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Investigation of the Hepatotoxic and Immunotoxic Effects of the Peroxisome Proliferator Perfluorodecanoic Acid

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13. ABSTRACT *(Maximum 200 words)*

Our research efforts of the past six months have involved evaluation of the immunotoxic and toxic effects of perfluorodecanoic acid (PFDA). Eight day exposure to PFDA caused thymic atrophy with marked thymocyte depletion as well as decreased spleen cellularity at 50 mg/kg. Con A-induced T cell mitogenic responses were decreased at 20 mg/kg PFDA, but no effect on B cell proliferation was observed. Antigen-induced proliferation was decreased in both 20 and 50 mg/kg PFDA-exposed animals, although delayed-type hypersensitivity reactions did not vary significantly from control animals. Interestingly, IL-2 activity of cell culture supernates was increased with PFDA exposure, although NK cell activity was similar to control and lymphocyte subsets (T_h and $T_{s/c}$ cells) were not affected. In addition, some other effects of PFDA were noted: increased hepatocellular hypertrophy and granularity with an increase in immature cell numbers; also an increase in the kidney to body weight of PFDA treated rats compared to control was observed.

14. SUBJECT TERMS
Perfluorodecanoic acid; immunotoxic; thymic atrophy; lympho-proliferation; delayed-type hypersensitivity; NK cell activity; lymphocyte subsets; interleukin 2

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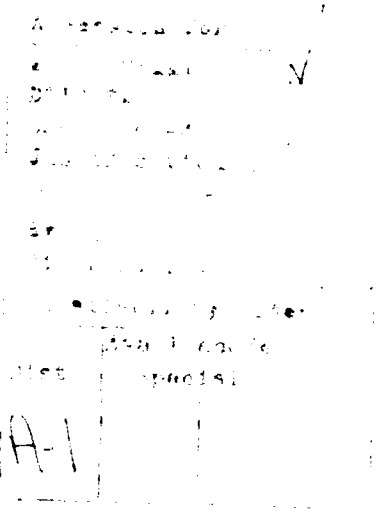

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I. STATEMENT OF WORK

The following is a list of original specific aims for which work has been completed or is ongoing since the beginning of the Grant funding period (October 1990).

A. Evaluation of the immunotoxic effects of PFDA.

We have completed most of the Tier I experiments associated with 8 day exposure *in vivo* and are beginning Tier I experiments to examine the 30 day exposure period to PFDA *in vivo*. We have also begun to titrate PFDA to evaluate immunologic responses *in vitro*.

1. Tier I

   a. Determine the morphologic changes of various lymphoid organs associated with PFDA exposure *in vivo*.

   b. Determine the effect of PFDA on T-cell and B-cell proliferation.

   c. Determine the effect of PFDA on T-cell function (DTH reaction).

   d. Determine the effect of PFDA on Natural Killer (NK) cell function.

   e. Determine the effect of PFDA on B-cell function (serum antibody production).

2. Tier II

   Some Tier II studies were initiated during the past few months to titrate antibodies and reagents that will be used for these studies.

   a. Determine the effects PFDA on lymphokine (IL-2) production.

   b. Determine the effects PFDA on lymphoid cell populations by measuring cell surface antigen expression.

B. Characterize the kinetics of the cell cycle of hepatocytes from PFDA-treated rats and hepatocytes exposed *in vitro* to PFDA using flow cytometry.

C. Evaluate changes in hepatocyte surface or cytoplasmic enzyme expression in PFDA-treated rats.

D. Examine the effects of PFDA upon hepatocyte surface igA receptor expression and serum IgA levels.

II. RESEARCH STATUS

The following is a detailed account of the rationale, methods, results, and significance of each specific aim.

A. Evaluation of the morphologic changes in various lymphoid organs (spleen, thymus, and lymph nodes), kidney and liver.

1. Rationale

Various chemicals have been shown to alter lymphoid organ morphology and histopathology. PFDA and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) cause thymic atrophy in treated animals (Andersen *et al.* 1981; Vos *et al.*, 1974). We examined the morphologic changes of several rat lymphoid organs (to define PFDA-associated organ immunotoxicity), as well as liver and kidney upon *in vivo* exposure. In addition, whole body and daily food intake were recorded.

2. Methods

Lymphoid organs, kidney and liver were removed aseptically, weighed (organ to body weight ratios calculated), and placed in culture media for other experiments. A small section was removed and fixed in formalin. Fixed tissues were dehydrated, embedded in paraffin, sectioned at 5-6 μm and stained with hematoxylin and eosin.

Data were analyzed initially by analysis of variance using the SAS

statistical package. If a significant treatment effect was found, the analysis was followed by Tukey's Studentized Range Test which was used to perform multiple means comparisons between treatments. A treatment group was considered to be significantly different from the control group (or from the associated pair-fed group) when $p < 0.05$. Il-2 data (section E.) were analyzed by multiple linear regression, also using SAS ($n = 3$).

"*" or "a" = significantly different from control

"b" = significantly different from pair-fed

3. Results

a. Body Weights

The weights of all animals (including normal controls) were recorded prior to PFDA or vehicle injection and after the 8 day exposure period. The change in animal weight for this 8 day period is reported in Figure 1. Both PFDA-treated and pair-fed Fischer 344 rats had significantly reduced body weights when compared to control rats. However, the body weights of the PFDA-treated rats, although somewhat lower, were not significantly different from their pair-fed controls.

b. Thymus Weights

The relative thymus weights (thymus to body wt. ratio) of 50 mg/kg PFDA-treated rats were significantly decreased compared to normal control rats, but were not significantly different from their pair-fed controls. In addition, the relative thymus weights of the 50 mg/kg pair-fed rats were also significantly less than normal control weights (Figure 2).

c. Spleen Weights

The relative spleen weights obtained from the 20 mg/kg and 50 mg/kg PFDA-treated rats were significantly decreased compared to that of both normal and pair-fed control rats (Figure 3).

d. Liver Weights

The relative liver weights obtained from both 20 mg/kg and 50 mg/kg PFDA-treated rats were significantly increased when compared to those obtained from normal and pair-fed control rats. The relative liver weight of the pair-fed (50 mg/kg) rat was also significantly decreased from normal controls (Figure 4).

e. Kidney Weights

The relative kidney weights of the 50 mg/kg PFDA-treated rats were significantly increased compared to that of both normal and pair-fed control rats (Figure 5).

f. Histopathology

Our studies revealed a moderate to marked thymocyte depletion in 50 mg/kg PFDA-treated rats, while pair-fed rats showed a decrease in overall size of the thymus, but normal anatomy and cellularity. The other consistent alteration in 50 mg/kg PFDA-treated rats was hepatocellular hypertrophy and increased granularity, as well as an increased number of immature hepatocytes. In contrast, the pair-fed rats exhibited only marked hepatocellular atrophy. Other changes noted in Table I were mild and inconsistent.

g. Daily Food Intake

The mean daily food intake was recorded for normal control, and PFDA-treated/Pair-fed rats. 50 mg/kg PFDA-treated rats ingested less food than either normal or 20 mg/kg PFDA-treated rats each day of the 8 day exposure period (Figure 6).

4. Significance

A single i.p. dose of PFDA produces hypophagia and severe weight loss, anorexia, thymic atrophy and marked dose-dependent hepatomegaly. Pair-fed control rats did not lose as much weight as PFDA-treated rats even though food intake was the same. Normal control rats exhibited an increase in weight over the 8 day period. These

results correlated with the overall weight changes previously described in rats exposed to PFDA (Van Rafelghem *et al.*, 1982; George and Andersen, 1986).

Fifty mg/kg PFDA-treated rats exhibited a decrease in the thymus to body weight ratio as reported previously by Olsen and Andersen (1983). This decrease in thymus weight was also associated with thymic atrophy and necrosis as reported by Andersen *et al.* (1981). The observed decrease in the relative spleen weights of PFDA-treated rats (20 and 50 mg/kg) extends observations by Harris *et al.* (1989) for mice exposed to 80 mg/kg (approximate LD_{50}). They reported decreases in the absolute and relative spleen weights in surviving animals. In addition, we noted a decrease in spleen cellularity of 50 mg/kg PFDA-treated rats upon isolation of single cell suspensions for our immunologic assays. These changes in overall spleen cell number occurred only in the 50 mg/kg PFDA-treated correlating with the observed histopathology of these rats and was not evident in the pair-fed controls.

There was a significant increase in the relative liver weights obtained from both 20 mg/kg and 50 mg/kg PFDA-treated rats compared to control confirming results reported by George and Andersen (1986). This increase in liver weight corresponds to both hypertrophy, increased granularity, and hyperplasia associated with hepatocellular peroxisome proliferation in PFDA-exposed animals. The marked hepatocellular atrophy of the 50 mg/kg pair-fed animals was the result of the pair-feeding regimen.

We observed an increase in the relative kidney weight of 50 mg/kg PFDA-exposed rats, although histopathology was inconclusive for overall changes. George and Andersen had previously reported an increase in the kidney to body weight ratios for rats exposed to 50 mg/kg PFDA, as did Harris *et al.* (1989) for mice. PFDA does not appear to induce peroxisome proliferation in the kidney as does di(2-ethylhexyl)phthalate,

a promoter for renal carcinomas in the rat (Kurokawa *et al.*, 1988).

B. Characterization of *in vivo* and *in vitro* effects of PFDA on lymphocyte proliferation.

1. Rationale

Lymphocyte proliferation induced by T- and B-cell specific mitogens is a common immunologic tool utilized in assessing a chemical's immunotoxicity. *In vitro* mitogenic stimulation is similar to antigen-induced lymphocyte proliferation, except that the response is polyclonal and not antigen specific. The mitogens concanavalin A (con A) and lipopolysaccharide (LPS), specific for T- and B-cells, respectively, and KLH (T-cell dependent antigen) were used to induce lymphocyte blast transformation (LBT) *in vitro* in our evaluation of PFDA-treated, pair-fed and control lymphoid cells.

2. Methods

a. *In vivo* administration of PFDA: Rats were sacrificed 8 days after i.p. injection of PFDA and spleens removed. Splenocyte suspensions of 2 X 10^5 cells/well in 96-well microculture plates were incubated with 2.0 μg/ml Con A or 10 μg/ml LPS (for mitogenic response), or 5.0 μg/ml KLH (for antigenic response) for 72 hours. [^{3}H]-thymidine incorporation during last 6 hours of cell culture of LBT was measured by liquid scintillation spectrophotometry.

b. *In vitro* lymphoproliferation experiments: Splenocytes were harvested from normal rats and cultured *in vitro* with various concentrations of PFDA. Splenocytes were stimulated with both Con A and LPS to induce lymphocyte proliferation and measured as described above.

3. Results

a. Effect of *in vivo* exposure to PFDA on mitogen-induced

lymphocyte proliferation

In vivo PFDA exposure significantly suppressed T cell proliferation in response to Con A at the low dose of 20 mg/kg compared to both normal and pair-fed control groups. However, at the high dose of 50 mg/kg PFDA *in vivo*, T cell proliferation in response to con A was not significantly decreased and almost equal that of control rats. In addition, the response of cells 50 mg/kg PFDA-treated rats was significantly increased compared to cells from pair-fed (50 mg/kg) rats (Figure 7a).

In vivo PFDA exposure significantly suppressed lymphocyte proliferative response to KLH compared to their respective pair-fed groups, but not when compared to controls. The response of the cells isolated from pair-fed animals to KLH exceeded that of the control group and was significantly increased in the 20 mg/kg pair-fed animals (Figure 7b).

In vivo PFDA exposure did not significantly alter B cell proliferation in response to LPS at either dose when compared to pair-fed and normal control rats (Figure 7c).

b. Effect of *in vitro* exposure to PFDA on mitogen-induced lymphocyte proliferation

The data presented here represent that of a single experiment. A dose-dependent suppression of the T cell proliferative response to Con A was observed without significant reduction of cell viability (Figure 8).

4. Significance

The results of the experiments performed to date point towards PFDA-induced alteration of T cell proliferation but not B cell. The mitogenic response of B cells from PFDA-exposed rats did not differ significantly from pair-fed or normal controls. T cell mitogenic responses were somewhat varied with respect to amount of PFDA injected *in vivo*,

the mitogen (Con A) or antigen (KLH) used, and pair-feeding regimen. This variation may stem from the complexity of interactions within the whole animal due to chemical exposure, loss of appetite, and weight reduction. *In vitro* exposure of lymphocytes to PFDA is not influenced by all these factors and resulted in a nice dose-dependent suppression of the lymphoproliferative response.

C. Evaluation of cell-mediated immune responses.

1. Rationale

Several assays measure cell-mediated immune response; delayed-type hypersensitivity (DTH) and the mixed lymphocyte reaction (MLR) are the most commonly used. These reactions are mediated by specific T cells: the T_{dth} cell for DTH response, and the cytotoxic T cell (T_c cell) for the MLR. An *in vivo* DTH response can be measured by foot pad swelling in response to antigen sensitization. For our purposes, the DTH reaction was preferred for evaluation of cell-mediated immunity in our multiple-assay-single-animal use protocol.

2. Methods

The technique described by Exon *et al.* (1986) was followed. Briefly, PFDA-naive were injected twice, subcutaneously (7 days apart) in the caudal tail fold with 1.0 mg/ml KLH antigen. KLH was injected into the left footpad and sterile vehicle (control) injected into the right footpad 24 hours prior to sacrifice, at which time footpad swelling was measured using calipers. Results were calculated as shown below:

$$\text{Footpad Thickness}_{expt} - \text{Footpad Thickness}_{ctrl} = \text{measure of DTH}$$

The 8 day experiment schedule allowed us to determine the effects of PFDA on the effector stage of the cell-mediated immune response, as this group of animals was immunized with KLH prior to PFDA exposure.

The proposed 30 day experiments will allow measurement of the DTH response during the induction phase because KLH immunization will follow PFDA exposure.

3. Results

A slight decrease in DTH response was observed with PFDA-treated rats, but this was not dose-dependent or statistically significant compared to control or pair-fed rats (Figure 9).

4. Significance

The 8 day experimental setup allowed the effector phase of the DTH proliferative response to KLH antigen *in vivo* be evaluated. PFDA perturbance of the *in vivo* cell-mediated immune reaction as measured by DTH response somewhat paralleled the antigen (KLH)-induced lymphoproliferative response measured *in vitro*. These results are not too surprising in that a proliferative response to the KLH antigen was being measured in both assays. PFDA caused a decrease in the DTH response compared to control although not significant, while pair-fed animals showed a slight but not significant increase in DTH response. Stress induced by pair feeding regimen in pair-fed control rats may have contributed to DTH response observed *in vivo*.

D. Examination of Natural Killer cell activity *in vivo* and *in vitro*.

1. Rationale

Natural killer (NK) cells provide a line of immunosurveillance against possible tumor cells. NK cell activity is closely correlated to the induction and release of interferon (IFN) and interleukin 2(IL-2). Thus the affects on NK function could be due to direct effects on NK activity or indirectly by activation of NK cells.

2. Methods

NK cell analysis was adopted from that reported by Slezak and Horan (1989). Briefly, PFDA-treated, pair-fed and normal rat splenocytes

were isolated by Ficoll-Paque. Mononuclear cells were suspended at a concentration of 1 x 10^7 cells/ml in complete medium, and were incubated overnight in 24-well tissue culture plates in a humidified incubator at 37°C, 5% CO_2. Nylon wool columns were prepared to further remove adherent cells and B cells. Nylon wool (6g) was placed into syringes (12ml) up to the 6ml mark, and syringes were autoclaved. Columns were washed with phosphate buffered saline (50ml), and pre-warmed complete medium was added to the columns. Cells were suspended at a concentration of 5 x 10^7cells/ml, and were run onto the column. Complete medium (1ml) was used to wash the cells onto the columns. Complete medium (2ml) was added to the top of each column. Columns were sealed with parafilm, and were incubated at 37°C, 5% CO_2 for one hour. Effector cells were eluted from each column with complete medium (30ml). Effector cell concentration was adjusted to 1 x 10^7 cells/ml, and serial dilutions were prepared at ratios of 1:2 and 1:4.

Cultures were prepared in 96-well microtiter plates. Effector cell:target cell ratios of 100:1, 50:1, and 25:1 were prepared by combining 100μl of the appropriate effector cell suspension with 100μl of the target cell suspension. YAC-1 target cells were stained with PKH-2 as per manufacturer's (Zynaxis Cell Science, Inc.) instructions and were suspended in complete medium at a concentration of 1 x 10^5 cells/ml. Plates were centrifuged at 50 x g for 10 minutes and were incubated at 37°C, 5% CO_2 for four hours. Cytotoxicity was

determined by exclusion of propidium iodide as assessed by flow cytometry and distinguished by green fluorescence (PKH-2) and red fluorescence (PI incorporation) of NK-lysed tumor cells at 488nm.

3. Results

The results shown in Table II are derived from a single experiment using this technique. The original method of evaluation of NK activity proposed in the grant did not allow clear separation of effector and targets cells for enumeration of target cell killing by rat NK cells. This preliminary study reveals that the NK cell activity of PFDA-treated Fischer 344 rats is similar to that of normal and pair-fed controls, although a slight decrease in NK killing was observed at both 20 and 50 mg/kg PFDA.

4. Significance

Peroxisome proliferators are a structurally diverse group of chemicals that have been found to induce hepatocellular carcinomas (Reddy and Azarnoff,1980; Warren *et al.*, 1980). The carcinogenic potential of PFDA and similar perhalogenated compounds raises the question of whether natural immune surveillance may be compromised upon exposure to these compounds. Our analysis of NK activity will provide further information regarding the relationship of these types of carcinogenic agents on the innate immune system.

E. *Evaluation of the effects of PFDA on lymphokine (IL-2) production.*

1. Rationale

Interleukin 2 (IL-2) is a lymphokine produced by the helper T-cell population following antigen or mitogen stimulation. *In vitro*, IL-2 enhances antibody synthesis by B-cells, produces cytotoxic T-cell proliferation and is essential for maintenance of long term T-cell culture lines. *In vivo* administration of IL-2 in mice accelerates allograft rejection and regression of established tumors. Based upon its immunoenhancing properties, IL-2 is now being examined as a possible immunotherapeutic agent. Thus it is important to evaluate the effects of PFDA on IL-2 production and activity due to the fundamental role IL-2 has in controlling the immune response.

2. Methods

Spleen cells were harvested from either normal, pair-fed or PFDA-treated rats and cultured in the presence of con A to stimulate IL-2 production. Cell culture supernates were harvested after 24 hours, and the IL-2 activity quantitated by *in vitro* proliferation of an IL-2-dependent cell line, CTLL-20. Proliferation was measured by ^{3}H-thymidine incorporation into newly synthesized cellular DNA. Culture supernatant IL-2 activity was compared to the response from a standard IL-2 preparation.

3. Results

IL-2 production in response to con A was elevated in cells isolated from PFDA-treated rats when compared to pair-fed and normal controls. IL-2 production in response to con A was decreased in cells isolated from pair-fed Fischer 344 rats. The treatment effect was determined to be significant ($p < 0.0002$) by multiple linear regression (Figure 10).

4. Significance

The IL-2 bioassay is a standard method of evaluating IL-2 production and activity. We report a significant increase in proliferative response of an IL-2-dependent cell line to cell culture supernates from PFDA-treated rats compared to controls. These results point toward a PFDA-induced increase in IL-2 production or activity within these supernates. These results are exciting and perplexing due to the regulatory role that IL-2 plays during an immune reaction. It is difficult to extrapolate from the results of this bioassay to the other methods of immune function analysis since there is some variation in the observed responses (DTH and lymphoproliferation assays). This enhancement of IL-2 production by PFDA deserves further evaluation beyond that proposed by using molecular biological techniques to better characterize increased protein production or expression of messenger RNA. We plan on coupling our IL-2 receptor (see section G.1.c) work with further studies to provide more continuity to our evaluation of this lymphokine.

F. Evaluation of humoral immunity.

1. Rationale

Altered antibody production and secretion by B-cells is a measure of chemically-induced immune dysfunction. Antigen stimulated B-cells secrete IgM as a primary response, but require T-cell help to elicit specific IgG and IgA antibody production for T-cell dependent antigens, like KLH. Thus changes in T-cell help or B-cell sensitivity may be altered upon PFDA exposure.

2. Methods

Enzyme-linked immunosorbent assay (ELISA) of serum antibodies in rats injected with KLH will be performed as described by Exon *et al.* (1986). Briefly, serum samples collected at sacrifice will be evaluated for IgM, IgG, and IgA antibodies by diluting serum in a phosphate buffered saline-Tween 20 solution and incubating in 96-well microtiter plates which have been coated with 2.0 mg/ml KLH for 1 hour at 37°C. Commercially available anti-rat IgM, IgG, or IgA antibody conjugated to alkaline phosphatase will be added and incubated for 1 hour at 37°C. The substrate for alkaline phosphatase, p-nitrophenol phosphate, will be added to each well after extensive washing and the color reaction that develops will be quantified by spectrophotometry at 405nm.

3. Results

Serum samples have been collected from PFDA-exposed, pair-fed

and normal control rats and will be evaluated in the near future.

G. Examination of lymphoid cell populations exposed to PFDA by measuring cell surface antigen expression.

1a. Rationale, lymphocyte subset antigens

Functional subsets of rat lymphocytes can be distinguished by their cell surface antigens. Recent development of monoclonal antibodies (mAbs) to rat lymphocyte antigens can distinguish T and B lymphocyte populations. T-lymphocytes can be further divided into subclasses of helper/inducer and suppressor/cytotoxic T-cells by mAbs as well. Alteration of these T-cell subsets in absolute numbers, ratio or function has been associated with certain autoimmune and immunodeficiency diseases. PFDA-induced thymic atrophy in rats as reported by Van Rafelghem *et al.* (1982) and confirmed by our experiments, warrants further investigation of the effects of PFDA on various lymphoid populations as determined through immunofluorescence and flow cytometry.

2a. Methods, lymphocyte subset antigens

Lymphocytes will be isolated from spleen, lymph nodes, and thymus of normal and PFDA-treated rats. The cell suspensions will be incubated with fluorochrome-conjugated mAbs against B-cells, helper T-cells and cytotoxic T-cells. These cells will be evaluated for the percent of cells expressing these cell surface markers and the density of antigen

(relative fluorescence) per cell by flow cytometry.

1b. Rationale, Ia antigen

The immune response is characterized by interactions of foreign antigens with the Major Histocompatibility Complex (MHC) antigens and various receptors on helper, effector, and suppressor T-cells. The MHC antigens is divided into two types: Class I, which are present on virtually every cell and Class II, which are present only on cells of the immune system. Although both classes are important, MHC Class II or Ia antigens, present in high density on accessory cells, are important in the recruitment of appropriate responder cells, and the regulation of an optimal response to foreign antigen. PFDA-induced alteration of Ia antigen by either decreased expression or its ability to interact with antigen and its presentation to receptors could affect this immunoregulatory mechanism.

2b. Methods, Ia antigen

Spleen cells from normal and PFDA-treated rats will be separated into adherent and nonadherent cell populations, incubated with PFDA (normal cells only) and fluorochrome-conjugated mAb against Ia antigen. These cells will be analyzed by flow cytometry as described previously.

1c. Rationale, IL-2 Rc

Cells of the immune system secrete soluble molecules which modulate the actions of the immune response. Cells that express

receptors for these secreted lymphokines are regulated by binding the soluble molecule and then transmitting the signal's regulatory action within the cell. The Interleukin 2 receptor (IL-2 Rc) binds the IL-2 molecule secreted by helper T-cells and stimulates effector B- and T-cell population proliferation. The IL-2 and IL-2 Rc interaction is essential for normal immune function and regulation. PFDA alteration of IL-2 Rc expression and/or its ability to transmit the IL-2 proliferation signal would cause severe immune dysfunction.

2c. Methods, IL-2 Rc

Spleen cells from normal and PFDA-treated rats will be isolated. Cells will be incubated with PFDA (normal cells only) and con A to induce IL-2 Rc expression. These cells will the be incubated with a commercially available fluorochrome-conjugated anti-IL-2 Rc antibody and analyzed by flow cytometry as described above.

3. Results

We have begun titration of monoclonal antibodies to the specific cell surface markers of interest. Table III contains the results of one experiment completed to date for T cell subset (T_h and $T_{s/c}$ cell) evaluation of splenocytes. There does not appear to be any difference in the percent of subset populations examined. We will be continuing our efforts in this area with the 8 day exposure period to PFDA and following with 30 day experiments.

H. Characterize the kinetics of the cell cycle of hepatocytes from PFDA-treated rats and hepatocytes treated *in vitro* with PFDA using flow cytometry.

I. Evaluate changes in hepatocyte surface or cytoplasmic enzymes expression in PFDA-treated rats Enzyme markers for initiated or preneoplastic cells.

J. Examine the effects of PFDA upon hepatocyte surface IgA receptor expression and serum IgA levels.

The objectives of this proposal are also to further characterize the toxic and putative preneoplastic effects of PFDA on rat hepatocytes, by evaluating changes in liver proteins, DNA ploidy, and surface IgA receptors upon *in vivo* and *in vitro* exposure to PFDA. These studies have been slow to start. Our attempts to duplicate the perfusion techniques of our fellow collaborators at Wright-Patterson AFB have met with some minor difficulties. Concerns have also been raised as to whether the liver cell-collagenase isolation methods might alter the cell surface markers (i.e. IgA receptor) we are interested in evaluating. Another isolation technique which does not involve enzymatic digestion hepatocellular architecture to isolate hepatocytes is being explored. As yet, specific protein candidates to which polyclonal or monoclonal antibodies will be produced is currently being defined. Dr. Witzmann's work is bringing us closer this specific aim, since he has found that PFDA induces a 2-D

protein pattern that is distinct from another peroxisome proliferator, clofibrate. Recovery of enough purified protein to develop these antibodies is another holdup to our efforts defined by Specific Aim I. As mentioned in Specific Aim F.4, we have collected serum samples from our 8 day *in vivo* experiments, but we have yet to evaluate them, this will help in our efforts defined in Specific Aim J. Upon perfection of our perfusion technique we will be able to isolate hepatocytes and look at IgA receptor expression.

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III. LIST OF WRITTEN PUBLICATIONS: PLANNED FOR SUBMISSION

Histopathology and lymphoid proliferation of PFDA-exposed Fischer 344 Rats (in preparation).

Natural killer cell activity of Fischer 344 rats exposed *in vivo* to perfluorodecanoic acid (in preparation).

IL-2 and IL-2 receptor changes upon exposure to perfluorodecanoic acid (in preparation).

Perfluorodecanoic acid induced changes in nuclear ploidy distributions determined by flow cytometry (in preparation).

IV. PROFESSIONAL PERSONNEL ASSOCIATED WITH RESEARCH EFFORT

A. Melinda J. Tarr, D.V.M., Ph.D., Principle Investigator, Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210

B. Donald E. Frazier, Jr., Research Associate

Dr. Frazier is a postdoctoral researcher responsible for coordination of experimental procedures, graduate student training, data collection and report writing. Dr. Frazier is fully supported by the grant.

C. Debra L. Nelson, B.S., M.S.

Ms. Nelson is a graduate student working towards her Ph.D. Ms. Nelson is fully supported by the grant.

V. INTERACTIONS: PRESENTATIONS AT CONFERENCES, MEETINGS, CONSULTANT AND ADVISORY FUNCTIONS

A. PFDA Roundtable Discussion Group Meeting #1. Participants of the collaborative research effort to understand the biology and toxicology of PFDA met to exchange ideas, present new data, and discuss future goals and directions of the PFDA project. October 1990.

B. Flow cytometric determination of natural killer cell activity isolated from fischer 344 rats exposed to perfluorodecanoic acid (PFDA). Frazier, D.E., Jr.,

Nelson, D.L., and Tarr, M.J. 30th Annual Meeting of the Society of Toxicology, Dallas, Texas, February 1991.

C. Evaluation of the histopathologic changes and mitogen-induced proliferative response of lymphoid tissue isolated from perfluorodecanoic acid (PFDA)-treated fischer 344 rats. Nelson, D.L., Frazier, D.E., Jr., and Tarr, M.J. 19th Conference on Toxicology, Dayton, Ohio, April 1991.

D. Flow cytometric analysis of hepatocellular changes in fischer 344 rats upon *in vivo* exposure to perfluorodecanoic acid (PFDA). Frazier, D.E., Jr., and Tarr, M.J. 19th Conference on Toxicology, Dayton, Ohio, April 1991.

E. PFDA Roundtable Discussion Group Meeting #2. Participants of the collaborative research effort to understand the biology and toxicology of PFDA met to exchange ideas, present new data, and discuss future goals and directions of the PFDA project. April 1991.

VI. DISCOVERIES, INVENTIONS, OR PATENT DISCLOSURES

None

VII. OTHER STATEMENTS OF ADDITIONAL INSIGHT TO PROGRAM MANAGER

In light of our efforts over the past 3 months, we feel we have made significant

inroads in our evaluation of the immunotoxic effects of PFDA. We are continuing our investigative efforts by initiating 30 day studies (the first will be finished in mid May 1991) as well as filling some data holes in our current 8 day studies. We have already begun some Tier II experiments. Experimental findings from our Tier I experiments prompted us to begin them sooner than anticipated. In that regard, we will be combining the proposed IL-2 studies (IL-2 production/activity and IL-2 receptor) and begin lymphocyte subset analyses. As mentioned earlier, the "experimental bugs" are being worked out for the hepatocyte studies and we expect data with the next round of experiments (mid May 1991). An interesting development in the area of peroxisome proliferators has been the finding that peroxisome proliferating compounds have been shown to bind to receptor molecules much like steroid hormones (Issemann and Green, 1990), thus it is possible that the effects of PFDA may be mediated through receptor binding and gene regulation similar to that reported for TCDD (Gasiewicz and Rucci, 1984).

Table I. Histopathology results of 8 day *in vivo* PFDA experiments.

	Thymus	Other Lymphoid Organs (spleen, L.N.)	Liver
Normal Control	NSML	NSML	NSML to slight diffuse vacuolar change
20 PFDA	NSML	NSML	Normal to mild hepato-cellular hypertrophy
50 PFDA	Mild to marked lymphoid depletion	Most had NSML; some showed mild lymphoid depletion	Normal to prominent hepatocellular hypertrophy & increased granularity; also increased no. of immature hepatocytes
20 pair fed	NSML	NSML	Normal to slight hepato-cellular atrophy
50 pair fed	NSML, but overall size smaller than control	NSML	Most had prominent hepatocellular atrophy

Other organs examined were kidney and intestine; these organs did not show any lesions that could be attributed to treatment (PFDA or pair feeding).

Table II. Percent NK cytotoxicity calculated from control, pair-fed control, and PFDA-treated Fischer 344 rats.

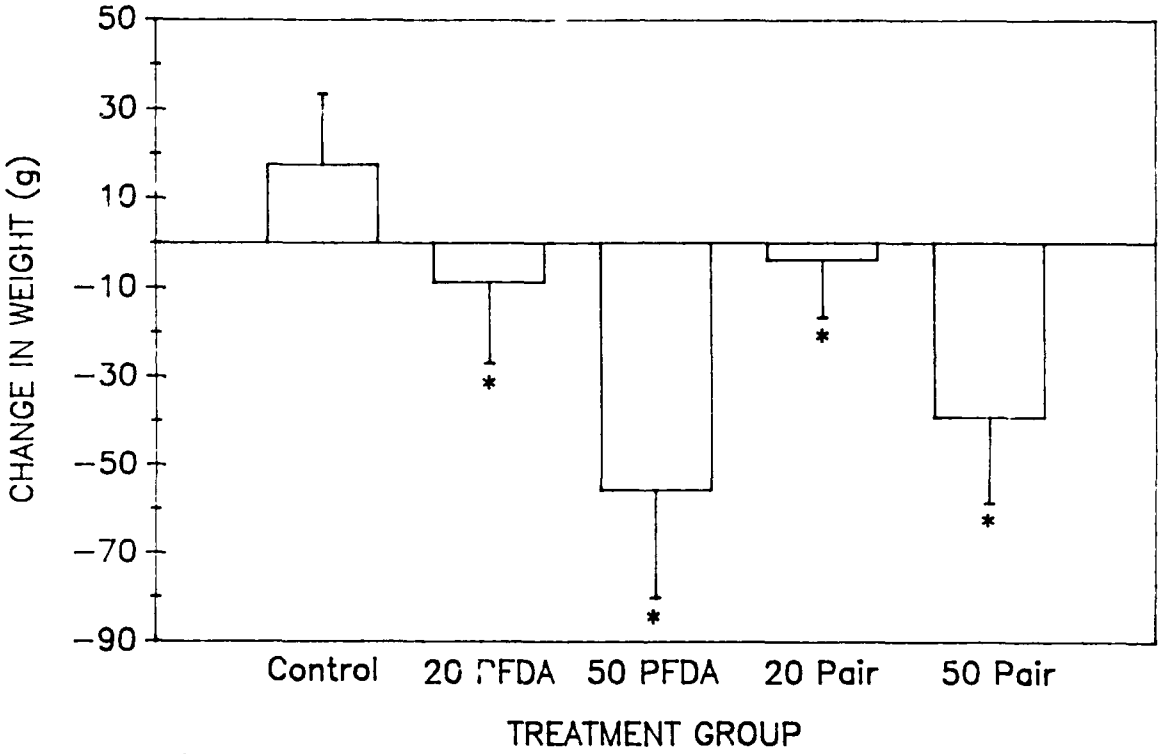
	% CYTOTOXICITY[a]		
	100:1	**50:1**	**25:1**
Control	45.2	21.0	14.8
20 mg/kg PFDA	24.2	19.7	12.6
50 mg/kg PFDA	25.9	15.2	9.4
Control	21.0	21.3	17.5
20 Pair-fed	24.5	23.0	18.5
50 Pair-fed	29.2	26.7	21.7

[a] % Cytotoxicity = (Q2/Q2 + Q4)specific - (Q2/Q2 + Q4)nonspecific x 100

Table III. T cell subset analysis of PFDA-exposed, pair-fed, and normal Fischer 344 rats.

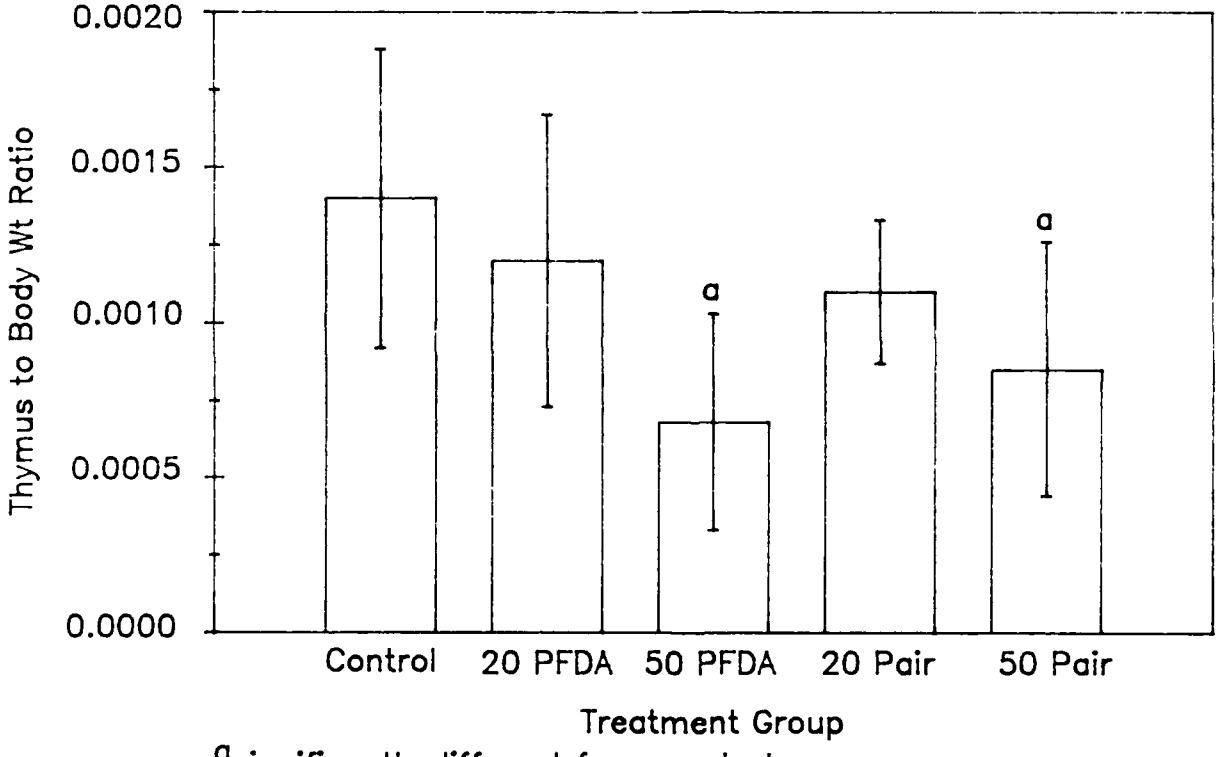
	$CD4^+$		$CD8^+$		$CD4^+CD8^+$	
	#1	#2	#1	#2	#1	#2
Control	28.8	27.5	35.9	36.1	14.0	18.2
20 PFDA	38.7	35.5	30.9	32.3	14.4	16.1
50 PFDA	37.0	38.8	30.0	28.4	16.1	17.6
Control	34.8	33.7	24.2	25.5	15.4	16.7
20 Pair-fed	37.5	33.3	22.1	30.6	12.9	11.1
50 Pair-fed	35.3	36.9	33.7	30.4	11.1	15.1

Figure 1. CHANGE IN BODY WEIGHT DURING 8 DAY EXPERIMENTS IN CONTROL, PFDA–TREATED AND PAIR–FED FISCHER 344 RATS



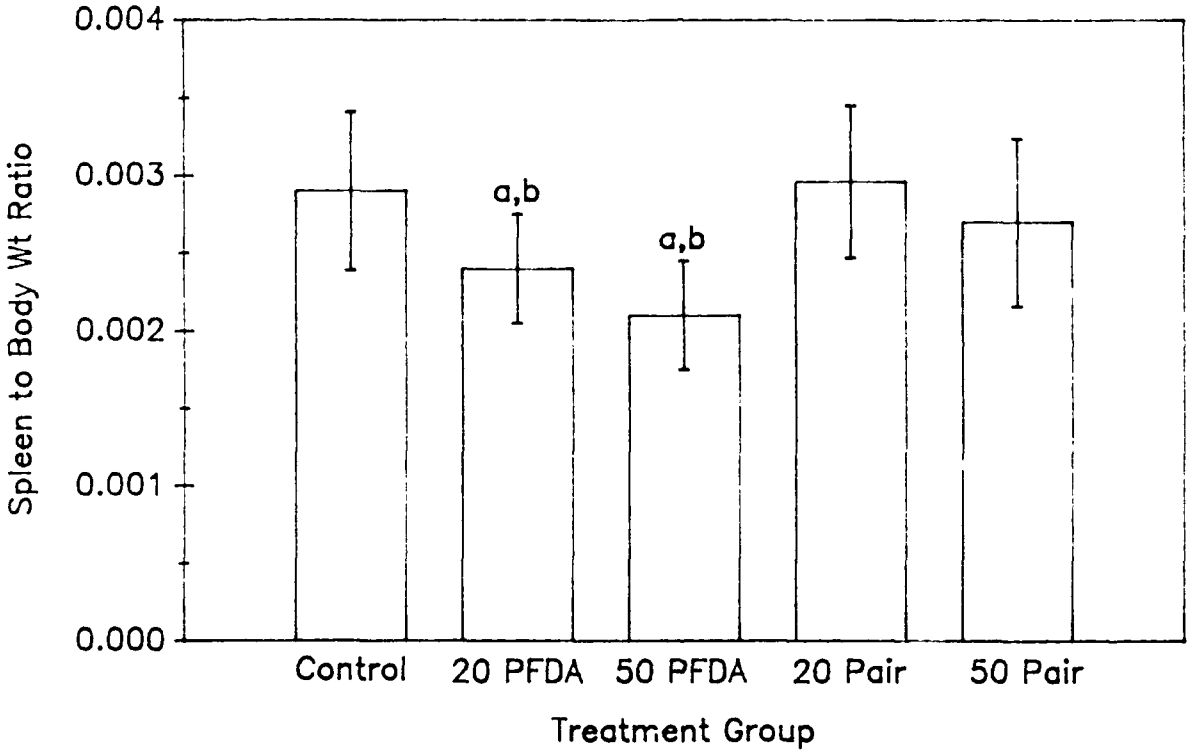
*significantly different from control

Figure 2. Thymus Weights of 8 day Control, PFDA–treated, and Pair–fed Control Fischer 344 Rats



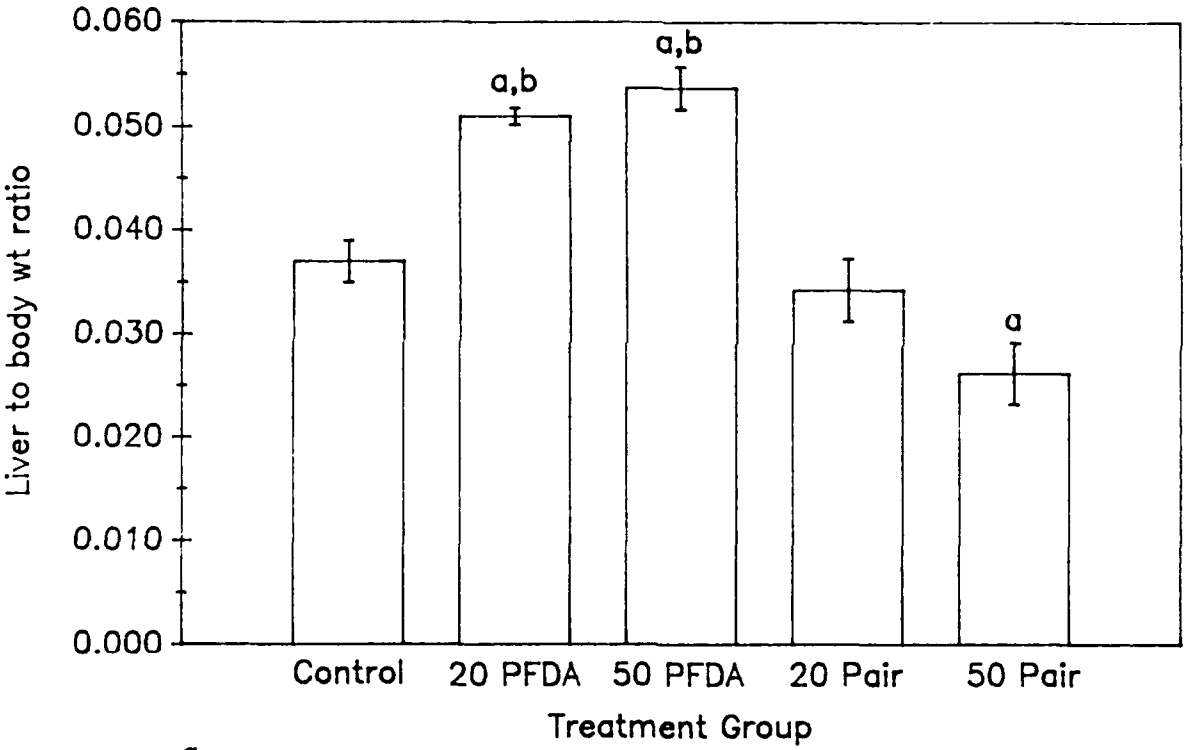
[a]significantly different from control

Figure 3. Spleen Weights of 8 day Control, PFDA–treated, and Pair–fed Control Fischer 344 Rats



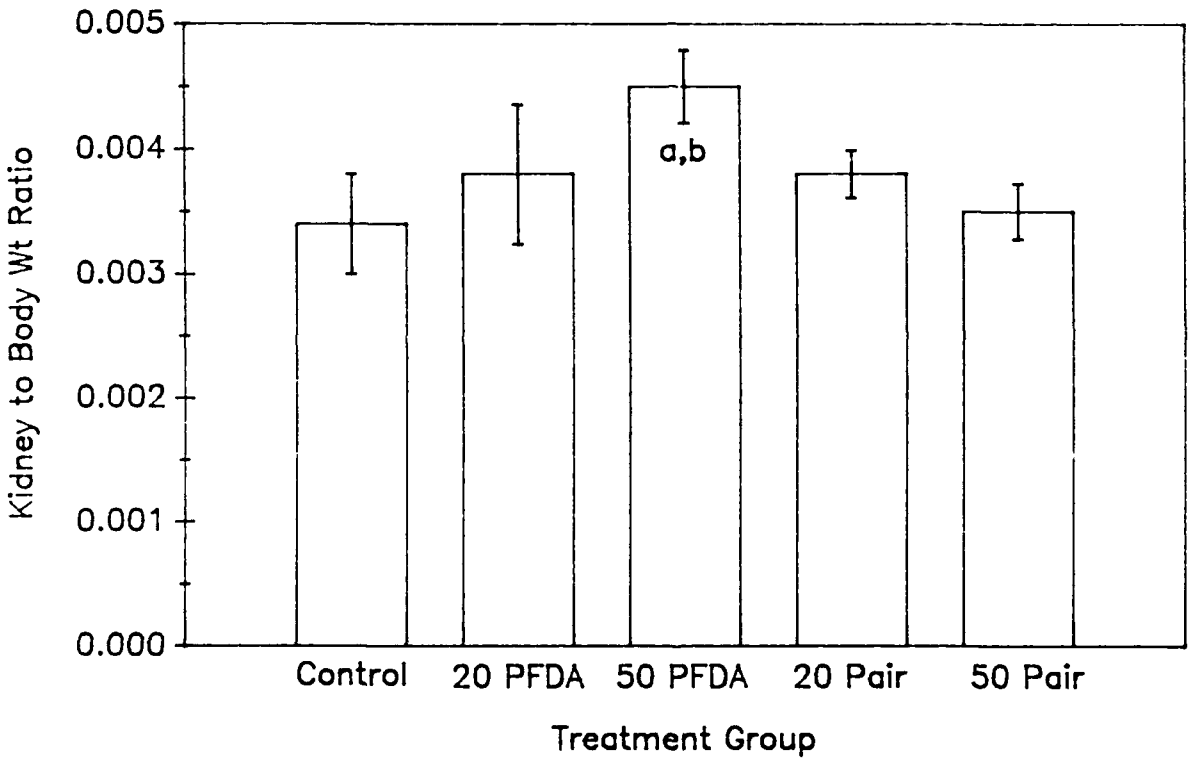
[a]significantly different from control
[b]significantly different from pair–fed

Figure 4. Liver Weights of 8 day Control, PFDA–exposed, and Pair–fed Control Fischer 344 Rats



[a]significantly different from control
[b]significantly different from pair–fed

Figure 5. Kidney Weights from 8 day Control, PFDA–treated, and Pair–fed Control Fischer 344 Rats



[a]significantly different from control
[b]significantly different from pair–fed

Figure 6. FOOD INTAKE DURING 8 DAY EXPERIMENTS IN CONTROL, AND PFDA/PAIR-FED FISCHER 344 RATS

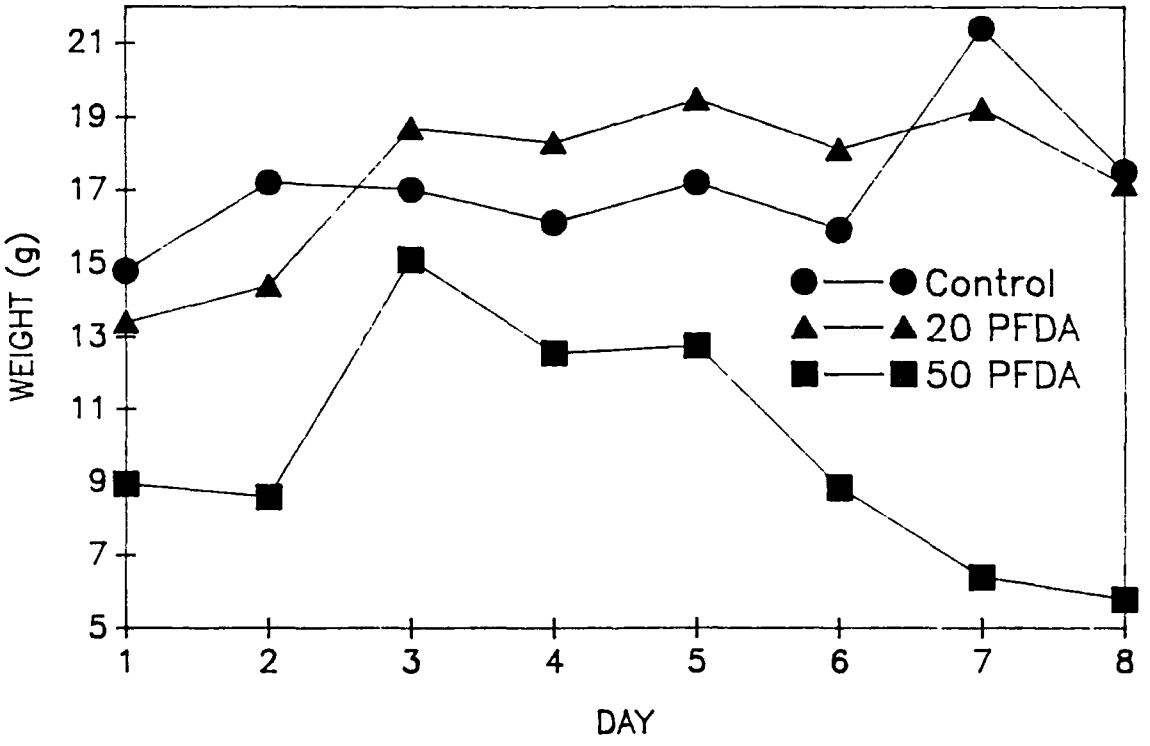
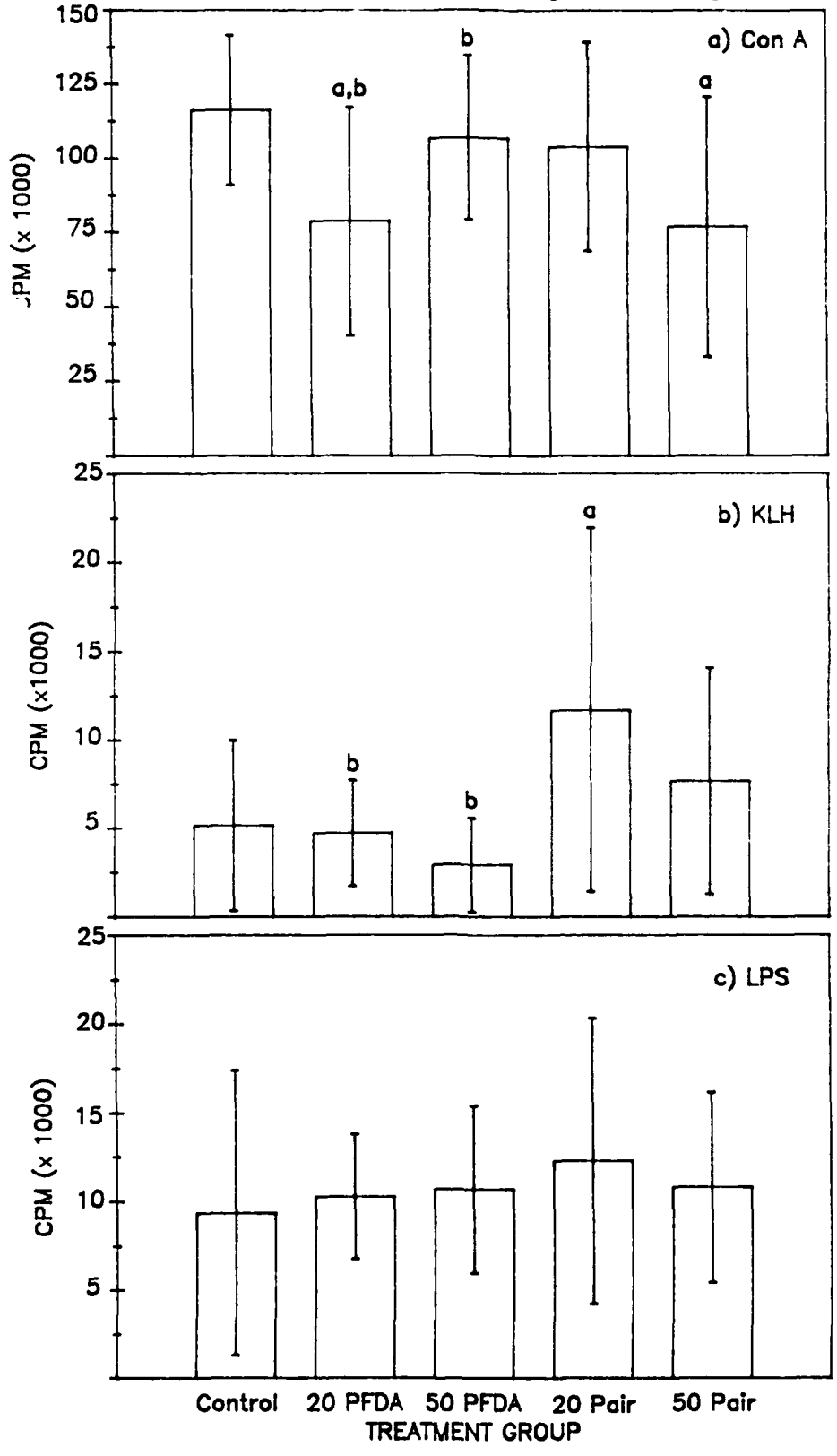
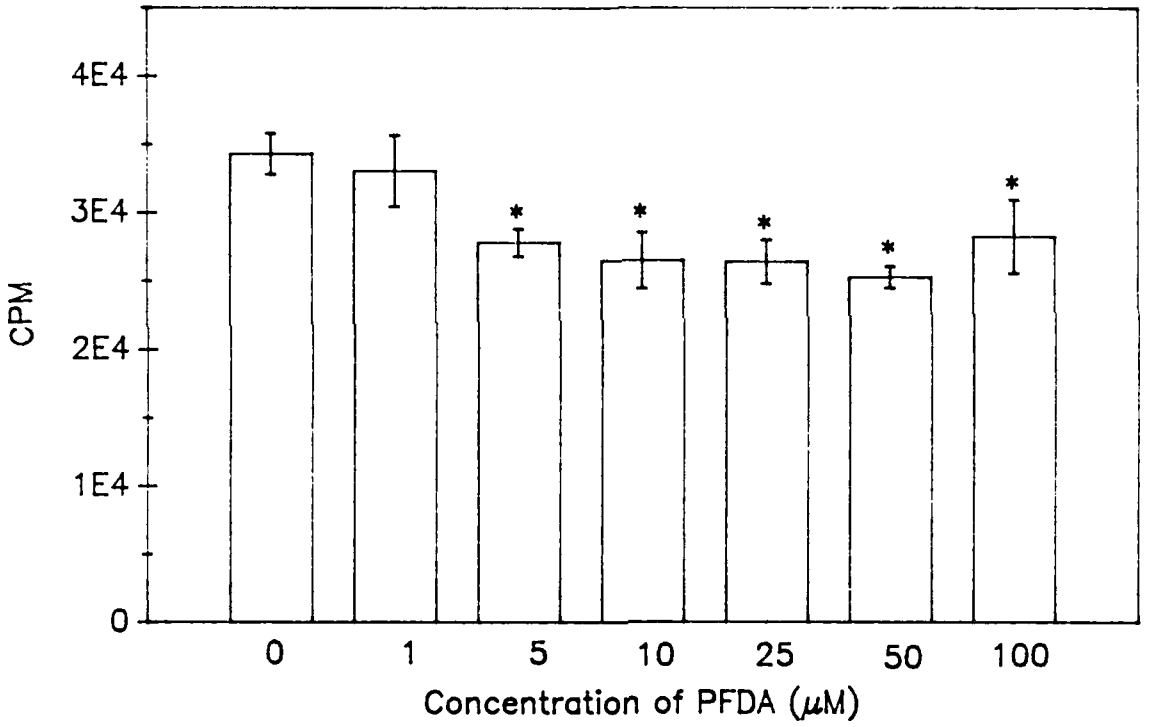


Figure 7. Lymphoproliferative Response of Cells Isolated From Control, Pair–fed, and PFDA–treated Rats in Response to Mitogen or Antigen



[a]significantly different from control
[b]significantly different from pair–fed

Figure 8. Alteration of Lymphocyte Proliferative Response to Con A with Addition of PFDA In Vitro



*significantly different from control

Figure 9. DTH Response in Control, PFDA–treated, and Pair–fed Fischer 344 Rats

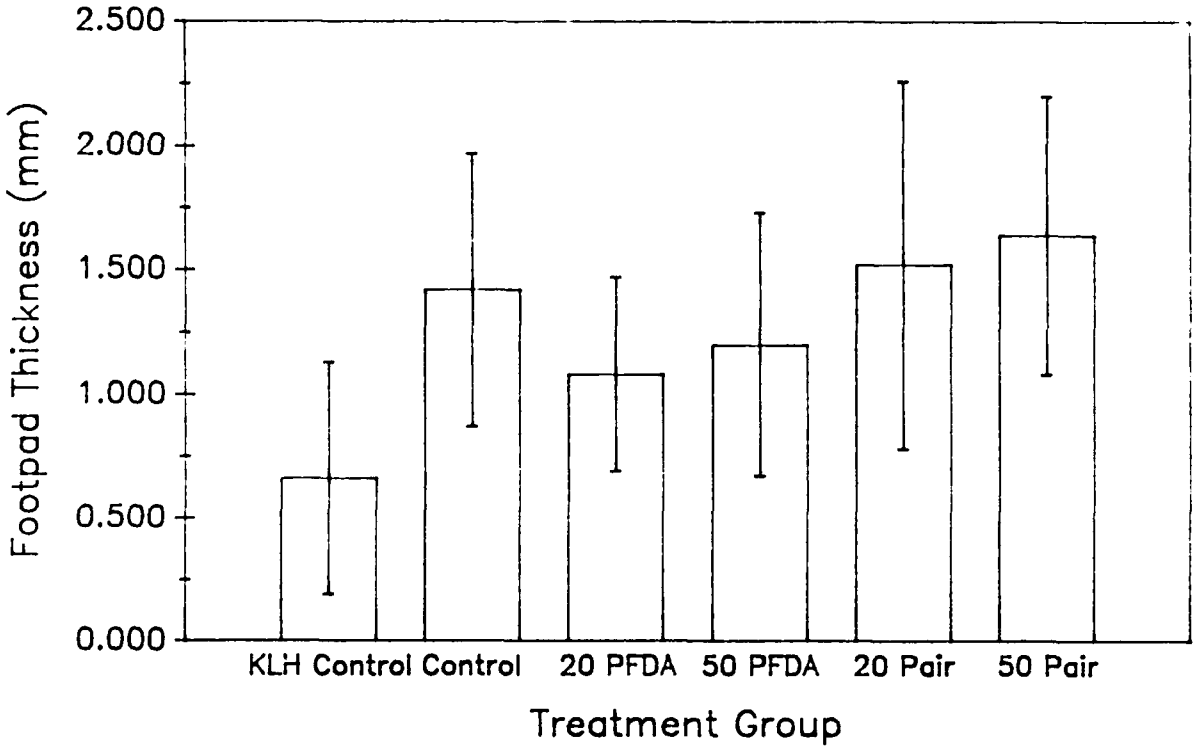


Figure 10. IL-2 Production in cells isolated from Control, PFDA-treated, and Pair-fed Control Fischer 344 Rats