SCIENTIFIC REPORT

Chiral Xenobiotics in the Environment: Opportunities for Research Progress

An Exploratory Workshop

Funded by the Life, Earth and Environmental Sciences (LESC) Standing Committee of the European Science Foundation

Hosted by the University of Birmingham, UK

At the Apollo Hotel, Hagley Road, Birmingham

26th - 28th March, 2006

Co-Convenors:

Dr. Stuart Harrad (Chair)
Division of Environmental Health & Risk Management,
School of Geography, Earth and Environmental Sciences,
University of Birmingham,
Birmingham, UK

Dr. Kai Bester
Waste and Waste Water Management
Institute for Environmental Analysis
University Duisburg-Essen
Essen, Germany

Prof. Dr. Heinrich Hühnerfuss
Institute of Organic Chemistry
University of Hamburg
Hamburg, Germany

Prof. Dr. Walter Vetter
Universität Hohenheim
Institut für Lebensmittelchemie (170b)
Stuttgart, Germany

1. Executive Summary

The exploratory workshop was held over 2 days, bringing together 19 experts (17 from Europe, 2 from North America) in various aspects of the application of chirality to furthering understanding of the environmental fate, behaviour, and relevance of chemicals. It consisted of four sessions each of approximately 3 hours duration and consisting of between 2 and 4 oral presentations by those with particular expertise in that area, followed by an open discussion of issues raised led by one of the co-convenors or other expert. The open discussions were each around 80 minutes in duration.

The four sessions were centred on:

- Technical developments in enantioselective analysis and their implications for research progress
- Issues related to persistent organic pollutants (POPs)
- Issues related to current use biocides
- Issues related to personal care products and pharmaceuticals

Presentations at the workshop underlined the unique environmental forensic capacity of chirality. Specific examples were:

- Source apportionment – comparison of chiral signatures of POPs in various environmental compartments have offered unique insights into the relative significance of different sources to a specific compartment of a given POP. Exploitation of the chiral properties of such POPs has provided *direct* source apportionment evidence as opposed to the indirect methods previously available.
- Direct proof of biotransformation – chirality was shown to offer direct evidence of biodegradation and metabolism of chiral pollutants. Such knowledge has hitherto been only available on the basis of indirect and thus less conclusive evidence.

A key issue for the future development of enantioselective analysis was the establishment of QA/QC schemes such as interlaboratory trials and certified reference materials. It was agreed that a concerted effort would be made to explore such opportunities. Furthermore, the need for

establishing a coherent international collaborative framework of researchers active in research involving aspects of chirality was recognised. It was agreed to establish a searchable on-line database of research groups with facilities and expertise in relevant areas.

The overall conclusion of the workshop was that chirality offers real and wide-ranging opportunities to generate genuinely new and useful knowledge. For example, it was emphasised that by measuring both the exposure (based on enantioselective degradation) to the respective enantiomers and their toxicity one would obtain a more accurate risk assessment for environmental regulation of chiral compounds. The next step was promulgating this belief beyond the cognoscenti. It was agreed that participants would contribute to a multi-authored review paper promoting the utility of chirality as a research tool, and establishing a benchmark for the correct nomenclature and terminology within the field. While participants agreed to actively pursue opportunities for collaborative research in this area, they recognised that establishing links to the toxiocological impacts of pollutants was a key component of delivering societally relevant research likely to attract funding.

2. Scientific Content of the Event

A detailed programme is given in section 4 of this report, and copies of all oral presentations made are available for download at:
http://www.gees.bham.ac.uk/research/popsnetwork/Conferences/ESFworkshop/index.htm

This section will highlight key issues raised during the four sessions around which the workshop was focused.

Session on "Analytical Measurement Issues, Potential for Development"

This opening session was viewed as key to the future development of the research area, owing to the fundamental need for more powerful, accurate, and reproducible enantioselective separation techniques. A number of speakers addressed delegates with reports of the state-of-the-art with

respect to enantioselective analysis. The potential of GCxGC in enantioselective analysis was examined, with the conclusion that while research to date shows this technique to have high potential for enhancing the separation of enantiomers from other achiral potential coeluters, it is of limited value in resolving enantiomer pairs, based on the current state of instrumental development. The utility of multi-dimensional (heart-cut") GC in the enantioselective analysis of chiral PCBs was also examined. The fact that no one chiral stationary phase (CSP) is capable of resolving all of the 19 stable atropisomers of PCBs into enantiomers was highlighted. The ability of multi-dimensional GC to facilitate all of these enantiomer pairs was demonstrated (albeit via a very time-consuming series of 4 separate injections), in the context of a study revealing congener- and species-specific differences in enantioselective metabolism. Other presentations demonstrated: (a) the capacity of MS-MS when coupled with LC (and to a far lesser extent GC, although the advent of a new generation of comparatively low-cost benchtop triple quadrupole instruments may reverse the balance) to reduce coelutions of enantiomers with achiral interferences, and to offer opportunities for enhanced QA/QC checks on data quality (e.g. via multiple reaction monitoring); and (b) the fact that chiral phase LC-MS/MS has provided significant novel insights into the environmental fate, behaviour and significance of an important class of brominated flame retardants, *viz* the 16 stereoisomers of hexabromocyclododecane. For example, recent research has demonstrated enantioselective accumulation of α-HBCD in fish, along with species-specific variations in enantiomer fractions (EFs).

The session then moved into open discussion. Particular foci were:

- The relative merits of the terminology possible for expressing chiral signatures, viz: enantiomer fraction (EF), enantiomer ratio (ER), and enantiomer excess (ee). Although EF was acknowledged as being the easiest to deal with with respect to statistical analysis, it was agreed that each parameter had its own merits and disadvantages, and that the preferred parameter was dependent on the scenario in which it was to be applied.
- The definition of what should be considered as a racemic signature. It was agreed that this was a complex issue, and that a simple universally applicable definition was not possible. Instead, it was recognised that researchers should base their definitions on knowledge of the standard deviation obtained for replicate analysis of real racemic samples (e.g. an Aroclor PCB formulation) at realistic concentration levels.

- The need for a range of reference materials certified for chiral signatures of chiral pollutants. Participants agreed that there were none currently available, although it was recognised that a paper existed reporting EFs of selected chiral PCBs and organochlorine pesticides in a range of commercially available reference materials. It was agreed that the potential for the CEC's IRMM to develop such materials would be explored by the IRMM participants. Another approach was to work with colleagues from RIVO/IVM in the Netherlands to obtain funding to stage an interlaboratory comparison of chiral signatures of key pollutants in previously characterised commercially-available reference materials. Those analysed in the above-mentioned paper were suggested as a possibility (Wong et al, 2002. Chemosphere, 49, 1339-1347; erratum: Hoekstra et al., 2005. Chemosphere 60, 1667).
- Establishing a database (ideally on-line and searchable) of researchers active in a specific area (e.g. current use biocides), and of the availability of individual enantiomer reference standards, and columns with non-commercially available CSPs, together with their potential applications. Participants agreed that this would be useful.

Session on Chiral POPs

This session centred on illustrations of the utility of chiral signatures as a powerful environmental forensic tool. Specific examples were:

- Source apportionment – comparison of chiral signatures of POPs in air, soil, water, sediments, and grass have offered unique insights into the relative significance of different sources to a specific compartment of a given POP. Exploitation of the chiral properties of such POPs has provided *direct* source apportionment evidence as opposed to the indirect methods previously available.
- Direct proof of biotransformation – chirality was shown to offer direct evidence of biodegradation and metabolism of chiral pollutants. Such knowledge has hitherto been only available on the basis of indirect and thus less conclusive evidence. Of particular interest was the observed variability in the direction of enantioselective degradation of chiral pollutants in soils, along with small-scale spatial variability in chiral signatures in soils. Both sets of observations raise important questions as to the processes involved in edaphic enantioselective degradation of POPs.

- Insights into the formation pathways of methylsulfonyl PCBs. Enantioselective analysis of these PCB metabolites (which are more persistent than the parent compounds), reveals that they are formed through an enantioselective metabolic process most likely facilitated via a single enzyme displaying high stereoselectivity. Furthermore, it was shown that in mammals only one of the enantiomers of some methylsulfonyl-PCBs is found. This raises the question as to whether the specific formation of that enantiomer is due to either: (a) only one of the enantiomers of the parent PCB is metabolised while the other is either retained or excreted, or (b) both enantiomers of the parent PCB are metabolised to the methylsulfonyl derivative, with subsequent enantioselective excretion or metabolism of the non-observed chiral methylsulfonyl-PCB enantiomer.
- The behaviour in sediments and biota of the chiral toxaphene compound B6-923 was reported. This compound is highly stable compared to other components of commercial toxaphene mixtures, comprising 0.38% of technical toxaphene, but up to 70% of the toxaphene found in soils and sediments. While racemic signatures were observed in contaminated sediments, evidence of enantioselective degradation was seen in fish. When such contaminated fish were placed in “clean” environments, one observes fast elimination (6 weeks) of both enantiomers, but with a fair degree of enantioselectivity towards the 2nd eluting enantiomer which is eliminated within 4 weeks. This information permits calculation of enantioselective elimination kinetics and thus half-lives of B6-923. Extrapolation of this facilitates estimation of the time taken for remediaton of the contaminated lake to clear – a very useful tool.
- It was also suggested that as healthy and unhealthy animals have been observed to display different chiral signatures of some POPs, that such information could constitute a biomarker of disease or nutritional status.

The session then moved into open discussion, addressing the following key issues:

- The need to understand better the influence of other xenobiotics on the extent and direction of enantioselective degradation was recognised, as were the factors causing differences in chiral signatures in soils even over short distances. A related issue was the perceived lack of knowledge regarding differences in enantioselective behaviour between aerobic and

anaerobic environments. Laboratory microcosm studies were suggested as one approach via which understanding of such issues could be enhanced.

- The role of temperature was discussed, and it was pointed out that it played an important role: at low temperatures, degradation/metabolism was slow but highly enantioselective; at high temperatures, it was faster, but less enantioselective. The possibility of researching the potential impact of climate change on pollutant behaviour was discussed, and a collaborative global soil and water monitoring study was mooted.
- It was agreed that there was a need to study possible enantioselective toxicity.

Session on Chiral Current Use Biocides

It was pointed out that around 30% of pesticides are chiral, and that their mode of action and environmental fate is frequently highly enantioselective. Knowledge of the enantioselectivity of such products is of great commercial interest since if only one enantiomer is active, then application of an enantiopure formulation can offer significant cost as well as environmental benefits. However, the extent and direction of enantioselective degradation is not always possible to predict. As an illustration, a study of the degradation of the halogenated acid BCAA in water from six rivers was discussed. While enantioselective degradation was observed at all locations, its direction and extent was variable, suggesting that there are different microbial communities working at different rates and different times. Other possible explanations for variable chiral signatures were cited as: enantioselective sorption to chiral components of humic materials, or even mineral surfaces. In this field, enantioselective differences in effects have the potential to be widely studied using metabolomics. This offers several benefits, including: (a) enhanced ability to determine the toxic mode of action; and (b) changes in endogenous metabolites often reflect toxicity. An important point was made that the effects of the racemate are not always a simple sum of the effects of the individual enantiomers; this suggests the existence of synergistic/antagonistic effects.

A focus on fungicides and herbicides followed. Here, the proportion of commercially available chemicals that are chiral is even higher, at 50%. A study of the enantioselective kinetics in soil of the fungicide metalaxyl, and the herbicides dichlorprop and mecoprop) was reported. Differences in the degradation rate of each enantiomer were observed not only between soils but also within

the same soil. It was found that there was a strong correlation between soil pH and the extent of enantioselective (but not the overall rate of) degradation. If such observations are applicable to other chiral pollutants, then the observed spatial differences in chiral signatures in soils may be at least partly attributable to variations in soil pH.

Open discussion for this session highlighted the need to be aware of the possibility of chiral metabolites arising from non-chiral parents. A good example of such a *prochiral* compound is lindane (γ-HCH). Also, while the need for controlled studies of microbially-mediated enantioselective transformation was strongly acknowledged, caution was urged when interpreting such experiments when conducted using single microbial cultures, given "real-world" microbial diversity. The fact that enantiomerisation (i.e. the conversion of one enantiomer to the other) occurs in the environment was also noted. A very important point was made that a key focus of future research activity should be studies of enantioselective toxicity, and the need to interface with toxicologists and molecular biologists was emphasised. There was also a continuation of discussions from previous sessions that related to the correct use of terminology.

Session on Personal Care Products and Pharmaceuticals

The first presentation in this session addressed the utility of chirality to trace the environmental fate of synthetic musk compounds (specifically HHCB and AHTN) within waste water treatment plants (wwtps). This is of great societal relevance, as these personal care fragrances are produced and used in huge quantities (currently 8,000 t yr^{-1}). As shown elsewhere for other chiral compounds, enantioselective studies permit the gathering of *direct* evidence of the relative significance and rate of various processes occurring within a wwtp. Eaxmples of such processes include: (a) sorption/desorption to sludge; (b) microbial oxidation; (c) abiotic oxidation; and (d) microbial reduction. This is a significant advance on the indirect evidence gathered by the only other feasible method, viz a complex mass balance. As pointed out in the opening session of the workshop, the benefits in terms of enhanced data quality of 2-dimensional GC-MS as opposed to single column GC-MS were underlined.

The second presentation dealt with the environmental fate of chiral pharmaceutical products. The relevance of this issue given the inexorable ageing of the population, was underlined. It is known that enzymatic transformation results in an excess of the 2*S* enantiomer of ibuprofen in humans, however, this enantioselectivity is switched to an excess of the 2*R* enantiomer in wwtps in Hamburg, Germany but *not* in Tromsø, Norway. Similarly puzzling switches in chiral signatures are observed in rivers 2 km downstream of Hamburg STPs. Similar observations are also evident for the hydroxy metabolite, ibuprofen-OH. Evidence was presented that the species-specific variation in chiral signatures reported previously for other chiral pollutants, was also observed for personal care products such as HHCB and AHTN.

It was stressed and agreed by participants that effective risk assessment of a given chemical must take into account the transformation pathways and the impacts of the transformation products as well as the parent compound. In this respect, enantioselective studies offer potentially very useful and novel insights. The point was made that when combined with mass balance studies, enantioselective information on the fate of chiral pollutants in wwtps could advance our understanding of the processes involved greatly, by e.g. permitting calculation of degradation half-lives and factors influencing these. Such enhanced knowledge had great potential for improving the design and performance of these facilities.

In open discussion, the need for agreement on common quantification criteria (e.g. acceptable signal:noise ratio) for reporting chiral signatures was noted. Further, it was suggested that the potential of LC-MS/MS using CSPs to resolve enantiomers could be better exploited in future.

The workshop closed with a summary of key points raised, and agreed actions. These are outlined in section 3.

3. Assessment of the results, contribution to the future direction of the field

At this early stage it is difficult to evaluate the likely impact of the workshop. This is despite the fact that all attendees were agreed that it provided an invaluable forum for discussion between key players in the field of important issues that require to be addressed if the full potential of chiral techniques is to be exploited.

Despite this, it is possible to identify areas of development that will potentially arise as a result of the workshop. Specifically, these are:

- Establishment of interlaboratory comparisons of measurements of chiral signatures. Related to this is the development of reference materials for which chiral signatures are certified.
- Production of a multi-authored review paper that will clearly define correct usage of terms relevant to chirality and enantioselective analysis, while promoting the unique environmental forensic capacity of chirality.
- Establishment of a database (ideally searchable on-line) containing details of research groups active in enantioselective research, including details of the availability of individual enantiomer reference standards, and non-commercially available CSPs.
- Development of collaborative research proposals covering various aspects of enantioselectivity and its environmental significance. It was recognised that the building of closer links between members of the “chiral” community and its extension were necessary if the field was to be advanced, and participants were urged to consider contributing research presentations on enantioselective topics to various international conferences. The special session on Chiral Xenobiotics to be held at the Dioxin 2006 symposium in Oslo between 20th and 25th August was highlighted.

4. Final Programme

Monday 27th March

9.00 – Welcome and introductions –Stuart Harrad (Overall Workshop Chair)

9.15 – Session on Analytical Measurement Issues, Potential for Development (Session Chair: Heinrich Hühnerfuss; Rapporteur: Stefan Voorspoels); Aims of Session

9.20 – Application of GCxGC to Enantioselective Studies – Peter Korytar

9.40 – Enantioselective separation of chiral PCBs by multidimensional gas chromatography techniques. Application to real samples - Maria José Gonzalez

10.00 – The Utility of GC-MS/MS in Enantioselective Studies –Walter Vetter, University of Hohenheim

10.20 – The Application of Chiral LC to the study of HBCDs in the Environment –Adrian Covaci, University of Antwerp

10.40 – Coffee

11.00 – Discussion of Issues, including QA/QC issues – establishing interlaboratory comparisons and agreed definition of what is racemic. (led by Heinrich Hühnerfuss)

12.30 Lunch

14.00 – Session on Chirality and POPs (Session Chair: Terry Bidleman; Rapporteur: Martin Preston); Aims of Session

14.05 – Chiral Xenobiotics as Tracers of Biogeochemical Processes –Terry Bidleman, Meteorological Service of Canada

14.40 – Chiral PCB methyl sulfone metabolites - an overview –Ake Bergman, Stockholm University

15.00 – The Behaviour of the Toxaphene Component B6-923 in Sediment and Biota –Walter Vetter, University of Hohenheim

15.20 – Tea

15.40 – Discussion of Issues (led by Terry Bidleman)

17.00 – Close

Tuesday 27th March

9.00 – Session on Current Use Biocides (Chair Walter Vetter; Rapporteur: Luisa Ramos-Bordajandi); Aims of Session

9.05 – Enantiomer-Specific Fate and Effects of Modern Chiral Pesticides - Wayne Garrison, National Exposure Research Laboratory, USEPA

9.55 - Enantioselective Degradation of Fungicides in Soils: Chiral Preference Changes with Soil pH –Ignaz Bürge, Swiss Federal Research Station

10.20 – Coffee

10.40 – Discussion of Issues (led by Walter Vetter)

12.00 – Lunch

13.00 – Session on Personal Care Products and Pharmaceuticals (Chair Kai Bester, University of Duisberg-Essen; Rapporteur: Karin Wiberg); Aims of Session

13.05 – Chirality as a Novel Tool for Understanding fate of Synthetic Musks in Sewage Treatment Plants –Kai Bester

13.30 – Chirality as Applied to Studies of Pharmaceuticals and Personal Care Products –Heinrich Hühnerfuss, University of Hamburg

14.20 – Discussion of Session Issues (led by Kai Bester) – includes tea @ 15.00

15.40 – Workshop Summary – Actions to be Taken Forward (Stuart Harrad)

16.00 – Close

5. Statistical Information on Participants

#	Name	Country	Sex	Young?
1	Stuart Harrad	UK	M	
2	Kai Bester	Germany	M	
3	Heinrich Hühnerfuss	Germany	M	
4	Walter Vetter	Germany	M	
5	Martin Preston	UK	M	
6	Stefan Voorspoels	Belgium	M	Yes
7	Volker Schurig	Germany	M	
8	Hai Pham Tuan	Germany	M	Yes
9	Adrian Covaci	Belgium	M	Yes
10	Lubomir Karasek	Czech Republic/ Belgium	M	Yes
11	Maria José Gonzalez	Spain	F	
12	Luisa Ramos-Bordajandi	Spain/ Belgium	F	Yes
13	Ignaz Buerge	Switzerland	M	Yes
14	Peter Korytar	The Netherlands/Slovakia	M	Yes
15	Ake Bergman	Sweden	M	
16	Karin Wiberg	Sweden	F	Yes
17	Terry Bidleman	Canada	M	
18	Wayne Garrison	USA	M	
19	René Juhler	Denmark	M	

6. Final List of Participants

#	Title/ Name	Contact Address	Tel.	Fax.	Email
1	Dr. Stuart Harrad	Division of Environmental Health & Risk Management, Public Health Building, School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK	+44 121 414 7298	+44 121 414 3078	S.J.Harrad@bham.ac.uk
2	Dr. Kai Bester	Institute for Environmental Analytical Chemistry, University Duisburg-Essen, Universitätsstr. 15 45141 Essen, Germany	+49 201 183 2764	(49) 201 183 3465	kai.bester@uni-essen.de
3	Prof. Heinrich Hühnerfuss	Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Pl 6, D20146 Hamburg, Germany	+49 40 42838 4240	+49 40 42838 2893	huehnerfuss@chemie.uni-hamburg.de
4	Prof. Walter Vetter	Universität Hohenheim, Institut für Lebensmittelchemie (170b), Garbenstr. 28, D-70599 Stuttgart, Germany	+49 711 459 4016	+49 711 459 4377	w-vetter@uni-hohenheim.de
5	Dr. Martin Preston	Department of Earth and Ocean Sciences, University of Liverpool, 4 Brownlow Street, Liverpool L69 3GP, UK	+44 151 794 4093	+44 151 794 5196	preston@liverpool.ac.uk
6	Mr. Stefan Voorspoels	Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium	+32 3 820 27 04	+32 3 820 27 22	stefan.voorspoels@ua.ac.be
7	Prof. Volker Schurig	Institut Organische Chemie Auf der Morgenstelle 18 D-72076 Tübingen, Germany	+49 7071 2976257	+49 7071 295538	volker.schurig@uni-tuebingen.de
8	Dr. Hai Pham Tuan	University of Hamburg, Institute of Organic Chemistry, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany	+49 40-42838-3159	+49 40-42838-2893	hai.pham-tuan@chemie.uni-hamburg.de
9	Dr. Adrian Covaci	Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium	+32-3-820 2704	+32-3-820 2722	Adrian.Covaci@ua.ac.be

10	Dr. Lubomir Karasek	European Commission Institute for Reference Materials and Measurements Directorate General Joint Research Centre (EC – JRC – IRMM), Food Safety and Quality Unit Retieseweg 111, B-2440 Geel, Belgium	+32 14 571 30	+32 14 571 343	Lubomir.KARASEK@cec.eu.int
11	Prof. Maria José Gonzalez	Institute of Organic Chemistry (CSIC), Department of Instrumental Analysis and Environmental Chemistry, Juan de la Cierva 3, 28006-Madrid, Spain	+34 91 562 2900	+34 91 564 4853	mariche@iqog.csic.es
12	Dr. Luisa Ramos-Bordajandi	European Commission Institute for Reference Materials and Measurements Directorate General Joint Research Centre (EC – JRC – IRMM), Food Safety and Quality Unit Retieseweg 111, B-2440 Geel, Belgium	+32-14-571 971	+32-14-571 548	Luisa.RAMOS-BORDAJANDI@cec.eu.int
13	Dr. Ignaz Buerge	Swiss Federal Research Station (agroscope), Plant Protection Chemistry, PO Box 185, CH-8820 Wädenswil, Switzerland	+41 44 783 63 83	+41 44 780 63 41	Ignaz.Buerge@faw.admin.ch
14	Dr. Peter Korytar	Netherlands Institute for Fisheries Research, P.O. Box 68, 1970 AB IJmuiden, The Netherlands	+31-255-564607	+31-255-564644	Peter.Korytar@wur.nl
15	Prof. Ake Bergman	Department of Environmental Chemistry, Stockholm University, SE-106 91, Stockholm, Sweden	+46-8-163997	+46-8-163979	Ake.Bergman@mk.su.se
16	Dr. Karin Wiberg	Department of Chemistry, Environmental Chemistry, Umeå University, SE-901 87 Umeå, Sweden	+46-90-786 5672	+46-90-128133	Karin.Wiberg@chem.umu.se
17	Prof. Terry Bidleman	Meteorological Service of Canada, Air Quality Research Branch, 4905 Dufferin Street, Toronto, M3H 5T4, Canada	+1 705-458-332	+1 705-458-3301	Terry.Bidleman@ec.gc.ca

18	Dr. Wayne Garrison	U.S.EPA National Exposure Research Laboratory, Ecosystems Research Division, 960 College Station Rd., Athens, GA 30605, USA	+1 706 355 8219	+1 706 355 8202	garrison.wayne@epamail.epa.gov
19	Dr. René Juhler	Geological survey of Denmark and Greenland, Oester Voldgade 10, 1350 Copenhagen K, Denmark	+45 3814 2000	+45 3814 2050	RKJ@GEUS.DK