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One of the most sensitive biochemical responses to the exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) is the induction of ethoxyresorufin-O-deethylase (EROD). To investigate interactions among TCDD and PCBs, rat hepatoma cells (H4IIE) were exposed to the mixtures of 2,4,5,2',4', 5'-hexachlorobiphenyl (PCB#153, *fg-ug* quantities) and ED50 doses of either TCDD, PCB#77 (3,4,3',4'-tetrachlorobiphenyl), or PCB#126 (3,4,5,3',4'-pentachlorobiphenyl) for 72 hours. EROD activities were measured by spectrofluorometry and dose-related synergistic effects observed in these binary mixtures. At higher concentrations, hover, PCB#153 was an antagonist to TCDD and PCB#126.

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ABSTRACT

One of the most sensitive biochemical responses to the exposure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs) is the induction of ethoxyresorufin-O-deethylase (EROD). To investigate interactions among TCDD and PCBs, rat hepatoma cells (H4IIE) were exposed to the mixtures of 2,4,5,2′,4′,5′-hexachlorobiphenyl (PCB#153, *fg-ug* quantities) and ED_{50} doses of either TCDD, PCB#77 (3,4,3′,4′-tetrachlorobiphenyl), or PCB#126 (3,4,5,3′,4′-pentachlorobiphenyl) for 72 hours. EROD activities were measured by spectrofluorometry and dose-related synergistic effects observed in these binary mixtures. At higher concentrations, however, PCB#153 was an antagonist to TCDD and PCB#126.

8 MAR 1993

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INTRODUCTION

PCBs are highly stable, lipophilic compounds which have been used in heat transfer liquids, plasticizers, lubricants, inks, wax extenders, flame retardants, and as dielectric fluids in capacitors and transformers (Safe, 1984). Due to their chemical stability, PCBs are widespread environmental contaminants in the air, water, sediments, fish, wildlife, and humans (Risebrough et al, 1968; Buckley, 1982).

TCDD and related halogenated aromatic hydrocarbons elicit common biologic and toxic effects in animal species (Leece et al., 1985). These effects includes body weight loss, thymic atrophy, hepatotoxicity, chloracne and other dermal lesions, and reproductive problems (Fishbein, 1974; Safe, 1990). Early signs of acute toxicity in most species exposed to PCBs are weight loss or reduced weight gain.

One of the most sensitive and earliest biochemical responses to halogenated hydrocarbons is induction of hepatic

microsomal enzymes of the mixed function oxidase system (cytochrome P450). EROD is the representative P450 system (P450IA1) which is induced by PCB exposures. Induction of this enzyme is caused by receptor-mediated action of TCDD and other planar chlorinated hydrocarbons such as PCBs. This receptor is a high affinity cytosolic protein designated the A*h* (aryl hydrocarbon) receptor (Roberts et al., 1985). The activity of 15 PCB congeners was measured as inducers of P450 systems in rat hepatoma H4IIE cell cultures (Sawyer et al., 1982). All the PCBs which had previously reported as enzyme inducers in rats also induced EROD in H4IIE cells. This study demonstrated that there was a correlation of EROD induction in both hepatoma cells and immature rat liver.

Few studies have been carried out on the mixture toxicity of polychlorinated hydrocarbons. When a mixture of PCB#153 and TCDD or other PCB congeners was exposed to mice, increased incidence of cleft palate was observed (Birnbaum et

al., 1985). Different strains of mice gave different results. No study has been carried out to show the effects of mixtures on hepatoma cells. This work was designed to study the chemical interactions between halogenated hydrocarbons.

METHODS

The H4IIE rat hepatoma cells obtained from the ATCC were cultured in D-MEM supplimented with vitamins, amino acids and 10% fetal bovine serum. Cells trypsinized at confluency were seeded in Petri dishes (10^6/plate) in 10 mL D-MEM. Cells were allowed to attach for 24 hours and then exposed to the appropriate chemicals dissolved in 100 *u*L isooctane (n=4). No effect from the vehicle was observed. Dosed cells were incubated for 72 hours and harvested with a cell scraper. Protein analysis was carried out by the BCA method, and EROD activities were measured spectrofluorometrically (550-nm excitation, 585-nm emission). EROD specific activities were determined against a standard curve calculated as pmoles of resorufin formed per milligram of protein per minute.

Concentration range of dosing solution for TCDD were 13.02-1040 *pg*/plate; for PCB#126 104 *pg* -125 *ng*/plate; and for PCB#77 18.7*ng* -117 *ug*/plate. In the mixture study, ED_{50} levels of either TCDD, PCB#126 or #77 were coexposed with various concentrations of PCB#153 (0.5 *pg* - 2.5×10^{8}). Controls and positive controls were employed throughout the experiments.

CONCLUSIONS

Figures 1-3 are the dose-response curves and figs 4-6 the probit analysis of TCDD, PCB#126 and #77, respectively. The ED_{50} of TCDD, PCB#126 and #77 were 68 *pg*, 3 *ng*, and 3 *ug* per plate, respectively. Results of the mixture studies are shown in figs 7-9. When PCB#153 alone was exposed to H4IIE, no EROD activities were observed. However, synergistic effects were detected when TCDD, PCB#126 or #77 was exposed with PCB#153. In all three exposures, PCB#153 acted as an agonist to each chemical. PCB#153 acted as an antagonist to TCDD when the PCB#153 concentration was higher than 50 *ug*/plate. A similar antagonistic effect was observed with PCB#126 when the concentration of PCB#153 was 2.5×10^5 *ng*/plate.

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DOSE–RESPONSE CURVE OF TCDD

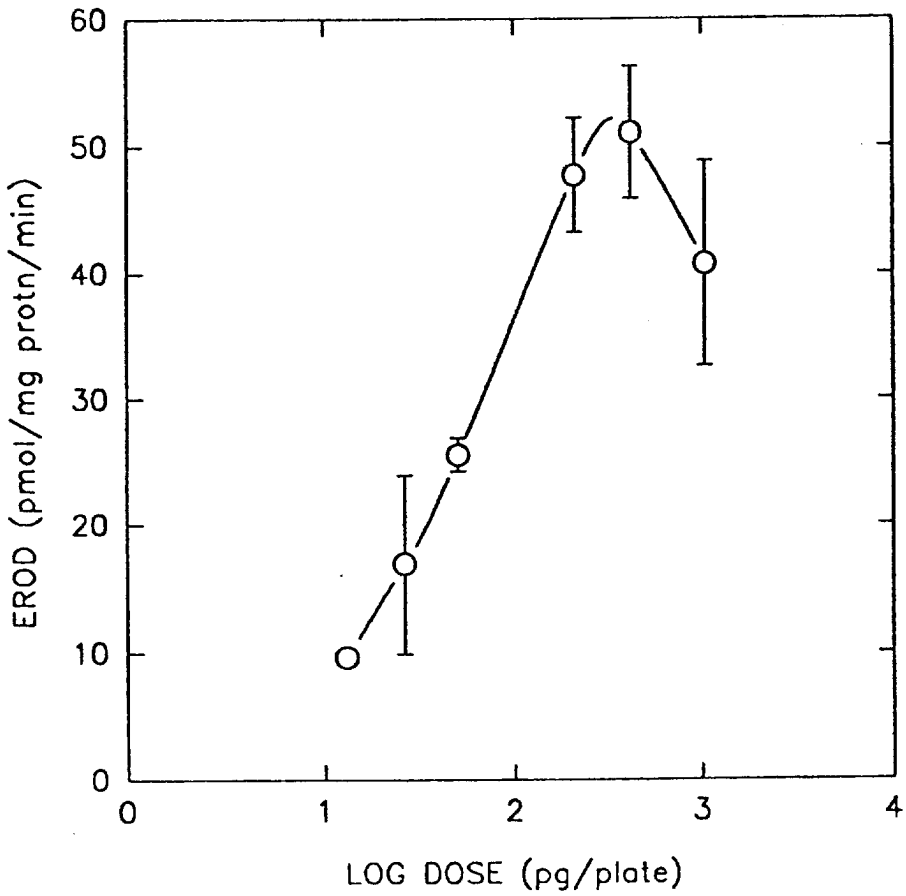

Fig 1

DOSE-RESPONSE CURVE OF PCB#126

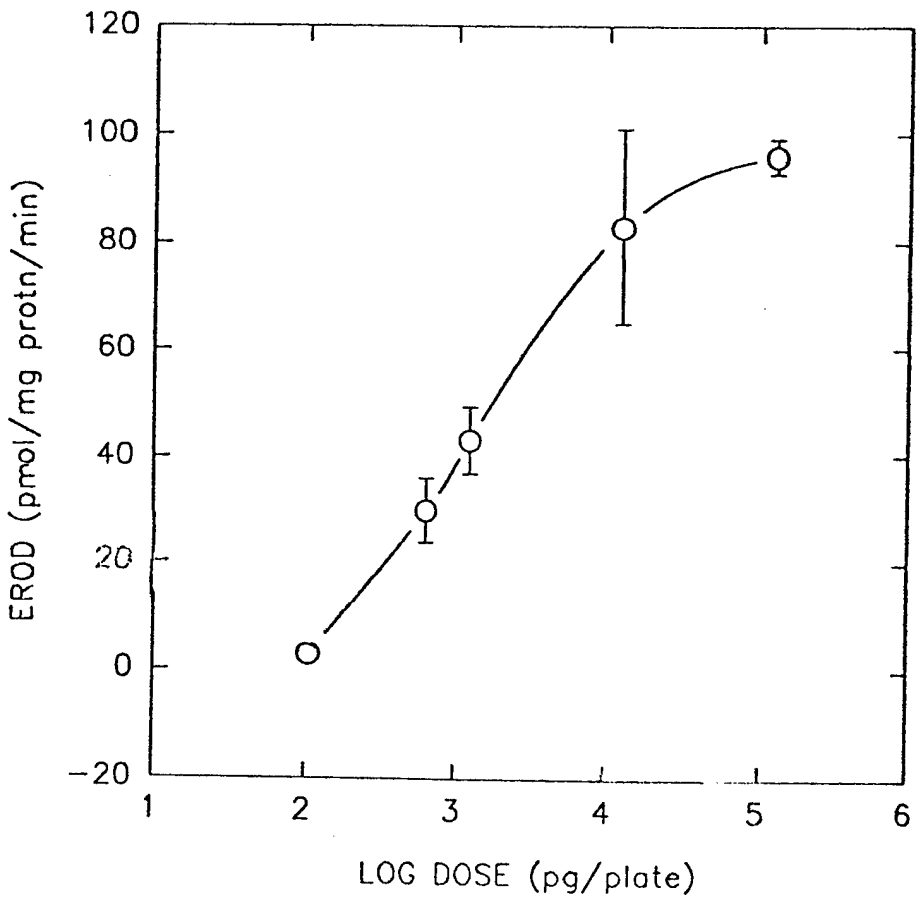

Fig. 7

DOSE-RESPONSE CURBE OF PCB#77

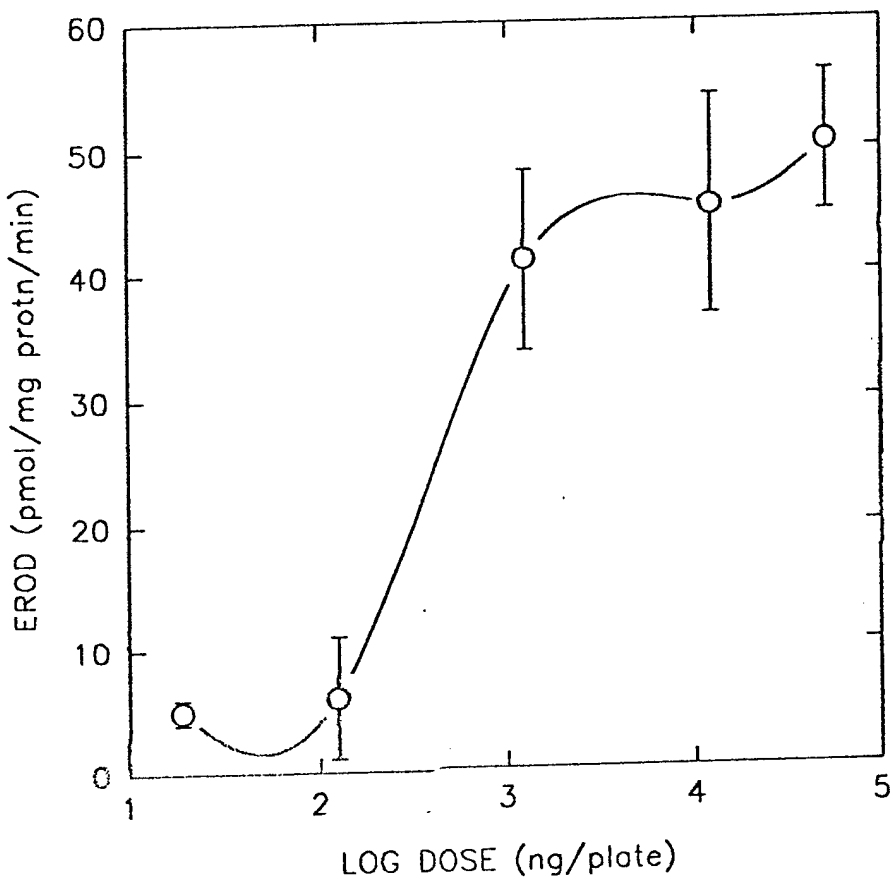

Fig 3

Probit Regression of H4IIE Cell
Dose-Response to TCDD

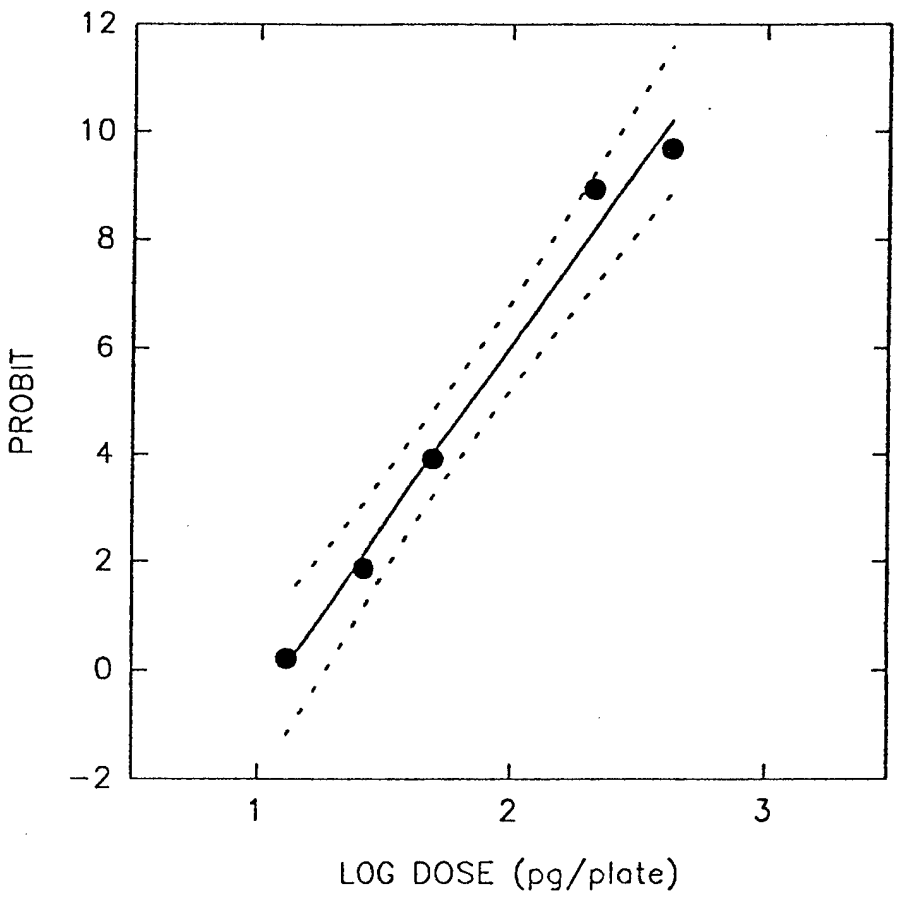

Fig 4

Probit Regression of H4IIE Cell Dose–Response to PCB#126

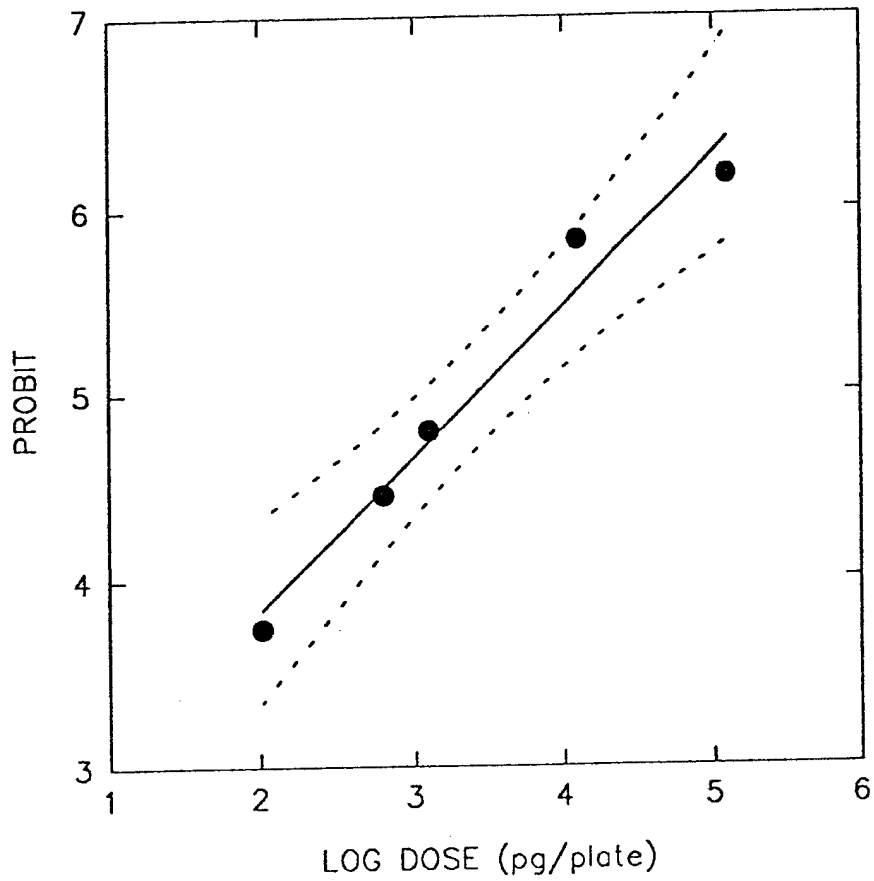

Fig 5

Probit Regression of H4IIE Cell
Dose–Response to PCB#77

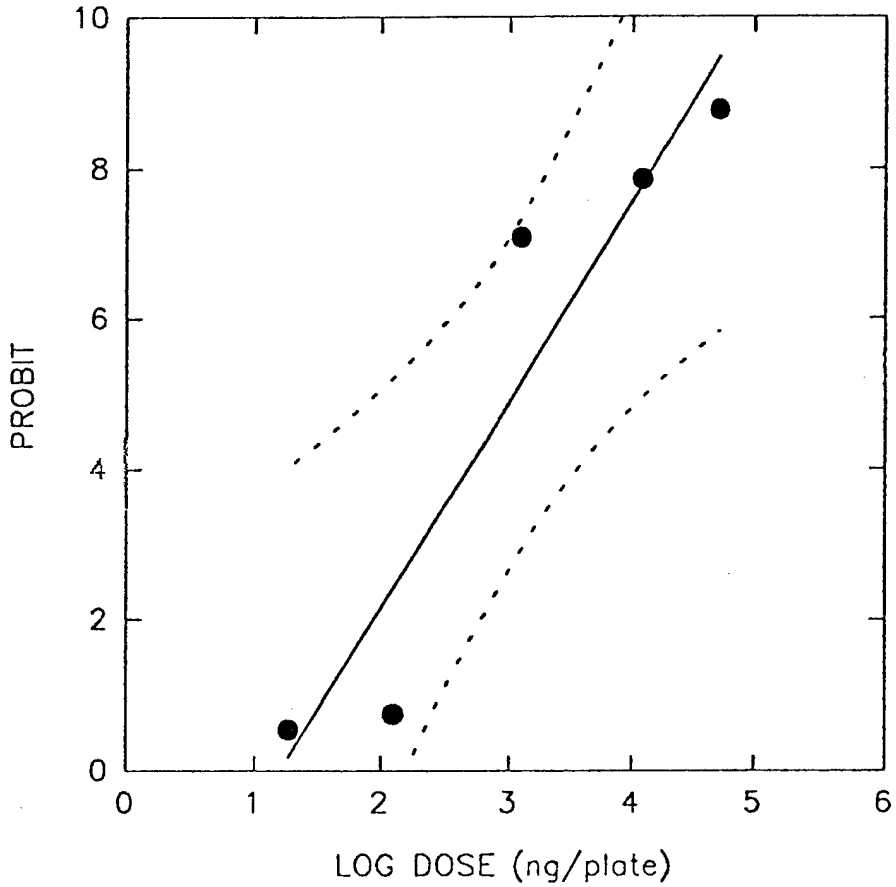

Fig. 6

GGTCDD153
2 11:14 Page 1

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TCDD & PCB#153

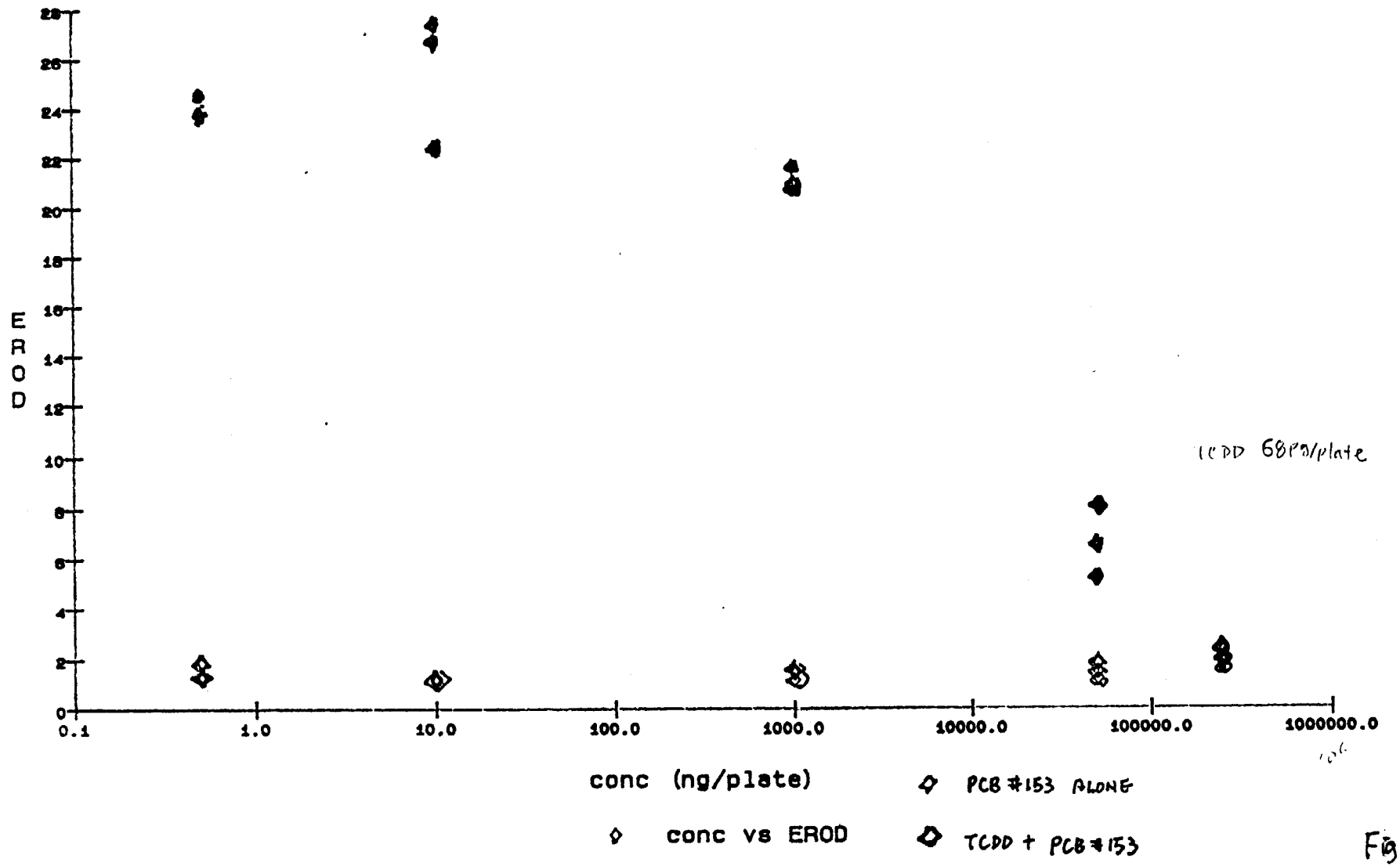

Fig 7

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2 7: 10 Page 1

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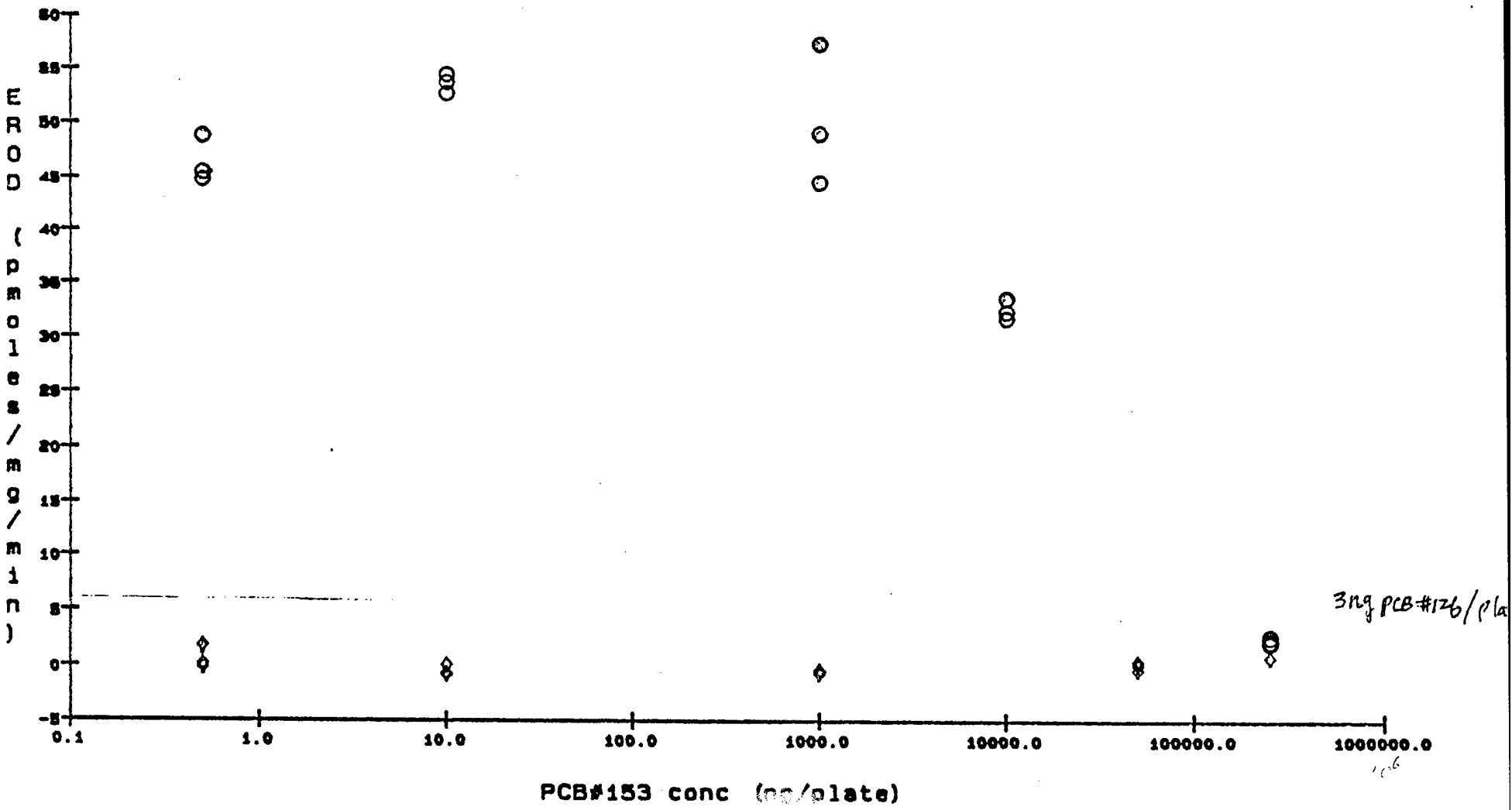
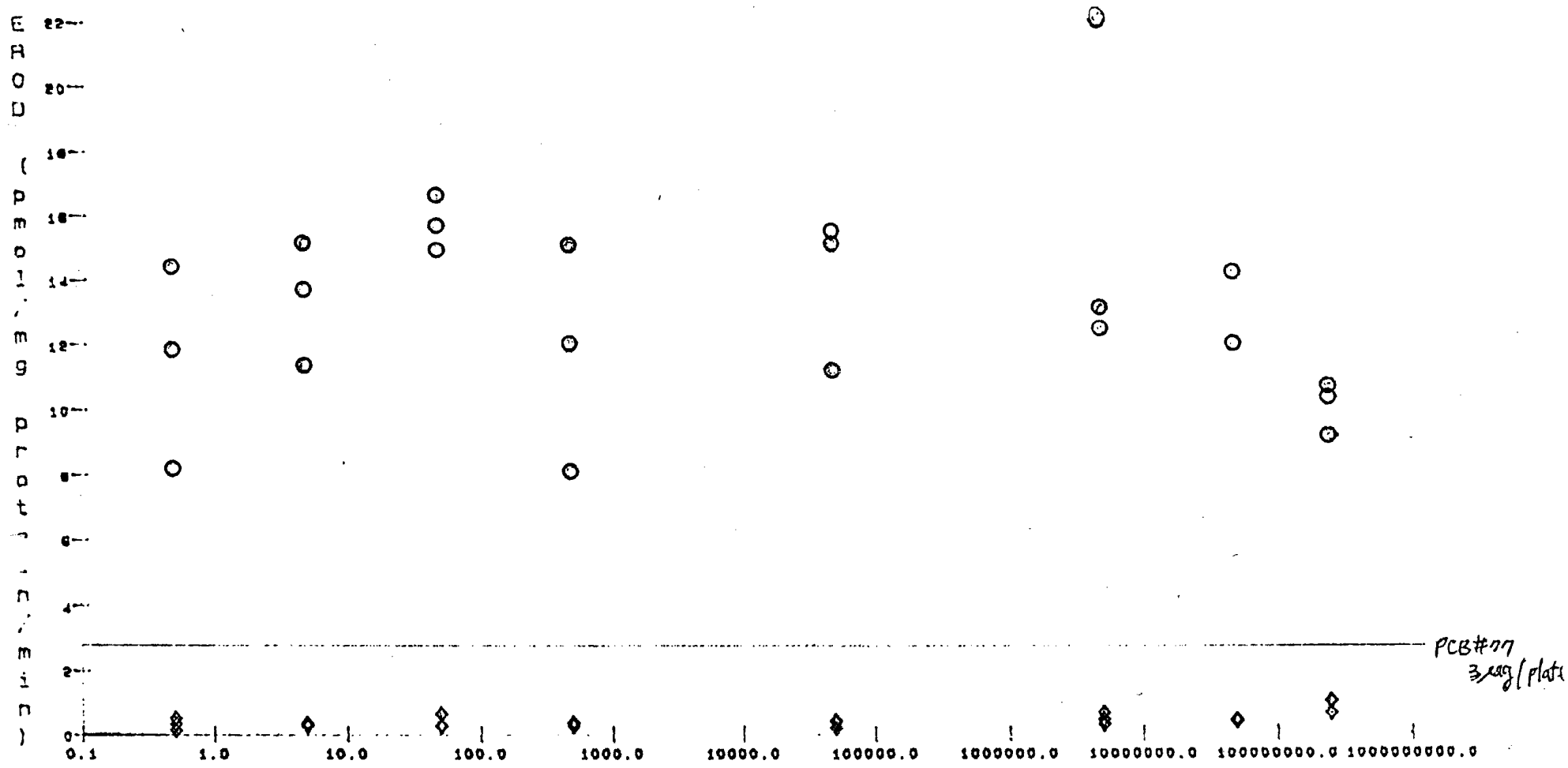
MIXTURE OF PCB#126 & PCB#153



◆ conc vs EROD PCB#153 ALONE
⊙ conc vs EROD PCB#153 + PCB#126

GMIXB21 22-AUG-9
2 23:4" Page 1

MIXTURE EFFECT OF PCB153 & "7"



◇ conc pcb 153 alone, pcb153 & "7" O

◆ conc vs EROD PCB #153 ALONE

⊙ conc vs EROD PCB #153 + PCB #77

Fig 9