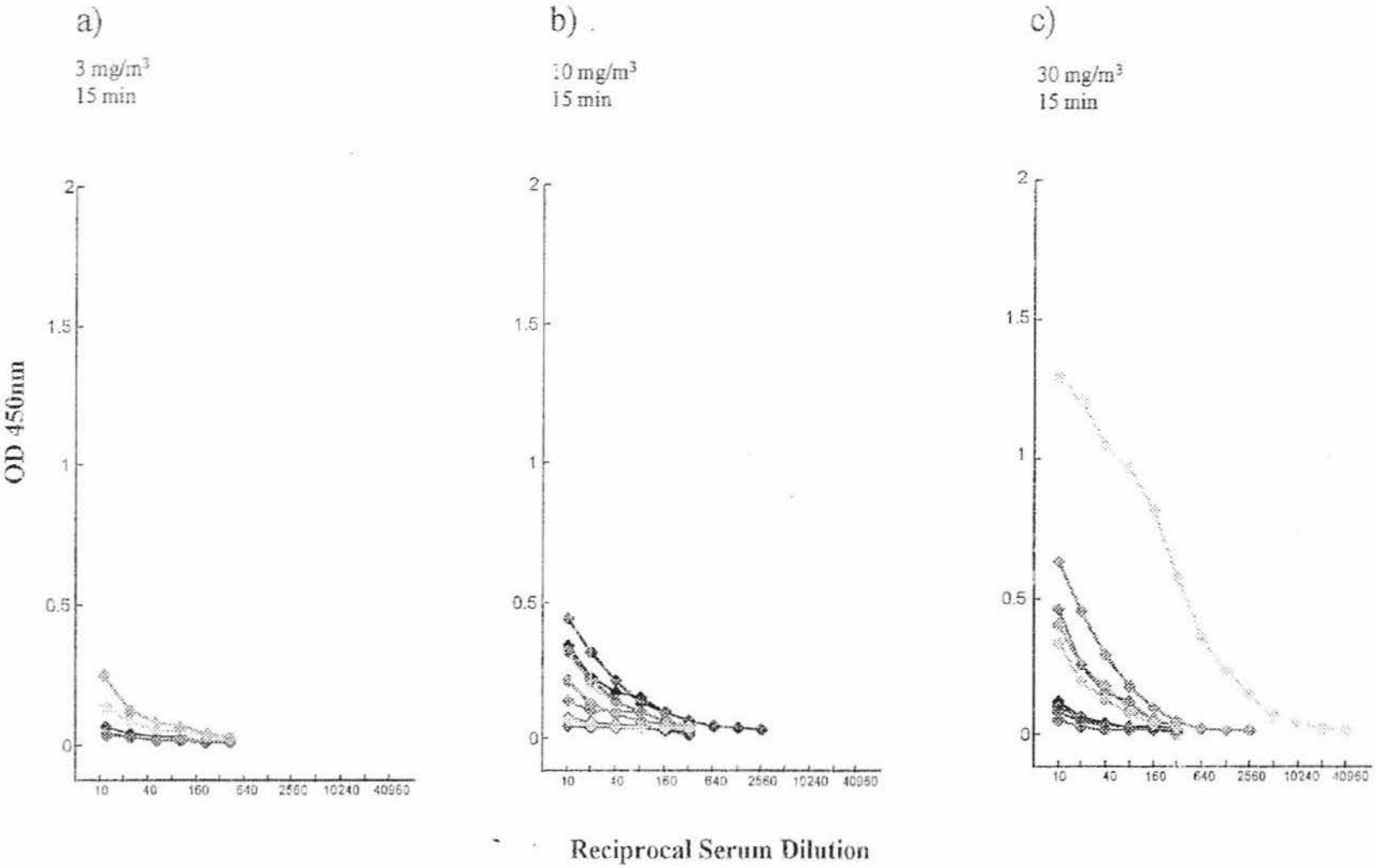

a)
3 mg/m³
15 min
b)
10 mg/m³
15 min
c)
30 mg/m³
15 min
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

Figure 2

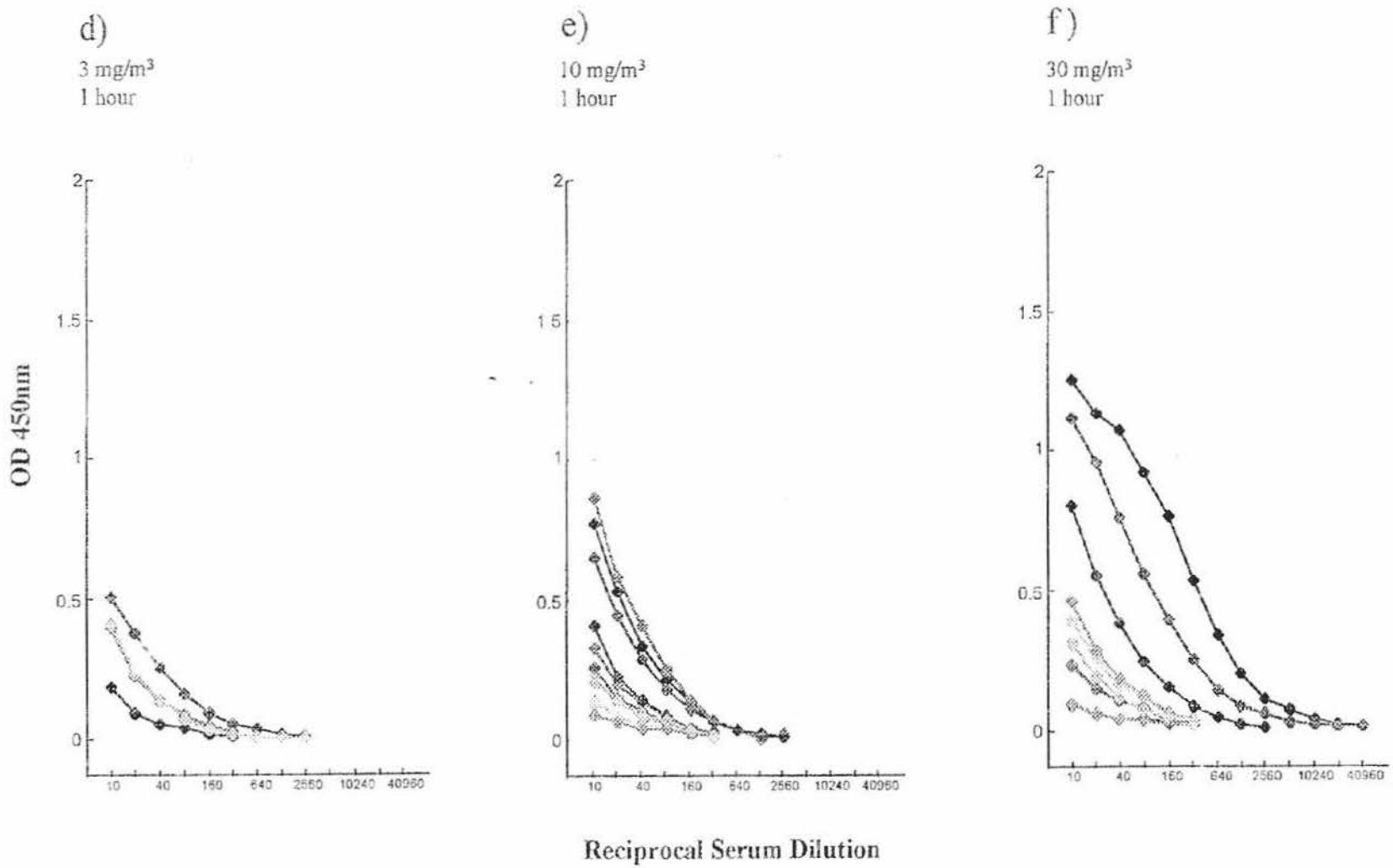

d)
3 mg/m³
1 hour
e)
10 mg/m³
1 hour
f)
30 mg/m³
1 hour
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

Figure 2

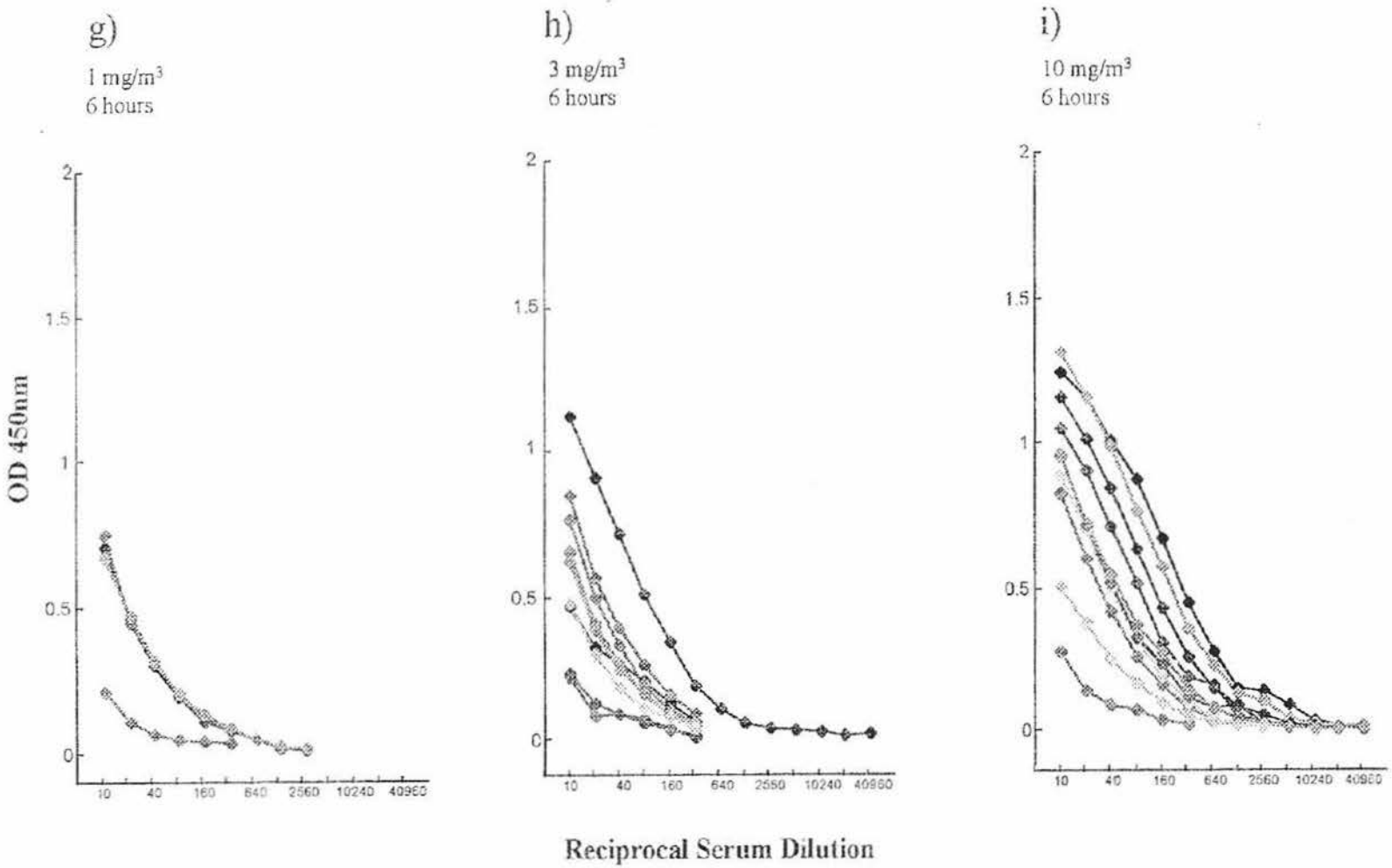

g)
1 mg/m³
6 hours
h)
3 mg/m³
6 hours
i)
10 mg/m³
6 hours
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

Figure 2

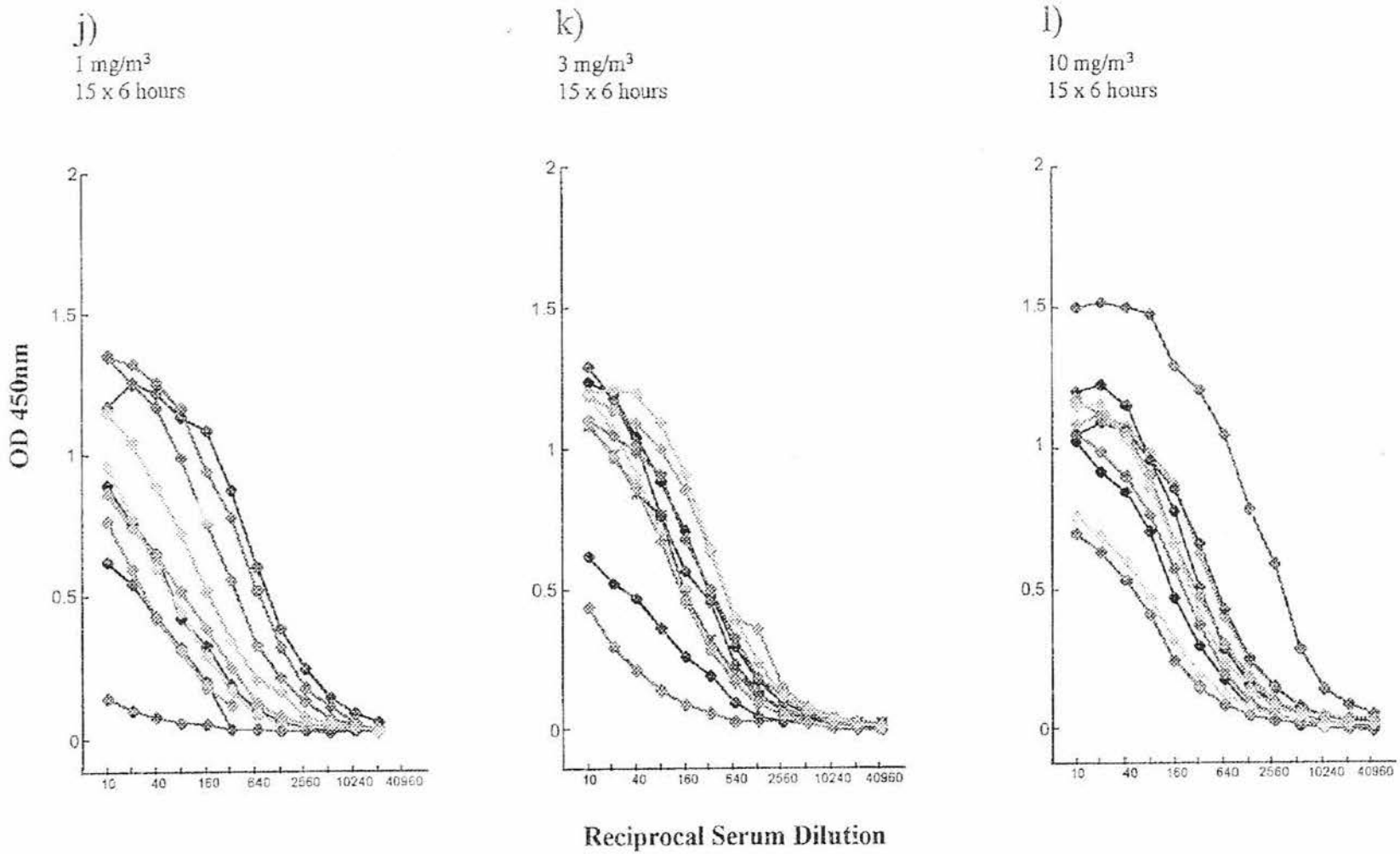

j)
1 mg/m³
15 x 6 hours
k)
3 mg/m³
15 x 6 hours
l)
10 mg/m³
15 x 6 hours
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

Figure 3

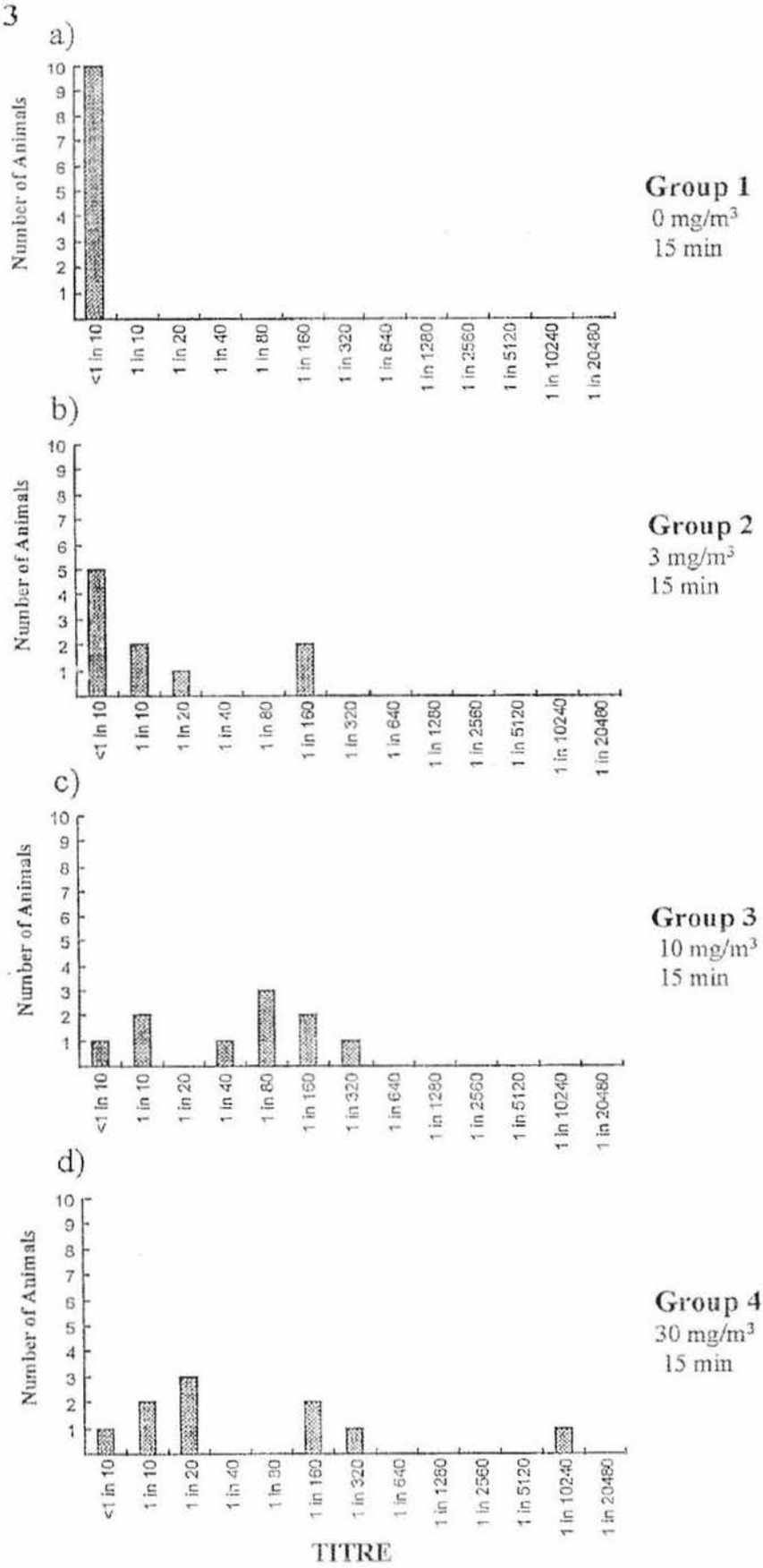
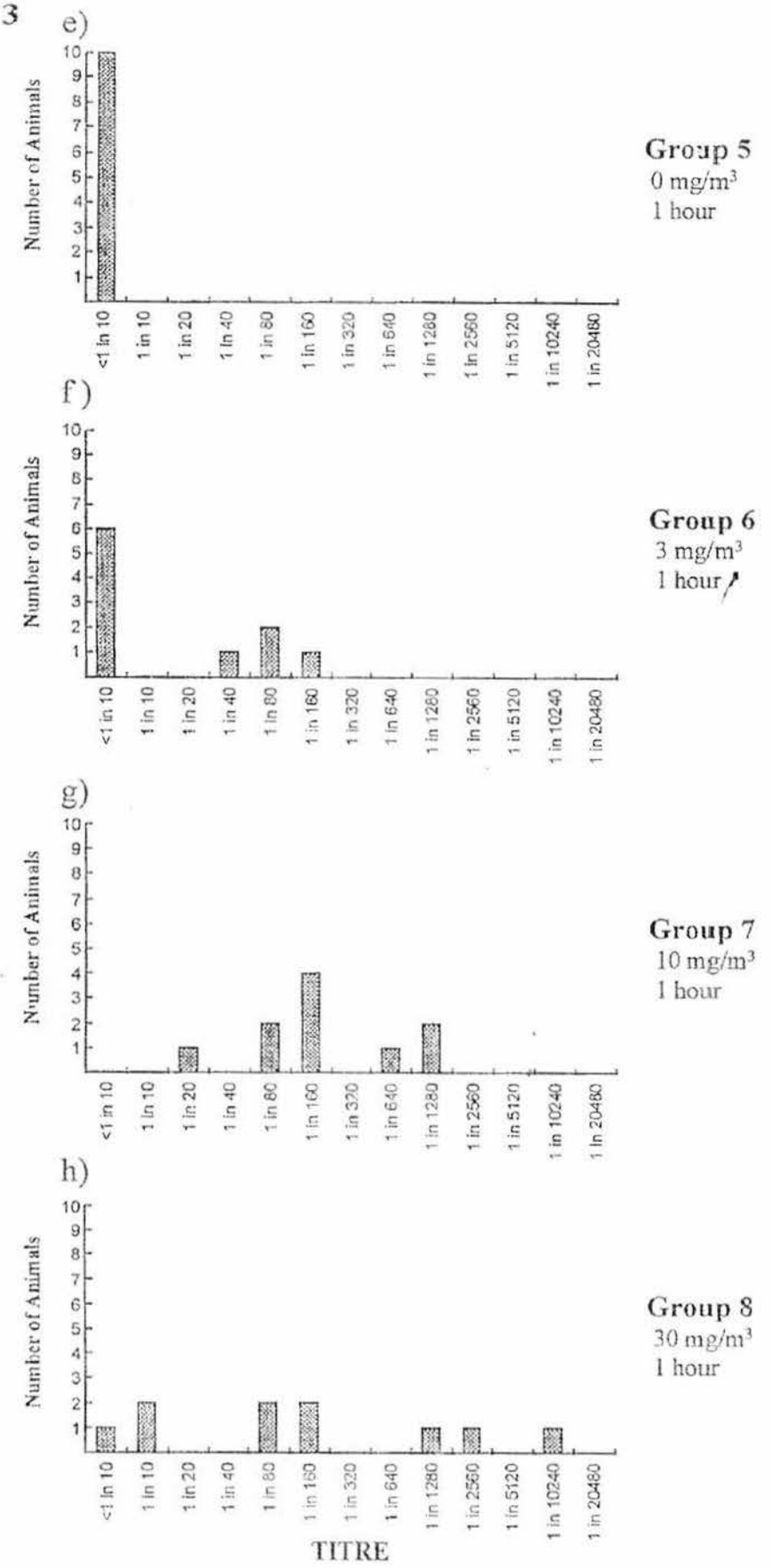
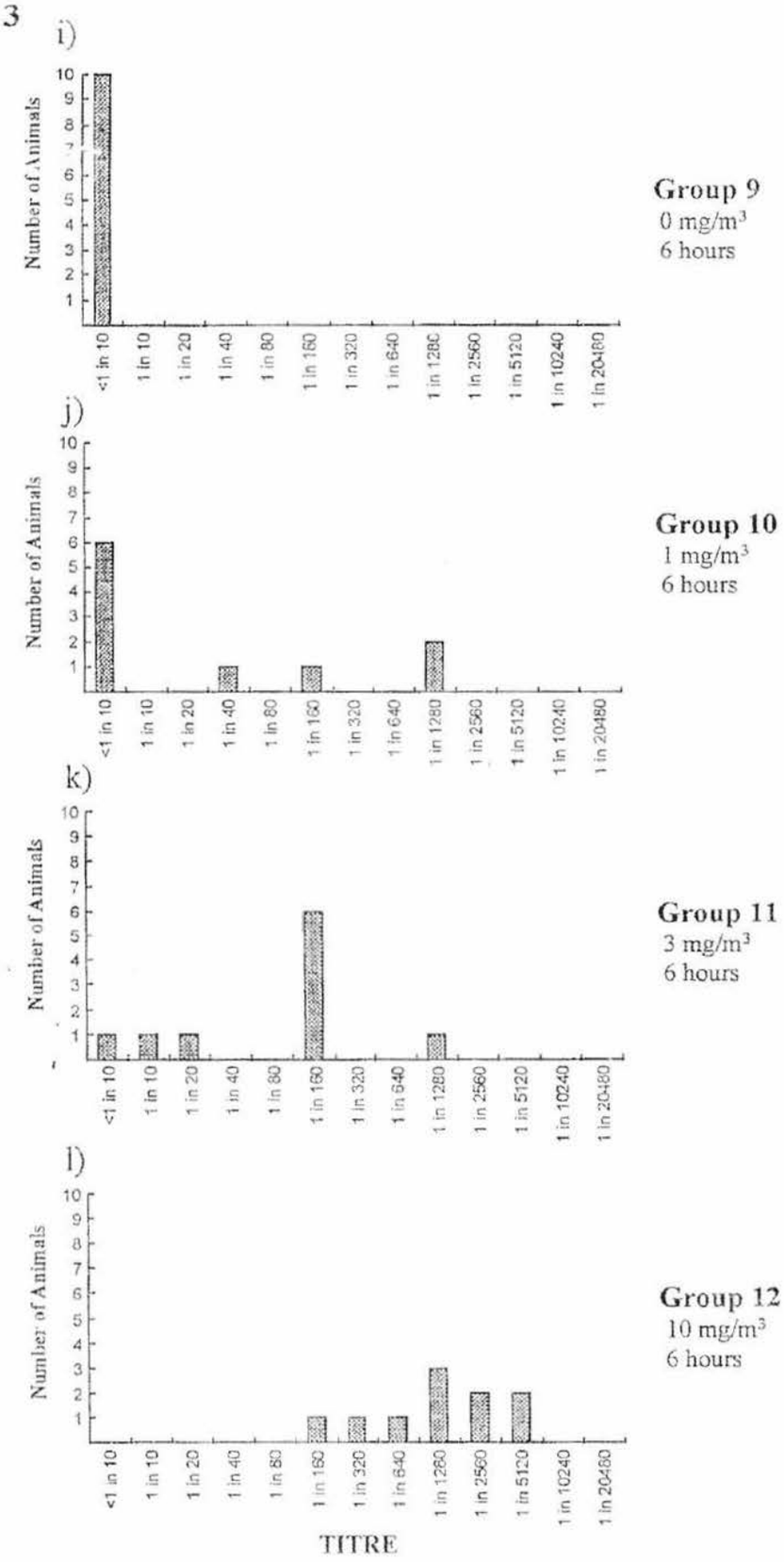

a)
Number of Animals
Group 1
0 mg/m³
15 min
b)
Number of Animals
Group 2
3 mg/m³
15 min
c)
Number of Animals
Group 3
10 mg/m³
15 min
d)
Number of Animals
Group 4
30 mg/m³
15 min
<1 in 10
1 in 10
1 in 20
1 in 40
1 in 80
1 in 160
1 in 320
1 in 640
1 in 1280
1 in 2560
1 in 5120
1 in 10240
1 in 20480
TITRE

Figure 3


e)
Number of Animals
Group 5
0 mg/m³
1 hour
f)
Number of Animals
Group 6
3 mg/m³
1 hour
g)
Number of Animals
Group 7
10 mg/m³
1 hour
h)
Number of Animals
Group 8
30 mg/m³
1 hour
<1 in 10
1 in 10
1 in 20
1 in 40
1 in 80
1 in 160
1 in 320
1 in 640
1 in 1280
1 in 2560
1 in 5120
1 in 10240
1 in 20480
TITRE

C 09

Figure 3


i)
Number of Animals
Group 9
0 mg/m³
6 hours
j)
Number of Animals
Group 10
1 mg/m³
6 hours
k)
Number of Animals
Group 11
3 mg/m³
6 hours
l)
Number of Animals
Group 12
10 mg/m³
6 hours
<1 in 10
1 in 10
1 in 20
1 in 40
1 in 80
1 in 160
1 in 320
1 in 640
1 in 1280
1 in 2560
1 in 5120
1 in 10240
1 in 20480
TITRE

10

Figure 3

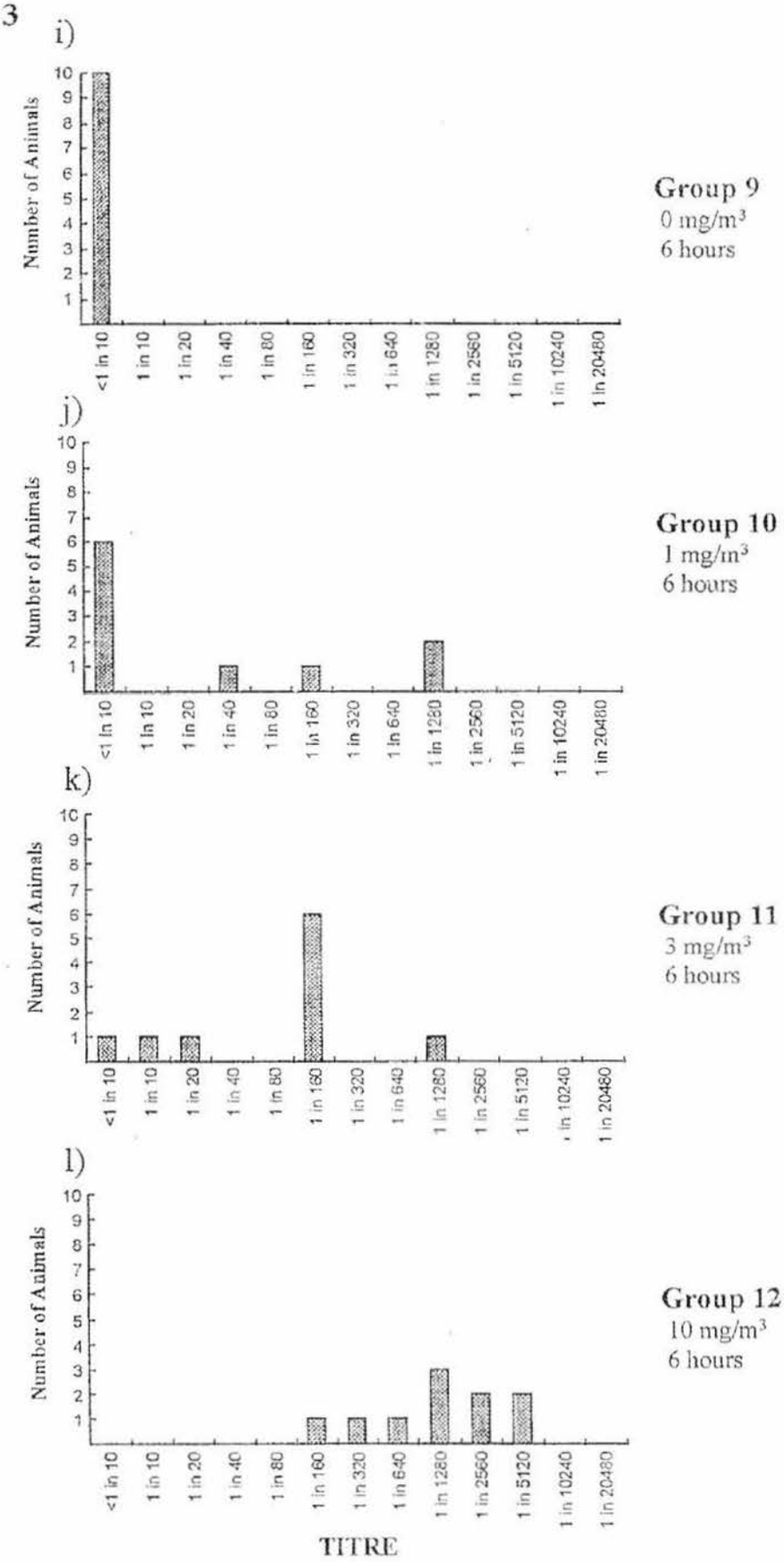

i)
Number of Animals
Group 9
0 mg/m³
6 hours
j)
Number of Animals
Group 10
1 mg/m³
6 hours
k)
Number of Animals
Group 11
3 mg/m³
6 hours
l)
Number of Animals
Group 12
10 mg/m³
6 hours
<1 in 10
1 in 10
1 in 20
1 in 40
1 in 80
1 in 160
1 in 320
1 in 640
1 in 1280
1 in 2560
1 in 5120
1 in 10240
1 in 20480
TITRE

Figure 3

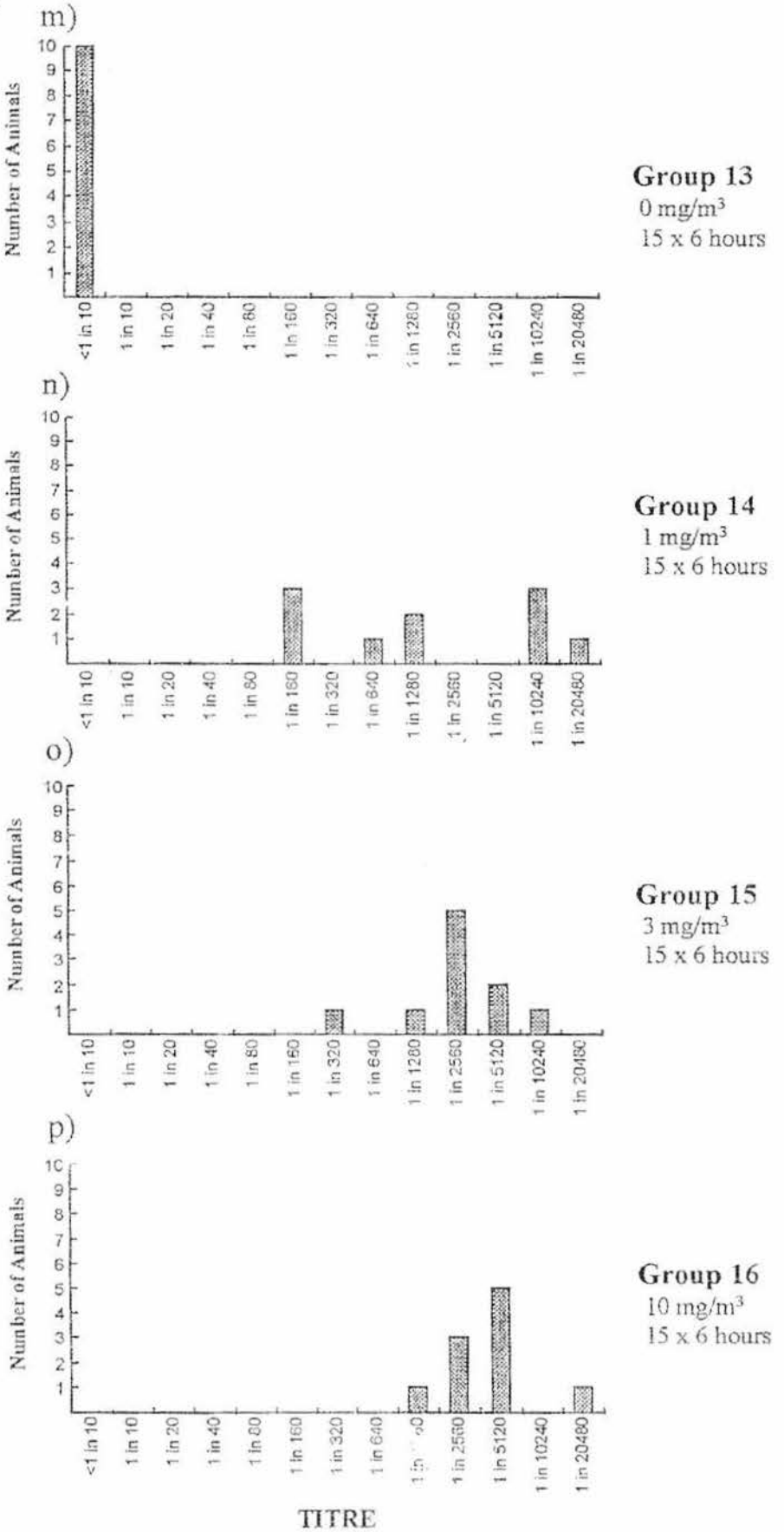
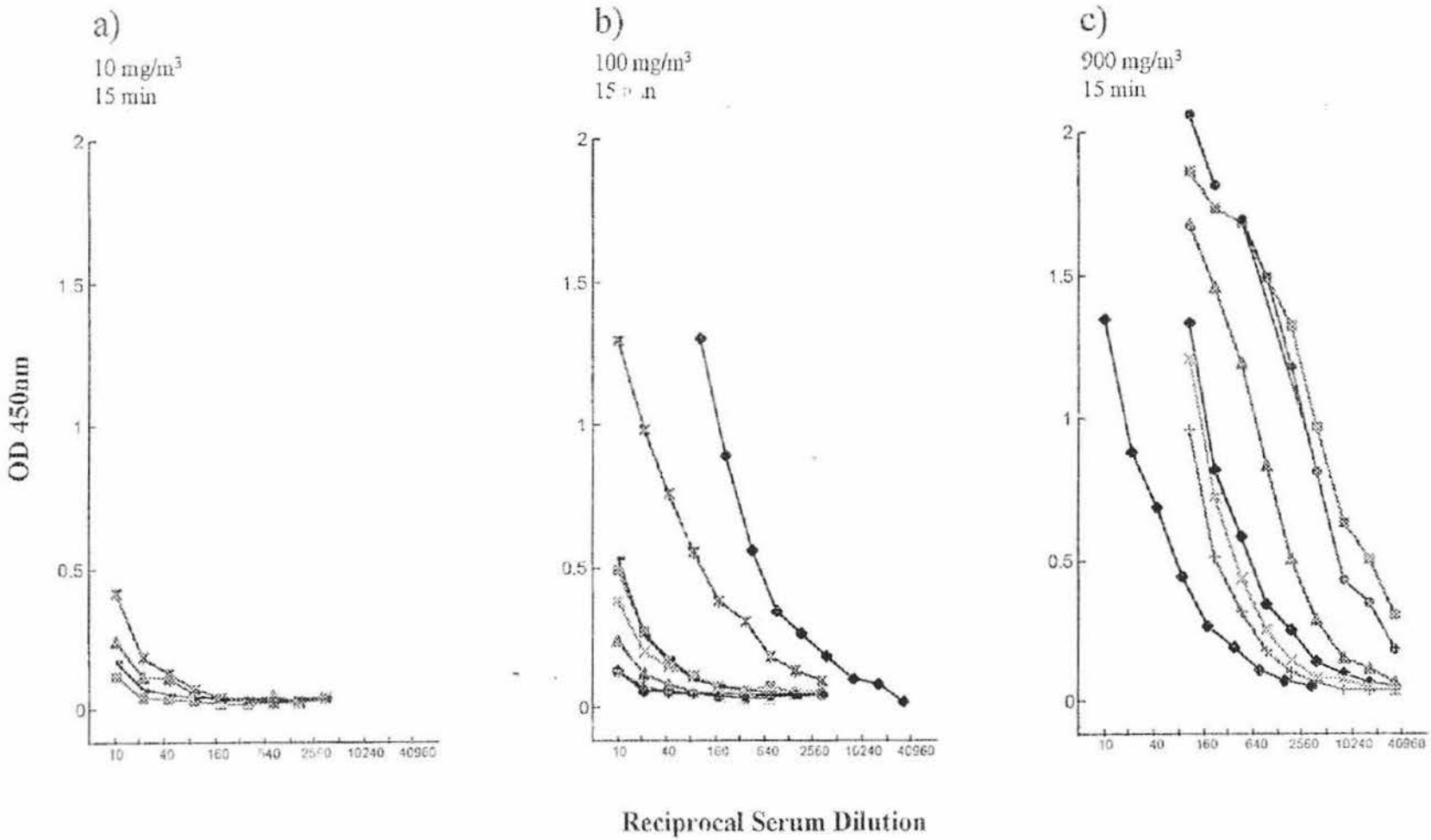

m)
Number of Animals
Group 13
0 mg/m³
15 x 6 hours
n)
Number of Animals
Group 14
1 mg/m³
15 x 6 hours
o)
Number of Animals
Group 15
3 mg/m³
15 x 6 hours
p)
Number of Animals
Group 16
10 mg/m³
15 x 6 hours
<1 in 10
1 in 10
1 in 20
1 in 40
1 in 80
1 in 160
1 in 320
1 in 640
1 in 1280
1 in 2560
1 in 5120
1 in 10240
1 in 20480
TITRE

Figure 4


a)
10 mg/m³
15 min
b)
100 mg/m³
c)
900 mg/m³
15 min
OD 450nm
2
1.5
1
0.5
0
10
40
160
640
2560
10240
40960
Reciprocal Serum Dilution

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