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Contamination of *Tris*(4-Chlorophenyl) Methane and *Tris*(4-Chlorophenyl) Methanol in Marine Mammals from Russia and Japan: Body Distribution, Bioaccumulation and Contamination Status

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Four seal species (*Phoca caspica*, *Phoca sibirica*, *Phoca hispida* and *Phoca largha*) and one whale (*Orcinus orca*) from Russia and Japan were examined to determine the body distribution, bioaccumulation and contamination status of *tris*(4-chlorophenyl) methane (TCPMe) and *tris*(4-chlorophenyl) methanol (TCPMOH). Lipid normalized concentrations of TCPMe and TCPMOH were comparable in various organs and tissues, implying that their body distribution is followed to the lipid-dependent accumulation, similar to that for other organochlorines. The highest body burden of these compounds was found in the blubber. Bioaccumulation potential of TCPMe and TCPMOH was high and comparable to PCBs and DDTs. Relatively higher concentrations of TCPMe and TCPMOH were observed in Caspian seal than in other seal species examined. TCPMOH concentration in killer whale was the highest of the marine mammals examined. Another peak detected had similar mass spectrum to TCPMOH, however, at a different retention time, suggesting the presence of an isomer of TCPMOH.

Keywords: *tris*(4-chlorophenyl) methane; *tris*(4-chlorophenyl) methanol; marine mammals; seal; killer whale.

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Introduction

Environmental contamination by *tris*(4-chlorophenyl) methane (Fig.1(a): TCPMe) and *tris*(4-chlorophenyl) methanol (Fig.1(b): TCPMOH) were first reported by Walker *et al.* (1989) and Jarman *et al.* (1992). Since then there have been several reports of environmental contamination by TCPMe and TCPMOH (Zook *et al.*, 1992; Rahman *et al.*, 1993; de Boer *et al.*, 1996; Muir *et al.*, 1996). Nevertheless, there is very little information about these compounds concerning their main sources, toxicity and distribution. Possible sources of TCPMe and TCPMOH are considered to be from technical DDT, dicofol and other agrochemicals as impurities, and synthetic high polymers and light-fast dyes for acrylic fibers (Jarman *et al.*, 1992; Buser, 1995). TCPMOH seems to be originated partly from the metabolite of TCPMe or *tris*(4-chlorophenyl) methylchloride (Jarman *et al.*, 1992; de Boer, 1997). While toxicity of TCPMe is still unclear, TCPMOH has been reported to induce the phases I and II drug metabolizing enzymes in the liver and to elicit anti-androgenic activity (Korner *et al.*, 1997; Poon *et al.*, 1997). The contamination of marine mammals by TCPMe and TCPMOH has been noted in a few reports from Europe and North America (e.g. Walker *et al.*, 1989; Jarman *et al.*, 1992; de Boer *et al.*, 1996). However, data on the environmental contamination by those compounds in Asian and nearby areas is not available.

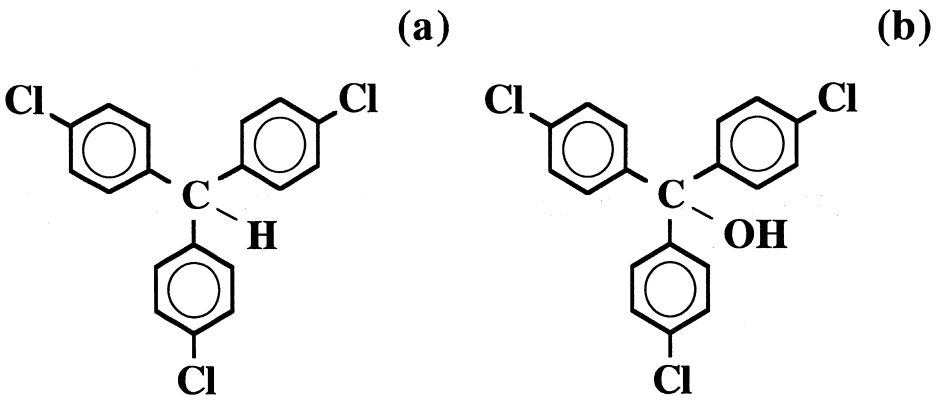

Fig. 1 Chemical structures of *tris*(4-chlorophenyl) methane (a) and *tris*(4-chlorophenyl) methanol (b).

The present study is aimed at examining body distribution and bioaccumulation of TCPMe and TCPMOH, and their contamination status in marine mammals from Russia and North Japan. In addition, we report the identification of an isomer of TCPMOH.

Materials and Methods

Sampling locations and other details of marine mammals analyzed in this study are given in Fig. 2. The tissue samples of blubber, muscle, liver, kidney, pancreas, brain, intestine, lung and heart from an adult male Caspian seal (*Phoca caspica*) were analyzed to elucidate the body distribution of TCPMe and TCPMOH. Blubber samples of Caspian seal, Baikal seal (*Phoca sibirica*), ringed seal (*Phoca hispida*), larga seal (*Phoca largha*) and killer whale (*Orcinus orca*) were analyzed to examine the magnitude of contamination. Prey fishes from Caspian Sea (*Rutilus sp.*), Russia, and the western coast of Hokkaido (*Pleurogrammus* sp.,

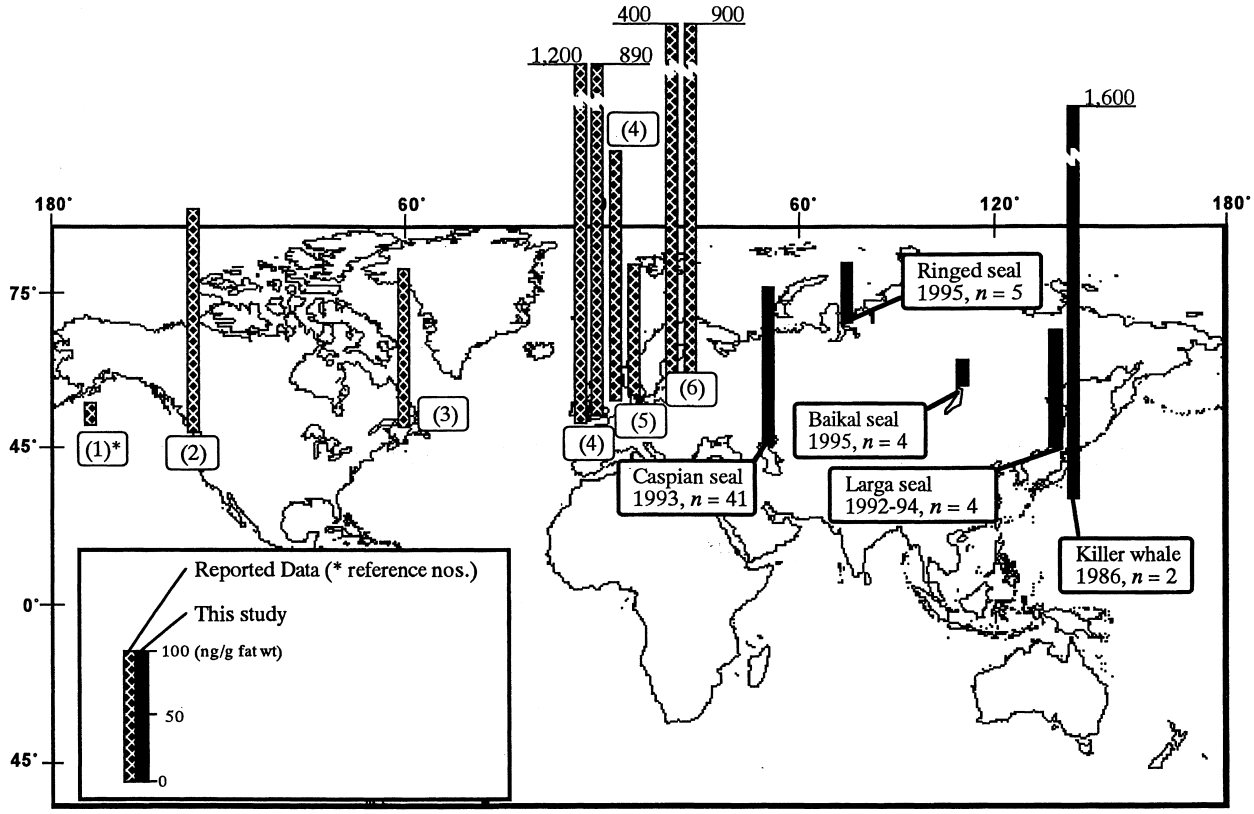

Fig. 2 Comparison of mean concentration of TCPMOH in the blubber of marine mammals from Russia and Japan with those of pinnipeds and cetaceans from various waters. Data was cited from (1) Jarman *et al.* (1992), (2) Walker *et al.* (1989), (3) Muir *et al.* (1996), (4) de Boer *et al.* (1996), (5) Rahman *et al.* (1993) and (6) Zook *et al.* (1992).

Teragra chalcogramma), Japan, were also homogenized and analyzed in each species.

Chemical analysis of TCPMe and TCPMOH as well as other persistent organochlorines such as polychlorinated biphenyls (PCBs), DDT and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs) chlordane compounds (CHLs) and hexachlorobenzene (HCB) were modified following the method of Tanabe *et al.* (1994). Four grams of seal blubber and 20 g of fish homogenate were ground with anhydrous sodium sulfate and extracted using a Soxhlet apparatus with a mixture of diethyl ether and hexane. The extract was added onto a 20 g Florisil packed glass column and then dried by passing nitrogen. Organochlorines adsorbed on Florisil were eluted with 150 ml of 20% water in acetonitrile to a separatory funnel containing hexane and water. After partitioning, the hexane layer was concentrated and then passed through a 8 g of activated Florisil for separation. The first fraction was eluted with hexane and contained PCBs, *p,p'*-DDE, *trans*-nonachlor and HCB, and the second fraction was eluted with 20% dichloromethane in hexane and contained other pesticides and TCPMe. For eluting TCPMOH, 50% dichloromethane in hexane was used as the third fraction.

Except for TCPMe and TCPMOH, the quantification of organochlorine residues was performed using a gas chromatograph (Hewlett-Packard 5890 series II) equipped with ECD (electron capture detector), a fused silica capillary column (DB-1; J&W Scientific, 0.25 mm i.d. and 30 m length) and a moving needle-type injection port (splitless and solvent cut mode, Shimazu, Japan). TCPMe and TCPMOH were identified and quantified using a gas chromatograph (Hewlett-Packard 5890 series II) coupled with electron impact-mass spectrometry (Hewlett-Packard 5972 series), in the total ion chromatogram (TIC) mode scanning from 50 to 550 Da and the selective ion monitoring (SIM) mode scanning m/z 311, 313, 346 and 348 for TCPMe and m/z 139, 251, 253, 362 and 364 for TCPMOH. The GC column used was a fused silica capillary (DB-1; J and W Scientific, 0.25 mm i.d. and 30 m length). The recoveries for this method were 90 ± 1.1% for TCPMe, 95 ± 2.7% for TCPMOH, 100 ± 6.1% for PCBs, 98 ± 3.2% for DDTs, 95 ± 4.3% for HCHs, 96 ± 4.8% for CHLs and 94 ± 7.3% for HCB.

Results and Discussion

Body distribution and bioaccumulation

To understand the body distribution of TCPMe and TCPMOH, eight different tissues of an adult male Caspian seal were examined (Fig. 3). On a fat weight basis, TCPMe and TCPMOH (as well as other organochlorines) were at similar concentrations in all organs and tissues, except brain. The logarithmic concentrations (wet weight basis) of TCPMe and TCPMOH in various organs and tissues, excluding brain, were correlated significantly with logarithmic lipid contents (TCPMe: $r^2 = 0.996$, $p < 0.001$; TCPMOH: $r^2 = 0.981$, $p < 0.001$). These results indicate clearly that the body distribution of TCPMe and TCPMOH is principally followed to the lipid-dependent accumulation, similar to that described for other organochlorines such as PCBs and DDTs.

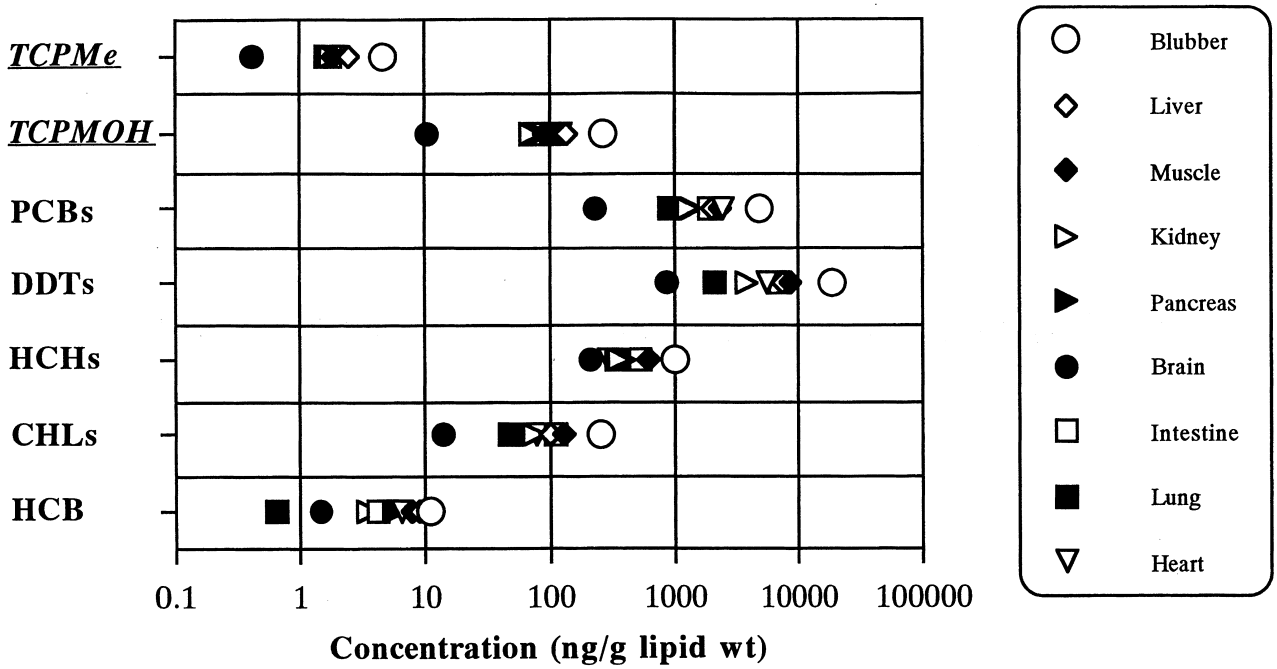

Fig. 3 Concentration of TCPMe, TCPMOH and other organochlorines in various organs and tissues of an adult male Caspian seal.

Based on these results, it is apparent that the blubber plays an important role as a reservoir of TCPMe and TCPMOH. In the Caspian seal, more than 95% of the total burdens of TCPMe, TCPMOH, and other organochlorines were present in the blubber, while this tissue comprised 47% of the total body weight. Similar patterns were observed for PCBs, DDTs, HCHs and HCB residues in other marine mammals (Tanabe *et al.*, 1981; Kim *et al.*, 1996). This result indicates that total body burdens of TCPMe and TCPMOH in marine mammals can be estimated from the concentrations in the blubber and the total weight of this tissue.

Fig. 4 shows the biomagnification factors (BMFs) of TCPMe and TCPMOH as well as other organochlorines in the Caspian and larga seal. BMF is the ratio of the contaminant concentration (on a whole body basis) in predator to that of their prey fish. Whole body basis concentration in the Caspian seal was estimated from the animal that was used for tissue partitioning study. The whole body concentrations of organochlorines in the larga seal were estimated from blubber concentrations and the total weight of this tissue, which was assumed to comprise 30% of body weight. BMFs of TCPMe and TCPMOH were comparable to those of PCBs and DDTs, which are known to highly amplify in marine mammals (Tanabe *et al.*, 1984).

The statistical analysis between TCPMe/TCPMOH and other organochlorines in 41 Caspian seals indicated that TCPMe and TCPMOH were correlated positively with PCBs and DDTs ($p < 0.001$), but less (or not) correlated with HCHs ($p < 0.01$) and HCB ($p > 0.05$), implying that the kinetics of TCPMe and TCPMOH is similar to those of PCBs and DDTs. This observation as well as higher BMFs of TCPMe and TCPMOH as indicated above, suggest the high bioaccumulative nature of TCPMe and TCPMOH in seals.

Contamination status

The mean concentration of TCPMOH in the blubber of pinnipeds and cetaceans from various waters are given in Fig. 2. Among the seals analyzed in this study, Caspian seal retained the highest concentration of TCPMOH. This level was comparable to those in harbor seal from the Puget Sound, USA (Walker *et al.*, 1989) and beluga whale from St. Lawrence Estuary (Muir *et al.*, 1996). While concentrations of TCPMOH in larga seal from the western coast of Hokkaido were similar to those in Caspian seal, apparently lower concentrations were observed in Baikal seal from the Lake Baikal and ringed seal from the Russian Arctic. The concentrations in Baikal seal and ringed seal were comparable to those in northern fur seal from the Northern North Pacific (Jarman *et al.*, 1992). Four species of seals analyzed in this study retained TCPMOH with much lower concentrations than in marine mammals from heavily polluted areas such as the Baltic Sea (Zook *et al.*, 1992) and the Wadden Sea (de Boer *et al.*, 1996). The differences in the concentrations among marine mammals from various locations might be due to the variations in the biological parameters such as age, sex, metabolic capacity and trophic level

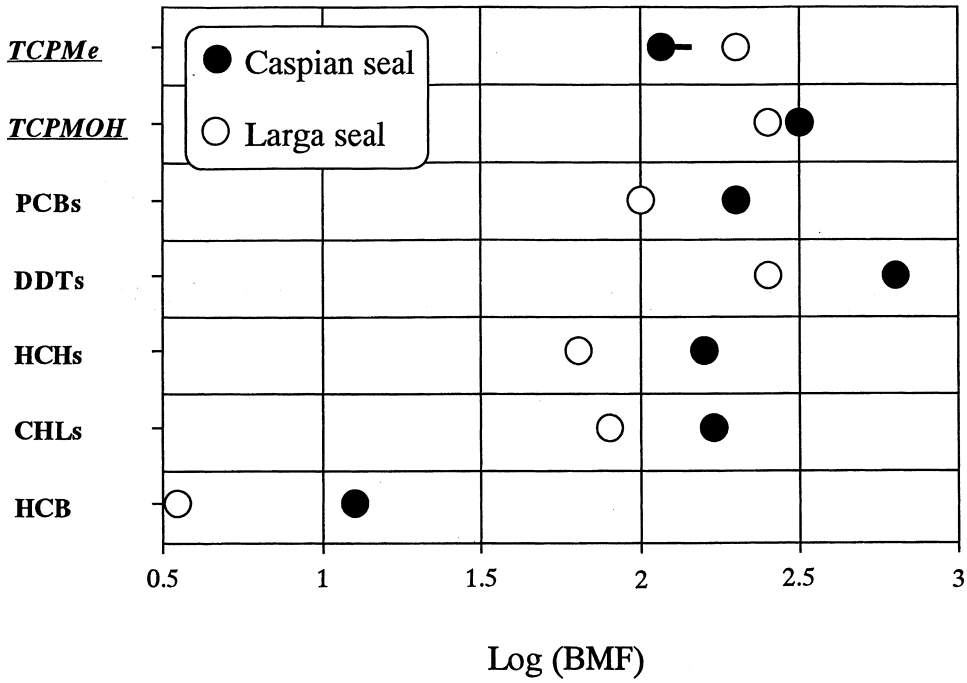

Fig. 4 Biomagnification factor (BMF) of TCPMe, TCPMOH and other organochlorines in adult male animals of Caspian seal and larga seal.

etc., in addition to the different status of industrial and human activities.

Due to the lack of analytical standard, data on TCPMe not only in marine mammals but also in other biota have been limited. The highest concentration of TCPMe was found in Caspian seal (2.0–88 ng/g fat wt.), followed by larga seal (1.6–17 ng/g), Baikal seal (0.5–9.3 ng/g) and ringed seal (0.4–0.9 ng/g). However, these concentrations were lower than those in beluga whale (42–1500 ng/g) from the St. Lawrence Estuary (Muir *et al.*, 1996).

TCPMOH concentration in the blubber of a killer whale was the highest among the samples examined, and comparable to those of marine mammals analyzed from

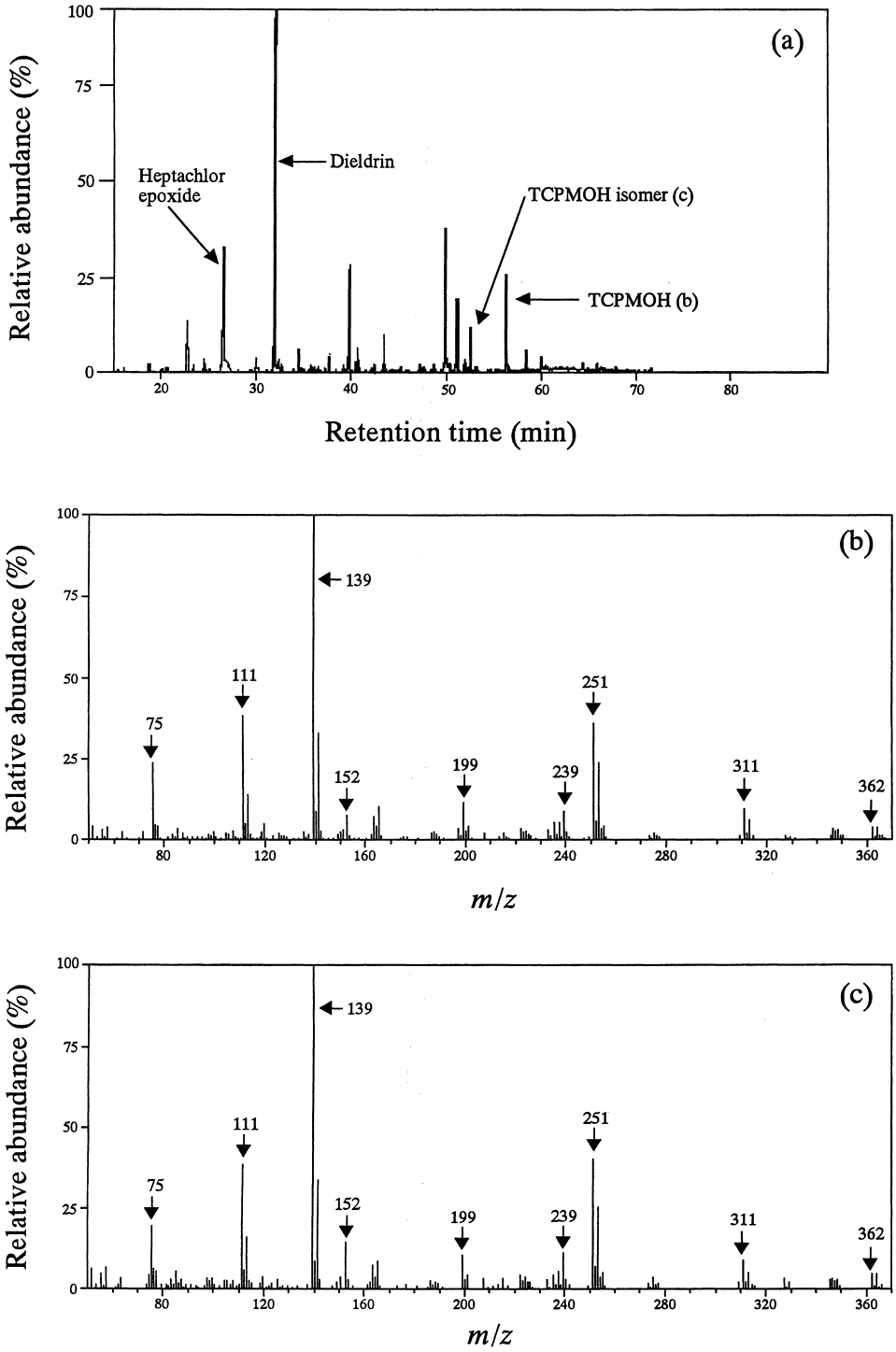

Fig. 5 Total ion chromatogram for fraction III of the blubber extract of killer whale (a), and mass spectrum of *tris*(4-chlorophenyl) methanol (b) and its isomer (c) for fraction III of the same extract.

the Baltic and the Wadden Seas (Fig. 2). The high accumulation of TCPMOH in killer whale might be attributable to its highest trophic position in the food web and a long life-span of this species. Interestingly, another peak was found on the mass chromatogram closer to the retention time of TCPMOH in the extract of killer whale and it had a similar mass spectrum characteristics to this contaminant (Fig. 5). As Buser (1995) reported the presence of 10 isomers of TCPMe which are structurally-related compounds to TCPMOH, the peak found in the extract of killer whale is expected to be a newly reported isomer of TCPMOH. The mean concentration of this isomer estimated based on the response of TCPMOH was 1.0 μg/g fat wt., about two-third of the TCPMOH concentration. However, this isomer was not detected in any other samples examined. TCPMe in the extract of the killer whale could not be identified and quantified due to the presence of an interfering peak at the same retention time.

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