Journal of Chromatography B, 878 (2010) 1277–1284

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

Chirality of organophosphorus pesticides: Analysis and toxicity☆

Mae Grace Nillos[a,b], Jay Gan[a,*], Daniel Schlenk[a]

[a] Department of Environment Sciences, University of California, Riverside, CA 92521, USA
[b] Department of Chemistry, University of the Philippines, Miag-ao, Iloilo 5023, Philippines

ARTICLE INFO

Article history:
Received 28 August 2009
Accepted 12 November 2009
Available online 18 November 2009

Keywords:
Chiral analysis
Enantioselective analysis
Stereoisomers
Enantiomers
Organophosphate insecticides
Organophosphorus compounds (OPs)

ABSTRACT

Although the importance of chirality in organophosphorus compounds (OPs) is well recognized in relation to their biological effects, as with most chiral pesticides, OPs are generally marketed, used and released to the environment as racemates (i.e., equimolar mixtures of enantiomers). In addition, research on enantioselective environmental fate and effects of chiral OPs is still limited, particularly in the evaluation of enantioselectivity in their environmental degradation. A large number of OPs are chiral compounds, and yet enantioselectivity in their environmental fate and effects is rarely addressed. This paper highlights the current state of knowledge on the environmental occurrence and behavior of chiral OP pesticides. Developments in enantioselective analytical techniques, specifically gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), as applied in the evaluation of enantiomer-specific fate and effects of chiral OPs, are also discussed.

Contents

1. Introduction

Scientists have long recognized the significance of chirality in the biological activity of a chemical agent. Until recently, the overwhelming emphasis has been on chiral drugs [1–3]. However, a significant proportion of current-use pesticides have active ingredients with chiral structures. A vast majority of which continues to be manufactured, used and hence released into the environment as mixtures of stereoisomers [4–8]. Studies into the stereoselective behavior of chiral pesticides only started in the early 1990s with most research efforts focused on understanding the environmental fate and effects of legacy chiral pesticides [6,7,9,10]. Results from such studies have shown that enantiomers of the same chiral compound can degrade at significantly different rates [6,11–19] and have very different toxicological characteristics in the environment [6,18,20–25].

Organophosphorus (with carbamate) pesticides are among the most important chemicals used for protection against agricultural and household pests [26]. Despite their selective phase-out, which began in the early 2000 in the U.S., organophosphorus insecticides remain among the most widely used pesticides [26–28]. It is estimated that OPs are worth nearly 40% of the global market and that they are expected to maintain dominance for some time into the future [29].

Chiral OPs can have a stereogenic center on the pentavalent phosphorus, a carbon, or a sulfur substituent [6,27,30] (Fig. 1).

☆ This paper is part of the special issue 'Bioanalysis of Organophosphorus Toxicants and Corresponding Antidotes', Harald John and Horst Thiermann (Guest Editors).

* Corresponding author. Tel.: +1 951 827 2712; fax: +1 951 827 3993.
E-mail address: jgan@ucr.edu (J. Gan).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.11.022

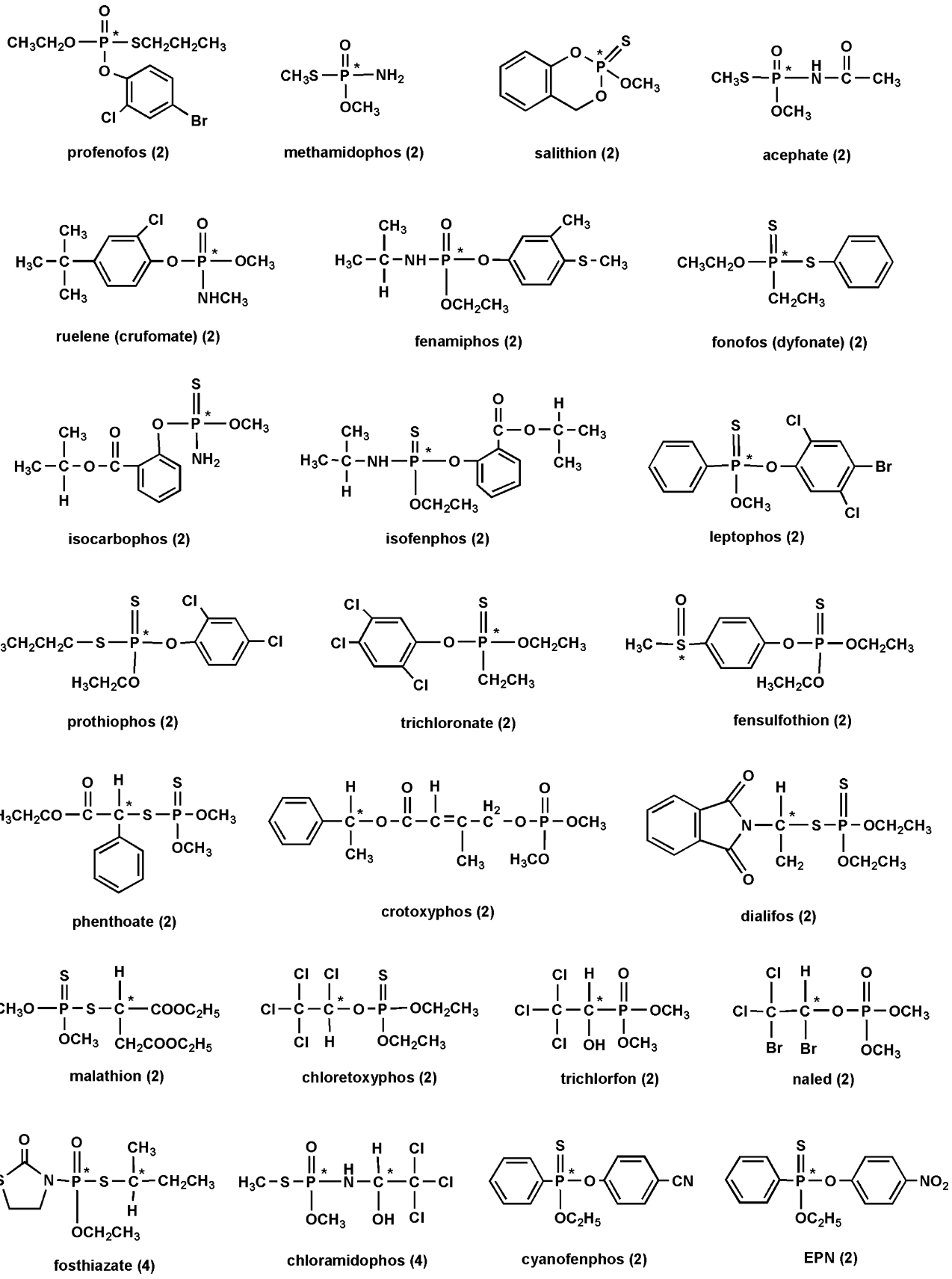

Fig. 1. Examples of chiral organophosphorus pesticides (* denotes chiral center; number of stereoisomer in each compound is indicated in parenthesis).

Table 1
Enantioselective gas chromatography (GC) analysis of chiral organophosphorus pesticides in literature.

Organphosphorus compound	CSP column	Detection	Comment	References
Chloretoxyphos	Chirasil-Dex	FID	Partial separation of enantiomers	[32]
HDCP[a]	Chirasil-Val	NPD	Satisfactory separation of enantiomers but low sensitivity	[50]
Isofenphos	Chirasil-Dex	FID	Partial separation of enantiomers	[32]
Naled	Chirasil-Dex	FID	Partial separation of enantiomers	[32]
Methamidophos[b]	Chirasil-Dex	FID	Partial separation of enantiomers	[32]
Ruelene[b]	Chirasil-Dex	FID/ECD ICP-MS	Complete separation of enantiomers; improved sensitivity with ICP-MS	[32,49]
Trichlorfon[b]	Chirasil-Dex	FID/ECD	Complete separation of enantiomers	[32]

[a] O-hexyl O-2,5-dichlorophenylphosphoramidate.
[b] Enantiomer separation attempted with Chirasil-Val, but none resolved under the used conditions.

Although the importance of chirality in OP compounds is well recognized in relation to their chemistry, biochemistry, biology and toxicology, as with most chiral pesticides, OPs are generally marketed and used as racemates (i.e., equimolar mixture of enantiomers) [5,6,27,31,32]. Enantioselectivity in degradation has been reported for several chiral OPs [3,17,30,39]. However, despite the significant increase in interest on chiral OP compounds in recent years, particularly in relation to their enantioselective toxicity to aquatic organisms, research on enantioselective environmental fate and effects of chiral OPs is in general still rather inadequate [18,23,33–39].

In order to address the need for enantiomer-specific toxicological information for chiral pesticides, several factors have to be considered, foremost of which is the availability of suitable analytical techniques and appropriate assay methods [40]. The separation and quantification of enantiomers is probably the biggest challenge in conducting toxicity assessment and analysis of chiral chemicals [41]. Enantiomer separation can only be achieved in chiral environments, mostly on chiral chromatographic columns containing chiral derivatives [10]. Moreover, the difficulty in chiral analysis is further complicated by the lack of enantiopure standards that are not easy to synthesize and purify. In addition, for compounds with labile chiral centers, enantiomers may undergo stereochemical inversion, which further complicates their analysis [42–45].

The past 30 years saw significant advances in chiral separation and synthesis techniques allowing for the chromatographic resolution and isolation of enantiomers, which in turn allowed for enantiomer-specific evaluation of an increasing number of chiral pesticides [2,6,7,10,40,46]. This paper highlights the current state of knowledge on the environmental occurrence and behavior of chiral OPs (Fig. 1). Developments in enantioselective analytical techniques, specifically gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) as applied in the evaluation of enantiomer-specific fate and effects of chiral OPs are also discussed.

2. Analysis of chiral OPs

Significant developments in enantiomeric separation techniques in recent decades helped facilitate the routine separation of enantiomers of environmentally important chiral pesticides for use in environmental assessment [6,7]. Of these, HPLC and GC, in conjunction with a suitable chiral-selective column are the most commonly used instrumentation in the analysis of chiral pesticides [10]. Chiral HPLC is ideal for preparative work and for laboratory experiments, while GC is more practical for analysis of environmental samples. In addition, both instruments can be coupled with mass spectrometry detectors for more sensitive quantitation and confirmatory detection of analytes. Capillary electrophoresis (CE) has also been explored and has shown a great potential for enantiomer resolution due to its high separation efficiency, versatility and low consumption of chiral selectors [9,10,17,47,48].

Separations of enantiomers of a number of chiral OPs by GC [32,49,50], HPLC [23,30,33,34,36–39,51–53] and CE [9,17,48,54,55] have been reported. These studies are summarized in Tables 1–3, and are discussed below.

2.1. Enantioselective GC analysis

Gas chromatography is primarily used in the analysis of volatile and thermally stable compounds. Chiral GC has the advantage of high efficiency, sensitivity and reproducibility. In addition, auxiliary systems, such as mass spectrometer (MS) and electron capture detector (ECD) can be coupled with chiral GC for the analysis of enantiomers in complicated matrices including environmental, biological and agricultural samples [18,19,56].

Table 2
Enantioselective high-performance liquid chromatography (HPLC) analysis of chiral organophosphorus pesticides in literature.

Organophosphorus pesticide	Number of stereoisomers	CSP column	References
Acephate	2	Chiralcel® OD	[51]
Crufomate (Ruelene)	2	Chiralpak® AD; Chiralcel® OD	[30]
Fenamiphos	2	Chiralpak® AD; Chiralpak® AS	[30,39]
Fonofos (Dyfonate)	2	Chiralcel® OJ	[23,30]
Isocarbophos	2	Chiralcel® OD	[37]
Isofenphos	2	Chiralcel® OD	[30]
Leptophos	2	(R,R)-Whelk-O1; (S,S)-Whelk-O1	[38]
Methamidophos	2	Chiralcel® OD	[30,34,51]
Phenthoate	2	Chiralcel® OD	[52,53]
Profenofos	2	Chiralcel® OJ	[23,30]
Prothiophos	2	Chiralcel® OJ	[30]
Trichloronate	2	Chiralcel® OD; Chiralcel® OJ	[30,34]
Crotoxyphos	2	Chiralcel® OJ	[23,30]
Dialifos	2	Chiralcel® OJ	[30]
Malathion	2	Chiralcel® OJ	[30]
Chloramidophos	4	Chiralpak® AD	[36]
Fosthiazate	4	Chiralpak® AD	[35]
Fensulfothion	2	Chiralpak® AD	[30]

Table 3
Enantioselective capillary electrophoresis (CE) analysis of chiral organophosphorus pesticides in literature.

Organphosphorus compound	Chiral selector	Buffer composition	Comment	References
Ruelene (Crufomate)	HP-β-CD (60 mM)	20% methanol, 100 mM SDS in 20 mM borate buffer (pH 9)	Adequate separation of enantiomers	[54]
	CM-β-CD (15 mM) + HP-β-CD (40 mM)	70 mM SDS in 20 mM borate buffer (pH 8.6)	Complete separation of enantiomers	[9]
	HP-β-CD (40 mM)	20% acetonitrile, 100 mM SDS in 20 mM borate buffer (pH 8.5)	Complete separation of enantiomers	[17]
Malathion	HP-β-CD (60 mM)	100 mM SDS in 20 mM borate buffer (pH 9)	Adequate separation of enantiomers	[54]
	CM-β-CD (50 mM) + HP-β-CD (50 mM)	10% methanol, 10 mM SDS in 20 mM borate buffer (pH 8.6)	Complete separation of enantiomers	[9]
	CM-β-CD (20 mM)	25 mM Tris buffer (pH 7)	Complete separation of enantiomers	[48]
Isomalathion	CM-β-CD (20 mM)	25 mM Tris Buffer (pH 7)	Separation of 3 of 4 enantiomers	[48]
Fensulfothion	CM-β-CD (12.5 mM) + HP-β-CD (45 mM)	70 mM SDS in 20 mM borate buffer (pH 8.6)	Complete separation of enantiomers	[9]
Phenthoate	CM-β-CD (20 mM)	25 mM Tris buffer (pH 7)	Complete separation of enantiomers	[48]
Phenamiphos	CM-β-CD (20 mM)	25 mM Tris buffer (pH 7)	Adequate separation of enantiomers	[48]
Fonofos	γ-CD (25 mM)	15% acetonitrile, 100 mM SDS in 20 mM borate buffer (pH 8.5)	Complete separation of enantiomers	[17]
Dialifos	HP-β-CD (60 mM)	10% methanol, 100 mM SDS in 20 mM borate buffer (pH 9)	Complete separation of enantiomers	[54]
Naled	CM-β-CD (10 mM)	25 mM borate buffer (pH 9)	Very reactive in aqueous solution	[48]
Pyraclofos	SC (100 mM)	50 mM SDS in methanol/acetonitrile (4:1, v/v)	Complete separation of enantiomers	[55]
Prothiofos	SC (75 mM) and γ-CD (20 mM)	Methanol/water/acetonitrile (5:4:1, v/v/v)	Possible adsorption on inner wall of capillary	[55]
Sulprofos	SC (50 mM) and γ-CD (10 mM)	Methanol/water/acetonitrile (5:4:1, v/v/v)	Possible adsorption on inner wall of capillary	[55]
Profenofos	SC (50 mM) and γ-CD (10 mM)	Methanol/water/acetonitrile (5:4:1, v/v/v)	Possible adsorption on inner wall of capillary	[55]

Methods for the enantioselective GC separation of a number of OPs were recently optimized using commercially available chiral columns including CP-Chirasil-Dex CB (heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin) and Chirasil-Val (L-valine-*tert*-butylamide) [32,49,50] (Table 1). Fidalgo-Used et al. [32] investigated the separation by GC with flame ionisation detection (FID) of the enantiomers of 13 OP pesticides, including seven compounds with a C chiral center (i.e., trichlorfon, malathion, malaoxon, naled, chlorethoxyphos, dialifos and phenoate), and six compounds with a P chiral center (i.e., methamidophos, isofenphos, ruelene, fenamiphos, leptophos and pyraclophos). No resolution was achieved on Chirasil-Val column. In contrast, baseline separation for the enantiomers of ruelene and trichlorfon, and partial separation for enantiomers of naled, chlorethoxyphos, methamidophos and isophenphos were achieved using Chirasil-Dex column (Table 1). In the case of the OPs with a C chiral center, the authors attributed the successful enantiomeric resolution of trichlorfon, naled and chloretoxyphos to the presence of chlorine or bromine substituents at the carbon atom bound to the asymmetric carbon in their structures as having some effect on the subtle stereoselective interactions with the β-cyclodextrin column [32]. Similarly, in the chiral OPs with a P chiral center, the presence of a chlorine atom in the phenyl ring together with an amide group attached to the phosphorus chiral center was thought to contribute to the adequate resolution for the enantiomers of ruelene [32]. Thus, the formation of an inclusion complex with a β-cyclodextrin chiral stationary phase (CSP) does not appear to be sufficient for achieving adequate separation of enantiomers of chiral OPs [32,50].

A solid-phase microextraction (SPME)-enantioselective GC procedure has also been developed and evaluated using two different detection systems: a classical electron capture detection (ECD) and a P-specific detection with inductively coupled plasma mass spectrometry (ICP-MS), resulting in enhanced separation and detection of enantiomers of ruelene in various spiked samples [50].

Studies on the enantiomeric separation of chiral OPs through GC are in general very limited despite the perceived advantage of high efficiency and sensitivity [32]. To date the GC resolution of chiral pesticides, especially OP compounds, is contingent with the selection of a suitable enantioselective stationary phase [10,32]. The mechanisms of enantiomeric resolution may involve a combination of multiple (and complex) associations between the analyte and CSP [32]. For compounds with high polarity or low vapor pressure, or heat-labile compounds, including many OPs, the need for derivatization can further complicate enantiomeric analysis by GC [9,47].

2.2. Enantioselective HPLC analysis

Compared to the other methods, significantly more studies have been devoted to developing and applying HPLC methods for the separation and analysis of stereoisomers of chiral pesticides. The advantage of using enantioselective HPLC methods is that individual enantiomers could be recovered following the separation, and subsequently used in toxicological experiments that require separated stereoisomers. In addition, due to the strongly polar nature and low volatility of most OPs, enantiomeric resolution of chiral OPs is often best achieved by enantioselective HPLC [57–59].

Chiral OPs have so far been successfully resolved using HPLC equipped with polysaccharide CSP columns [23,30,33,34,36–39,51–53] (Table 2). The mechanisms of chiral recognition on HPLC CSP columns are extensively reviewed in Huhnerfuss and Shah [10] and Ali et al. [59].

Ellington et al. [30] described the enantiomeric separation of 12 OPs, including eight compounds with a P chiral center, i.e., isofenphos, methamidophos, profenofos, crufomate (ruelene), pro-

Table 4
Enantioselective insecticidal toxicity of some chiral organophosphorus pesticides.

Organophosphorus pesticide	Active enantiomer[a]	Comment	References
Acephate	*R*-		[5]
EPN	*R*-	Similar selectivity for oxon	[5,31]
Cyanofenphos	*R*-	Similar selectivity for oxon; interspecies difference in magnitude of selectivity	[5]
Fonofos (Dyfonate)	*R*-	Opposite selectivity for oxon; active enantiomer: *S*-	[64,65]
Profenofos	*R*-	Opposite selectivity of acetylcholinesterase inhibition *in vitro*; active enantiomer: *S*-	[5,67]
Isofenphos	(+)-	Similar selectivity for oxon	[5,31]
Methamidophos	*R*-		[5]
Salithion	*S*-	Similar selectivity for oxon	[5,31]
Malathion	*R*-(+)-	Similar selectivity for oxon; results based on diethyl derivatives of malathion and malaoxon	[3,68]
Phenthoate	(+)	Reported interspecies difference in selectivity	[31,52]

[a] Based on LD_{50} values on target insects unless indicated otherwise; if information is available, active enantiomer is identified based on its absolute configuration.

thiophos, fonofos, fenamiphos and trichloronate, three compounds with a C chiral center, i.e., crotoxyphos, dialifos and malathion, and one compound with a S chiral center, i.e., fensulfothion, on commercially available polysaccharide enantioselective HPLC columns using alkane–alcohol mobile phases. Reversed-phase enantioselective HPLC on a Pirkle-type stationary phase has also been evaluated for the separation of a series of 14 O-ethyl, O-phenyl, and N-isopropyl phosphoroamidothioate enantiomers containing a phosphorus atom as the chiral center [57].

However, due to the inherent limited resolving power of the conventional HPLC techniques, optimization of the enantiomer resolution generally involves complex procedures or numerous experiments leading to the consumption of large amounts of solvents and samples [9,47]. Nevertheless, at present enantioselective HPLC is still the preferred method for determination of ionic, polar, or heat-labile chiral pesticides, particularly chiral OPs [7,9,47].

Enantiomeric HPLC separation has been successfully applied in the recovery of individual enantiomers of various chiral OPs for the purpose of subsequent toxicological evaluation [18,23,33–39,60]. In addition, enantioselective HPLC analysis has also been used in the environmental degradation study of chiral OPs [52,53].

2.3. Enantioselective CE analysis

As with enantioselective GC and HPLC, enantiomeric separation by CE requires a chiral-selective agent (e.g., cyclodextrins), commonly as additive to the background electrolyte [47,48]. Next to enantioselective HPLC, chiral OPs are generally separated by CE techniques (Table 3) [7]. For instance, the enantiomers of malathion, ruelene and dialifos were adequately separated by hydroxypropyl-β-cyclodextrin (HP-β-CD), β-cyclodextrin (β-CD) and/or γ-cyclodextrin (γ-CD) through CE in micellar electrokinetic chromatography (MEKC) mode [54]. The enantiomeric resolution of malathion and ruelene was improved through dual-cyclodextrin electrokinetic capillary chromatography (dual-CECC) and micellar electrokinetic capillary chromatography (MECC), respectively [9]. Dual-CECC uses combined native and charged cyclodextrins, while in MECC a mixture of surfactants and cyclodextrin (with or without the addition of an organic modifier) is employed [9]. Other chiral OPs that have been resolved by enantioselective CE include fonofos [17], fensulfothion [9], phenthoate [48], pyraclofos, prothiofos, sulprofos and profenofos [55].

Ali et al. [47] provides an extensive review on the application of CE to enantioselective determination of chiral environmental pollutants, from electrophoresis conditions to chiral recognition mechanisms, including parameters that can be varied to optimize enantiomer resolution.

At present, enantioselective CE is not yet applicable for routine resolution of chiral pesticides. Reproducibility of results is a major problem in CE analysis [47]. Other limitations in the application of CE in the analysis of chiral pesticides include the consumption of the chiral selector as it is used in the background electrolyte. Another major disadvantage is that unlike HPLC, it cannot be coupled with chiroptical detectors (e.g., polarimeter and circular dichroism) due to the presence of the chiral selector in the background electrolyte [47].

Overall, enantioselective HPLC is still the best choice for the chiral resolution of chiral OPs, mainly due to its versatility, ease of operation, selectivity, efficiency and reproducibility [59].

3. Importance of chirality in OP insecticide toxicity

As potent acetylcholinesterase (AChE) inhibitors, OPs are expected to have the same mode of action in target and non-target organisms [26,31,61]. They are highly toxic to invertebrates and mammals but are generally less persistent in the environment than the organochlorine pesticides that preceded them [26]. Currently, OPs pose a risk to non-target organisms and ecological systems mainly through their acute toxicities. Although mostly marketed and used as racemic mixtures, chirality can be found in a significant number of OPs having a center of asymmetry on P, C, or S [5–7,34]. In addition, certain OPs such as those possessing thioether structures (e.g., fenthion) may undergo asymmetric induction resulting in the formation of a chiral metabolite [62]. For an extensive discussion on the stereochemistry of OP agrochemicals, the reader is referred to Sasaki [5].

3.1. Enantioselective insecticidal activity

The significance of stereochemistry in the mode of action of chiral OPs is evident in the stereospecificity of their insecticidal activity [5,31]. Table 4 summarizes the enantioselective insecticidal activity of a number of chiral OP compounds.

Organophosphorus compounds like fonofos and malathion which contains a P=S moiety requires metabolic activation by oxidative desulfuration to the P=O (oxon) form for enzyme inhibition to take place [63,64] (Fig. 1). *R*-(−)-Fonofos was reported to have higher biological activity *in vivo* than the (*S*)-(+)-fonofos to housefly, mosquito fly and mice [5]. In contrast, direct interaction of fonofos–oxon enantiomers with AChE indicated the *S*-oxon as 2.6–12.2 times more toxic than the corresponding *R*-oxon to the same species [65]. The opposite selectivities between fonofos and its oxon form were explained through the *in vitro* metabolic activation study of fonofos in rat liver microsomes where *R*-fonofos was observed to predominantly convert to the *S*-oxon [65].

Similarly, Leader and Casida [66] observed that the (+)-profenofos was 2–48 times more potent than the (−)-profenofos as an *in vitro* inhibitor of bovine erythrocyte and housefly head AChE, even though the (−)-enantiomer was 4–23 times more toxic than the (+)-enantiomer to adult housefly and mice *in vivo*. This is due to the preferential sulfoxidation of (−)-profenofos *in vivo*, resulting in a metabolite that is up to 34 times more inhibitory to AChE

Table 5
Enantioselective activity of some chiral organophosphorus pesticides to non-target organisms.

Organophosphorus pesticide	Selectivity	Optical activity detection[a]	Test species	Comment	References
Methamidophos	(+)>(−)	CD	*Daphnia magna*	Acute toxicity; *in vitro* inhibition of electric eel and bovine erythrocyte acetylcholinesterase inhibition indicate opposite selectivity: (−)>(+)	[33]
Fonofos (Dyfonate)	(−)>(+)	LPD	*Daphnia magna* and *Ceriodaphnia dubia*	Acute toxicity and *in vivo* acetylcholinesterase inhibition	[18,23]
Profenofos	(−)>(+)	LPD	*Daphnia magna* and *Ceriodaphnia dubia*	Acute toxicity and *in vivo* acetylcholinesterase inhibition	[18,23]
	(−)>(+)	LPD	*Oryzias latipes*	*In vivo* acetylcholinesterase inhibition; *in vitro* inhibition of electric eel and human recombinant acetylcholinesterase indicate opposite selectivity: (+)>(−)	[23]
	R-(−)>*S*-(+)		Mouse	Acute toxicity; *in vitro* inhibition acetylcholinesterase indicate opposite selectivity: *S*-(+)>*R*-(−)	[31,67]
Crotoxyphos	(+)>(−)	LPD	*Daphnia magna*	*In vivo* acetylcholinesterase inhibition	[23]
	(+)>(−)	LPD	*Oryzias latipes*	*In vivo* acetylcholinesterase inhibition; *in vitro* inhibition of electric eel and human recombinant acetylcholinesterase indicate opposite selectivity: (−)>(+)	[23]
Trichloronate	(−)>(+)	LPD	*Daphnia magna* and *Ceriodaphnia dubia*	Acute toxicity	[34]
Fenamiphos	(+)>(−)	LPD	*Daphnia magna*	Acute toxicity	[39]
Leptophos	(+)>(−)	LPD	*Daphnia magna*	Acute toxicity	[38]
Isocarbophos	(+)>(−)	LPD	*Daphnia magna*	Acute toxicity	[37]
Malathion	(*R*)>(*S*)			*In vitro* inhibition of human and bovine erythrocyte acetylcholinesterase of oxon	[63]
Fosthiazate	pk1 > pk2 > pk4 > pk3	CD and LPD	*Daphnia magna*	Acute toxicity; *in vitro* inhibition of electric eel acetylcholinesterase not stereoselective; has 4 stereoisomers due to presence of P and C chiral centers; stereoisomers identified based on elution order on Chiralpak® AD column	[35]
Chloramidophos	pk3 > pk2 > pk1 > pk4	CD	*Daphnia magna*	Acute toxicity; has 4 stereoisomers due to presence of P and C chiral centers; stereoisomers identified based on elution order on Chiralpak® AD column	[36]

[a] CD: circular dichroism detector; LPD: laser polarimeter detector.

[67]. The same study also showed the preferential detoxification of (+)-profenofos.

In the case of malathion and malaoxon, chirality is due to an asymmetric carbon center in the thiosuccinyl substituent [5] (Fig. 1). The enantiomers of malathion are also selective in their effects, with the *R*-(+)-enantiomer being more acutely toxic than the *S*-(+)-enantiomer to a variety of anthropods and also to rats [31,68]. In another chiral OP with an asymmetric carbon atom, phenthoate, interspecies difference in its enantioselective toxicity has been reported [31,52]. In most target insects, as well as in mice, (+)-phenthoate is more acutely toxic. On the other hand, the (−) enantiomer is more effective against houseflies [31].

3.2. Enantioselective non-target toxicity

In the previous section we noted that for most chiral OPs, only one of the two enantiomers is often responsible for most or all of the racemate's insecticidal activity (Table 4). The other enantiomer, although inert to the target pest, may end up as an undesirable chemical load to the environment and could be toxic to non-target organisms [6,18]. While OPs are expected to have the same mode of action in both target and non-target organisms [26,31,61], the magnitude of stereoselectivity in enzyme inhibition may vary and the preferred configuration could reverse between animal species or between *in vitro* and *in vivo* determinations for the chiral members of its class [5,31,63,66].

A number of studies have appeared over the past decade to address the enantioselective toxicity of chiral OPs to aquatic organisms [18,23,33–39]. The most recent findings on the enantioselective toxicity of chiral OPs to non-target species are summarized in Table 5.

Similar to the observations for the enantioselectivity of fonofos and profenofos in insecticidal activity, opposite selectivities have been observed for methamidophos, crotoxyphos and profenofos between the *in vivo* acute toxicity and AChE inhibition and the *in vitro* assays for non-target aquatic species [18,23,33] (Table 5). Like profenofos, methamidophos also contains a sulfur substituent to the chiral P in its structure, and may be subject to similar bioactivation processes as previously described here for profenofos [5,67] (Fig. 1).

The (−) enantiomers of fosthiazate were previously shown to have 20-fold higher nematicidal activity than the (+) enantiomers [5]. Fosthiazate contains both a phosphorus chiral center and a carbon chiral center (Fig. 1), but absolute configuration has not been determined [5,35]. Lin et al. [35] resolved the enantiomer pairs of fosthiazate on a Chiralpak® AD column, and distinguished them through both circular dichroism detector and optical rotation detector. The respective enantiomer pairs are (−) and (+) for the first (pk1) and third (pk3) eluting peaks, and (−) and (+) for the second (pk2) and fourth (pk4) eluting peaks, if optical rotation is used to identify the fosthiazate stereoisomers. The opposite is observed with circular dichroism. Thus the authors elected to label

the stereoisomers based on their elution order on the Chiralpak column (Table 5) [35].

It is important to re-emphasize the highly column-specific (CSP-dependent) resolution of many chiral compounds on HPLC [18,69–71]. Researchers must therefore exercise caution, particularly when comparing their toxicity results with that from previous studies that used different chromatographic conditions and optical activity detection methods. At most, these studies may be used to support the occurrence of enantioselectivity in a given effect, but not to ascertain the enantiomer-specific effect. The absolute configuration is a more definitive and consistent identification method. However, in the absence of authentic enantiopure standards the optical detection method and column used for resolving the enantiomers should be qualified.

Inhibitory activity of enantiomers and racemic mixtures of the insecticide fenamiphos to horse serum butyrylcholinesterase (BChE), and its toxicity to *Daphnia pulex* has also been reported. Both enantiomers and the racemate showed significant differences in horse serum BChE inhibition, with the (+)-fenamiphos as more inhibitory than the (−)-fenamiphos and the racemate [39]. In general, however, AChE has been shown to be more stereoselective versus BChE towards the toxic enantiomers of various chiral OP agents [72].

3.3. Biotransformation and environmental degradation

Likely due to their relatively short environmental persistence, there has been little interest in the possible enantioselective transformations of chiral OPs in the environment [26]. Nevertheless, enantioselective degradation of a few chiral OPs has been reported [5,30,35,39].

Partially purified enzyme from *E. coli* was reported to quickly degrade crotoxyphos enantiomers at different rates [30]. Ruelene was also found to degrade selectively in a variety of soils [3]. However, fonofos exhibited non-selective enantiomer losses in a laboratory study with aerobic soil slurries [17]. Similarly, no significant difference in the dissipation of (+)-fenamiphos, (−)-fenamiphos, and racemate was observed in selected soils and natural waters [39].

In contrast, the asymmetric induction (bioconversion of an achiral compound to a chiral compound) has been observed in the metabolic sulfoxidation of microorganisms of the OP insecticide phorate [5]. The use of purified FAD-containing monooxygenase (FMO) and cytochrome P-450 (CYP) isozymes isolated from mouse liver further showed the stereospecificity of oxidation of phorate to the sulfoxide metabolite, with FMO preferentially catalyzing the formation of the (−)-phorate sulfoxide, and the CYP isozymes preferentially catalyzing the formation of the (+) sulfoxide metabolite [5].

Fenthion is another achiral OP compound that undergoes asymmetric sulfoxidation [62,73]. Similar to phorate, fenthion is predominantly converted into (+)-fenthion sulfoxide when incubated with human tissue microsomes [74]. Gadepalli et al. [62] developed an efficient synthesis method of both enantiomers of fenthion sulfoxide, as well as the enantiomers of fenoxon sulfoxide, which allowed for the evaluation of the contribution of FMO-catalyzed sulfoxidation of fenthion in AChE inhibition. Results of *in vitro* assays suggested minimal contribution of sulfur stereochemistry to electric eel and human recombinant AChE inhibition of fenthion sulfoxide isomers [62]. In contrast, fenoxon sulfoxide was stereoselective, with the (*R*)-(+)-fenoxon sulfoxide primarily responsible for AChE inhibition [62].

Overall, stereospecific metabolism may influence the dose–response relationship of chiral OPs. For example, in studies involving the oxidative bioactivation of profenofos, *in vitro* experiments indicated that (+)-profenofos was the more potent inhibitor of AChE. However, the (−) enantiomer was found to be more toxic to mice *in vivo*. Incubation with liver microsomes followed by quantification of AChE inhibition indicated that the individual enantiomers reacted in an opposite manner. The (−)-profenofos underwent preferential metabolic activation and the metabolite formed was 34 times more inhibitory to AChE. In contrast, the (+)-profenofos was preferentially detoxified and its capacity to inhibit AChE was reduced 2-fold [67]. Such shifts in enantioselectivity in AChE inhibition following metabolism were attributed to either differences in rates of oxidation, or the intrinsic potency of metabolite enantiomers.

Finally, the occurrence of non-enzymatic isomerization may also influence the activity and contribute to the unintended effects of chiral pesticides in the environment. For instance, isomalathion is produced by thermal or photochemical isomerization of the widely used OP insecticide, malathion. This molecular rearrangement greatly enhances the inhibitory potency of the parent insecticide toward AChE, owing to an increase in the electrophilicity of phosphorus [75]. In addition, a consequence of the isomerization of malathion is the creation of a new asymmetric center at phosphorus in addition to the existing chiral carbon center in the diethyl thiosuccinyl substituent resulting in four stereoisomers [75,76]. Differences in post-inhibitory kinetics have been observed for AChE inactivated by the stereoisomers of isomalathion [76].

4. Summary and conclusion

Organophosphorus insecticides continue to be an important chemical protection option against agricultural and household pests in the world. The successful enantiomeric resolution of a good number of chiral OPs has made possible the evaluation of enantioselectivity in their activity to both target and non-target species. In comparison, enantioselectivity in their environmental degradation has not been well studied. This is probably due to their relatively low environmental persistence or the lack of reliable techniques for the analysis of chiral OPs in environmental samples. Chiral GC methods have the advantage of high efficiency, sensitivity and reproducibility. However, due to the polar nature and low vapor pressure of most OP pesticides, the need for derivatization complicates enantiomeric analysis of OPs using GC. As such, enantiomeric resolution of chiral OPs is currently best achieved by using the less sensitive enantioselective HPLC approach. To this end, efforts are on the way to improve both techniques through coupling with more sensitive detectors, including, mass spectrometry detectors for more sensitive quantitation and confirmatory detection of analytes. In addition, CE methods have also been explored and are considered to have a promising potential in enantiomer resolution of chiral OPs.

In conclusion, evidence of enantioselectivity in the environmental fate and effects of chiral OPs points to the need for considering enantiomers individually when assessing the environmental risks of chiral OPs. This is contingent with the availability of suitable and reliable analytical techniques.

References

[1] S. Mason, Trends Pharmacol. Sci. 7 (1986) 20.
[2] G.M. Tombo, D. Bellus, Angew. Chem. 30 (1991) 1193.
[3] A.W. Garrison, Green Chem. 6 (2004) G77.
[4] A. Williams, Pestic. Sci. 46 (1996) 3.
[5] M. Sasaki, in: N. Kurihara, J. Miyamoto (Eds.), Chirality in Agrochemicals, Wiley, Chichester, England, 1998, p. 85.
[6] A.W. Garrison, Environ. Sci. Technol. 40 (2006) 16.
[7] C. Wong, Anal. Bioanal. Chem. 386 (2006) 544.
[8] E. Ulrich, Chimica Oggi 25 (2007) 37.
[9] V.C. Anigbogu, H. Woldeab, A.W. Garrison, J.K. Avants, Int. J. Environ. Anal. Chem. 83 (2003) 89.
[10] H. Huhnerfuss, M.R. Shah, J. Chromatogr. A 1216 (2009) 481.

[11] K. Moller, C. Bretzke, H. Huhnerfuss, R. Kallenborn, J.N. Kinkel, J. Kopf, G. Rimkus, Angew. Chem. 106 (1994) (1994) 882.
[12] S. Tanabe, P. Kumaran, H. Iwata, R. Tatsukawa, N. Miyazaki, Mar. Pollut. Bull. 32 (1996) 27.
[13] M.D. Muller, H. Buser, Environ. Sci. Technol. 31 (1997) 1953.
[14] H. Buser, M. Muller, Environ. Sci. Technol. 32 (1998) 626.
[15] T. Bidleman, R. Falconer, Environ. Sci. Technol. 33 (1999) 206.
[16] D. Lewis, A. Garrison, K. Wommack, A. Whittemore, P. Steudler, J. Melillo, Nature 401 (1999) 898.
[17] J. Jarman, W. Jones, L. Howell, A.W. Garrison, J. Agric. Food Chem. 53 (2005) 6157.
[18] W. Liu, J. Gan, D. Schlenk, W. Jury, PNAS USA 102 (2005) 701.
[19] S. Qin, R. Budd, S. Bondarenko, W. Liu, J. Gan, J. Agric. Food Chem. 54 (2006) 5040.
[20] W. McBlain, Life Sci. 40 (1987) 215.
[21] P. Hoekstra, B. Burnison, T. Neheli, D. Muir, Toxicol. Lett. 125 (2001) 75.
[22] P. Wang, S. Jiang, D. Liu, H. Zhang, Z. Zhou, J. Agric. Food Chem. 54 (2006) 1577.
[23] M.G. Nillos, G. Rodriguez-Fuentes, J. Gan, D. Schlenk, Environ. Toxicol. Chem. 26 (2007) 1949.
[24] B.J. Konwick, A.W. Garrison, M.C. Black, J.K. Avants, A.T. Fisk, Environ. Sci. Technol. 40 (2006) 2930.
[25] J.P. Overmyer, D.R. Rouse, J.K. Avants, A.W. Garrison, M.E. Delorenzo, K.W. Chung, P.B. Key, W.A. Wilson, M.C. Black, J. Environ. Sci. Health Part B 42 (2007) 471.
[26] N. Scholz, W. Hopkins, Environ. Toxicol. Chem. 25 (2006) 1185.
[27] M. Sasaki, Phosphorus Sulfur Silicon 183 (2008) 291.
[28] F. Spurlock, M. Lee, in: J. Gan, F. Spurlock, P. Hendley, D. Weston (Eds.), Synthetic Pyrethroids: Occurrence and Behavior in Aquatic Environments, ACS Symposium Series 991, ACS, Washington, DC, 2008, p. 3.
[29] B. Singh, A. Walker, FEMS 30 (2007) 428.
[30] J. Ellington, J. Evans, K. Prickett, W. Champion, J. Chromatogr. A 928 (2001) 145.
[31] N. Kurihara, J. Miyamoto, G.D. Paulson, B. Zeeh, M.W. Skidmore, R.M. Hollingworth, H.A. Kuiper, Pure Appl. Chem. 69 (1997) 2007.
[32] N. Fidalgo-Used, E. Blanco-Gonzales, A. Sanz-Medel, Talanta 70 (2006) 1057.
[33] K. Lin, S. Zhou, C. Xu, W. Liu, J. Agric. Food Chem. 54 (2006) 8134.
[34] W. Liu, K. Lin, J. Gan, Chirality 18 (2006) 1.
[35] K. Lin, F. Zhang, S. Zhou, W. Liu, J. Gan, Z. Pan, Environ. Toxicol. Chem. 26 (2007) 2339.
[36] S. Zhou, K. Lin, H. Yang, L. Li, W. Liu, J. Li, Chem. Res. Toxicol. 40 (2007) 400.
[37] K. Lin, W. Liu, L. Li, J. Gan, J. Agric. Food Chem. 56 (2008) 4273.
[38] J. Yen, C. Tsai, Y. Wang, Ecotoxicol. Environ. Safety 55 (2003) 236.
[39] Y. Wang, K. Tai, J. Yen, Ecotoxicol. Environ. Safety 57 (2004) 346.
[40] B. Waldeck, Pharmacol. Toxicol. 93 (2003) 203.
[41] S. Gorog, Trends Anal. Chem. 26 (2007) 12.
[42] B. Testa, P.A. Carrupt, J. Gal, Chirality 5 (1993) 105.
[43] V.S. Lamzin, Z. Dauter, K.S. Wilson, Curr. Opin. Struct. Biol. 5 (1995) 830.
[44] M. Reist, P.A. Carrupt, E. Francotte, B. Testa, Chem. Res. Toxicol. 11 (1998) (1998) 1521.
[45] V. Wsol, L. Skalova, B. Szotakova, Curr. Drug Metab. 5 (2004) 517.
[46] I. Agranat, H. Caner, J. Caldwell, Nat. Rev. 1 (2002) 753.
[47] I. Ali, V.K. Gupta, H.Y. Aboul-Enein, Crit. Rev. Anal. Chem. 38 (2008) 132.
[48] C. Garcia-Ruiz, G. Alvarez-Llamas, A. Puerta, E. Blanco, A. Sanz-Medel, M.L. Marina, Anal. Chim. Acta 543 (2005) 77.
[49] N. Fidalgo-Used, M. Montes-Bayon, E. Blanco-Gonzalez, A. Sanz-Medel, J. Anal. Atom. Spectrom. 21 (2006) 876.
[50] N. Diaz-Alejo, E. Vilanova, J. Chromatogr. 622 (1993) 179.
[51] C.E. Kientz, J.P. Langerberg, J. High Resolut. Chromatogr. 14 (1991) 461.
[52] Z.Y. Li, Z.C. Zhang, L. Zhang, L. Leng, Bull. Environ. Contam. Toxicol. 79 (2007) 153.
[53] Z.Y. Li, Z.C. Zhang, Q.L. Zhou, Q.M. Wang, R.Y. Gao, Q.S. Wang, J. AOAC Int. 86 (2003) 521.
[54] Ph. Schmitt, A.W. Garrison, D. Freitag, A. Kettrup, J. Chromatogr. A 792 (1997) 419.
[55] L. Huang, J. Lin, L. Xu, G. Chen, Electrophoresis 28 (2007) 2758.
[56] W. Liu, J. Gan, Agric. Food. Chem. 52 (2004) 755.
[57] R.Y. Gao, G.S. Yang, H.Z. Yang, Z.Y. Chen, Q.S. Wang, J. Chromatogr. A 763 (1997) 125.
[58] I.H. Hardt, C. Wolf, B. Gehrcke, D.H. Hochmuth, B. Pfaffenberger, H. Huhnerfuss, J. High Resolut. Chromatogr. 17 (1994) 859.
[59] I. Ali, K. Saleem, I. Hussain, V.D. Gaitonde, H.Y. Aboul-Enein, Sep. Purif. Rev. 38 (2009) 97.
[60] R.Y. Gao, H.F. Wang, Z.Y. Chen, H.Z. Yang, Q.S. Wang, Chromatographia 51 (2000) 322.
[61] J. Battershill, P. Edwards, M. Johnson, Food Chem. Toxicol. 42 (2004) 1279.
[62] R.S.V. Gadepalli, J.M. Rimoldi, F.R. Fronczek, M.G. Nillos, J. Gan, X. Deng, G. Rodriguez-Fuentes, D. Schlenk, Chem. Res. Toxicol. 20 (2007) 257.
[63] O.P. Rodriguez, G.W. Muth, C.E. Berkman, K. Kim, C. Thompson, Bull. Environ. Contam. Toxicol. 58 (1997) 171.
[64] P. Lee, R. Allahyari, T.R. Fukuto, Pestic. Biochem. Physiol. 8 (1978) 146.
[65] P. Lee, R. Allahyari, T.R. Fukuto, Pestic. Biochem. Physiol. 8 (1978) 158.
[66] H. Leader, J. Casida, J. Agric. Food Chem. 30 (1982) 546.
[67] K. Wing, A. Glickman, J. Casida, Science 219 (1983) 63.
[68] A. Hassan, W. Dauterman, Biochem. Pharmacol. 17 (1968) 224.
[69] W. Liu, J. Gan, S. Qin, Chirality 17 (2005) S127.
[70] Y. Jin, W. Wang, C. Xu, Z. Fu, W. Liu, Aquat. Toxicol. 88 (2008) 146.
[71] Y. Jin, J. Chen, L. Sun, W. Wang, L. Zhou, W. Liu, Z. Fu, Chemosphere 74 (2009) 1238.
[72] O. Cohen, C. Kronman, L. Raveh, O. Mazor, A. Ordentlich, A. Shafferman, Mol. Pharmacol. 70 (2006) 1121.
[73] O. Bawardi, J. Rimoldi, D. Schlenk, Pestic. Biochem. Physiol. 88 (2007) 321.
[74] B. Furnes, D. Schlenk, Toxicol. Sci. 78 (2004) 196.
[75] S. Jianmongkol, B.R. Marable, C.E. Beckman, T.T. Talley, C.M. Thompson, R.J. Richardson, Toxicol. Appl. Pharmacol. 155 (1999) 43.
[76] J.T.T. Doorn, C. Thompson, R. Richardson, Chem. Res. Toxicol. 14 (2001) 807.