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Accumulation of toxic organic pollutants in the blue crab Callinectes sapidus (aromatic hydrocarbons, gas chromatography, polychlorinated biphenyls, Virginia)

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ACCUMULATION OF TOXIC ORGANIC POLLUTANTS IN THE BLUE CRAB CALLINECTES SAPIDUS

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ACCUMULATION OF TOXIC ORGANIC POLLUTANTS IN THE BLUE CRAB *CALLINECTES SAPIDUS*

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ABSTRACT

Levels of toxic organic pollutants (TOP) were examined in the blue crabs, Callinectes sapidus, of Virginia waters. Alkyl substituted polynuclear aromatic hydrocarbons (ASPAH), thought derived from weathered petroleum, were the most common organic pollutants present. Unsubstituted aromatics, heterosubstituted aromatics, polychlorinated biphenyls (PCB), and DDT metabolites were also detected by the analytical procedure employed.

Highest levels of ASPAH were found in crabs from the heavily industrialized Hampton Roads area of the James River. Lower concentrations were detected in crabs from the Pocomoke Sound, the upper James and the Rappahannock River sampling sites. PCB levels were elevated in samples taken from both James River stations.

Levels of TOP were generally higher in crabs sampled in June than in September of 1981.

Concentrations of pollutants in the tissues and tissue lipid content were correlated. Highest levels of both were detected in the hepatopancreas, followed by egg, ovary and muscle.

TOP levels in field samples and in-vivo biotransformation of the model compound benzo(a)pyrene (B(a)P) in the hepatopancreas, during in-laboratory experiments, suggested that differences may exist in the uptake and elimination of pollutants as a function of the sex and maturity of the crabs. It appeared that the metabolism of aromatic hydrocarbons varied with the molt stage of the crabs.

A variety of polar metabolites of tritium labeled B(a)P were detected in the hepatopancreas of blue crabs, when examined by normal and reverse phase high performance liquid chromatography.

In conclusion, to accurately assess the levels and effects of TOP in biota from the field, several factors must be considered. Among these are the species and tissues examined, the sex, maturity and condition of the individuals selected for analysis, and the location and season of sampling.

ACCUMULATION OF TOXIC ORGANIC POLLUTANTS IN THE BLUE CRAB *CALLINECTES SAPIDUS*

I. INTRODUCTION

Prior to the industrial revolution, when the human population was low and its byproducts were relatively nontoxic and degradable, serious environmental degradation from chemicals was rare and localized. However, with the exponential growth of the population chemical pollution in many areas has reached formidable proportions.

Aquatic systems have long been used as receptacles of waste. Unwanted materials were discharged into waterways to be carried away, and diluted by the currents. Oceans were seen as having unlimited assimilative capacity in which pollutant levels could never become a problem. As the population grew, the shortcomings of these philosophies became evident. Communities downstream of waste dischargers reaped the rewards sown by those upriver. Fish kills and oil slicks appeared in isolated areas of the oceans. Especially affected were the estuaries, transition zones between freshwater and marine habitats, and foci for man's activities due to their value as ports, transportation routes and fishing grounds. They function as nursery and spawning areas for many types of biota and often contribute substantially to the productivity of the surrounding coastal areas. An important factor supporting this richness is the ability of the estuary to act as a sink or trap for nutrients and other materials that enter it. Unfortunately this property applies to pollutants as well.

An estuary exhibiting the characteristics described above is the Chesapeake Bay located on the east coast of the United States (Figure

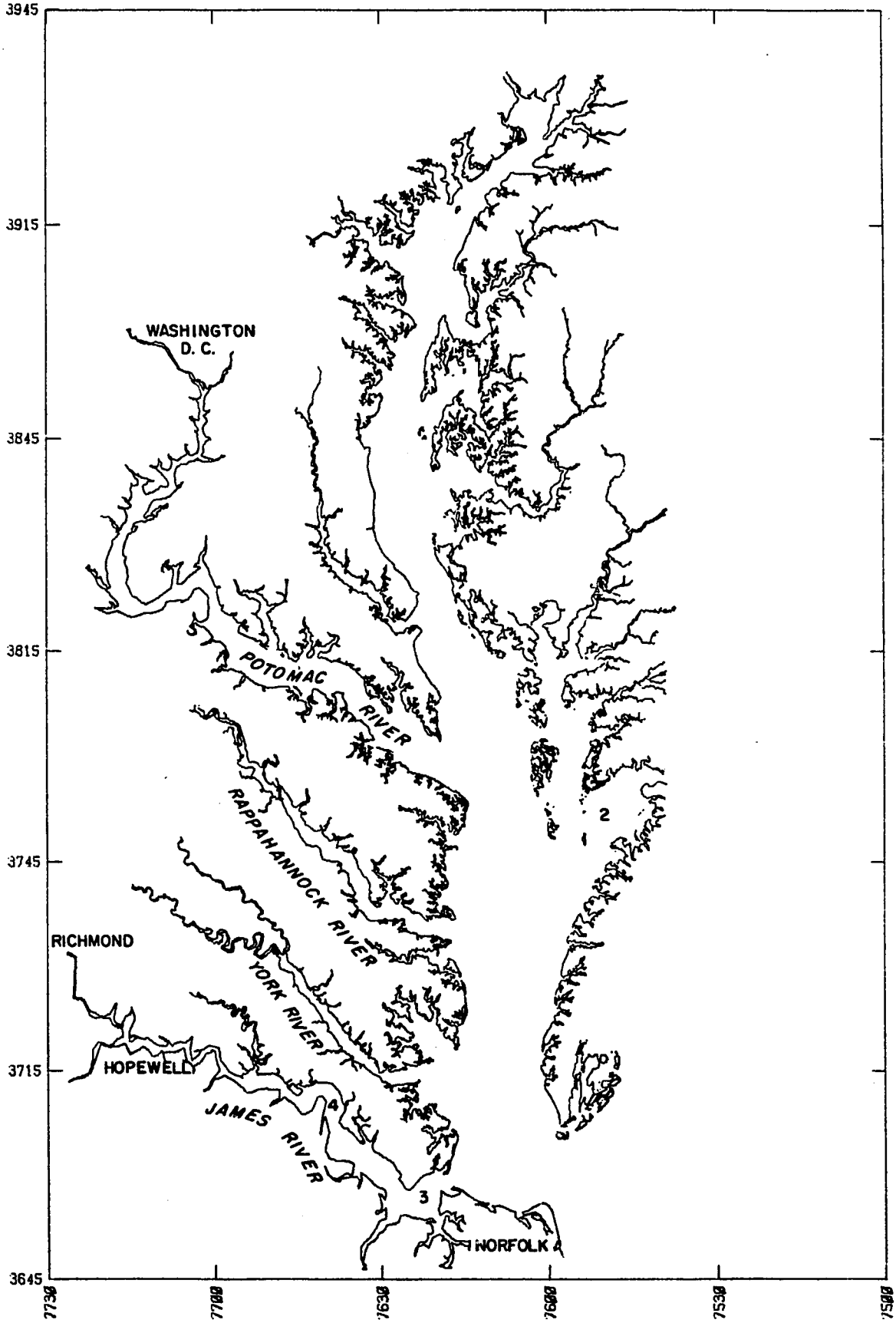
1). It and its tributaries are bordered by several large population and industrial centers, e.g., Washington, D.C., Norfolk, Va., and Baltimore, Md. Effluents that enter the Bay from these sources include storm sewer discharges, industrial wastes, sewage treatment plant discharges and losses from fossil fuel production, usage and shipment. Runoff from agricultural areas contribute significant loads of sediment, nutrients and pesticides.

This work focuses on those components of the effluent which are persistent anthropogenic organics exhibiting moderate to high toxicity. These toxic organic pollutants (TOP) encompass several classes including the polynuclear aromatic hydrocarbons (PAH), the polychlorinated biphenyls (PCB), and the chlorinated pesticides.

Much work has centered on the acute toxicities of TOP to biota. Substances responsible are the lighter water soluble components (Rice et al., 1975; Templeton et al., 1975).

Interest recently has begun to focus on the effects due to chronic exposure, particularly to the persistent water insoluble moieties. Research has shown that the polynuclear aromatic hydrocarbons, polychlorinated biphenyls, and the chlorinated pesticides accumulate to high levels in the tissues of marine organisms (Ellgenhausen et al., 1980; McLeese et al., 1980; Chiou et al., 1975). This accumulation is due to the nonpolar nature of these pollutants which associate, via weak Van der Waal forces, with biological lipids. In addition, an increase in entropy is experienced upon partitioning of the hydrophobic substances from the water to the lipids. This is due to the high degree of ordering of the water molecules, thus low entropy, required for the dissolution of the

Figure 1. The Chesapeake Bay Estuary is depicted. Sampling locations are shown: (1) Upper Rappahannock River; (2) Pocomoke Sound; (3) Lower James River; and (4) Upper James River.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nonpolar substance in water compared to lipids (Ben-Naim, 1974). The tendency to accumulate in the lipids has been quantified (Ellgenhausen et al., 1980; Veith et al., 1979) by means of an octanol/water partitioning coefficient. This coefficient estimates the relative solubilities of the hydrophobic compound in question in the two substances. The octanol serves as a surrogate for the biotic lipids.

Sequestering these compounds into the lipid reserves minimizes their availability to deleteriously interfere with the normal metabolism of the organism. Reinert and Bergman (1974) found that when the lipid stores contained within the fatty tissues of fish decreased, DDT residues redistributed themselves among the lipids of the nervous system causing death.

In addition, TOP partition from the water into aquatic sediments (Ho and Karim, 1978; Baker, 1980). As in biotic lipids, the bonds involved are generally weak. However, once again the decrease in water ordering and subsequent increase in entropy of the system determine the position of the equilibrium. Although this sorption may decrease the bioavailability of the relatively insoluble compounds, the sediments may act as a reservoir, releasing pollutants to the overlying water for many years (Fletcher et al., 1981; Macko et al., 1981; Varanasi and Gmur, 1981).

Biomagnification of pollutants along foodchains has also been examined (Ellgenhausen et al., 1980; Thomann, 1981; Petrocelli and Anderson, 1975). Most early work suggested that lipophilic compounds accumulated to higher and higher levels as one moved up the food chain. However, numerous exceptions have been noted. It is now

generally felt that individual relationships must be examined more closely.

Levels of TOP have been monitored in a variety of aquatic organisms around the world (Anderlini et al., 1981; Amico et al., 1979; Basturk et al., 1980). Many methods have been employed for the identification and quantification of these compounds. The most popular methods use organic solvents to extract the TOP from tissues, followed by some form of chromatography to separate the complex mixture of pollutants from biogenic compounds. Some of the detection systems utilized in these studies include: flame ionization and electron capture (Bjorseth and Eklund, 1979; Chesler et al., 1978), electron impact and chemical ionization mass spectrometry (Dahlgren and Abrams, 1982; Kuehl, 1977) and spectrophotometric detectors (Krahn et al., 1981; Litten et al., 1982).

Although lipophilic pollutants appear to accumulate in most of the aquatic organisms examined; the levels, types and toxicity of the compounds are a function of many factors. The results of any monitoring study are a function of the species and locations sampled.

Molluscs have commonly been used as monitoring organisms in pollutant studies due to their sedentary existence and poor ability to biotransform xenobiotics (e.g, Farrington et al., 1983). The purpose of those studies was more to determine the distribution of the pollutants in the environment than to examine organism-pollutant interactions, as in the present research.

In this study the blue crab, Callinectes sapidus, was chosen for monitoring since it is an important, wide-ranging estuarine organism

and supports a valuable commercial fishery on the East and Gulf coasts of the United States.

Many factors influence the bioaccumulation of xenobiotics. For example, the maturity and size of the organism influence characteristics such as the lipid content, surface area and habits of the animal (Wharfe and Van den Broek, 1978). Seasonal effects have been shown to be important (Fletcher et al., 1981; Edgren et al., 1981; Fossato and Canzonier, 1976). Sex and condition of the specimen may have a profound influence (Phillips, 1978; Hubert and Ricci, 1981) on the accumulation of pollutants. These may, in the case of the blue crab for example, involve mobilization of lipid stores and subsequent sequestering and elimination via the eggs. This was found to be the case by Roberts and Leggett (1980) with the compound Kepone.

Differences in biotransformations of xenobiotics as a function of season and sex have been observed by Chambers and Yarbrough (1976). Migration patterns of the biota may affect the exposure of mobile organisms to pollutants (Pastel et al., 1980). This is possible in the case of the blue crabs inhabiting the Chesapeake Bay. Also, the choice of tissues examined is crucial to the results obtained depending on the route of entry, subsequent binding and storage, and ultimate biological fate of the pollutants (Minchew et al., 1980; Sheridan, 1975; Mix et al., 1982). Many pollution studies neglect the above mentioned parameters (Huggett, 1981).

To conduct a study of pollutant loads in the blue crabs of the Chesapeake Bay, one must understand the basic life history of the organism. The biology of the crab has been reviewed by several authors (Tagatz, 1978; Van Engel, 1958; Williams, 1965). The

crustacean is able to tolerate a wide range of temperature and salinity (Tan and Van Engel, 1966). Callinectes sapidus inhabits polyhaline to oligohaline coastal areas from Nova Scotia to Uruguay (Williams, 1965). It is an omnivore, consuming fish, plant material, molluscs and other crustaceans (Laughlin, 1982).

In the Chesapeake Bay, eggs develop in the high salinity water at the mouth of the Bay and hatch into zoeae after about 11 to 14 days (Sandoz and Rogers, 1944). These undergo six to eight molts, passing through seven larval stages and then metamorphose into megalopae, a more crablike form (Costlow, 1965). This stage lasts from one to three weeks, and is followed after a molt by the juvenile Callinectes stage. Environmental factors, e.g., temperature, salinity, food availability and water movements are crucial for the survival of the larvae and recruitment of juveniles to the estuary (Sulkin et al., 1980). Larvae are particularly susceptable to the toxic effects of pollutants such as Kepone and methoxychlor (Bookhout, 1976; 1980). Although the number of eggs spawned by a single female may exceed 2,000,000 only two of these on the average will survive to become mature crabs (Van Engel, 1958).

The male Callinectes sapidus continues to molt and grow throughout its life span, believed to be a maximum of three years. However, the female undergoes a terminal maturing molt, thus preventing further growth. Only at the time of this last molt, when the female is in the soft crab stage, can it mate. Sperm is stored by the female and, although mating usually occurs in July through September, eggs normally are not extruded until the following spring. Additional spawnings by a single crab have been reported (Williams,

1965). Eggs are attached to the female on setae on the posterior appendages, the swimmerets, and remain there until hatching. The egg clumps, or sponges, initially appear orange in color. As development continues the yolk is utilized and the eggs become dark brown or black in color.

As noted, larval development requires high salinity water. Females, after mating in the low salinity reaches of the rivers feeding the Chesapeake or the upper Bay itself, migrate to the Bay mouth where the eggs are extruded. The larvae exhibit a planktonic form of existence, but the juveniles are believed to utilize the bottom currents in their migration towards the lower salinity areas, where they continue their growth (Sulkin et al., 1980).

In that many pesticides have been formulated to control insects, it is not surprising that nontarget arthropods, such as the crustaceans, are particularly sensitive to their effects (Schimmel et al., 1979).

TOP express their toxicity via a number of physiological mechanisms. A common mode is through damage to cellular membranes (Hutchinson et al., 1979; Bastian and Toetz, 1982; Packham et al., 1981). This is reasonable, as the membranes consist largely of lipids. Symptoms of pollutant exposure have been reviewed by Sindermann (1979) and Meyers and Hendricks (1982), these include skeletal anomalies, neoplasia, genetic abnormalities, ulcers, fin rot and shell erosion. The causes may be the direct action of the pollutants or indirect, e.g., lowering of resistance and subsequent viral or bacterial attack due to immune response suppression.

Alterations of liver and pancreatic tissues of fish have been noted following exposure to various Arochlor mixtures (Hansen et al., 1974; Freeman et al., 1982) and crude oil extracts (Eurell and Haensly, 1981). Hepatocellular lipid vacuolization and damage to gill membranes have been observed (Malins, 1982). Benzo(a)pyrene, naphthalene and Arochlor 1254 disturbed neurotransmitter levels in channel catfish (Fingermann and Short, 1983). Maslova (1981) showed that DDT altered cholinesterase activity and Ghiasuddin and Matsumura (1979) noted that Ca^{++} regulation in crustacean nerves was also affected. Detrimental effects of TOP on developing eggs and young of aquatic organisms have also been observed (Hose et al., 1982; McKeown, 1981).

Changes in behavioral responses, such as reflexes, by DDT were shown by Gdovsky and Flerov (1979) to occur in fish. Such alterations may put organisms at a disadvantage in predator/prey relationships (Stehn et al., 1976). Pheromone communication is important in the reproductive behavior of blue crabs (Gleeson, 1980); it is hypothesized that petroleum may interfere with the ability of the crab to detect the chemical signals.

Not only is the damage done by TOP serious and the number of dangerous substances immense, the possibility for interactions between the various components of the pollutant load are likely (Conney and Burns, 1972).

As mentioned, the potential for harm by pollutants is lessened by sequestering them into lipid storage sites. Organisms may also excrete them unaltered via the feces, urine, respiratory and

integumentary surfaces (Harding et al., 1981; Lee et al., 1976; Varanasi and Gmur, 1981).

Another method of minimizing the burden of bioaccumulated compounds is by biotransformation of the nonpolar xenobiotics to water soluble metabolites and subsequent excretion. An important mechanism by which this is accomplished is via the mixed function oxygenase enzyme system (MFO). This system has been most thoroughly studied in mammals in relation to the metabolism of drugs. Later it was found that the MFO system biotransformed a wide range of compounds, including organic pollutants. Current work suggests that the enzyme system consists of a closely related family of enzymes each exhibiting a specificity for a select group of substances (Paine, 1981).

At first it was believed that the oxygenase system was absent in aquatic biota. However, recently it has been found in bony fish (Stegeman, 1977), elasmobranchs (James and Bend, 1980), polychaete worms (Lee and Singer, 1980), molluscs (Moore et al., 1980), echinoderms (Malins and Roubal, 1982) and crabs (Singer and Lee, 1977). An explanation of this disparity involves the lower activity of the system in aquatic biota compared with terrestrial forms hinging perhaps on the requirements of the latter to conserve water, while the former may excrete a dilute urine (Paine, 1981). In addition, the optimal assay conditions for the two groups differ, this resulted in underestimates of activity in aquatic forms when methods designed for mammalian systems were used.

The system is responsible for a range of oxidative transformations, among these: hydroxylation, O-dealkylation, N-dealkylation, deamination, sulphoxidation and N-oxidation (Paine,

1981). The mixed function oxygenase system in invertebrates consists of the cytochrome P-450 (a haem containing protein), phospholipid and NADPH cytochrome P-450 reductase (Lee, 1981). It requires oxygen, Mg^{++} as a cofactor, and a substrate to be oxidized, such as benzo(a)pyrene (B(a)P).

Basicly, the substrate reacts with the oxidized P-450, (Fe^{+++})-P-450, to form the complex (Fe^{+++})-P-450-substrate. This complex is reduced by an electron from NADPH cytochrome P-450 reductase. The product of this reduction is (Fe^{++})-P-450-substrate.

The reduced complex reacts with oxygen and another electron, thought to come from NADPH, to form an unstable superoxide intermediate (Lee, 1981). The intermediate dissociates to form water, oxidized P-450 and hydroxylated substrate.

A host of positional isomers may be produced by the incorporation of the hydroxyl (Solbakken et al., 1980). The hydroxylated product may be conjugated with various indogenous substrates, such as glucose, glucuronic acid, glutathione and sulphate, forming water soluble, easily excreted moieties (Malins, 1977; Chambers and Yarbrough, 1976). Conversely, the primary metabolites may be further oxidized by the MFO system to diols, quinones, triols, tetrols or combinations of these.

The hydroxylated metabolites exhibit mutagenic and cytotoxic potential (Selkirk and McLeod, 1982; Gmur and Varanasi, 1982). In addition, certain of these are easily metabolized to the more oxidized forms, some of which are extremely mutagenic. Thus, elucidation of the metabolite profiles is critical for the assessment of the toxic potential of procarcinogens. This has important implications for the

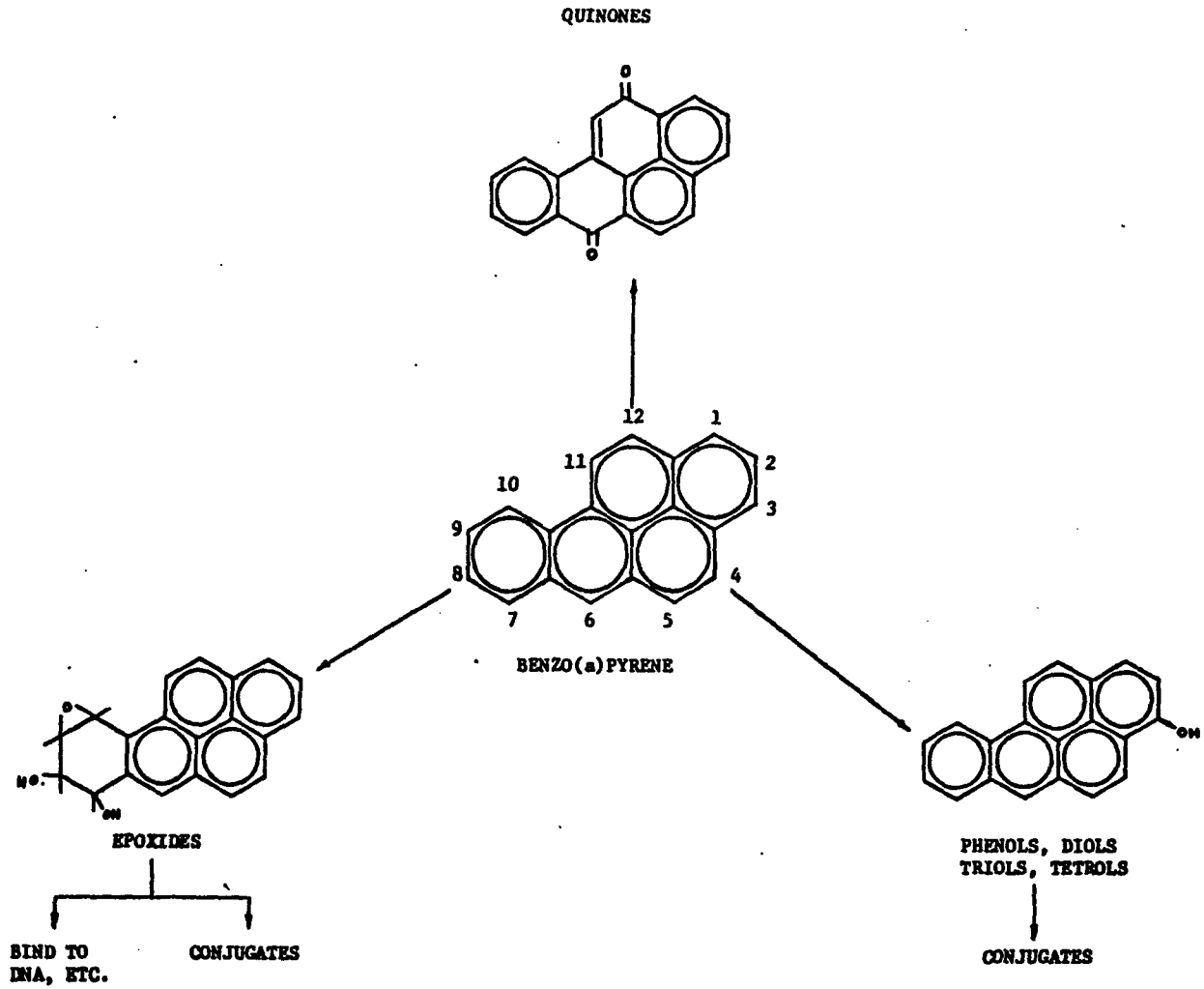
aquatic organisms as well as their human consumers. The enhanced toxicity of the metabolites compared with the parent compounds is, initially, surprising. It is probable that during the evolution of the system exposure to pollutants was negligible. Therefore, the oxygenases were not prepared for the onslaught of chemicals they now encounter and are performing a function they were not originally designed for. As a result, they may generate products that are detrimental to the survival of the organism.

As an example, benzo(a)pyrene is rather inert in its unmetabolized form. However, it is lipophilic and bioaccumulated as are other aromatic hydrocarbons. It may be metabolized to the 7,8-diol-9,10-epoxide which is an extremely potent mutagen. The mechanism of mutagenesis is believed to involve an electrophilic attack by a carbonium ion, formed via the opening of the strained epoxide ring, on an electron rich species, such as DNA (Selkirk and McLeod, 1982). Varanasi et al., (1982) demonstrated that metabolites of B(a)P covalently bound to proteins and DNA of English sole, Parophrys vetulus. The pathway and several of the possible products of B(a)P metabolism are shown in Figure 2.

Much work has been done with mammalian systems but little utilizing invertebrates and most of them using in-vitro methods. Although in-vivo experiments are more difficult to control, they are valuable since intact cell metabolism of substrates has been shown (Selkirk and McLeod, 1982) to differ from that in cell free in-vitro systems.

Elevation of enzyme activity following exposure to substrates has been noted in terrestrial organisms and fish (Paine, 1981; Nava

Figure 2. Typical products of the <u>in-vivo</u> biotransformation of benzo(a)pyrene. These include B(a)P phenols, quinones, epoxides, diols, triols, tetrols and conjugates.


QUINONES
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11
2
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9
8
4
7
6
5
BENZO(a)PYRENE
O
HO
OH
EPOXIDES
BIND TO DNA, ETC.
CONJUGATES
OH
PHENOLS, DIOLS TRIOLS, TETROLS
CONJUGATES

and Engelhardt, 1982). This phenomenon has important implications since it may allow organisms inhabiting polluted areas to more quickly purge themselves of lipophilic compounds (Spies et al., 1982). Induction has been noted in human cigarette smokers (Marshall et al., 1979). Conversely, it may make the organism more prone to cancers due to the production of electrophilic metabolites.

Workers employing mammalian systems have classified enzyme inducers into two groups: the phenobarbital and the polynuclear aromatic hydrocarbon types. The former includes many of the drugs and pesticides in use today (Matthews and Kato, 1979). The latter are also known as the methylcholanthrene inducers. When originally discovered, the MFO system was thought to contain a single cytochrome, P-450, so named because of its absorption of ultraviolet (UV) light at 450 nm. However, it is now believed that at least two separate forms exist, corresponding to the inducer classifications mentioned above. More detailed work shows that an absorption maximum may occur at 448 nm, indicating the presence of a second form of the cytochrome. This cytochrome, P-448 is thought to be that responsible for the metabolization of the PAH and the very toxic dioxins. The PCB exhibit a mixed response due, in part, to the number and variety of isomers that occur (Paine, 1981). The toxicity of PCB and dioxins has been correlated with their ability to induce the MFO enzyme system (Stallings et al., 1979). The strongest inducers, e.g., the tetrachlorodibenzodioxins, are some of the most toxic compounds known (Safe et al., 1982).

Many chlorinated compounds exhibit low water solubility and high bioaccumulation. This accumulation is selective in the PCB series.

The more chlorinated members are preferentially retained due to their lower water solubility and the difficulty associated in the biotranformation of the sterically hindered sites (Guiney and Peterson, 1980; McLeese et al., 1980; Hansen et al., 1976).

Increases in activity caused by the phenobarbital and the methylcholanthrene inducers have not always been differentiated in aquatic species (Spies et al., 1982). Some species of marine organisms exhibit no detectable increase in MFO activity after exposure to either type of inducer (Malins, 1977). Lee (1981) reported spectral changes suggesting induction in blue crabs after exposure to Arochlor 1254. Induction in the polychaete, Nereis virens, (Lee et al., 1981) and channel catfish, Ictalurus punctatus, (Fingerman et al., 1983) have been reported. Obviously, different forms or stages of development of the system exist among aquatic organisms.

In addition to biotransforming lipophilic xenobiotics to more polar water soluble moieties, MFO is responsible for performing many oxidative reactions involving endogenous compounds such as steroid hormones (Blumberg, 1978). It is for this function that the enzymes are thought to have evolved.

The molting hormone in the blue crab is believed to be B-ecdysone (Krishnakumaran and Schneiderman, 1970; Horn et al., 1968; Borst and O' Connor, 1967). The same substance is synthesized in insects by the hydroxylation of ecdysone by the cytochrome P-450 system (Richards, 1981).

As mentioned, crabs possess the MFO system and are capable of biotransforming xenobiotics to more polar metabolites (Corner et al.,

1973; Lee et al., 1976). High levels of MFO have been found utilizing in-vitro assays of stomach tissue from male and female blue crabs (Singer and Lee, 1977). High activity has been reported in the green gland of Callinectes sapidus. Oxygenase activity in this organ varies as a function of the ecdysial cycle. At the time of ecdysis activity is considerably lower than that found in the premolt condition. However, it quickly increases again after molting to levels exceeding that found prior to the event (Singer and Lee, 1977). This correlation of the molt cycle to fluctuations of MFO activity, coupled with the research mentioned previously concerning the link between cytochrome P-450 and ecdysone production, may help explain the high toxicity of TOP to juvenile crustaceans (Singer and Lee, 1977). It may also explain why aromatic hydrocarbons and PCB have been implicated as agents preventing limb regeneration, decreasing the growth increment and inhibiting molting (Fingerman and Fingerman, 1977, 1979, 1980; Cantelmo et al., 1981).

Finally, in-vivo work (Lee et al., 1976) suggests that the hepatopancreas, a storage and digestive organ in the crab, is the major site of PAH storage and metabolism. It has been reported that the in-vivo assays utilizing hepatopancreas microsomes showed lower activity than those of green gland due, perhaps, to the presence of endogenous inhibitors, present in the form of digestive fluids (Singer and Lee, 1977).

OBJECTIVES

The objectives of the research reported here were:

1) To examine the levels of toxic organic pollutants in the blue crabs populating Virginia waters.

2) To determine if the life history and biology of the blue crab exerts a significant influence upon the levels of pollutants detected in the field samples collected. Parameters considered include: sex, maturity and spawning condition. In addition, to examine the distribution of TOP among the tissues of the crabs.

3) To determine whether the biotransformation of pollutants is a function of the sex, maturity and molt stage of the crab.

4) To examine elimination rates and metabolite profiles in order to understand the pharmacokinetics of persistent organic xenobiotics in these organisms.

II. TOXIC ORGANIC POLLUTANTS IN BLUE CRABS COLLECTED FROM THE FIELD

A. METHODS

Major field sampling of blue crab populations for toxic organic pollutants occurred in June and September 1981, during the Virginia Institute of Marine Science (VIMS) Crustaceology Survey of Virginia waters, utilizing a 30 foot semi-balloon otter trawl equipped with a 1/4 inch liner and a tickler chain. Crabs collected were separated by sex and maturity criteria. Males, adult females and juvenile females were recognized by the shape of the abdominal carapace; T-shaped, rounded, and triangular, respectively. The maturity of males was ascertained by examination of the proximal segments of the abdomen: they are tightly secured in the immature crab. A fifth group contained female crabs with attached egg masses (berried crabs). All specimens utilized in this portion of the project were in the intermolt condition. Supplementary samplings of adult male crabs from the lower James River station were conducted in July, August, October and November 1981.

The entire hepatopancreas and a subsample of the body musculature were removed from each crab. Ovaries were removed from adult females and egg masses saved from berried crabs. Each sample analyzed consisted of tissues pooled from up to 15 crabs of each of the above mentioned classifications, if sufficient specimens were available. Sirota et al. (1983) determined that PAH concentrations exhibited considerable variability from individual to individual in lobster (Homarus americanus) exposed to creosoted timber. Therefore

it was felt that the pooled sample approach would provide the most representative view of the pollutant levels in the crabs present in the field. This sampling regime was repeated at the sampling locations shown in Figure 1. Stations chosen represent diverse geographical areas subject to a range of anthropogenic inputs. These included: the lower James River, one to five miles from its mouth (an area of high industrial activity); the upper James, 23 to 27 miles from its mouth (located between Norfolk and Richmond, and encompassing the turbidity maximum of the James River Estuary); the Rappahannock River, 25 to 35 miles from its mouth; and the Pocomoke Sound, an area on the bay side of the eastern shore of Virginia (hypothesized to be low in persistent pollutant input).

All glassware utilized in this work, prior to usage, was washed in soapy water, rinsed with tap water, deionized water, 3N HCl, and again with deionized water. It was then rinsed sequentially with toluene, methanol, acetone and methylene chloride. Glassware was dried in an oven at 400^{o}C after rinsing. All solvents were purchased from Burdick and Jackson, Muskegon Mi., and rated for pesticide analyses.

Procedures used in the chemical analysis of the samples followed closely that employed by Bieri et al. (1982) for the determination of toxic organics in oysters. After thawing, samples were ground in a Virtis Tissue Homogenizer. The addition of a small amount of methylene chloride extracted water was necessary for homogenization of the muscle samples.

The homogenates were transferred to stainless steel pans and wet weights of the tissues obtained, after which the samples were refrozen

in preparation for freeze-drying. Wet weights of muscle tissues were determined prior to homogenization.

Samples were lyophilized for 36 hours in a freeze-dryer and reweighed to determine the percentage of water in the various tissues. The dry homogenates were broken up and a subsample removed from each and charged to extraction thimbles. It was found that if a sample was finely ground the volume of the tissues was reduced and the glass frits of the thimbles quickly became blocked by fines during extraction. Initially up to eight grams of tissue were used from each sample. However, this was reduced to approximately two grams in subsequent work for tissues of high lipid content such as hepatopancreas, ovary and egg.

The thimbles were placed in soxhlet extractors equipped with 500 ml round bottom flasks, filled with 300 ml methylene chloride and gently refluxed for 24 hours with electric heating mantles. Preliminary work revealed that no significant increases in yield were obtained by extracting for longer periods. Extraction efficiency was approximately 70%, based on yields of aromatic standards, S9 (phenyl ether, dibenzothiophene, atrazine, dibutyl phthalate, malathion, fluoranthene, o,p′-DDD, benzo(e)pyrene and decachlorobiphenyl) and S3 (2,2′-binapthyl, p-quaterphenyl and decachlorobiphenyl). The relatively low efficiency was a function of the varied compound types targeted for examination. Results were not corrected for recovery.

The extract was reduced in volume on a rotary evaporator and the concentrate and several methylene chloride rinses of the extraction flask transferred to a graduated centrifuge tube. The volume in the tube was blown down in a water bath with purified nitrogen to nine ml.

All water baths used were maintained at 40°C. Three ml of the extract was transferred to a preweighed disposable aluminum pan, the solvent allowed to evaporate overnight, and the residue weighed to assess the amount of extractable lipids present in the tissues.

Due to the relatively high levels of biogenic material coextracted with the toxics, cleanup steps were necessary prior to gas chromatography so that the low level TOP were not obscured.

The remaining 6 ml of sample were picked up in a syringe and injected onto a gel permeation chromatographic column (GPC; Autoprep Model 1001; Analytical Bio Chemistry Laboratories, Inc.) equipped with a 5.4 ml injection loop. Biobeads S-X8 resin was employed in the column. Methylene chloride eluted the sample components. Elution volumes were determined with aromatic standard mixtures (S9, previously described, and S8 consisting of naphthalene, biphenyl, hexamethylbenzene, phenanthrene, pyrene, chrysene, perylene and benzo(ghi)perylene). Retention differed from column to column depending on the batch and packing technique.

In GPC, separation is a function of molecular size. Small molecules are able to enter the pores of the resin and are eluted last. Compounds of relatively high molecular weight, such as certain biogenic molecules, pass through the column quickly. Thus these molecules may be separated from the compounds of interest.

The volume containing the toxic organic compounds was collected, reduced in volume on a rotavap, transferred with solvent rinses to a graduated tube and blown down with nitrogen to a volume of 0.2 ml.

The "cleaned" sample was injected on a high performance liquid chromatograph (HPLC) containing a normal phase preparative column

(Whatman Partisil-10 PAC Magnum 9; amino and cyano functional groups bonded to silica gel). The program used is shown in Figure 3A. Solvent A was hexane and Solvent B isopropanol. This program allowed the separation of the aliphatics and polar compounds, not examined in this phase of the project, from the desired aromatics. The aromatic fraction was collected, reduced to 0.2 ml as previously described, diluted to 0.5 ml with toluene and blown down with nitrogen to 0.2 ml.

The sample was then ready for injection onto a Varian 2700 gas chromatograph (GC). These GC contained injection port splitters and were modified to accept glass capillary columns. The columns were constructed at VIMS and coated with SE 52 stationary phase according to the procedures outlined by Grob (1979) and Grob et al. (1982). Detection was by flame ionization (FID). Detector temperature was 280°C and the injection port was maintained at 275°C. Chlorinated compounds were also quantitated by FID after identification by GC/MS. Although not as sensitive as electron capture detection, it was possible to detect several PCB isomers and metabolites of DDT. Response of the detector to these compounds is a function of the degree and pattern of chlorination. Reported response factors of the pollutants detected varied but generally were 0.5 or more, relative to the response exhibited by biphenyl (Hutzinger et al., 1974; Onuska and Comba, 1980). Carrier gas was helium, detector gases were hydrogen and air. Chromatographs were equipped with septum purges and makeup gas capability. All gases employed were high purity. Flows varied depending on the column and GC used and were adjusted for optimum response.

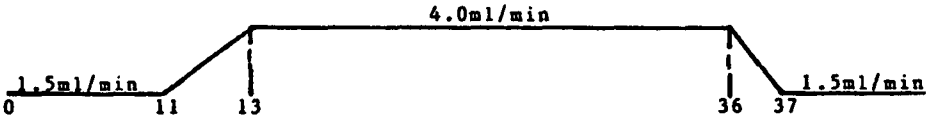
Figure 3. HPLC programs utilized for the separation of lipophilic pollutants from polar biogenic compounds (A), and for the identification of radiolabeled B(a)P metabolites (B).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
Employing a Whatman Partisil-10 PAC Magnum 9

A)PROGRAM USED IN THE ANALYSIS OF FIELD SAMPLES FOR TOP.

Solvent A, n-Hexane Solvent B, Isopropanol

Flow Rate
Initially:
1.5ml/min

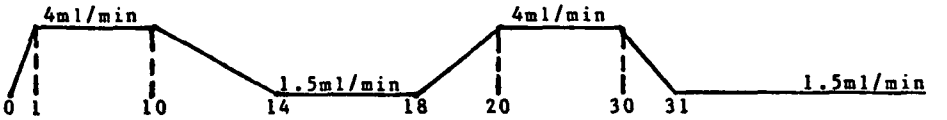


Solvent
Composition
Initially:
0 %B

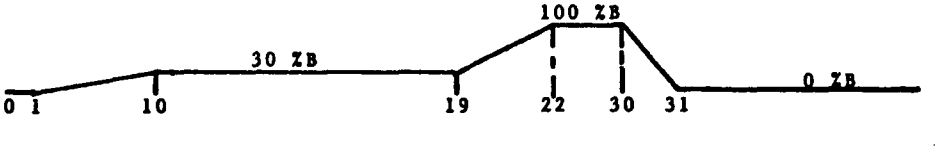
B)PROGRAM USED FOR THE ANALYSIS OF B(a)P METABOLITES

Solvents: A, n-Hexane B, 50/50 (v/v) isopropanol/methanol

Flow rate:
Initially
1.5ml/min



Solvent
Composition:
Initially
0 %B



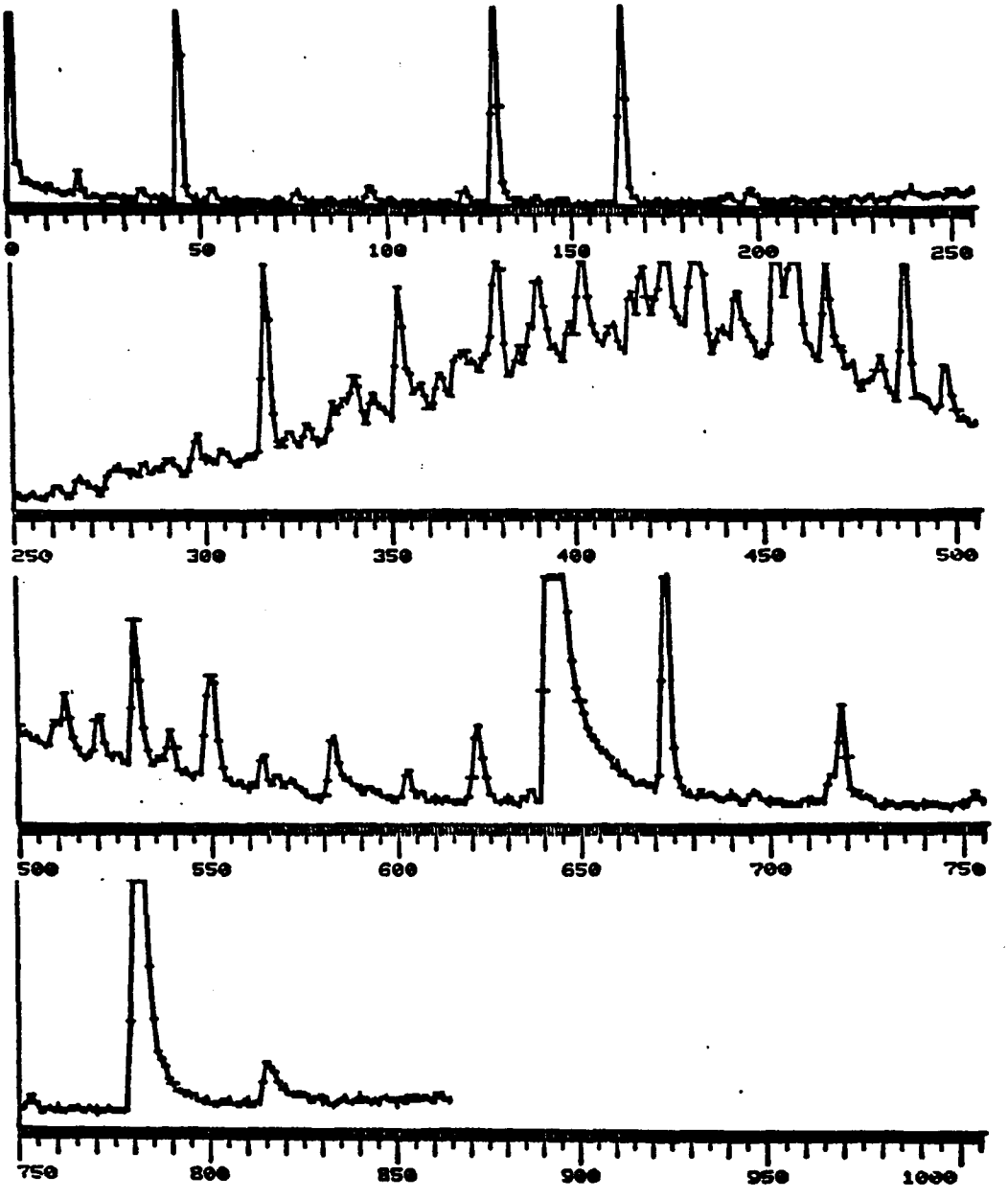
All injections employed the hot needle technique described by Grob and Rennhardt (1980) and were injected in the splitless mode. The splitter was opened after the solvent front passed through the column, flushing the injection port. Column oven temperature programming was employed extending from 75°C to 280°C at 6°C/min.

Initial identification of compounds in selected samples was accomplished with the use of a Varian 2700 GC, with a capillary column, coupled to a Dupont Model 21-492B mass spectrometer and 21-094B Data System. The temperature program described above was employed for the GC/MS runs. Electron impact (70 electron volts) was the chosen method of fragmentation. A reconstructed ion chromatogram of a sample is shown in Figure 4.

Samples were chosen for GC/MS characterization based on their complexity, and included two from both the lower and upper James River and one from both the Rappahannock River and Pocomoke Sound sites. Aromatic retention indices (ARI) for the compounds in the GC/MS runs were assigned based on their retention time in relation to those of the S8 standard components (Bieri et al., 1980). The indices were used to identify the compounds in the remaining samples, which were coinjected with S8 standard and 1,1′-binaphthyl for quantitation. Data from the GC were recorded on a Houston Instruments strip recorder as well as digitized by A/D converters and fed to a HP 3354B data system for data reduction and presentation.

Statistical analyses were conducted on a Prime 850 computer utilizing software programs from the Statistical Package for the Social Sciences, Second Edition, (Nie et al., 1975).

Figure 4. Reconstructed ion chromatogram of a hepatopancreas sample from adult female blue crabs from the upper James River. Sample was cleaned up as described in the text prior to injection into the GC/MS.


0
50
100
150
200
250
250
300
350
400
450
500
500
550
600
650
700
750
750
800
850
900
950
1000

B. RESULTS

As noted in the methods section, sponge crabs were to be collected from four locations: an upper James River, lower James River, Rappahannock River and a Pocomoke Sound site. Unfortunately adult females with extruded eggs were impossible to obtain at all the required stations and dates. Insufficient numbers preclude detailed statistical analysis of this group although trends are noted.

Table 1 presents the compounds identified in the samples selected for GC/MS characterization. Eighty two compounds were identified from the mass spectra (this figure includes isomers). Eight more were characterized by their molecular weight and alkylation structure but were not positively identified. Twenty nine compounds in the table could not be identified by their mass spectra but appear to be of possible anthropogenic origin. Additionally, two chlorinated compounds were observed which could not be identified. Numerous biogenic compounds were still present in the purified extracts but these were not of interest in this research.

Compounds identified were grouped into categories, based on probable source and chemical characteristics, for statistical analysis. Alkyl substituted polynuclear aromatic hydrocarbons (ASPAH), most likely of a petroleum derived origin, were summed in one such group. Only those compounds positively identified and present in quantities exceeding 5 ug kg^{-1} were included in this sum. Results of this are shown in Table 2. Another group contained the polychlorinated biphenyls. The nonsubstituted polynuclear aromatic hydrocarbons, probably of a pyrolytic origin, were not present in high

TABLE 1

Compounds identified in selected samples by GC/MS and their relative retention indices. The three most abundant fragment ions in the mass spectra of the unidentified compounds are given. The aromatic retention indices have been calculated by interpolation and normalization of compound retention time with that of the retention times of the two encompassing S8 standard components.

AROMATIC RETENTION INDEX	COMPOUND NAME
-10.9	Benzaldehyde
0	Naphthalene (S8 Standard)
5.5	Unknown (91,77,103)
10.6	Unknown (57,70,91)
20.0	Benzothiazole
37.0	Cis-4-phenyl-3-butene-2-one
38.8	Unknown (85,145,103)
50.2	Unknown (95,109,180)
74.0	Unknown (152,147,54)
90.0	Trans-4-phenyl-3-butene-2-one
92.8	Unknown (85,55,56)
100	Biphenyl
119.3	Hexamethylbenzene (S8-Standard)
126.3	Acenapthene
135.9	Dibenzofuran
137.6	Bibenzyl
140.0	C3-Naphthalene
140.6	Unknown-sulfur compound
144.2	C3-Naphthalene
148.4	C3-Naphthalene
152.9	Fluorene/C3-Naphthalene
153.9	C3-Naphthalene
160.8	Unknown (71,111,56)
163.6	1,1'-Biphenyl-2-amine
173.8	Unknown (177,248,191)
176.2	C4-Naphthalene
178.8	C2-168
178.9	C5-Naphthalene
182.8	1-Methyl-fluorene
185.0	C2-Dibenzofuran
185.4	C3-154/C2-Dibenzofuran
186.0	Unknown (82,169,105)
190.3	C4-Naphthalene
192.0	C2-Dibenzofuran
193.5	Dibenzothiophene
193.6	C3-154/C2-Dibenzofuran
200	Phenanthrene (S8-Standard)
207.2	Unknown (253,331,254)
208.0	Unknown (165,92,210)
212.7	C2-Fluorene
213.5	C3-Acenaphthene/C2-Fluorene
215.3	C2-Fluorene
217.2	C2-Fluorene
220.9	C2-Fluorene
223.8	Methyl-dibenzothiophene
226.1	C4-Fluorene
230.1	C4-154/C3-Dibenzofuran
230.5	Methyl-dibenzothiophene
234.6	Methyl-dibenzothiophene
237.1	3-Methyl-phenanthrene
238.6	Methyl-phenanthrene
243.3	Methyl-178/C5-154/C4-Dibenzofuran
245.3	Methyl-phenanthrene
245.4	Unknown (105,192,179)
249.9	C3-Fluorene
254.1	C5-Fluorene
255.0	C2-Dibenzothiophene
256.6	C2-Dibenzothiophene
260.0	C5-Fluorene
262.9	C2-Dibenzothiophene
264.9	Phthalate
267.8	Unknown (55,69,88)
269.0	C2-Dibenzothiophene
274.1	Unknown (151,242,91)
275.7	Unknown-ester (88,101,69)
278.8	C2-Phenanthrene
280.4	C2-Dibenzothiophene
284.7	Unknown (234,233,115)
285.4	Fluoranthene
289.7	Unknown (193,207,226)
290.7	C3-Dibenzothiophene
292.6	Cl-5-Biphenyl/Unknown
295.6	Unknown (55,67,81)
300	Pyrene (S8-Standard)
303.7	C2-202
310.7	Cl-5-Biphenyl
316.8	Chlorinated compound (406,408,404)
320.9	Unknown (79,238,220)
325.9	p,p'-DDE
328.2	Cl-5-Biphenyl
333.8	C4-178
343.5	Cl-6-Biphenyl
345.2	Cl-5-Biphenyl
351.6	p,p'-DDD
355.7	Cl-6-Biphenyl
359.2	Cl-6-Biphenyl
361.1	Cl-6-Biphenyl
366.4	Unknown (243,187,91)
368.2	Unknown (243,69,107)
371.2	Unknown (79,57,95)
372.5	Cl-6-biphenyl
374.7	1,1'-Binaphthyl (Internal Standard)
375.5	Cl-6-Biphenyl
377.3	Phthalate
377.9	Benzo(b)napthol2,1,d)thiophene
381.3	Unknown (55,69,81)
385.3	Cl-7-Biphenyl
387.7	Cl-7-Biphenyl
400	Chrysene (S8-Standard)
407.2	Unknown (292,207,209)
414.9	Cl-7-Biphenyl
420.5	Unknown (79,91,67)
429.1	Phthalate
445.0	2,2'-Binapthyl (S3-Standard)
467.0	Chlorinated compound (107,109,499)
491.8	Benzo(e)pyrene
494.6	Benzo(a)pyrene
495.2	Unknown (69,81,95)
500	Perylene (S8-Standard)
503.6	Cl-10-Biphenyl (S3-Standard)
566.6	p-Quaterphenyl (S3-Standard)
591.2	Unknown (282,331,141)
600	Benzo(g,h,i)perylene (S8-Standard)

TABLE 2

Levels of alkylated polynuclear aromatic hydrocarbons identified in tisues from the crabs examined. Only those compounds present in amounts greater than 5 ug kg^{-1} are included in this total. Those samples containing no identifiable compounds in quantities exceeding 5 ug kg^{-1} are denoted as having 'Trace' levels. N/A: Sample not available for analysis; Lost: Sample lost during analysis.

COLLECTION SITE	DATE	SEX/MATURITY OF CRABS IN SAMPLE	TISSUE	ALKYLATED PAH CONC.
Lower James	June	Adult Female	Hepatopancreas	524
			Muscle	5
			Ovary	71
		(Sponge)	Yellow Sponge	188
		(Sponge)	Brown Sponge	435
		(Sponge)	Black Sponge	18
		(Sponge)	Hepatopancreas	465
		(Sponge)	Muscle	8
		(Sponge)	Ovary	Trace
		Adult Male	Hepatopancreas	231
			Muscle	Trace
		Juvenile Female	Hepatopancreas	Trace
			Muscle	17
		Juvenile Male	Hepatopancreas	144
			Muscle	104
	July	Adult Male	Hepatopancreas	167
			Muscle	61
	August		Hepatopancreas	721
			Muscle	45
	September	Adult Female	Hepatopancreas	382
			Muscle	Trace
			Ovary	244
		Adult Male	Hepatopancreas	28
			Muscle	50
		Juvenile Female	Hepatopancreas	N/A
			Muscle	N/A
		Juvenile Male	Hepatopancreas	394
			Muscle	450
	October	Adult Male	Hepatopancreas	292
			Muscle	Lost
	November		Hepatopancreas	399
			Muscle	Trace
Upper James	June	Adult Female	Hepatopancreas	1052
			Muscle	273
			Ovary	515
		Adult Male	Hepatopancreas	74
			Muscle	88

		Juvenile Female	Hepatopancreas	9
			Muscle	Trace
		Juvenile Male	Hepatopancreas	9
			Muscle	8
	September	Adult Female	Hepatopancreas	28
			Muscle	Trace
			Ovary	9
		Adult Male	Hepatopancreas	Trace
			Muscle	16
		Juvenile Female	Hepatopancreas	Trace
			Muscle	Trace
		Juvenile Male	Hepatopancreas	Trace
			Muscle	Trace
Pocomoke Sound	June	Adult Female	Hepatopancreas	395
			Muscle	Trace
			Ovary	9
		Adult Male	Hepatopancreas	90
			Muscle	5
		Juvenile Female	Hepatopancreas	28
			Muscle	Trace
		Juvenile Male	Hepatopancreas	1094
			Muscle	70
	September	Adult Female	Hepatopancreas	Trace
			Muscle	Trace
			Ovary	195
		(Sponge)	Hepatopancreas	Trace
		(Sponge)	Muscle	Trace
		(Sponge)	Ovary	Trace
		(Sponge)	Yellow Sponge	Lost
		Adult Male	Hepatopancreas	Trace
			Muscle	Trace
		Juvenile Female	Hepatopancreas	23
			Muscle	97
		Juvenile Male	Hepatopancreas	145
			Muscle	19
Rappahannock	June	Adult Female	Hepatopancreas	Lost
			Muscle	Trace
			Ovary	8
		Adult Male	Hepatopancreas	9
			Muscle	Trace
		Juvenile Female	Hepatopancreas	21
			Muscle	10
		Juvenile Male	Hepatopancreas	373
			Muscle	6
	September	Adult Female	Hepatopancreas	Lost
			Muscle	Trace
			Ovary	90
		Adult Male	Hepatopancreas	Lost
			Muscle	199
		Juvenile Female	Hepatopancreas	196
			Muscle	62
		Juvenile Male	Hepatopancreas	228
			Muscle	109

concentrations and were treated separately. Unresolved envelopes or complexes (UCM) were present in many of the samples. These consist of large numbers of chromatographically indistinguishable isomers, possibly petroleum derived. The areas of these were estimated by utilizing a BASIC computer program (UCM26, C. Smith) which calculated the size of the envelope relative to the resolved peaks above it. The total quantities of the latter were subsequently calculated and the UCM area determined.

Four-way analysis of variance (4x4x2x2) was run with the level of ASPAH as the dependent variable. Independent variables used were sampling site; month of sampling (June or September); tissue type (hepatopancreas or muscle only, since these were present in all sex/maturity groups); and the sex/maturity of the crabs sampled.

Due to three empty cells in the matrix only two-way interactions and main effects were assessed.

Examining Table 3 only the month-organ interaction was found significant at the 0.05 alpha level. When cell totals for this interaction were calculated, it was obvious that the level of alkyl substituted aromatics identified in the hepatopancreas were lower in September than those observed in June. However, values in the muscle tissue were nearly double in September versus June. The interaction between sex/maturity-organ possessed a F significance of 0.06. The ratio of quantities of substituted aromatic hydrocarbons in hepatopancreas to that in muscle was 92 in adult females, compared to 3 in juvenile males and 1.4 in adult males and juvenile females. This represented an interaction of degree rather than direction, as in the previous case.

TABLE 3

Results of the Four-Way Analysis of Variance (4x4x2x2) with the concentration of alkyl substituted aromatic hydrocarbons detected in the blue crabs sampled as the dependent variable. The second column shows the results when the data were adjusted for two extreme values by substituting the medians of concentration values measured for crabs at the same site and in the same tissue (groups involved: adult females from the upper James and juvenile males from the Pocomoke Sound sampled in June).

ANALYSIS OF VARIANCE

ALKYL SUBSTITUTED AROMATIC HYDROCARBON CONCENTRATION BY
MONTH (MONTH OF SAMPLING)
SITE (SAMPLING SITE)
SMATUR (SEX/MATURITY CLASSIFICATION)
ORGAN (TISSUE TYPE SAMPLED)

SOURCE OF VARIATION	SIGNIFICANCE OF F	SIGNIFICANCE OF F (ADJUSTED FOR EXTREMES)
MAIN EFFECTS	0.02	0.00
MONTH	0.17	0.32
SITE	0.89	0.27
SMATUR	0.03 *	0.01 **
ORGAN	0.00 **	0.00 **
TWO-WAY INTERACTIONS	0.08	0.00
MONTH-SITE	0.12	0.04 *
MONTH-SMATUR	0.09	0.00 **
MONTH-ORGAN	0.03 *	0.03 *
SITE-SMATUR	0.33	0.21
SITE-ORGAN	0.87	0.81
SMATUR-ORGAN	0.06	0.00 **
EXPLAINED	0.03	0.00

DUE TO EMPTY CELLS HIGHER ORDER INTERACTIONS WERE SUPPRESSED.

The main effect of tissue type was found to be highly significant (0.00) by ANOVA. Deviations from the grand mean, i.e, the mean calculated from all individual cell values, are shown in Table 4. Hepatopancreas burdens were higher than that of muscle. Chromatograms of the `cleaned up' extracts of hepatopancreas and muscle, from a group of juvenile male crabs from the Pocomoke Sound, are shown in Figures 5 and 6, respectively. Higher levels of aromatics are obvious in the former tissue.

The sex/maturity class effect on the level of ASPAH of the crabs exhibited an F significance of 0.03. Adult female and juvenile males possessed much higher amounts of resolved alkyl substituted aromatics than adult males and juvenile females.

Neither site nor month of sampling exerted a strong effect on the levels of the above mentioned compounds. Adult males sampled in July, August, October and November from the lower James were not included in the ANOVA since the other sex/maturity groups were not present for these months; inclusion would result in an incomplete matrix. However, levels appeared higher than in crabs from the other sites in either June or September.

When the data of Table 2 were examined closely it was observed that two extreme values were present (for the upper James adult females and the Pocomoke Sound juvenile males sampled in June). The effects of these values were assessed by replacing them with the medians of their respective station and organ type. The data were then re-examined, the results of which are shown in the same tables as the original analyses.

TABLE 4

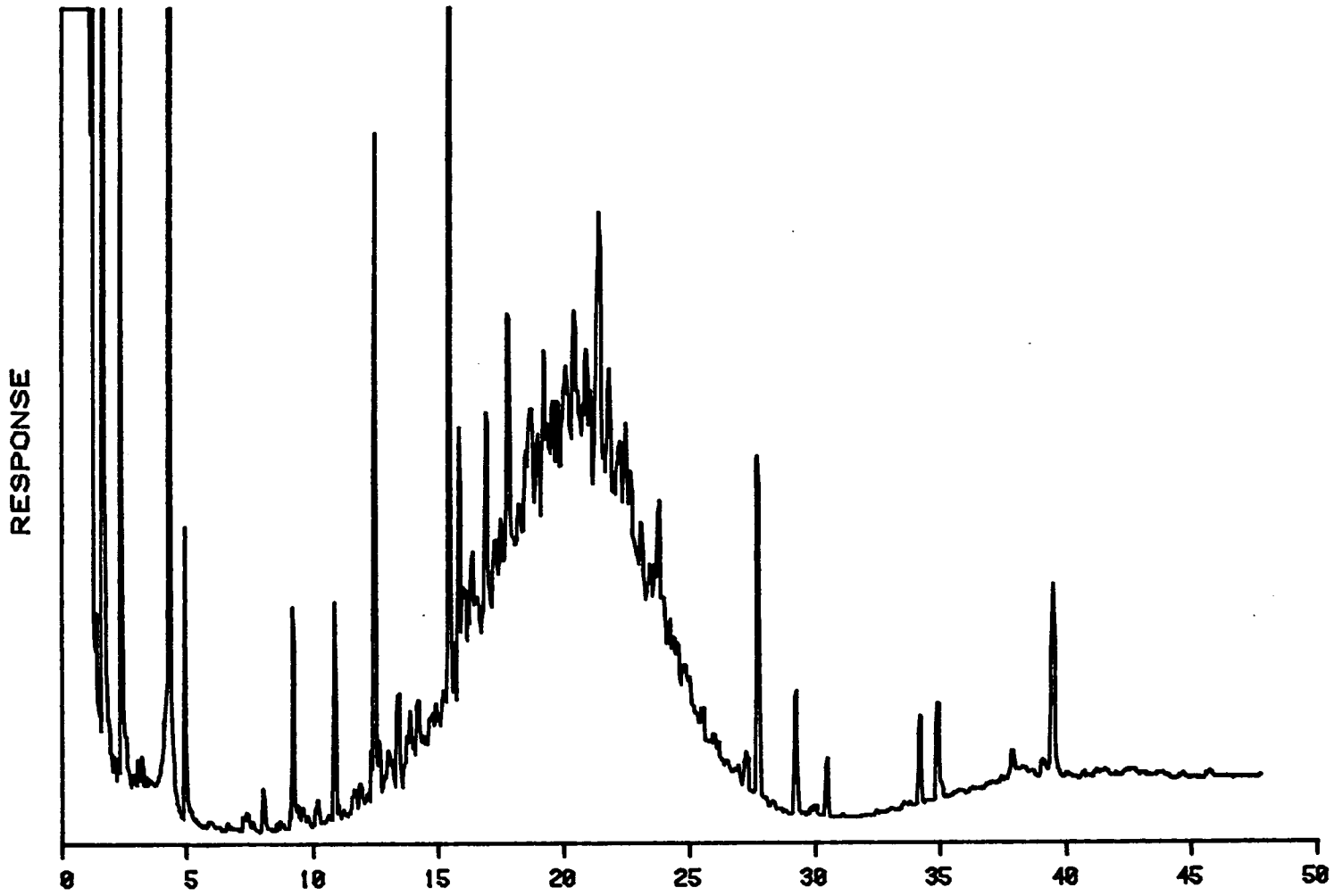
Results of the Multiple Classification Analysis of the concentrations of alkyl substituted aromatic hydrocarbons detected in the crabs sampled. The deviations from the grand mean have been adjusted to compensate for the effects the independent variables not under consideration exert on the means of the groups. The second column of deviations have been adjusted for two extreme concentration determinations, from the upper James and the Pocomoke Sound (adult female and juvenile male groups respectively, sampled in June). Median concentration values obtained from similar tissue and site were substituted.

MULTIPLE CLASSIFICATION ANALYSIS
ALKYL SUBSTITUTED AROMATIC HYDROCARBON CONCENTRATION BY
MONTH (MONTH OF SAMPLING)
SITE (SAMPLING LOCATION)
SMATUR (SEX-MATURITY CLASSIFICATION)
ORGAN (TISSUE SAMPLED)

GRAND MEAN=119.9

VARIABLE AND CATEGORY	N	ADJUSTED FOR INDEPENDENTS	
		DEVIATION FROM GRAND MEAN	DEVIATION FROM GRAND MEAN (ADJUSTED FOR EXTREMES)
MONTH			
JUNE	31	31.5	15.5
SEPTEMBER	28	-34.9	-17.2
SITE			
POCOMOKE SOUND	16	2.7	-42.0
UPPER RAPPAHANNOCK	13	-5.9	9.8
UPPER JAMES	16	-22.9	-8.2
LOWER JAMES	14	28.6	48.3
SMATUR			
ADULT FEMALE	14	77.2	91.7
ADULT MALE	15	-67.5	-51.8
JUVENILE MALE	16	74.5	29.8
JUVENILE FEMALE	14	-90.0	-70.3
ORGAN			
HEPATOPANCREAS	28	76.3	60.5
MUSCLE	31	-68.9	-54.6

Figure 5. Chromatogram (FID detection) of the aromatic fraction of a hepatopancreas extract from juvenile male crabs from the Pocomoke Sound.



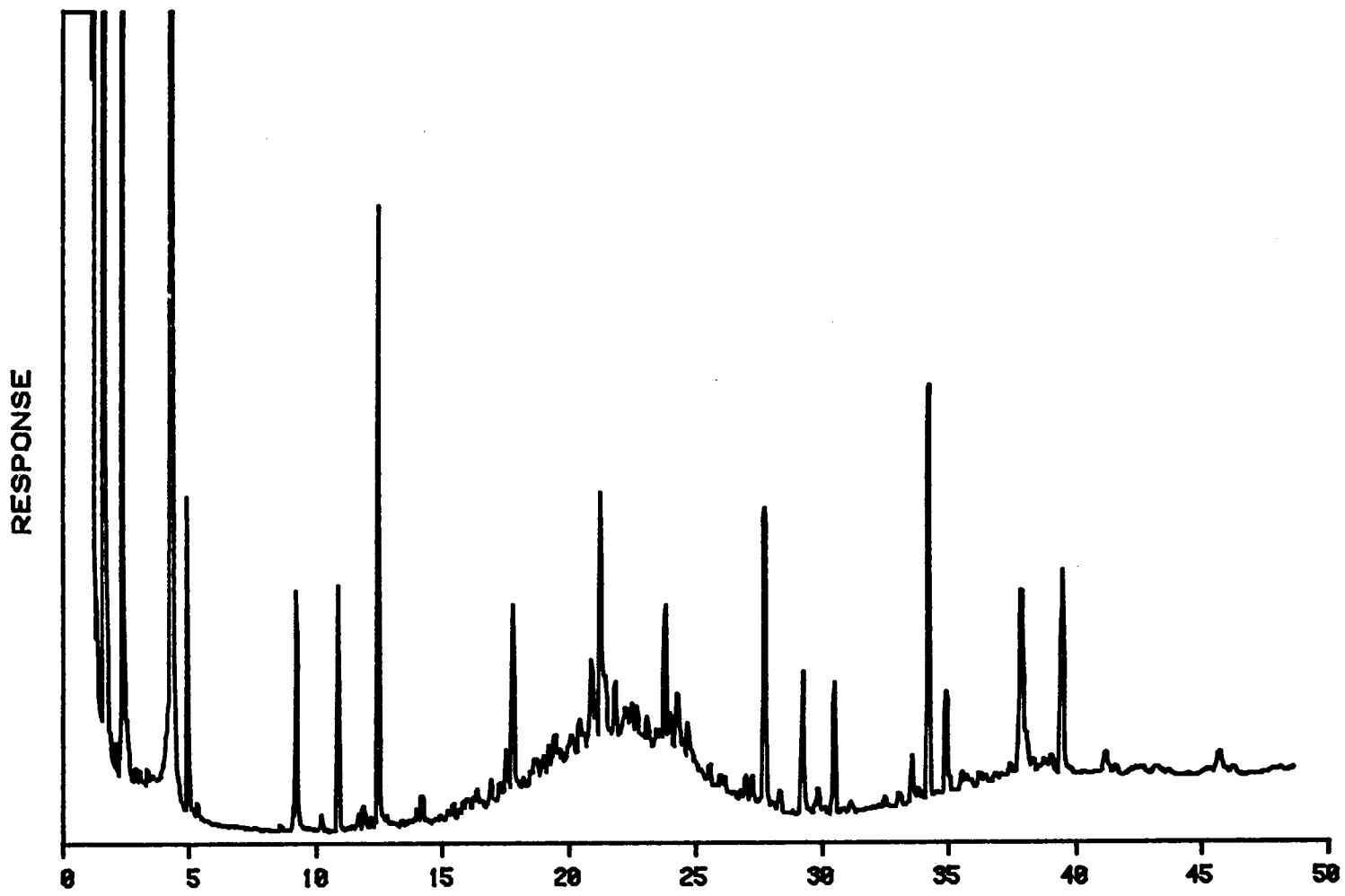
SAMPLE: PH43

RAW FILE: RR43

SCALE FACTOR: .2

PLOT SPEED: 2

Figure 6. Chromatogram (FID detection) of the aromatic fraction of a muscle extract from juvenile male crabs from the Pocomoke Sound.


RESPONSE
0
5
10
15
20
25
30
35
40
45
50
SAMPLE: PM41
RAW FILE: RR41
SCALE FACTOR: .2
PLOT SPEED: 2

The Multiple Classification Analysis (MCA) results of the adjusted data were generally similar. However, the outlying value detected at the Pocomoke Sound station contributed substantially to this site total. Substitution of the median value greatly reduced the mean, making it the lowest site in regards to ASPAH levels. The F probability of the null hypothesis of no significant site effects was 0.27. An alteration in the means was also observed for the sex/maturity classification. The mean total for the juvenile male group was greatly lowered by the substitution. This decreased the F significance for the main effect of the sex and maturity of the crab on ASPAH levels to 0.01. Three of the changes in the interactions were of interest. The month-sex/maturity interaction F significance decreased to 0.00. Cell totals showed higher values in September than in June for the juvenile crabs, suggesting that growth processes might result in accumulations of ASPAH in these crabs. The site-month interaction F significance decreased to 0.04. Cell totals showed higher levels present in June, for the upper James and the Pocomoke Sound sites, than September. The converse was true for the lower James and the Rappahannock stations. The final obvious change in the F significances was observed for the sex/maturity-organ interaction; it decreased to 0.00. Ratios of concentrations between hepatopancreas and muscle ranged from approximately one in the upper James to more than four at the Pocomoke Sound and Rappahannock stations.

Sample size in pollutant studies generally are small due to the laborious and expensive techniques involved. Therefore, they are vulnerable to the effects of extreme values. Nonparametric statistics, rather than parametric ones, such as ANOVA, are not as

subject to the problems presented by such values. Normally more information may be obtained by employing the latter. However, care must be exercised in examining the data before making conclusions.

Since ovaries were only available in the adult female group they were considered separately. Differences in levels of substituted aromatics in muscle, hepatopancreas and ovary for adult females without extruded eggs were examined by One-Way Analysis of Variance and were found to be significant. Ovary values were intermediate between the hepatopancreas and the lower muscle burdens; however, employing Duncan's Multiple Range Test (DMRT) ovary was not judged to differ from either in regards to alkyl substituted aromatic hydrocarbon concentration at the 0.05 level of significance (muscle and hepatopancreas were declared significantly different).

Differences in levels of alkyl substituted polynuclear aromatics in muscle and hepatopancreas between adult females without and with egg masses were not statistically examined due to the low sample size of the latter; however, by inspection they appeared similar. Ovary burdens in the latter group were much lower. Levels in the egg mass themselves surpassed muscle and ovary values but not hepatopancreas.

Polychlorinated biphenyls were also examined by Four-Way Analysis of Variance (4x4x2x2). No interactions were found significant at the 0.05 level, although the month-sex/maturity, month-organ and sex/maturity-organ interactions exhibited F significances of less than 0.09, as shown in Table 5. The pattern for the month-organ interaction was similar to that observed with the ASPAH.

Organ type was the only main effect judged to be significant for the polychlorinated biphenyls (0.00). As in the case of the

TABLE 5

Results of the Four-Way Analysis of Variance (4x4x2x2) with the concentration of polychlorinated biphenyls detected in the crabs sampled as the dependent variable. Only organ type was declared significant at the 0.05 level.

ANALYSIS OF VARIANCE

POLYCHLORINATED BIPHENYL CONCENTRATION BY
MONTH (MONTH OF SAMPLING)
SITE (SAMPLING SITE)
SMATUR (SEX/MATURITY CLASSIFICATION)
ORGAN (TISSUE TYPE SAMPLED)

SOURCE OF VARIATION	SIGNIFICANCE OF F
MAIN EFFECTS	0.01
MONTH	0.33
SITE	0.10
SMATUR	0.28
ORGAN	0.00 **
TWO-WAY INTERACTIONS	0.16
MONTH-SITE	0.18
MONTH-SMATUR	0.07
MONTH-ORGAN	0.06
SITE-SMATUR	0.76
SITE-ORGAN	0.23
SMATUR-ORGAN	0.08
EXPLAINED	0.04

DUE TO EMPTY CELLS HIGHER ORDER INTERACTIONS WERE SUPPRESSED.

substituted aromatics, hepatopancreas contained much higher concentrations of PCB than muscle tissue, as shown in Table 6. Also, polychlorinated biphenyls were present at higher levels in June than in September, but not significantly. The effect of sampling site on PCB concentration in blue crabs was strong compared to ASPAH (probability of the null hypothesis 0.10 and 0.89, respectively). Examination of the deviations from the grand mean show that crabs from the upper James River site contained similar PCB concentrations to the lower James. Crabs from the Rappahannock River and Pocomoke Sound stations had lower levels. The order of the sex/maturity groups in regards to PCB burdens was the same as that observed for the substituted aromatics, except that levels in juvenile males were much lower than from those in adult females.

The results of the statistical analysis of PCB levels in the ovaries of females without extruded eggs were essentially the same as those described for the alkyl substituted hydrocarbons. Ovary burdens higher than muscle but lower than those in hepatopancreas were found. DMRT did not declare the concentration of PCB in ovary to be significantly different from either tissue at the 0.05 level.

Similarly, PCB in the adult females with extruded eggs showed muscle and hepatopancreas concentrations comparable with those in unladen crabs. Levels in the ovaries of the former group were lower than those of their nonsponge possessing compatriates. PCB content of the sponge itself was higher than concentrations in muscle and ovary but lower than hepatopancreas, as was the case with the alkyl substituted aromatics.

TABLE 6

Results of the Multiple Classification Analysis of the concentrations of polychlorinated biphenyls present in the crabs sampled. Deviations from the grand mean for each classification have been adjusted for the effects of the independent variables not under consideration.

MULTIPLE CLASSIFICATION ANALYSIS

POLYCHLORINATED BIPHENYL CONCENTRATION BY
MONTH (MONTH OF SAMPLING)
SITE (SAMPLING LOCATION)
SMATUR (SEX-MATURITY CLASSIFICATION)
ORGAN (TISSUE SAMPLED)

GRAND MEAN=10.4

VARIABLE AND CATEGORY	N	ADJUSTED FOR INDEPENDENTS DEVIATION FROM GRAND MEAN
MONTH		
JUNE	31	2.0
SEPTEMBER	28	-2.2
SITE		
POCOMOKE SOUND	16	-7.2
UPPER RAPPAHANNOCK	13	-3.2
UPPER JAMES	16	6.3
LOWER JAMES	14	4.0
SMATUR		
ADULT FEMALE	14	7.5
ADULT MALE	15	-2.1
JUVENILE MALE	16	-0.9
JUVENILE FEMALE	14	-4.2
ORGAN		
HEPATOPANCREAS	28	8.0
MUSCLE	31	-7.2

As mentioned, relatively low levels of nonsubstituted aromatics were detected in the samples. Compounds identified included: benzo(a)pyrene, benzo(e)pyrene, acenaphthene, fluorene, fluoranthene, phenanthrene, perylene and pyrene. Heterosubstituted compounds were observed, such as: dibenzofuran, dibenzothiophene, benzaldehyde, benzothiazole, cis and trans-4-phenyl-3-butene-2-one and 1,1'-biphenyl-2-amine. By inspection it was apparent that the concentrations of these compounds in the hepatopancreas were higher than those in muscle tissue. Greater amounts were found in September than in June. Highest levels were observed in samples taken from the lower James, while lowest were seen at the Pocomoke and Rappahannock River sites. Finally, adult males had the highest amounts of these compounds and juvenile females the least. An MCA summary is shown in Table 7.

Unresolved complexes (UCM) were evident in many of the samples. Areas of these are presented in Table 8, along with other pertinent data. UCM were more numerous in June than September, the lower James than elsewhere, and in adult females and juvenile males. They were found in 41% of the hepatopancreas and 19% of the muscle tissue samples.

Low levels of DDD, DDE and two unidentified chlorinated compounds were detected. Their occurrence and concentrations are shown in Table 9 along with the data on the polychlorinated biphenyls. In addition, phthalates were detected in some of the crabs sampled. Due to their ubiquitous presence in the environment (Musial et al., 1981; Russell and McDuffie, 1983), and thus the possibility of sample contamination, they were not examined in detail.

TABLE 7

Results of the Multiple Classification Analysis of the concentrations of nonsubstituted aromatic hydrocarbons present in the crabs sampled. The deviations have been adjusted to compensate for the effects of the independent variables not under consideration.

MULTIPLE CLASSIFICATION ANALYSIS

NONSUBSTITUTED AROMATIC HYDROCARBON CONCENTRATION BY
MONTH (MONTH OF SAMPLING)
SITE (SAMPLING LOCATION)
SMATUR (SEX-MATURITY CLASSIFICATION)
ORGAN (TISSUE SAMPLED)

GRAND MEAN=19.9

VARIABLE AND CATEGORY	N	ADJUSTED FOR INDEPENDENTS DEVIATION FROM GRAND MEAN
MONTH		
JUNE	31	-6.1
SEPTEMBER	28	6.7
SITE		
POCOMOKE SOUND	16	-5.3
UPPER RAPPAHANNOCK	13	-5.8
UPPER JAMES	16	-1.9
LOWER JAMES	14	13.6
SMATUR		
ADULT FEMALE	14	0.3
ADULT MALE	15	5.2
JUVENILE MALE	16	0.9
JUVENILE FEMALE	14	-6.9
ORGAN		
HEPATOPANCREAS	28	7.9
MUSCLE	31	-7.2

TABLE 8

Estimated areas of the unresolved envelopes present in the crabs sampled. Values given are expressed in ug kg^{-1}. Envelopes were most common in the hepatopancreas of crabs taken from the James River.

COLLECTION SITE	DATE	SEX/MATURITY OF SAMPLE	TISSUE	ESTIMATED UCM AREA ug kg^{-1}
Lower James	June	Adult Female	Hepatopancreas	4850
		(Sponge)	Yellow Sponge	6010
		(Sponge)	Brown Sponge	1390
		(Sponge)	Hepatopancreas	5000
		Juvenile Male	Hepatopancreas	1130
			Muscle	1170
	July	Adult Male	Hepatopancreas	3510
	August	Adult Male	Hepatopancreas	9130
	September	Adult Female	Hepatopancreas	4880
		Juvenile Male	Hepatopancreas	7470
			Muscle	7360
	October	Adult Male	Hepatopancreas	3060
Upper James	June	Adult Female	Hepatopancreas	5600
			Muscle	2300
		Adult Male	Hepatopancreas	1840
			Muscle	2260
Pocomoke Sound	June	Adult Female	Ovary	3600
		Juvenile Female	Muscle	880
		Juvenile Male	Hepatopancreas	9820
			Muscle	2020
	September	Juvenile Male	Hepatopancreas	440
Rappahannock	June	Juvenile Male	Hepatopancreas	4080
	September	Juvenile Female	Muscle	720
		Juvenile Male	Hepatopancreas	920

TABLE 9

Levels of DDD, DDE, PCB and two unidentified chlorinated compounds found in tissue samples from blue crabs. Compounds detectable but present at levels less than 5 ug kg^{-1} are shown as 'Trace' amounts. The symbol '+' signifies that additional PCB isomers were detected but at concentrations below 5 ug kg^{-1} and were not included in the total listed.

COLLECTION SITE	DATE	SEX/MATURITY OF SAMPLE	TISSUE	COMPOUND (ug kg^{-1}) TRACE<5.0 ug kg^{-1}
Lower James	June	Adult Female	Hepato.	PCB-40+
			Ovary	PCB-7
		(Sponge)	Hepato.	PCB-47+
		(Sponge)	Yellow	PCB-29+
				DDE-Trace
		(Sponge)	Brown	PCB-37
		(Sponge)	Black	PCB-27
				Chlor. cpd.-9
		Adult Male	Hepato.	PCB-23
		Juvenile Female	Hepato.	PCB-19
				Chlor. cpd.-Trace
			Muscle	PCB-10+
				Chlor. cpd.-10
		Juvenile Male	Hepato.	PCB-5
			Muscle	PCB-9
	July	Adult Male	Hepato.	PCB-58
			Muscle	PCB-6
	August		Hepato.	PCB-27+
				DDD-Trace
				Chlor. cpd.-22
			Muscle	PCB-31+
	September	Adult Female	Hepato.	PCB-36
			Ovary	PCB-12
		Adult Male	Hepato.	PCB-31
			Muscle	PCB-Trace
				DDE-7
		Juvenile Male	Hepato.	DDE-Trace
			Muscle	PCB-45
				DDE-Trace
	October	Adult Male	Hepato.	PCB-44+
	November		Hepato.	PCB-29
			Muscle	PCB-Trace
Upper James	June	Adult Female	Hepato.	PCB-131
			Muscle	PCB-9
			Ovary	PCB-48
				DDE-6
				Chlor. cpd.-9

		Adult Male	Hepato.	PCB-27+
			Muscle	PCB-Trace
		Juvenile Female	Hepato.	PCB-7
		Juvenile Male	Hepato.	PCB-33
	September	Adult Female	Hepato.	PCB-6
			Ovary	PCB-22
		Adult Male	Hepato.	PCB-8+
			Muscle	PCB-7
		Juvenile Female	Hepato.	PCB-26
		Juvenile Male	Hepato.	PCB-18
				Chlor. cpd.-6
Pocomoke Sound	June	Adult Female	Hepato.	PCB-23
		Adult Male	Hepato.	PCB-Trace
			Muscle	DDE-Trace
				Chlor. cpd.-27
		Juvenile Male	Hepato.	PCB-9
			Muscle	Chlor. cpd.-14.5
	September	Adult Female	Hepato.	Chlor. cpd.-39.2
			Ovary	PCB-Trace
		(Sponge)	Hepato.	PCB-Trace
		(Sponge)	Ovary	PCB-7
		Juvenile Female	Hepato.	PCB-22+
				Chlor. cpd.-Trace
		Juvenile Male	Muscle	PCB-Trace
			Muscle	Chlor. cpd.-15.8
Rappahannock	June	Adult Male	Hepato.	PCB-12
		Juvenile Female	Hepato.	PCB-Trace
		Juvenile Male	Hepato.	PCB-26+
				DDD-Trace
	September	Adult Female	Muscle	PCB-Trace
			Ovary	PCB-34
		Adult Male	Muscle	15
		Juvenile Male	Hepato.	PCB-9
				Chlor. cpd.-14.2
			Muscle	PCB-Trace

The chromatograms of samples run during the early phases of the project exhibited several large peaks. Three of these peaks corresponded to internal standards with which the samples were spiked to determine percent recovery. In addition, a large phthalate ester peak was present in some, caused by an impurity in the septum of the GC injector. This peak was eliminated in later work by employing a different lot of septa and baking the septa for a longer period of time in order to drive off volatile contaminants.

Extractable Lipid and % Water Data

Percent methylene chloride extractable lipid material and percent water content of the tissues, utilized in the radiotracer study and from representative tissue samples from the field sampling, were determined. This data is summarized in Table 10.

Considering all independent variables together, except tissue type, it was found that muscle tissue contained significantly more water per gram (Scheffe Test, 0.05 alpha level) than hepatopancreas or ovary (Table 10A). The water content in the egg mass exceeded the average muscle tissue value. Differences in extractable lipid were significant between all organ pairs examined; highest in hepatopancreas, followed by ovary and muscle. The egg mass value was intermediate between hepatopancreas and ovary.

When differences in percent water content were examined within the sex/maturity groups, all pertinent tissue types exhibited significant differences (Table 10B). In regards to percent

TABLE 10

Summary of % water content and % methylene chloride extractable lipid data in the crab tissues analyzed. The results of the ANOVA and Scheffe analyses for significant differences in these parameters between tissue types in: (A) all sex/maturity groups together; and (B) within individual groups are presented.

A. All Sex/Maturity Groups Considered

	% WATER CONTENT			% EXTRACTED LIPID		
ORGAN	N	MEAN	STANDARD DEVIATION	N	MEAN	STANDARD DEVIATION
Hepatopancreas	76	70.4	8.4	56	34.0	11.5
Muscle	25	80.5	4.3	26	4.3	1.6
Ovary	9	66.1	4.9	7	18.3	5.9
Egg	1	86.9	N/A	1	26.6	N/A
ANOVA F Probability	(0.00)			(0.00)		
Pairs Found Significantly Different By Scheffe (0.05):						
Muscle/Ovary				Muscle/Ovary		
Hepatopancreas/Muscle				Hepatopancreas/Muscle		
				Hepatopancreas/Ovary		

B. Within Individual Groups

Adult Females Only:	
ANOVA F Probability (0.00)	(0.00)
Pairs Found Significantly By Scheffe (0.05), N In Brackets:	
Hepatopancreas [19]/Ovary[7]	Hepatopancreas[12]/Muscle[6]
Muscle[6]/Ovary	Hepatopancreas/Ovary[5]
Hepatopancreas/Muscle	
Only Hepatopancreas And Muscle Analyzed For The Groups Below N In Brackets [Hepatopancreas,Muscle]	
Adult Males Only:	
ANOVA F Probability (0.00) [21,6]	(0.00) [13,6]
Juvenile Females Only:	
ANOVA F Probability (0.00) [18,6]	(0.00) [18,6]
Juvenile Males Only:	
ANOVA F Probability (0.01) [7,5]	(0.00) [6,6]

extractable lipids, all pairs were significantly different, except muscle and ovary in the adult female group.

No significant differences in either % water or % lipid content of muscle tissue were detected by ANOVA between adult males and adult females or between juvenile males and females, in the intermolt condition. The percent water content in the hepatopancreas of adult males was significantly lower than that of adult females (F probability 0.03); and molting juvenile females possessed significantly more water in the hepatopancreas than nonmolting juvenile males or females (F probability 0.00). Muscle samples for the molting group were not examined, but it was suspected that they possessed substantially greater water content than the intermolt juveniles.

III. UPTAKE, BIOTRANSFORMATION AND ELIMINATION OF TRITIUM LABELED BENZO(a)PYRENE IN BLUE CRABS

A. METHODS

Adult male, adult female and juvenile female blue crabs of intermolt condition were obtained on the VIMS Crustaceology Survey of the York River, Virginia, in September 1982. These specimens were supplemented by additional crabs purchased from a local fisherman. Approximately 100 crabs were maintained in a recirculated water system constructed in the Demonstration Building at VIMS prior to the commencement of the experiments. The system consisted of two 4x8 foot fiberglass tanks for holding specimens, a 3x10 foot fiberglass algae tank with transparent side ports, and two circular fiberglass tanks each containing a wood filter box filled with clean oyster shell. The filters were covered with plastic screening and filter floss which removed large particulate matter from the water. The oyster shell provided a large surface area for the growth of bacteria and other organisms and functioned as a biological filter, breaking down wastes and other materials.

Prior to the commencement of the experiment, juvenile female crabs exhibiting signs of imminent molting were obtained from a York River fisherman. Water was pumped from the York River to the holding tanks, each containing approximately 208 liters. Salinity was 17 ± 1 PPT and the temperature $23\pm1^{\circ}C$.

Water was circulated through a PVC protein skimmer by a March Model TE-5C-MD Magnetic Drive Corrosive Solution Pump capable of

pumping 1010 gallons per hour. An identical pump was used to transport the filtered water in the sumps to the algae tank, which contained numerous glass jars. These jars provided an additional surface area for the growth of algae which utilized the nutrients from the breakdown of wastes produced in the system. Water was provided to the rest of the system by gravity flow. Pump flows were balanced by adjustable valves.

During the exposure period of the experiment only one holding tank was used. Water was recirculated in this tank by a single pump and was not filtered. Supplemental aeration was provided with air stones.

The holding tank was sheltered from direct fluorescent lighting to reduce photo-oxidation of the B(a)P. Crabs to be exposed to the radiolabeled aromatic were moved to the holding tank prior to exposure. Peeler crabs were separated from intermolt crabs by placing the former in a plastic cage to prevent cannibalism. Since the crabs occupied the same tank the setup insured that all crabs used were exposed to the same level of B(a)P, under identical conditions.

Fifty mCi of tritium labeled B(a)P dissolved in hexane were obtained from New England Nuclear (Boston, Mass.). The compound was nonspecifically labeled and the activity was 70.5 Ci/mmol. The benzo(a)pyrene was redissolved in 10 ml ethanol carrier and mixed with 1000 ml distilled water in a one liter flask equipped with a magnetic stirring bar. Sufficient radiolabeled B(a)P was released into the exposure tank such that a concentration of 0.9 ug l^{-1} would be obtained under ideal conditions. Measurement of the activity of the tank water after addition, in the presence of the crabs, was found to

be 199,100 DPM or approximately 0.3 ug l^{-1} (based on a counting efficiency of 19.4%). This level remained constant through the remainder of the exposure period.

The lower initial activity was believed to be a result of, in part, the adsorption of the B(a)P on the fiberglass walls of the exposure tank. Glass aquaria have been the most common enclosures used in hydrocarbon experiments. However, fiberglass tanks have also been used when large volumes of water were required (Mac and Seelye, 1981). Significant adsorption of polynuclear aromatic hydrocarbons on glass surfaces from water occurs and it is expected that a fiberglass surface would be even more prone to adsorb due to its chemical and physical nature. However, since it was necessary to obtain identical exposure conditions for all groups of crabs, the tank was found adequate. Another reason for the lower activity of the water was the removal of the radiolabeled B(a)P by the crabs themselves, both by actual uptake and by adsorption to their outer surfaces.

The exposure period lasted 36 hours at which time the tank water was passed through two activated charcoal filter cartridges. The filtration lowered the activity of the water sufficiently to allow unrestricted disposal of the tank water according to NRC requirements. The holding area was flushed with river water, to prevent residual radiolabeled material from confounding the elimination patterns of the crabs, and refilled. The entire recirculated system was reassembled and normal filtration resumed. The exposed crabs were distributed between the two holding tanks and held for an additional 10.5 days for depuration studies. To reduce the reabsorption of radiolabeled B(a)P and its metabolites after elimination by the crabs the water was

changed after the fifth day of the depuration period. The activity of the water was monitored daily and never surpassed 3% of the initial exposure level.

Crabs received food in the form of chopped grey trout (Cynoscion regalis), also obtained from the York River, on Days 5 and 7.

Three samples, consisting of two crabs each (except for the newly molted group, which consisted of single crabs) from each study group were removed after 24 and 36 hours of exposure; and after 60, 156 and 252 hours of depuration. Hepatopancreases were immediately removed, placed in foil pans and wet weights determined. This organ was chosen since it was found by Lee et al. (1976) to be the major site of in-vivo xenobiotic metabolism and storage. Care was taken to remove the organ in its entirety.

Samples were frozen and freeze-dried without Virtis homogenization, due to their small size, and a dry weight obtained. A two gram aliquot of each sample was charged to a fritted glass thimble and extracted as described for the field samples.

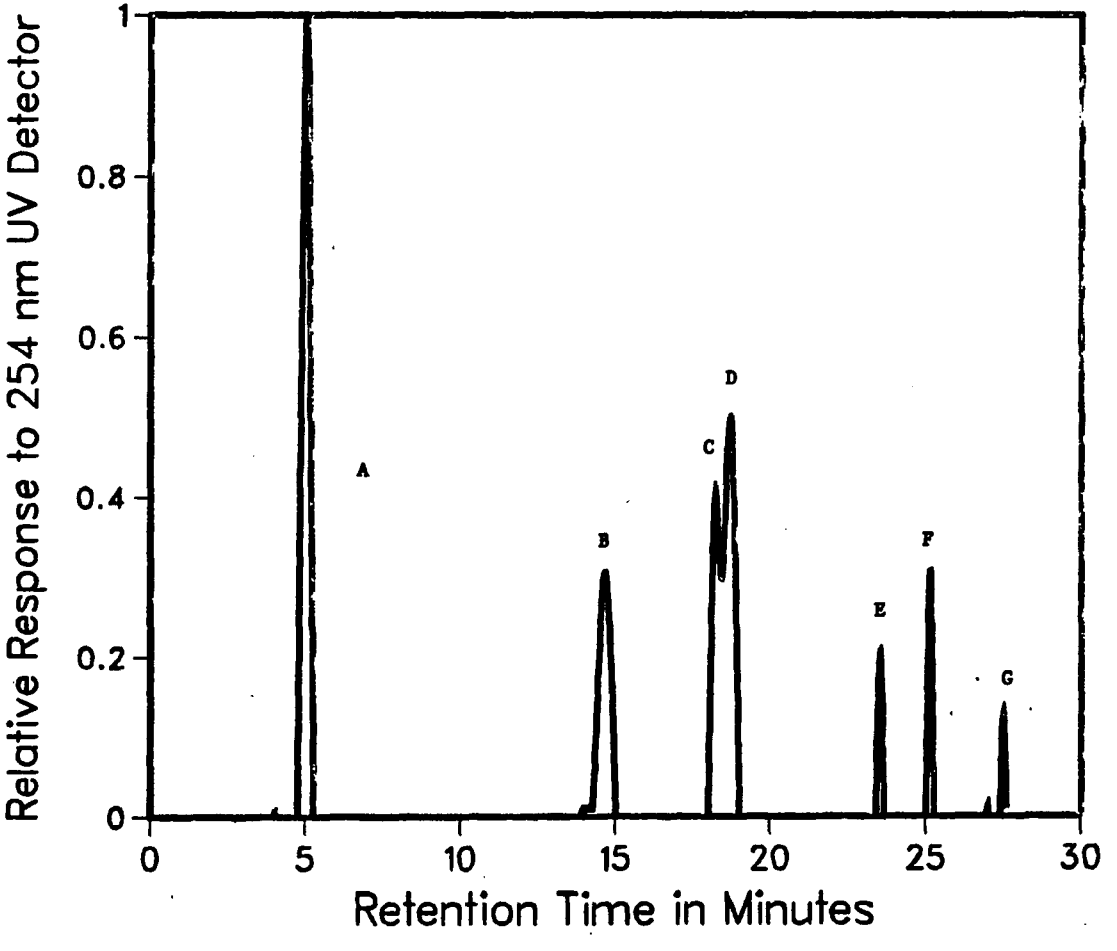
The extract was subdivided into three fractions: 1) to assay for the percent methylene chloride extractable lipids; 2) to assay total extractable radioactivity; and 3) for metabolite profile examination. This last fraction, from each of the extracted replicates, was pooled and run on the GPC as a single sample. GPC parameters were the same as described for field samples. Both the high molecular weight and the aromatic fractions were saved. The latter fraction contained the parent B(a)P as well as the hydroxylated metabolites (up to and including the 7,8,9,10-tetrol); the former contained most of the conjugates and other high molecular weight material to which the

labeled compound became associated. Retention of the conjugates in the high molecular weight fraction was confirmed using standards and chromatographing on the HPLC (solvent methanol, flow rate 1.5 ml/min.) using an All-Tech C18 reverse phase column (Alltech Associates) and a 254 nm UV detector.

The aromatic fraction was blown down to 0.2 ml and run on the HPLC employing the normal phase column described previously. The solvent program used is shown in Figure 3B. Solvent A was hexane while Solvent B was a 50/50 mixture (v/v) of isopropanol and methanol. The program was designed using authentic metabolite standards obtained from Dr. David Longfellow, National Cancer Institute, Chemical Carcinogen Reference Repository. A reconstruction of a HPLC run employing B(a)P, the 3-hydroxy, 4,5-dihydroxy, 7,8-dihydroxy, 9,10-dihydroxy, 7,8,9-trihydroxy, and 7,8,9,10-tetrahydroxy metabolites of B(a)P is shown in Figure 7. The effluent from the HPLC was collected in 10 ml scintillation vials over the course of the program at 30 second intervals. This effluent was combined with toluene cocktail (Liquifluor, New England Nuclear), made up to a volume of 10 ml with toluene and assayed on a Beckman Liquid Scintillation Counter. The high molecular weight fraction was similarly treated. However, due to the high color quenching present, only a portion of this extract was counted. All samples were placed in the dark for 48 hours prior to counting to reduce chemoluminescence. The samples were coinjected with the authentic standards at concentrations which were detectable with a 254 nm UV detector to confirm the retention times of the <u>in-vivo</u> metabolites produced by the crabs themselves.

Figure 7. Results of a HPLC analysis of a mixture of B(a)P metabolite standards (further details are available in the text) is presented. Standards are: (A) B(a)P; (B) 3-hydroxy B(a)P; (C) B(a)P-trans-4,5-dihydrodiol; (D) B(a)P-trans-7,8-dihydrodiol; (E) B(a)P -trans-9,10-dihydrodiol; (F) B(a)P-7, trans-8,9-trihydrotriol; (G) B(a)P-7,8,9,10-tetrahydrotetrol.

HPLC Analysis of Metabolite Standards



The counts per minute (CPM) obtained from the liquid scintillation counter were corrected to disintegrations per minute (DPM) by quench correction using a tritium labeled water standard in conjunction with the channels ratio method.

The DPM obtained from the HPLC fraction collections were then entered into a SPSS Graphics Linechart (Hull and Nie, 1981) Program producing a DPM versus time plot. This plot allowed the identification of many of the metabolites produced via their retention time. Results were expressed on a per hepatopancreas basis.

Two 50 mg aliquots of each freeze dried sample were also wetted with distilled water and solubilized in NCS Tissue Solubilizer overnight. To these were added 10 ml Aquasol-2 Universal LSC Cocktail and the samples stored 48 hours in the dark to reduce chemoluminescence before counting. Quench correction was accomplished as described for the extracted aliquots. Triplicates of each category of sample were averaged in order to determine the uptake and depuration rates for the various groups of blue crabs.

B. RESULTS

Turbid conditions were observed in the exposure tank within 24 hours of the onset of the experiment due to the accumulation of waste products; since filtration could not be employed during the exposure period. Adsorption of B(a)P on suspended particulate material may have altered uptake from that in a system in which the labeled material was strictly in the dissolved phase.

As noted in the methods section, a low level of activity was detected in the water during the exposure period. It was hypothesized that this activity was due to depuration by the crabs, rather than desorption of tritium labeled B(a)P from the walls of the tank. This conclusion was borne out by placing one of the exposed crabs in a clean aquarium and monitoring the activity of the water over time. Changes in activity in the aquarium paralleled those in the large system.

Table 11 shows the results of the whole tissue activity determinations. Data were expressed on a DPM per gram of hepatopancreas, as well as on the DPM burden of the entire organ. The latter figure was obtained by multiplying the per gram figure by the total amount of tissue dissected (dry weight basis) from the specimens used in that particular sample; this result was then divided by the number of crabs utilized. In this way a total tissue burden and an estimate of concentration were obtained.

Three replicates of each sex-molt group were taken on the desired sampling days. To ensure that sufficient specimens were available for examination on the later depuration intervals only one

TABLE 11

Quantities of total radiolabeled material present in the hepatopancreases of crabs examined during in-laboratory exposures to 0.9 ug l^{-1} tritium labeled B(a)P. The crabs were exposed for 36 hours and allowed to depurate. Samples were taken at the intervals noted. Values are in ug x 10^{4} for comparison with the data obtained by Lee et al. (1976). The upper values are expressed on a total tissue burden while the lower are on a concentration (per gram) basis.

TIME	NEWLY MOLTED FEMALE	INTERMOLT ADULT FEMALE	INTERMOLT ADULT MALE	INTERMOLT JUVENILE FEMALE	LEE ET AL. INTERMOLT JUVENILES
Standard deviations given in parentheses					
24 HOUR EXPOSURE	972(293) 824(353)	2204(1475) 1486(1351)	1742(1682) 891(855)	1107(183) 483(105)	270(90) -
48 HOUR EXPOSURE	-	-	-	-	590(210) -
48 HOUR DEPURATION	-	-	-	-	300(18) -
60 HOUR DEPURATION	1355(187) 672(34)	653(109) 315(80)	515(219) 222(45)	315(65) 266(61)	-
144 HOUR DEPURATION	-	-	-	-	210(16) -
156 HOUR DEPURATION	569(76) 363(160)	400(101) 298(67)	196(35) 81(43)	136(46) 91(45)	-
252 HOUR DEPURATION	-	189(180) 245(216)	100(25) 44(20)	17(4) 16(4)	-
288 HOUR DEPURATION	-	-	-	-	320(74) -
480 HOUR DEPURATION	-	-	-	-	40(26) -

sample, consisting of three crabs, was taken from each group at the conclusion of the exposure period (36 hours). Owing to the large variation in values obtained between the replicates at the 24 hour sampling it was not felt that this was adequate to describe the 36 hour activity level. These data points were therefore not included in the results.

Data obtained on the other intervals were examined for significant differences by One-Way Analysis of Variance. Significant differences were further explored using Duncan's Multiple Range Test at the 0.05 alpha level. The results of these analyses are shown in Table 12.

Although large differences in the means for the sex-molt groups were apparent after 24 hours of exposure (see Table 11 and Figure 8) no significant differences were found on either a concentration or total burden basis. This was due to the large range of values obtained in the replicates for the adult groups. The trends in the means are nonetheless of interest.

As seen in Figure 8, on a mean total tissue burden, adult intermolt females had the highest levels; followed by: adult intermolt males, juvenile intermolt females, and juvenile molting females. When expressed on a concentration basis, juvenile molting females possessed levels greater than those of juvenile intermolt females and approaching those of adult intermolt males.

Sixty hours into the depuration period all molting juvenile female crabs had undergone ecdysis and exhibited the rounded ventral abdomen characteristic of the adult female. The terms juvenile molting female and newly molted female both describe this group.

TABLE 12

Results of the One-Way Analysis of Variance for differences in activities measured in the hepatopancreases of intermolt adult females, males, juvenile females and molting juvenile females after 24 hours of exposure to tritium labeled B(a)P and 60, 156 and 252 hours of depuration (following 36 hours of exposure). No molting juvenile females were available at the 252 hour depuration interval.

	SIGNIFICANCE OF F ON A TOTAL TISSUE BASIS	SIGNIFICANCE OF F ON A CONCENTRATION BASIS
24 HOUR EXPOSURE	0.54	0.54
60 HOUR DEPURATION	0.00 **	0.00 **

Groups found to differ by DMRT (0.05):

Total Tissue Basis
Adult intermolt females from juvenile intermolt females; juvenile molting females from all groups

Concentration Basis
Juvenile molting females from all groups

156 HOUR DEPURATION	0.00 **	0.01 **

Groups found to differ by DMRT (0.05):

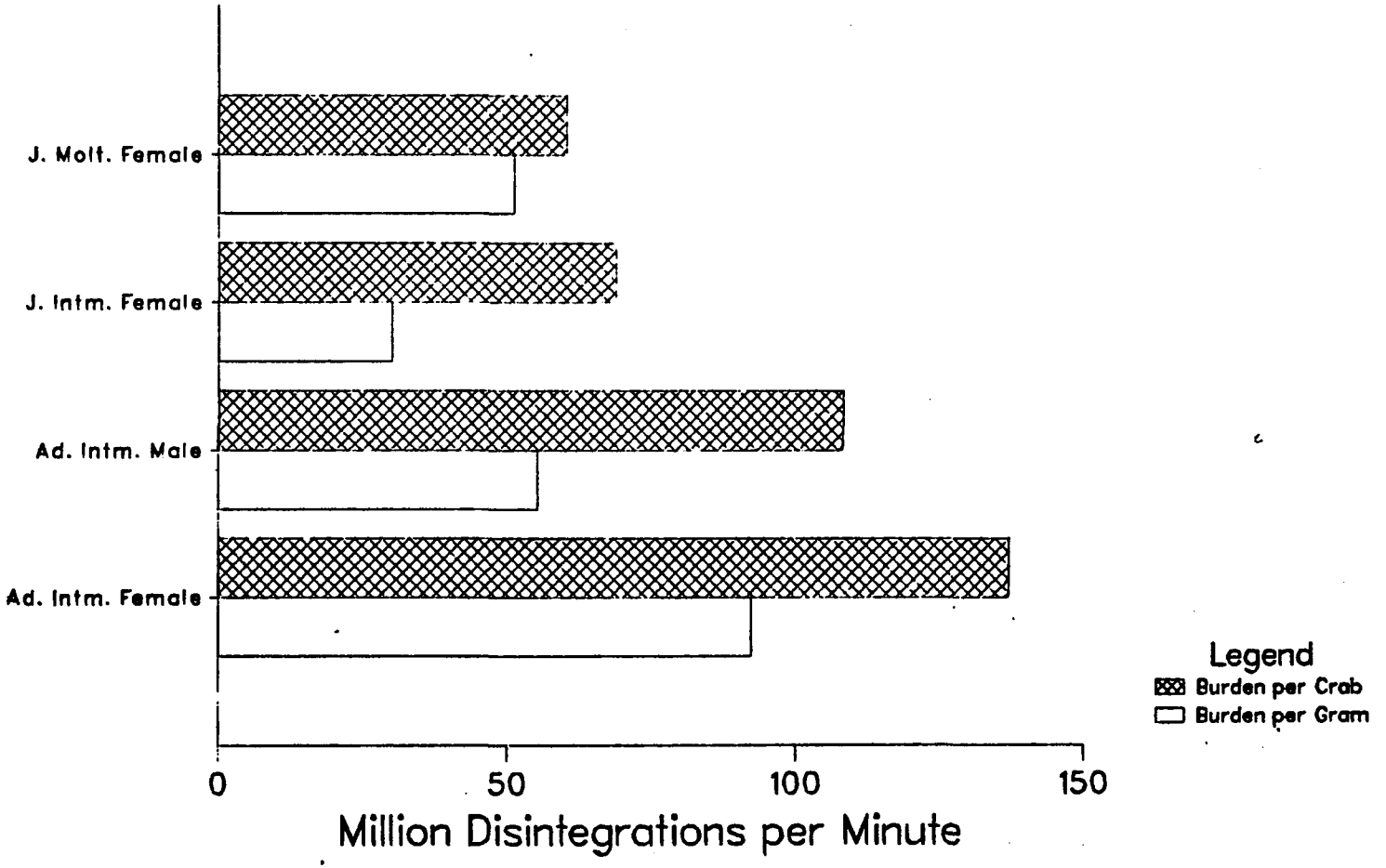
Total Tissue Basis
Adult intermolt females from intermolt adult males and juvenile females; juvenile molting females from all groups

Concentration Basis
Adult intermolt females from intermolt adult males and juvenile females; juvenile molting females from intermolt adult males and juvenile females.

252 HOUR DEPURATION	0.21	0.12

Figure 8. Activity present in the hepatopacreases of blue crabs after 24 hours of exposure to 0.9 ug l^{-1} of tritium labeled B(a)P (ethanol carrier) dissolved in the water.

Level of Radiolabeled B(a)P in Hepatopancreas: 24 Hr Uptake



Activities in all groups, except the juvenile molting females, greatly decreased from earlier levels. The latter crabs were found to have levels higher than the molting juvenile females sampled after 24 hours of exposure (note that the exposure period lasted 36 hours). Differences between the sex-molt classes were found to be highly significant (0.00 on both a concentration and total burden approach). DMRT declared the burdens in intermolt adult males and intermolt juvenile females to be similar. However, amounts in juvenile intermolt females were found to be statistically lower (0.05 alpha level) than adult intermolt females. Molting juvenile females possessed much higher levels then any of the aforementioned groups.

Only the concentrations (per gram basis) in the molting juvenile females were declared different from the others by DMRT.

After 156 hours of depuration, molting juvenile female crabs were still found to have substantially higher amounts of the radiolabel than the other classes, based on the total amount contained in the hepatopancreas. Adult intermolt females contained lower quantities than the above mentioned group but significantly higher than the homogeneous (by DMRT) intermolt adult males and juvenile females.

Concentrations in the intermolt adult males and juvenile females were found similar but both were declared to be statistically lower than the intermolt adult females and molting juvenile female crabs, the means of which were not found to be different by DMRT as seen in Table 12.

No molting juvenile females were examined after 252 hours of depuration. Due to the high variation in the replicates of the adult

intermolt females none of the remaining sex-molt groups were found to differ significantly, although the means appeared to vary.

The mean values for the classes sampled during the depuration period, on a DPM per hepatopancreas basis, as well as on a DPM per gram basis, are graphed in Figures 9 and 10, respectively. Slopes, based on activity values, computed per gram of hepatopancreas, with time were similar to those based on a total hepatopancreas burden, except in the case of the adult intermolt females. In general, the slopes of the lines for the intermolt groups did not differ greatly. However, the slope of the molting juvenile female group was much steeper.

Linecharts of the HPLC fraction collection assays for the determination of B(a)P and metabolites expressed as total hepatopancreas burden per crab are shown in Figures 11 to 22. The data points shown were based on 30 second interval collections, except for the intermolt juvenile females and the 60 hour depuration runs. These were the first samples to be examined, and as such, extra care was taken in order to insure that adequate resolution of the metabolites was obtained. In these runs fractions were taken every 15 seconds during periods where metabolite standards were eluted. In several of these runs the unmetabolized B(a)P peak appeared split. This was thought to be a result of coelution with the toluene sample solvent. In later work methylene chloride was utilized as the solvent and this effect was reduced.

Figures 11 to 14 show metabolite profiles for the sex-molt-maturity categories after 24 hours of exposure to radiolabeled benzo(a)pyrene. Highest levels of unmetabolized B(a)P were observed

Figure 9. Activity present within the hepatopancreases of the test groups after 60, 156 and 252 hours of depuration. The determination was based on the activity of the entire hepatopancreas following a 36 hour exposure to tritium labeled B(a)P.

B(a)P Depuration Based on Total Hepatopancreas Burden

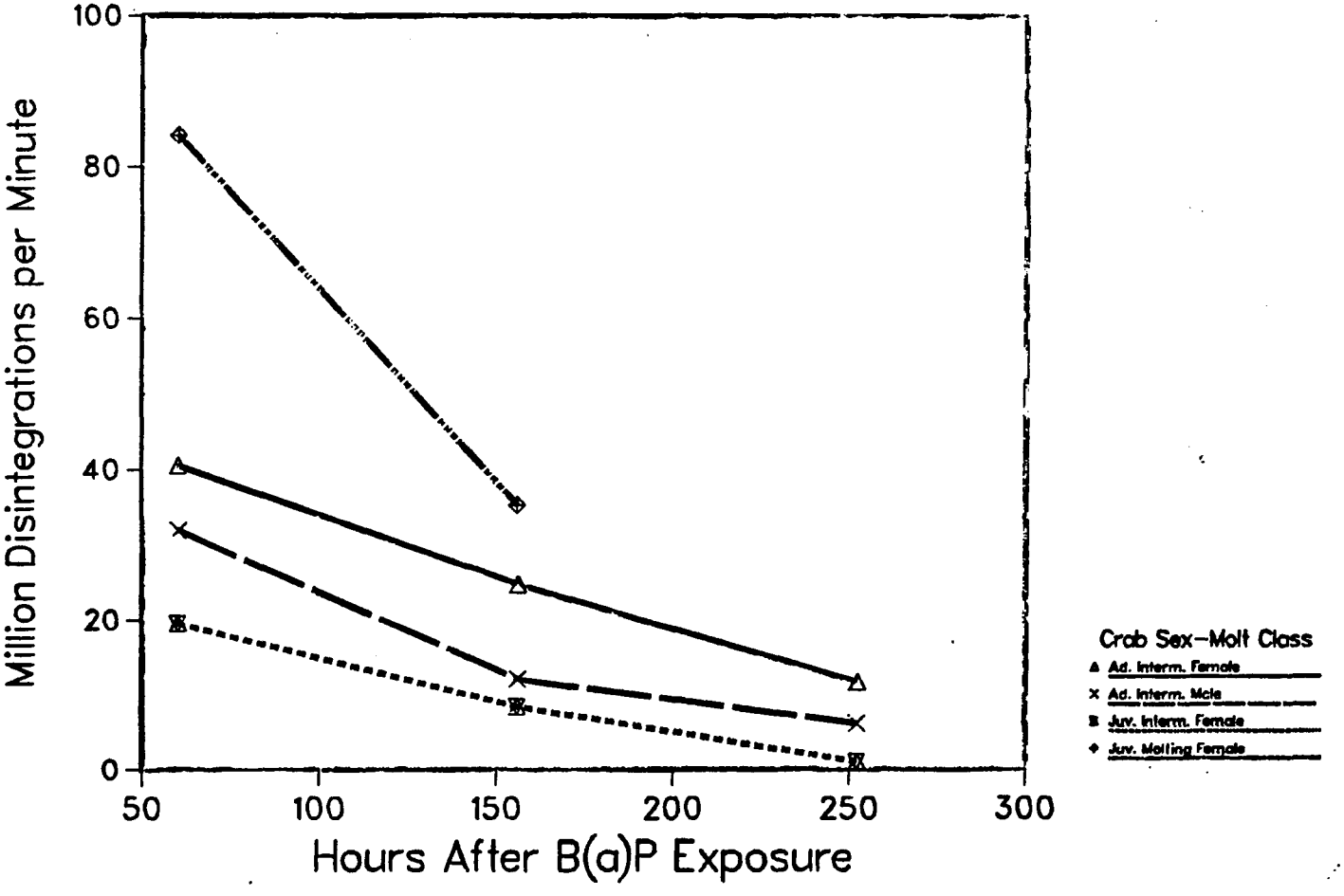


Figure 10. Activity present within the hepatopancreases of the test groups after 60, 156 and 252 hours of depuration. The determination was based on the activity per gram following a 36 hour exposure to tritium labeled B(a)P.

B(a)P Depuration Based on DPM per Gram

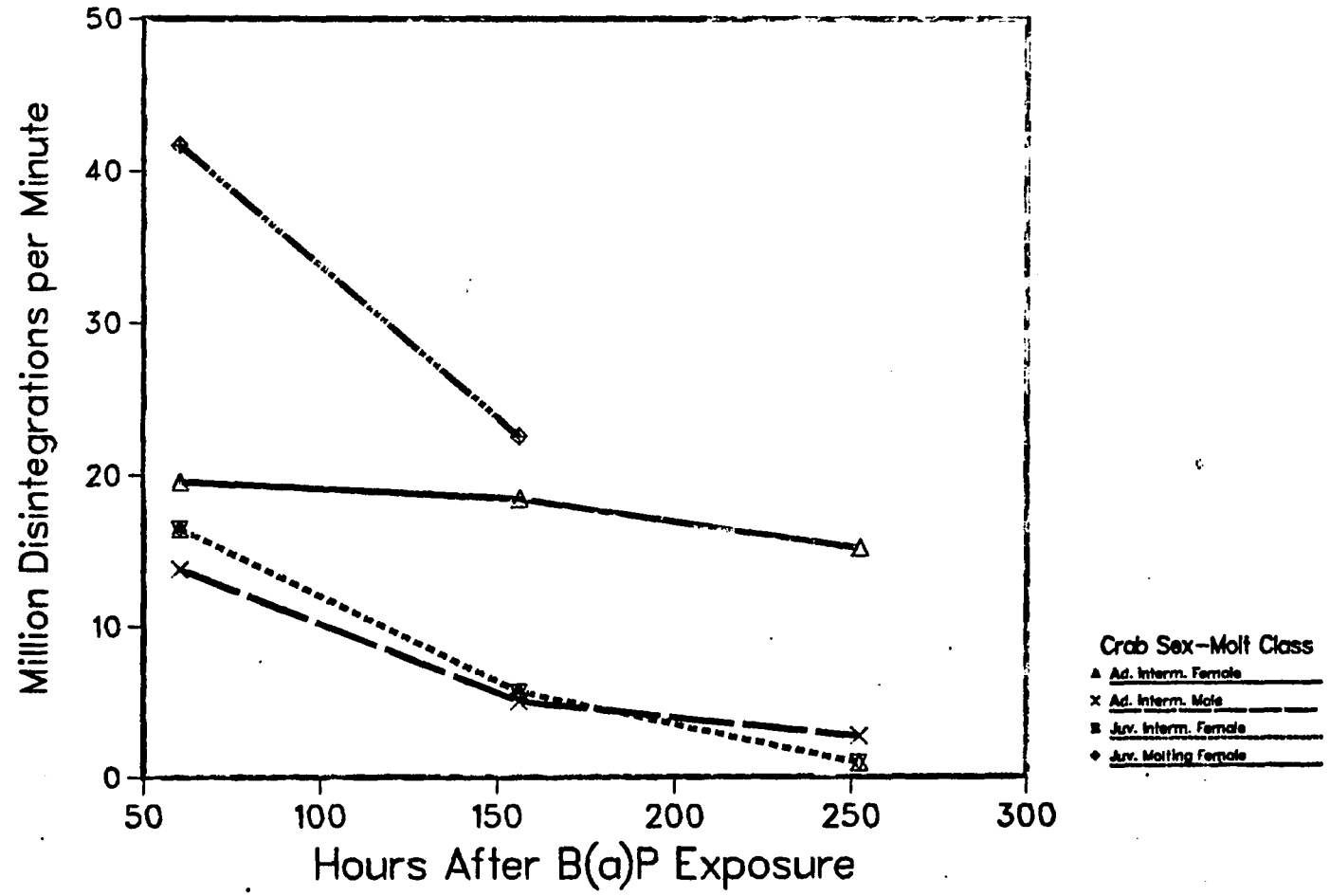
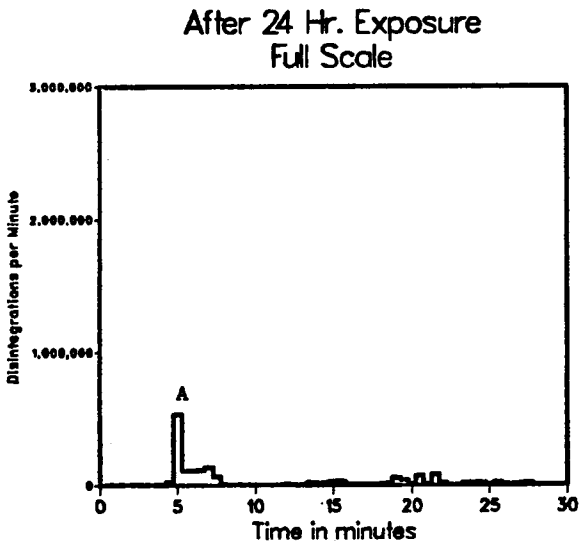


Figure 11. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt females sacrificed after a 24 hour exposure to tritium labeled B(a)P. Values in this and the following figures are based on the activity present in the entire hepatopancreas. The first chromatogram shows a full scale presentation while the second has been enlarged. Tentative identification of the compounds present based on coelution with metabolite standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol reported by Gmur and Varanasi (1982).

Metabolite Profile: Adult Intermolt Females



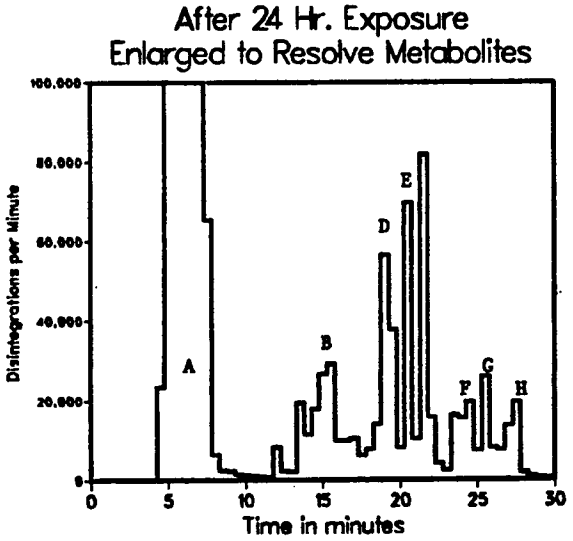
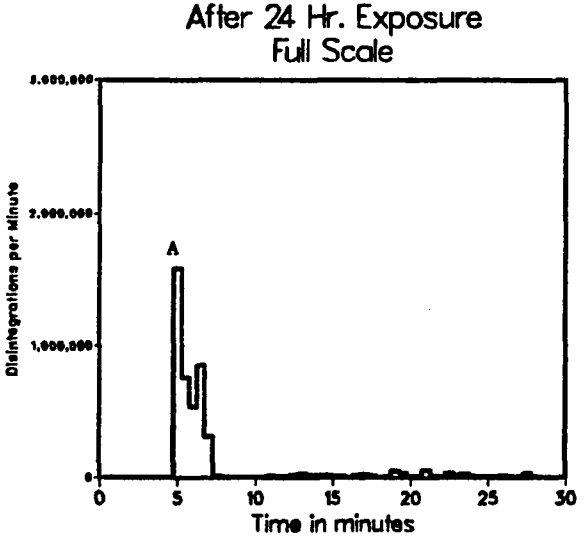


Figure 12. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt males sacrificed after a 24 hour exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Adult Intermolt Males



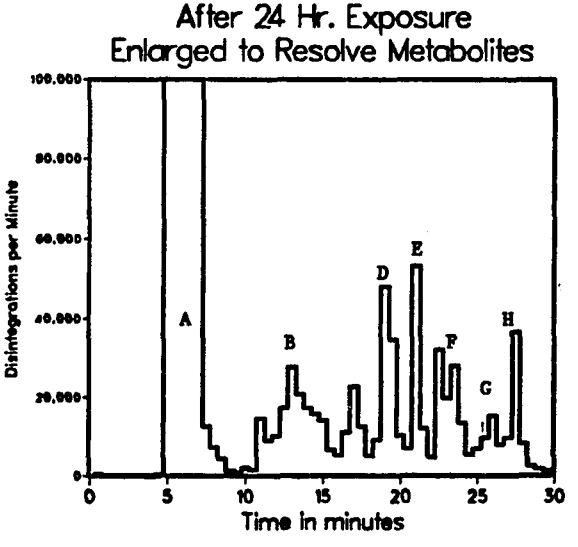
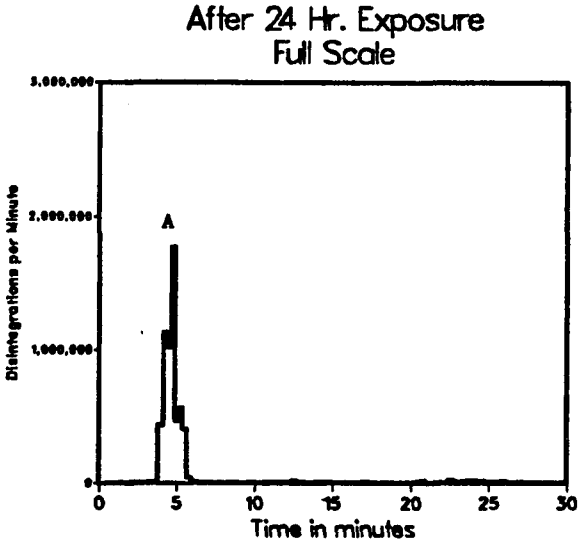


Figure 13. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile intermolt females sacrificed after a 24 hour exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; and (G) B(a)P-7,8,9-triol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Juvenile Intermolt Females



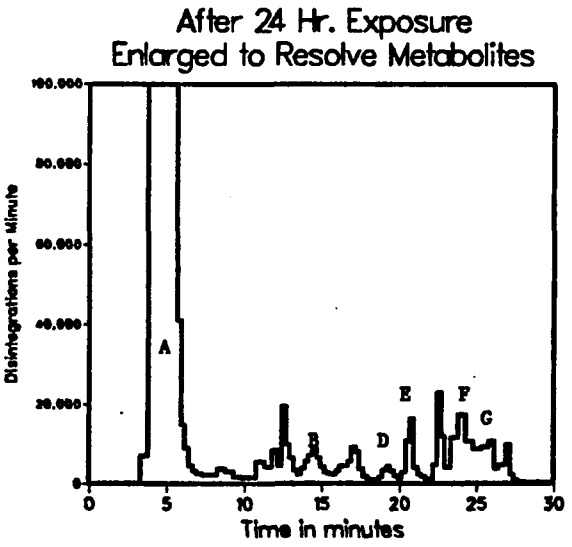
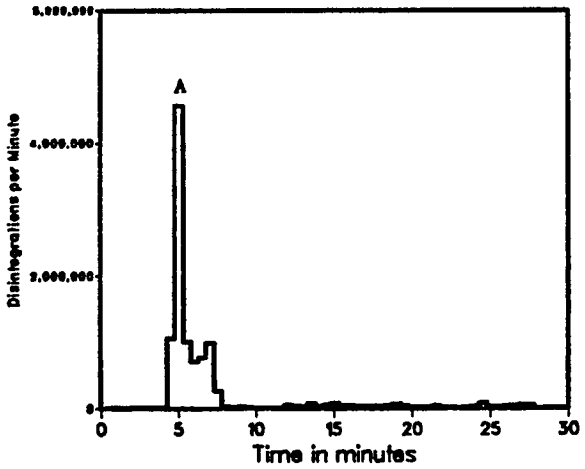


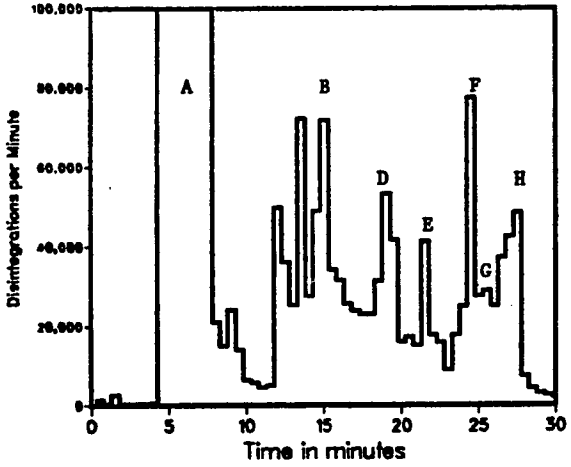
Figure 14. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile molting females sacrificed after a 24 hour exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Juvenile Molting Females

After 24 Hr. Exposure
Full Scale



After 24 Hr. Exposure
Enlarged to Resolve Metabolites



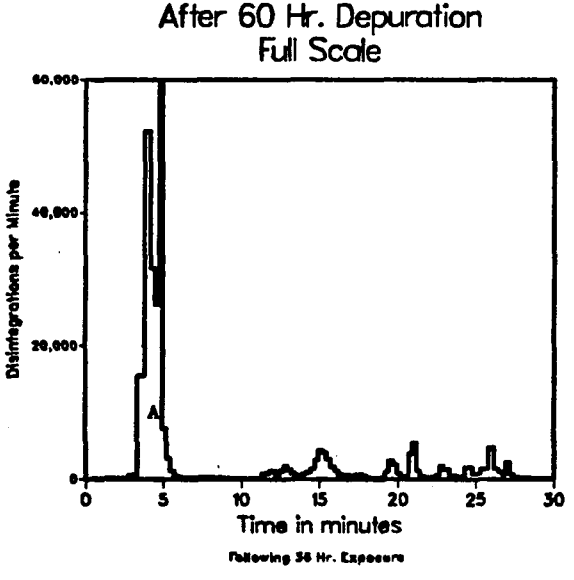
in the molting juvenile female crabs. Adult intermolt females had much lower amounts, while intermolt adult males and juvenile females exhibited intermediate loads. Significant levels of B(a)P phenols were observed in all groups, highest quantities in the molting juvenile females. Peaks were present coeluting with the 7,8- and 9,10-diol, as well as the 7,8,9-triol standards. A peak suspected to be a 7,8,9,10-tetrol was present in the samples, however, in the juvenile intermolt female sample this peak appeared at a retention time shorter than the standard used. In this sample, intervals in the vicinity of suspected metabolites were examined every 15 seconds as previously mentioned, providing greater resolution. A large peak was present eluting after the 7,8-diol and prior to the 9,10-diol. A similar peak was seen by Gmur and Varanasi (1982) in English sole. They hypothesized that it was the nonvicinal 3,9-diol.

Figures 15 to 18 depict the values obtained from the HPLC runs of hepatopancreas extract from crabs allowed to depurate for 60 hours after exposure to the tritium labeled B(a)P. Again molting juvenile females possessed much higher levels of the unmetabolized compound. Note that the scale factor in Figure 18 is much larger than in the other figures, for the same depuration interval. Sizable peaks corresponding to B(a)P phenols and the 7,8-diol, 9,10-diol and suspected 3,9-diol appeared in all samples examined. A small peak is evident in the adult intermolt male group eluting with the 7,8,9,10-tetrol standard. A labeled moiety with a retention time similar to the 7,8,9-triol was present in all but the molting juvenile group.

The extract from molting juvenile females after 156 hours of exposure was examined and the results presented in Figure 19. The

Figure 15. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt females sacrificed after 60 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol. Peak E is thought to correspond to the 3,9-diol reported by Gmur and Varanasi (1982).

Metabolite Profile: Adult Intermolt Females



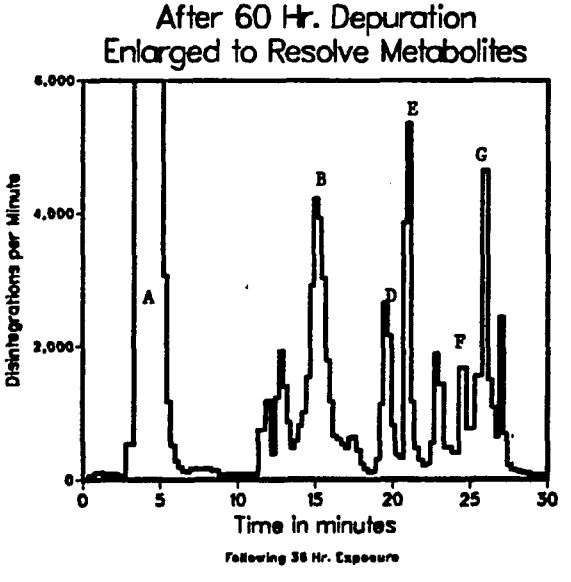
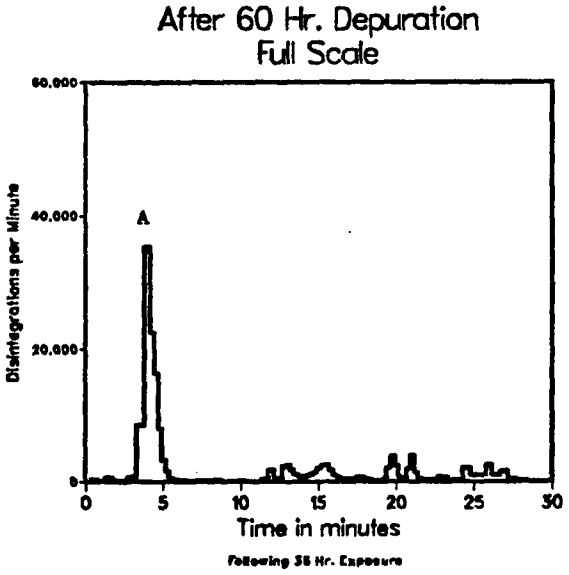


Figure 16. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt males sacrificed after 60 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol; (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol reported by Gmur and Varanasi (1982).

Metabolite Profile: Adult Intermolt Males



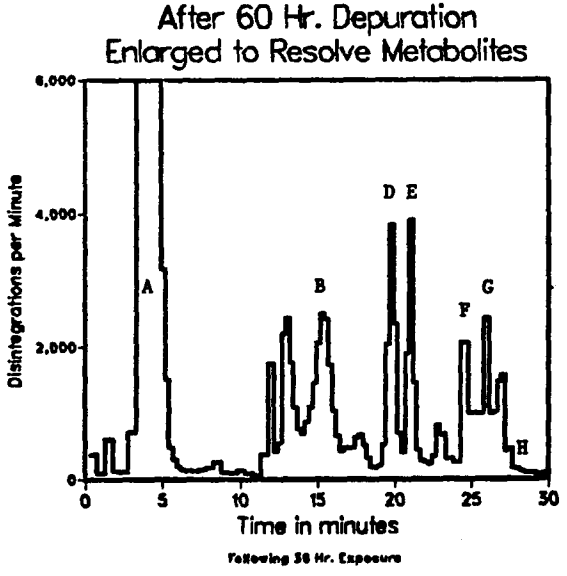
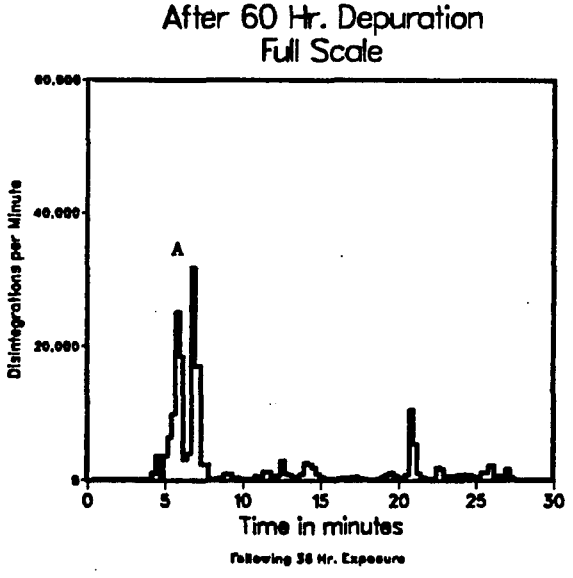


Figure 17. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile intermolt females sacrificed after 60 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (F) B(a)P-9,10-diol; and (G) B(a)P-7,8,9-triol. Peak E is thought to correspond to the 3,9-diol reported by Gmur and Varanasi (1982).

Metabolite Profile: Juvenile Intermolt Females



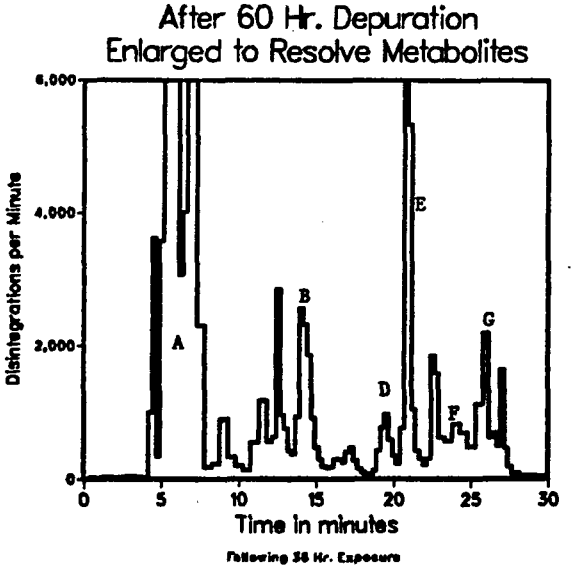
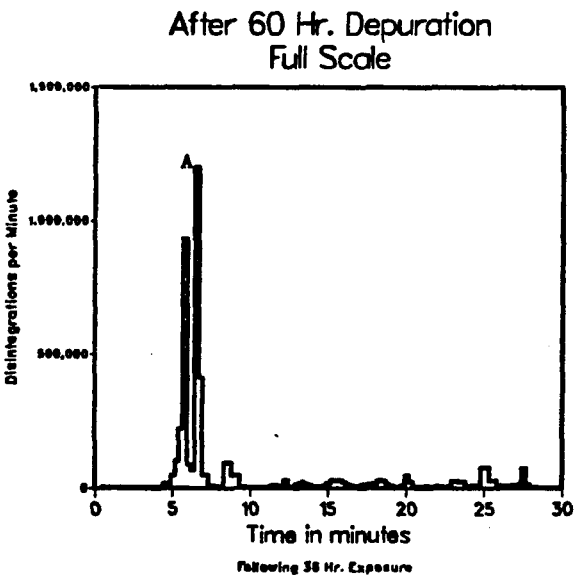


Figure 18. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile molting females sacrificed after 60 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; and (F) B(a)P-9,10-diol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Juvenile Molting Females



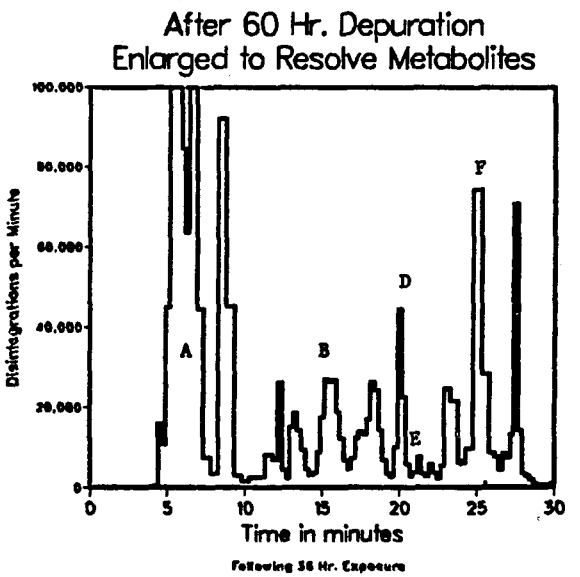
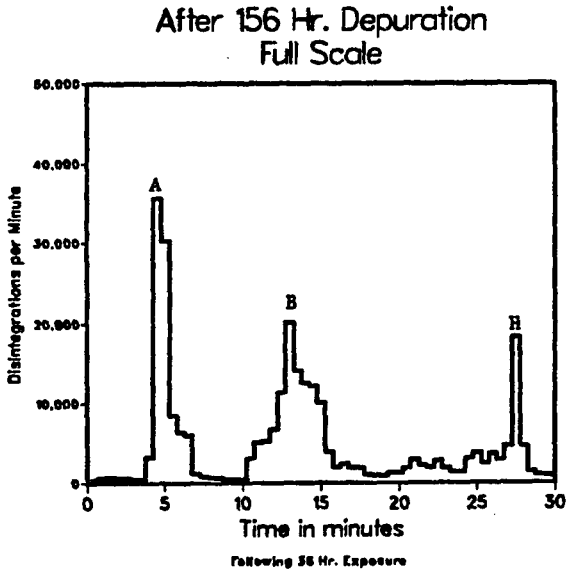
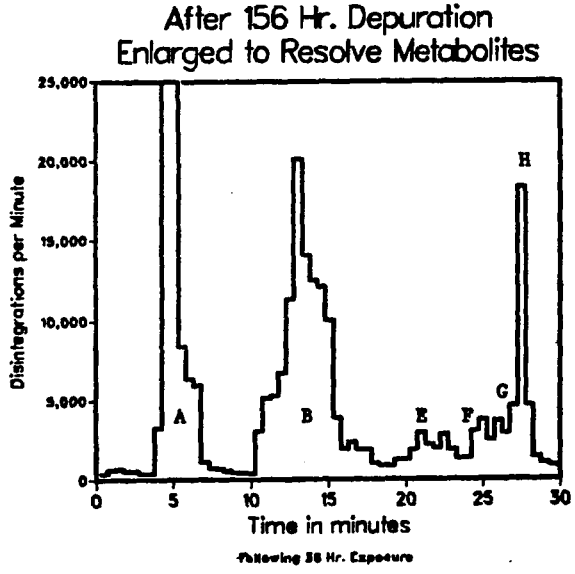


Figure 19. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile molting females sacrificed after 156 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (F) B(a)P-9,10-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Juvenile Molting Females





quantity of unmetabolized B(a)P was similar to that in the intermolt juveniles after 60 hours of depuration; however, the broad phenol peak was much larger. No peak with a retention time similar to 7,8-diol was observed. A shoulder of a peak appears where the 9,10-diol elutes. A large peak is present corresponding to the retention time of the 7,8,9,10-tetrol standard.

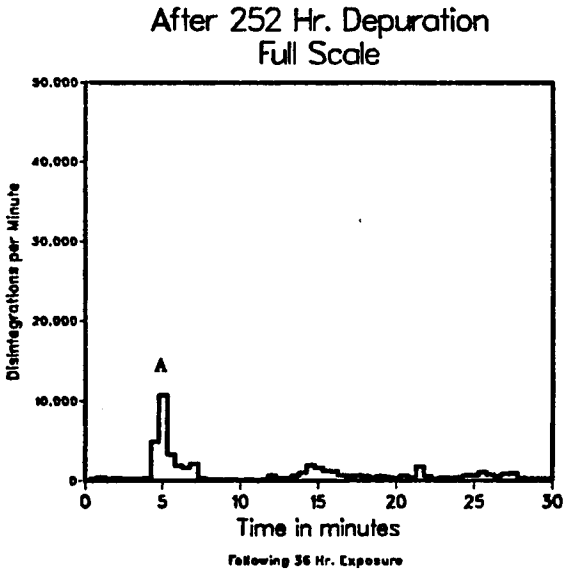
Figures 20 to 22 display the metabolite profiles after 252 hours of depuration. Notable here was the observation that the unmetabolized B(a)P peak for adult intermolt males was essentially unchanged from those allowed to depurate for only 60 hours. Levels in intermolt juvenile and adult females decreased from those earlier observed, but at a slower rate than initially. The suspected phenol, diol, and triol peaks in the adult intermolt males and females were similar. A compound eluting with a retention time similar to the 7,8,9,10-tetrol was observed, however, as mentioned above its presence was suspect.

Two peaks eluting prior to the phenols were commonly observed in the samples analyzed. These were hypothesized to be quinones based on their position in the chromatographic run (Varanasi and Gmur, 1981).

Extraction efficiencies based on the activity in the methylene chloride extract versus that measured by assaying the levels present in whole tissues, ranged from 5.2 to 63.7% with a mean of $29.2 \pm 18.4\%$. Significant color quenching was present in the extracts. Therefore, aliquots were assayed to minimize this effect. The portion of the extracted radioactivity present in the large molecular weight fraction separated on the GPC was determined. Values ranged from 15.3 to 104.8% of the extractable activity, with a mean of $56.6 \pm 23.7\%$.

Figure 20. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt females sacrificed after 252 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Adult Intermolt Females



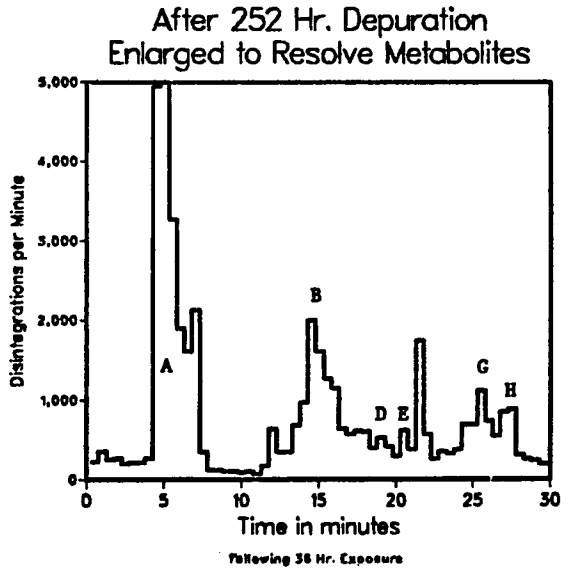
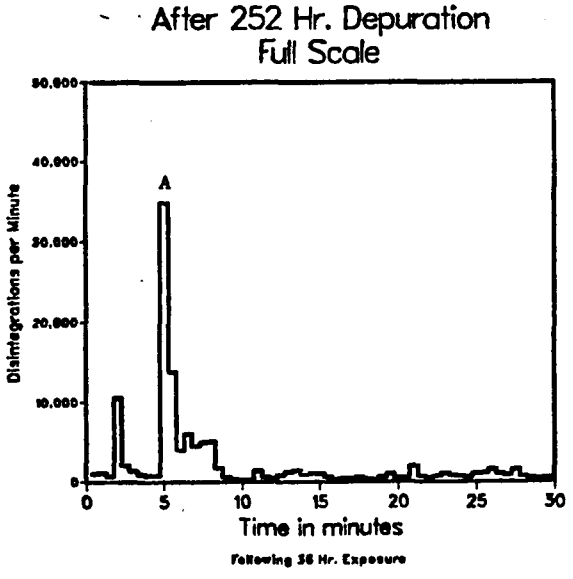


Figure 21. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of adult intermolt males sacrificed after 252 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (D) B(a)P-7,8-diol; (G) B(a)P-7,8,9-triol; and (H) B(a)P-7,8,9,10-tetrol. Peak E is thought to correspond to the 3,9-diol.

Metabolite Profile: Adult Intermolt Males



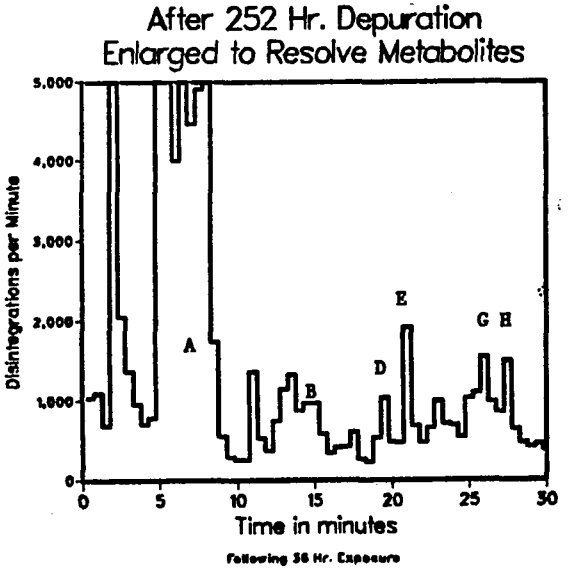
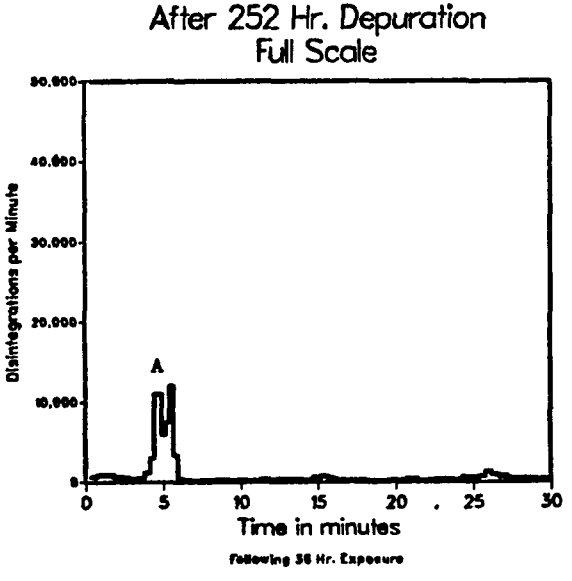
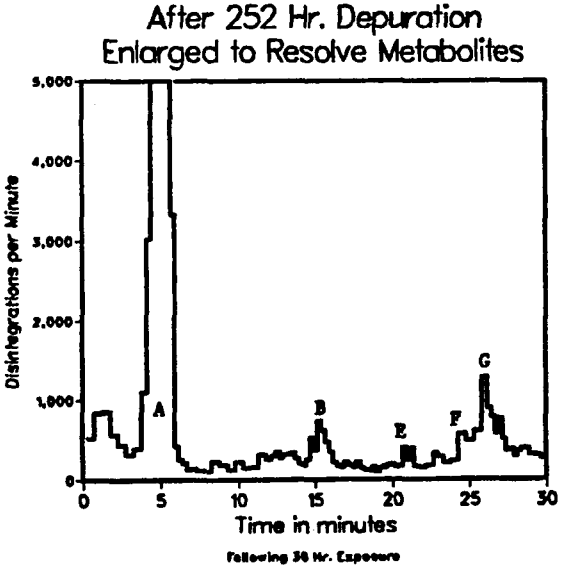


Figure 22. Radiochromatograms produced by fraction collection of the HPLC effluent from the analysis of the hepatopancreas extract of juvenile intermolt females sacrificed after 252 hours of depuration following 36 hours of exposure to tritium labeled B(a)P. Tentative identification of the metabolites based on coelution with standards include: (A) B(a)P; (B) B(a)P phenol; (F) B(a)P-9,10-diol; and (G) B(a)P-7,8,9-triol. Peak E is thought to correspond to the 9,10-diol.

Metabolite Profile: Juvenile Intermolt Females





Efficiencies were acceptable considering the polar nature of the hydroxylated metabolites and conjugates, and the relatively nonpolar extracting solvent, methylene chloride.

IV. DISCUSSION

Toxic organic pollutants are widespread in the environment. Their presence has been detected in all compartments be it air (Funcke et al., 1982), water (Thomas et al., 1980), soil (McGill and Rowell, 1980) or biota (Mironov et al., 1981). The purpose of this research was to establish the distribution and fate of these compounds within a population of an important estuarine inhabitant, the blue crab, Callinectes sapidus, of the Chesapeake Bay estuarine system.

Predominant among the toxic organics detected in these organisms were the alkyl substituted polynuclear aromatic hydrocarbons, e.g., the methylated phenanthrenes. These compounds are found in appreciable quantities in fossil fuels (Sinkkonen, 1982). Thus their presence in the estuarine environment is symptomatic of a release, be it natural or anthropogenic (Mille et al., 1982). No significant natural oil seeps are present in the Chesapeake Bay so it may be assumed that this contamination is related to human activity.

The Chesapeake Bay and contiguous waters are highly utilized for a variety of purposes. Among the major contributors to the burden of TOP are sewage treatment plants. Although facilities releasing sewage effluent into the Bay have been upgraded, their impact is still significant. Contributors to these waste treatment facilities include industries as well as municipalities. It was estimated that industrial waste at the Hopewell (Virginia) Regional Plant made up 85% of the flow (Virginia State Water Control Board, 1980). Many industrial concerns release effluents with little or no pretreatment

of their toxic wastes. Inadequate domestic unit sewage systems often discharge untreated wastes directly to the environment.

In addition to land based sources, boat traffic contributes significantly to the levels of pollution. Spills and losses of fossil fuels through accidents and normal operation of recreational boats, large tankers and freighters are common.

Shaw and Connell (1982) and Matsumoto and Hanya (1981) determined that uptake of pollutants in organisms was proportional to the concentrations present in their surrounding environment. Since the blue crab is a resident of a polluted environment it was expected that it would accumulate significant levels of contaminants.

Substantial concentrations of aromatic pollutants were noted by Bieri et al. (1982) in their examination of organic compounds in sediment and oysters taken in the vicinity of highly urbanized and industrialized areas. Blue crabs were sampled in the present study near one of these same sites, Hampton Roads, located at the confluence of the James River with the Chesapeake Bay. This is a center of commercial and military activity, major installations include: Newport News Shipbuilding and Drydock Company, Virginia Chemicals Company, Virginia Electric and Power Company (VEPCO), U.S. Navy and Army bases, a major coal loading facility, a creosote factory on the Elizabeth River (a tributary of the lower James) and several sewage treatment facilities. Concentrations of ASPAH detected in crabs collected from this site reflect the high level of pollution present.

Bieri et al. (1982) generally found highest TOP levels at the mouth of rivers. This effect may be caused by the heightened boat traffic at these sites. The lower solubility of hydrocarbons in

saline water (Rossi and Thomas, 1981; Herrmann and Huebner, 1982) than fresh and the lower sediment carrying capacity of the current as it passes into the Bay may contribute.

A second site on the James River, near Hog Island, was also examined. This station was 25 miles downriver from heavily industrialized Hopewell, Virginia. Richmond is approximately 90 miles upstream. Although the area surrounding this station is not as urbanized as the lower James, several large installations are present in the immediate area, such as the Dow Badische Corporation, Anheuser-Busch Inc., several sewage treatment plants and a VEPCO nuclear power plant. This station was in the vicinity of the James River turbidity maximum (Lunsford, 1981). Compounds such as aromatic hydrocarbons and PCB, which associate with sediment particles due to their low water solubility may concentrate here. This was found to be the case with the hydrophobic compound Kepone, released into the James River estuary at Hopewell (Nichols and Trotman, 1977). However, low levels of ASPAH were detected from blue crabs sampled from this upper river site, indicative of the lower hydrocarbon input, compared with the Hampton Roads station.

Initially, the crabs sampled from the Pocomoke Sound appeared to possess ASPAH concentrations higher than those at the upper James site. This was found to be a function of one heavily contaminated sample. It was thought that this sample included crabs which had recently been exposed to a large volume of petroleum shortly before sampling. This conclusion was reached due to the presence of a large quantity of alkyl substituted aromatics, particularly dibenzothiophenes. Vassilaros et al. (1981) reported that aromatic

sulfur heterocyclics were strongly bioaccumulated by aquatic organisms following oil spills.

When the value noted above was replaced by the median of tissue levels of all groups obtained at the site, the mean value for the Pocomoke Sound dropped below those of the other sites. Sources of anthropogenic material on the eastern shore of Virginia are mainly agricultural. Therefore, it was hypothesized that the major input of ASPAH was from boat associated activities.

Crabs sampled from the final station, located 35 miles upriver from the mouth of the Rappahannock River, near Tappahannock, Virginia, possessed ASPAH concentrations similar to those seen on the upper James. Anthropogenic input was relatively low (Virginia State Water Control Board, 1980), limited to a few sewage treatment plants and marinas. Industrial activity was not highly developed. FMC formally operated a plant in the vicinity but closed its facility in 1978. The only other industrial concern listed in the Virginia Water Quality Inventory (State Water Control Board, 1980) was the Safeguard Corporation which operated near the area sampled.

The results of the Four-Way ANOVA did not indicate significant differences between crabs sampled in 1981 from the four above mentioned sites. This suggested that, although highest levels of alkyl substituted aromatic hydrocarbons were present in blue crabs inhabiting urbanized areas, the Bay population as a whole had substantial body burdens. Obviously the entire Chesapeake Bay has a TOP problem. Migration of crabs may have obscured differences in pollution burdens between stations. However, as shown by the in-laboratory experiments, uptake and clearance of ASPAH is rapid.

Therefore, migration was unlikely to affect the hydrocarbon patterns observed. It might have influenced the pattern of more persistent compounds, such as PCB.

Unresolved envelopes, consisting of chromatographically indistinguishable hydrocarbon isomers are thought to be of a petroleum origin. Indeed, when Jones et al. (1983) incubated coastal sediments spiked with North Sea crude oil, unresolved complex envelopes developed. It was hypothesized that the UCM consisted of aerobically biodegraded alkylaromatic hydrocarbons. Therefore, their occurrence should coincide with that of the ASPAH. This was found to be the case. Highest concentrations were evident from crabs taken from the lower James, followed by the Pocomoke Sound, upper James and Rappahannock, respectively.

The nonsubstituted aromatic hydrocarbons are thought to originate predominantly from pyrolytic sources (Hites et al., 1977), although they are present in fossil fuels in low concentrations and have been associated with oil spills (Mix and Schaffer, 1983). Pancirov and Brown (1977) found only minor amounts of these compounds in aquatic organisms from the Chesapeake and Raritan bays. These compounds were detected in blue crabs in much lower concentrations than the ASPAH. Bieri et al. (1982) found higher levels of nonsubstituted aromatics than ASPAH in Bay sediments and oysters, although levels in the latter were much lower than in the former. Highest levels of nonsubstituted aromatics were found in the area of the developed lower James River (Table 7). Concentrations determined in samples from the upper James exceeded those from both the Pocomoke Sound and Rappahannock River

stations. Distributions were in agreement with the level of urban and industrial development present in these areas.

The polychlorinated biphenyls, unlike the ASPAH and nonsubstituted aromatics which may be produced in natural processes, are exclusively synthetically produced. As such they are good markers of anthropogenic activity. PCB exist as a family of isomers in commercial preparations (209 isomers are possible, ranging up to ten chlorines on the biphenyl molecule). Chlorine content is usually between 40 and 60 percent. Their distribution in the environment is a function of the physical and chemical properties of the individual isomers. Water solubility decreases with increasing chlorine substitution. Their accumulation within biota is also determined by the number of chlorines present. Generally the higher chlorinated isomers are preferentially taken up and are more difficult to metabolize (Goldstein, 1980; McLeese et al., 1980). Shaw and Connell (1982) found that PCB isomers with seven or more chlorines were taken up in estuarine organisms to a lesser extent than the five and six chlorine compounds, due to steric considerations. Chlorination pattern is also important in determining the biological fate of PCB. The absence of chlorines on two adjacent carbons greatly facilitates the biotransformation processes (Guiney and Peterson, 1980).

These chemical factors strongly influenced the levels of PCB detected in the crabs analyzed. Biphenyls possessing from five to seven chlorines predominated. Concentrations, uncorrected for the decreased sensitivity shown chlorinated compounds by the FID detector, ranged from nondetectable to 131 ug kg^{-1} (based on the summation of the quantities measured for the individual isomers) in the

hepatopancreas on a dry weight basis. The mean was 21 ug kg^{-1}. This was in agreement with the low levels found in blue crabs from the Maryland portion of the Bay by Eisenburg et al. (1980). Reid (1983) reported Arochlor 1254 residues averaging only 10 ug kg^{-1} in crabs from Long Island Sound but values 40 times higher in Raritan Bay.

It is interesting to note the distribution of PCB burden by sampling site. ANOVA declared the probability of the hypothesis of no difference between sampling sites to be 0.10 (much less than the 0.89 observed with the ASPAH). Consideration of the mean concentrations detected in the crabs showed that higher levels were present in samples from the James River than from either the Rappahannock or Pocomoke Sound. Levels were comparable between the upriver and the lower site. Thus, the primary source of the PCB seemed centered in or near the James River. This same conclusion was not apparent with the ASPAH. The possibility exists that there was a considerable upriver input; however, this was not conclusive due to the high residence time of PCB (resistance to biotransformation) in crabs. Crabs at the upper station may have been supplemented by migrants from the industrialized lower James which had retained their PCB burden.

Metabolites of the pesticide p,p′-DDT were detected in several of the samples. The use of DDT has been banned since 1970. However, Bieri et al. (1982) detected significant concentrations of p,p′-DDT, along with PCB, in sediment from a mid-bay station in their examination of toxic organic compounds in the Chesapeake Bay. They hypothesized that their presence was due to a recent dumping incident, or a release from the deterioration of a container previously discarded containing the compounds, since metabolites of p,p′-DDT were

not detected. In the crabs analyzed in this research both p,p′-DDE and p,p′-DDD were found, although the former was much more common. No parent DDT was detected. Residues in blue crabs were more commonly encountered in samples from the lower James. The predominance of DDE over DDD was in agreement with other workers (Giam et al., 1972; Eder et al., 1976; Sheridan, 1975) examining levels in marine and estuarine organisms. Murphy (1970) determined that DDE was preferentially accumulated over DDD in aquatic organisms at high salinities when both metabolites were present in the water in identical concentrations.

Two unidentified chlorinated compounds were detected in the tissues of the blue crab. Compounds exhibiting retention times similar to these compounds appeared in 12 of the 83 samples analyzed. They were encountered most frequently in the Pocomoke Sound.

Higher quantities of PCB, alkyl substituted aromatic hydrocarbons and unresolved envelope components were found in June than in September. A spring maximum has been noted by other researchers (Olsson et al., 1978; Edgren et al., 1981). Water runoff from urban areas is a significant source of pollutant input, especially hydrocarbons (Gschwend and Hites, 1981), and may contribute to this pattern. The problem often becomes critical during the spring when heavy rains cause direct discharge of contaminated overflow directly into waterways (Herrmann and Huebner, 1982). An example of this occurred in the Hampton Roads area just prior to the June field sampling. Heavy rains resulted in excessive pressure at a pumping station, releasing 20,800 gallons of sewer water into the James and forcing the closing of a five mile section of the river to shellfish harvesting. Heavy rains also cause soil erosion with attendant

leaching and transportation of material, including pollutants, to rivers and the Bay. Erosion is particularly acute where the land has been denuded of vegetation, often these are developed areas having the highest levels of pollutants.

Seasonal variations in pollutant levels have been correlated with biological parameters as well. Changes in lipid content and feeding habits were cited as possible explanations (Reinert and Bergman, 1974; Phillips, 1978). No significant differences in methylene chloride extractable lipids were found between crabs collected in June and September. However, Rouse (1972) reported lower glycogen levels in late summer than spring in the blue crab and increased molting frequency with warmer temperatures.

Water temperature exerts an influence on the levels of xenobiotics in poikilothermic marine organisms. Varanasi et al. (1981) force-fed naphthalene to juvenile starry flounder and reported 2 to 15 times as much present in the tissues of fish held at $4^{o}C$ as those at $12^{o}C$. After one week fish maintained at $4^{o}C$ contained 26 to 34 times greater concentrations than those at $12^{o}C$. James et al. (1979) found activities of mixed function oxygenase to be much higher at warmer temperatures. Therefore, differences in concentrations of pollutants may be due to differences in biotransformation rates at varying temperatures. Microbial and physical degradation of pollutants in the aquatic environment may have been lower in June as well. Lowered water temperatures in the Bay probably contributed to the higher pollutant levels observed in crabs collected in June.

The nonsubstituted aromatic hydrocarbons were present in higher concentrations in the tissues of blue crabs sampled in September than

June. As noted, quantities were low, making detailed conclusions unwise. Contributing to the levels detected was the compound perylene, thought to be at least partially derived from the decomposition of terrestrial material under reducing conditions. Its formation is a relatively slow process and appreciable quantities have been detected in deep sea sediments off isolated coastlines (Tissier and Saliot, 1981). Therefore, perylene may not have been contributed to the crabs by an exclusively anthropogenic source.

A very strong correlation of PCB and alkyl substituted aromatic hydrocarbons with tissue type was found. The null hypothesis of no difference in concentration for these substances between hepatopancreas and muscle had an F probability of less than 0.01 (Four Way ANOVA). This preferential accumulation agreed with the results of other researchers working with crustaceans (Palmork and Solbakken, 1980; Tarshis, 1981). Concentrations of unresolved envelope components and nonsubstituted aromatic hydrocarbons also followed this pattern. Higher lipid content was observed in the hepatopancreas (mean 34%) compared to muscle (mean 4%) and contributed to the higher levels of the lipophilic pollutants observed in the former tissue. Pillay and Nair (1973) reported similar lipid figures in their biochemical examination of decapod crustaceans.

Another factor contributing to the elevated pollutant load of the hepatopancreas is its function as the major storage and detoxification site in the crab. Lee et al. (1976) found hydrocarbons were quickly accumulated in the hepatopancreas of blue crabs after exposure in both food and water. It is reasonable to expect that after exposure to xenobiotics some of the initial pollutant burden in the hepatopancreas

might be redistributed to other tissues of the crab, such as the muscle. Owing to the relatively low lipid content of muscle it is improbable that high concentrations would be built up. However, due to the large contribution the muscle tissue makes to the mass of the organism, and the low MFO activity it possesses, levels are generally not negligible and may be expected to persist for a substantial period of time. Varanasi et al. (1982) noted that unmetabolized benzo(a)pyrene was transported from the liver to the ovary in English sole. Although greater quantities were found in the liver, a larger percentage of the radioactivity in the gonad after 168 hours was associated with unconverted B(a)P. Lee et al. (1976) reported a greater amount of radiolabeled hydrocarbon associated activity in the muscle than the ovary, based on total tissue burden. The redistribution of residues to sites where the pollutant is less accessable to biotransformation by metabolizing enzymes may have been partially responsible for the biphasic elimination observed in organisms after exposure to xenobiotics. A possible manifestation of this effect was the large interaction between month of sampling and tissue seen in the blue crabs examined in this study (F probability of no interaction effect for ASPAH was 0.03 and 0.06 for PCB). Levels in muscle tissue relative to hepatopancreas increased in September, compared with those in June. This retention of xenobiotics in metabolically isolated reserves was supported by the decrease in biotransformation rate apparent during the in-laboratory exposures conducted in this study and by others (Tarshis, 1981; Guiney and Peterson, 1980).

The sex and maturity of the crabs had a strong influence on the levels of ASPAH. Adult females and juvenile males had higher concentrations than either adult males or juvenile females. Although this distribution was strongly influenced by two samples containing high ASPAH loads, replacement of these with median values did not alter the order of the means. No significant differences in lipid levels between the two adult groups or between the juvenile classifications were detected. Therefore, simple partitioning into lipids does not appear to be the reason. However, lipid material was not further characterized. It is possible that different classes of lipids may be present; pollutants may partition preferentially into some of these.

Interactions between month-sex/maturity and tissue-sex/maturity were suggested by the data (F significances 0.09 and 0.06 respectively). Adult females contained more ASPAH in hepatopancreas relative to muscle than the other groups (this may also have been affected by the recency of petroleum exposure). Juvenile females were the only group that showed an increase in ASPAH in September compared with June. When the above mentioned extreme values were removed, juvenile males also exhibited an increase in September. A possible explanation for this phenomenon is related to the molt cycle. Juvenile crabs molt at a much higher frequency than mature crabs (adult females do not molt). In-laboratory experiments suggested that the biotransformation of xenobiotics was reduced during ecdysis. This might have resulted in increases in ASPAH in juveniles, especially during their early growth phases.

Sex and maturity of the blue crabs sampled exerted less of an influence on the levels of PCB detected (probability of the hypothesis of no difference between groups 0.28). The same interactions as in the ASPAH were appreciable for the PCB (month-sex/maturity 0.07, tissue-sex/maturity 0.08)

When cell frequencies for the month-sex/maturity groups were examined it was seen that PCB concentrations in juvenile and adult males were similar in the June and September samplings. An increase was found in juvenile females and a decrease in adult females between the two sampling dates. This may be explained by the difficulty involved in the metabolization of PCB with five or more chlorines. Levels would be expected to either remain stable or to increase over the summer. The decrease in the concentrations in the adult females in September might be due to the partitioning of residues into the egg masses. Roberts and Leggett (1980) hypothesized that egg extrusion was a principle route of Kepone elimination from adult females in the Chesapeake Bay, primarily via loss from the ovary. However, they noted elevated Kepone concentrations in muscle and hepatopancreas after extrusion, and thought them to be derived from the resorption of egg associated residues. This compound is, as is the highly chlorinated PCB, extremely resistant to biotransformation. Schimmel et al. (1979) exposed blue crabs to Kepone for 28 days. After a 28 day depuration period no loss of the compound was detected. The sex/maturity-tissue interaction, in the current study, for the PCB was due primarily to the higher ratio of hepatopancreas to muscle in the adult females compared to the other groups. This was similar to the pattern seen with the ASPAH. Due to the rapid biotransformation of

the hydrocarbons, egg extrusion is thought to be less important for aromatic hydrocarbon elimination than for the persistent chlorinated compounds.

Concentrations of ASPAH in ovaries of adult females without extruded eggs were intermediate between those of hepatopancreas and muscle in the same crabs. This was expected when the lipid content of the ovary was considered. Percent methylene chloride extractable lipid of ovary, on a dry weight basis, was 18.3% versus 34.0% in hepatopancreas, and 4.3% in muscle. Unfortunately, since adult females with extruded eggs were not available at most sampling sites on the dates required, sample size was small. Analysis of the eggs from the crabs obtained revealed concentrations greater than ovary but less than hepatopancreas. Thus extrusion could result in reductions in total lipophilic pollutant body burdens. This was in agreement with the relative order hypothesized on a percent lipid consideration (eggs contained 26.6% lipid). Niimi (1983) found a similar partitioning of contaminants into eggs of fish based on lipid considerations. Crabs with extruded eggs contained lower ASPAH residues in the ovary than females without eggs, although sample size was small. This was supported by the work of Roberts and Leggett (1980). Data for the PCB paralleled that of the ASPAH.

Several heterosubstituted aromatic hydrocarbons were detected in the crabs. Dibenzofuran, benzothiazole and dibenzothiophene were common environmental contaminants. Benzaldehyde was detected in several samples. It is used in the manufacture of dyes, perfumes and solvents. However, it has also been found as a contaminant in the

toluene used as a solvent in this research. Cis and trans-4-phenyl-3-butene-2-one, precursors in the production of plastics and intermediates in the synthesis of steroids and vitamins (The Merck Index, 1976) were detected. It may also be produced by the reaction of benzaldehyde with acetone under basic aqueous conditions (Pine et al., 1980). Acetone was used in several phases of the project to rinse glassware.

In addition, 1,1′-biphenyl-2-amine was found in the tissues of blue crabs. The isomer 1,1′-biphenyl-4-amine, formerly used in the rubber industry as an antioxidant (Parkes, 1976), is a potent carcinogen.

Results of the laboratory exposure of test groups of adult intermolt males and females, juvenile intermolt and molting females to less than one ug l^{-1} tritium labeled benzo(a)pyrene confirmed that significant bioaccumulation of hydrocarbons from low environmental levels is possible. Due to the ability of the crab to eliminate these aromatic compounds (Lee et al., 1976) the high levels of alkyl substituted aromatic hydrocarbons in the crabs sampled from the field were thought to be the result of a very recent contact with petroleum.

Table 11 shows the total activity values in hepatopancreas obtained from the analysis of nonextracted tissue. Values were expressed as micrograms of tritium labeled material, including both B(a)P and metabolites, per tissue, as well as on a per gram basis. These results were compared with those obtained by Lee et al. (1976) which also appear in the table. Lee and his associates employed only juvenile crabs (sex not indicated) in his work on uptake of B(a)P from water. Higher uptake values were obtained in the present study after

24 hours of exposure to B(a)P than after 48 hours in the experiments of Lee et al. (1976). They reported no further increases in uptake after 48 hours in preliminary experiments. Obana et al. (1983) found levels of B(a)P accumulated from water to reach a maximum in short necked clams after 24 hours.

All test groups exhibited comparable, elevated uptake of B(a)P. Several factors may have contributed to this discrepancy with the data of Lee et al. (1976). As noted in the results section, significant particulate matter was present in the exposure tank. Association of the B(a)P with this material may have led to enhanced uptake. Also, a greater volume of carrier per microgram of B(a)P was employed in this study to ensure optimal solution and dispersion of the compound in the holding tank. This carrier may have facilitated the uptake of B(a)P. Lee et al. (1976) reported an approximately 20% loss of the radiolabeled hydrocarbon from the aquaria utilized in his experiments, attributing half of this loss to uptake by the crabs. Numbers of crabs per liter of water were comparable in the two studies. In this research adult crabs as well as juveniles were used. The adults possessed greater capacity for storage of the B(a)P via their larger size. Losses from the tank water were correspondingly higher. The specific activity of the radiolabeled compound was also considerably higher than that employed in the earlier study (70.5 Ci/mmol versus 25.2 Ci/mmol), allowing the use of a lower B(a)P concentration (0.9 ug l^{-1} versus 2.5 ug l^{-1}). McLeese et al. (1980) reported greater uptake efficiency at lower concentrations in American lobster for PCB. Harding and Vass (1979) noted linear uptake over a range of concentrations.

If the 24 hour uptake value for juvenile intermolt females was expressed on a ug kg^{-1} basis a value of about 50 ug kg^{-1} was obtained. Of this 50 ug kg^{-1} an appreciable portion of the radiolabeled material was in the form of polar metabolites, and thus would not be detected using conventional nonpolar hydrocarbon detection procedures. The blue crab tissue samples examined from the field in this study had identifiable ASPAH concentrations as high as 1094 ug kg^{-1}. The accompanying unresolved envelope had an area of approximately 9820 ug kg^{-1}. Some of the components comprising this envelope were probably hydrocarbon metabolites, although many were removed from the extract examined via the preparative HPLC step employed. Obana et al. (1983) reported a bioconcentration factor for B(a)P in clams of about 3000. Obviously the value for blue crabs is much lower. Spacie et al. (1983) noted lower B(a)P concentrations in bluegill than expected, based on either partition coefficients or rate constants in in-laboratory exposures. Anthracene behaved much more ideally. They hypothesized that the differences were due to high biotransformation of B(a)P. Data from field studies (Reid, 1983; Eisenberg et al., 1980) suggest blue crabs possess lower pollutant burdens than many other organisms.

Levels of radiolabeled material in adult males and females after 24 hours of exposure were higher than concentrations in either intermolt or newly molted juvenile females. Respiration rates for adults are greater, due to the lower surface area attendent with larger size, resulting in passage of greater water volumes through the gills (Lewis and Haefner, 1978). This may have resulted in greater exposure to water borne B(a)P and a subsequent elevation in uptake

over the smaller crabs. Kamimura (1980) determined that uptake of heavy metals in shellfish increased as a function of increases in soft tissue weight, especially midgut gland. Adult crabs possess a larger lipid reserve, thus greater quantities per crab may be accumulated. To investigate this possibility, activity was calculated on a per gram basis. Concentrations in newly molted females were similar to adult intermolt males suggesting that tissue size was important in regards to burdens.

Initially, it was felt that juvenile males and females would be exposed to similarly polluted environments in the field, and would possess similar patterns of uptake and elimination. Therefore, in order to reduce the experiment to a manageable size only the juvenile female group was utilized in the laboratory exposures. Examination of the field data collected on the concentrations of pollutants in blue crabs from the Chesapeake Bay estuary causes this hypothesis to be doubted.

Similar loads of radiolabeled entities were detected in the juvenile intermolt and newly molted females after 24 hours of exposure to tritium labeled B(a)P. When the data were expressed on a concentration rather than a total organ burden basis the molting group exhibited higher levels. Differences in percent methylene chloride extractable lipid between these groups were not large (F significance 0.38). Lawrence (1976) noted in Cancer pagurus the ability to resorb entire body components rather than altering lipid concentration during periods of interrupted feeding. Decreases in total lipid content at ecdysis were also observed. No differences in percent lipid content

or hepatopancreas dry weight between juvenile intermolt and newly molted females were found in the present study.

Crabs experience an oxygen debt during ecdysis (Lewis and Haefner, 1976), which must be compensated for, resulting in high respiration rates following molting. In addition, blue crabs actively drink copious volumes of water, increasing their volume at the time of molt. Scheffe analysis of the differences in water content of the hepatopancreas between juvenile intermolt and newly molted females were significant at the 0.05 level. These factors could contribute to the higher concentrations of B(a)P accumulated in the newly molted group.

Research by Varanasi et al. (1981) with flounder has shown that biotransformation of B(a)P is rapid. Samples taken of liver showed that only 2% of the labeled material present was in the form of the parent compound 24 hours after exposure. Singer and Lee (1977) suggested that an interruption in mixed function oxygenase activity in the green gland of the female blue crab occurred at the time of the proecdysial fast, several days prior to ecdysis. This decrease in MFO activity may have contributed to the higher B(a)P concentrations found in the newly molted female test group compared with the intermolt juveniles. Within 48 hours after ecdysis enzyme activity increased to levels exceeding that prior to molting.

Examination of the metabolite profiles for the sex/maturity classifications studied supported the contention that the high activity found in the newly molted female group may indeed be caused by a decrease in xenobiotic transformation potential. A large percentage of the radiolabeled material was still in the form of the

parent compound for the above mentioned group compared with the intermolt groups.

Extraction efficiencies based on total tissue radioactivity were rather low compared with what is commonly presented as percent recovery for spiked samples. However, much of the radiolabeled material was in the form of highly polar material and might have been closely associated with biogenic material, precluding its efficient extraction with a relatively nonpolar solvent (Varanasi et al., 1981). Varanasi et al. (1980) found metabolites of B(a)P covalently bonded to electron rich material such as DNA in fish. Haddock et al. (1983) determined that the length of time of contact of an anthracene spike with a sediment sample affects extraction efficiency of the spike. The recovery values given in the literature for compounds often are higher than those obtained for the actual environmental pollutants present in the samples.

Approximately 50% of the radiolabeled material extracted after 24 hours of exposure, in this study, was associated with the high molecular weight GPC fraction, into which the conjugate standards tested were separated (the glucopyranosiduronic and sulfate conjugates). Corner et al. (1973) reported the presence of glucose and sulfate conjugates in spider crabs after exposure to naphthalene. After exposing English sole for 24 hours to radiolabeled B(a)P and naphthalene via sediment, Varanasi and Gmur (1981) found 55% of the B(a)P in the form of water soluble conjugates in the liver. In that ethyl acetate, a slightly more polar solvent than methylene chloride, was employed, direct comparisons were not possible. However, it was

apparent in both studies that B(a)P was quickly and extensively metabolized.

In the blue crabs examined from the field B(a)P was only encountered at very low concentrations, presumably due to its suitability as a substrate for the MFO system. Mix et al. (1982) found appreciable concentrations of B(a)P in bay mussels. Molluscs, however, possess low levels of MFO activity (Malins, 1977).

Although B(a)P concentrations in the field samples examined in the present research were low, many polynuclear aromatic hydrocarbons were detected in appreciable quantities. Varanasi and Gmur (1981) found naphthalene to be less extensively biotransformed than B(a)P in sole. The phenomenon of differential metabolism between hydrocarbons was observed by Spacie et al. (1983) as well. Bluegill sunfish were found to metabolize anthracene to a much smaller extent than B(a)P. Differential uptake of xenobiotics also contributes to the profile of pollutants in biota. For example, Vassilaros et al. (1981) reported that sulfur analogs of aromatic hydrocarbons exhibit greater water solubility, although the octanol/water partitioning coefficients are similar. The result is greater availability of these heterocycles compared to the nonsubstituted compounds.

Based on the retention time of authentic metabolite standards co-chromatographed with the blue crab hepatopancreas extracts, compounds corresponding to B(a)P phenols, diols, triols and tetrols appeared. Varanasi and Gmur (1981) in their study of B(a)P metabolism in the English sole found 50% of the organic solvent extractable metabolites in bile to be more polar than the 9,10-diol. In addition, they

detected several isomers of 7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxy-B(a)P after glucuronidase treatment of the sample. The existence of several tetrol isomers may explain the discrepancy in retention time between the suspected tetrol found in this study and the standard employed. Identification of the metabolites is tentative. Varanasi et al. (1982) noted that unknown radiolabeled metabolites may coelute with the standards. They suggested that for further verification a second column and solvent system could be employed.

Lee et al. (1976) found the 3-hydroxy to be the major B(a)P phenol formed in blue crabs, with minor amounts of 9-hydroxy present. The normal phase HPLC program employed in this research was unable to separate the various B(a)P phenol isomers so the identities of these could not be confirmed. The program was able, however, to resolve the more polar products. Peaks were detected corresponding to the retention times of the 7,8- and 9,10-diols. These have been confirmed as dominant B(a)P metabolites in marine organisms (Varanasi and Gmur, 1981). The 3,9-diol and quinones may also have been produced by the crabs.

After 60 hours of depuration, activity values were similar to those of Lee et al. (1976), as seen in Table 11. A large amount of the tritiated material had been eliminated. Activity in newly molted females was higher than that measured in this group after 24 hours of exposure. Obviously, radiolabeled hydrocarbon had accumulated in the final twelve hours of the exposure period in excess of the amount that the crabs were able to eliminate in the succeeding depuration interval. At this time concentrations of radiolabeled material were substantially higher in the newly molted class than any of the

intermolt test groups. A very large proportion of the activity was associated with the high molecular weight/conjugate fraction suggesting that biotransformation of B(a)P was occurring at a rapid rate. Proportions of the unmetabolized B(a)P to the total activity in the tissue were similar for all groups. This further suggested that large amounts of B(a)P had begun to be metabolized in the newly molted crabs. Singer and Lee (1977) reported recovery of MFO activity in the green gland within 48 hours of molting.

Activity in the intermolt crabs substantially declined after 60 hours of depuration. Although proportions of parent compound to total activity were similar to that in the newly molted crabs, concentrations of primary metabolites extracted were about an order of magnitude lower. Total activity in these groups did not decrease to the same extent. Elimination of the radiolabeled B(a)P via biotransformation appeared to have slowed. This biphasic elimination pattern has been reported for PCB by Guiney and Peterson (1980) in yellow perch and rainbow trout. They estimated the half-life of 2,5,2′,5′-tetrachlorobiphenyl during the initial elimination phase, for whole body, to be 1.6 days. The half-life for the compound based on the rate of the secondary elimination phase was calculated as in excess of one year. Tarshis (1981) found a similar phenomenon for the elimination of naphthalene in crayfish. Lee et al. (1976) noted large quantities of parent hydrocarbon in the fecal material of blue crabs. This suggested that much of the organic xenobiotics accumulated were quickly voided, unchanged by crabs. A similar situation may be responsible for the rapid depuration of such compounds by molluscs, which possess little MFO activity. Solbakken and Palmork (1981)

reported high concentrations of unmetabolized phenanthrene in the green gland of Norway lobster. They hypothesized that this compound was being eliminated via vacuoles, or formed bodies, produced by the cells of this organ. Lee et al. (1976) reported all activity associated with the green gland in his experiments was in the form of highly polar metabolites.

After 156 hours of depuration hepatopancreas burdens had decreased further in the test groups. Elimination rates for the intermolt groups were substantially lower than initially, following the biphasic pattern. The rate was similar for the intermolt crabs examined after 252 hours. When calculated on a total tissue burden little decrease was noted, especially in adult intermolt females.

The activity in the newly molted females had decreased greatly after 156 hours of depuration, suggesting that elimination was still in the initial rapid phase and that biotransformation potential had recovered. Presentation of the data on a burden per tissue produced a similar picture.

By the conclusion of the depuration period (252 hours) conjugated metabolites had increased to 80% of the extracted radioactivity. Varanasi et al. (1981) also found an increase in the conjugate share of extractable radioactivity with time. No newly molted female crabs were available for examination on the 252 hour depuration interval. It was expected that these would have reached the slow, secondary elimination phase. Activity had decreased to levels approaching that in the intermolt groups.

Examination of the metabolite profile of the newly molted females after 156 hours of depuration showed a very large phenol peak,

rivaling the parent B(a)P peak (Figure 19). It appears that this peak also includes those hypothesized to be quinones, these appeared to elute prior to the B(a)P phenol in the other radiochromatograms. This was the only example observed in which the levels of the primary metabolites extracted exceeded that of the parent compound. It suggested that metabolism of the xenobiotic was very rapid at this time.

Decreases in activity in adult intermolt males from 156 to 252 hours of depuration were a function of losses of metabolite associated tritium rather than the parent compound. B(a)P concentrations in juvenile and adult intermolt females were similar. However, higher amounts of both conjugates and primary metabolites were retained in the adult females explaining in part the elevated activities measured.

The higher uptake of B(a)P from water and slower elimination of activity burden in adult intermolt females was paralleled by this group's position in the hierarchy of the pollutant concentrations observed in the samples from the Chesapeake Bay estuary.

In addition to uptake of xenobiotic compounds from water, aquatic organisms may accumulate toxics via food. Although bioconcentration factors are higher for uptake from the former, levels in food may often be orders of magnitude higher (McLeese et al., 1980). Lee et al. (1976) exposed blue crabs via both the water and food. Similar biotransformation of hydrocarbons was reported, regardless of mode of entry. Palmork and Solbakken (1980) administered their dosages orally, using DMSO as a solvent, and obtained higher tissue levels than Lee and his group. No food studies were conducted in the present project.

V. CONCLUSIONS

Levels of toxic organic pollutants were examined in the blue crabs of Virginia waters. The entire population had significant concentrations of alkyl substituted polynuclear aromatic hydrocarbons. It was hypothesized that petroleum releases connected with commercial shipping and recreational boating were major contributers. Lippson (1983) reported oil deliveries in Hampton Roads in 1974 totaled 24 million tons. Deliveries on the Eastern Shore, involving the Nanticoke and Wicomico Rivers were in excess of one million tons. It was also reported that Hampton Roads in 1974 ranked third in the United States in the value of foreign tonnage handled. Although the Bay is healthier than many of the other major east coast estuaries, pollution is widespread. PCB were detectable at all sites examined but were highest at the upper and lower James River stations. Nonsubstituted aromatics, as well as DDT metabolites, were found but at low levels.

Higher concentrations of most pollutants were present in blue crabs collected in June than September. Major factors influencing this were thought to be spring runoff, carrying large quantities of TOP into the aquatic system; lower water temperatures, resulting in depressed xenobiotic metabolism; and extrusion of eggs during the course of the summer, reducing burdens, particularly of poorly metabolized lipophilic compounds, in adult females (as suggested by Roberts and Leggett (1980) for the reduction of Kepone residues in crabs) which exhibited some of the highest burdens.

Differences in distribution of TOP within blue crabs appeared to be related to the lipid content of the tissues. Highest levels were found in hepatopancreas, followed by eggs, ovary and muscle. This was supported by the research of others (Insalaco et al., 1982; Tarshis, 1981; McLeese et al., 1980).

In-laboratory exposures of blue crabs to B(a)P and the results of the field sampling suggested that differences in uptake and elimination of xenobiotics occurred between adults and juveniles and intermolt and molting crabs. Biotransformation of pollutants appeared retarded during the molting process, although uptake continued. Clearance of nonchlorinated hydrocarbons in intermolt crabs was rapid and displayed a biphasic pattern as suggested by Lee et al. (1976).

Due to the significant influence on TOP concentrations found in this study of sampling season, specimen sex, maturity, and condition (in addition to site, species and tissue studied) it is suggested these factors be carefully considered in the design of pollutant monitoring programs if valid results are to be obtained. Many studies of pollutant levels report results only on a wet weight basis. Significant differences in water content between the sex and molt groups examined in this study were found. These differences may exert a confounding influence if their effects are not considered.

In many cases pollutants consist of large numbers of similar components, as in the case of the hydrocarbons in petroleum and the PCB isomers in Arochlor mixtures. Often these groups are considered as single entities for detection and hazard assessment purposes. However, many of the components behave differently in the physical environment, as well as in biological systems. Selective uptake and

elimination occurs as a function of such factors as water solubility, partition coefficients and suitability as substrates for xenobiotic biotransformation enzyme systems. Care must be exercised in regards to these often overlooked parameters in future studies and decisions pertaining to pollutants in the environment.

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