# FAST AND EFFECTIVE TRANSFORMATION OF TOXAPHNE BY SUPERREDUCED VITAMIN B12 AND DICYANOCOBALAMIN

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## Introduction

The chloropesticide toxaphene (Camphechlor, Melipax) has been used in high quantities since 1945 [1,2]. Toxaphene has been classified as a persistent organohalogen pollutant (POP) and belongs to the "dirty dozen" [3]. Due to its heavy use in the 1960s and 1970s, several environmental compartments were significantly contaminated with toxaphene. For instance, concentrations in polluted areas could exceed 1 mg/kg sediment [4]. Recently, it was shown that cultures of the isolated bacterium *Dehalospirillum multivorans* transformed toxaphene in a similar way as anaerobic sediment and soil samples [5]. The potential of *D. multivorans* for the anaerobic transformation of organohalogens was previously demonstrated for chloroethenes (PCE and TCE) [6]. The reactive dehalogenase of *D. multivorans* contains a corrinoide co-factor [7]. In this study two corrinoids cyanocobalamin (vitamin B12, CCA) and dicyanocobinamide (DCC) were used in their superreduced forms (i. e. those having the central atom Co in the oxidation state +I) [8]. Interestingly, a very fast transformation of toxaphene was observed with superreduced DCC. Unfortunately, the transformation occurred so fast that no transformation products could be identified. For this reason, we performed further experiments with the less reactive superreduced vitamin B12 ($CCA_s$). $CCA_s$ has previously been used for the transformation of PCBs, hexachlorobenzene, chloroform, and chloroethanes [9-12].

## Material and Methods

**Chemicals:** Melipax (in original packaging) was found in a garden shed in Jena (Germany). Toxaphene (Hercules Inc.) was obtained from L. Alder (BfR, Berlin). B6-923 was isolated from an environmental sample by HPLC. A reference standard of B6-923 (commercially distributed by Dr. Ehrenstorfer, Augsburg, Germany) was obtained from H. Parlar. Other POP standards were from Dr. Ehrenstorfer or LGC Promochem (Wesel, Germany). The internal standard perdeuterated α-HCH ($\alpha$-PDHCH) was previously synthesized [13]. Cyanocobalamin (CCA) and dicyanocobinamide (DCC) were obtained from Fluka (Neu-Ulm, Germany).

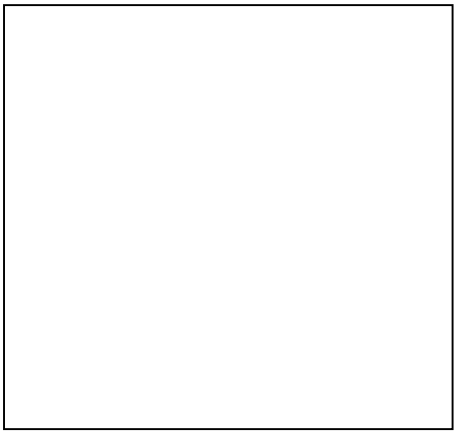

**Figure 1:** Structure of cyanocobalamin (vitamin B12)

The corrinoids CCA and DCC have the same structure except the lower axial substituent ($Co_\alpha$) which is the bonded base 5,6-dimethylbenzimidazole in the case of CCA and cyano in the case of DCC (which has no base). The axial ligand influences the redox potential of the corrinoid: the redox potential of the "base of" corrinoid (DCC) is higher than the redox potential of the "base on" corrinoid (CCA).
Superreduced corrinoids (0.1 - 5 µmol in the case of DCC; 1 µmol – 1 mmol in the case of CCA) were prepared with Ti(III)citrate [14] in 10 mL-vials; the end point of the reduction was observed by the change of colour of the solution. The vials were kept in a glove box kept under strictly anaerobic conditions [14].

**Experimental performance, sample preparation and analysis:** In an anaerobic glove box, 5 mL of superreduced corrinoids (see above) were pipetted into 10 mL-vials, 80 µg Toxaphene, 55 - 220 µg Melipax or 18 ng B6-923 were added in 5 µL *n*-hexane. After a reaction phase of 2 h, 6 h, and 1, 2, 3, 6, and 7 days, the vials were opened, the internal standard *α*-PDHCH was added and the entire sample was immediately extracted with 2 x 10 mL *n*-hexane (ultrasonic bath, 5 min). The combined *n*-hexane extracts were filtered through $Na_2SO_4$, and the volume was adjusted to 2 mL (technical toxaphene) or 1 mL (B6-923). 1 µL of each extract was analyzed by GC/ECD [15].
**Gas chromatography in combination with mass spectrometry (GC/MS):** GC/MS measurements were performed with a Varian CP-3800 GC coupled to a Varian 1200 triplequadrupole MS. Helium 5.0 was used as carrier gas. The injector and transfer line temperatures were set at 230 °C and 280 °C, respectively. The scan rate was set at 4 cycles/s, and the filament emission was set at 150 µa.
GC-analysis were performed with a Factor Four CP-Sil 8MS column (30 m x 0.25 mm i.d. x 0.25 µm $d_f$, Varian). The GC oven temperature program started at 60 °C (hold time 2 min), which then was raised at 20 °C/min to 180 °C, raised at 1.5 °C/min to 250 °C (hold time 2 min) and finally at 5 °C/min to 290 °C/min (hold time 1.33 min). The total run time was 60 min. Injections were performed in splitless mode (split opened after 2 min), using a pressure pulse at 40 psi for the initial 1.5 min. A constant flow rate of 1.2 mL/min was used throughout the measurements.
In the negative ion chemical ionization mode (GC/NICI-MS), the electron energy was set at 150 eV. The ion source temperature was set at 200 °C. Methane 4.5 was used as the reagent gas at ~8.8 Torr, the SIM peak width was set at 0.7 u.
Initial GC/NICI-MS full scan experiments were carried out with m/z 30-500 to detect as much products during the degradation as possible. The chloride masses m/z 35 and m/z 37 were consulted for the determination of the total area of CTTs in a sample. The detector voltage was set at 1500 V. In the GC/NICI-MS-SIM mode, the following ions of tetra- to hexachloro-CTTs were monitored at 4 cycles/s: m/z 235.0, 236.0, 237.0, 238.0, 239.0, 240.0, 241.0, 258.9, 260.9, 262.9, 269.0, 270.0, 271.0, 272.0, 273.0, 274.0, 275.0, 276.0, 277.0, 278.0, 302.9, 303.9, 304.9, 305.9, 306.9, 307.9, 308.9, 309.9, 310.9 and 311.9. The detector voltage was set at 1500 V.

**Results and Discussion**

Incubation of technical toxaphene (Melipax) with $DCC_s$ was accompanied with a very effective transformation of toxaphene. Within 6 h more than 90% of Melipax was eliminated (**Figure 2**).

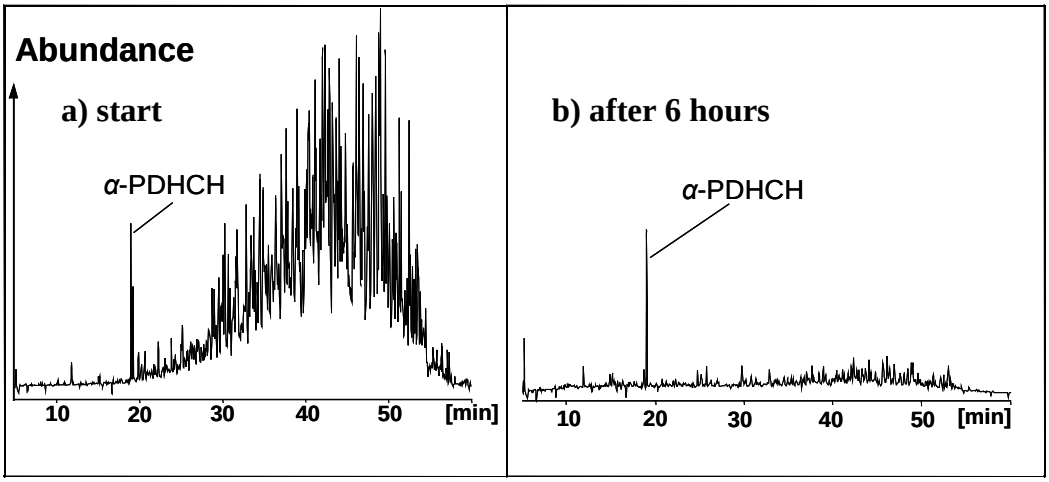


**Figure 2:** GC/ECD chromatograms of (a) Melipax and (b) Melipax after a 6h-treatment with DCC. The peak of the internal standard *α*-PDHCH is marked in both chromatograms

However, no preferential enrichment of certain CTTs in Melipax was observed which is in contrast with transformation of toxaphene with the anaerobic bacterium *D. multivorans* [5]. This was confirmed by the reaction of individual B6-923 (18 ng) with $CCA_s$. B6-923 (also known as Hx-Sed) is the most persistent toxaphene compound in anaerobic media such as soil, sediment and sewage sludge [5,16]. Incubation with 0.1 µmol $DCC_s$ resulted in a ~90% transformation of B6-923 within 2 hours (data not shown). Therefore, the velocity of the transformation of B6-923 was comparable with Melipax.

Three toxaphene concentrations (55 - 220 µg, corresponding with 11 – 44 mg/L) gave a slightly lower transformation rates for the highest concentration (about 90% instead of ~95% in the lower-concentrated solutions). The high recovery rate of the internal standard along with the observation that reactions without Ti(III)citrate allowed to recover CTTs quantitatively prove that low amounts of Melipax detected after treatment with $DCC_s$ are due to abiotic transformation.

Because of the very fast and unselective transformation of CTTs with $DCC_s$ subsequent experiments were performed with superreduced vitamin B12 ($CCA_s$). Treatment of toxaphene with $CCA_s$ resulted in a significantly slower transformation rate. More than 100-fold of the concentration of the corrinoid ($CCA_s$ instead of $DCC_s$) and a reaction time of eight days instead of six hours were required to obtain the same degree of CTT transformation. These experiments clarified that Ti(III)citrate alone did not degrade CTTs but the superreduced corrinoids.

GC/MS analysis was used for the detection of individual CTTs at shorter retention time. Most of these hexachloro-CTTs were also found in the starting mixture, but some new CTTs originally not present in toxaphene were detected after three days (**Figure 3**).

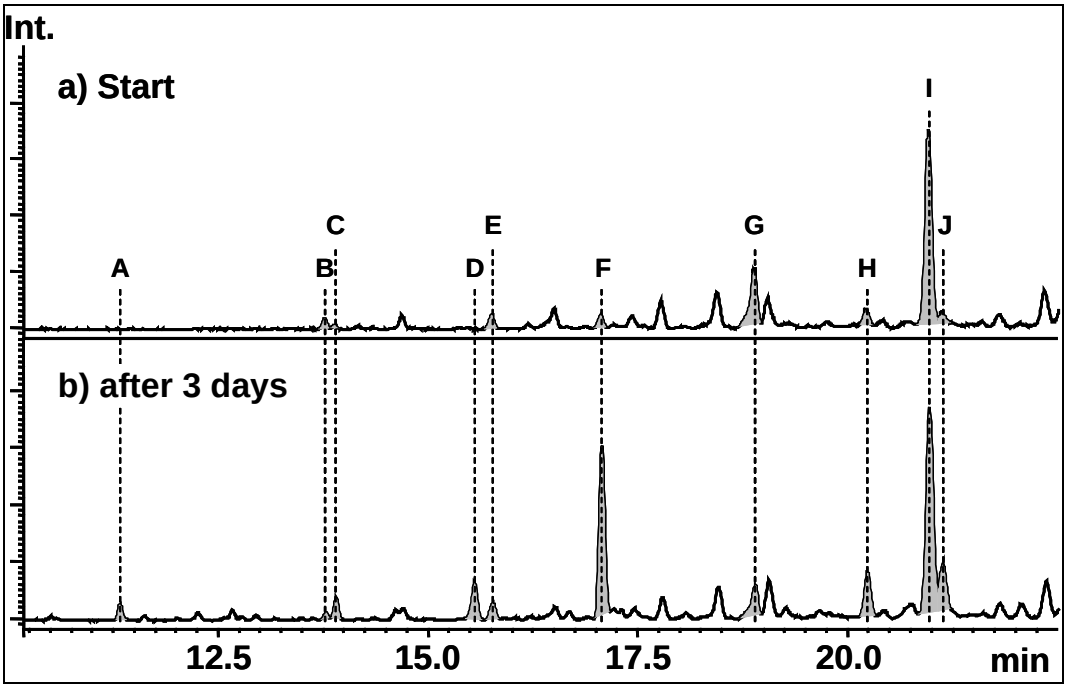


**Figure 3:** GC/NICI-MS-SIM chromatograms of hexachloro-CTTs (m/z 300-315) in (a) toxaphene and (b) toxaphene after a 3-day-treatment with superreduced vitamin B12 ($CCA_s$)

Some of these peaks underwent significant changes in their intensities whereas other CTTs did only vary slightly. **Figure 4** shows the relative changes in the intensities of selected peaks. While some peaks were formed in the first phase (day 3) of the experiment (peak denoted A and D in **Figures 3** and **4**), all studied peaks were lower concentrated at the end of the study (14 days) than after three days. This may explain why this effect was not observed in the transformation experiments performed with the more effective $DCC_s$ (see above) [8]. GC/ECNI-MS studies clarified that ~50% of the transformation products identified so far were hexachlorobornenes (A, C, F, G and J) and hexachlorobornanes/hexachlorocamphenes (B, D, E, H and I), respectively.

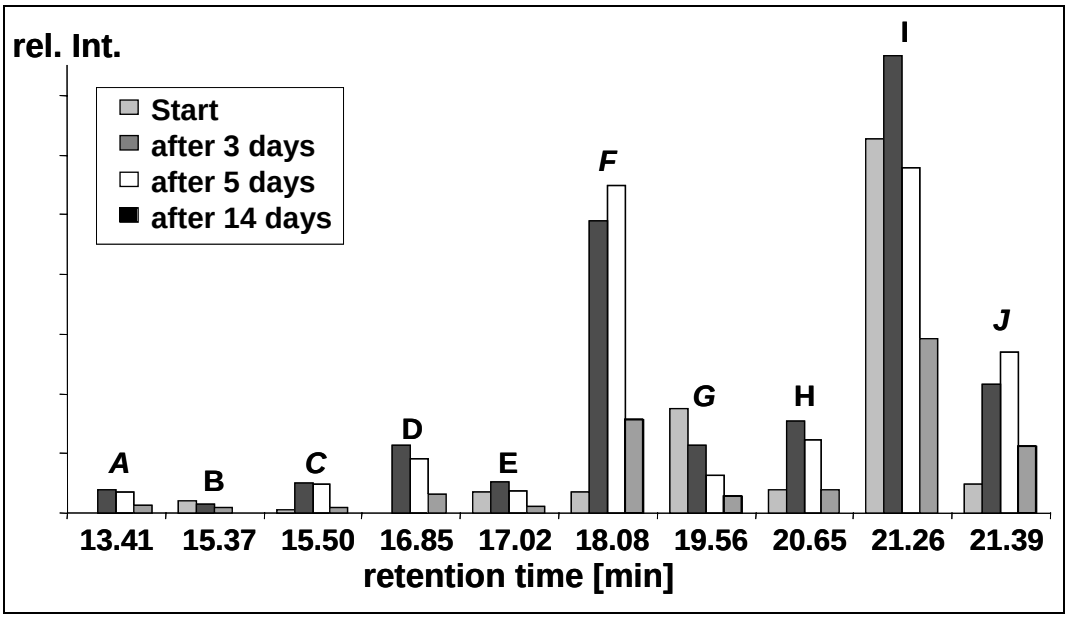


**Figure 4:** Time-dependent formation and transformation of selected hexachloro-CTTs during the treatment of toxaphene with superreduced vitamin B12 ($CCA_s$) (for peak labelling see **Figure 3)**

However, the sum of the transformation products was much lower than the amounts transformed in the effective process with superreduced corrinoids (**Figure 5**). Therefore, we cannot exclude that other transformation pathways next to dechlorination and dehydrochlorination may take place during the treatment of toxaphene with superreduced corrinoids.

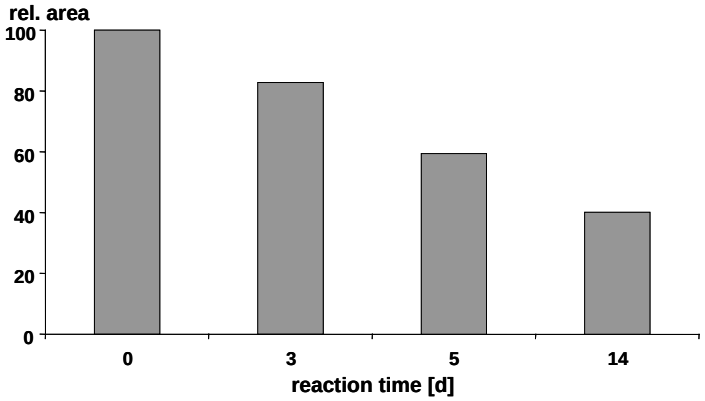


**Figure 5:** Transformation profile of toxaphene with superreduced vitamin B12 ($CCA_s$). The relative amount (% of the initial area of m/z 35 and m/z 37) of toxaphene was detected in dependence of the reaction time.

**Conclusions**

Reaction with superreduced corrinoids ($CCA_s$, $DCC_s$) allowed for a significant transformation of toxaphene. The efficiency of the toxaphene transformation with $DCC_s$ was higher than with any method previously described. While the transformation speed will be improved with a more potential corrinoid such as superreduced DCC in the case of toxaphene, less reactive corrinoids are more suitable to study the transformation pathway as was shown by using superreduced CCA in this study.

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