Long-chain haloalkanes are incorporated into fatty acids by *Rhodococcus rhodochrous* NCIMB 13064

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The fatty acid composition of the cellular lipids of *Rhodococcus rhodochrous* NCIMB 13064 grown on various long-chain haloalkanes has been investigated and the influence of halogen substituents, carbon chain length and the position of halogen substitution in the growth substrate explored. Of the total fatty acids present in cells grown on 1-chloro-, 1-bromo- and 1-iodohexadecane, 75, 90 and 81%, respectively, were substituted in the ω -position by the corresponding halogen but only 1% of the fatty acids present after growth on 1-fluorotetradecane were fluorinated in this position. The extent of the halofatty acid incorporation with different halogen substituents in the growth substrate appears to reflect the degree to which oxygenase attack is restricted to the non-halogenated end of the haloalkane. Studies of the fatty acid composition of cells after growth on a series of 1-chloroalkanes containing an even number of carbon atoms between C₁₀ and C₁₈ indicated chlorofatty acid incorporation from C₁₂ to C₁₈ substrates at levels ranging from 21% with C₁₂ to 75% with C₁₆. The chlorofatty acids formed by initial oxidation of the chloroalkane were chain-lengthened or chain-shortened by from two to eight carbon atoms, with accompanying desaturation in some instances. Substantial quantities of a methyl-branched C_{19:0} chlorofatty acid were also present with several chloroalkane substrates. When the fatty acid composition of cells after growth on 1-bromoalkanes containing an odd number of carbon atoms between C₁₁ and C₁₇ was examined, the incorporation of bromofatty acids was observed with C₁₃, C₁₅ and C₁₇ substrates; a maximum of 76% was recorded for the C₁₅ bromoalkane. As with even chain-length chloroalkanes, both chain-lengthening and -shortening occurred predominantly via two-carbon units so that most bromoacids present possessed an odd number of carbon atoms. When 1-bromododecane or 2-bromododecane were substrates, overall incorporations of bromofatty acids into the lipid fraction were very similar, demonstrating that the position of halogen substitution in the haloalkane was not critical in determining the extent of incorporation of the haloacids into cellular lipids. The results of the study indicate a mechanism by which degradation products of chlorinated paraffins could enter the biological food chain.

Keywords: *Rhodococcus rhodochrous*, long-chain haloalkanes, ω -halofatty acids, cellular lipids

INTRODUCTION

Mycobacterium spp. grown on long-chain chloroalkanes, such as 1-chlorohexadecane, as sole carbon source directly incorporate the compounds as the corresponding ω -chlorofatty acids into the cellular lipid fraction of the

organism. Transformation into chlorinated fatty acids of both longer and shorter chain length, and desaturation occur such that ω -chlorofatty acids comprise as much as 85% of the total fatty acid content of the organism (Murphy & Perry, 1983). Similarly, 1-chloroalkanes are incorporated in substantial quantities into the cellular

fatty acid fraction of the filamentous fungi *Cunninghamella elegans* and *Penicillium zonatum*, and the yeast *Candida lipolytica* (Murphy & Perry, 1984). The fate of organic chlorine in the growth substrate not incorporated into the lipid fraction was not investigated in these studies but clearly, for the chloroalkane to act as a carbon source, such organisms must be capable of at least partial catabolism of the carbon chain of the chloroalkane.

A new insight into this area arose from recent work on *Rhodococcus rhodochrous* NCIMB 13064, an organism capable of utilizing a wide range of haloalkanes as sole carbon source (Curragh *et al.*, 1994). Whilst catabolism of short chain chloroalkanes by this organism is initiated by a hydrolytic dehalogenase, it appears that attack on chloroalkanes with a chain length above C_{10} is initiated by an oxygenase at the non-dehalogenated end of the molecule, leading to the formation of ω -chlorofatty acids. These compounds are degraded by subsequent β -oxidation to 4-chlorobutyric acid which is chemically lactonized with the elimination of chloride to form γ -butyrolactone. Notwithstanding the ability of the bacterium to dehalogenate the substrate completely, analysis of the cellular fatty acid composition after growth on 1-chlorohexadecane indicated that ω -chlorofatty acids constituted approximately 75% of the total fatty acid content with $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$ acids predominating. This surprising accumulation of halolipids in an organism potentially capable of total mineralization of the haloalkane is not only of considerable significance in the biological treatment of industrial effluents, but has more general environmental implications.

In this paper we explore the influence of carbon chain length, the nature of the halogen substituent and the position of halogen substitution in the haloalkane growth substrate on the fatty acid composition of the cellular lipids of *R. rhodochrous* NCIMB 13064.

METHODS

Organism and culture. *Rhodococcus rhodochrous* NCIMB 13064 was grown routinely in 500 ml conical flasks containing 200 ml medium of the following composition ($g\ l^{-1}$): $NaH_2PO_4 \cdot 2H_2O$, 1.6; Na_2HPO_4 , 1.4; $(NH_4)_2SO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; K_2SO_4 , 0.5. The pH of the solution was adjusted to 7.0 and the medium supplemented with a trace element solution ($10\ ml\ l^{-1}$) containing ($g\ l^{-1}$): $Na_2EDTA \cdot 2H_2O$, 12.0; $FeSO_4 \cdot 7H_2O$, 2.0; $CaCl_2$, 1.0; $ZnSO_4 \cdot 7H_2O$, 0.4; $MnSO_4 \cdot 4H_2O$, 0.4; $CuSO_4 \cdot 5H_2O$, 0.1. Haloalkane (1 mmol) was added and the flasks, stoppered with cotton wool, were incubated on an orbital shaker (200 r.p.m.) at 25 °C except when certain ω -bromoalkanes were employed as substrates when an incubation temperature of 30 °C was used to ensure that the compounds remained in the liquid phase and were adequately dispersed in the culture medium.

Chemicals. All alkanes, chloroalkanes, iodoalkanes and most bromoalkanes were obtained from Aldrich. Purity was in all cases > 95% and in many instances > 98%. 1-Bromopentadecane (purity > 98%) and 1-bromoheptadecane (purity > 97%) were acquired from Fluka. 1-Fluorotetradecane (purity > 96%) was obtained from Fluorochem. None of the halogenated alkanes employed as substrates contained halo-

genated impurities at levels above the limit of detection using gas chromatography/mass spectrometry, i.e. 0.5%.

Determination of fatty acid composition of cellular lipids.

When growth on the haloalkane substrate was complete in the early stationary phase, cells (5 g wet wt) were harvested by centrifugation, washed with petroleum ether (b.p. 40–60 °C, $3 \times 20\ ml$) to remove residual substrate and cellular fatty acids were converted directly to the corresponding methyl esters by the transesterification technique of Dunlap & Perry (1967). Wet cells (1 g wet wt) were suspended in 14% BF_3 in methanol/benzene (9:1, v/v, 4 ml) and heated for 12 h at 60 °C in a water bath in a glass-stoppered tube. After cooling, water (8 ml) was added and the mixture extracted with petroleum ether (b.p. 40–60 °C, $2 \times 10\ ml$). The extracts were combined, washed with water (10 ml) and concentrated to 1 ml.

Fatty acid methyl esters (FAME) were identified and quantified by gas chromatography/mass spectrometry on a Hewlett Packard 5890 gas chromatograph linked to a HP 5970 mass selective detector controlled by an HP 300 series computer. The gas chromatograph was fitted with a CP-SIL-19 fused silica WCOT column (25 m $\times$ 0.25 mm) with 7% cyanopropyl, 7% phenyl, 85% methyl, 1% vinyl-polysiloxane as bonded phase. Helium was used as the carrier gas at a flow rate of 0.75 $ml\ min^{-1}$. In the acquisition of chemical ionization spectra, methane was bled into the source at a flow rate of 0.25 $ml\ min^{-1}$. After a splitless injection of the sample, the oven temperature was held at 40 °C for 1 min, then programmed at 10 °C min^{-1} up to 300 °C and held at this temperature for 10 min. Ion currents between m/e 30 and 500 were monitored. Individual FAME were identified from their electron impact (EI) and chemical ionization (CI) mass spectra and retention times (Murphy & Perry, 1983). To quantify each FAME, the total ion current in the electron impact mode was expressed as a percentage of the sum of the ion currents associated with all the FAME in the extract with an abundance greater than 1% of the total. In Tables of fatty acid composition in Results and Discussion, only fatty acids present at concentrations > 1% are listed.

RESULTS AND DISCUSSION

Effect of halogen substituent

The fatty acid composition of *R. rhodochrous* cells harvested after growth on ω -haloalkanes containing different halogen substituents is shown in Table 1. As previously reported (Curragh *et al.*, 1994), extensive incorporation of chlorofatty acids into the lipid fraction was observed after growth on 1-chlorohexadecane. Surprisingly, the proportion of halofatty acids present was even greater after growth on hexadecanes with the sterically bulkier bromo- and iodo-substituents. Substantial incorporation was observed in each case into the $C_{14:0}$, $C_{16:0}$, $C_{16:1}$ and $C_{18:1}$ fractions with the overall proportion of halofatty acids exceeding 90% when 1-bromohexadecane was substrate. With increased size of the halogen substituent there was evidence of a trend toward greater accumulation of halofatty acids of shorter chain length. Thus, whilst the lipid fraction of 1-chlorohexadecane grown cells contained approximately 17% of the $C_{14:0}$ chloroacid, the corresponding iodoacid comprised 54% of the fraction in 1-iodohexadecane-grown cells.

Because neither 1-fluorohexadecane nor 1-bromo and 1-iodo-tetradecanes were commercially available, the effect

Table 1. Fatty acid composition of *R. rhodochrous* after growth at 25 °C on different ω -haloalkanes

Values are expressed as the percentage of total fatty acid content. Abbreviations: Non Hal, non-halogenated fatty acid; F, ω -fluorofatty acid; Cl, ω -chlorofatty acid; Br, ω -bromofatty acid; I, ω -iodofatty acid; Me, methyl-branched fatty acid.

Fatty acid	Growth substrate							
	1-Fluorotetradecane		1-Chlorohexadecane		1-Bromohexadecane		1-Iodohexadecane	
	Non Hal	F	Non Hal	Cl	Non Hal	Br	Non Hal	I
C _{8:0}	2.5	—	—	4.7	—	—	—	—
C _{10:0}	5.3	—	—	3.8	—	—	—	—
C _{12:0}	—	—	—	—	—	—	—	—
C _{14:0}	39.4	1.0	1.2	16.6	—	27.7	—	53.8
C _{14:1}	2.9	—	—	—	—	—	—	—
C _{15:0}	—	—	2.1	1.6	1.1	4.1	1.3	—
C _{16:0}	7.5	—	15.3	27.7	4.6	22.3	12.6	9.7
C _{16:1}	1.0	—	3.0	10.3	—	16.0	—	12.7
C _{17:0}	—	—	—	—	—	7.3	—	1.1
C _{18:1}	25.7	—	3.7	10.0	1.4	9.8	2.4	3.6
C _{19:0} Me	12.6	—	—	—	2.7	1.9	2.8	—
C _{20:0}	—	—	—	—	—	1.1	—	—
C _{20:1}	2.1	—	—	—	—	—	—	—
Total	99.0	1.0	25.3	74.7	9.8	90.2	19.1	80.9

of fluorine substitution could not be compared directly with that of other halogens in alkanes of the same carbon chain lengths. Nevertheless, since only a relatively small difference between the overall levels of halofatty acid incorporation was observed with 1-haloheptadecane and 1-halotetradecane as growth substrates (Table 2), 1-fluorotetradecane was employed as a substitute for 1-fluorohexadecane as growth substrate in the comparison in Table 1. The sole fluorofatty acid detected in 1-fluorotetradecane-grown cells was the C_{14:0} compound and represented only 1 % of the total fatty acids. When considered in the light of the results with other haloalkanes, this suggests that the size of the halogen substituent in the growth substrate may be one of the most important factors in determining the extent of halofatty acid incorporation. Paradoxically, the presence of a large halogen substituent actually appears to increase the likelihood that a fatty acid will be incorporated into the cellular lipid fraction, a finding which could be construed as implying a highly unusual substrate specificity for the process. However, factors influencing the initial formation of halofatty acids from the haloalkane must also be considered in interpreting this finding. Curragh *et al.* (1994) concluded that the degradation of ω -chloroalkanes with chain lengths of C₁₂ and above by *R. rhodochrous* was initiated mainly by oxygenase attack at the non-halogenated end of the alkane molecule. This conclusion does not rule out the possibility that the specificity of the oxygenase may be sufficiently relaxed for it to be capable of attacking the halogenated end of the molecule as well. Other investigators (Omori & Alexander, 1978; Yokota *et al.*, 1986) have attributed release of chloride from 1,9-dichlorononane by resting cells of several *n*-

alkane-utilizing bacteria to this type of enzyme action. Such attack would be particularly favoured if the halogen substituent has relatively minor steric effects as in the case of fluorine. Indeed, it is quite conceivable that in *R. rhodochrous* the strong polarization of the C–F bond associated with electron withdrawal by the fluorine substituent can result in attack predominantly focused on the fluorinated end of the fluorotetradecane molecule with consequent defluorination and the formation of a non-substituted fatty acid. Thus, the failure to observe appreciable incorporation of fluorine into the lipid fraction during growth of *R. rhodochrous* on 1-fluorotetradecane may be attributable to the absence of significant fluorofatty acid formation during growth on this substrate. Similarly, the differing incorporation of ω -chloro, -bromo and iodofatty acids into cellular lipids may merely reflect the degree to which oxygenase attack is restricted to the non-halogenated end of the haloalkane rather than any intrinsic selectivity in utilization of the acids in lipid synthesis.

Effect of chain length

Table 2 shows the fatty acid composition of *R. rhodochrous* cells harvested after growth on a series of ω -chloroalkanes with even numbers of carbon atoms. Chlorofatty acids were incorporated into the lipid fraction during growth on haloalkanes between C₁₂ and C₁₈ at levels ranging from 21 % with C₁₂ to 75 % at C₁₆. No chlorofatty acids were detected after growth on C₁₀ or lower alkanes. As previously reported by Curragh *et al.* (1994) using 1-chlorohexadecane as substrate, the chlorofatty acid formed by initial oxidation of the alkane appears, in each

Table 2. Fatty acid composition of *R. rhodochrous* after growth at 25 °C on ω -chloroalkanes with even number carbon chains between C₁₀ and C₁₈

Values are expressed as the percentage of total fatty acid content. Abbreviations: Non Cl, non-chlorinated fatty acid; Cl, ω -chlorofatty acid; Me, methyl-branched fatty acid.

Fatty acid	Growth substrate									
	1-Chlorodecane		1-Chlorododecane		1-Chlorotetradecane		1-Chlorohexadecane		1-Chlorooctadecane	
	Non Cl	Cl	Non Cl	Cl	Non Cl	Cl	Non Cl	Cl	Non Cl	Cl
C _{8:0}	—	—	—	—	—	—	—	4.7	—	—
C _{10:0}	3.5	—	—	—	—	—	—	3.8	—	—
C _{12:0}	1.3	—	—	6.5	1.0	3.2	—	—	—	—
C _{14:0}	8.0	—	9.6	—	9.7	26.7	1.2	16.6	—	—
C _{15:0}	6.5	—	2.2	—	1.0	—	2.1	1.6	—	—
C _{16:0}	29.7	—	39.9	—	9.5	1.5	15.3	27.7	16.1	27.4
C _{16:1}	1.1	—	—	—	1.7	—	3.0	10.3	1.8	3.2
C _{17:0}	5.0	—	—	—	—	—	—	—	—	—
C _{18:0}	1.8	—	—	—	—	—	—	—	—	—
C _{18:1}	12.3	—	8.6	9.4	6.5	17.3	3.7	10.0	4.0	11.7
C _{19:0} Me	18.2	—	15.6	5.1	4.7	5.0	—	—	17.5	18.3
C _{19:1}	7.3	—	—	—	—	1.0	—	—	—	—
C _{20:0}	1.7	—	3.1	—	—	2.1	—	—	—	—
C _{20:1}	3.6	—	—	—	2.8	1.5	—	—	—	—
C _{22:0}	—	—	—	—	2.3	—	—	—	—	—
C _{22:1}	—	—	—	—	—	2.5	—	—	—	—
Total	100	0	79.0	21.0	39.2	60.8	25.3	74.7	39.4	60.6

case, to be converted to chlorofatty acids of both longer and shorter chain length. With C₁₂ and C₁₄ chloroalkanes, chain lengthening predominated, whilst with the C₁₈ chloroalkane, chain shortening prevailed. The C₁₆ chloroalkane was subject to both processes in approximately equal measure. Desaturation also occurred readily, leading to the formation of the C_{18:1} chlorofatty acid with C₁₂ and C₁₄ chloroalkanes and both C_{16:1} and C_{18:1} chlorofatty acids with C₁₆ and C₁₈ chloroalkanes.

A methyl-branched C_{19:0} chlorofatty acid was present when C₁₂, C₁₄ and C₁₈ chloroalkanes were substrates; it was particularly abundant in 1-chlorooctadecane-grown cells where, together with its non-halogenated analogue, it constituted 36 % of the total fatty acids. It is interesting to note that where such methyl-branched chloroacids were found, substantial quantities of the unsaturated chlorofatty acids with a carbon chain one carbon shorter were present. This is consistent with the results of King & Perry (1975) which indicated that the C₁₀ methyl group in branched C₁₇, C₁₈ and C₁₉ fatty acids was derived by addition of the methyl group from *S*-adenosylmethionine across the double bond of the Δ^9 unsaturated homologue.

Although longer chain ω -chloroalkanes with an odd carbon chain length were not available commercially, the corresponding bromo-compounds with chain length between C₁₁ and C₁₇ were employed as growth substrates for *R. rhodochrous*. However, the higher melting points of the

longer chain bromoalkanes necessitated growth at 30 °C rather than the temperature of 25 °C utilized for growth on chloroalkanes. Bromofatty acids were incorporated into the lipid fraction during growth on 1-bromoalkanes between C₁₃ and C₁₇ (Table 3). Maximum incorporation of 76 % was observed at C₁₅, a finding consistent with the C₁₆ maximum recorded for even carbon chain length 1-chloroalkanes (Table 2). As with the latter series, chain lengthening and chain shortening occurred in two-carbon units so that most bromoacids present possessed an odd number of carbon atoms. Desaturation resulted in the accumulation of substantial quantities of the C_{17:1} and C_{19:1} bromoacids after growth on several of the bromoalkanes. The formation of a methyl-branched C_{18:0} bromoacid in large quantities in cells grown on C₁₃, C₁₅ and C₁₇ bromoalkanes mirrored the biosynthesis of methyl-branched C_{19:0} chloroacid from the even carbon chain length chloroalkane-grown cells.

Although, for the reason outlined above, it was not practical to conduct growth experiments at temperatures less than 30 °C with all the bromoalkanes, it was possible to employ both C₁₁ and C₁₅ 1-bromoalkanes as growth substrates at 25 °C. Significantly increased bromoacid incorporation was apparent with both substrates at the lower temperature with total bromoacid content for C₁₁ 1-bromoalkane-grown cells rising from 0 % at 30 °C to 12.7 % at 25 °C, and for C₁₅ 1-bromoalkane-grown cells from 76.1 % at 30 °C to 83.9 % at 25 °C. In view of the

Table 3. Fatty acid composition of *R. rhodochrous* after growth at 30 °C on ω -bromoalkanes with odd number carbon chains between C₁₁ and C₁₇

Values are expressed as the percentage of total fatty acid content. Abbreviations: Non Br, non-brominated fatty acid; ω Br, ω -bromofatty acid; Me, methyl-branched fatty acid.

Fatty acid	Growth substrate							
	1-Bromoundecane		1-Bromotridecane		1-Bromopentadecane		1-Bromoheptadecane	
	Non Br	Br	Non Br	Br	Non Br	Br	Non Br	Br
C _{7:0}	—	—	—	1.6	—	—	—	—
C _{9:0}	3.9	—	8.5	5.9	—	—	—	—
C _{11:0}	5.8	—	4.1	—	—	—	—	—
C _{13:0}	—	—	9.3	10.7	—	1.1	—	—
C _{14:0}	6.2	—	1.7	—	—	1.3	1.6	—
C _{15:0}	9.7	—	10.0	3.3	21.9	34.3	10.3	37.0
C _{15:1}	—	—	—	—	1.0	4.3	—	2.3
C _{16:0}	31.5	—	11.5	—	—	—	8.8	—
C _{16:1}	2.6	—	—	—	—	—	—	—
C _{17:0}	5.0	—	3.3	—	—	—	5.8	1.2
C _{17:1}	2.0	—	1.8	6.0	—	11.4	—	14.2
C _{18:0}	1.0	—	—	—	—	—	—	—
C _{18:0} Me	1.3	—	—	7.9	—	18.0	—	11.3
C _{18:1}	6.1	—	1.3	—	—	—	1.6	—
C _{19:0}	—	—	—	1.2	—	—	—	—
C _{19:0} Me	7.0	—	1.8	—	1.0	—	3.3	—
C _{19:1}	14.1	—	7.6	2.5	—	5.7	1.4	—
C _{20:0} Me	1.0	—	—	—	—	—	1.2	—
C _{20:1}	2.8	—	—	—	—	—	—	—
Total	100	0	60.9	39.1	23.9	76.1	34.0	66.0

well documented effects of temperature on the composition of lipids in cell membranes, such variation is not unexpected.

Effect of position of halogen substitution

An investigation was conducted into the effect on fatty acid composition of *R. rhodochrous* cells of altering the position of halogen substitution in the haloalkane growth substrate from the terminal position to the sub-terminal position using 1-bromododecane and 2-bromododecane as growth substrates (Table 4). The overall incorporation of bromofatty acids into the lipid fraction was very similar with the two substrates, suggesting that the position of halogen substitution in the haloalkane was not critical in determining the extent of incorporation of haloacids into the cellular lipids. The principal difference in composition between the lipids derived from growth on the two substrates was the presence of (ω -1)-bromofatty acids in the 2-bromododecane-grown cells instead of the ω -bromofatty acids of the 1-bromododecane-grown cells. No 2-bromofatty acids could be detected in lipids from *R. rhodochrous* cells grown on 2-bromododecane, indicating either that the oxygenase was unable to attack the 1-position of 2-bromododecane or that, if attack does occur, the 2-bromododecanoic acid so formed is rapidly

metabolized by β -oxidation with the two-carbon fragment containing the bromine atom being eliminated as bromoacetic acid.

Concluding remarks

The apparent ease with which a wide range of long-chain haloalkanes are assimilated into the component lipids of the haloalkane-degrading organism *R. rhodochrous* has important implications for the biological treatment of haloalkane-containing effluent. Harvesting and safe disposal of biomass after such treatment may be necessary to prevent potentially toxic haloanalogues of normal fatty acids entering the biological food chain.

In the wider context there is increasing evidence that chlorofatty acids are widespread in the environment, particularly in fish lipids. Hitherto, the determination of organochlorine components in lipids has employed techniques specifically targeted on various man-made pollutants such as the organochlorine insecticides and polychlorinated biphenyls or has involved saponification of the triglyceride fraction which results in the elimination of chlorine as chloride from most chlorinated aliphatic compounds. However, recent investigations using enzymic and other non-destructive techniques have

Table 4. Fatty acid composition of *R. rhodochrous* after growth at 25 °C on 1-bromododecane and 2-bromododecane

Values are expressed as the percentage of total fatty acid content. Abbreviations: Non Br, non-brominated fatty acid; Br, ω -bromofatty acid; (ω -1) Br, (ω -1)-bromofatty acid; Me, methyl-branched fatty acid.

Fatty acid	Growth substrate			
	1-Bromododecane		2-Bromododecane	
	Non Br	Br	Non Br	(ω -1) Br
C _{8:0}	—	1.6	—	—
C _{10:0}	1.2	1.2	—	—
C _{12:0}	5.3	7.8	—	5.0
C _{14:0}	6.0	1.4	7.7	2.9
C _{15:0}	3.3	—	4.8	—
C _{16:0}	26.8	1.2	27.9	2.3
C _{16:1}	5.0	—	2.8	—
C _{17:0}	—	—	2.3	—
C _{17:0} Me	1.6	—	1.9	—
C _{17:1}	—	—	1.4	—
C _{18:0} Me	—	—	1.5	—
C _{18:1}	13.2	11.7	5.8	16.9
C _{19:0} Me	7.4	1.0	10.3	2.2
C _{19:1}	1.4	—	1.4	—
C _{20:0} Me	—	—	2.9	—
C _{20:1}	2.9	—	—	—
Total	74.1	25.9	70.7	29.3

demonstrated that a substantial proportion of organically bound chlorine in fish lipids is associated with fatty acids present in triglycerides. Thus, Wesén *et al.* (1992) have shown that fatty acids comprised 90% of the 1200 p.p.m. extractable organically bound chlorine present in eel lipids. Similarly, a large proportion of the organically bound chlorine in bivalves has been identified as halogenated fatty acids (Wesén *et al.*, 1995). As yet there is no evidence as to whether these chlorinated lipids are of natural or anthropogenic origin.

Long-chain fatty acid chlorohydrins have been identified as natural products in a species of edible jellyfish (White & Hager, 1977) and could possibly enter the marine food chain from such a source. Certain chlorinated fatty acids are known to be formed in pulp mill bleaching processes (Leach & Thakore, 1977) and observations referred to by Sundin *et al.* (1993) suggest that they can be incorporated into fish lipids. Another possible man-made source of such compounds are the chlorinated paraffins widely used as plasticizers and contained in lubricants, paints and fire retardants; the total world production is estimated to be of the order of 300 000 tonnes per year (Houghton, 1991). Investigations to date have not indicated significant bioaccumulation of these compounds as such (Campbell & McConnell, 1980), nor given evidence of appreciable

toxicity to fish or birds (Madeley & Birtley, 1980). Nevertheless, chloroparaffins, although incapable of sustaining microbial growth alone, can undergo co-metabolic degradation by *n*-alkane-degrading bacteria leading to the production of a variety of chlorinated long-chain fatty acids (Omori *et al.*, 1987). A proportion of such acids can be further degraded in mixed culture with mineralization of between 15 and 60% of chlorine, depending on the type of chlorinated paraffin under study. However, some of the chlorinated fatty acids formed appear resistant to further breakdown. The work described in this paper indicates a mechanism by which such bacterial degradation products could enter the biological food chain.

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REFERENCES

- Campbell, I. & McConnell, G. (1980). Chlorinated paraffins and the environment. 1. Environmental occurrence. *Environ Sci & Technol* **14**, 1209–1214.
- Curragh, H., Flynn, O., Larkin, M. J., Stafford, T. M., Hamilton, J. T. G. & Harper, D. B. (1994). Haloalkane degradation and assimilation by *Rhodococcus rhodochrous* NCIMB 13064. *Microbiology* **140**, 1433–1442.
- Dunlap, K. R. & Perry, J. J. (1967). Effect of substrate on the fatty acid composition of hydrocarbon-utilizing microorganisms. *J Bacteriol* **94**, 1919–1923.
- Houghton, K. L. (1991). Chlorinated paraffins. In *Encyclopedia of Chemical Technology*, 4th edn, vol. 6, pp. 78–87. Chichester: J. Wiley & Sons.
- King, D. H. & Perry, J. J. (1975). Characterization of branched and unsaturated fatty acids in *Mycobacterium vaccae* strain JOBS. *Can J Microbiol* **21**, 510–512.
- Leach, J. M. & Thakore, A. N. (1977). Compounds toxic to fish in pulp mill waste streams. *Prog Water Technol* **9**, 787–798.
- Madeley, J. R. & Birtley, R. D. N. (1980). Chlorinated paraffins and the environment. 2. Aquatic and avian toxicology. *Environ Sci & Technol* **14**, 1215–1221.
- Murphy, G. L. & Perry, J. J. (1983). Incorporation of chlorinated alkanes into fatty acids of hydrocarbon-utilizing mycobacteria. *J Bacteriol* **156**, 1158–1164.
- Murphy, G. L. & Perry, J. J. (1984). Assimilation of chlorinated alkanes by hydrocarbon-utilizing fungi. *J Bacteriol* **160**, 1171–1174.
- Omori, T. & Alexander, M. (1978). Bacterial dehalogenation of halogenated alkanes and fatty acids. *Appl Environ Microbiol* **35**, 867–871.
- Omori, T., Kimura, T. & Kodama, T. (1987). Bacterial cometabolic degradation of chlorinated paraffins. *Appl Microbiol Biotechnol* **25**, 533–557.
- Sundin, P., Wesén, C., Mu, H. & Odham, G. (1993). Are chlorinated fatty acids in fish lipids of natural origin? *Abstracts of Posters at the 1st International Conference on Naturally-produced Organohalogenes*, pp. 31–33. Delft, The Netherlands, September, 1993.
- Wesén, C., Mu, H., Kvernheim, A. L. & Larsson, P. (1992). Identification of chlorinated fatty acids in fish lipids by partitioning studies and by gas chromatography with Hall electrolytic conductivity detection. *J Chromatogr* **625**, 257–269.

Wesén, C., Mu, H., Sundin, P., Ringstad, O. & Odham, G. (1995). Occurrence of halogenated fatty acids in bivalve lipids. In *Naturally-produced Organohalogenes*, pp. 307–316. Edited by A. Grimvall & E. W. B. de Leer. Dordrecht: Kluwer Academic Publishers.

White, R. H. & Hager, R. H. (1977). Occurrence of fatty acid chlorohydrins in jellyfish lipids. *Biochemistry* **16**, 4944–4948.

Yokata, T., Fuse, H., Omori, T. & Minoda, Y. (1986). Microbial dehalogenation of haloalkanes mediated by oxygenase or halohydrinase. *Agric Biol Chem* **50**, 453–460.

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