# DEVELOPMENT AND APPLICATIONS OF COMPOUND-SPECIFIC CHLORINE ISOTOPE ANALYSIS OF ORGANOCHLORINES: THE CASES OF PCDDs IN U.S. BALL CLAY AND DDT IN THE BALTIC ENVIRONMENT

Holmstrand H[1,2], Mandalakis M[3], Zencak Z[2], Gadomski D[4], Andersson P[1], Gustafsson Ö[2]

[1]Laboratory for Isotope Geology (LIG), Swedish Museum of Natural History, 10405 Stockholm, Sweden; [2]Department of Applied Environmental Science (ITM), Stockholm University, 10691 Stockholm, Sweden; [3]Environmental Chemical Processes Laboratory (ECPL), University of Crete, 71409 Ampelokipi-Heraclion (Crete), Greece; [4]IEH Laboratories & Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

## Introduction

Compound-specific chlorine isotope analysis (CSIA-Cl) was developed and applied as an off-line technique in order to study processes and fate of semi-volatile organochlorines (OCls) in the environment[1]. Conventional techniques for CSIA-$\delta^{37}$Cl, at the time of our method development, required the sample to contain >50 µg of chloride, e.g. corresponding to 100 µg DDT (DDT and DDE pertains to the *p,p'*-isomers throughout the text)[2]. Organochlorine compounds are usually present at low levels (ng/g dw) in environmental matrices, complicating the sampling and extraction prior to CSIA-Cl. Increased sensitivity in CSIA-Cl was accomplished by the use of preparative capillary gas chromatography (pcGC) to harvest enough high-purity sample material from a complex environmental matrix, in combination with thermal-ionization mass spectrometry (TIMS) instead of conventional dual-inlet isotope-ratio mass spectrometry (dual-inlet IRMS)[3]. Samples containing ~5 µg of chlorine have been analyzed so far, with potential for smaller samples.

Chlorine has two stable isotopes, $^{37}$Cl and $^{35}$Cl, which constitute ~24% and ~76% of the natural abundance, respectively. The proportions of these isotopes in a sample is expressed in per mil units relative to the chlorine isotope composition of standard mean ocean chloride (SMOC), which is used as the isotopic reference material:

$$\delta^{37}\mathrm{Cl}(‰)=(R_{\mathrm{Sample}}-R_{\mathrm{Ref}})/R_{\mathrm{Ref}}\cdot 1000‰$$

The measured ratios of $^{37}$Cl/$^{35}$Cl in samples and the reference material are denoted $R_{\mathrm{Sample}}$ and $R_{\mathrm{Ref}}$, respectively. Observed values of $\delta^{37}$Cl in natural and anthropogenic compounds usually fall in the range -10 to +10‰. Differences in the isotopic reaction rates, i.e. $^{37}$Cl tend to react slower than $^{35}$Cl due to the stronger chemical bonds formed by the heavier isotope (termed the kinetic isotope effect, KIE), and isotopic chemical-equilibrium constants impose variations on the order of 0.1-10‰ in the $\delta^{37}$Cl of OCls that have undergone various processes, or stem from different sources. Enzymatic chlorination, generally considered as the main reaction pathway in the production of natural OCls, has been shown to yield highly depleted chlorine isotope signatures with $\delta^{37}$Cl <-10‰, whereas abiotic chlorination (e.g. industrial synthesis or natural abiotic chlorination) appears to consistently result in $\delta^{37}$Cl in the range –7 to +4‰[4-6]. This has been hypothesized to be useful in the distinction between the natural and anthropogenic OCls.

The accuracy of the method for isotope analysis, as well as for the preceding procedures of pcGC, were confirmed by analysis of DDT with a known isotope composition from analysis with dual-inlet IRMS[1, 7]. The developed method of CSIA-Cl was used to elucidate the origin of polychlorinated dibenzo-*p*-dioxins (PCDDs) in US. ball clay from the Mississippi Embayment (exhibiting the putative natural formation pattern), based on the chlorine-isotope signature of abiotic vs. biotic chlorination processes[8]. Furthermore, the degradation of DDT in the Baltic Sea was studied by CSIA-Cl of DDT and DDE from the blubber of the Baltic Grey seal (*Halichoerus grypus*)[9]. The fraction of remaining non-degraded DDT in the Baltic Sea, relative to the quantity of DDT that was released into the environment, was calculated with a Rayleigh isotope-distillation model, based on the kinetic isotope effect during dehalogenation of DDT.

### Materials and Methods

Samples for method development and control were chemically pure DDT (from Aldrich and BDH) and octachloro-dibenzo-*p*-dioxin (OCDD, from Cambridge Isotope Laboratories). The samples of ball clay have been described by Gadomski et al.[10], and the CSIA-Cl was performed on pooled subsamples from 7.0-9.4 m depth in core D. Additional samples were analyzed for the conventional radiocarbon date of the total organic carbon. These samples were sent directly to the accelerator mass-spectrometry facility NOSAMS in Woods Hole (MA, USA). Seal blubber for CSIA-Cl of DDT and DDE was collected from two specimens of Grey seal that were sent to the Swedish Museum of Natural History after having been found dead at the Swedish east coast.

The clay samples were Soxhlet extracted and purified in a multistep column with silica-gel modified with KOH, $H_2SO_4$ and $Na_2SO_4$, respectively. The seal blubber was extracted in a Wallenberg perforator using acetonitrile. Acetonitrile has the advantage of being only partially miscible with the lipids of the blubber, and the continuous partitioning between the lipid and acetonitrile phases was maintained for five days in order to ensure efficient extraction. The blubber extract was re-extracted two times, and was then treated with $H_2SO_4$ in order to remove remaining lipids. Columns with silica-gel were used to isolate fractions containing the target compounds DDT and DDE.

The individual target compounds from ball clay and seal blubber were isolated from the purified extracts by means of preparative capillary gas chromatography (pcGC). Repetitive injections (n>100) were used to harvest enough material for CSIA-Cl (14 µg OCDD, >100 µg DDT and DDE). The samples were transferred to borosilicate-glass tubes, which were evacuated and sealed after addition of CuO. The sealed tubes were heated to 630° in order to combust the samples, thus converting the organically bound chlorine to inorganic chloride in the form CuCl. The liberated chloride was dissolved, purified, and converted to CsCl*(aq)* salt for use in TIMS.

After determination of the chloride concentration in the CsCl solution, 1-3 µg of chloride was loaded on a tantalum filament, together with graphite powder from an ethanol suspension, dried, and inserted to the ion source of the TIMS instrument. Ionization was accomplished by a small current through the filament, thus generating heat and ionizing the CsCl. The intensities of the ion beams m/z 301 ($^{133}Cs_2{}^{35}Cl^+$) and m/z 303 ($^{133}Cs_2{}^{37}Cl^+$) are proportional to the chlorine isotope composition of the sample, and were used to obtain the R for calculation of $\delta^{37}Cl$.

### Results and Discussion

The method of sealed-tube combustion of OCls in conjunction with TIMS was validated by analysis of a DDT sample (from Aldrich), which had been analyzed with dual-inlet IRMS[1]. The two methods resulted in overlapping values (-4.42 ± 0.46‰ for TIMS and -4.34 ± 0.25‰ for dual-inlet IRMS, all uncertainties given for 1$\sigma$). The lower precision of TIMS is an acceptable trade-off for the improvement in sensitivity by a factor 10-20. Both precision and sensitivity in TIMS are limited by the preparation of samples after sealed-tube combustion, and can thus be improved.

The complete method of CSIA-Cl relies on pcGC to isolate the target compounds. Isotope fractionation is known to occur in the separation column of GC systems, and the potential problems associated with this were investigated by an experiment were the eluting peaks were partially collected and analyzed for their isotope composition[11]. A set of samples were prepared by injecting a DDT standard of known isotope composition, and collecting samples consisting of the front and end halves of the eluting peaks, respectively. The normal operation of the pcGC procedures was checked by collecting whole peaks. The latter would not be influenced by isotope fractionation within the column, but rather in the injector as a consequence of thermal degradation of the injected DDT. It was found that no net isotope fractionation, relative to initially injected material, could be observed in the sample where whole peaks had been collected (-3.2 ± 0.1‰ vs. –3.6 ± 0.8‰, respectively). However, splitting of the eluting peaks induced shifts as large as ±2‰. This simulates the scenario where completely resolved peaks are unattainable. Hence, pcGC preserves the isotope composition of the sample when operated properly.

Several reports on elevated concentrations of PCDDs in modern sediments, as well as clay deposits of high age, have fueled speculations of whether potent natural sources of PCDDs exist[10,12-14]. The congener pattern of these samples, along with very low levels of polychlorinated dibenzofurans, dominance of 1,2,3,7,8,9-hexachloro-dibenzo-*p*-dioxin (among the 2,3,7,8-substituted HxCDD congeners) and high levels of octachloro-dibenzo-*p*-dioxin (OCDD), has been referred to as the "natural formation pattern", and could potentially be a clue to the surplus in the global PCDD budget.

The putative natural formation pattern does not match that of any known anthropogenic source. CSIA-Cl, together with analysis of TOC radiocarbon and pyrogenic black carbon, was deployed in a multidisciplinary effort to establish the origin of the extremely high levels of PCDDs in ball clay from the Mississippi Embayment[8]. The conventional radiocarbon date of the clay from three cores, at the depths 7.0-11.3 m, fell in the range 14700 years B.P. to >48000 years B.P. (i.e. non detectable $^{14}$C content). Hence, the origin of the PCDDs at higher depths than these top samples for radiocarbon is natural, as the PCDDs pre-dates the industrial era. CSIA-Cl of OCDD, the most abundant and least toxic congener, gave a $\delta^{37}$Cl of –0.2 ± 0.2‰. Combined, these results suggest that the high levels of PCDDs in the ball clay are of natural abiotic origin[4]. Potential mechanisms of formation include participation of the clay minerals as catalysts, serving to increase the reaction rate and to stabilize the products.

DDT is one of the most prominent pollutants of the Baltic Sea, causing harmful effects to wildlife. The product of the natural attenuation of DDT is DDE, which is a more recalcitrant and toxic compound than DDT. DDE is presently the most abundant substance of the DDx group (i.e. DDT and its metabolites) in the Baltic environment. The extent of DDT degradation in the environment has not been unambiguously determined. Ratios of DDT/DDE in different matrices have been used to constrain the remaining fraction of non-degraded DDT in the environment, but the results are highly influenced by the type of matrix analyzed[15]. The use of CSIA-Cl could provide an independent estimate of the extent of environmental degradation of DDT, based on the differences in reaction rates among isotopes. DDT from the blubber of two Grey seals was analyzed to provide a data point on the contemporary chlorine isotope composition of DDT in the environment[9]. The seal was considered to be an integrating sampler in the Baltic Sea, by direct uptake of DDT through partitioning with water, and by bioaccumulation as the result of feeding habits. The obtained $\delta^{37}$Cl of –0.69 ± 0.21‰ was enriched relative to the assumed initial value of –4.34‰, which would then correspond to the chlorine isotope composition of the DDT that was released into the environment. The difference between these two values was consistent with a kinetic isotope effect during degradation. A Rayleigh isotope distillation model was used to calculate the extent of the degradation (f) that would match the initial parameters, for a chosen isotope fractionation factor ($\alpha$). The calculations indicated that, for Baltic Sea, 7 ± 2% of the released *p,p'*-DDT is still present in the environment.

It is clear that CSIA-Cl is a promising tool for studies of sources and fate of OCls in the environment, with the ability to extract information that cannot be obtained by conventional analysis of concentrations. Furthermore, chlorine isotope analysis is a powerful complement to CSIA techniques of radiocarbon, stable carbon and hydrogen isotopes.

## Acknowledgements

We thank Anna Roos (Swedish Museum of Natural History) for facilitating sampling of seal blubber, and Maria Unger (Stockholm University) for assistance with extraction of target compounds from seal blubber. This study was supported financially by the Swedish Strategic Environmental Research Foundation (MISTRA Idestöd, contract no. 2002-057), and through a senior research fellowship to Ö.G. from the Swedish Research Council (VR contract No. 629-2002-2309) and a Ph.D. position to H.H. at the Swedish Museum of Natural History.

## References

1. Holmstrand H, Andersson P, Gustafsson Ö. *Anal Chem* 2004;76;2336.
2. Holt BD, Sturchio NC, Abrajano TA, Heraty LJ. *Anal Chem* 1997;69;2727.
3. Xiao YK, Lu H, Zhang C, Wang Q, Wei H, Sun A, Liu W. *Anal Chem* 2002;74;2458.
4. Reddy CM, Xu L, Drenzek NJ, Sturchio NC, Heraty LJ, Kimblin C, Butler A. *JACS* 2002;124;14526.
5. Drenzek NJ, Tarr CH, Eglinton TI, Heraty LJ, Sturchio NC, Shiner VJ, Reddy CM. *Org Geochem* 2002;33;437.
6. Jendrzejewski N, Eggenkamp HGM, Coleman ML. *Appl Geochem* 2001;16;1021.
7. Reddy CM, Drenzek NJ, Eglinton TI, Heraty LJ, Sturchio NC, Shiner VJ. *Environ Sci Pollut R* 2002;9;183.
8. Holmstrand H, Gadomski D, Mandalakis M, Tysklind M, Irvine RL, Andersson P, Gustafsson Ö. *Environ Sci Technol* 2006;In press, available online ASAP.
9. Holmstrand H, Zencak Z, Mandalakis M, Andersson P, Gustafsson Ö. *Environ Sci Technol* 2006;submitted manuscript.
10. Gadomski D, Tysklind M, Irvine RL, Burns PC, Andersson R. *Environ Sci Technol* 2004;38;4956.
11. Holmstrand H, Mandalakis M, Zencak Z, Gustafsson Ö, Andersson P. *J Chromatogr A* 2006;1103;133.
12. Ferrario JB, Byrne CJ, Cleverly DH. *Environ Sci Technol* 2000;34;4524.
13. Gaus C, Papke O, Dennison N, Haynes D, Shaw GR, Connell DW, Muller JF. *Chemosphere* 2001;43;549.
14. Rappe C, Anderson R, Bonner M, Cooper K, Fiedler H, Howell F, Kulp SE, Lau C. *Chemosphere* 1997;34;1297.
15. Olsson M, Bignert A, Eckhell J, Jonsson P. *Ambio* 2000;29;195.