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Detection of Dibenzothiophene in Mussel, *Mytilus edulis*, as a Marker of Pollution by Organosulfur Compounds in a Marine Environment

S. Kira, T. Izumi, and M. Ogata

Department of Public Health, Okayama University Medical School, Okayama 700, Japan

Organosulfur compounds are minor components of crude oil and of some fuel oils. Although the quantity of these compounds depends on the source of production, generally it ranges from 0.002 to nearly 30% in crude oil, found as sulfur containing hydrocarbons (NAKAMURA and KASHIMOTO,1977), and 1600 ppm in #2 fuel oil (DILLON et al,1978). In a field study, these compounds were found in benthic organisms after an oil spills(GRAHL-NIELSEN et al,1978). Researchers have presented several papers on the accumulation of these compounds in eels and short-necked clams(OGATA et al,1980a) and have also identified dibenzothiophene(DBT) through gas chromatography-mass spectrometry(GC-MS)(OGATA and MIYAKE,1980b) and capillary GC-MS (OGATA and MIYAKE,1980c) in biota samples after experimental exposure to crude oil suspension. Moreover mussels are a well-known bilogical monitor of marine pollutants which scientists call "the mussel watch"(GOLDBERG,1975). Many investigators have reported the susptibility of this organism to petroleum hydrocarbons(LEE et al, 1972) and poly-nuclear aromatic hydrocarbons(DUNN and YOUNG,1976). But few reports have been published on organosulfur compound contamination in field mussel samples. In the present study, the authors identified several organosulfur compounds through GC-MS, and measured the levels of DBT through GC-flame photometric detector(GC-FPD), in both mussels and in water of the environment. The calculated concentration ratio of DBT in mussels to that in water ranged up to 500 in the field sample and 800 or higher after an experimental exposure. The estimated biological half-life of DBT from field mussel samples was about 9 days in clean sea water.

EXPERIMENTALS

Collection of samples. Mussels were collected in the Seto Inland Sea of Japan shown in Fig.1. Sampling site A is a port surrounded by a large industrial area including petrochemicals operations, oil refineries, an automobile plant, a power plant and an iron manufacturer. Sampling sites B and G are small fishery ports, sites C and D are commercial and fishery ports where boats and ships come and go frequently. Sampling sites E and F are far from the industrial area and are considered relatively clean. But an accidental oil spill(about 9 KL of fuel oil) had occured at site F six days before the sampling in 1981. The dotted area indicates the spread of the oil membrane at the accident. The sampling was duplicated in the spring of 1982.

Exposure and dissipation experiments. A group of mussels from site E was used for the laboratory exposure experiment after acclimation

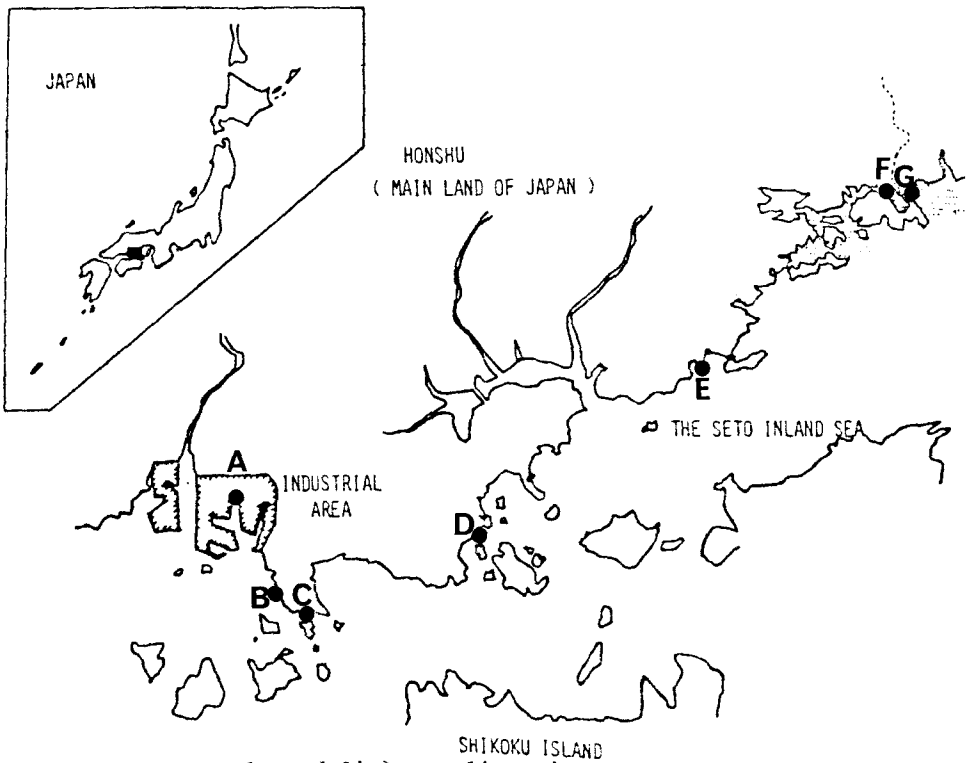

Fig.1 Mussel(Mytilus edulis) sampling sites
dotted area: see text.

in a filtered clean sea water tank. As an exposure experiment, about 1 kg of mussels was dipped in a crude oil suspension(100mg/L) and aerated throughout the experiment. A group of mussels was taken out at scheduled periods, days 2,4 and 8 of the experiment. For the dissipation experiment, levels of DBT were measured in the mussels during the acclimation period prior to the exposure study.

Analysis of samples. Analysis procedure for the organosulfur compounds was essentially based on our previous report(OGATA et al, 1979). Levels of DBT were measured by GC-FPD. In the case of the measurement by FPD, the amount of organosulfur compounds correlated to the square of the peak height on the chromatogram(NAKAMURA and KANEMOTO,1978). Identification of DBT was carried out by GC-MS. Analysis procedure and GC conditions were summarized as below.

Sample preparation: Approximately 10-30 g of mussel flesh was homogenized with distilled water and hydrolyzed with 2 N KOH-ethanol solution maintaining a temperature of 100°C for one hour. Hydrolyzed samples were extracted with n-hexane three times and applied on a column(the upper layer was 5 g activated almina and the lower layer was 10 g silica gel moistened with 5% v/w water). The first 100 mL eluate of n-hexane was condensed and partitioned against equal volume acetonitrile. An acetonitrile layer was concentrated to a suitable volume for the GC analysis. In case of water, 500 mL of sample was used without homogenization and hydrolysis in the above process.

GC conditions: GC-FPD. Instrument:Hitachi 163-FPD. This filter was adjusted at 394 nm specific for sulfur compounds. Column:3% GE SE-52 on chromosorb WAWDMCS, 1.5 m X 3 mm i.d. Oven temp.:100-260°C, 7.5°C/min. Carrier gas:N_2, 40 mL/min. Data processor:Shimadzu, Chromatopac C-R1B. GC-MS with packed column:2% OV-17 on chromosorb WAWDMCS, 2 m X 3 mm i.d. Ionization:EI, 20eV. Acceleration:3 kV. Instrument:Hitachi M-80 + M-003 data processor. GC-MS with capillary column: ShiLanox SCOT OV-17, 30 m X 0.3 mm i.d. Ionization:EI,20eV. Acceleration:3.5 kV. Instrument:Shimadzu auto GC-MS 6020 + SCAP 1123 data processor.

All solvents, almina and silica gel were the pesticide analysis grade manufactured by WAKO PURE CHEMICAL CO.,Japan. DBT standard was from TOKYO KASEI CO.,Japan. Crude oil used for the exposure study was a mixture of the Arabian Light Crude oil and the Zubea Crude oil (4:1).

RESULTS and DISCUSSION

Detection and identification of DBT in mussels. Figure 2 shows a typical chromatogram of mussel extract by GC-FPD. By the present analysis method, DBT was separated clearly from other organosulfur compounds even under a parts per billion level. Figure 3 and 4 show the GC-MS patterns by packed and capillary column respectively. GC-MS single ion monitor as shown in Figure 3 indicated the presence

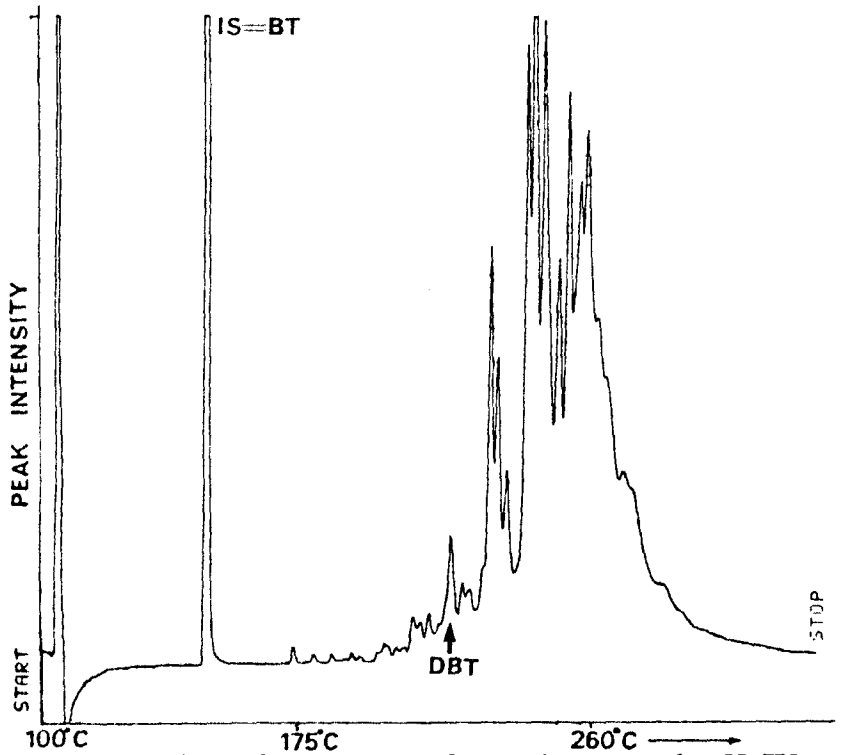

Fig.2. A typical chromatogram of mussel extract by GC-FPD. IS=Internal standard. BT=Benzothiophene.

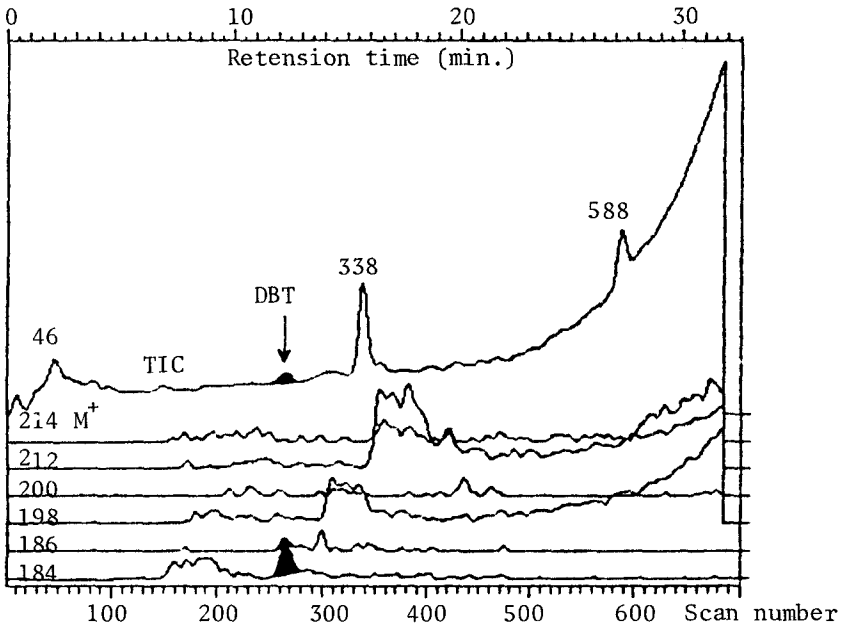

Fig. 3. A GC-MS chromatogram of mussel extract by packed column

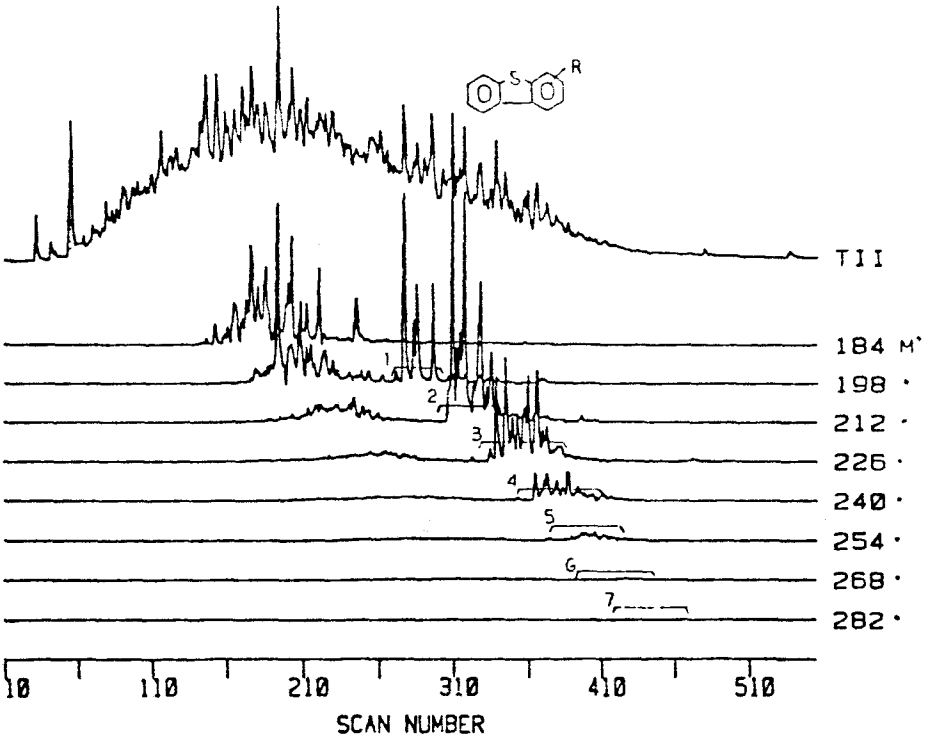

Fig. 4. A GC-MS chromatogram of mussel extract by capillary column.

of DBT, by simultaneous detection, of M^+ 184 and 186. These were originated from ^{32}S and ^{34}S respectively. By the use of a capillary column, as shown in Figure 4, GC-MS chromatogram also indicated the co-existence of the alkyl derivatives of DBT showing mass weight of 184+14n(n=carbon numbers of alkyl chain"R"). Thereafter DBT was picked out as a marker compounds of organosulfur components in the following experiments.

Measurement of DBT in the field samples. Table 1 shows the levels of DBT in mussels and water collected at each sampling site. The levels ranged from under 0.1 to above 800 ppb in the mussels and 0.08 to 0.15 in the water samples. The highest concentration was observed in mussels from a port where continuous oil spills from ships and boats was suspected. Although a water sample from the area where oil spill accident had occurred(site F) showed the highest level, the mussels contained only 3.4 ppb of DBT. The calculated concentration ratio of DBT in the field mussel samples ranged from 22.7 to 531.3. The lowest ratio was observed in the sample from site F. These results suggested that the accumulation of DBT in the field mussel does not reflect accidental pollution but indicates continuous pollution by organosulfur compounds in the marine environment.

TABLE 1. Levels of dibenzothiophene(DBT) in the field samples.

Sampling sites	Levels in mussel[a] 1981.3	1982.3	Levels in water[b] 1981.3	Concentration ratio[c]
A	42.5 ppb	NA	0.08 ppb	531.3
B	NA	66.0	NA	NA
C	169.0	809.0	NA	NA
D	NA	73.0	NA	NA
E	14.7	NA	0.12	122.5
F	3.4	ND	0.15	22.7
G	35.6	34.0	NA	NA

a): Detected in 10-30 g mussel flesh. Detection limit=1.0 ppb.
b): Detected in 500 mL water. Detection limit=0.05 ppb.
c): Calculated from the sample collected in March 1981.
NA= not available. ND=not detected=under detection limit.

Half-life of DBT in the field mussel samples. As the field mussel samples from sampling site E, which was to be used for the exposure experiment, contained measurable levels of DBT, the dissipation experiment was carried out. Figure 5 shows the change of the levels of DBT in mussels kept in a tank supplied with filtered sea water. No DBT was previously detected inthe sea water. DBT in the mussels dissipated as time elapsed and became to be under detection limit after 20 days. The calculated half-life was about 9 days by a simple curve method fit to the equation of $Ct=C_0e^{-at}$ where "Ct","C_0" and "a" represent a level of DBT after "t" days, the initial level and the dissipation factor respectively. The obtained half-life was longer than that of other hydrocarbons reported by FOSSATO and CANZONIER (FOSSATO and CANZONIER,1976) who calculated 2.7 or 3.6 days.

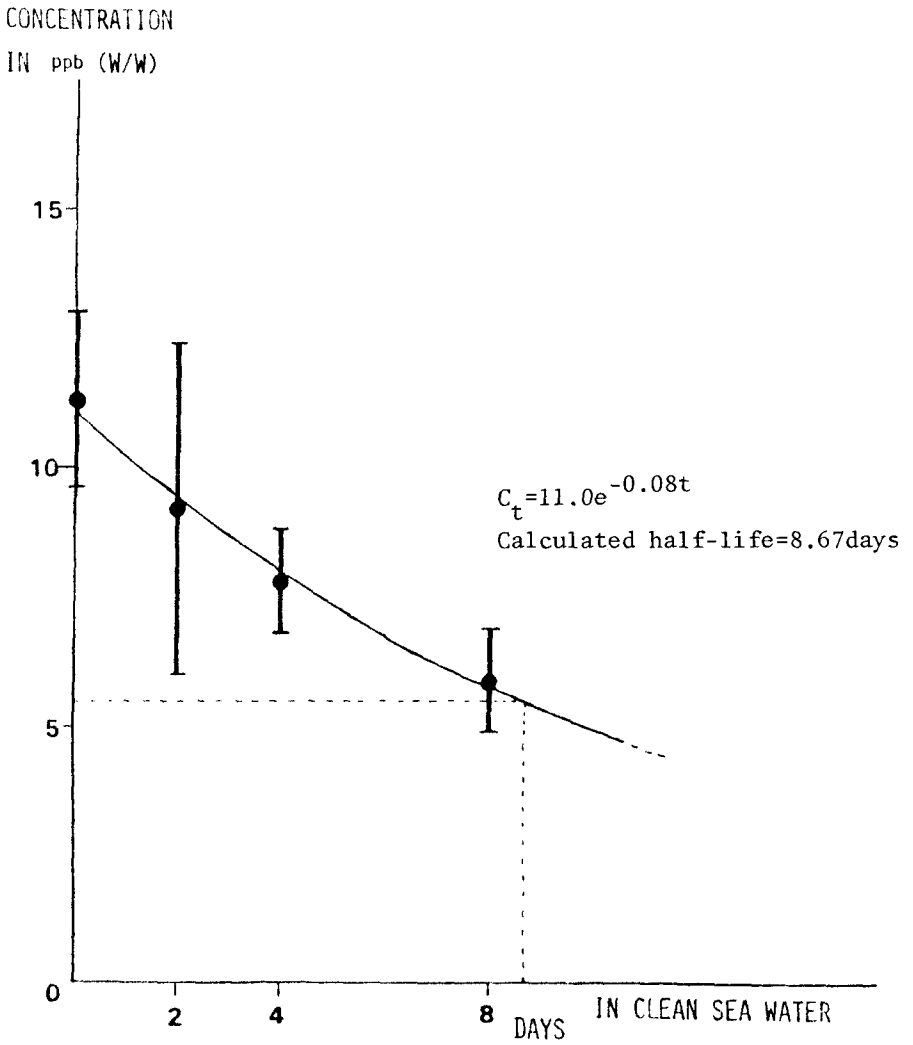

Fig.5. Dissipation of DBT from field mussels in clean sea water.

Experimental exposure of mussels to a crude oil suspension. Figure 6 shows the accumulation curves of DBT in mussel, eel and short-necked clam samples. Accumulation of the compound in mussel was approximately 600 and 800 times higher than the levels in water after 4 and 8 days exposure, respectively. The obtained concentration ratio of 800 after 8 days exposure was close to that of petroleum hydrocarbons which was 1000(FOSSATO and CANZONIER,1976). Comparing the data with those of eels and short-necked clams in our previous report(OGATA et al,1980a), mussels showed a much higher accumulation than these organisms under similar exposure conditions. If the present data were applied to the field mussel samples whose concentration ratio exceeded 500, the marine environment should

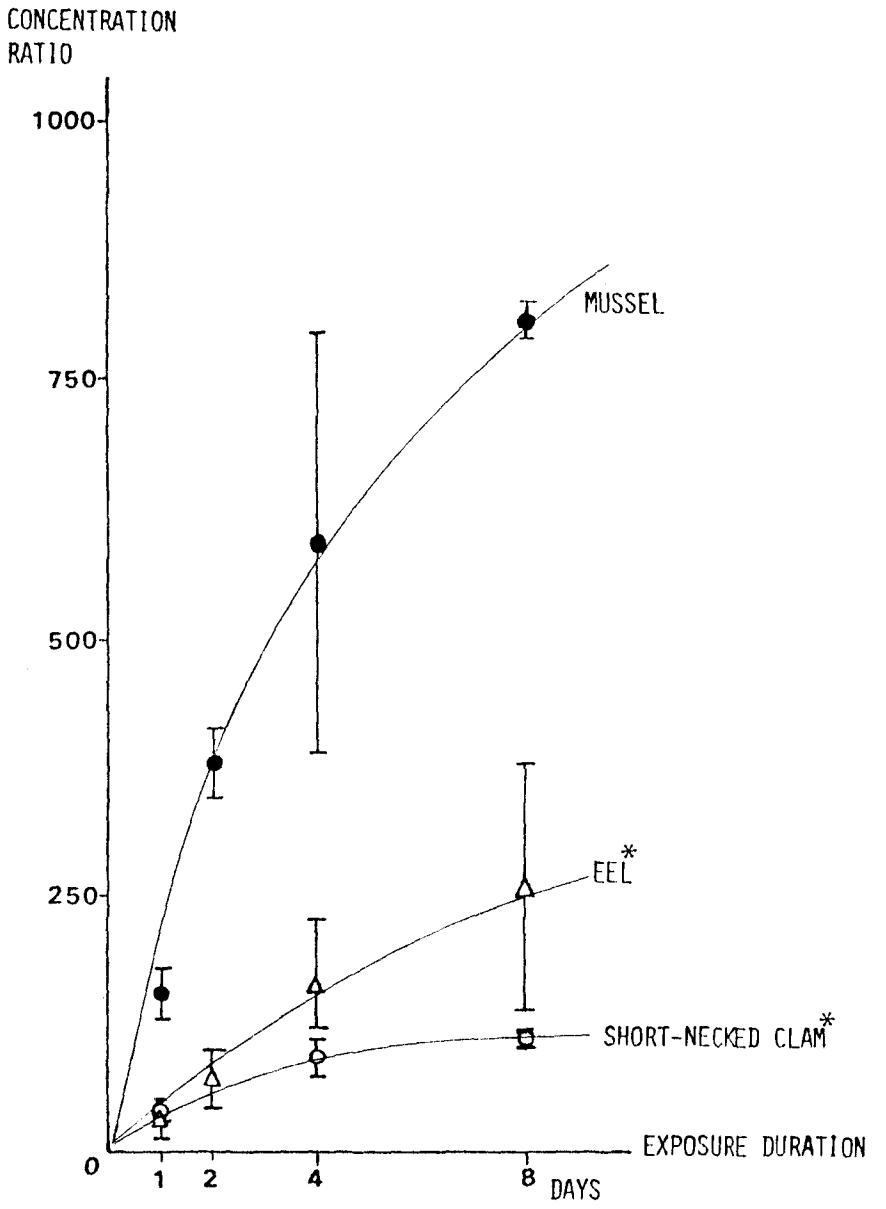

Fig.6. Accumulation curves of DBT in mussel,eel and short-necked clam.
*Data are originated from our previous work(OGATA et al, 1980a)

have been polluted by oil for 4 days or longer, however, the measured levels of DBT was low in the field mussel samples.

In conclusion, an initial detection of DBT by GC-FPD, instead of measuring all the organosulfur compounds in mussels is a possible means of monitoring prolonged pollution by oil,such as crude oil or some fuel oils,which contain organosulfur compounds.

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