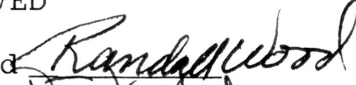
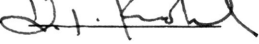


THE EFFECT OF DIETARY FATS ON THROMBOTIC METABOLITES
IN HUMAN PLASMA

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APPROVED

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**THE EFFECT OF DIETARY FATS ON THROMBOTIC METABOLITES
IN HUMAN PLASMA**

INTRODUCTION

Some dietary saturated fatty acids may be linked to coronary heart disease (1,2) which causes 50% of all deaths in the United States (3). These saturated fatty acids tend to increase low density lipoprotein (LDL) level in plasma. LDL transports cholesterol from the liver to peripheral tissues through arteries. Atherosclerosis is initiated when fatty material, particularly cholesterol, infiltrates the intima of the arteries and eventually restricts the arterial lumen. Coronary heart disease is linked not only with atherosclerosis but arterial thrombosis, the formation of a blood clot within the artery. It is usually the result of endothelial damage, which can be caused by chronic hypercholesterolemia, hypertension, wall shear forces, etc. Damage to the vessel wall causes a chain of reactions resulting in the formation of thrombin, which converts fibrinogen to fibrin. The fibrin molecules clump together and trap passing blood platelets in an insoluble network, forming a blood clot. The platelets use arachidonic acid to synthesize and secrete lipids called prostaglandin endoperoxides and thromboxane A_2 (4,5), which reinforce the response to the initial stimulus, the damage to the vessel wall. Formation of a thrombus can obstruct the flow of blood if it becomes large enough, leading to infarction (death of living tissue resulting from obstruction of circulation due to a thrombus). Arachidonic acid is derived from dietary linoleic acid

(6); arachidonic acid is then converted to the prostaglandin endoperoxides, most of which are converted to thromboxane A₂ (7,4,5).

The ratio of thromboxane A₂ and prostacyclin (PGI₂) seems to determine the tendency towards thrombosis (6). Thromboxane A₂ is a vasoconstrictor which has a prothrombotic effect and induces platelet aggregation. The vasodilator prostacyclin inhibits platelet aggregation and is antithrombotic.

The type and amount of a person's dietary fatty acids have been shown to affect the tendency towards thrombogenesis. Diets which are high in polyunsaturated fats tend to reduce the risk of thrombogenesis, and long-chain saturated fats tend to enhance the risk (7,8). An exception to this rule seems to be the tropical oil palm oil, the second largest selling vegetable oil in the world market (9). It contains 50% saturated fatty acids (primarily palmitic acid), 40% oleic acid, and 10% linoleic acid (3) and shows an uncharacteristic antithrombotic effect despite its high content of saturated fat. It has been speculated that the reason for this is the presence of unusual triglyceride species in palm oil and possibly also the composition of its nontriglyceride fraction. Palm oil has some triglyceride species that are completely saturated, whereas most natural triglycerides have a saturated fatty acid in their 1- and/or 3-positions with a polyunsaturated fatty acid in the 2-position (9).

The aim of this study was to determine the effects that specific dietary fats have on the concentrations of thromboxane B₂

and 6-keto-prostaglandin F_{1a} in human plasma. Thromboxane B_2 is a stable intermediate of thromboxane A_2 , which has a short half-life (30 sec). It will be measured in conjunction with 6-keto-prostaglandin F_{1a} , a degradation product of PGI_2 (10), to give the effect of the diets on these antagonistic metabolites.

MATERIALS AND METHODS

Dietary fats tested were butter, refined palm oil, crude palm oil, sunflower oil, Parkay margarine, and a blend of 80% palm oil and 20% sunflower oil. Six groups of five men between the ages of 30 and 60 were given a different dietary fat for six weeks. For selection of the study, the subjects had to have a physical examination, electrocardiogram, and blood chemistry profile to eliminate the use of subjects with possible health problems. The subjects also had to have blood cholesterol levels between 130 and 240 mg/dl. Between each diet period was a six week "wash out" period. Rotations continued until all groups had been given all six diet fats being tested. Only three rotations were able to be measured for this study. During each diet period 40% of each participant's total calories came from fat of which 60% was the test fat. Therefore 24% of total calorie intake was from the test fat, which replaced natural fats in ice cream, milk, cookies, muffins, and other foods. A nutritionist planned the individual diets for the subjects to correspond to their habitual diet, and each subject recorded a seven-day food record for each diet period. Plasma samples were taken the week immediately before the test

period, to be used as a baseline, and the fifth and sixth week of the test period.

Biotecx Thromboxane B₂ [¹²⁵I] Direct and 6-Keto-Prostaglandin F_{1a} [¹²⁵I] Direct Radioimmunoassay Kits were used on the human plasma. Plasma was collected into a tube in which aspirin solution was immediately placed. Reagents and samples were brought to room temperature after storage at 2-8 C. For the standards, two tubes each were used. Total activity and Non-Specific Binding (NSB) tubes were also counted twice. For Thromboxane B₂ the standards were 0,1,5,10,25,50, and 100 pg/0.1 ml; 6-Keto Prostaglandin F_{1a} also had two tubes of 200 pg/0.1 ml. Total counts tubes contained 100 ul of the tracer [¹²⁵I]. NSB tubes contained 100 ul of the zero standard and 100 ul of the tracer. Standards had 100 ul of the appropriate concentration and samples had 100 ul of the appropriate subject's plasma. All tubes except the total activity and NSB tubes contained 100 ul of the corresponding antiserum. The tubes were incubated at 2-8 C overnight. One ml of Precipitating Reagent was pipetted into all tubes except the total activity tubes. Tubes were vortexed and centrifuged at 1000 X G for ten minutes. The supernatant was then decanted from all tubes except the total activity tubes. Tubes were left inverted for five minutes, and then the remaining drops were absorbed with a tissue paper. Tubes were counted in a gamma scintillation counter for one minute.

RESULTS

The average counts per minute (CPM) were measured for all

tubes. The average CPM of the NSB tubes was subtracted from all measurements, giving corrected activity. The corrected activity for all tubes was divided by the total activity to give the %Binding. The corrected counts for each sample was divided by the average corrected counts for the zero standard. This gave corrected binding. The concentration of the standards was plotted logarithmically against the linear percent binding of the standards. From this, the percent binding of the samples was plotted, allowing extrapolation of sample concentrations (Fig. 1a). Once sample concentrations were determined, they could be plotted for each subject for all three diet periods.

Each subject had three baseline concentrations for both metabolites since baselines were taken for all three diet periods. These concentrations were averaged for a mean concentration for both metabolites for each subject. Then each mean concentration for all subjects were averaged for a population mean concentration, with one standard deviation (Fig. 1-44). This concentration and its deviation were averaged to give a range of habitual concentrations for the population, and this was plotted for each man's metabolite concentration for a particular diet; in each figure the term "Dietary Response" refers to the average of the baseline figures subtracted from the averages of the fifth and sixth weeks (Fig. 45-56). Thus, both metabolites had graphs showing all the test fats and their effect on the concentration of the metabolite for all of the men on that test fat. From this, t-tests were run on each graph to see if the test fats had

significant effects on the metabolite concentrations compared to the average baseline concentrations.

DISCUSSION

Of all of the test fats, refined palm oil, butter, and the sunflower/palm oil blend did not show a significant effect on thromboxane B₂. All test fats showed significant effects on 6-keto-prostaglandin F_{1a}. This is contrary to Rand's study of palm oil (1988) using the aorta loop technique in which thromboxane formation was significantly reduced while prostacyclin production was unaffected (11).

Of the remaining three fats, sunflower and Parkay showed significant increases of thromboxane B₂. Of these two fats, sunflower oil had the greater effect. Crude palm oil increased thromboxane B₂ concentrations significantly.

6-keto-prostaglandin F_{1a} concentrations were significantly lowered by butter, Parkay, crude and refined palm oil. The sunflower oil and sunflower/palm oil blend had the least significance on decreasing 6-keto-prostaglandin F_{1a} concentrations. These results show that sunflower oil, crude palm oil, and Parkay would increase tendency towards thrombosis since they all increased thromboxane concentrations while decreasing 6-keto-prostaglandin F_{1a} concentrations. Effects of both refined palm oil and butter on thromboxane concentrations were not confirmed but they did lower 6-keto-prostaglandin F_{1a} concentrations; this might still increase

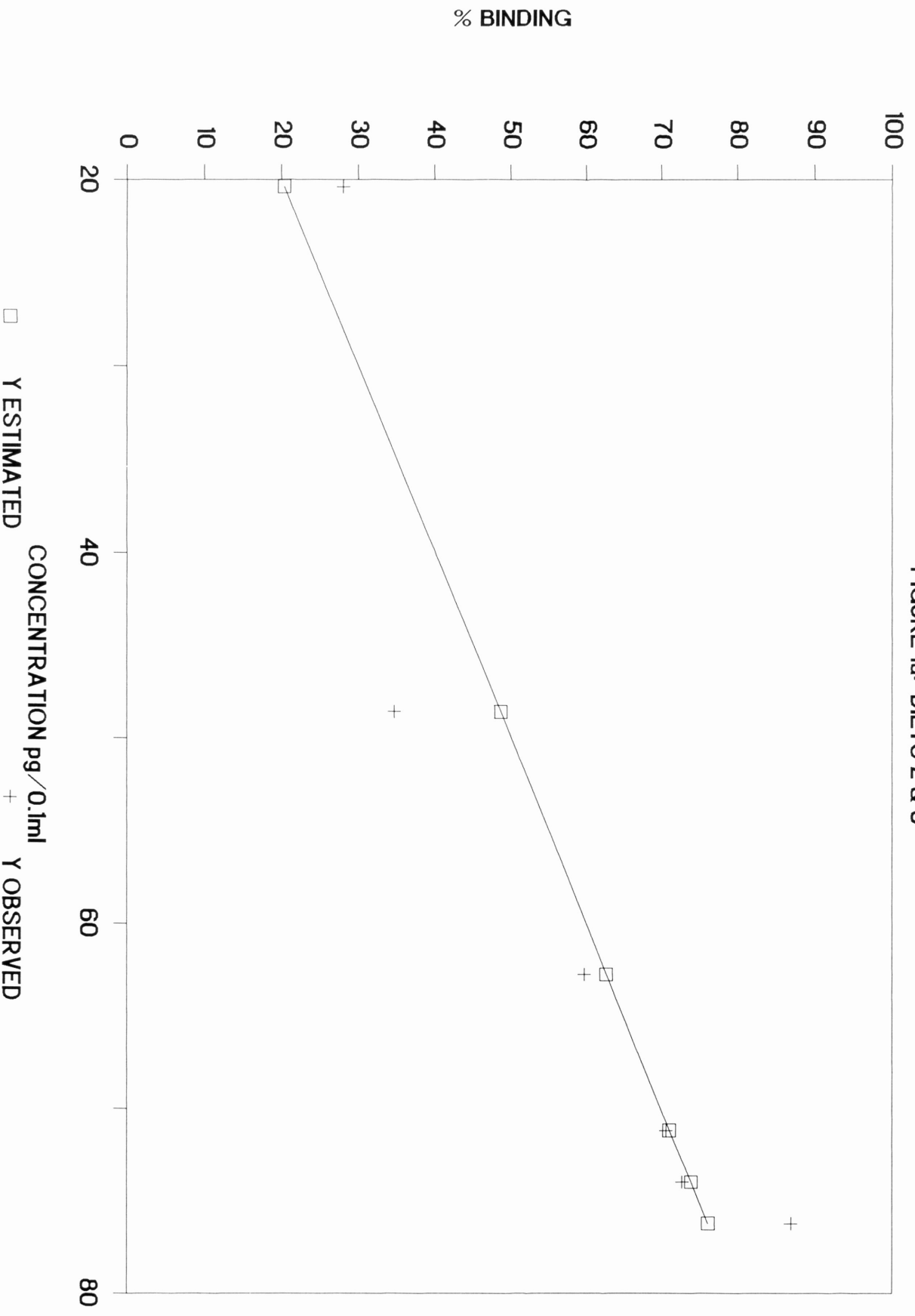
tendency toward thrombogenesis, since 6-keto-prostaglandin F_{1a} is a vasodilator. The sunflower palm oil blend also had this effect although it was not as significant as refined palm oil and butter.

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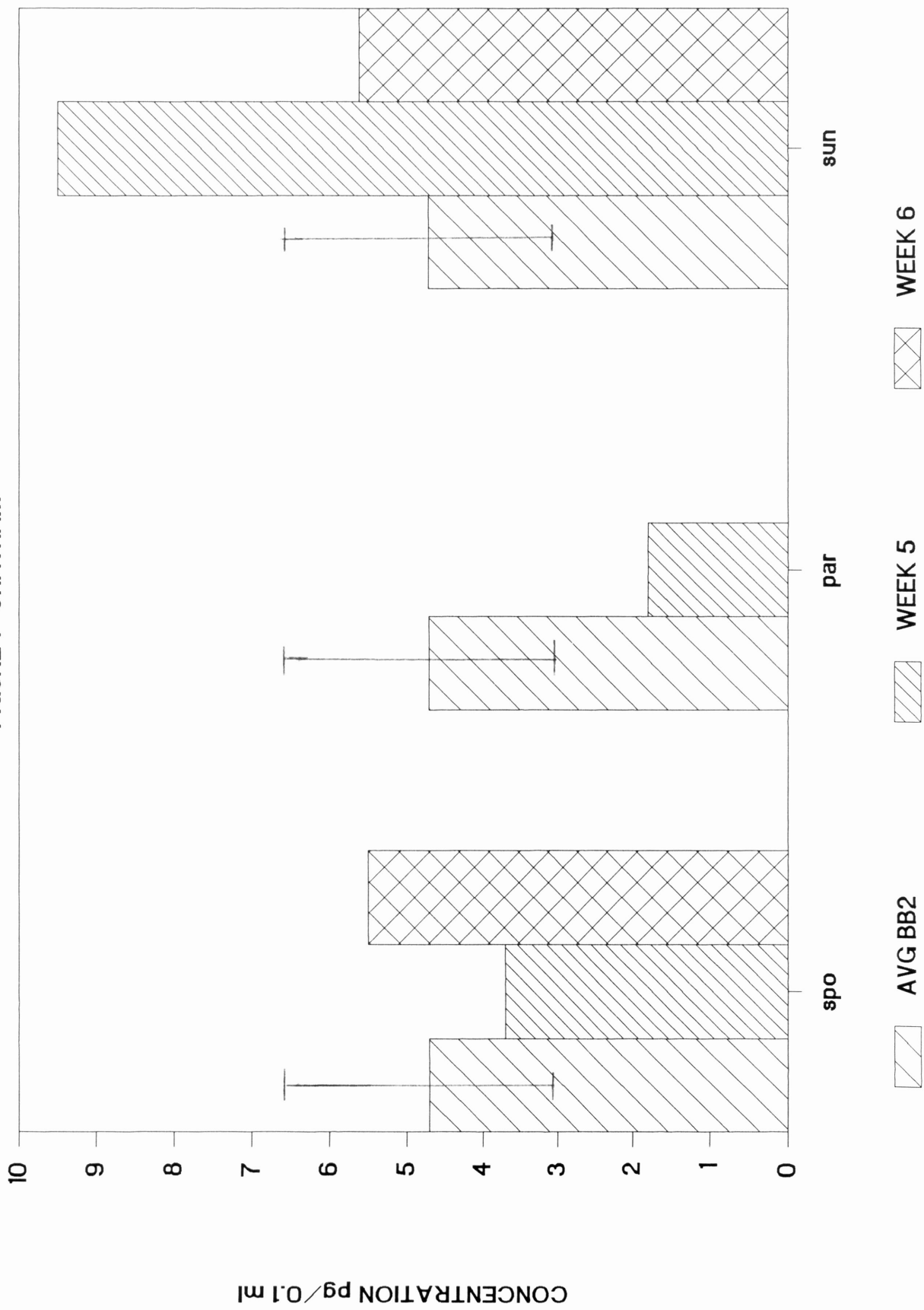
STANDARDS: THROMBOXANE B2

FIGURE 1a: DIETS 2 & 3



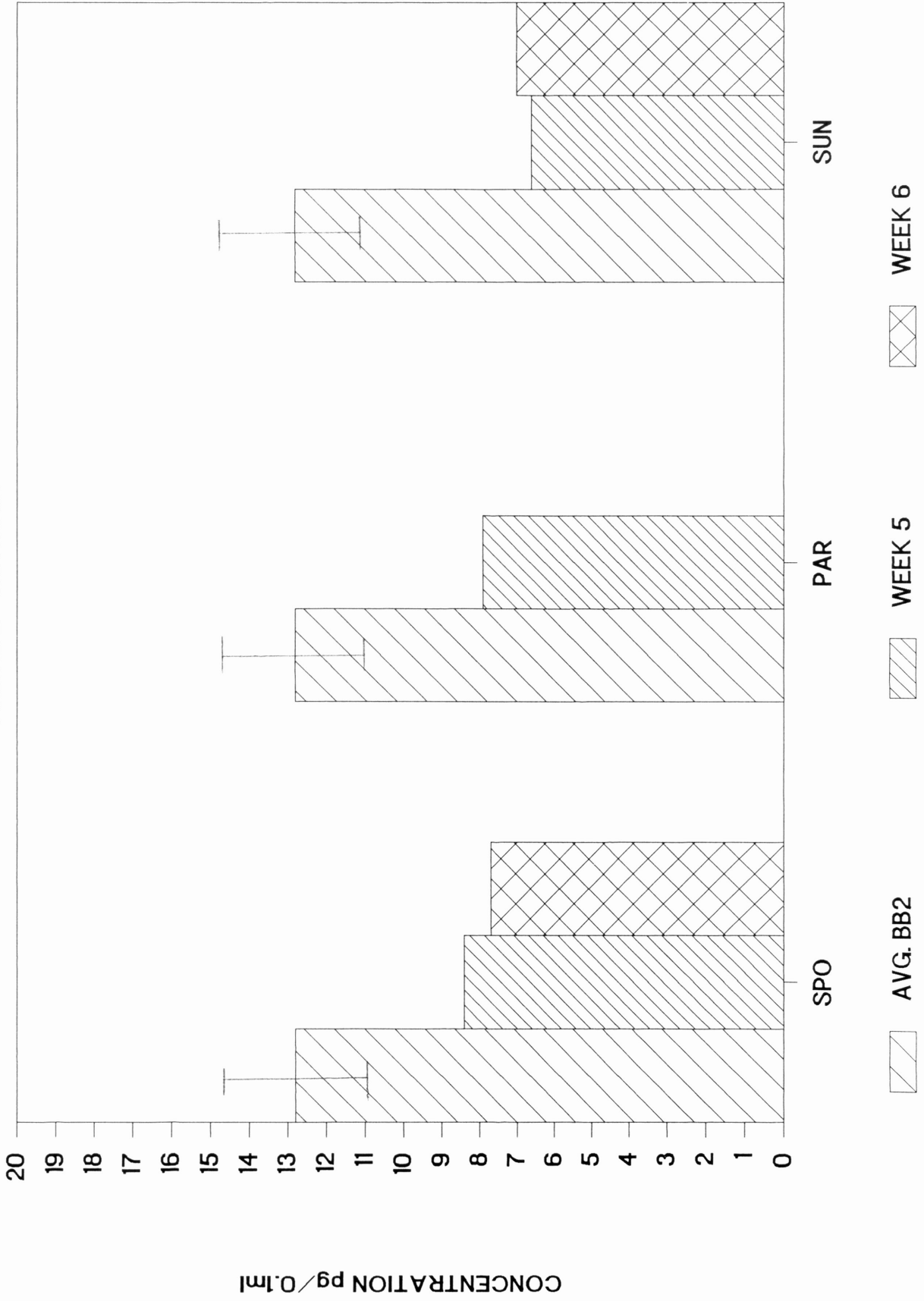
THROMBOXANE B2

FIGURE 1: CHATHAM



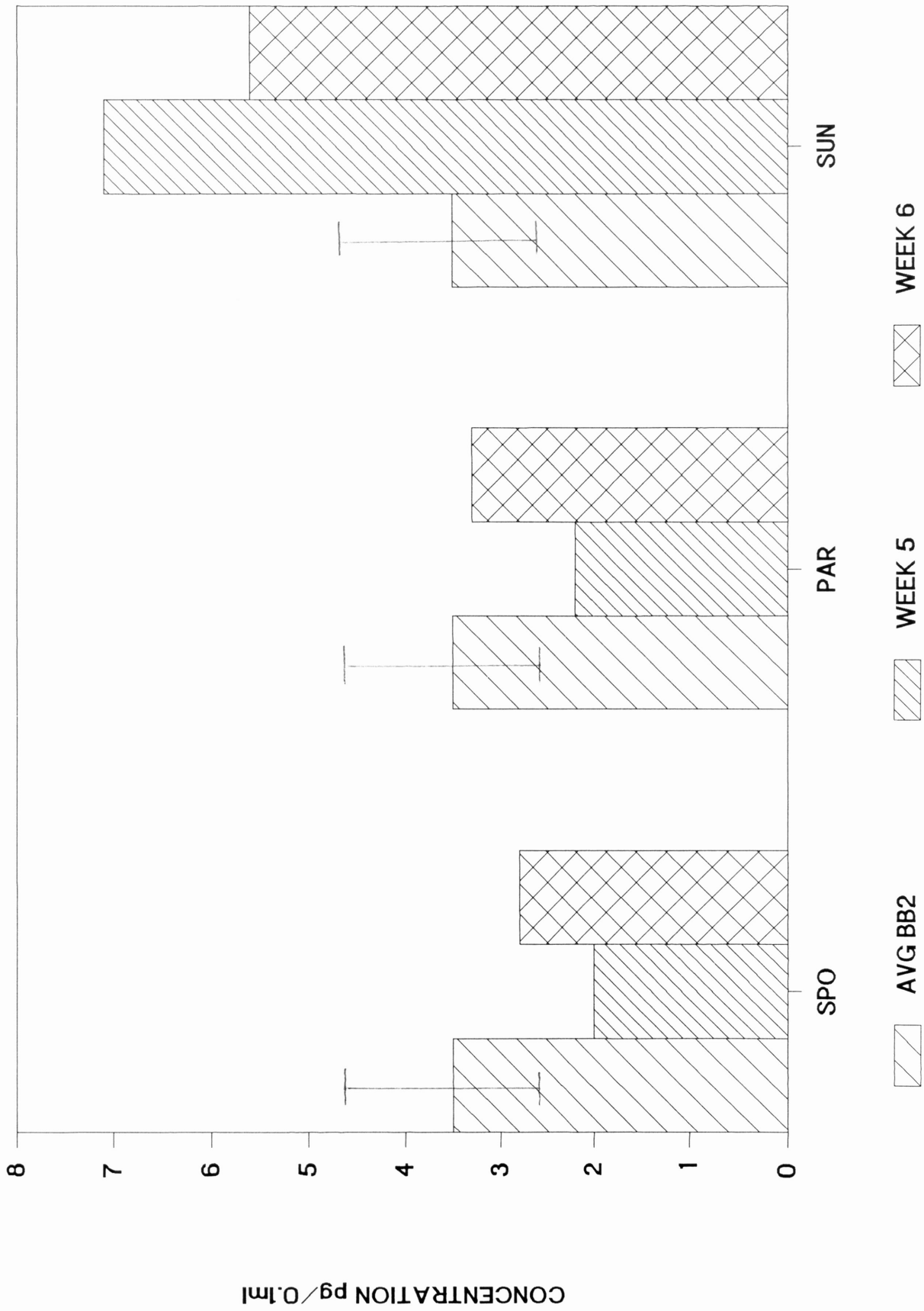
6-KETO-PROSTAGLANDIN F1a

FIGURE 2: CHATHAM



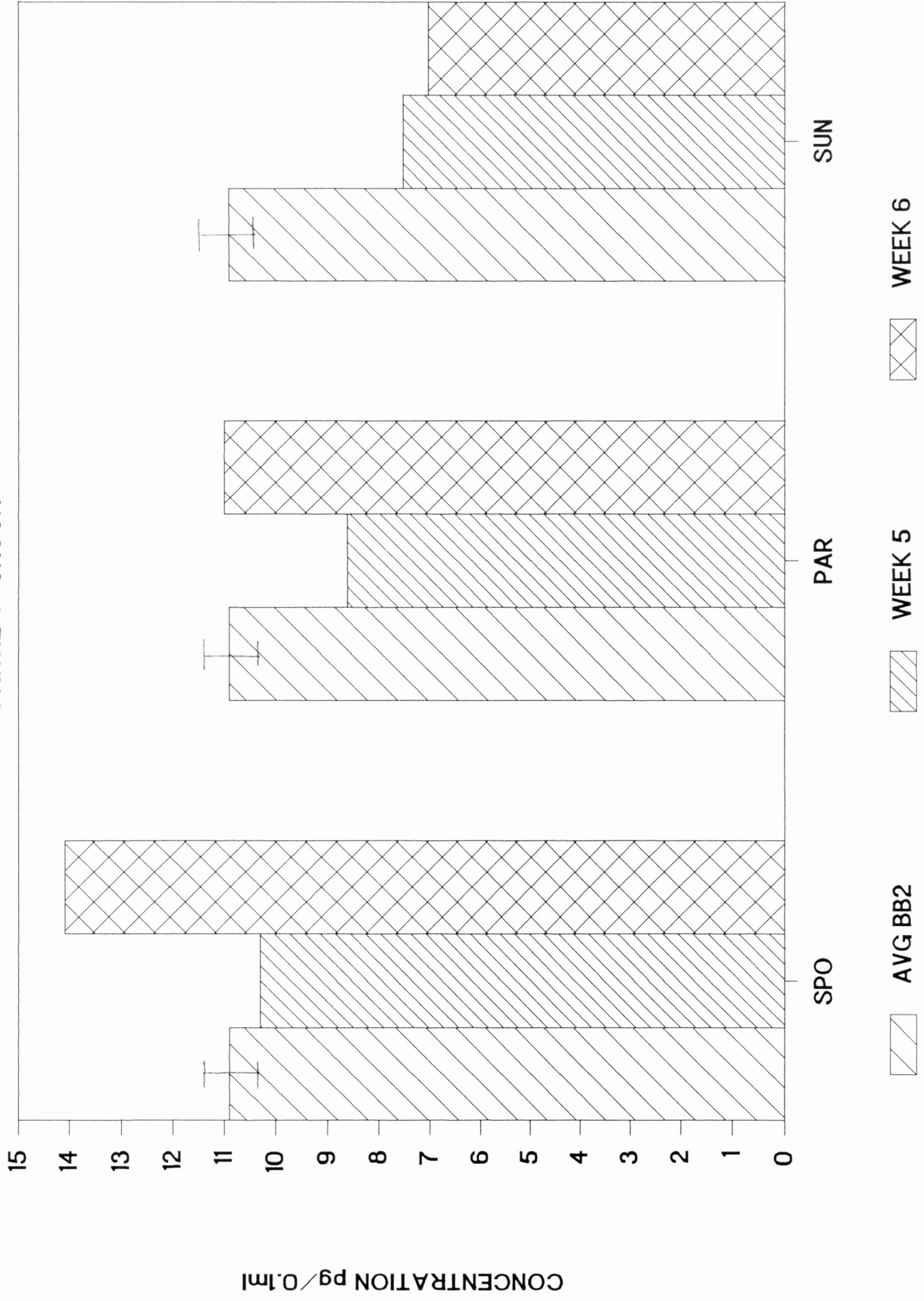
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FIGURE 3: CROOK



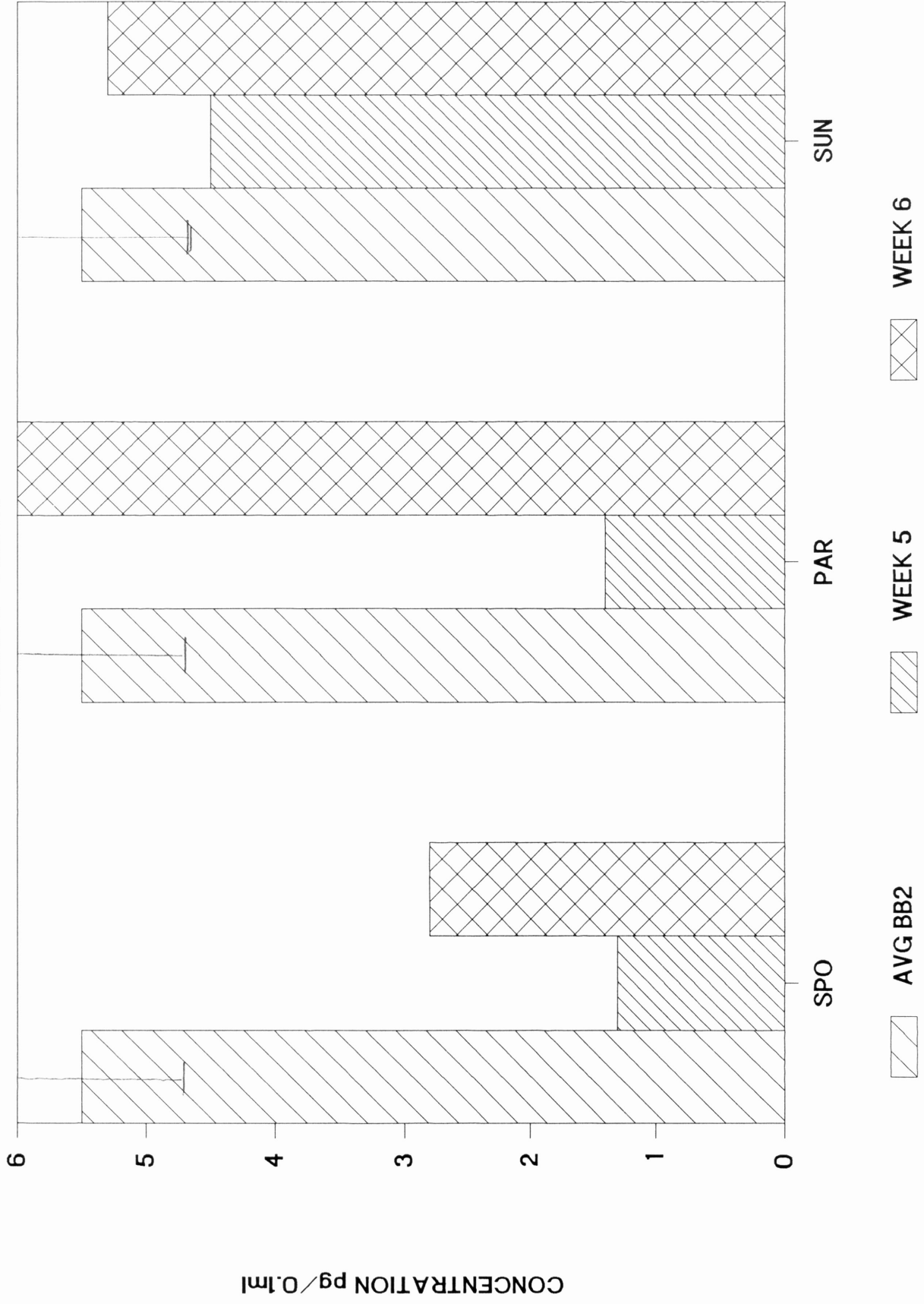
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FIGURE 4: CROOK



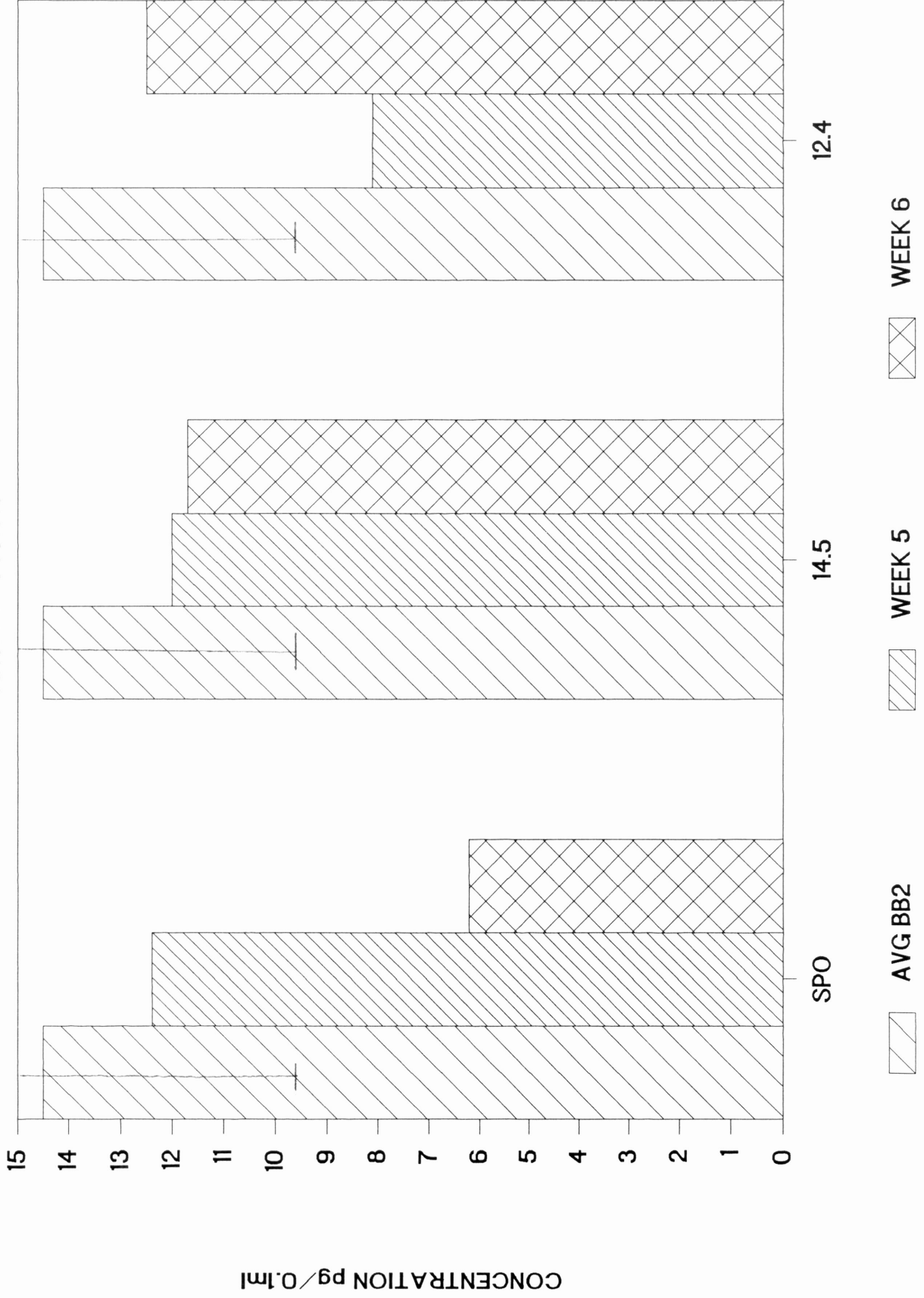
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FIGURE 5: CUSICK



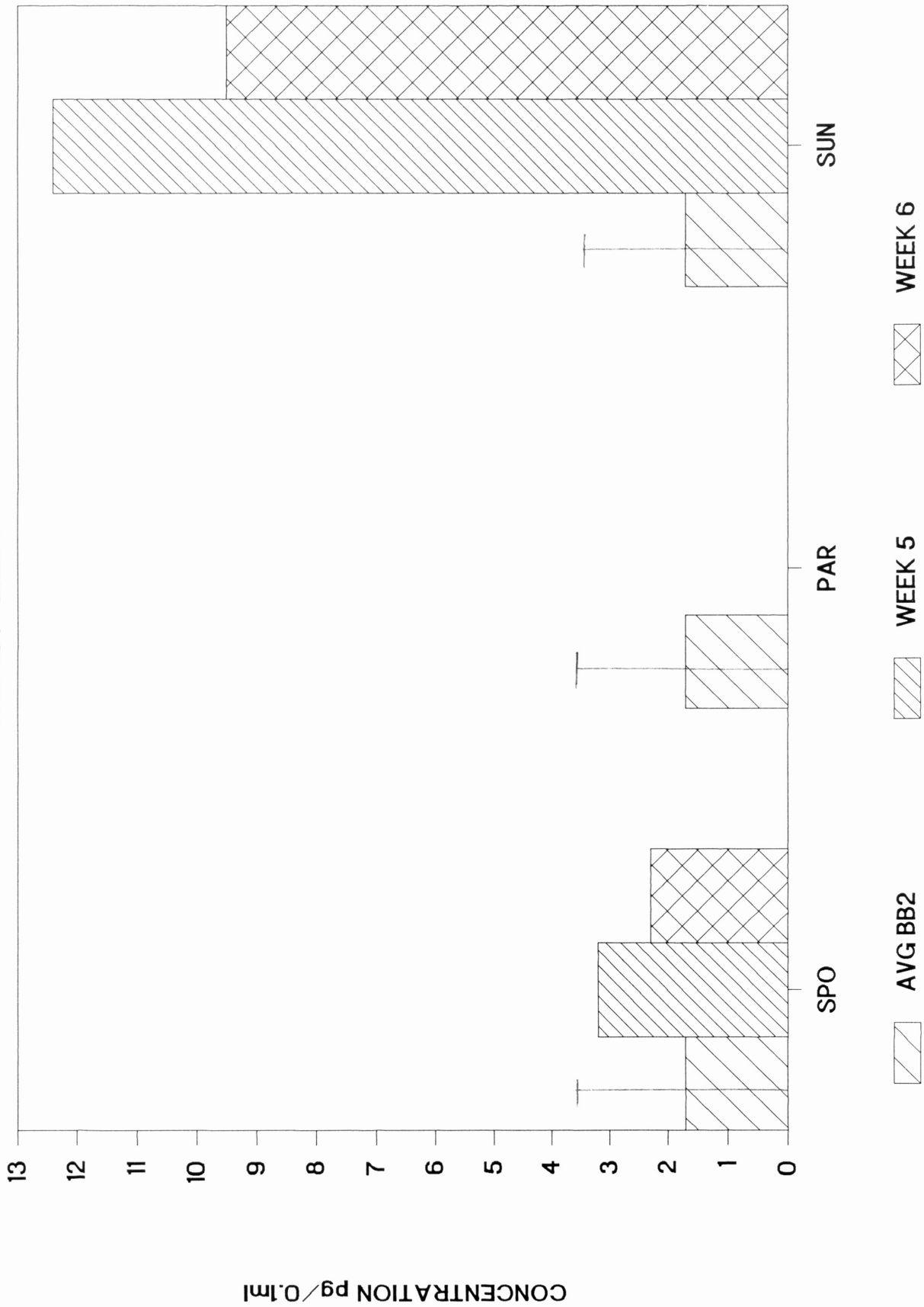
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FIGURE 6: CUSICK



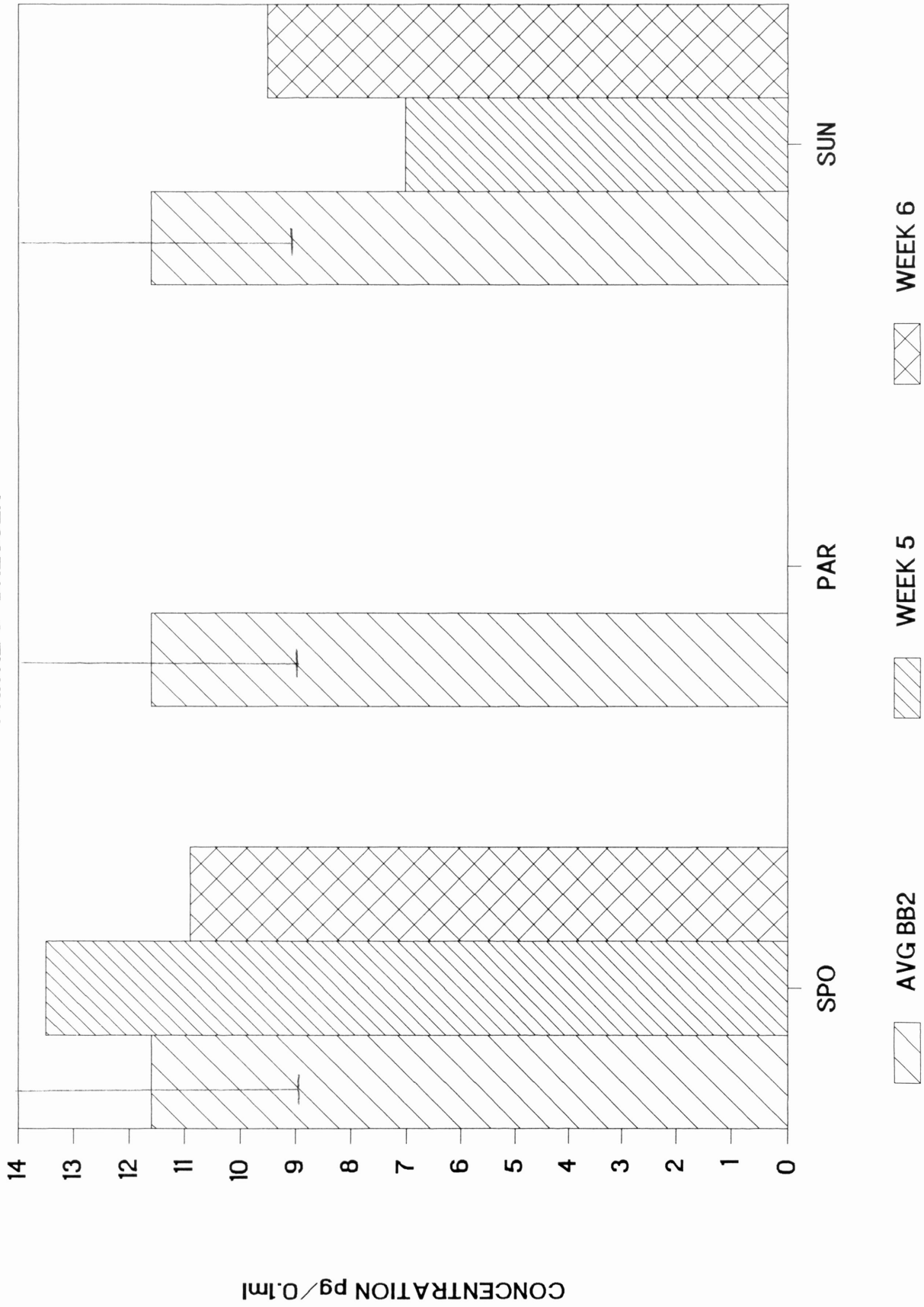
THROMBOXANE B2

FIGURE 7: DRESSER



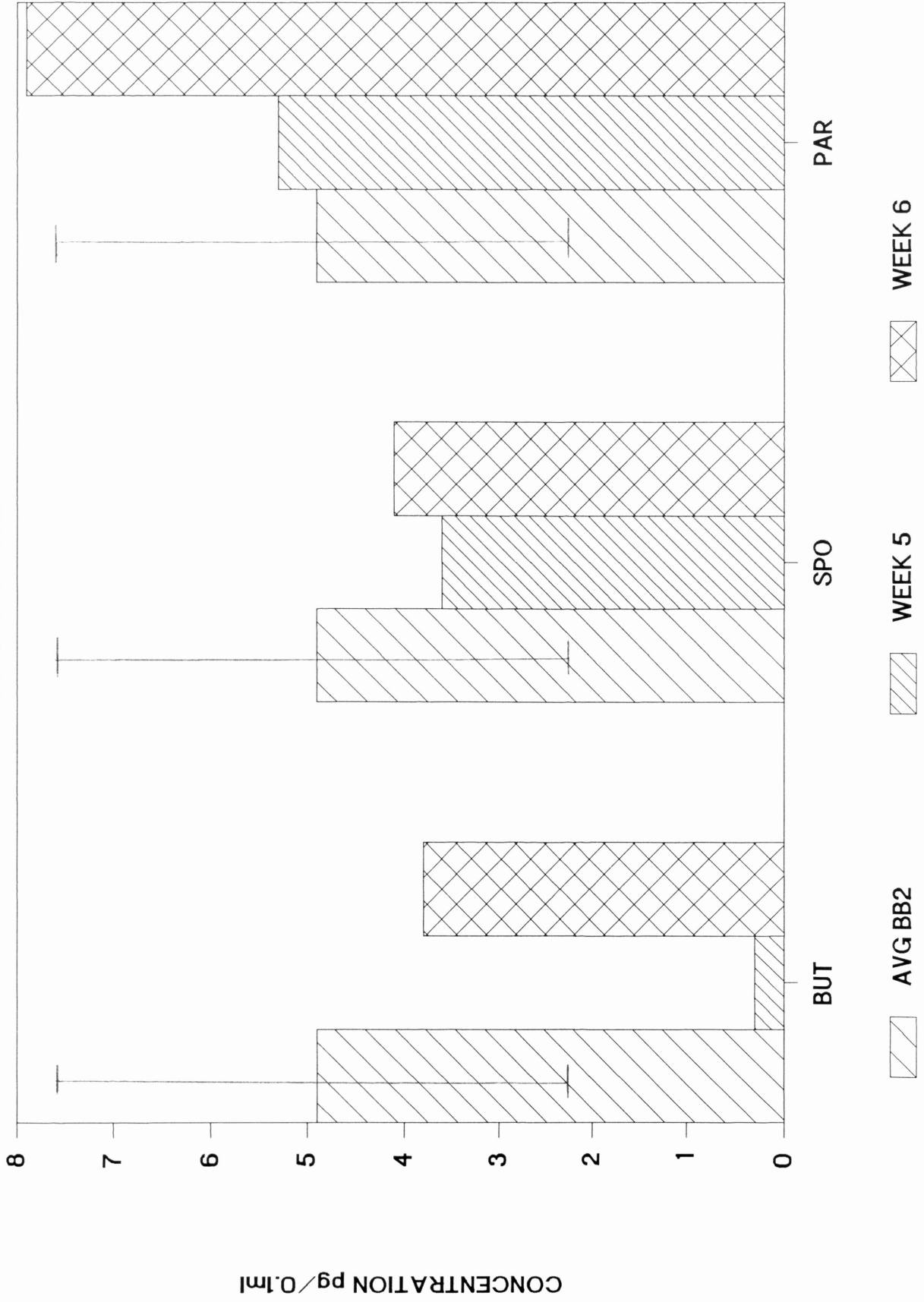
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FIGURE 8: DRESSER



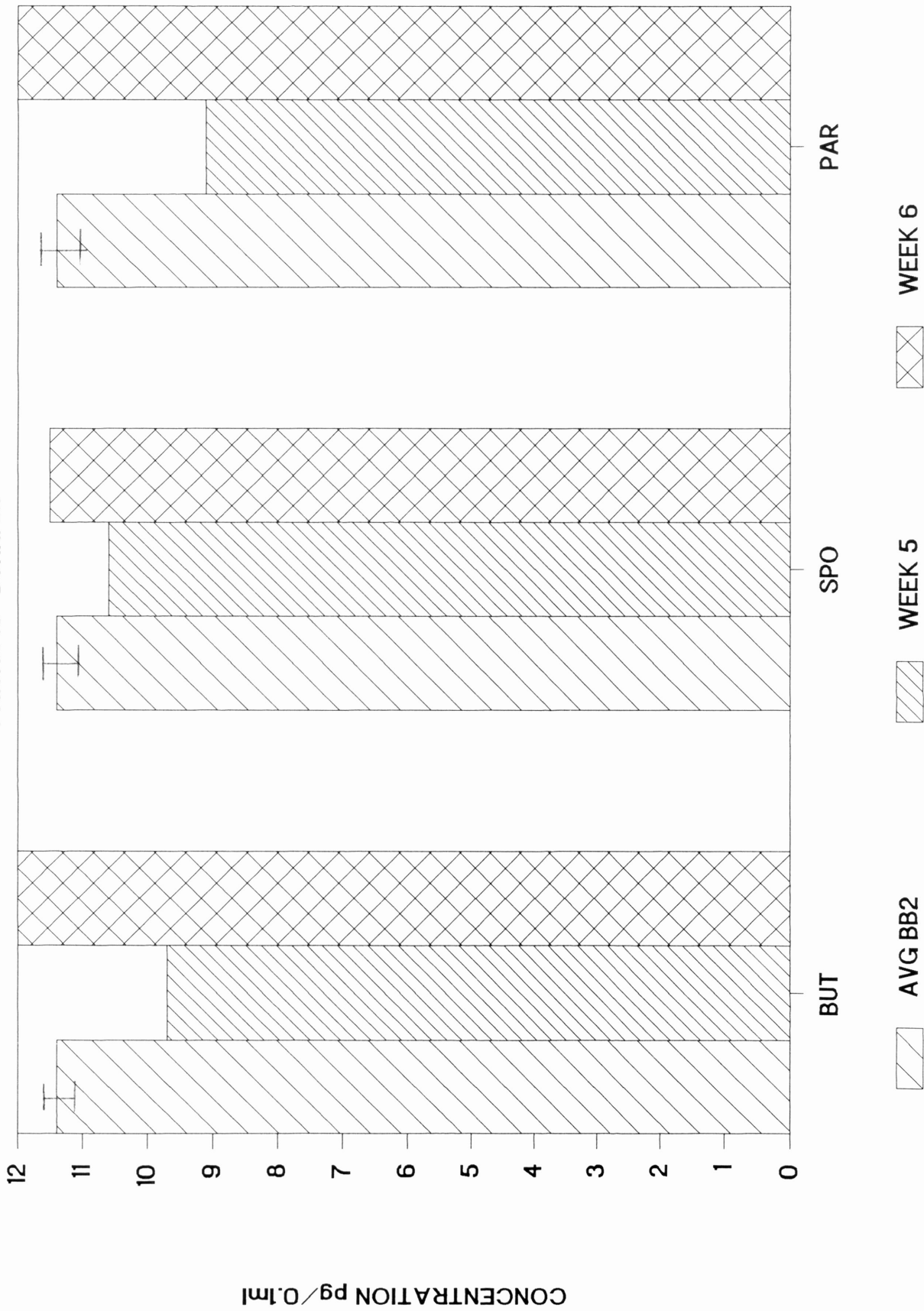
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FIGURE 9: DUNHAM



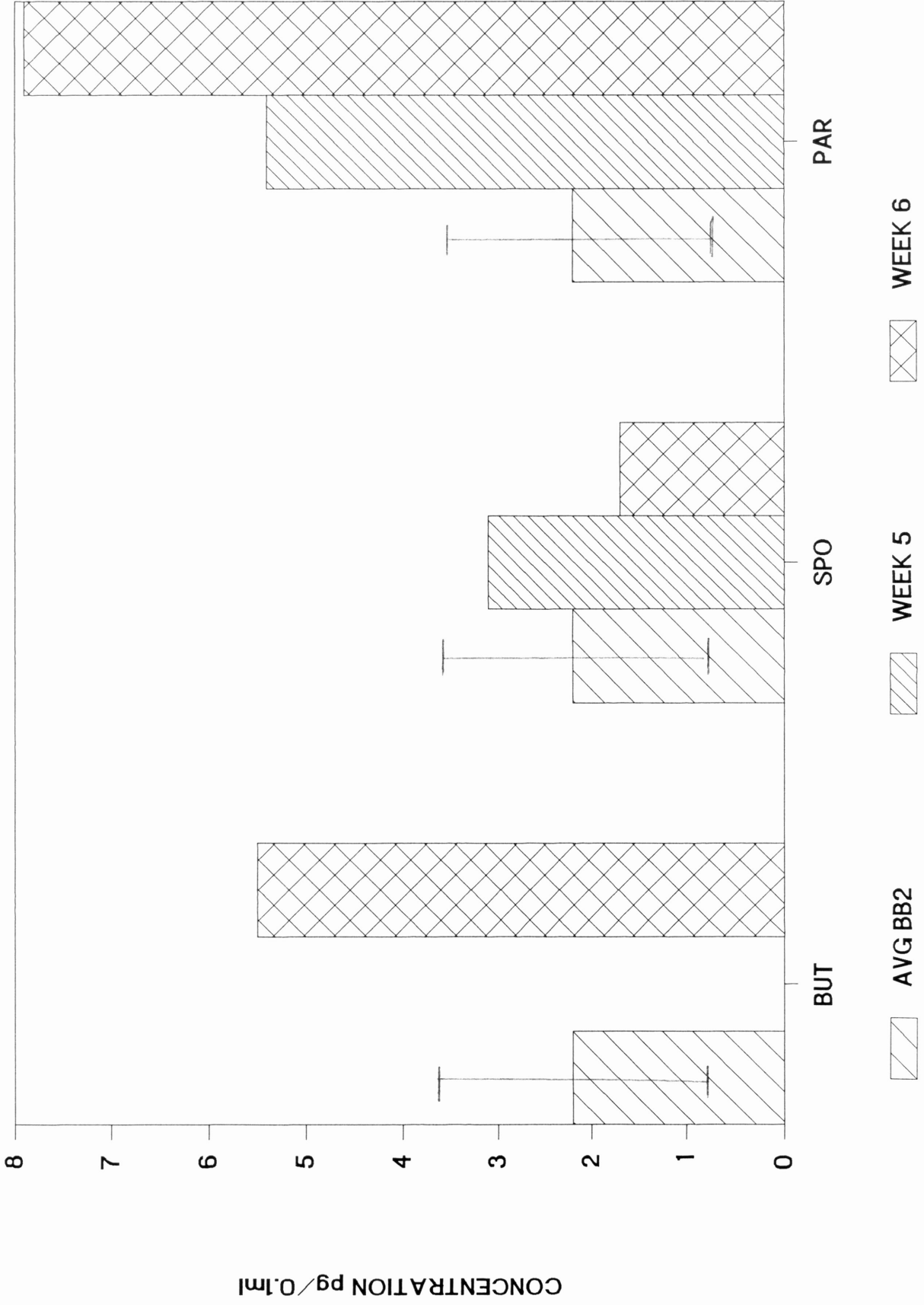
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FIGURE 10: DUNHAM



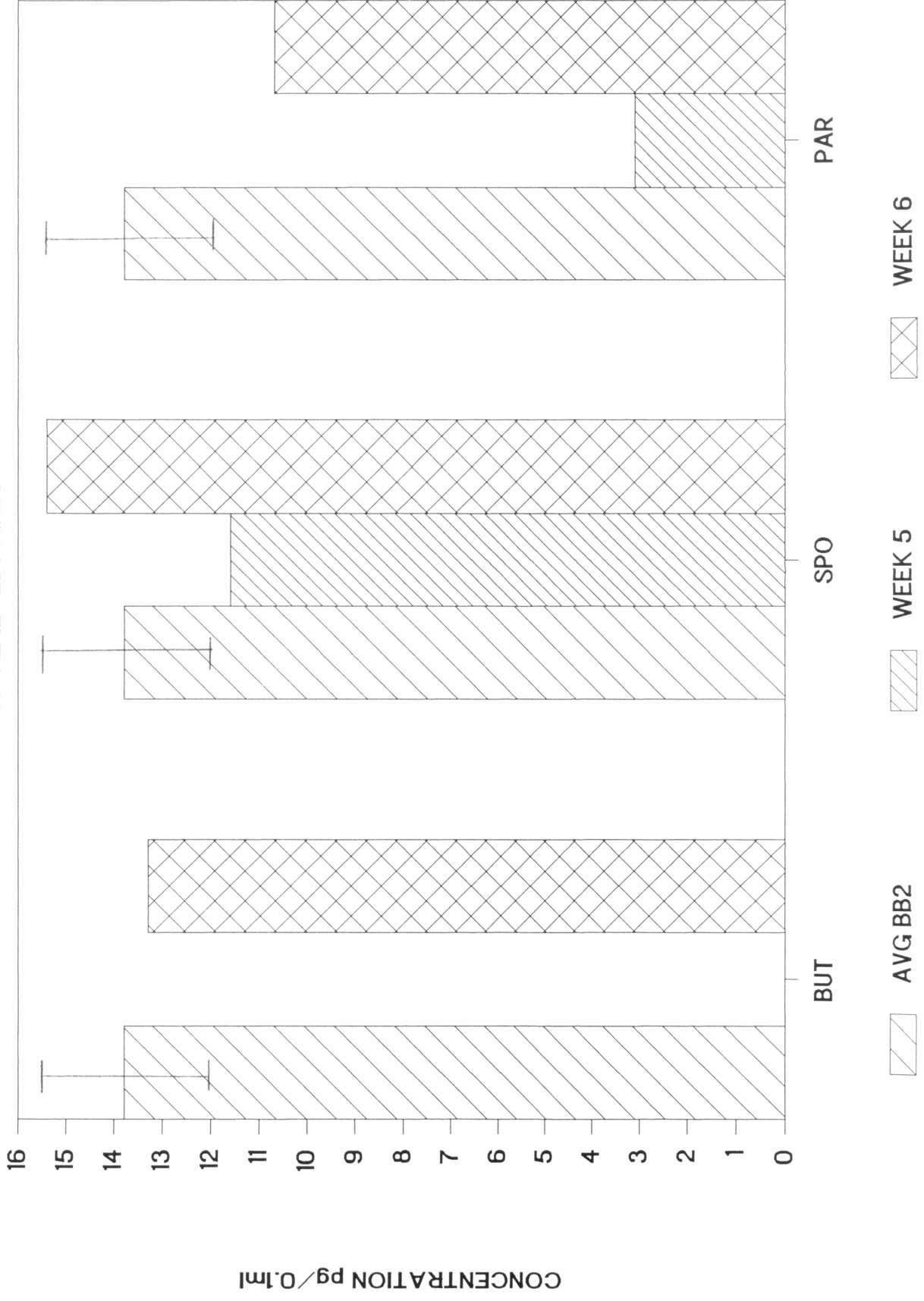
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FIGURE 11: EDWARDS



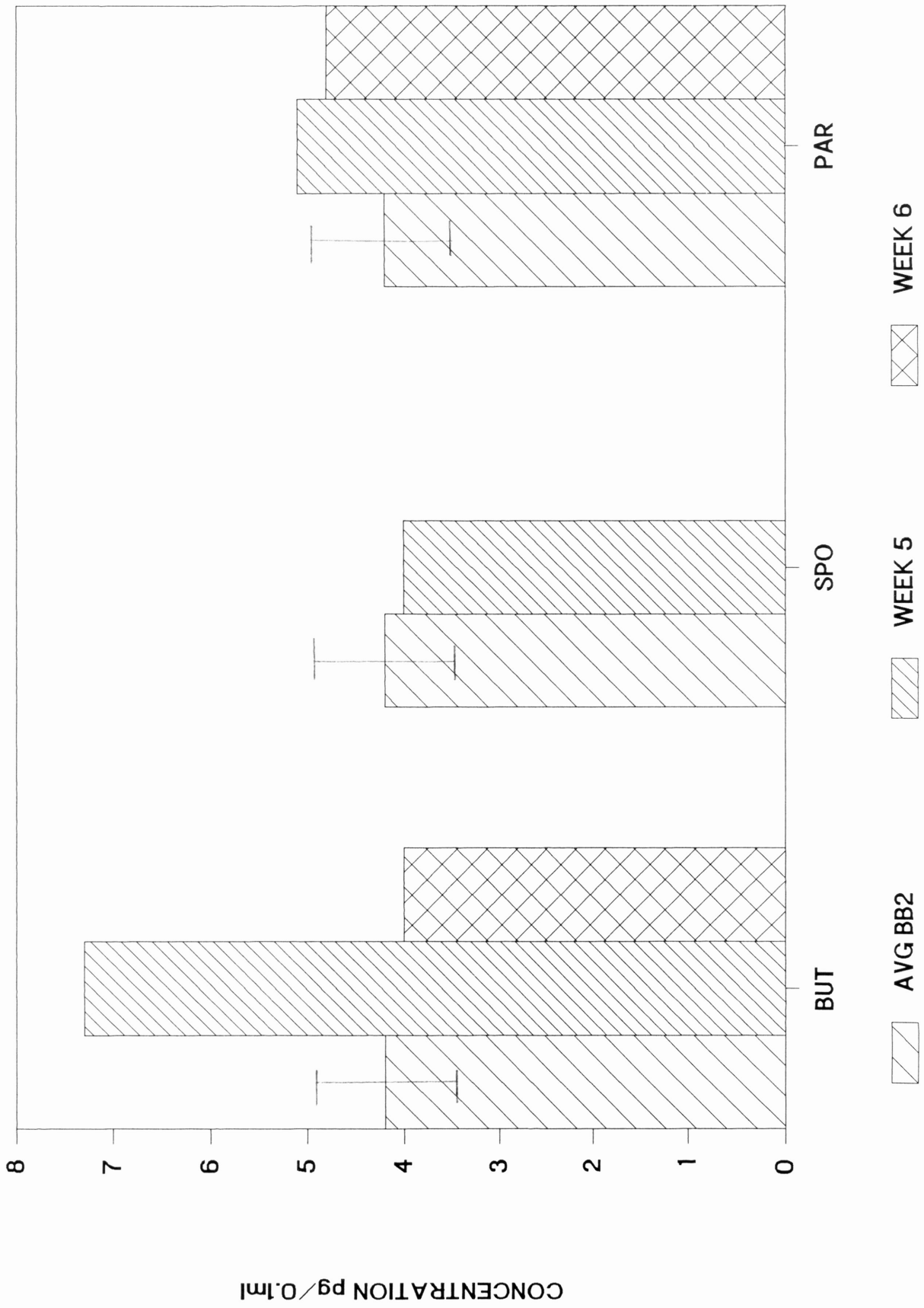
6-KETO-PROSTAGLANDIN F1a

FIGURE 12: EDWARDS



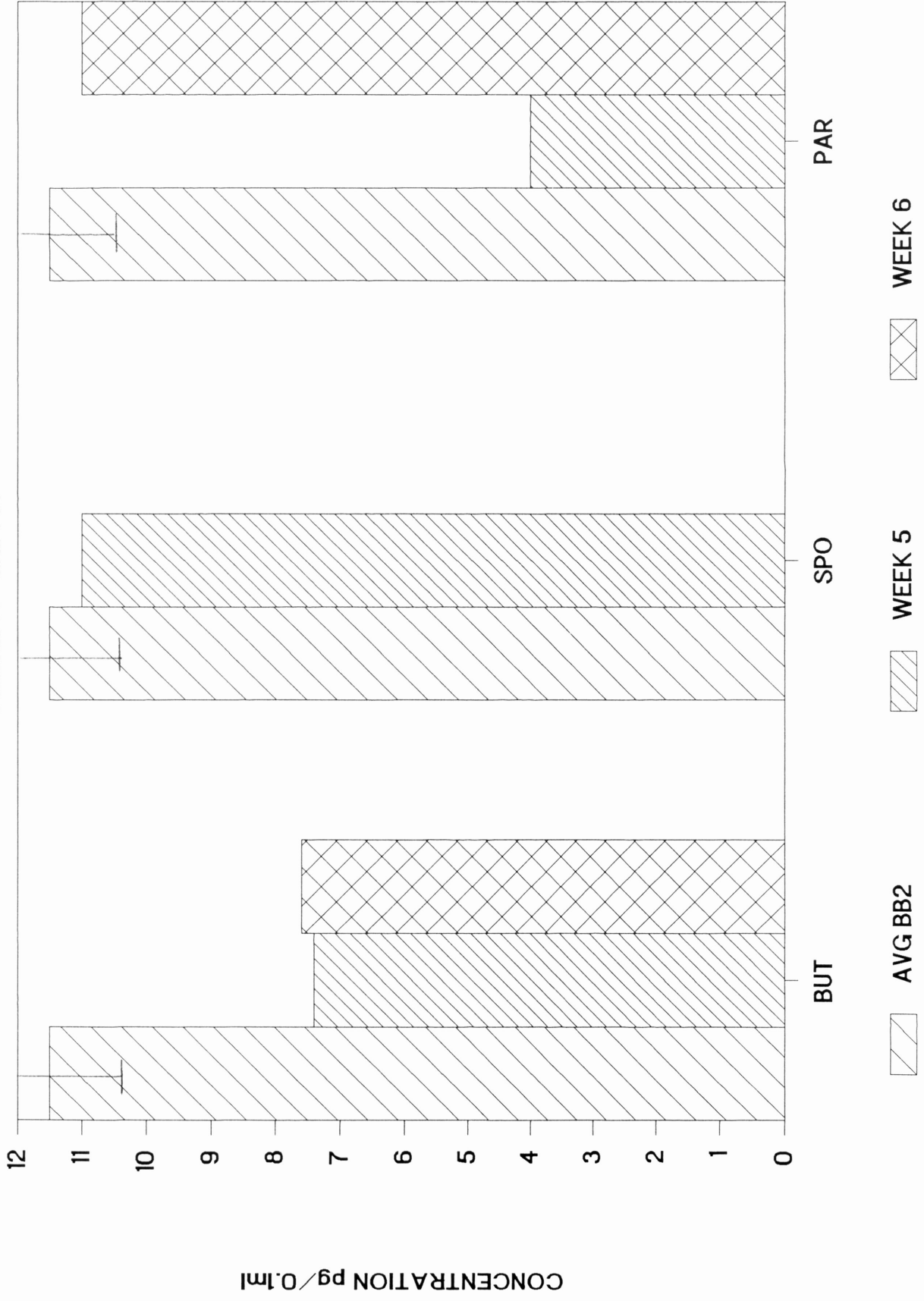
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FIGURE 13: GRIFFIN



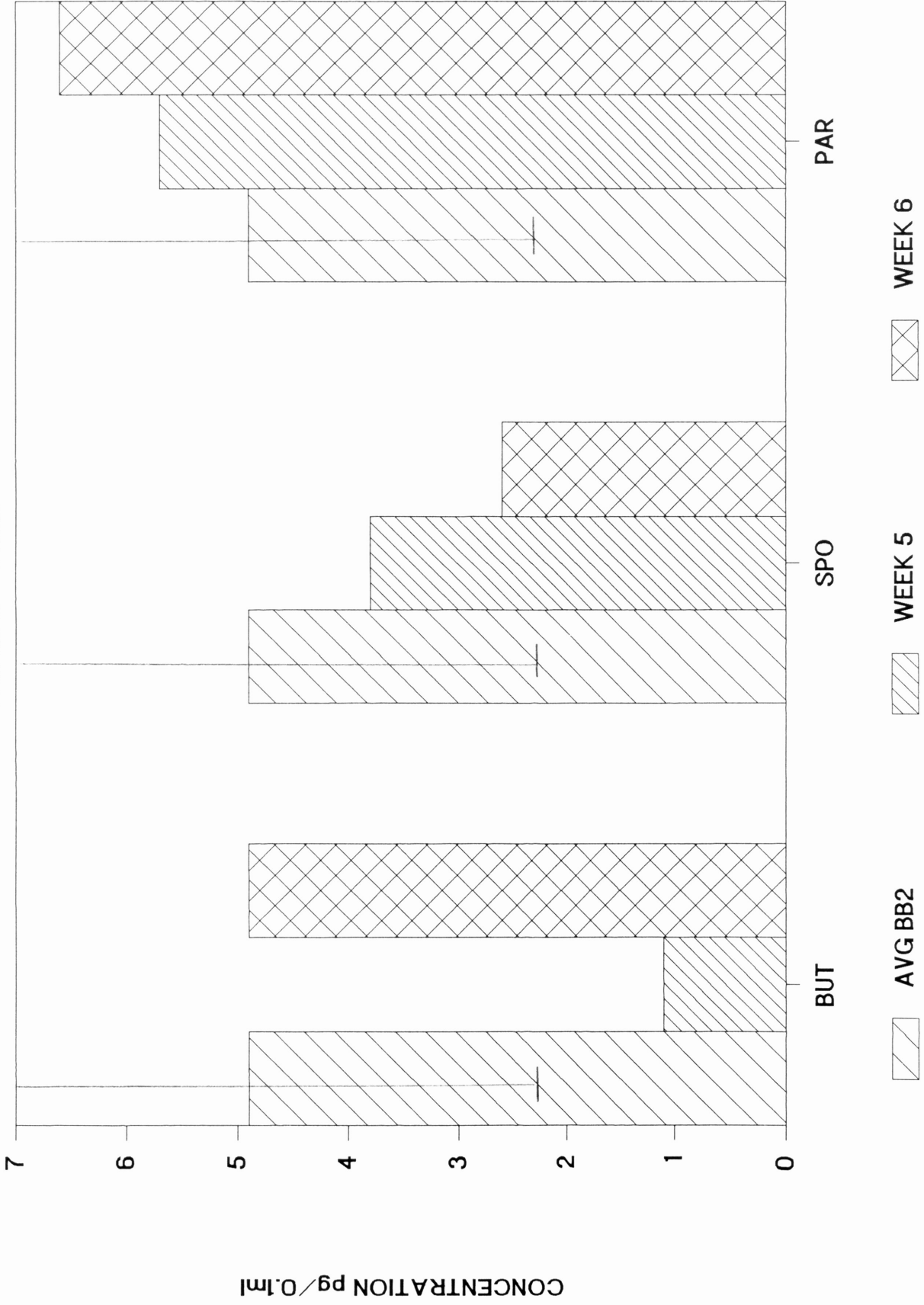
6-KETO-PROSTGLANDIN F1a

FIGURE 14: GRIFFIN



THROMBOXANE B2

FIGURE 15: HAVEN



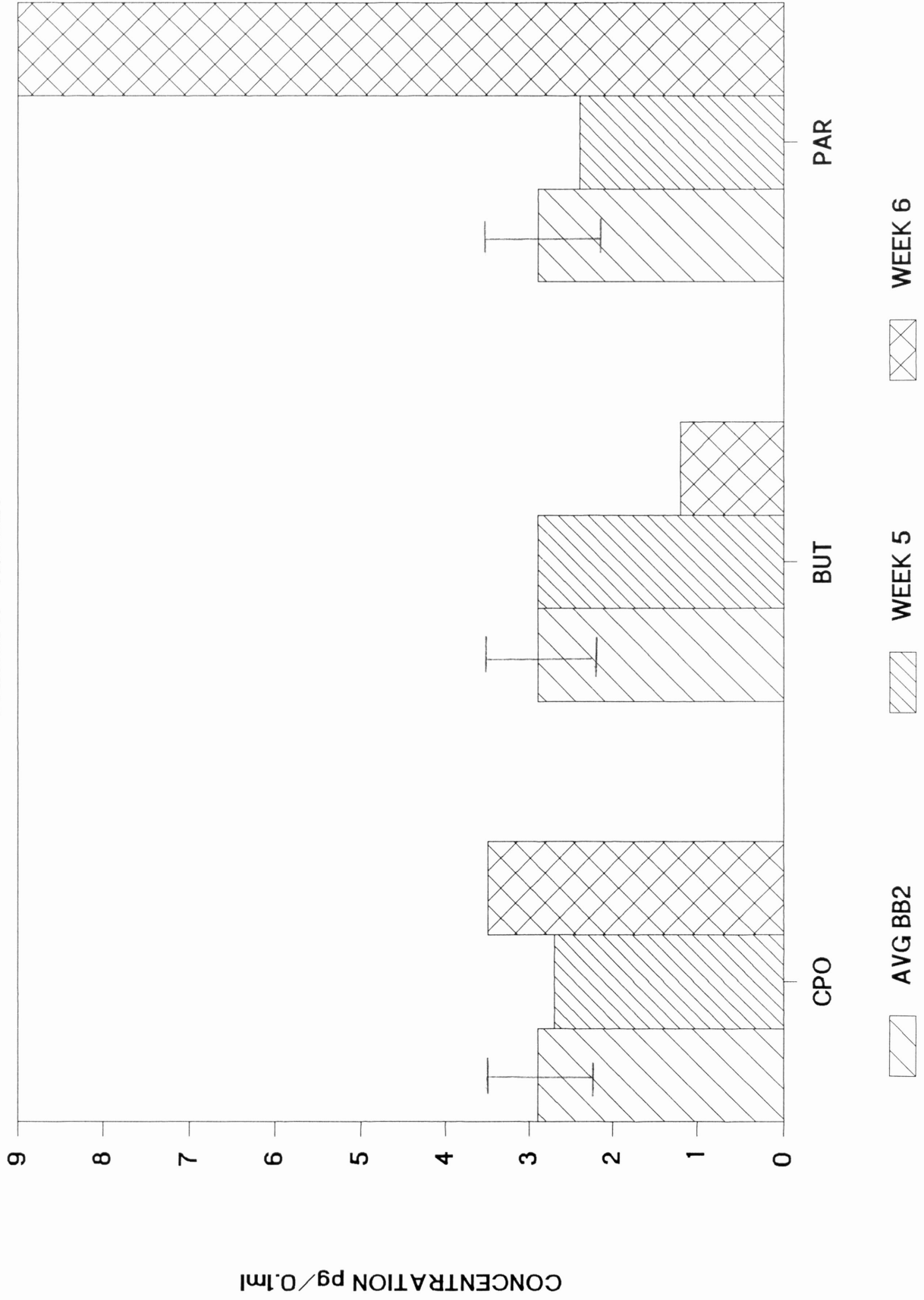
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FIGURE 16: HAVEN



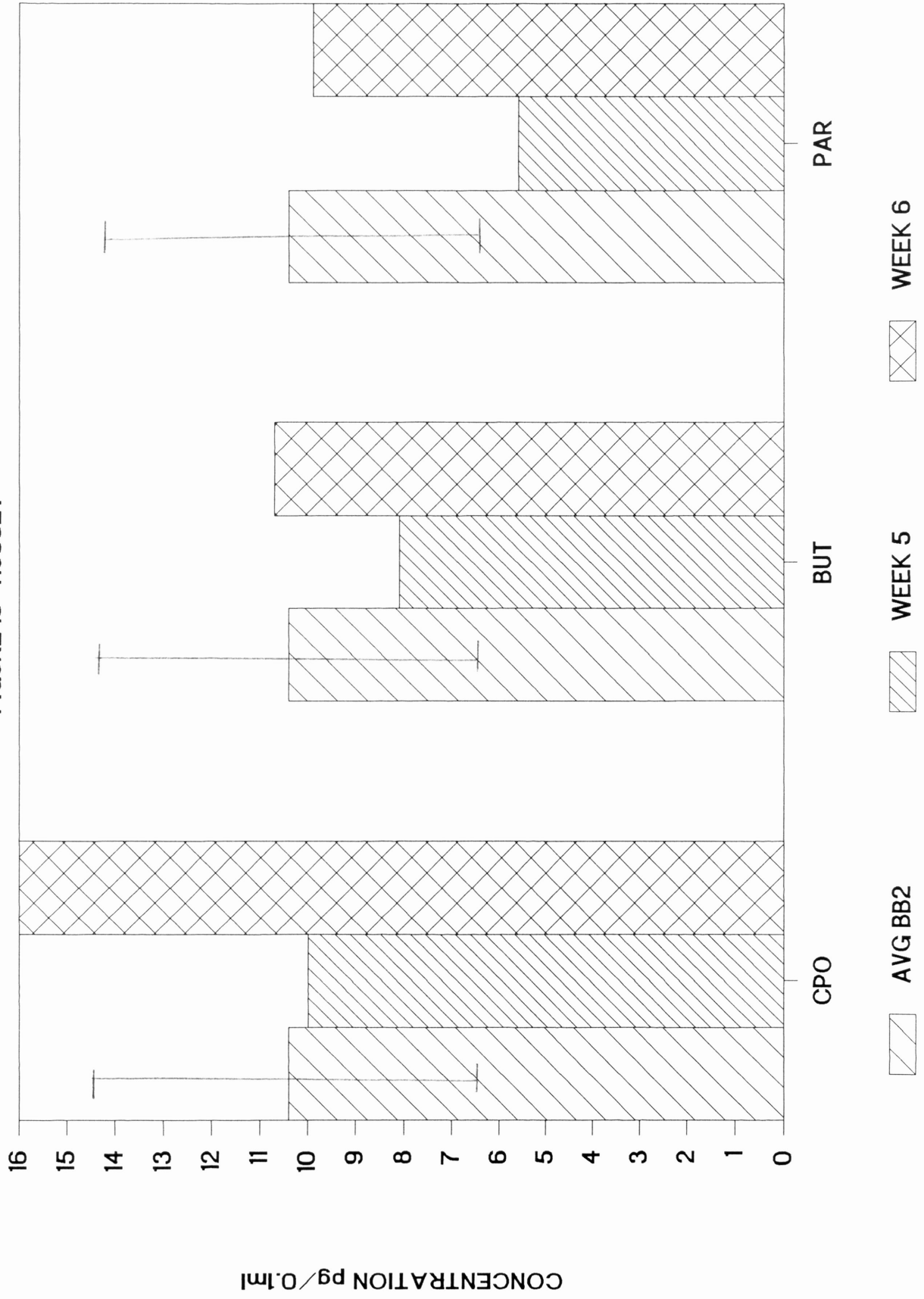
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FIGURE 17: HUSSEY



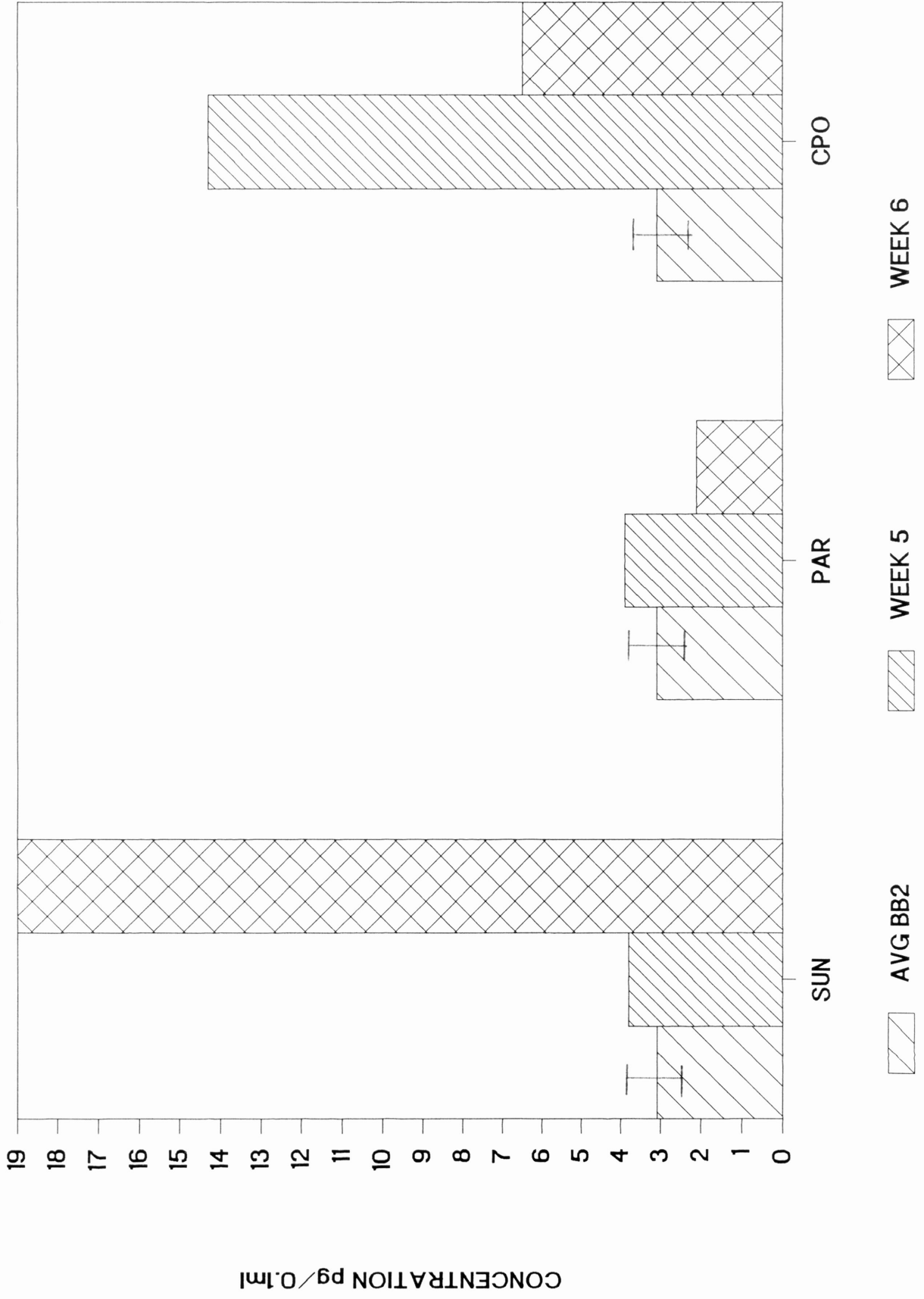
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FIGURE 18: HUSSEY



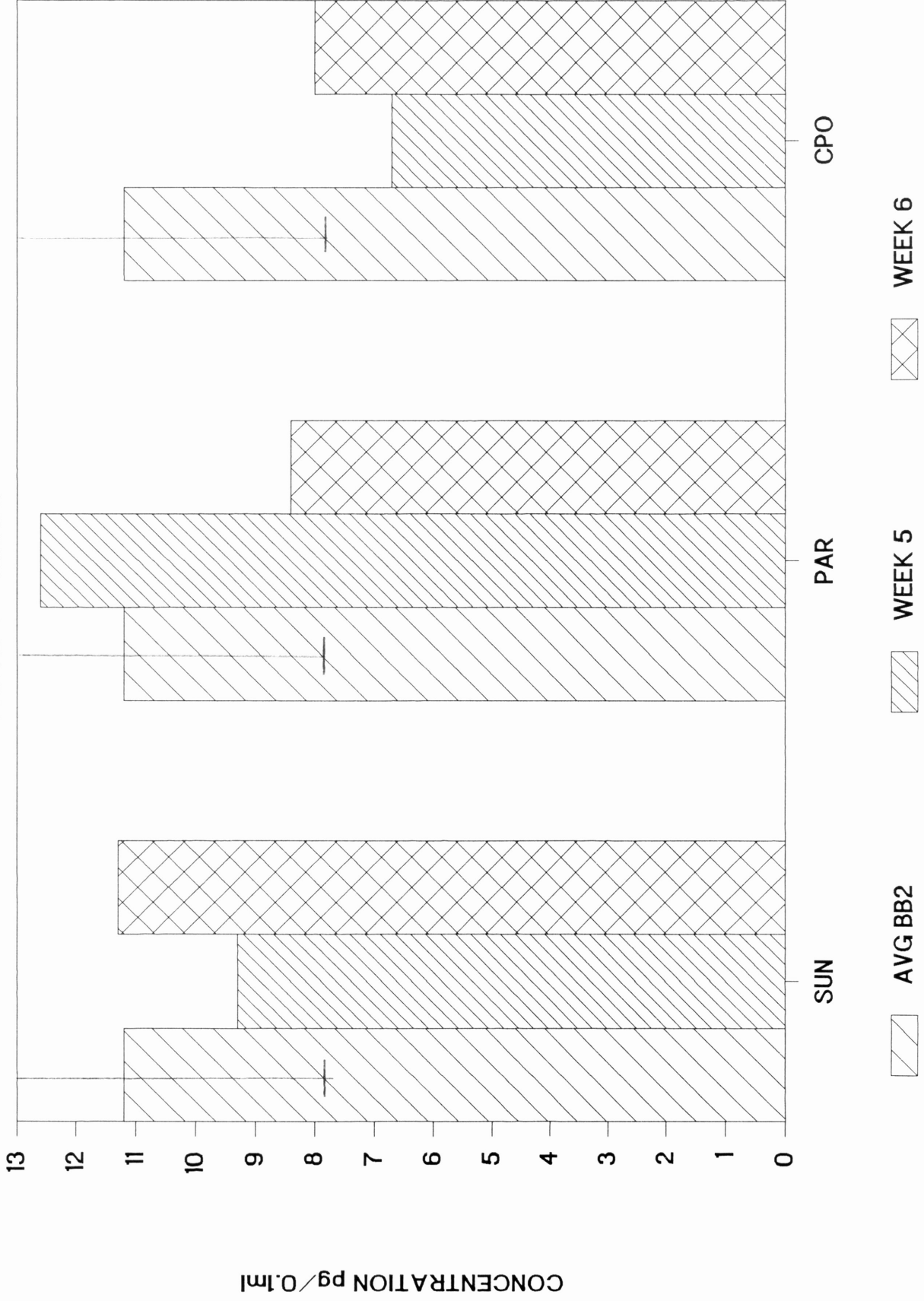
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FIGURE 19: JENKINS



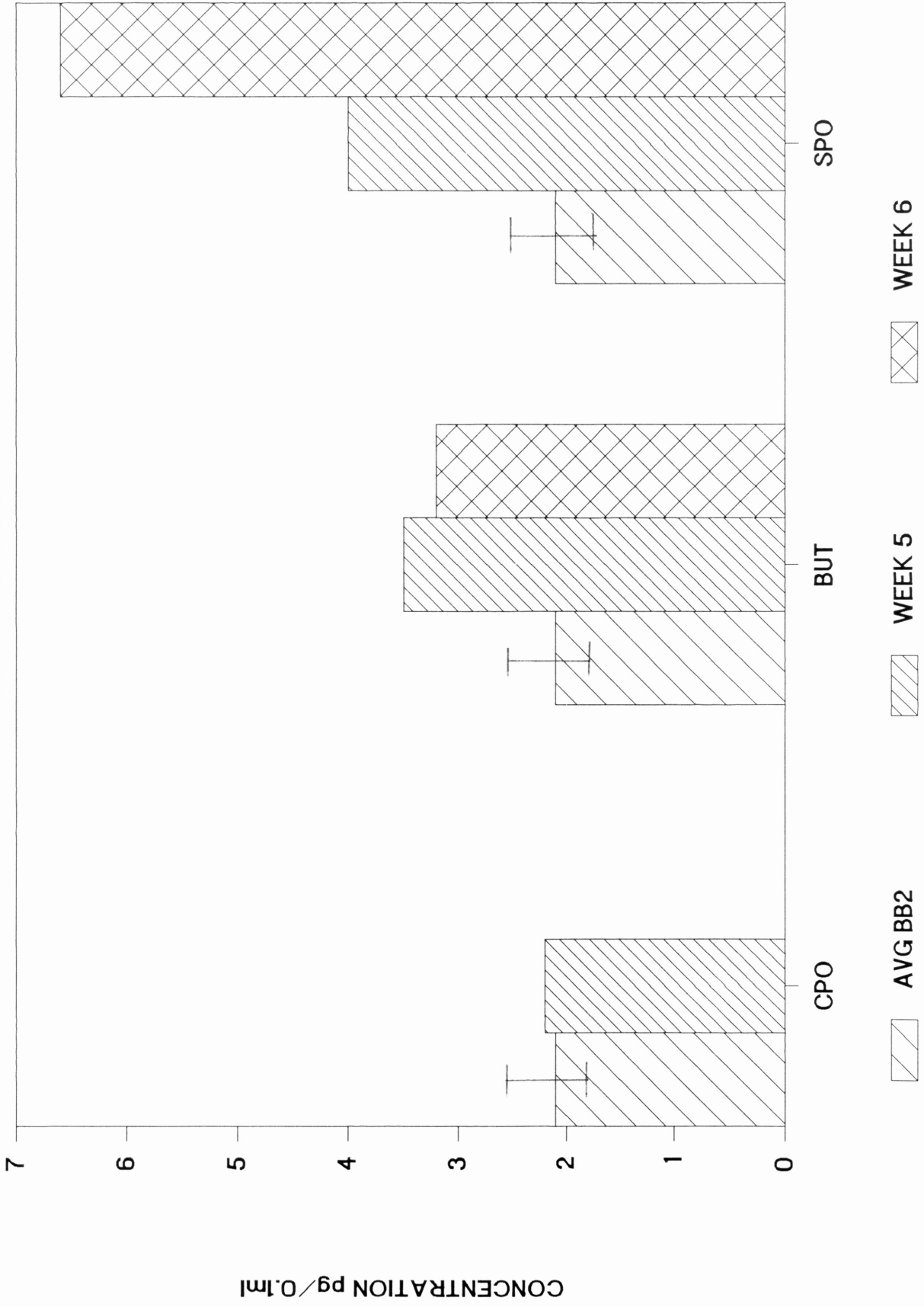
6-KETO-PROSTAGLANDIN F1a

FIGURE 20: JENKINS



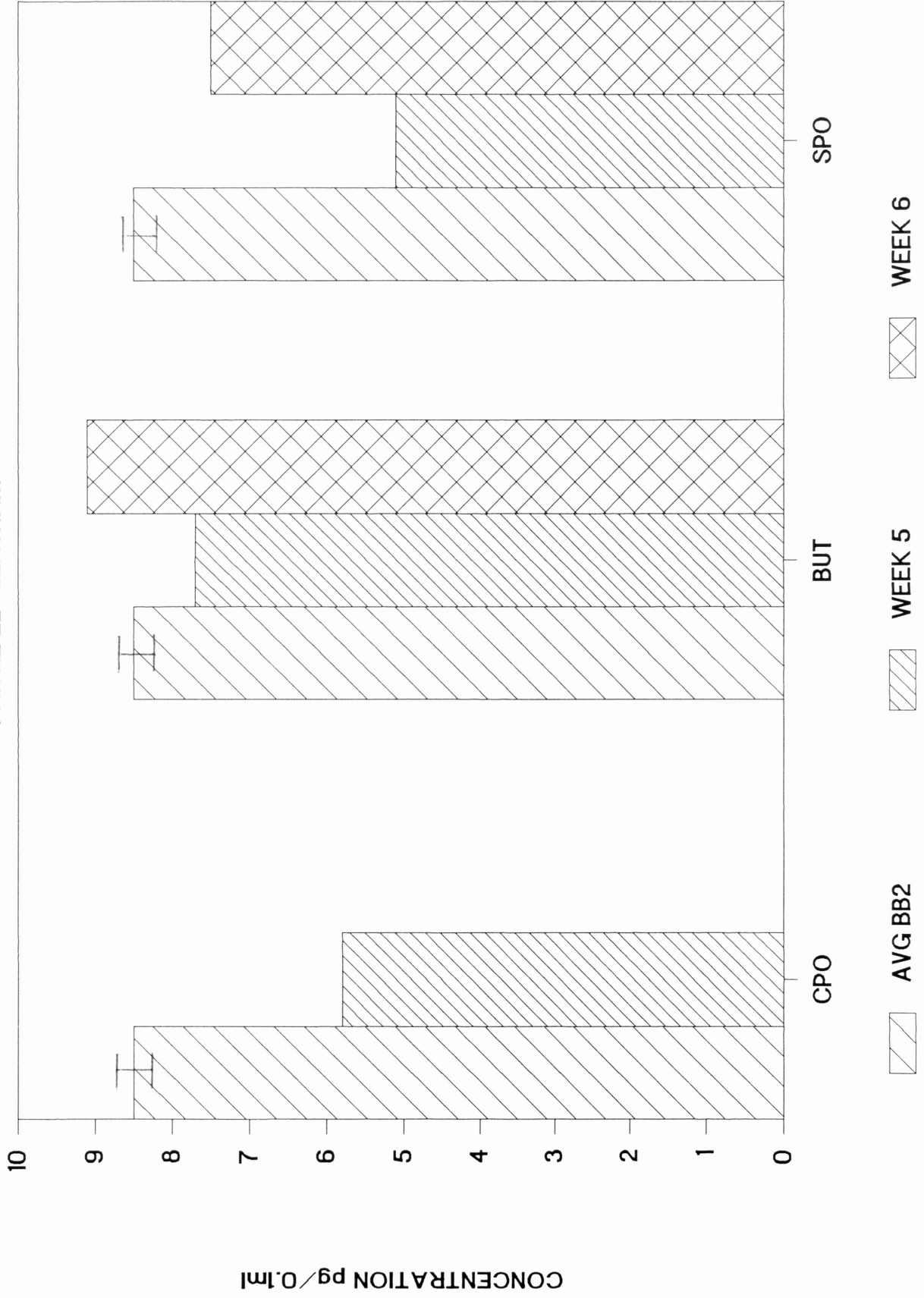
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FIGURE 21: LEATHAM



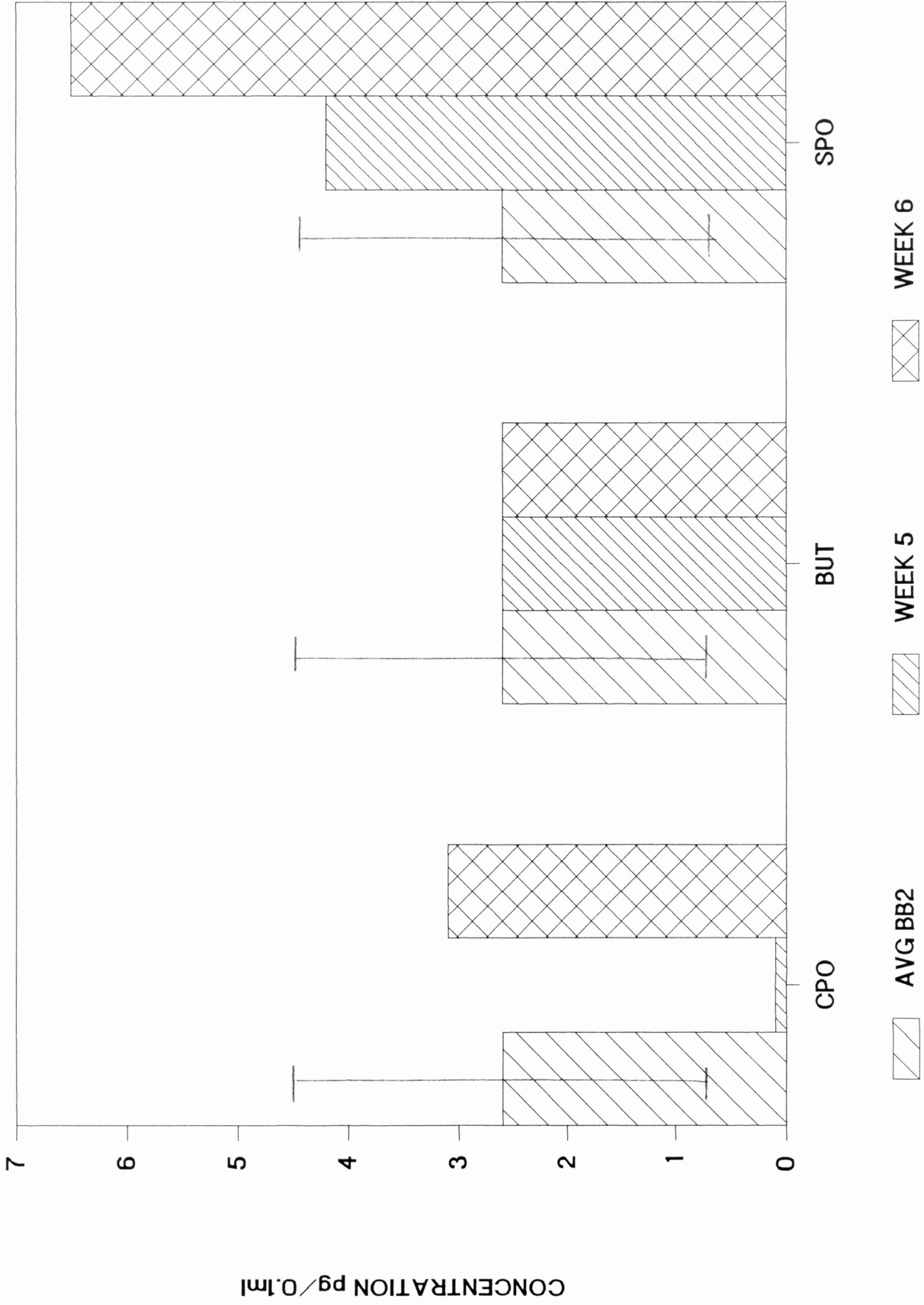
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FIGURE 22: LEATHAM



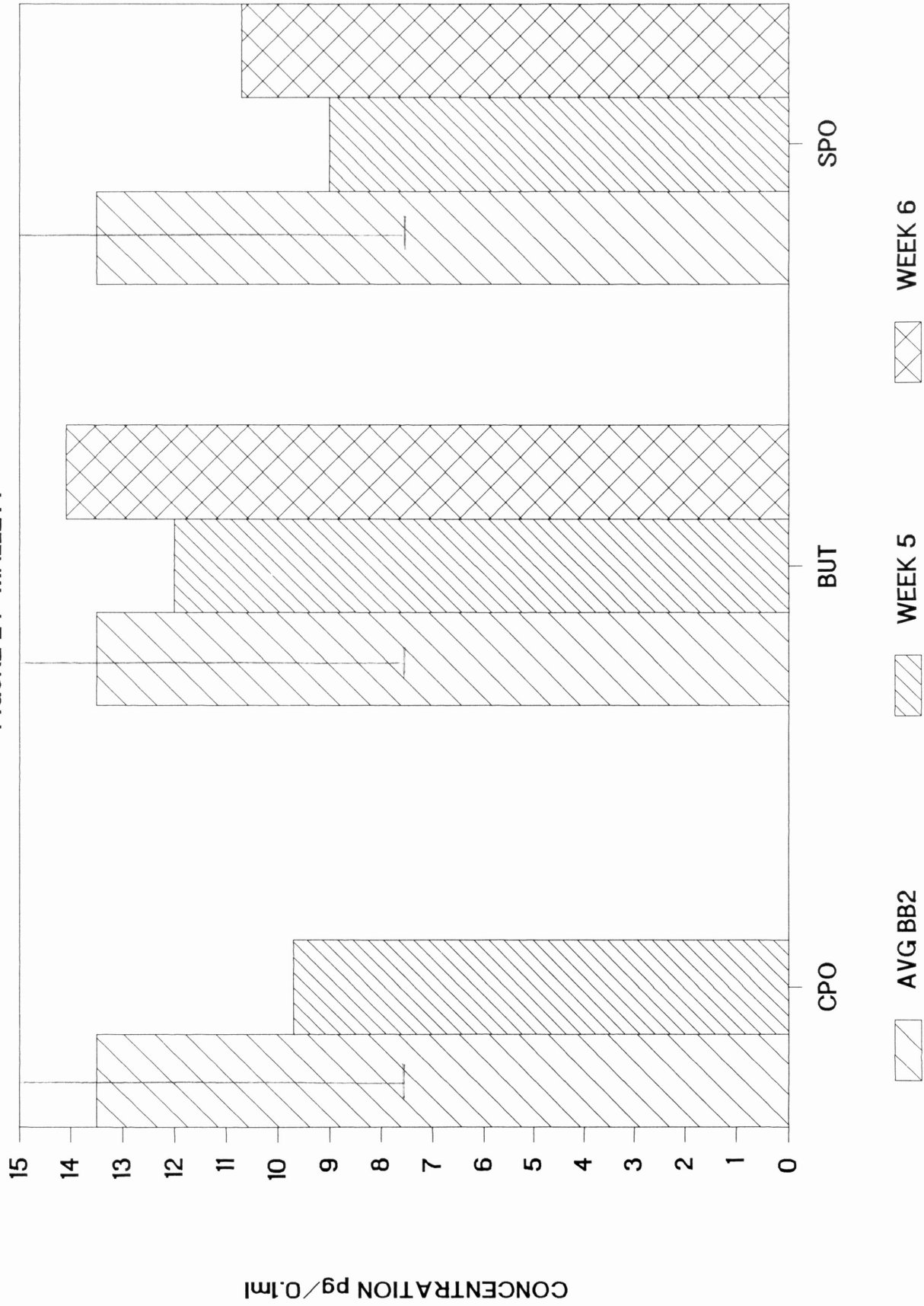
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FIGURE 23: MALLETT



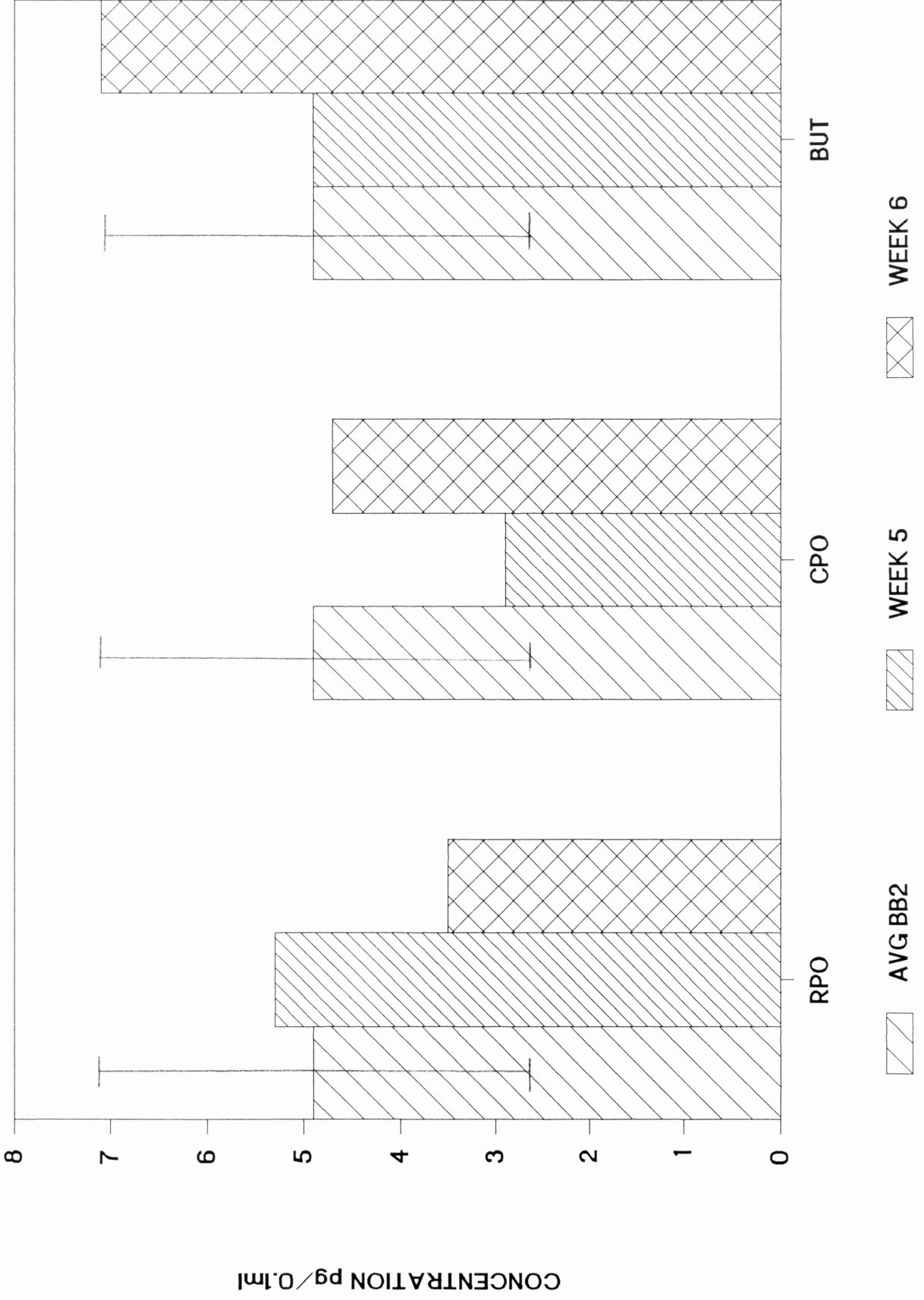
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FIGURE 24: MALLETT



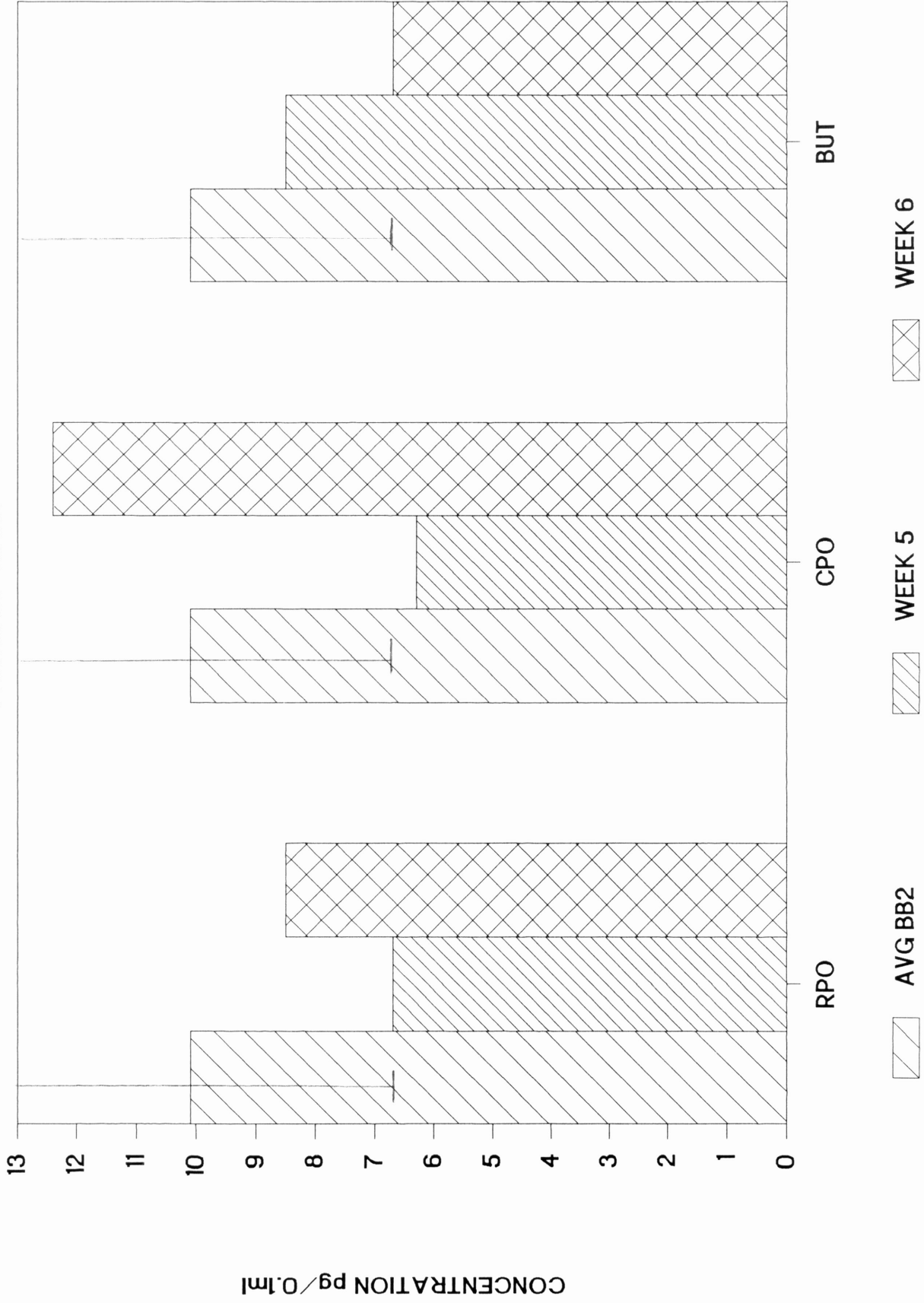
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FIGURE 25: MANN



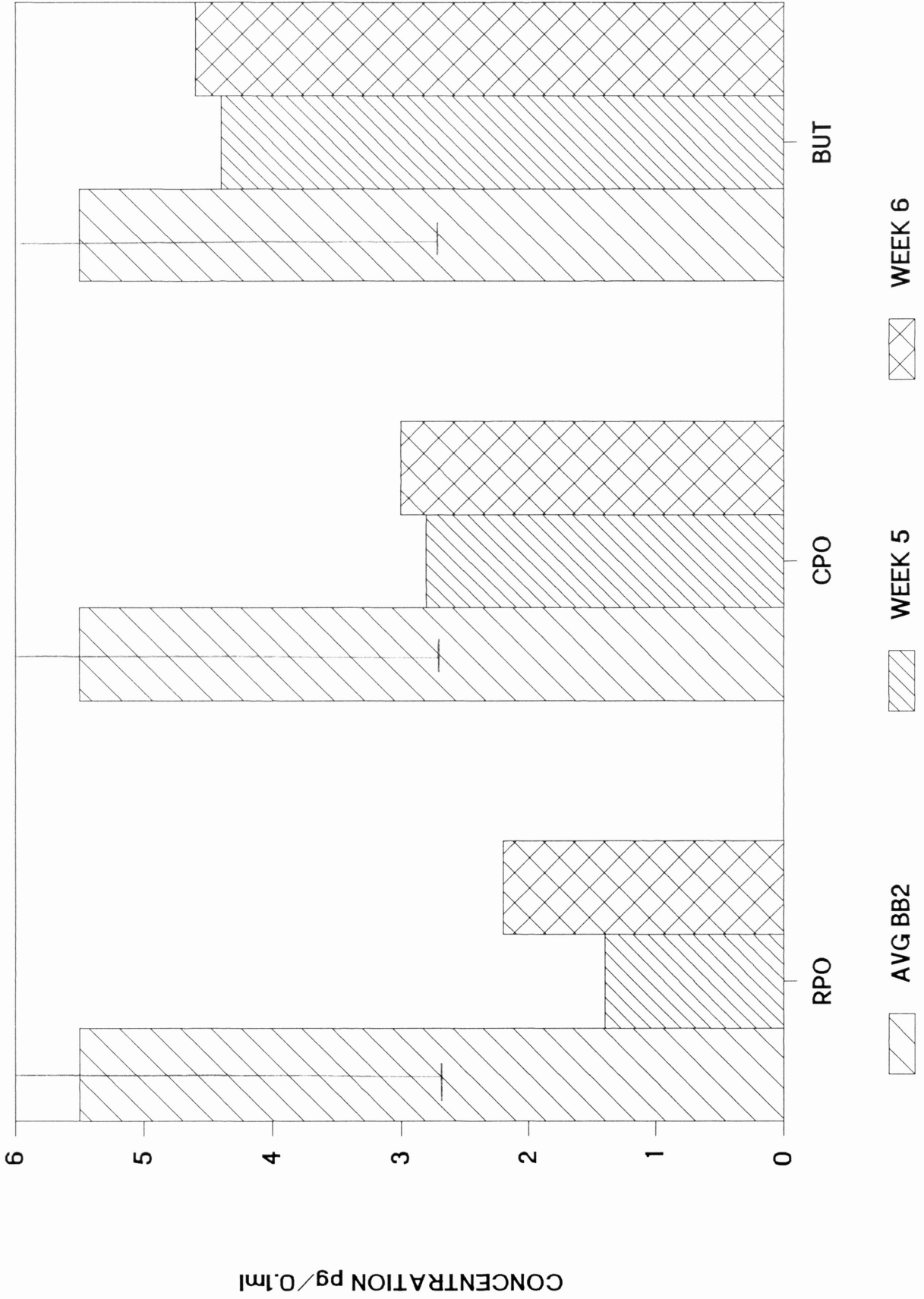
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FIGURE 26: MANN



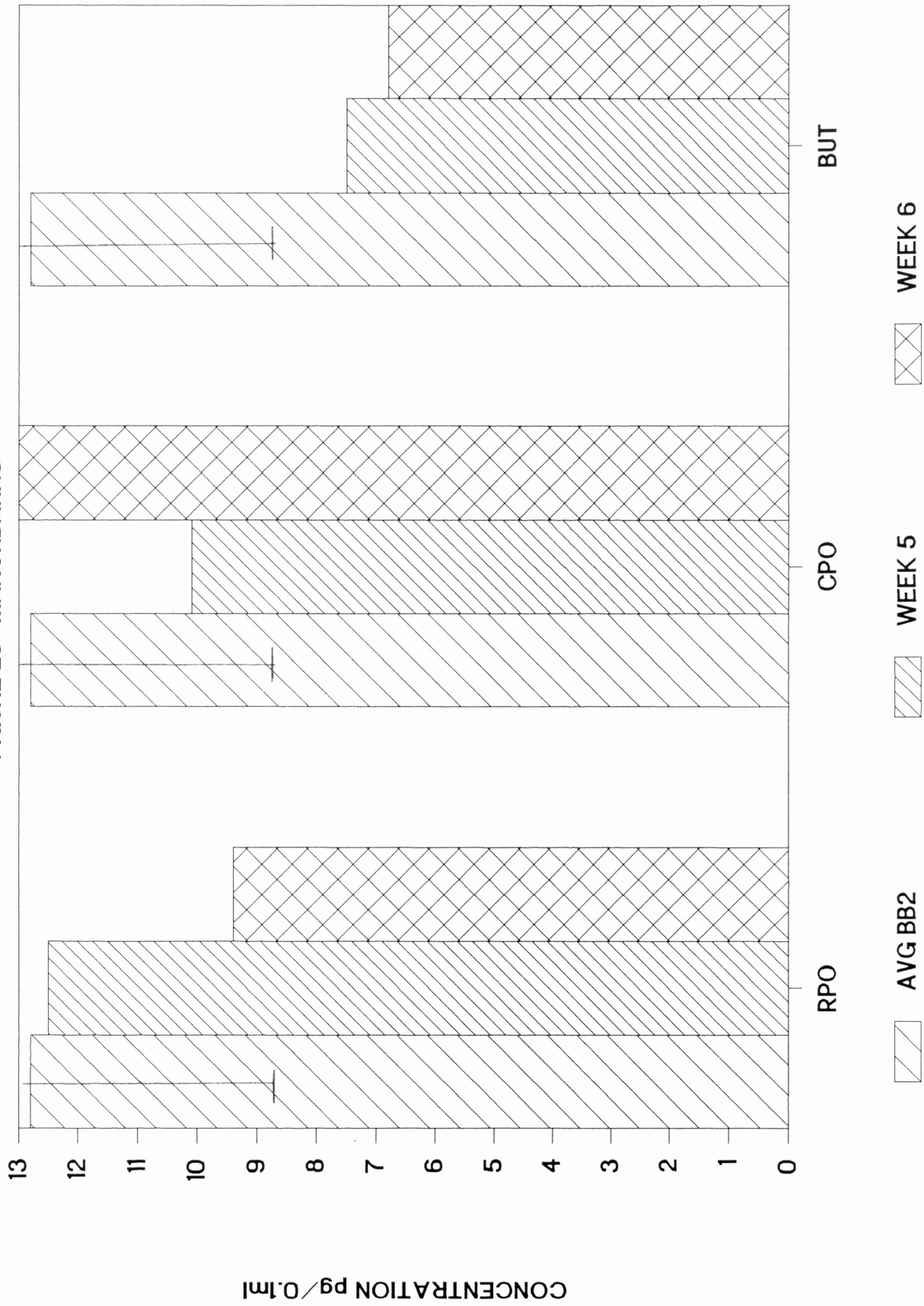
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FIGURE 27: MARCHBANKS



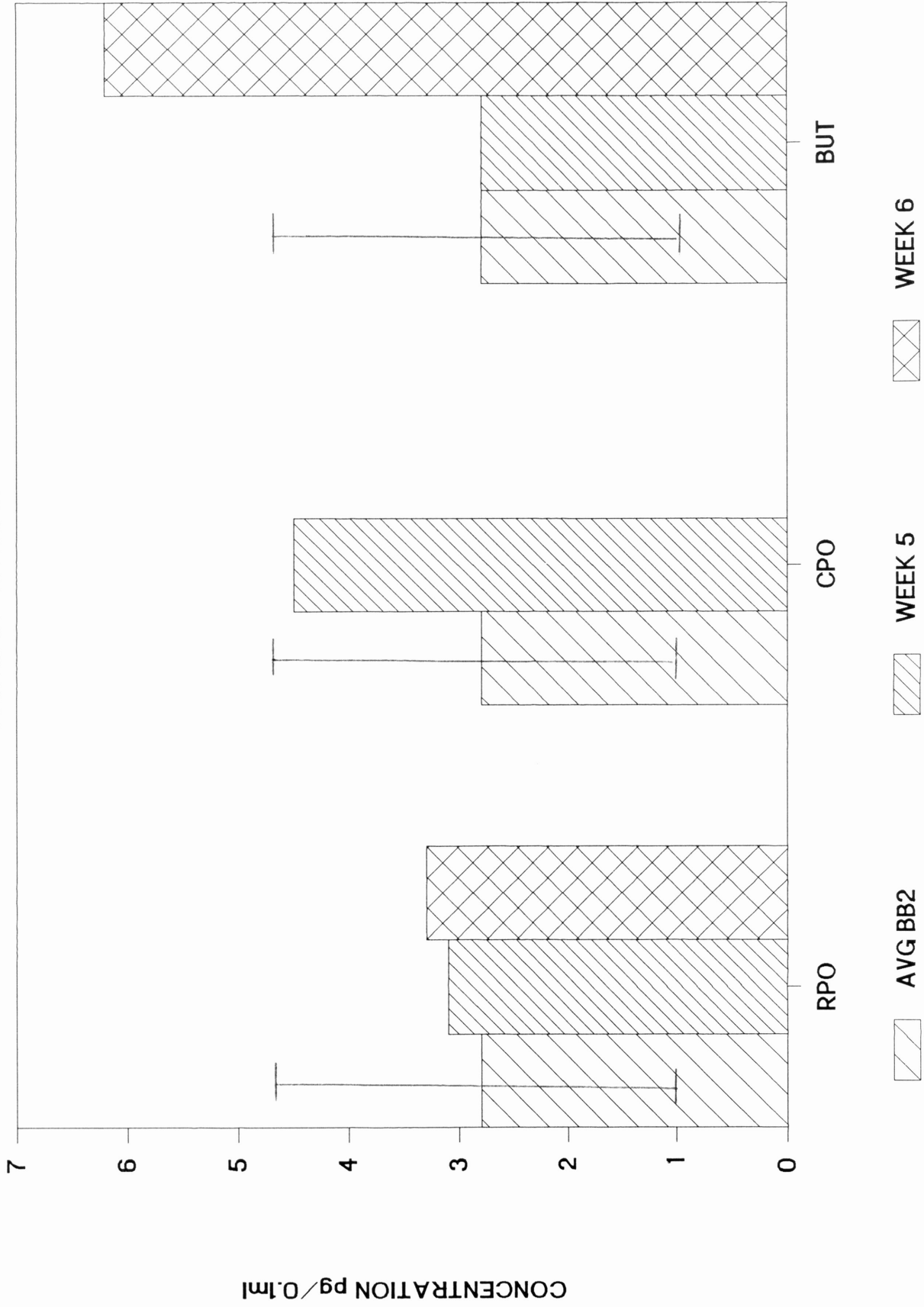
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FIGURE 28: MARCHBANKS



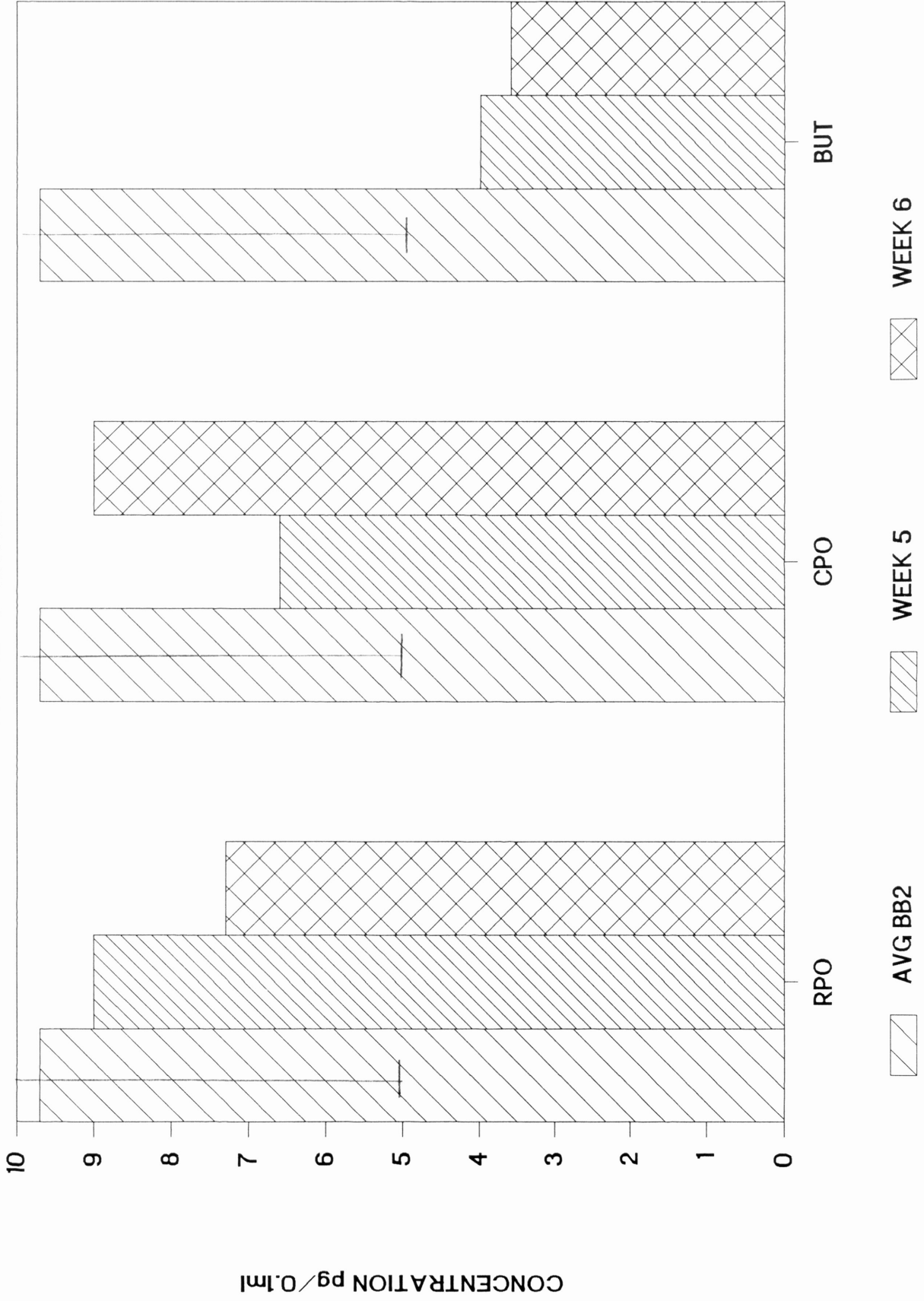
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FIGURE 29: MORGAN



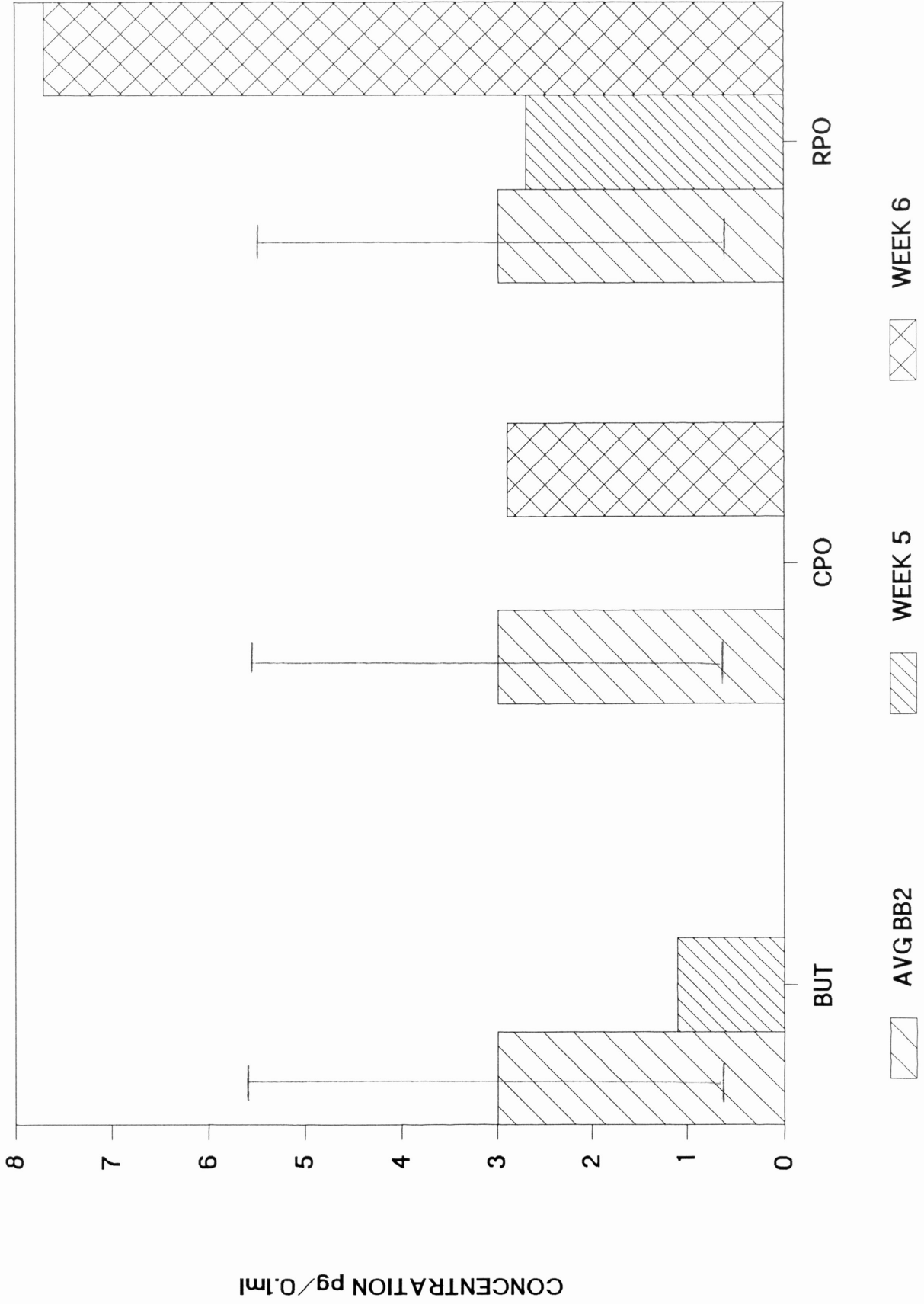
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FIGURE 30: MORGAN



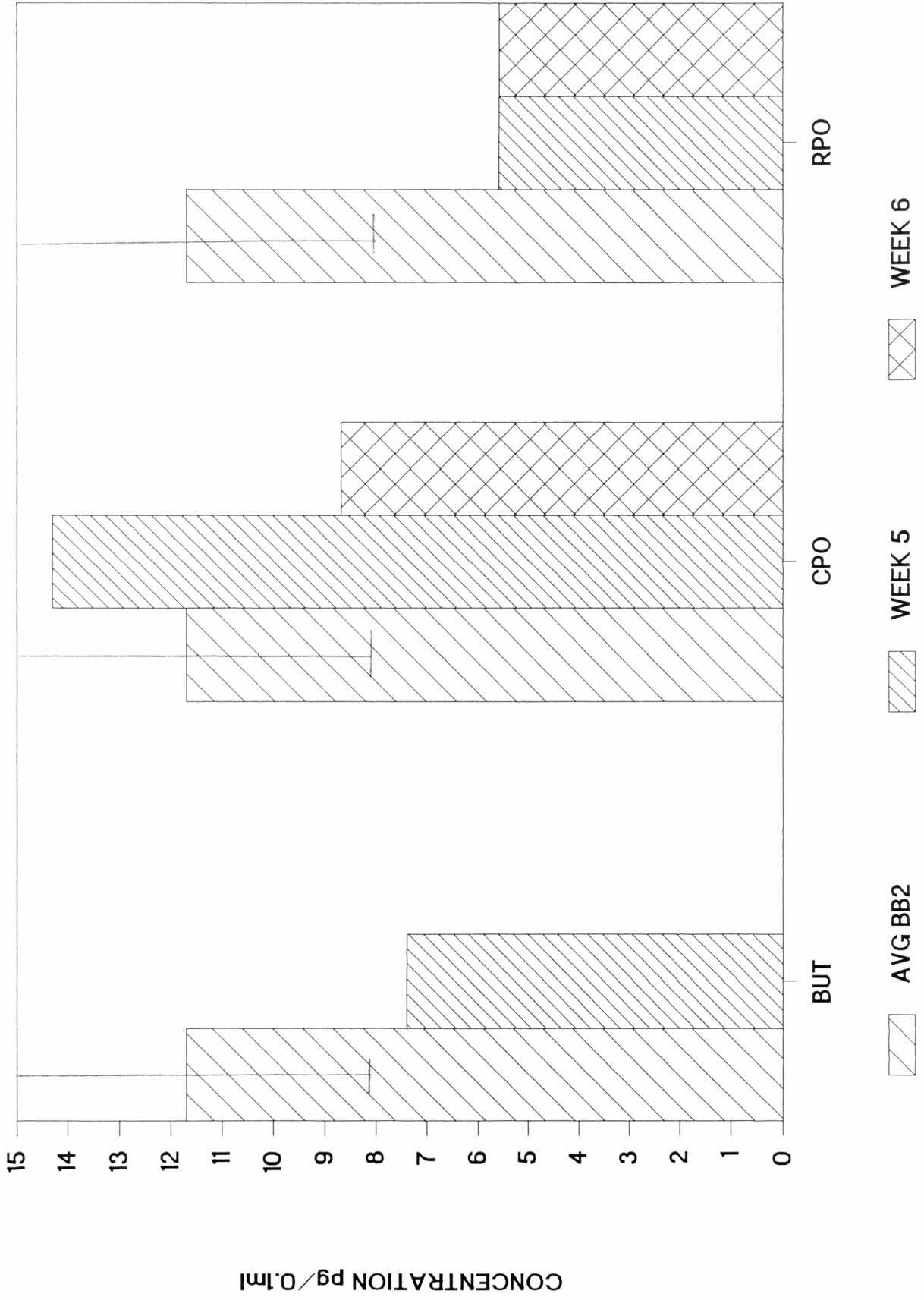
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FIGURE 31: MORRIS



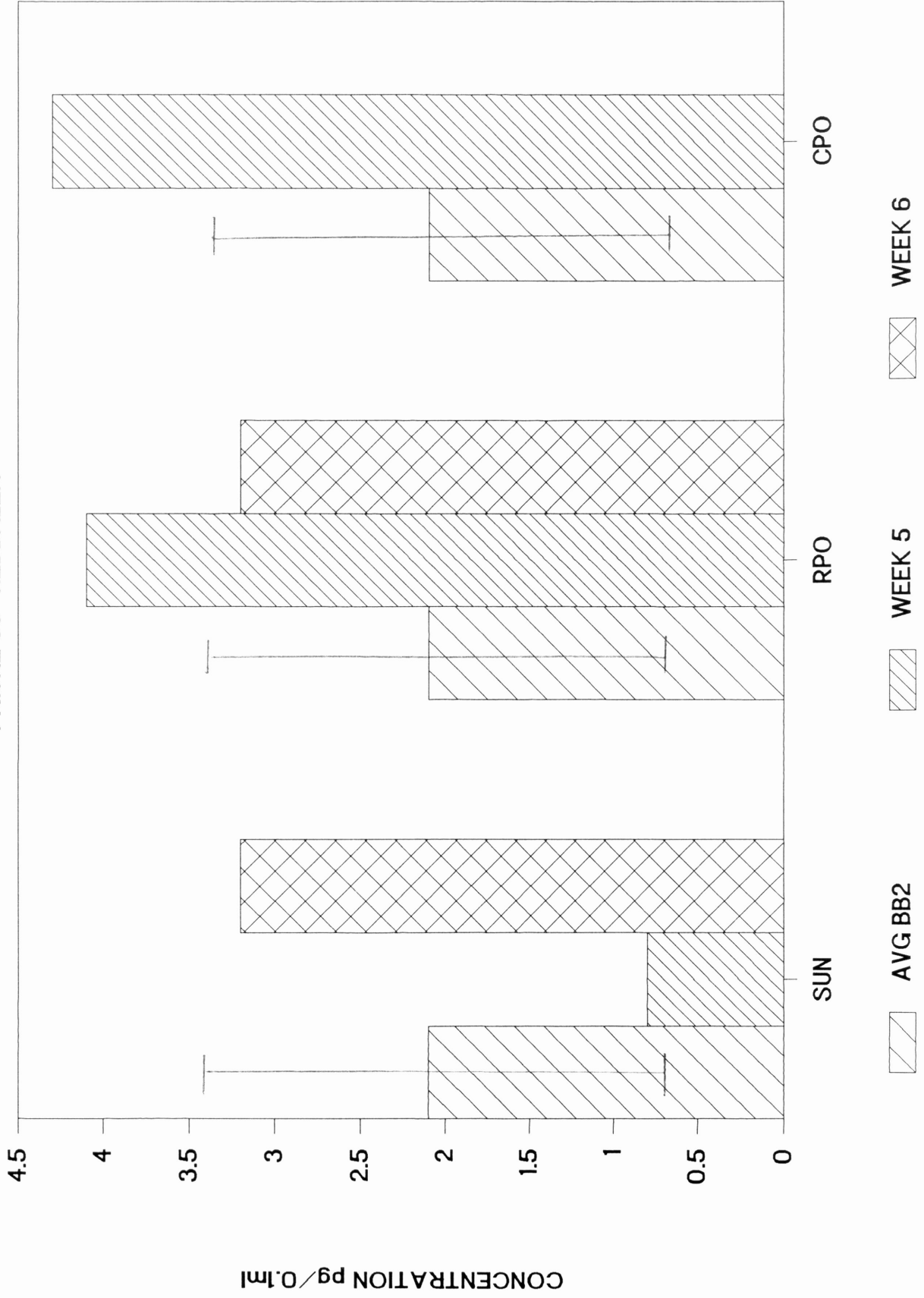
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FIGURE 32: MORRIS



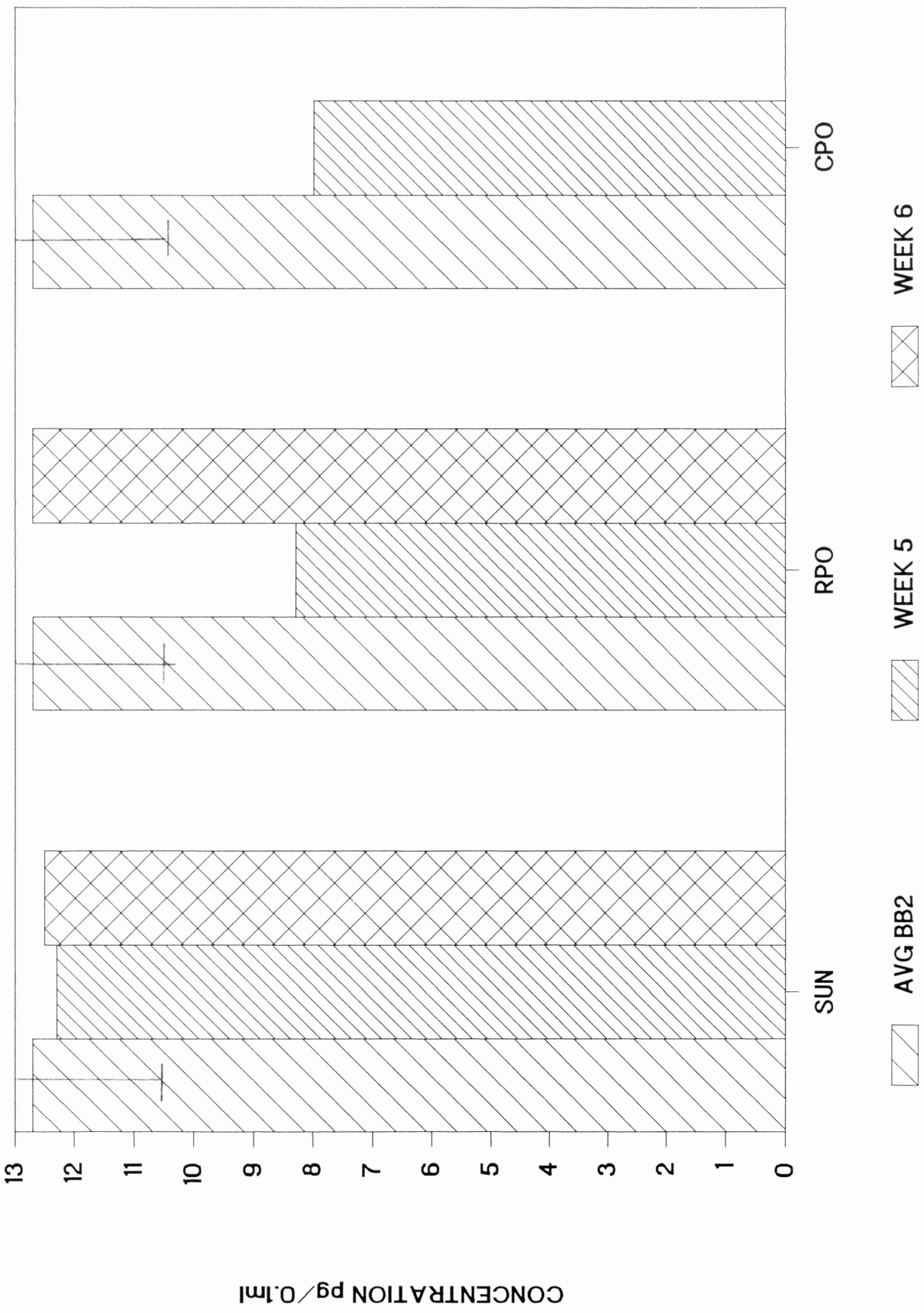
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FIGURE 33: NEDBALEK



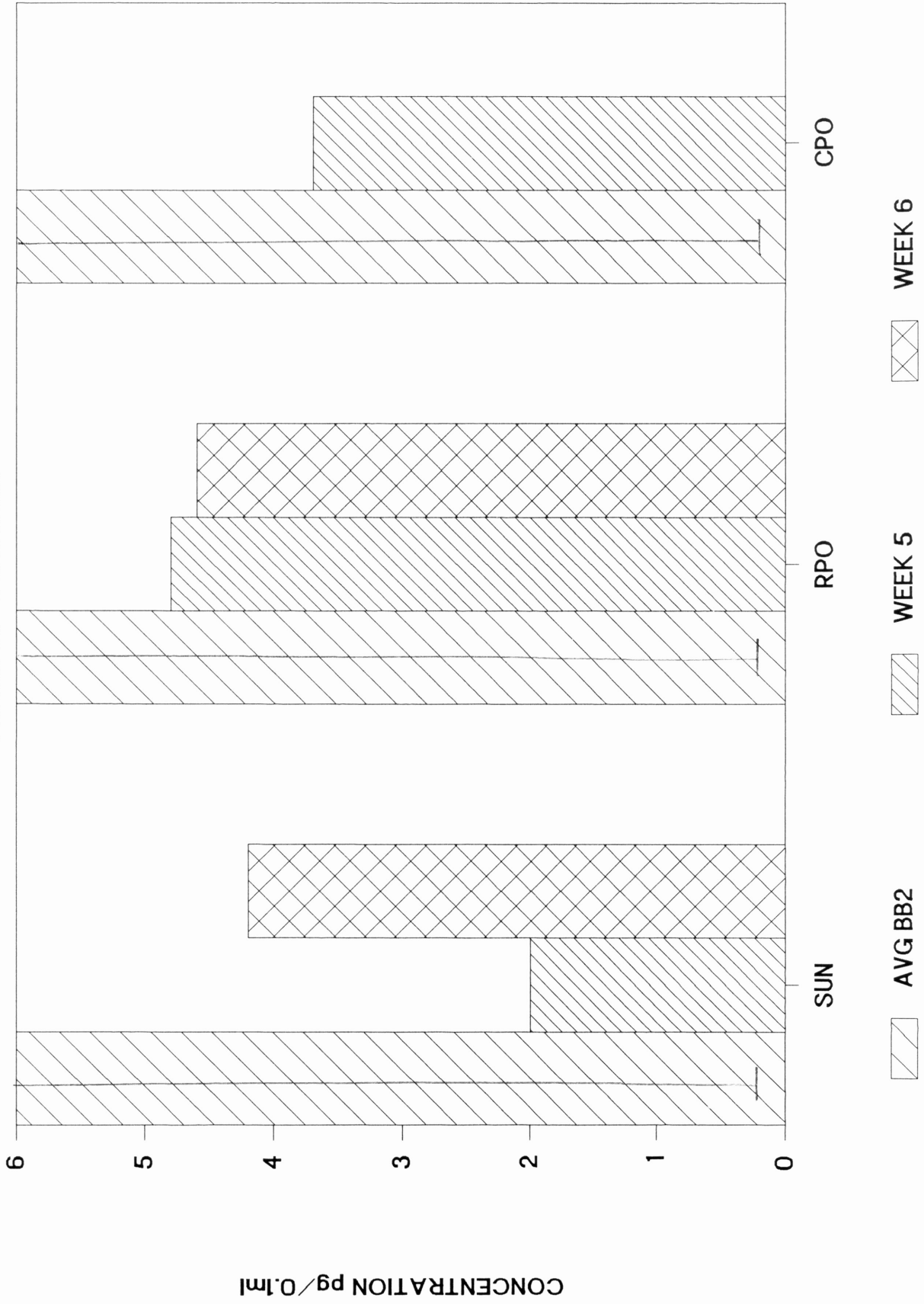
6-KETO-PROSTAGLANDIN F1a

FIGURE 34: NEDBALEK



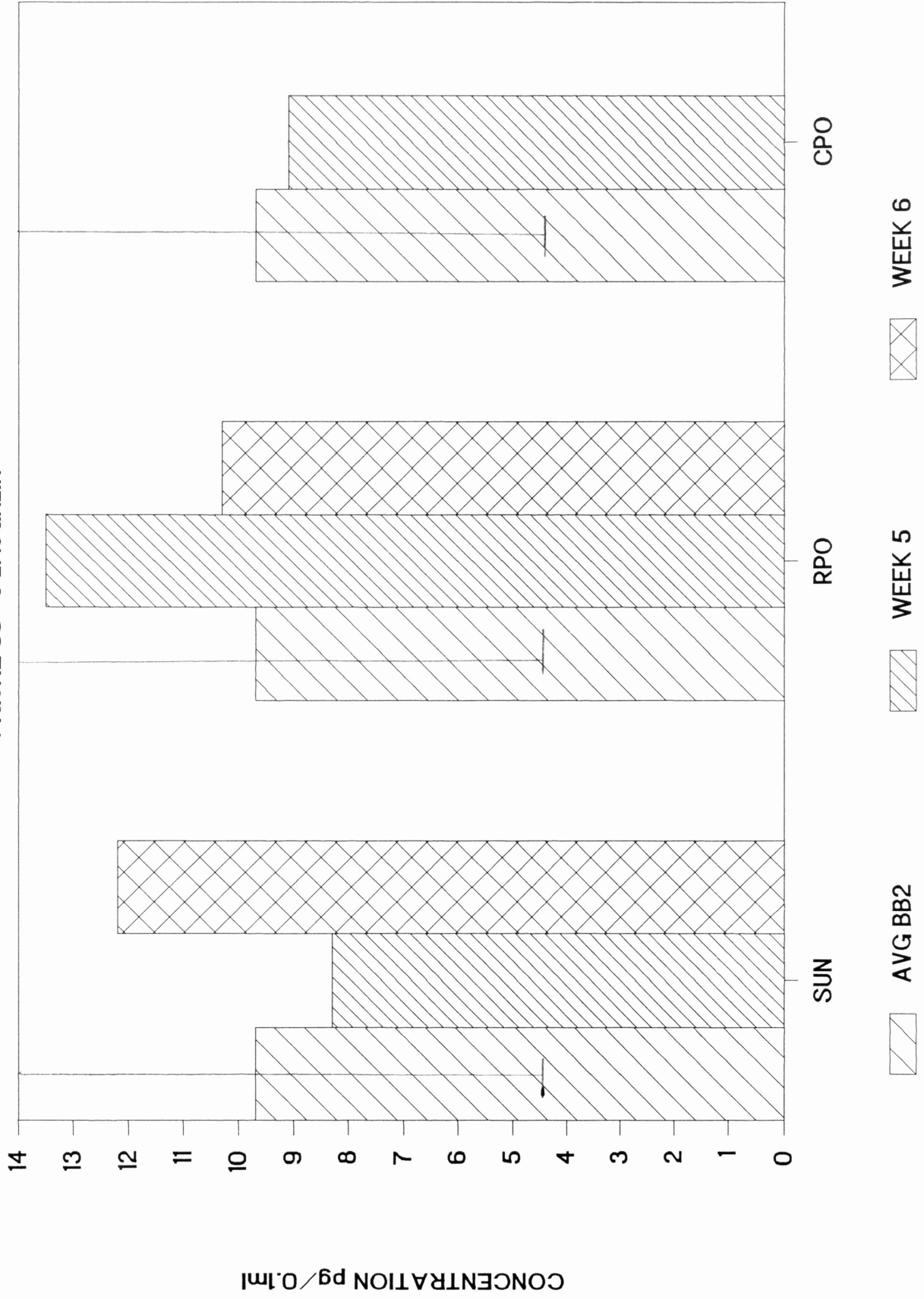
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FIGURE 35: O'LAUGHLIN



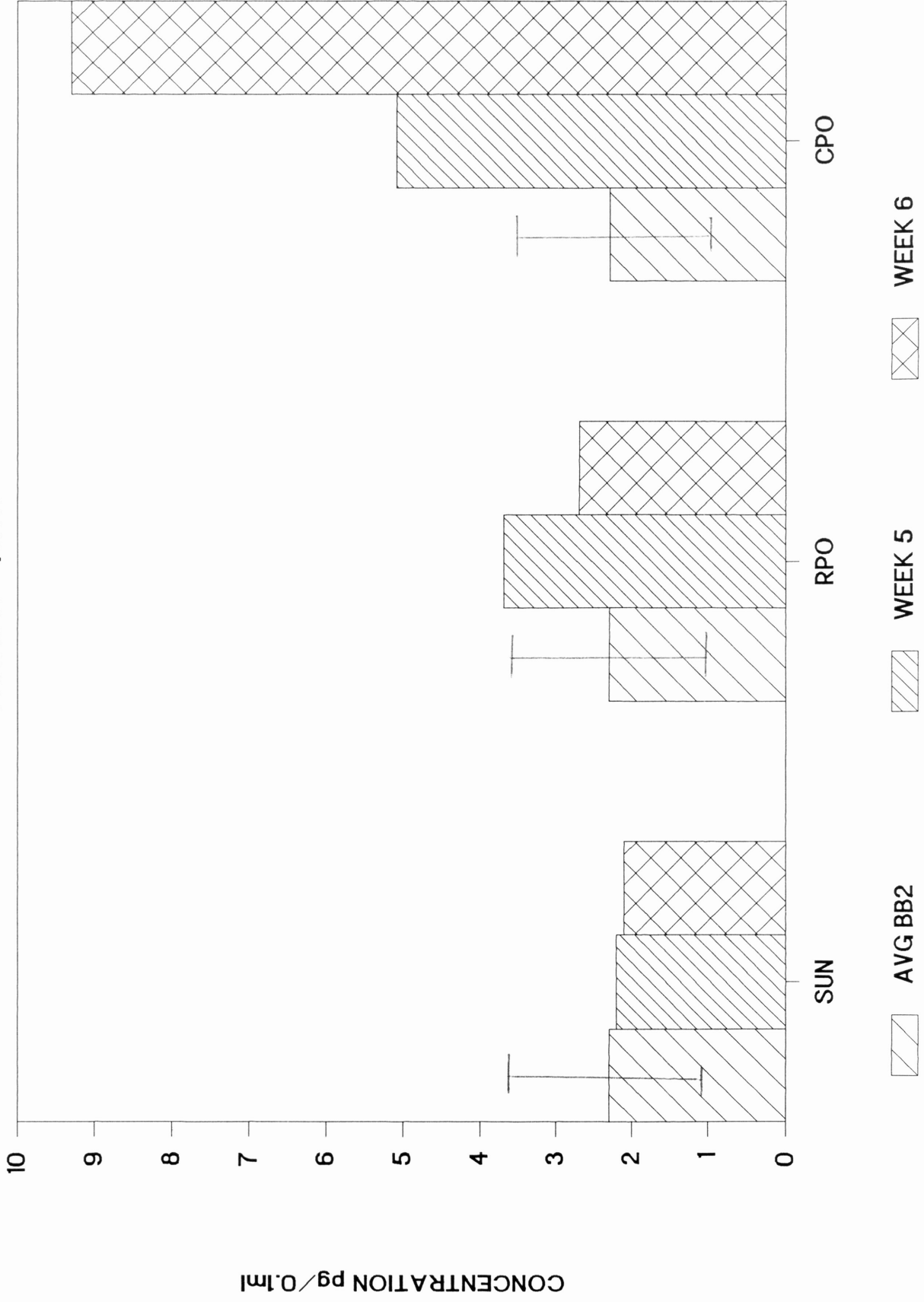
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FIGURE 36: O'LAUGHLIN



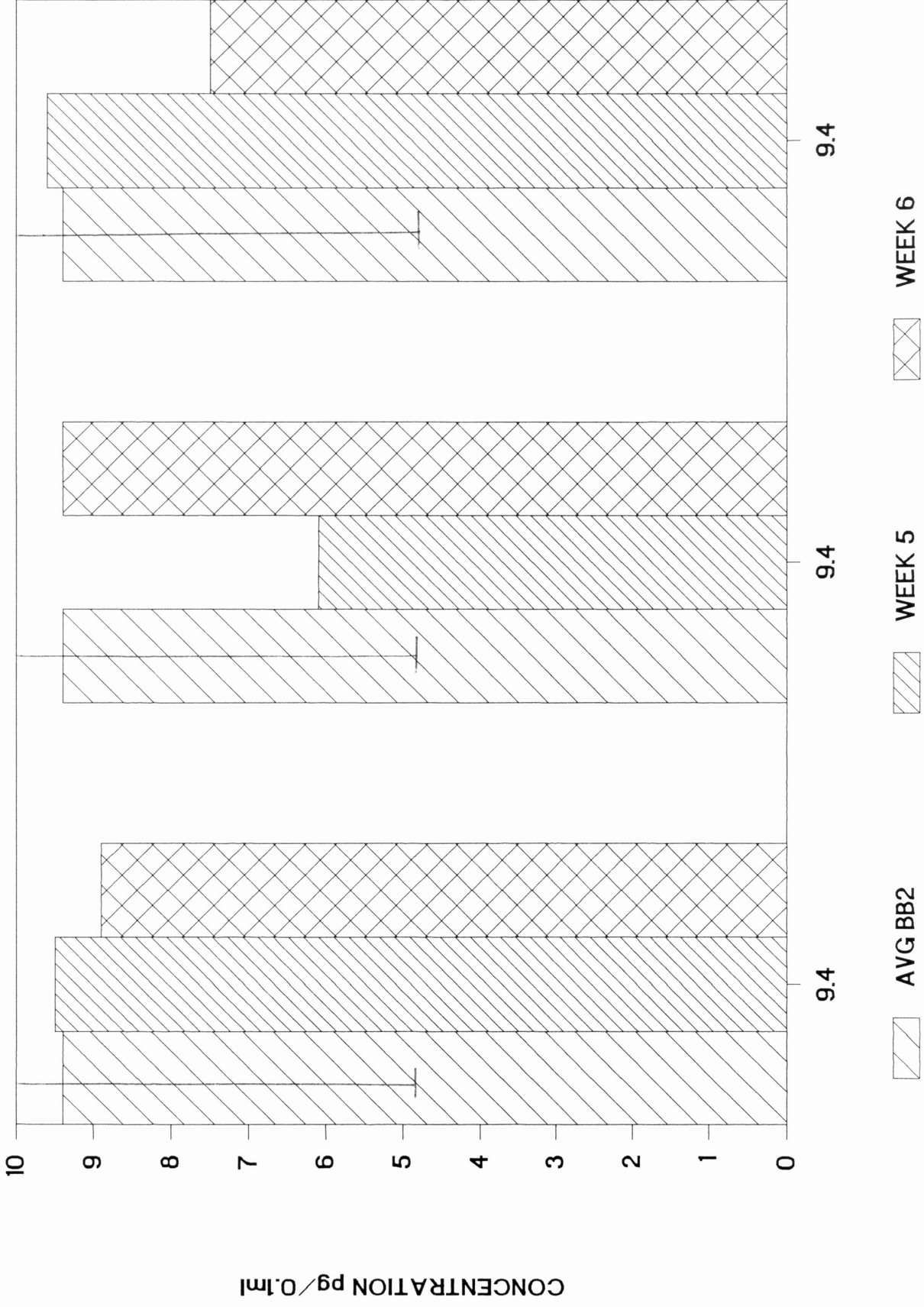
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FIGURE 37: QUICK



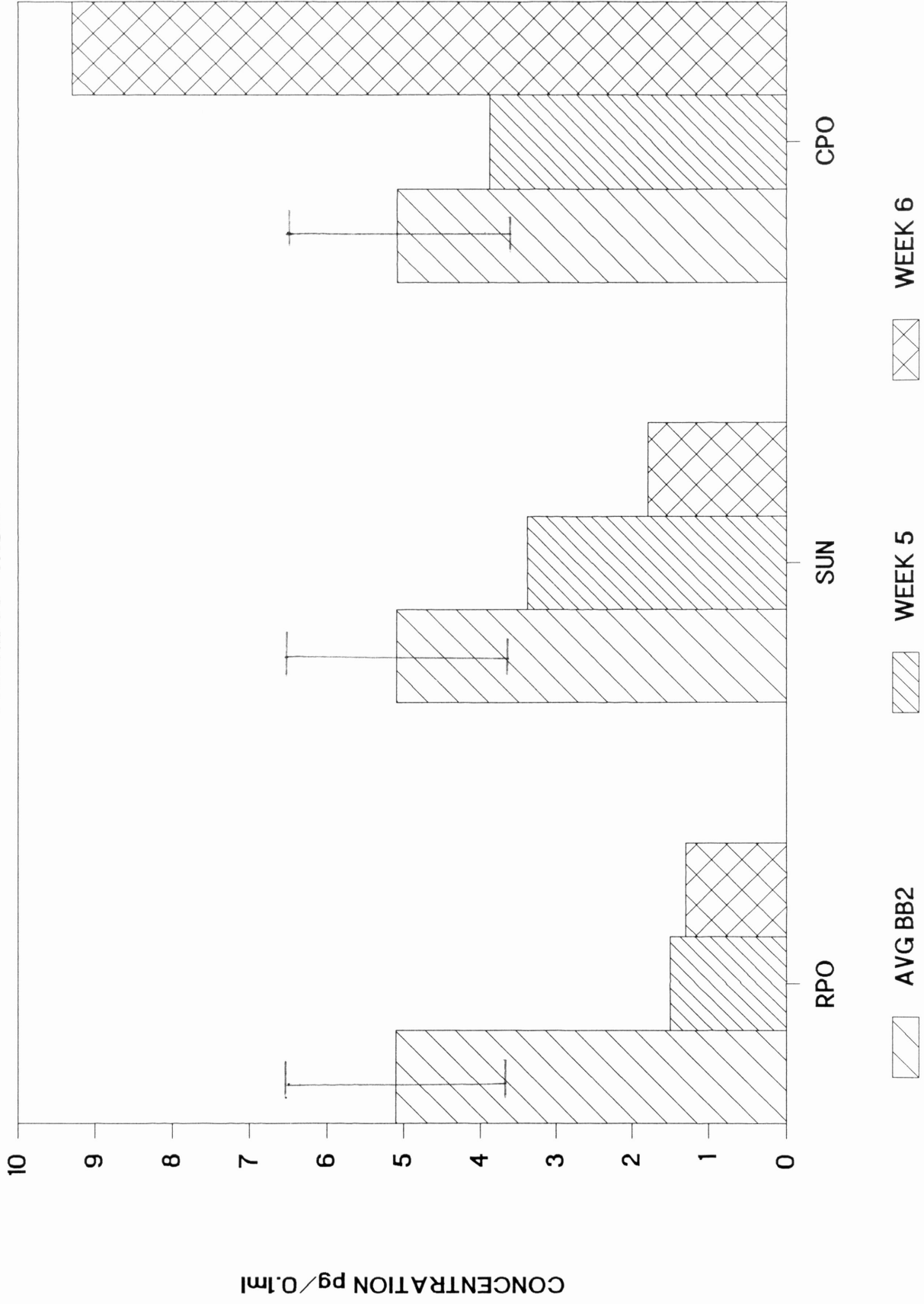
6-KETO-PROSTAGLANDIN F1a

FIGURE 38: QUICK



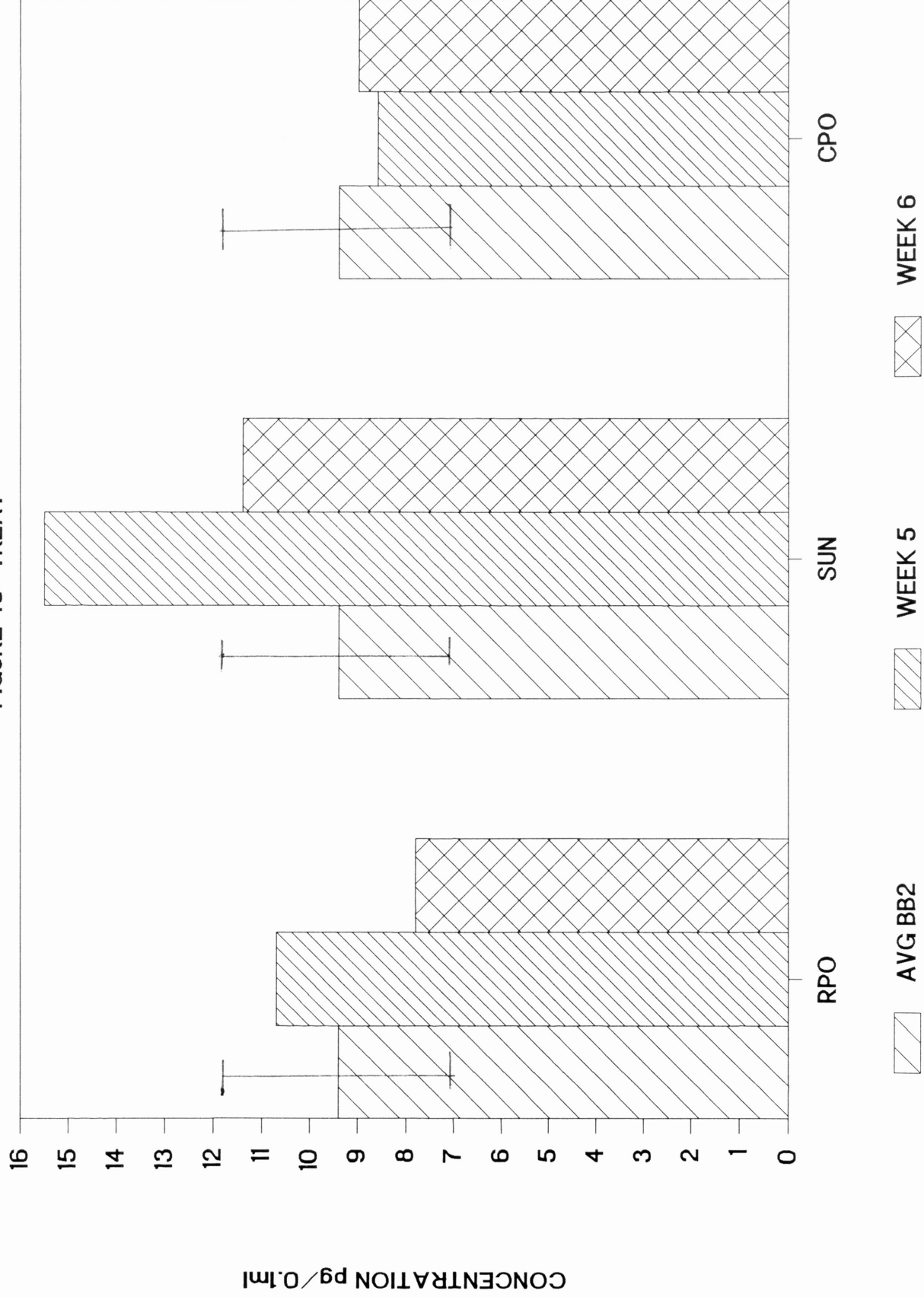
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FIGURE 39: TREAT



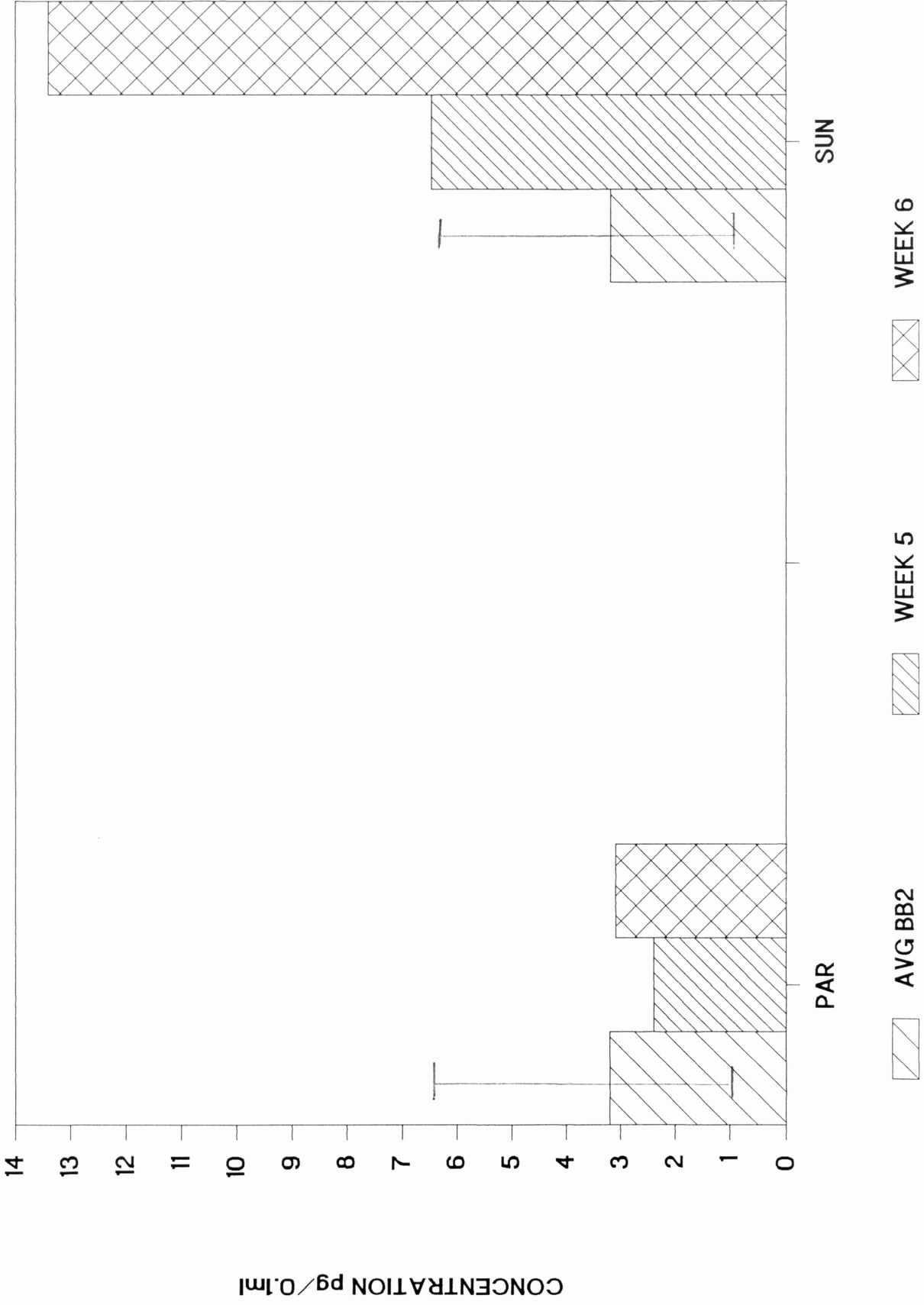
6-KETO-PROSTAGLANDIN F1a

FIGURE 40: TREAT



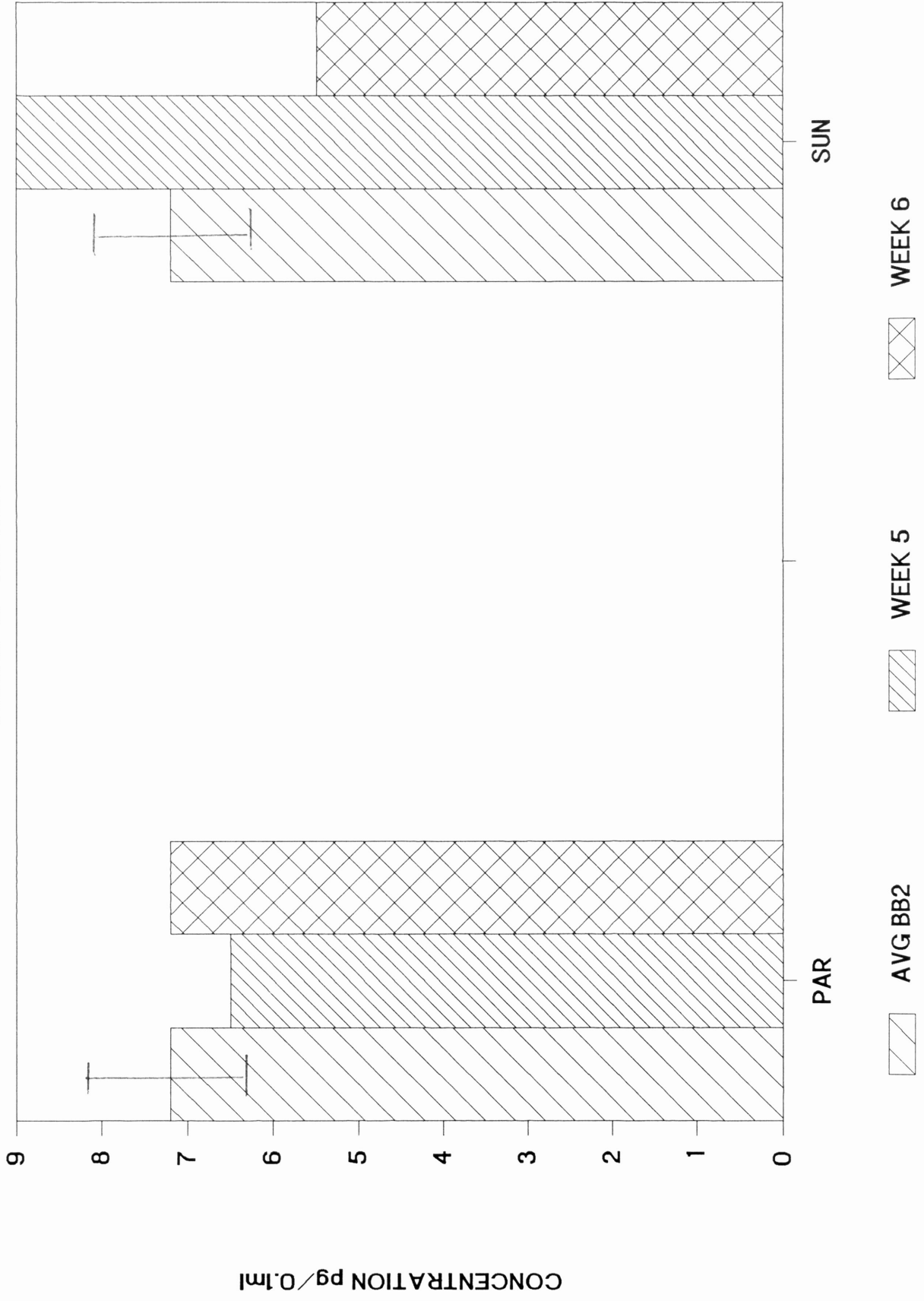
THROMBOXANE B2

FIGURE 41: WEAVER



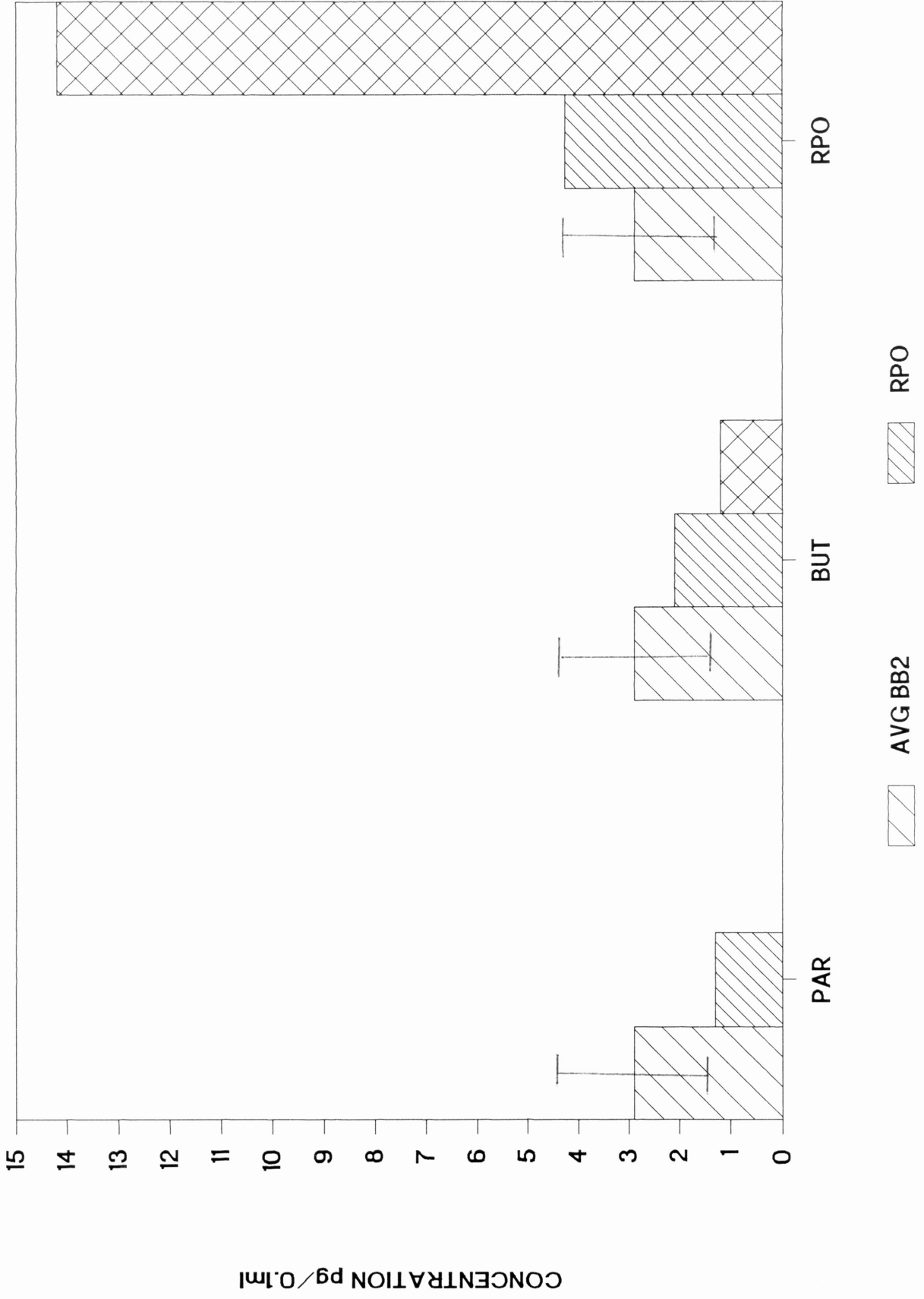
6-KETO-PROSTAGLANDIN F1a

FIGURE 42: WEAVER



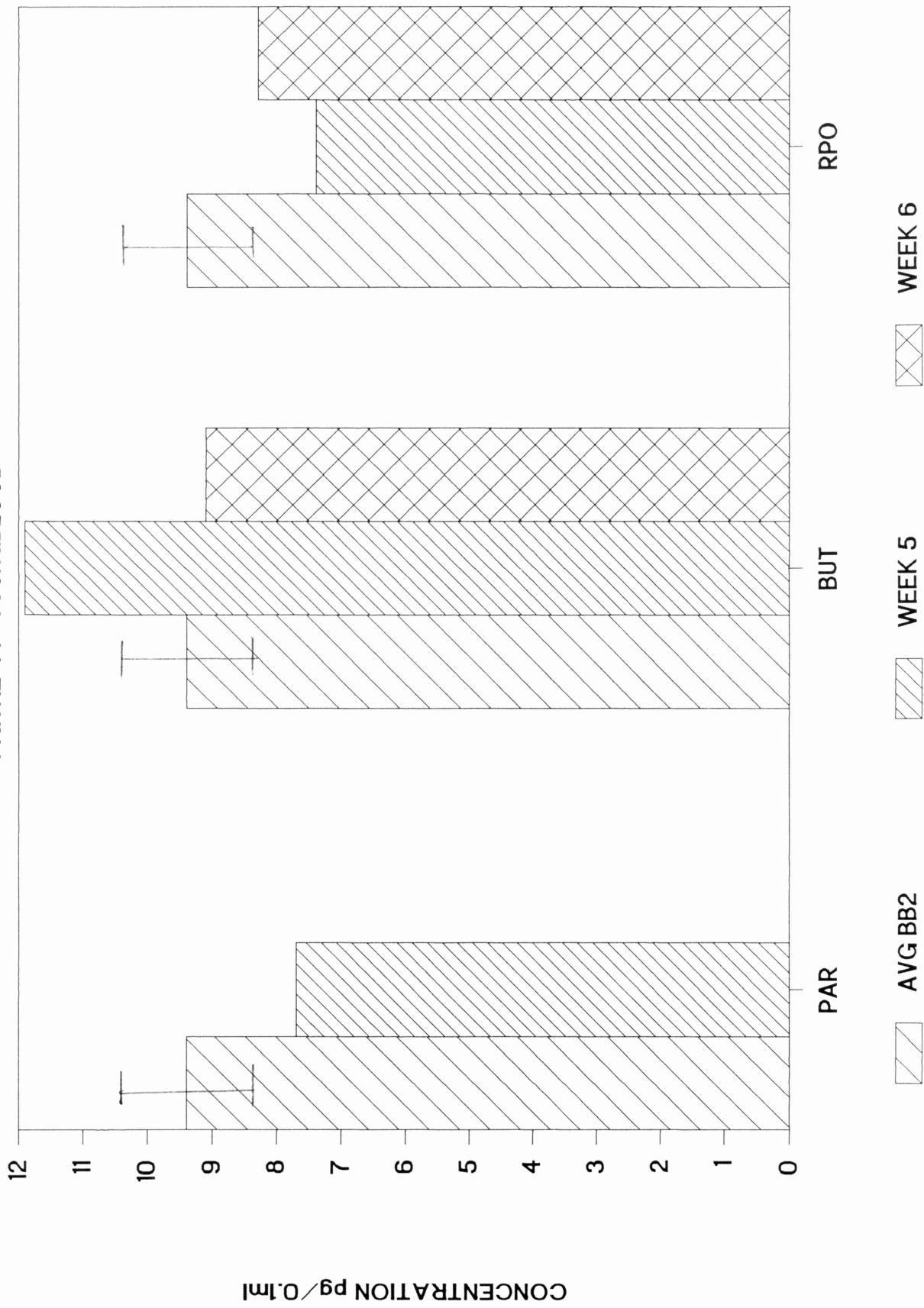
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FIGURE 43: YOUNGBLOOD



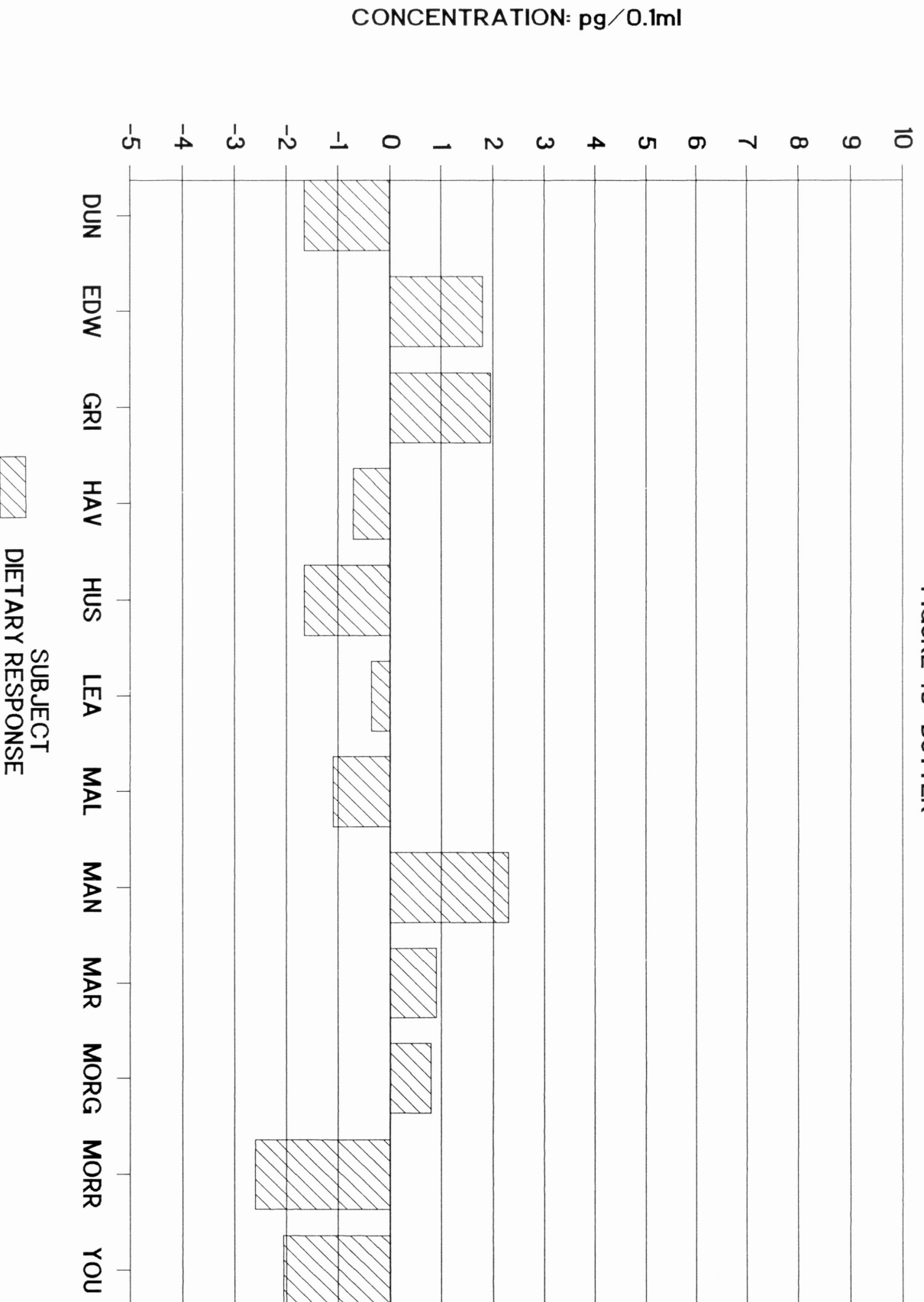
6-KETO-PROSTAGLANDIN F1a

FIGURE 44: YOUNGBLOOD



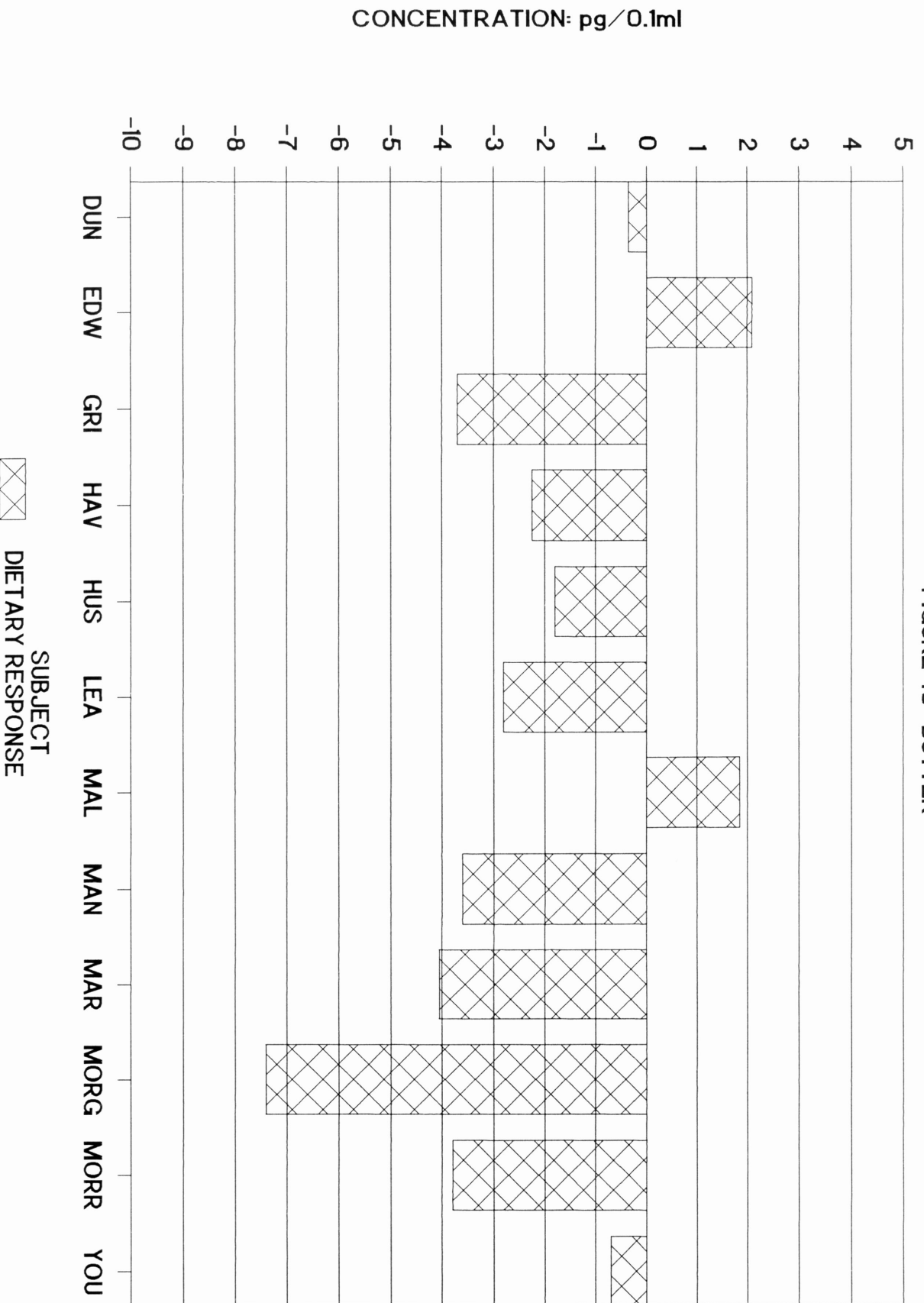
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FIGURE 45: BUTTER



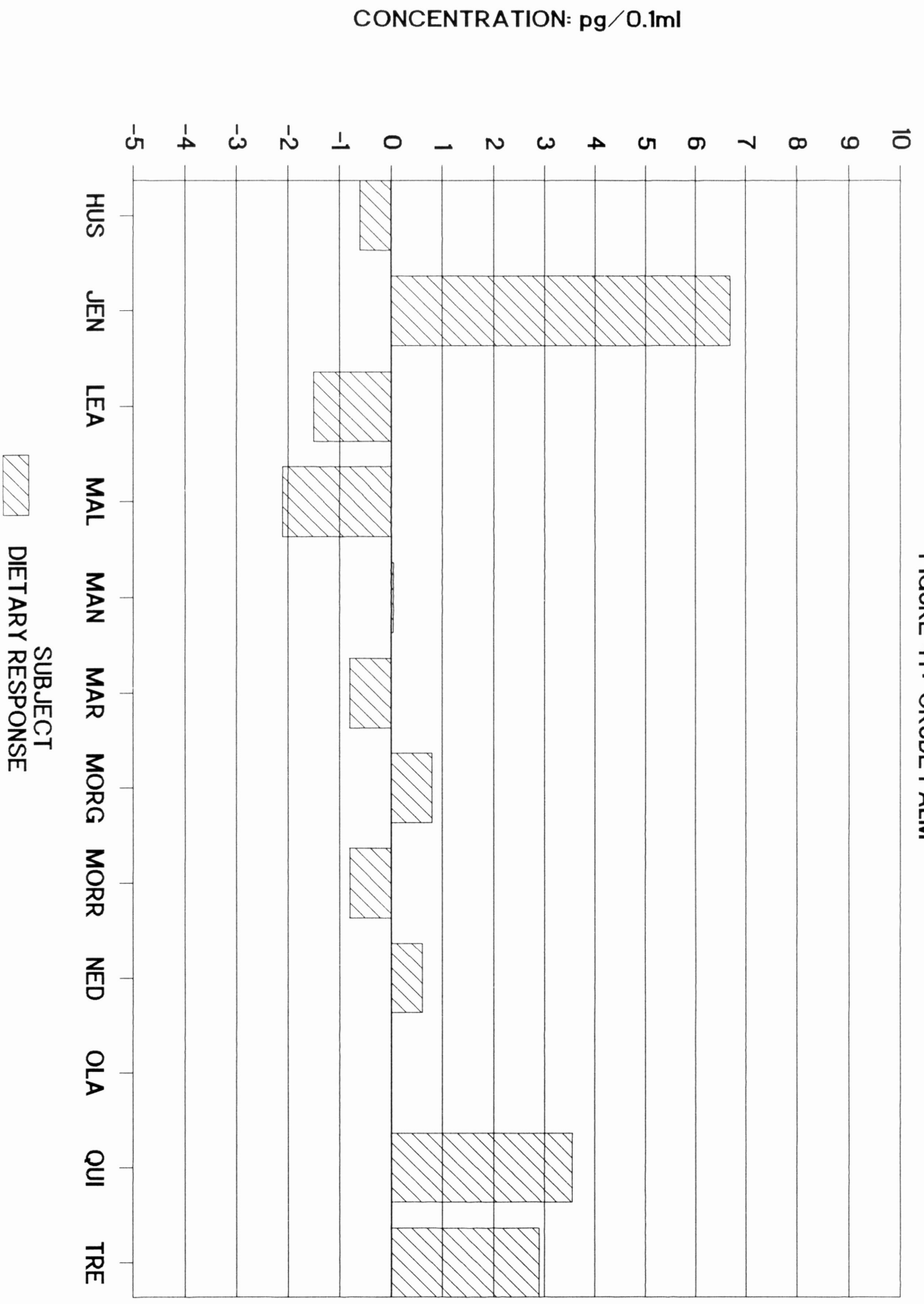
6-KETO-PROSTAGLANDIN F1a

FIGURE 46: BUTTER



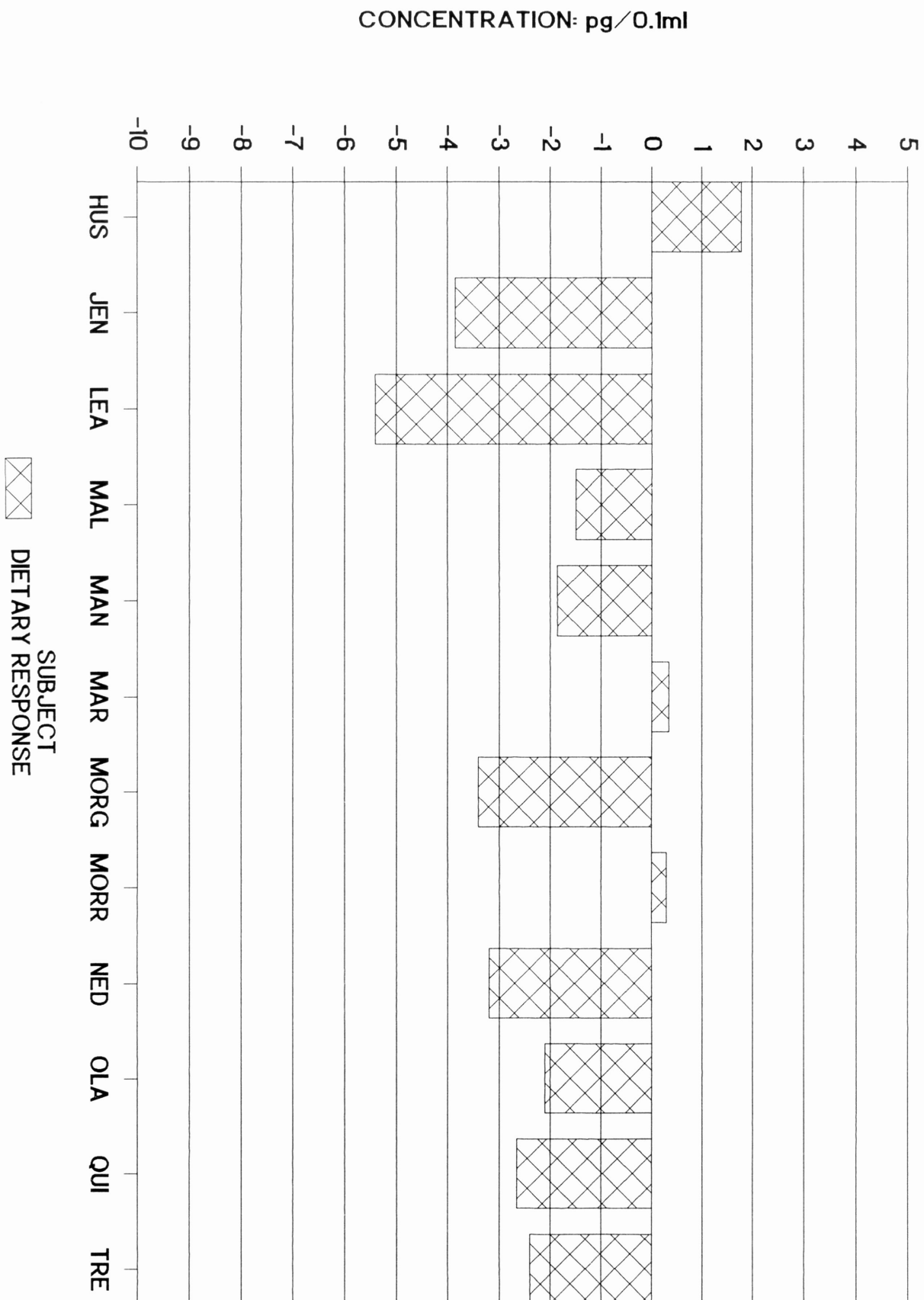
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FIGURE 47: CRUDE PALM



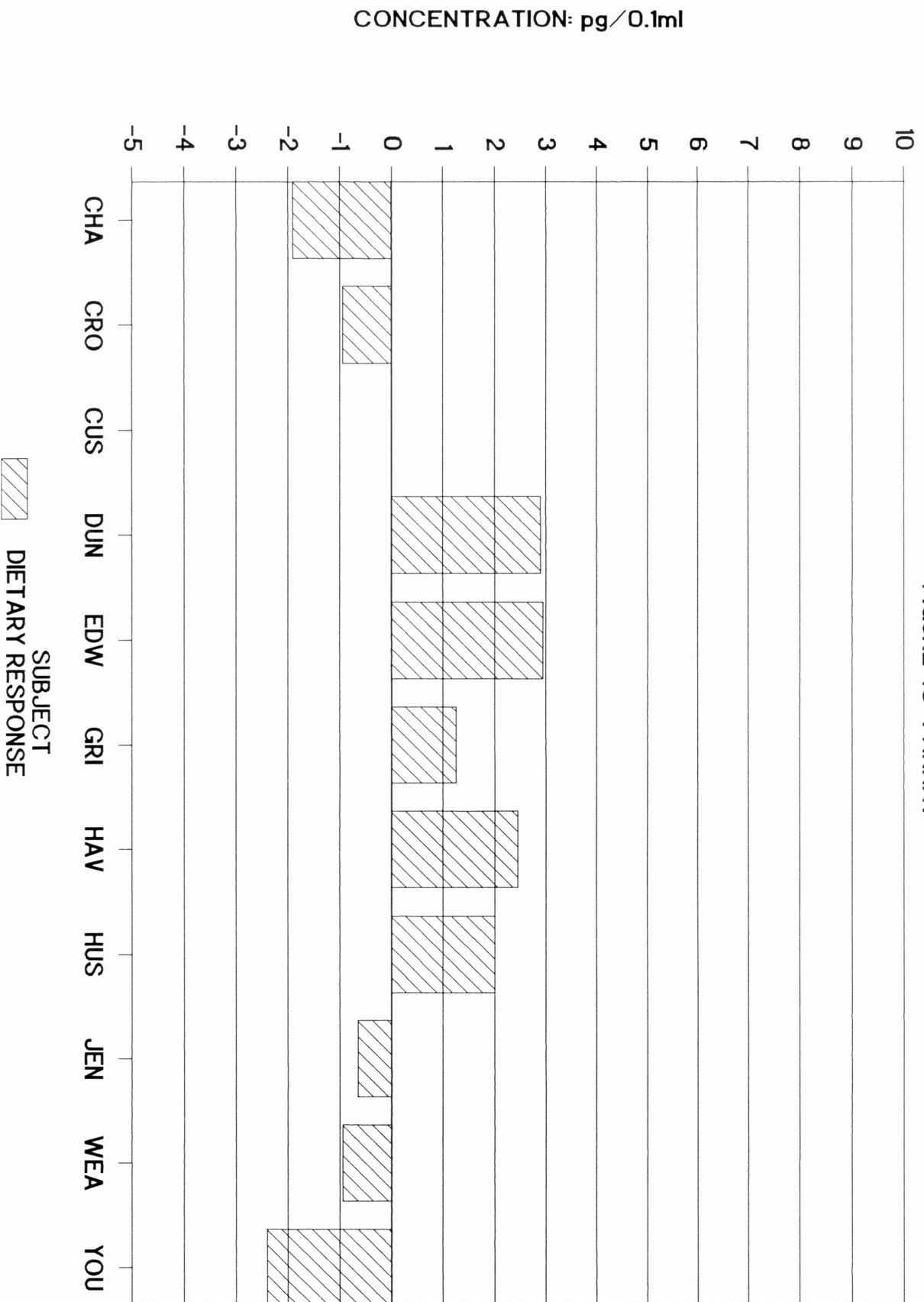
6-KETO-PROSTAGLANDIN F1a

FIGURE 48: CRUDE PALM



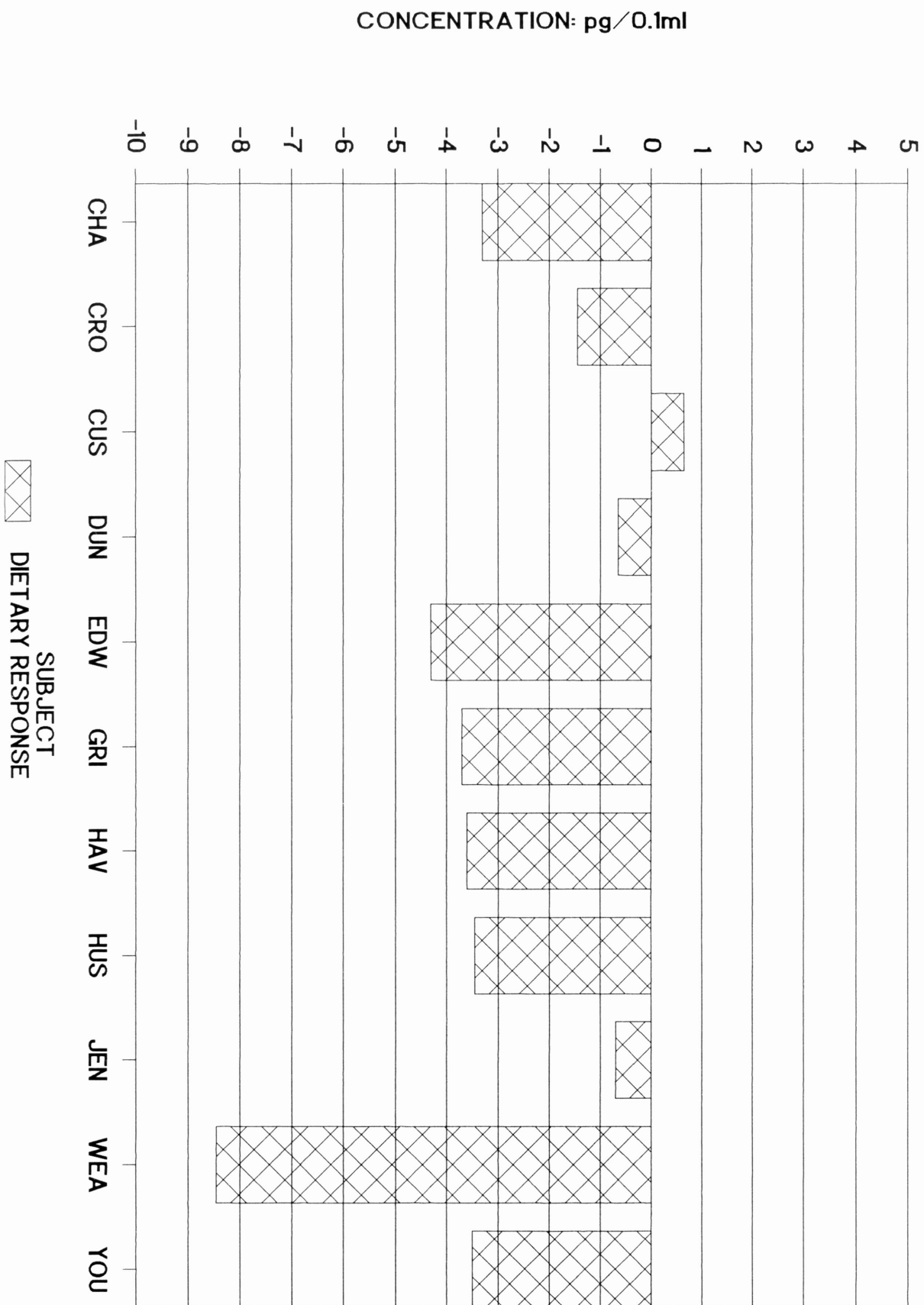
THROMBOXANE B2

FIGURE 49: PARKAY



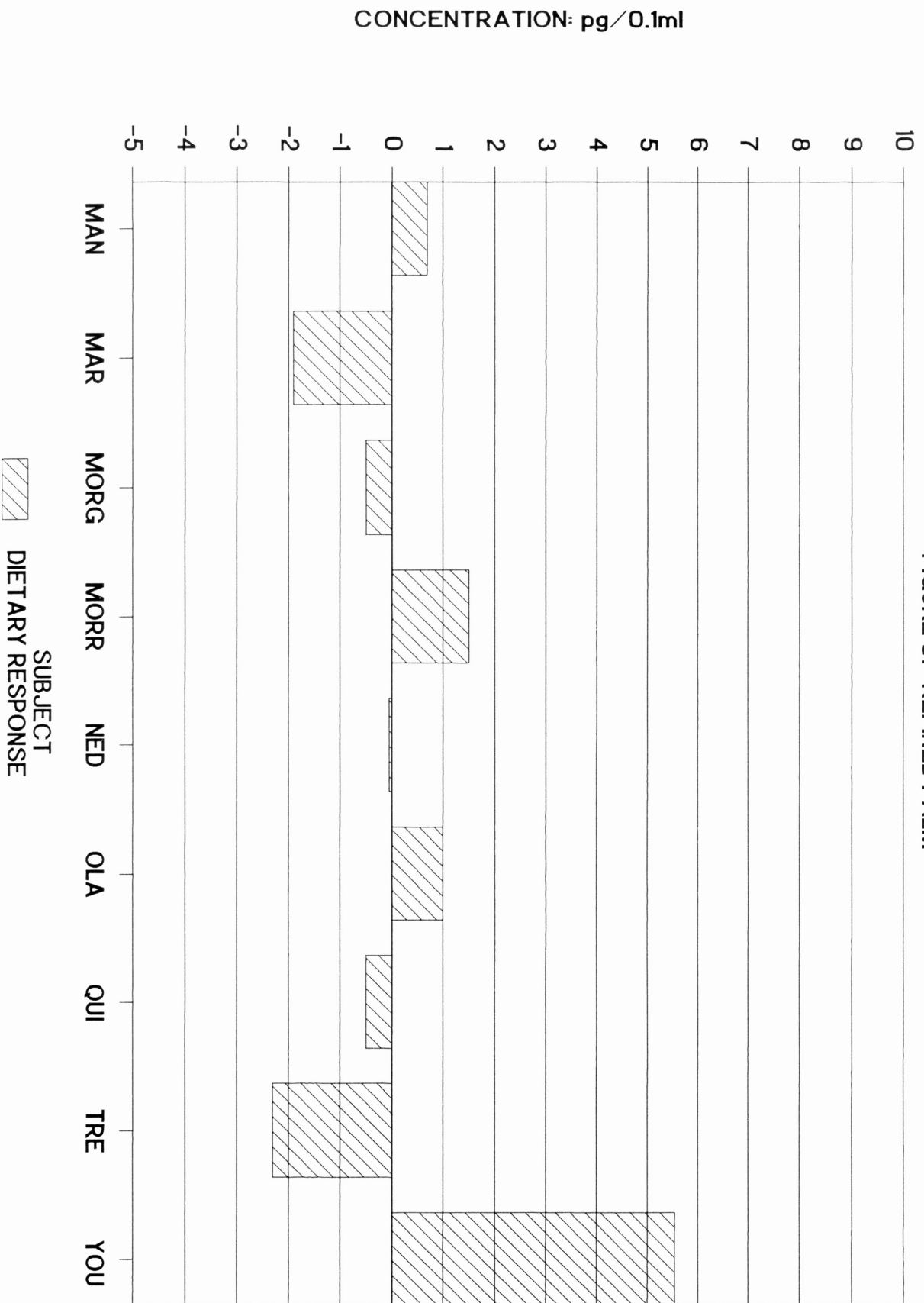
6-KETO-PROSTAGLANDIN F1a

FIGURE 50: PARKAY



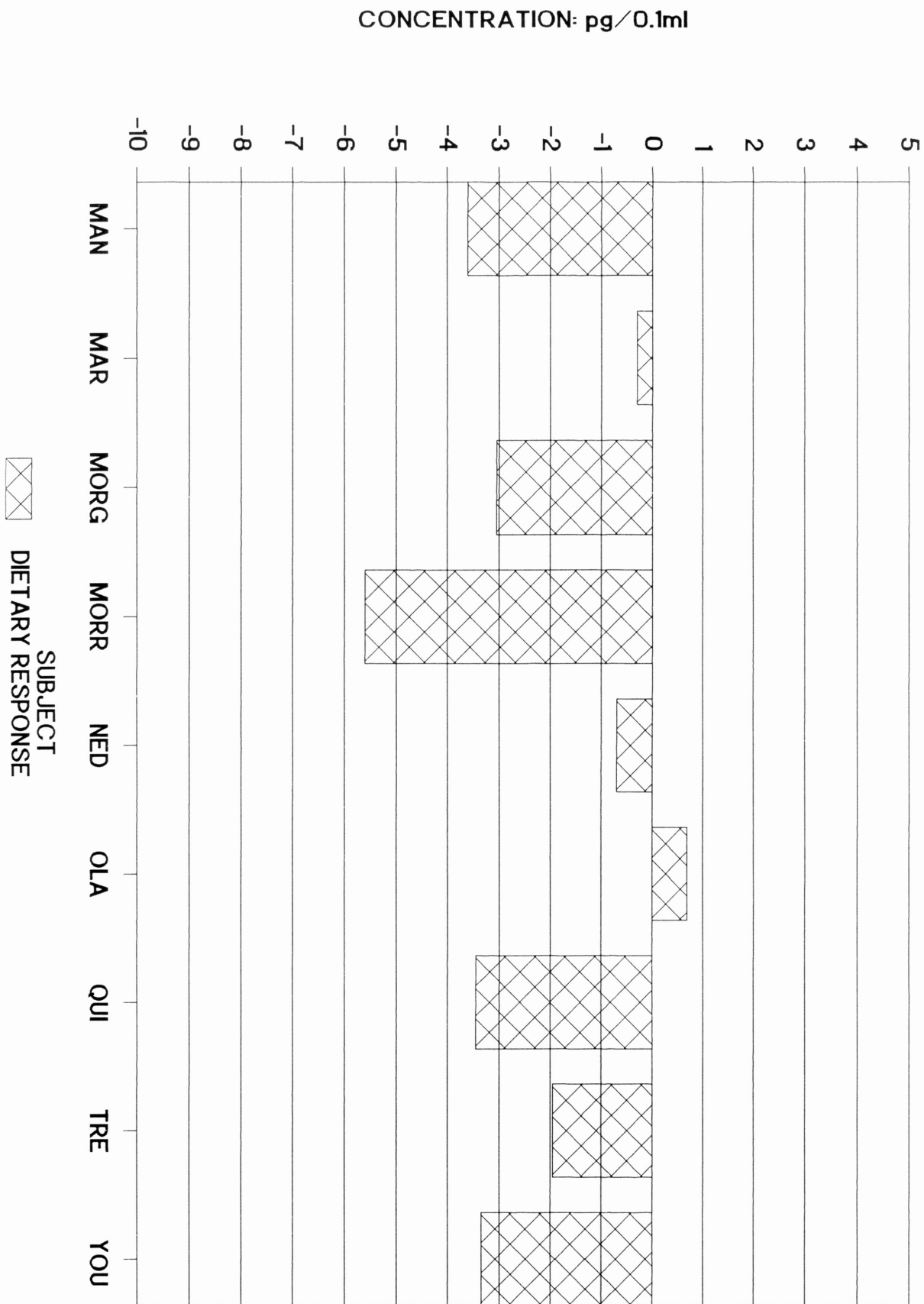
THROMBOXANE B2

FIGURE 51: REFINED PALM



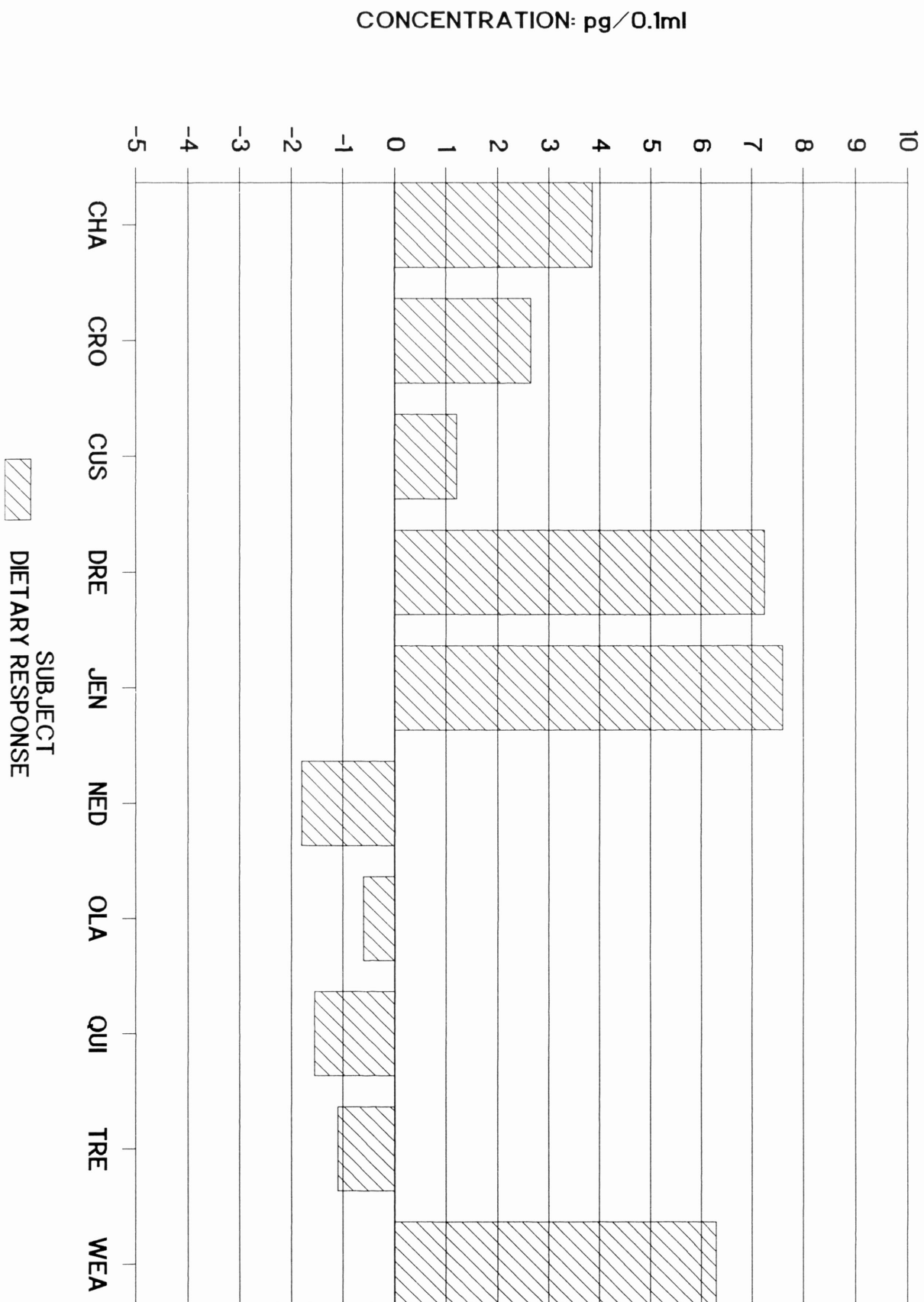
6-KETO-PROSTAGLANDIN F1a

FIGURE 52: REFINED PALM



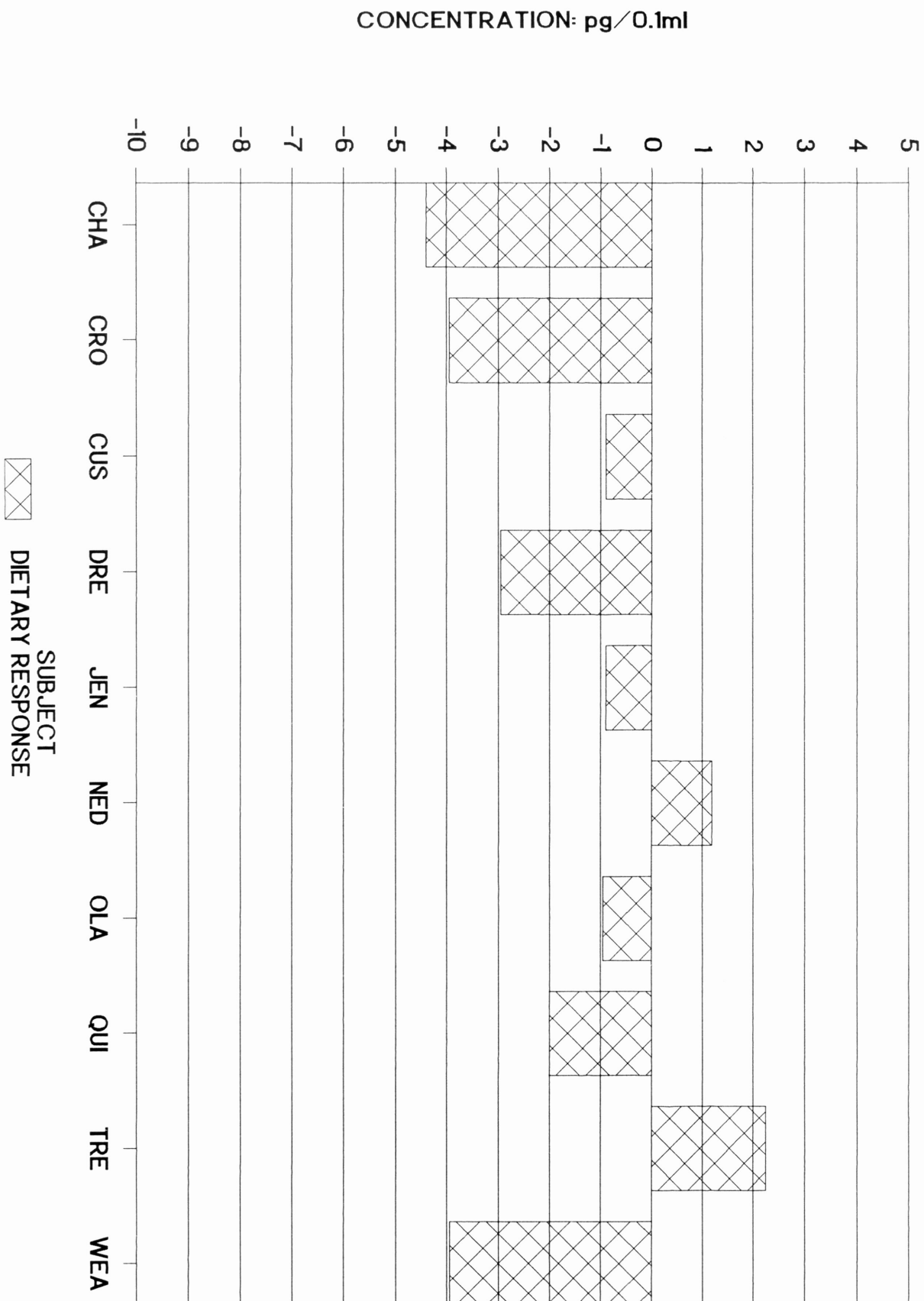
THROMBOXANE B2

FIGURE 53: SUNFLOWER



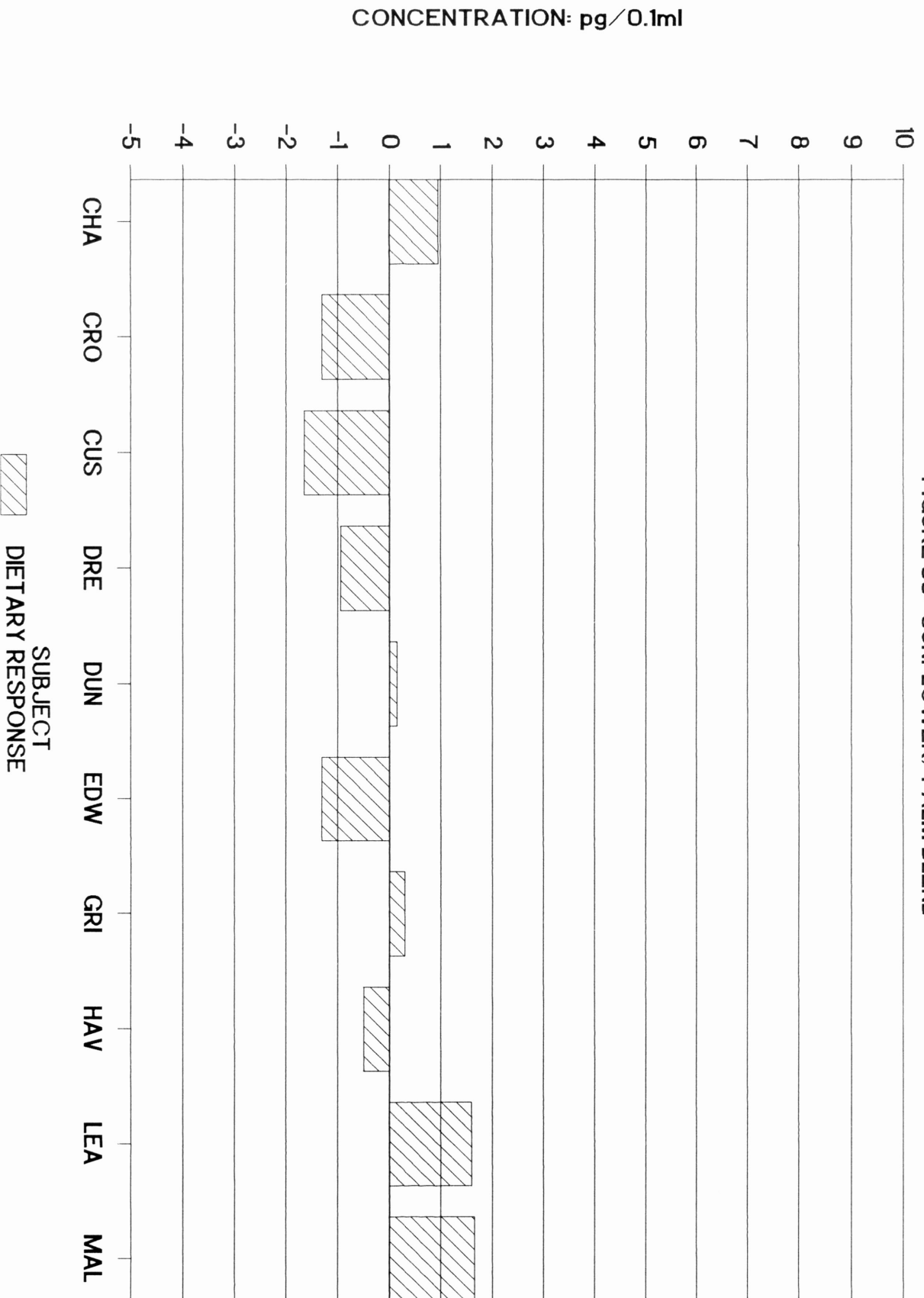
6-KETO-PROSTAGLANDIN F1a

FIGURE 54: SUNFLOWER



THROMBOXANE B2

FIGURE 55: SUNFLOWER/PALM BLEND



6-KETO-PROSTAGLANDIN F1a

FIGURE 56: SUNFLOWER/PALM BLEND

