ACCUMULATION, METABOLISM, AND RELEASE OF BENZO(a)PYRENE FROM SEA WATER BY MEMBERS OF THE GENUS PENAEUS

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

William George Horine

December, 1975

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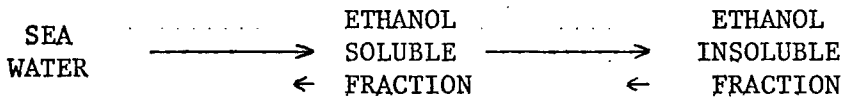
ABSTRACT

The accumulation of the carcinogen benzo(a)pyrene from sea water by member of the genus Penaeus, as well as its release and possible metabolism by the subject animal were studied. Radiolabeling techniques were used in which the concentrations of the tritiated carcinogen-pollutant in the ethanol soluble and ethanol insoluble fractions of the animals were measured. Accumulation occurred rapidly, reaching a concentration at least 150 times the ambient sea water concentration after only 180 minutes of incubation. This uptake was linear and was of a passive nature, probably the result of a partitioning of the benzo(a)pyrene into the nonpolar and lipid fractions of the animals. The accumulation into the ethanol soluble fraction was found to be some two orders of magnitude faster than the accumulation into the ethanol insoluble fraction of the animals.

The release of the compound from the tissues, both ethanol soluble and ethanol insoluble, was linear also, and probably of a passive nature. However, this rate of release from the shrimp was some thirty times slower than the rate of uptake. In fact, after a preincubation of one hour in 10 parts per billion benzo(a)pyrene followed by 24 hours of incubation in clean sea water, the benzo(a)pyrene concentration in the ethanol soluble fraction of the animals was still approximately 10 times original ambient benzo(a)pyrene concentration. In the same study, benzo(a)pyrene was seen to continue to increase in concentration in the ethanol insoluble fraction in the first four hours, thereafter

remaining constant for the duration of the 24 hours release period. The following scheme of benzo(a)pyrene movement was proposed:



Chromatographic studies indicated that less than 1% of the accumulated benzo(a)pyrene was metabolized by the shrimp. The entry of benzo(a)pyrene into both the marine food web at the level of the Penaeid shrimp and into the human diet are discussed.

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INTRODUCTION

INTRODUCTION

There is under way a serious decline in the quality of the marine environment (Blumer, 1970; Blumer et al., 1972). This decline is in large part a result of the entry of petroleum and its derivatives into the waters adjacent to areas of industrial activity, crude oil movement, or heavy population. This entry has been estimated to occur at a rate of 1 million (Blumer et al., 1972) to 3 million (Charter et al., 1973) metric tons per annum.

Petroleum is composed of a surprisingly large number of compounds in an extremely wide range of boiling points and structural configurations. Aliphatic and olefinic hydrocarbons predominate by weight and for the most part are within the metabolic capabilities of most animals. However, they are metabolized at a much slower rate than nutrient compounds such as monosaccharides and amino acids (Blumer, 1970). Many of these compound types and several of those in the class known as aromatics are of such physical characteristics (boiling point, density) that they are readily lost through volatilization to the air. The higher boiling aromatics, on the other hand, are among the most persistent and refractory of all the components of crude oil. This is true not only from the standpoint of biological removal but from one of physical and chemical removal (Andelman and Suess, 1970). The polycyclic aromatic hydrocarbons (PAH), composed of two or more fused benzene rings, have been shown to be ubiquitous and extremely persistent when the number of rings exceeds three (Suess, 1970). Table 1 gives a partial list of

detected sources of PAH. The wide distribution of PAH in the human environment and diet becomes ominous since several of these compounds have been found to be linked to animal carcinogenesis (Miller, 1970).

Two early workers found that repeated application of coal tar to the skin of mice and rabbits resulted in the induction of epitheliomas (Yamagiwa and Ichikawa, 1918). Since that time, the isolation (Cook et al., 1933; Kennaway and Heiger, 1930) and biological testing of the several PAH found in nature has proceeded quickly and fruitfully. Carcinogenesis due to PAH has been demonstrated in mammals, birds, fish, reptiles, and amphibians (Clark and Diamond, 1971; Diamond and Clark, 1970; Miller, 1970; Sims, 1967). Mutagenicity has been noted in Drosophila (Fahmy and Fahmy, 1973). Toxicity of PAH has been studied in the marine copepod, Calanus helgolandicus (Lee et al., 1972) and in mice (Gerarde, 1960). Each study showed a definite lethal effect at concentrations as low as 4 parts per billion (ppb). In an in vitro study, it was found that there was a positive correlation of cytotoxicity with the ability of the cells of man, mouse, and hamster to metabolize benzo(a)pyrene, one of the most potent PAH studied (Gelboin et al., 1968).

Metabolism of PAH, benzo(a)pyrene in particular, has also been shown to be essential to the process of tumor induction (Cookson et al., 1971; Gelboin et al., 1969; Miller, 1970; Schoental, 1964; Cavalieri and Calvin, 1971a; Cavalieri and Calvin, 1971b; Bowden, et al., 1974). This induction is thought to occur during the reaction

TABLE 1

SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS

SOURCES	REFERENCE
Tobacco smoke condensate	A
DL-menthol (cigarette additive) pyrolysis	A
Polluted air	A
Exhaust from gasoline engines	A
Roasted coffee and coffee substitutes	A
Baked bread and biscuits	A
Smoked foods	A
Oranges	A
Commercial wax	A
Marine flora	A
Marine fauna	A
Greenland Cod fish	B
Greenland Mollusc	B
Greenland Mussel	B
Italian Mussel	B
Italian Sardine	B
French Atlantic Oyster	B
French Channel Oyster	B
Alabama Oyster	B
Virginia Oyster	B
California Barnacle	B
Marine algae	B
Surface waters (Europe)	B

References: A- Fishbein et al., 1970
B- Andelman and Suess, 1970

catalyzed by aryl hydrocarbon hydroxylase, a microsomal enzyme (Sims, 1967). This hydroxylation occurs via an epoxide intermediate (Cookson et al., 1971) and usually results in the formation of vicinal hydroxyl moieties. The epoxide formed may be the carcinogenically reactive site, since this functional group has been shown to have cancer-inducing ability (Pederson and Hershberger, 1974).

In the case of metabolically incapable species, while the immediate danger of tumor induction is absent, there remains the possibility of the sequestration of benzo(a)pyrene and other PAH in an unchanged form in the lipid fraction of the subject animal with a subsequent transferral of this hazardous material to the next level of the food chain. This process could be expected to occur since it has been noted that chlorinated pesticides are transferred in this manner (Johnson et al., 1971). In this case, the pesticide is highly insoluble in water. As long as this compound type and others of similar solubility are suspended, there exists a tendency for it to be partitioned into any substance in which its solubility is greater. Biological material, with its high lipid content serves as just such a solvating medium (Butler, 1969; Butler, 1971). In fact, in the adult American oyster, Crassostrea virginica, there exists an almost linear relationship between the percentage of body weight as lipid and the accumulation of petroleum hydrocarbons (Stegeman and Teal, 1971). These authors reported a concentration factor in the animal tissues of three thousand after 49 days of exposure of the animals to contaminated seawater containing No.2 fuel oil at a concentration of 106 ppb. This "biological magnification" results in a sharp increase in contaminant concentration in the

absorbing species and a threat to organisms further up the food chain.

The study of the fate of benzo(a)pyrene and other PAH in the tissues of marine animals has been extensive (Lee et al., 1972; Lee et al., 1973; Corner et al., 1973; Suess, 1970; Pederson and Hershberger, 1974; Anderson et al., 1974). Most of these studies have concentrated on the long term accumulation of petroleum derived hydrocarbons by the adult of the subject species. Characteristically, there is an initial period of rapid uptake which appears to reach an equilibrium only after a considerable time, usually one to several days. In cases where the animal is subsequently placed in clean water, there is a decrease in tissue content of the hydrocarbon, though the loss preceeds much more slowly than did the uptake. This loss usually does not result in the total elimination of the hydrocarbon. This process is similar to one described for chlorinated pesticides (Lowe et al., 1971; Grzenda et al., 1970; Macek et al., 1970). No information is available on the accumulation of this compound into the earlier life stages of marine invertebrates. This type of information is required in order to assess the environmental impact of this compound and others like it on the marine ecosystem and accumulation of this compound into foods for human consumption. It is particularly important to study these earlier life stages since (i) They are more sensitive as compared to the adult forms to any type of toxicant in the environment; (ii) Their larger surface to volume ratio as compared to the adult forms is more

conducive to rapid accumulation of any compound that is available to them; and, (iii) The earlier life stages, which usually exist as zooplankton, represent an extremely large biomass capable of phenomenal levels of accumulation.

The movement of PAH into the diet of man has been the center of much conjecture, the main avenue of entry being the consumption of cured and cooked foods (Bryan and Lower, 1970). The movement of PAH into marine animals which are eaten by man from the water environment, where it is apparently ubiquitous (see Table 1), has been little investigated. Thus, the accumulation of benzo(a)pyrene by a commercially important marine species such as the Penaeid shrimp was examined in the present studies.

One of the most commercially important species in the region of the Gulf of Mexico is the brown shrimp, Penaeus aztecus. There are several other Penaeid species of commercial value, though their habitats vary widely. Of greatest importance to this study is the fact that a large portion of the life cycle of Penaeid shrimp is spent in the estuary, the site of greatest contamination of marine waters. For example, the brown shrimp (Penaeus aztecus) spawn several miles out to sea. Shortly after hatching, in the early postlarval stages, the shrimp migrate into the estuaries where the relative safety and abundant food supply provide maximum probability of survival. The young shrimp remain here for 4 to 6 weeks until an overall length of 90 to 100 mm is reached. It is during this period that the greatest

rate of growth is observed and that accumulation of pollutants is potentially greatest. On reaching 100 mm in length, the individuals return to the open sea to continue their maturation process and to reproduce (Cook and Lindner, 1970). The developing Penaeid shrimp, then, are confronted by a contaminant laden environment when they are most likely to absorb and be affected by it.

The purpose of the study undertaken was to determine the rate and amount of accumulation of benzo(a)pyrene by the relatively early developmental stages of several species of Penaeid shrimp, to ascertain how it is handled in their tissues, and to determine whether such compounds are retained to any great extent when the animal has been removed from exposure to the contaminant.

STATEMENT OF PROBLEM

STATEMENT OF PROBLEM

The purpose of this study was to determine the mode and rate of entry, release, and metabolism of a ubiquitous carcinogenic hydrocarbon, benzo(a)pyrene (Figure 1) into the tissues of the commercially important members of the genus Penaeus.

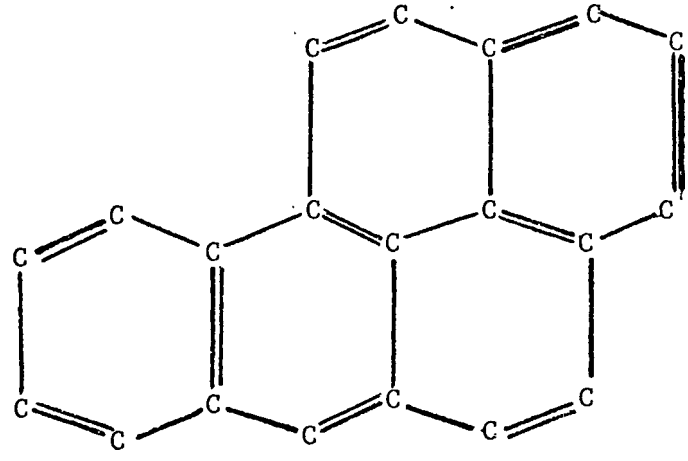


Figure 1: Chemical structure of benzo(a)pyrene

MATERIALS AND METHODS

MATERIALS AND METHODS

Postlarvae of the species Penaeus setiferus, Penaeus stylorostris, and Penaeus vannamei were obtained at various ages (2 days to 56 days) from Ralston Purina Company, Crystal River, Florida. Penaeus californiensis were obtained from the University of Arizona. Uniformly labelled tritiated benzo(a)pyrene was obtained from Amersham Searle at a specific activity of 4-6 mCi/mmol. Unlabelled benzo(a)pyrene was obtained from the Sigma Chemical Company.

Upon receipt, animals were immediately placed in fresh, clean sea water, aerated, and allowed to equilibrate at room temperature for at least 18 hours. If animals maintained longer than 24 hours before use, they were placed in aquaria at a salinity of 28 parts per thousand (o/oo) and at a temperature of 25°C and fed brine shrimp nauplii. Just prior to each experiment, animals were isolated so as to allow each group (flask) of animals to acclimate for at least 4 hours at 28 o/oo and 26°C in a Dubnoff Metabolic Shaker shaking at 80 oscillations per minute. Each group (flask) consisted of a variable number of shrimp (5 to 20) in 25 ml of Instant Ocean artificial sea water contained in a 50 ml Erlenmeyer flask. Initiation of experiments consisted of the addition by Eppendorf pipette (Brinkmann Instruments) of varying volumes of 75% ethanol in which the tritiated and unlabeled benzo(a)pyrene was dissolved. The concentration of radiolabel was constant in all experiments at 0.1μCi/ml ambient sea water. The ambient water concentrations of benzo(a)pyrene were

confirmed by sampling 200 µl of the ambient solutions immediately after initiation and immediately prior to termination and counting of this sample in a Beckman LS - 150 Scintillation counter. Termination consisted of the filtration of the animals from the labelled ambient medium, washing with two 20 ml aliquots of 28 o/oo sea water, and then placing all the animals from one flask into a 28 ml screw cap vial containing 20 ml of 75% ethanol. This marked the beginning of the ethanol extraction period which continued in all cases for 6 days or more in the dark. At the end of this period, 1 ml of ethanol extract was removed for scintillation counting. In early experiments, a second ethanol extraction was made to confirm that at least 95% of all ethanol extractable material was being extracted by the first ethanol extraction. The animals were then removed from the ethanol extract and washed with 25 ml of 75% ethanol, blotted, and weighed. After weighing, the animals from each flask were separated into two approximately equal groups. One-half of the animals from each flask was blotted, weighed, and placed into an aluminum pan. These were dried for 48 hours at 100°C and reweighed. The other half of the animals from each flask were blotted, weighed, and placed in scintillation vials with 1 ml of Soluene 100 (Packard) and solubilized for 3 days in the dark at room temperature. Scintillation fluid (Liquifluor, New England Nuclear) was then added, the vials placed in the dark for an adaption period of 3 days and counted. The compartment that is extractable by ethanol is designated as the

ethanol soluble fraction of the animals while the material remaining was designated the ethanol insoluble fraction. It was determined by total lipid extraction of the animals after the ethanol extraction that at least 99% of the lipid in the animal had been removed by the first ethanol extraction. The lipid extraction was performed according to the procedure of Folch et al. (1957).

With each experiment, groups of 100 animals were filtered, blotted, weighed, dried, and reweighed. A similar group was then filtered, weighed, extracted in ethanol for 3 days, blotted, reweighed, dried, and weighed again. This manipulation gave a good estimate of ethanol extracted wet weight as compared to non-ethanol extracted wet weight, both of which values were used in the calculation of the concentrations of benzo(a) pyrene accumulated by the animal.

Five types of experiments were performed during this study:

(1) Supporting or pilot experiments required to determine a valid and reliable experimental procedure.

(2) Accumulation of benzo(a)pyrene with increasing time of incubation;

(3) Accumulation with increasing ambient concentration of benzo(a)pyrene;

(4) Release of tritiated benzo(a)pyrene into clean sea water with increasing times of depuration (release); and

(5) Chromatography of ethanol extracts and total lipid extracts for determination of metabolic alteration of benzo(a)pyrene.

Pilot experiments performed included studies designed to:

(a) Determine toxicity of benzo(a)pyrene and vehicles used to dissolve it when administered to the several Penaeid species used;

(b) Assess the loss of the highly water insoluble benzo(a)pyrene due to adsorption to the walls of the incubating vessel;

(c) Reveal any interspecific differences in accumulation among the various species used; and

(d) Show any differences in accumulation resulting from differences in animal weight.

Experiments involved with the accumulation of benzo(a)pyrene in relation to increasing times of incubation were initiated and terminated as previously mentioned. The times of incubation in the benzo(a)pyrene containing medium were 1, 3, 10, 30, 90, and 180 minutes.

Experiments in which the ambient concentrations of benzo(a)pyrene were varied were initiated and terminated as previously mentioned. The concentrations used were 2, 4, 8, 10, and 20 parts per billion (ppb).

Release experiments consisted of incubating the animals in a 10 ppb ^{3}H- benzo(a)pyrene contaminated medium for 60 minutes, followed by a transfer of the animals, by filtration, into flasks containing clean seawater. The animals were allowed to remain in this second ambient solution for varying periods of time before termination. The amount of radiolabel remaining in the animals

at the end of the 60 minutes of incubation in the contaminated sea water and at 4, 8, 16, and 24 hours in clean sea water was determined. The amount of radiolabeled benzo(a)pyrene remaining in the animals' tissues on a per gram wet weight basis after any given time increment in the clean sea water indicated the loss of the benzo(a)pyrene contaminant from the animal.

Chromatographic studies were done in order to detect and identify any products of animal alteration of the subject compound. Thin layer chromatography was carried out first according to manufacturer's methods for purifying the benzo(a)pyrene label. This involved the use of n-hexane as the solvent on prepared plastic backed plates coated with Silica gel G in a 250 μ layer (Eastman Kodak). A second series of chromatography studies utilized the method of Schaad (1970) with chloroform as the solvent on the above mentioned plates. Paper chromatography was performed using the method reported by Pietzch (Schaad, 1970). In this case, Whatman No. 1 Chromatography paper was used in a descending technique with methanol: water (9:1) as the solvent system. The volume applied to the origin of the thin layer plate was always 30 μl, the plates having been activated for 30 minutes at 100°C. After development, chromatograms were sequentially cut into 5 mm lengths, each segment being placed in a separate scintillation vial containing Liquifluor. Rf's were calculated using ultraviolet light to visualize the spots and also through the use of scintillation methods. Spots were identified using authentic standards in co-chromatographic techniques.

In all cases, experimental flasks were those that contained animals in the presence of benzo(a)pyrene in the surrounding medium. Control flasks were those that contained animals in the absence of benzo(a)pyrene and in the presence of appropriate concentrations of the dissolving vehicle. The control flasks were used primarily as indices of animal viability.

RESULTS

RESULTS

The pilot studies performed to determine the toxicity of benzo(a)pyrene to members of the genus Penaeus indicated that there exists an LD_{50} for a concentration of 40 parts per billion after 60 minutes of incubation. As a result of this finding, benzo(a)pyrene concentrations were limited to 20 parts per billion. A 95% survival rate was obtained in all control flasks, thus any deaths occurring in the experimental flasks was the result of benzo(a)pyrene toxicity. No significant deaths were noted in either set of flasks.

Studies undertaken to determine the loss of benzo(a)pyrene through adsorption to the flask walls showed that after 12 hours, approximately 25% of the original label administered to a flask containing sea water was lost. In order to minimize this loss, the maximum incubation period was set at 60 minutes in the same medium. Longer incubation times involved a transfer to newly pipetted medium every 60 minutes. The percentage of label lost to adsorption after 60 minutes was less than 1%.

Although there were no two experiments in which the same size animals from two different species were compared with regard to their ability to accumulate benzo(a)pyrene, it is thought that the mechanism of uptake is similar in each case. This may be seen on comparison of Figures 2, 3, and 4. The differences in slope of the curves seen in these experiments are the result of the widely varying sizes of the animals used.

Experiments concerned with the uptake of benzo(a)pyrene with respect to increasing time of incubation showed a linear accumulation into the ethanol soluble fraction with time up to 180 minutes (Figures 2, 3, and 4). This may be seen by comparison of the data points with the regression line calculated from those points. The benzo(a)pyrene concentration of the ambient solution for all time variable experiments was 1.98×10^{-8} M (moles per liter). The greatest accumulation attained of benzo(a)pyrene into the ethanol soluble fraction of the animals was 3×10^{-9} moles per gram wet weight. This indicates that the concentration factor in the tissues of these animals for this particular compound is at least 150 times the ambient concentration. In these studies, no saturation of the uptake mechanism was reached.

The accumulation of benzo(a)pyrene into the ethanol insoluble fraction of the animals with respect to increasing incubation time was also linear (Figures 5, 6, and 7). The total accumulation of benzo(a)pyrene by these species of Penaeid shrimp is shown in Figures 8, 9, and 10. These Figures show the sum of the ethanol soluble and ethanol insoluble accumulation. The great similarity that exists between the ethanol soluble uptake and the total uptake is due to the relatively small uptake into the ethanol insoluble fraction, calculated to be less than 1% of the accumulation into the ethanol soluble fraction.

FIGURE 2

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL SOLUBLE FRACTION WITH TIME

ANIMAL: *Penaeus californiensis*

AVERAGE ANIMAL WEIGHT: .0038 ± .0004 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK ± THE STANDARD ERROR OF THE MEAN

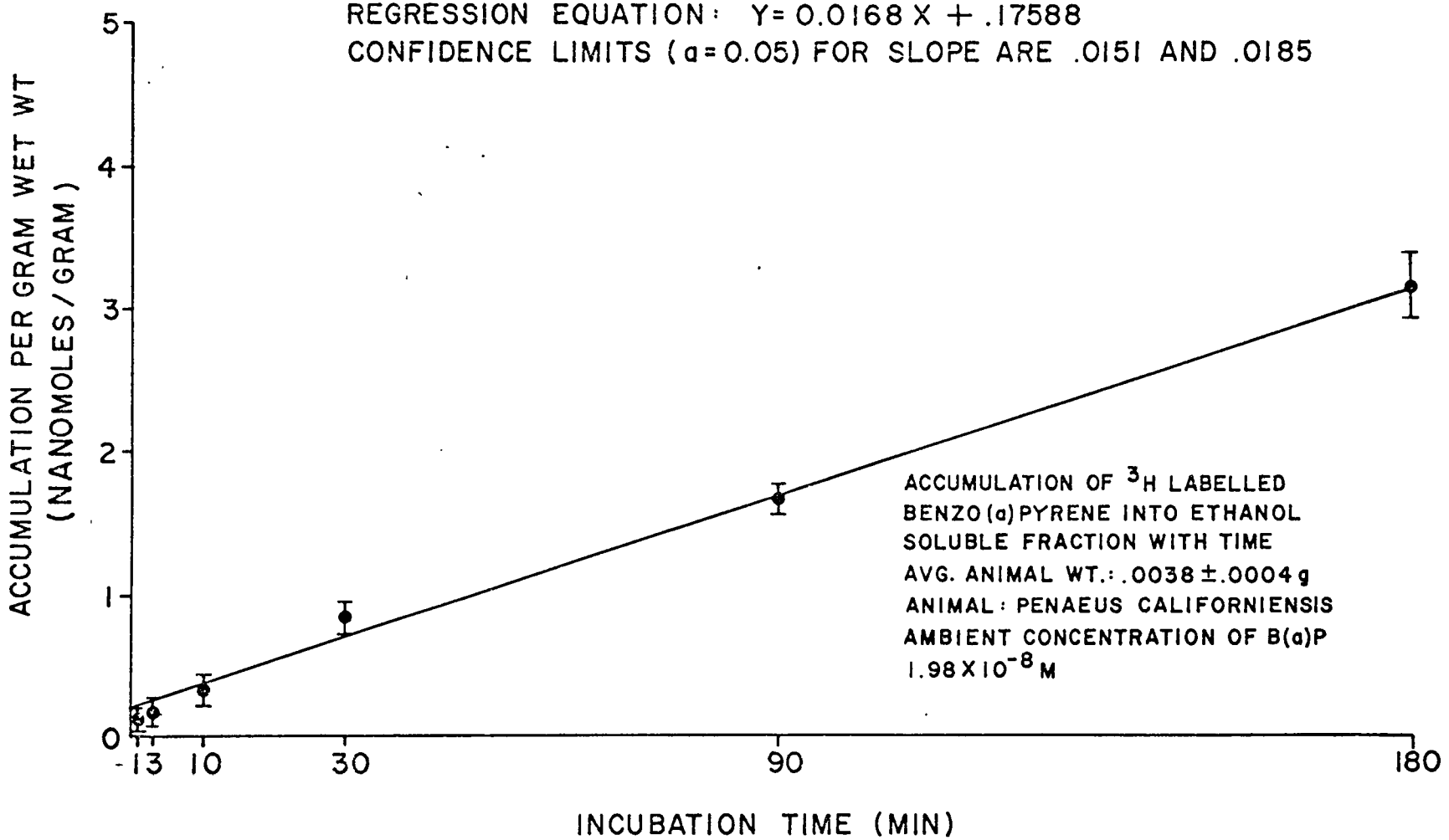

REGRESSION EQUATION: Y= 0.0168 X + .17588
CONFIDENCE LIMITS (α=0.05) FOR SLOPE ARE .0151 AND .0185
ACCUMULATION PER GRAM WET WT (NANOMOLES/GRAM)
0
1
2
3
4
5
-13
10
30
90
180
INCUBATION TIME (MIN)
ACCUMULATION OF ^{3}H LABELLED BENZO(a)PYRENE INTO ETHANOL SOLUBLE FRACTION WITH TIME
AVG. ANIMAL WT.: .0038 ± .0004 g
ANIMAL: PENAEUS CALIFORNIENSIS
AMBIENT CONCENTRATION OF B(a)P
1.98×10^{-8} M

FIGURE 3

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL SOLUBLE FRACTION WITH TIME

ANIMAL: Penaeus setiferus

AVERAGE ANIMAL WEIGHT: .0160 $\pm$ .001 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

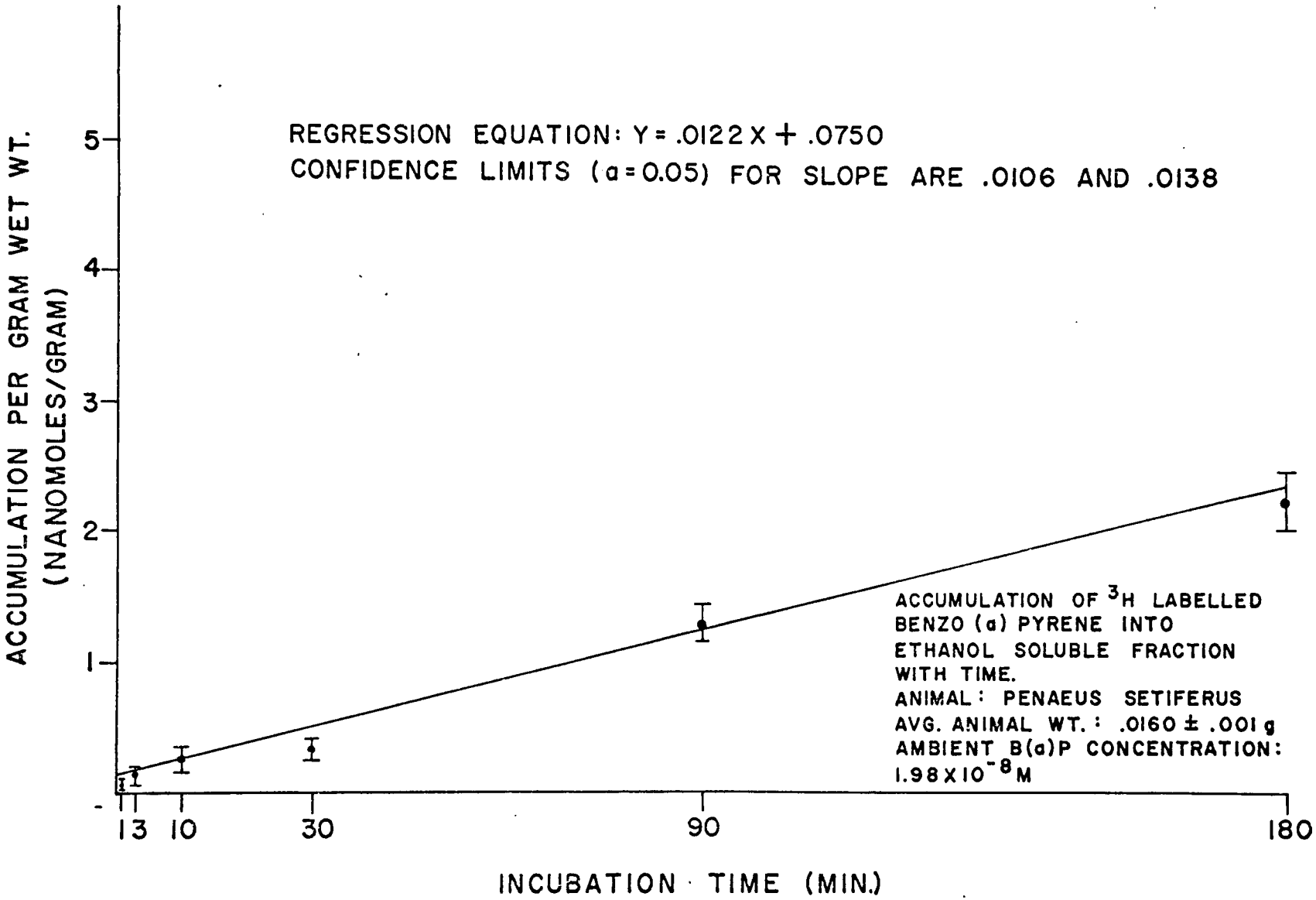

REGRESSION EQUATION: Y = .0122 X + .0750
CONFIDENCE LIMITS (α = 0.05) FOR SLOPE ARE .0106 AND .0138
ACCUMULATION PER GRAM WET WT. (NANOMOLES/GRAM)
5
4
3
2
1
-
13
10
30
90
180
INCUBATION TIME (MIN.)
ACCUMULATION OF ^{3}H LABELLED BENZO (a) PYRENE INTO ETHANOL SOLUBLE FRACTION WITH TIME.
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0160 ± .001 g
AMBIENT B(a)P CONCENTRATION: 1.98×10^{-8} M

FIGURE 4

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL SOLUBLE FRACTION WITH TIME

AVERAGE ANIMAL WEIGHT: .0063 $\pm$ .0001 g

ANIMAL: *Penaeus setiferus*

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

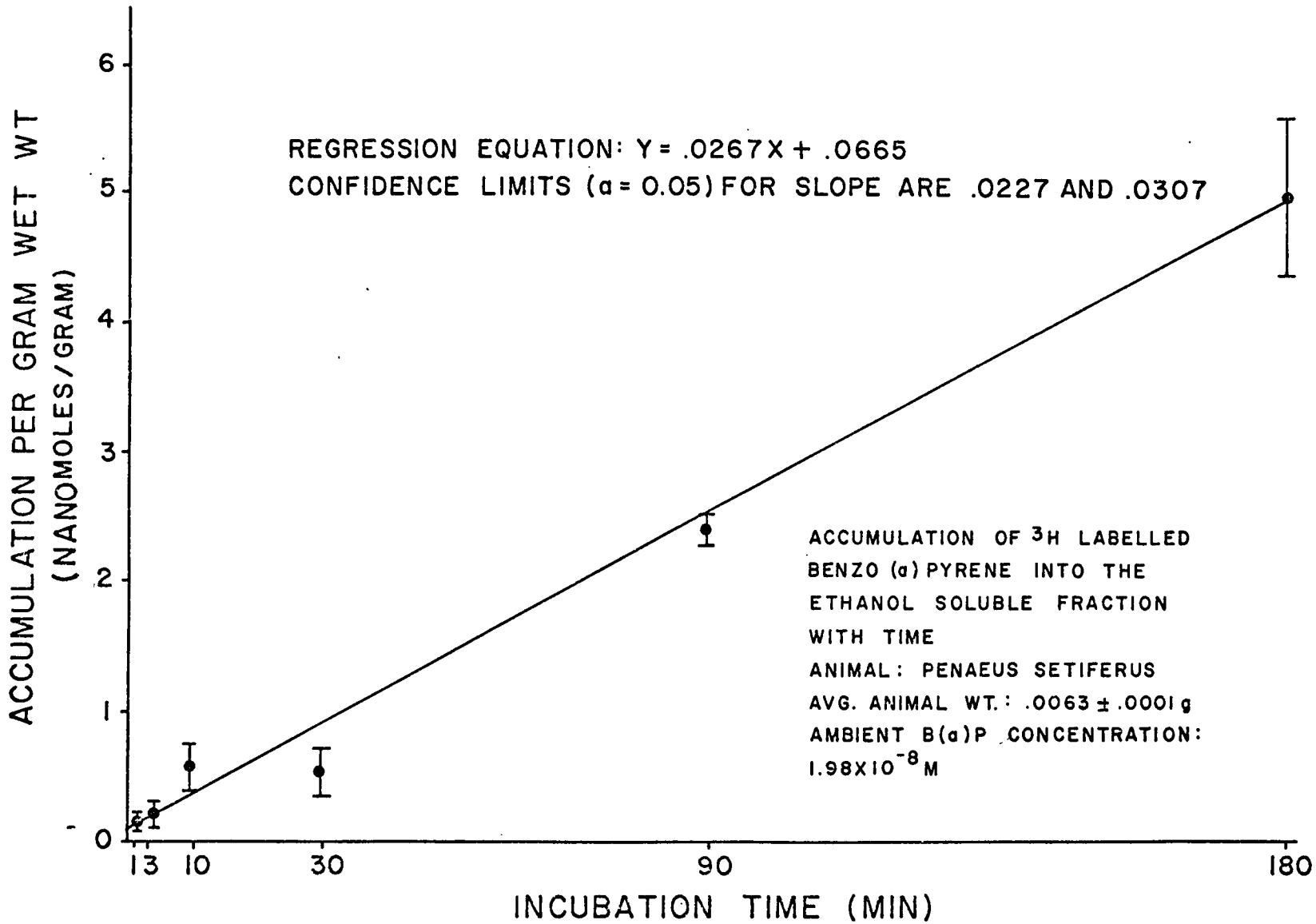

REGRESSION EQUATION: Y = .0267X + .0665
CONFIDENCE LIMITS (α = 0.05) FOR SLOPE ARE .0227 AND .0307
ACCUMULATION OF ^{3}H LABELLED BENZO (a) PYRENE INTO THE ETHANOL SOLUBLE FRACTION WITH TIME
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0063 ± .0001 g
AMBIENT B(a)P CONCENTRATION: 1.98×10^{-8} M
ACCUMULATION PER GRAM WET WT (NANOMOLES/GRAM)
0
1
2
3
4
5
6
13
10
30
90
180
INCUBATION TIME (MIN)

FIGURE 5

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTION WITH TIME.

ANIMAL: Penaeus californiensis

AVERAGE ANIMAL WEIGHT: .0038 $\pm$ .0004 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH OF THE POINTS REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

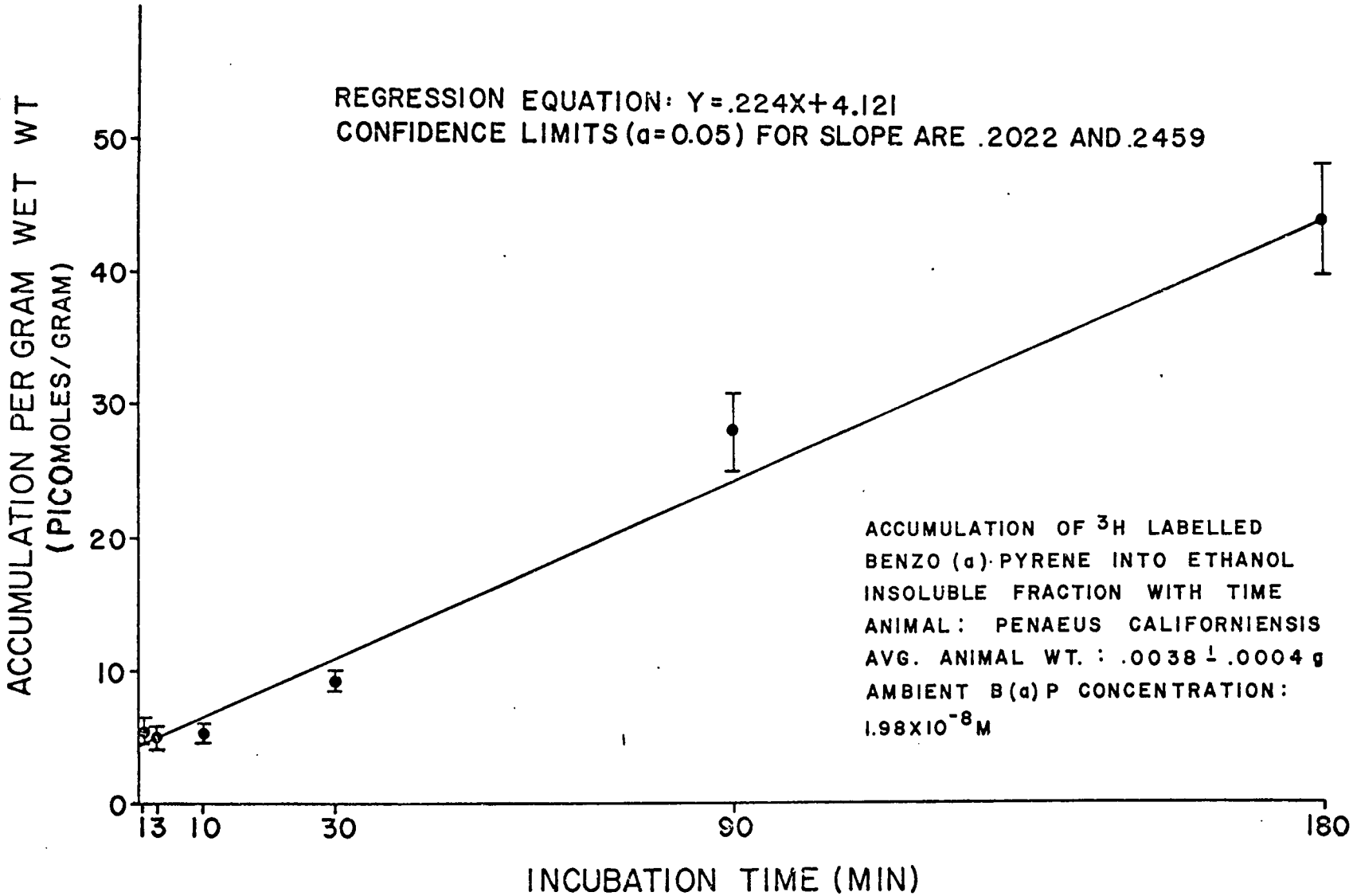

REGRESSION EQUATION: Y=.224X+4.121
CONFIDENCE LIMITS (α=0.05) FOR SLOPE ARE .2022 AND .2459
ACCUMULATION PER GRAM WET WT (PICOMOLES/GRAM)
0
10
20
30
40
50
13
10
30
90
180
INCUBATION TIME (MIN)
ACCUMULATION OF ^{3}H LABELLED BENZO (a) PYRENE INTO ETHANOL INSOLUBLE FRACTION WITH TIME
ANIMAL: PENAEUS CALIFORNIENSIS
AVG. ANIMAL WT. : .0038 ± .0004 g
AMBIENT B(a)P CONCENTRATION: 1.98×10^{-8} M

FIGURE 6

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTION WITH TIME

ANIMAL: *Penaeus setiferus*

AVERAGE ANIMAL WEIGHT: .0160 $\pm$ .001 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

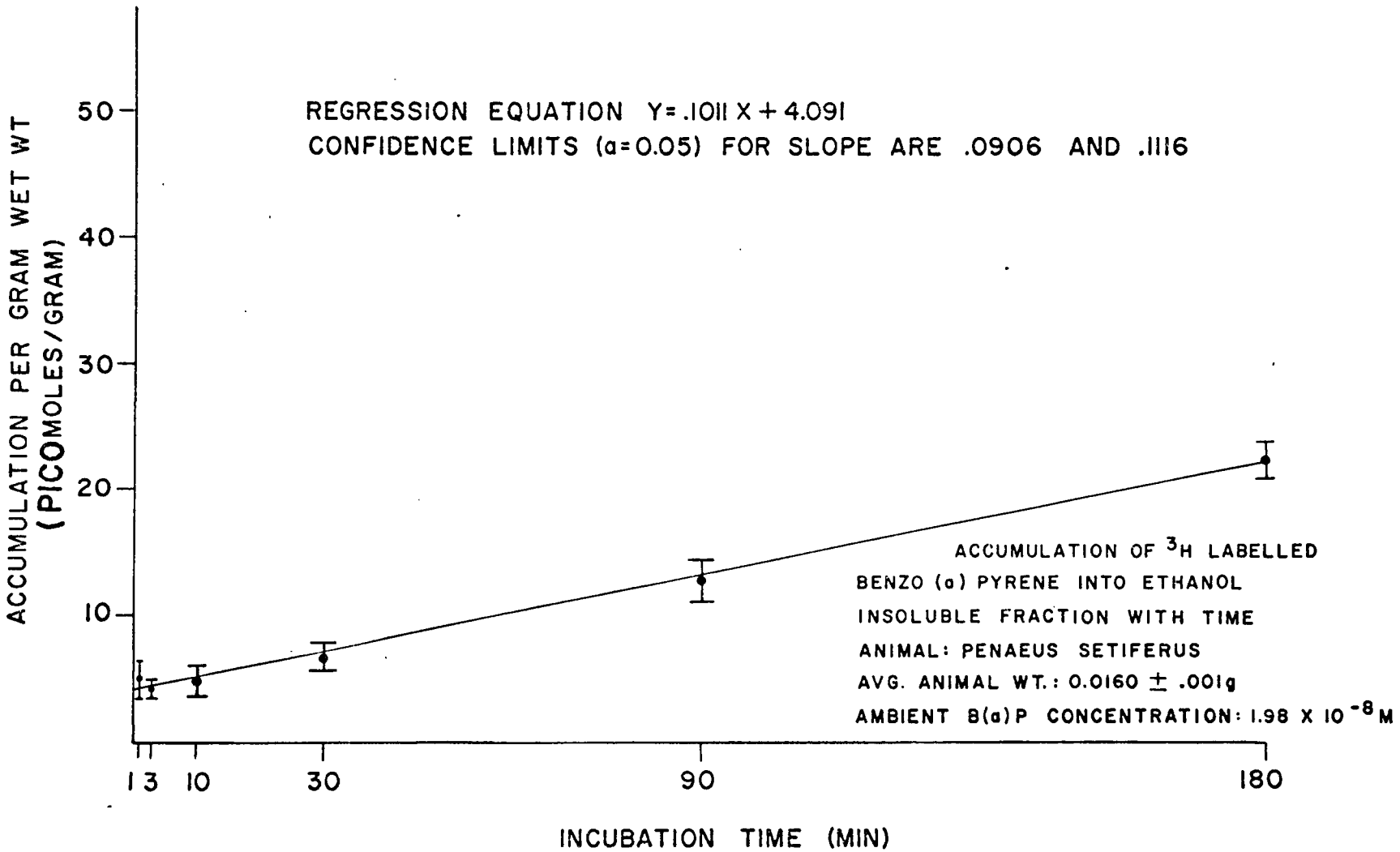

REGRESSION EQUATION Y=.1011 X + 4.091
CONFIDENCE LIMITS (α=0.05) FOR SLOPE ARE .0906 AND .1116
ACCUMULATION PER GRAM WET WT (PICOMOLES/GRAM)
50
40
30
20
10
ACCUMULATION OF ^{3}H LABELLED
BENZO (a) PYRENE INTO ETHANOL
INSOLUBLE FRACTION WITH TIME
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: 0.0160 ± .001g
AMBIENT B(a)P CONCENTRATION: 1.98 X 10^{-8} M
1 3 10 30 90 180
INCUBATION TIME (MIN)

FIGURE 7

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTION WITH TIME

ANIMAL: Penaeus setiferus

AVERAGE ANIMAL WEIGHT: .0063 ± .0001 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK ± THE STANDARD ERROR OF THE MEAN

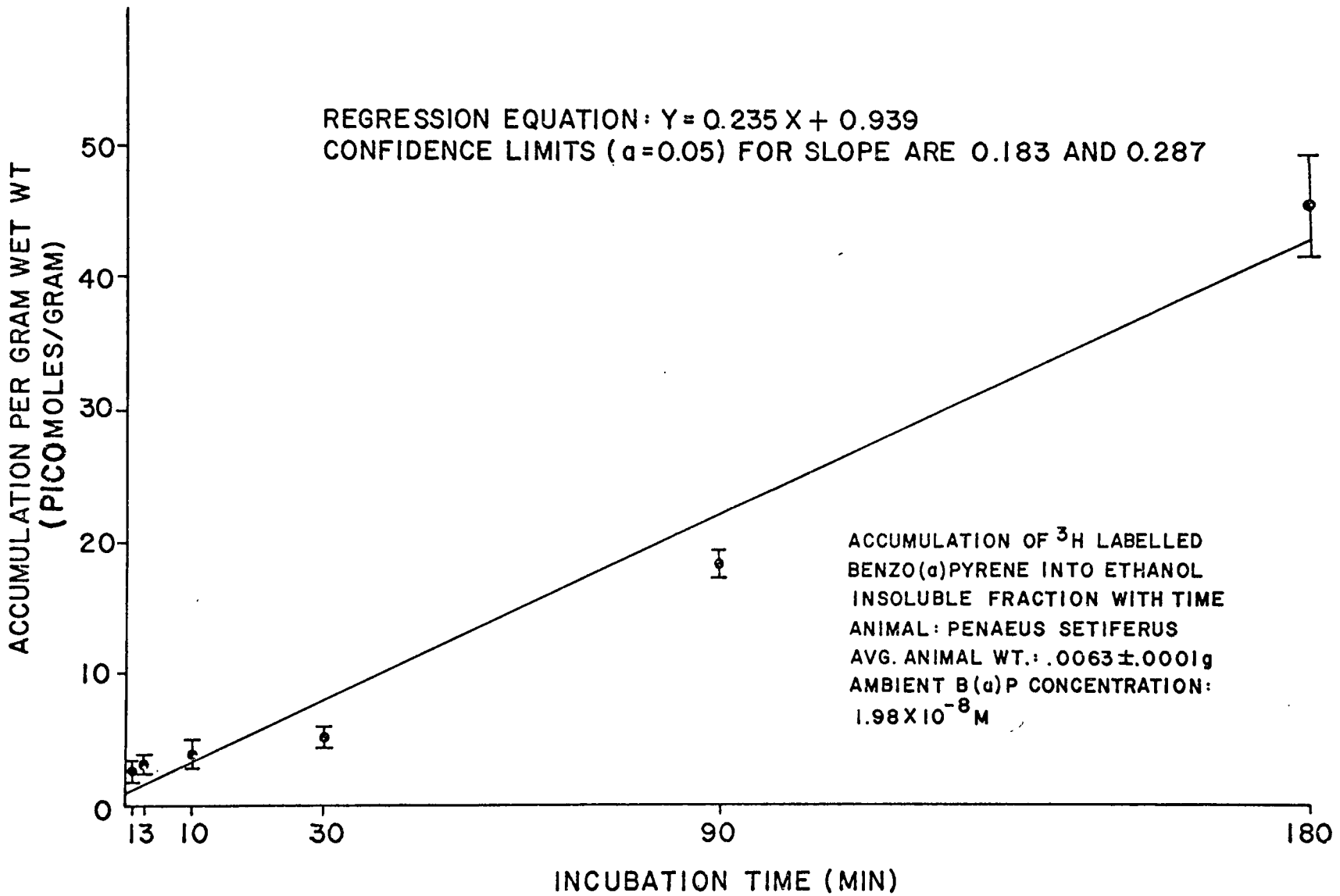

REGRESSION EQUATION: Y = 0.235 X + 0.939
CONFIDENCE LIMITS (α=0.05) FOR SLOPE ARE 0.183 AND 0.287
ACCUMULATION PER GRAM WET WT (PICOMOLES/GRAM)
0
10
20
30
40
50
13
10
30
90
180
INCUBATION TIME (MIN)
ACCUMULATION OF ^{3}H LABELLED BENZO(a)PYRENE INTO ETHANOL INSOLUBLE FRACTION WITH TIME
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0063±.0001g
AMBIENT B(a)P CONCENTRATION: 1.98×10^{-8} M

FIGURE 8

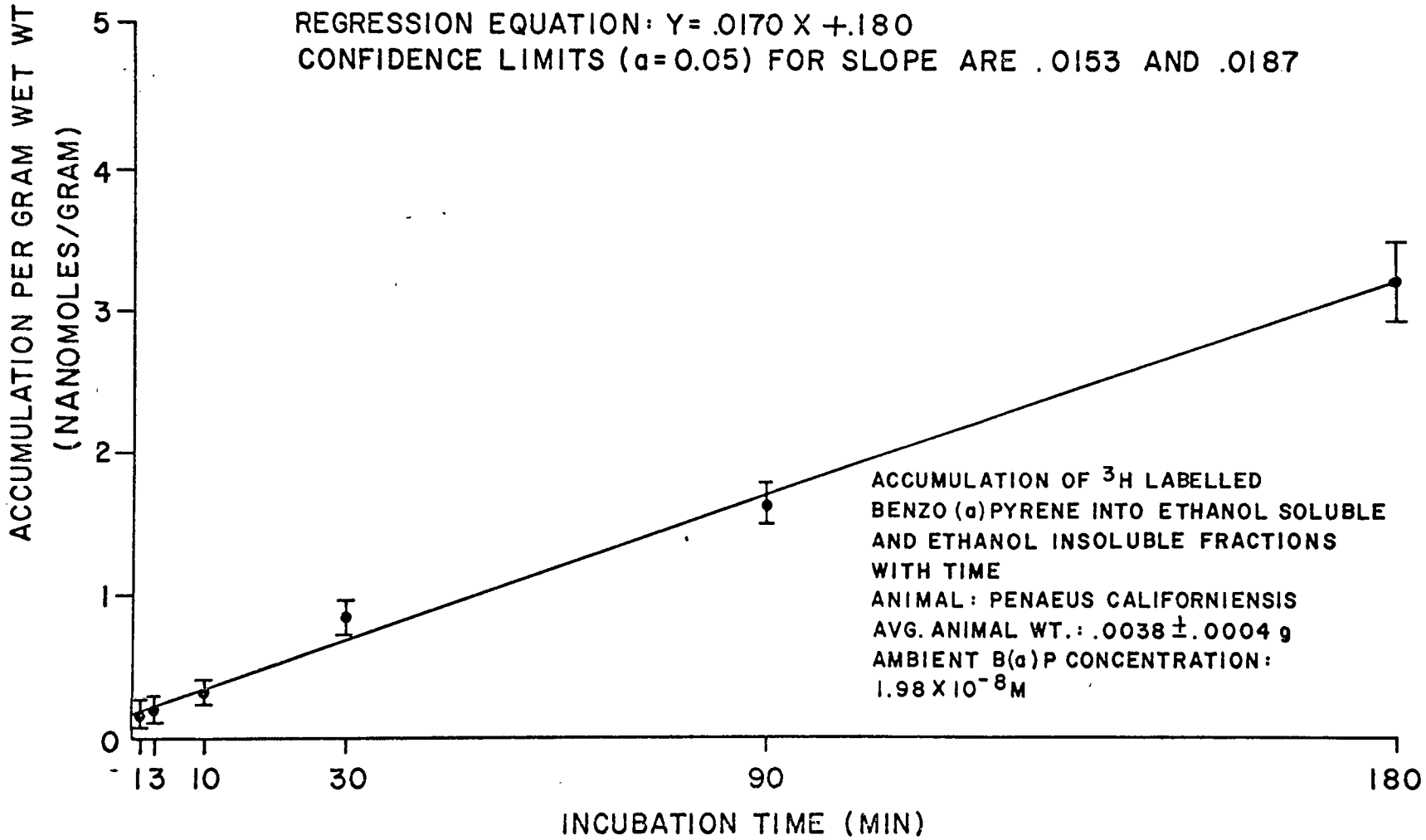
ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTIONS WITH TIME.

ANIMAL: *Penaeus californiensis*

AVERAGE ANIMAL WEIGHT: .0038 ± .0004 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK ± THE STANDARD ERROR OF THE MEAN


REGRESSION EQUATION: Y= .0170 X +.180
CONFIDENCE LIMITS (a=0.05) FOR SLOPE ARE .0153 AND .0187
ACCUMULATION PER GRAM WET WT (NANOMOLES/GRAM)
0
1
2
3
4
5
13
10
30
90
180
INCUBATION TIME (MIN)
ACCUMULATION OF ^{3}H LABELLED BENZO (a) PYRENE INTO ETHANOL SOLUBLE AND ETHANOL INSOLUBLE FRACTIONS WITH TIME
ANIMAL: PENAEUS CALIFORNIENSIS
AVG. ANIMAL WT.: .0038 ± .0004 g
AMBIENT B(a)P CONCENTRATION: 1.98×10^{-8} M

The accumulation of benzo(a)pyrene into the ethanol soluble fraction of the shrimp with respect to a varying ambient concentration of benzo(a)pyrene is shown in Figure 11. Again, the relation appears to be linear upon comparison of the data points with the calculated regression line. No saturation of this mechanism is evident for concentrations up to 20 parts per billion. The accumulation of benzo(a)pyrene into the ethanol insoluble fraction is shown in Figure 12. Figure 13 shows the total accumulation into the ethanol soluble and ethanol insoluble fractions with varying ambient concentration. Here, as with the time variable experiments, the ethanol insoluble accumulation is negligibly small (less than 1%).

Release (depuration) of benzo(a)pyrene from the ethanol soluble fraction was found to begin immediately upon the animals' transfer to uncontaminated sea water and appears to be linear for at least the time period tested (24 hours). Figure 14 shows the data from one such experiment. Attempts to carry this depuration series any further than 24 hours resulted in excessive mortality of the animals (8% at 32 hours). Even at 24 hours, signs of morbidity (disorientation, paralysis) had begun to appear in some experimental groups. Of note in this group of experiments was the fact that the release of the benzo(a)pyrene back into the sea water was markedly slower than its original accumulation. Taking into consideration the accumulation of 1.0 nanomole per gram wet weight in a period of 60 minutes as

FIGURE 9

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTIONS WITH TIME

ANIMAL: Penaeus setiferus

AVEPAGE ANIMAL WEIGHT: .0160 ± .001 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE FOR 6 FLASKS, 10 ANIMALS PER FLASK ± THE STANDARD ERROR OF THE MEAN

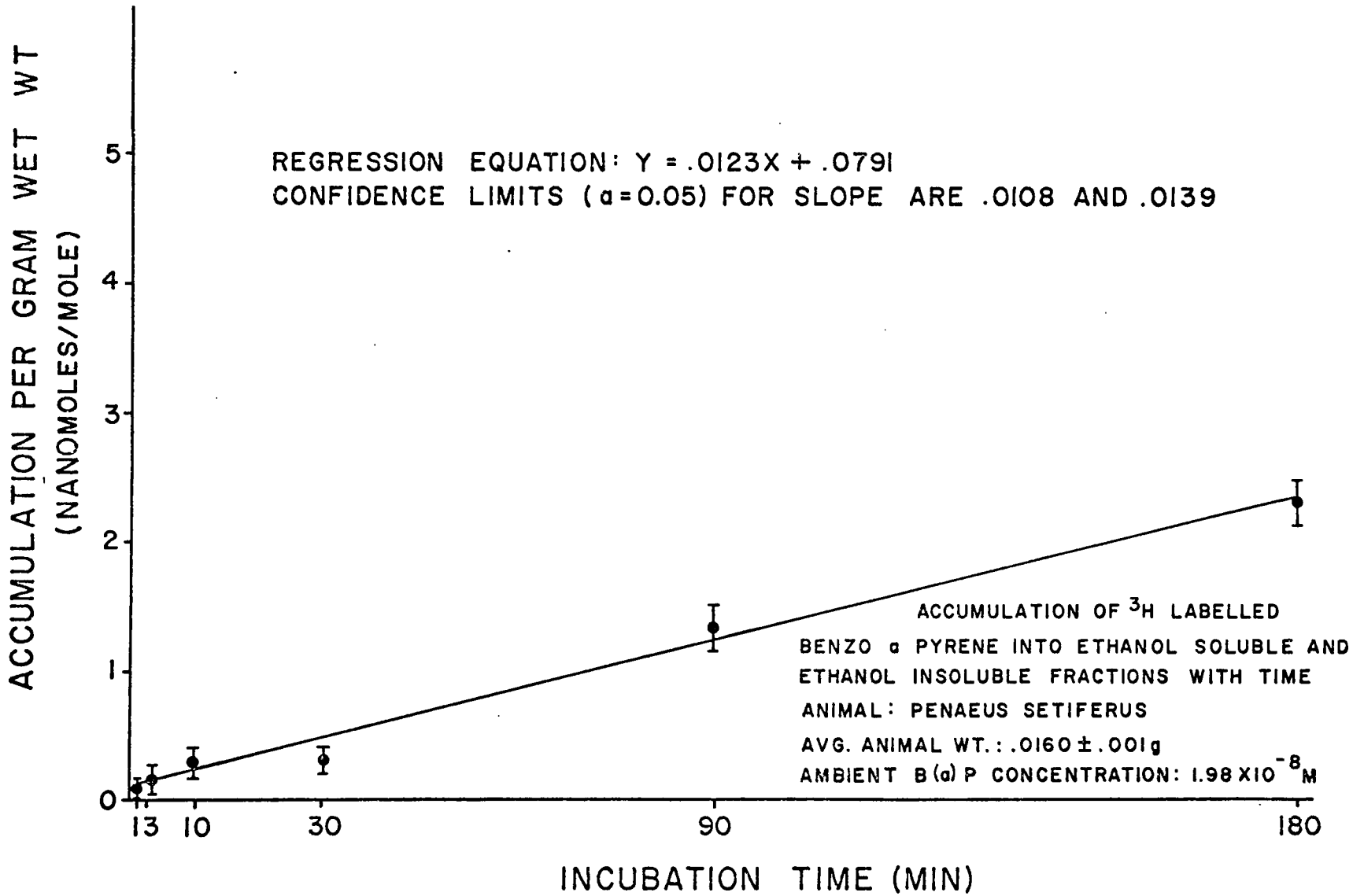

REGRESSION EQUATION: Y = .0123X + .0791
CONFIDENCE LIMITS (α=0.05) FOR SLOPE ARE .0108 AND .0139
ACCUMULATION OF ^{3}H LABELLED
BENZO α PYRENE INTO ETHANOL SOLUBLE AND
ETHANOL INSOLUBLE FRACTIONS WITH TIME
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0160 ± .001g
AMBIENT B(α)P CONCENTRATION: 1.98×10^{-8} M
ACCUMULATION PER GRAM WET WT (NANOMOLES/MOLE)
0
1
2
3
4
5
13
10
30
90
180
INCUBATION TIME (MIN)

FIGURE 10

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL SOLUBLE AND ETHANOL INSOLUBLE FRACTIONS WITH TIME

ANIMAL: *Penaeus setiferus*

AVERAGE ANIMAL WEIGHT: .0063 ± .0001 g

AMBIENT BENZO(a)PYRENE CONCENTRATION: 1.98×10^{-8} M

EACH POINT REPRESENTS THE MEAN VALUE OF 6 FLASKS, 10 ANIMALS PER FLASK ± THE STANDARD ERROR OF THE MEAN

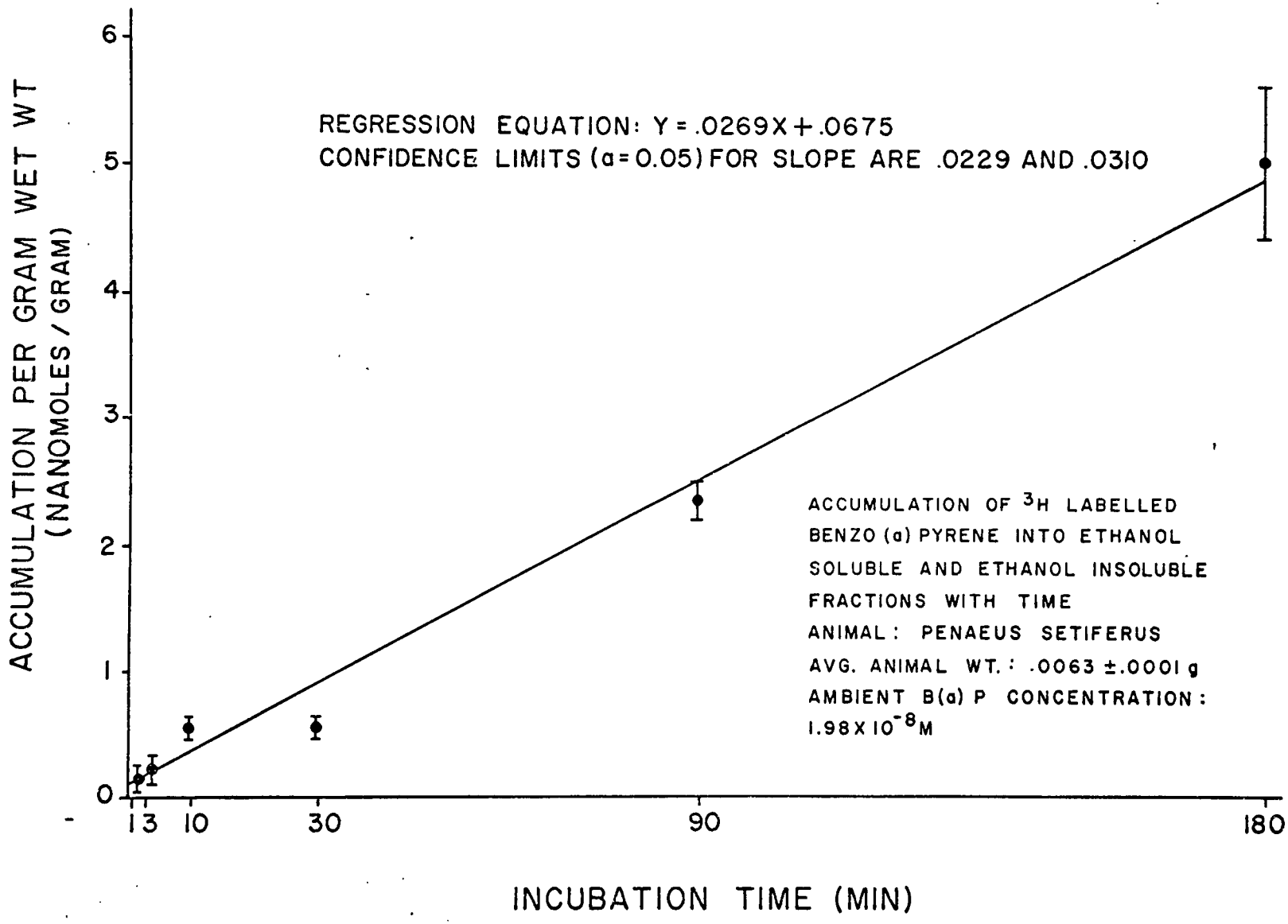

REGRESSION EQUATION: Y = .0269X + .0675
CONFIDENCE LIMITS (α = 0.05) FOR SLOPE ARE .0229 AND .0310
ACCUMULATION OF ^{3}H LABELLED BENZO (a) PYRENE INTO ETHANOL SOLUBLE AND ETHANOL INSOLUBLE FRACTIONS WITH TIME
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0063 ±.0001 g
AMBIENT B(a) P CONCENTRATION: 1.98×10^{-8} M
ACCUMULATION PER GRAM WET WT (NANOMOLES / GRAM)
0
1
2
3
4
5
6
1
3
10
30
90
180
INCUBATION TIME (MIN)

FIGURE 11

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL SOLUBLE FRACTION WITH INCREASING AMBIENT CONCENTRATION

ANIMAL: *Penaeus stylorostris*

AVERAGE ANIMAL WEIGHT: .0003 g

INCUBATION TIME: 60 min

EACH POINT REPRESENTS THE MEAN VALUE FOR 8 FLASKS, 50 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

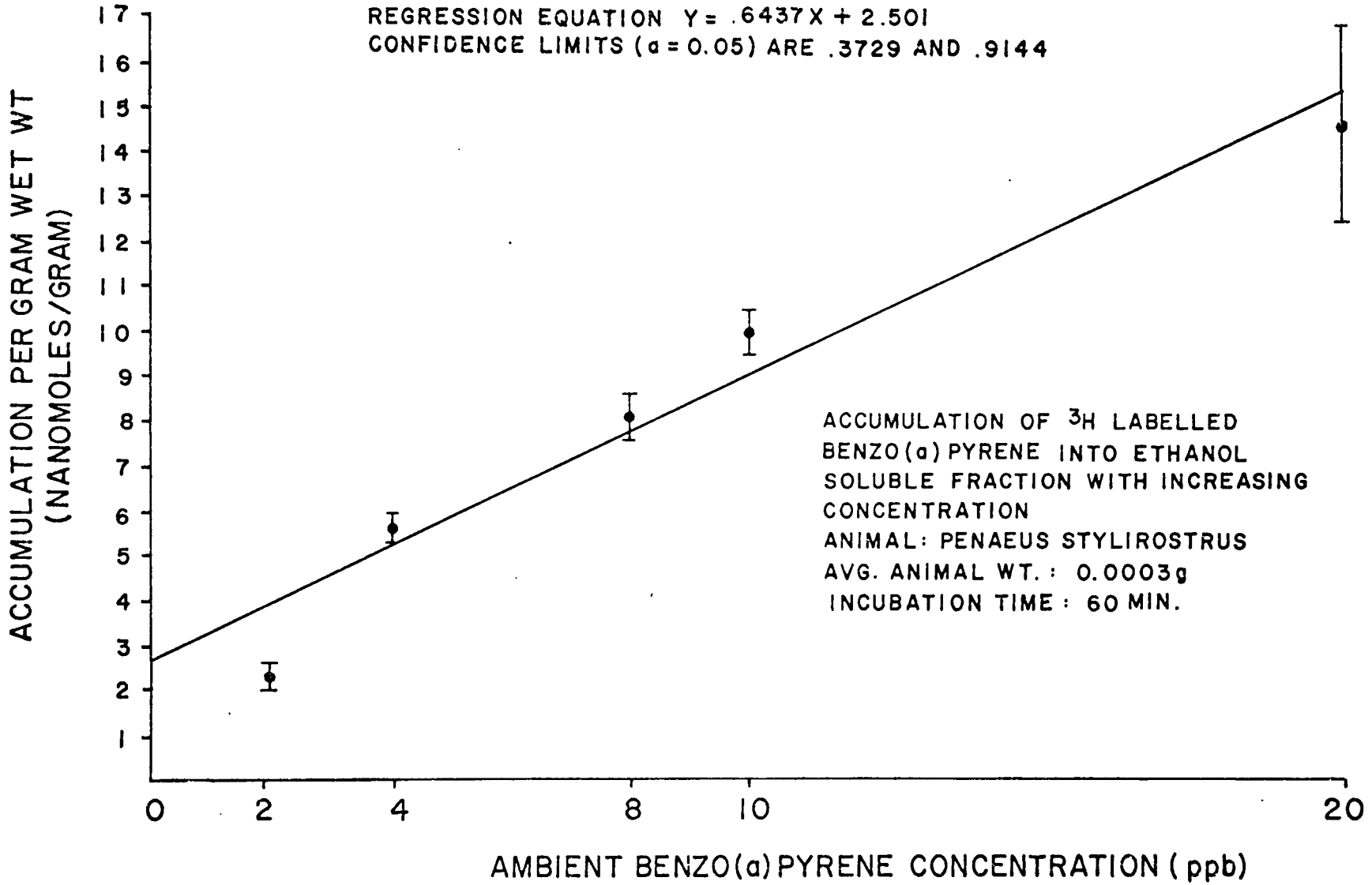

REGRESSION EQUATION Y = .6437X + 2.501
CONFIDENCE LIMITS (α = 0.05) ARE .3729 AND .9144
ACCUMULATION OF ^{3}H LABELLED BENZO(a) PYRENE INTO ETHANOL SOLUBLE FRACTION WITH INCREASING CONCENTRATION
ANIMAL: PENAEUS STYLIROSTRUS
AVG. ANIMAL WT. : 0.0003g
INCUBATION TIME : 60 MIN.
ACCUMULATION PER GRAM WET WT (NANOMOLES/GRAM)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
0 2 4 8 10 20
AMBIENT BENZO(a) PYRENE CONCENTRATION (ppb)

FIGURE 12

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE FRACTION WITH INCREASING AMBIENT CONCENTRATION

ANIMAL: *Penaeus stylorostris*

AVERAGE ANIMAL WEIGHT: .0003 g

INCUBATION TIME: 60 min

EACH POINT REPRESENTS THE MEAN VALUE FOR * FLASKS, 50 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

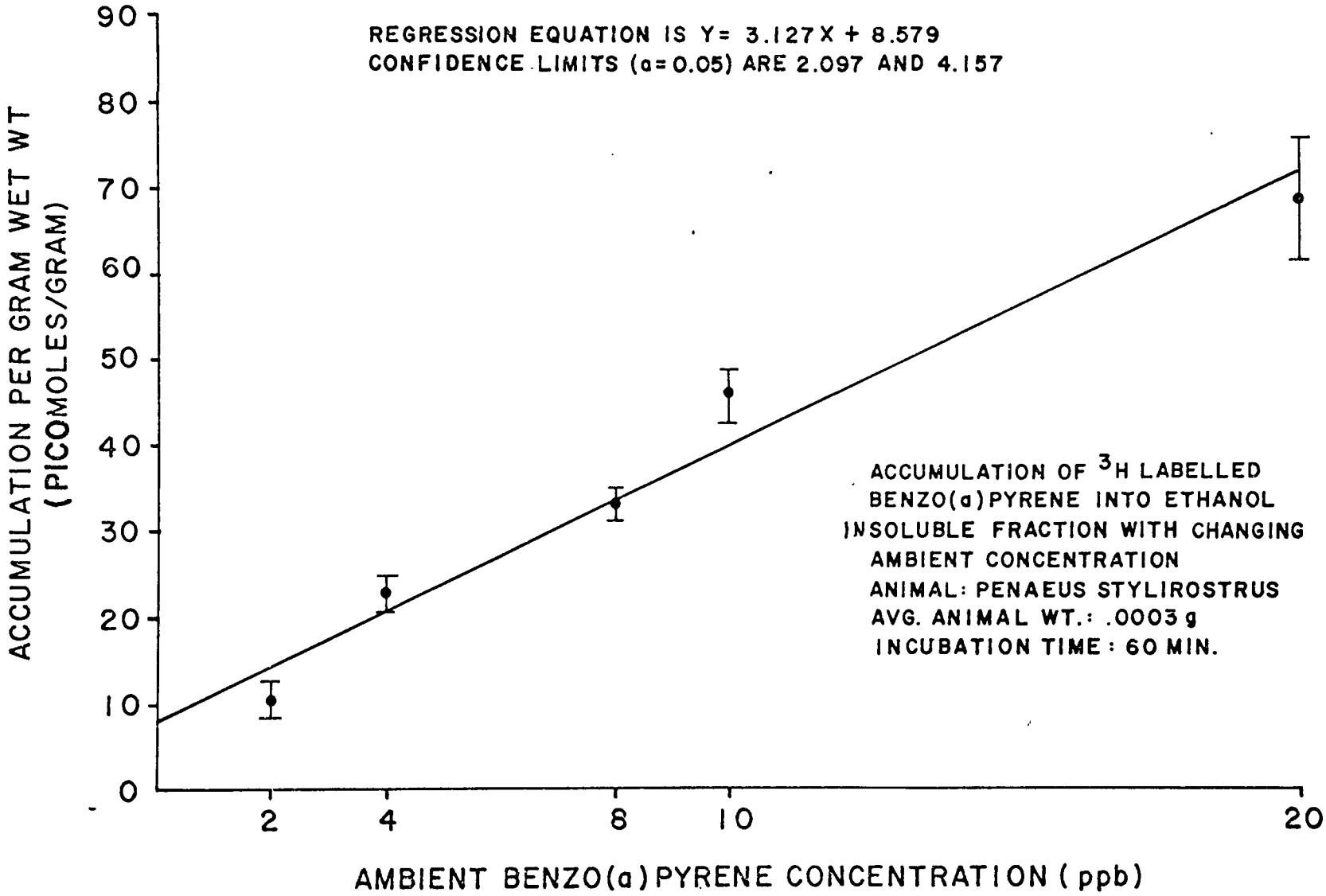

REGRESSION EQUATION IS Y= 3.127X + 8.579
CONFIDENCE LIMITS (α=0.05) ARE 2.097 AND 4.157
ACCUMULATION OF ^{3}H LABELLED BENZO(a)PYRENE INTO ETHANOL INSOLUBLE FRACTION WITH CHANGING AMBIENT CONCENTRATION
ANIMAL: PENAEUS STYLIROSTRUS
AVG. ANIMAL WT.: .0003 g
INCUBATION TIME: 60 MIN.
ACCUMULATION PER GRAM WET WT (PICOMOLES/GRAM)
0
10
20
30
40
50
60
70
80
90
2
4
8
10
20
AMBIENT BENZO(a)PYRENE CONCENTRATION (ppb)

FIGURE 13

ACCUMULATION OF ^{3}H LABELED BENZO(a)PYRENE INTO THE ETHANOL INSOLUBLE AND ETHANOL SOLUBLE FRACTIONS WITH INCREASING AMBIENT CONCENTRATION

ANIMAL: *Penaeus stylorostris*

AVERAGE ANIMAL WEIGHT: .0003 g

INCUBATION TIME: 60 min

EACH POINT REPRESENTS THE MEAN VALUE FOR 8 FLASKS, 50 ANIMALS EACH $\pm$ THE STANDARD ERROR OF THE MEAN

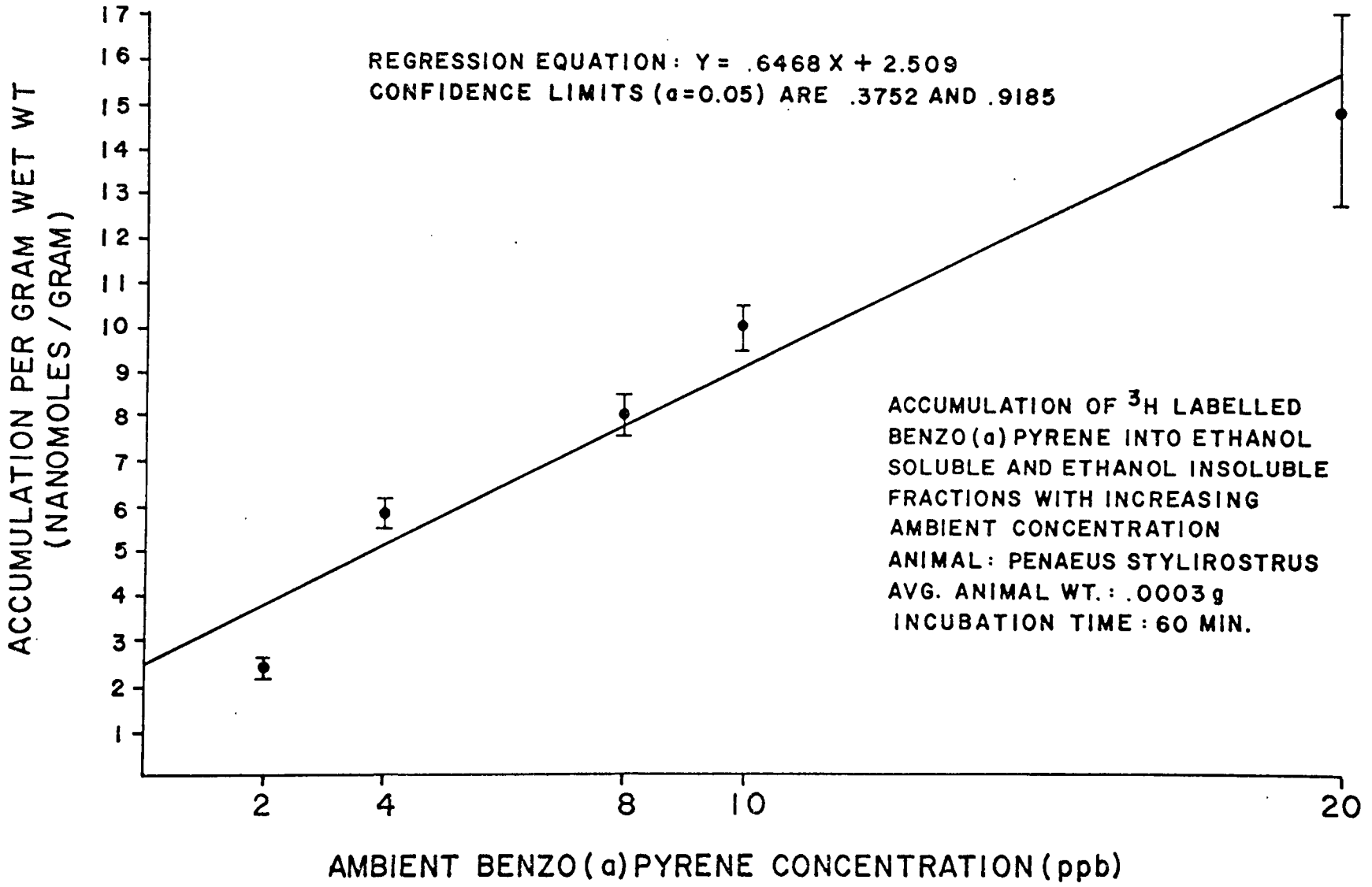

REGRESSION EQUATION: Y = .6468 X + 2.509
CONFIDENCE LIMITS (α=0.05) ARE .3752 AND .9185
ACCUMULATION OF ^{3}H LABELLED BENZO(a) PYRENE INTO ETHANOL SOLUBLE AND ETHANOL INSOLUBLE FRACTIONS WITH INCREASING AMBIENT CONCENTRATION
ANIMAL: PENAEUS STYLIROSTRUS
AVG. ANIMAL WT.: .0003 g
INCUBATION TIME: 60 MIN.
ACCUMULATION PER GRAM WET WT (NANOMOLES / GRAM)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
2
4
8
10
20
AMBIENT BENZO(a) PYRENE CONCENTRATION (ppb)

FIGURE 14

RELEASE OF ^{3}H LABELED BENZO(a)PYRENE FROM THE ETHANOL SOLUBLE FRACTION WITH INCREASING DEPURATION TIMES INTO CLEAN SEA WATER.

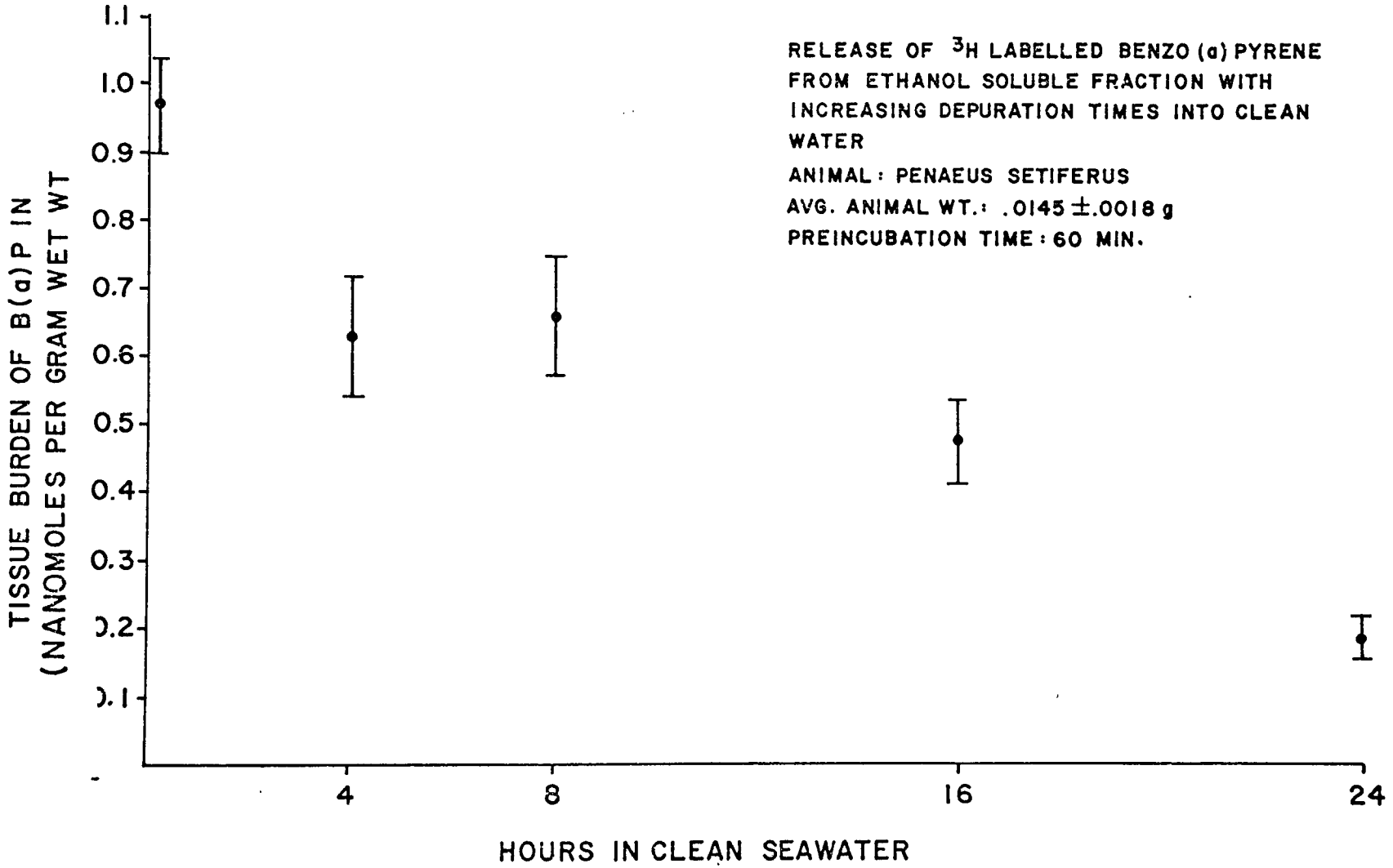
ANIMAL: Penaeus setiferus

AVERAGE ANIMAL WEIGHT: .0145 $\pm$ .0018 g

PREINCUBATION TIME: 60 min

EACH POINT REPRESENTS THE MEAN VALUE OF 8 FLASKS, 5 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

INCUBATION CONCENTRATION: 1.98×10^{-8} M


RELEASE OF ^{3}H LABELLED BENZO (a) PYRENE FROM ETHANOL SOLUBLE FRACTION WITH INCREASING DEPURATION TIMES INTO CLEAN WATER
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0145 ±.0018 g
PREINCUBATION TIME: 60 MIN.
TISSUE BURDEN OF B(a)P IN (NANOMOLES PER GRAM WET WT
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
4
8
16
24
HOURS IN CLEAN SEAWATER

compared to the release into the water of approximately 0.8 nanomoles per gram wet weight after 24 hours, a "factor of temporal partition" may be calculated. In this case it is 0.033, meaning that the rate of release of this compound from this compartment of the animals' tissues is some thirty times slower than its rate of uptake.

Of interest in the case of the depuration studies is the fact that the uptake into the ethanol insoluble fraction appears to continue even though the ambient concentrations of benzo(a)pyrene have been reduced to zero. This is seen in Figure 15. The concentration of benzo(a)pyrene in this fraction increased some threefold during the first four hours of depuration. Thereafter, this level of concentration remains surprisingly constant for the remaining 20 hours of the 24 hour depuration period. At 24 hours, the concentration of benzo(a)pyrene in the ethanol insoluble fraction, shown in Figure 15 to be near 35 picomoles per gram wet weight, still exceeds the original ambient concentration of the compound by a factor of 2.

The possibility of metabolism of benzo(a)pyrene by the Penaeid shrimp tested was not supported by any of the chromatographic data obtained. Thin layer chromatograms of ethanol extracts of the animals, when developed in n-hexane, revealed the presence of only one radioactive locus in the mobile phase. This spot had an Rf of approximately 0.19. The pure, unlabeled compound revealed a spot on UV irradiation which had an Rf of 0.21. This discrepancy, first thought to denote some metabolic alteration of benzo(a)pyrene proved to be the result of an impeding effect imposed on the extracted benzo(a)pyrene by the

FIGURE 15

RELEASE OF ^{3}H LABELED BENZO(a)PYRENE FROM THE ETHANOL FRACTION WITH INCREASING DEPURATION TIMES INTO CLEAN SEA WATER.

ANIMAL: Penaeus setiferus

AVERAGE ANIMAL WEIGHT: .0145 $\pm$ .0018 g

PREINCUBATION TIME: 60 min

EACH POINT REPRESENTS THE MEAN VALUE OF 8 FLASKS, 5 ANIMALS PER FLASK $\pm$ THE STANDARD ERROR OF THE MEAN

INCUBATION CONCENTRATION: 1.98×10^{-8} M

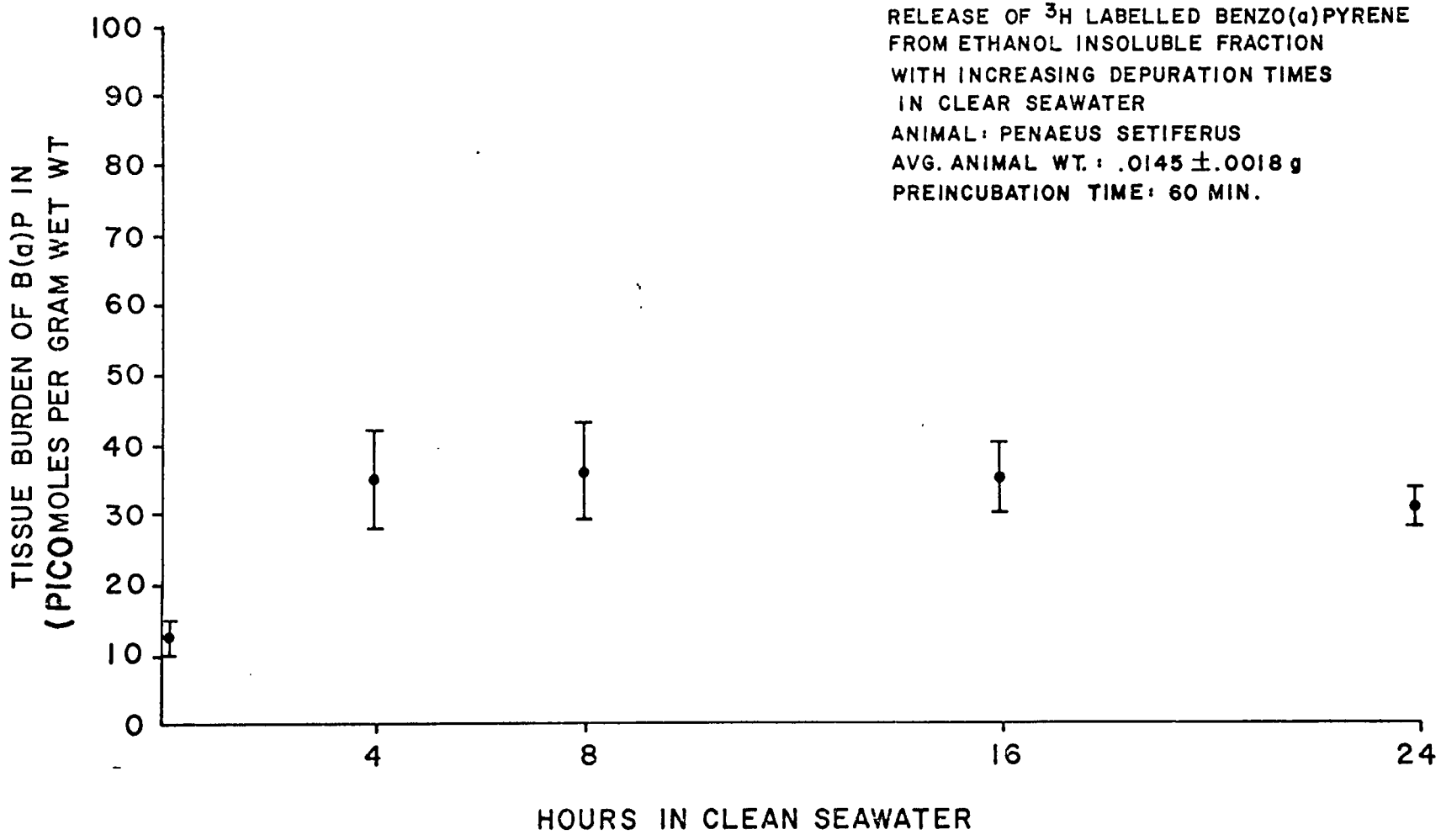

RELEASE OF ^{3}H LABELLED BENZO(a)PYRENE
FROM ETHANOL INSOLUBLE FRACTION
WITH INCREASING DEPURATION TIMES
IN CLEAR SEAWATER
ANIMAL: PENAEUS SETIFERUS
AVG. ANIMAL WT.: .0145 ± .0018 g
PREINCUBATION TIME: 60 MIN.
TISSUE BURDEN OF B(a)P IN
(PICOMOLES PER GRAM WET WT
100
90
80
70
60
50
40
30
20
10
0
4
8
16
24
HOURS IN CLEAN SEAWATER

TABLE 2

DESCENDING PAPER CHROMATOGRAPHY

SEGMENT NO. (cm)	A CPM 30 µl ETHANOL EXTRACT +1 µl HOT	% OF TOTAL	B CPM 30 µl ETHANOL EXTRACT +1 µl HOT	% OF TOTAL	C CPM 30 µl ETHANOL EXTRACT ALONE	% OF TOTAL
1	310	1.0	252	0.8	101	1.0
11	310	1.0	20	0.1	2	0.1
3	15	0.1	16	0.1	2	0.1
4	24	0.1	9	0.1	2	0.1
5	24	0.1	20	0.1	1	0.1
6	27	0.1	40	0.1	1	0.1
7	27	0.1	40	0.1	5	0.1
8.	54	0.2	50	0.1	9	0.1
9	64	0.2	74	0.3	18	0.3
10	87	0.3	85	0.3	27	0.4
11	123	0.4	127	0.4	57	0.8
12	201	0.6	191	0.6	87	1.2
13	289	0.9	266	0.9	106	1.5
14	317	1.0	307	1.0	112	1.5
15	601	1.9	613	2.0	318	4.4
16	1753	5.4	1686	5.6	528	7.2
17	14316	44.0	13767	45.8	4420	60.6
18	13640	41.9	12206	40.6	1485	20.3
19	321	1.0	246	0.8	19	0.3
20	44	0.1	47	0.2	5	0.1
21	9	0.1	11	0.1	1	0.1

Note: Solvent system was Methanol:Water (9:1).

other ethanol soluble constituents of the animal tissue. Many of these constituents appeared to be pigments since the extracts bore a definite orange-pink color. It was noted that a small percentage of the radioactivity placed at the origin of the chromatograms remained there after development in both thin layer and paper chromatography. The same phenomenon was observed on chromatography of the commercially supplied labeled benzo(a)pyrene. Table 2 shows the result of the development of 30 µl of the ethanol extract as compared to 30 µl of the same extract plus 1 µl of a dilute (2 ppb) solution of the commercially supplied labeled compound. This table shows the descending paper chromatography of the compounds in a solvent system of methanol: water (1:1). It is probable that the counts remaining at the origin are not the result of metabolic alteration of the benzo(a)pyrene but rather are due to some photooxidation product of the original compound. This type of degradation had been noted by Andelman and Suess (1970).

DISCUSSION

DISCUSSION

The data concerned with the accumulation of benzo(a)pyrene with varying times of incubation shows this accumulation to be linear for times up to 180 minutes in all three experiments reported, with a maximum concentration in the animal that is at least 150 times the ambient concentration. This linearity is similar to that seen by Stegeman and Teal (1973) in their study of a wide range of petroleum hydrocarbons and their absorption by the adult Japanese oyster, Crassostrea gigas. This pattern of accumulation was also seen in adult oysters when exposed to the chlorinated hydrocarbon pesticide, DDT (Butler, 1969). In this study, it was reported that the American oyster (Crassostrea virginica) was capable of attaining a magnification factor of 70,000 X after 30 days of incubation in the DDT contaminated medium.

The exceedingly high tissue concentration of benzo(a)pyrene seems to suggest an active process. However, a more probable explanation for the magnification is the partitioning of the poorly water soluble benzo(a)pyrene into the lipid rich tissues of the shrimp, particularly those extracted by ethanol. This agrees with the finding of Stegeman and Teal (1973) that hydrocarbons are accumulated from sea water in direct relation to the lipid content of the absorbing animal. The difference in slope from one experiment to another is probably attributable to both differences in animal size and animal nutritional states and not to any interspecific differences in accumulation mechanism. The nutritional states of the

animals were variable and beyond our control until they were received.

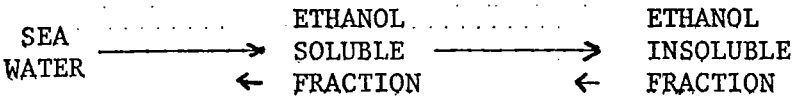
The movement of benzo(a)pyrene into the ethanol insoluble fraction of the shrimp was also linear. However, this accumulation occurs at a rate some two orders of magnitude slower than that seen for ethanol soluble material. It is possible that this ethanol insoluble fraction could represent a metabolic alteration of the compound by the shrimp. This could result in the non-accessibility of the labeled compound to ethanol extraction. However, this possibility is remote since all known animal metabolites of benzo(a)pyrene are more water and ethanol soluble than the parent compound. Further, chromatographic analysis of total lipid and ethanol soluble extracts indicated that less than 1% of the accumulated label had been altered, and this is thought to be due to the photooxidation of the benzo(a)pyrene rather than any animal activity. It is more likely that the ethanol insoluble fraction represents incorporation into a tissue lipid compartment that is non ethanol extractable. For example, this ethanol insoluble fraction could represent benzo(a)pyrene incorporation into structural elements of the cell membrane. The possibility of replacement of membrane lipids by multi-ring compound is put forth by Schoental (1964). In this review, she discusses the substitution of polycyclic aromatic hydrocarbons for cholesterol in the membrane.

Studies concerned with the accumulation of benzo(a)pyrene with respect to a varying ambient concentration offer much the same conclusions. The linear increase of accumulation with an increasing

ambient concentration suggests a passive mechanism of uptake. If an active process were responsible for this accumulation, one would expect to observe a leveling off of the rate of uptake as carrier sites become saturated and a maximum rate of transport is reached. This is not seen in this study using benzo(a)pyrene concentrations up to 20 parts per billion and incubation periods of up to 60 minutes. The accumulation into the ethanol insoluble fraction with increasing ambient concentration is linear also, though two orders of magnitude lower than the uptake into the ethanol soluble fraction. In support of these findings, the uptake of hydrocarbons from sea water by many animals has been found to be a passive process, mainly dependent on diffusion (Blumer, using adult oysters, 1972; Anderson et al., using adult shrimp, 1974; Lee et al., using adult mussels, 1971).

The release of benzo(a)pyrene from the ethanol soluble fraction of these postlarval shrimp is also linear, though proceeding at a rate some thirty times slower than the uptake into the same compartment. Thus, if benzo(a)pyrene accumulation were allowed to continue to an equilibrium, complete depuration of the contaminant in a completely hydrocarbon free environment would require an extremely long period. However, a complete depuration is unlikely since Blumer (1970) reported that adult oysters placed in clean, flowing sea water after having been removed from the site of an oil spill still contained small amounts of all the original contaminants one month later. This is supported by the observation in the present study that benzo(a)pyrene concentration in the ethanol insoluble fraction continues to increase

for approximately four hours and then remains constant for an additional 20 hours after removal of the animal from the contaminated sea water. The fact that the ethanol insoluble fraction continues to accumulate benzo(a)pyrene even after the water concentration of the contaminant has been reduced to zero strongly suggests that the movement of benzo(a)pyrene into Penaeid shrimp follows the scheme:



The chromatographically immobile portion of the ethanol and total lipid extracts could represent the result of metabolic alteration of the benzo(a)pyrene, though this fraction represents less than 1% of the totally extractable material. However, it is more likely that it represents the product of photooxidation of benzo(a)pyrene outside the animal itself. Evidence for this possibility is seen in the fact that these compounds were found in the same relative percentages in chromatograms of the commercialllly supplied labeled benzo(a)pyrene. Should the animal be capable of metabolism, however, it represents a small if not insignificant contribution to removal of the benzo(a)pyrene from the animal.

Thus, there remains the problem of the magnification and persistence of the polycyclic aromatics, benzo(a)pyrene in particular, in the tissues of the Penaeid shrimp. It is evident from the accumulation studies reported here that this compound is extracted very efficiently from sea water by Penaeid postlarvae. This process occurs in such a

way that the final tissue concentration in the entire animal after three hours of exposure exceeds the sea water concentration of the contaminant by at least 150 times. The finding that the benzo(a)pyrene is only poorly metabolized if at all by these animals could have serious consequences where man is concerned. If the compound were altered greatly by the commercially important species of the genus Penaeus, it would be considerably more water soluble in the altered form and much more easily lost, though this would pose a carcinogenic threat to the shrimp themselves. Since the benzo(a)pyrene is almost negligibly metabolized, it remains and is concentrated in the shrimp's tissues in an unaltered form and still bearing all its native carcinogenicity until it is consumed by individuals higher in the marine food web or by man.

Such a scheme of biological magnification of a potent carcinogen by the Penaeid shrimp, a highly desirable food animal, can only signify that a very dangerous portion of man's offal is possibly being returned to him by one of his favorite foods. The contribution made by such a scheme of movement to the increase in human cancer cannot presently be assessed, but it generally assumed that since carcinogens show no dose- response relationships (i.e. can cause cancer as a result of reaction of one carcinogen molecule with one host cell) all possible means of entry of these compounds into the human diet should be investigated and, if possible, controlled. The pathway seen in this study indicates that such a program of investigation would be highly justified.

SUMMARY

SUMMARY

The accumulation, release, and possibility of metabolic alteration of the environmentally ubiquitous carcinogen, benzo(a)pyrene, by several species of the genus Penaeus from sea water was studied. The accumulation was found to occur rapidly, reaching a magnification factor of at least 150 X after 180 minutes of incubation of the animals in the contaminated medium. This accumulation was shown to be of a passive nature and to proceed linearly as both incubation time and contaminant concentration were increased. The accumulation into the ethanol soluble fraction was approximately two orders of magnitude greater than that into the ethanol insoluble fraction. The release of the compound benzo(a)pyrene was found also to proceed in a linear fashion, though some thirty times slower than the previously observed uptake. There was a certain persistent concentration of contaminant left in the ethanol insoluble fraction after 24 hours of incubation of the animals in contaminant free sea water. In addition, it was seen that the ethanol insoluble fraction continued to absorb the tritiated compound while the ethanol soluble fraction of the animals was seen to lose the compound steadily. This suggested the existence of a pathway of movement of the compound similar to:

SEA WATER ⇄ ETHANOL SOLUBLE FRACTION ⇢ ← ETHANOL INSOLUBLE FRACTION

It was suggested by thin layer and paper chromatography that there

was no metabolic alteration of the subject compound by any of the species studied. The possibility of transmission of this carcinogen from the marine environment to the human diet by the commercially important Penaeid shrimp was discussed.

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