

## Theoretical modelling in combination with empirical investigations as a new tool for ecotoxicological evaluations of chiral pollutants.

### Chlorinated bornanes as an example.

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#### Introduction

Structure activity relationship (SAR) modelling approaches are already an important tool in modern biochemical research. Today, quality structure activity relationship (QSAR) considerations are essential for modern effect assessment of chiral drugs<sup>1</sup>. However, for chiral environmental pollutants it is still difficult to use QSAR approaches for ecotoxicological evaluations. The mode-of-action for chiral xenobiotics is not as well known as for chiral drugs. In addition, pollutants may compete with natural agents or other chemicals. Nevertheless, for a non-stereoselective evaluation of possible ecotoxic effects SAR modelling is well established<sup>2</sup>. SAR is based on physicochemical properties and calculated quantum mechanic descriptors. These parameters are not stereoselective and can, therefore, not describe the stereoselectivity of biochemical processes. However, already in 1956, Pfeiffer postulated a rule for chiral drugs, which is still accepted as important for pharmacological drug evaluation<sup>3</sup>. He stated '*the better the drug-receptor match, the greater the drug potency and the higher the enantiomeric ratio (ER)*'. This rule can easily be transferred to chiral ecotoxicology. Thus, it can be assumed for environmental processes: The better the diastereomeric complex build by enantiomer and receptor match, the greater is the environmental hazardous potential and the higher the enantiomeric excess. According to this postulation, an ER in environmental samples, different from the enantiomeric distribution of the technical product, indicates an ecotoxic potential. Furthermore, SAR research is based on the assumption that for a stable chemical high persistence is to be found in the environment. For a first approach these two postulates (1. modified Pfeiffers rule, 2. stability postulate) will be combined with non-stereoselective SAR calculations in order to predict the stereochemical properties of chiral xenobiotics. According to this theory, high environmental levels of low chlorinated bornanes with calculated high stability should be considered as a result of degradation from higher chlorinated congeners. At the other hand, high concentration levels of high chlorinated congeners with calculated high stability properties indicate passive bioaccumulation. For further evaluation, these basic considerations will be compared to analytically derived enantiomeric distributions. Possible scenarios are described in table 1 and will be discussed in the presentation (table 1).

#### Material and methods

The structure activity relationship (SAR) for 40 chlorinated bornanes (compounds of the technical toxaphene, CTTs) was modelled using the software package Alchemy 2000 (Tripos Inc., St. Louis, MO, USA). Descriptors were calculated by molecular mechanics (MM3) and semi-empirical quantum mechanics (MOPAC, AM1) methods. The following descriptors were chosen: *Heat of formation ( $\Delta H_f$ )*: semi-empirically calculated by MOPAC AM1 (Austin Model 1) as well as by MM3 (molecular mechanics calculation). For quality control, the  $\Delta H_f$  parameters derived from these two methods were compared. *Lipophilic properties*: Calculation of the Octanol-water

distribution was performed with the program SciLogP based on three-dimensional structure properties. *HOMO*: The 'highest occupied molecular orbital', as the negative ionisation potential was determined. *LUMO*: The 'lowest unoccupied molecule orbital' as the negative electron affinity was calculated. *Hardness*: The hardness [ $HA = 1/2[(HOMO-LUMO)]$ ] was calculated as a measure for the reactivity or polarisability of the molecule. *Dipole*: The AM1 MOPAC and MM3 method were used to calculate dipole moments based on atomic charges.

Table 1: Scenarios for the degradation and accumulation of chiral CTTs. RA = racemic distribution, EE = enantiomeric excess

Calculate d Stability	chlorination	Presence in the environment	ER	Explanation
High	High	High concentration	RA	Achiral accumulated in the food web
High	High	High concentration	EE	Achiral accumulated in the food web <sup>*,+</sup>
High	High	Low or not present	RA	Minor constituent in the technical mixture
High	High	Low or not present	EE	Degradation product or minor constituent of the technical mixture <sup>*</sup>
High	Low	High concentration	RA	Achiral accumulated in the food web
High	Low	High concentration	EE	Final degradation product.
Low	Low	High concentration	RA	Achiral accumulated in the food web <sup>#</sup>
Low	Low	High concentration	EE	Degradation product, further degradation expected <sup>#</sup>
Low	High	High concentration	RA	Achiral accumulated in the food web <sup>#</sup>
Low	High	High concentration	EE	Achiral accumulated in the food web <sup>*,+,#</sup>
Low	High	Low or not present	RA	Degraded into metabolites
Low	High	Low or not present	EE	Intermediate, further degraded <sup>*</sup>

<sup>\*</sup>) Possible EE in the technical toxaphene mixture can not be excluded. <sup>+</sup>) Minor degradation can be assumed. <sup>#</sup>) A local source nearby the sampling site should be considered

## Results and discussions

Vetter and Scherer published for the first time SAR calculations in order to assess the persistence of chlorinated bornanes<sup>4</sup>. Based on these results the authors described a 'bridge and exo' rule for the most persistent chlorinated bornanes in the environment. For more detailed information on the environmental fate of CTTs please refer to a recently published review<sup>5</sup>. The following energetic properties were found for persistent chlorinated bornanes based on the here presented SAR calculations: High LUMO (low electron affinity); Low  $\Delta H_f$  (low sterical energy) within each isomer group and high hardness (low reactivity). Reductive dechlorination and

dehydrochlorination processes have experimentally been shown to be important in abiotic degradation and biochemical metabolism. Most of the compounds found to degrade and thus not isolated from environmental samples have a geminal dichloro-substitution and show the highest structural energy (thermodynamic instability) within their respective isomer groups. In addition, LUMO is located at the carbon atoms with geminal dichloro-groups, approached during reductive dechlorination. The same carbons have the highest positive atomic charge in the main C1-C6 ring. In general, LUMO, HA, location of LUMO and C2-charge, seem to be important descriptors for kinetic controlled lability and degradation. Hardness (HA) versus the charge for those with geminal dichloro-groups and C2 for those without geminal dichloro-groups is given in figure 1.

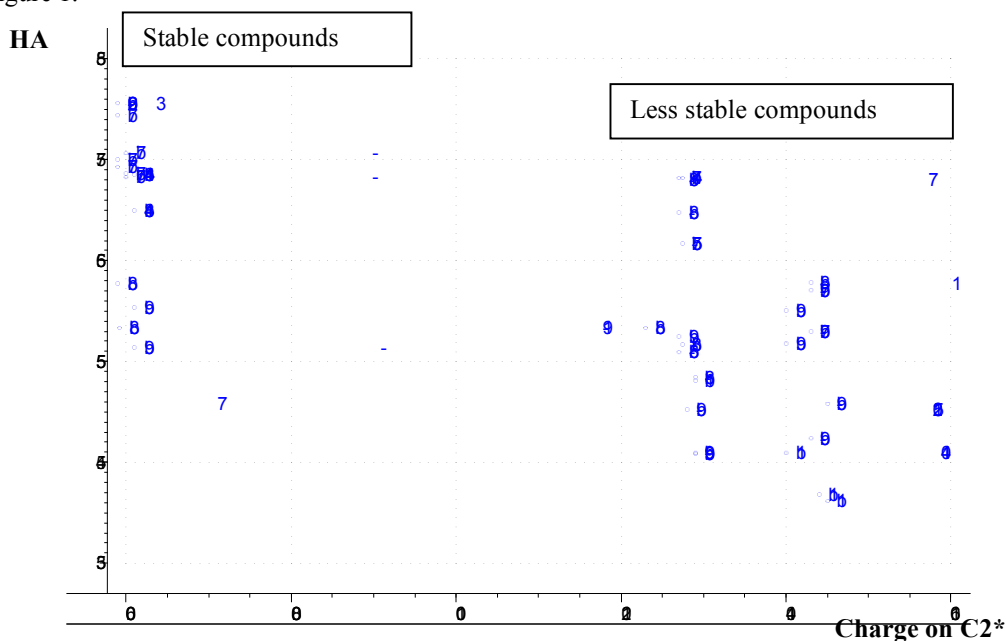


Figure 1: Relationship between Gasteiger atomic charge on carbons with geminal dichloro-group and hardness of 40 CTTs. \*B8-2229: charge on C5, B9-2006, b9-2009 and B10-1981: charge on C3.

Figure 1 reveals two main groups characterised as stable and less stable compounds. LUMO and HA alone describe the lability of the compounds. The compounds B6-923 and B7-1001 have the highest HA (figure 2) and are experimentally isolated in high concentration levels from sediment and sewage sludge<sup>5</sup>. These two compounds are also found to be major metabolites during reductive dechlorination in biota<sup>5,6</sup>. LUMO and HA should therefore be considered as important descriptors for the final metabolites (B6-923 & B7-1001) in strong reducing environment such as anaerobic sediments and biota. Based on analytical methods using modified cyclodextrin capillary columns and gas chromatographic separation, the enantiomer separation of ten CTTs is reported<sup>5</sup>. The most abundant congeners in the environment are the congeners B8-1413 and B9-1679. Although high concentrations were determined no enantiomeric excess were found for these congeners compared to the technical product<sup>5</sup>. Thus, according the scenarios described in table 1, for these congeners an achiral accumulation in the environment can be assumed. The following

congeners express ERs in the environment unlike the technical product: B6-923, B7-1715, B8-1412, B8-2229, B9-718, B9-1025. High levels of B6-923 are found in sediment samples with considerable enantiomeric excess. Therefore, this congener can be considered as a degradation product of higher chlorinated congeners<sup>7,8</sup>.

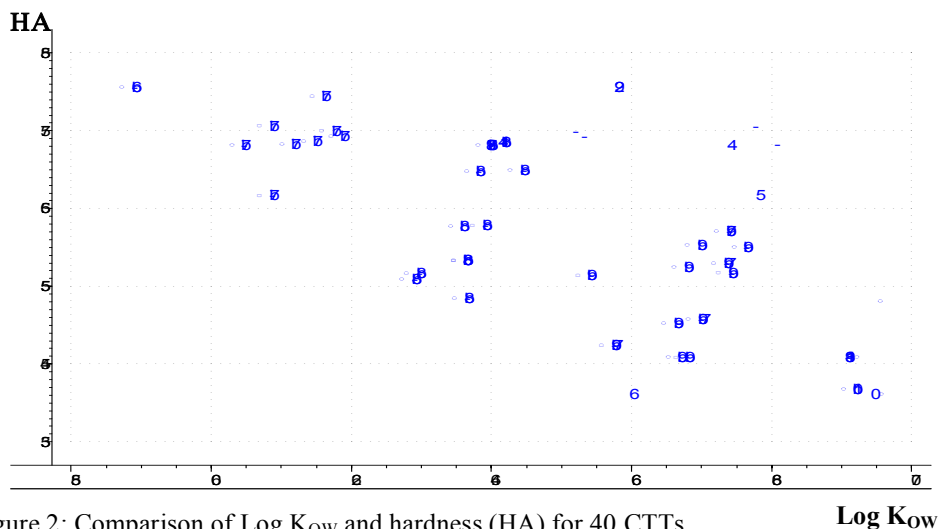


Figure 2: Comparison of Log Kow and hardness (HA) for 40 CTTs.

## Acknowledgement

The support of A. Smalås and A. Sckancke (University of Tromsø) is highly appreciated. We are grateful for the financial support from the Norwegian Research council (122151/730: Congener and enantiomer selective properties of selected toxaphenes).

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