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13. ABSTRACT *(Maximum 200 words)*

This report outlines our progress regarding toxicological studies of perfluoro-*n*-octanoic acid (PFOA) and perfluoro-*n*-decanoic acid (PFDA). These Air Force chemicals belong to a class of compounds known as peroxisome proliferators (PP). Many PP cause hepatotoxicity and carcinogenesis in rodents. The mechanisms are unknown and represent an active area of research.

Recent studies have demonstrated specific effects of PFDA treatment on hepatic phospholipid and carbohydrate metabolism. PFDA alters hepatic glucose transport. PFDA-treated rats showed rates of glucose transport which were 2-fold less than controls. PFDA treatment also caused a 3-fold increase in liver diacylglycerol (DAG); PFOA had no effect. This DAG is derived from phosphatidylcholine via phospholipase C activity. Since DAG stimulates protein kinase C (PKC), these data suggest that the hepatotoxic effects of PFDA may be initiated through a PKC response. Accordingly, experiments are in progress to investigate the effects of PFDA on liver PKC activity; preliminary data are presented herein. In other studies, preliminary data indicate that the influence of PFDA on phospholipid metabolism occurs at doses well below the LD_{50}. Also, in studies involving C8-C11 perfluorocarboxylic acids, only chain lengths ≥ C9 affect phospholipid metabolism. These data suggest that the metabolic effects may be related to the compound's ability to diffuse into membranes. This research furthers our understanding of the mechanisms involved in the hepatotoxicity associated with these Air Force chemicals.

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I. INTRODUCTION/RESEARCH OBJECTIVES

The primary objective of this research program is to gain a better understanding of the toxicological mechanisms associated with peroxisome proliferator compounds. To date, our work has predominately focused on the perfluorocarboxylic acids and the hepatotoxicity associated with perfluoro-*n*-octanoic acid (PFOA) and perfluoro-*n*-decanoic acid (PFDA). Newly proposed studies for our laboratory will expand the breadth of this work to include other classes of peroxisome proliferators.

These studies involve the application of nuclear magnetic resonance (NMR) spectroscopy *in vivo* and strive to further our understanding of the effects that peroxisome proliferators have on endogenous liver metabolism. NMR data obtained from tissue *in vivo* and complementary data from biochemical assays provide information about specific metabolic processes and the impact of treatment on these metabolic pathways. These experiments provide new insight toward our understanding of the mechanisms involved in the hepatotoxicity associated with this class of compounds. Additionally, this research expands the applicability of the NMR technique in the field of toxicology.

II. PUBLICATIONS

Full Journal Publications

C.M. Goecke, B.M. Jarnot, and N.V. Reo. "Effects of the Peroxisome Proliferator, Perfluoro-n-decanoic Acid, on Hepatic Gluconeogenesis and Glycogenesis: A ^{13}C NMR Investigation." *Chem. Research Toxicol.* **7**, 15-22 (1994).

N. V. Reo, C. M. Goecke, L. Narayanan, and B. M. Jarnot. "Effects of Perfluoro-*n*-octanoic Acid, Perfluoro-*n*-decanoic Acid, and Clofibrate on Hepatic Phosphorus Metabolism in Rats and Guinea Pigs *in Vivo*." *Toxicol. Appl. Pharmacol.* **124**, 165-173 (1994).

C. M. Goecke-Flora, J. F. Wyman, B. M. Jarnot, and N. V. Reo. "Effects of the Peroxisome Proliferator Perfluoro-*n*-decanoic Acid on Glucose Transport in the Perfused Rat Liver." *Chem. Research Toxicol.* (in press; preprint attached).

Published Abstracts/Presentations

C. M. Goecke, N. V. Reo, J. Wyman and B. M. Jarnot: "Effects of Perfluoro-*n*-Decanoic Acid on Hepatic Glucose Transport." *The Toxicologist* **14** (1), (1994). Society of Toxicology, Annual Meeting, Dallas, TX, March 1994.

N. V. Reo and K. B. Kling: "Effects of the Peroxisome Proliferator, Perfluorodecanoic Acid, on Liver Protein Kinase C Activity." International Society for the Study of Xenobiotics, *ISSX Proceedings*, **6**, 282 (1994). Presented at the Sixth North American ISSX Meeting, Raleigh, NC, October 1994.

N. V. Reo, M. Adinehzadeh, and L. Narayanan: "Concentration of Liver Diacylglycerol (a Cellular Second Messenger) is Increased by the Peroxisome Proliferator, Perfluorodecanoic Acid." International Society for the Study of Xenobiotics, *ISSX Proceedings*, **6**, 281 (1994). Presented at the Sixth North American ISSX Meeting, Raleigh, NC, October 1994.

Manuscript in Preparation

N. V. Reo, L. Narayanan, and M. Adinehzadeh. "Induction of Liver Phospholipase C Activity and Elevation of Diacylglycerol by the Peroxisome Proliferator, Perfluorodecanoic Acid." Will be submitted to *Xenobiotica*.

III. PERSONNEL

Katrina Leigh, M.S. – Laboratory Technician (formerly Katrina Kling)

Carol Goecke-Flora – Ph.D. student who was supported by an ASSERT grant until March 1994. Carol completed all requirements for the Ph.D. degree in Biomedical Sciences in March 1994.

Dissertation Title: Hepatotoxicity of Perfluorocarboxylic Acids: A ^{19}F-, ^{13}C-, and ^{31}P-NMR Investigation.

On June 1, 1994 Carol began working in the laboratory as a Postdoctoral Research Associate and is currently supported by the AFOSR grant.

Mehdi Adinehzadeh – Ph.D. student (3rd year). Mehdi was supported by a graduate stipend from the AFOSR grant until May 31, 1994. He took a leave of absence in the summer to visit family in Iran, and returned to the laboratory in September 1994. Since September, Mehdi has been supported by the ASSERT grant.

IV. INTERACTIONS WITH AIR FORCE AND OTHER DOD LABORATORIES

Our laboratory has participated in an active ongoing collaboration with Capt. Bruce Jarnot, Ph.D. of the Toxicology Division, Armstrong Laboratory, Wright-Patterson AFB. During the past year we have meet on a regular basis to discuss data and plan experiments. Unfortunately, Dr. Jarnot left the Air Force in August 1994. We have, however, maintained a working

relationship with the toxicology laboratory at WPAFB. In particular, Capt. John Lipscomb, Ph.D. has been helping our laboratory with liver microsome preparations and we regularly share equipment in his laboratory.

Our laboratory has also collaborated with Lt. Commander John Wyman of the Naval Medical Research Institute (NMRI), Toxicology Detachment, Wright-Patterson AFB. Dr. Wyman is an expert in rat liver perfusion and has assisted us in a study to investigate the effects of PFDA on hepatic glucose transport (*vide infra*). This work culminated into a manuscript that was recently accepted for publication (Dr. Wyman is a co-author). Dr. Wyman retired from the Navy in June 1994, and he is presently working for Mantech at WPAFB. He regularly communicates with our laboratory, and remains a resourceful colleague.

V. RESEARCH ACCOMPLISHMENTS

[Tables, figures, and figure legends are included at the rear of this report]

During the past year our research efforts have focused on further characterizing the effects of PFDA on hepatic metabolism. Specifically, we were interested in its effects on glucose and glycogen metabolism, and phospholipid metabolism. Previous studies in our laboratory have demonstrated various effects of PFDA on these cellular processes:

(1) PFDA inhibits liver glycogen synthesis

(2) PFDA causes a significant increase in the concentration of liver phosphocholine (PCho)

(3) PFDA induces liver phospholipase C (PLC) activity

(4) PFDA inhibits CTP: phosphocholine cytidylytransferase activity in liver cytosolic fractions

All of these effects were not observed following PFOA treatment. Thus PFDA seemed to be unique and prompted further studies to investigate the mechanisms of action for these biological activities. These studies are outlined below.

A. ***Effects of PFDA Treatment on Hepatic Glucose Transport.*** Our data concerning the influence of PFDA on hepatic carbohydrate metabolism suggested that PFDA may inhibit glucose transport. Therefore, a study was initiated to measure glucose transport activity in perfused livers from PFDA-treated (50 mg/kg) and pair-fed control rats. The results of this study substantiated our prediction and showed that PFDA significantly inhibits glucose transport in

liver. Percent hepatic glucose extraction is 1.8-fold greater in controls than in PFDA-treated rats. A manuscript has been accepted for publication in *Chemical Research in Toxicology* and is scheduled to appear in the January/February 1995 issue. Details of this study can be obtained from the attached pre-print.

B. ***Effect of PFDA-treatment on Liver Diacylglycerol Concentration and Protein Kinase C Activity.*** The influence of PFDA on hepatic phosphocholine concentration, phospholipase C activity, and cytidylytransferase activity suggests that this compound alters liver phospholipid metabolism. These data led to the hypothesis that PFDA may stimulate a phosphatidylcholine-specific phospholipase C and causes an elevation in the concentration of diacylglycerol (DAG). This, in turn, can activate protein kinase C (PKC) and influence various cellular processes. Thus our efforts during the past year have focused on the effects of PFDA on liver DAG concentration and PKC activity. These studies are outlined below.

(1) Diacylglycerol Study

Methods. Lipid extracts were prepared by the methods of Bligh and Dyer (Can. J. Biochem. Physiol. 37: 911, 1959). Livers were homogenized in chloroform/methanol (1:2 v/v; 3 ml/g tissue) and the phases were separated by centrifugation at 5000 x g for 5 min. The chloroform phase was analyzed for *sn*-1,2-diacylglycerol using an assay kit purchased from Amersham Corp. (#RPN-200). This assay involves the enzyme-catalyzed phosphorylation of DAG with [γ-^{32}P]ATP to yield radiolabeled phosphatidic acid. The [^{32}P]phosphatidic acid is separated from unreacted ATP using Amprep™ minicolumns with a silica sorbent (Amersham #RPN 1906) and then quantified by liquid scintillation spectrometry. All samples were run in triplicate; the standard error in triplicate assays was <10 %.

Results and Discussion. Liver DAG concentrations determined in PFOA-treated, PFDA-treated, and corresponding control groups are depicted in Figure 1. The results show that PFDA treatment causes a 3-fold increase in this liver metabolite as compared to control ($p = 0.0002$). In contrast, PFOA treatment has no effect.

Our data indicate that PFDA activates a phosphatidylcholine-specific phospholipase C (shown previously) and, consequently, causes a significant increase in both the DAG and PCho pools. DAG is a key metabolic intermediate since it is a precursor for both phospholipid and triglyceride synthesis. Thus the increase in this metabolite may explain the elevation in liver triglycerides, free fatty acids, and fatty acyl-CoA following PFDA exposure that has been reported by others (George, *et al.*, Toxicol. Appl. Pharmacol. 85: 169, 1986; Olson, *et al.*, Toxicol.

Appl. Pharmacol. 70: 362, 1983). More important, however, is the well-known role of DAG as a second messenger in the activation of PKC. Elevated DAG concentrations can activate PKC and affect many cellular processes. The effect of PFDA on phospholipid metabolism may provide a mechanism by which this compound influences a variety of cellular responses including cell proliferation, hepatomegaly, and induction of cytochrome P450 enzymes. Thus our research provides strong evidence for a mechanistic model of PFDA-induced toxicity that involves phosphatidylcholine degradation, DAG elevation, and subsequent activation of PKC. Such cellular events can have profound effects on metabolic processes and may constitute a probable mechanism for the hepatotoxicity associated with PFDA.

Interestingly, Thurman and coworkers have recently reported that certain peroxisome proliferators, including PFOA, can stimulate liver microsomal PKC activity (Bojes, *et al.*, Toxicol. Lett. 62: 317, 1992; Toxicol. Appl. Pharmacol. 126: 233, 1994). They hypothesize that the ability of peroxisome proliferators to stimulate PKC activity may be related to their potency for hepatic tumorigenesis. Studies are in progress in our laboratory to measure liver PKC activity in PFDA-treated rats. Such data also suggest that PFDA mat <u>not</u> be unique but, rather, may share a common mechanism for biological activity with other peroxisome proliferators.

(2) <u>Protein Kinase C Study</u>

The aim of these experiments is to measure and compare liver PKC activity in PFDA-treated and pair-fed control rats. Measurements are being conducted in liver cytosolic and microsomal fractions. The studies are not yet complete due to various problems that we have encountered with the enzyme assay. Preliminary results, however, are detailed below.

Methods. Male Fischer-344 rats (231 - 251 g) were paired according to similar body weights. Treated rats received a single intraperitoneal injection of PFDA (50 mg/kg in propylene glycol/H_2O, 1:1 v/v), while controls were dosed with an equal volume of vehicle. Body weights and food consumption were monitored daily, and control animals were given the same amount of food that their paired partners had consumed on the previous day (pair-feeding). All animals were fasted 16-24 hr prior to experiments. At 5 days posttreatment, rats were anesthetized with halothane (5% induction, 1% maintenance) and livers were perfused *in situ* via the portal vein with ~35 ml of ice-cold Tris HCl buffer solution (pH 7.5). Once the tissues were completely blanched, they were removed and homogenized in buffer (4 ml/g liver) using a Potter-Elvehjem Teflon/glass homogenizer. Cytosolic and microsomal fractions were isolated by standard differential centrifugation techniques. Microsomes were reconstituted in Tris

buffer (1 ml/g liver). All samples were stored at -20 °C in 50% glycerol, and assayed for PKC activity within 12 hrs.

Enzyme Assay PKC enzyme activity was measured using an assay system purchased from Amersham Corp. (#RPN 77). This assay is based on the PKC-catalyzed transfer of the γ-phosphate of ATP to the threonine group on a peptide that is specific for PKC. An appropriate control was run to account for calcium-phospholipid-independent kinase activity. The reaction was initiated with the addition of [γ-^{32}P]ATP and the samples were incubated at 25 °C for 19 min. The radiolabeled phosphorylated peptide was separated on binding paper. The papers were washed 4 times with acetic acid and the remaining radioactivity was then quantified by liquid scintillation spectrometry. The assay was run in triplicate and data exceeding a SEM of 12% was eliminated. The amount of phosphate transferred (pmol) was calculated from the specific radioactivity of the [^{32}P]ATP. Protein content was estimated using the BCA protein assay reagent (Pierce Co., #23235). PKC activity is expressed as pmol of phosphate transferred/min/mg protein.

Results and Discussion. Figure 2 depicts the PKC activity in both cytosolic and microsomal fractions from PFDA-treated and control groups. Greater enzyme activity is observed in cytosolic fractions than in microsomes for both groups (paired t-test; $p \leq 0.02$). There is no difference, however, in comparison between treated versus control groups in either cytosolic or microsomal fractions ($p < 0.05$).

These preliminary data suggest that the elevation in liver DAG following PFDA treatment (*vide infra*) does not stimulate PKC activity under this specific experimental protocol. Further studies are necessary in order to interpret the significance of the DAG/PKC response in PFDA-treated rats. There are two problems that concern us at the present time: (1) we are uncertain about the accuracy and dependability of the enzyme assay system and, (2) it is important that we measure PKC activity at shorter times post-dose. With regard to this latter concern, it has been reported that continuous stimulation of PKC by elevated endogenous DAG levels may subsequently lead to downregulation of the enzyme (Brooks, *et al.*, J. Biol. Chem. 268: 23868, 1993). It is known that prolonged treatment with phorbol esters selectively downregulates the expressed levels of PKC [Wilkinson, *et al.*, TIPS 15: 53, 1994). Thus, it is possible that by 5 days post-dose the stimulatory effect of high DAG concentrations on PKC may have subsided and is no longer observed.

Studies are currently in progress to measure PKC activity at 5 and 10 hours post dose with PFDA. We are also proceeding with various experiments to ensure that the assay system is reliable.

C. Studies Which Further Characterize the Effects of PFDA on Liver Metabolism

PFDA has a dramatic influence on liver phospholipid metabolism that is manifested in a significant increase in the concentration of PCho and DAG. The elevation in PCho can easily be observed by ^{31}P NMR studies of rat liver *in vivo*. We have been intrigued by the fact that these effects seem to be specific to PFDA, and are not observed following treatment with PFOA. Is this effect due to an acute toxic poisoning by PFDA? Will this effect only be observed at very high dose, or is it a biological response characteristic of this compound? Is this activity dependent upon the carbon chain length of the perfluorocarboxylic acid? Such information may provide insight regarding the mode of action for this biological activity.

To address these questions and further characterize the biological activity of perfluorocarboxylic acids, two additional studies have been initiated: (1) obtain dose-response data for PFDA regarding its hepatic effects, and (2) investigate the possible relationship between carbon chain length and the effects on liver PCho metabolism. Details of these studies and preliminary data are outlined below.

(1) Dose-Response for PFDA Treatment

The purpose of this investigation is to evaluate the effects on liver phospholipid metabolism following treatment with various doses of PFDA, specifically doses below the 30-day-LD_{50}. These studies will determine if the metabolic effects previously observed with treatment at a dose of 50 mg/kg (i.e., increases in liver PCho and DAG) are the result of an acute toxic reaction. Such an effect might only be manifested at very high doses and may not represent a characteristic response of PFDA exposure.

Methods. Male Fischer-344 rats (200-250 g) were given a single ip. injection of PFDA at one of the following doses: 25, 15, or 5 mg/kg. Food consumption and body weight were monitored daily. Pair-fed controls were administered an equal volume of vehicle solution. All animals were fasted 12-18 hours prior to NMR experiments. On day 5 posttreatment, ^{31}P NMR spectra of livers were obtained from rats *in vivo* and analyzed for the integrated peak intensities in the phosphomonoester (PME) region of the spectra. Acquisition methods and data analyses for NMR experiments have been described in previous publications from our laboratory and will not be reiterated here. At the completion of the NMR experiments, the livers were freeze-clamped and stored under liquid N_2 for subsequent analyses of fatty acyl CoA-oxidase (FAO) activity. This enzyme is the rate limiting enzyme of the peroxisomal fatty acid oxidizing system and serves as a specific marker for peroxisome proliferation.

Assay for Fatty Acyl CoA-Oxidase Activity. Livers were homogenized in a 10% (w/w) sucrose solution containing 3 mM imidazole (pH 7.4). A peroxisome-enriched fraction was prepared using standard methods of centrifugation, and FAO activity was measured spectrophotometrically as described by Small, *et al.* (Biochem J., 227: 205, 1985). This method uses palmitoyl-CoA as substrate and leucodichlorofluorescin (leuco-DCF) as a chromophore coupled to H_2O_2 production. The assay was carried out in a semi-microcuvette at 23 °C and a final volume of 1 ml. The rate of oxidation of leuco-DCF was monitored continuously for approximately 10 min. using a Beckman DU-50 spectrophotometer. After correction for the rate of autoxidation of dye, the maximum enzyme activity was calculated from the initial slope of the absorption versus time plot (linear regression).

Results and Discussion. Figure 3 depicts the PME signal intensities from the ^{31}P NMR spectra of liver *in vivo* from PFDA-treated and corresponding control rats. Data are shown for two doses: 15 and 25 mg/kg. Studies involving the 5 mg/kg dose are ongoing at the present time and preliminary data are not yet available. The results clearly show that PFDA treatment, at either dose, causes a significant increase in the concentration of liver PME's as compared to corresponding controls ($p \leq 0.003$). No significant differences were noted between the PME signal intensities of the two PFDA-treated groups (15 vs. 25 mg/kg). Previous studies from our laboratory have confirmed that the signal intensity in the PME region of the ^{31}P spectrum is predominately due to PCho. Interestingly, the elevation in the PME signal intensity following treatment with 15 or 25 mg/kg, were very similar to that previously reported for the 30 day LD_{50} dose of 50 mg/kg. Thus the effect of PFDA treatment on the level of liver PCho occurs well <u>below</u> the LD_{50} dose and does <u>not</u> appear to be dose dependent.

Measurements of FAO activity are not complete at the present time; however, preliminary data indicate that PFDA treatment, at both 15 and 25 mg/kg, causes a large induction (3 to 5 fold) in enzyme activity as compared to corresponding controls.

These results indicate that low doses of PFDA (below the LD_{50}) are sufficient to cause both peroxisome proliferation and a significant increase in the level of liver PCho. Thus the effects observed on hepatic phospholipid metabolism are probably a characteristic response of treatment and not due to a severe acute toxicity. In addition, peroxisomal induction and the effects on phospholipid metabolism may be correlated processes in response to PFDA treatment. Studies using a dose of 5 mg/kg are currently in progress and will help to corroborate this interpretation.

(2) Carbon Chain Length Study

In previous studies we demonstrated that rats treated with PFDA show a significant increase in the signal intensity in the PME region of the ^{31}P NMR spectrum of liver *in vivo*. This signal was attributed to an increase in PCho. This effect, however, was not observed in rats treated with PFOA. Although PFOA and PFDA are structurally very similar, differing only by a two carbon chain-length, these data show that PFDA exerts a distinct effect on hepatic phospholipid metabolism. These results prompted a study to evaluate the importance of carbon chain length with regard to the induced metabolic effects of these peroxisome proliferators.

Methods. Perfluorononanocid acid (PFNA), a nine-carbon carboxylic acid, and perfluoroundecanoic acid (PFUA), an eleven-carbon carboxylic acid, were investigated with regard to their effects on hepatic phospholipid metabolism. This was accomplished by measuring the signal intensity of the PME peak in the ^{31}P NMR spectrum of liver from treated and pair-fed control rats. Three doses were investigated for PFNA: 50, 100, and 150 mg/kg. The PFUA compound was found to display a much greater potency for toxicity and, consequently, studies employed a dose of 50 mg/kg only. NMR measurements were made on 3 and 5 days posttreatment for both compounds. The effects of PFNA and PFUA on hepatic peroxisomal FAO activity was also measured to determine whether these compounds are peroxisome proliferators. FAO activity was determined spectrophotometrically as described above.

Results and Discussion. Preliminary data showing the effect of PFNA and PFUA treatments on hepatic peroxisomal FAO activity at 3 and 5 days posttreatment are shown in Table 1. In general, PFNA- and PFUA-treated rats show approximately 5-fold greater activity than controls on both days post-dose. These data indicate that the C9 and C11 perfluorocarboxylic acids are peroxisome proliferators, similar to PFOA and PFDA.

Figure 4 shows the integrated NMR signal intensities of the PME region for PFNA- and PFUA-treated and control rats at different doses and days posttreatment. Upon cursory review, PFNA- and PFUA-treated rats show a 2 to 3-fold increase in the PME signal intensities in comparison to controls at all doses and days posttreatment. This increase appears to be dose and time dependent in PFNA-treated rats. High resolution ^{31}P NMR spectra from liver extracts prepared from PFNA- and PFUA-treated rats show an increase in the intensity of a resonance at 6.4 ppm, which is attributed to PCho. Liver PCho concentrations were calculated from the spectra of liver extracts by adding a known amount of phosphocreatine (PCr) to the sample and normalizing the integrated NMR signal intensities to the PCr peak. The results reveal that the

levels of PCho in PFNA- and PFUA-treated rats are significantly greater than control ($p \leq 0.05$) at all doses and days posttreatment (Table 2). The PCho level observed at 3 days post-dose with PFUA is 1.6-fold greater than control. This is slightly less than the effect observed for PFNA treatment, which shows a *ca.* 3-fold increase in PCho for doses ranging from 50 to 150 mg/kg. On day 5 posttreatment, PFNA-treated rats show a *ca.* 6- to 7-fold increase in PCho while PFUA-treated rats show a 3-fold increase compared to control.

In summary, data from this study and previous work indicate that the C8, C9, C10 and C11 perfluorocarboxylic acids are all peroxisome proliferators in rodent. In addition, treatment with either PFNA, PFDA or PFUA (C9 to C11) results in a significant increase in the concentration of liver PCho as measured by ^{31}P NMR spectroscopy. PFOA-treated rats, however, do not show this increase in liver PCho. Thus, the effects observed on hepatic phospholipid metabolism (namely, increases in PCho and probably DAG as well) are not unique to PFDA, but rather display a characteristic chain length dependence. This finding suggests that such biological activity may be related to the lipophilic character of these perfluorocarboxylic acids. In other words, the impact on phospholipid metabolism may result from the ability of the compound to embed itself in the cell membrane; only compounds with a chain length $\geq$ C9 may possess this ability.

VI. OVERALL CONCLUSIONS

During the past year, this research program has focussed on the perfluorocarboxylic acids and the effects they have on endogenous phospholipid and carbohydrate metabolism in liver. In summary, these studies have demonstrated that carbon chain lengths ranging from C9 to C11 can impact phospholipid metabolism by causing a significant increase in liver PCho. PFOA (C8) does not induce this response. The increase in PCho has been shown to signify phospholipase C activity which, in turn, causes an increase in liver DAG. These data suggest that PKC may also be activated, and studies are currently in progress to address this issue. PFDA also inhibits glucose transport in liver and subsequently affects carbohydrate metabolism. The common denominator in these results may involve a specific interaction of the perfluorocarboxylic acids with the plasma membrane. The toxic response may be dependent upon the compounds lipophilic character and its ability to disrupt phospholipid metabolism and membrane structure/function.

Future research plans will expand the scope of this project to include other peroxisome proliferator compounds. Through the use of NMR spectroscopy and standard biochemical assays, these studies will probe specific metabolic pathways and examine the impact of peroxisome proliferators on hepatic metabolism. This investigative strategy will delineate the

metabolic effects exerted by these compounds and aid in developing a clearer understanding of the hepatotoxic mechanisms at play.

TABLE 1

Effect of PFNA or PFUA Treatments on Hepatic Peroxisomal Fatty Acyl Oxidase Activity (Mean ± SEM)[a]

Group	Dose	Day Posttreatment	
		Day 3	Day 5
Control	vehicle	1.027 ± 0.13 (n = 3)	1.006 ± 0.167 (n = 4)
C_9-PFA	50 mg/kg	5.128 ± 0.092 (n = 2)	4.356 ± 0.618 (n = 2)
C_{11}-PFA	50 mg/kg	4.762 (n = 1)	4.807 ± 1.16 (n = 4)

[a]Measured in nmole/min/mg protein at 25 °C.

TABLE 2

Liver Phosphocholine Levels (Means ± SE) in μmol/g Tissue for PFNA and PFUA Treated and Control rats at Various Doses and Days Postdose[a]

	PFNA (mg/kg)			PFUA (mg/kg)	Control
Dose	50	100	150	50	vehicle
Day 3	3.49 ± 0.54 (n = 3)	4.21 ± 1.16 (n = 2)	4.29 ± 0.79 (n = 3)	1.72 ± 0.44 (n = 3)	1.31 ± 0.14 (n = 2)
Day 5	4.70 ± 1.36 (n = 3)	4.03 ± 0.28 (n = 2)	———	2.11 ± 0.46 (n = 2)	0.68 ± 0.13 (n = 2)

[a]Concentrations were calculated from ^{31}P NMR spectra of liver extracts.

FIGURE LEGENDS

Figure 1: Liver *sn*-1,2 diacylglycerol concentrations measured in rats treated with PFDA (50 mg/kg), PFOA (150 mg/kg), and corresponding pair-fed controls (vehicle dosed). Values are mean ± SD (number of rats per group is denoted in the figure). PFOA-treated and corresponding controls were examined on day 3 post-dose. PFDA-treated and corresponding controls were examined on day 5 post-dose. PFDA treatment causes a 3-fold increase in liver DAG concentration as compared to control ($p = 0.0002$). PFOA treatment has no effect.

Figure 2. Rat liver protein kinase C activity in cytosolic and microsomal fractions. Measurements were made at 5 days post-dost with either PFDA (50 mg/kg) or vehicle solution (control). Error bars denote the SE. Data are not different between treated versus control groups.

Figure 3. ^{31}P NMR spectral intensities (mean ± SE) for the PME peak from rat liver spectra *in vivo*. Measurements were made 5 days post-dose for both PFDA-treated (X-hatched) and corresponding pair-fed control groups (hatched). Data are shown for 15 and 25 mg/kg doses, and n=4 for all groups. The NMR signal intensities are relative to the integrated area for the entire ^{31}P spectrum set equal to 100. The asterisks denote a significant difference relative to corresponding controls ($p \leq 0.003$).

Figure 4. ^{31}P NMR spectral intensities (means ± SE) for the PME peak from rat liver spectra *in vivo* for PFNA- (open bars), PFUA-treated (cross-hatched) and corresponding control groups (solid bars). Data are shown for each group at 1 and 3 days posttreatment. Data for PFNA-treated rats are shown at 3 different doses (50, 100, and 150 mg/kg) while data for PFUA-treated rats are shown for 50 mg/kg only. The numbers of animals in each group are given below the group labels on the x-axis. NMR signal intensities are relative to the integrated area for the entire ^{31}P spectrum set equal to 100.

Liver *sn*-1,2 Diacylglycerol

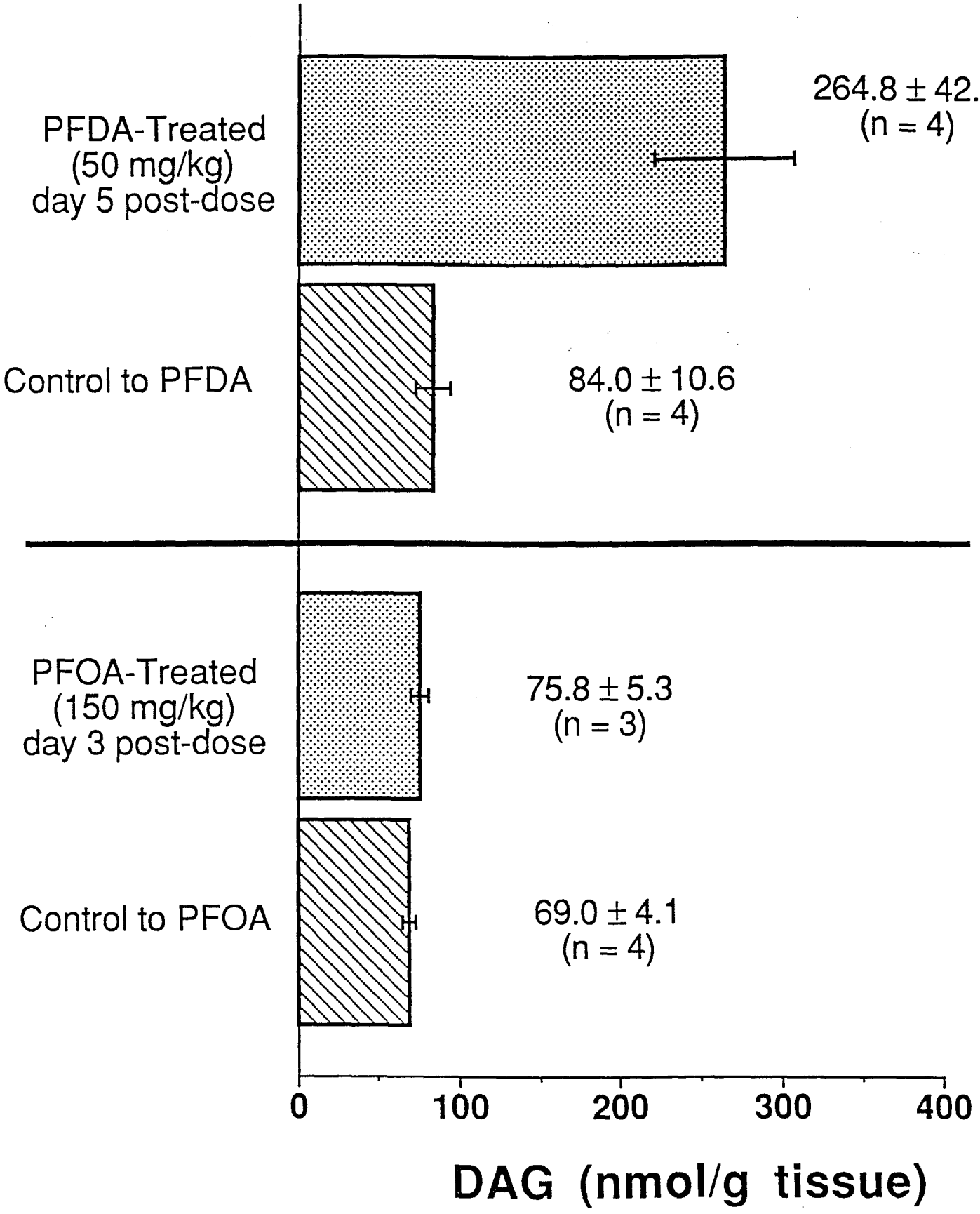

Figure 1

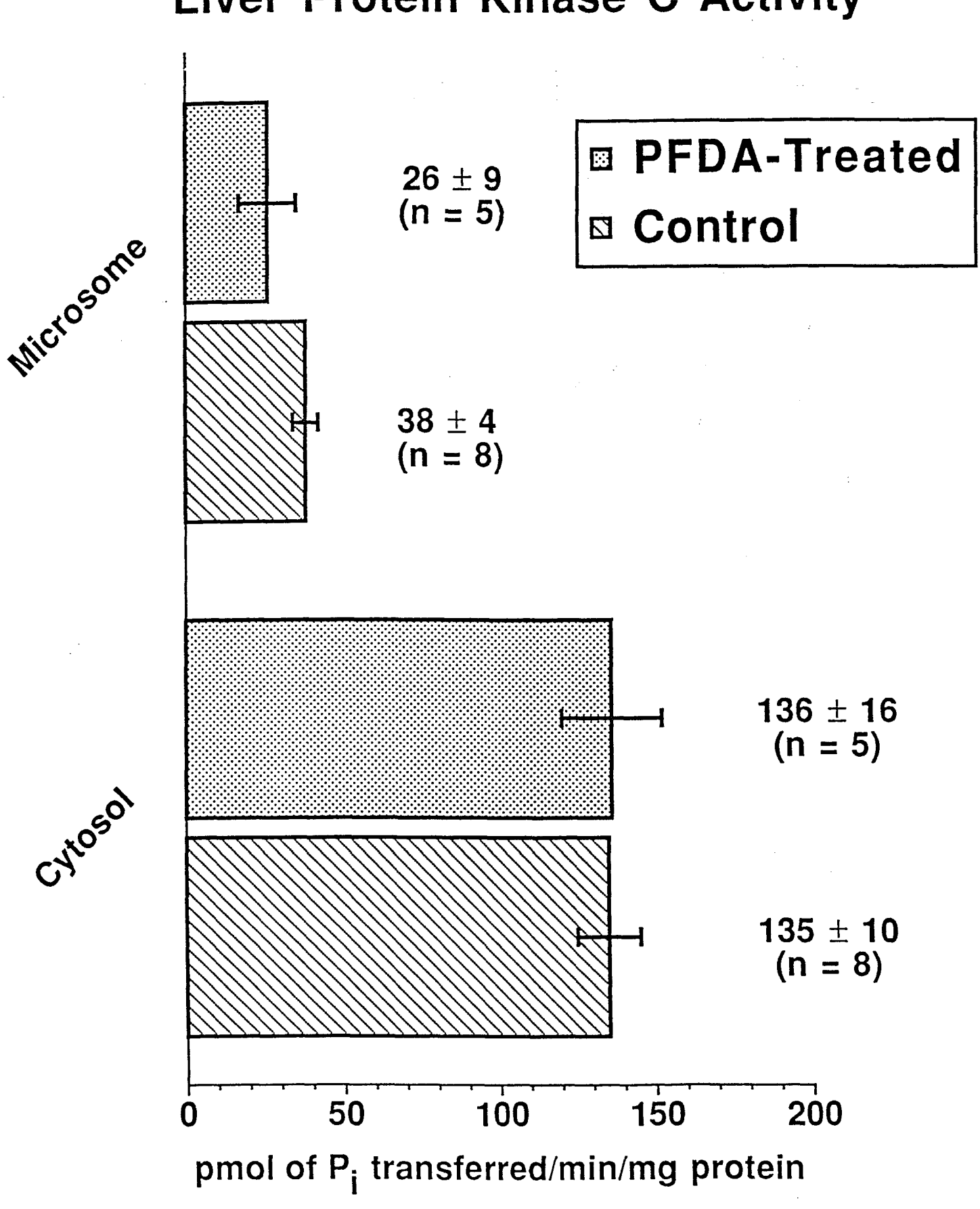
Liver Protein Kinase C Activity
PFDA-Treated
Control
Microsome
26 ± 9
(n = 5)
38 ± 4
(n = 8)
Cytosol
136 ± 16
(n = 5)
135 ± 10
(n = 8)
0
50
100
150
200
pmol of P_i transferred/min/mg protein

Figure 2

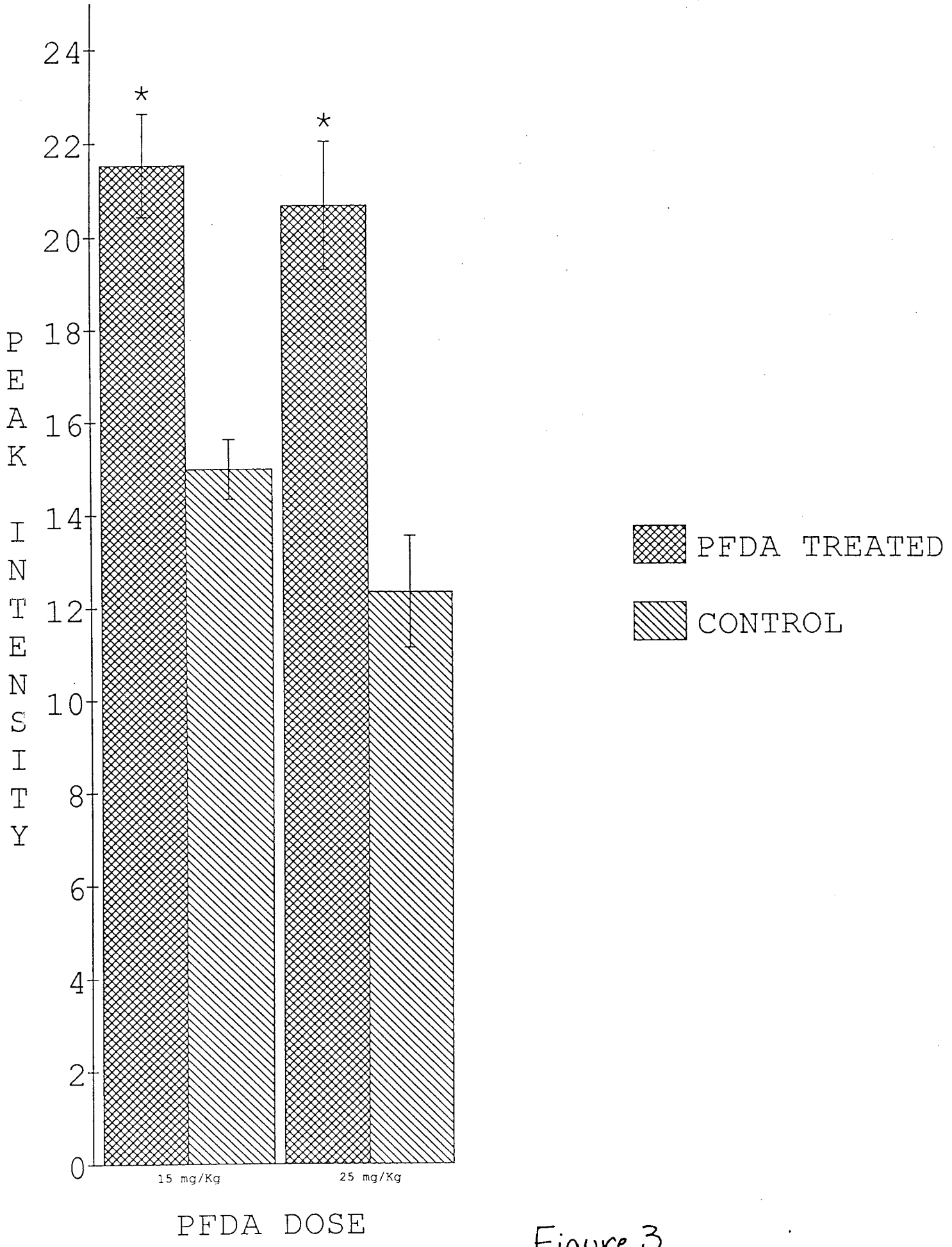
24
22
20
18
16
14
12
10
8
6
4
2
0
PEAK INTENSITY
*
*
15 mg/Kg
25 mg/Kg
PFDA DOSE
PFDA TREATED
CONTROL

Figure 3

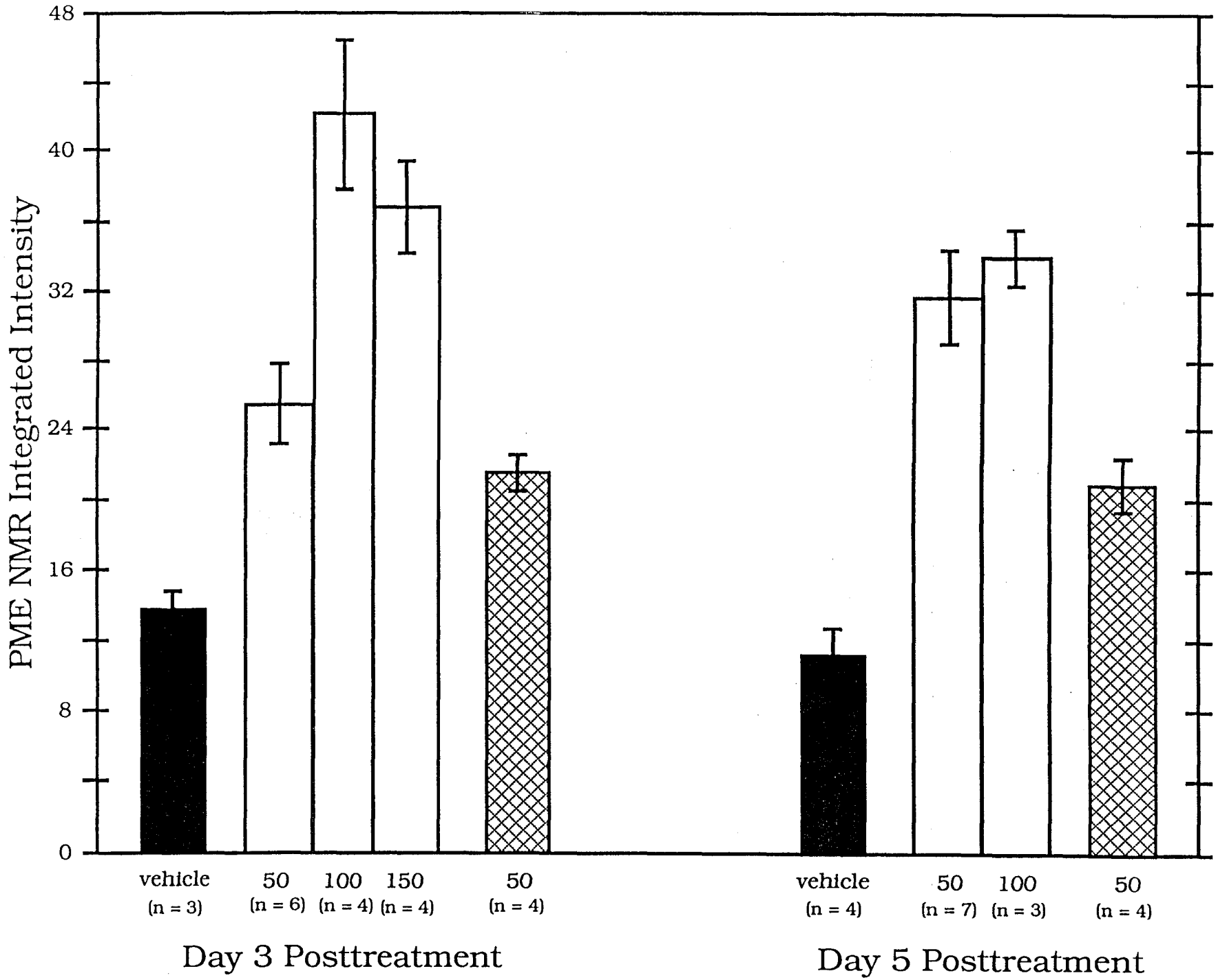
PME NMR Integrated Intensity
48
40
32
24
16
8
0
vehicle
(n = 3)
50
(n = 6)
100
(n = 4)
150
(n = 4)
50
(n = 4)
vehicle
(n = 4)
50
(n = 7)
100
(n = 3)
50
(n = 4)
Day 3 Posttreatment
Day 5 Posttreatment

Figure 4