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RESEARCH ARTICLE

BIOACCUMULATION STUDY OF ANTHRACENE IN A FEW TISSUES OF RASBORA DANICONIUS

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ABSTRACT

Chronic exposure of the freshwater fish Rasbora daniconius to PAH Anthracene showed bioaccumulation in varying concentrations in the gill, liver, intestine and kidney tissues. Bioaccumulation was assessed with the help of GCMS in terms of wet weight of tissue. Maximum bioaccumulation was found in liver tissue, followed by gills, kidney and intestine.

Key Words: Rasbora daniconius, Anthracene, Chronic exposure, Bioaccumulation, GCMS.

INTRODUCTION

Anthracene, a polyaromatic hydrocarbon (PAH), is so commonly used that its presence in air, water and soil has become ubiquitous. Anthracene is a pale yellow crystalline solid, having chemical formula C14H10. It is obtained from coal tar. It is used for industrial production of alizarin, a natural red pigment from plant, as well as in wood preservatives, insecticides and surface coatings. Anthracene of MERCK make was used for the present work. Anthracene, after its various uses, finds its way into air, water and soil media, thereby polluting them, and tends to bioaccumulate and bioconcentrate in tissues of biota. This leads to acute as well as chronic deleterious effects on their tissues, and may prove to be fatal at higher doses. There is abundant literature available on the effects of various pollutants on fish. These include pesticides, heavy metals, etc. Various methods have been used with this aim in view. Different workers have studied the toxic and bioaccumulative effects of PAHs (Ron Van der Oost *et al.,* 1991), (C. Porte and J. Albaigés, 1993), (John E. Stein *et al.,* 1995), (P. Baumard *et al.,* 1998), (Estefania EscartÍn and Cinta Porte, 1999), (Tarja Hyötyläinen, Aimo Olkari, 1999), (P.J. Ruddock *et al.,* 2002), (Rainer Lohmann *et al.,* 2004), (Ichiro Takeuchi *et al.,* 2009), etc. PAHs have been seen to accumulate in liver and muscle of eel Anguilla anguilla, and also to cause gill lesions and liver and spleen tumors (C.A. Oliveira Ribeiro *et al.,* 2005). The present work deals with the study of the degree of bioaccumulation of the PAH Anthracene in kidney, intestine, liver and gill tissues of the freshwater fish Rasbora daniconius after chronic exposure to a safe dose of Anthracene at intervals of every 5 days, over a period of 30 days.

MATERIALS AND METHODS

Freshwater fish Rasbora daniconius were obtained from local freshwater bodies.

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Factors such as availability, size, weight, ease of stocking and handling, etc. were considered for the choice of fish species for the present study. The fish were acclimatized in dechlorinated tap water for about 10 days at room temperature. Characteristics of dilution water were analyzed and have been described in Table 1. Anthracene is not soluble in water. A stock of the test solution was prepared using 1 mg /ml of Anthracene with Dichloromethane (MERCK) as an organic solvent and then diluted for use as per requirement. The fish were exposed to varying concentrations of such solution of Anthracene in Dichloromethane, by performing acute bioassays, at 24, 48, 72 and 96 hours intervals. Anthracene of MERCK make was used for the present work. A sub lethal concentration, based on the LC50 values obtained during acute bioassay, was arrived at. The incipient LC50 is recommended as the most useful single criterion of toxicity evaluation (J.B. Sprague, 1969). Based on these values, a sub lethal concentration of the substance under study was determined, and further chronic toxicity tests were carried out on the test fish over a period of 30 days, with samples taken at intervals of 5, 10, 15, 20, 25 and 30 days. Many references are available in literature where such method of toxicity evaluation has been used. Test fish have been exposed to pollutants for 24, 48, 72 and 96 hours (G. Brenniman *et al.,* 1976), (Nuno M. Fragoso *et al.,* 2006). Percent mortality was noted for these different concentrations every 24, 48, 72 and 96 hours.

Based on these observations, the LC50 values, NOEC and 95% confidence interval were calculated using probability graph paper using standard methods in literature (Litchfield, J.T. Jr. and F. Wilcoxon, 1949) and used for determining sub lethal concentrations of Anthracene for chronic toxicity testing. The test organisms were exposed to such a safe, sub lethal 0.016 µg l-1 concentration of Anthracene, repeated every 24 hours, using a continuous flow through method using an indigenously designed flow through unit for dosing (Fig.1). Appropriate controls were maintained at all stages of the experiment. The dosing unit consists of two Perspex cylindrical reservoirs kept one above the other. The upper reservoir is closed at the top with a rubber bung and is airtight.

At the bottom, it has an inlet on one side for flow of solution and a glass tube is fitted in the central hole. An annular Perspex ring of smaller diameter is fixed to the bottom of the upper reservoir on the lower side. The lower reservoir has a stop cock acting as an outlet near the bottom and an air vent at the top. Capacity of the dosing unit is 10 litres. The upper reservoir is filled with test solution having known concentration of Anthracene and the upper hole is closed with the rubber bung. Liquid starts flowing to the lower reservoir through the inlet tube. When liquid in the lower reservoir rises to the rim of the annular ring and into the glass tubing, a tension is created, resulting in break of the flow. A continued discharge through the outlet brings down the liquid level and the flow starts again, when the liquid level falls below the rim. This creates an automatic make and break of flow in the lower reservoir. The stop cock was adjusted to discharge the required flow of 7 ml/ min.

Table 1, Characteristics of Dilution Water

Parameters	Values *
Temperature ° C	25-27
pH	7.5-8.2
Total Alkalinity as $CaCO_3$	156-190
Total Hardness as $CaCO_3$	142-172
Ca Hardness as $CaCO_3$	80-94
Mg Hardness as $CaCO_3$	62-78
Dissolved Oxygen	6.9-7.3
Calcium as Ca	32-38
Magnesium as Mg	14-18
Sodium as Na	36-38
Potassium as K	2-4
Chloride	126

(*All the values are expressed as mg/L except temperature and pH.)

As soon as the liquid level in the lower reservoir drops below the rim of the annular ring, air enters through the 'air vent' and instant flow from the upper reservoir starts. This cycle repeats as the liquid level touches and breaks from the annular ring continuously, keeping the level of liquid in the lower reservoir at almost constant height. Experiments were carried out using a 20 litre rectangular glass aquarium. Experiments were continued for a time interval of 5, 10, 15, 20, 25 and 30 days only. The dosing unit was normally filled up with dilution water and required concentration of Anthracene solution was added to it. The same concentration of Anthracene solution was also added to the aquarium at the beginning of the experiment.

The flow rate was so adjusted that as to allow a flow of 20 litres of solution through the aquaria in 24 hours. The incoming solution was delivered at the bottom of the aquarium through a funnel so as to avoid short circuiting. An overflow arrangement at the top of the aquarium was provided to maintain a constant volume in the aquarium. 20 numbers of fish were introduced in the aquarium for each concentration of Anthracene. Feeding and replacements of fresh solutions were followed as per details reported in literature (Murty, A.S., 1986). The test fish were sacrificed at intervals of every 5 days up to 30 days, and their tissues were collected for bioaccumulation study. Tissues were fixed in Buoin's fixative prior to further processing. Fixed tissues were thoroughly washed, weighed, macerated and then extracted in Dichloromethane by clean up using Celite 545 filter aid medium, as indicated in literature (Shanta Satyanarayan and Ramakant, 2004).

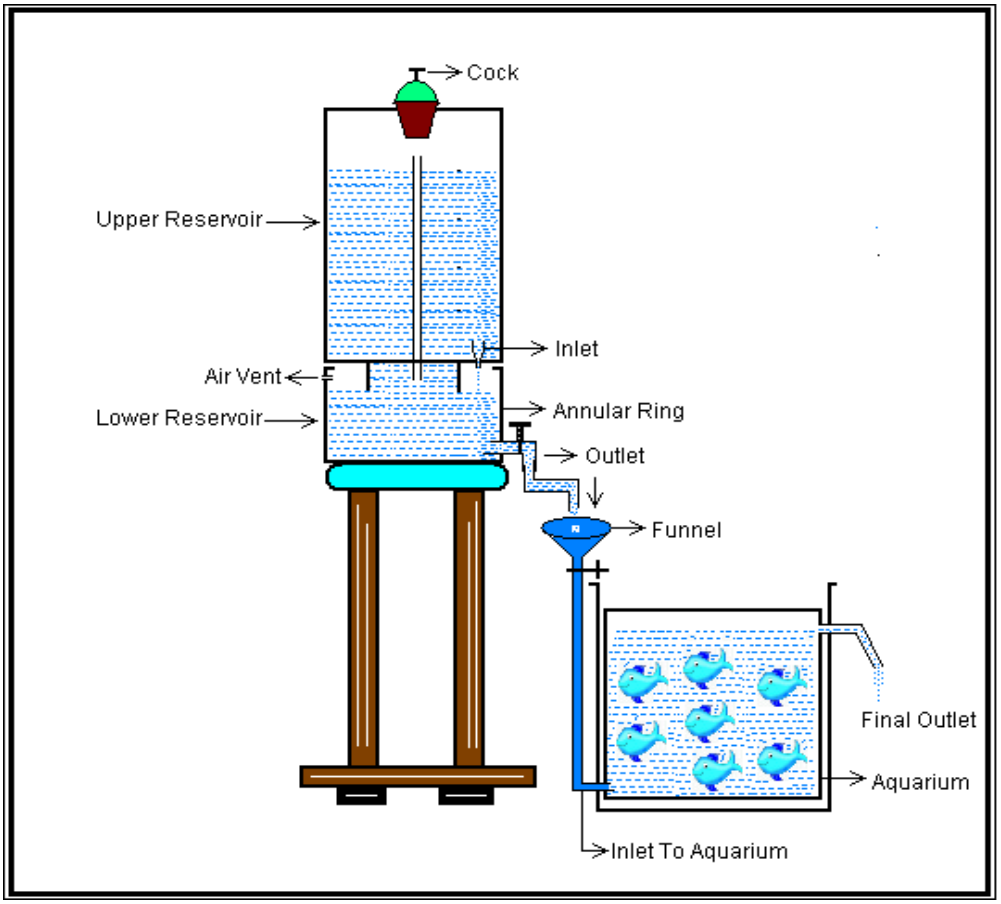


Fig.1. Schematic Diagram of the Experimental Set-up for Chronic Bioassay Studies

Tissue extracts were concentrated by evaporation, collected in stoppered KD tubes and analyzed for their Anthracene content using GC-MS (Gas Chromatography- Mass Spectrophotometry). The use of GC and GC-MS to estimate the degree of bioconcentration has been widely accepted (Laura Maack and William C. Sonzogni, 1988). It can identify a wide variety of compounds in many different matrices and in the presence of interfering compounds (Larry H. Keith, 1979).
For this purpose, a GC-MS/MS (Gas Chromatograph – Mass Spectrometer) of make Thermo Scientific, USA and model TSQ Quantum MS Trace GC Ultra with a Capillary column TR5-MS (30m x 0.25mm i.d.,0.25 µm) was used. Mass spectra, Standard chromatogram and calibration curves were obtained for pure Anthracene, as shown in Fig. 2 to Fig. 4. This was followed by analysis of samples.

RESULTS AND DISCUSSION

Tissue extracts showed increasing concentrations of Anthracene over intervals of 5 days. Figures 5 to 8 show a few chromatograms for Anthracene in liver, gill, kidney and intestine tissues of Rasbora. Based on these observations, concentrations of Anthracene were calculated in terms of µg g-1 wet weight of the tissues. These calculations have been shown in Table 2, and the same have been represented graphically in Graph 1.

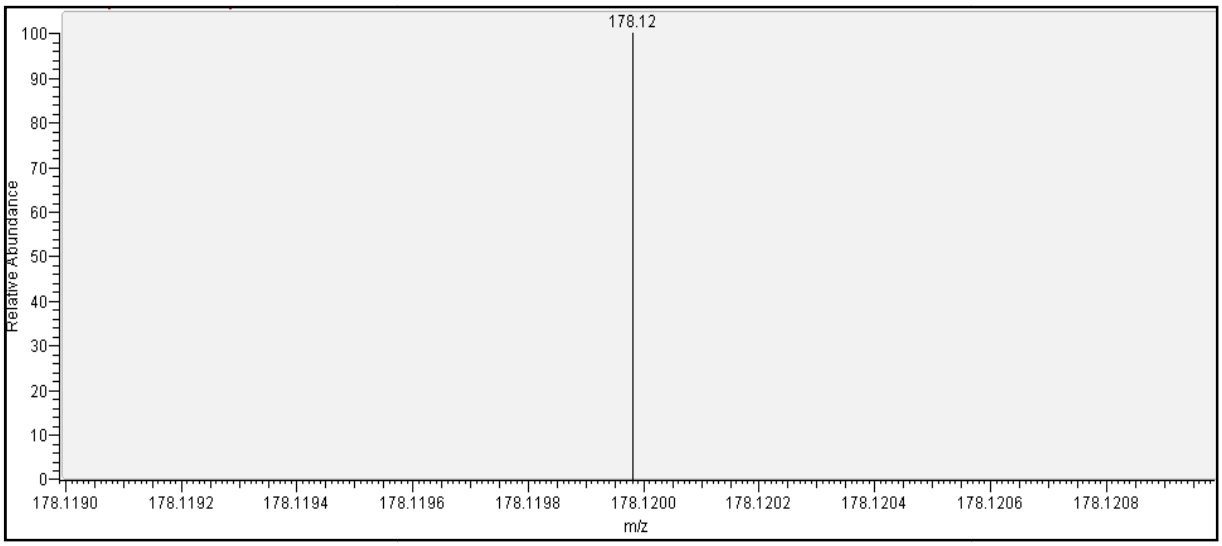


Fig. 2. Mass spectra of Anthracene

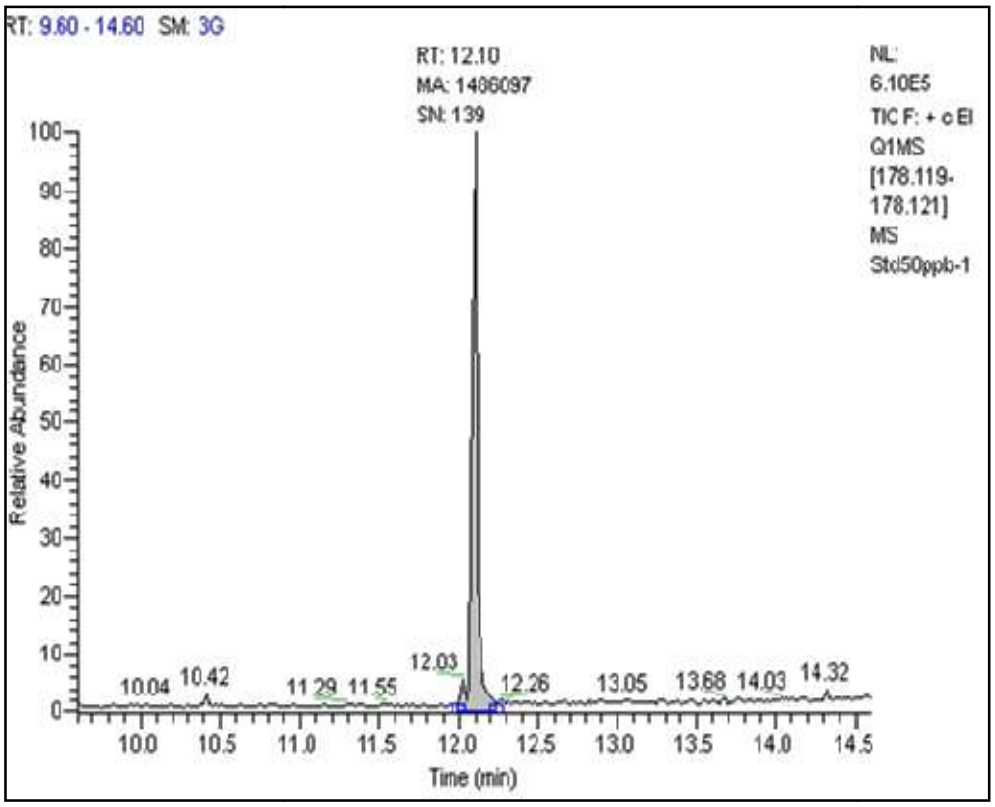


Fig. 3. Standard Chromatogram of Anthracene

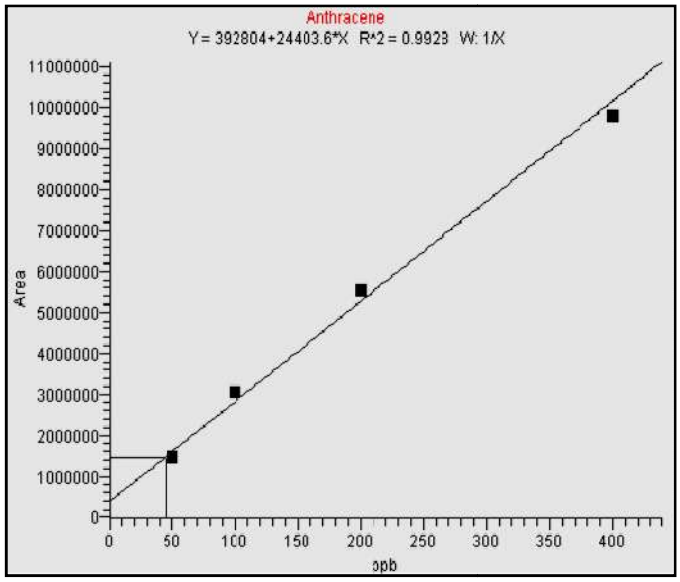


Fig. 4. Calibration curve of Anthracene

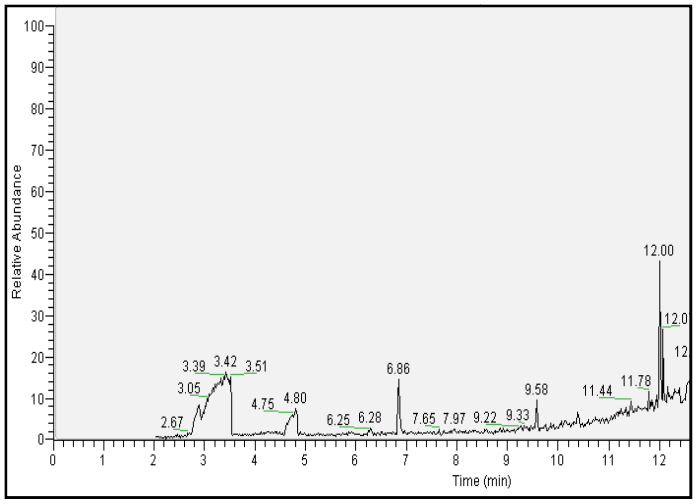


Fig. 5. Chromatogram showing Anthracene in extract of *Rasbora* Liver (30 Days)

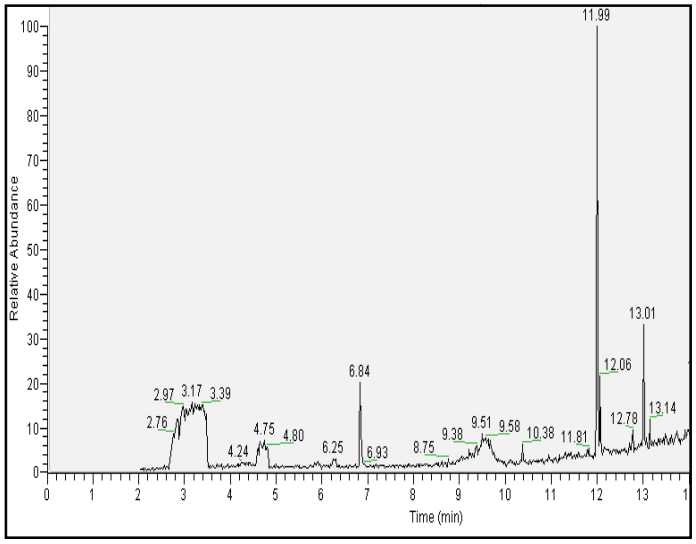


Fig. 6. Chromatogram showing Anthracene in extract of *Rasbora* Gill (20 Days)

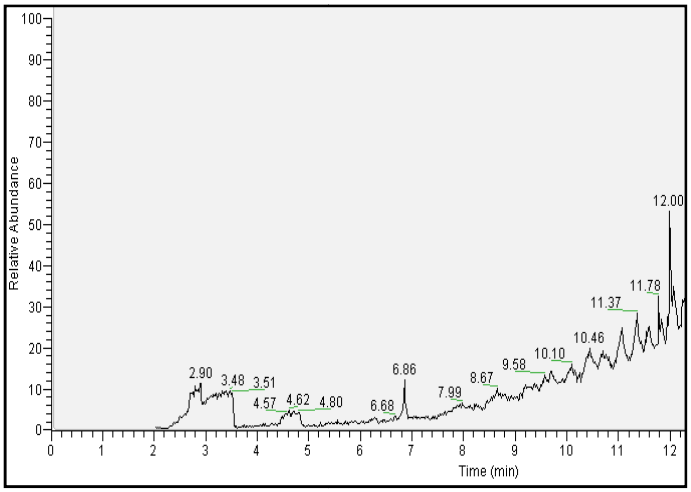


Fig. 7. Chromatogram showing Anthracene in extract of *Rasbora* Kidney (30 days)

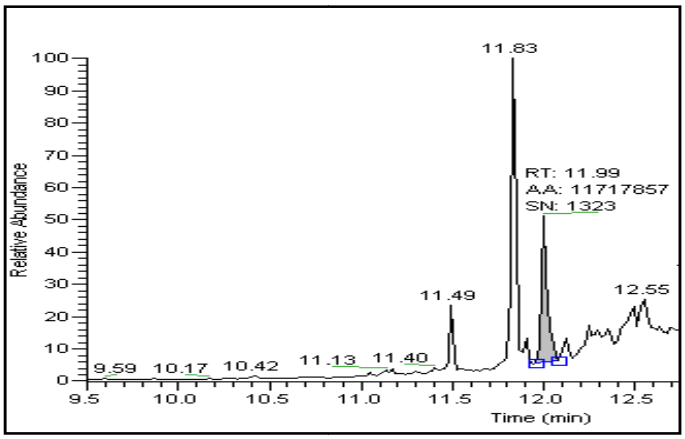
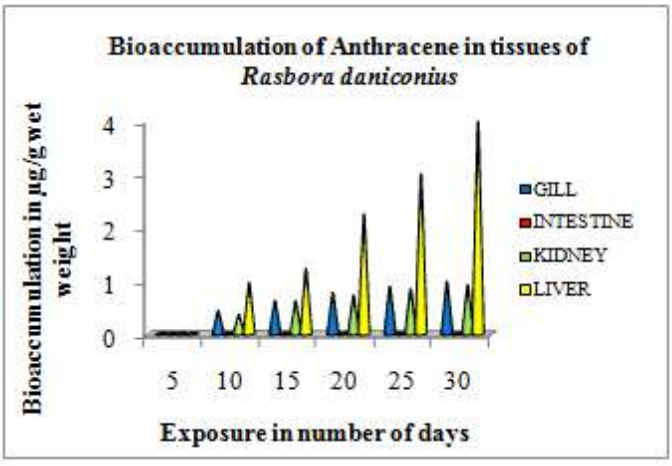


Fig. 8. Chromatogram showing Anthracene in extract of *Rasbora* Intestine (30 Days)

Table 2. Bioaccumulation of Anthracene in *Rasbora daniconius*

Exposure time, days	Values in µg g^{-1} wet weight of the tissue			
	Gill	Intestine	Kidney	Liver
5	ND	ND	ND	ND
10	0.44	ND	0.36	0.96
15	0.63	0.01	0.61	1.22
20	0.77	0.02	0.72	2.24
25	0.89	0.03	0.84	2.99
30	0.98	0.03	0.92	3.97



Graph1. Bioaccumulation of Anthracene in *Rasbora daniconius*

Conclusions

The concentration of Anthracene was highest in the liver, i.e.0.96 µg g-1, at the end of 5 days, and the trend continued over the complete period of study till Anthracene was detected to be 3.97 µg g-1 in the liver of Rasbora at the end of 30 days. There was a gradual yet low build up of Anthracene in the gill and kidney, as compared to the constant and significant build up in the liver. Accumulation of Anthracene in the intestine tissue, which was initially detected at 0.01 µg g-1 only at the end of 15 days, did not show significant increase, and was only 0.03 µg g-1 at the end of 30 days as compared to build up in other tissues. This indicates that Anthracene is probably transferred through the metabolic pathways of biotransformation for disposal through the excretory products of the kidney from gills, it is absorbed through the intestine but accumulates in the liver tissue to a high extent. This also indicates the probable lipophilicity of Anthracene for lipids in the liver.

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