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Identification of Metabolites Formed During Degradation of β- and δ-Hexachlorocyclohexane (HCH) by *Sphingobium indicum* B90A

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ABSTRACT

HCH (hexachlorocyclohexane) was once widely used as an insecticide until its environmental persistence was recognized and its use was discontinued or banned in many countries. Nevertheless, environmental problems surface now from former production and dump sites, in particular with the more stable β-HCH and δ-HCH endangering ground and surface waters. In the present study, several polar hydroxy metabolites formed during degradation of β- and δ-HCH by the soil microorganism *Sphingobium indicum* B90A (formerly *Sphingomonas paucimobilis* B90A(1,2,3)), were identified and characterized. The two metabolites from **β-HCH** were identified as a **pentachlorocyclohexanol** and a **tetrachlorocyclohexanediol**. Four additional hydroxy metabolites were observed from **δ-HCH** and identified also as a **pentachlorocyclohexanol** and a **tetrachlorocyclohexanediol**, structurally different from those of β-HCH, and an unsaturated **tetrachloro-2-cyclohexen-1-ol** and a **trichloro-2 cyclohexene-1, 4-diol**. The latter cyclohexenol and cyclohexenediol are likely formed via **δ-PCCH**, initially formed from δ-HCH, rather than by dehydrochlorination of initially hydroxylated compounds. Such data will aid in future investigations on the degradation of HCH in environmental studies. The data suggests that hydroxylation proceeded via an **S_N2 type reaction with substitution of equatorial-Cls and formation of axial-OH substituted compounds**. The study also revealed that equatorial-Cl in β- and δ-HCH are more reactive toward hydroxylation with the enzymes of *S. indicum* B90A, which is in contrast to the general dehydrochlorination of HCHs where axial-Cls are far more reactive.

HCH ISOMERS

HCH isomers (α-, β-, γ- and δ) differ with respect to the relative orientation of the chlorine atoms (axial or equatorial) bound to carbon atoms as well as with respect to physical chemical properties and persistence. In general, **presence of axial chlorine atoms furnish available sites for enzymatic degradation**.

RESULTS AND DISCUSSION

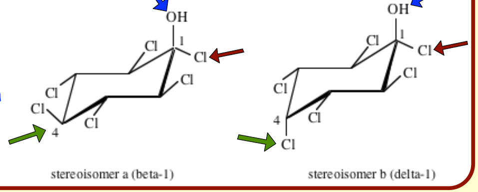
CHLORIDE-HCH MASS BALANCE

S. indicum B90A aerobically degrades α-, β-, γ- and δ -HCH within 24h (γ > α > δ > β). However, mass balance analysis showed that **α-and γ-HCH** are completely mineralized (**5-6 chlorides released per HCH molecule degraded**), but for **β-and δ-HCH** only **2-3 chlorides were released per HCH molecule degraded**, indicating incomplete mineralization.

PENTACHLOROCYCLOHEXANOL- beta-1; delta-1

EI mass spectra of **beta-1** and **delta-1** were quite similar but showed some differences in relative abundance of certain fragments ions. The two compounds appeared to be stereoisomers. This identified beta-1 and delta-1 as two isomeric pentachlorocyclohexanols.

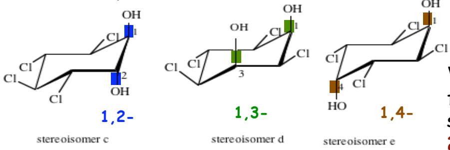
- ❖**Hydroxylation at equatorial Cl(1)**
- ❖**Substitution of -OH in axial position**
- ❖**Epimers w.r.t. C(4)**



stereoisomer a (beta-1)   stereoisomer b (delta-1)

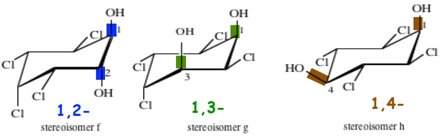
TETRACHLOROCYCLOHEXANEDIOL-beta-2;delta-2

EI mass spectra of **beta-2** and **delta-2** were also similar appeared to be stereoisomers. They were identified as two isomeric tetrachlorocyclohexanediol.



stereoisomer c   stereoisomer d   stereoisomer e

Stereoisomers of beta-2



stereoisomer f   stereoisomer g   stereoisomer h

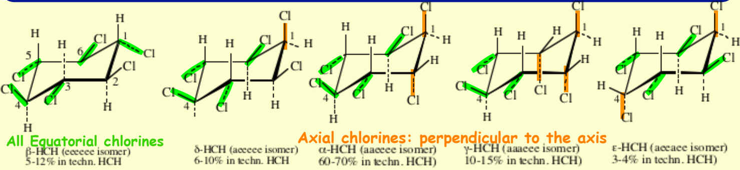
Stereoisomers of delta-2

With **S_N2 type mechanism** for formation, many possible stereoisomers of both **beta-2** and **delta-2** can be formed with **-OH in 1,2-, 1,3- and/or 1,4-diaxial position**.

CONCLUSIONS

1. Equatorial chlorides in β-and δ-HCHs are more susceptible towards hydroxylation than the general dehydrochlorination of axial chlorides in α- and γ-HCH.
2. delta-1 and delta-2 are not precursors for delta-3 and delta-4 respectively.
3. delta-3 and delta-4 can result from the formation of δ-PCCH from δ-HCH via elimination of (trans diaxial HCl elimination) axial Cl and followed by subsequent hydroxylation of equatorial Cl by substitution of -OH

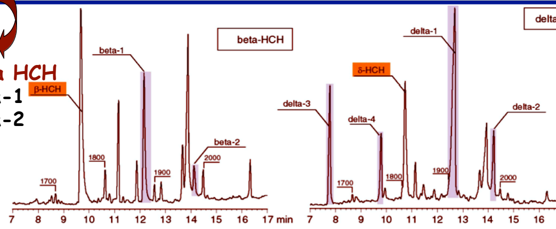
Consequently, **γ-HCH (aaaeee) and α-HCH (aaeeee) are more easily degradable than δ-HCH (aeeeee) and β-HCH (eeeeee),** as they have less equatorial chloro-substituents



**Technical Hexachlorocyclohexane: Mixture of α-HCH, β-HCH, γ-HCH and δ-HCH.
β-HCH and δ-HCH have thermodynamically more stable configurations**

FORMATION OF HYDROXYLATED METABOLITES

Beta HCH
Beta-1
Beta-2



Delta HCH
Delta-1
Delta-2
Delta-3
Delta-4

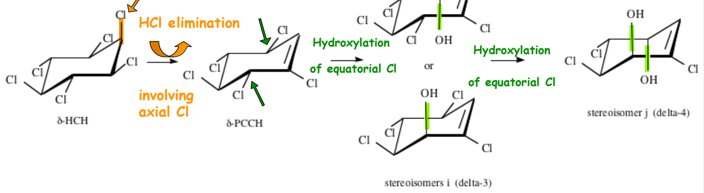
EI TIC GC-MS chromatograms of isolates from the incubation of β-HCH (left) and δ-HCH (right) with *S. indicum* **B90A** soil bacteria. 60-min incubated samples, analyzed as acetates. Retention indices relative to n-alkanes. DB-5 column, 80-120-280˚C, 5˚/min

- 2 major metabolites from β-HCH; 4 metabolites from δ-HCH
- small differences between β- and δ-HCH metabolites: stereoisomers

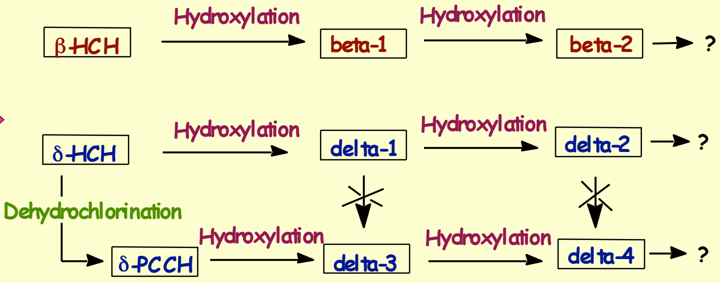
TETRACHLOROCYCLOHEXENOL delta 3

TRICHLOROCYCLOHEXENEDIOL delta 4

Two additional metabolites of δ-HCH were identified as **tetrachlorocyclohexenol and trichlorocyclohexenediol** which could be only formed by **initial HCl elimination of δ-HCH to δ-PCCH** by a simple antiperiplanar trans HCl elimination involving the axial Cl followed by **hydroxylation of the equatorial Cl** in allylic position would yield the delta -3 and delta-4.



Proposed configurations of delta-3 and delta-4, tetrachlorocyclohexenol and trichlorocyclohexenediol, and their formation from δ-HCH via δ-PCCH



Proposed pathways of degradation of β- and δ-HCH in *S. indicum* B90A

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