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Biological Monitoring of Ambient Water Quality: the Case for using Bivalves as Sentinel Organisms for Monitoring Petroleum Pollution in Coastal Waters

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Concepts of lipid/water equilibration to explain bio-concentration of hydrocarbons were tested *in situ* and seem to hold for petroleum mixtures commonly encountered in coastal waters. K_{bef} in bivalve lipids was constant at approximately 2×10^6 when concentrations were between 1 and 400 $\mu g\ l^{-1}$. These and related studies on the factors controlling body burden in bivalves were used to formulate a strategy for monitoring levels of chronic oil pollution necessary for water quality management.

Introduction

Receiving water quality standards are used as the pollution control strategy in coastal marine waters in Victoria, Australia and similar approaches have been proposed in other countries. Use of this strategy generates a requirement for routine monitoring of water quality to assure that policy objectives are attained. Many organic pollutants occur in marine waters at concentrations of ng l^{-1} or less. Methods for the direct analysis of such concentrations in seawater are being developed but the time and expense of handling the large volumes of seawater required prohibit the routine use of water analyses in many monitoring programmes. Some marine animals are known to concentrate pollutants in their body tissues to many times ambient water concentrations. This observation has encouraged the search for biological indicators of water quality.

Several species have been suggested as possible sentinel organisms based on their ability to take up and eliminate a particular set of compounds. Bivalves have proved to be promising candidates since they actively concentrate several classes of pollutants and are attached filter feeders. Before sentinel organisms can be useful in water quality management, a relationship between the tissue content of the indicator species and ambient environmental levels of pollutant must be established. Progress toward this goal has been made for monitoring petroleum hydrocarbons and related contaminants. Several programmes have been reported

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to use both indigenous and transplanted populations of bivalves to study pollutant distributions in coastal waters (National Academy of Sciences, 1980). This article examines the potential for using bivalves, and mussels in particular, as biological indicators of water quality in relation to monitoring strategies. Using our studies of petroleum hydrocarbons in coastal waters in Victoria, Australia, we hope to demonstrate that within defined limits some bivalves are exceedingly useful both as quantitative and qualitative indicators of ambient water quality.

Petroleum hydrocarbons in bivalves

For reasons summarized in the mussel watch concept (Goldberg *et al.*, 1978) we chose the marine mussel *Mytilus edulis* as our indicator species to detect low levels of petroleum pollution in Victorian coastal waters. We collected mussels from stations near to known or suspected sources of oil pollution and other more remote sites for comparison. Mussels were solvent extracted, subjected to chemical clean-up procedures to separate hydrocarbons from other body lipids and then fractionated by column chromatography procedures to yield both saturated and unsaturated hydrocarbon extracts. Extracts were then analysed by gas chromatography and coupled excitation-emission fluorescence spectroscopy. These methods were detailed enough to yield quantitative data on the amount of hydrocarbons in animal tissues and to facilitate the identification of general sources of petroleum contamination based on oil type. Procedural details and examples of source identification were described earlier (Burns & Smith, 1977).

Using this approach we pinpointed areas where mussels indicated high levels of oil being discharged into coastal waters. Repetitive site sampling showed areas which appeared to suffer chronic discharges. Analysis of sediments and seawater confirmed our conclusions based on mussels for both source assignment and in estimating the seriousness of chronic pollution problems (Burns & Smith, 1979).

Mussels showed an order of magnitude difference of two in body burden between animals from clean and polluted sites. We noted a gradient in body burden in animals collected further away from point sources, and we noted changes in chemical composition of hydrocarbons in various components of the ecosystem which hinted at processes controlling the distributions of various hydrocarbon fractions in the coastal environment.

Thus, mussels showed great potential for marine monitoring programmes. However, since discharge regulation in these waters is based on ambient seawater quality, we needed to examine the parameters controlling body burden in bivalves. We attempted to synthesize available data from field and laboratory studies on petroleum hydrocarbons in bivalves and determine if results fit a reasonable uptake model based on lipid/water partitioning. We identified aspects of the model that should be satisfied for bivalves to be useful indicators and made field measurements to confirm crucial aspects.

Relation to surrounding waters

Published laboratory studies on the dynamics of uptake and depuration of hydrocarbons in oysters, mussels and clams (Anderson, 1973; Stegeman & Teal, 1973; Fossato & Canzonier, 1976) yield a general pattern. When placed in seawater spiked with sublethal doses the animals take up hydrocarbons at an initially high rate and accumulate them to a maximum concentration, C_{max} [Figure 1(a)]. At C_{max} either the animal's tissues are saturated or an equilibrium has been reached between the concentration in animal tissues and the

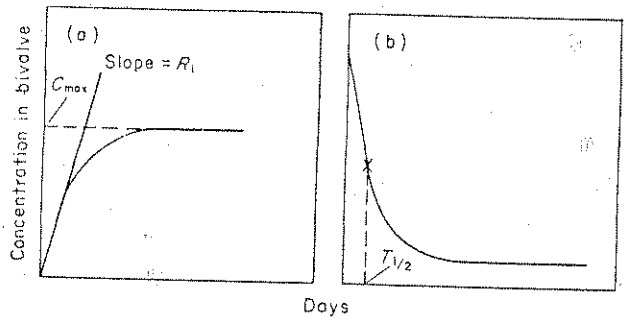

Figure 1. Generalized curves for (a) uptake and (b) depuration of petroleum hydrocarbons by bivalves exposed to a fixed concentration in seawater and then transferred to clean seawater. R_i is the initial rate of uptake. C_{max} is the maximum concentration obtained by the bivalves. $T_{\frac{1}{2}}$ is the time to lose half of the body burden in the initial exponential phase of equilibration.

surrounding water. When placed in clean water the animals will depurate to some background level [Figure 1(b)]. The initial rate of uptake (R_i) time to equilibrium and C_{max} depend on several factors.

Both R_i and C_{max} depend on the concentration of hydrocarbons in exposure water. Stegeman & Teal (1973) measured R_i for the uptake of Number 2 fuel oil by oysters and showed R_i is proportional to water concentration up to 450 μg l^{-1}. Greater concentrations caused the oysters to cease filtering. These authors also showed that the C_{max} resulting from exposure to one oil concentration was different in populations with different lipid contents. When results were calculated on the basis of uptake per gram body lipid, the same concentrations were reached in the two populations.

Uptake and depuration appear to be passive processes resulting from the absorption of hydrocarbons from contaminated food and biochemical equilibration between seawater and body lipids. But these laboratory experiments and other field experiments involving transplanting bivalves to areas of different chronic pollution levels have shown equilibration to be at least a two-phase process. The initial phase is characterized by rapid uptake or elimination of about 90% of body burden in less than 3 weeks (Fossato, 1975; Di Salvo *et al.*, 1975). Data for this initial phase fit a simple exponential function with a half-life of hydrocarbons in body tissues of approximately $3\frac{1}{2}$ days. The *final* phases are characterized by a very slow change in body burden. Numerous transplant experiments have shown that animals taken from chronically-heavily polluted areas will retain at least 10% of their body burden of hydrocarbons for several months. Various physiological mechanisms can be postulated for why this hydrocarbon residue does not equilibrate as rapidly as the major body burden. These include concepts of differential partitioning of hydrocarbons between various tissue components and the relative rates of transfer and degradation of hydrocarbons once incorporated into various lipid stores.

Analysis of bivalves taken from a variety of polluted areas indicate there is a maximum level to which the animals accumulate petroleum hydrocarbons. If accumulation is related to lipid content then this level should correspond to the lipid saturation point. For the mixed petroleum products we encountered in Australian coastal waters the maximum level shown in mussel tissues was approximately 30 mg hydrocarbons per gram of body lipid. This value was exceeded only at stations showing visible oil pollution and may have been due to ingestion of oil droplets. Comparison with literature values and rough conversion of results

expressed on dry or wet weight basis to lipid weights confirms this saturation level (Ehrhardt, 1972; Ehrhardt & Heineman, 1975; Fossato & Siviero, 1974).

Several authors have proposed models for the bio-concentration of hydrocarbons in animal tissues based on equilibration of hydrocarbons between animal lipids and surrounding waters (Hamelink *et al.*, 1971; Neely *et al.*, 1974; Bransen *et al.*, 1975; Chlou *et al.*, 1977). The simplest model assumes bio-concentration is independent of other constituents in seawater and defines a bio-concentration factor (K_{bcf}) at equilibrium as the concentration of hydrocarbons in animal tissues divided by concentration in surrounding waters. Metcalf (1975) reviewed the concepts of bio-concentration of lipid soluble toxicants in aquatic organisms and emphasized that K_{bcf} is inversely related to the water solubility of pollutants.

Laboratory experiments measuring the uptake of petroleum hydrocarbons in bivalve tissues suggested K_{bcf} is on the order of 2×10^6 for oil mixtures commonly encountered in coastal environments (Stegeman & Teal, 1973; Fossato & Canzonier, 1976). If K_{bcf} is constant for a given compound or mixture of lipid soluble compounds and if equilibrium is reached in a relatively short time, then it should be possible to measure the level of oil pollution in mussel lipids and to calculate the level in surrounding waters.

As mentioned above, a similarity in chemical composition of hydrocarbons in bivalves and seawater is necessary for identification of pollution sources. Since these previous studies suggest these major criteria are met for monitoring petroleum hydrocarbons we set out to test the specific aspects of the partition model that were relevant to developing effective monitoring strategies.

Testing the equilibration model *in situ*

Seawater and mussels collected simultaneously from the same location in Victorian coastal waters showed that if hydrocarbons are present in a 'dissolved' form (those that pass through a glass fibre filter and onto a resin absorption column) then the g.c. pattern of mussel extracts will look qualitatively similar to the seawater extract. If the hydrocarbons are primarily in the 'particulate' form (those trapped on the glass filter) then the mussels show a relative enrichment of the lower boiling compounds in both the saturated and unsaturated hydrocarbon fractions. Risebrough *et al.* (1979) report similar patterns in samples collected off California.

Our field studies in Victorian coastal waters showed that the majority of hydrocarbons (except in moderately polluted areas) appear to be 'particulate' (Burns & Smith, 1980). Thus the qualitative g.c. pattern in mussels was usually slightly lower boiling range than seawater in the same area. Often the pattern in mussels appeared much more degraded than that from water with the resolved paraffin peaks less in proportion to the mixture of unresolved hydrocarbons peculiar to petroleum products.

Bivalves appear to have little ability to metabolize petroleum hydrocarbons and thus modify the g.c. pattern of oil incorporated into body tissues only very slowly (Lee *et al.*, 1972; Moore, 1979). This adds further support to the usefulness of mussels in qualitatively reflecting oil pollution in the water column and in source identification.

To gain an estimate of the amount of time required by mussels to equilibrate to ambient water concentrations in the field, we selected two coastal bay stations in Victoria that had relatively consistent but different oil pollution levels. Mussels were sampled at each station with some placed in polypropylene mesh bags and transferred to the opposite site. After 51 days both native and transplanted mussels were resampled. Site I is a navigation beacon near the main discharge of an oil refinery on Port Philip Bay. Levels of oil pollution are

usually much higher than at Site II which is the refinery pier in Westernport Bay. All experimental mussels were subtidal to minimize any contributions from surface slick material. Results of hydrocarbon analyses on these samples are shown in Table 1. Levels at both stations fluctuate slightly but the data show that neither set of transplanted mussels had reached complete equilibrium after 51 days. Mussels taken from Site I (high pollution) and moved to Site II (moderate pollution) were still depurating. Mussels from Site II moved to Site I were still taking up hydrocarbons.

TABLE 1. Petroleum hydrocarbon content of mussels transplanted between two stations of chronic high and moderate levels of oil pollution

Sample and date	Saturates mg g^{-1} lipid	unsaturates mg g^{-1} lipid	Total		Substituted benzene derivatives mg g^{-1} lipid
			µg g^{-1} dry	mg g^{-1} lipid	
Site II, 19 Dec. 1977	4·6	2·3	837	6·9	0·0
Trans. from Site I	2·8	2·8	818	5·6	0·3
Site II, 10 Feb. 1978	2·0	1·3	454	3·3	0·0
Site I, 19 Dec. 1977	3·4	6·0	1421	9·4	1·2
Trans. from Site II	4·2	5·3	1354	7·5	0·9
Site I, 10 Feb. 1978	4·2	6·4	1700	10·6	1·3

Gas chromatograms of these samples showed that Site I was contaminated with a series of substituted benzene derivatives which could be distinguished above a large background of degraded crude oil present at this station (confirmed by g.c.m.s. analysis). Thus the per cent unsaturated hydrocarbon content of Site I samples was greater than at Site II. Table 1 shows that mussels tended to equilibrate quantitatively with hydrocarbon levels in ambient water and changed the chemical composition of their body burden to reflect ambient sources of contamination.

Mussels transplanted from Site II to Site I showed an average net change in hydrocarbon content of: -8 µg (g lipid)$^{-1}$ day^{-1} (saturates); $+59$ µg (g lipid)$^{-1}$ day^{-1} (unsaturates); $+51$ µg (g lipid)$^{-1}$ day^{-1} (total). Mussels moved from Site I to Site II showed an average net change of: -12 µg (g lipid)$^{-1}$ day^{-1} (saturates); -61 µg (g lipid)$^{-1}$ day^{-1} (unsaturates); -73 µg (g lipid)$^{-1}$ day^{-1} (total). Rates of uptake and discharge of the substituted benzene contaminants were identical over the 51 days. Using these average rates of change, the time to equilibrium for both processes in these field conditions were estimated at approximately 90 days. However, since the rate of change in concentration is slower as the animals approach even the first stage of equilibrium, this is a minimum estimate.

Long-term site monitoring was conducted off the refinery pier in Westernport Bay. Tidal mixing is extremely rapid in this area and related monitoring results have shown the refinery effluent is well mixed in receiving waters (Burns & Smith, 1979). Table 2 shows that levels of hydrocarbons in mussels from the pier were consistent when expressed on a lipid weight basis, regardless of season, with a few obvious exceptions. Once, unusually low levels in mussels occurred when the refinery had shut down for maintenance (2 November 1977). Within 3 months of resuming discharges, oil levels in mussels off the pier were up to previous values. Other exceptions were unusually high values which reflected recent small oil spills (26 October 1977 and 21 September 1978). The analysis of animals collected on 24 September 1978 affirmed that the majority of body burden equilibrates to average levels in 3–4 days. Similar results were noted at other monitoring stations (Burns & Smith, 1979, 1980).

TABLE 2. Petroleum hydrocarbon content of mussels from refinery wharf in Westernport Bay (Australia) per gram lipid and dry weights

Date	Lipid wt % of dry wt	Petroleum hydrocarbons	
		μg g^{-1} dry	mg g^{-1} lipid
4 July 1975	16·8	735	4·4
12 December 1975	15·0	689	4·5
12 February 1976	15·0	570	3·8
28 April 1976	11·9	685	3·5
28 April 1976[a]	15·7	1186	6·4
2 November 1977	10·9	158	1·4
2 November 1977[a]	8·2	156	2·1
29 April 1977	15·5	660	4·2
8 June 1977	17·6	970	5·4
26 October 1977	15·9	1975	12·3
19 December 1977	11·0	837	6·9
10 February 1978	14·0	454	3·3
21 September 1978	15·2	1242	8·2
25 September 1978	12·9	721	5·5
3 October 1978	12·4	603	4·9
11 October 1978	13·5	529	4·5

[a]Samples taken below the low tide line. All other samples were intertidal.

TABLE 3. Hydrocarbon content of different size classes of mussels sampled from the same stations

Average shell length (cm)	μg g^{-1} dry	mg g^{-1} lipid
Corio Bay 25 March 1976		
7·8	989	6·6
5·1	837	5·6
Hobsons Bay 6 October 1977		
6·0	1423	12·4
3·4	1703	14·9

Preliminary evidence on selective size class analysis showed no consistent trend in body burden with size (Table 3). Additional data from a variety of areas would help clarify any variation due to size, age, seasonality and other parameters.

To test the equilibrium model *in situ*, we collected samples of mussels at various stations in Victorian coastal waters at the same time as water samples. Analytical results are given in Table 4. K_{bcf} was calculated assuming the mussels were in equilibrium with surrounding waters. This field data indicated that K_{bcf} for petroleum hydrocarbons (in our analyses in the C_{10}–C_{40} paraffin boiling range encompassing diesel oils, fuel oils, light crudes and lube oils) varies from 10^5 to 10^7 in mussel lipids. Similar K_{bcf} were seen by Risebrough *et al.* (1979) in their California data.

Figure 2 is a graph of K_{bcf} *vs.* water concentration and demonstrates a rather complex relationship. For oil concentrations below 1 μg l^{-1} in seawater, K_{bcf} is highly variable. But above 1 μg l^{-1} K_{bcf} appears to be relatively constant. We then added data available from the literature for oysters and mussels (Stegeman & Teal, 1973; Fossato & Canzonier, 1976) and made data comparable to ours by assuming per cent lipid of wet weights were similar to our mussels based on the average of 185 separate analyses. Expressing the concentration of oil in mussel

TABLE 4. Petroleum hydrocarbon content of mussels and seawater sampled simultaneously in three areas of Australian coastal water. K_{bcf} calculated assuming equilibrium and is the ratio of mg g^{-1} lipid in mussels to µg l^{-1} in water. Overall average is $4{\cdot}8 \times 10^6$

Sample, station and date	*Hydrocarbons* Water µg l^{-1}	Mussels mg g^{-1} lipid	K_{bcf}
Corio Bay			
Shell Bcn 9			
28 September 1977	22·6	12·1	$5{\cdot}3 \times 10^5$
Silo Bcn 4			
28 September 1977	1·0	3·3	$3{\cdot}3 \times 10^6$
Ripp. Bcn 1			
27 September 1977	0·2	2·7	$1{\cdot}3 \times 10^7$
Hope. Bcn 15			
27 September 1977	1·0	3·1	$3{\cdot}1 \times 10^6$
Hobsons Bay			
Webb Bcn 18			
6 October 1977	9·7	12·5	$1{\cdot}1 \times 10^6$
Pt. Melb Bcn 8			
4 October 1977	8·6	4·8	$5{\cdot}5 \times 10^5$
Pl. Pile Altona			
11 October 1977	0·3	0·6	$2{\cdot}0 \times 10^6$
Fawkner Bcn			
4 October 1977	1·9	0·3	$1{\cdot}6 \times 10^5$
Westernport Bay			
Stony pt.			
24 October 1977	0·3	2·7	$9{\cdot}0 \times 10^6$
Esso Wharf			
27 October 1977	0·7	3·9	$5{\cdot}5 \times 10^6$
BP Wharf			
26 October 1977	0·8	12·4	$1{\cdot}5 \times 10^7$
21 September 1978	0·5	8·2	$1{\cdot}6 \times 10^7$
25 September 1978	0·25	5·5	$2{\cdot}5 \times 10^7$
3 October 1978	0·15	4·9	$4{\cdot}9 \times 10^7$
11 October 1978	0·15	4·5	$4{\cdot}5 \times 10^7$

lipids as a function of seawater concentration between 1 and 400 µg l^{-1} (plotted in Figure 3) gave a highly significant linear relationship (correlation coefficient is 0·86 with $P<0{\cdot}001$). Over this range the concentration in mussel lipids was proportional to the amount accommodated in surrounding water. Average K_{bcf} calculated from the data points was 2×10^5. This is a surprisingly consistent relationship considering the data encompass a variety of oil products in oysters and mussels from both field and laboratory experiments.

At seawater concentrations above 400 µg l^{-1} mussel lipids appear to saturate and body burden is no longer proportional to water concentrations.

The variability in K_{bcf} when water concentrations are below 1 µg l^{-1} could be caused by several factors such as pulse events of oil pollution in the environment and errors in measuring these low levels of hydrocarbons in seawater. Much disagreement exists on the accuracy of various methods for sampling and analysis of hydrocarbons in the low µg l^{-1} seawater concentration range. Direct solvent extraction requires handling large volumes of seawater and ultra-pure extraction solvents. To circumvent these problems several authors have suggested the concentration of seawater organics on various filters and resins. Several reports detail the distribution of halogenated hydrocarbons in ocean water using this technique. We and other workers have applied absorption methods to analysing petroleum hydrocarbons (e.g. Risebrough *et al.*, 1979; Burns & Smith, 1980). But calibrations are

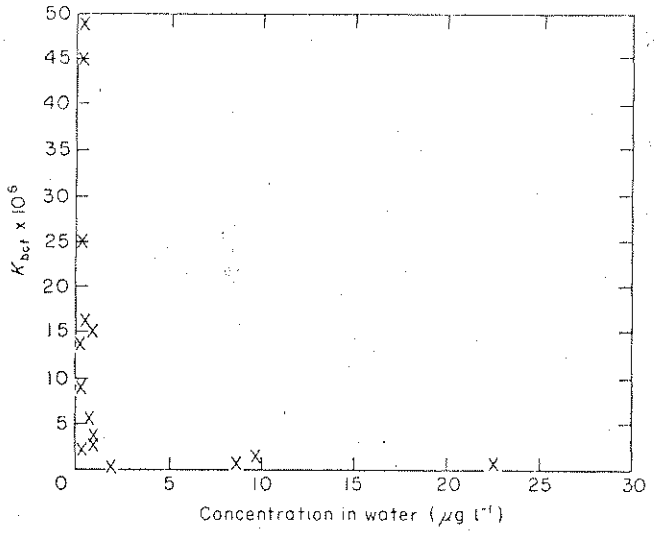

Figure 2. Graph of K_{bcf} (ratio of concentration of hydrocarbons in mussel lipids to water concentration) against water concentration. Scatter graph indicated a complex relationship where K_{bcf} appeared relatively constant when water was above 1 $\mu g\ l^{-1}$ but showed great variability when water was below 1 $\mu g\ l^{-1}$.

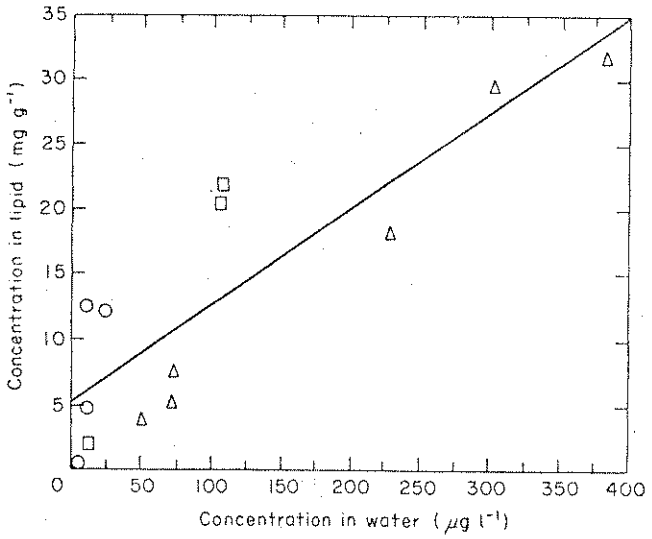

Figure 3. Graph of petroleum hydrocarbons in mussels (mg g^{-1} lipid) against water concentration ($\mu g\ l^{-1}$) for field data above 1 $\mu g\ l^{-1}$ (○). Data of Stegeman & Teal (1973) for oysters are shown by (□). Data of Fossato & Canzonier (1976) for mussels were converted to lipid basis by assuming a similar % lipid to wet wt as the Australian data (△). Correlation coefficient is 0·86 with $P<0{\cdot}001$. Average K_{bcf} is 2×10^5.

usually done by spiking seawater with hydrocarbons at concentrations greater than 1 μg l^{-1}. Our data indicate there may be some significance to a relationship between K_{bcf} and water concentration below 1 μg l^{-1}. Since resin absorption employs similar concentration mechanisms as bio-concentration in animal lipids (partition equilibria) perhaps these methods are not quantitative for these low concentrations of oil in seawater. Boehm & Quinn (1976) noted that the concentration of dissolved organic matter (DOM) affects the efficiency with which bivalves accumulate individual hydrocarbons and oil mixtures. If DOM changes the equilibrium dynamics between animal tissues and seawater, then the effects would surely be most visible at these relatively low pollution levels.

Monitoring strategies

Our studies in Victorian coastal waters and other laboratory experiments and monitoring efforts show that analysis of mussels and some other bivalves can provide valuable information on the relative distributions of petroleum hydrocarbons in coastal environments. The animals qualitatively reflect what they are exposed to in water and can be used to estimate sources and types of contamination. Within defined limits mussel analyses can be used to estimate the relative concentration of oil in ambient waters. Long-term monitoring produces a record of chronic low-level contamination indicative of average water quality. Sudden inputs such as from small oil spills show up as spikes over the average concentrations.

Uptake and depuration of the majority of body burden in mussels occurs relatively quickly for petroleum hydrocarbons which appear to have a half life in body tissues of the order of days. Thus repetitive site sampling of indigenous populations is a feasible method of identifying areas subject to chronic contamination. Expression of results on a lipid weight basis and sampling animals of similar size and reproductive state helps reduce the variability of body burden data within populations. Areas subject to fluctuating inputs show greater variation in mussel data than areas receiving continuous discharges.

The concepts of a simple lipid/water equilibration to explain bio-concentration seem to hold for the bivalves and types of petroleum cited in these studies. K_{bcf} in mussel lipids appears to be relatively constant (at approximately 2×10^5) over the range of seawater concentrations between 1 and 400 μg l^{-1}. The water quality standard in Victorian coastal waters was set in the low μg l^{-1} range for petroleum hydrocarbons in accordance with guidelines provided by the United States Environmental Protection Agency (1976). Mussel analyses provide the analytical tool whereby levels of chronic oil pollution in the range necessary for water quality management can be routinely monitored.

If discharges to the coastal environment are so great as to cause receiving waters to exceed 400 μg l^{-1}, mussels would show tissue saturation and their usefulness to reflect quantitatively relative water quality would be limited. Conservative estimates for the solubility of petroleum oils in seawater lie in the range of 0·5 to 10 mg l^{-1} (Boylan & Tripp, 1971; Anderson, 1975). Thus relative water quality at these high oil pollution levels would be apparent as visible slicks and oil films. Methods for measuring oil concentrations in seawater in the low μg l^{-1} range typical of most coastal waters and open ocean areas are still in the research stage and are not yet within the capability of most agencies responsible for routine monitoring of water quality in coastal areas.

Thus we developed a strategy for monitoring oil pollution in coastal waters as follows. After surveys of indigenous populations of mussels have identified major sources of contamination and areas of special concern, then transplants of clean mussels to particular sites can be used to detail distributions. Further assessment of environmental quality is achieved by analysis

of seawater and sediments together with biochemical and biological assessment of organism and community health (Burns & Smith, 1979).

Similar reasoning suggests that bivalves should be useful sentinel organisms for other classes of contaminants, and strong evidence supports their usefulness for qualitative assessment of water quality (National Academy of Sciences, 1980). But long equilibration times, lack of evidence for depuration and dependence of body burden on size, age and exposure time suggests bivalves may indicate the history of pollution in an area rather than ambient levels of toxicants such as some heavy metals and halogenated hydrocarbons. Thus analysis of indigenous populations should be replaced by transplant experiments where the rate of uptake of these toxicants over specific time periods could be related to ambient water quality (Metcalf, 1975; Majori & Petronio, 1973).

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