AN UPDATE OF THE KNOWLEDGE ON Q1, A $C_9H_3Cl_7N_2$ COMPOUND, THAT HAS BEEN IDENTIFIED AS A NATURAL BIOACCUMULATIVE ORGANOCHLORINE

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Introduction

About 20 years after their ban, organohalogen compounds still belong to the most harmful anthropogenic contaminants[1]. Therefore, regular global monitoring of organohalogen residues in food and the environment are of importance. During such studies on the pollution of seal blubber from the Southwest of Africa, an unknown heptachloro compound labeled Q1 was recently identified[2]. Application of HRMS provided the information that Q1 has the molecular formula $C_9H_3Cl_7N_2$ [3]. It became also evident that Q1 is a natural organochlorine compound[3,4]. The highest level (>1 mg/kg) detected in an environmental sample was recently measured in blubber of a bottlenose dolphin from Australia[5]. This and results from other samples suggest that Q1 is a persistent bioaccumulative natural organochlorine. Here we present new data on Q1 which support this assumption.

Methods and Materials

Samples.

Blubber of Antarctic elephant seal was collected in 1993/4 at the Jubany station[6]. Origin of the ringed seal sample has been described in an earlier publication[7]. The sample of farmed Norwegian salmon was kindly donated by L. Alder (BgVV Berlin, Germany), SRM 1588 cod liver oil was from Promochem (Wesel, Germany).

Sample clean-up. After addition of the internal standard perdeuterated α-HCH, combined microwave-assisted extraction and gel-permeation chromatography was performed with ethyl acetate/cyclohexane (1:1, v:v)[8]. Additionally, adsorption chromatography on 3 g silica (deactivated with 30% water, w:w, in a 1 cm i. d. glass column) was carried out[8]. Some samples were cleaned by acid digestion, liquid-liquid partitioning into n-hexane, sulphuric acid treatment and adsorption chromatography on silica[7].

Gas chromatography with electron capture detection. Sample extracts were analyzed on a Hewlett-Packard 5890 series II gas chromatograph equipped with two 50 m x 0.25 capillary columns (CP-Sil 2 and CP-Sil 8/C18) and two ECDs in parallel[2]. Quantitation of Q1 was carried out by using the response factor of trans-nonachlor as reference[2].

Gas chromatography with electron capture negative ion mass spectrometry. Identification of unknown and confirmation of known organochlorine compounds was performed on a Hewlett-Packard 5890 series II/5989B GC/MS system. A ß-BSCD column consisting of 25% randomly *tert.*-butyldimethylsilylated ß-cyclodextrin diluted in PS086 (BGB Analytik, Adliswil, Switzerland) was installed in the GC oven. The column parameters were: 30 m length, 0.25 µm internal diameter, and 0.20 µm film thickness. In the full scan mode m/z 50 through m/z 650 were monitored. Further parameters were published elsewhere[4]. In the SIM mode 6 m/z values, respectively, were run in three time windows. The second consisted of the Q1-selective m/z

values 384, 386, 388 as well as m/z values 302, 442, and 444 for nonachlor isomers. A solution with identical ECD abundance of trans-nonachlor and Q1 in ECD provided a 4.2 times higher response of Q1 in ECNI-MS[9].

Results and Discussion

An extensive search of the literature gave no entry for compounds with the molecular formula $C_9H_3Cl_7N_2$ (expect the unstable reaction intermediate 4-dichloro-N-dichloromethylene-N'-trichloromethylbenzamidin, CAS 65866-99-1[10,11]. Looking for a plausible structure for Q1 two publications on halogenated bipyrrols were interesting to us. The first component, a hexabromo-2,2'-bipyrrol, had been identified in marine bacteria (*Chromobacterium sp.*) in the 1970s[11]. Formation of halogenated (bi)pyrrols in terrestrial and marine media was not unexpected due to the high reactivity of these heterocycles during electrophilic substitution[12]. A similar compound with the molecular formula $C_{10}H_6Br_4Cl_2N_2$ was identified in bird eggs from Canada in 1999. This compound was found to be a 1,1'-dimethyl-2,2'-bipyrrol[13,14]. Several fragment ions in the mass spectra of Q1 support that our heptachloro compound Q1 has also a bipyrrol backbone[9].
Except the type of halogens, the three discussed compounds differ only by one or two methyl groups at bipyrrol-nitrogens. However, the suggested structural variant for Q1 (**Figure 1**) is surprising. Since all protons on Q1 are on one methyl group[3], (at least) one of the nitrogens must be substituted with Cl or it has three bonds to ring carbons. Note that N-Cl bonds are usually instable although this cannot be generally ruled out in the presence of further Cl.

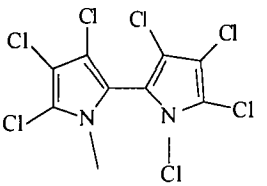

Figure 1: Proposed (isomeric) structure of Q1

With the help of a program of the Syracuse Research Corporation[15], $\log K_{OW}$ values between 5.9 and 6.2 were calculated for structural variants of Q1[16]. These $\log K_{OW}$-values are in the range of other bioaccumulative organochlorines. Due to the lipophilic character of Q1 it was not astonishing that the compound has been detected in the selected human milk samples of Faroean women that regularly consumpted fish and whale blubber[9]. The levels in these samples were 12 – 230 µg/kg human milk[9]. These levels are in the range of typical residue limits for chloropesticides and PCBs in food in Europe and the USA, and which exceed tolerable levels of chloropesticides in human milk[9].
In our earlier work, it was reported that Q1 was more abundant in the Southern Hemisphere than above the equator. This is unique for organochlorines since other organochlorines such as PCBs, DDT and other chlorpesticides are more abundant in the Northern than in the Southern Hemisphere[17]. Particularly, the high abundance of Q1 in samples from the Antarctic (biota and air) was remarkable (see **Figure 2a**). Therefore, it cannot be ruled out that certain organisms in the Antarctic are able to synthesize Q1. On the other hand, we did not detect Q1 in samples from Spitsbergen (see **Figure 2b**). This was also valid for eggs of birds of prey from the Antarctic (presence of Q1[18]) and the Arctic (Q1 not detectable[19]). Note that the ratio of Q1 and trans-nonachlor is a good measure of Q1's relevance in samples. In the Southern Hemisphere, ECNI-MS abundance of Q1 usually exceeds trans-nonachlor while in all samples from the Northern Hemisphere investigated so far, trans-nonachlor was more abundant. However, Q1 was detected in more and more sample extracts from the Northern Hemisphere during the last months. We found

Q1 in farmed salmon from Norway (**Figure 3a**) but in SRM 1588 cod liver oil, Q1 was around the detection limit (**Figure 3b**). Our results illustrate that Q1 might be more distributed than we know at present. This also points towards higher Q1 levels in samples from coastal areas.

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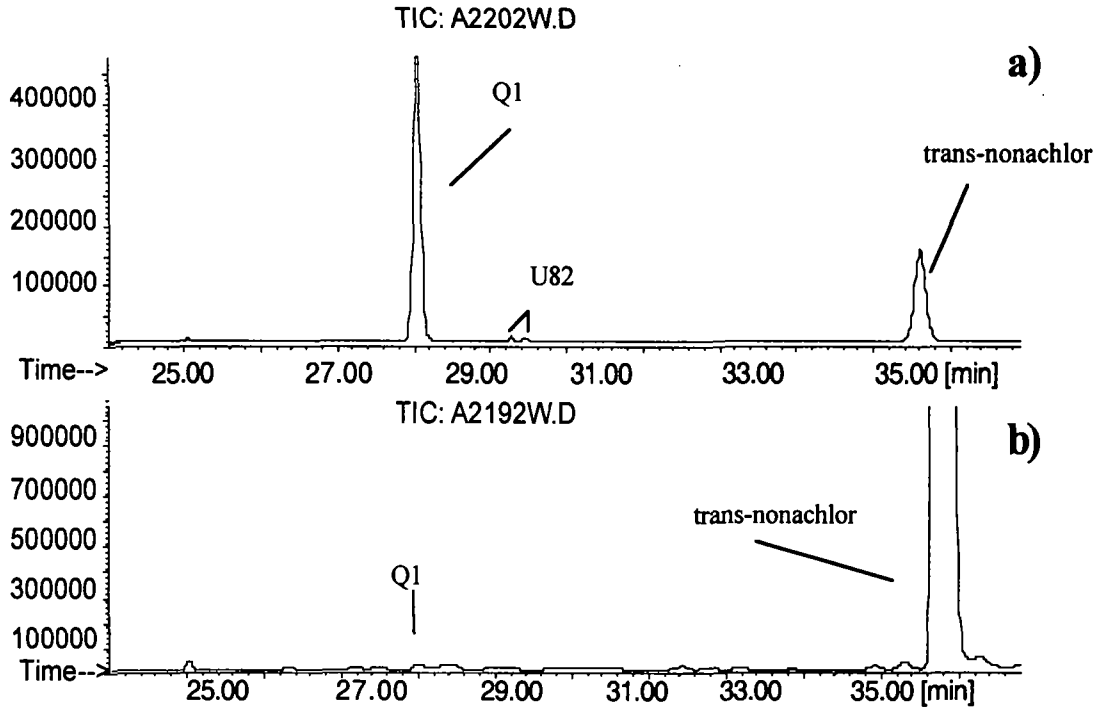

Figure 2: GC/ECNI-SIM chromatograms of a brain sample of (a) Antarctic elephant seal and (b) a blubber sample of Arctic ringed seal. In the SIM mode we recorded m/z 384/386/388 for Q1, m/z 302 for U82, m/z 302/442/444 for trans-nonachlor.

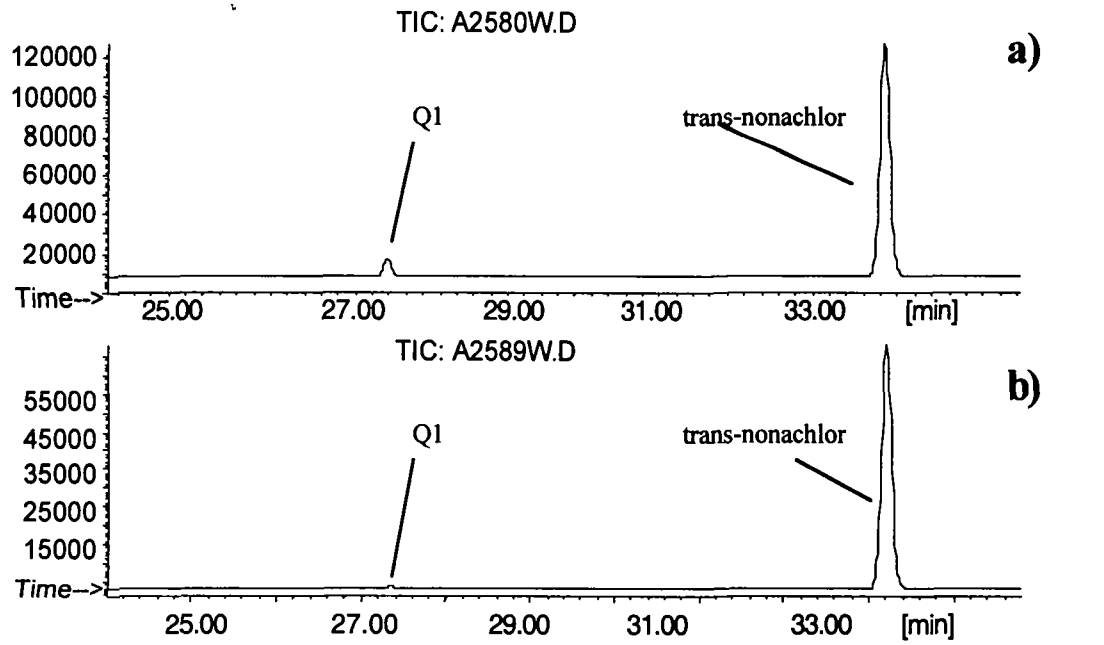

Figure 3: GC/ECNI-SIM chromatogram of (a) farmed salmon from Norway and (b) cod liver oil SRM 1588. Same m/z values recorded as in Figure 2.