

DOW CORNING

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December 13, 2001

MR 53883

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Re: For Your Information Submission:
49 FR 46741 (November 28, 1984) [OPTS-84013; FRL-2725-1]
58 FR 28511 (May 14, 1993) [OPPTS-82040; FRL-4271-1]
TSCA Section 8(d) Health and Safety Data Reporting

Dear Sir:

The enclosed information is submitted on behalf of Dow Corning Corporation, Midland, Michigan, 48686-0994, on a For-Your-Information (FYI) basis as a follow-up to submissions made concerning hexamethyldisiloxane (HMDS) and decamethylcyclopentasiloxane (DMCPS), which chemical substances were the subject of a health and safety data rule issued under Section 8(d) of the Toxic Substances Control Act (TSCA) and with an effective date of June 14, 1993 (sunset date June 30, 1998); and octamethylcyclotetrasiloxane (OMCTS), which chemical substance was the subject of health and safety data rule issued under Section 8(d) of TSCA and with an effective date of December 28, 1984 (sunset date December 28, 1994), as codified at 40 CFR 716 (Health and Safety Data Reporting). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

Chemical Substances:

107-46-0 Hexamethyldisiloxane (HMDS, L₂)
541-02-6 Decamethylcyclopentasiloxane (DMCPS, D₅)
556-67-2 Octamethylcyclotetrasiloxane (OMCTS, D₄)

Contain NO CBI



FYI-02-001420



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Title of Report:

NON-REGULATED STUDY: IN VITRO EFFECTS OF SILOXANES ON
HUMAN IMMUNE CELLS

Dow Corning Corporation
2000-10000-49256
June 25, 2001

Manufacturer:

Dow Corning Corporation
2200 West Salzburg Road
Midland, Michigan 48686-0994

For purposes of this For-Your-Information (FYI) submission, the general INTERNAL designation on the attached health and safety report is waived by Dow Corning.

If you require further information regarding this submission, please contact Dr. Rhys G. Daniels, Senior Regulatory Compliance Specialist, Product Safety and Regulatory Compliance, at 989-496-4222 or at the address provided herein.

Sincerely,



Patrick W. Langvardt
Director of Health and Environmental Sciences
(989) 496-4626

RGD01381

**DOW CORNING
TECHNICAL REPORT**

Report No: 2000-I0000-49256

Authors: John Looney, Mark Utell and Kathleen Plotzke

Department: Health & Environmental Sciences; Toxicology

Supervisor: Patrick W. Langvardt

Location: Midland Corporate, Michigan USA

Date: June 25, 2001

Title: Non-Regulated Study: In Vitro Effects of Siloxanes on Human Immune Cells

Distribution

Full Report

K. P. Plotzke

Title Page and Abstract

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FINAL REPORT-June 25, 2001

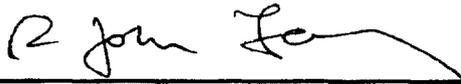
***IN VITRO* EFFECTS OF SILOXANES ON HUMAN IMMUNE CELLS**

R. JOHN LOONEY, M.D. – PRINCIPAL INVESTIGATOR

**ALLERGY, IMMUNOLOGY AND RHEUMATOLOGY DIVISION
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ARCHIVAL OF RESEARCH RECORDS

All records resulting from this study will be retained by the Allergy, Immunology and Rheumatology Unit of the Department of Medicine, University of Rochester Medical Center, Rochester, New York 14642, for a minimum of 5 years after acceptance of the Final Report or transferred to the sponsor upon written request. Good clinical laboratory procedures were used, i.e. Standard Operating Procedures for all technical procedures were followed.



6/28/01

R. John Looney, M.D., Principal Investigator

Date

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ABSTRACT

The *in vitro* immunological effects of siloxanes (D₄= octamethylcyclotetrasiloxane, D₅ = decamethylcyclopentasiloxane, and HMDS = hexamethyldisiloxane) were investigated using cultured human immune cells (PBMCs = peripheral blood mononuclear cells). In serum-free medium the cyclic siloxanes D₄ and D₅ completely inhibited PHA-induced proliferation of PBMCs at concentrations greater than 10 μ M/ml. In cultures with serum-free medium D₄ and D₅ also inhibited proliferation of PBMCs induced by tetanus toxoid or alloantigens. In contrast, even at > 1000 μ M the linear siloxane HMDS had no effect on proliferation. The inhibitory effect of D₄ and D₅ was completely reversed by addition of even small amounts of serum or plasma to the serum-free medium. The component(s) of serum responsible for reversing the effects was contained in the lipoprotein fractions of serum (VLDL, LDL, and HDL). Intralipid®, a lipid preparation used for hyperalimentation, had the same effects as serum lipoproteins. Moreover, mixing the linear siloxane HMDS with D₄ or D₅ substantially reversed the inhibitory effects of these cyclics. Inhibition of proliferation in serum-free medium appears to be due to a toxic effect of D₄ and D₅, because viability of PBMCs assessed by trypan blue exclusion was <20% when cultured with 10 μ M of these cyclic siloxanes for 24 hr in serum-free medium. Culturing PBMCs and D₄, D₅, or HMDS, with or without serum, was not associated with production of TNF α . These findings suggest that exposure of cells to high levels of cyclic siloxanes may be deleterious under conditions in which other lipophilic substances are not also present. Extrapolation to *in vivo* relevance would, of course, need to consider issues of metabolism and the effects of plasma or serum. Our results also suggest that the toxic effects of cyclic siloxanes may also be reversed by linear siloxanes such as HMDS. The toxic effects of D₄ and D₅ described are unlikely to be relevant systematically since the high levels of lipids in plasma and tissues would certainly neutralize these potential effects of D₄ and D₅.

INTRODUCTION

The University of Rochester in collaboration with Dow Corning Corporation has undertaken an extensive research program to study the pharmacokinetics, metabolism,

and immune and clinical effects in humans of siloxanes delivered by the respiratory tract, gastrointestinal tract, or skin. This work was funded in part by the Silicone Environmental Health and Safety Council of North America.

Silicones are used in skin care products, anti-perspirants, anti-gas agents, and other consumer products. Exposure to these materials occurs via the respiratory or gastrointestinal tract, or skin. In this Final Report, we are providing data on the *in vitro* effects of siloxanes on immune cells.

The standard operating procedures (SOPs) related to the *in vitro* immunology assays are in Appendix A.

BACKGROUND

The term silicone identifies a large class of individual chemical substances with widely differing properties and molecular size. The three small molecules (D4, D5, and HMDS) evaluated in this study are siloxanes, chemicals with the general structure $O_xSi(CH_3)_y$. Siloxanes also include high molecular weight compounds such as polydimethylsiloxanes. Silicones are widely used in consumer products and medical devices. Epidemiological studies have so far failed to establish an association between silicone breast implants and autoimmune disease (1-3). Nevertheless, the effect of silicones on the immune system remains an area of active investigation. In addition to their use in medical devices such as breast implants, shunts, and joint replacements, silicones are used in skin care products, anti-perspirants, anti-gas agents, and other consumer products. Thus exposure to silicones in consumer products occurs via the respiratory or gastrointestinal tract or skin.

An immunological adjuvant is a substance that enhances the specific immune response to an antigen. In animal experiments some silicone materials have been demonstrated to behave as immunological adjuvants (4-8). In these studies, antigens emulsified with silicones and injected into mice or rats induced a more robust immune response than antigen injected alone. Separate administration of antigen and silicone did not result in an

enhanced immune response (5). The mechanism(s) responsible for the effects of adjuvants are varied and may include serving as a reservoir for antigen that would otherwise be rapidly cleared, promoting ingestion by macrophages or other antigen presenting cells, and recruiting macrophages to the site of antigen application or activating macrophages so that they become more effective antigen-presenting cells (9). Exposure to silicone materials via the respiratory tract (10), gastrointestinal tract or skin, as might be seen with consumer products, has not been shown to serve as an immunological adjuvant.

Animal models have also been used to investigate the effects of injected silicones on adjuvancy and autoimmunity. Immune response to antigens emulsified in silicones is enhanced (11,12). Thus, under certain circumstances silicones can act as immune adjuvants (12). However, no adjuvant effects are seen when antigen and silicones are injected separately, indicating that adjuvancy is dependent on antigen and silicone being in the same compartment. Rat thyroglobulin combined with silicone gel, a mixture made up primarily of PDMS, and injected into susceptible rats induced autoantibodies but no autoimmune lesion (13). Bovine type II collagen mixed with silicone gel, but not silicone gel itself, induced arthritis in the DA strain of rat (14). Implanted silicone had no effect on the course or severity of murine type II-collagen induced arthritis or on lupus in MRL mice (15,16). Nevertheless, as one expert recently stated "There is, as yet, no evidence that any silicone product, acting alone, can facilitate the induction of autoimmune disease in animals." (17)

In preliminary studies to investigate the effects of siloxanes on immune cells in tissue culture, we observed profound differences depending on what tissue culture medium was used. In serum-containing medium no effect was seen, while in serum-free medium there appeared to be a profound inhibitor effect of D₄ or D₅. The present study was undertaken to document this phenomenon, and to investigate the factors in serum that prevent this apparent toxicity. The effect of siloxanes on cells in tissue culture has been previously reported. In these studies medium contained fetal calf serum, and γ -cyclodextrin served as

a transport vehicle for siloxanes to deliver these hydrophobic compounds to cells (18). When siloxanes were delivered to cells using γ -cyclodextrin, there was loss of cell viability as assayed using MTT, and some leakage of LDH indicating disruption of the cytoplasmic membrane. Effects of sublethal doses of siloxanes included depletion of glutathione and induction of IL-6 secretion.

OBJECTIVES

The objective of the present study was to determine the *in vitro* effects of three siloxanes (D₄ = octamethylcyclotetrasiloxane, D₅ = decamethylcyclopentasiloxane, and HMDS = hexamethyldisiloxane) on human immune cells (PBMCs = peripheral blood mononuclear cells).

PERSONNEL

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Paul E. Morrow, Ph.D. - Emeritus Professor of Environmental Medicine

Ellen Miles - Quality Assurance Officer; Technical Associate

TEST MATERIAL

Siloxanes were provided by Dow Corning; D₄ lot number LL084732 (99.9% pure), D₅ lot number LL014002 (96% pure), and HMDS (99.9% pure) lot number AA065009.

MATERIALS AND METHODS. (See Appendix A for Standard Operating Procedures.)

Preparation of Test Material. For the *in vitro* studies siloxanes were diluted in absolute ethanol and then added to the cultures. For each final concentration of siloxane a separate mixture of siloxane and ethanol was used so that the final concentration of ethanol in the cultures remains constant (0.1%). Dodecane was obtained from Aldrich; sodium dodecylsulfate (SDS) was obtained from BioRad; albumin and gammaglobulin were obtained from Sigma.

Isolation of Peripheral Blood Mononuclear Cells (PBMC). Buffy coat blood was purchased from the Red Cross was used as the source for PBMCs. The buffy coat, diluted 1:1 in phosphate buffered saline and approximately 35 ml, was layered over 12.5 ml of room temperature ficoll-hypaque (Lymphoprep, Gibco, Grand Island, NY) in a 50 ml polypropylene centrifuge tube and then spun at 400 g for 30 min., at room temperature. The mononuclear cells at the top of the gradient were pipetted off and washed twice in PBS, resuspended in AIMV medium (a serum-free medium from Gibco), counted and adjusted to the proper concentration.

Proliferative Assays. For phytohemagglutinin (PHA) induced proliferation, quadruplicate cultures of PBMCs (0.5×10^6 /ml final concentration) in AIMV were established in a 96-well flat bottom tissue culture plate with an optimal concentration of PHA (Sigma Chemical Co.), and were incubated at 37° C and 7.5% CO₂ for 3 days in a waterjacketed tissue culture incubator. For the last 6 hrs. 1 μ Ci of ³H methylthymidine (NEN, Boston, MA) was added in 50 μ L of medium. Wells were harvested on a Micromate Harvester (Packard, Meriden, CT) and incorporated radioactivity was measured using a Matrix 96 Counter (Packard). For allogeneic stimulation, 2×10^6 /ml irradiated stimulator cells were mixed with 1×10^6 /ml PBMCs and incubated for 5 days. Wells were then pulsed with radiolabeled thymidine and incorporated radioactivity measured as above. Stimulator cells were PBMCs from four different donors mixed to form one lot. For tetanus toxoid induced proliferation, 1×10^6 /ml PBMCs were incubated with tetanus toxoid for 7 days before measuring the incorporation of radioactive thymidine.

In vitro Production of Cytokines. PBMCs in AIMV were stimulated with LPS (lipopolysaccharide) or siloxanes and incubated in a 24 well plate at 1×10^6 for 48 hrs. The wells were harvested and centrifuged, and the supernatants were frozen at -20°C until assayed. Tumor necrosis factor alpha ($\text{TNF}\alpha$) was assayed by ELISA using antibody pairs and recombinant cytokine standards from PharMingen (San Diego, CA). The supernatant was aspirated and frozen at -20°C until assayed by ELISA.

Serum, plasma, and serum components. Frozen aliquots of serum and plasma from a single donor (RJL) were used in these experiments. Lipoprotein fractions from fasting plasma from this same donor, prepared by ultracentrifugation, were a gift from Dr. Janet Sparks (Associate Professor of Pathology and Laboratory Medicine, University of Rochester). Serum from a patient with x-linked agammaglobulinemia (prior to treatment with immunoglobulin) was a gift from Dr. John Leddy (Professor of Medicine, University of Rochester). X-linked agammaglobulinemia is a disease where affected individuals fail to make immunoglobulins.

Viability assay. Cell viability was measured by trypan blue exclusion. At the indicated time points 20 μl was removed from each well and mixed with an equal volume of trypan blue (Gibco). The total number of cells and the non-viable (blue) cells were counted using a hemocytometer.

RESULTS. (Figures 1-10 are representative results.) See Appendix B for additional data and for data in tabular form.)

Effect of Siloxanes on PHA-Induced Proliferation of Peripheral Blood Mononuclear Cells (PBMCs). (See Fig. 1.)

When PBMCs were cultured in serum-free medium, the cyclic siloxanes D_4 and D_5 completely inhibited PHA-induced proliferation, as assayed by ^3H -thymidine

incorporation, at approximately 10 μM . In contrast the linear siloxane HMDS had no effect on PHA-induced proliferation even at $> 1000 \mu\text{M}$.

Effects of Serum and Plasma on Inhibition of Proliferation by Siloxanes. (See Fig. 2.)

To see if the inhibition of PHA-induced proliferation was due to the use of serum-free medium, varying dilutions of serum or plasma were added to cultures. As expected addition of D_4 or D_5 to serum-free cultures completely blocked proliferation while HMDS at this concentration had no effect. However, both serum and plasma were potent inhibitors of the effects of the cyclic siloxanes, i.e. PHA-induced proliferation was restored to normal levels by 1% or less of either serum or plasma.

Comparison of D_4 to Dodecane and Sodium Dodecyl Sulfate (SDS). (See Fig. 3.)

The cyclic siloxanes are hydrophobic materials. Therefore, the effects of D_4 were compared to the effects of dodecane that was similarly dissolved in ethanol and added to cultures. As can be seen in Fig. 3, even in the absence of serum, dodecane had no effect on proliferation. In contrast, SDS which is often used as a positive control in assays of cytotoxicity, e.g. to determine 100% release of ^{51}Cr , was a potent inhibitor of PHA-induced proliferation. On a molar basis, D_4 was about 10 times as potent as SDS in serum-free conditions. When 1% or 10% serum were added to cultures, the effects of D_4 but not SDS were completely inhibited.

Effect of Serum Components on Inhibition of Proliferation by Siloxanes. (See Fig. 4.)

Experiments using specific serum components or use of serum deficient in specific components were carried out to determine the nature of the protective factor(s) in serum and plasma. As can be seen in the top panel of Fig. 6, addition of gammaglobulin to serum-free medium had no protective effect, and conversely, agammaglobulinemic serum was as effective as normal serum. Addition of albumin was also ineffective compared to serum. Results with lipoprotein fractions of serum gave strikingly different results. Each lipoprotein fraction was capable of completely reversing the effects of D_4 . In contrast,

"infranant" which was the serum left over after removal of all lipoprotein fractions had no effect on the inhibition of PHA-induced proliferation by D₄.

Effect of Intralipid on Inhibition of Proliferation by Siloxanes. (See Fig. 5.)

The effects of serum components strongly suggested that lipoproteins were the serum/plasma factors that reversed the effects of cyclic siloxanes. To further substantiate the importance of lipid in these protective effects, Intralipid was used as a substitute source of lipid. As little as 10 µg/ml Intralipid essentially reversed the effects of a similar amount of D₄ or D₅.

Effect of HMDS on D₄ and D₅ Toxicity. (See Fig. 6.)

Linear and cyclic siloxanes are miscible. Therefore, the effects of premixing HMDS with D₄ or D₅ was investigated. In these experiments HMDS was mixed with either D₄ or D₅ in ETOH before dilution into culture medium. As can be seen in Fig. 6, HMDS was able to substantially reverse the inhibitory effects of both D₄ and D₅ on PBMC proliferation.

Effect of Siloxanes on Viability. (See Fig. 7.)

The mechanism for the effects of D₄ or D₅ was apparent in all of the above experiments when cultures were examined using an inverted scope at the time the radioactive thymidine was added. Wells with no proliferation were also devoid of viable cells. To document these effects of cyclic siloxanes, cell counts and viability by trypan blue exclusion were performed, less than 20% of the input cells were alive in the well containing D₄ or D₅, i.e. were present and able to exclude trypan blue. Even small amounts of serum protected cells from these toxic effects.

Effect of Siloxanes on Tetanus toxoid- and Alloantigen-Induced Proliferation. (See Fig. 8 and 9.)

The effects of D₄ and D₅ on other stimuli that induce proliferation of PBMCs were also investigated. As can be seen in Fig. 8 and 9, D₄ or D₅ also completely inhibited stimulation of PBMCs under these serum-free conditions.

Effect of Siloxanes on TNF α Production. (See Fig. 10.)

To determine if siloxanes either by themselves or interacting with serum components are able to induce inflammatory cytokines, PBMCs were cultured for 24 hrs with either LPS as a positive control or varying concentrations of siloxanes with 0%, 1%, or 10% serum. Supernatants were then harvested and assayed for TNF α by ELISA. LPS alone induced over 400 pg/ml of TNF, but under no condition did any of the siloxanes produce significant TNF secretion.

DISCUSSION.

The data presented in this report indicates that in the absence of serum D₄ and D₅ are toxic to PBMCs, inhibiting proliferation induced by PHA, tetanus toxoid or alloantigens and leading to loss of cell viability. These effects of D₄ and D₅ are completely prevented by low concentrations of serum. The serum factors responsible for protection are lipoproteins. Other sources of biocompatible lipids, i.e. Intralipid, similarly prevent the toxic effects of D₄ and D₅, as did the non-toxic siloxane HMDS.

As noted in several of the figures, human serum or plasma and lipoproteins can by themselves have some inhibitor effects on lymphocyte stimulation. This phenomena has been previously observed and may have several different mechanisms (19-21).

The toxic effects of D₄ and D₅ described in this reports are unlikely to be relevant systemically since the high levels of lipids in plasma would certainly neutralize the effects of D₄ and D₅ in blood. In addition, the levels of D₄ and D₅ required for the *in vitro* effects in serum-free medium are much higher than we have observed in human blood or plasma in our previous controlled exposures (10). Nevertheless, our observation may be relevant under certain experimental conditions. For example, if D₄ or D₅ are injected subcutaneously or into a body cavity, or when large quantities of D₄ or D₅ are ingested, or if droplets of D₄ or D₅ impact onto the respiratory mucosa, the local concentration of lipid may be insufficient to neutralize the effects of D₄ or D₅, and the resultant cellular damage may lead to inflammation or fibrosis. Even in these experimental circumstances, any lipophilic component of formulations would be expected to have mitigating effects.

REFERENCES.

1. Hochber, M.C., Perlmutter, D. The association of augmentation mammoplasty with connective tissue disease, including systemic sclerosis (scleroderma): a meta-analysis. *Curr Top Microbiol Immunol* 210:411-17, 1995.
2. Perkins, L.L., Clark, B.D., Klein, P.J., Cook, R.R. A meta-analysis of breast implants and connective tissue disease. *Ann Plast Surg* 35: 561-70, 1995.
3. Wang, O. A critical assessment of the relationship between silicone breast implants and connective tissue diseases. *Regul Toxicol Pharmacol* 23:74-85, 1996.
4. Naim, J.O., Lanzafame, R.J., van Oss, C.J. The adjuvant effect of silicone gel on antibody production in rats. *Immunol Invest* 22:151-61, 1993.
5. Klykken, P.C., White, K.L. The adjuvancy of silicones: dependency on compartmentalization. *Curr Top Microbiol Immunol* 210: 113-21, 1995.
6. Hill, S.L., Landavere, M.G., Rose N.R. The adjuvant effect of silicone gel, and silicone elastomer particles in rats. *Curr Top Microbiol Immunol* 210: 123-37, 1995.
7. Klykken, P.C., Galbraith, T.W., Kolesar G.B., Jean P.A., Woolhiser M.R., Elwell M.R., Burns-Naas L.A., Mast R.W., McCay J.A., White K.L. Jr., Munson A.E. Toxicology and humoral immunity assessment of octamethylcyclotetrasiloxane (D4) following a 28-day whole body vapor inhalation exposure in Fischer 344 rats. *Drug & Chemical Toxicology*. 22(4):655-77, 1999
8. Nicholson, J.J. 3rd., Hill S.L., Frondoza C.G., Rose N.R. Silicone gel and octamethylcyclotetrasiloxane (D4) enhances antibody production to bovine serum albumin in mice. *Journal of Biomedical Materials Research*. 31(3):345-53, 1996
9. Singh, M., O'Hagan, D. Advances in vaccine adjuvants. *Nature Biotechnology* 17: 1075-81, 1999.
10. Looney, R.J. Frampton, M.W., Byam, J., Kenaga, C., Speers, D., Cox, C., Mast, R., Klykken, P.C., Morrow, P.E., Utell, M.J. (1998) Immune Effects of Respiratory Exposure of Human Volunteers to Octamethylcyclotetrasiloxane (D₄). *Toxicological Sciences* 44: 214-220, 1998.
11. Nicholson, J.J., Hill, S.L., Frondoza, C.G., Rose, N.R. Silicone gel and octamethylcyclotetrasiloxane (D4) enhances antibody production to bovine serum albumin in mice. *Journal of Biomedical Materials Research*. 31(3):345-53, 1996

12. Klykken, P.C., White, K.L. The adjuvancy of silicones: dependency on compartmentalization. *Current Topics in Microbiology & Immunology*. 210:113-21, 1996
13. Naim, J.O., Lanzafame, R.J., van Oss, C.J. The effect of silicone-gel on the immune response. *Journal of Biomaterial Science, Polymer Edition* 7: 123-32, 1995.
14. Naim, J.O., Ippolito, K.M., Lanzafame, R.J., van Oss, C.J. Induction of type II collagen arthritis in the eDA rat using silicone gel as adjuvant. *Current Topics in Microbiology and Immunology* 210: 103-11, 1996.
15. Schaefer, C.J., Knapp, T., Andrews, A., Delustro, F., Wooley, P.H. Silicone implantation induces antibodies to silicone-bound proteins during murine type II collagen-induced arthritis. *Arth Rheu* 39(9): abstract # 144, S50, 1996.
16. Schaefer, C.J., Wooley, P.H. The influence of silicone implantation on murine lupus in MRL mice. *Arth Rheu* 39(9): abstract # 145, S51, 1996.
17. Rose, N.R. The silicone breast implant controversy: the other courtroom. *Arth Rheu* 39(10): 1615-18, 1996.
18. Felix, K., Janz, S., Pitha, J., Williams, J.A., Mushinski, E.B., Bornkamm G.W., Potter M. Cytotoxicity and membrane damage *in vitro* by inclusion complexes between gamma-cyclodextrin and siloxanes. *Current Topics in Microbiology & Immunology*. 210:93-9, 1996.
19. Cuthbert, J.A., Lipsky, P.E. Immunoregulation by low density lipoprotein in man. Inhibition of mitogen-induced T lymphocyte proliferation by interference with transferrin metabolism. *J. of Clin. Invest.* 73: 992-1003, 1984.
20. Soyland, E., Nenseter, M.S., Braathen, L., Drevon, C.A. Very long chain n-3 and n-6 polyunsaturated fatty acids inhibit proliferation of human T-lymphocytes *in vitro*. *European J. Clin. Invest.* 23: 112-121, 1993.
21. Caspar-Bauguil, S., Tkaczuk, J., Haure, M.J., Durand, M., Alcouffe, J., Thomsen, M., Salvayre, R., Benoit, H. Mildly oxidized low-density lipoproteins decrease early production of interleukin 2 and nuclear factor kappaB binding to DNA in activated T-lymphocytes. *Biochemical J.* 337: 269-274, 1999.

Figure 1. Effect of Siloxanes on PHA-induced Proliferation of Peripheral Blood Mononuclear Cells (PBMCs). PBMCs were cultured in AIMV (serum-free medium) with the concentrations of D₄, D₅, and HMDS indicated on the abscissa. The proliferative response to PHA was assayed after 3 days in culture using incorporation of ³H-thymidine. Mean for quadruplicate assays is indicated as CPM (counts per minute) on the Ordinate.

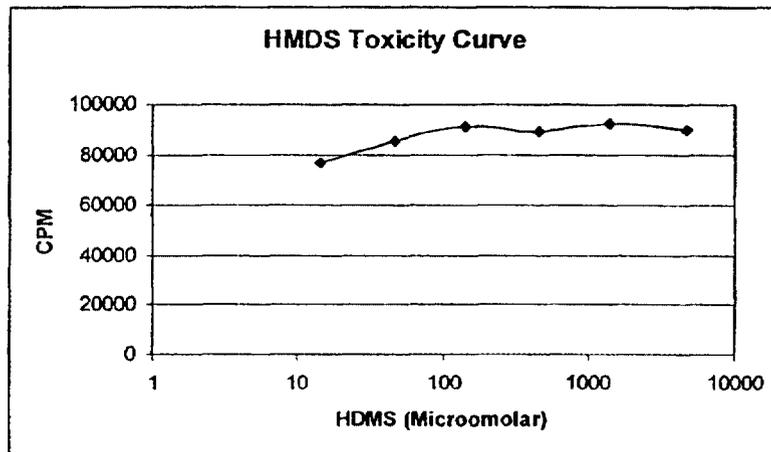
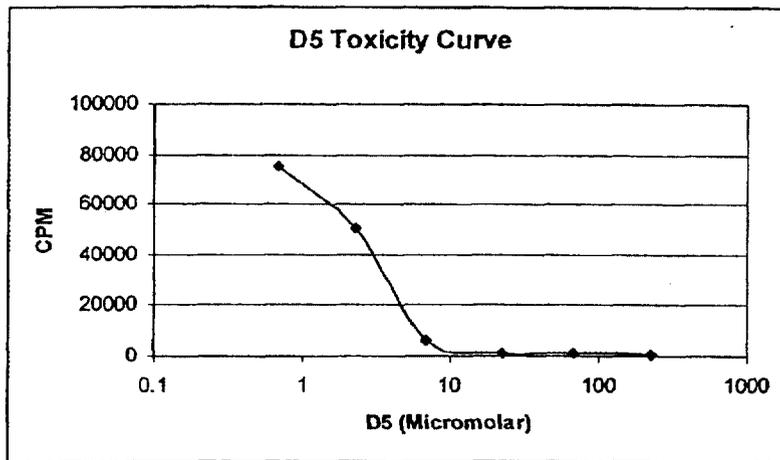
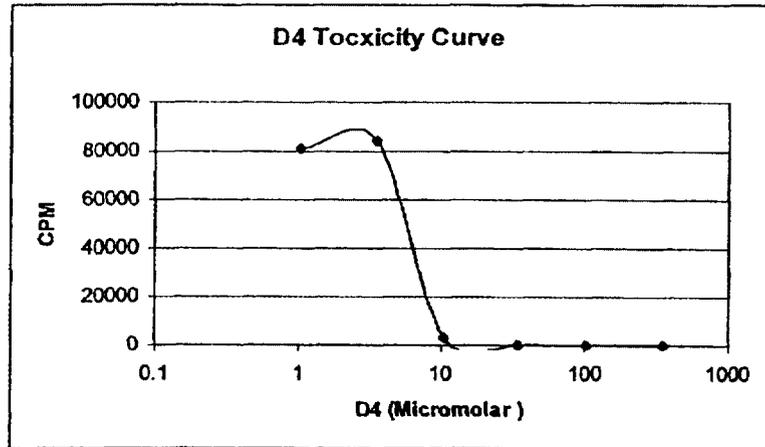
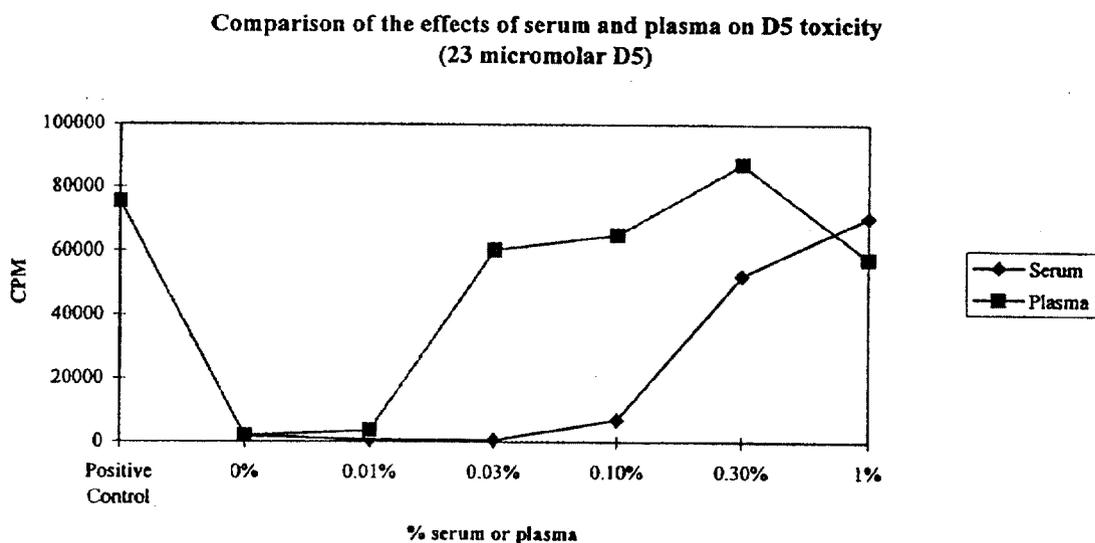
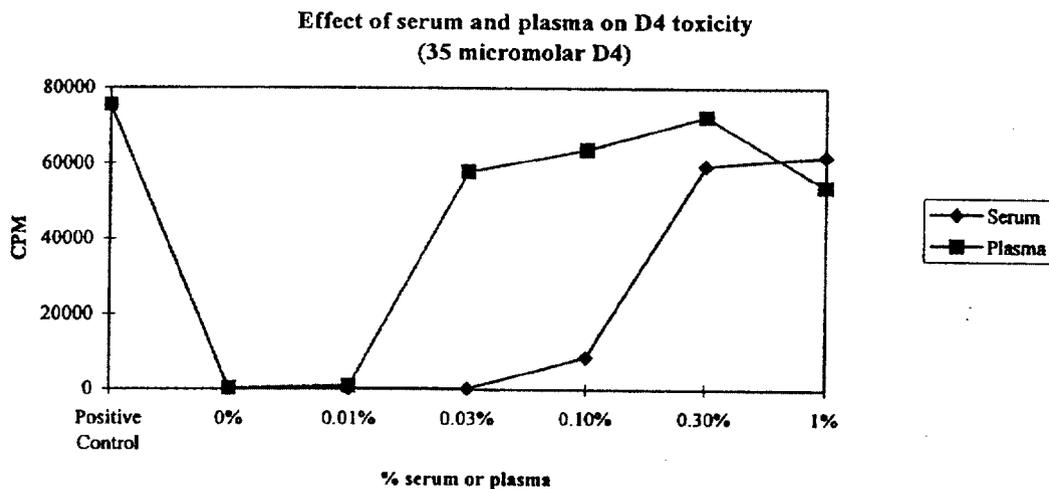


Figure 2. Effects of Serum and Plasma on Inhibition of Proliferation by Siloxanes. PBMCs were cultured in AIMV (serum-free medium) with 35 μ M D₄, 23 μ M D₅ or 47 μ M HMDS and varying concentrations of serum or plasma as indicated on the abscissia. The proliferative response to stimulation with PHA was assayed after 3 days in cultures using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the ordinate.



Effect of serum and plasma on the toxicity of HMDS
(47 micromolar HMDS)

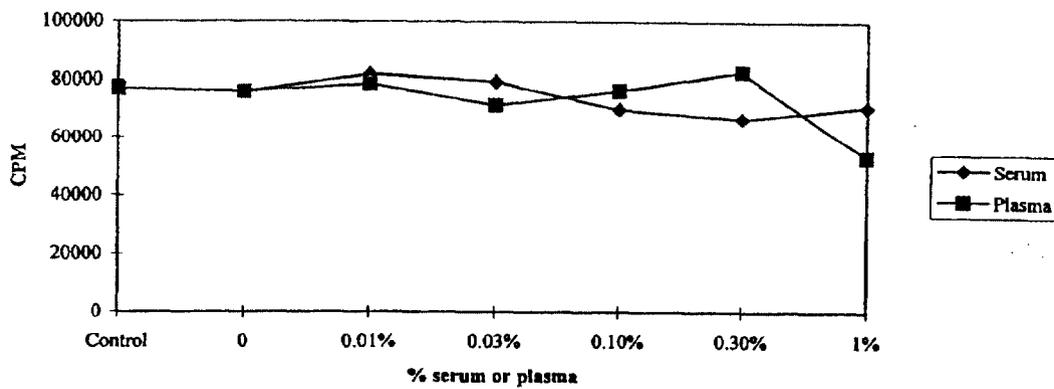
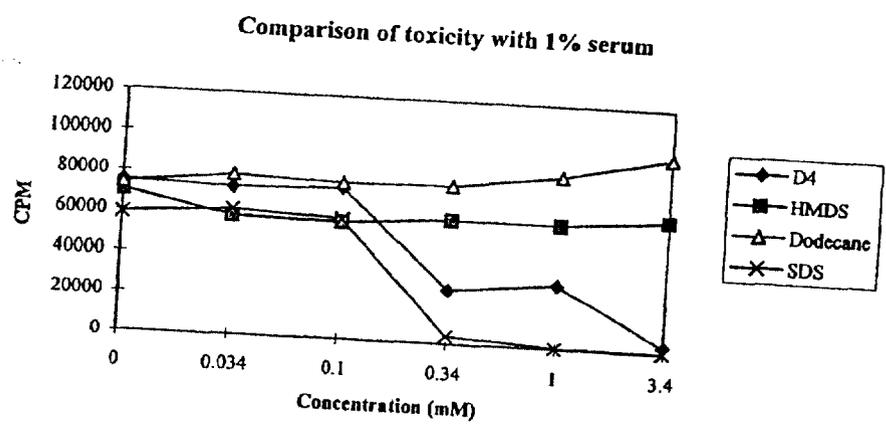
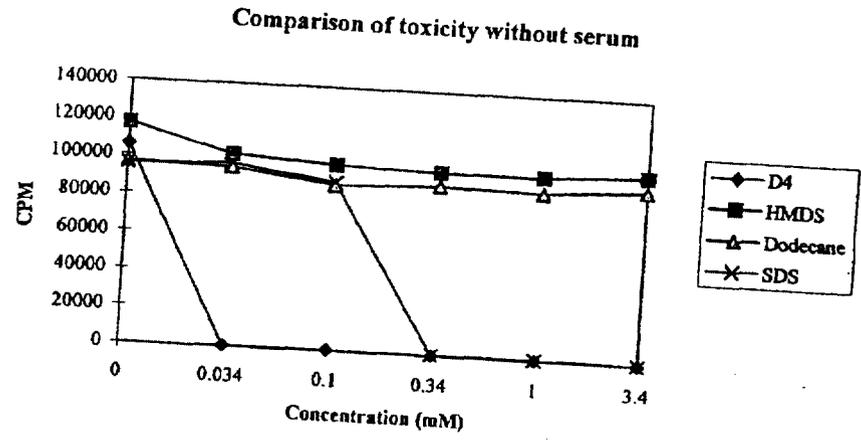


Figure 3. Comparison of D4 to Duodecane and Dodecyl Sulfate (SDS). PBMCs were cultured in AIMV (serum-free medium) with varying concentrations of D₄, HMDS, duodecane, and SDS as indicated on the abscissa. The proliferative response to stimulation with PHA was assayed after 3 days in culture using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the ordinate. The proliferative response without serum, with 1% serum, and with 10% serum are shown in the three separate graphs.



Comparison of toxicity with 10% serum

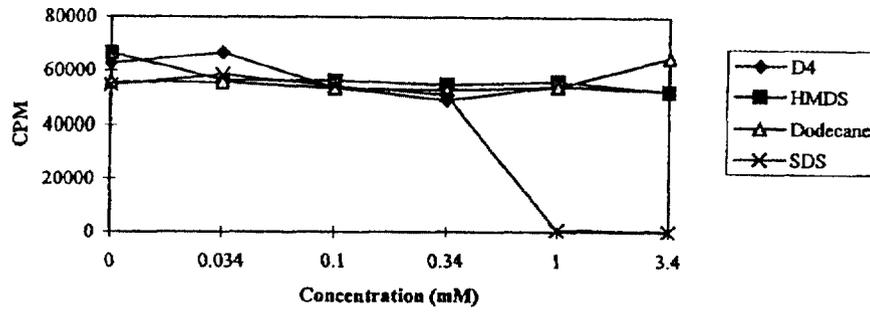


Figure 4. Effect of Serum Components on Inhibition of Proliferation by Siloxanes. PBMCs were cultured in AIMV (serum-free medium) with 35 μ M D₄ and varying concentration of serum or serum components as indicated on the abscissa. The proliferative response to stimulation with PHA was assayed after 3 days in culture using incorporation of 3H-thymidine and is indicated as CPM (counts per minute) on the ordinate.

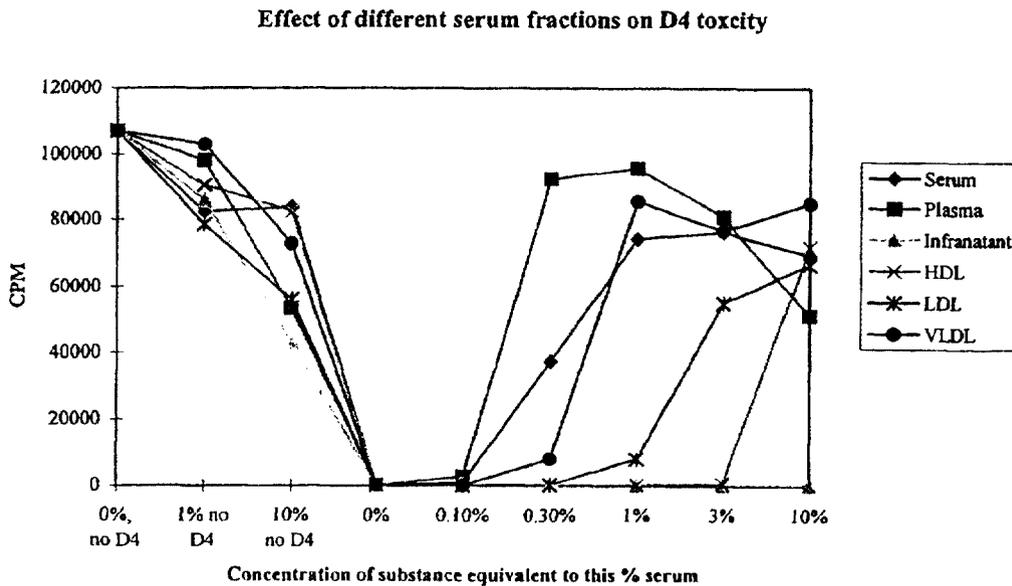
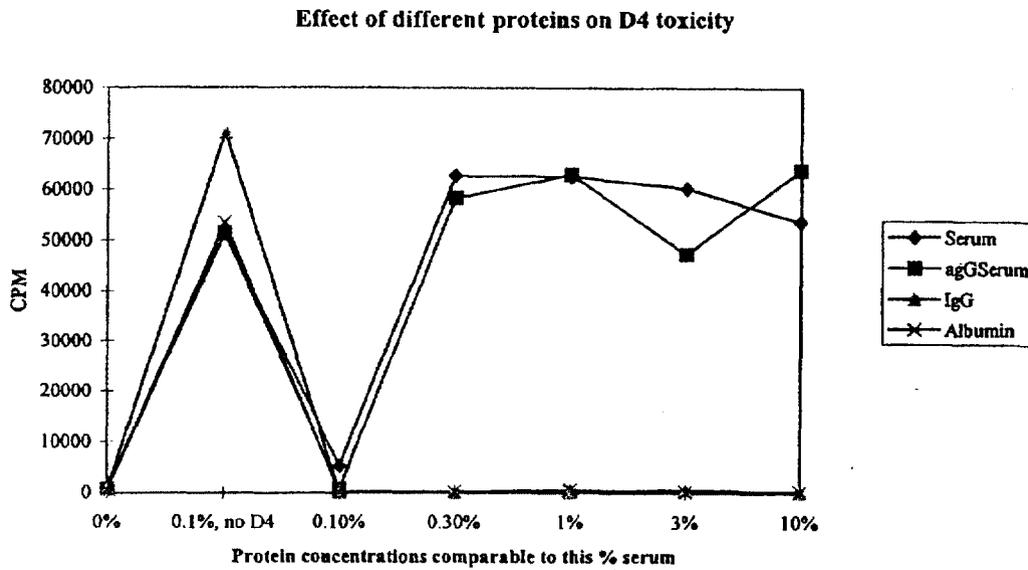


Figure 5. Effect of Intralipid on Inhibition of Proliferation by Siloxanes. PBMCs were cultured in AIMV (serum-free medium) with medium only, 35 μ M D₄, 23 μ M D₅, or 47 μ M HMDS and varying concentrations of Intralipid as indicated on the abscissa. The proliferative response to stimulation with PHA was assayed after 3 days in culture using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the ordinate.

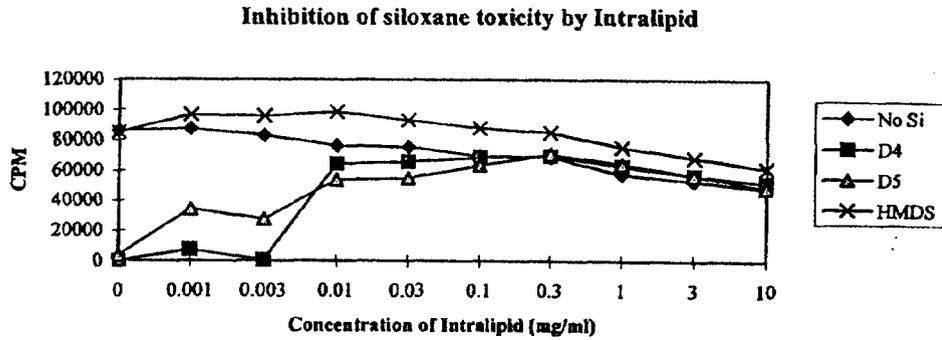


Figure 6. Effect of HMDS on D₄ and D₅ toxicity. PBMCs were cultured in AIMV (serum-free medium) with the concentrations of D₄, D₅, and HMDS indicated on the abscissa. The proliferative response to PHA was assayed after 3 days in culture using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the Ordinate.

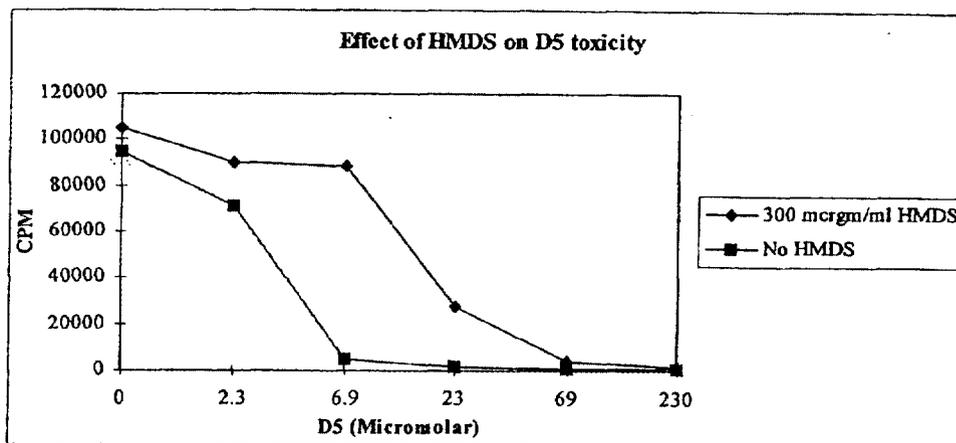
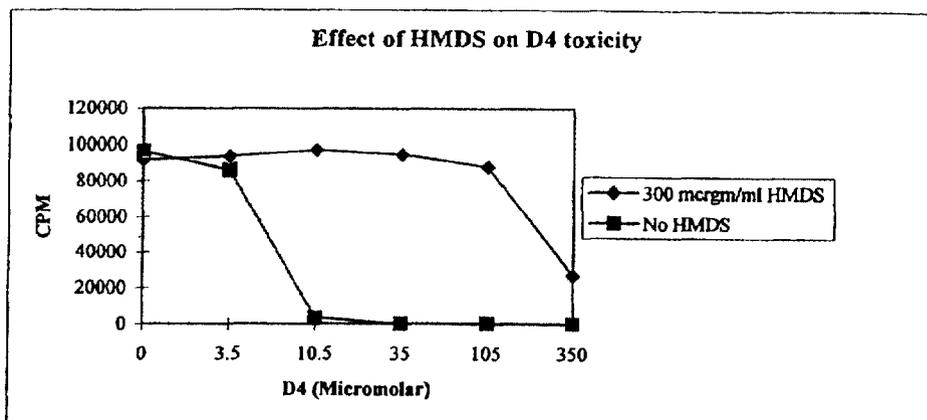
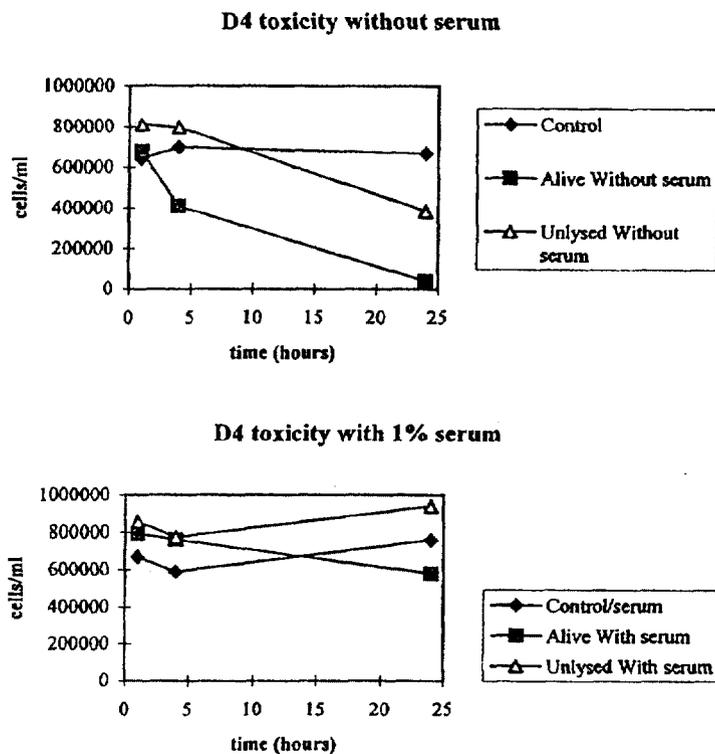
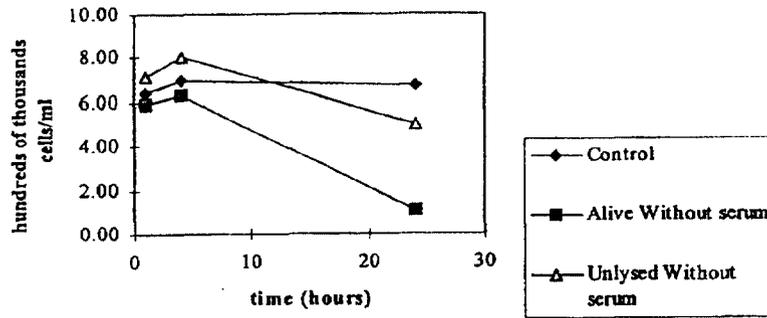


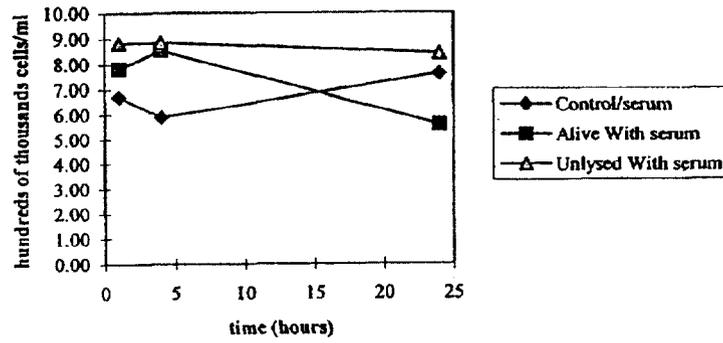
Figure 7. Effect of Siloxanes of Cell Viability. PBMCs were cultured in AIMV (serum-free medium) with 35 μ M D₄, 23 μ M D₅ or 47 μ M HMDS and varying concentrations of serum or plasma as indicated on the abscissia. Cells were then cultured in AIMV alone or AIMV with 1% serum as indicated by the headings. Cell viability and total number of cells were determined using trypan blue exclusion and counting on a hemocytometer immediately after starting the cultures and at 24 and 48 hr.



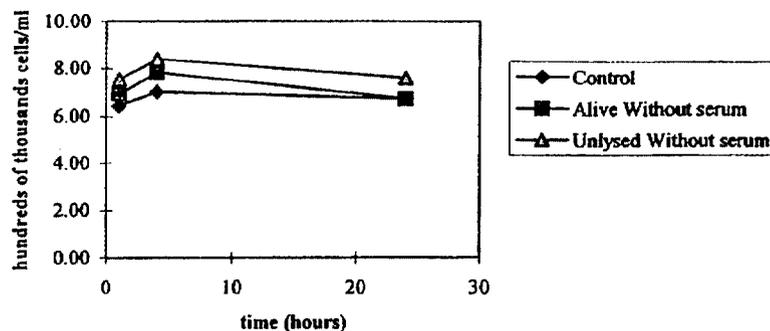
D5 toxicity without serum



D5 toxicity with 1% serum



HMDS toxicity without serum



HMDS toxicity with 1% serum

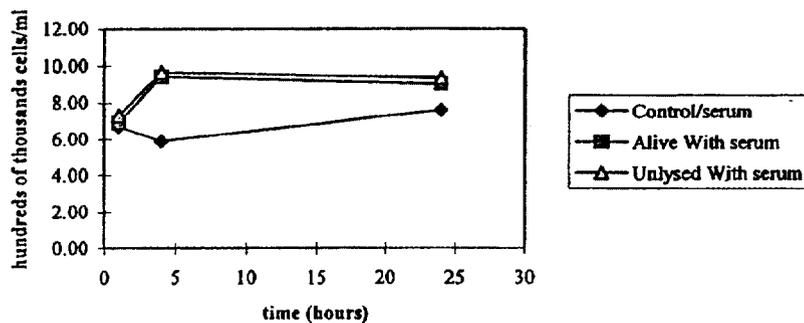


Figure 8. Effect of Siloxanes on Tetanus Toxoid-Induced Proliferation of PBMCs in the absence and presence of different concentrations of serum. PBMCs were cultured in AIMV (serum-free medium) with the concentrations of D₄ and D₅, indicated on the abscissa. The proliferative response to stimulation with tetanus toxoid was assayed after 7 days in culture using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the Ordinate.

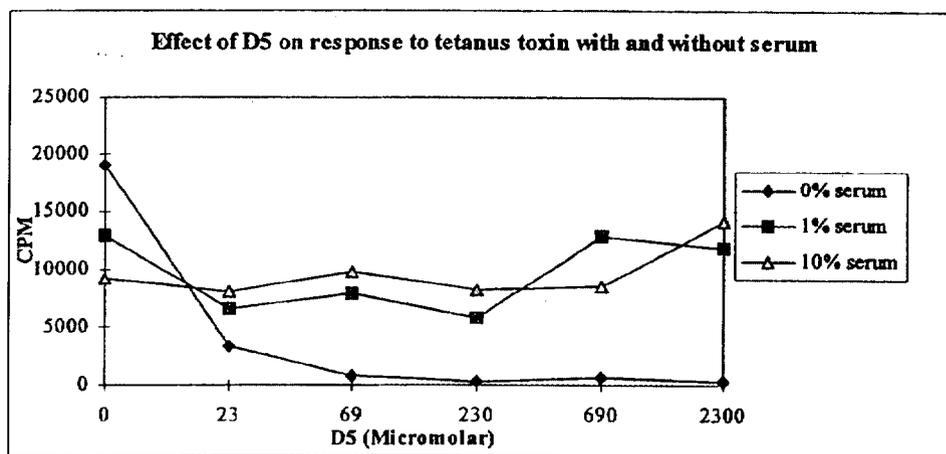
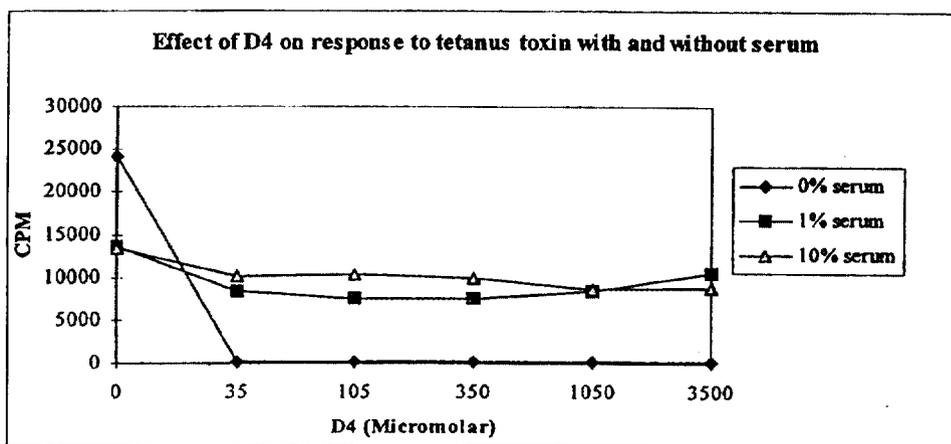


Figure 9. Effect of Siloxanes on Alloantigen-Induced Proliferation of PBMCs in the absence and presence of different concentrations of serum. PBMCs were cultured in AIMV (serum-free medium) with the concentrations of D₄ and D₅ indicated on the abscissa. The proliferative response to stimulation with allogeneic PBMCs was assayed after 7 days in culture using incorporation of ³H-thymidine and is indicated as CPM (counts per minute) on the Ordinate.

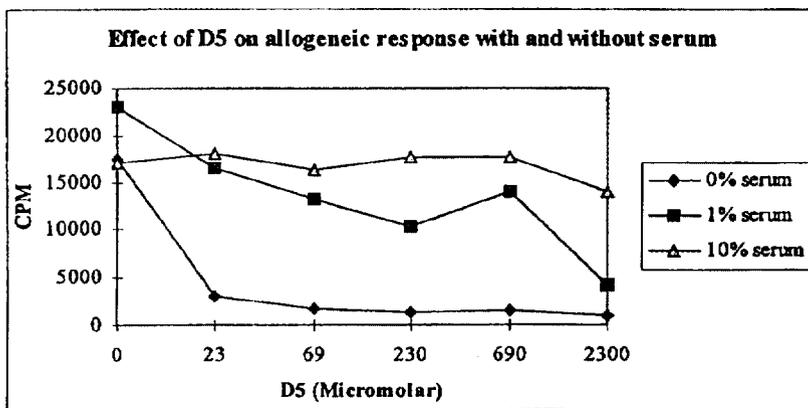
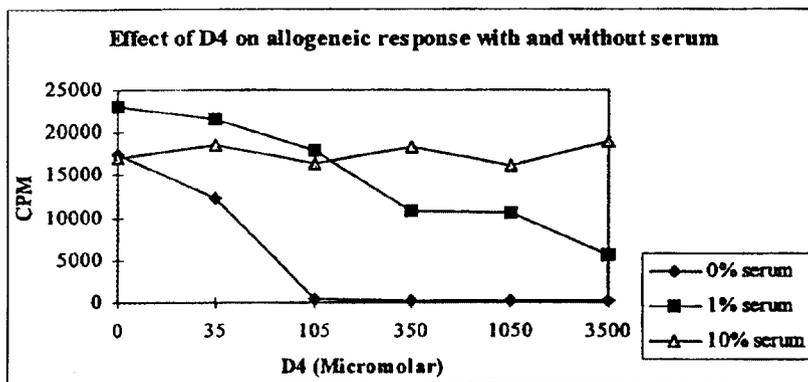
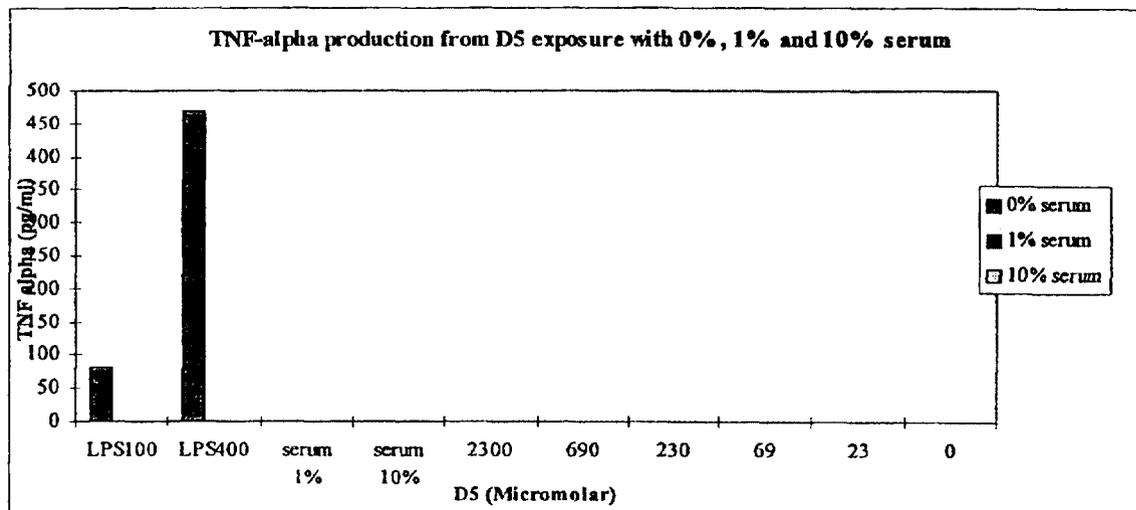
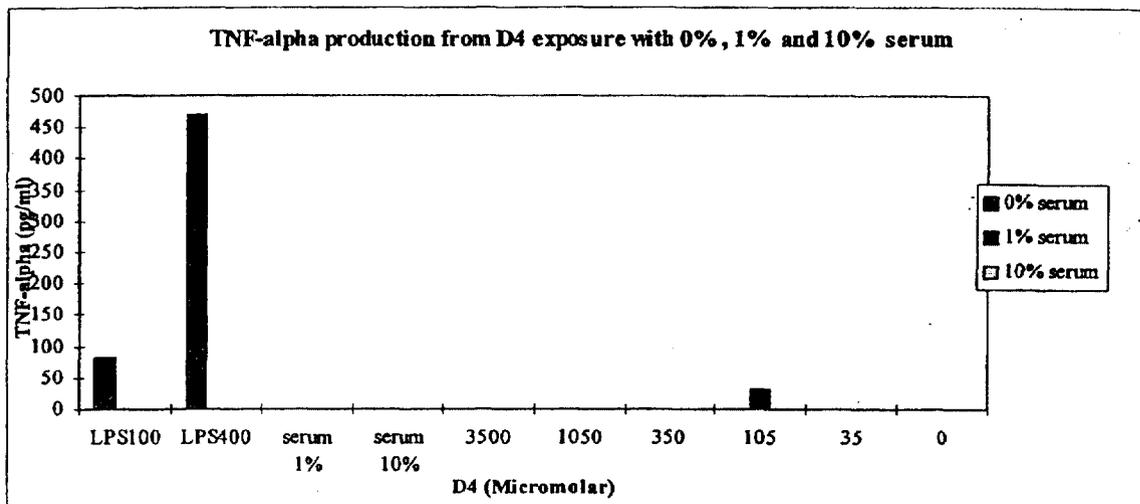
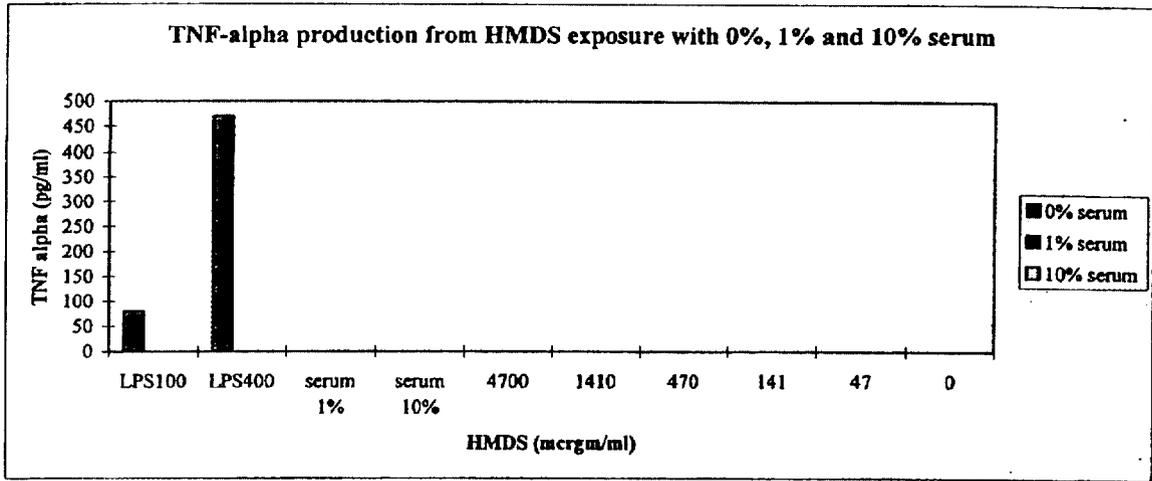


Figure 10. Effects of Siloxanes on Tumor Necrosis Factor Production Alpha (TNF α) in the absence and presence of different concentrations of serum. PBMCs were cultured with the concentrations of D₄, D₅, and HMDS indicated on the abscissa in AIMV (serum-free medium) alone or AIMV with either 1% or 10% serum. After 24 hrs supernatants were collected and assayed for TNF α by ELISA. The concentration of TNF α is shown on the ordinate. Lipopolysaccharide (LPS) at 100 or 400 ng/ml was used as a positive control





Appendix A:
Standard Operating Procedures



Buffy Coat SOP

Order a buffy coat from the Red Cross (50 Prince st, Rochester, 241-4186) to be drawn and delivered at room temperature within 4 hours of use. Dilute the buffy coat 1:1 in PBS and overlay 35 ml onto 12.5 ml room temperature Lymphoprep (cat #100-1969, VWR) in a 50 ml tube. Spin the tubes at 1350 RPM for 45 minutes at room temperature in a centrifuge (Damon/IEC model DPR-6000). Alternatively, one may underlay 12.5 ml Lymphoprep under 35 ml of the diluted buffy coat already in the 50 ml tube, but this takes somewhat longer.

Remove the lymphocytes at the interface of the serum and Lymphoprep, transfer to another tube and dilute at least 1:1 with PBS. Spin the cells at 1000 RPM for 10 minutes in a 4° centrifuge. Discard the supernatant and resuspend cells to a total volume of 10 ml. Dilute an aliquot of the cell suspension 1:100 and count on a hemacytometer (American Optical Bright-line hemacytometer). Dilute the 10ml of cells further in PBS (to a total of around 50 ml) and spin again at 1000 RPM for 10 min at 4°. Resuspend the cells in media to the concentration needed for the assay being used.



SOP

Preparation of siloxane solutions for *in vitro* studies

The following chart should be used to make the siloxane solutions:

Concentration desired	Siloxane added to ETOH (final vol=1ml)	D4/ETOH added to 5 ml media
2.5 µg/ml	2.5 µl	20 µl
3 µg/ml	3 µl	20 µl
10 µg/ml	10 µl	20 µl
20 µg/ml	20 µl	20 µl
30 µg/ml	30 µl	20 µl
100 µg/ml	100 µl	20 µl
300 µg/ml	300 µl	20 µl
1000µg/ml	500 µl	40 µl

The pure siloxane is added to 100% ethanol in the amount indicated for a total volume of 1 ml, and then an aliquot of this solution (last column above) is added to 5 ml media. The concentration of this solution at this point is four times the concentration desired- the solution should be diluted 1:4 in the plate (24- or 96-well, depending on the assay). If a more dilute solution is needed, the ethanol/siloxane solution may be diluted 1:10, and 20 µl would be added to 5 ml media. Use the same chart for solutions containing a mixture of siloxanes- for example, for 10 µg/ml D4 with 300 µg/ml HDMS, combine 10 µl D4 and 300 µl HDMS, and add ethanol to 1 ml.

If a series of different dilutions is required, each solution of media/ethanol/siloxane will be made uniquely, not by removing an aliquot of a higher concentration of siloxane in media and diluting it, but by following the instructions above for each and every concentration needed. This is because at higher concentrations, micelles form resulting in a heterogeneous mixture. Also, the concentration of ethanol in each sample needs to remain constant (0.1%), to eliminate this as a variable in the results.

Solutions should be made immediately before use.

RJD



PHA toxicity curve

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml in AIMV. Make siloxane solutions as per protocol for desired concentration. Draw a plate diagram ahead of time- the number of columns for the components listed below is relative to the number of siloxane solution concentrations you want to test.

In a 96-well flat-bottom plate add:

- 100 μ l AIMV to top 4 wells of each column, plus one for a no-siloxane control
- 100 μ l 1:1000 PHA to bottom 4 wells of each column used (including no-siloxane control)
- 50 μ l of each siloxane solution concentration to a single column
- 50 μ l AIMV to the no-siloxane positive control column
- Add 50 μ l PBMCs to all wells

Incubate 3 days at 37° and 7.5% CO₂ in a humidified water jacketed incubator.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H³ methyl thymidine (NEN #NET027A) in AIMV to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packard Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5 mg/ml in AIMV.

AIMV: order from VWR, Gibco cat # 12055-091.

Effect of serum and plasma on the toxicity of siloxanes, measured by the inhibition of proliferation of T-cells induced by PHA

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml in AIMV. Make siloxane solutions as per protocol for desired concentrations. Make solutions of 4% and 40% serum and plasma in media (ratios of 40 μ l serum or plasma in 1 ml media and 400 μ l serum or plasma plus 600 μ l media, respectively). Serum and plasma used is from frozen aliquots from John Looney.

In 96-well flat-bottom plates add:

- 50 μ l AIMV to top 4 wells
- 50 μ l 1:500 PHA to bottom 4 wells
- 50 μ l of each siloxane solution to each of three columns
- 50 μ l AIMV to a no-siloxane positive control column
- 50 μ l of each serum solution and each plasma solution to each column of a given siloxane solution. The remaining column receives 50 μ l AIMV as a negative control.
- Add 50 μ l PBMCs to all wells

Incubate 3 days.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H^3 methyl thymidine (NEN #NET027A) in media to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packard Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5 mg/ml in media.

AIMV: order from VWR, Gibco cat # 12055-091.

Alternative: Plasma and serum titration with a constant siloxane concentration

Prepare serum and plasma concentrations of 4% and 1.2%- 4% as directed above, and for the 1.2% add 12 μ l serum for each ml of media. Dilute the 4% 1:10 for 0.4%, and dilute the 0.4% for 0.04%. Dilute the 1.2% 1:10 for 0.12%. Each of these will be diluted 1:4 into the plate. Make siloxane solutions as per the directions above. Into the plate:

- 50 μ l of each serum or plasma concentration to each well, one column for each siloxane concentration tested
- 50 μ l media, one column for each plate as a negative control
- 50 μ l siloxane concentration to each well, one column for every serum/plasma concentration, plus one column without serum/plasma
- 50 μ l PHA to the bottom 4 wells of every column
- 50 μ l media to the top 4 wells of every column
- 50 μ l cells (as prepared above) to every well

Incubate and harvest as stated above.

Comparison of toxicities of D₄, HMDS, Sodium dodecyl sulfate (SDS), and Dodecane

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml. Make siloxane solutions as per protocol for 1000, 300, 100, 30 and 10 μ g/ml.

Make a solution of dodecane (Aldrich #D22,110-4) at the same molarity of D₄ (3.2M) by adding 27 parts ethanol to 73 parts dodecane, and filter sterilize. Then, make the concentrations as per the siloxane solution protocol.

For the SDS (Bio-Rad #161-0302), begin with a stock solution of 10% SDS in distilled deionized water - this is 0.34M. Dilute this 1:25 in ethanol. This will be the highest concentration used, 3.4 mM after 1:4 dilution into the plate. Dilute 3 parts of this with 7 parts ethanol for 1mM. Dilute the 3.4 mM 1:10 for 0.34mM and again for 0.034mM, in ethanol. Dilute the 1mM 1:10 in ethanol for a final set of concentrations of 3.4, 1, 0.34, 0.1, and 0.034 mM.

Make solutions of 4% and 40% serum in media (ratios of 40 μ l serum in 1 ml media and 400 μ l serum plus 600 μ l media, respectively). Serum used is from frozen aliquots from John Looney.

There will be 6 plates total: 2 plates for each of 0% serum, 1% serum and 10% serum. For each set of 2 plates, 6 columns will be used for different concentrations of SDS, HMDS, Dodecane and D₄ - the concentrations listed above, and a positive control column without the substance.

In 96-well flat-bottom plates add:

- 50 μ l AIMV to top 4 wells of each column
 - 50 μ l 1:500 PHA to bottom 4 wells of each column
 - 50 μ l of each concentration of each test substance
 - 50 μ l AIMV to a no-siloxane positive control column
 - 50 μ l media, 4% serum or 40% serum to the appropriate plates.
- Add 50 μ l PBMCs to all wells

To clarify the above: Usually it is easiest to designate plates as 0% serum, one as 1% serum and a third as 10% serum. The bottom of each plate would contain the PHA. Each plate would contain two test substances, 6 columns for each: 5 different concentrations as outlined above, plus a no-test substance positive control.

Incubate 3 days at 37° and 7.5% CO₂ in a humidified water jacketed incubator.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H³ methyl thymidine (NEN #NET027A) in AIMV to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packad Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5 mg/ml in media.

AIMV: order from VWR, Gibco cat #12055-091



Effect of Agammaglobulin Serum, Immunoglobulin G and Albumin on D₄ toxicity, as compared to normal serum, as exemplified by proliferative response to PHA

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml in AIMV. Make D₄ solution (10 µg/ml) as per protocol for desired concentration. Make solutions of 4%, 1.2%, 0.4%, 0.12%, and 0.04% normal serum and agammaglobulin serum (Normal serum used is from frozen aliquots donated by John Looney and the agammaglobulin serum is from a clinical sample obtained from John Leddy's lab). These will be diluted 1:4 when added to the plate. The 4% is made by adding 40 µl to each ml media. Three parts of this are added to 7 parts media for the 1.2% solution, and both of these are diluted 1:10 for the remaining concentrations. Make the following IgG (Sigma #I-4506) concentrations: 4, 1.2, 0.4, 1.12 and 0.04 mg/ml (these values correspond to the physiological levels in the serum concentrations above). Also make the following albumin (Sigma #A-8763) concentrations: 20, 6, 2, 0.6 and 0.2 mg/ml (these values also correspond to normal physiological levels).

In a 96-well flat-bottom plate add:

- 50 µl AIMV to top 4 wells
- 50 µl 1:500 PHA to bottom 4 wells
- 50 µl of the D₄ solution to each plate
- 50 µl AIMV to a no-siloxane positive control column (with a median amount of protein or sera)
- 50 µl sera or protein per well of one column of each siloxane concentration
- Add 50 µl PBMCs to all wells

Incubate 3 days at 37° and 7.5% CO₂ in a humidified water jacketed incubator.

For the last 6 hours of the incubation, add 50 µl of 20 µCi/ml H³ methyl thymidine (NEN # NET027A) in media to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest 50 µl from the non-PHA wells to test for tumor necrosis factor by ELISA (see ELISA protocol). Then harvest the plate onto glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packard Matrix 96 Direct Beta Counter model #A9609*.

*counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5mg/ml in media.

AIMV: order from VWR, Gibco cat # 12055-091

Intralipid assay

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml in AIMV. Make 10 μ g/ml siloxane solutions as per the siloxane solution protocol. Prepare solutions of 40 mg/ml and 12 mg/ml Liposyn II (this is a fat emulsion solution obtained from the Strong Memorial Hospital Pharmacy in 50 ml aliquots) by mixing 2 ml Liposyn II with 8 ml AIMV for the 40 mg/ml, and 3 ml of this diluted with 7 ml AIMV. Dilute these 1:10 (300 μ l with 2.7 ml media) serially to make solutions of 4, 1.2, 0.4, 0.12, 0.04, 0.012 and 0.004 mg/ml. In conjunction with the Liposyn II, a titration of serum with the siloxanes should be set up as a positive control. Refer to the alternative at the bottom of the protocol 'Effect of serum and plasma on the toxicity of siloxanes measured by the inhibition of proliferation of T-cells induced by PHA'.

In a 96-well flat-bottom plate add:

- 100 μ l AIMV to top 4 wells of each column
- 100 μ l 1:1000 PHA to bottom 4 wells of each column used (including no-siloxane control)
- 50 μ l of each solution concentration to a single column
- 50 μ l AIMV to a no-siloxane positive control column
- Add 50 μ l PBMCs to all wells

Incubate 3 days at 37° and 7.5% CO₂ in a humidified water jacketed incubator.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H³ methyl thymidine (NEN # NET027A) in media to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packard Matrix 96 Direct Beta Counter model #A9609*.

*counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5mg/ml in media.
AIMV: order from VWR, Gibco cat # 12055-091



Effect of serum on toxicity of siloxane

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 2×10^6 /ml in AIMV. Make siloxane solutions as per protocol for desired concentrations. Make solutions of 4% and 40% serum in media (ratios of 40 μ l serum in 1 ml media and 400 μ l serum plus 600 μ l media, respectively). Serum used is from frozen aliquots from John Looney.

In 96-well flat-bottom plates add:

- 50 μ l AIMV to top 4 wells of each column
- 50 μ l 1:500 PHA to bottom 4 wells of each column
- 50 μ l of each siloxane solution to each of three columns (for 0%, 1%, and 10% serum)
- 50 μ l AIMV to a no-siloxane positive control column, one for each serum concentration
- 50 μ l of AIMV, 4% or 40% serum to appropriate columns.
- Add 50 μ l PBMCs to all wells

Incubate 3 days.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H^3 methyl thymidine (NEN #NET027A) in AIMV to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packard Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

PHA: phytohemagglutinin Sigma cat #L9132, stock solution at 5 mg/ml in media.
AIMV: order from VWR, Gibco cat #12055-091



Effect of serum on the inhibition of proliferative response of T cells by siloxanes when stimulated by tetanus toxin

Isolate cells from a buffy coat as per buffy coat protocol. Set cells at a concentration of 4×10^6 /ml in AIMV. Make siloxane solutions as per protocol for desired concentration. Make solutions of 4% and 40% serum in media (ratios of 40 μ l serum in 1 ml media and 400 μ l serum plus 600 μ l media, respectively). Serum used is from frozen aliquots from John Looney.

In a 96-well flat-bottom plate add:

- 100 μ l AIMV to top 4 wells of each column of three plates
- 100 μ l 1:400 tetanus toxin to bottom 4 wells of each column, all plates
- 50 μ l 40% serum to one plate, all wells
- 50 μ l 4% serum to another plate, all wells
- 50 μ l AIMV to the third plate, as a no-serum negative control
- 50 μ l AIMV to the first and seventh column of each plate (as a no-siloxane control)
- 50 μ l of each siloxane solution concentration to one column of each plate. (To keep things clear, designate the left side of each plate D4 and the right side D5, and add the concentrations lowest to highest. The column labels will be 0, 10, 30, 100, 300 and 1000 μ g/ml for the D4 side and repeat for the D5 side.)

Add 50 μ l PBMCs to all wells

Incubate 7 days.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H^3 methyl thymidine (NEN #NET027A) in media to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packad Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

tetanus toxin: Sigma cat #L9132, stock solution at 5.2 mg/ml in media.
AIMV: order from VWR, Gibco cat # 12055-091.

Effect of serum on the inhibition of proliferation of T cells by siloxane, allogeneic lymphocytes used as stimulant

Isolate cells from two buffy coats as per buffy coat protocol. Set one set of cells at a concentration of 2×10^6 /ml in AIMV. Prepare the other set of cells as feeders as per the feeder cell protocol (don't freeze them), and set at a concentration of 8×10^6 /ml.

Make siloxane solutions as per protocol for desired concentrations.

Make solutions of 4% and 40% serum in media (ratios of 40 μ l serum in 1 ml media and 400 μ l serum plus 600 μ l media, respectively). Serum used is from frozen aliquots from John Looney.

In 96-well flat-bottom plates add:

50 μ l AIMV to top 4 wells

50 μ l feeder cells to the bottom 4 wells

50 μ l per well of each siloxane solution to each of three columns (one for each serum concentration)

50 μ l per well AIMV to a no-siloxane positive control column

50 μ l per well of each serum solution to one column for every siloxane concentration. One column for each Siloxane solution receives 50 μ l AIMV for a no-serum negative control.

Add 50 μ l PBMCs to all wells

Incubate 5 days.

For the last 6 hours of the incubation, add 50 μ l of 20 μ Ci/ml H^3 methyl thymidine (NEN #NET027A) in media to each well.

Freeze plate at -20° for up to three days, if needed.

Thaw plate and harvest onto a glass filter using a Packard Micromate 196 96-well plate harvester model #B9614 and let filter dry for at least 4 hours. Count filter on a Packad Matrix 96 Direct Beta Counter model #A9609*.

*Counter is calibrated once per month.

AIMV: order from VWR, Gibco cat # 12055-091.

SOP #LVIII

TNF ELISA Protocol (Enzyme-linked immunoabsorbance assay)

1. Dilute purified anti-TNF capture monoclonal antibody (Pharmingen #18631D) to 2µg/ml in coating solution. Add 50µl to wells of an enhanced protein binding ELISA plate (Corning easy wash; cat. #25805-96). Cover plate and incubate overnight at 4°C. Wash 2X with PBS/tween. For each wash, wells are filled with PBS/tween and allowed to stand for at least 1 minute prior to aspirating or dumping. Pound plate on paper towels as a final step.
2. Block with PBS/10% fetal bovine serum at 200 µl per well. Cover plate and incubate at room temperature for 2 hours. Wash 2X with PBS/tween.
3. Add standards (Pharmingen #19761T) and samples at 100 µl per well (diluted in PBS/10%FBS). Dilute samples 1:20 and/or 1:60- 1:20 will be sufficient for most samples, for samples exceeding the highest standard at this dilution, perform the assay again at 1:60. Cover and incubate at 4°C overnight. Wash 4X with PBS/tween.
4. Dilute biotinylated anti-TNF detecting monoclonal antibody (Pharmingen #18642D) to 1µg/ml in PBS/10%FBS and add 100µl to each well. Cover and incubate at room temperature for 45 minutes. Wash 6X with PBS/tween.
5. Dilute avidin-peroxidase (Sigma #A-3151) 1:400 in PBS/10%FBS and add 100 µl to each well. Cover and incubate at room temperature for 30 minutes. Wash 8X with PBS/tween.
6. Thaw ABTS substrate within 5 minutes of use. Add 10 µl of H₂O₂ per 10 ml of substrate and vortex. Immediately add 100 µl per well and allow to develop at room temp. Read plate at OD 405 nm on a ELISA plate reader (Bio-rad model 3550-UV).

Coating solution: 0.1 M NaHCO₃, pH 8.2 stable indefinitely at 4°C.
PBS solution: 80.0g NaCl, 11.6g Na₂HPO₄, 2.0g KH₂PO₄, 2.0g KCl; volume to 10L; pH to 7.0. Stable indefinitely at 4°C.
PBS/tween (Tween from VWR #JTX251-7) Add 0.5ml Tween-20 to 1L PBS from above. Stable indefinitely at room temperature.
Substrate solution: Add 150mg 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma #A-1888) to 500ml 0.1 M citric acid in ddH₂O. pH to 4.35 with NaOH pellets. Aliquot 11ml and store at -20°C.

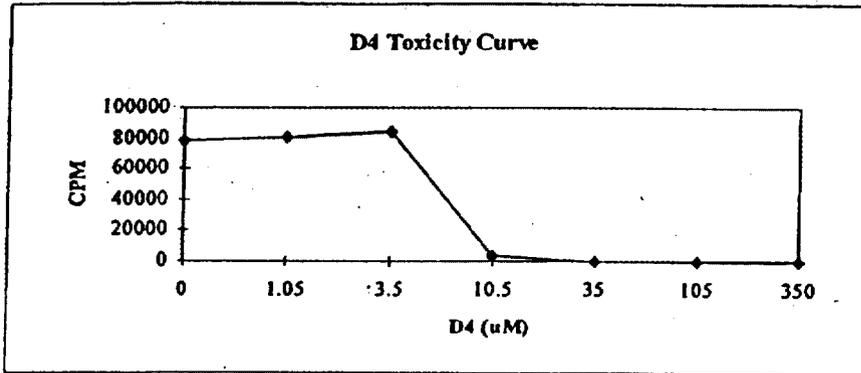
Appendix B:
Data from *In Vitro* Assays



B1. Cyclic Siloxanes Inhibit PHA-Induced Proliferation of Peripheral Blood Mononuclear Cells (PBMCs).

2/26/97

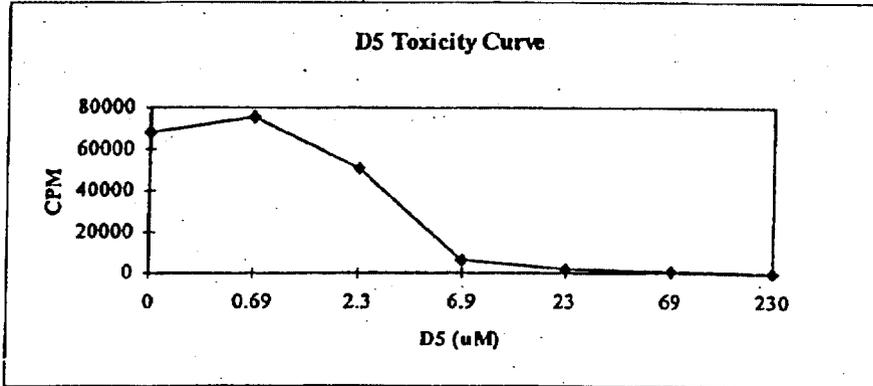
	[D4]						
	0	1.05	3.5	10.5	35	105	350
PHA	78906	81042	84560	3074	161	216	210
% Error	13.7%	11.1%	5.2%	109.0%	10.7%	53.8%	20.7%
Background	120	150	180	84	62	100	118
% Error	25.2%	25.2%	31.8%	30.1%	32.7%	23.1%	24.2%



Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



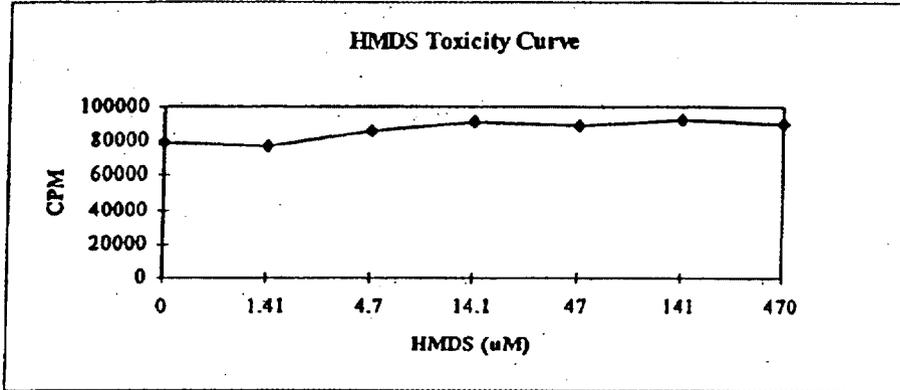
[D5]	2/26/97						
	0	0.69	2.3	6.9	23	69	230
PHA	68475	75567	50809	6060	1515	1116	438
% Error	21.0%	2.8%	23.7%	37.5%	44.1%	40.2%	61.6%
Background	170	157	124	140	68	75	76
% Error	21.4%	22.4%	40.5%	18.7%	53.1%	20.1%	33.4%



Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



[HMDS]	2/26/97						
	0	1.41	4.7	14.1	47	141	470
PHA	79546	76441	85658	90832	88997	92124	89756
% Error	21.1%	14.0%	9.4%	3.6%	3.4%	3.7%	6.9%
Background	142	178	187	177	160	151	149
% Error	22.1%	12.5%	19.9%	28.4%	41.1%	41.2%	14.0%

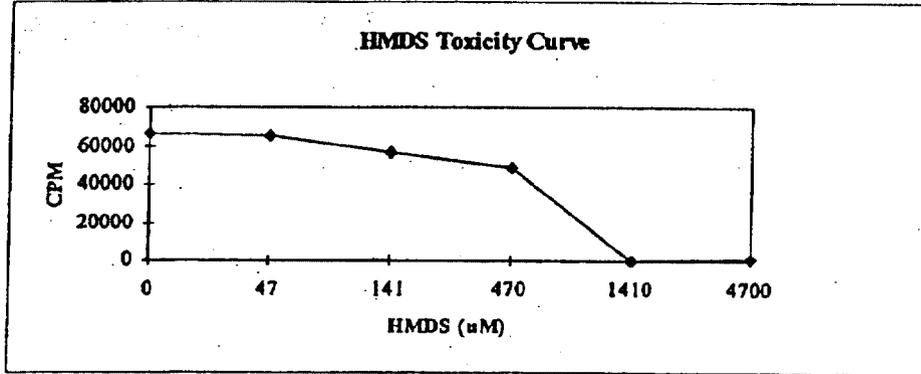


Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



1/8/97

	[HMDS]					
	0	47	141	470	1410	4700
PHA	66408	65706	56550	49120	396	976
%error	9.9%	3.4%	18.9%	36.1%	50.8%	23.8%
background	239	188	234	211	146	174
% Error	29.8%	36.4%	35.9%	34.8%	28.2%	15.6%



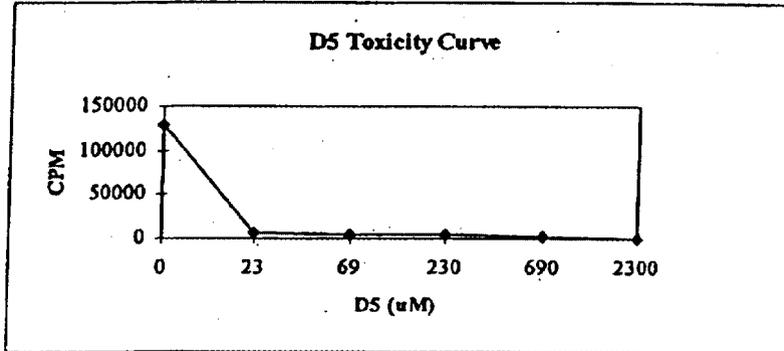
Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



2/3/97

[D5]

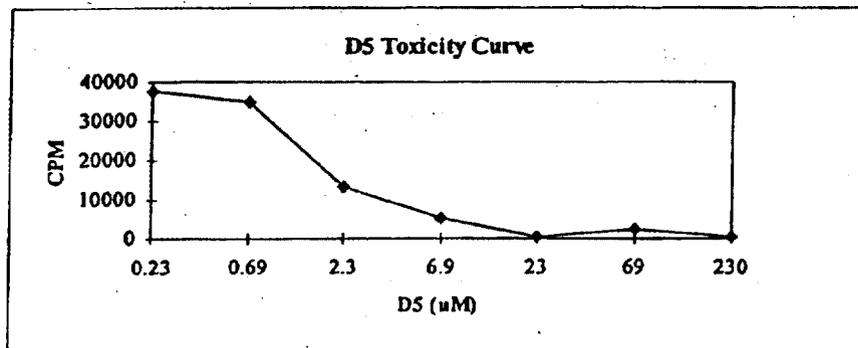
	0	23	69	230	690	2300
PHA	128000	5399	4961	3504	2597	561
% Error	7.2%	51.2%	21.9%	44.9%	60.9%	40.0%
Background	649	179	136	136	112	93
% Error	20.1%	8.2%	45.3%	11.8%	30.3%	21.5%



Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



[DS]	02/07/97						
	230	69	23	6.9	2.3	0.69	0.23
PHA	502	2448	485	5112	13209	34723	37628
%Error	29.5	45.8	36.9	27.1	25.5	10.1	9.5
Background	157	137	135	710	158	112	145
% Error	23.6%	7.5%	23.1%	44.6%	47.2%	45.0%	47.4%

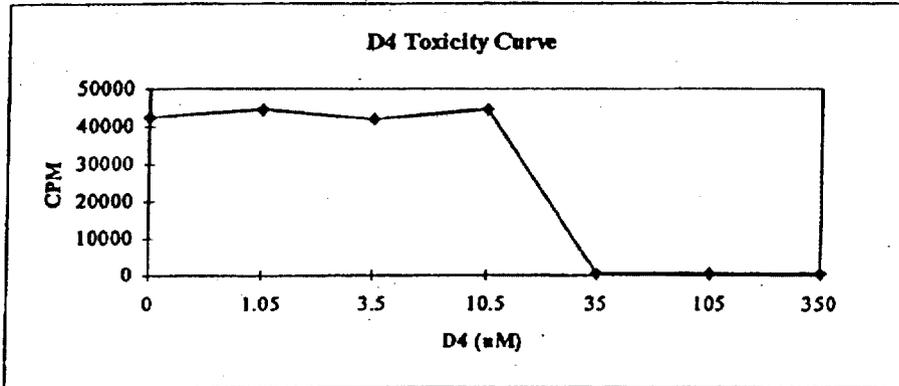


Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



2/21/97 [D4]

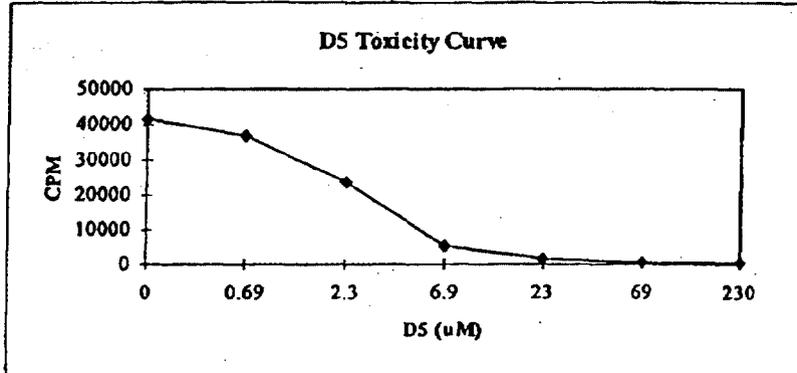
	0	1.05	3.5	10.5	35	105	350
PHA	42357	44298	41963	44426	313	604	343
% Error	4.6%	10.1%	4.9%	2.0%	13.2%	76.6%	21.0%
Background	196	204	247	262	145	126	48
% Error	13.1%	14.8%	15.3%	22.3%	24.7%	11.4%	35.8%



Values are the mean of 4 wells in CPM
%error=(standard deviation)x 100



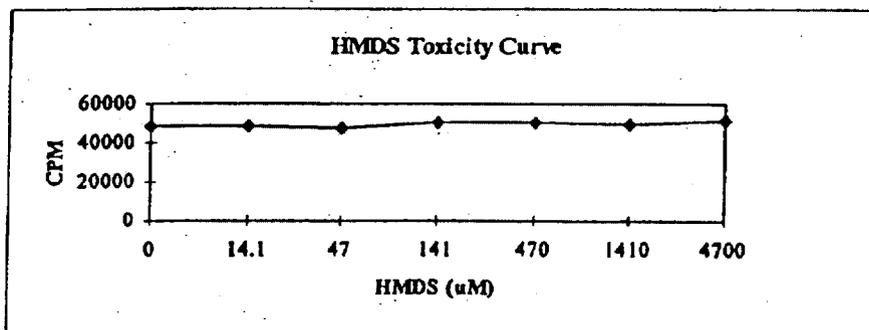
[DS]	2/21/97						
	0	0.69	2.3	6.9	23	69	230
PHA	41761	36886	23858	5302	1326	537	405
% Error	9.7%	0.5%	15.3%	30.8%	25.4%	32.6%	17.5%
Background	265	264	445	173	146	109	42
% Error	15.5%	27.1%	43.2%	13.3%	41.7%	39.6%	34.2%



Values are the mean of 4 wells in CPM
%error=(standard deviation)x100



[HMDS]	2/21/97						
	0	14.1	47	141	470	1410	4700
PHA	48991	48482	47529	50518	50078	49926	51333
% Error	1.5%	6.8%	0.6%	2.9%	2.5%	4.0%	1.2%
Background	245	254	286	267	333	308	340
% Error	18.7%	11.6%	18.8%	18.1%	16.5%	13.4%	31.6%

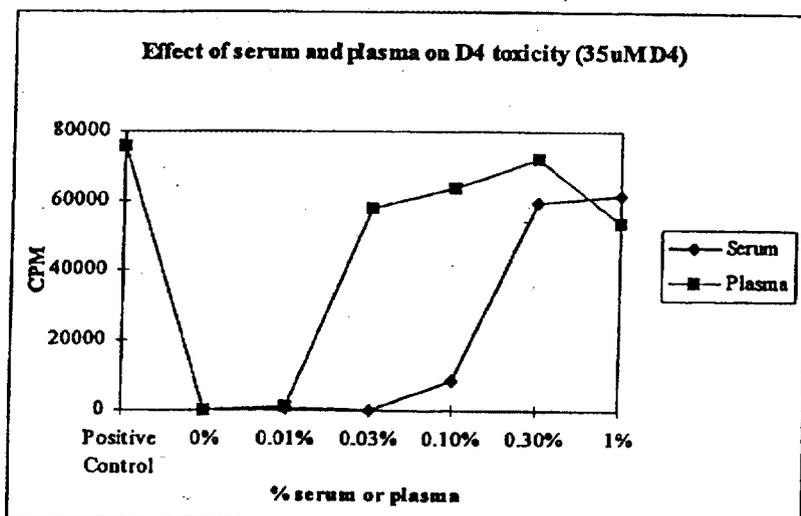


Values are the mean of 4 wells in CPM
%error=(standard deviation)x100

B2. Serum and Plasma Protect PBMCs from the Effects of Siloxanes

	35uM D4	2/26/97					
	Positive Control	0%	0.01%	0.03%	0.10%	0.30%	1%
Serum	75556	243	299	255	8683	59346	61834
% Error	6.2%	7.1%	86.6%	24.9%	75.5%	5.9%	8.2%
Background	99	366	210	285	221	133	125
% Error	18.1%	68.1%	35.4%	33.2%	22.4%	21.6%	9.1%

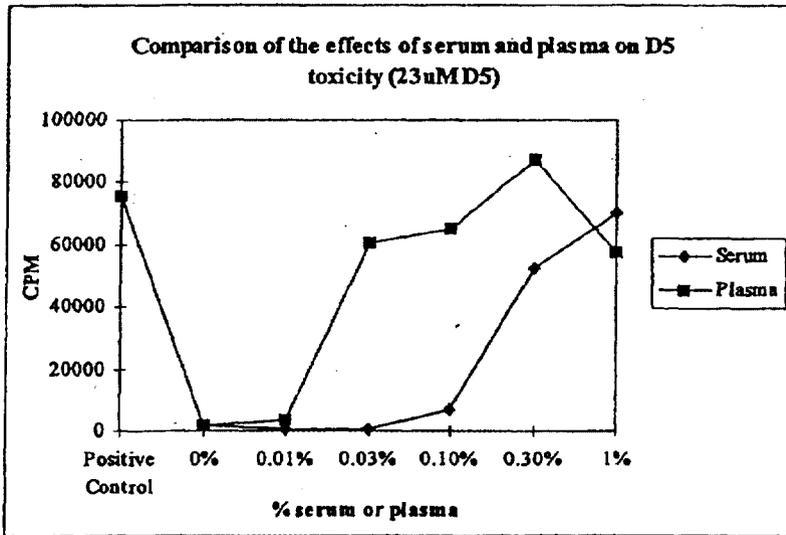
	35uM D4	2/26/97					
	Positive Control	0%	0.01%	0.03%	0.10%	0.30%	1%
Plasma	75556	243	914	57825	63599	72339	53945
% Error	6.2%	7.1%	43.3%	2.6%	3.9%	5.6%	14.4%
Background	99	366	173	285	149	172	284
% Error	18.1%	68.1%	54.0%	10.3%	30.7%	23.4%	30.3%



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

	23uM D5	2/26/97					
Positive Control	0%	0.01%	0.03%	0.10%	0.30%	1%	
Serum	75565	1911	653	654	6844	52335	70515
% Error	3.9%	19.1%	16.2%	21.0%	49.9%	8.6%	3.3%
Background	178	102	67	85	122	137	91
% Error	19.4%	27.3%	11.7%	31.2%	25.7%	22.9%	34.1%

	23uM D5	2/21/97					
Positive Control	0%	0.01%	0.03%	0.10%	0.30%	1%	
Plasma	75565	1911	3448	60398	65021	87289	57657
% Error	3.9%	19.1%	53.6%	12.0%	4.0%	16.3%	11.5%
Background	178	102	147	112	110	74	160
% Error	19.4%	27.3%	78.4%	10.2%	20.4%	12.0%	11.8%

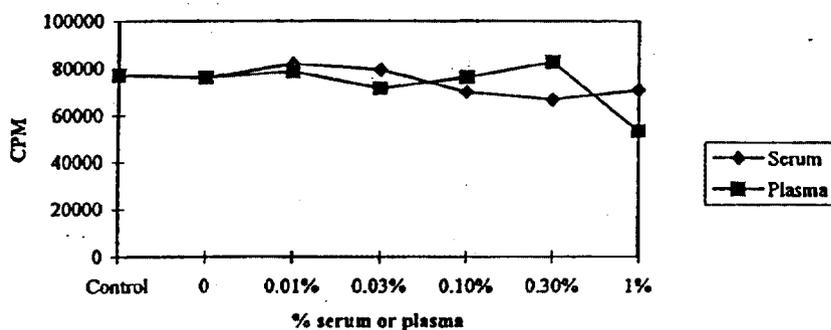


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

		47uM HMDS		2/26/97				
		Control	0	0.01%	0.03%	0.10%	0.30%	1%
Serum		77099	76006	81806	79361	69916	66679	70773
	% Error	20.1%	8.0%	3.6%	7.2%	13.7%	4.9%	11.6%
Background		193	263	240	327	245	132	159
	% Error	16.8%	36.9%	35.8%	20.5%	45.5%	33.4%	12.5%

		47uM HMDS		2/26/97				
		Control	0	0.01%	0.03%	0.10%	0.30%	1%
Plasma		77099	76006	78616	71435	76292	82581	53173
	% Error	20.1%	8.0%	9.3%	16.9%	4.9%	3.6%	5.4%
Background		193	263	147	174	158	142	203
	% Error	16.8%	36.9%	40.5%	37.4%	22.5%	28.0%	33.7%

Effect of serum and plasma on the toxicity of HMDS
(47uM HMDS)



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

12/04/96

[D4]

	0	35	105	350	1050	3500
1% serum	47238	41919	39428	46029	47021	46307
% Error	7.4%	7.2%	6.5%	3.0%	7.0%	4.4%
10% serum	ND	47122	38189	36113	37083	31436
% Error		6.3%	10.7%	12.9%	15.1%	38.2%

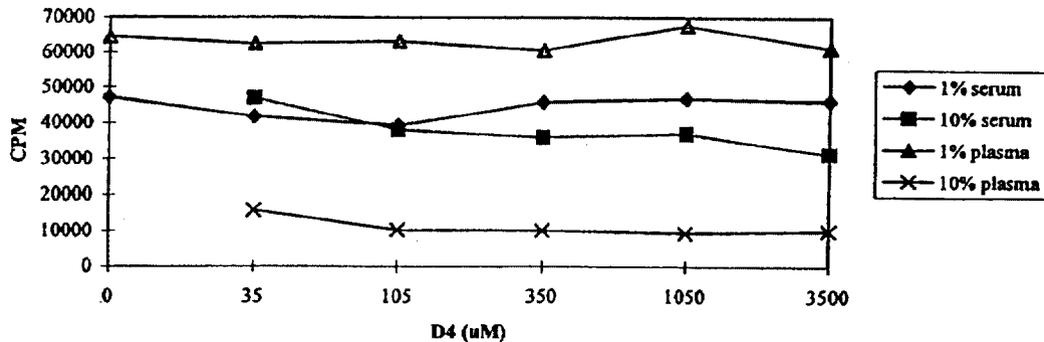
	0	35	105	350	1050	3500
1% plasma	64343	62546	63187	60658	67687	61336
% Error	2.6%	7.2%	4.4%	3.8%	6.6%	9.9%
10% plasma	ND	15804	10292	10185	9425	10065
% Error		28.0%	30.0%	13.1%	32.0%	24.9%

Background

	0	35	105	350	1050	3500
1% serum	241	213	213	394	414	487
% Error	11.2%	36.8%	7.3%	24.5%	33.7%	54.6%
10% serum	ND	174	146	185	218	1146
% Error		26.6%	19.1%	15.9%	22.5%	55.7%

	0	35	105	350	1050	3500
1% plasma	233	222	187	258	257	271
% Error	28.9%	11.0%	16.6%	24.2%	14.7%	9.1%
10% plasma	ND	208	148	168	143	212
% Error		30.9%	28.5%	35.6%	40.1%	42.6%

Comparison of the effects of plasma and serum on D4 toxicity



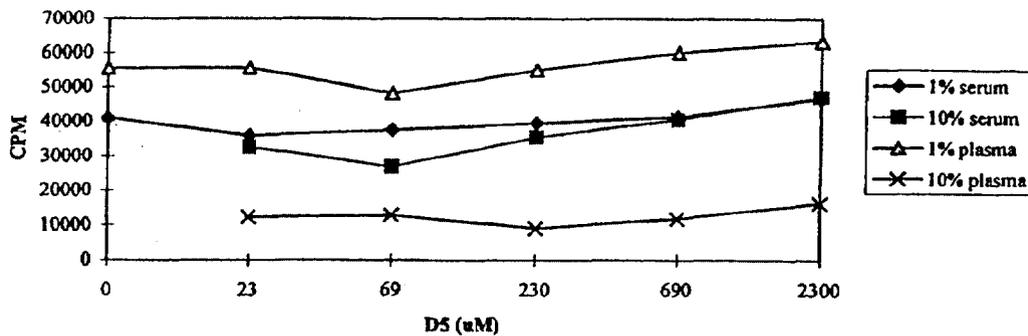
ND - Not Done

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



12/4/96	[D5]	0	23	69	230	690	2300
1% serum		41035	35818	37582	39497	41414	46506
% Error		11.0%	19.3%	9.5%	3.9%	10.3%	9.1%
10% serum		ND	32504	26849	35408	40606	47095
% Error			40.4%	49.2%	31.2%	23.2%	6.8%
1% plasma		55447	55700	48207	54959	60149	63316
% Error		14.1%	5.8%	19.4%	8.3%	5.2%	5.9%
10% plasma		ND	12186	12797	9047	11793	16227
% Error			23.9%	66.6%	28.2%	24.7%	23.7%
Background							
1% serum		159	280	252	552	565	471
% Error		41.2%	25.0%	9.7%	30.1%	17.7%	15.7%
10% serum		ND	188	147	186	406	435
% Error			17.1%	33.5%	17.7%	72.7%	46.5%
1% plasma		248	236	257	347	332	625
% Error		41.3%	22.7%	26.7%	29.6%	25.8%	26.4%
10% plasma		ND	271	177	197	193	269
% Error			6.7%	28.5%	36.1%	59.7%	2.6%

Comparison of the effects of plasma and serum on D5 toxicity



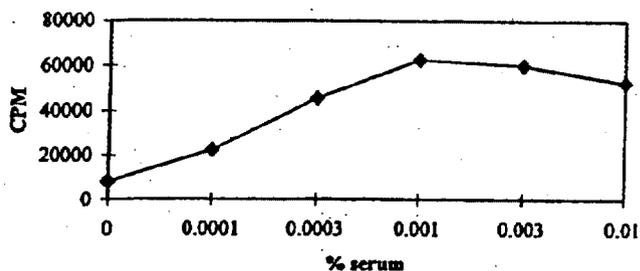
ND - Not Done

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



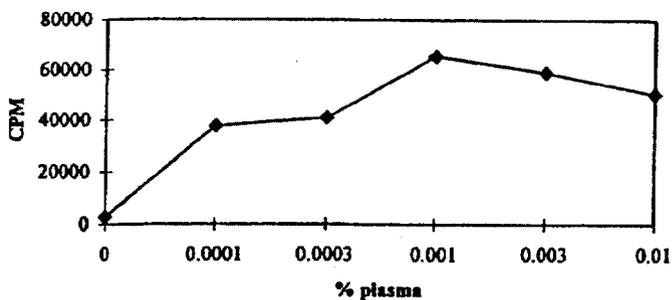
Serum	35uM D4		12/4/96			
	0%	0.01%	0.03%	0.10%	0.30%	1%
PHA	8215	22264	45523	62500	60014	52564
% Error	16.4%	29.2%	45.8%	48.2%	16.5%	33.1%
Background	125	198	384	344	446	251
% Error	32.9%	59.5%	37.5%	16.7%	25.5%	21.6%

Inhibition of D4 (35uM) toxicity by serum



Plasma	35uM D4		12/4/96			
	0%	0.01%	0.03%	0.10%	0.30%	1%
PHA	2803	37458	40978	65427	59077	50526
% Error	56.8%	14.8%	25.2%	30.0%	32.0%	26.6%
Background	110	189	320	376	345	363
% Error	21.5%	59.4%	34.4%	16.3%	18.4%	13.0%

Inhibition of D4 (35uM) toxicity by plasma



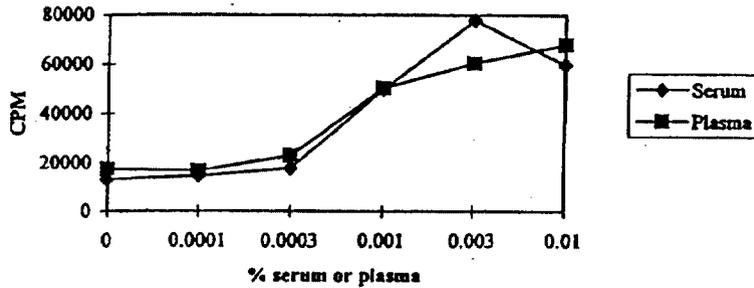
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	23uM D5		12/4/96			
	0%	0.01%	0.03%	0.10%	0.30%	1%
Serum	13005	14637	17724	49611	77765	59735
% Error	18.3%	38.0%	22.2%	34.1%	4.4%	24.3%
Background	212	175	303	702	1075	297
% Error	22.9%	49.6%	52.9%	26.2%	32.8%	18.2%

	23uM D5		12/4/96			
	0%	0.01%	0.03%	0.10%	0.30%	1%
Plasma	17472	16792	22861	50201	60370	67795
% Error	21.0%	19.5%	26.8%	35.4%	24.3%	16.0%
Background	321	232	303	541	641	580
% Error	74.2%	86.4%	18.1%	23.7%	40.2%	23.8%

Effect of serum and plasma on D5 toxicity

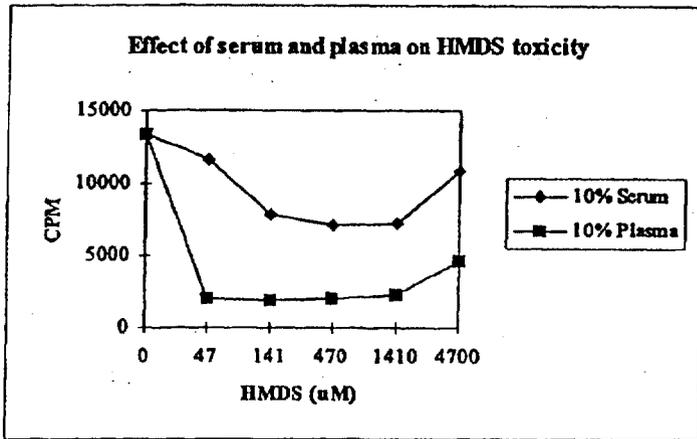


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	[HMDS]	1/8/97				
	0	47	141	470	1410	4700
10% Serum	13428	11659	7929	7088	7192	10922
% Error	22.5%	23.9%	10.0%	30.2%	31.1%	20.1%
Background	99	183	137	260	152	182
% Error	29.1%	53.4%	26.5%	115.2%	8.7%	17.8%

	[HMDS]					
	0	47	141	470	1410	4700
10% Plasma	13428	2035	1863	1965	2312	4654
% Error	22.5%	39.5%	10.6%	35.9%	25.7%	32.6%
Background	99	235	264	189	184	204
% Error	29.1%	27.3%	34.3%	48.5%	32.8%	19.6%

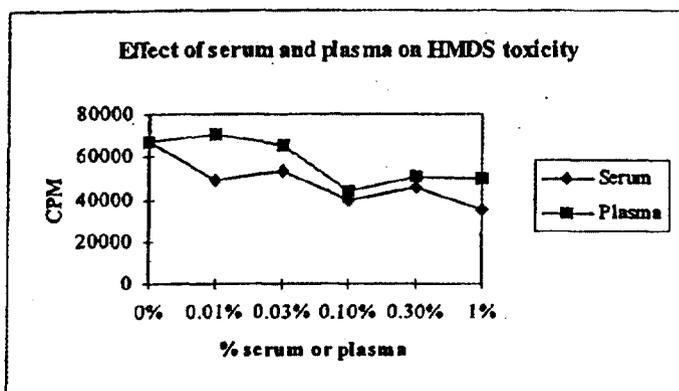


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)×100



	47uM HMDS		1/8/97			
	0%	0.01%	0.03%	0.10%	0.30%	1%
Serum	66926	48673	53404	39666	45377	35250
% Error	12.2%	41.9%	24.9%	22.7%	29.5%	24.8%
Background	212	131	180	233	154	334
% Error	20.4%	19.1%	27.1%	12.0%	8.9%	48.6%

	47uM HMDS		1/8/97			
	0%	0.01%	0.03%	0.10%	0.30%	1%
Plasma	66926	70837	65637	44178	50691	49888
% Error	12.2%	7.3%	9.2%	50.4%	26.0%	15.4%
Background	212	219	257	186	330	163
% Error	20.4%	11.8%	22.9%	11.4%	87.1%	35.1%

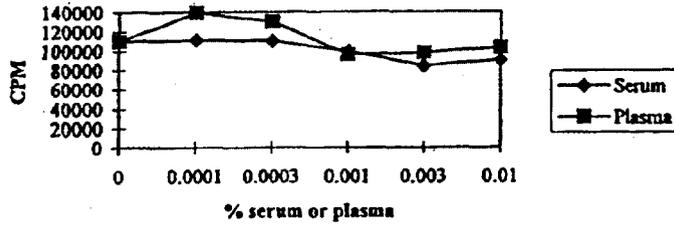


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	1410uM HMDS		1/30/97			
	0	0.01%	0.03%	0.10%	0.30%	1%
Serum	110000	111000	110000	98992	83764	89727
% Error	5.9%	4.4%	9.6%	6.8%	9.4%	6.4%
Background	664	331	321	227	155	121
% Error	12.2%	13.2%	17.8%	20.6%	37.0%	23.0%
	0	0.01%	0.03%	0.10%	0.30%	1%
Plasma	110000	139000	130000	95599	97339	103000
% Error	5.9%	6.8%	6.8%	12.4%	2.8%	5.8%
Background	664	409	331	212	137	107
% Error	12.2%	2.3%	26.4%	10.3%	41.0%	21.1%

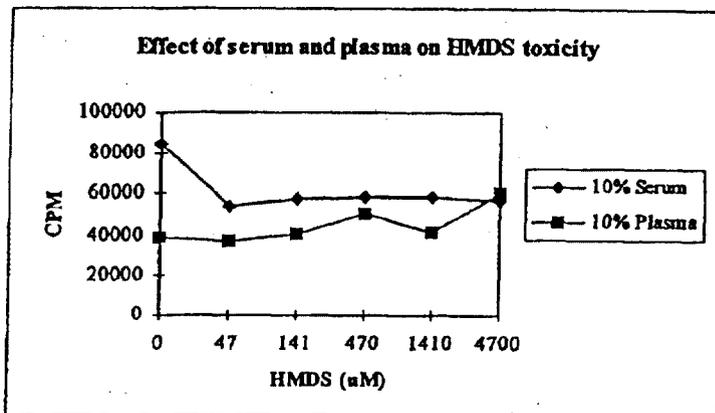
Comparison of the effects of serum and plasma on the toxicity of HMDS



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

	[HMDS]		1/30/97			
	0	47	141	470	1410	4700
10% Serum	84374	53951	57336	58923	58283	57065
% Error	17.6%	12.0%	31.3%	18.9%	25.2%	32.2%
Background	186	204	136	127	119	200
% Error	52.5%	61.3%	63.3%	42.1%	40.8%	77.2%

	[HMDS]		1/30/97			
	0	47	141	470	1410	4700
10% Plasma	38929	37298	40935	50082	41735	60462
% Error	27.2%	29.6%	29.0%	12.4%	32.4%	18.3%
Background	240	269	141	205	221	338
% Error	45.1%	65.6%	35.1%	21.8%	26.4%	80.4%

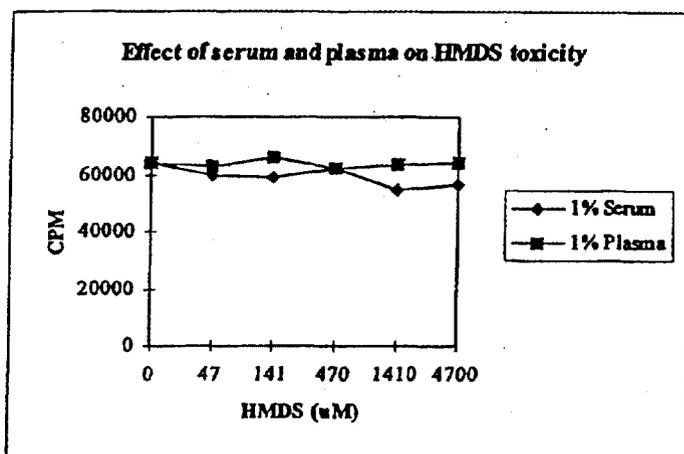


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	[HMDS] 2/7/97					
	0	47	141	470	1410	4700
1% Serum	63873	59368	58751	62250	54789	56246
% Error	9.8%	12.0%	7.5%	11.9%	8.7%	4.8%
Background	91	60	52	114	80	44
% Error	25.7%	38.8%	15.6%	25.4%	32.0%	4.3%

	[HMDS]					
	0	47	141	470	1410	4700
1% Plasma	63873	62632	66349	62063	63197	64225
% Error	9.6%	9.8%	10.6%	11.8%	6.2%	19.7%
Background	91	55	82	79	84	56
% Error	25.7%	13.6%	58.5%	20.1%	67.3%	26.5%



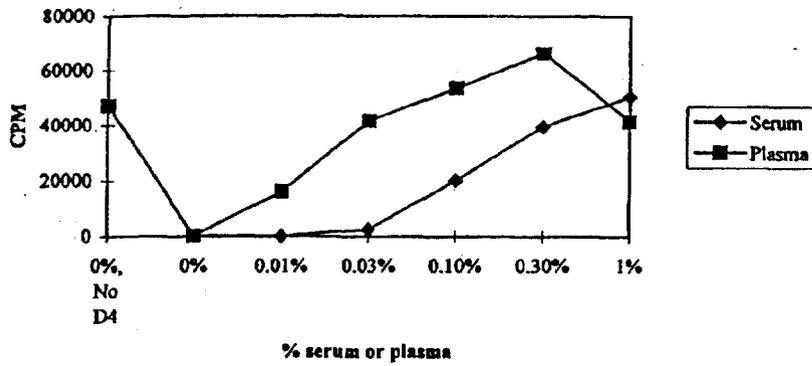
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	35uM D4		2/21/97				
	0%, No D4	0%	0.01%	0.03%	0.10%	0.30%	1%
Serum	47119	255	256	2585	20194	39548	50494
% Error	7.3%	22.1%	7.8%	57.1%	29.9%	3.6%	2.8%
Background	253	95	123	834	2807	262	132
% Error	25.7%	57.7%	29.6%	47.8%	32.5%	12.4%	21.0%

	35uM D4		2/21/97				
	0%, No D4	0%	0.01%	0.03%	0.10%	0.30%	1%
Plasma	47119	255	16110	41721	53550	66269	41477
% Error	7.3%	22.1%	17.8%	4.7%	4.1%	5.4%	23.3%
Background	253	95	2407	845	130	108	81
% Error	25.7%	57.7%	57.8%	34.3%	6.2%	23.7%	22.6%

Comparison of the effects of serum and plasma on D4 toxicity

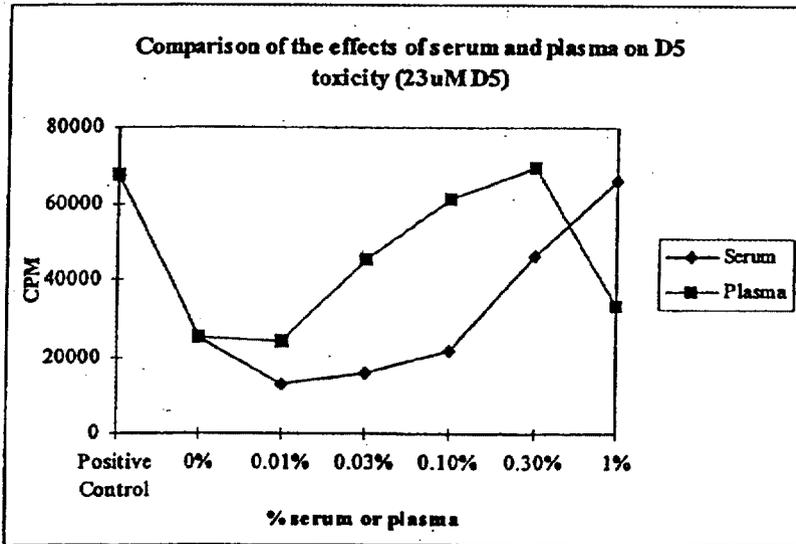


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	23uM D5		2/21/97				
	Positive	0%	0.01%	0.03%	0.10%	0.30%	1%
	Control						
Serum	67405	24971	13112	15935	21710	46276	65889
% Error	8.5%	14.6%	4.9%	29.8%	18.5%	5.9%	1.5%
Background	557	318	326	687	664	481	380
% Error	18.2%	20.5%	27.3%	18.6%	12.6%	7.2%	9.0%

	23uM D5		2/21/97				
	Positive	0%	0.01%	0.03%	0.10%	0.30%	1%
	Control						
Plasma	67405	24971	24189	45465	61145	69441	33418
% Error	8.5%	14.6%	24.5%	5.9%	7.4%	7.6%	26.6%
Background	557	318	627	459	368	412	141
% Error	18.2%	20.5%	15.1%	5.8%	28.4%	23.1%	40.5%



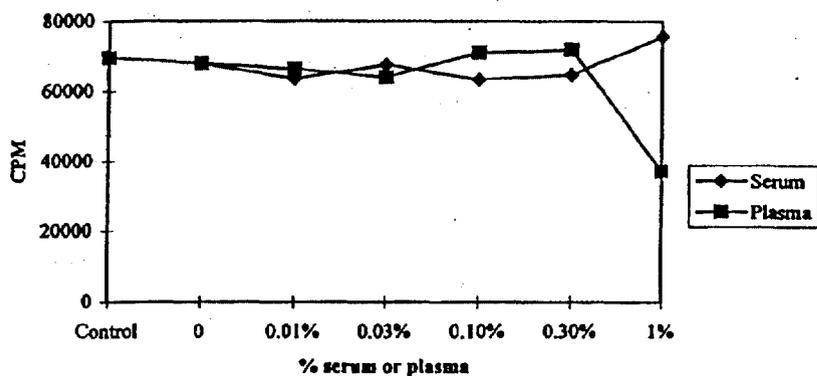
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



		47uM HMDS		2/21/97				
		Control	0	0.01%	0.03%	0.10%	0.30%	1%
Serum		69687	67946	63701	67688	63384	64763	75723
% Error		5.7%	3.0%	4.9%	5.9%	5.6%	5.5%	1.8%
Background		524	558	588	481	379	254	197
% Error		14.8%	10.1%	28.9%	4.3%	15.4%	13.7%	23.6%

		47uM HMDS		2/21/97				
		Control	0	0.01%	0.03%	0.10%	0.30%	1%
Plasma		69687	67946	66450	64092	71025	71969	37210
% Error		5.7%	3.0%	6.4%	5.2%	6.5%	8.2%	29.9%
Background		524	558	309	269	224	308	308
% Error		14.8%	10.1%	9.9%	14.8%	33.0%	31.5%	23.9%

Effect of plasma and serum on HMDS toxicity (47uMHMDS)



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

B3. Effects of Serum on D4 and SDS Inhibition of PHA-Induced Proliferation.

	5/16/97					
0%serum	0	0.034	0.1	0.34	1	3.4
D4	106000	121	243	129	138	167
% Error	3.2%	20.0%	60.0%	17.7%	18.8%	27.1%
HMDS	117000	102000	98769	97211	97377	99981
% Error	4.8%	3.2%	5.1%	7.8%	5.4%	4.8%
Dodecane	96541	95184	87802	90000	88906	92223
% Error	3.0%	4.4%	11.5%	7.7%	5.2%	9.1%
SDS	95693	97218	89422	403	403	139
% Error	2.4%	4.4%	5.4%	49.1%	65.3%	25.2%
1%serum	0	0.034	0.1	0.34	1	3.4
D4	75031	73201	74906	26833	31671	3330
% Error	6.1%	3.3%	4.5%	110.2%	112.4%	154.5%
HMDS	70394	58998	58092	61280	61145	65145
% Error	8.4%	8.9%	6.1%	7.0%	7.6%	7.8%
Dodecane	73890	79400	77480	78319	85256	96247
% Error	2.2%	9.6%	8.2%	3.9%	6.6%	11.1%
SDS	58995	62205	59570	3907	230	99
% Error	8.4%	1.7%	3.7%	32.0%	89.1%	58.0%
10%serum	0	0.034	0.1	0.34	1	3.4
D4	62648	66651	53413	49030	54133	52315
% Error	5.2%	13.2%	12.1%	11.3%	15.2%	14.7%
HMDS	66483	56487	56206	54757	55905	52251
% Error	8.5%	8.3%	9.7%	9.7%	8.5%	8.6%
Dodecane	55551	55564	53328	52862	53806	64972
% Error	8.9%	7.9%	21.3%	16.0%	8.5%	9.6%
SDS	54447	58382	54039	51010	624	151
% Error	9.0%	17.7%	8.9%	9.2%	26.8%	42.3%

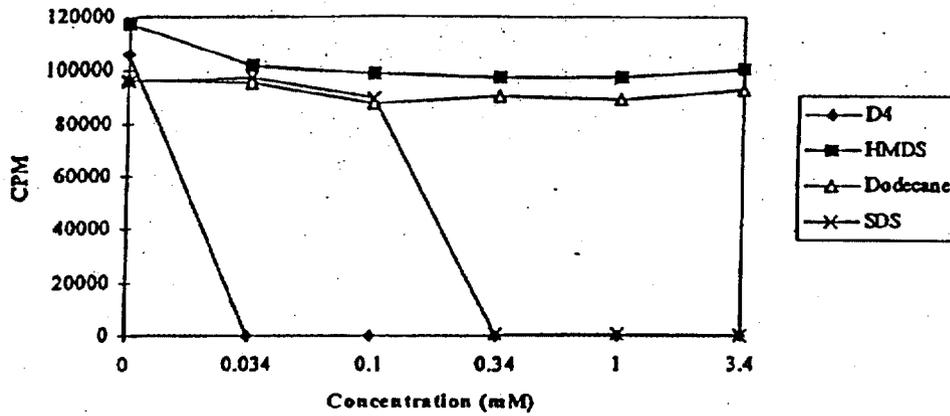
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



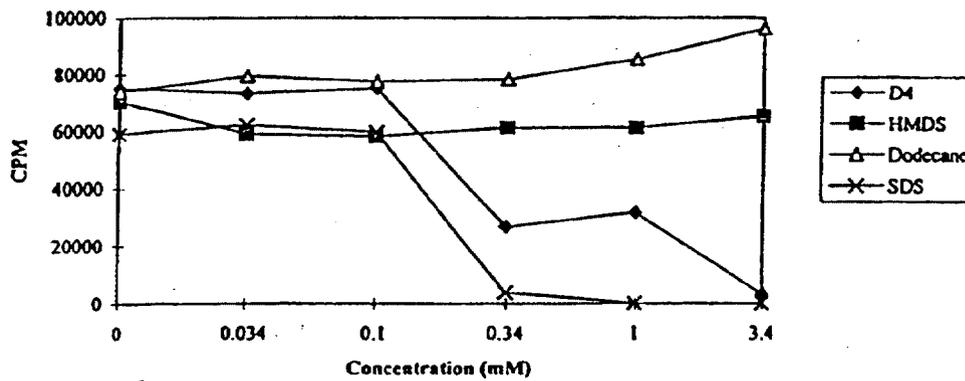
5/16/97	Background					
	0	0.034	0.1	0.34	1	3.4
D4	715	93	103	146	136	127
% Error	12.7%	36.3%	28.9%	60.0%	47.4%	76.3%
HMDS	632	553	594	554	540	764
% Error	19.6%	18.5%	20.8%	23.9%	15.2%	64.9%
Dodecane	626	553	774	797	611	694
% Error	23.6%	21.8%	16.3%	8.0%	12.0%	12.1%
SDS	541	482	482	63	43	21
% Error	27.1%	22.2%	32.6%	57.7%	63.4%	13.0%
D4	88	90	94	308	299	438
% Error	17.9%	31.5%	26.2%	60.2%	51.1%	111.7%
HMDS	78	86	170	160	211	240
% Error	31.9%	35.6%	94.5%	66.8%	53.4%	77.6%
Dodecane	182	118	97	88	99	117
% Error	43.4%	42.8%	39.4%	21.9%	9.5%	33.0%
SDS	165	129	174	267	55	54
% Error	77.3%	54.1%	97.0%	85.8%	32.4%	22.4%
D4	92	77	81	52	92	82
% Error	30.2%	22.6%	8.7%	15.6%	41.2%	36.6%
HMDS	317	146	156	363	100	96
% Error	89.5%	99.9%	91.7%	153.9%	77.3%	61.1%
Dodecane	86	56	65	77	65	72
% Error	28.1%	17.7%	30.1%	11.3%	26.7%	9.9%
SDS	156	94	108	107	77	137
% Error	46.0%	29.9%	53.9%	49.0%	20.7%	44.2%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

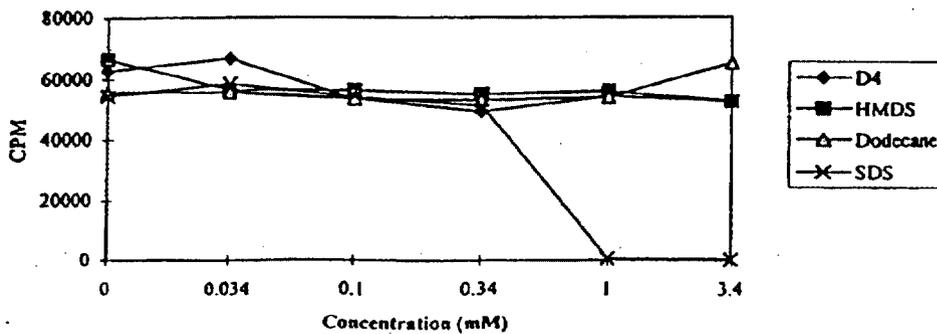
Comparison of toxicity without serum



Comparison of toxicity with 1% serum



Comparison of toxicity with 10% serum



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



	10/3/97					
0%serum	0	0.034	0.1	0.34	1	3.4
D4	39284	127	69	43	53	65
% Error	8.0%	50.2%	39.1%	22.5%	62.9%	33.0%
HMDS	44097	42398	42036	43848	44548	43253
% Error	4.6%	1.9%	3.0%	4.4%	2.5%	6.2%
Dodecane	41970	36897	35696	38166	37298	34596
% Error	9.0%	9.2%	10.7%	10.1%	8.2%	7.8%
SDS	36419	39789	30790	164	139	139
% Error	7.4%	6.6%	13.4%	23.9%	93.3%	41.1%
1%serum						
D4	38708	39858	37252	27032	26725	27433
% Error	9.4%	12.1%	9.1%	29.2%	38.6%	35.2%
HMDS	42739	38034	37355	39454	40172	38617
% Error	14.4%	3.6%	3.6%	3.9%	5.8%	6.0%
Dodecane	43708	40216	40355	41411	40277	40412
% Error	4.3%	4.7%	7.3%	6.2%	1.1%	7.3%
SDS	42153	40620	39687	113	79	73
% Error	5.2%	8.7%	2.2%	34.8%	39.4%	36.8%
10%serum						
D4	37514	34445	34305	32062	32056	32554
% Error	3.7%	2.2%	5.4%	10.0%	9.0%	3.8%
HMDS	32533	34006	34391	34487	35082	35817
% Error	7.4%	4.2%	8.7%	11.0%	6.2%	9.3%
Dodecane	33545	34959	34377	35627	35654	38186
% Error	4.9%	2.5%	5.5%	7.1%	3.6%	3.5%
SDS	37484	36616	36724	40122	108	122
% Error	3.3%	4.1%	5.1%	8.2%	27.9%	34.7%

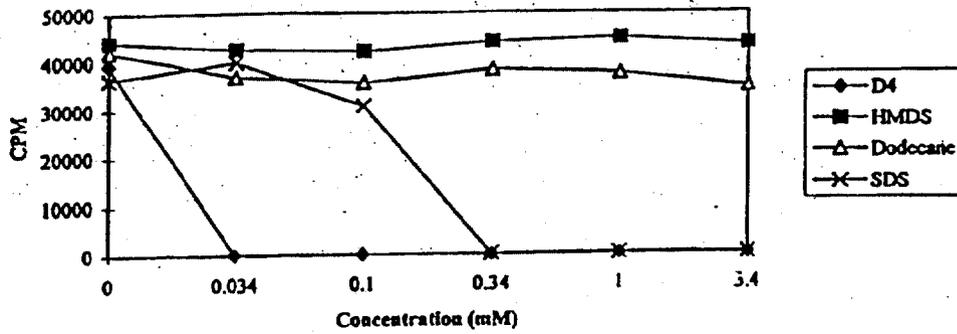
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



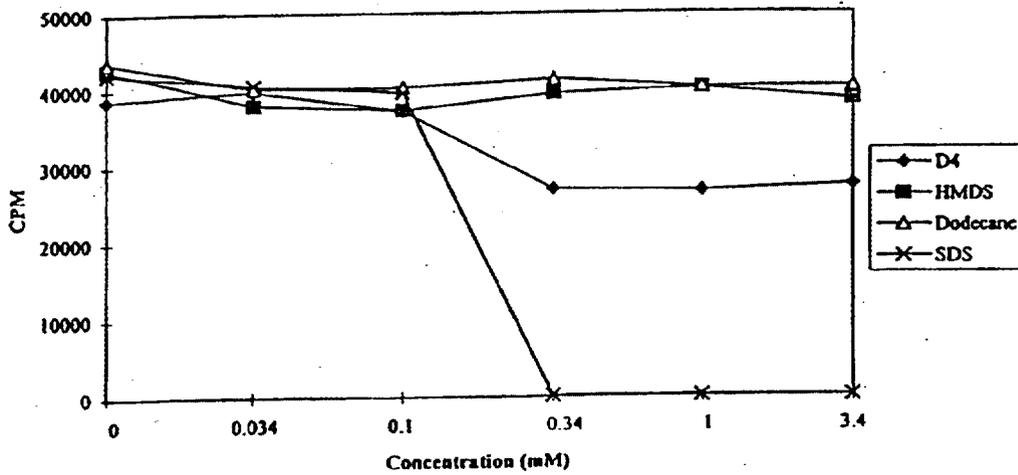
10/3/97	Background					
	0	0.034	0.1	0.34	1	3.4
D4	312	55	51	75	97	96
% Error	17.0%	39.6%	34.8%	41.4%	61.3%	58.1%
HMDS	303	262	216	227	281	324
% Error	30.9%	47.3%	11.9%	30.5%	28.1%	17.2%
Dodecane	505	489	502	468	401	474
% Error	29.6%	33.7%	24.4%	27.3%	21.2%	24.5%
SDS	372	224	188	100	92	106
% Error	30.4%	28.2%	26.6%	43.3%	35.9%	53.6%
D4	63	90	95	131	108	80
% Error	12.9%	29.7%	30.5%	24.4%	23.3%	30.0%
HMDS	129	107	77	84	92	90
% Error	44.3%	37.4%	33.1%	35.5%	48.6%	48.2%
Dodecane	142	94	83	114	106	111
% Error	16.0%	23.9%	27.9%	6.3%	8.9%	37.8%
SDS	102	87	70	53	39	40
% Error	48.6%	40.8%	55.3%	34.9%	31.9%	47.1%
D4	106	87	65	95	73	104
% Error	56.9%	35.9%	20.4%	45.8%	28.8%	37.8%
HMDS	78	77	73	59	85	66
% Error	8.9%	48.0%	28.3%	25.6%	32.5%	52.8%
Dodecane	133	80	76	90	134	115
% Error	102.4%	51.3%	48.1%	26.2%	42.5%	13.6%
SDS	79	76	64	77	40	29
% Error	11.9%	61.6%	56.1%	50.4%	23.0%	33.8%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

Comparison of toxicity without serum



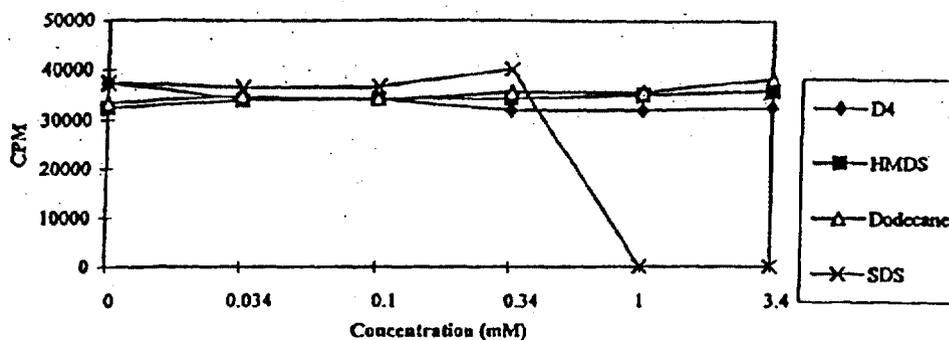
Comparison of toxicity with 1% serum



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



Comparison of toxicity with 10% serum



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



B4. Protection of PBMCs from Siloxanes by Plasma Components.

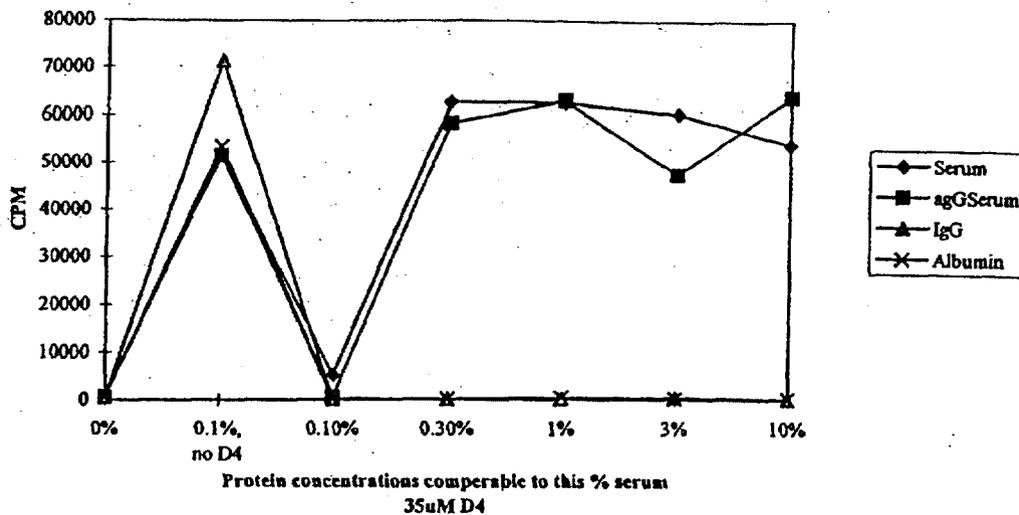
11/11/96 PHA proliferation: effect of proteins on D4 toxicity
D4 at 35uM

	0%	0.1%, no D4	0.10%	0.30%	1%	3%	10%
Serum	554	51359	5274	62739	62615	60267	53759
% Error	65.3%	17.8%	32.5%	5.9%	0.9%	5.7%	11.0%
Background	145	212	609	972	177	156	156
% Error	15.3%	25.7%	13.6%	21.8%	15.9%	17.7%	29.5%
	0%	0.1%, no D4	0.10%	0.30%	1%	3%	10%
ayGSerum	554	51359	702	58323	63046	47352	63825
% Error	65.3%	17.8%	57.5%	5.0%	9.3%	15.1%	7.1%
Background	145	212	1065	541	168	181	300
% Error	15.3%	25.7%	87.6%	20.7%	15.9%	14.7%	13.0%
	0	0.1 IgG, no D4	0.01	0.03	0.1	0.3	1
IgG	554	71302	183	227	256	560	219 mg/ml
% Error	65.3%	5.6%	28.1%	8.5%	19.7%	57.3%	10.9%
Background	145	307	207	180	238	170	114
% Error	15.3%	18.3%	47.1%	35.3%	57.5%	45.7%	10.1%
	0	0.5 Alb, no D4	0.05	0.15	0.5	1.5	5 mg/ml
Albumin	554	53445	216	268	583	292	290
% Error	65.3%	10.7%	31.5%	25.2%	107.0%	36.6%	32.1%
Background	145	494	186	190	113	106	96
% Error	15.3%	10.3%	58.3%	33.0%	45.0%	50.2%	41.0%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



Effect of different proteins on D4 toxicity



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



11/22/96 D4 at 35 uM and D5 at 23 uM
No siloxane

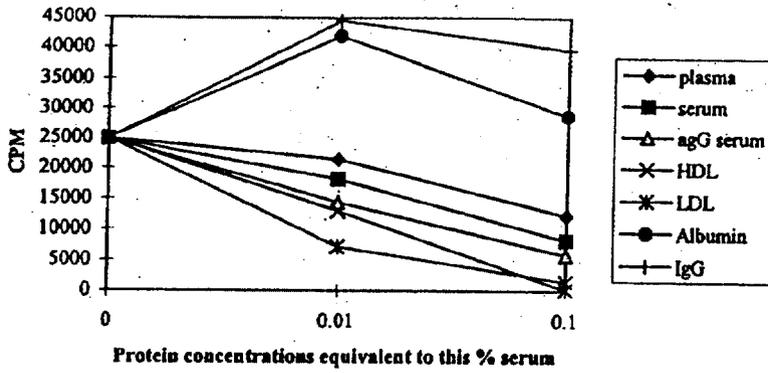
	0	1%	10%
plasma	24893	21435	12248
% Error	7.8%	10.1%	23.0%
background	210	129	72
% Error	14.7%	34.2%	48.6%
serum	24893	18169	8232
% Error	7.8%	17.4%	20.9%
background	210	70	65
% Error	14.7%	46.2%	24.9%
agG serum	24893	14372	5802
% Error	7.8%	7.4%	28.5%
background	210	58	99
% Error	14.7%	22.7%	64.7%
	0	0.03	0.3 mg/ml
HDL	24893	13002	179
% Error	7.8%	3.6%	40.4%
background	210	194	48
% Error	14.7%	28.4%	40.4%
	0	0.04	0.4 mg/ml
LDL	24893	7177	1504
% Error	7.8%	13.6%	34.9%
background	210	71	42
% Error	14.7%	15.6%	11.8%
	0	0.5	5 mg/ml
Albumin	24893	41962	28737
% Error	7.8%	0.3%	5.1%
background	210	633	917
% Error	14.7%	19.1%	8.2%
	0	0.1	1 mg/ml
IgG	24893	44491	39637
% Error	7.8%	1.3%	5.4%
background	210	268	311
% Error	14.7%	13.4%	18.5%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

B35



Effects of serum proteins without siloxane



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

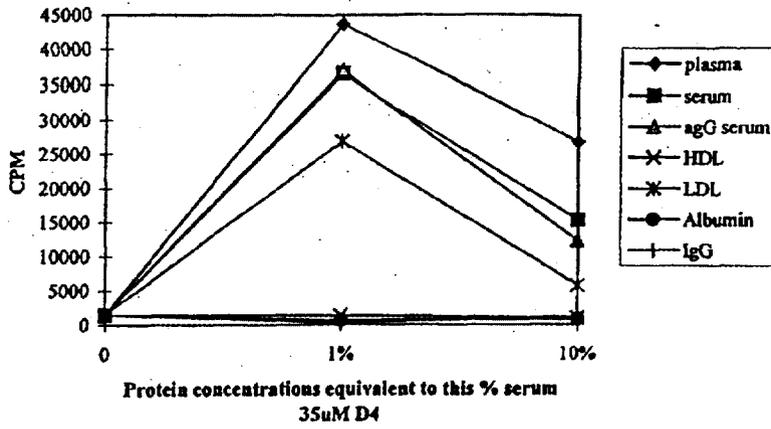


11/22/96	D4	0	1%	10%
plasma		1517	43668	26748
% Error		148.3%	4.3%	49.2%
background		332	362	395
% Error		55.6%	60.0%	77.8%
serum		1517	36558	15334
% Error		148.3%	4.7%	48.0%
background		332	278	156
% Error		55.6%	32.1%	25.1%
agG serum		1517	37045	12322
% Error		148.3%	2.1%	32.2%
background		332	216	231
% Error		55.6%	25.4%	42.7%
HDL		0	0.03	0.3 mg/ml
% Error		1517	1413	1007
background		332	235	130
% Error		55.6%	60.8%	14.0%
LDL		0	0.04	0.4 mg/ml
% Error		1517	26876	5752
background		332	410	95
% Error		55.6%	96.5%	29.7%
Albumin		0	0.5	5 mg/ml
% Error		1517	665	809
background		332	123	136
% Error		55.6%	38.2%	80.0%
IgG		0	0.1	1 mg/ml
% Error		1517	236	1267
background		332	126	102
% Error		55.6%	73.6%	13.2%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



Effect of serum proteins on D4 toxicity



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

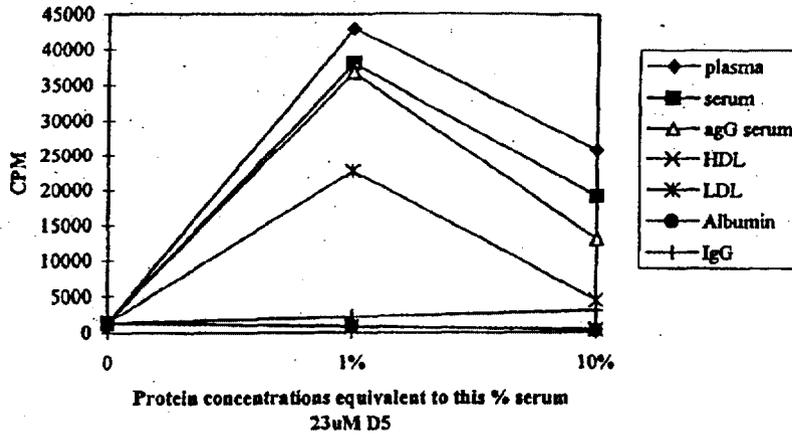


	0	1%	10%
11/22/96 D5			
plasma	1340	42896	25703
% Error	47.2%	2.6%	45.6%
background	156	131	71
% Error	12.2%	44.6%	15.6%
serum	1340	37980	19076
% Error	47.2%	8.2%	15.4%
background	156	90	108
% Error	12.2%	30.8%	40.8%
agG serum	1340	36719	13015
% Error	47.2%	1.4%	19.9%
background	156	231	182
% Error	12.2%	27.1%	25.4%
HDL	0	0.03	0.3 mg/ml
% Error	47.2%	35.0%	58.5%
background	156	115	88
% Error	12.2%	17.2%	36.1%
LDL	0	0.04	0.4 mg/ml
% Error	47.2%	3.7%	8.9%
background	156	253	82
% Error	12.2%	13.1%	27.4%
Albumin	0	0.5	5 mg/ml
% Error	47.2%	70.6%	28.4%
background	156	116	70
% Error	12.2%	19.7%	37.8%
IgG	0	0.1	1 mg/ml
% Error	47.2%	26.2%	37.1%
background	156	163	151
% Error	12.2%	15.4%	41.6%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



Effect of serum proteins on D5 toxicity

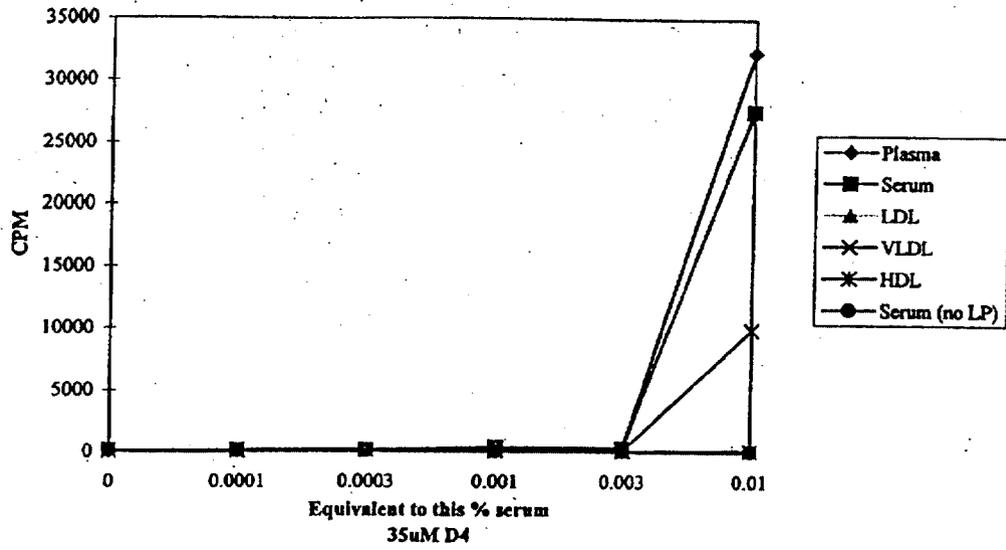


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

	12/31/96 35 uM D4					
	0%	0.01%	0.03%	0.10%	0.30%	1%
Plasma	143	168	188	223	402	32278
% Error	36.4%	36.2%	32.1%	33.3%	41.4%	4.2%
background	130	180	173	222	416	472
% Error	36.4%	51.3%	20.2%	75.3%	57.2%	59.0%
Serum	144	132	154	378	343	27563
% Error	58.0%	52.0%	50.0%	122.8%	56.9%	3.0%
background	112	108	130	131	168	319
% Error	30.7%	14.9%	60.7%	42.0%	36.6%	16.3%
LDL	199	174	118	204	115	267
% Error	46.3%	45.4%	33.5%	25.1%	36.2%	30.2%
background	261	87	235	238	193	210
% Error	9.1%	18.5%	72.0%	35.8%	20.4%	36.8%
VLDL	83	126	117	129	125	9932
% Error	26.3%	33.1%	44.2%	19.6%	54.5%	14.3%
background	51	89	94	95	106	1426
% Error	31.8%	41.8%	66.1%	36.7%	33.2%	17.5%
HDL	47	76	111	86	91	154
% Error	48.7%	37.4%	73.6%	54.6%	23.0%	24.9%
background	42	47	54	65	74	178
% Error	63.3%	19.7%	51.8%	45.0%	42.4%	72.3%
Serum (no LP)	151	62	91	106	86	98
% Error	30.3%	55.7%	18.1%	25.6%	42.2%	54.6%
background	94	56	76	102	91	114
% Error	47.6%	35.7%	18.8%	61.1%	22.2%	46.5%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

Effect of different serum fractions on D4 toxicity

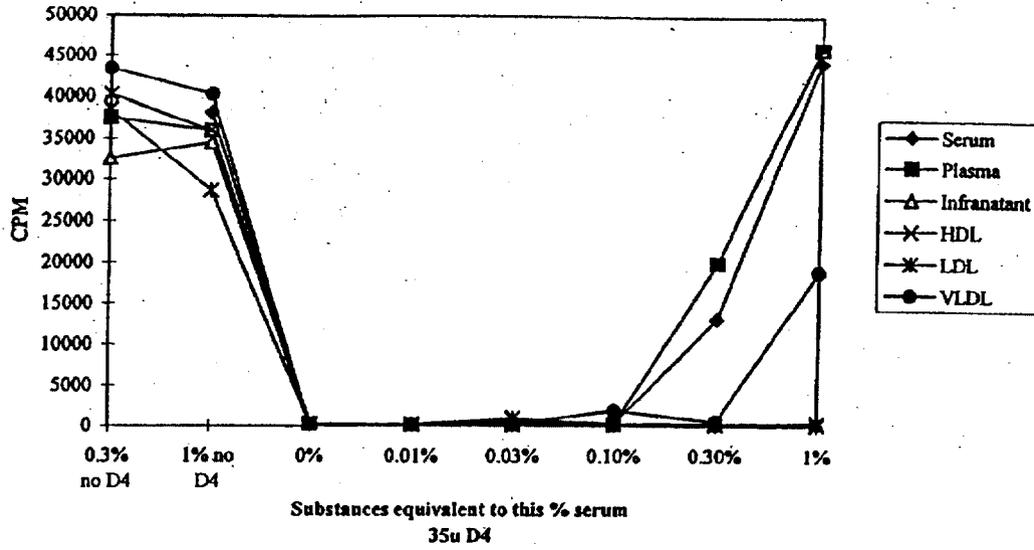


Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

	1/8/97							
	0.3% no D4	1% no D4	35uM D4 0%	0.01%	0.03%	0.10%	0.30%	1%
Serum		38160	311	217	205	320	13144	44284
% Error		9.1%	17.2%	13.5%	40.9%	36.1%	22.4%	5.9%
background		170	136	189	205	201	236	132
% Error		21.7%	39.6%	24.7%	28.0%	41.1%	13.2%	6.2%
Plasma	37529	36033	311	218	268	212	19907	45928
% Error	5.3%	16.5%	17.2%	46.4%	51.3%	28.9%	19.3%	9.0%
background	213	184	136	164	167	139	280	237
% Error	27.9%	11.6%	39.6%	15.1%	61.8%	47.8%	19.9%	62.6%
Infranantant	32533	34515	311	249	611	355	371	452
% Error	8.5%	7.4%	17.2%	30.7%	101.0%	28.7%	52.1%	47.5%
background	178	150	136	282	229	182	226	131
% Error	5.3%	19.8%	39.6%	12.8%	18.0%	35.7%	36.1%	37.5%
HDL	40257	36035	311	193	181	246	281	199
% Error	3.2%	9.4%	17.2%	8.3%	29.5%	34.6%	61.0%	26.6%
background	252	220	136	299	195	424	402	242
% Error	26.3%	13.0%	39.6%	37.1%	34.2%	24.9%	20.5%	27.4%
LDL	38514	28692	311	345	1024	403	200	540
% Error	5.5%	31.4%	17.2%	16.1%	71.1%	50.9%	42.6%	30.6%
background	254	279	136	158	165	197	178	114
% Error	7.0%	18.5%	39.6%	32.8%	31.7%	60.9%	20.1%	28.7%
VLDL	43393	40288	311	278	362	1961	599	18879
% Error	2.4%	4.0%	17.2%	28.1%	75.1%	162.0%	13.7%	19.1%
background	244	317	136	116	168	134	135	237
% Error	24.6%	45.8%	39.6%	35.7%	49.6%	23.4%	33.0%	21.4%

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

Effects of different serum fractions on D4 toxicity



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



Dow Corning Report # 2000-I0000-49256
INTERNAL

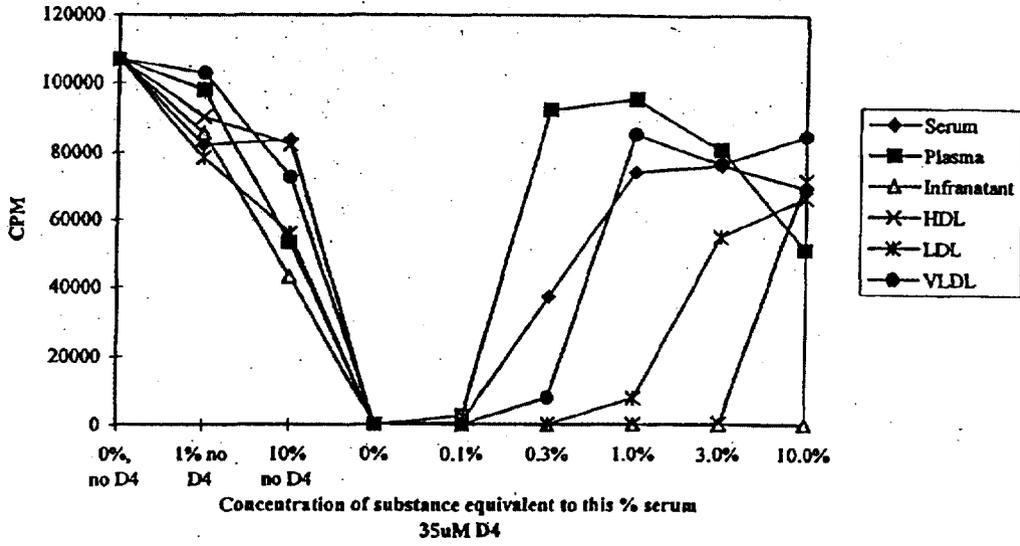
	1/30/97 35uM D4									
	0%, no D4	1% no D4	10% no D4	0%	0.1%	0.3%	1.0%	3.0%	10.0%	
Serum	107000	82322	83729	222	931	37423	74081	76074	69280	
% Error	4.8%	3.7%	9.9%	101.4%	109.0%	41.2%	4.0%	11.4%	7.3%	
background	532	75	58	79	472	558	127	313	139	
% Error	12.1%	52.8%	18.6%	63.4%	174.8%	61.3%	24.0%	140.1%	28.6%	
Plasma	107000	98071	53379	222	2745	92542	95575	80716	51276	
% Error	4.8%	5.0%	13.0%	101.4%	28.1%	3.7%	4.6%	15.8%	8.8%	
background	532	86	221	79	150	365	107	125	217	
% Error	12.1%	46.0%	46.1%	63.4%	25.0%	12.5%	36.0%	50.2%	21.8%	
Infranant	107000	85853	43230	222	242	66	165	205	266	
% Error	4.8%	6.6%	5.5%	101.4%	102.1%	16.9%	24.4%	22.3%	20.9%	
background	532	149	75	79	46	47	74	54	41	
% Error	12.1%	52.1%	37.4%	63.4%	36.9%	26.3%	28.2%	19.0%	40.7%	
HDL	107000	90507	82460	222	75	327	276	615	71629	
% Error	4.8%	8.9%	10.2%	101.4%	33.7%	78.9%	34.5%	25.6%	3.7%	
background	532	516	586	79	36	36	45	57	572	
% Error	12.1%	20.6%	19.1%	63.4%	14.6%	7.2%	21.7%	69.7%	33.6%	
LDL	107000	78504	56006	222	154	345	7968	54924	66447	
% Error	4.8%	10.3%	16.9%	101.4%	18.5%	84.7%	60.4%	7.8%	18.3%	
background	532	511	129	79	41	58	206	288	227	
% Error	12.1%	806.0%	30.8%	63.4%	26.9%	46.5%	43.4%	16.5%	34.9%	
VLDL	107000	103000	72722	222	136	8093	85417	76432	84778	
% Error	4.8%	9.0%	11.6%	101.4%	11.0%	58.0%	4.2%	22.7%	5.3%	
background	532	549	443	79	73	152	408	321	327	
% Error	12.1%	18.3%	16.9%	63.4%	22.1%	34.1%	46.9%	6.8%	22.0%	

Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

B45



Effect of different serum fractions on D4 toxicity



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

B46



INTERNAL

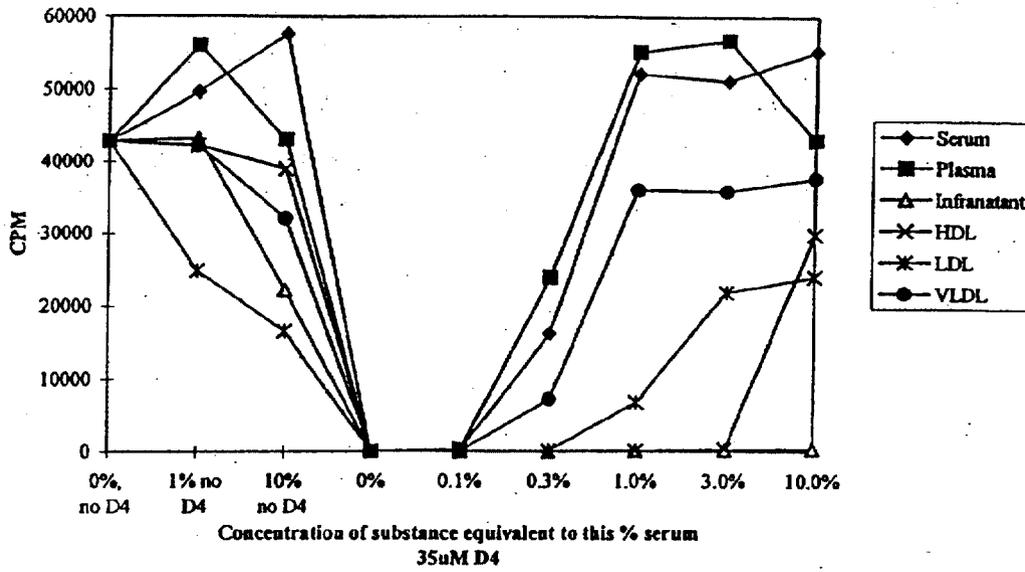
	2/7/97 35uM D4								
	0%, no D4	1% no D4	10% no D4	0%	0.1%	0.3%	1.0%	3.0%	10.0%
Serum	42967	49622	57606	89	289	16289	52133	51129	55208
% Error	3.6%	4.4%	6.6%	23.1%	21.9%	44.1%	5.4%	3.4%	5.1%
background	107	95	153	73	63	220	132	78	116
% Error	26.7%	59.5%	29.0%	33.0%	30.8%	40.0%	50.4%	45.4%	52.3%
Plasma	42967	56000	43123	89	345	23993	55177	56783	43025
% Error	3.6%	1.6%	3.5%	23.1%	56.9%	18.0%	4.6%	13.5%	3.8%
background	107	150	99	73	58	319	85	114	95
% Error	26.7%	23.7%	36.2%	33.0%	15.6%	13.2%	26.9%	75.8%	48.7%
Infranantant	42967	43263	22258	89	85	88	188	290	399
% Error	3.6%	1.6%	9.5%	23.1%	41.4%	54.9%	69.7%	108.4%	79.6%
background	107	73	138	73	102	446	59	101	85
% Error	26.7%	25.7%	22.9%	33.0%	43.7%	47.1%	77.9%	69.5%	41.8%
HDL	42967	42305	38965	89	155	90	151	369	29919
% Error	3.6%	8.6%	5.2%	23.1%	79.3%	37.3%	68.2%	93.7%	6.8%
background	107	158	144	73	89	62	62	56	65
% Error	26.7%	12.4%	10.5%	33.0%	58.3%	50.9%	35.7%	25.6%	28.4%
LDL	42967	24874	16561	89	107	176	6862	21942	24134
% Error	3.6%	8.0%	17.1%	23.1%	30.0%	35.8%	25.3%	6.6%	10.7%
background	107	142	89	73	120	76	143	89	95
% Error	26.7%	10.7%	19.8%	33.0%	29.0%	4.4%	24.5%	46.0%	19.9%
VLDL	42967	42265	32099	89	96	7285	36138	35932	37715
% Error	3.6%	3.4%	2.2%	23.1%	22.2%	59.8%	4.1%	5.6%	3.8%
background	107	144	117	73	87	193	96	83	82
% Error	26.7%	19.0%	5.5%	33.0%	31.2%	28.2%	34.7%	16.7%	62.2%

Values are the mean of 4 wells in CPM
 $\% \text{error} = (\text{standard deviation} / \text{mean}) \times 100$

B47

★

Effect of different serum fractions on D4 toxicity



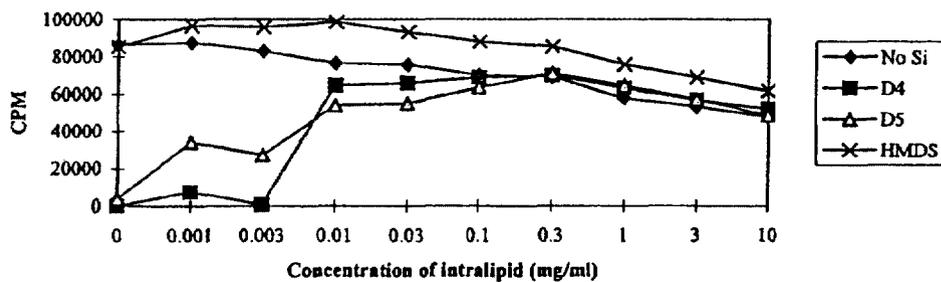
Values are the mean of 4 wells in CPM
 %error=(standard deviation/mean)x100

B5. Protection of PBMCs from Siloxanes by Intralipid.

2/26/97

	Concentration of lipid (mg/ml)									
	0	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
No Si	86384	87450	83010	76571	75701	69889	69160	57694	53235	47911
% Error	12.7%	7.9%	15.1%	6.7%	5.5%	9.0%	13.6%	5.9%	5.1%	19.4%
background	199	171	170	215	231	254	296	252	200	141
% Error	15.1%	17.2%	19.6%	10.6%	9.2%	26.8%	28.0%	31.2%	29.6%	21.6%
D4 (35uM)	202	7447	768	64622	65899	68912	69783	63095	56844	51990
% Error	13.8%	28.8%	113.3%	9.6%	6.8%	11.0%	7.9%	6.7%	9.3%	13.0%
background	199	193	100	129	218	260	330	249	282	231
% Error	16.4%	35.7%	32.6%	26.3%	19.4%	4.3%	9.3%	18.8%	32.3%	12.1%
D5 (23uM)	3729	34197	27425	53848	54912	63597	71083	64628	57077	48649
% Error	22.9%	9.2%	15.4%	11.1%	4.0%	12.0%	11.8%	7.9%	11.8%	3.1%
background	99	257	158	226	229	300	312	294	231	186
% Error	34.6%	37.0%	30.8%	9.7%	19.7%	12.0%	19.8%	16.7%	51.5%	24.6%
HMDS (47uM)	85262	96729	95933	98584	93259	87938	85283	75757	68915	61683
% Error	23.8%	4.9%	4.4%	7.5%	3.5%	7.4%	4.1%	4.2%	7.9%	7.7%
background	195	185	183	217	238	268	381	248	229	184
% Error	28.5%	34.8%	10.5%	26.7%	33.7%	37.5%	28.0%	16.3%	22.9%	16.1%

Inhibition of siloxane toxicity by intralipid



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

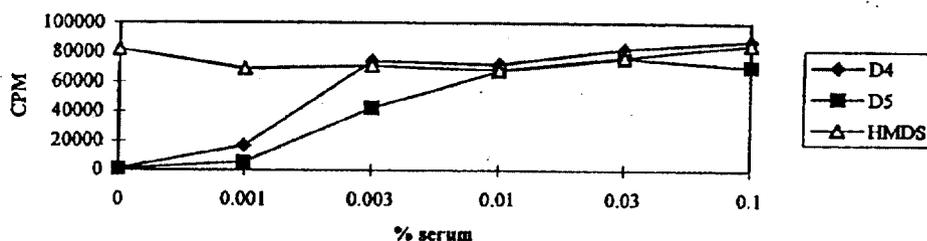


2/26/97

Concentration of serum

	0%	0.10%	0.30%	1%	3%	10%
D4 (35uM)	780	16667	74169	72080	82568	88168
% Error	18.1%	110.3%	11.3%	8.0%	2.1%	8.5%
background	88	68	100	98	83	92
% Error	40.2%	53.8%	41.8%	38.1%	33.3%	17.6%
D5 (23uM)	850	5357	42377	67591	76116	70711
% Error	48.2%	80.2%	20.8%	4.6%	10.1%	6.7%
background	66	155	84	138	120	158
% Error	79.5%	17.7%	50.4%	33.5%	24.8%	33.9%
HMDS (47uM)	82100	69062	71126	68316	76707	85554
% Error	10.4%	10.0%	4.1%	3.7%	6.9%	3.3%
background	146	105	86	83	102	113
% Error	17.8%	10.8%	36.6%	33.1%	4.3%	34.3%

Inhibition of siloxane toxicity by serum



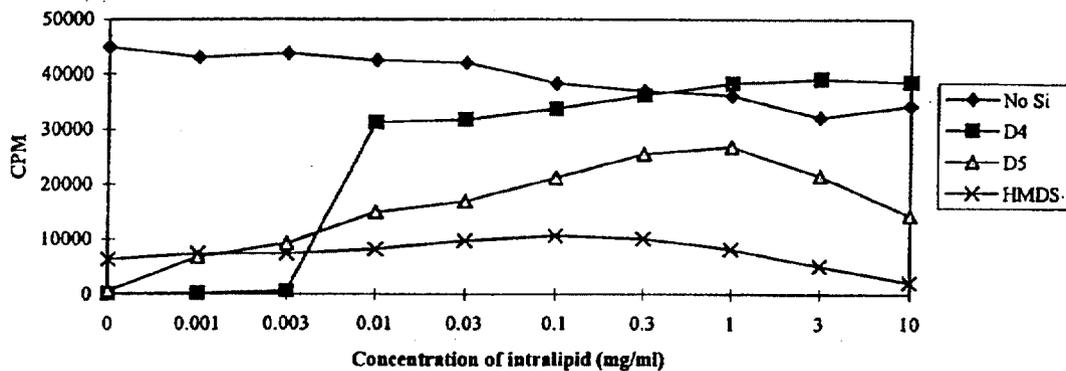
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

B50



2/21/97	Concentration of lipid (mg/ml)									
	0	0.001	0.003	0.01	0.03	0.1	0.3	1	3	10
No Si	44906	43058	43727	42490	41999	38254	36938	36066	32125	34211
% Error	5.1%	3.7%	3.2%	2.8%	3.4%	5.6%	4.3%	5.7%	6.8%	4.4%
background	212	254	220	237	244	344	307	275	227	141
% Error	9.1%	8.0%	12.7%	22.4%	19.9%	39.3%	16.3%	15.6%	19.4%	11.2%
D4 (35uM)	163	227	631	31263	31727	33674	36117	38343	39092	38606
% Error	9.7%	20.0%	32.8%	3.9%	2.8%	7.0%	3.3%	3.3%	2.5%	3.1%
background	139	227	221	403	269	302	315	327	257	158
% Error	25.5%	23.6%	43.0%	9.6%	30.8%	22.9%	14.7%	4.7%	16.9%	10.0%
D5 (23uM)	662	6817	9234	14912	16976	21120	25460	26754	21520	14319
% Error	12.6%	10.9%	7.1%	10.7%	6.3%	3.7%	9.9%	7.1%	7.2%	7.7%
background	50	119	151	165	154	207	233	223	145	86
% Error	23.8%	30.2%	25.4%	37.2%	29.4%	36.7%	54.5%	66.4%	42.7%	52.1%
HMDS (47uM)	6354	7409	7391	8190	9748	10602	10068	8184	5123	2188
% Error	13.1%	5.9%	8.2%	10.2%	1.6%	17.9%	5.0%	5.9%	13.4%	20.0%
background	80	73	72	65	67	83	93	74	47	26
% Error	96.1%	102.4%	108.4%	78.1%	51.1%	49.9%	52.3%	25.9%	60.3%	100.3%

Inhibition of siloxane toxicity by intralipid



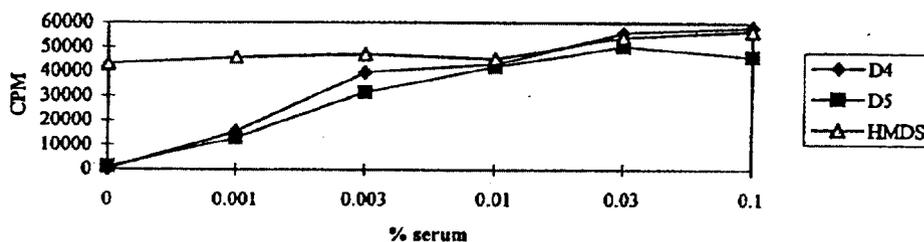
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



2/21/97

	Concentration of serum					
	0%	0.10%	0.30%	1%	3%	10%
D4 (35uM)	270	15300	39572	42976	55949	58331
% Error	37.7%	93.0%	10.8%	3.1%	5.5%	2.6%
background	41	726	167	92	92	122
% Error	41.7%	98.1%	35.4%	14.3%	28.7%	16.8%
D5 (23uM)	1197	12717	31512	42000	50439	46231
% Error	45.1%	19.0%	3.7%	10.9%	3.3%	8.0%
background	61	316	119	92	134	111
% Error	80.5%	18.5%	21.7%	35.0%	31.5%	18.9%
HMDS (47uM)	43228	45864	47158	45413	54080	56628
% Error	2.9%	9.0%	4.2%	5.7%	7.1%	3.0%
background	238	166	156	145	170	217
% Error	13.0%	13.7%	17.0%	6.7%	14.8%	8.0%

Inhibition of siloxane toxicity by serum



Values are the mean of 4 wells in CPM
 %error=(standard deviation/mean)x100

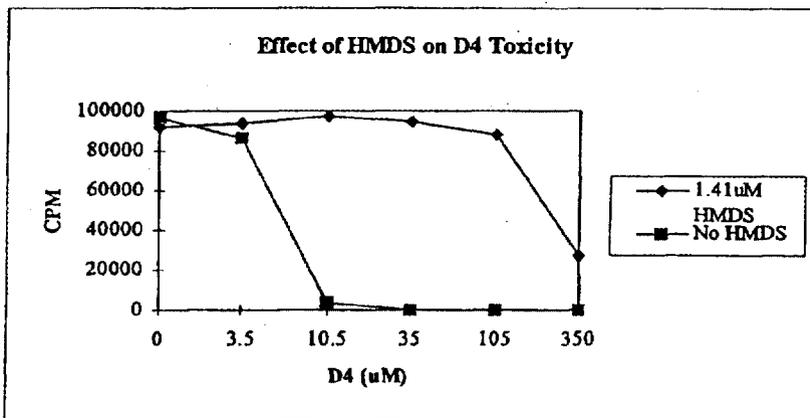


B6. Effect of HMDS on D4 and D5 Toxicity

Siloxanes were mixed together before being added to the media

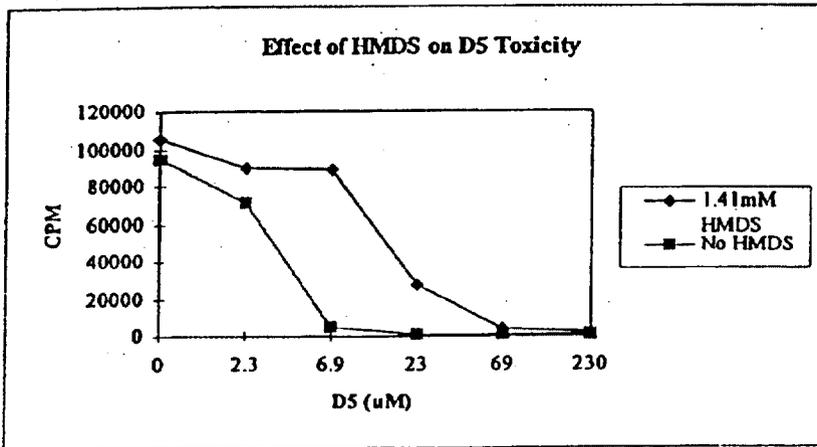
1.41mM HMDS	3/19/97 D4 titration					
	0	3.5uM	105uM	35uM	105uM	350uM
	91562	93668	97182	94672	87861	27243
% Error	15.2%	11.1%	7.5%	12.7%	8.3%	91.3%
background	447	475	820	949	1223	1727
% Error	26.7%	11.0%	46.3%	46.3%	55.4%	46.5%

No HMDS	3/19/97 D4 titration					
	0	3.5uM	105uM	35uM	105uM	350uM
	96189	85915	3596	126	386	242
% Error	13.6%	21.9%	132.3%	38.8%	110.7%	24.8%
background	1692	1182	674	588	766	371
% Error	68.3%	60.4%	67.4%	87.3%	89.4%	56.9%



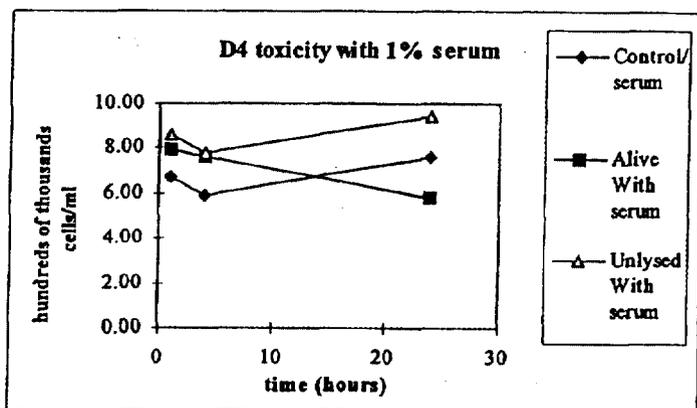
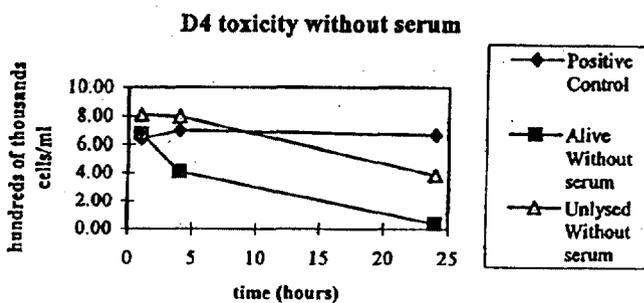
1.41mM HMDS	D5 titration					
	0	2.3	6.9	23	69	230
HMDS	105000	89966	88442	27660	3842	1930
% Error	7.1%	6.7%	10.5%	35.4%	50.1%	38.0%
background	525	659	1025	1073	610	668
% Error	8.5%	18.4%	36.3%	47.2%	52.8%	49.6%

No HMDS	D5 titration					
	0	2.3	6.9	23	69	230
No HMDS	94832	71442	4478	1397	705	974
% Error	10.8%	12.1%	23.2%	52.1%	79.1%	39.5%
background	938	1394	605	378	407	460
% Error	38.7%	28.7%	83.0%	59.4%	39.8%	84.4%



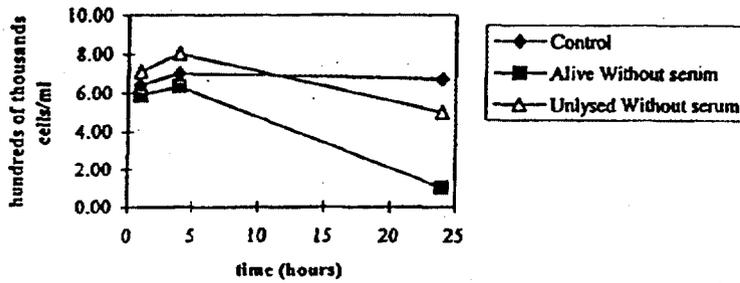
B7. Effect of Siloxanes on Cell Viability

D4	3/4/97	Values x 100,000 = cells/ml					
		Positive		Alive		Unlysed	
Mean:		Control	Control/serum	Without serum	With serum	Without serum	With serum
1		6.43	6.70	6.78	7.75	8.10	8.58
4		7.00	5.90	4.10	7.63	7.98	7.75
24		6.70	7.60	0.40	5.80	3.85	9.40
StDev:							
1		0.40	1.12	1.02	1.25	0.83	1.30
4		0.34	1.64	0.34	1.03	0.94	0.95
24		1.35	1.42	0.08	0.91	0.58	1.35

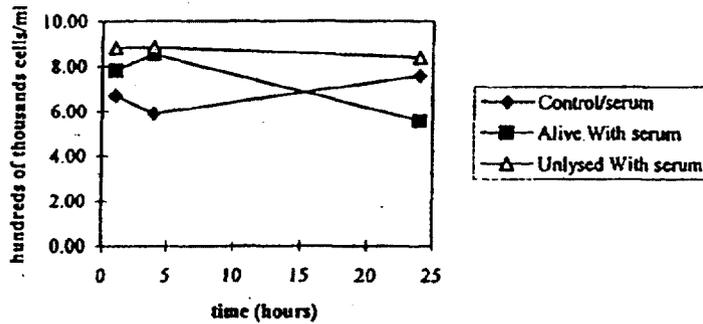


D5	3/4/97	Values x 100,000 = cells/ml				
Mean:	Control	Control/serum	Alive	With serum	Unlysed	With serum
			Without serum		Without serum	
1	6.43	6.70	5.88	7.83	7.10	8.83
4	7.00	5.90	6.35	8.55	8.03	8.85
24	6.70	7.60	1.05	5.58	5.00	8.40
StDev:						
1	0.40	1.12	1.27	0.22	1.66	0.21
4	0.34	1.64	0.78	0.68	0.79	0.69
24	1.35	1.42	0.71	0.81	1.13	1.53

D5 toxicity without serum

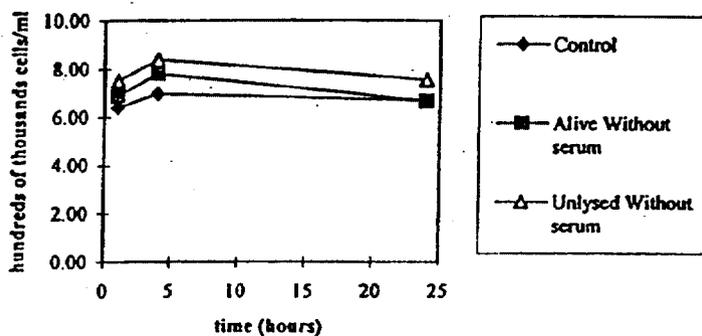


D5 toxicity with 1% serum

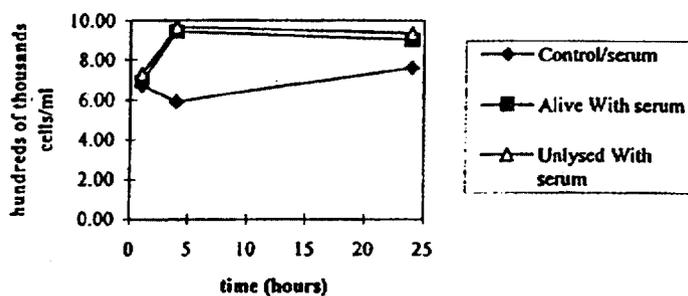


HMDS	3/4/97	Values x 100,000 = cells/ml				
Mean:	Control	Control/serum	Alive		Unlysed	
			Without serum	With serum	Without serum	With serum
1	6.43	6.70	6.90	6.90	7.53	7.30
4	7.00	5.90	7.83	9.43	8.40	9.68
24	6.70	7.60	6.70	9.03	7.58	9.35
StDev:						
1	0.40	1.12	1.14	1.13	1.22	1.14
4	0.34	1.64	0.75	0.76	0.74	1.01
24	1.35	1.42	0.78	1.26	0.80	1.14

HMDS toxicity without serum



HMDS toxicity with 1% serum

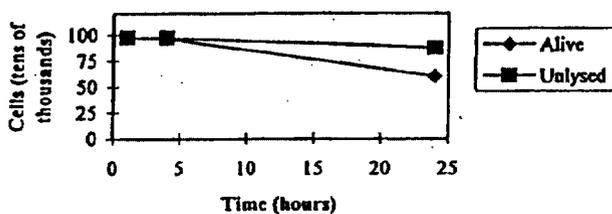


Values x 100,000 = cells/ml

3/27/97 No siloxane

Mean:	Alive	Unlysed
1	9.70	9.73
4	9.60	9.70
24	6.00	8.75
StDev:		
1	0.70	0.67
4	1.20	1.22
24	1.40	1.81

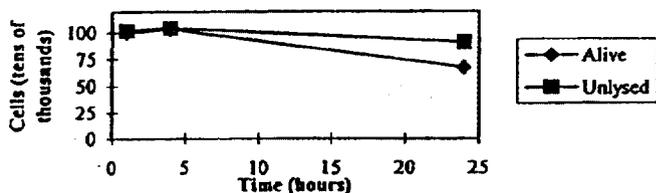
Control cells with no siloxane



3/27/97 141uM HMDS

Mean:	Alive	Unlysed
1	10.00	10.20
4	10.30	10.50
24	6.70	9.10
StDev:		
1	0.80	0.80
4	0.60	0.60
24	1.10	1.00

Toxicity of 141uM HMDS

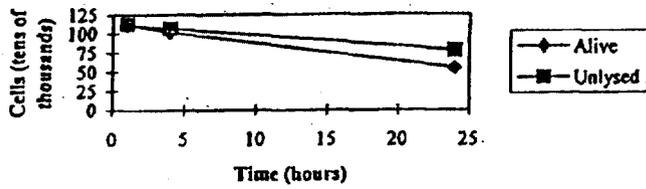


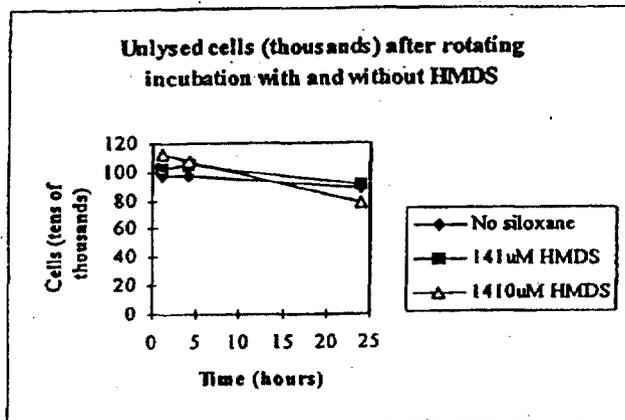
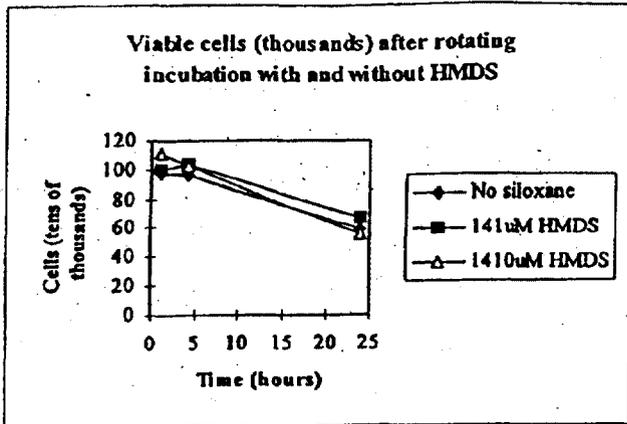
3/27/97 1.41mM HMDS

Mean:	Alive	Unlysed
1	11.13	11.25
4	10.15	10.65
24	5.48	7.80

StDev:	Alive	Unlysed
1	0.97	0.99
4	0.66	0.74
24	0.72	1.10

Toxicity of 1.41mM HMDS

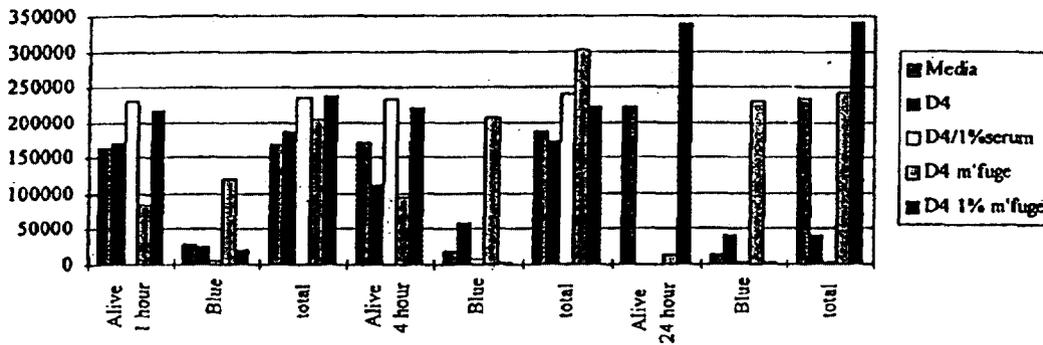




Values x 100,000 = cells/ml

6/28/97	1 hour			4 hour			24 hour		
Mean:	Alive	Blue	total	Alive	Blue	total	Alive	Blue	total
Media	1.63	0.28	1.68	1.70	0.18	1.88	2.23	0.13	2.35
D4	1.70	0.25	1.88	1.13	0.60	1.73	0.00	0.40	0.40
D4/1%serum	2.30	0.05	2.35	2.33	0.08	2.40	0.00	0.03	0.03
D4 m'fuge	0.85	1.20	2.05	0.95	2.08	3.03	0.13	2.30	2.43
D4 1% m'fuge	2.18	0.20	2.38	2.20	0.03	2.23	3.40	0.03	3.43
StDev:									
Media	0.13	0.17	0.32	0.37	0.10	0.43	0.97	0.10	1.01
D4	0.24	0.19	0.19	0.38	0.29	0.66	0.00	0.18	0.18
D4/1%serum	0.24	0.06	0.30	0.43	0.10	0.42	0.00	0.05	0.05
D4 m'fuge	0.39	0.22	0.49	0.45	0.54	0.50	0.13	0.59	0.67
D4 1% m'fuge	0.40	0.08	0.34	0.87	0.05	0.83	1.18	0.05	1.16

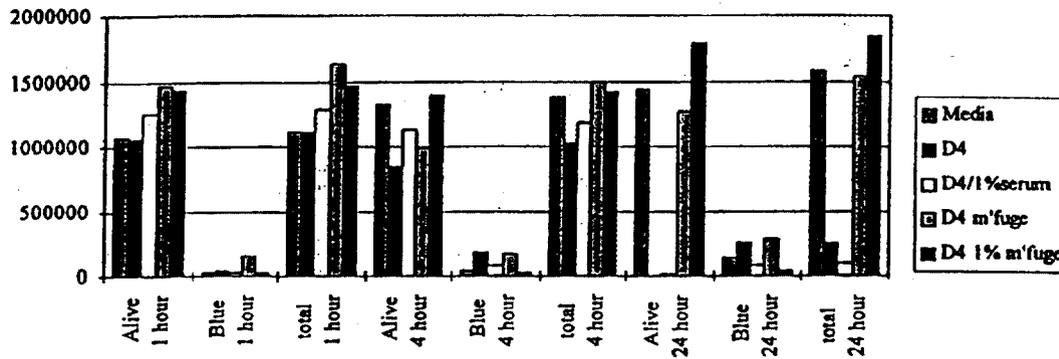
Cytotoxicity of microfuged and unmicrofuged D4, with and without serum



Values x 100,000 = cells/ml

7/2/97	1 hour	1 hour	1 hour	4 hour	4 hour	4 hour	24 hour	24 hour	24 hour
Mean:	Alive	Blue	total	Alive	Blue	total	Alive	Blue	total
Media	10.73	0.35	11.08	13.28	0.48	13.75	14.40	1.38	15.78
D4	10.60	0.48	11.08	8.40	1.88	10.28	0.00	2.53	2.53
D4/1%serum	12.50	0.33	12.83	11.20	0.88	11.83	0.18	0.88	1.05
D4 m'fuge	14.70	1.60	16.30	9.85	1.75	15.00	12.65	2.75	15.40
D4 1% m'fuge	14.30	0.30	14.60	13.93	0.33	14.25	17.93	0.48	18.40
StDev:									
Media	0.99	0.17	1.08	1.79	0.19	1.80	1.51	0.89	2.04
D4	3.39	0.25	3.47	0.41	0.46	0.36	0.00	0.88	0.88
D4/1%serum	1.60	0.25	1.73	0.67	0.45	0.91	0.17	0.21	0.37
D4 m'fuge	4.83	0.76	4.62	5.94	0.58	2.67	3.34	1.10	3.99
D4 1% m'fuge	6.33	0.08	6.34	3.70	0.22	3.84	4.43	0.29	4.50

Cytotoxicity of microfuged and unmicrofuged D4, with and without serum

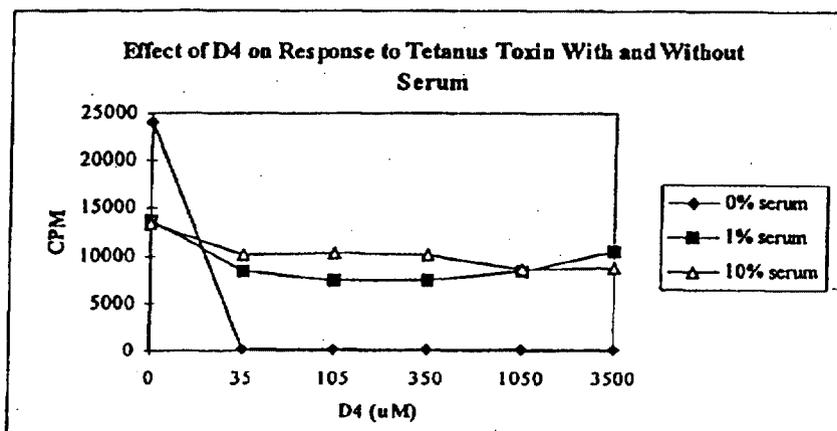


B8. Siloxanes Inhibit Tetanus Toxoid-Induced Proliferation of PBMCs

Tet 4/25/97

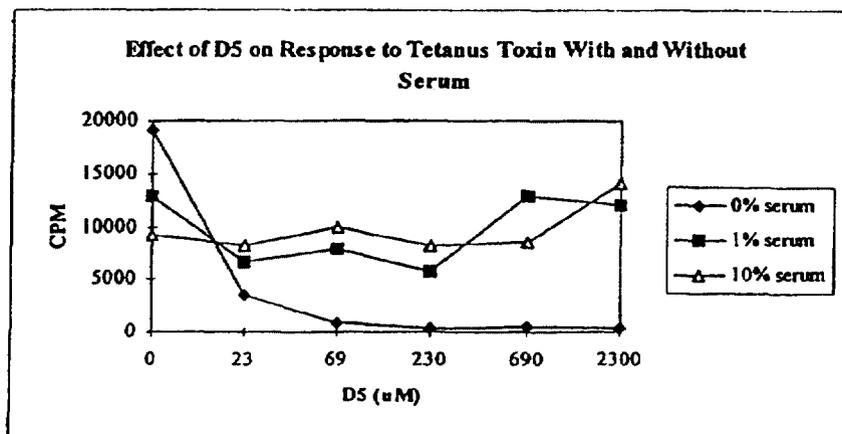
D4

	0	35	105	350	1050	3500
0% serum	24069	205	218	192	230	236
% Error	8.9%	15.8%	19.2%	33.4%	8.1%	26.4%
1% serum	13659	8376	7515	7513	8341	10564
% Error	16.7%	12.5%	4.9%	10.2%	16.9%	17.7%
10% serum	13278	10189	10265	10034	8643	8818
% Error	7.3%	24.5%	27.8%	42.1%	47.6%	13.9%



D5

	0	23	69	230	690	2300
0% serum	19100	3408	864	264	572	369
% Error	5.2%	23.1%	28.0%	29.6%	60.4%	11.1%
1% serum	12880	6549	7840	5773	12886	11967
% Error	28.0%	25.9%	20.7%	47.4%	33.6%	26.2%
10% serum	9203	8132	9874	8259	8477	14115
% Error	18.6%	12.6%	32.7%	33.6%	17.6%	17.7%



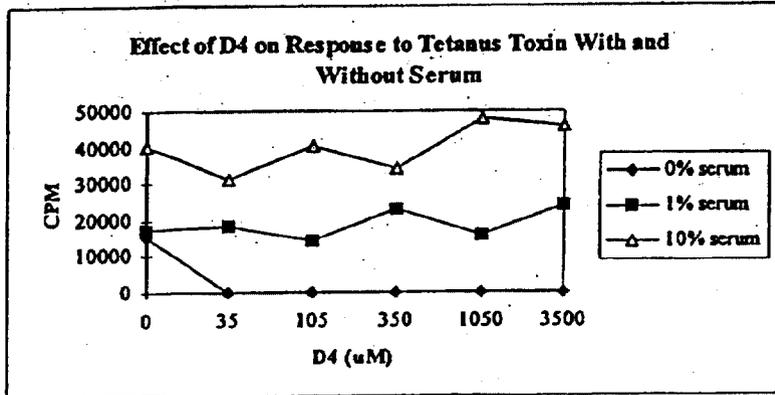
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



11/7/97

D4

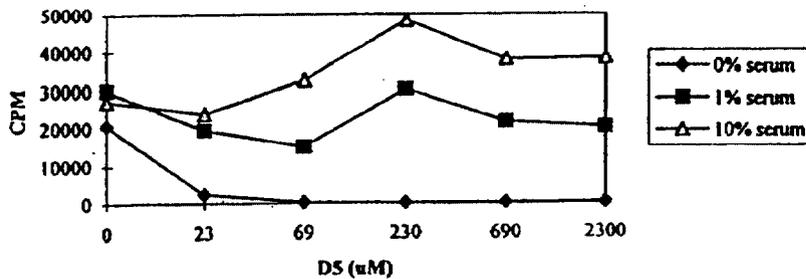
	0	35	105	350	1050	3500
0% serum	15142	115	136	114	140	223
% Error	46.7%	24.8%	22.8%	22.3%	17.2%	31.4%
1% serum	17559	18559	14382	22809	16039	24082
% Error	55.2%	35.7%	26.1%	73.9%	10.6%	67.3%
10% serum	40069	31316	40501	34003	47940	45964
% Error	35.6%	28.9%	12.3%	44.7%	22.6%	11.6%



D5

	0	23	69	230	690	2300
0% serum	20697	2451	327	130	252	266
% Error	6.4%	55.1%	79.4%	46.8%	93.4%	52.9%
1% serum	30054	19480	14931	30230	21781	20266
% Error	20.0%	36.9%	16.7%	22.0%	22.7%	50.4%
10% serum	27232	23824	32479	48365	37976	38200
% Error	32.6%	18.7%	25.3%	6.6%	47.0%	53.1%

Effect of D5 on Response to Tetanus Toxin With and Without Serum



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)×100

B64

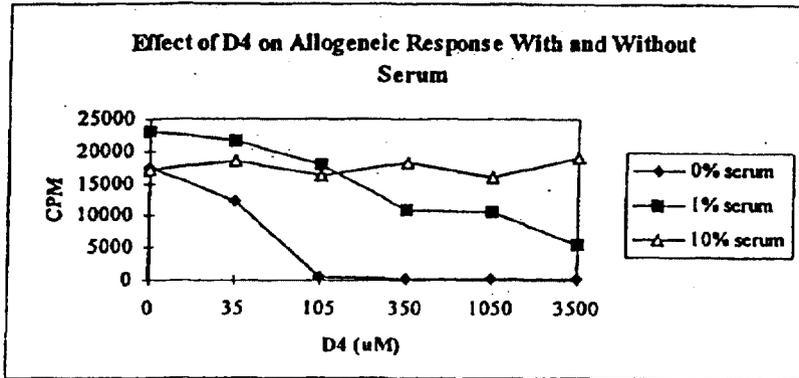


B9. Siloxanes Inhibit Alloantigen-Induced Proliferation of PBMCs

4/11/97

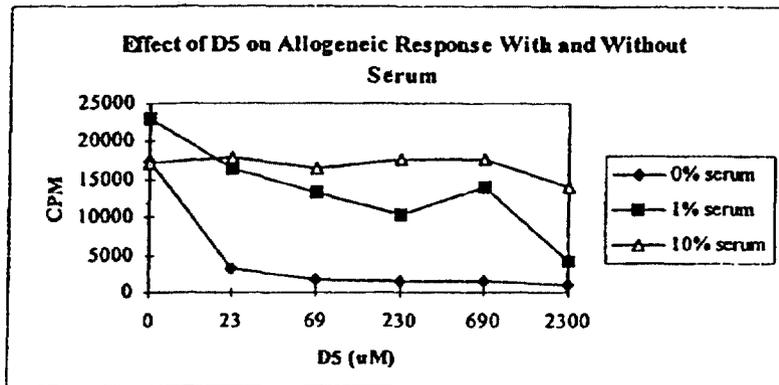
D4

	0	35	105	350	1050	3500
0% serum	17391	12374	446	269	269	252
% Error	6.5%	6.9%	48.4%	26.7%	25.9%	36.1%
1% serum	23003	21649	17945	10837	10571	5684
% Error	3.0%	7.0%	6.0%	20.0%	15.0%	18.0%
10% serum	17094	18430	16293	18341	16109	19001
% Error	10.9%	5.8%	10.0%	16.5%	7.3%	13.7%



D5

	0	23	69	230	690	2300
0% serum	17391	3134	1787	1349	1462	997
% Error	6.5%	8.6%	7.9%	18.3%	7.2%	12.3%
1% serum	23003	16449	13185	10317	13879	4241
% Error	3.0%	5.0%	12.0%	11.0%	11.0%	26.0%
10% serum	17094	17942	16375	17539	17672	14037
% Error	10.9%	14.7%	10.6%	10.2%	12.8%	70.0%



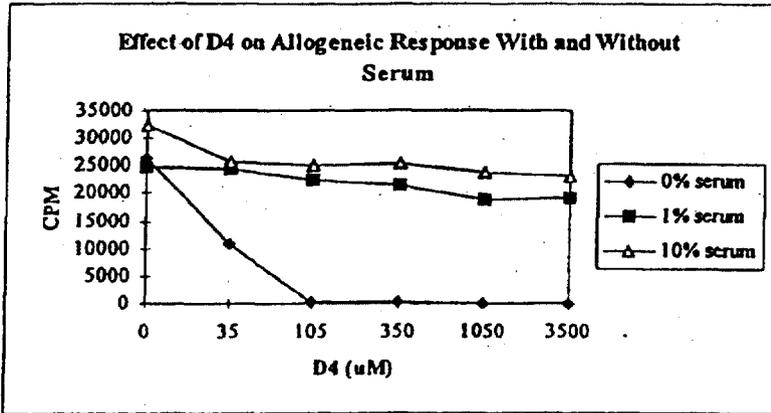
Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100



4/25/97

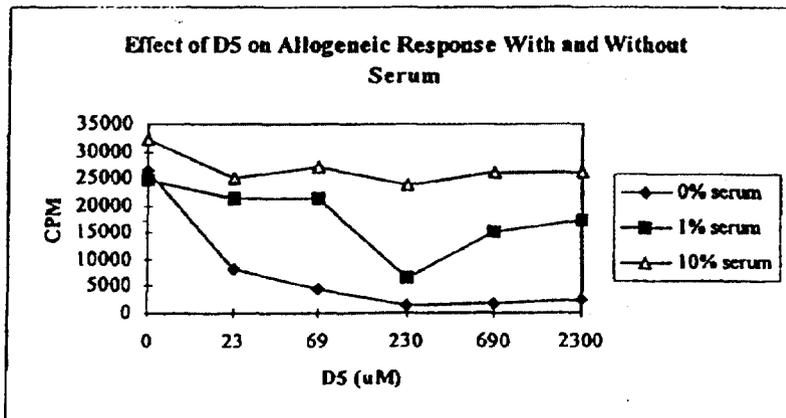
D4

	0	35	105	350	1050	3500
0% serum	26564	10936	424	200	157	162
% Error	6.0%	13.5%	57.2%	31.4%	23.4%	32.3%
1% serum	24835	24422	22527	21608	18670	19098
% Error	5.6%	14.4%	5.8%	18.8%	19.5%	17.8%
10% serum	32195	25675	25242	25549	23861	23035
% Error	7.7%	7.0%	10.0%	8.7%	6.9%	13.2%



D5

	0	23	69	230	690	2300
0% serum	26564	8232	4508	1426	1842	2404
% Error	6.0%	15.7%	15.6%	33.2%	16.8%	24.2%
1% serum	24835	21136	21129	6366	14990	17038
% Error	5.6%	6.2%	7.1%	8.6%	12.3%	18.1%
10% serum	32195	25193	27141	23588	25944	25997
% Error	7.7%	11.7%	18.8%	5.8%	11.2%	6.3%



Values are the mean of 4 wells in CPM
%error=(standard deviation/mean)x100

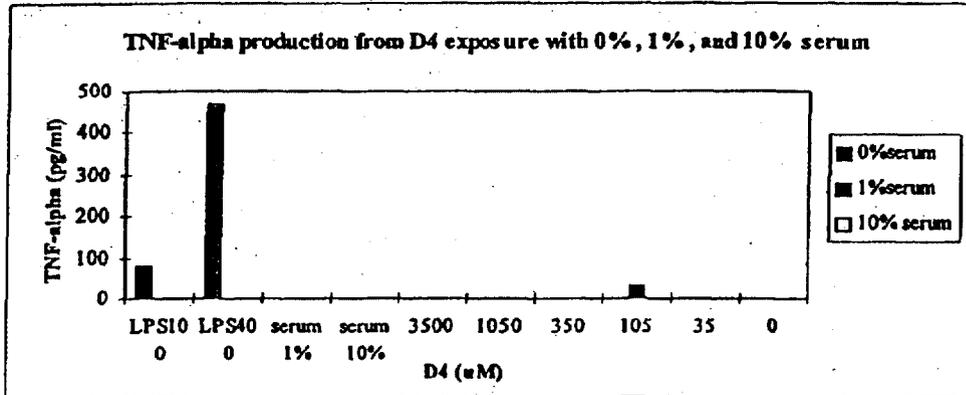
B66



B10. Siloxanes Do Not Induce the Production of Tumor Necrosis Factor

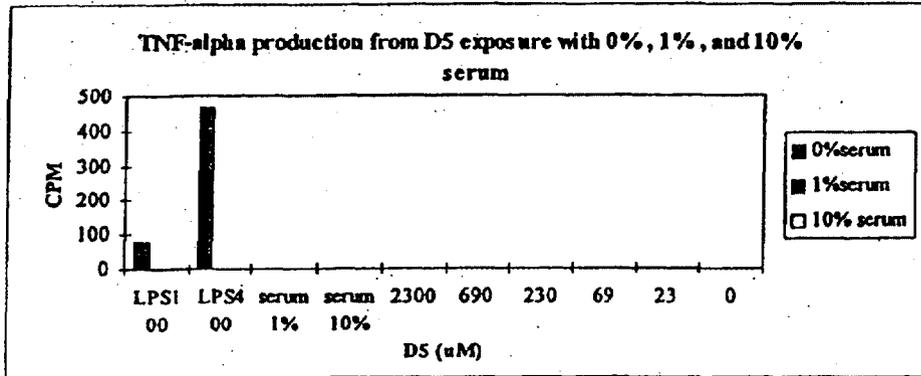
D4 TNF Started 3/19/97

	LPS100	LPS400	serum 1%	serum 10%	3500	1050	350	105	35	0
0%serum	80	470	0	0	0	0	0	0	0	0
1%serum					0	0	0	32	0	0
10% serum					0	0	0	0	0	0



D5 TNF Started 3/19/97

	LPS100	LPS400	serum 1%	serum 10%	2300	690	230	69	23	0
0%serum	80	470	0	0	0	0	0	0	0	0
1%serum					0	0	0	0	0	0
10% serum					0	0	0	0	0	0



HMDS TNF Started 3/19/97

	LPS100	LPS400	serum 1%	serum 10%	4700	1410	470	141	47	0
0%serum	80	470	0	0	0	0	0	0	0	0
1%serum					0	0	0	0	0	0
10% serum					0	0	0	0	0	0

