The Inducibility of P-450IA mRNA Expression by PCB Mixtures, PCB Congeners, and a PAH in Hudson River Tomcod and Hogchoker

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ABSTRACT

Previous experiments showed that Atlantic tomcod exposed to clean sediments artificially spiked with two PAH's, fluoranthene and phenathrene, showed elevated levels of P450IA mRNA. Intraperitoneal injection of ß-naphthoflavone, also a PAH, elicited this response in tomcod, as well (Wirgin et al. in press). In this study, two PCB congeners, 3,3'4,4' tetrachlorinated biphenyl and 2,3,3'4,4' polychlorinated biphenyl, and a PCB mixture, Aroclor 1254, were analyzed for their ability to induce P450IA mRNA expression in tomcod. In addition, the kinetics of depuration and P450IA mRNA induction by ß-naphthoflavone was studied further in both tomcod and another bottom-dwelling fish, the hogchoker. P450IA induction in tomcod injected with ß-naphthoflavone was shown to begin around 26 hours after injection and continued until about 5 days after injection. Induction was found to be statistically significant at 5 days after injection, with a significant decrease in expression between 5 and 7 days. Neither PCB congener elicited a significant response. P450IA mRNA expression in Aroclor-injected tomcod was found to be induced at 3 days after injection. Expression at 5 days post-injection was noticeably decreased. Contaminated sediments from Foundry Cove did not significantly induce the P450IA mRNA response in eleven tomcod. However, five of the eleven fish showed very significant levels of induction, whereas the other six showed no increase in induction at a confidence level of 95%. Hogchokers injected with ß-naphthoflavone showed no signs of elevated P450IA mRNA expression after one day. Several polymorphisms in the structure of P450IA mRNA were found in tomcod exposed to Aroclor 1254 and ß-NF. Therefore, it was found that flat, coplanar compounds, for example, PAH's induce P450IA mRNA expression in tomcod, but that non-coplanar compounds, such as the PCB congeners used in this experiment, do not elicit the measured response. Also, there appears to be some species variation in response to these compounds.

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INTRODUCTION

The Hudson River's proximity to a large urban center has made this waterway popular for domestic water use, moderately sized commercial fisheries, and recreation. Historically, the Hudson River has also been used as a dumping ground for both organic and inorganic pollutants (Horn et al., 1979; Limburg, 1988). Some of the organic compounds polluting the river include dioxins, polycyclic aromatic hydrocarbons (PAH's), and PCB mixtures (Aroclors) (Limburg, 1988). The contamination of this important natural resource has caused the closure of commercial fisheries for several important Hudson River species, such as the striped bass. In addition, bioaccumulation and spread of the pollutants throughout the food chain has caused wide-spread concern.

Atlantic tomcod (<u>Microgadus tomcod</u>) from the Hudson River display extremely high liver cancer rates compared to tomcod from more pristine aquatic environments (Cormier, 1986; Cormier et al., 1989; Dey et al., 1986). More than 50% of spawning one-year-old and more than 90% of two-year-old tomcod exhibit hepatocellular carcinomas. These tomcod spend their entire lifespan within the confines of the lower Hudson River (Dew et al., 1976; Klaud et al., 1988; McLaren et al., 1988). They are bottom-dwellers and so are exposed readily to contamination from river sediment. These fish have very high lipid levels in their liver, which contribute to bioaccumulation of fat-soluble pollutants, such as polychlorinated biphenyls (PCB's) (Klauda et al., 1981). The hogchoker, another bottom-dwelling Hudson River fish, shares the same ecological niche with the tomcod, yet does not display the high levels of liver cancer exhibited by tomcod.

Previously, it has been shown that Hudson tomcod liver tumor DNA contains an activated K-*ras* oncogene (Wirgin et al., 1989), as well as a high rate of mutational change at another oncogene locus, c-*abl* (Wirgin et al., 1990). High concentrations of PCB Aroclor mixtures were reported in tomcod livers (Klauda et al., 1981). An inability to detect PAH's in tomcod livers suggests that these compounds may be rapidly metabolized to a form which potentially is reactive with tomcod DNA (Dey et al.,1986). It has been shown in other fish species that metabolic activation of some xenobiotics, such as polycyclic aromatic hydrocarbons, by the cytochrome monooxygenase system is required for the carcinogenic activity of the compounds (Jerina et al., 1984; Stegeman et al., 1987; Kleinow et al., 1987). The cytochrome P450 family of proteins has shown to play a role in this

metabolic activation.

It has previously been found in this lab that tomcod taken from the Hudson River and sacrificed immediately have an elevated level of P450IA mRNA expression compared to fish kept in clean water for several days (Wirgin et al, in press). Expression of P450IA mRNA in fish immediately sacrificed after removal from the Hudson was approximately ten times higher than that of tomcod kept in clean water for >twenty days. Fish which were kept in clean water for eight hours still showed significant levels of P450IA mRNA expression compared to the fish depurated for twenty days, but this group showed 75% less P450IA mRNA expression compared to the group sacrificed immediately after being taken out of the Hudson. Tomcod depurated for twenty days and reintroduced into the Hudson for two to three days exhibited elevated expression of P450IA mRNA. In separate experiments, depurated Hudson River tomcod were injected with a polycyclic aromatic hydrocarbon, ß-naphthoflavone, dissolved in corn oil. The results from these experiments demonstrated that P450IA mRNA expression could be induced by deliberate injection of a PAH. Other experiments were done in which the tomcod were exposed to clean sediments spiked with two other PAH's. These tomcod also demonstrated elevated P450IA mRNA levels. The fact that both natural Hudson River agents and laboratory-administered PAH's induced this response suggests that the presence of PAH's in the Hudson may be causing this induced response in the Hudson River tomcod.

In order to identify the Hudson River factors which are inducing P450IA mRNA expression in tomcod, we injected one of two specific PCB congeners or a PCB mixture (Aroclor 1254) into tomcod and then analyzed for P450IA mRNA expression in Northern blot analyses. In addition, previous results with ß-naphthoflavone were further explored by injection of ß-naphthoflavone into tomcod to determine how quickly the response was induced and how long it took for the levels of P450IA mRNA expression to return to basal levels. In addition, depurated tomcod were exposed to contaminated sediments present at the Foundry Cove area and the livers were analyzed for levels of P450IA mRNA expression. Finally, ß-NF injections were performed on hogchoker (Trinectus maculatus), another Hudson River fish sharing the same ecolological niche as tomcod in the Hudson, and P450IA mRNA expression levels were determined.

MATERIALS AND METHODS

Animal collections and treatments: Tomcod and hogchokers were collected from the Hudson River in March and April, 1990. Tomcod were collected by bottom-trawling off Manhattan with the help of Normandeau Associates. Hogchokers were obtained by bottom-trawling also with the help of Normandeau Associates. The fish were returned to the laboratory and maintained in artificial sea water (5ppt) at a constant temperature; the tomcod were kept at 4° C and the hogchokers were kept at 15° C.

The PCB Aroclor mixture and specific PCB congeners obtained from Ultra Scientific were dissolved in corn oil and injected inside the peritoneum of the depurated fish. For each kilogram of body mass, one milligram of 3,3'4,4' TCB (PCB+) or 2,3,3'4,4' PCB (PCB-), twenty-five milligrams of Aroclor 1254, or 100 mg ß-naphthoflavone was injected. Seven depurated tomcod were injected intraperitoneally with PCB-; of these, four tomcod were sacrificed after three days of exposure to the chemical and three tomcod were sacrificed after five days. Seven depurated tomcod were injected with PCB+ and sacrificed after four days. Twenty-eight depurated tomcod were injected with Aroclor 1254. Of these, seven were sacrificed one day post-injection, four were sacrificed three days after injection, four were sacrificed five days post-injection, four were sacrificed after seven days, four were sacrificed after eleven days and five were sacrificed nineteen days post-injection. Twenty-two depurated tomcod were injected with ß-naphthoflavone. Four were sacrificed four hours after injection, four were sacrificed eight hours post-injection, four were sacrificed twenty-six hours after injection, four of the ß-NF injected tomcod were sacrificed three days after injection, three were sacrificed after five days and three were sacrificed after seven days. Seven depurated hogchokers were injected with ß-NF and sacrificed after one day. Five hogchokers were kept in clean water for twenty days without injection of the chemical. Four hogchokers were sacrificed two hours after being removed from the Hudson at Manhattan. For a positive control, we used ß-NF injected tomcod sacrificed after twenty-six hours, which were previously shown to contain greatly induced levels of P450IA mRNA. For negative controls, depurated fish were either left uninjected or had corn oil injected into the abdomen. Livers were immediately frozen upon their removal from sacrificed fish and stored at -80° C.

RNA Isolation: The livers were frozen by immersion in liquid nitrogen, homogenized, and RNA was then extracted using the RNAzol method (Chomczynski and Sacchi, 1987). Approximately 20 mg of frozen liver tissue was homogenized in a glass-teflon homogenizer in 1.2 mL of the RNAzol reageant. The homogenate was then poured into Eppendorf tubes. DNA and proteins were removed by the addition of one-tenth volume chloroform (120 uL). Each tube was vortexed for fifteen seconds, then placed on ice for fifteen minutes. The tubes were then spun in a microcentrifuge at 14,000 RPM for 15 minutes at 4° C, which generated two phases. The top aqueous layer contained the RNA and was transferred to new tubes while the bottom layer containing DNA and protein was discarded. The volume of the top layer was typically 1.2 mL. We then added one-half volume of isopropanol (600 uL). This mixture was left overnight at -70° C to precipitate the ribonucleic acid. The next morning, the tubes were centrifuged as described above and the supernatants were discarded. The pellets were air-dried, then resuspended in diethylpyrocarbonate (DEPC)-treated water (typically about 100 uL). One-tenth volume of 5M NaCl (10 uL), and then three volumes of 100% ethanol (330 uL) were added to the resuspended RNA. The tubes were again left for at least one hour at -70° C to reprecipitate the RNA. The supernatant was discarded after spinning the RNA precipitate down for fifteen minutes in a microcentrifuge at the conditions described above; the pellets were then air-dried. The RNA pellets were once again resuspended in

DEPC-treated water (typically about 50 - 150 uL). Concentrations and relative purity of the RNA samples were determined by reading the samples in the spectrophotometer at 260 and 280 nanometers. The RNA concentration was the reading at A_{260} X 40 X dilution factor. The A_{260}/A_{280} ratios for relatively pure samples were between 1.6 and 1.8 for tomcod RNA and between 1.8 and 2.0 for hogchoker RNA samples.

Gel Electrophoresis and Northern Blotting: Approximately 10 µg of total RNA was added onto each lane of the gel. Before loading, RNA samples were denatured in a solution of glyoxal (13.8% by volume of total denaturing mixture), 0.1M NaH_2PO_4 (to a final concentration of 10.3 mM), and dimethylsulfoxide (41.8% by volume). The tubes were incubated in a water bath at 50° C for one hour to denature the RNA. In the mean time, a 1% agarose gel made in 10 mM NaH_2PO_4 buffer was prepared. To each glyoxylated RNA sample, a volume of loading buffer and tracking dye equivalent to the original volume of RNA used for the sample was added. Each sample was loaded into a well in the gel. The gel was typically run at about 21 volts overnight with a pump to recirculate the 10 mM NaH_2PO_4 buffer. The separated RNA samples were then transferred by northern blotting onto ZetaBind filters overnight in 20 X SSC buffer. After northern blotting, the filter was washed in 6 X SSC and then UV Stratalinked to fix the RNA to the filter. The tracking dye was removed from the filter by incubation for 30 min in agitating 0.1 X SSC + 0.1% SDS solution at 65° C.

Prehybridization: Before hydribidization, filters were initially incubated in 6 X SSC buffer for five minutes at room temperature. A prehybridization solution of 6.0 mL double-distilled water, 4.5 mL 20 X SSC, 0.75 mL 1M NaH_2PO_4, 3.0 mL 50X Denhardt's solution, and 0.15 gm glycine was heated to 65° C in a water bath. One mL of sheared, boiled salmon sperm DNA was added to this solution before pouring the entire mixture into a plastic seal-a-meal bag containing the soaked filter. Air bubbles were removed from the bag as much as possible, and then the bag was sealed using a heat sealer. The sealed bag was incubated in the 65° C bath for at least three hours. During this time, a hybridization solution was prepared (see below). At the end of the three hours, the prehybridization solution was taken out of the bag by cutting a hole in the edge of the bag and squeezing out the liquid.

Hybridization and Autoradiography: Each filter was hybridized to a nick-translated ^{32}P-labeled P450IA cDNA probe isolated from 3-methylcholanthrene-induced rainbow trout (Haasch et al, 1989). The nick-translated radioactive cDNA probe was added to a 0.8 X TE buffer solution behind a radioactivity shield and boiled for five minutes. This boiled probe solution was then added to a 65_0 C hybridization solution containing: 9 mL double-distilled water, 4.5 mL of 20 X SSC, 1.2 mL IM NaH_2PO_4, 0.6 mL 50 X Denhardt's solution and 1.5 gm of dextran sulfate (fully dissolved before the probe was added). This whole mixture was then swirled and quickly added to the bag containing the filter with a 10 mL serological pipet. Air bubbles were removed as much as possible and the bag was resealed with a heat sealer and was put into another plastic bag to prevent leakage of radioactive material. The filter was hybridized at 65° C overnight. In the morning, the radioactive solution was removed and placed in a radioactive waste container. The filter was rinsed three times (5 min each) in 2 X SSC + 0.1 % SDS at room temperature; the rinse solution was discarded in a radioactive waste container. The filter was then washed in a final wash of 0.5 X SSC + 0.1% SDS for thirty minutes at 65° C. The final wash was removed and the filter was air-dried and checked for counts. The dried filter was wrapped in plastic wrap and placed in a cassette with Cronex Lightning Plus intensifying screens (DuPont). Kodak XAR film

was placed in the cassette and the cassette was put up for exposure at -70° C for 2-6 days.

Autoradiograms of each filter were analyzed for strength of signal using a laser densitometer (LKB Instruments). The area underneath the curve in the densitometer reading directly relates to the strength of the signal, and therefore the amount of P450IA mRNA present. Therefore, we quantified levels of P450IA mRNA in each lane of the autoradiograph by densitometry.

Each filter was subsequently stripped of the P450IA probe by soaking in boiling double-distilled water for about a half-hour. Each filter was rehybridized with a radioactively-labelled cDNA actin probe isolated from carp. The actin probe was used to ensure that equal amounts of mRNA were initially added to each lane in the gel. Levels of P450IA mRNA were expressed by unit of total mRNA. Densitometer readings of the actin mRNA and P450IA mRNA from different filters were standardized according to an internal standard which was present on each filter. The P450IA/actin mRNA ratios for each sample were then analyzed statistically to detect any P450IA mRNA level significantly higher than basal expression levels.

RESULTS

PAHs: Tomcod injected with ß-naphthoflavone expressed higher levels of P450IA mRNA than depurated tomcod or tomcod injected with corn oil. This agrees with results obtained previously by this lab. Induction of expression of P450IA mRNA was statistically significant at 26 hours after injection of the chemical at a confidence level of 95%. The average value for the ß-NF-26 hour group was 1.6 (n=4), compared to a mean value of 0.10 (n=11) for the negative controls. In addition, there was a significant increase in expression between eight hours post-injection and twenty-six hours after injection of ß-NF. The mean P450/actin ratio for the ß-NF-8 hour group was 0.16 (n=4) compared to the previously stated value of 1.61 for the ß-NF-26 hour group. Figure 1 compares the mean levels of expression between the various treatment groups. Figure 3 shows the autoradiogram of the filter hybridized with P450IA cDNA probe from trout.

Analysis of another group of sixteen tomcod injected with ß-naphthoflavone showed that peak expression of P450IA mRNA occurred at 5 days after injection. No difference was seen in P450IA mRNA levels between depurated tomcod and depurated tomcod intraperitoneally injected with corn oil. Expression increased tenfold between the injection time and 5 days, and then significantly decreased between 5 and 7 days (Figures 2 and 4). The average level of P450IA/actin expression for the 5 day period was 1.6 (n=3), whereas the average P450IA/actin ratio for the depurated tomcod and depurated tomcod injected with corn oil was 0.08 (n=4). Therefore, peak induction at day 5 was approximately 20 fold. P450IA/actin ratios for depurated tomcod and tomcod injected with corn oil were combined as

negative controls, since the average values for each group (0.08 and 0.06 respectively) did not differ significantly at a confidence level of 95%. Statistical analysis was conducted using the Dunnett's t ($t_{0.025}$=2.84) at a confidence level of 95%.

PCB Congeners and Aroclor 1254: Neither PCB congener elicited a significant response. P450IA/actin ratios were approximately zero for both congener-injected fish and negative controls. For example, the average P450IA/actin ratio for negative controls was 0.08 (n=4), whereas the average P450IA/actin ratio for 3,3',4,4' tetrachlorinated biphenyl was 0.10 (n=7), not a significant difference at a confidence level of 95% ($t_{0.025}$=2.48). The average P450IA/actin ratio for 2,3,3'4,4' polychlorinated biphenyl was 0.07 (n=4), again, not significantly different from that of the negative controls.

Aroclor 1254 did induce a significant increase in P450IA mRNA expression 3 days post-injection. There was about a thirteen-fold increase in P450IA mRNA expression between the negative controls and the 3-day Arochor samples, for $t_{0.025}$ = 2.94. There was also a significant six-fold decrease in expression between 3 days and 5 days (see Figure 5). Expression from 7 to 19 days post-injection was not significantly higher than the negative controls (see Figure 7). In addition, there were several polymorphisms detected in some of the gel lanes from these Aroclor-treated fish (see Figure 8). The polymorphisms were seen as bands which hybridized with the P450IA probe, but were located a small distance away from the location of most of the P450IA bands. This indicates a slight variation in mRNA length and/or base sequence in the P450IA mRNA.

Because ß-naphthoflavone, a polycyclic aromatic hydrocarbon, induced a strong response in the tomcod, another group of depurated tomcod were exposed to natural Hudson River sediments from Foundry Cove presumed contaminated with several pollutants. It is suspected that besides heavy metal contamination, there may be some PCB/PAH contamination in this sediment, so we were interested in investigating the usefulness of the P450IA mRNA induction in tomcod as a sentinel of environmental condition. Statistically, the contents of the Foundry Cove sediments did not significantly cause induction of P450IA mRNA expression in tomcod at the 95% confidence level. However, five of the eleven fish did express a very high expression level while the other six did not display any remarkable P450IA mRNA expression (see Figure 9). The magnitude of the P450IA/actin

ratio for these five tomcod, with a mean of 0.10, is a ten-fold increase over that of the negative controls, which had a mean of 0.01 (n=1). Further experiments may demonstrate a significant level of expression of P450IA mRNA expression from tomcod in this region of the Hudson River.

Hogchoker share the same ecological niche with tomcod, and so may be exposed to the same environmental contaminants. However, hogchoker do not display the elevated liver cancer rates seen in tomcod from the Hudson. In addition, we found that P450IA mRNA expression was not induced in hogchokers by ß-naphthoflavone - a substance which induced a significant response in tomcod. At a confidence level of 95% ($t_{0.025}$ = 2.78), none of the P450IA/actin ratios of the hogchoker were significant after exposure to ß-NF for one day (see figures 10 and 11).

DISCUSSION

The fact that a PAH induces an elevated P450IA mRNA response in tomcod, a fish which displays high liver cancer rates in the Hudson River, but does not induce the same response in another species which does not have high liver cancer, suggests a relation between the P450IA mRNA expression and liver cancer. It has already been found that tomcod liver tumor DNAs show activation of the oncogene K-*ras*, a finding frequently related to the presence of cancer. It is also known that the P450 enzyme(s) are involved in carcinogenic activation of several compounds, such as polycyclic aromatic hydrocarbons (PAH's). Elevated P450IA mRNA expression caused by exposure to contaminants probably corresponds to elevated levels of the P450IA enzymes. This remains to be seen. However, it does appear that there is a definite relation between exposure to polycyclic aromatic hydrocarbons and possibly polychlorinated biphenyls in tomcod and P450IA mRNA expression, as demonstrated in this experiment and others.

Our results with ß-naphthoflavone - injected tomcod agree with those found by Haasch, et al. (1989) in rainbow trout injected with ß-naphthoflavone in that this polycyclic aromatic hydrocarbon induces P450IA mRNA in both species. However, the time course for induction differs between the two species. Our results showed peak expression five days after injection of ß-NF into tomcod, with the levels returning almost to basal levels seven days after treatment; the rainbow trout in the study by Haasch, et al. showed peak expression eighteen hours after treatment, with a return to basal levels by forty-eight hours post-injection. The same cDNA

probe was used in both experiments. Thus, it appears that there is some difference among species in the kinetics of P450IA mRNA induction and depuration.

In a study by Gooch, et al. (1989), the PCB congener 3,3'4,4'-tetrachlorobiphenyl (TCB+) was found to induce P450IA mRNA, as well as EROD activity and P450E protein, in scup. Their study also found that the congener 2,3,3'4,4'-pentachlorobiphenyl (PCB-) did not induce this response. Our study found that neither of these congeners caused an induction of P450IA mRNA in tomcod. The difference in results between the two studies suggests that there are variations between species in how the animal deals with polychlorinated biphenyls. This conclusion is also supported by our finding that P450IA mRNA was induced in tomcod injected with ß-NF while there was no such induction in hogchoker.

The probable induction of P450IA mRNA expression in tomcod by an actual contaminated sediment (Foundry Cove) demonstrates that P450IA mRNA induction in tomcod may be very useful as a sentinel of ecological perturbation of the Hudson River. The detection of elevated P450IA mRNA is very sensitive and occurs quickly once exposure occurs.

Aroclor mixtures may contain very small amounts of polycyclic aromatic hydrocarbons. However, they are primarily composed of non-coplanar PCB's. The induction of P450IA mRNA expression by Aroclor 1254 may indicate that the small amount of PAH in the PCB mixture can induce the response; or it may show that non-coplanar compounds may cause this reaction also. This was not supported by our results, however.

CONCLUSIONS

These results suggest several future experiments. First of all, it remains to be seen if the elevated P450IA mRNA levels in the tomcod translate to elevated levels of the P450IA enzyme.s Second, the livers used for RNA isolation need to be checked for the presence of PCB's. The polymorphisms observed in several of the tomcod also present another interesting facet for further research since it is entirely possible that PCB's may cause mutations in P450 DNA resulting in slightly different P450 mRNA, which may in turn code for different P450 proteins.

ACKNOWLEDGEMENTS

I wish to thank John Waldman and Betsy Blair for making this research possible. I would also like to thank Dr. Ike Wirgin and Dr. Guat-Lian Kreamer for invaluable assistance, advice, and direction. And thanks to the tomcod and hogchokers for donating their livers, even if they weren't quite done with them yet.

FIGURE 1

	Autoradiogram JB8: BNF 4h - 26h					
		correction factor P450		2.54		
		correction factor actin		1.6		
		1	2	3	4	5
treatment	sample	JB8/P	JB8/A	corrected JB8/P	corrected JB8/A	B8P/JB8A
pos. control	147	2.23	1.17	5.66	1.87	3.03
corn oil control	204	0.06	0.92	0.14	1.47	0.10
corn oil control	205	0.05	0.39	0.13	0.63	0.20
corn oil control	210	0.20	2.03	0.51	3.24	0.16
corn oil control	211	0.12	2.56	0.31	4.09	0.08
corn oil control	212	0.07	1.17	0.18	1.88	0.09
corn oil control	217	0.11	1.11	0.28	1.78	0.16
corn oil control	218	0.06	1.40	0.14	2.24	0.06
corn oil control	219	0.19	2.30	0.49	3.68	0.13
int. std.	87	0.29	0.29	0.73	0.46	1.58
cleaned out	198	0.07	0.73	0.19	1.17	0.16
cleaned out	199	0.03	0.65	0.06	1.04	0.06
cleaned out	200	0.10	1.04	0.25	1.67	0.15
ßNF-4hr	206	0.05	2.22	0.12	3.56	0.03
ßNF-4hr	207	0.10	2.34	0.26	3.74	0.07
ßNF-4hr	208	0.08	1.56	0.20	2.49	0.08
ßNF-4hr	209	0.09	1.56	0.22	2.50	0.09
int. std.	174	0.10	0.64	0.26	1.03	0.25
ßNF-8hr	213	0.12	3.77	0.30	6.03	0.05
ßNF-8hr	214	0.14	0.93	0.35	1.48	0.23
ßNF-8hr	215	0.39	1.60	0.99	2.56	0.39
ßNF-8hr	216	0.04	0.60	0.09	0.95	0.10
ßNF-26hr	220	0.87	0.91	2.20	1.46	1.51
ßNF-26hr	221	1.49	1.45	3.78	2.32'	1.63
ßNF-26hr	222	0.38	1.00	0.96	1.60	0.60
ßNF-26hr	223	1.62	0.67	4.10	1.08	3.82

1 Optical density readings (OD) for levels of P450IA mRNA

2 Optical density readings (OD) for levels of B-actin mRNA

3 P450IA mRNA OD divided by correction factor for P450IA on autoradiograph JB 8 (2.54)

4 Actin mRNA OD divided by correction factor for B-actin on autoradiograph JB 8 (1.6)

5 Corrected P450IA mRNA OD divided by corrected B-actin mRNA OD

FIGURE 2

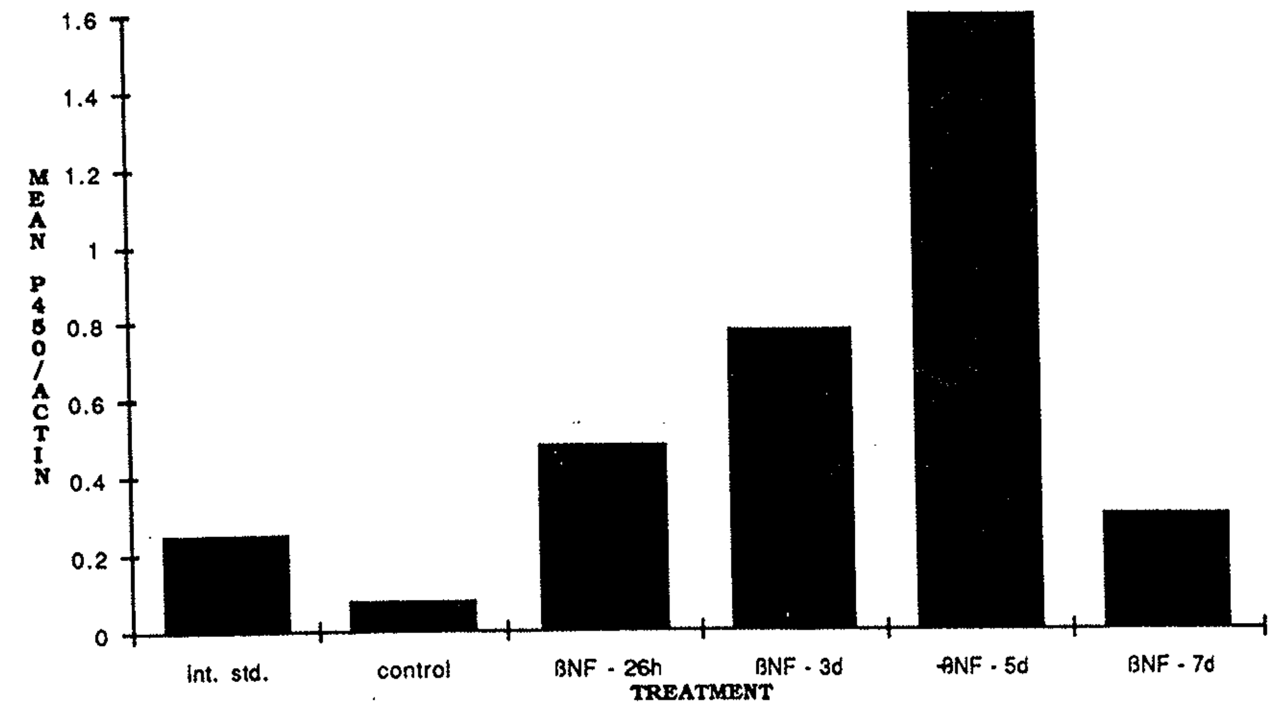


FIGURE 3

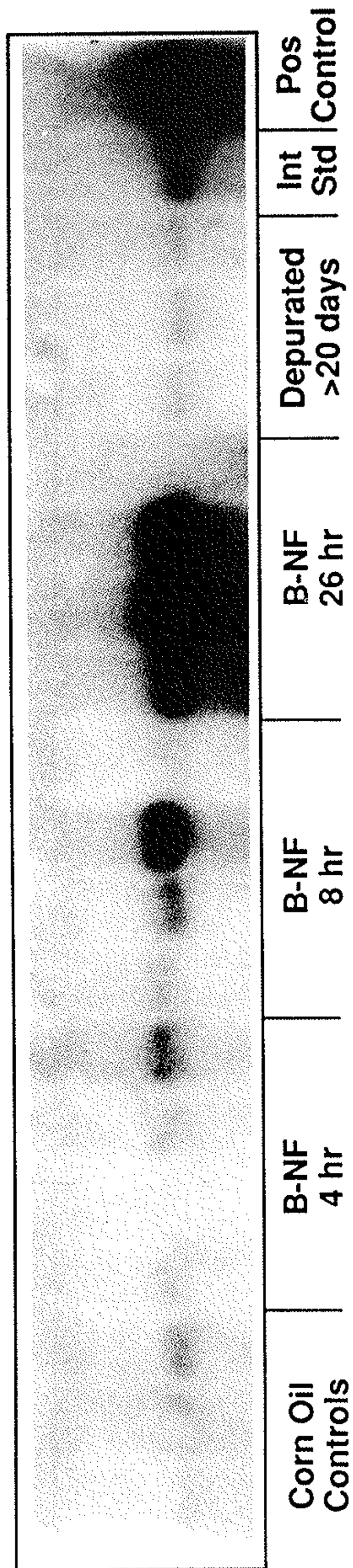


FIGURE 4

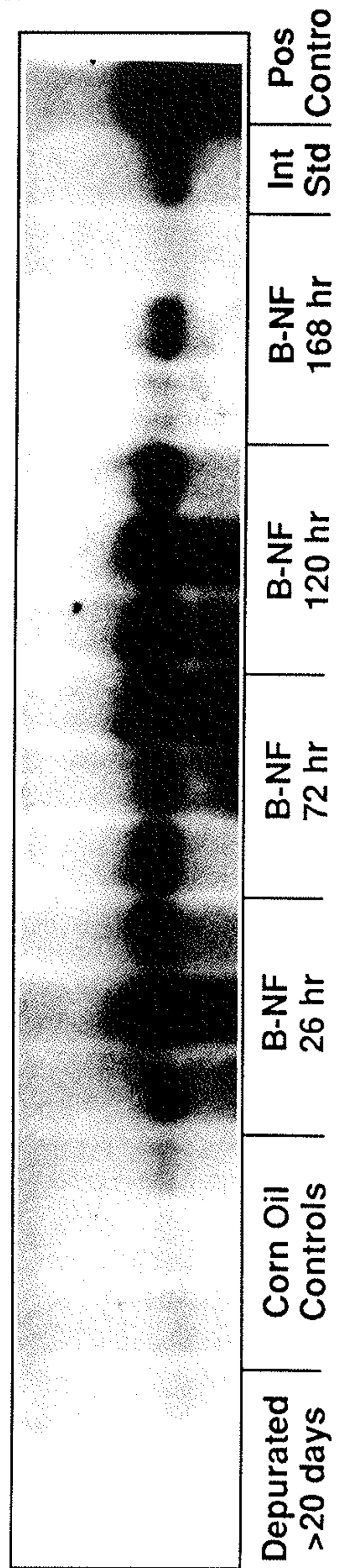


FIGURE 5

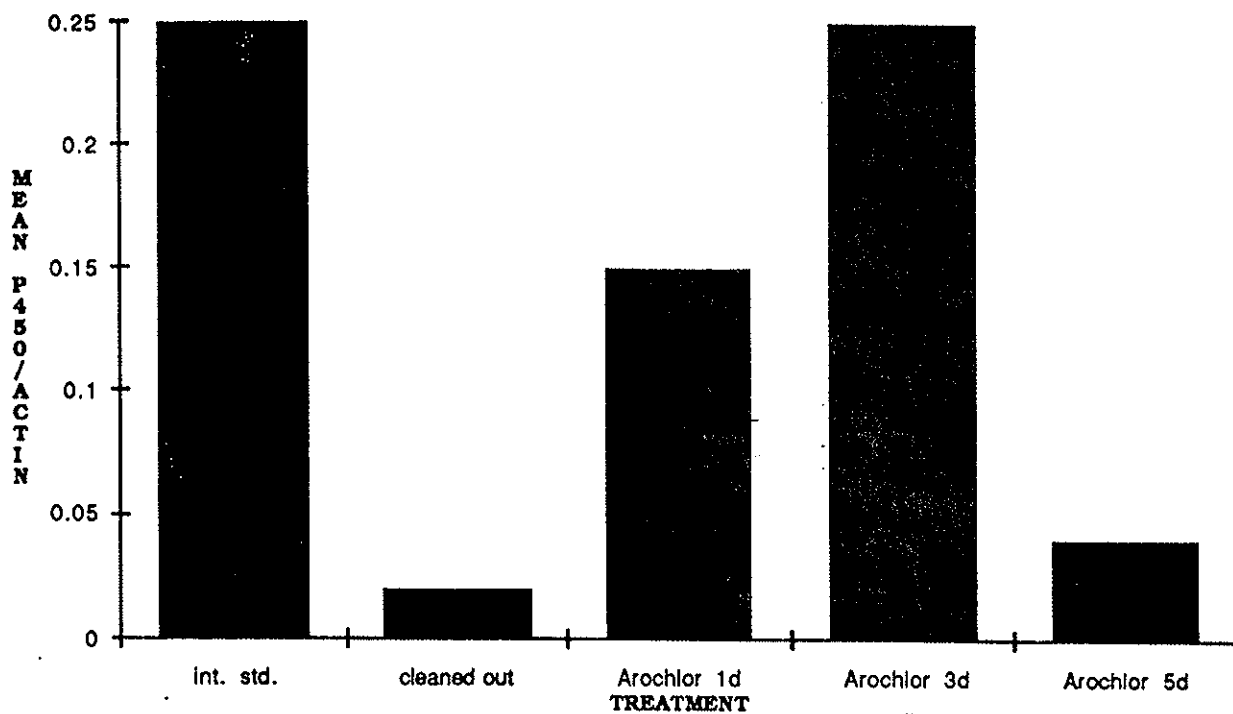


FIGURE 6

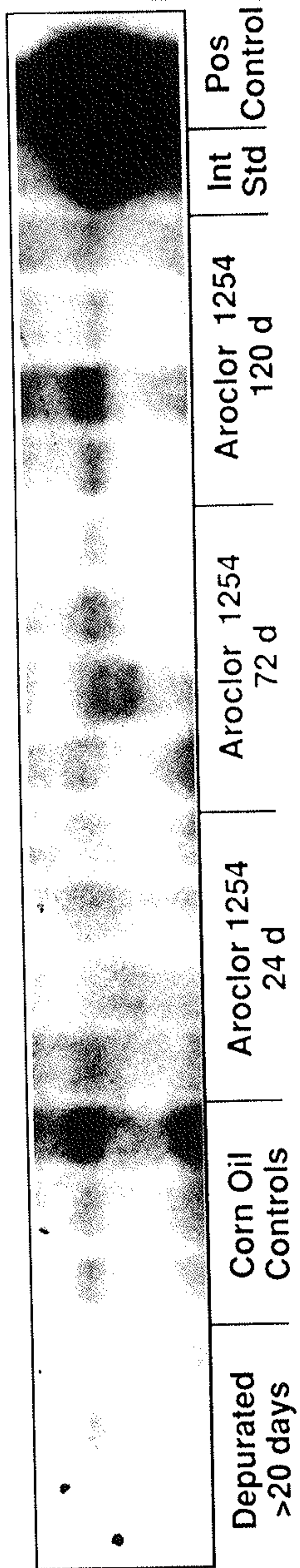


FIGURE 7

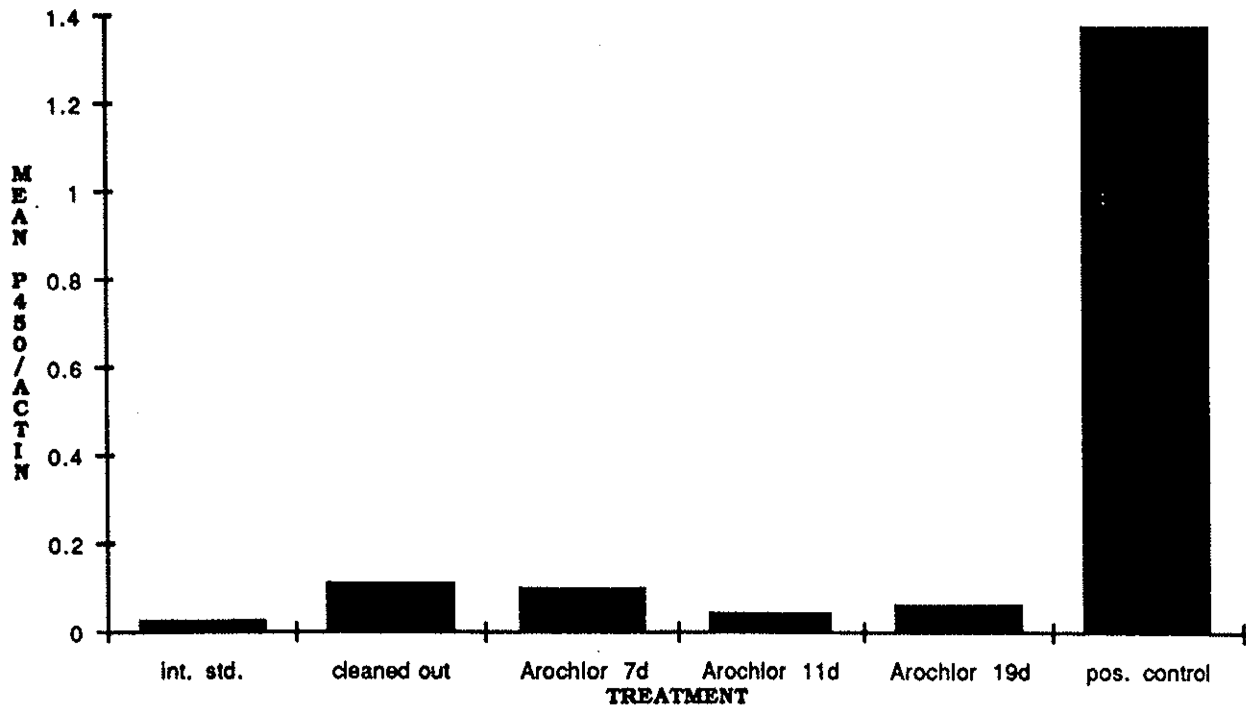


FIGURE 8

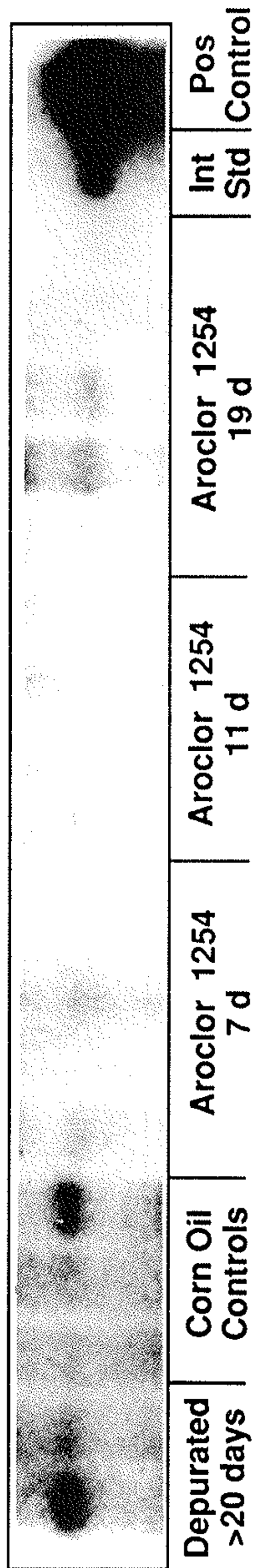


FIGURE 9

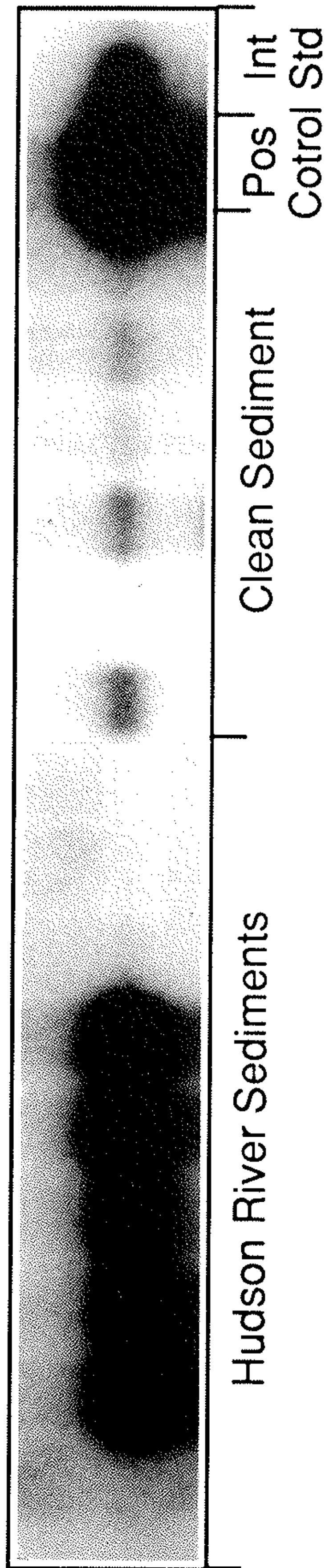


FIGURE 10

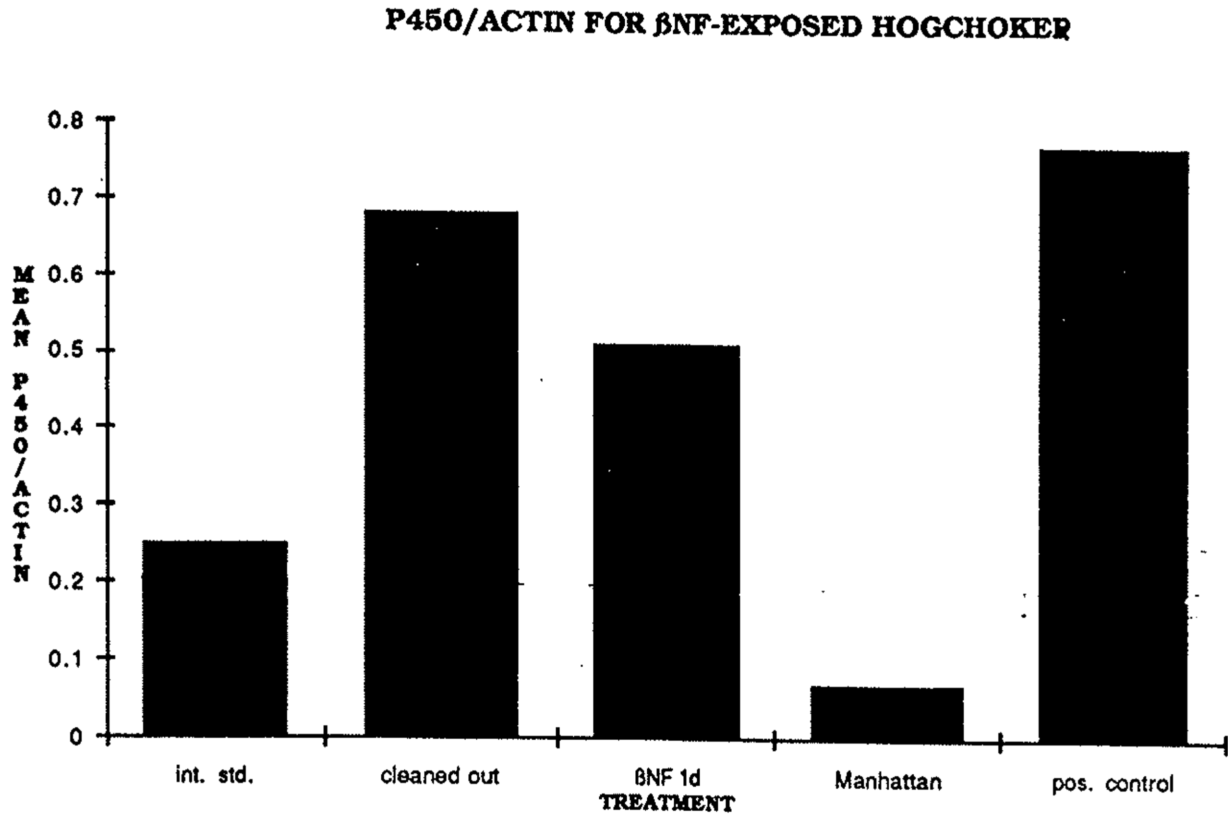
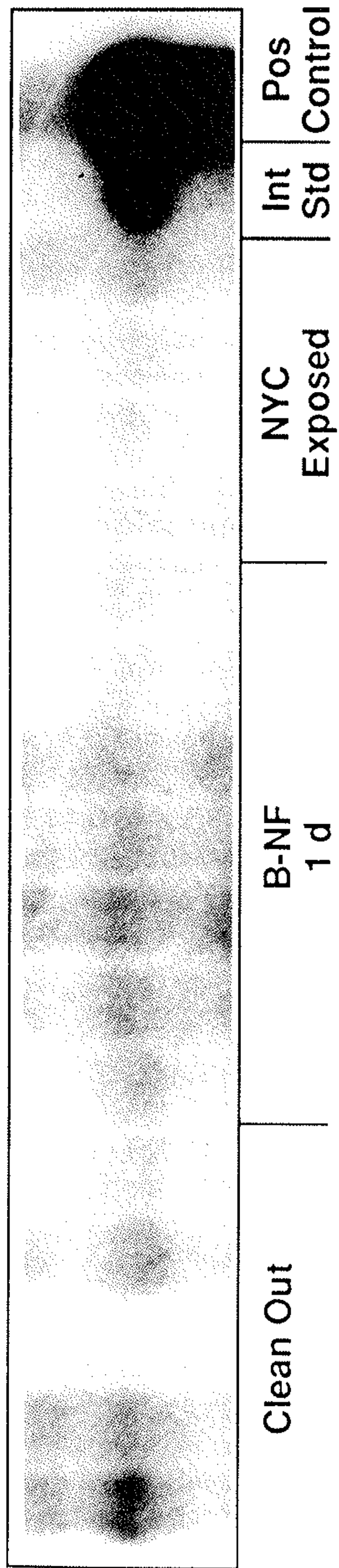


FIGURE 11



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