OFFICE COPY

R & D REPORT

DOW CHEMICAL U.S.A.

RESTRICTED · for use within The Dow Chemical Company only.

CRI NUMBER

LABORATORY REPORT CODE: HET K-1711-(22) Part I of II

DATE ISSUED: Sept. 22, 1978

DEPARTMENT: Toxicology Research Department, Health & Environmental Research

LAB. NO.: 1 1 7 1 | PROBLEM NO.: 0 0 0 7 7 0 0

TITLE: Stimulation of Lymphocyte Transformation in Splenic Cultures of Animals Exposed to Vinyl Chloride

38 PAGES IN FULL REPORT

AUTHOR(S): R. P. Sharma, R. J. Kociba, H. O. Yakel, and P. J. Gehring

AUTHOR(S) SIGNATURE(S): R.P. Sharma 9/21/78 H.O. Yakel 9/20/78 P.J. Gehring 9/20/78

REVIEWER'S SIGNATURE: BAS Shuey 21 Sept 78

This report is: ☐ INTERIM ☒ FINAL and mainly: ☒ NEW ☐ REVIEW

DATA REFERENCES (book and page):

NBK 21-5, NBK 21-6, NBK 21-7, NBK 21-9

(Refer also to earlier related reports and publications.)

PATENT STATUS: ☐ disclosure submitted ☐ case filed ☒ no patent action required

DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

Immunologic responses in mice and rabbits exposed by inhalation to 10, 100 or 1000 ppm vinyl chloride (VC), 6 hr/day, 5 days/week for up to 8 weeks were investigated. Other than altered immunological responses, the only effects attributable to VC exposure occurred in mice and rabbits exposed to 1000 ppm; a slight increase in the spleen weight of mice and thymus weight of rabbits was noticed at this highest exposure level. Increased DNA synthesis was evident in cultured lymphocytes from spleens of mice after 2, 4 and 8 weeks of VC exposure. The response of these lymphocytes to phytomitogens, i.e., phytohemagglutinin and pokeweed mitogen, as measured by ^{3}H-thymidine incorporation into cells in culture, were several-fold increased. In mice and rabbits, VC exposure was not associated with any consistent change in serum immunoglobulins. Mice and rabbits immunized with tetanus toxoid and Freund's complete adjuvant revealed no effect of VC exposure on skin reactivity to tuberculin, or the number of antibody producing cells in lymph nodes. The results indicated that VC exposure caused stimulation of splenic lymphocyte transformation, indicative of an enhancement of immune reactivity. Enhanced immunological reactivity may be related to the presence of immune complexes observed previously in certain VC-exposed workers.

DISTRIBUTION: See Back Page

FORM C-46439

R&S 022476

RESTRICTED: THIS PAGE SHOULD FOLLOW THE DOW SUMMARY PAGE AND BE ATTACHED TO COPIES MAINTAINED ONLY IN THE TOXICOLOGY RESEARCH ARCHIVES AND QUALITY ASSURANCE UNIT FILES.

LOCATER SHEET

TOXICOLOGY RESEARCH LABORATORY
HEALTH AND ENVIRONMENTAL RESEARCH
DOW CHEMICAL U.S.A.

FILE NO. HET K-1711-22

DEAD STORAGE NO. 1540

TITLE OF REPORT: Stimulation of Lymphocyte Transformation in Splenic Cultures of Animals Exposed to Vinyl Chloride

DATE REPORT ISSUED: September 22, 1978

AUTHOR(S): R.P. Sharma, R.J. Kociba, H.O. Yakel, P.J. Gehring

NOTEBOOK(S) - (NUMBER AND PAGE(S): Inclusive

1) NBK 21-5
2) NBK 21-6
3) NBK 21-7
4) NBK 21-9
5)
6)

SPECIMENS:

1)
2)
3)
4)

PATHOLOGY NUMBERS:

ADDITIONAL DOCUMENTS:

1)
2)

LOCATION:

ALL DATA ARE SIGNED, DATED, IDENTIFIED BY PROJECT FILE NUMBER, LOGICALLY ORGANIZED, AND ADEQUATELY BOUND IN COMPLIANCE WITH GOOD LABORATORY PRACTICES PROCEDURES.

Study Director R.P. Sharma PJG Date 9-14-78

21 March 1978

STIMULATION OF LYMPHOCYTE TRANSFORMATION IN SPLENIC CULTURES OF ANIMALS EXPOSED TO VINYL CHLORIDE

R. P. Sharma, R. J. Kociba, H. O. Yakel and P. J. Gehring

Toxicology Research Laboratory
Health and Environmental Research
Dow Chemical U.S.A.
Midland, Michigan 48640

September 22, 1978

STIMULATION OF LYMPHOCYTE TRANSFORMATION IN SPLENIC CULTURES OF ANIMALS EXPOSED TO VINYL CHLORIDE

ABSTRACT

Immunologic responses in mice and rabbits exposed by inhalation to 10, 100 or 1000 ppm vinyl chloride (VC), 6 hr/day, 5 days/week for up to 8 weeks were investigated. Other than altered immunological responses, the only effects attributable to VC exposure occurred in mice and rabbits exposed to 1000 ppm; a slight increase in the spleen weight of mice and thymus weight of rabbits was noticed at this highest exposure level. Increased DNA synthesis was evident in cultured lymphocytes from spleens of mice after 2, 4 and 8 weeks of VC exposure. The response of these lymphocytes to phytomitogens, i.e., phytohemagglutinin and pokeweed mitogen, as measured by ^{3}H-thymidine incorporation into cells in culture, were several-fold increased. In mice and rabbits, VC exposure was not associated with any consistent change in serum immunoglobulins. Mice and rabbits immunized with tetanus toxoid and Freund's complete adjuvant revealed no effect of VC exposure on skin reactivity to tuberculin, or the number of antibody producing cells in lymph nodes. The results indicated that VC exposure caused stimulation of splenic lymphocyte transformation, indicative of an enhancement of immune reactivity. Enhanced immunological reactivity may be related to the presence of immune complexes observed previously in certain VC-exposed workers.

STIMULATION OF LYMPHOCYTE TRANSFORMATION IN SPLENIC CULTURES OF ANIMALS EXPOSED TO VINYL CHLORIDE

Exposure to vinyl chloride (VC) has been associated with various health problems in man and animals. In workers exposed to high levels of VC, hepatic fibrosis associated with or leading to portal hypertension and angiosarcoma of the liver has been described (Berk et al., 1976). Dermal induration, acro-osteolysis, splenomegaly and thrombocytopenia have also been observed (Harris and Adams 1967; Lange et al., 1974). In animals, VC exposure has been reported to cause several types of tumors (Maltoni and LeFemine, 1974).

In a recent report Ward et al., (1976) suggested the presence of aberrant immune complexes in workers exposed to vinyl chloride. The incidence and the level of these complexes were correlated with the level of vinyl chloride exposure as well as other manifestations of toxicity. An increase in immunoglobulins and a corresponding reduction in peripheral T-lymphocytes was suggested. In addition, the presence of circulating immune complexes (inferred by the presence of mixed cryoglobulins and decreased values of complement) and autoantibodies against various tissue components were demonstrated in exposed workers. On the basis of their findings, Ward et al. (1976) suggested that the "vinyl chloride disease" may be an immune complex disorder and the immune response may be initiated by the adsorption of vinyl chloride or its metabolite on the tissue or plasma proteins.

In another related report, Page et al. (1976) demonstrated elevated levels of carcinoembryonic antigen (CEA) in the plasma of workers exposed to vinyl chloride. Lange et al. (1974) have reported both increases and decreases of different fractions of immunoglobulins in 10 patients suffering from VC-disorders and on the basis of their findings excluded the possibility of an autoimmune phenomenon.

Based on these contradictory reports, we investigated the effects of vinyl chloride exposure on the immune system of mice and rabbits. For the evaluation of immune reactivity in individuals and animals, the response of lymphocytes in culture, particularly blast formation in the presence of antigenic substances, has been suggested as a useful index (Dutton and Eady, 1964). Sensitized lymphocytes have been shown to play a very significant role in the production of autoimmune diseases (Orgad and Cohen, 1974). The findings that T- and B-lymphocytes can be selectively transformed in vitro by different lectins (Janossy and Greaves, 1977; Stockman et al., 1971; Andersson et al., 1972) have provided a working model to evaluate the responsiveness of these lymphocytes in cell cultures. Our results described in this report suggest that VC exposure caused stimulation of lymphocyte transformation in cultures, but had no appreciable effect on the immune responses induced by a simultaneous challenge to other antigens.

METHODS

Vinyl chloride monomer (obtained from Matheson Gas Products, Joliet, Ill.) of 99.9% minimum purity was used for exposure of mice and rabbits. The exposures were conducted in inhalation chambers (6'x6'x8'). The concentration of VC was controlled to provide atmospheric concentrations of 0 (control), 10, 100 and 1000 ppm, 6 hr/day, 5 days/week, for durations of up to 8 weeks. The time-weighted averages of vinyl chloride concentration in the chamber, as determined by a gas chromatograph are described in Table 1.

Male, CD-1 mice (approximately 7 weeks old and weighing 35 g each at the beginning of exposure) were obtained from Charles River (Wilmington, Mass.) and acclimated for 2 weeks with the surroundings. The animals were housed 4 to a cage, identified by ear punch, and were allowed free access to food and water except during the exposure period. No food or water was allowed during the exposure. After 2 weeks of acclimation, male White New Zealand rabbits, weighing approximately 3 kg each, were also exposed to vinyl chloride in groups of 5 each. The rabbits were housed individually. The rabbits were removed from the chambers to different cages when not being exposed. Food and water was allowed ad libitum except during exposure. All animals were weighed once a week.

A selected group of mice (4 per exposure level) and all rabbits were injected with an antigen after 4 weeks of exposure. The antigen was a

1:1 mixture of tetanus toxoid (Jen-Sal, Kansas City, MO) and Freund's complete adjuvant (GIBCO, Grand Islands, New York) injected at a volume of 0.4 ml per rabbit and 0.02 ml per mouse in their foot-pad. Injection of the antigen was repeated 2 weeks later. Blood was collected from the ear vein of rabbits at the beginning of the exposure, just prior to the antigen injections, and at the end of the exposure period (except at the time of sacrifice, when blood could be collected immediately after decapitation). Groups of mice (4 animals/group/VC level) were sacrificed at 2, 4 and 8 weeks of exposure and blood samples obtained.

Hematology and Serum Immunoelectrophoresis. Total erythrocyte and leukocyte counts and differential leukocyte counts were performed on all blood samples using routine methods. The concentration of immunoglobulins, IgG in rabbits and total Ig in mice, was determined by immunoelectrophoretic techniques modified from Bjerrum et al. (1973). The details of the technique are described elsewhere (Sharma et al, 1977).

Immunologic and Serologic Evaluations. In rabbits, the skin sensitivity to tuberculin was determined 10 days after the first and second antigen challenge. Tuberculin (Jen-Sal, Kansas City, MO), in doses of 0.1 ml, was injected intradermally in a shaven area of the flank and the diameter of reaction measured 24 and 48 hours after this injection. The tetanus anti-toxin titers in the serum of antigen-challenged animals were estimated by an indirect hemagglutination procedure. Sheep erythrocytes (SRBC) were incubated for 1 hour with 1:200 of tetanus toxoid solution,

washed, and added as indicator to serially diluted serum in microtiter plates. The highest serum dilution in which complete hemagglutination occurred after 2 hours of incubation was used as an endpoint.

Necropsy and Organ Collection. All rabbits were thoroughly evaluated for any lesions at necropsy. Selected organs, i.e., liver, kidney, spleen, thymus, brain, heart, adrenals and popliteal lymph nodes were weighed and organ/body weight ratios determined. All these organs, and also lungs, were saved in 10% buffered formalin for histopathologic evaluation. The tissues were processed, stained with hematoxylin-eosin, and examined microscopically. In mice, liver, kidney, spleen and thymus were weighed and these organs, except spleen, were fixed for histological examination. The spleen was saved in sterilized saline solution for lymphocyte cultures.

From rabbits, one popliteal lymph node was frozen for evaluation of plasma cells (see below) and part of the spleen was saved in saline for cultures.

Splenic Lymphocyte Cultures. The cultures were performed as described elsewhere in detail (Sharma et al. 1977). The spleens were washed with sterilized saline and mashed in saline with a forceps. Subsequently, the agglutinated cells and large particles were removed by passing the cells through a series of hypodermic needles with the resulting cell suspension transferred to a glass tube. After separating the cells by

centrifugation, the cells were suspended in culture medium (RPMI-1640 supplemented with glutamine, penicillin and streptomycin), an aliquot counted electronically and the concentration of cells adjusted to approximately $8x10^6$ cells/ml. Aliquots of this suspension were transferred to microculture plates, more media with or without mitogenic lectins was added, and the system incubated at 37°C in humidified air containing 5% carbon dioxide. Optimal concentrations of phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were used. After 50-52 hours of incubation, 0.5 μCi of ^{3}H-thymidine (New England Nuclear, Boston, Mass.) was added to each culture and incubation continued for an additional 16 hours. The cells were then harvested and the radioactivity counted using a liquid scintillation spectrometer. Each culture was performed in triplicate and their values were averaged after corrections for quenching. Stimulation indices for PHA and PWM were determined for each spleen by dividing the disintegrations/min. in the presence of the mitogen by those in the system containing no mitogen.

Fluorescent Evaluation of Rabbit Popliteal Lymph Nodes. The plasma cells in frozen lymph nodes were observed after the sections were incubated with a fluorescein conjugated swine anti-rabbit IgG. The cells were examined in ultraviolet light using a fluorescent microscope (Leitz, Inc. Rockleigh, N. J.) and scored as described previously (Street and Sharma, 1975).

Statistical Evaluation. The values are represented as means together with the respective standard deviations or standard errors. The animal

and organ weights were compared by using a Dunnett's test (Steel and Torrie, 1960). Other parameters were compared by using a "t" test or analysis of variance. For all tests, a predetermined value of $p<0.05$ was used to determine statistical significance.

RESULTS

In mice, vinyl chloride exposure for 8 weeks produced no observable effects on growth, or weights of liver, kidney or thymus (Table 2). Spleen weights were increased at the highest level of exposure, 1000 ppm. This effect was noticed after 2 weeks of exposure and persisted throughout the exposure. A slight reduction in liver/body weight ratios after 8 weeks of exposure to 1000 ppm VC may be attributable to greater body weights in those mice as compared to controls. The body weights of mice exposed to 1000 ppm VC were consistently higher than controls after 4 weeks of exposure when the body weights of all animals in that group were considered (data not shown in the table). No differences were seen in the hematological parameter of any group exposed to VC (data not shown). At the time of sacrifice, there were no gross lesions observed in any of the organs that could be related to the exposure. Subsequent histological examination of selected tissues revealed no differences.

In rabbits, a significant increase in the thymus weight was observed in the group exposed to 1000 ppm VC (Table 3). This was the only organ that showed any weight change in this species. No hematologic alterations or microscopic lesions in any organs were detected.

Vinyl chloride exposure caused stimulation of spontaneous lymphocyte transformation in mouse splenic cultures. This effect was observed at the highest exposure level, 1000 ppm of VC, after 2 weeks of exposure.

After 4 and 8 weeks of exposure, an increased transformation of lymphocytes prepared from the exposed animals was observed at all levels of VC (Figures 1-3). The stimulation indices for PHA were significantly increased in mice exposed to 1000 ppm VC for 2 weeks (Figure 4). At subsequent time intervals, however, there was no definite dose response, since in the mice exposed to 1000 ppm VC the indices were lower than for those exposed to the two lower levels. The stimulation indices at these points were, however, consistently greater than those observed for controls (Figures 5 and 6).

For all groups of mice, the DNA synthesis by lymphocytes in culture decreased with time. Such findings were consistent with those generally observed in growing animals. This was consistent in all exposure groups including control, the latter showing a considerable drop in the stimulating indices by PHA and PWM.

In groups of control mice that were challenged with the antigen mixture (tetanus toxoid and adjuvant), the spontaneous lymphocyte transformation was enhanced as compared to the groups not challenged with antigen and sacrificed simultaneously. However, the effect of VC on lymphocyte transformation was not evident in these animals (Table 4). Only a slight and nonsignificant difference was observed at low levels of VC exposure. The values decreased at the highest exposure level of vinyl chloride. A similar trend was also seen in the stimulatory effect of PHA and PWM.

The trend in splenic lymphocyte cultures from antigen-challenged rabbits was similar to that observed for mice (Table 4). The synthesis of DNA in unstimulated lymphocyte cultures was higher in those prepared from rabbits exposed to 10 and 100 ppm of VC than for control. The cultures prepared from rabbits exposed to 1000 ppm VC did not incorporate as much thymidine as those prepared from rabbits exposed to lower levels. The stimulation indices were not influenced in a consistent manner, the PHA induced stimulation showing a decrease at the highest level of VC exposure whereas an increase at low concentrations followed with a gradual decline at high concentrations of vinyl chloride was observed for PWM stimulation index. These results for rabbits were consistent with those obtained for mice injected with the antigen mixture (Table 4).

Skin reactivity to tuberculin in sensitized rabbits (injected with Freund's complete adjuvant) did not reveal any conclusive trend. The results are shown in Table 5. Ten days after the first antigen injection a significant decrease in the response of rabbits exposed to 10 ppm VC occurred while a significant increase occurred in those exposed to 1000 ppm. No differences in the skin test were observed when the skin test was repeated 10 days after the second antigen challenge.

Tetanus anti-toxin titers in the serum of antigen injected rabbits and mice are shown in Table 6. Vinyl chloride did not produce any effect on these parameters. Table 6 also includes data on the fluorescence evaluation of plasma cells in rabbit popliteal lymph nodes. Again, no influence of vinyl chloride exposure was evident.

Table 7 shows the relative amounts of immunoglobulins in mouse serum at different intervals of VC exposure. After 2 and 4 weeks of exposure, an increase was noticed in VC-exposed animals as compared to control values. A significant increase was noted at the 10 ppm VC level at 4 weeks of exposure. After 8 weeks of exposure, the control animals showed high values of immunoglobulins in their serum and this was the reason that the values in other treatment groups were significantly decreased. Similar data for immunoglobulin (IgG fraction) in rabbits are shown in Table 8. Again, the results are inconsistent and inconclusive. The group of rabbits exposed to the highest level of VC had somewhat lower preexposure serum IgG values. At the end of 8 weeks of VC exposure, however, the same group showed a significant rise in this immunoglobulin level as compared to the control group.

DISCUSSION

The results reported here suggest that vinyl chloride stimulated the immune responsiveness of rabbits and mice. The enhanced responsiveness developed rapidly upon exposure to high levels while 4 weeks of repeated exposure to lower levels (10 ppm) were needed to elicit a discernible response. Blast formation of lymphocytes in splenic cell cultures, as evidenced by an increased incorporation of tritiated thymidine into cellular materials, was the most sensitive indication of increased immune responsiveness. Vinyl chloride exposure at all levels increased the spontaneous lymphocyte transformation in splenic cell cultures, the effect being similar to that produced by injection of antigenic substances. The stimulating effect of two plant lectins, phytohemagglutinin and pokeweed mitogen, was also increased, further indicating an enhanced immune reactivity in these VC exposed animals.

The stimulating effect of vinyl chloride exposure on immune responsiveness does not appear to be a nonspecific phenomenon. It is unlikely that this phenomenon is stress induced since stress is generally known to cause immunosuppression. The decrease in lymphocyte transformation in splenic cultures observed in the control group of mice with increasing time interval appears to be consistent with stress induced immune suppression, since the exposure environment may provide some stress.

The immunologic responsiveness of the animals to other antigens was not influenced by exposure to VC. In fact, antigenic stimulation of immune

responses in our studies masked the stimulatory effect of vinyl chloride exposure. However, there was no suppression or interference of antigen induced immune effects observed when the animals were exposed to vinyl chloride, and there was an additive effect, if any.

The immunostimulatory effect of VC was not always dose-related. After a 2-week exposure period only mice exposed to 1000 ppm VC showed such an effect. After 4 and 8 weeks of exposure, the effect was seen at all levels of VC exposure. However, the effect in mice exposed to 1000 ppm VC for 8 weeks was less pronounced than observed in mice exposed for shorter durations. This suggests that with continued exposure to high levels of VC, the initial increase of immune responsiveness may reverse either as a result of adaption or a direct depressive effect of high levels of VC exposure.

The metabolism of vinyl chloride has been shown to be a saturable process (Watanabe et al., 1976). A dose-related saturable binding of vinyl chloride has also been shown in rat liver (Watanabe et al., 1977). Also, protein alkylating metabolites of vinyl chloride in an in vitro liver microsomal system have been demonstrated (Kappus et al., 1976). In view of these, it is not surprising that the immunostimulating effect of vinyl chloride is saturable since it too may possibly be mediated subsequent to biotransformation of VC in the body. It has been speculated that chloroethylene oxide is the reactive intermediate in the biotransformation of vinyl chloride. Formation of this alkylating intermediate,

although not conclusively shown, has been hypothesized as a possible proximate carcinogen. Alkylation of a normal body protein by such a chemical may produce an antigenic determinant and the resultant immunostimulation. Whether such possibility exists needs to be investigated further.

The immunostimulatory effects of vinyl chloride agree with the recent reports of Ward et al. (1976) that the vinyl chloride syndrome may involve an autoimmune phenomenon. The studies reported by these workers included patients with clinical effects of VC exposure. In our animal studies, the effects are shown after a relatively short duration of exposure. In these animal studies, an increased reactivity of lymphocytes, including those of "T" cells occurred since PHA is somewhat selective to mouse T-cells. A stimulation of T-cells has been reported to be an indication of an autoimmune phenomenon (Orgad and Cohen, 1974), a finding also consistent with the hypothesis proposed by Ward et al. (1976).

The immune stimulation caused by vinyl chloride exposure may be considered similar to the immune modulation caused by levamisole (Renoux et al., 1976). This chemical, which was first introduced as an animal anthelmintic, has been attempted as a therapeutic adjunct in cancer patients and has been shown to prolong the disease-free interval in patients with lung and breast cancer and malignant melanoma (Rojas et al., 1976; Wilkins and Olkowski, 1977). At the same time, this chemical enhances survival of allogeneic tumors in animal models and also in some patients. In the case of vinyl chloride, its carcinogenic property may be distinct from

its immune effects, but the latter may enhance the carcinogenic process once initiated even if not actually caused by it.

Other reports involving the immunostimulating properties of different chemicals have recently appeared. Renoux et al. (1976) reported an immunostimulating property of propylene glycol, a common solvent and vehicle for medicinal preparations. Goodman and Weigle (1977) have reported a dose-related lymphocyte stimulation by 2-mercaptoethanol and α-thioglycerol. These chemicals have a thiol moiety in common in their chemical structure, a property also shared by the molecule of levamisol (Renoux and Renoux, 1977). Two major metabolites of vinyl chloride, i.e., thiodiglycolic acid and N-acetyl-S-(2-hydroxyethyl)-cysteine, have been described (Watanabe et al., 1976), and are a result of glutathione conjugation by this chemical. It is possible that these metabolites may somehow be involved in the immunostimulatory property of vinyl chloride.

Written by:

R. P. Sharma RPS 9-20-78
R. P. Sharma

R. J. Kociba

H. O. Yakel 9/20/78
H. O. Yakel

P. J. Gehring 9/20/78
P. J. Gehring

Reviewed by:

B A Schwetz 21 Sept 78
B. A. Schwetz

REFERENCES

Andersson, J., Moller, G., and Sjoberg, O. (1972). Selective induction of DNA synthesis in T- and B-lymphocytes. Cell. Immunol. 4:381-393.

Berk, P. D., Martin, J. F., Young, R. S., Creech, J., Salikoff, I. J., Falk, H., Watanabe, P., Popper, H., and Thomas, L. (1976). Vinyl chloride associated liver disease. Ann. Int. Med. 84:717-731.

Bjerrum, O. J., Ingild, A., Lowenstein, H. and Weeke, B. (1973). Carbamylated antibodies used for quantitation of human IgG. A routine method. In, Quantitative Immunoelectrophoresis (Axelsen, N. H., Kroll, J. and Weeke, B., Eds) pp. 145-148. Universiters Forlaget, Oslo.

Dutton, R. W. and Eady, J. D. (1964). An in vitro system for the study of the mechanism of antigenic stimulation in the secondary response. Immunology 7:40-53.

Goodman, M. G., and Weigle, W. O. (1977). Lymphocyte stimulation by 2-merceptoethanol (2-ME) and α-thioglycerol (αTG). Federation Proc. 36:1288.

Harris, D. K. and Adams, W. G. F. (1967). Acroosteolysis occurring in men engaged in the polymerization of vinyl chloride. Brit. Med. J. 3:712-714.

Janossy, G. and Greaves, M. F. (1971). Lymphocyte activation. Response of T- and B-lymphocytes to phytomitogens. Clin. Exp. Immunol., 9:483-498.

Kappus, H., Bolt, H. M., Buchter, A. and Bolt, W. (1976). Liver microsomal uptake of ^{14}C vinyl chloride and transformation to protein alkylating metabolites in vitro. Toxicol. Appl. Pharmacol., 37:461-471.

Lange, C. E., Juhe, S., Stein, G. and Veltman, G. (1974). The so-called vinyl chloride disease - an occupational systemic sclerosis? Internat. Arch. Arbeitzmed., 32:1-32.

Maltoni, S., and LeFemine, G. (1974). Carcinogenicity bioassays of vinyl chloride, 1. Research plan and early results. Environ. Res., 7:387-405.

Orgad, S. and Cohen, I. R. (1974). Autoimmune encephalomylitis: Activation of thymus lymphocytes against syngeneic brain antigens in vitro. Science 183:1083-1085.

Page, M., Theriault, L. and Delorme, F. (1976). Elevated CEA levels in polyvinyl chloride workers. Biomedecine 25:279.

Renoux, G., Renoux, M., Teller, M. N., McMahon, S., and Guillaumin, J. M. (1976). Potentiation of T-cell mediated immunity by levamisole. Clin. Exp. Immunol. 25:288-296.

Renoux, M. L., de Montis, G., Roche, A. and Hemon, D. (1976). Experimental study of the study of a polyvitaminic preparation on humoral antibody response. Evidence for an immunostimulating activity of propylene glycol. La Nouvelle Presse Medicale 5:2053-2056.

Renoux, G. and Renoux, M. (1977). Roles of imidazole or thiol moiety on the immunostimulant action of levamisole. In, Control of Neoplasia by Modulation of the Immune System (Chirigos, M.A., Ed), pp. 67-80, Raven Press, New York. 603 pp.

Rojas, A. F., Mickiewicz, E., Feierstein, J. N. (1976). Levamisole in advanced human breast cancer. Lancet 1:211-215.

Sharma, R. P., Kociba, R. J. and Gehring, P. J. (1977). Immunotoxicologic studies involving 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice and rabbits. (in manuscript)

Steel, R. G. D. and Torrie, H. H. (1960). Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York.

Street, J. C. and Sharma, R. P. (1975). Alteration of induced cellular and humoral responses by pesticides and chemicals of environmental concern: Quantitative studies of immunosuppression by DDT, Aroclor 1254, carbamyl, carbofuran and methylparathion. Toxicol. Appl. Pharmacol., 32:587-602.

Stockman, G. D., Gallagher, M. T., Heim, L. R., South, M. N., and Trentin, J. J. (1971). Differential stimulation of mouse lymphoid cells by phytohemagglutinin and pokeweek mitogen (35410). Proc. Soc. Exp. Biol. Med. 136:980-982.

Ward, A. M., Udnoon, S., Watkins, J., Walker A. E., and Darke, C. S. (1976). Immunological mechanisms in the pathogenesis of vinyl chloride disease. Brit. Med. J. 1:936-938.

Watanabe, P. G., McGowan, G. R., and Gehring, P. J. (1976). Fate of ^{14}C vinyl chloride after single oral administration in rats. Toxicol. Appl. Pharmacol. 36:339-352.

Watanabe, P. G., Zempel, J. A., Pegg, D. G., and Gehring, P. J. (1977). Hepatic macromolecular binding following exposure to vinyl chloride. Toxicol. Appl. Pharmacol. (in press).

Wilkins, S. A., and Olkowski, Z. L. (1977). Immunocompetence of cancer patients treated with levamisole. Cancer 39:487-493.

TABLE 1

EXPOSURE CHAMBER ANALYSES FOR VINYL CHLORIDE[a]

	VC Concentration, ppm		
Desired Concentration	10	100	1000
Concentration determined (mean±S.D.)	9.9±0.64	101.3±4.8	982.8±34.9
Percent of exposure days within 10% of mean analytical concentration	87	98	100
Number of analyses (days x analytical determinations per day)	45x4	45x4	45x4

[a]VC concentrations were determined on a Varian 2400 gas chromatograph equipped with a flame ionization detector. The column used was 3' x 1/8" stainless steel, Porpak QS, 80/100 mesh, maintained at a temperature of 120°C (injector and detector at 260°C and 225°C, respectively). Carrier gas was He, 30 ml/min. Samples of 1 ml were analyzed and compared with an appropriate standard curve prepared simultaneously.

TABLE 2

ORGAN AND BODY WEIGHTS OF MICE EXPOSED TO VINYL CHLORIDE FOR DIFFERENT DURATIONS

Duration of Exposure	Level of Exposure (ppm)	Body Weight (g)	Organ Weights and Their Relation to Body Weight: Thymus (g)	Thymus (g/100g)	Spleen (g)	Spleen (g/100g)	Liver (g)	Liver (g/100g)	Kidney (g)	Kidney (g/100g)
Preexposure	0	35±2	0.05±0.01	0.14±0.01	0.10±0.01	0.27±0.01	1.91±0.07	5.42±0.28	0.54±0.05	1.53±0.20
2 weeks	0	36±3	0.04±0.02	0.12±0.03	0.09±0.01	0.24±0.00	1.98±0.30	5.46±0.46	0.59±0.09	1.64±0.18
	10	35±5	0.05±0.01	0.14±0.03	0.08±0.02	0.24±0.03	1.93±0.41	5.48±0.47	0.58±0.09	1.67±0.20
	100	36±2	0.04±0.01	0.12±0.02	0.09±0.02	0.23±0.04	1.99±0.23	5.49±0.35	0.57±0.06	1.58±0.16
	1000	35±4	0.04±0.01	0.10±0.02	0.13±0.01*	0.38±0.07*	2.09±0.29	5.92±0.29	0.64±0.08	1.83±0.05
4 weeks	0	34±2	0.05±0.01	0.15±0.04	0.07±0.02	0.21±0.05	1.75±0.15	5.15±0.23	0.55±0.07	1.63±0.21
	10	38±4	0.05±0.02	0.14±0.03	0.10±0.03	0.25±0.05	2.29±0.22*	5.98±0.45*	0.66±0.08	1.74±0.29
	100	35±1	0.05±0.01	0.13±0.04	0.08±0.01	0.22±0.02	2.06±0.19	5.92±0.45*	0.58±0.06	1.66±0.15
	1000	37±2	0.05±0.01	0.14±0.03	0.11±0.01*	0.30±0.04*	2.11±0.11*	5.69±0.29	0.64±0.05	1.73±0.12
8 weeks	0	38±4	0.04±0.01	0.11±0.03	0.09±0.03	0.23±0.06	2.09±0.33	5.57±0.37	0.67±0.11	1.78±0.18
	10	42±2	0.03±0.01	0.07±0.02	0.10±0.01	0.23±0.03	2.46±0.21	5.90±0.42	0.76±0.04	1.83±0.10
	100	42±3	0.05±0.01	0.13±0.04	0.10±0.01	0.23±0.05	2.30±0.08	5.51±0.30	0.70±0.06	1.67±0.12
	1000	42±1	0.05±0.02	0.11±0.05	0.11±0.01	0.26±0.03	2.02±0.15	4.82±0.26*	0.64±0.06	1.53±0.14

Mean ± SD of 4 animals per group.

*Indicate significant deviation from control ($p<.05$, Dunnett's test).

TABLE 3

BODY AND ORGAN WEIGHTS OF RABBITS EXPOSED TO VINYL CHLORIDE FOR 8 WEEKS

	Exposure to Vinyl Chloride, ppm			
	0 Control	10	100	1000
Body Weight (kg)	3.4±0.3	3.1±0.2	3.3±0.3	3.5±0.4
Organ weight, g, (organ/body wt. g/100g) for				
Liver	63.07±9.05 (1.88±0.16)	54.69±7.55 (1.79±0.22)	61.44±5.05 (1.88±0.20)	64.61±8.87 (1.86±0.15)
Kidneys	15.51±1.61 (0.46±0.03)	13.02±1.20 (0.43±0.03)	14.66±1.96 (0.44±0.03)	15.56±1.45 (0.45±0.03)
Brain	9.26±0.67 (0.28±0.02)	9.33±0.55 (0.31±0.03)	9.19±0.60 (0.28±0.01)	9.82±0.79 (0.28±0.03)
Heart	5.81±0.35 (0.17±0.01)	5.81±0.42 (0.19±0.01)	6.18±0.18 (0.19±0.02)	6.43±0.95 (0.19±0.02)
Thymus	3.63±0.89 (0.11±0.02)	2.41±0.77 (0.08±0.02)	4.59±0.78 (0.14±0.01)	5.15±0.96* (0.15±0.02)*
Spleen	1.78±0.55 (0.05±0.02)	1.51±1.00 (0.05±0.03)	1.44±0.37 (0.04±0.02)	1.12±0.26 (0.03±0.01)
Adrenals	0.38±0.9 (0.01±0.00)	0.46±0.15 (0.01±0.00)	0.50±0.11 (0.02±0.00)	0.41±0.08 (0.01±0.00)
Popliteal Lymph Nodes	0.40±0.08 (0.01±0.00)	0.37±0.05 (0.01±0.00)	0.40±0.05 (0.01±0.00)	0.40±0.14 (0.01±0.00)

Mean ± SD of 5 animals per group.

*Indicate significant deviation from control ($p<.05$, Dunnett's test).

TABLE 4

INCORPORATION OF TRITIATED THYMIDINE IN SPLENIC LYMPHOCYTES OF MICE AND RABBITS EXPOSED TO VINYL CHLORIDE AND CHALLANGED WITH A MIXTURE OF TETANUS TOXOID AND FREUND'S ADJUVANT[a]

	Mice			Rabbits		
Vinyl Chloride Exposure ppm	DPM/10^6 Cells Without any Mitogen	Stimulation Index[b] PHA	PWM	DPM/10^6 Cells Without any Mitogen	Stimulation Index[b] PHA	PWM
0	11,736±3,584	8.1±4.8	6.5±2.9	36,926±5,489	15.9±1.3	3.3±0.4
10	17,629±2,353	6.8±0.9	11.4±2.0	59,809±7,444*	14.2±2.8	8.0±1.5*
100	12,406±3,195	11.0±3.1	11.9±2.2	67,286±13,991*	13.5±1.5	5.2±1.4
1000	5,774±1,645	5.5±0.9	4.1±0.5	46,026±9,248	12.0±1.2*	4.1±0.5

[a]Animals were injected with a mixture of tetanus toxoid and Freund's complete adjuvant, 2 and 4 weeks prior to their sacrifice. The values are Mean ± SE. Four animals per group of mice, whereas 5 rabbits per group were averaged.

[b]Stimulation index = dpm in the presence of a mitogen divided by the dpm without any mitogen.

*Indicate significant differences from respective control group.

TABLE 5

SKIN REACTIVITY TO TUBERCULIN IN RABBITS EXPOSED TO VINYL CHLORIDE AND SENSITIZED WITH FREUND'S ADJUVANT

Conc. of Vinyl Chloride, ppm	Primary Skin Response[a]		Secondary Skin Response	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.
0	20.8±3.3	15.8±1.4	20.0±2.6	15.6±3.9
10	7.8±1.7*	11.0±1.5*	17.6±1.6	18.2±2.0
100	14.2±2.1	16.6±1.2	21.6±0.5	16.8±1.6
1000	26.2±1.9	25.6±1.0*	21.4±0.8	15.8±1.4

[a]Primary and secondary response is 10 days after the first and second challange of adjuvant, respectively. The values are millimeters of diameter of skin reaction. Mean ± SE of 5 animals per group.

TABLE 6

SERUM ANTITETANUS HEMAGGLUTINATION TITERS IN RABBIT AND MOUSE AND THE EVALUATION OF PLASMA CELLS IN RABBITS EXPOSED TO VINYL CHLORIDE

Exposure Conc. of Vinyl Chloride ppm	Serum Antitetanus Titers			Rabbit Popliteal Lymph Node fluorescence Score[c]
	Rabbit[a]		Mouse[b]	
	Primary	Secondary	Secondary	
0	10.4±2.4	17.6±3.9	5.0±1.0	11.0±0.6
10	6.4±1.0	11.2±2.0	4.0±0.0	10.8±1.1
100	12.8±4.8	8.6±2.1	6.0±1.2	10.6±0.5
1000	6.0±1.3	13.6±5.0	4.0±0.0	11.4±1.7

[a]Primary and secondary titers are 2 weeks after the first and second antigen challange, respectively.

All values are means ± SE of mean for 5 samples per group.

[b]Mean ± SE of 4 samples per group, 2 weeks after the second antigen challange.

[c]Evaluation of popliteal lymph node by observing 10 random fields in a blind random fashion under a microscope. See Methods for details.

TABLE 7

SERUM IMMUNOGLOBULIN IN MICE EXPOSED TO VINYL CHLORIDE

Vinyl Chloride Conc., ppm	Preexposure	Serum Ig at[a] 2 weeks	4 weeks	8 weeks
0	0.8±0.1	1.2±0.1	0.9±0.1	2.1±0.2
10	-	1.2±0.1	1.6±0.3*	1.2±0.2*
100	-	1.2±0.1	1.2±0.2	1.7±0.3
1000	-	1.8±0.3	1.4±0.3	1.7±0.1*

[a]Values indicated are the ratios of total serum Ig to that in normal pooled mouse serum. Mean ± SE of 4 animals per group.
*Indicate significant deviation from control at $p<.05$.

TABLE 8

SERUM IgG IN RABBITS EXPOSED TO VINYL CHLORIDE

Vinyl Chloride Conc., ppm	IgG, mg/ml, at Preexposure	4 weeks	6 weeks	8 weeks
0	17.4±2.1	18.3±2.2	25.6±1.7	27.2±1.0
10	13.5±1.9	14.6±2.1	21.2±1.6*	20.6±3.6
90	20.3±2.8	15.1±1.5	16.8±2.5*	25.1±2.2
1000	12.3±1.4*	19.9±1.8	14.0±1.9*	25.9±1.4*

Mean ± SE of 5 observations per group.

*Indicates statistically significant difference from respective control group at $p<.05$.

LEGENDS TO FIGURES

FIGURE 1 Thymidine uptake by lymphocyte cultures prepared from mice exposed for 2 weeks to vinyl chloride. Average of 4 animals, the cells cultured in triplicate. Values significantly different from controls are marked with an asterisk ($p<0.05$).

FIGURE 2 Thymidine uptake by lymphocyte cultures prepared from mice exposed for 4 weeks to vinyl chloride. Average of 4 animals, the cells cultured in triplicate. *Indicates values significantly different from respective control ($p<0.05$).

FIGURE 3 Thymidine uptake by lymphocyte cultures prepared from mice exposed for 8 weeks to vinyl chloride. Average of 4 animals, the cultures performed in triplicate. *Indicates values significantly different from controls at $p<0.05$.

FIGURE 4 Response of mouse splenic lymphocytes to PHA and PWM after 2-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicate values significantly different from controls ($p<0.05$). Stimulation index = dpm in the presence of a mitogen divided by the dpm without any mitogen.

FIGURE 5 Response of mouse splenic lymphocytes to PHA and PWM after 4-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicate values significantly different from controls at $p<0.05$. Stimulation index = dpm in the presence of a mitogen divided by the dpm without any mitogen.

FIGURE 6 Response of mouse splenic lymphocytes to PHA and PWM after 8-week exposure to vinyl chloride. All values are average of 4 animals, the cells cultured in triplicate. Vertical bars represent standard error of mean and * indicate values significantly different from controls at $p<0.05$. Stimulation index = dpm in the presence of a mitogen divided by dpm without any mitogen.

Figure 1

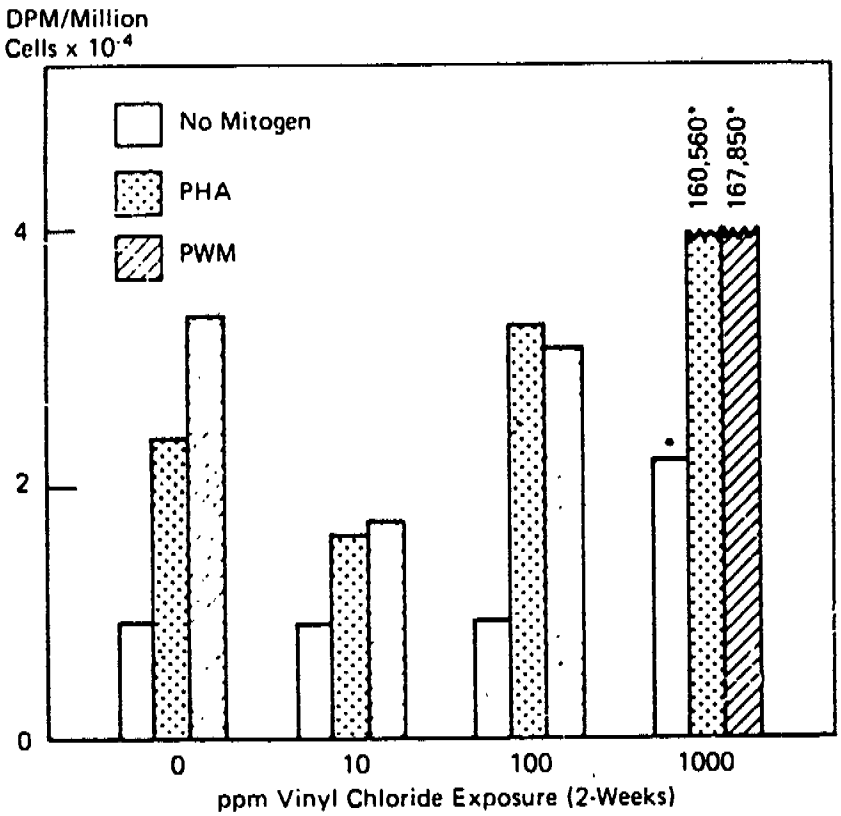

Figure 2

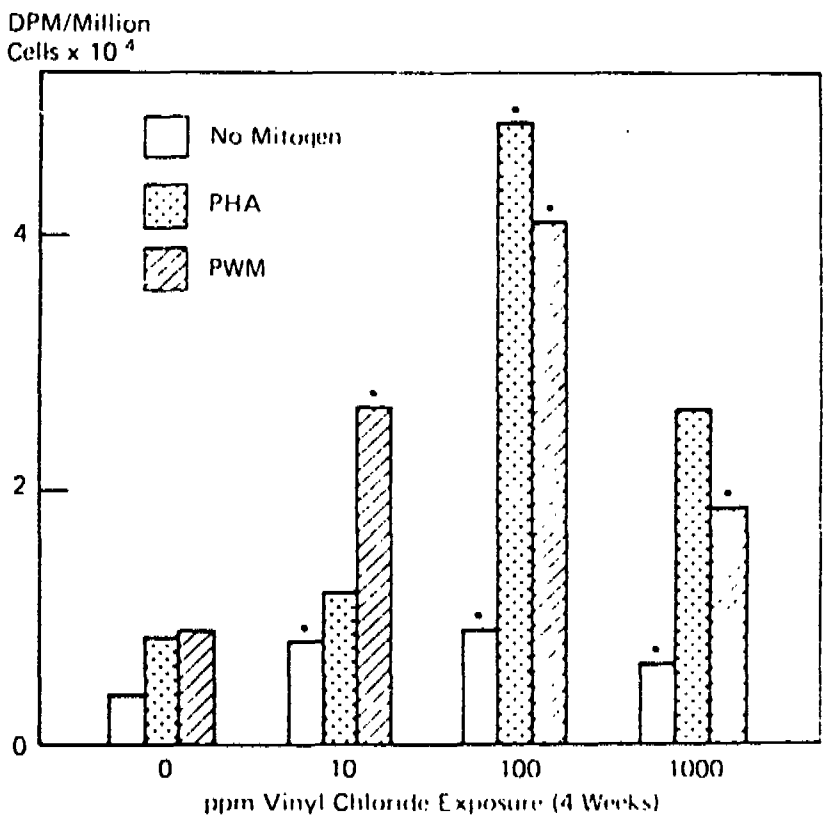

Figure 3

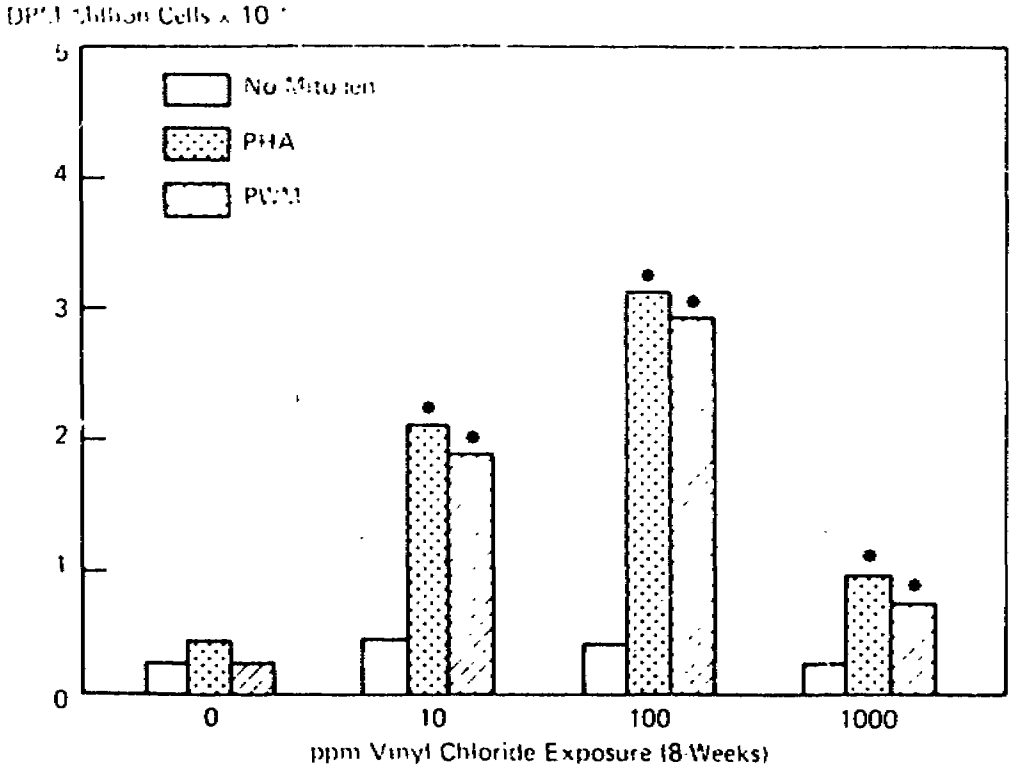

Figure 4

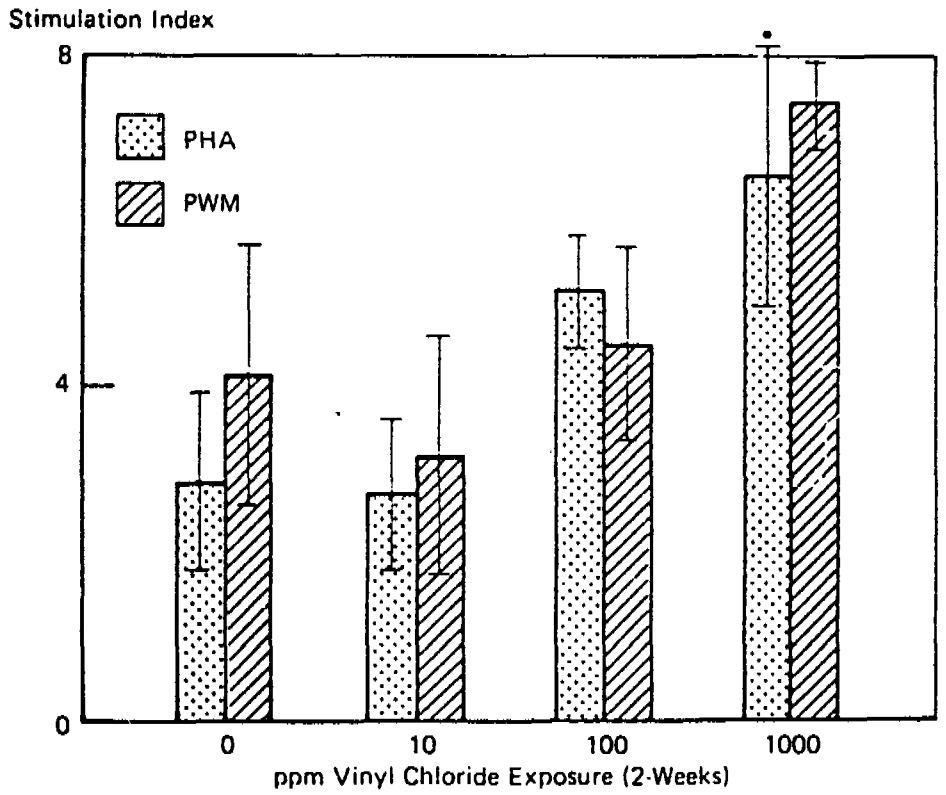

Figure 5

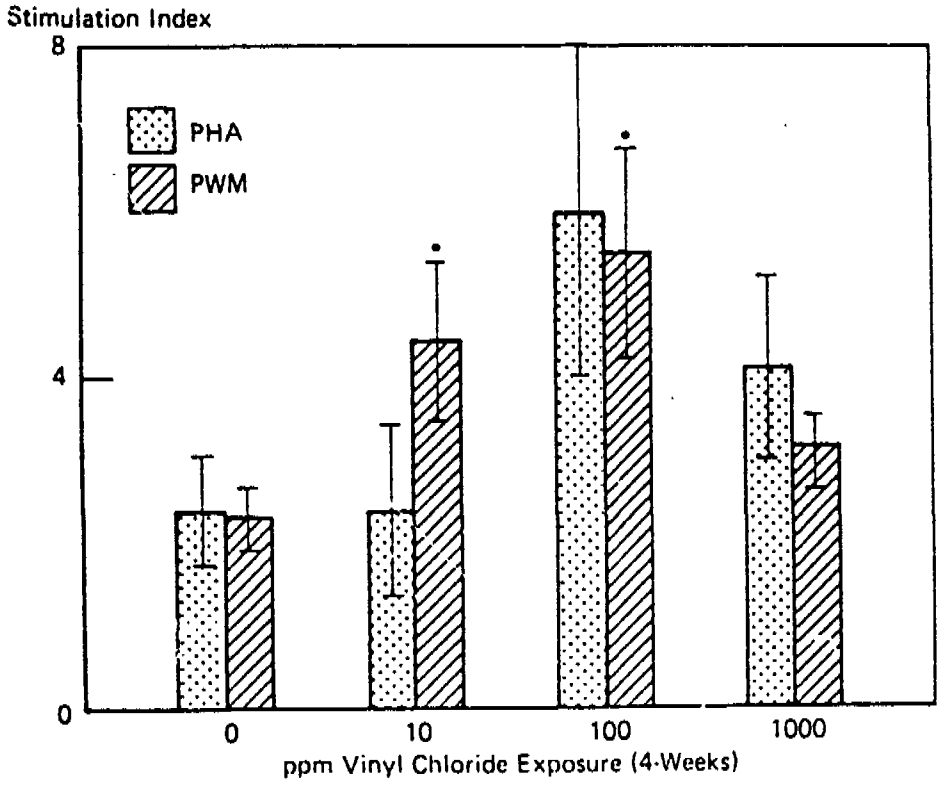

Figure 6